年報 Annual Report 2011

近畿大学医学部消化器内科学教室

医局員集合写真

第6回　Kinki GUT Club　平成24年1月15日

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2011 年 Annual Report の発刊にあたって

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1.はじめに

2011 年の教育、研究、診療の実績をお届けします。近畿大学医学部に消化器内科学教室が新設されたのは平成 11 年 4 月であります。従って平成 24 年 3 月でやっと丸 13 年が経過したことになります。開設当初は医局のスペースも 2 部屋のみでスタッフ 8 名、研修医 6 名、計 14 名での出発でありました。現在では狭山の本院に籍を置くスタッフは約 40 名、また堺病院や奈良病院もそれなりに人材・設備共に整いつつあります。

しかしながら、新設私立医大の宿命かもしれませんが、2004 年より始まった新臨床研修医制度の余波をまともに受けて消化器内科も毎年 5～6 人から 12～13 人入局していた入局者数が最近では激減し、また次第に開業あるいは結婚退職、郷里への U ターンなどの退職者も増え、現在はなお厳しい状況に置かれているというのが現状です。ただし、ここ数年は 2-4 人の入局がコンスタントに続いており、良い傾向が見え始めました。今後の更なる消化器内科学教室の発展のために医局員一同が一致団結して診療、研究、教育活動に専念していかなければならない重要な時期であると考えております。

2.診療活動

別添えの資料をご覧頂ければ一目瞭然でありますが、消化器内科の年間の入院及び外来収入、及びそれを合計した総収入は平成 11 年の開設初年度は約 8 億程度でありましたが、平成 23 年には 30 億円を超える収入となっており、病院経営にも多大の貢献をしております。平成 23 年度は病院全体も約 15 億の黒字決算となる見込みですが、消化器内科の貢献も大きいと考えております。また一日平均入院患者数も年間を通して 80 人前後、平均在院日数も 8 日を切っており極めて多忙な診療活動を行っていることがおわかり頂けると思います。腹部超音波検査の件数も確実に右肩上がりであり、内視鏡の件数も総件数が平成 22 年度は 20,125 例と着実に上昇を示しております。また、肝癌に対するラジオ波治療（RFA）の総件数も多く、日経新聞や朝日新聞、読売新聞、週刊朝日等にも度々取り上げられ、総件数としては連続 7 年以上、日本国内の 2 位（内

科と外科の件数、及び転移性肝癌を含めて）に位置づけられるという実績を残しております（581 ページ参照)。ラジオ波は平成 11 年 6 月より開始し、平成 23 年 12 月末の時点で総件数 4,000 例に達しており、5 年生存率は 70%強であり、手術とほぼ同等の治療成績が得られております。インターフェロン治療の実績でも PEG インターフェロン・リバビリン併用療法の実績は全国ランキング 5 位以内でした（2 社の合計分)。現在、C 型肝炎治療を積極的に行っており、大阪南部から C 型肝炎・肝癌を根絶したいと願っています。

平成 15 年度に導入した早期胃癌に対する内視鏡的粘膜下層切開剥離術（ESD）も確実に症例数が増え、今後も益々増え続けていくものと考えております。もちろん、ESD 関連の研究論文も少しずつ増えていっております。また、高度先進医療としての大腸 ESD も樫田教授の尽力で平成 22 年より開始され、症例も増加しています。また従来より行っていた胆膵グループによる超音波内視鏡検査の件数も増加しています。これを機に平成 23 年 11 月 27 日には近畿消化器内視鏡ライブコースも近畿大学で開催され、300 人の参加者を得て成功裏に終わりました。平成 23 年度には内視鏡室が光学治療センターに格上げとなり、スペースの拡充も予定されております。さらには腹部超音波室も拡充され 1 階へ移動し、平成 23 年 10 月 3 日から稼働を開始しました。これらのことは病床数の増加とともに消化器内科にとっては今後ますます発展する素地ができあがりつつあると考えます。

御承知のように大和川以南は一般に「南大阪」と呼ばれておりますが、その南大阪の人口は約 260 万にも達しております。その 260 万人の医療圏の中で大学医学部は近畿大学のみであります。その意味でこの 260 万人の方々の健康を守り病からの回復の重責を我々こそが担っているのだという自負と自覚をもってこれからも良い医療を行っていきたいと考えております。

3.教育活動

教育は当然のことながら大学医学部の役割の極めて根幹を占める重要な部分であります。消化器内科学は消化器コースの内の肝臓の責任科であり、肝臓のユニットを 1 週間担当している他、上部消化管、下部消化管、胆膵のユニットや臨床腫瘍コースならびに画像診断のコースでも講義を担当しております。更には病因・病態のコースの 3 週間のうち 1 週間の責任科として大変多忙な教育活動を行っております。5 年生 6 年生のクリニカルクラークシップも例年 6

年生を常時 6 人程度受け入れており、講義や総括など充実した bed side 教育となるよう全力を尽くしております。

平成 20 年 10 月から（私の意志ではありませんでしたが）病院長に任ぜられましたのでその公務のために教育活動の多くの部分を北野准教授、松井講師はじめ多くの講師の先生方にご負担をおかけすることになってしまい、申し訳なく思っております。消化器コース及び病因・病態コースあるいは日々のクリニカルクラークシップ等の教育活動では決して手を抜かず積極的に行っていくつもりですので何卒ご容赦下さい。この紙面をお借りして感謝とお詫びを申し上げたいと思います。

3. 研究活動

（1）論文業績

英文論文の発表は 1999 年消化器内科の設立当初は一桁台でありましたが、年と共に確実に増加し、3 年目からは平均 20 編以上の英文論文がコンスタントに出るようになりました。2010 年の英文論文数は 51 編に達しました。残念ながら 2011 年は 47 編にとどまりましたが､何とか毎年 50 編の大台を超えたいと祈念しております。また 13 年間の総インパクトファクターは 1149.677 点であり英文総論文数は 384 編ですので、近畿大学消化器内科のような小さな所帯の教室としてはまずまずの結果を残せているのではないかと思っております。来年以降も最低、英文原著論文は 50 編以上を目標に頑張っていきたいと考えておりますので教室員の皆様の自覚と更なる奮闘を期待致しております。

（2）厚生労働省科学研究費補助金事業研究班の活動

平成 22 年度に採択された厚労科研（がん臨床部門）**「進行・再発肝細胞癌に対する動注化学療法と分子標的薬併用による新規治療法の確立を目指した臨床試験（Phase III）ならびに効果を予測する biomarker の探索研究」**（工藤班）の主任研究者として日本発のエビデンスを創出すべく、努力しています。また平成 23 年度には厚労科研（難病･がん等の疾患分野の医療の実用化部門）**「慢性ウイルス性肝疾患の非侵襲的線化評価法の開発と臨床的有用性の確立」**（工藤班）の主任研究者としても採択され、多くの大学との協同研究を開始しています。またその他にも下記の厚労科研の分担研究者として教室の先生方に実務を担当して頂いております。この場をお借りして感謝申し上げます。

① 「血小板低値例へのインターフェロン治療法の確立を目指した基礎および臨床的研究」（西口班）（厚労科研）
② 「抗悪性腫瘍薬による肝炎ウイルス再活性化の調査とその対応に関する研究」（池田班）（国立がん研究センターがん研究補助金）
③ 「初発肝細胞癌に対する肝切除とラジオ波焼灼両方の有効性に関する多施設共同研究」（國土班）（厚労科研）
④ 「肝がんの新規治療法に関する研究」（本多班）（厚労科研）
⑤ 「多発肝のう胞症に対する治療ガイドライン作成と試料バンクの構築」（大河内班）（厚労科研）
⑥ 「進行肝胆膵がんの治療法の開発に関する研究」（奥坂班）（国立がん研究センターがん研究補助金）
⑦ 「C 型肝炎ウイルス（ＨＣＶ）陽性進行肝臓がん症例に対するテーラーメイドがんペプチドワクチンの第Ⅱ相単盲検無作為割付群間比較臨床試験」（文科省知的クラスター事業）

（3）今後の研究の方向性

今年の消化器内科の論文も一覧するとやはりまだまだ Impact factor の高い雑誌に掲載されているのは少ないようです。やはり Impact factor 15 点以上の雑誌を目指すには prospective な比較試験など中・長期的な視野に立った研究計画を組んで質の高い臨床研究を進めて行くことが現時点での我々に課せられた最も大きな課題と考えております。臨床試験については 2008 年 9 月 11 日に大阪府より認証を受けた NPO 法人「日本肝がん臨床研究機構（JLOG）」を中心に現在 7 つの prospective study が走っております。そのうち 3 つが厚労科研に採択されたため現在では 4 つの前向き試験を行っております。これからも世界へ向けて発信できるような成果を出して行くつもりでおります。もちろん、retrospective な解析研究で新しいデータを publish していくという努力も今後も続けていかなければなりません。

また基礎研究の分野でも京都大学から赴任された西田直生志准教授、櫻井俊治講師、萩原智講師を中心に積極的に研究を進めて頂いており、今後の publication を期待しております。

もう一つの重要な点は私が常日頃申し上げておりますように症例観察の重要性であります。臨床においては一例一例がたとえ同じ病名であったとして

も一例として同じ症例はありません。同じ病気でも一つとして全く同一であるということはなく、何か異なるメッセージを発信しているのです。そのことを的確にキャッチすることにこそ意味があるという目で一例一例の患者さんを注意深く診療し観察していくことこそが最も大事であると考えています。そのような注意深い観察から新しい臨床的な発見も生まれてきますし、また逆にそのような観察眼が生まれる素地としては臨床家として真面目に臨床と向き合って最高の level に到達している必要があります。そのような点で日々の臨床の現場には”clinical pearl”とでも言うべきものがあちこちに転がっている、まさに宝の山であります。そのような理由で症例観察に基づいたケースレポートを書くということも極めて、その本人の勉強になることはもちろんのこと、今後の新しい疾患概念の確立、新しい治療法の着想などに結びつき得る重要な姿勢であると思われます。残念ながら、ケースレポートは最近の Impact factor 重視主義の多くの Journal から採用されない傾向にはありますが、それでも short report や Letter to the Editor などとしては採用されますので業績をあげるという目的ではなく、症例をキチンと観察・整理して document していくという姿勢に立つことは重要であります。すなわち症例の観察研究を報告することは我々、アカデミアに籍を置く者に課せられた使命であると自覚すべきと考えております。

ここまで読まれた方は最初に私が述べたような大規模な前向きな比較試験を行うべきということと症例の観察研究とでは全く正反対の次元の違うことを述べているように思われるかもしれません。しかしこの 2 つは臨床を知り尽くし、かつ、臨床をじっくり真面目にやっている医師にしかできないことであるという点で共通していることであります。基礎研究あるいは臨床に結びつくかもしれない基礎研究までは MD ではなくとも PhD でも実行可能なことであり、その field ではしばしば PhD の方が quality の高い研究成果を上げ得るかも知れません。しかしながら、臨床の疑問点にもとづいた基礎研究もしくは本当に臨床に直結するような基礎研究や症例の観察研究、および大規模臨床試験などはその価値を知り得る MD にしかできないことであることは間違いありませんし、それらを遂行し得るのは患者さんと日々正面から向き合っている最高水準の医師にしかできない研究であります。そのような点でこの二つは決して矛盾するものではありませんし、両方ともに臨床家こそがやるべき研究であります。

以上、述べた 2 つの異なったアプローチは、我々の教室の研究の方向性として今後も積極的に実行して行きたいと思っております。繰り返しになりま

すが、臨床的な発想に基づく、あるいは臨床に本当に必要な基礎的データを抑えるという研究は、大変重要ですのでそれらは引き続き継続していかなくてはなりません。

2009年に立ち上げた日本肝がん分子標的治療研究会（第1回研究会: 2010年1月16日、参加者450人）は年2回開かれております。肝癌はこれからは分子標的治療の時代ですので永井先生が分子標的薬の研究でゲノム生物学教室で頑張ってくれているのは大変私にとって嬉しい限りです。特許も出願することが出来ましたし、Impact Factorが7以上の雑誌にもこの分野で2-3編通りました。臨床的ニーズに基づいた基礎研究で成果を上げることほどエキサイティングなことはありません。是非とも近畿大学から肝がんに関して臨床に貢献できる基礎的エビデンスを次々と発信して行きたいと心から願っています。

(3) Research Conference

現在消化器内科では定期の各グループの臨床カンファレンスに加え、毎週火曜日の早朝の1時間みっちりとResearch Conferenceを行っております。このカンファレンスでは全て英語でPresentationからDiscussionまでを行っております。ほとんど1年を通じて海外からの留学生がおりますし、特筆すべき点としてこれまではアジアの留学生が中心でしたが平成22年はイタリア人のDr. Lorenzoがapplyしてできたことです。これも日本における肝細胞癌研究のleading centerとしてヨーロッパの国からも認知され始めている証拠であると思いますので大変喜ばしいと思っております。平成23年には世界で最も古い歴史のあるイタリアボローニャ大学のProf. Bolondiの教室からDr. Albertoがやってきて3か月の研修を終えて帰りました。そのような留学生にも配慮してResearch Conferenceは英語で行っておりますが、やはりこのEnglish Research Conferenceというのが消化器内科が行っているカンファレンスの中でも最も重要であると考えております。もちろん、このカンファレンスへの出席は本人の自発的意欲に基づくものではありますが、毎週多くの教室員に参加して頂いております。以下にこの数年の出席率を示しますが、出席率の高い医局員ほどやはり研究に対するactivityが高い傾向にあると感じておりますので今後も引き続き積極的に参加して頂きたいと思っております。

副次的な効果としてこのカンファレンスを通じて海外で英語でDiscussionできる英語力や自信も自然と磨かれるものと確信しております。

English Research Conference 出席状況

教室員	2008		2009		2010		2011	
	出席回数	出席率	出席回数	出席率	出席回数	出席率	出席回数	出席率
工藤	27/27	100	20/20	100	29/29	100	23/23	100
樫田	−	−	−	−	12/19	63	20/23	87
北野	25/27	93	16/20	80	21/29	72	21/23	91
西田							5/5	100
松井	22/27	81	18/20	90	23/29	79	23/23	100
上嶋	6/27	22	3/20	15	12/29	41	7/23	30
鄭	23/27	85	15/19	79	26/29	90	9/9	100
櫻井	−	−	−	−	17/19	89	20/23	87
南	19/24	79	1/2	50	−	−	13/14	93
井上	17/25	68	16/20	80	25/29	86	21/23	91
萩原	11/24	46	5/20	25	9/29	31	11/23	48
矢田	7/11	64	14/19	74	26/29	90	14/23	61
北井	15/27	56	8/20	40	15/29	52	13/23	57
朝隈	16/27	59	12/20	60	11/29	38	15/23	65
坂本	19/27	70	1/2	50	12/19	63	11/23	48
小牧	18/27	67	13/20	65	20/29	69	11/20	55
永井	18/19	95	17/20	85	14/29	48	12.5/23	54
上田	15/27	56	6/20	30	11/29	38	1/9	11
川崎	23/27	85	6/20	30	4/29	14	6/23	26
早石	10/19	53	9/19	47	5/29	17	8/23	35
田北	1/2	50	13/20	65	15/29	52	7/23	30
今井	−	−	9/15	60	18/29	62	10/23	43
永田	−	−	13/15	87	16/29	55	12/23	52
有住	−	−	7/15	47	15/29	52	17/23	74
鎌田	−	−	11/15	73	15/29	52	10/23	43
高山	−	−	4/7	57	−	−	13/14	93

宮田	−	−	9/15	60	16/29	55	14.5/23	63
峯	−	−	9/15	60	17/29	59	17/23	74
足立	−	−	−	−	−	−	8/14	57
大本	−	−	−	−	−	−	12/14	86
門阪	−	−	−	−	−	−	12/14	86

4.学会活動および海外における活動

2011 年における国内の学会発表については 102 演題、国際学会の発表については 66 演題、海外特別講演は 33、国内特別講演は 75 でありました。私自身の海外出張は 2011 年は 20 回とこれもやや例年より多くなっております。

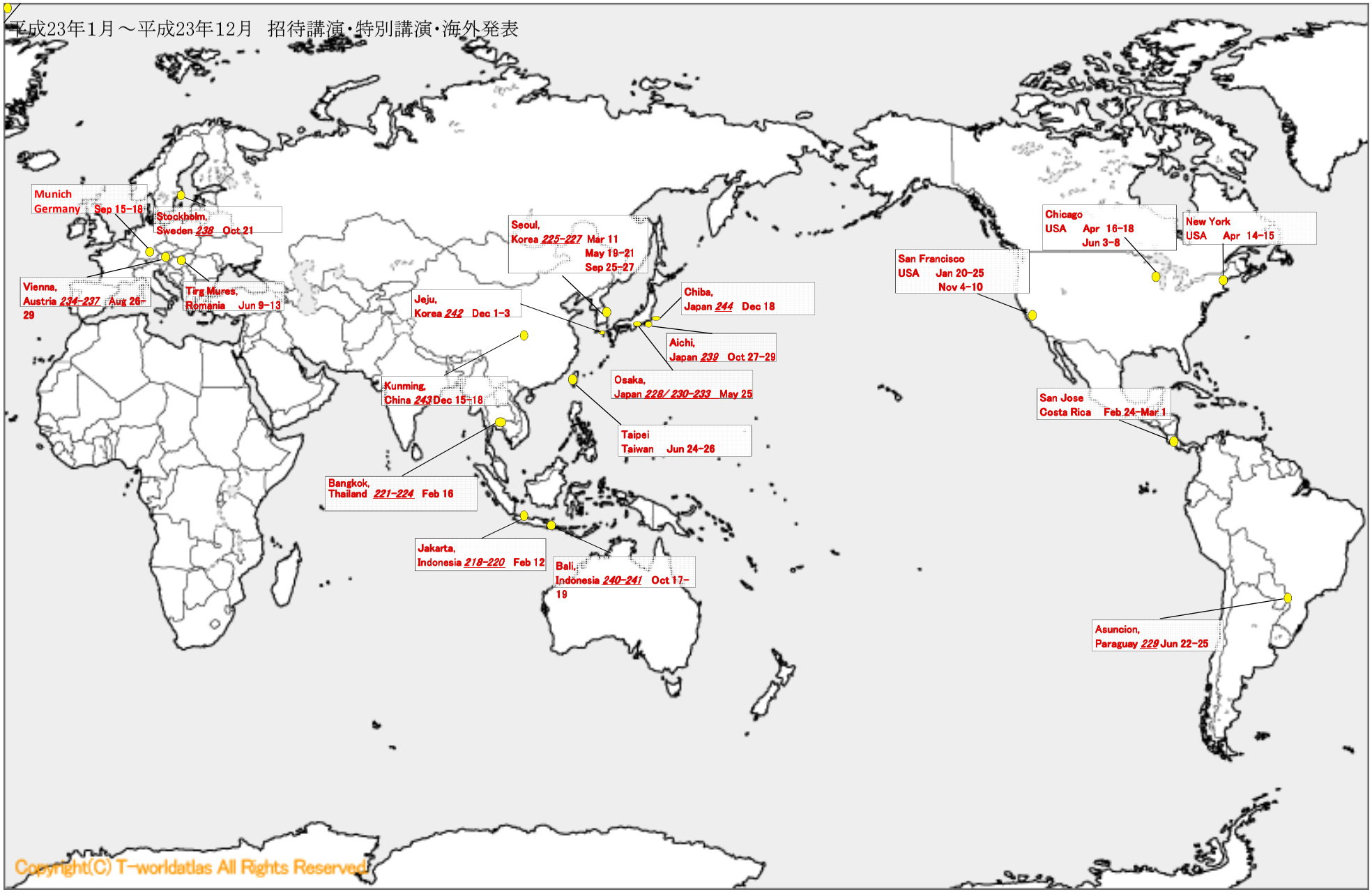
2011 年

1. 1 月 20 日-25 日　ASCO-GI に出席
   (San Francisco, USA)
2. 2 月 12 日-14 日　世界超音波医学会（WFUMB）
   Center of Excellence (COE) Workshop にて 3 Lecture
   (Jakarta, Indonesia)
3. 2 月 15 日-16 日　世界超音波医学会（WFUMB）
   Asia Project Workshop にて 3 Lecture
   (Bangkok, Thailand)
4. 2 月 24 日-3 月 1 日　世界超音波医学会（WFUMB）理事会
   (San Jose, Costa Rica)
5. 3 月 11 日-12 日　Asan Medical Center, Liver Institute Opening Symposium
   Invited Speaker
   (Seoul, Korea)
6. 4 月 14 日-15 日　米国超音波医学会（AIUM）
   名誉会員賞（Honorary Fellow）受賞式出席
   (New York, USA)
7. 4 月 16 日-18 日　GIDEON Steering Committee Meeting 出席
   (Chicago, New York, USA)
8. 5 月 19 日-21 日　韓国超音波医学会（KSUM）

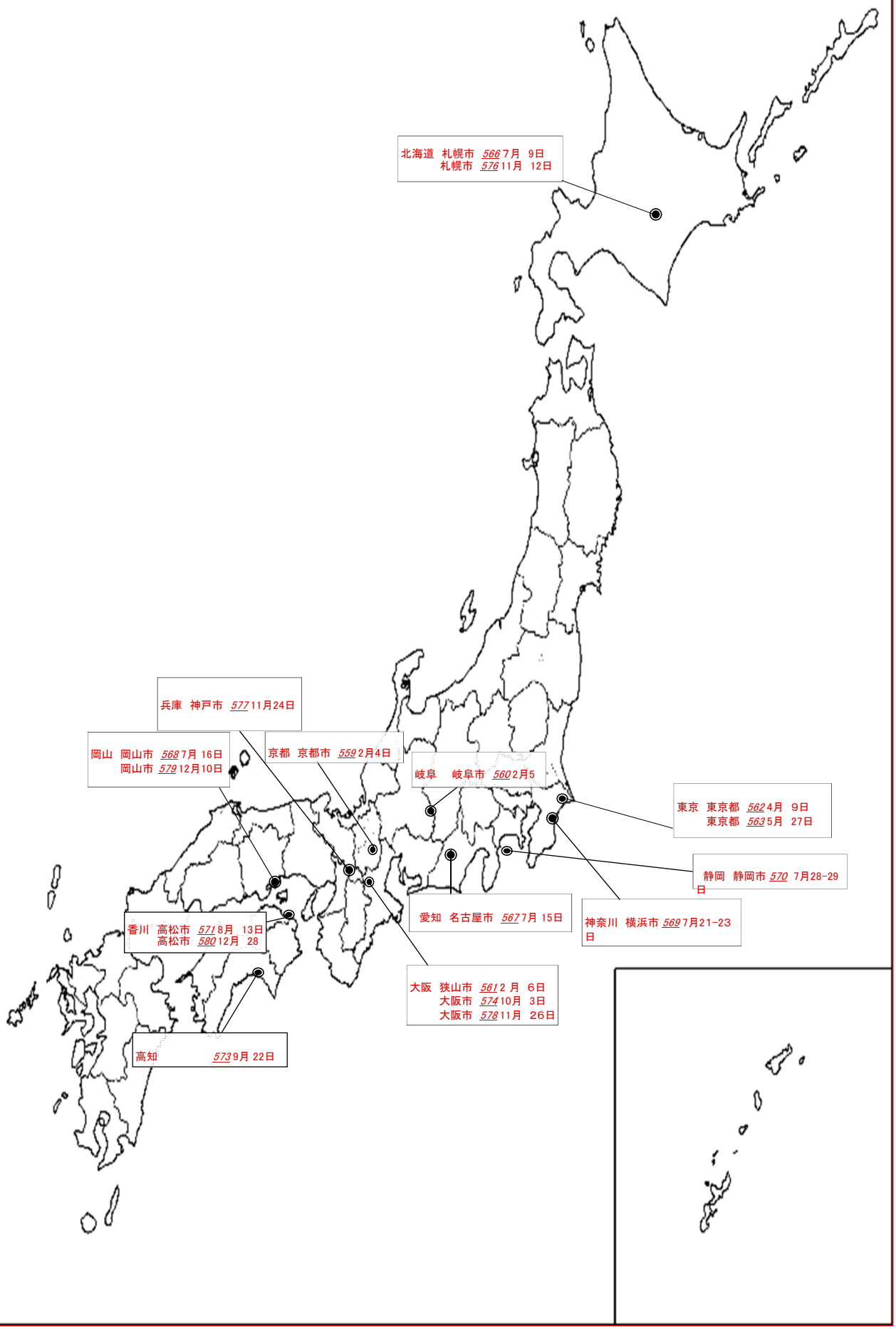
	招待講演 (Seoul, Korea)
9. 6 月 3 日-8 日	ASCO 参加 Advisory Board Meeting 出席 (Chicago, USA)
10. 6 月 9 日-13 日	世界超音波医学会（WFUMB） Center of Excellence (COE) Workshop 招待講演 2 題 (Tirg Mures, Romania)
11. 6 月 24 日-26 日	台湾肝臓学会（Taiwan Association of Study of the Liver: TASL） (Taipei, Taiwan)
12. 8 月 24 日-30 日	世界超音波医学会（WFUMB）学術会議 3 Lecture, 2 Chair, 2 Council meeting, 1 General Assembly President として参加 (Vienna, Austria)
13. 9 月 1 日-4 日	国際肝癌学会（International Liver Cancer Association）, Council Member として参加 2 Lectures, 1 Chair (Hong Kong, China)
14. 9 月 15 日-18 日	EOB Liver Forum 1 Lecture (Munich, Germany)
15. 9 月 25 日-27 日	Yonsei University Samsung Medical Center (Seoul, Korea)
16. 10 月 23 日-26 日	United European Gastroenterology Week (UEGW) ヨーロッパ消化器病週間 2 Lectures, 1 Chair (Stockholm, Sweden)

17. 11 月 4 日-10 日	American Association of the Study of Liver (AASLD) 米国肝臓学会 2 oral presentations (San Francisco, USA)
18. 11 月 18 日-21 日	Asian Federation for the Societies of Ultrasound in Medicine and Biology (AFSUMB) Council Meeting Workshop にて 3 Lecture Site inspection (Bali, Indonesia)
19. 12 月 1 日-3 日	Asia-Pacific Association of Study of the Liver (APASL) アジア太平洋肝臓学会 Single Topic Conference on HCC Lecture (Jeju, Korea)
20. 12 月 17 日-18 日	3rd Conference International Contrast Ultrasound Imaging 1 Lecture (Kunming, China)

平成23年1月～平成23年12月　招待講演・特別講演・海外発表



平成23年1月～平成23年12月　招待講演・特別講演



5. 学会主催

今年の学会主催としては 7 月 1 日-3 日に「第 2 回アジア太平洋肝がん専門家会議（Asia-Pacific Liver Cancer Expert Meeting: APPLE）をハイアットリージェンシー大阪にて開催させて頂き、約 500 名の参加者を得て盛大裏に行われました。Invited Speaker は別表の如くです。また 8 月 20 日に日本消化器病学会近畿支部第 95 回例会を開催させて頂き、約 1,000 名の参加者を得て、成功裏に終わりました。また翌日の 8 月 21 日はウイルス肝炎と肝がんの理解のための市民公開講座を大阪国際交流センターにて開催させて頂きました。幕内雅敏先生、國土典宏先生、泉並木先生、仁科亜季子さんをゲストに迎え、約 1,000 名の市民の皆様にご参加頂き、大成功に終わりました。この時の様子は朝日新聞紙面（610-611 ページ参照）をご覧ください。また、11 月 27 日は第 1 回近畿消化器内視鏡ライブコースを開催し、胃の ESD、大腸の ESD、総胆管結石の除去、膵の造影 EUS の症例などでライブデモを行いました。約 300 名の参加者がありました。

別表: Invited speakers at the 2nd APPLE Meeting (alphabetical order)

Western Speakers (18)	Asian Speakers (20)	Japanese Speakers (21)
Jacques Belghiti (France)	Subrat Acharya (India)	Yasuhiro Asahina
Adrian Michael Di Bisceglie (USA)	Oidov Baatarkhuu (Mongolia)	Yasuaki Arai
Luigi Bolondi (Italy)	Ding-Shinn Chen (Taiwan)	Takafumi Ichida
John FP Bridges (USA)	Pei-Jer Chen (Taiwan)	Tomoaki Ichikawa
Jordi Bruix (Spain)	Ann-Lii Cheng (Taiwan)	Namiki Izumi
Richard S. Finn (USA)	Pierce K.H. Chow (Singapore)	Shuichi Kaneko
Jean-Francois H. Geschwind (USA)	Kwang-Hyub Han (Korea)	Kazuhiko Koike
Peter R. Galle (Germany)	Yun Hwan Joseph Kim (Korea)	Shigehiro Kokubu
Riccardo Lencioni (Italy)	Jeong Min Lee (Korea)	Norihiro Kokudo
Josep M. Llovet (Spain/USA)	Sung-Gyu Lee (Korea)	Masatoshi Kudo
Vincenzo Mazzaferro (Italy)	Laurentius A. Lesmana (Indonesia)	Masatoshi Makuuchi
Valerie Paradis (France)	Shi-Ming Lin (Taiwan)	Osamu Matsui
Lewis R. Roberts (USA)	Sheng-Nan Lu (Taiwan)	Kazuto Nishio
Riad Salem (USA)	Joong-Won Park (Korea)	Kiwamu Okita
Myron E. Schwartz (USA)	Young Nyun Park (Korea)	Takuji Okusaka
Morris Sherman (Canada)	Hunchul Rhim (Korea)	Masao Omata
Augusto Villanueva (Spain)	Hui-Chuan Sun (China)	Michiie Sakamoto
Andrew X. Zhu	Hee Jung Wang (Korea)	Shuichiro Shiina
	Sheng-Long Ye (China)	Kenichi Takayasu
	Jian Zhou (China)	Ryosuke Tateishi
		Kazuomi Ueshima

6. 留学生受け入れ

留学生の受け入れですが、1999 年から 2000 年にかけて中国上海から

Ding Hong 先生（丁　紅）（上海医科大学)、2001 年には中国広州から Wen YL 先生（文　艶玲）（中山医科大学)、2002 年には中国広州から Zheng RQ 先生（鄭栄琴）（中山医科大学)、2003 年には中国重慶より Zhou Pei（周　佩）（人民解放軍重慶病院)、2004 年にはカンボジアより Ly Sokhey 先生、2005 年にはタイから Worawan Chinamnan 先生、同じく 2005 年に若干時期を違えてインドから Kaushal Madan 先生（All India Institute of Medical Science: AIIMS)、2007 年 Kunal Das 先生を受け入れました。2008 年 Yu Xia（北京、中国)、2009 年 Md. Nadiruzzaman（バングラディシュ)、2010 年 Lorenzo Andreana（イタリア）が来ていました。またエジプトから Alshimaa 先生も来られました。2011 年にはマレーシアから Hadzri 先生が来られましたし、またイタリア　ボローニャ大学からも Alberto 先生が来られました。このように毎年、留学生が日中友好協会、笹川財団や日本消化器病学会、日本超音波医学会のフェローシップ留学生あるいは自国での fund をもって私どもの教室を希望して頂き、受け入れてきました。また来年度以降も先生方にはご迷惑をお掛けするかと思いますが、これも国際交流、アジアや世界への日本の貢献、各々の英語力に磨きをかけるという意味で有益と思いますので何卒御理解・御協力のほどお願い申し上げます。

7. 人事について

冒頭でも述べましたが、2003 年までの入局者は毎年 5、6 名～12、13 名と大学内でも最も多くの入局者がおりましたが、2004 年に新臨床研修医制度が開始されてからの入局者、すなわち 2006 年の入局者は 2 名に留まり、2007 年の入局者も 1 名に留まりました。2008 年には 8 名もの入局者が入って来られました。2010 年には 3 名の新入局者とともに 4 月 1 日には樫田博史先生に臨床教授として来て頂きました。2011 年は 3 名の研修医が入局し、2012 年には 2 名が入局予定です。反面、2-3 人の方が医局を離れました。従いまして依然、医局としての体制は大変厳しい状況にあります。このような状況の中で南大阪では大阪大学や大阪市大、和医大、奈良医大などがそれぞれの大学に人を引き上げているという状況のため、消化器内科医が激減し、南大阪の多くの公的病院では消化器内科医がほとんどゼロの状態が続いております。そのあおりで近医からの紹介患者や外来患者数は激増し、消化器内科の診療にも大きな負担がかかっております。しばらくはこのような状況が続くものと思われるますので、本

学ならびに分院の奈良病院、堺病院ともに結束して一人でも多くの人に入局して頂き、教育・研究・診療を円滑に行っていきたいと考えております。

8. NPO 法人「日本肝がん臨床研究機構（Japan Liver Oncology Group）」の活動

1. **JLOG 0801 trial**「肝癌早期診断のための多施設共同無作為化比較試験（Sonazoid-Enhanced LivEr Cancer Trial for Early Detection (SELECTED Study)）」
2. **JLOG 0901 trial** 「進行・再発肝細胞癌に対する動注化学療法と分子標的薬併用による新規治療法の確立を目指した臨床試験（Phase III）ならびに効果を予測する biomarker の探索研究（Randomized Controlled Trial Comparing Efficacy of Sorafenib versus Sorafenib In combination with Low dose cisplatin/fluorouracil hepatic arterial InfUSion chemotherapy in Patients with Advanced Hepatocellular Carcinoma And Explolatory Study of Biomarker Predicting Its Efficacy (SILIUS Phase III trial)）」

→**2010 年より厚労科研に移行**（厚生労働省科学研究費補助金　厚生労働省科学研究費補助金事業研究班（がん臨床部門）平成 23 年度「進行・再発肝細胞癌に対する動注化学療法と分子標的薬併用による新規治療法の確立を目指した臨床試験（Phase III）ならびに効果を予測する biomarker の探索研究」（工藤班））

3. **JLOG 0902 trial**「早期肝癌診断における EOB-MRI の有用性に関する多施設共同研究（Diagnosis of Early LIver Cancer Through EOB-MRI(DELICATE Study)）」
4. **JLOG 1001 trial** 「切除不能肝細胞癌に対する肝動脈化学塞栓療法（TACE）とソラフェニブの併用療法第 II 相臨床試験（Phase II study: Transcatheter Arterial Chemoembolization Therapy In Combination with Sorafenib (TACTICS Study)）」
5. **JLOG 1002 trial**「慢性肝疾患における非侵襲的弾性検査法を用いた肝線維化評価予測に関する研究(Assessment of Liver FIBROsis by Real-time Tissue ELASTography in Chronic Liver Disease (FIBROELAST Study))」

→**2011 年より厚労科研に移行**（厚生労働省科学研究費補助金事業研究班（難病・

がん等の疾患分野の医療の実用化部門）平成 23 年度「慢性ウイルス性肝疾患の非侵襲的線化評価法の開発と臨床的有用性の確立」（工藤班））

6. **JLOG 1003 trial**「非侵襲的弾性検査法を用いた肝線維化度評価によるウイルス性肝炎患者における肝発癌・門脈圧亢進症の発現予測（Prediction of Incidence of Liver Cancer or porTal Hypertension in Patients with Viral Hepatitis by Use of Real-time Tissue Elastography (PICTURE Study)）」

→**2011 年より厚労科研に移行**（厚生労働省科学研究費補助金事業研究班（難病・がん等の疾患分野の医療の実用化部門）平成 23 年度「慢性ウイルス性肝疾患の非侵襲的線化評価法の開発と臨床的有用性の確立」（工藤班））

7. **JLOG 1004 trial**「インスリン抵抗性を合併する C 型代償性肝硬変患者を対象とした BCAA 顆粒製剤の肝細胞癌抑制効果に関する第 III 相臨床試験（BCAA Granule for patients with Hepatitis C-related Liver Cirrhosis and Insulin Resistance On the Effect of Reduction of Carcinogenic RisK in the Liver(Phase III study)(BLOCK Study)）」

9. おわりに

この年報を作成にあたりましては例年の如く、教授秘書、医局秘書の秘書連合軍の 15 名の皆様に全面的に編集をして頂き大変感謝を致しております。また、医局員の皆様にも大変この一年お世話になりました。この一年間も大変なハードワークではありましたが、無事皆様の頑張りにより乗り切ることができました。この場をお借りして深く感謝申し上げます。2010 年には病床数も 76 床から 85 床への増床、念願の一病棟まるまる消化器内科が占めるという状態が実現しましたし、腹部超音波室も拡充されました。光学治療センターの拡充計画も決定しておりますので何卒昨年以上にモチベーションを上げて頂いて日本一、あるいは世界一の消化器内科学教室へ育つようにご尽力頂きたいと思います。2012 年も教育・診療・研究において、特に英文論文、新しい研究の立ち上げ（種蒔き）ということについては 2011 年以上に積極的に取り組んでいきたいと考えておりますので医局員全員が共通の価値観と消化器内科の将来の方向性に対するベクトルを共有し、心を一つにして邁進して頂きたいと祈念・期待しております。

2012 年 4 月　大阪狭山にて

2011 年度表彰式一覧

- **Highest Impact Factor Award 2011（最高インパクトファクター賞）**

1 位　工藤可苗　7.338（Clin Cancer Res）
2 位　坂本洋城　5.608（Gastrointest Endosc）

※　3 位　永井知行　5.225（Mol Cancer Ther）
※　工藤正俊　6.882（Am J Gastroenterol）

- **Most Numbers of Paper Award 2011（最多英文論文発表賞）**

1 位　櫻井俊治　3 本（Pancreatology, Oncology×2）
2 位　北野雅之　2 本（Pancreatology, Digest Endosc）
2 位　南　康範　2 本（Int J Hepatol, World J Gastroenterol）
2 位　坂本洋城　2 本（Gastrointest Endosc, Pancreatology）

※　工藤正俊　12 本

- **Total Highest Impact Factor Award 2011（累積最高インパクトファクター賞）**

1 位　坂本洋城　7.736（2 本）
2 位　工藤可苗　7.338（1 本）

※　3 位　櫻井俊治　7.204（3 本）
※　工藤正俊　28.714（12 本）

- **最多入院受持患者賞**

1 位　宮田　剛　306 人
2 位　矢田典久　282 人

※　3 位　今井　元　227 人

- **最多緊急内視鏡賞**

1 位　今井　元　26 件
2 位　鎌田　研　22 件

※　3 位　有住忠晃　21 人

- **最多外来患者診療賞**

1 位　萩原　智　1,471 人
2 位　松井繁長　1,026 人

※ 3 位　矢田典久　998 人
※ 工藤正俊　1,281 人

工藤正俊（くどうまさとし）

（平成 24 年 3 月 1 日更新）



昭和 29 年	愛媛県西条市生まれ
昭和 53 年	京都大学医学部 卒業
同	京都大学医学部附属病院 勤務（研修医）
昭和 54 年	神戸市立中央市民病院内科 勤務（研修医）
昭和 55 年	同 消化器内科 医員
昭和 60 年	同 消化器内科 副医長
昭和 62 年	カリフォルニア大学留学（デービスメデイカルセンター）
平成元年	神戸市立中央市民病院消化器内科 副医長 復職
平成 4 年	同 消化器内科 医長
平成 9 年	近畿大学医学部第２内科学 助教授
平成 11 年	近畿大学医学部消化器内科学 教授 現在に至る
	Website（ www.med.kindai.ac.jp/shoukaki/ ）
（現在の併任）	近畿大学医学部附属病院病院長（平成 20 年 10 月 1 日～現在）
	高度先端総合医療センター長（平成 22 年 10 月 1 日-現在）
	光学治療センター長（平成 22 年 10 月 1 日-現在）
	中央臨床検査部長（平成 22 年 10 月 1 日-現在）
	心身医療センター長（平成 23 年 4 月 1 日-現在）
	近畿大学医学部奈良病院消化器内科 教授（兼務）
	近畿大学医学部堺病院消化器科 教授（兼務）
	神戸市立中央市民病院消化器内科 顧問（兼務）

主な所属学会

日本消化器病学会（財団評議員・指導医・専門医）、日本肝臓学会（理事・指導医・専門医・治験支援委員会委員長・倫理委員会副委員長・肝移植検討委員会委員）、日本消化器内視鏡学会（評議員・指導医・専門医）、日本超音波医学会（理事・指導医・専門医・国際交流委員会委員長）、日本内科学会(評議員・認定内科医)、日本核医学会(評議員・専門医)、日本肝癌研究会（常任幹事・追跡調査委員長・取扱規約委員長・肝癌治療効果判定基準作成委員会委員長・事務局代表)、日本臨床腫瘍学会、日本肝移植研究会（世話人)、肝血流動態イメージ研究会（幹事)、日本腹部造影エコー・ドプラ診断研究会(事務局・世話人)、肝癌治療シミュレーション研究会(副代表幹事・企画委員)、超音波治療研究会（常任世話人)、日本肝がん分子標的治療研究会（代表世話人・事務局代表)、日本消化器内視鏡財団(評議員)、日本癌学会(評議員)、米国肝臓学会（AASLD）（肝癌部門企画運営委員: Steering Committee of hepatobiliary malignancy)、米国消化器病学会（AGA)、世界肝臓学会 （IASL)、欧州肝臓学会（EASL）、米国消化器内視鏡学会（ASGE)など。

国際学会役員・委員

- 世界超音波医学会（WFUMB） President（理事長）
- アジア超音波医学会（AFSUMB）Treasurer（財務担当理事）
- 世界肝癌学会（ILCA）理事（Founding Board Member, Governing Board Council Member）
- 米国肝臓学会（AASLD）肝癌部門運営委員会委員（Steering Committee Member）
- 世界保健機構（WHO）Blue Book「Classification of the Tumor」改訂委員（平成 21 年 5 月 1 日）
- ウイルス肝炎研究財団 日米医学協力研究会肝炎専門部会研究員
- International Liver Thought Leadership Study (ILCS), Council member

受賞

- 米国核医学会 Berson-Yalow Award 受賞（平成元年 6 月）
- 日本対がん協会がん研究助成奨励賞 受賞（平成 4 年 3 月）
- 日本消化器病学会奨励賞 受賞（平成 4 年 4 月）
- 日本核医学会賞 受賞（平成 5 年 10 月）
- 米国超音波医学会（AIUM）学会賞受賞（平成 15 年 6 月 4 日）
- ボローニャ大学医学部医学会名誉会員賞（平成 18 年 9 月 15 日）
- フィリピン超音波医学会名誉会員（Honorary Member of PSUCMI）（平成 20 年 3 月 19 日）
- アジア太平洋消化器病学会（APDW）OKUDA Award 受賞（平成 20 年 9 月 13 日）
- 北米放射線学会 Certificate of Merit 受賞（平成 20 年）
- インド肝臓学会 Madangopalan Award 受賞（平成 21 年 3 月 28 日）
- 北米放射線学会 Cum Laude 賞受賞（平成 21 年 12 月）（7000 編の論文中上位 10 編に採択）
- 日本肝臓学会「日本肝臓学会機関誌 Highest Citation 賞」受賞（平成 22 年 6 月）
- JISAN Lecture Award Presented by Korean Society of Ultrasound in Medicine（平成 22 年 5 月）
- 米国超音波医学会名誉会員賞（AIUM Honorary Member Award）受賞（平成 23 年 4 月）
- 韓国超音波医学会名誉会員賞（KSUM honorary Award）受賞（平成 23 年 5 月）
- 日本肝臓学会「日本肝臓学会機関誌 Highest Citation 賞」受賞（平成 23 年 6 月）（2 回目）
- Romanian Society of Ultrasound in Medicine and Biology (SRUMB) Honorary Award 受賞（平成 23 年 6 月）
- 北米放射線学会 Certificate of Merit 受賞（平成 23 年 11 月）（2 回目）

著書（単著）

- Contrast Harmonic Imaging in the Diagnosis and Treatment of Liver Tumors （Springer-Verlag　2003）
- 肝腫瘍における造影ハーモニックイメージング（医学書院　2001）

編集

- 松井　修，**工藤正俊**,編集：消化器疾患の造影エコーUp Date．南江堂，東京，2003.
- **工藤正俊**，編集：肝細胞癌治療の最近の進歩，消化器病セミナー97，へるす出版，東京，2004.
- 河田純男，白鳥康史，**工藤正俊**，榎本信幸，編集，小俣政男，監修：肝疾患 Review 2004，日本メディカルセンター，東京，2004.
- 河田純男，白鳥康史，**工藤正俊**，榎本信幸，編集，小俣政男，監修：肝疾患 Review 2006-2007,日本メディカルセンター，東京，2006.
- 河田純男，横須賀收，**工藤正俊**，榎本信幸，編集，小俣政男，監修：肝疾患 Review 2008-2009，日本メディカルセンター，東京，2008.
- 河田純男，横須賀收，**工藤正俊**，榎本信幸，編集，小俣政男，監修：肝疾患 Review 2010-2011，日本メディカルセンター，東京，2010.
- 幕内雅敏，菅野健太郎，**工藤正俊**，編集：今日の消化器疾患治療指針　第3版，医学書院，東京，2010.
- **工藤正俊**，泉　並木，編集：症例から学ぶ ウイルス肝炎の治療戦略．　（株）診断と治療社，東京，2010.
- **工藤正俊**，編集：肝細胞癌の分子標的治療，アークメディア，東京，2010.
- 山雄健次，**工藤正俊**，編集：見逃し、誤りを防ぐ!肝・胆・膵癌画像診断アトラス，羊土社，東京，2010.
- **工藤正俊**，編集：朝倉内科学，「肝胆膵疾患」，朝倉書店，東京，2010.
- **工藤正俊**，編集：医学のあゆみ「肝癌の分子標的治療」，医歯薬出版株式会社，東京，2011.
- **工藤正俊**，編集:「肝細胞がん診療の進歩：Up-To-Data」,最新医学社，大阪，2011.

学術雑誌編集委員

- Editor-in-Chief

  World Journal of Hepatology (China)
  Liver Cancer (Karger)

- Guest Editor-in-Chief

Intervirology	Vol 47,	2004
Intervirology	Vol 48,	2005
Oncology	Vol 72,	2007
Digestive Deases	Vol 25,	2007
Oncology		2008
Intervirology		2009
Oncology		2010
Pancreatology		2011
Digestive Disease		2011
Digestion		2011
Oncology		2011

- Associate Editor

Journal of Oncology (Germany)
Ultrasound in Medicine and Biology (New York)
World Journal of Gastroenterology (China)
World Journal of Radiology (China)
肝胆膵画像編集幹事（医学書院, 東京）
肝胆膵編集委員（アークメディア, 東京）

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Hepatology Research (Tokyo)
Journal of Gastroenterology (Tokyo)
International Journal of Clinical Oncology (Tokyo)
Ultrasound in Medicine and Biology (New York)
Hepatology International (New York)
Liver International (New York)
Open Hepatology Journal

- Journal Referee

J Clin Oncol(18.970) , Lancet Oncol (17.764) , Gastroenterology(12.032), Hepatology(10.885) , J Hepatol(9.334), Oncologist(5.826), Am J Gastroenterol(6.882), Endoscopy(6.096), Clin Exp Metastas(4.113), Cancer Sci(3.846) , Expert Rev Mol Diagn(4.652) , Eur Radiol(3.594) , Liver Int(3.840), J Gastroenterol(3.610) , Eur J Clin Invest(2.736), J Nucl Med(7.022), J Gastroen Hepatol(2.410), Oncology-Basel (International Journal of Cancer Research and Treatment)(2.538), Ultrasound Med Biol(2.493) , Acta Paediatr(1.955), Hepatol Int(2.963) , Eur J Gastroen Hepat (1.598) , J Hepato-Bil-Pan Scu (1.963), Hepatol Res(1.857), Int J Clin Oncol(1.437), Jpn J Clin Oncol(1.856), Internal Med(1.037), J Clin Ultrasound(0.808), Biomark Med(1.247), Hepato-Gastroenterol(0.677), Ann Nucl Med(1.386), Expert Review of Anticancer Treatment(0), J Cancer Res Ther (0.825), CSR National Registry(0), J Gastrointest Liver (1.434), Cancer Informatics(0), Expert Review of Proteomics and Future Oncology(0)
日本核医学会雑誌「核医学」(東京)
日本消化器病学会雑誌(東京), 日本肝臓学会誌「肝臓」(東京)
日本超音波医学会機関誌「超音波医学」 (東京)
日本内科学会英文誌 「Internal Medicine」(Tokyo)
日本老年医学会誌
日本消化器内視鏡学会誌
「最新医学」(大阪)

研究業績

- 発表論文（英文） 404 編（総 IF=1,254.523）（内、著書分担執筆 20 編）
- 発表論文（和文） 970 編（内、著書・分担執筆 235 編）
- 特別講演（国内学会） 583 件
- 特別講演（国際学会） 250 件
- 学会発表（国内学会） 1,637 件
- 学会発表（国際学会） 313 件

科学研究費等外部資金の獲得状況

- 文部科学省科学研究費補助金　　基盤研究(A)　1 件（総額 1,000 万円）
  基盤研究(B)　6 件（総額 2,311 万円）
  基盤研究(C)　10 件（総額 1,210 万円）
  挑戦的萌芽研究　2 件（総額　200 万円）
- （主任研究者）　（　150 万円）
  「肝細胞癌の発癌・進展の分子機序: 造影超音波クッパー相と遺伝子発現を用いた融合解析」
- （分担研究者）　（　50 万円）
  「肝細胞癌のソラフェニブ著効例における感受性規定遺伝子変異の探索」（主任研究者　西尾和人）
  知的クラスター創生事業（がんペプチドワクチン）　1 件（総額　10 万円）
- 車両財団がん研究助成金　1 件（総額　100 万円）
- 学会奨励研究補助金　6 件（総額　530 万円）
- 医師会・民間医学振興財団等研究補助金　32 件（総額 2,089 万 5 千円）
- 国立がん研究センターがん研究開発費（分担研究者）　（　245 万円）
  「抗悪性腫瘍薬による肝炎ウイルス再活性化の調査とその対応に関する研究」（班長　池田公史）
- 国立がん研究センターがん研究開発費（分担研究者）　（　12 万円）
  「進行肝胆膵がんの治療法の開発に関する研究」（班長　奥坂拓志）

- 厚生労働省科学研究費　**主任研究者**　3 件（総額 1 億 3,675 万円）
  1.（がん臨床研究事業）
  「進行・再発肝細胞癌に対する動注化学療法と分子標的薬併用による新規治療法の確立を目指した臨床試験（Phase III）ならびに効果を予測する biomarker の探索研究」
  2.（難病・がん等の疾患分野の医療の実用化研究事業）
  「慢性ウイルス性肝疾患の非侵襲的線化評価法の開発と臨床的有用性の確立」

- 厚生労働省科学研究費　**分担研究者**　27 件（総額 3,175 万円）
  1.（肝炎等克服緊急対策研究事業）
  「血小板低値例へのインターフェロン治療法の確立を目指した基礎および臨床的研究」（班長　西口修平）
  2.（がん臨床研究事業）
  「初発肝細胞癌に対する肝切除とラジオ波焼灼両方の有効性に関する多施設共同研究」（班長　國土典宏）
  3.（肝炎等克服緊急対策研究事業）
  「肝がんの新規治療法に関する研究」（班長　本多政夫）
  4.（難治性疾患克服研究事業）
  「多発肝のう胞症に対する治療ガイドライン作成と試料バンクの構築」（班長　大河内信弘）
  5.（難病・がん等の疾患分野の医療の実用化研究事業）
  「慢性ウイルス性肝疾患患者の情報収集の在り方等に関する研究」（班長　相崎英樹）

ガイドライン策定委員会委員

- 「科学的根拠に基づく肝癌診療ガイドライン」（日本肝臓学会編），金原出版
- 「「慢性肝炎の治療ガイドライン」（日本肝臓学会編），文光堂
- 「肝癌診療マニュアル」（日本肝臓学会編），医学書院
- 「肝癌治療効果判定基準」（日本肝癌研究会取扱い規約委員会編），肝臓
- 臨床病理「肝癌取り扱い規約」（日本肝癌研究会編）
- Clinical Practice Guidelines for Hepatocellular Carcinoma, Japan Society of Hepatology, Hepatology Research
- General Rules for the Clinical and Pathological Study of Primary Liver Cancer, 3rd English Version, Liver Cancer Study Group of Japan, Kanehara, Tokyo, 2010
- Response Evaluation criteria in the Cancer of the Liver (RECICL), Liver Cancer Study Group of Japan, Hepatology Research

特許取得

発明の名称：　ソラフェニブの効果予測方法
出願番号：　特願 2011-104275
出願日：　2011 年 5 月 9 日
発明者：　荒尾徳三、松本和子、西尾和人、工藤正俊
出願人：　学校法人近畿大学

発明の名称：　N 型糖鎖を利用した膵臓癌の診断方法
公開番号：　特許公開 2009-270996
公開日：　2009 年 11 月 19 日
発明者：　荒尾徳三、松本和子、西尾和人、坂本洋城、北野雅之、工藤正俊
出願人：　住友ベークライト株式会社

全国規模の学会・研究会事務局

・ 日本肝癌研究会（**事務局**・追跡調査委員長）
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・ NPO 法人日本肝がん臨床研究機構（**理事長・事務局**）
・ 日本肝がん分子標的治療研究会（**代表世話人・事務局**）

全国規模の研究会世話人・役員

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・平成 9 年–現在　京都肝疾患懇話会世話人
・平成 9 年–現在　肝臓分子生物学研究会
・平成 11 年–平成 18 年　肝代謝コロキウム世話人
・平成 11 年–現在　大阪肝胆膵懇話会世話人
・平成 11 年–現在　南大阪肝胆膵疾患研究会世話人
・平成 11 年–現在　南大阪消化器病懇話会世話人
・平成 11 年–現在　南大阪肝疾患研究会世話人
・平成 11 年–現在　消化器ラウンドテーブルディスカッション世話人

・平成 11 年–平成 18 年　泉州肝臓病研究会世話人
・平成 11 年–平成 18 年　大阪肝炎ミーティング世話人
・平成 12 年–現在　大阪肝臓病談話会世話人
・平成 12 年–現在　関西経皮内視鏡的胃瘻造設術研究会世話人
・平成 12 年–現在　肝疾患座談会 in Kyoto 世話人
・平成 12 年–現在　近畿肝癌談話会常任幹事
・平成 13 年–現在　関西肝血流動態イメージ研究会世話人
・平成 16 年–現在　あおい肝臓研究会世話人
・平成 18 年–現在　大阪肝臓ミーティング世話人
・平成 19 年–現在　近畿・超音波内視鏡研究会顧問

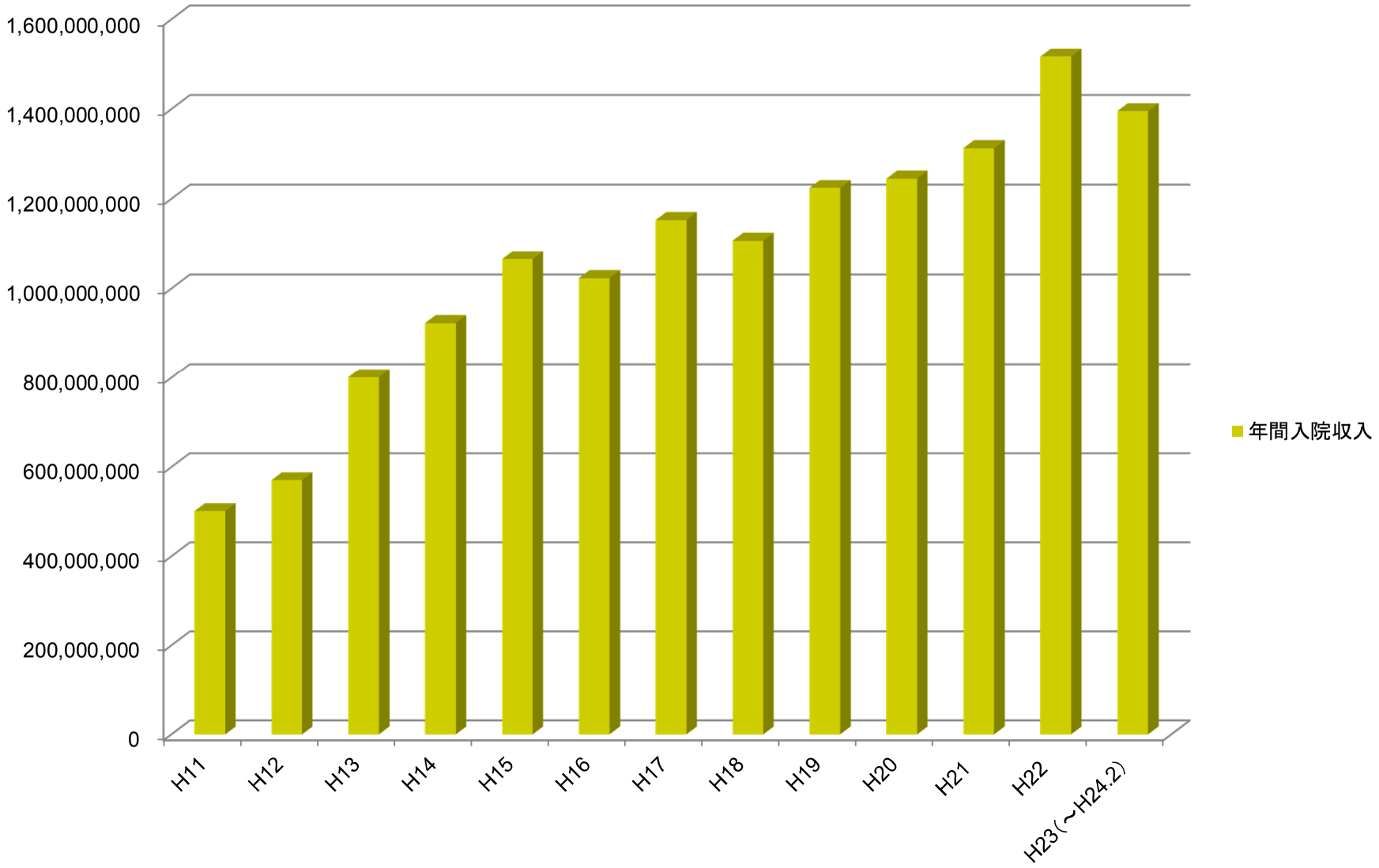
消化器内科学教室業績抜粋

	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	計
英文論文 (著書・分担執筆を含む) (Impact Factor)	8 40.281	12 36.067	27 56.229	13 18.596	22 48.048	21 68.517	25 56.834	15 35.094	31 93.084	45 139.421	26 94.146	51 127.498	48 124.201	39 221.667	383 1159.683
和文論文 (著書・分担執筆を含む)	37	41	43	34	31	54	45	39	46	73	81	130	65	80	799
海外学会発表	2	9	4	6	24	23	14	14	17	26	20	35	66	11	271
国内学会発表	46	56	71	113	105	79	69	52	79	87	66	96	102	25	1046
海外特別講演	0	11	4	11	8	18	16	25	18	36	34	43	35	6	265
国内特別講演	37	40	40	52	37	38	39	27	36	39	62	93	75	17	632
単著教科書			1		1 (英文)										2

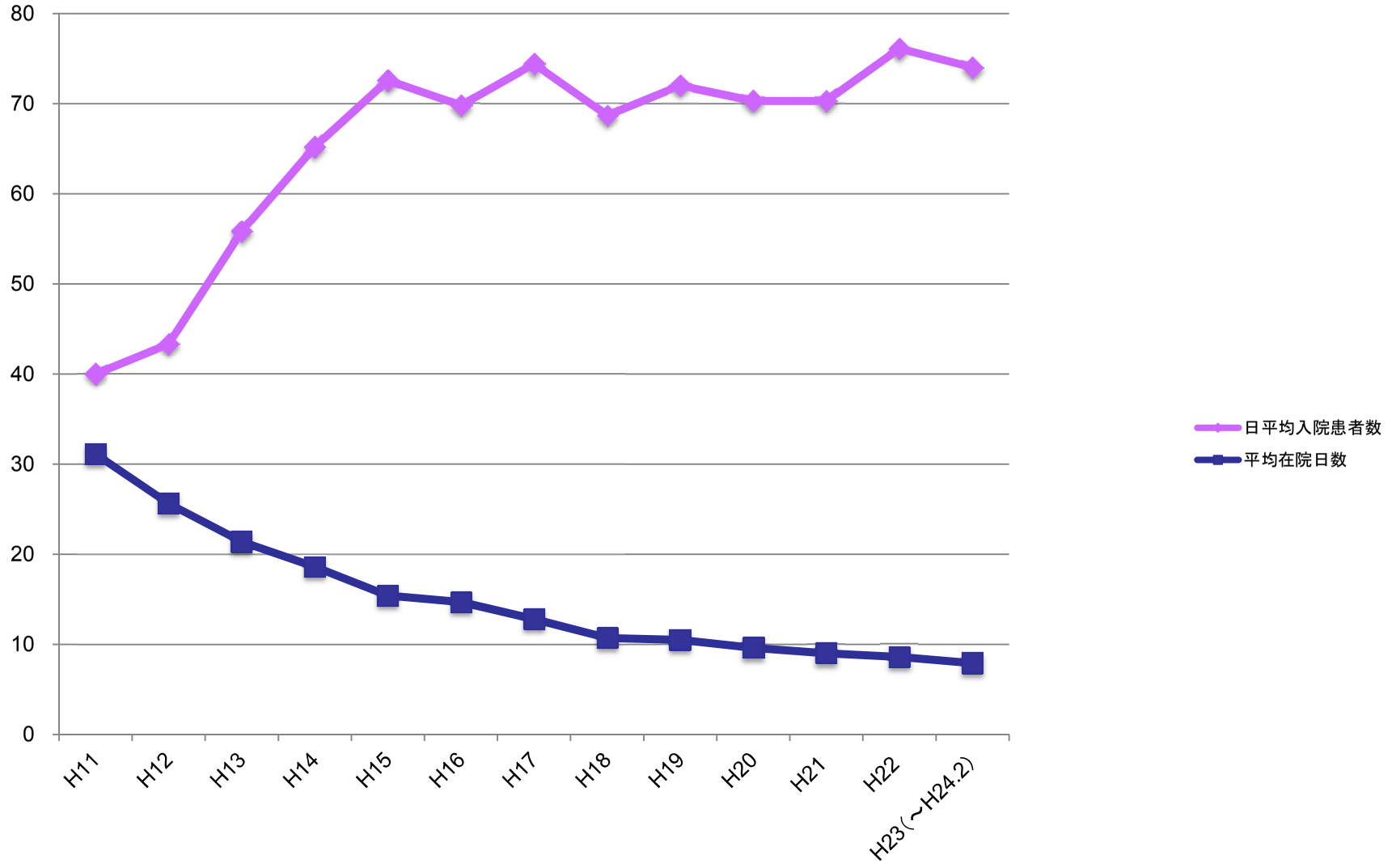
消化器内科年度別診療実績

	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23 (～H24.2)
稼働床	40	44	44	44	60	78	78	77	76	73	85	84	84
稼働率	107.2%	98.5%	126.7%	148.2%	121.0%	89.5%	95.3%	89.2%	94.7%	96.3%	91.8%	89.9%	88.1%
日平均 入院患者数	40	43.3	55.8	65.2	72.6	69.8	74.4	68.7	72.0	70.3	70.3	76.1	74.0
平均 在院日数	31.1	25.6	21.4	18.6	15.4	14.7	12.8	10.7	10.5	9.6	9	8.6	7.9
年間 入院収入	501,570,188	570,616,464	801,199,124	923,171,333	1,065,481,449	1,023,271,279	1,152,778,111	1,106,484,453	1,224,122,968	1,244,806,271	1,312,812,506	1,516,925,835	1,395,479,987
年間 外来収入	314,641,639	334,517,979	386,084,329	530,035,297	635,562,806	649,876,475	818,049,485	966,247,389	1,013,910,559	1,257,804,553	1,432,350,698	1,464,645,183	1,341,129,451
消化器内科 年間収入	816,211,827	905,134,443	1,187,283,453	1,453,206,630	1,701,044,255	1,673,147,754	1,970,827,596	2,072,731,842	2,238,033,527	2,502,610,824	2,745,163,204	2,981,571,018	2,736,609,438

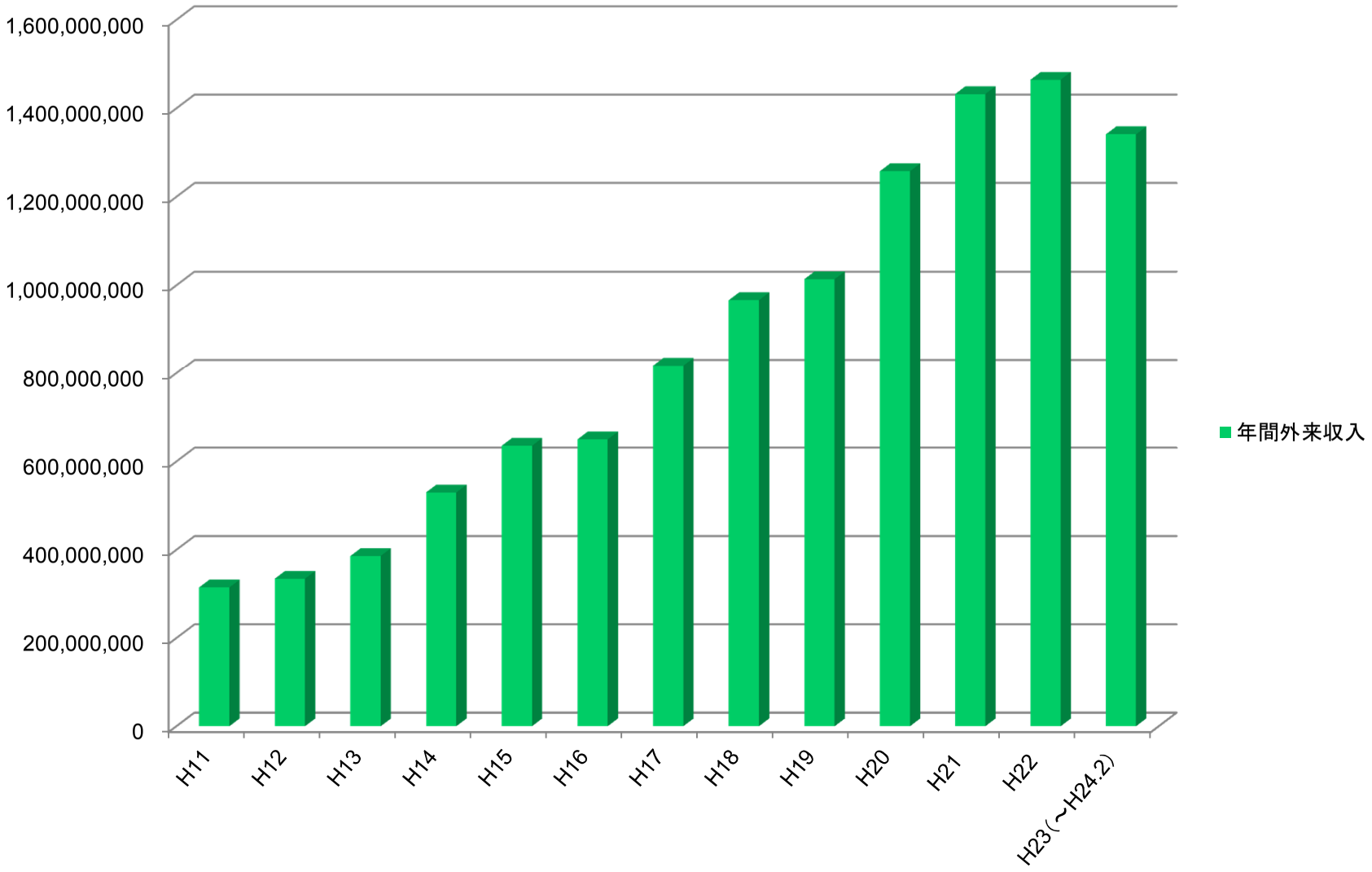
消化器内科年間入院収入



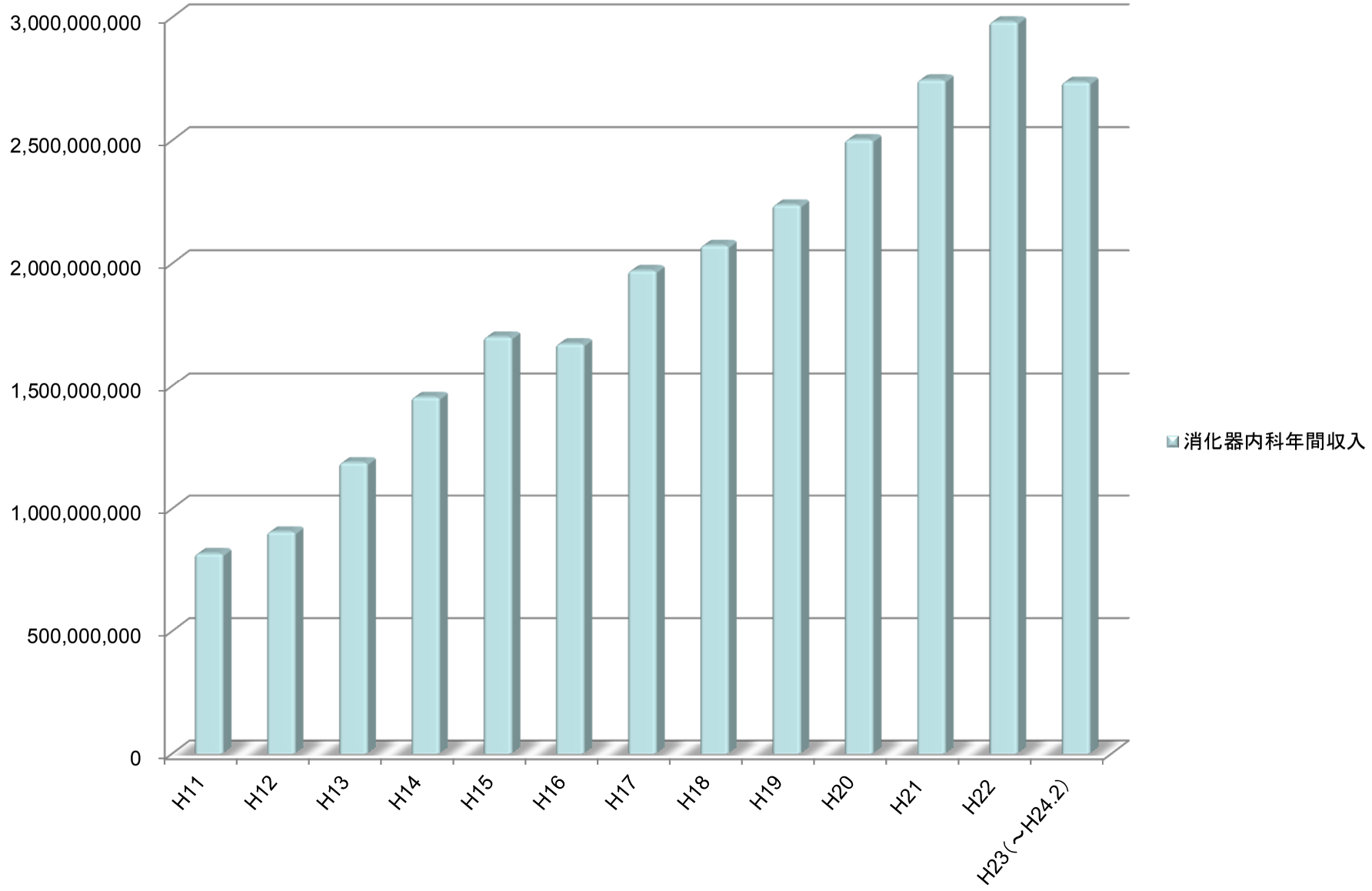
消化器内科入院診療実績



消化器内科年間外来収入



消化器内科年間総収入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H11
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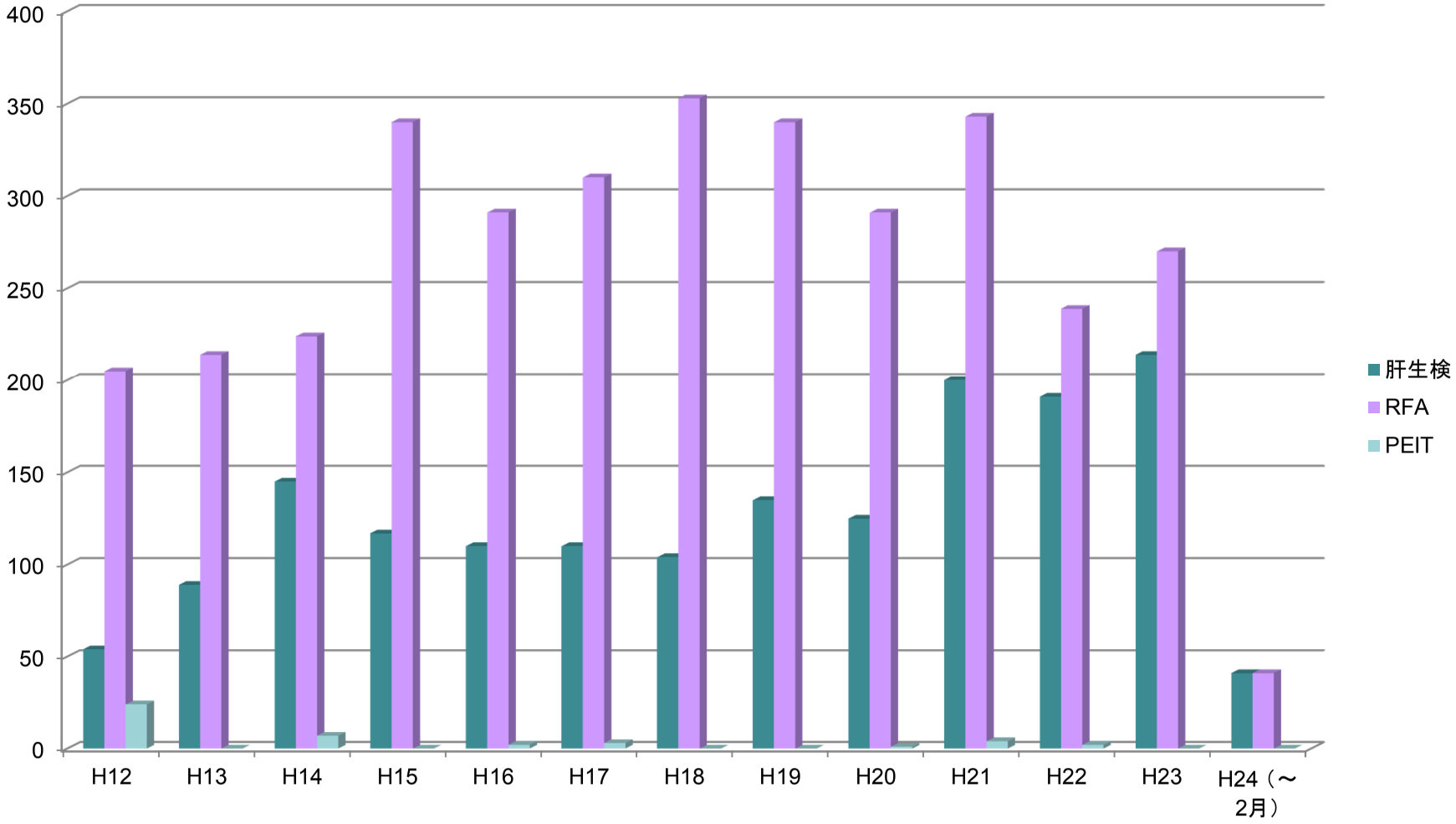
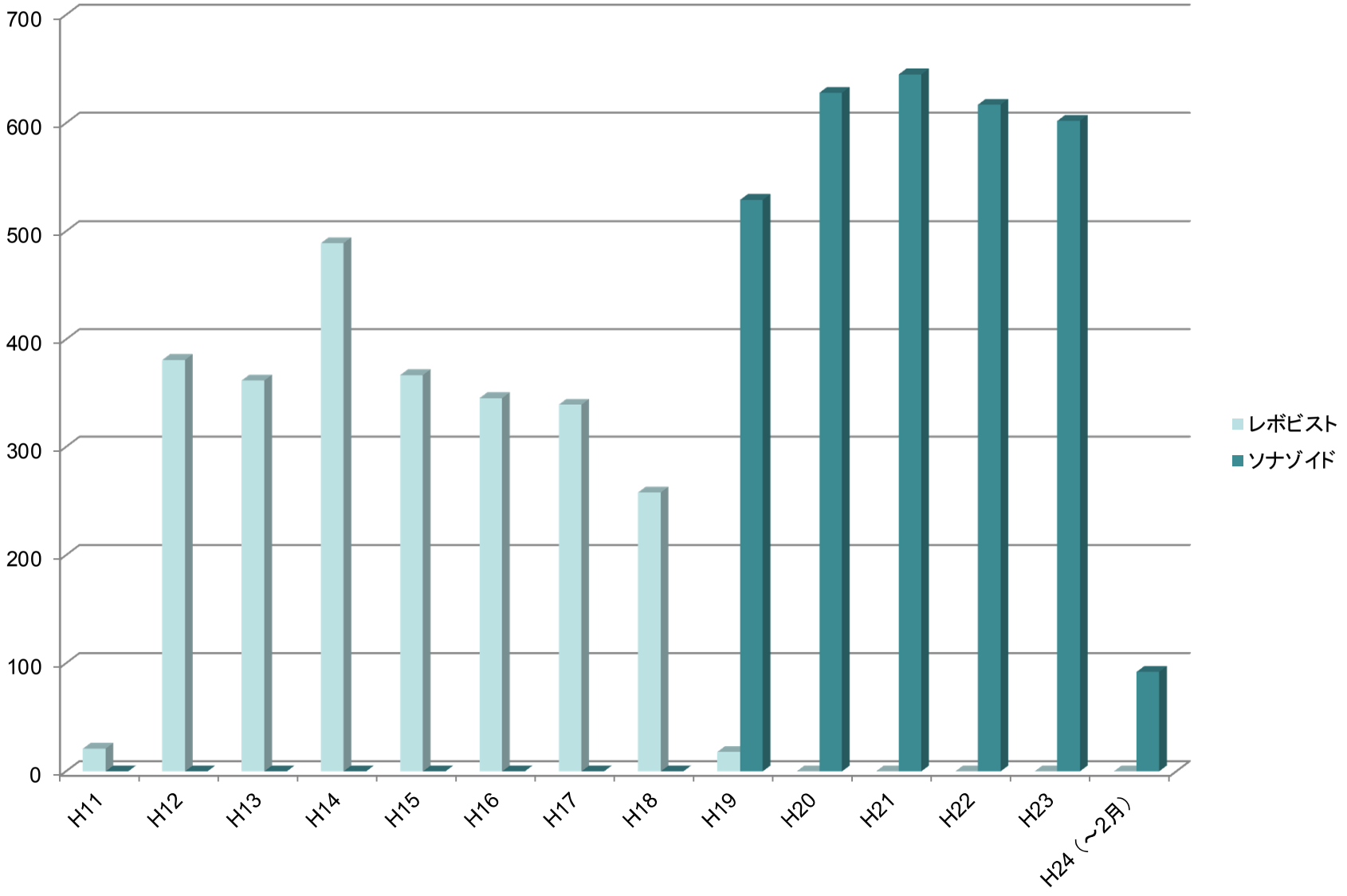
H19
H20
H21
H22
H23(～H24.2)
消化器内科年間収入

経皮的局所治療・肝生検総件数



腹部造影エコー検査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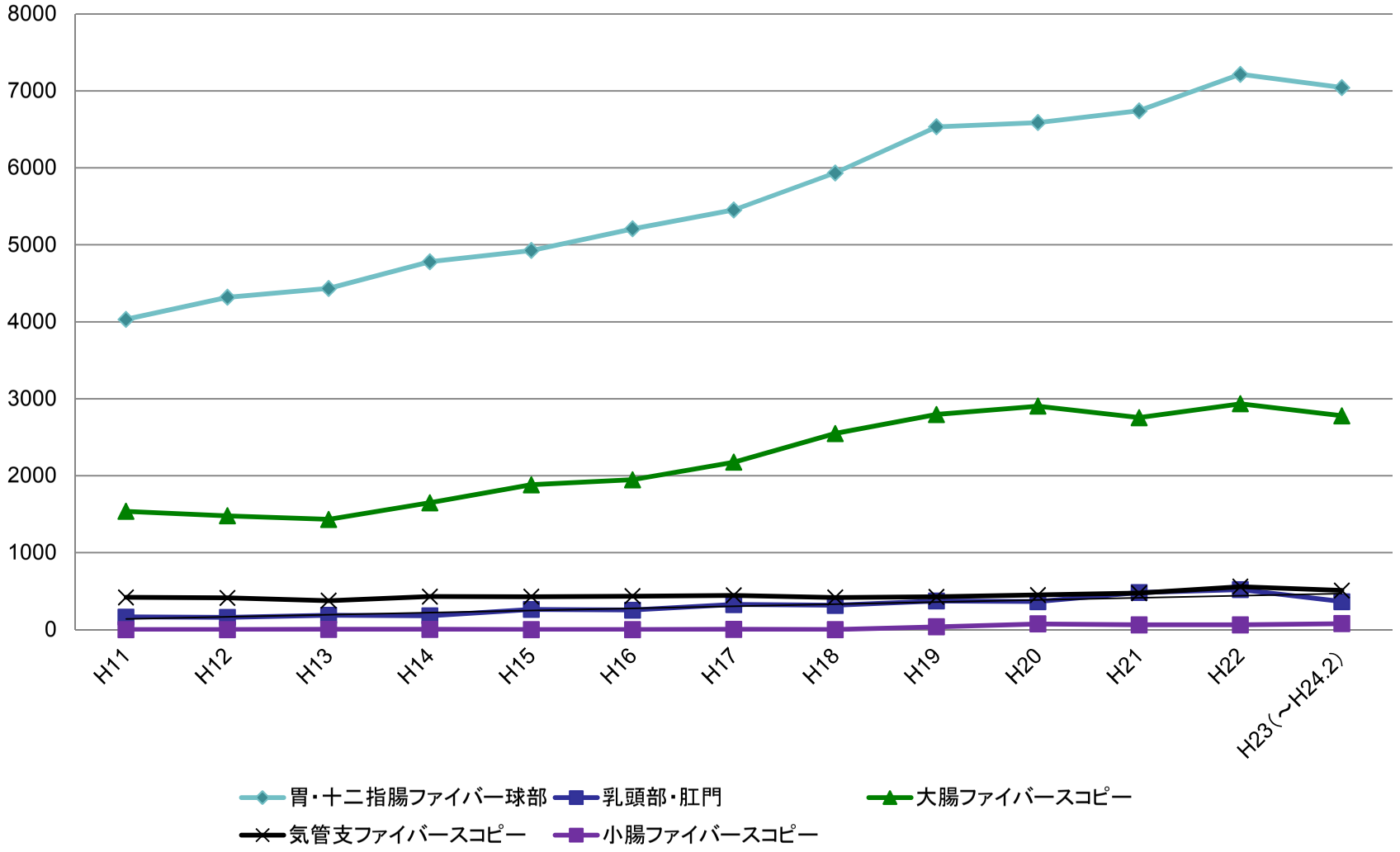
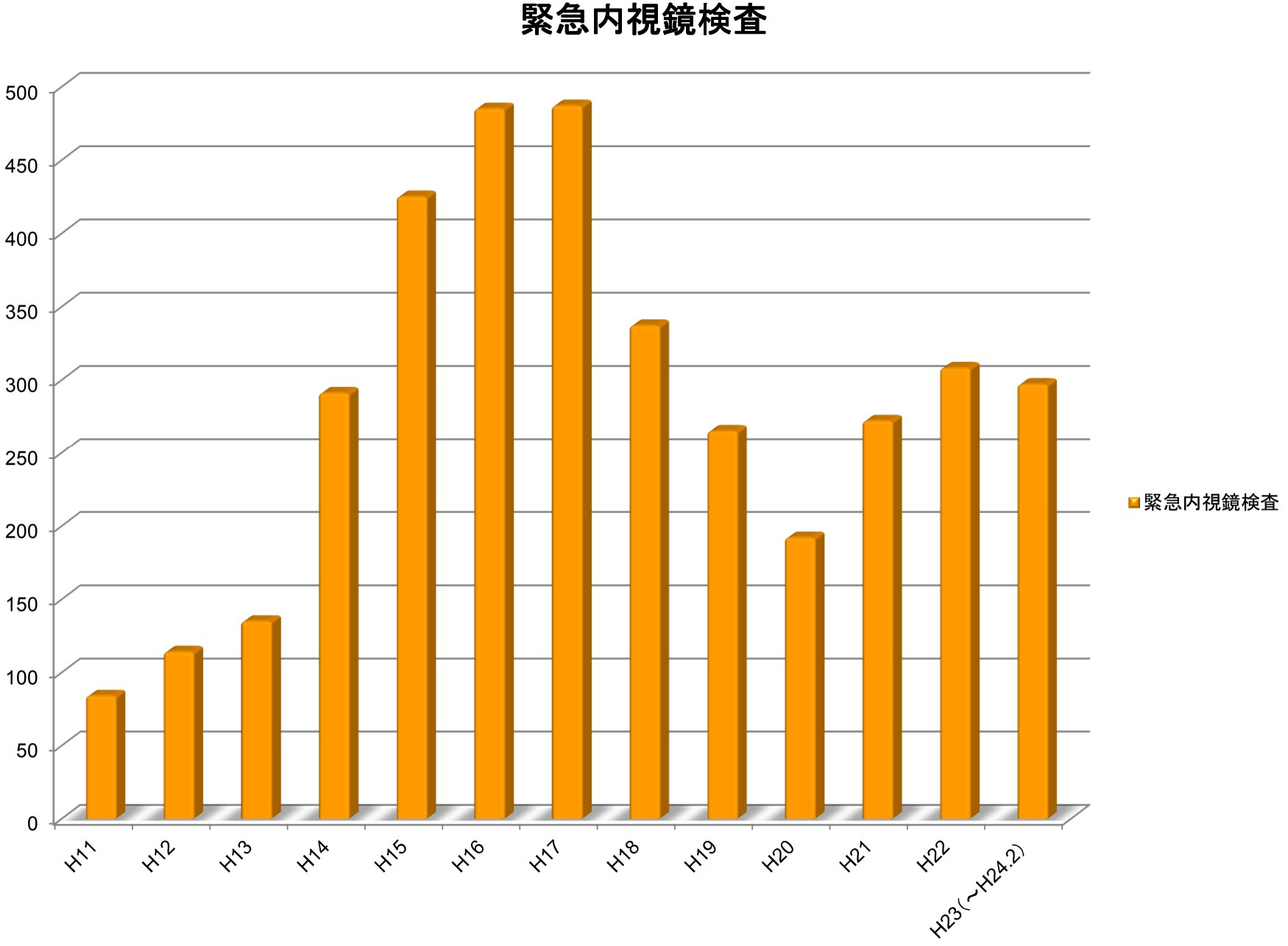
H20
H21
H22
H23
H24（～2月）
レボビスト
ソナゾイド

内視鏡部年報

検査	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23(～H24.2)
胃・十二指腸ファイバー球部	4032	4318	4434	4780	4926	5208	5453	5934	6534	6588	6742	7215	7043
乳頭部・肛側	165	160	187	181	263	254	327	316	373	365	482	520	365
大腸ファイバースコピー	1537	1479	1433	1648	1883	1947	2175	2548	2796	2904	2754	2934	2779
気管支ファイバースコピー	420	412	376	429	428	434	445	417	426	450	475	558	508
小腸ファイバースコピー	1	0	3	3	0	0	4	0	37	75	62	63	78
計(スクリーニング)	6155	6369	6433	7041	7500	7843	8404	9215	10166	10382	10515	11290	10773
胃生検	1509	1650	1851	1908	2005	2101	2473	2533	2412	2261	2175	2134	1849
大腸生検	1028	970	831	829	861	869	952	1143	1088	987	932	1001	874
気管支生検	245	222	227	265	260	296	281	314	302	314	345	393	331
小腸生検	1	0	0	2	0	0	1	0	11	8	12	18	19
計	2783	2842	2909	3004	3126	3266	3707	3990	3813	3570	3464	3546	3073
胆道ドレナージ	43	43	60	66	121	96	151	122	141	142	234	225	133
乳頭切開	15	19	14	11	44	44	46	44	48	46	74	132	84
乳頭バルーン拡張術	0	6	8	2	1	0	9	11	13	5	5	9	1
結石除去	16	24	15	17	38	49	55	51	62	65	86	130	67
食道静脈瘤結紮術	72	79	51	62	97	100	75	58	81	83	62	69	69
硬化療法	2	9	13	13	23	12	7	4	5	12	11	7	14
EISL	-	22	52	56	47	54	40	27	22	11	26	31	33
食道ブジー	116	137	124	152	210	308	252	246	234	308	284	269	230
APC	6	8	22	23	25	32	24	52	72	65	68	32	26
異物除去	6	2	3	11	14	17	14	11	18	21	14	20	24
胃ポリペクトミー	12	18	18	16	7	12	9	1	2	0	1	2	5
大腸ポリペクトミー	257	300	296	344	397	111	89	66	41	20	22	42	44
EMR(胃)	27	24	64	73	70	73	90	100	52	127	52	39	33
ESD(胃)	-	-	-	-	36	32	52	51	71	82	98	109	103
EMR(大腸)	-	-	-	-	-	290	373	470	443	458	467	484	423
緊急内視鏡検査	84	114	135	291	425	485	487	337	265	192	272	308	297
凝固止血バイポーラ	35	48	50	91	91	68	89	52	51	32	63	63	74
色素撒布法	216	291	459	724	1194	1470	1582	1909	1753	1731	1891	1937	1858
トロンビン被覆療法	81	88	113	193	198	236	294	369	387	319	326	282	305
アルト被覆療法	41	41	52	97	91	96	180	161	190	144	182	130	132
経皮内視鏡的胃瘻造設術	-	14	18	10	15	23	33	28	54	33	69	74	79
超音波内視鏡(EUS)	48	77	181	170	205	249	293	379	505	523	622	895	934
超音波内視鏡(大腸)	-	17	28	6	8	5	5	0	1	0	1	0	4
計	1077	1381	1776	2428	3357	3836	4203	4498	4511	4419	4930	5289	4972

消化管内視鏡検査件数年次推移

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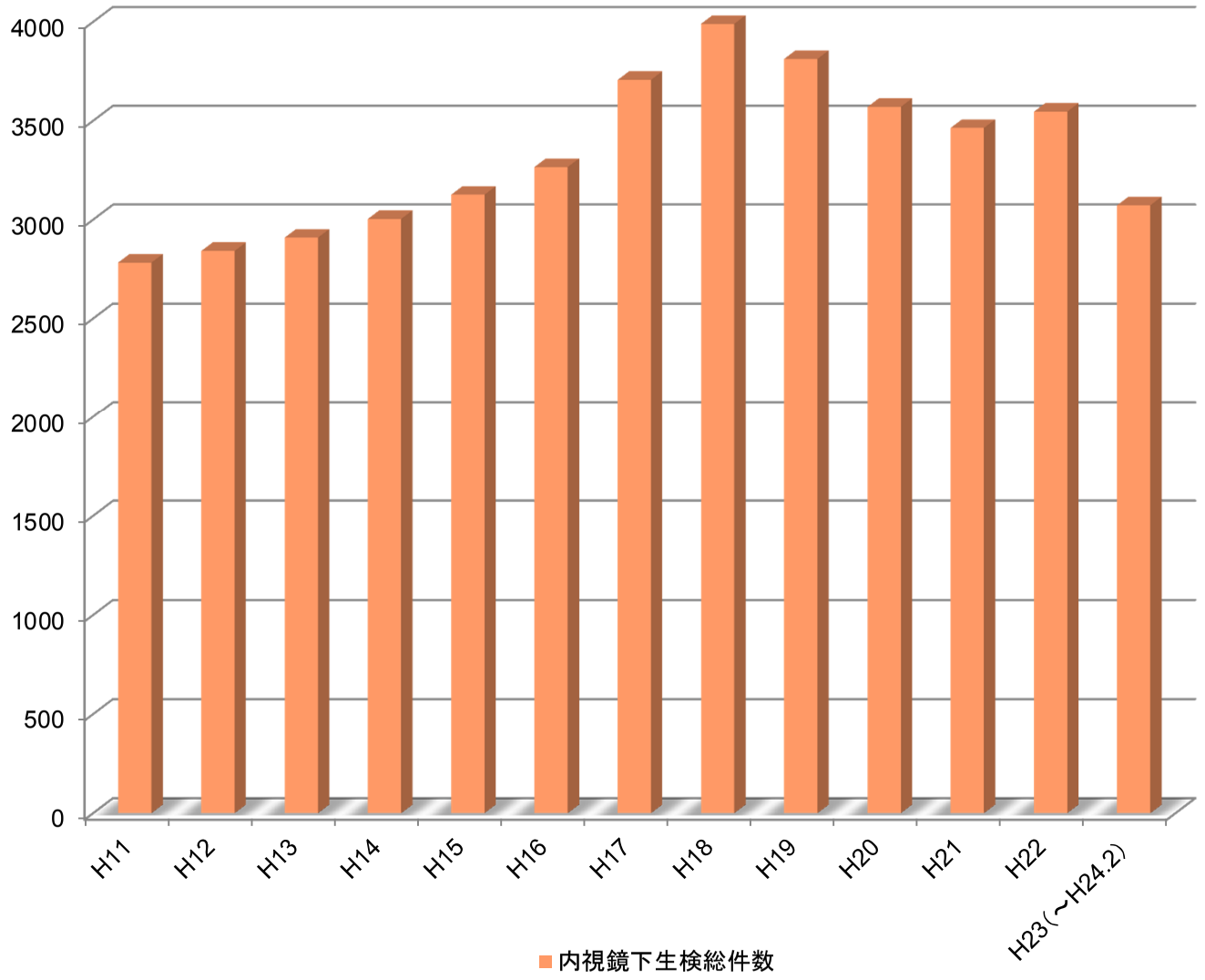
350
300
250
200
150
100
50
0
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
H23(～H24.2)
緊急内視鏡検査

内視鏡下生検総件数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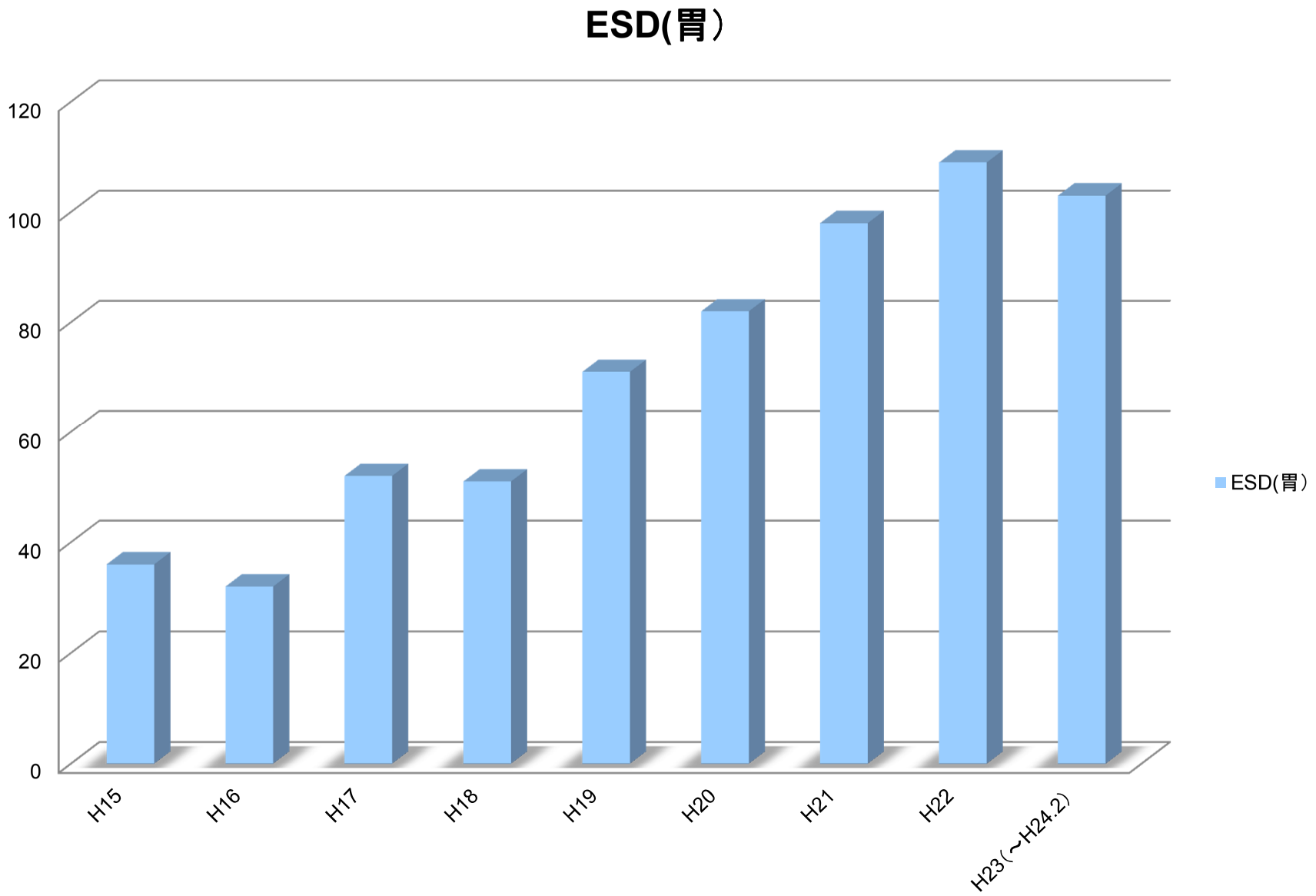
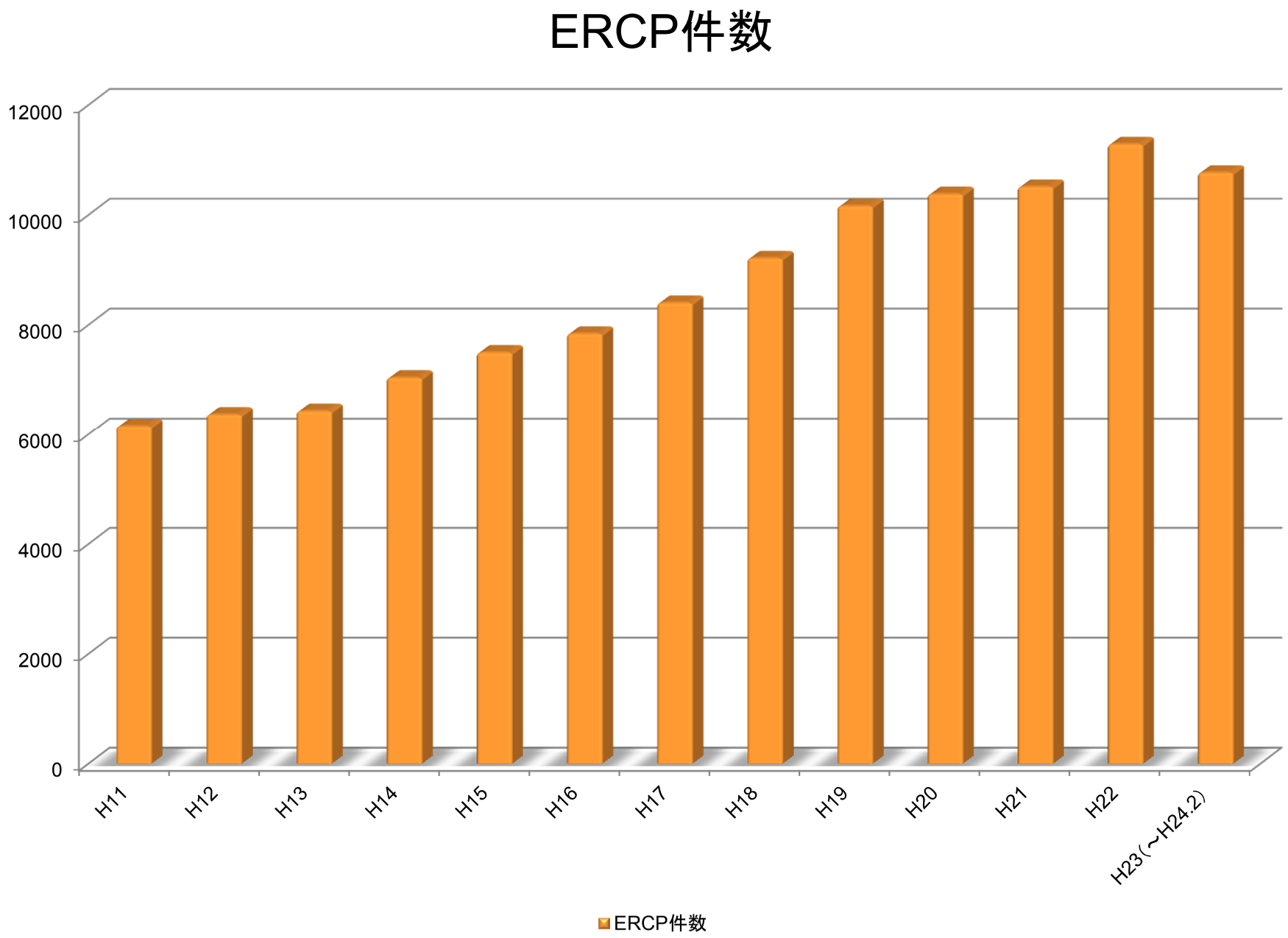
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
H23(～H24.2)
内視鏡下生検総件数

早期癌に対する内視鏡的粘膜下層切開剥離術

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H11
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H14
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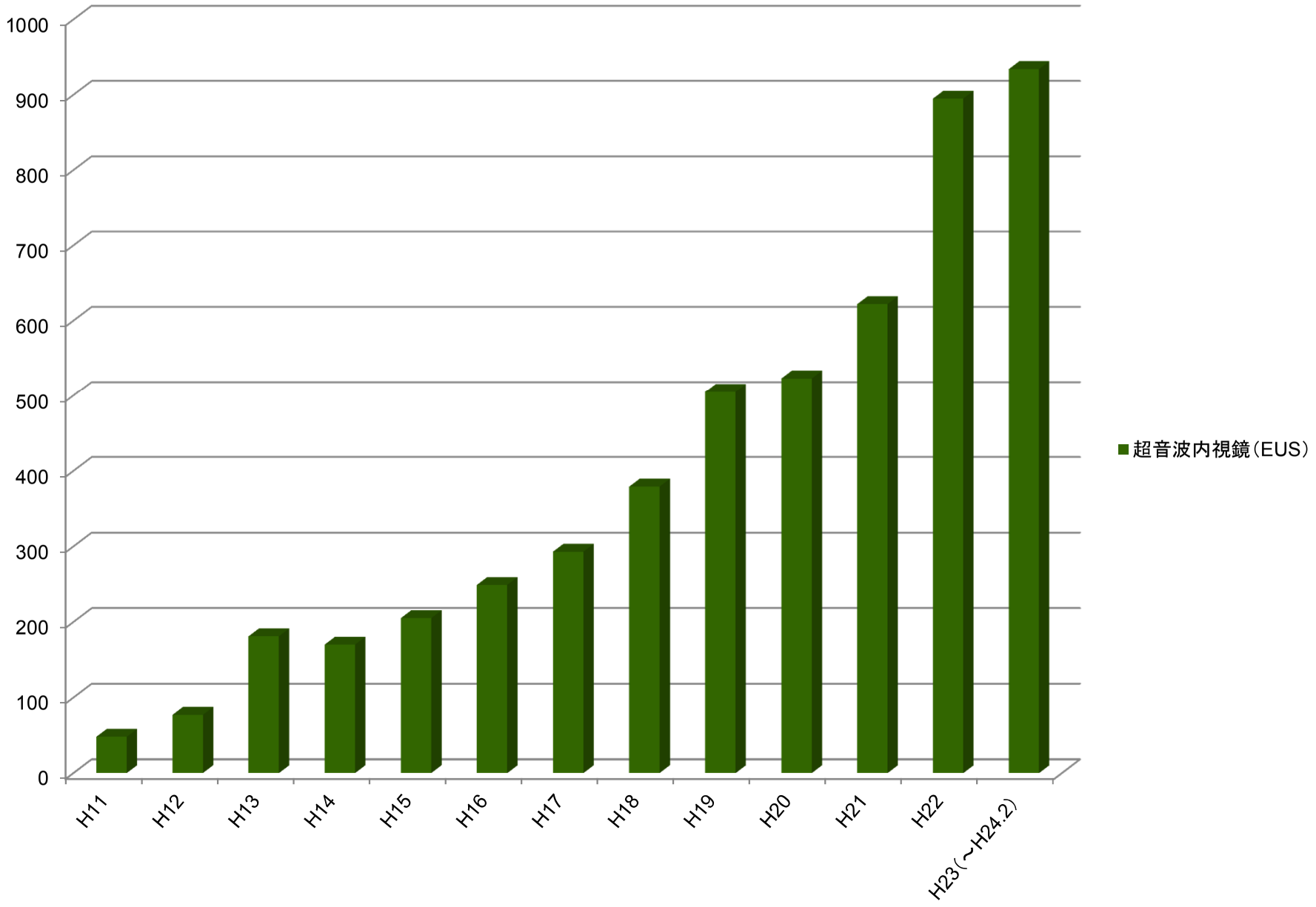
H20
H21
H22
H23(～H24.2)
ERCP件数


超音波内視鏡（EUS）
1000
900
800
700
600
500
400
300
200
100
0
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
H23（～H24.2）
超音波内視鏡（EUS）

近畿大学　消化器内科学教室医局員

（平成 24 年 3 月現在）

主任教授	工藤正俊	S53	肝臓・消化器・肝癌の診断と治療
教授（内視鏡部）	樫田博史	S58	下部消化管
准教授	汐見幹夫	S55	上部・胆膵内視鏡（関空クリニック所長・教授兼務）
	北野雅之	H2	消化管全般・胆膵疾患
	西田直生志	S60	肝臓病学・肝癌の分子生物学
講師	松井繁長	H3	食道静脈瘤止血・上部消化管
	(医局長)		
医学部講師	上嶋一臣	H7	慢性肝炎・肝癌の治療
	(病棟医長)		
	櫻井俊治	H7	上部消化管・分子生物学
	南　康範	H9	肝疾患・消化器一般
	萩原　智	H10	肝疾患・消化器一般
	井上達夫	H11	肝疾患・消化器一般
	矢田典久	H11	肝疾患・消化器一般
	坂本洋城	H12	胆膵疾患・消化器一般
	朝隈　豊	H14	上部消化管・消化器一般
	北井　聡	H14	肝疾患・消化器一般
助教	畑中絹世	H13	肝疾患の腹部超音波検査
	川崎正憲	H15	消化器内視鏡・消化器一般
	永井知行	H16	ゲノム生物学
	永田嘉昭	H16	消化器一般
	今井　元	H17	胆膵疾患・消化器一般
	早石宗右	H18	肝疾患・消化器一般
	有住忠晃	H19	肝疾患・消化器一般
	鎌田　研	H19	胆膵疾患・消化器一般
	峯　宏昌	H19	消化器一般
	宮田　剛	H19	胆膵疾患・消化器一般
	高山政樹	H19	消化器一般
	足立哲平	H20	肝疾患・消化器一般
	大本俊介	H20	消化器一般

	門阪薫平	H20	胆膵疾患・消化器一般
非常勤	仲谷達也	H3	仲谷クリニック
	中岡良介	H8	山本病院内科
	福田信宏	H10	朝日大学附属村上病院　消化器内科
	市川　勉	H13	内海町いちかわ診療所
	黒木恵美	H12	肝疾患・消化器一般
	岡田無文	H13	消化器一般
	柴田千栄	H15	肝疾患・消化器一般
	上田泰輔	H15	肝疾患・消化器一般
大学院 4 年	田北雅弘	H15	肝疾患・消化器一般
	早石宗右	H18	肝疾患・消化器一般
大学院 3 年	永田嘉昭	H16	消化器一般
	今井　元	H17	胆膵疾患・消化器一般
	有住忠晃	H19	肝疾患・消化器一般
	鎌田　研	H19	胆膵疾患・消化器一般
	宮田　剛	H19	胆膵疾患・消化器一般
	高山政樹	H19	消化器一般
大学院 2 年	峯　宏昌	H19	消化器一般
大学院 1 年	足立哲平	H20	肝疾患・消化器一般
	大本俊介	H20	消化器一般
	門阪薫平	H20	胆膵疾患・消化器一般

実験助手　鏡　郁子

臨床試験コーディネーター（CRC）

小川佳良子

教授秘書　藤田真紀

井上真由美

村橋亜季

弓削公子

坂上浩美

上田由未子

小田智裕子

日本肝癌研究会　田村利恵

	前原なつみ
医局秘書	胡桃由佳
	朝隈　智
	林　直子

院外出張

辻　直子	S60	近畿大学堺病院　准教授・科長
山本典雄		近畿大学堺病院　臨床助教
奥村直巳	H21	近畿大学堺病院　臨床助教
高場雄久		近畿大学堺病院
梅原康湖	H12	近畿大学堺病院
川崎俊彦	S58	近畿大学奈良病院消化器内分泌内科　准教授
岸谷　譲	S62	近畿大学奈良病院消化器内分泌内科　講師
宮部欽生	H14	近畿大学奈良病院消化器内分泌内科　診療講師
豊澤昌子	H12	近畿大学奈良病院消化器内分泌内科　診療助教
茂山朋広	H17	近畿大学奈良病院消化器内分泌内科　診療助教
奥田英之	H19	近畿大学奈良病院消化器内分泌内科　診療助教
木下大輔	H20	近畿大学奈良病院消化器内分泌内科　臨床助教
秦　康倫	H21	近畿大学奈良病院消化器内分泌内科　臨床助教
水野成人	S61	近畿大学奈良病院消化器内分泌内科　非常勤医師
加藤玲明	H11	近畿大学奈良病院消化器内分泌内科　非常勤医師
宮本容子	H12	近畿大学奈良病院消化器内分泌内科　非常勤医師
林　道友		近畿大学奈良病院消化器内分泌内科　非常勤医師
鍋島紀滋	S61	天理よろづ相談所病院
山本健二		岡本クリニック
井上良一	S43	吉川病院内科
南野達夫	S55	なんの医院
中里　勝		上ヶ原病院
川端一史	H 元年	川端内科クリニック
米田　円	H 元年	米田内科胃腸科
渡邉和彦	H3	結核予防会大阪府支部相談診療所
森村正嗣	H3	森村医院
遠田弘一	H7	
遠田由紀		
亀山千晴	H7	育和会記念病院
谷池聡子	H7	串本病院
工藤可苗	H12	近畿大学ゲノム生物学
仲谷達也	H3	仲谷クリニック
福永豊和	H4	北野病院消化器内科
由谷逸朗	S62	高石藤井病院

中岡良介	H8	山本病院内科
福田信宏	H10	朝日大学附属村上病院　消化器内科
小川　力	H11	高松赤十字病院　消化器内科
坂口康浩	H11	河崎内科病院
永島美樹	H12	桃坂クリニック
冨田崇文	H14	冨田病院
坂本康明	H15	（医）坂本クリニック
市川　勉	H13	内海町いちかわ診療所
齊藤佳寿	H14	庄内余目病院
高橋俊介	H14	市立堺病院
西尾　健	H14	南堺病院
末冨洋一郎	H8	末冨放射線科医院
梅原　泰	H11	辻　腎太郎クリニック
鄭　浩柄	H8	神戸市立中央センター中央市民病院
小牧孝充	H7	富田林病院

消化器内科学教室業績一覧（2011年）

I. 英文論文

1. 2011 Nagai T, Arao T, Furuta K, Sakai K, Kudo K, Kaneda H, Tamura D, Aomatsu K, Kimura H, Fujita Y, Matsumoto K, Saijo N, **Kudo M**, Nishio K: Sorafenib inhibits the hepatocyte growth factor-mediated epithelial mesenchymal transition in hepatocellular carcinoma. **Mol Cancer Ther** 10: 169-177, 2011. **(IF=5.225)**

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3. 2011 Kudo K, Arao T, Tanaka K, Kaneda H, Matsumoto K, Tamura D, Aomatsu K, Velasco M, Fujita Y, Saijo N, **Kudo M**, Nishio K: Antitumor activity of BIBF 1120, a vascular endothelial growth factor-2 inhibitor and use of VEGR2+pTYR+leucocyte as a pharmacodynamic biomarker. **Clin Cancer Res** 17: 1373-1381, 2011. **(IF=7.338)**

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7. 2011 Yokosuka O, Kurosaki M, Imazeki F, Arase Y, Tanaka Y, Chayama K, Tanaka E, Kumada H, Izumi N, Mizokmi M, **Kudo M**: Management of hepatitis B: Consensus of Japan Society of Hepatology 2009. **Hepatol Res** 41: 1-21, 2011. **(IF=1.857)**

8. 2011 Katsube T, Okada M, Kumano S, Hori M, Imaoka I, Ishii K, **Kudo M**, Kitagaki H, Murakami T: Estimation of liver function using T1 mapping onGd-EOB-DTPA-enhanced magnetic resonance imaging. **Invest Radiol** 46: 277-283, 2011. **(IF=4.665)**

9. 2011 **Kudo M**, Yamao K, Shimosegawa T: The prognosis of patients with pancreatic cancer is extremely poor. Prefece. **Pancreatology** 11: 1-2, 2011. **(IF=2.128)**

10. 2011 Sakurai T, **Kudo M**, Fukuta N, Nakatani T, Kimura M, Park AM, Munakata H: Involvement of angiotensin II and reactive oxygen species in pancreatic fibrosis. **Pancreatology** 11: 7-13, 2011. **(IF=2.128)**

11. 2011 Kitano M, **Kudo M**, Sakamoto H, Komaki T: Endoscopic ultrasonography and contrast-enhanced endoscopic. **Pancreatology** 11: 28-33, 2011. **(IF=2.128)**

12. 2011 Komaki T, Kitano M, Sakamoto H, **Kudo M**: Endoscopic ultrasonography-guided biliary drainage: evaluation of a choledochoduodenostomy technique. **Pancreatology** 11: 47-51, 2011. **(IF=2.128)**

13. 2011 Sakamoto H, Kitano M, Komaki T, Imai H, Kamata K, **Kudo M**: Endoscopic ultrasound-guided neurolysis in pancreatic cancer. **Pancreatology** 11: 52-58, 2011. **(IF=2.128)**

14. 2011 Minami Y, **Kudo M**: Radiofrequency ablation of hepatocellular carcinoma: A literature review. **Int J Hepatol** 2011: 9pages (104685), 2011. **(IF=0.000)**

15. 2011 Eguchi S, Kanematsu T, Arii S, Omata M, **Kudo M**, Sakamoto M, Takayasu K, Makuuchi M, Matsuyama Y, Monden M, for the Liver Cancer Study Group of Japan: Recurrence-free survival more than 10 years after liver resection for hepatocellular carcinoma. **Brit J Surg** 98: 552-557, 2011. **(IF=4.444)**

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17. 2011 **Kudo M**: Molecular targeted therapy for hepatocellular carcinoma: bench to bedside. **Digest Dis** 29: 273-277, 2011. **(IF=1.000)**

18. 2011 **Kudo M**: Signaling pathway and molecular-taegeted therapy for hepatocellular carcinoma. **Digest Dis** 29: 289-302, 2011. **(IF=1.000)**

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20. 2011 **Kudo M**: Future treatment option for hepatocellular carcinoma: a focus on brivanib. **Digest Dis** 29: 316-320, 2011. **(IF=1.000)**

21. 2011 Ueshima K, **Kudo M**, Takita M, Nagai T, Tatsumi C, Ueda T, Kitai S, Ishikawa E, Yada N, Inoue T, Hagiwara S, Minami Y, Chung H, Sakurai T: Des-γ-carboxyprothrombin may be a promising biomarker to determine the therapeutic efficacy of Soraenib for hepatocellular carcinoma. **Digest Dis** 29: 321-325, 2011. **(IF=1.000)**

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III. 和文論文

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4. 2011 北野雅之, 小牧孝充, 坂本洋城, 今井 元, 鎌田 研, 工藤正俊, 安田武生, 竹山宜典: 膵疾患診断における造影ハーモニック超音波内視鏡検査の有用性. 膵臓 26: 23-28, 2011.

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14. 2011 工藤正俊: 序論. 特集: 肝細胞がん診療の進歩: up-to-date, 最新医学 66: 5, 2011.

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16. 2011 井上達夫, 工藤正俊: 肝細胞がんの診断・治療アルゴリズム. 特集: 肝細胞がん診療の進歩: up-to-date, 最新医学 66: 43-51, 2011.

17. 2011 工藤正俊: 新規分子標的薬の開発状況と肝がん診療の今後の展望. 特集: 肝細胞がん診療の進歩: up-to-date, 最新医学 66: 101-107, 2011.

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31. 2011 坂本洋城, 北野雅之, 門阪薫平, 工藤正俊: 胆・膵-胆膵病変の診断・治療における EUS の有用性. インナービジョン 26: 50-52, 2011.

32. 2011 小川光一, 福永 潔, 竹内朋代, 川岸直樹, 工藤正俊, 大河内信弘: 本邦における多発肝嚢胞症のアンケート調査. 肝臓 52: 709-715, 2011.

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朝隈　豊，櫻井俊治，松井繁長，工藤正俊：画像強調による大腸腫瘍の精密診断．消化器内視鏡 23：790-796，2011.

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42. 2011　樫田博史，筑後孝章，池原伸直，浜谷茂治：鋸歯状病変の特徴と診断（２）臨床の立場から．臨牀消化器内科 26：1613-1622，2011.

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46. 2011 樫田博史，若村邦彦，工藤進英：大腸 Endocytoscopy．病理と臨床 29：997-1004，2011.

47. 2011 樫田博史：EMR/ERMR―局注，スネアリングの達人になるために．消化器内視鏡 23：289-295，2011.

48. 2011 坂本洋城，北野雅之：研修医からの質問 Q&A． 臨床腫瘍プラクティス 7：442，2011.

IV. 招待講演・特別講演（海外）

1. **Kudo M**: Special lecture “Double contrast US for surveillance of hepatoma.” World Federation for Ultrasound in Medicine and Biology (WFUMB) Centre of Excellence Workshop, Jakarta, Indonesia, February 12, 2011.

2. **Kudo M**: Special lecture “Sonazoid-enhanced US for hepatoma: Value of defect re-perfusion of imaging.” World Federation for Ultrasound in Medicine and Biology (WFUMB) Centre of Excellence Workshop, Jakarta, Indonesia, February 12, 2011.

3. **Kudo M**: Special lecture “Contrast enhanced endoscopic ultrasound value in the diagnosis of small pancreatic cancer.” World Federation for Ultrasound in Medicine and Biology (WFUMB) Centre of Excellence Workshop, Jakarta, Indonesia, February 12, 2011.

4. **Kudo M**: Special lecture “Double contrast US for surveillance of hepatoma.” Innovative Practive in Ultrasound With Live Demonstration, Bangkok, Thailand, February 16, 2011.

5. **Kudo M**: Special lecture “Sonazoid enhanced US for the management of liver cancer.” Innovative Practive in Ultrasound With Live Demonstration, Bangkok, Thailand, February 16, 2011.

6. **Kudo M**: Special lecture “Diagnosis of pancreatic tumors by EUS-FNA and CE-EUS.” Innovative Practive in Ultrasound With Live Demonstration, Bangkok, Thailand, February 16, 2011.

7. **Kudo M**: Special lecture “Interventional US for pancreatic malignancy.” Innovative Practive in Ultrasound With Live Demonstration, Bangkok, Thailand, February 16, 2011.

8. **Kudo M**: Special lecture “Treatment guideline of hepatocellular carcinoma: Asian perspective.” Asan Liver Center Opening Symposium, Seoul, Korea, March 11, 2011.

9. Kashida, H: Intubation technique for colonoscopy. 10th Yokohama International Endoscopy Conference with Live Demonstration, March 12-13, Yokohama, Japan.

10. **Kudo M**: Special Focus Session “Update on endoscopic USG: how much for

imaging, needling, or therapy?" The 42nd Annual Congress of the Korean Society of Ultrasound in Medicine (KSUM), Seoul, Korea, May 20, 2011.

11. **Kudo M**: Special Invited Lecture "Sonazoid-enhanced US in the management of HCC" The 42nd Annual Congress of the Korean Society of Ultrasound in Medicine (KSUM), Seoul, Korea, May 20, 2011.

12. **Kudo M**: Luncheon "Hepatocellular carcinoma: prevention and treatment by interferon." The Japanese Society for Interferon and Cytokine Research-The Japanese Society for Macrophage Molecular and Cell Biology (JSICR-MMCB) 2011, Osaka, Japan, May 25, 2011.

13. **Kudo M**: Sonazoid-enhanced US for liver tumors: Value of reinjection technique. 14th National Conference of the Romanian Society of Ultrasound in Medicine and Biology Ultrasound(SRUMB), Tirgu Mures, Romania, June 10-12, 2011.

14. **Kudo M**: Contrast-enhanced and interventional EUS for pancreatobiliary tumors. 14th National Conference of the Romanian Society of Ultrasound in Medicine and Biology Ultrasound(SRUMB), Tirgu Mures, Romania, June 10-12, 2011.

15. **Kudo M**: Special Invited Lecture "Sonazoid-enhanced US in the management of HCC" XV Congress of the Latin American Federation for Ultrasound in Medicine and Biology (FLAUS), Asuncion, Paraguay, June 22-25, 2011.

16. **Kudo M**: Special Lecture "RECICL." The 2nd Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE), Osaka, Japan, July 1-3, 2011.

17. **Kudo M**: Special Lecture "EVOLVE-1 trial." The 2nd Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE), Osaka, Japan, July 1-3, 2011.

18. **Kudo M**: Special Lecture "Current situation of HCC management in Japan." The 2nd Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE), Osaka, Japan, July 1-3, 2011.

19. **Kudo M**: Special Lecture "Design and conduct of clinical trials for the design and rational of clinical trials for the combination of hepatic arterial infusion chemotherapy with molecular targeted agents." The 2nd Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE), Osaka, Japan, July 1-3, 2011.

20. Ueshima K: “Targeted agents and TACE/HAIC combination trial.” The 2nd Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE), Osaka, Japan, July 1-3, 2011.

21. Kitano M: Interventional EUS and its future perspectives. The International Workshop for the 80th Anniversary of Foundation of Konkuk University Medical Center and Opening of Global Digestive Disease Center, July 9-10, Konkuk University, Korea.

22. Kashida H: Keynote address: Concept of de novo colorectal cancer. International Symposium “Clinical implications of colorectal cancer of the denovo origin”, 81st Annual Meeting of Japan Gastroenterological Endoscopy Society, Aug. 17-19, Nagoya, Japan.

23. **Kudo M**: US-guided ablation of HCC. “US-guided tumor therapy”, 13th World Congress of the world federation for ultrasound in medicine and biology (WFUMB) 2011, Vienna, Austria, August 26-29, 2011.

24. **Kudo M**: Benefit and nonsense in oncologic follow-up imaging and the role of US. “US in tumor follow-up and palliation”, 13th World Congress of the world federation for ultrasound in medicine and biology (WFUMB) 2011, Vienna, Austria, August 26-29, 2011.

25. **Kudo M**: Interventional EUS for pancreatic tumours. “Abdominal 7-Gallbladder, pancreas and spleen”, 13th World Congress of the world federation for ultrasound in medicine and biology (WFUMB) 2011, Vienna, Austria, August 26-29, 2011.

26. **Kudo M**: Future prospects of ultrasound diagnosis for diffuse liver disease. Symposium “Hitachi Aloka Medical Ltd.: The next generation in high-resolution imaging technology and elastography”, 13th World Congress of the world federation for ultrasound in medicine and biology (WFUMB) 2011, Vienna, Austria, August 26-29, 2011.

27. Kitano M: Special educational event discussing “EUS techniques.” Evening seminar “Pancreatico biliary techniques”, October 2, Singapore.

28. **Kudo M**: “Establishing treatment algorithms for hepatocellular carcinoma: What tests do we need?” , Symposium “When East meets West: Management of hepatocellular carcinoma”, 19th United European Gastroenterology Week (UEGW), Stockholm, Sweden, October 24, 2011.

29. **Kudo M**: Hepatocellular carcinoma: Recent development of molecular targeted agent. JSCO university "Hepatobiliary and pancreas cancers", The 49th Annual Meeting of Japan Society of Clinical Oncology, Aichi, Japan, October 27-29, 2011.

30. **Kudo M**: Lecture "Interventional EUS for pancreatobiliary tumors." 9th ABDA teaching course in conjunction with AFSUMB Workshop, Bali, Indonesia, November 17-19, 2011.

31. **Kudo M**: Lecture "Sonazoid-enhanced US in the management of HCC." 9th ABDA teaching course in conjunction with AFSUMB Workshop, Bali, Indonesia, November 17-19, 2011.

32. Kitano M: State of the Art Talk "Contrast enhanced imaging", 2nd Annual Cook Medical ans RPA Hospital Live Workshop, November 21, 2011, Sydney, Australia.

33. **Kudo M**: Clinical staging systems for HCC. Symposium "HCC staging and response assessment", APASL 2nd Hepatocellular carcinoma conference (APASL STC), Jeju, Korea, December 1-3, 2011.

34. **Kudo M**: Diagnosis of gross pathology of HCC: Value of Kupffer phase of Sonazoid-enhanced US. 3rd Asia-Pacific Conference on Ultrasound Contrast Imaging, 13th International Symposium on Ultrasound Contrast Imaging, Kunming, China, December 15-18, 2011.

35. **Kudo M**: Molecular targeting therapy. Session "HCV and HCC I", The 7th APASL single topic conference, Chiba, Japan, December 18, 2011.

V. 招待講演・特別講演（国内）

1. 樫田博史：大腸腫瘍の精密診断と、それに基づいた治療について．第 262 回広島胃と腸疾患研究会，平成 23 年 1 月 8 日，広島.

2. 樫田博史：潰瘍性大腸炎に対するペンタサ錠 4g の評価．大腸病態治療研究会，平成 23 年 1 月 22 日，大阪.

3. 樫田博史：大腸内視鏡挿入法．日本消化器内視鏡学会　第 13 回近畿消化器内視鏡ガイドライン講習会，平成 23 年 1 月 23 日，大阪.

4. 北野雅之：特別講演「Therapeutic EUS の現状と将来展望」，第 18 回京滋消化器内視鏡治療勉強会，平成 23 年 1 月 27 日，ホテルグランヴィア京都，京都.

5. 工藤正俊：特別講演「肝癌診療ガイドラインと最新治療：分子標的治療の位置付けを中心に」，第 164 回滋賀肝・胆・膵勉強会，京都センチュリーホテル，京都，平成 23 年 2 月 4 日.

6. 工藤正俊：特別講演「肝細胞癌診療の新しいパラダイム」，ウイルス肝炎講習会，岐阜県医師会館，岐阜，平成 23 年 2 月 5 日.

7. 工藤正俊：特別講演「ウイルス性肝炎」，平成 22 年度「肝がん撲滅運動」，大阪狭山市さやかホール，大阪，平成 23 年 2 月 6 日.

8. 鄭　浩柄：生活習慣から来る肝臓病．　平成 22 年度「肝がん撲滅運動」，平成 23 年 2 月 6 日，大阪狭山市さやかホール，大阪.

9. 井上達夫：診断．　平成 22 年度「肝がん撲滅運動」，平成 23 年 2 月 6 日，大阪狭山市さやかホール，大阪.

10. 上嶋一臣：内科的治療．　平成 22 年度「肝がん撲滅運動」，平成 23 年 2 月 6 日，大阪狭山市さやかホール，大阪.

11. 朝隈　豊：講演「食道」，第 398 回大阪胃研究会，平成 23 年 2 月 9 日，エーザイ株式会社，大阪.

12. 坂本洋城：造影 EUS（パワードプラとハーモニック）．　第 3 回インターベンショナル EUS 九州研究会，平成 23 年 3 月 12 日，ホテルクリオコート博多，福岡.

13. 坂本洋城：胆管ドレナージ術.　第3回インターベンショナルEUS九州研究会，平成23年3月12日，ホテルクリオコート博多，福岡.

14. 北野雅之：特別講演「コンベックス超音波内視鏡のコツ」，北野病院超音波内視鏡カンファレンス，平成23年3月5日，財団法人田附興風会 医学研究所北野病院，大阪.

15. 松井繁長：講演「内視鏡治療」，近畿大学医学部附属病院がんセンター第3回市民公開講座，平成23年3月5日，近畿大学医学部大講堂，大阪.

16. 樫田博史：麻痺性イレウス.　シリーズ「重篤副作用疾患別対応マニュアル」　日経ラジオ社　「薬学の時間」日本薬剤師会，平成23年3月8日放送（インターネット配信も有り）

17. 松井繁長：学術講演「早期食道・胃癌の最新の診断と治療～薬剤起因性消化管障害対策も踏まえて～」，松原市医師会学術講演会・第52回症例検討会，平成23年3月12日，松原市医師会館，大阪.

18. 坂本洋城：特別シンポジウム 手技の現状と新しい展開「造影EUS（パワードプラとハーモニック)」，第3回インターベンショナルEUS九州研究会，平成23年3月12日，ホテルクリオコート博多，福岡.

19. 坂本洋城：特別シンポジウム 手技の現状と新しい展開「胆管ドレナージ術」，第3回インターベンショナルEUS九州研究会，平成23年3月12日，ホテルクリオコート博多，福岡.

20. 樫田博史：大腸腫瘍の内視鏡診断と治療－最近の動向．第53回くすのき会，平成23年3月16日，神戸，兵庫.

21. 樫田博史：大腸腫瘍に対する内視鏡治療の適応と限界．第4回 Sayama Guys，平成23年3月18日，大阪狭山市，大阪.

22. 樫田博史：病診連携からみた大腸ポリープの診断と取り扱い．第3回泉南地区病診連携を考える会，平成23年3月24日，泉佐野，大阪.

23. 樫田博史：抗血栓薬・NSAIDs 服用患者における消化管病変と内視鏡．FGIDs治療講演会，平成23年3月26日，堺，大阪.

24. 坂本洋城：講演，平成23年4月2日，ホテル阪急インターナショナル，大阪.

25. 工藤正俊：シンポジウム 超音波医療の最前線「消化器領域の超音波診療最前線」，第28回日本医学総会，東京国際フォーラム，東京，平成23年4

月 9 日.

26. 工藤正俊: お昼の勉強会 Aplio が創る超音波の新潮流「肝癌の造影超音波」, 第 84 回日本超音波医学会総会, グランドプリンスホテル新高輪, 東京, 平成 23 年 5 月 27 日.

27. 工藤正俊: 日本超音波医学会と消化器関連学会と連携: 問題点は何か？特別企画「日本超音波医学会の役目は何か？（他の超音波関連学会との連携)」, 日本超音波医学会第 84 回学術集会, 平成 23 年 5 月 27 日-29 日, グランドプリンスホテル新高輪, 東京.

28. 鎌田　研: 胆嚢疾患に対する造影ハーモニック EUS を用いたダイナミックイメージング.　特別企画「胆膵超音波診断の最前線」, 日本超音波医学会第 84 回学術集会, 平成 23 年 5 月 27 日-29 日, グランドプリンスホテル新高輪, 東京.

29. 工藤正俊: 特別講演「コンセンサスに基づく肝細胞癌診断アルゴリズム」, 第 15 回 TCEL MR meeting, 東京コンファレンスセンター, 東京, 平成 23 年 5 月 28 日.

30. 樫田博史：高齢化社会における、安全な大腸内視鏡検査と治療の在り方：便潜血検査、内視鏡前処置から ESD まで. 第 56 回福岡ブロック大腸精検懇話会, 平成 23 年 6 月 3 日, 福岡.

31. 松井繁長: 特別講演「上部消化管癌の最新の内視鏡診断と治療」, 第 3 回南河内消化器カンファレンス, 平成 23 年 6 月 11 日, ラブリーホール, 大阪.

32. 樫田博史: 特別講演「下部消化管腫瘍の診断と治療-通常観察から最新医療まで」, 第 3 回南河内消化器カンファレンス, 平成 23 年 6 月 11 日, ラブリーホール, 大阪.

33. 樫田博史：下部消化管腫瘍－内視鏡的診断・治療の最前線－. 日本消化器病学会近畿支部第 36 回教育講演会,　平成 23 年 6 月 18 日, 神戸,　兵庫.

34. 樫田博史：大腸内視鏡：スコープ挿入から診断・治療まで. 第 4 回大腸内視鏡研究会,　平成 23 年 6 月 24 日, 京都.

35. 樫田博史：大腸における EMR と ESD. 第 7 回茨城内視鏡治療研究会, 平成 23 年 7 月 5 日, つくば, 茨城.

36. 工藤正俊: ワークショップ”肝炎・肝癌対策との比較で胃炎・胃癌対策を考える”「わが国の肝炎・肝癌対策について」, 第 16 回 JAPANGAST Study

Group, シャトレーゼ・ガトーキングダム・サッポロ, 北海道, 平成 23 年 7 月 9 日.

37. 工藤正俊: 特別講演「肝癌に対する分子標的治療の現状と Ongoing Trial」, 肝細胞癌ソラフェニブ治療研究会, 名古屋東急ホテル, 愛知, 平成 23 年 7 月 15 日.

38. 北野雅之: 特別講演「膵のう胞性病変の診断」, 第 37 回熊本消化器治療内視鏡研究会, 平成 23 年 7 月 15 日, ホテル日航熊本, 熊本.

39. 工藤正俊: 特別講演「肝癌診療の新しいパラダイム」, 第 28 回烏城消化器カンファレンス, 岡山プラザホテル, 岡山, 平成 23 年 7 月 16 日.

40. 工藤正俊: 肝臓癌における mTOR 阻害剤開発の現状. シンポジウム「PI3K/Akt/mTOR 経路阻害剤の基礎と臨床」, 第 9 回日本臨床腫瘍学会学術集会, 平成 23 年 7 月 21 日-23 日, パシフィコ横浜, 神奈川.

41. 工藤正俊: シンポジウム・特別発言「超音波を用いた肝細胞癌治療の更なる進歩」, 第 47 回日本肝癌研究会, 平成 23 年 7 月 28 日-29 日, 静岡県コンベンションアーツセンター, 静岡.

42. 樫田博史：病診連携にも役立つ, 大腸病変の内視鏡診断・治療の話. 第 27 回西大寺邑久地区消化器内視鏡懇話会, 平成 23 年 7 月 29 日, 岡山.

43. 工藤正俊: 特別講演「肝疾患最近の話題」, 香川ベアネットカンファレンス, 全日空ホテルクレメント高松, 香川, 平成 23 年 8 月 13 日.

44. 坂本洋城: ミニレクチャー「膵管ドレナージ」, 第 81 回日本消化器内視鏡学会総会, 平成 23 年 8 月 19 日, 名古屋国際会議場, 愛知.

45. 北野雅之: 講師「超音波内視鏡による診断と治療」, 日本消化器病学会近畿支部第 37 回教育講演会, 平成 23 年 8 月 20 日, 大阪国際交流センター, 大阪.

46. 樫田博史: 基調講演「大腸分光内視鏡所見分類の問題点」, シンポジウム「下部消化管疾患における画像強調内視鏡の活用法」, 日本消化器病学会近畿支部第 95 回例会, 平成 23 年 8 月 20 日, 大阪国際交流センター, 大阪.

47. 峯　宏昌: ディベートセッション「消化管セッション」, 日本消化器病学会近畿支部第 95 回例会, 平成 23 年 8 月 20 日, 大阪国際交流センター, 大阪.

48. 小牧　孝充: ディベートセッション「胆膵セッション」, 日本消化器病学

会近畿支部第95回例会，平成23年8月20日，大阪国際交流センター，大阪.

49. 矢田典久：ディベートセッション「肝臓セッション」，日本消化器病学会近畿支部第95回例会，平成23年8月20日，大阪国際交流センター，大阪.

50. 樫田博史：大腸疾患のトレンド：鋸歯状腺腫から ESD まで．第353回浜松消化器病研究会，平成23年9月1日，浜松，静岡.

51. 矢田典久：ミニレクチャー「肝エラストグラフィー：各モダリティ―における測定原理と結果の解釈」，第 2 回大阪消化器画像・IVR 研究会，平成23年9月2日，ブリーゼプラザ，大阪.

52. 樫田博史：大腸腫瘍の診断と治療：表面型病変を中心に．第54回奈良県大腸疾患勉強会，平成23年9月3日，奈良.

53. 北野雅之：特別講演「胆膵疾患に対するEUS-FNAとInterventional EUS」，第16回湖南消化器勉強会，平成23年9月8日，ライズヴィル都賀山，滋賀.

54. 工藤正俊：特別講演「肝癌治療の現状と今後の展開」，第 7 回西濃がん診療研究会学術講演会，大垣フォーラムホテル，岐阜，平成23年9月10日.

55. 工藤正俊：特別講演「肝硬変・肝癌の治療ガイドラインと発癌抑制」，リーバクト配合顆粒発売15周年講演会，城西館，高知，平成23年9月22日.

56. 工藤正俊：特別講演「C型慢性肝炎の最新の治療」，C型肝炎治療学術講演会，ホテルグランヴィア大阪，大阪，　平成23年10月3日.

57. 樫田博史：大腸腫瘍：診断・治療の基礎と最近の話題（1）NBI の原理と使い方．堺市医師会消化器談話会，平成23年10月8日，堺，大阪.

58. 樫田博史：大腸鋸歯状病変に関する臨床的課題．ワークショップ「大腸鋸歯状病変の内視鏡診断と治療」第53回日本消化器病学会大会，第19回日本消化器関連学会週間 JDDW 2011（第82回日本消化器内視鏡学会総会），平成23年10月20日-23日，福岡国際会議場，福岡.

59. 上嶋一臣：進行・再発肝細胞癌に対する動注化学療法と分子標的薬併用による新規治療法の確立を目指した厚生労働省科学研究費による多施設共同研究．　ブレックファーストセミナー「肝動注化学療法-Japanese experience から World Standard へ向けて-」，第19回日本消化器関連学会週間 JDDW 2011（第15回日本肝臓学会大会），平成23年10月20日-23日，福岡国際会議場，福岡.

60. 工藤正俊: 特別講演「造影超音波は肝癌診療をどう変えたか？」, 肝炎・肝硬変治療レクチャーミーティング, 平成 23 年 10 月 28 日, 川崎日航ホテル, 神奈川.

61. 工藤正俊: 特別講演「造影超音波は肝癌診療をどう変えたか？」, 第 11 回北海道腹部造影エコー・ドプラ診断研究会, 平成 23 年 11 月 12 日, 第一三共株式会社札幌支店, 北海道.

62. 樫田博史：こんなに便利、消化管超音波. 第 6 回広島消化管超音波研究会, 平成 23 年 11 月 15 日, 広島.

63. 樫田博史：上部消化管症状に対する診療の進め方 ～逆流性食道炎から小腸疾患まで～. Nexium Symposium in Kaizuka, 平成 23 年 11 月 17 日, 貝塚, 大阪 .

64. 樫田博史：実戦大腸内視鏡：検出、画像強調から ESD まで. 第 7 回実地医家の実戦内視鏡研究会, 平成 23 年 11 月 19 日, 大阪.

65. 工藤正俊: 特別講演「肝癌診療の新しいパラダイム」, 腹部超音波オープンカンファレンス特別講演会, 平成 23 年 11 月 24 日, 神戸市立医療センター中央市民病院, 兵庫.

66. 坂本洋城: 症例提示. Japan Biliary Top Runners' Meeting 2011, 平成 23 年 11 月 25 日, TKP 品川カンファレンスセンター, 東京.

67. 工藤正俊: 特別講演「分子機序に基づく癌治療戦略」, 第 53 回大阪肝穿刺生検治療研究会, 平成 23 年 11 月 26 日, ホテルグランヴィア大阪, 大阪.

68. 松井繁長: 特別講演「NSAIDs 潰瘍の最近の話題」, 第 20 回りんくう消化器病研究会, 平成 23 年 11 月 26 日, ベルビューガーデンホテル関西空港, 大阪.

69. 樫田博史：大腸内視鏡のスキルアップ：診断から治療まで～EMR/ESD の現状と展望～. 第 2 回滋賀消化管研究会, 平成 23 年 12 月 3 日, 大津, 滋賀.

70. 樫田博史：大腸鋸歯状病変の症例呈示と臨床的取扱い. 消化管 臨床と病理の会, 平成 23 年 12 月 5 日, 大阪狭山, 大阪.

71. 工藤正俊: 基調講演「肝癌治療アルゴリズムと治療法の選択」, 第 39 回日本肝臓学会西部会, 平成 23 年 12 月 10 日, 岡山コンベンションセンター, 岡山.

72. 北野雅之: 特別講演「コンベックス型 EUS による診断と EUS-FNS の基本手技」, 第 1 回九州 ERCP-EUS テクニカルセミナー, 平成 23 年 12 月 10 日, FFB ホール, 福岡.

73. 樫田博史：消化器医からみた　抗血栓薬使用患者の取扱い. 第 25 回日本冠疾患学会学術集会　セミナー「進化し続ける抗血栓療法～循環器と消化器の立場から～」, 平成 23 年 12 月 17 日,　大阪.

74. 松井繁長: 講演「NSAIDs 潰瘍の現状と今後の展望」,　阪南総合医学談話会, 平成 23 年 12 月 21 日, リーガロイヤルホテル堺, 大阪.

75. 工藤正俊: 特別講演「肝疾患最近の話題」, 東四国ベアネットカンファレンス, 平成 23 年 12 月 28 日, 全日空ホテルクレメント高松, 香川.

VI. 学会発表（海外シンポジウム）

1. 2011 Ueshima K, Kudo M, Tanaka M, Kumada T, Minami Y: Phase I/II study of Sorafenib in combination with low-dose cisplatin and fluorouracil intra-arterial infusion chemotherapy. International Session “Hepato-biliary-pancreatic Cancer”, The 9th Annual Meeting of Japanese Society of Medical Ocology, Yokohama, Japan, July 21-23, 2011.

2. 2011 Sakamoto H, Kitano M, Kudo M: Conmparative study between EUS-guided celiac plezus neurolysis and EUS-guided broad neurolysis. VTR シンポジウム「Interventional EUS の最前線」, 第 81 回日本消化器内視鏡学会総会, Aichi, Japan, August 18, 2011.

3. 2011 Kitano M: Recent advanced in diagnostic EUS. Symposium “Advances in pancreaticobiliary endoscopy”, Asian Pacific Digestive Week (APDW) 2011, October 1-4, 2011, Singapore.

VII. 学会発表（海外一般演題）

過去の年報への記載漏れ分

1. 2010 Kitano M, Imai H, Komaki T, Kamata K, Sakamoto H, **Kudo M**: EUS-guided gallbladder drainage for treatment of acute cholecystitis and obstructive jaundice. 2010 Digestive Disease Week, Louisiana, USA, May 1-5.

2011

1. 2011 Venook A, Lencioni R, Marrero JA, **Kudo M**, Nakajima K, Ye SL: First interim results of the Global Investigation of therapeutic DEcisions in hepatocellular carcinoma (HCC) and Of its treatment with sorafeNib (GIDEON) study: Oncologists and non-oncologists appear to use sorafenib (Sor) differently in the management of HCC. 2011 Gastrointeritinal Cancers Symposium (ASCO-GI 2011), San Francisco, USA, January 20-22.

2. 2011 Bae SH, Yoon SK, Ye SL, Marrero J, Lencioni R, Venook A, Nakajima K, **Kudo M**: GIDEON (Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafeNib) interim results: Child-Pugh status subgroup analysis. The 21st Conference of the Asian Pacific Association for the Study of the Liver (APASL), Bangkok, Thailand, February 17-20.

3. 2011 Geschwind JF, Lencioni R, Marrero J, Venook A, Ye S-L, Nakajima K, **Kudo M**: Worldwide trends in locoregional therapy for hepatocellular carcinoma (HCC): first interim analysis of the Global Investigation of therapeutic DEcisions in HCC and Of its treatment with sorafeNib (GIDEON) study. SIR 36th Annual Scientific 2011 Meeting, Chicago, USA, March 26-31.

4. 2011 Bridges JFP, BPharm GG, **Kudo M**, Okita K, Han KH, Ye SL, Blauvelt BM: Stakeholder involvement in priority setting of strategies to improve liver cancer control policy in Asia. 46th Annual Meeting of the European Association for the Sudy of the Liver (EASL) , Berlin, Germany, March 31-April 3.

5. 2011 Bronowicki JP, Lencioni R, Venook A, Marrero JA, **Kudo M**, Ye SL, Nakajima K, Cihon F, Papandreou C: GIDEON (Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafeNib) study first interim results, Sorafenib dosing across regions and disease

subgroups. 46th Annual Meeting of the European Association for the Sudy of the Liver (EASL), Berlin, Germany, March 31-April 3.

6. 2011 Kimura H, Sakamoto H, Nagai T, Kudo K, Furuta K, Arao T, Kitano M, **Kudo M**, Nishio K: Serum concentrations of Angiogenesis-related molecules in Patients with Pancreatic Cancer. AACR 102th Annual Meeting 2011, Florida, USA, April 2-6, 2011.

7. 2011 Nagai T, Arao T, Matsumoto K, Kudo K, Hagiwara S, Sakurai T, Ueshima K, Haji S, **Kudo M**, Nishio K: Expression levels of EMT-related genes in hepatocellular carcinoma. AACR 102th Annual Meeting 2011, Florida, USA, April 2-6, 2011.

8. 2011 Asakuma Y, Matsui S, Kawasaki M, Sakurai T, Kashida H, **Kudo M**: Prevention of delayed bleeding after endoscopic submucosal dissection (ESD) for gastric tumors. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

9. 2011 Sakamoto H, Kitano M, Kamata K, **Kudo M**: EUS-guided broad plexus-neurolysis over the superior mesenteric artery. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

10. 2011 Sakamoto H, Kitano M, Kamata K, Matsui S, Asakuma Y, **Kudo M**: Contrast enhanced harmonic EUS imaging of submucosal tumor of gastrointestinal tract. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

11. 2011 Matsui S, **Kudo M**, Okada M, Asakuma Y, Kawasaki M, Sakurai T, Kashida H: Evaluation of the response to chemotherapy in advanced gastric cancer by contrast-enhanced harmonic EUS. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

12. 2011 Kawasaki M, Asakuma Y, Matsui S, Sakurai T, Kashida H, **Kudo M**: The usefulness of *helicobacter pylori* eradication therapy for the healing artifical gastric ulcer after endoscopic submucosal dissection for early gastric cancer. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

13. 2011 Inoue T, **Kudo M**, Komuta M, Sakamoto M, Okada M, Murakami T:

Assessment of hepatobilliary phase Gd-EOB-DTPA-Enhanced MRI for HCC and borderline lesions and comparison of detection ability versus MDCT. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

14. 2011 Sakurai T, **Kudo M**, Ueshima K, Matsui S, Kashida H, Karin M: P38alpha inhibits liver fibrogenesis and consequent hepatocarcinogenesis by curtailing accumulation of reactive oxygen species. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

15. 2011 Kitano M, Imai H, Kamata K, Komaki T, Sakamoto H, **Kudo M**: EUS-guided gallbladder drainage as an alternative treatment for malignant biliary obstruction after unsuccessful ERCP: Outcomes of long term follow-up. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

16. 2011 Izumi N, Kaneko S, Nishiguchi S, **Kudo M**, Sata M, Omata M: Peginterferon alfa-2a (40KD) plus ribavirin for the treatment of patients with chronic hepatitis C and compensated liver cirrhosis in Japan. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

17. 2011 Kamata K, Kitano M, **Kudo M**, Imai H, Komaki T, Sakamoto H: Dynamic imaging of gallbladder diseases by contrast-enahanced harmonic EUS. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

18. 2011 Kamata K, Kitano M, **Kudo M**, Imai H, Komaki T, Sakamoto H: Role of EUS in detection and follow-up of intraductal papillary mucinous neoplasms and concomitant invasive carcinomas. Digestive Disease Week (DDW) 2011, Chicago, USA, May 7-10, 2011.

19. 2011 **Kudo M**, Izumi N, Sakamoto M, Matsuyama Y, Ichida T, Nakashima O, Matsui O, Ku Y, Kokudo N, Makuuchi M, for the Liver Cancer Group of Japan: Improved survival in patients with hepatocellular carcinoma over 30 years in Japan: Analysis of nationwide prospective registry of 148,161 patients. American Society of Clinical Oncology (ASCO) 2011 Annual Meeting, Chicago, USA, June 3-7, 2011.

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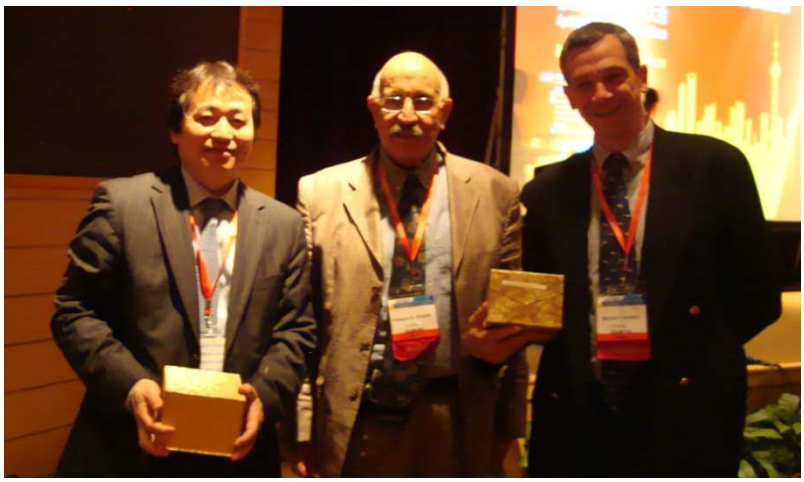

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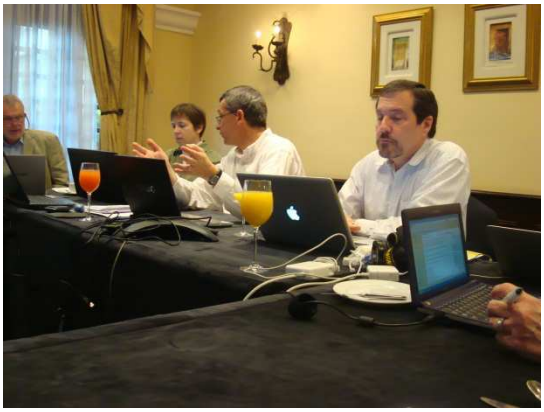

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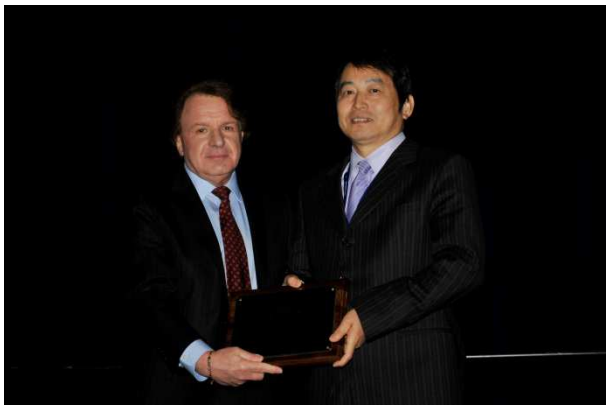
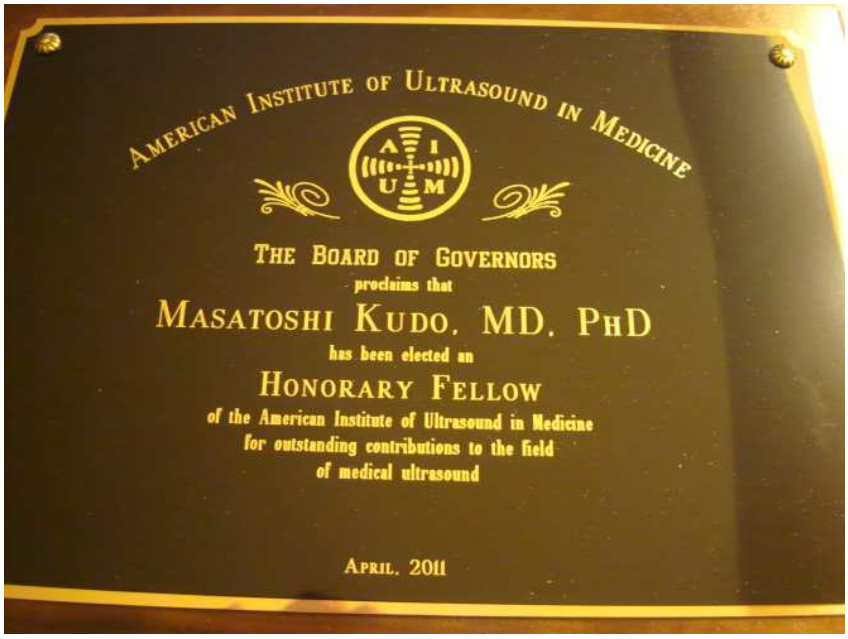


米国超音波医学会（AIUM）
名誉会員証受賞



WFUMB COE Lecture,
June 9-13, 2011
Tirg Mures, Romania


Norbert
Gritzmann


CASA VLAD DRACU
Sec. XV. Între 1431-1436, reședința lui Vlad Dracu, membru al Ordinului Dragonului. În 1431 aici s-a născut Vlad Țepeș Drăculea.
DAS HAUS VLAD DRACU
Haus aus dem XV Jahrhundert. War zwischen 1431-1436 Sitz des Vlad Dracu, Mitglied des Drachen Ordens. Hier wurde 1431 Vlad Țepeș Drăculea geboren.
THE VLAD DRACUL-HOUSE
Built in the 14th-15th century. Between 1431 and 1436 residence of Vlad Dracu, member of Dragon Order. Vlad Țepeș Draculea was born here in 1431.
1


RESTAURANT
TERASA
BAR
TERASA INTERIOARA

ドラキュラの生家

台湾肝臓学会
Invited Lecture
June 24-26, 2011
Taipei, Taiwan

Prof. Morris Sherman
Prof. Dinn Chin Chenと

世界超音波医学会（WFUMB）
学術会議 General Assembly

Aug 28, 2011
Vienna, Austria

WFUMB Executive Bureau Meeting

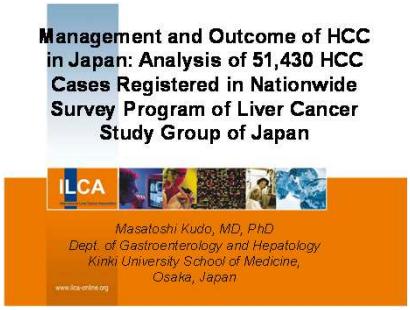
この日よりWFUMBのpresident
に就任


AUSTRIA CE
VIENNA
2011
WFUMB

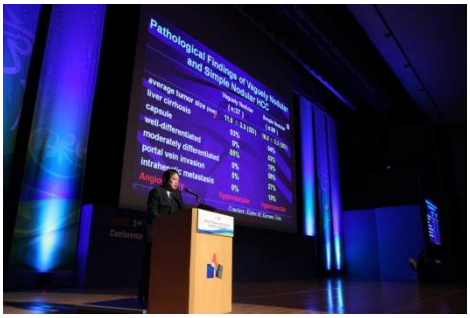

AUSTRIA CENTE
VIENNA
2011
WFUMB
EUROSON
ULTRASCHALL


WFUMB Education Committee Meeting
San Jose: Costa Rica: 25-26 February 2011

国際肝癌学会（ILCA）
Sep 1-4, 2011
Hong Kong, China


Management and Outcome of HCC in Japan: Analysis of 51,430 HCC Cases Registered in Nationwide Survey Program of Liver Cancer Study Group of Japan
ILCA
Masatoshi Kudo, MD, PhD
Dept. of Gastroenterology and Hepatology
Kinki University School of Medicine,
Osaka, Japan


UEGW
STOCKHOLM 2011


Pathological Findings of Vaguely Nodular and Simple Nodular HCC
average tumor size (mm)
liver cirrhosis
capsule
well-differentiated
moderately differentiated
portal vein invasion
intrahepatic metastasis


possible
prognos
APASL 2nd Hepatocellular Carcinoma Conference (9th APASL STC)


INTERNATIONAL LIVER CANCER ASSOCIATION
Fifth ILCA Annual Conference
2 - 4 September 2011
Hong Kong
ILCA


ILCA


ILCA

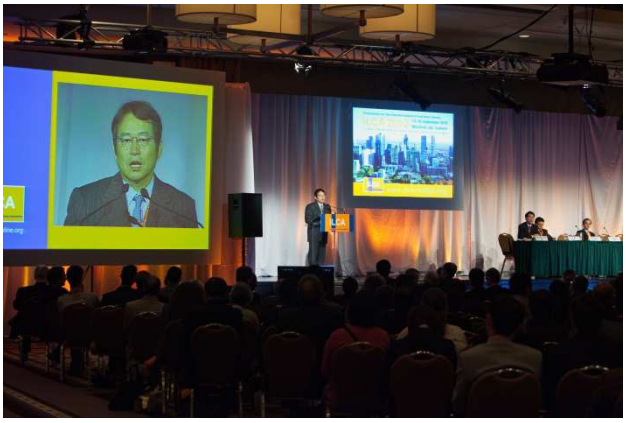

ILCA


INTERNATIONAL LIVER CANCER ASSOCIATION
Fifth ILCA Annual Conference
2 - 4 September 2011
Hong Kong


INTERNATIONAL LIVER CANCER ASSOCIATION
Fifth ILCA Annual Conference
2 - 4 September 2011
Hong Kong
ILCA


ILCA 2010
ILCA


ILCA
General Session 4:
Locoregional Therapies




Yong Park[1-3], Sang Hoon Ahn[1-4],
[1-4], Chae Yoon Chon[1-3] and Do Young Kim[1-3]*
[2]Institute of Gastroenterology, Yonsei University College of Medicine;
[4]Brain Korea 21 Project for Medical Science, Seoul, Korea.
Masatoshi Kudo
Riad Salem

Yonsei大学にて
Sep 25-27
Seoul, Korea

AFSUMB Council Meeting,
Nov 18-21, 2011,
Bali, Indonesia

3rd Conference International Contrast Ultrasound Imaging
Dec 17-18, 2011
Kunming, China

第3回日本肝がん分子標的治療研究会　平成23年1月15日 下関にて

Dr. Alshimaa食事会

平成23年3月28日
医局にて

鄭先生送別会

平成23年4月26日
堺東にて


PEUGEOT

二見さん送別会

平成23年5月28日
栂・美木多にて

The 2nd Asia-Pacific Primary Liver Cancer Expert Meeting

平成23年7月1日-3日
Hyatt Regency Osakaにて

Prof. Adrian M Di Bisceglie (USA)

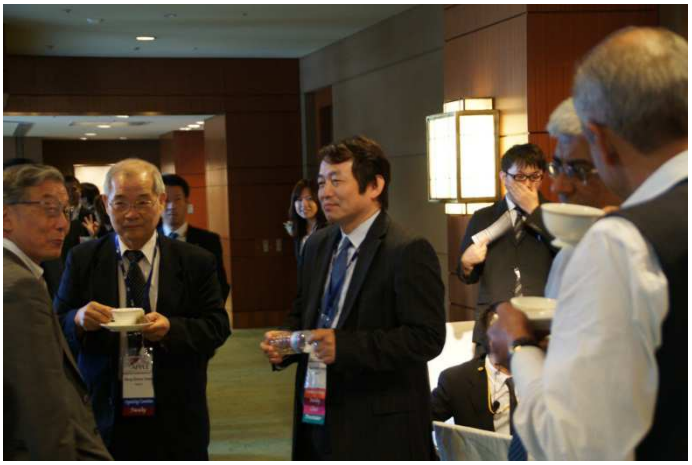
Prof. Morris Sherman (Canada)

神代正道教授（日本）

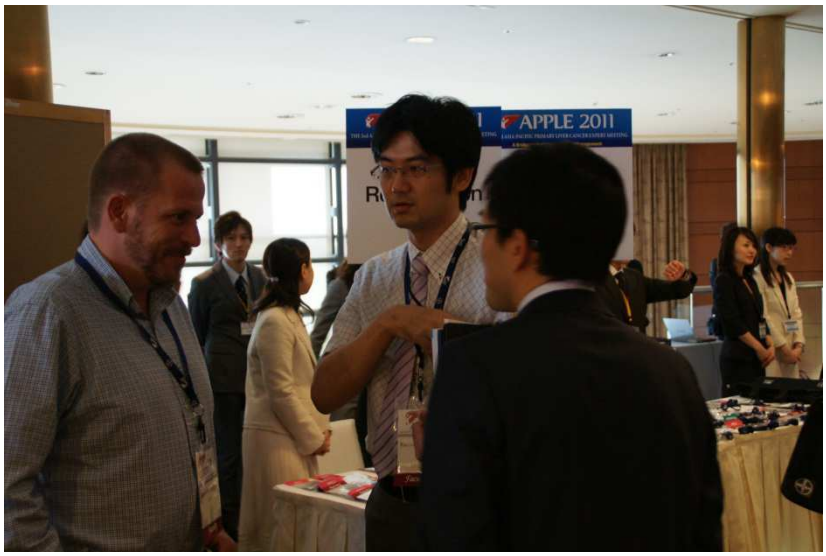
Prof. Jordi Bruix (Spain)

Prof. Kwang-Hyub Han (Korea)

Prof. Byung Ihn Choi (Korea)
Prof. Joong-Won Park (Korea)



Prof. Ding-Shinn Chen (Taiwan)



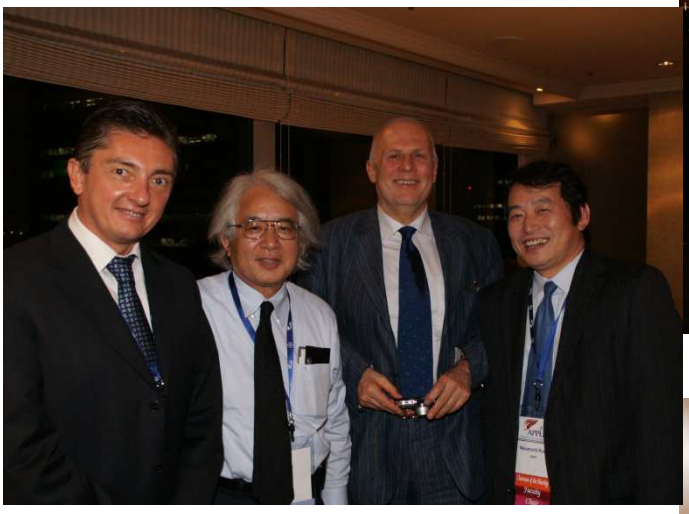
Prof. John FP Bridges (USA)


HYATT
REGENCY
OSAKA

大阪城をのぞむホテルニューオオタニにて
Faculty Dinner

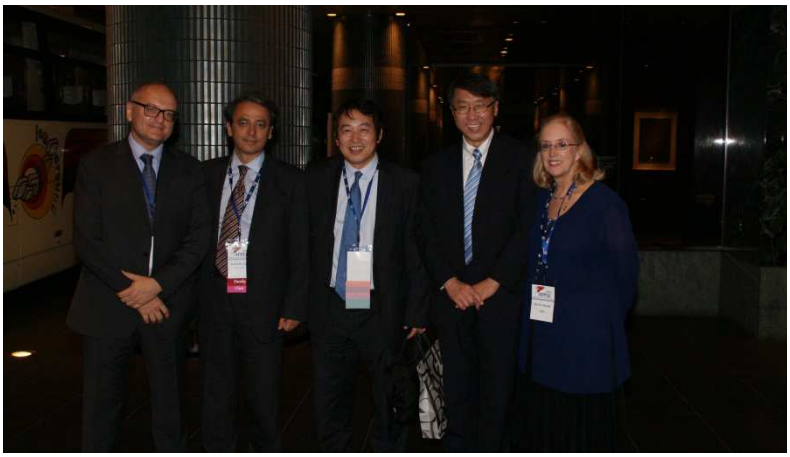
Prof. Fabio Piscaglia (Italy)
Prof. Riad Salem (USA)

Prof. Vincenzo Mazzaferro (Italy)



Prof. Riccardo Lencioni (Italy)
Prof. Masao Omata (Japan)
Prof. Luigi Bolondi (Italy)

Prof. Lewis Roberts (USA)



Prof. Vincenzo Mazzaferro (Italy)
Prof. Josep M Llovet (Spain)
Prof. Andrew X Zhu (USA)
Prof. Barri Blauvelt (USA)


Meet the Professor 3
Breakfast Seminar


Meet the Professor Breakfast Seminar
"New trends of TACE"
(Conventional, Beads, DCB, Yttrium 90)
Pref. Riccardo Lencioni (Italy)
Pref. Riad Salem (USA)
Pref. Yun Hwan Kim (Korea)
Moderator Yasuaki Arai, M.D. (Japan)


July 1-3, 2011
Masatoshi Kudo


Masatoshi Kudo


2011
APPLE
THE 2nd ASIA-PACIFIC
PRIMARY LIVER CANCER
EXPERT MEETING
mRECIST for HCC
Professor Riccardo Lencioni
2011
APPLE
THE 2nd ASIA-PACIFIC
PRIMARY LIVER CANCER
EXPERT MEETING


The 2nd APPLE Meeting, Osaka, July 1-3, 2011


Kazuomi Ueshima
Yonson Ku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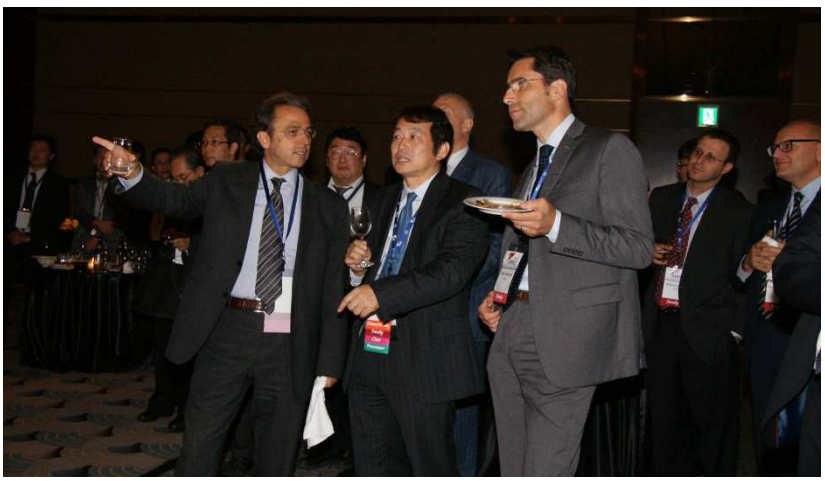
OSAKA

近大養殖マグロ解体ショー

Prof. Jordi Bruix (Spain)
Prof. Josep M Llovet (USA)
Prof. Vincenzo Mazzaferro (Italy)



左から
Prof. Young Nyun Park (Korea)
Prof. Sheng-Long Ye (China)
Prof. Valerie Paradis (France)
Prof. Masatoshi Kudo (Japan)
Prof. Kwang-Hyub Han (Korea)
Prof. Oidov Baatarkhuu (Mongolia)

Prof. Peter R Galle (Germany)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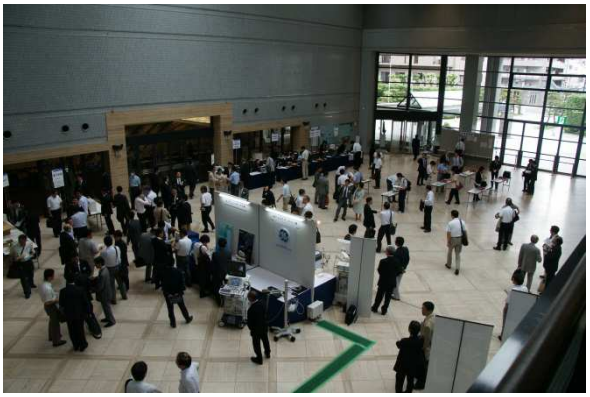
implantable port assist system
erapy is one of the methods of chemotherapy, which delivers a
to obtain a high local concentration of the drug in the tumor
chemotherapeutic agents infused by this method are most
ced hepatocellular carcinoma in Japan.


THE 2nd ASIA-PACIFIC
2011
APPLE
Asia-Pacific Primary Liver Cancer Expert Meeting
Hyatt Regency Osaka | Osaka, Japan

日本消化器病学会近畿支部第95回例会

平成23年8月20日
大阪国際交流センターにて




座長
辻 直子


司会者
樫田博


座長


司会者


座長


座 長


STAFF
次演者


JSGE
1898
日本消化器病学会近畿支部第95回例会
2011年8月20日(土) 大阪国際交流センター


JSGE
1898
日本消化器病学会近畿支部
第95回例会
会長 工藤 正俊（近畿大学消化器内科）
会期 2011年8月20日(土)
会場 大阪国際交流センター

ウイルス肝炎と肝がんの理解のための市民公開講座

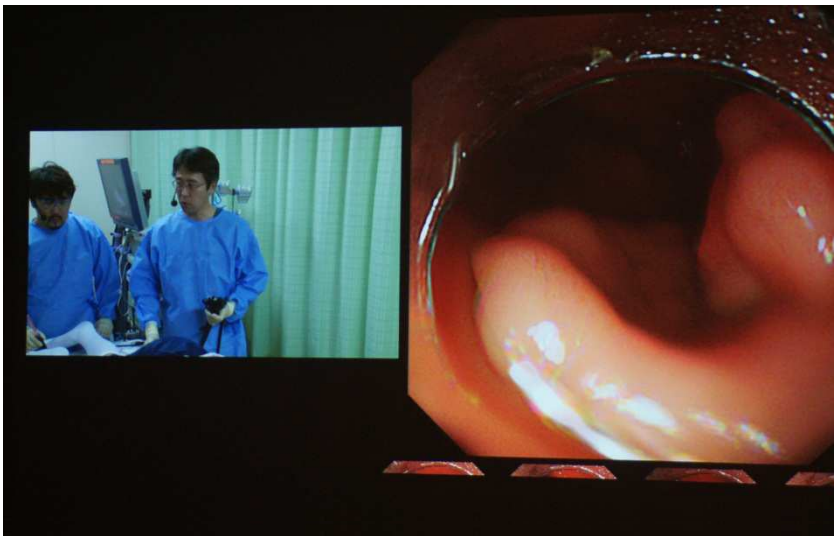
平成23年8月21日
大阪国際交流センターにて

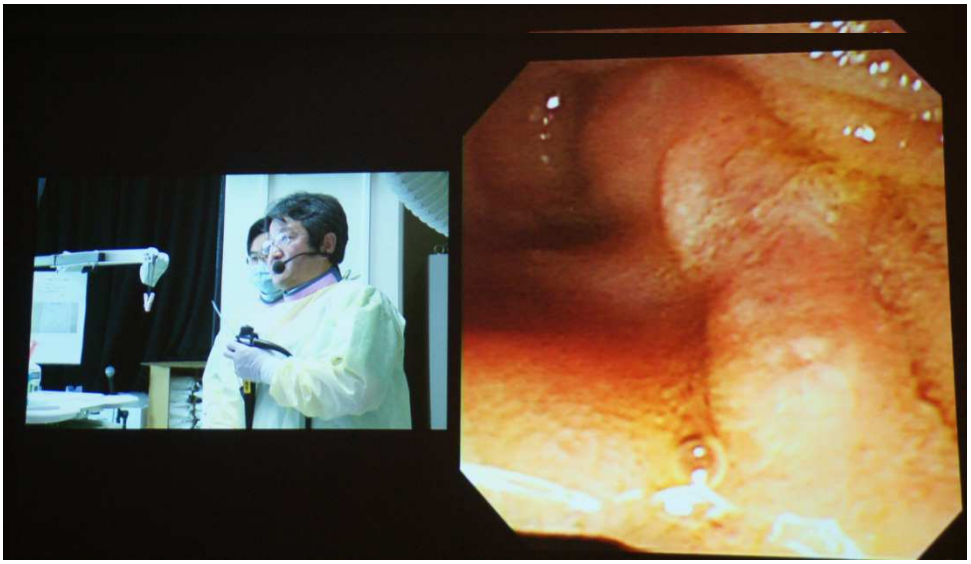

司会者

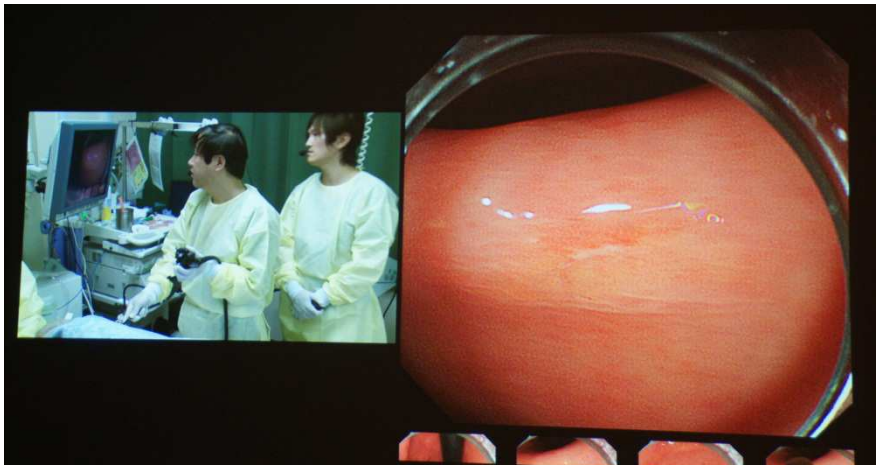
仁科　亜季子さん

第1回近畿消化器内視鏡ライブコース

平成23年11月27日
近畿大学医学部にて





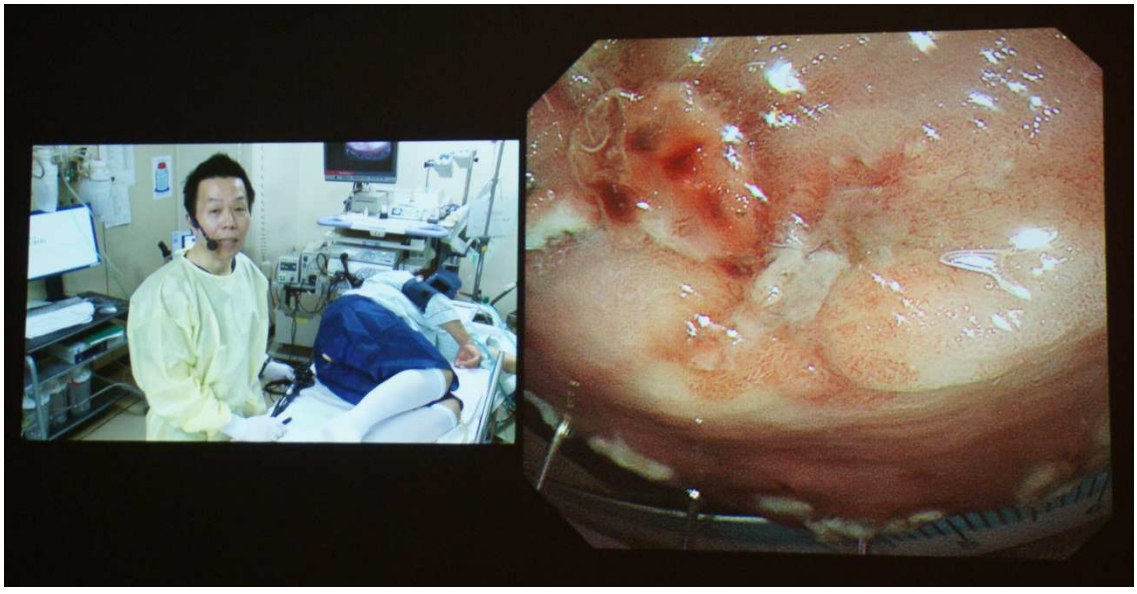


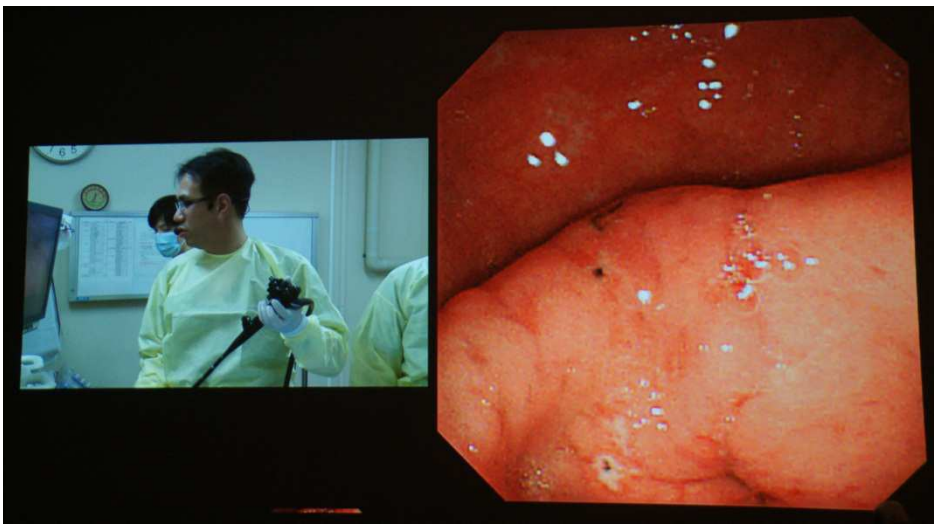
松井繁長先生

豊永高史先生（神戸大学）

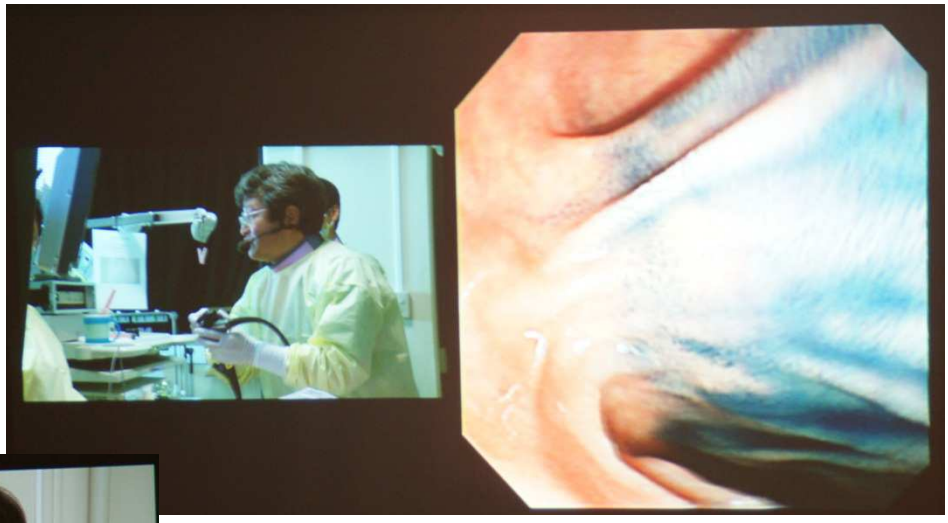
八隅　秀二郎先生（北野病院）

樫田博史先生

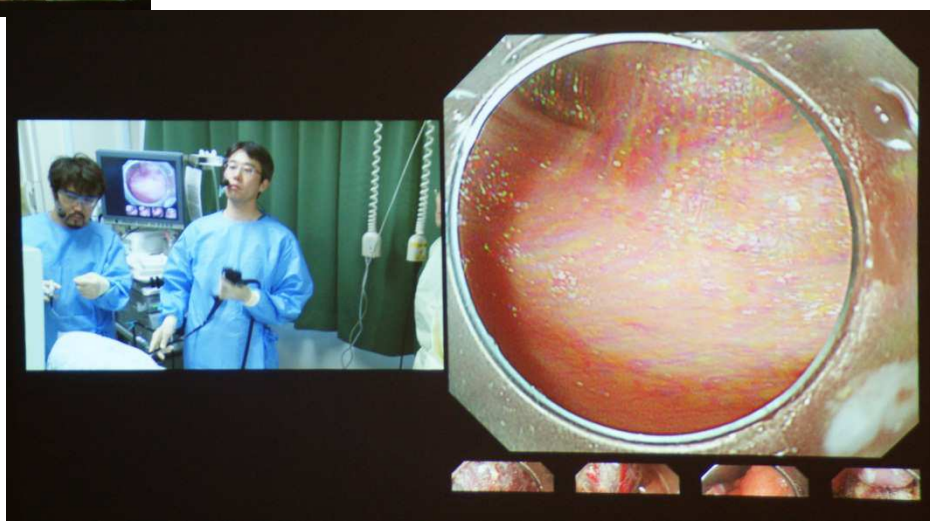




北野雅之先生



八隅　秀二郎先生（北野病院）

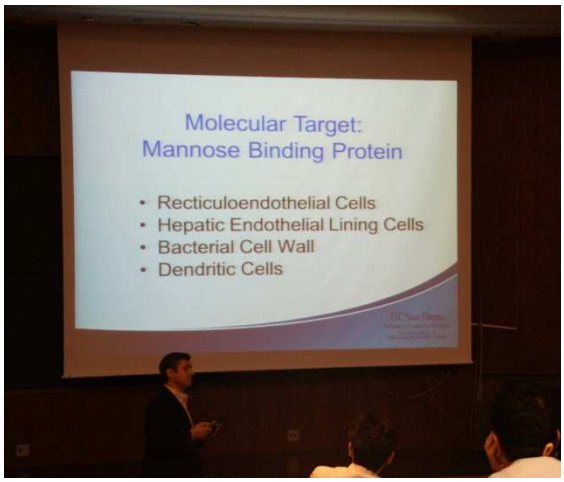


豊永高史先生（神戸大学）


Boston Scientific
OLYMPUS

Dr. Vera Special Lecture

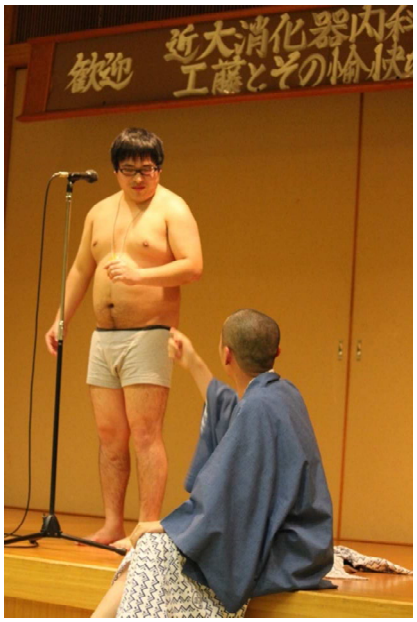
平成23年12月5日
近畿大学医学部にて



平成23年度医局旅行

平成23年12月10日-11日
長島スパーランド

近大消化器内科医局旅行
～工藤とその愉快な仲間たち～

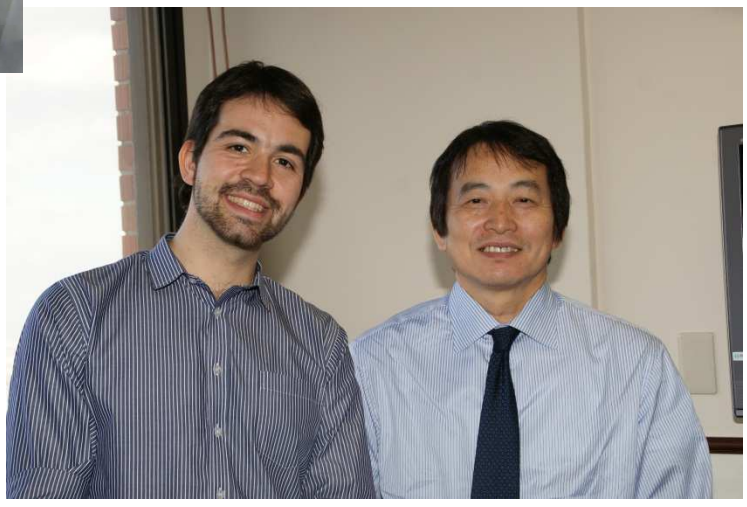


新入局予定　千品君

新入局予定　田中さん

Dr. Alberto

医局にて



イタリアのボローニャ大学
からの留学生
Dr. Alberto

別刷
新聞・雑誌・報道等

DOI:10.1158/1535-7163.MCT-10-0544

Molecular Cancer Therapeutics

Sorafenib Inhibits the Hepatocyte Growth Factor–Mediated Epithelial Mesenchymal Transition in Hepatocellular Carcinoma

Tomoyuki Nagai, Tokuzo Arao, Kazuyuki Furuta, et al.

Mol Cancer Ther 2011;10:169-177. Published online January 10, 2011.

DOI:10.1158/1535-7163.MCT-10-0544

Preclinical Development

Molecular Cancer Therapeutics

Sorafenib Inhibits the Hepatocyte Growth Factor–Mediated Epithelial Mesenchymal Transition in Hepatocellular Carcinoma

Tomoyuki Nagai[1,2], Tokuzo Arao[1], Kazuyuki Furuta[1], Kazuko Sakai[1], Kanae Kudo[1,2], Hiroyasu Kaneda[1], Daisuke Tamura[1], Keiichi Aomatsu[1], Hideharu Kimura[1], Yoshihiko Fujita[1], Kazuko Matsumoto[1], Nagahiro Saijo[3], Masatoshi Kudo[2], and Kazuto Nishio[1]

Abstract

The epithelial mesenchymal transition (EMT) has emerged as a pivotal event in the development of the invasive and metastatic potentials of cancer progression. Sorafenib, a VEGFR inhibitor with activity against RAF kinase, is active against hepatocellular carcinoma (HCC); however, the possible involvement of sorafenib in the EMT remains unclear. Here, we examined the effect of sorafenib on the EMT. Hepatocyte growth factor (HGF) induced EMT-like morphologic changes and the upregulation of SNAI1 and N-cadherin expression. The downregulation of E-cadherin expression in HepG2 and Huh7 HCC cell lines shows that HGF mediates the EMT in HCC. The knockdown of SNAI1 using siRNA canceled the HGF-mediated morphologic changes and cadherin switching, indicating that SNAI1 is required for the HGF-mediated EMT in HCC. Interestingly, sorafenib and the MEK inhibitor U0126 markedly inhibited the HGF-induced morphologic changes, SNAI1 upregulation, and cadherin switching, whereas the PI3 kinase inhibitor wortmannin did not. Collectively, these findings indicate that sorafenib downregulates SNAI1 expression by inhibiting mitogen-activated protein kinase (MAPK) signaling, thereby inhibiting the EMT in HCC cells. In fact, a wound healing and migration assay revealed that sorafenib completely canceled the HGF-mediated cellular migration in HCC cells. In conclusion, we found that sorafenib exerts a potent inhibitory activity against the EMT by inhibiting MAPK signaling and SNAI1 expression in HCC. Our findings may provide a novel insight into the anti-EMT effect of tyrosine kinase inhibitors in cancer cells. *Mol Cancer Ther; 10(1); 169–77. ©2011 AACR.*

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third largest cause of cancer-related death in the world annually (1). Recurrence, metastasis, and the development of new primary tumors are the most common causes of mortality among patients with HCC (2). Sorafenib (Nexavar; Bayer HealthCare Pharmaceuticals Inc.) is a small molecule that inhibits the kinase activities of Raf-1 and B-Raf in addition to VEGFRs, PDGFR-β (platelet-derived growth factor receptor β), Flt-3, and c-KIT (3). Two recent randomized controlled trials reported a clinical benefit of single-agent sorafenib in extending overall survival in both Western and Asian patients with advanced unresectable HCC (4, 5). The potential action mechanisms that lead to these clinical benefits are thought to include antiangiogenic effects and sorafenib's characteristic inhibitory effect on Raf-1 and B-Raf signaling.

Meanwhile, growing evidence indicates that the epithelial mesenchymal transition (EMT), a developmental process by which epithelial cells reduce intercellular adhesions and acquire fibroblastoid properties, has important roles in the development of the invasive and metastatic potentials of cancer progression (6–8). To date, numerous clinicopathologic studies have shown positive correlations between the expressions of the transcription factors SNAI1 (snail homologue 1/SNAIL) and SNAI2 (snail homologue 2/Slug), which are key inducible factors of the EMT, and poor clinical outcomes in breast, ovary, colorectal, and lung cancer; squamous cell carcinoma; melanoma, and HCC (reviewed in ref. 6).

Generally, the activation of a wide variety of ligands including FGF (fibroblast growth factor), TGF-β–BMPs (bone morphogenetic protein), Wnt, EGF (epidermal growth factor), VEGF, and HGF (hepatocyte growth factor) and its receptor can upregulate the expression of EMT-regulating transcription factors, including SNAI1, SNAI2, ZEB1, ZEB2, and TWIST (6). Among them, HGF (also known as scattering factor) activates

Authors' Affiliations: Departments of [1]Genome Biology, [2]Gastroenterology and [3]Medical Oncology, Kinki University School of Medicine, Japan

Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: Kazuto Nishio, Department of Genome Biology, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. Phone: 81-72-366-0221 (ext 3150); Fax: 81-72-367-6369; E-mail: knishio@med.kindai.ac.jp

doi: 10.1158/1535-7163.MCT-10-0544

DOI:10.1158/1535-7163.MCT-10-0544

the Met signaling pathway, thereby increasing the invasive and metastatic potentials of the cells and allowing the survival of cancer cells in the bloodstream in the absence of anchorage (9). In addition, HGF is well known as a potent angiogenic cytokine, and Met signal activation can modify the microenvironment to facilitate cancer progression (9). Therefore, the HGF-Met signaling pathway is regarded as a promising therapeutic target, and many molecular targeted drugs are under clinical development (10). In HCC, the mRNA levels of HGF and Met receptor are markedly increased compared with those in normal liver (11). A high serum HGF concentration is associated with a poor prognosis for overall survival after hepatic resection, and the serum level of HGF represents the degree of the carcinogenic state in the livers of patients with C-viral chronic hepatitis and cirrhosis (12–14). Thus, we examined the effect of sorafenib on the HGF-Met–mediated EMT in HCC.

Materials and Methods

Reagents

Sorafenib was provided by Bayer HealthCare Pharmaceuticals Inc. U0126, wortmannin (Cell Signaling Technology), and human HGF (R&D Systems) were purchased from the indicated companies. The structures of compounds are shown in Supplementary Figure 1.

Cell culture

The human HCC cell lines HepG2 and Huh7 were maintained in Dulbecco's modified Eagle's (DMEM) medium (Sigma) supplemented with 10% FBS, penicillin, and streptomycin (Sigma) in a humidified atmosphere of 5% CO_2 at 37°C. The cell lines were obtained from the Japanese Collection of Research Bioresources and were grown in culture for less than 6 months.

Scratch assay

The method used for the scratches assay has been previously described (15). Briefly, the cells were plated onto 24-well plates and incubated in DMEM containing 10% FBS until they reached subconfluence. Scratches were introduced to the subconfluent cell monolayer, using a plastic pipette tip. The cells were then cultured with DMEM containing 10% FBS at 37°C. After 24 hours, the scratch area was photographed using a light microscope (IX71; Olympus). The wound distance between edge to edge were measured and averaged from 5 points per 1 wound area, using DP manager software (Olympus). The 2 wound areas were evaluated in an experiment and the experiment was done in triplicate.

Migration assay

The migration assays were done using the Boyden chamber methods and polycarbonate membranes with an 8-μm pore size (Chemotaxicell), as previously described (15). The membranes were coated with fibronectin on the outer side and dried for 2 hours at room temperature. The cells to be analyzed (2×10^4 cells/well) were then seeded onto the upper chambers with 200 μL of migrating medium (DMEM containing 0.5% FBS), and the upper chambers were placed into the lower chambers of 24-well culture dishes containing 600 μL of DMEM containing 10% FBS or with 10 ng/mL of HGF or with HGF and 10 μmol/L of sorafenib. After incubation for 36 hours (HepG2) and 24 hours (Huh7), the media in the upper chambers were aspirated and the nonmigrated cells on the inner sides of the membranes were removed using a cotton swab. The cells that had migrated to the outer side of the membranes were fixed with 4% paraformaldehyde for 10 minutes, stained with 0.1% Giemsa stain solution for 15 minutes, and then counted using a light microscope. Migrated cells were averaged from 5 fields per 1 chamber and 3 chambers were used on 1 experiment. The experiment was done in triplicate.

Morphologic analysis

HepG2 and Huh7 cells (2×10^4 and 1×10^4 cells/well, respectively) were seeded in 6-well tissue culture dishes. After 24 hours of incubation, the cells were stimulated with 10 ng/mL of HGF or control PBS. When the inhibitors were used, the cells were exposed to each inhibitor for 3 hours before the addition of HGF. After 48 hours, the cells were analyzed using a light microscope. The experiment was done in triplicate.

Western blot analysis

The following antibodies were used in this study: phospho-Met (Y1349), Met, phospho-AKT (S473), AKT, phospho-p44/42 mitogen-activated protein kinase (MAPK), SNAI1/Snail, E-cadherin, N-cadherin, vimentin, β-actin antibody horseradish peroxidase–conjugated secondary antibody (Cell Signaling Technology), and fibronectin (Santa Cruz Biotechnology). All the experiments were done at least in duplicate. The Western blot analysis was done as described previously (16). The data were quantified by automated densitometry using Multigauge Ver. 3.0 (Fujifilm). Densitometric data were normalized by β-actin in triplicate and the average was shown above the Western blot as a ratio of control sample.

Real-time reverse transcription PCR

The real-time reverse transcription PCR (RT-PCR) method has been previously described (17). Briefly, 1 μg of total RNA from the cultured cells was converted to cDNA using a GeneAmp RNA-PCR kit (Applied Biosystems). Real-time RT-PCR amplification was done using a Thermal Cycler Dice (Takara) in accordance with the manufacturer's instructions under the following conditions: 95°C for 6 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Glyceraldehyde 3-phosphate dehydrogenase (GAPD) was used to normalize the expression levels in the subsequent quantitative analyses. To amplify the target genes, the following primers were purchased from TaKaRa: *CDH1*, forward 5′-TTA AAC

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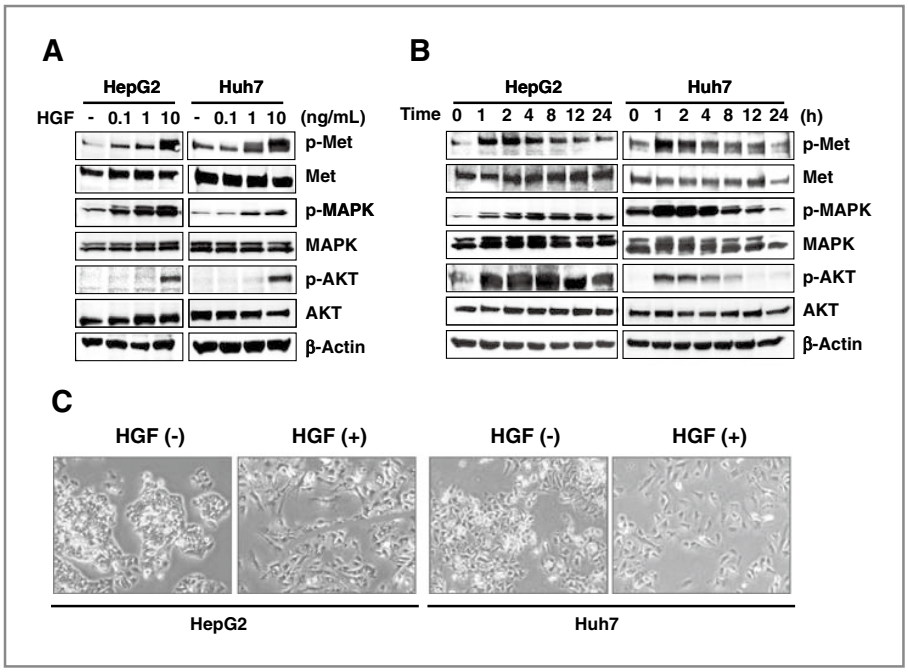

Figure 1. HGF stimulates the Met signaling pathway and induces morphologic changes in HCC. A, HGF stimulation (0, 0.1, 1, and 10 ng/mL) dose-dependently increased the phosphorylation of Met, MAPK, and AKT in the HCC cell lines HepG2 and Huh7. The results of a Western blot analysis are shown. β-Actin was used as a loading control. The serum-starved cells were stimulated with HGF for 60 minutes and then collected for analysis. B, time-course analysis of HGF stimulation. The HCC cells were stimulated with 10 ng/mL of HGF for 0, 1, 2, 4, 8, 12, and 24 hours. The results of a Western blot analysis are shown. C, HGF-mediated morphologic changes included cell scattering and the elongation of the cell shape that are characteristic of the EMT. The HepG2 and Huh7 cells were stimulated with or without 10 ng/mL of HGF for 48 hours and then photographed (magnification × 200).

TCC TGG CCT CAA GCA ATC-3′ and reverse 5′-TCC TAT CTT GGG CAA AGC AAC TG-3′; *CDH2*, forward 5′-CGA ATG GAT GAA AGA CCC ATC C-3′ and reverse 5′-GGA GCC ACT GCC TTC ATA GTC AA-3'; *SNAI1*, forward 5′-TCT AGG CCC TGG CTG CTA CAA-3′ and reverse 5′-ACA TCT GAG TGG GTC TGG AGG TG-3′; *SNAI2*, forward 5′-ATG CAT ATT CGG ACC CAC ACA TTA C-3′ and reverse 5′-AGA TTT GAC CTG TCT GCA AAT GCT C-3′; *VIM*, forward 5′-TGA GTA CCG GAG ACA GGT GCA G-3′ and reverse 5′-TAG CAG CTT CAA CGG CAA AGT TC-3′; *FN1*, forward 5′-GGA GCA AAT GGC ACC GAG ATA-3′ and reverse 5′-GAG CTG CAC ATG TCT TGG GAA C-3′; and *GAPD*, forward 5′-GCA CCG TCA AGG CTG AGA AC-3′ and reverse 5′-ATG GTG GTG AAG ACG CCA GT-3′.

Small interfering RNA transfection

Three different sequences of small interfering RNA (siRNA) targeting human SNAI1 (Hs_SNAI1_9785, 9786, and 9787) and those of 2 scramble control siRNAs were purchased from Sigma Aldrich Japan. The transfection methods have been previously described (17).

Statistical analysis

The statistical analyses were done using Microsoft Excel (Microsoft) both to calculate the SD and to test for statistically significant differences between the samples using a Student t test. A value $P < 0.05$ was considered statistically significant.

Results

To examine the activity of HGF-Met signaling in HCC cells, we examined the expressions of phospho-Met, Met, phospho-AKT, AKT, phospho-MAPK, and MAPK in the HepG2 and Huh7 cell lines, using Western blotting. The phosphorylation levels of Met, AKT, and MAPK were dose-dependently increased by HGF stimulation (Fig. 1A). A time-course analysis showed that the phosphorylation levels of Met, AKT, and MAPK peaked at 1 to 2 hours after HGF stimulation and gradually recovered to the baseline values at 4 hours later (Fig. 1B). These results indicated that Met signaling is actually capable of being activated in response to HGF in HCC cells.

From a morphologic aspect, the EMT is characterized by an increase in cell scattering and an elongation of the cell shape (18). To evaluate whether HGF mediates the morphologic change that is characteristic of the EMT in HCC cells, cellular morphology was examined after HGF stimulation. HGF clearly mediated both cell scattering and the elongation of the cell shape in HepG2 and Huh7 cell lines (Fig. 1C). These data indicate that HGF mediates

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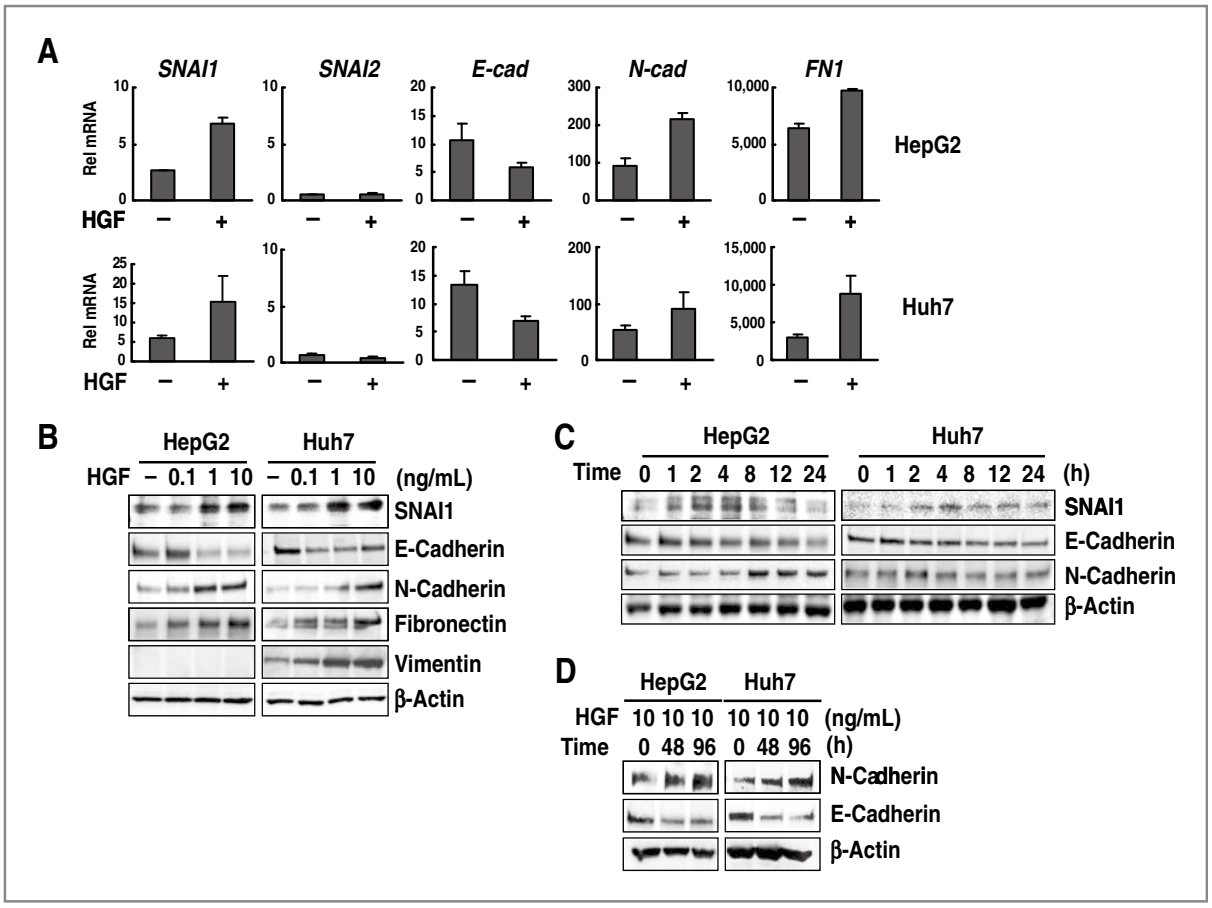

Figure 2. HGF upregulates SNAI1 expression and induces cadherin switching in HCC. A, changes in the mRNA expressions of the EMT-related genes *SNAI1/Snail*, *SNAI2/Slug*, *E-cadherin/CDH1*, *N-cadherin/CDH2*, and *fibronectin/FN1* were determined using real-time RT-PCR. The HepG2 and Huh7 cells were stimulated with or without 10 ng/mL of HGF for 2 hours (SNAI1 and SNAI2) or 48 hours (*E-cad*, *N-cad*, and *FN1*). Rel mRNA, normalized mRNA expression levels (target genes/GAPD $\times 10^4$); E-cad, E-cadherin; N-cad, N-cadherin. B, the HGF-mediated protein expression changes in SNAI1, E-cadherin, N-cadherin, fibronectin, and vimentin were determined using a Western blot analysis. The HepG2 and Huh7 cells were stimulated with HGF at the indicated dose (0, 0.1, 1, or 10 ng/mL) and collected for analysis after 4-hour stimulation for SNAI1 and 72 hours for the others. C, the cells were stimulated with 10 ng/mL of HGF for the indicated time course (0, 1, 2, 4, 8, 12, or 24 hours) and used for analysis. β-Actin was used as a loading control. D, Western blot analysis of E-cadherin and N-cadherin. The cells were stimulated with 10 ng/mL of HGF for 0, 48, and 96 hours and then analyzed.

the morphologic changes that are compatible with the induction of the EMT in HCC cell lines.

Because SNAI1 and SNAI2 are considered to be master regulators of the EMT, changes in the mRNA expression levels of EMT-related genes in response to HGF stimulation were evaluated using real-time RT-PCR (Fig. 2A). HGF stimulation upregulated SNAI1 mRNA expression by more than 2-fold, whereas the baseline expression of SNAI2 was very low compared with that of SNAI1 and did not respond to HGF in either of the HCC cell lines that were examined. Cadherin switching, which is characterized by the downregulation of E-cadherin and the upregulation of N-cadherin, is known as one of the most pivotal cellular events in the EMT (19). Cadherin switching was clearly observed on the basis of mRNA levels after HGF stimulation. The mesenchymal marker fibronectin was also upregulated (Fig. 2A).

Consistent with the mRNA changes, HGF stimulation dose-dependently upregulated the protein expression of SNAI1, N-cadherin, fibronectin, and vimentin and downregulated the expression of E-cadherin in both cell lines (Fig. 2B). Vimentin expression of HepG2 was not detected (baseline vimentin mRNA was also extremely low; data not shown). A time-course analysis showed that HGF upregulated the SNAI1 expression at 2 hours after stimulation and that the expression level recovered to the baseline value at 24 hours thereafter (Fig. 2C). Cadherin switching after HGF stimulation was observed at 8 hours later in HepG2 cells and 48 hours later in Huh7 cells (Fig. 2C and D). Generally, upregulation of SNAI1 is

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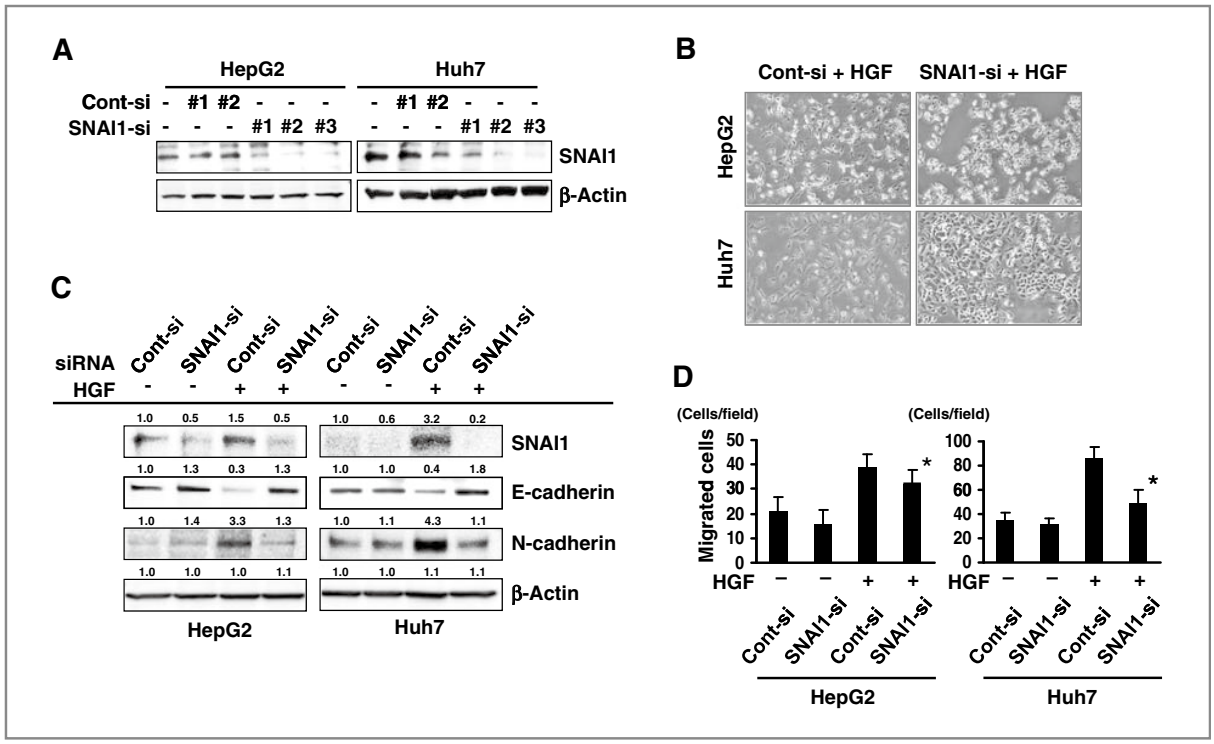

Figure 3. SNAI1 is required to induce the HGF-mediated EMT in HCC cells. A, knockdown of HGF-mediated SNAI1 expression using siRNA. Three sequences of SNAI1-siRNA (1, 2, and 3) were used. The HepG2 and Huh7 cells were treated with or without 50 nmol/L of each siRNA for 48 hours and then were stimulated with 10 ng/mL of HGF. SNAI1-siRNA #2 was effective and was used in subsequent experiments. B, SNAI1 knockdown canceled the HGF-mediated morphologic changes. The HepG2 and Huh7 cells were treated with 50 nmol/L of siRNA for 48 hours and were then stimulated with 10 ng/mL of HGF in all 4 panels. C, SNAI1 suppression by siRNA strongly canceled the HGF-mediated downregulation of E-cadherin and the upregulation of N-cadherin in both HepG2 and Huh7 cells. The cells were treated with 50 nmol/L of siRNA for 48 hours and were analyzed using a Western blot analysis. Densitometric data are shown above the Western blot. D, the siRNA knockdown of SNAI1 inhibited the HGF-mediated cellular migration. The siRNA-transfected HepG2 and Huh7 cells were evaluated using migration assay. The migration assays were conducted using the Boyden chamber methods as described in Materials and Methods. *, $P < 0.05$ (Cont-si vs. SNAI1-si with HGF); Cont-si, control-siRNA; SNAI1-si, SNAI1-targeting siRNA.

observed within few hours, but cadherin switching occurs around 24 hours later after stimulation (20, 21), consistent with our result. These results indicate that HGF meditates the induction of SNAI1, cadherin switching, and the EMT in HCC cells.

Besides SNAI1 and SNAI2, other transcription factors of several genes also have the potential to repress E-cadherin and to induce the EMT; these factors include ZEB1/TCF8, ZEB2/SMAD interacting protein 1, TWIST, E47/TCF3, and TCF4/E2-2 (6). Therefore, we examined whether SNAI1, among several EMT-inducible genes, has a central role in the HGF-mediated EMT in HCC cells. Three sequences of SNAI1-siRNA (1, 2, and 3) were used. A Western blot showed that both sequences 2 and 3 of SNAI1-siRNA completely suppressed the HGF-mediated upregulation of SNAI1 in the HepG2 and Huh7 cells (Fig. 3A); thus, the #2 SNAI1-siRNA was used in the following experiments: The siRNA knockdown of SNAI1 canceled the morphologic changes observed in HepG2 cells undergoing HGF-mediated EMT, whereas the control-siRNA did not (Fig. 3B). Similar results were obtained in Huh7 cells, indicating that SNAI1 is required for the morphologic changes observed in HGF-mediated EMT. Similarly, the siRNA knockdown of SNAI1 strongly canceled the HGF-mediated downregulation of E-cadherin and the upregulation of N-cadherin in both HepG2 and Huh7 cells (Fig. 3C). Those of mRNA expression changes were relatively correlated with the results of Western blot, except for N-cadherin in Huh7 cells (Supplementary Fig. 2A). Regarding the cellular migration, the siRNA knockdown of SNAI1 inhibited the HGF-mediated cellular migration (Fig. 3D). Collectively, these results indicate that SNAI1 is required to induce the HGF-mediated EMT in HCC cells.

In general, SNAI1 expression is regulated by ligand-receptor signal transduction through a downstream signal pathway that includes the Smad, MAPK, AKT, and GSK3 pathways (6, 22, 23). Sorafenib has been shown to inhibit RAF-MAPK signaling in HCC cells (24). Accordingly, we hypothesized that sorafenib might downregulate SNAI1 expression by inhibiting RAF-MAPK signaling, which is a unique activity of sorafenib. As

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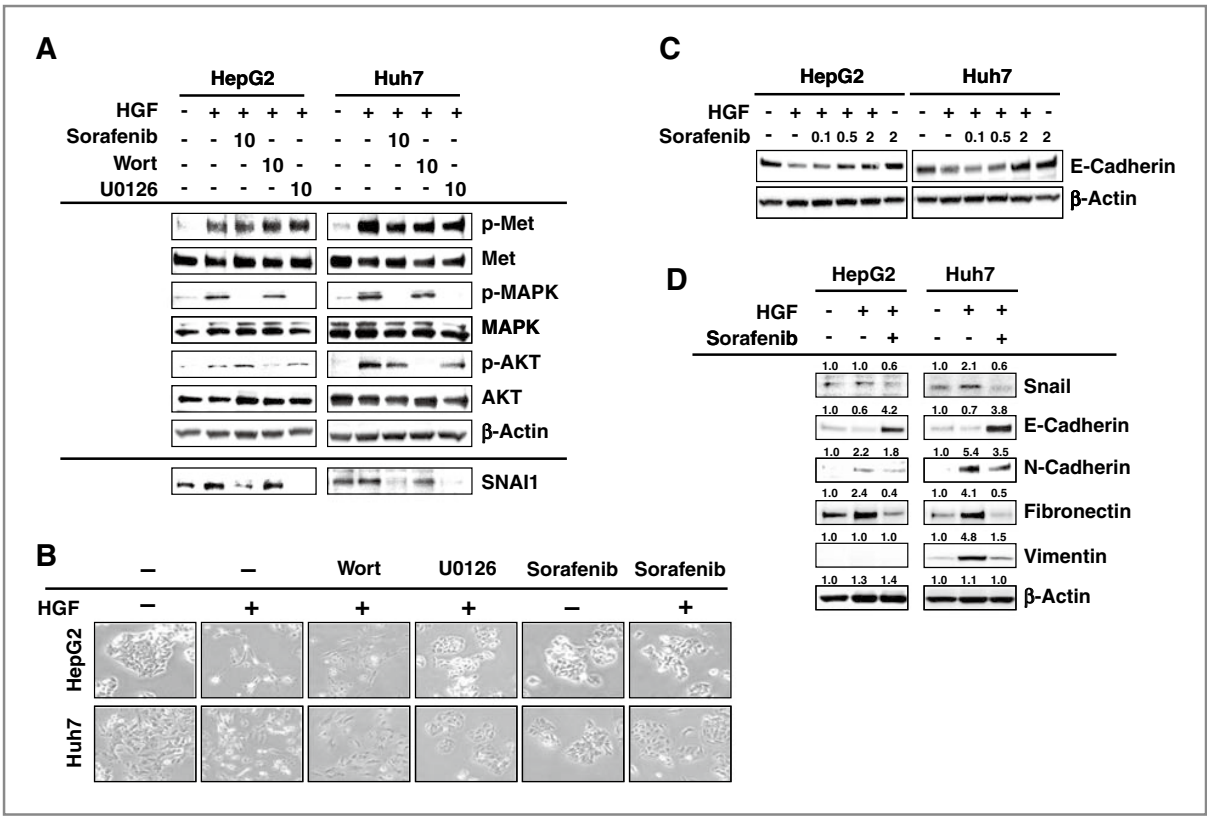

Figure 4. Sorafenib downregulates SNAI1 expression in HCC. A, as expected, sorafenib and the MEK inhibitor U0126 inhibited the HGF-mediated phosphorylation of MAPK, but the PI3K inhibitor wortmannin did not. Of note, SNAI1 expression was markedly downregulated by sorafenib and U0126. The HepG2 and Huh7 cells were exposed to 10 μmol/L of sorafenib or wortmannin or U0126 for 3 hours and were then stimulated with 10 ng/mL of HGF for 60 minutes. Wort, wortmannin. B, the HGF-mediated morphologic changes were canceled by sorafenib and U0126 but not by wortmannin in the HCC cells. The cells were exposed to sorafenib or wortmannin or U0126 for 48 hours with or without HGF (10 ng/mL) and then photographed. C, HGF-mediated downregulation of E-cadherin was canceled by sorafenib. The cells were stimulated with HGF (10 ng/mL) and treated with sorafenib at indicated concentration for 48 hours. D, HGF-mediated cadherin switching and upregulation of fibronectin and vimentin were canceled by sorafenib in the HCC cell lines. The cells were cultured with or without 2 μmol/L of sorafenib for 72 hours, with or without HGF (10 ng/mL), and then were analyzed using Western blot analysis. Densitometric data are shown above the Western blot.

expected, sorafenib and the MEK inhibitor U0126 (10 μmol/L) markedly inhibited the HGF-induced phosphorylation of MAPK, but the PI3K inhibitor wortmannin (10 μmol/L) did not. In contrast, only wortmannin inhibited the phosphorylation of AKT (Fig. 4A). Notably, SNAI1 expression was strongly downregulated by sorafenib and U0126 but not by wortmannin (Fig. 4A). These results showed that sorafenib downregulated SNAI1 expression via MAPK signaling. Meanwhile, we examined the HGF- and sorafenib-mediated expression changes of *SNAI2*, *ZEB1*, *ZEB2*, and *TWIST* using real-time RT-PCR and Western blot (Supplementary Fig. 3). Baseline and expression changes of SNAI2 and TWIST were very low compared with SNAI1, and the expression changes of ZEB1 and ZEB2 seemed not to be significant. Collectively, we considered that SNAI2, TWIST, ZEB1, and ZEB2 are not likely to be involved in the effect of HGF and sorafenib on EMT in this cell lines. Then, we examined the activity of sorafenib on HGF-mediated morphologic changes in HCC cells. HGF stimulation mediated the cell scattering and spindle-shaped changes, and these effects were clearly canceled by sorafenib and U0126, but not by wortmannin, in both HepG2 and Huh7 cells (Fig. 4B). These results were consistent with the results of Western blotting. To show whether sorafenib cancels the effect of HGF-mediated downregulation of E-cadherin, we examined the Western blot in dose–response analysis. Downregulation of E-cadherin was clearly canceled by sorafenib in a dose-dependent manner (Fig. 4C). Time-course analysis showed that HGF-mediated downregulation of E-cadherin was also canceled by sorafenib (Supplementary Fig. 4). HGF stimulation downregulated E-cadherin expression and upregulated N-cadherin, vimentin, and fibronectin in HCC cells; however, these effects were canceled by sorafenib in both HCC cell lines (Fig. 4D and Supplementary Fig. 2B). The mRNA data of N-cadherin in Huh7 cells were not correlated with protein level. These results show that sorafenib inhibits the

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RAF-MAPK pathway, thereby downregulating SNAI1 and inhibiting the EMT in HCC.

Because sorafenib inhibits the HGF-mediated EMT in HCC cells, we next examined whether the inhibitory effect of sorafenib on the EMT leads to an inhibition of cellular migration in HCC cells. A scratch assay revealed that HGF stimulation increased cellular migration by about 2- fold in both HCC cell lines; however, sorafenib significantly inhibited this effect to the baseline levels (Fig. 5A). Similarly, a migration assay using the Boyden chamber method revealed that sorafenib canceled HGF-mediated cellular migration in both cell lines (Fig. 5B). These results suggest that sorafenib actually inhibits the cellular migrating phenotype of the EMT in HCC cells. The combination of migration data with siRNA and sorafenib (Fig. 3D and Fig. 5B) suggests that inhibitory effects of sorafenib on migration may be mediated by Snail downregulation in some tumors (e.g., Huh7) but not in others (e.g., HepG2). It is assumed that the inhibitory activity of sorafenib on the cellular migrating phenotype is due to its inhibitory effect of Raf-MAPK signaling pathway (Fig. 4A and B). Regarding HGF-dependent PI3K-AKT signaling pathway, wortmannin weakly inhibited the wound closure in Huh7 cells and to the same extent by sorafenib in HepG2 cells (Supplementary Fig. 5). In contrast, wortmannin has no effect on Snail levels or on HCC morphology changes (Fig. 4A and B). Collectively, we speculate that activation of HGF-dependent PI3K-AKT pathway may not be involved in SNAI1 induction or morphologic change but at least partially involved in cell migration independent of Raf-MAPK-SNAI1 signaling.

Taken together, these results indicate that sorafenib inhibits the HGF-mediated EMT, which is characterized by cadherin switching, morphologic changes, and an increase in the cellular migrating phenotype, by inhibiting Raf-MAPK signaling, resulting in the downregulation of SNAI1 in HCC cells (Fig. 6).

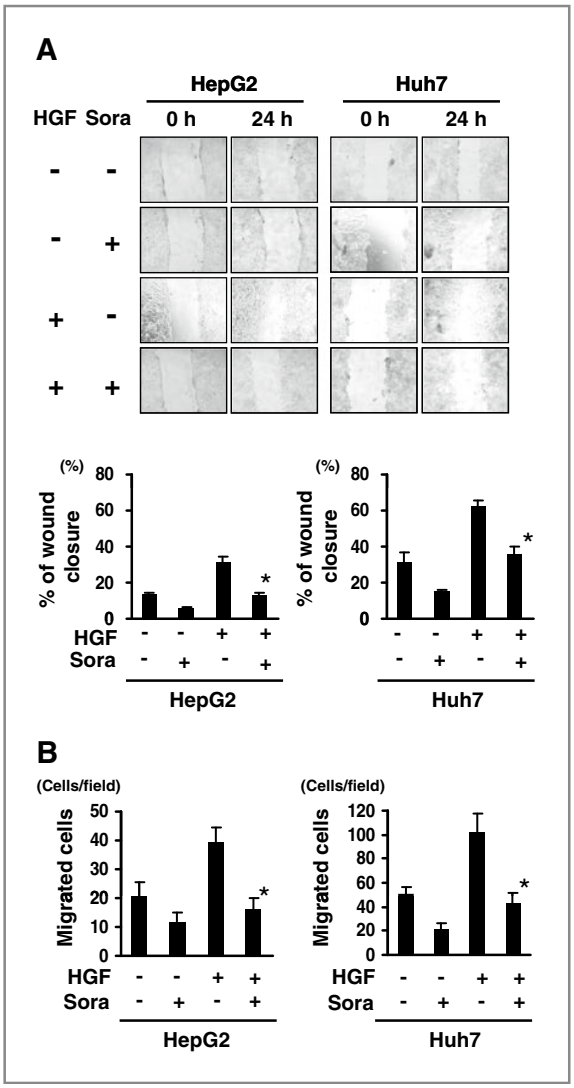

Figure 5. Sorafenib inhibits HGF-mediated cellular migration in HCC cells. A, a scratch assay revealed that HGF stimulation increased the cellular migration by about 2-fold, but sorafenib almost completely canceled the effect. The subconfluent HepG2 and Huh7 cells were scratched with a plastic pipette tip and incubated under the indicated conditions (control, 10 ng/mL of HGF; and HGF, 10 μmol/L of sorafenib). The scratch area was photographed and measured. The experiment was done in triplicate. *, sorafenib (−) versus (+), $P < 0.05$. B, migration assay using the Boyden chamber method revealed that sorafenib almost completely canceled the HGF-mediated cellular migration in both HCC cell lines. The cells were incubated under the indicated conditions: control, 10 ng/mL of HGF; and HGF, 10 μmol/L of sorafenib. *, sorafenib (−) versus (+), $P < 0.05$. Sora, sorafenib.

Discussion

Recent accumulating evidence has shown that the EMT is involved in drug sensitivity to several anticancer agents (25). Within this topic, the most intensively investigated drugs have been endothelial growth factor receptor (EGFR)–targeting drugs for the treatment of lung cancer. A clinical trial has revealed that lung cancer cells with strong E-cadherin expression exhibit a significantly longer time to progression after EGFR-TKI (tyrosine kinase inhibitor) treatment (26). Other studies on EGFR-targeting drugs have shown that mesenchymal type lung cancer cells exhibit an EMT-dependent acquisition of PDGFR, FGF receptor, and TGF-β receptor signaling pathways (27), and integrin-linked kinase is a novel target for overcoming HCC resistance to EGFR inhibition (28). Regarding sensitivity to gemcitabine, mesenchymal type cancer cells are reportedly associated with gemcitabine resistance in pancreatic cancer cells (29). The mechanism of resistance to gemcitabine has been shown to involve the activation of Notch signaling, which is mechanistically linked with the mesenchymal chemoresistance phenotype of pancreatic cancer cells (30). Thus, baseline cellular characteristics based on the EMT phenotype might be useful not only as prognostic biomarkers for a malignant phenotype but also as predictive markers

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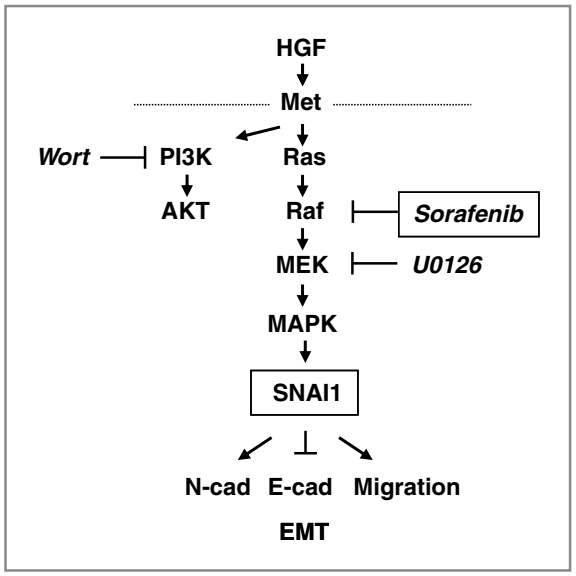

Figure 6. Diagram of the proposed mechanism by which sorafenib inhibits the EMT. Sorafenib inhibits the HGF-mediated EMT, which is characterized by morphologic changes, cadherin switching, and an increase in the cellular migrating phenotype. The anti-EMT effect of sorafenib occurs through the downregulation of SNAI1 by the inhibition of MAPK phosphorylation in HCC cells. Wort, wortmannin; N-cad, N-cadherin; E-cad, E-cadherin.

of sensitivity to anticancer agents. In this study, we focused on the signaling pathway responsible for inducing the EMT and showed that the multitarget TKI sorafenib downregulates SNAI1 by inhibiting Raf-MAPK signaling, thereby inhibiting the HGF-mediated EMT in HCC cells. Our findings may provide a novel insight into the actions of TKIs and their anti-EMT effects.

The mechanisms underlying the SNAI1-induced metastatic and aggressive phenotypes of cancer cells have recently been intensively investigated in both basic and clinical research studies. A novel aspect of the activity of SNAI1 is its involvement in immunosuppression. The SNAI1-induced EMT mediates regulatory T cells and impairs dendritic cells, accelerating cancer metastasis not only by enhancing invasion but also by inducing immunosuppression (31). A complex of histone deacetylase (HDAC) and SNAI1 plays an essential role in silencing E-cadherin (32), suggesting that the use of HDAC inhibitors to inhibit SNAI1 function might represent a promising therapeutic approach. On the other hand, large-scale clinical data on SNAI1 expression and the prognosis of patients with HCC were recently reported (33) and the overexpression of SNAI2 and/or TWIST was correlated with a worse prognosis. In contrast, no such significant differences were observed in samples that overexpressed SNAI2. The coexpression of Snail and TWIST was correlated with the worst prognosis for HCC (33). This evidence suggests that SNAI1 might be a useful therapeutic target for oncology. Our findings showed that sorafenib completely canceled the HGF-mediated SNAI1 induction in HepG2 and Huh7 cells. This activity of sorafenib, in addition to sorafenib's anti-angiogenic effects, might contribute to a clinical benefit against metastatic and aggressive phenotypes in patients with HCC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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mentioned in the recent editorial by McColl and Gillen (4). Howden and Kahrilas state that, "in summary, there is no clear clinical- or clinical trial-evidence of undue difficulty in reducing or discontinuing PPI treatment in GERD patients, apart from those with erosive esophagitis" (2). This is not correct as a placebo-controlled trial of discontinuation of PPIs in patients on long-term therapy was performed a few years ago (5). Most patients participating had gastroesophageal reflux disease (GERD) as the indication for the PPI and GERD patients had significantly more difficulties discontinuing PPI therapy as compared with patients with other indications (5). Exclusion criterion for participation was erosive esophagitis (5). In the articles mentioned above (2,3) there is no disagreement with the last paragraph of the editorial, that "PPI treatment remains an important, valuable and safe intervention for a multitude of patients with appropriate indications" (1). Finally, it is somewhat surprising that authors that are chosen to write the editorial of a study showing that PPI therapy can induce dyspeptic symptoms have strong and multiple conflicts of interest with the pharmaceutical companies producing PPIs (1).

CONFLICT OF INTEREST

Guarantor of the article: Einar Bjornsson MD, PhD.

Financial support: None.

Potential competing interests: None.

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Withdrawing PPI Treatment

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To the Editor: Our editorial (1) sought to highlight both the strengths and limitations of the study by Niklasson *et al.* (2), of which Dr Bjornsson was a co-author. We presume that Dr Bjornsson would agree that the results of controlled studies should not be overinterpreted, which is precisely what we had observed with the previously reported study of Reimer *et al.* (3) and what we caution of here. We do not dispute that some (although by no means all) investigators have shown rebound acid hypersecretion following PPI withdrawal and that such an effect is biologically plausible. Rather, our concern regards the clinical relevance of this phenomenon. Furthermore, although the observations of Reimer *et al.* (3) and Niklasson *et al.* (2) might be explained on the basis of rebound acid hypersecretion, readers should understand that neither study actually measured this phenomenon.

Regarding our stated conflicts of interest, readers are free to make of them what they choose. We welcome and adhere to the Journal's policy of making a declaration of all relevant financial relationships mandatory ("strong" and otherwise); this helps to ensure transparency and objectivity. However, a thoughtful reading of our editorial would conclude that we advocate minimizing and withdrawing PPI treatment whenever appropriate, hardly a viewpoint steeped in bias. To re-state our main argument, this is generally easily accomplished in clinical practice.

CONFLICT OF INTEREST

Dr Howden has been a consultant for Takeda Pharmaceuticals North America, Takeda Global Research and Development, Santarus, XenoPort, Schering-Plough Healthcare, Novartis Consumer Health, Novartis Oncology, Procter & Gamble, Eisai, Otsuka, and Boehringer Ingelheim. He has received speaking honoraria from Takeda Pharmaceuticals North America, Otsuka, and Novartis. He has received research support for an investigator-initiated project from AstraZeneca. Dr Kahrilas has been a consultant for AstraZeneca, Xenoport, ARYx Therapeutics, Eisai, EndoGastric Solutions, Novartis, Movetis, and Revalesio. He has received research support for investigator-initiated studies from the National Institutes of Health and Reckitt Benckiser Group plc.

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Double-Contrast Ultrasound: A Novel Surveillance Tool for Hepatocellular Carcinoma

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This letter underwent AJG editorial review.

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To the Editor: Hepatocellular carcinoma (HCC) is the third most common cause of

Table 1. Results of surveillance by double contrast US: B-mode US vs. double contras US

No.	Sex	Age	Virus	Location	Size (mm)	B-mode US	CEUS Kupffer phase	Double-contrast US	Pathological diagnosis
1	M	64	HCV	S6	6×6	Not detected	Defect	Positive	HCC
2	M	53	HCV	S8	7×7	Not detected	Defect	Positive	HCC
3	M	76	HCV	S6	8×8	Not detected	Defect	Positive	HCC
4	F	72	HCV	S7	8×7	Not detected	Defect	Positive	HCC
5	M	68	HBV	S5	8×8	Not detected	Defect	Positive	HCC
6	M	72	HCV	S2	9×8	Not detected	Defect	Positive	HCC
7	M	71	HCV	S3	10×9	Not detected	Defect	Positive	HCC
8	M	70	HBV	S8	10×10	Not detected	Defect	Positive	HCC
9	M	68	HCV	S2	10×7	Not detected	Defect	Positive	HCC
10	F	75	HCV	S6	11×11	Not detected	Defect	Positive	HCC
11	M	67	HCV	S6	11×10	Not detected	Defect	Positive	HCC
12	M	73	HBV	S7	12×11	Not detected	Defect	Positive	HCC
13	M	74	HCV	S5	12×11	Not detected	Defect	Positive	HCC
14	F	69	HCV	S2	12×10	Not detected	Defect	Positive	HCC
15	M	70	HCV	S6	12×11	Not detected	Defect	Positive	HCC
16	M	76	HCV	S8	13×12	Not detected	Defect	Positive	HCC

CEUS, contrast-enhanced US; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; US, ultrasound.

cancer death worldwide. Practice guidelines in the West (1) and East (2) recommend ultrasound (US) surveillance as a first-line test. However, despite the performance of periodic surveillance, some HCCs are still detected at advanced stages because of the coarse liver parenchyma. Furthermore, even HCCs detected at early stages, such as single nodular HCCs smaller than 3 cm, still show high annual recurrence rates (15–20%) after resection or ablation (3). These phenomena are attributed to the tumor biology of HCCs, which frequently metastasize via the portal vein even when they are less than 2 cm (4). Detection of much smaller HCC nodules that do not yet have microsatellites or vascular invasion is an urgent clinical need.

In 2007, Sonazoid, a second-generation US contrast agent, was approved for routine clinical use in Japan. The most important property of this agent is that it allows very stable Kupffer phase imaging for at least 60 min, which is tolerable for multiple scanning in addition to real-time imaging. From December 2007 to November 2009, Kupffer phase surveillance was performed for 292 consecutive patients with hepatitis B- or C-related cirrhosis, who are at very high risk for HCC. At the outpatient clinic, 0.01 ml/kg of Sonazoid was injected, followed by entire liver scanning at the Kupffer phase. Among the 292 patients, 27 Kupffer defects that were not detected by B-mode US were detected by Kupffer phase surveillance. Of these defects, 16 hypervascular nodules (5.5%) were confirmed as HCC by re-injecting Sonazoid at the Kupffer phase (double-contrast US) (5). All 16 nodules were proven to be HCC histologically, with a size range of 6–13 mm (**Table 1**). After resection ($n=2$) or radiofrequency ablation ($n=14$), none of these nodules showed local recurrence or intrahepatic recurrence during a median follow-up period of 2.3 years. Only one HCC nodule located at the subphrenic region was missed during detection by double-contrast US. The sensitivity of detecting B-mode US-undetectable hypervascular HCC was 94% using double-contrast US.

In conclusion, Kupffer phase surveillance of the cirrhotic liver followed by re-injection of Sonazoid (double-contrast US) is a novel technique in the surveillance program for detecting small hypervascular HCCs that are in a completely curable state. Based on these findings, a prospective randomized phase III multicenter controlled trial comparing B-mode and double-contrast US surveillance for virus-related cirrhotic patients is now ongoing (http://www.clinicaltrials.com; NCT 00822991).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Incidence Reduction Following Colonoscopic Polypectomy

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To the Editor: In Dr Sandler's editorial (1) in which he reviewed the current controversy in screening colonoscopy, he stated that the National Polyp Study (NPS) finding that colonoscopic polypectomy reduces colorectal cancer (CRC) incidence has not been replicated (2). This is an inaccurate statement. An incidence and mortality reduction similar to that observed by NPS was replicated in two other studies of post polypectomy patients that showed a 67% incidence reduction and an 88% mortality reduction, respectively (3,4). The studies that he cited as having a similar design to the NPS in fact had different designs with respect to the initial colonoscopy that identified the adenoma patients. In the NPS, all patients referred to participating clinical centers for initial colonoscopy prospectively had a protocol colonoscopy that reached the cecum, all polyps detected were removed, and all colonoscopies were performed by experienced endoscopy investigators. Those patients identified as having adenomas at this initial examination were eligible for the NPS. The studies cited by Sandler (1) had adenomas identified from community-based practices and then, 1 year later, had a clearing colonoscopy performed by experienced endoscopy investigators. Interval cancers attributable to missed lesions are not uncommon in community-based practice (5). When the missed cancers of the first non-protocol colonoscopy were excluded, the post-polypectomy CRC rate dropped from 1.8 to 0.96 per 1000 person years of follow-up, which is very similar to that of the NPS (0.6 per 1000). The CRC incidence reduction observed in the NPS compared with a simulated cohort of adenoma patients without their adenomas removed (90%) and compared with the general population Surveillance, Epidemiology and End Results rate (76%) was probably achieved as a result of the NPS design and methodology, which included rigorous baseline clearing with a 13% repeat for inadequate preparation.

There are three separate but related questions: first, does removal of adenomas reduce the incidence and mortality of CRC; second, what is the precise magnitude of this reduction; and third, what is the benefit of screening colonoscopy in the general population, of whom only a proportion have adenomas. The long-standing belief in the concept of the adenoma–carcinoma sequence and that its interruption reduces CRC incidence and mortality is supported by many studies, including the NPS (2–4,6). However, the precise magnitude of the colonoscopy effect in the general population has not been clearly established, and will not be established until completion 10 or 15 years hence of the European and American screening colonoscopy randomized controlled trials (RCTs). Data from the colonoscopy RCTs will also provide a comparison of the colonoscopy effect with the recently reported sigmoidoscopy effect (6). The NPS supports the importance of finding and removing adenomas with any screening method in addition to detecting early-stage cancers. The best method to do this needs to be established.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Appropriate Response to Influenza A (H1N1) Virus Vaccination in Patients With Inflammatory Bowel Disease on Maintenance Immunomodulator and/or Biological Therapy

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To the Editor: In April 2009 an outbreak of the novel influenza A (H1N1) virus infection occurred in Mexico and has assumed pandemic proportions soon. After initial controversial data, vaccines directed toward the influenza A (H1N1) virus have proven to be safe and efficient to prevent the complications of the infection.

Patients with inflammatory bowel diseases (IBD—Crohn's disease (CD), ulcerative colitis) on immunosuppressive therapy are at increased risk for various infections, some of which can be prevented by immunization. Inactivated influenza vaccination

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Clinical Cancer Research

Antitumor Activity of BIBF 1120, a Triple Angiokinase Inhibitor, and Use of VEGFR2 $^{+}$pTyr^{+} Peripheral Blood Leukocytes as a Pharmacodynamic Biomarker *In Vivo*

Kanae Kudo, Tokuzo Arao, Kaoru Tanaka, et al.

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Clinical Cancer Research

Antitumor Activity of BIBF 1120, a Triple Angiokinase Inhibitor, and Use of VEGFR2$^+$pTyr$^+$ Peripheral Blood Leukocytes as a Pharmacodynamic Biomarker *In Vivo*

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Abstract

Purpose: BIBF 1120 is a potent, orally available triple angiokinase inhibitor that inhibits VEGF receptors (VEGFR) 1, 2, and 3, fibroblast growth factor receptors, and platelet-derived growth factor receptors. This study examined the antitumor effects of BIBF 1120 on hepatocellular carcinoma (HCC) and attempted to identify a pharmacodynamic biomarker for use in early clinical trials.

Experimental Design: We evaluated the antitumor and antiangiogenic effects of BIBF 1120 against HCC cell line both *in vitro* and *in vivo*. For the pharmacodynamic study, the phosphorylation levels of VEGFR2 in VEGF-stimulated peripheral blood leukocytes (PBL) were evaluated in mice inoculated with HCC cells and treated with BIBF 1120.

Results: BIBF 1120 (0.01 μmol/L) clearly inhibited the VEGFR2 signaling *in vitro*. The direct growth inhibitory effects of BIBF 1120 on four HCC cell lines were relatively mild *in vitro* (IC_{50} values: 2–5 μmol/L); however, the oral administration of BIBF 1120 (50 or 100 mg/kg/d) significantly inhibited the tumor growth and angiogenesis in a HepG2 xenograft model. A flow cytometric analysis revealed that BIBF 1120 significantly decreased the phosphotyrosine (pTyr) levels of VEGFR2$^+$CD45^{dim} PBLs and the percentage of VEGFR2$^+$pTyr$^+$ PBLs *in vivo*; the latter parameter seemed to be a more feasible pharmacodynamic biomarker.

Conclusions: We found that BIBF 1120 exhibited potent antitumor and antiangiogenic activity against HCC and identified VEGFR2$^+$pTyr$^+$ PBLs as a feasible and noninvasive pharmacodynamic biomarker *in vivo*. *Clin Cancer Res; 17(6); 1373–81. ©2010 AACR.*

Introduction

A number of antiangiogenic inhibitors have been studied in clinical settings, some of which have clearly exhibited a clinical benefit in oncology. Consequently, VEGFs and VEGF receptors (VEGFR) are now well-validated targets in cancer therapy (1). In hepatocellular carcinoma (HCC), 2 recent randomized controlled trials for HCC have reported a clinical benefit of single-agent sorafenib for extending the overall survival in both Western and Asian patients with advanced unresectable HCC (2, 3). On the basis of the clear results of these trials, sorafenib is presently regarded as the standard therapy for HCC.

Because antiangiogenic inhibitors may achieve therapeutic levels long before toxicities arise compared with conventional cytotoxic chemotherapies, identifying pharmacodynamic biomarkers that accurately reflect the effects of the drug on its known targets are needed (4, 5). Therefore, a wide variety of biomarkers of antiangiogenic inhibitors have been proposed and intensively investigated, including plasma proteins, angiogenesis-related signaling, immunohistochemistry of endothelial cell markers for evaluating microvessel density (MVD), circulating endothelial progenitor/cells, and functional imaging such as dynamic contrast-enhanced MRI and molecular imaging using positron emission tomography (6). These candidate biomarkers have been evaluated and characterized as prognostic, pharmacodynamic, or response-predictive markers. Although the utility of biomarkers for evaluating MVD was highly anticipated, these markers were not predictive for clinical response in patients treated with bevacizumab (7). Regarding growth factors and cytokines, the plasma VEGF level has been shown to be neither a pharmacodynamic nor a predictive biomarker of antiangiogenic drugs (7, 8), although the plasma VEGF level is a well-known prognostic biomarker (9–11). Plasma-soluble VEGFR2, on the other hand, may be a promising and specific biomarker of

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

A wide variety of biomarkers of antiangiogenic inhibitors have been proposed and intensively investigated; however, no biomarkers have been validated for routine clinical use and a new pharmacodynamic biomarker is needed. We have shown in this study that (i) BIBF 1120, a VEGF receptor 2 (VEGFR2) inhibitor, exhibited potent antitumor and antiangiogenic activity against hepatocellular carcinoma *in vivo* and (ii) $VEGFR2^{+}pTyr^{+}$ peripheral blood leukocytes (PBL) were useful pharmacodynamic biomarker *in vivo*. Our findings indicate the clinical utility of $VEGFR2^{+}pTyr^{+}$ PBLs as a feasible, noninvasive, and VEGF signal-specific biomarker of VEGFR2 tyrosine kinase inhibitors for use in early clinical trials.

antiangiogenic drugs for evaluating their effects (12, 13). Indeed, we have shown that soluble VEGFR2 was certainly decreased by BIBF 1120 treatment in a phase I trial; however, this decrease was observed at a relatively late stage, 8 to 29 days after the start of treatment (14). These results suggest that soluble VEGFR2 is not a rapid-responding biomarker for monitoring effects of antiangiogenic drugs. As no other biomarkers have been validated for routine clinical use, a new pharmacodynamic biomarker is needed.

BIBF 1120 is a potent triple angiokinase inhibitor that inhibits VEGFR1, 2, and 3, fibroblast growth factor receptors (FGFR), and platelet-derived growth factor receptors (PDGFR). *In vitro* studies have shown that VEGFR2 tyrosine kinase activity was potently inhibited by BIBF 1120 (IC_{50} = 21 nmol/L) and was also active against VEGFR1 and 3 (IC_{50} = 34 and 13 nmol/L, respectively; ref. 15). BIBF 1120 dose dependently inhibited the growth of various human tumor xenografts and tumor angiogenesis *in vivo* studies, consistent with the potent inhibition of VEGF signaling (15). BIBF 1120 also exhibited a relatively strong direct growth inhibitory effect on cancer cell lines, influencing 9 of 14 acute myeloid leukemia cell lines in a colony formation assay with an IC_{50} value of less than 1 μmol/L (16).

We previously reported the antitumor activity of VEGFR2 tyrosine kinase inhibitors (TKI) against non–small cell lung cancer and gastric cancer, identifying a biomarker and the mode of action (17–19). In the present study, we focused on the antitumor activity of BIBF 1120 against HCC, which is hypervascular in nature. In addition, to identify a pharmacodynamic biomarker, we examined the phosphorylation levels of VEGFR-positive peripheral blood leukocytes (PBL) as a surrogate tissue in an *in vivo* model.

Materials and Methods

Compounds

BIBF 1120 was provided by Boehringer Ingelheim Pharma GmbH & KG. 5-Fluorouracil (5FU; Sigma-Aldrich) and an epidermal growth factor receptor (EGFR) TKI, AG1478 (Biomol International), were purchased from the indicated companies.

Cell lines and cultures

HepG2, HLF, HLE, and Huh7 (human hepatoblastoma and HCC cell lines, respectively) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS (Gibco BRL). HUVECs (human umbilical vein endothelial cells) were purchased from Kurabo and were maintained in Humedia-EG2 (Kurabo) medium with 2% FBS, 2 ng/mL of VEGF-A (R&D Systems), 10 ng/mL of EGF, 5 ng/mL of FGF, 10 μg/mL of heparin, and 1 μg/mL of cortisol. These cells were cultured in an atmosphere of 5% CO_2 at 37°C.

In vitro growth inhibition assay

The growth inhibitory effects of BIBF 1120 on the HepG2, HLF, HLE, and Huh7 cell lines were examined using an MTT assay as previously described (17, 18). The optical density was measured at 570 nm. Three independent experiments were conducted.

Western blot analysis

The antibodies used for the Western blot analysis were anti-KDR (IBL), anti–phospho (p)-VEGFR2 (Tyr1175), anti-VEGFR1, anti-p44/42 MAPK (mitogen-activated protein kinase), anti–p-p44/42 MAPK, anti–c-Kit, anti-PDGFRβ, anti-FGFR1, 2, and 3, horseradish peroxidase–conjugated secondary antibody (Cell Signaling Technology), and anti–β-actin (Santa Cruz Biotechnology). The methods have been previously described (18). Two independent immunoblotting experiments were conducted.

Tube formation assay

HUVECs were cultured without VEGF-A for 24 hours. A total of 40 μL of Matrigel (BD Bioscience) and 20 μL of PBS were mixed and incubated in 96-well plates. After the gel had solidified, a 100-μL volume of HUVECs (2×10^4 cells/well) was seeded onto the plates with 20 ng/mL of VEGF-A and the indicated concentration of BIBF 1120. The 96-well plates were then incubated for 4 hours. Capillary morphogenesis was evaluated under a microscope (Olympus). This assay was carried out in 3 independent experiments.

Real-time reverse transcriptase PCR

The method has been previously described (17). The primers used for real-time reverse transcriptase PCR (RT-PCR) are shown in Supplementary Table 1. *GAPD* was used to normalize the expression levels in the subsequent quantitative analyses.

Flow cytometric analysis for HUVECs

HUVECs were seeded on 6-well plates without VEGF-A for 24 hours. After exposure to BIBF 1120, AG1478, or 5FU for 3 hours, the cells were stimulated with 20 ng/mL of VEGF-A for 30 minutes. The flow cytometric procedure was carried out according to the manufacturer's protocols,

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using the Fixation/Permeabilization Kit (BD Biosciences); the data were obtained using a FACSCalibur flow cytometer (BD Biosciences). Anti-phosphotyrosine (pTyr) antibody (P-Tyr-100; Cell Signaling) was used to detect the phosphorylation levels.

Flow cytometric analysis for PBLs in the *in vivo* model

In the *in vivo* model, about 0.5 to 1 mL of peripheral blood was obtained from treated mice and 20 ng/ mL of VEGF was added to the whole blood samples for 20 minutes. The red cells were then lysed using a lysis buffer (155 mmol/L NH_4Cl, 10 mmol/L $NaHCO_3$, and 1 mmol/L EDTA2Na, pH 7.3) for 10 minutes, and leukocytes were fixed and permeabilized using a Fixation/Permeabilization Kit for analysis. The following antibodies were used: anti-mouse CD45-PerCP, anti-mouse Flk-1-PE (BD Biosciences), anti-pTyr (P-Tyr-100; Cell Signaling), and Alexa Fluor Mouse IgG1 Isotype Control (BD Parmingen). The analysis was carried out using the WinMDI software (20).

HCC xenograft model

Nude mice (BALB/c nu/nu; 6-wk-old females; CLEA Japan Inc.) were used for the *in vivo* studies and were cared for in accordance with the recommendations for the handling of laboratory animals for biomedical research, compiled by the Committee on Safety and Ethical Handling Regulations for Laboratory Animal Experiments, Kinki University. The ethical procedures followed and met the requirements of the United Kingdom Coordinating Committee on Cancer Research Guidelines.

Mice were subcutaneously inoculated with a total of 6×10^6 HepG2 cells. Two weeks after inoculation, the mice were randomized according to tumor size into 3 groups to equalize the mean pretreatment tumor size among the 3 groups ($n = 6$ in each group). The mice were then treated with BIBF 1120 (50 mg/kg/d, p.o.), BIBF 1120 (100 mg/kg/d, p.o.), or the vehicle control (saline, p.o.) for 14 days (Fig. 3A–C). On day 14, the mice were euthanized, blood samples were collected by cardiac puncture, and tumor specimens were collected for immunohistochemistry. The tumor volume was calculated as the length $\times$ width$^2 \times 0.5$ and was assessed every 2 to 3 days.

Immunohistochemical analysis

A mouse anti-CD31 monoclonal antibody (1:100; BD Biosciences) was used to detect the endothelial cells. The paraffin-embedded samples were cut into 4-μm sections, deparaffinized, and placed in a preheated antigen retrieval solution (Dako) in a steamer for 10 minutes. All the samples were then blocked in 3% H_2O_2 in methanol for 15 minutes and rinsed with PBS. The slides were then placed in a Sequenza slide staining system (Thermo Fisher Scientific) and blocked in 1% normal goat serum for 20 minutes. The slides were incubated overnight at 4°C with the CD31 antibody. A standard avidin–biotin peroxidase complex assay was then carried out using the ABC Elite Kit (Vector Laboratories). The slides were developed with 3,3′-diaminobenzidine (DAB; Zymed Laboratories) and counterstained with 10% hematoxylin. Microvessel density (MVD) was quantified by measuring the number of CD31-positive endothelial cells in the tumors. Ten random fields per tumor sample at 200× magnification were captured and saved for computer-assisted image analysis using the ImageJ software package (21). An algorithm for color deconvolution was used to segregate the brown DAB-positive CD31 endothelial cells and the blue tumor cells. Thresholds were adjusted to remove background and nonspecific signals. MVD was reported as the average ratio of CD31-positive cells to tumor cells.

Statistical analysis

The statistical analyses were carried out using Microsoft Excel (Microsoft) to calculate the SD and to test for statistically significant differences between the samples using a Student's t test. A value of $P < 0.05$ was considered statistically significant.

Results

BIBF 1120 potently inhibits VEGFR2 signaling in HUVECs

We evaluated the inhibitory effect of BIBF 1120 at various concentrations (0.0001–10 μmol/L) on VEGFR2 signaling, using HUVECs stimulated with 20 ng/mL of VEGF. BIBF 1120 at a concentration of 0.01 μmol/L completely inhibited the phosphorylation of VEGFR2 and MAPK in HUVECs (Fig. 1A). BIBF 1120 at a concentration of 0.01 μmol/L partially inhibited tube formation in HUVECs stimulated with VEGF, whereas BIBF 1120 at a concentration of 1 μmol/L completely inhibited tube formation (Fig. 1B). These data indicate that BIBF 1120 potently inhibits VEGFR2 signaling in endothelial cells.

Flow cytometry detects BIBF 1120–induced inhibition of pTyr levels

To detect the BIBF 1120–induced inhibition of pTyr levels by flow cytometry, the VEGF-induced pTyr levels of proteins in HUVECs were evaluated after exposure to BIBF 1120, the EGFR TKI AG1478 as a TKI control, or 5FU as a cytotoxic drug control. The controls agents were used to show that another target of TKI did not induce (AG1478) or to exclude the possibility that nonspecific effects such as cytotoxic cellular responses were not induced (5FU). Flow cytometry revealed that the VEGF-induced pTyr levels in HUVECs were significantly inhibited by BIBF 1120 at concentration of 1 and 5 μmol/L but not by AG1478 or by 5FU (Fig. 1C and D). This flow cytometric method is considered a feasible means of detecting the inhibition of VEGF-induced pTyr levels induced by VEGFR2 TKIs.

Growth inhibitory effects and expression status of targeted receptors in HCC cell lines *in vitro*

To evaluate the expression status of the putative targeted receptors of BIBF 1120 in the 4 HCC cell lines and HUVECs as a control, we examined the protein expression levels of VEGFR1, VEGFR2, FGFR1, FGFR2, FGFR3, PDGFRβ, and

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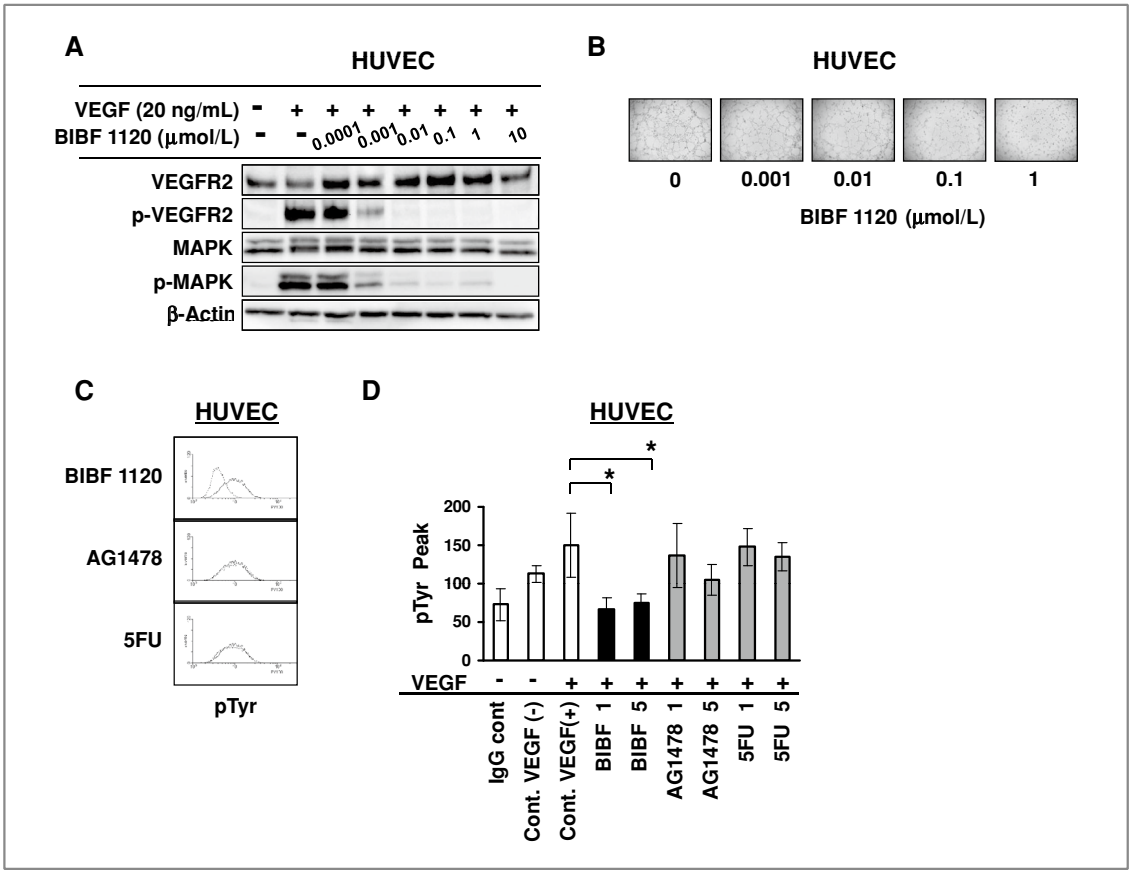

Figure 1. Inhibition of VEGFR2 signaling by BIBF 1120 and detection of the inhibition of pTyr by flow cytometry in HUVECs. A, the inhibition of VEGFR2 and MAPK phosphorylation by BIBF 1120 was determined using a Western blot analysis. HUVECs cultured in a medium containing 2% FBS were exposed to BIBF 1120 (0.0001–10 μmol/L) for 3 hours, stimulated with 20 ng/mL of VEGF for 15 minutes, and lysed for analysis. B, effect of BIBF 1120 on the inhibition of tube formation. HUVECs were seeded with 20 ng/mL of VEGF-A and exposed to BIBF 1120 (0.001–1 μmol/L) on Matrigel-layered 96-well plates for 4 hours. Capillary morphogenesis was evaluated under a microscope. This assay was conducted in 3 independent experiments. C and D, HUVECs were seeded on 6-well plates without VEGF-A for 24 hours. After exposure to BIBF 1120, AG1478, or 5FU for 3 hours, the cells were stimulated with 20 ng/mL of VEGF-A for 30 minutes. The inhibition of pTyr level was detected by flow cytometry with an anti-pTyr antibody. Note that only BIBF 1120 significantly inhibited the VEGF-induced phosphorylation levels of tyrosine. This assay was conducted in 3 independent experiments; bars, SD. *, $P < 0.05$.

c-Kit (the kinase activities of which are reportedly inhibited by BIBF 1120 (15) and p-VEGFR2, MAPK, and p-MAPK by Western blotting. The protein expression of these receptors were not highly upregulated in any of the HCC cell lines, except for PDGFRβ in HLE and HLF cells (Fig. 2A). A comparable expression level of MAPK was observed among the cell lines, and an increase in p-MAPK expression was observed in HLE cells. The mRNA expression levels of the target receptors *VEGFR1*, *VEGFR2*, *VEGFR3*, *PDGFRA*, *PDGFRB*, *FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4* were determined using real-time RT-PCR in the HUVEC line and the HCC cell line. Higher receptor expression levels were observed for *VEGFR2* in HUVECs, *PDGFRB* in HLE and HLF, *FGFR1* in HUVECs and HLE, *FGFR3* in HepG2, and *FGFR4* in Huh7 (Fig. 2B). The expression levels were consistent with the Western blotting results.

We next evaluated the direct growth inhibitory activity of BIBF 1120 in 4 HCC cell lines *in vitro*. The IC_{50} value of BIBF 1120 for the HLE, HLF, HepG2, and Huh7 cell lines were 2.7 ± 1.7, 2.7 ± 0.5, 5.3 ± 0.6, and 4.3 ± 0.9 μmol/L, respectively (Fig. 2C). These results indicate that the direct growth inhibitory activity of BIBF 1120 against HCC cells was relatively mild (IC_{50}: 2–5 μmol/L).

BIBF 1120 potently inhibits tumor growth and angiogenesis of HCC xenografts *in vivo*

Next, we examined the antitumor and antiangiogenic effects of BIBF 1120 *in vivo*. Mice inoculated with HepG2

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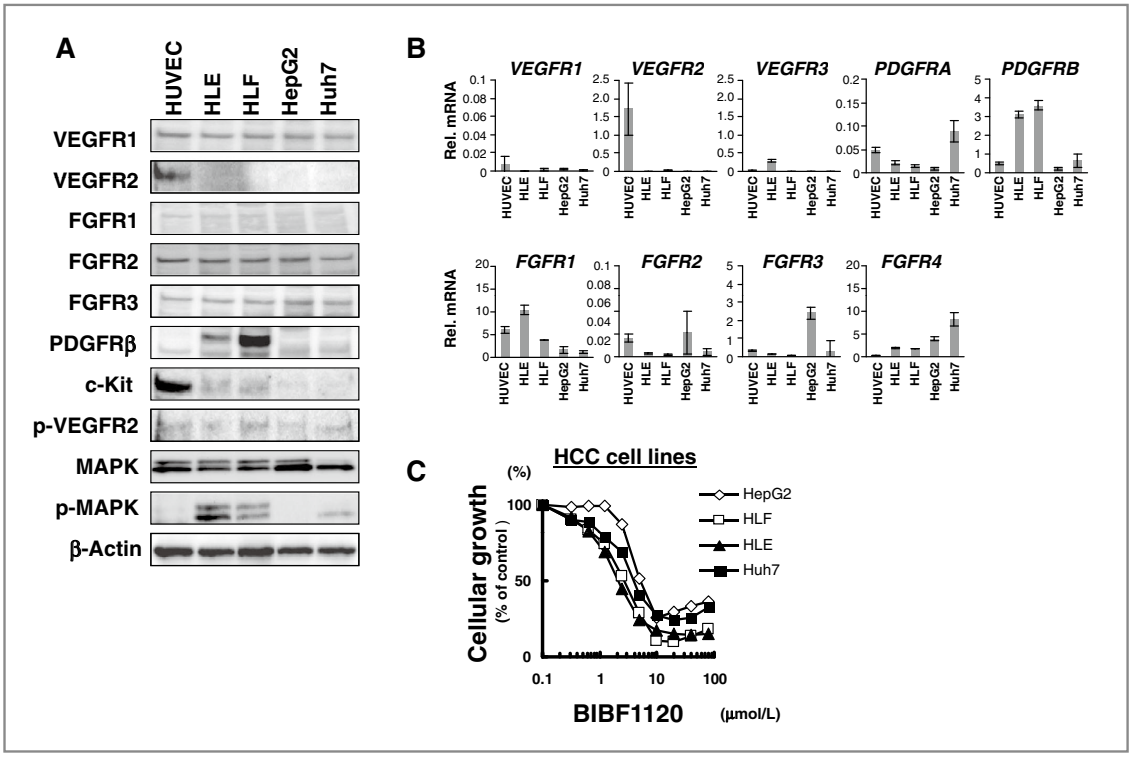

Figure 2. Expression levels of target receptors and sensitivity to BIBF 1120 in HCC cell lines. A, Western blot analysis of the expression levels of VEGFR1, VEGFR2, FGFR1, FGFR2, FGFR3, PDGFRβ, c-Kit, p-VEGFR2, MAPK, p-MAPK, and β-actin in HCC cell lines and HUVECs as a control. B, the mRNA expression levels of *VEGFR1*, *VEGFR2*, *VEGFR3*, *PDGFRA*, *PDGFRB*, *FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4* were determined using real-time RT-PCR. Rel mRNA, mRNA expression levels normalized using *GAPD* (target gene/GAPD $\times 10^3$). C, *in vitro* growth inhibitory effect of BIBF 1120 in 4 HCC cell lines by an MTT assay; bars, SD of 3 independent experiments. This assay was conducted in 3 independent experiments.

cells were orally given a low (50 mg/kg/d) or high (100 mg/kg/d) dose of BIBF 1120, or vehicle alone, for 2 weeks (Fig. 3A). The mean tumor volumes on day 14, for each group of mice, were as follows: vehicle alone, 1,367 ± 634 mm^3; 50 mg/kg/d, 488 ± 489 mm^3; and 100 mg/kg/d, 572 ± 556 mm^3. Both doses of BIBF 1120 significantly inhibited tumor growth ($T/C = 0.36$ and 0.42, respectively), indicating that BIBF 1120 has a potent antitumor activity against HCC *in vivo* (Fig. 3B). Body weight loss was not observed after the administration of BIBF 1120 at either dose (Supplementary Fig. S1). The CD31 staining of tumor tissues showed that BIBF 1120 administration also significantly inhibited tumor angiogenesis (Fig. 3C). Combined with the observation of the direct growth inhibitory activity against HCC *in vitro*, these findings suggest that the antitumor activity of BIBF 1120 *in vivo* mainly result from the drug's antiangiogenic activity, which blocks VEGF signaling.

VEGFR2$^+$pTyr$^+$ PBLs are a pharmacodynamic biomarker *in vivo*

VEGFR2$^+$CD45^{dim} PBLs are generally regarded as circulating endothelial cells (22); therefore, we hypothesized that VEGFR2$^+$CD45^{dim} PBLs might be useful as a biological biomarker of VEGFR2 TKIs. The effects of BIBF 1120 on the pTyr levels of VEGFR2$^+$CD45^{dim} PBLs and the percentage of VEGFR2$^+$pTyr$^+$ PBLs was examined *in vivo* (Fig. 4A). Murine blood samples were obtained from tumor-bearing, BIBF 1120–treated mice, as described previously. The pTyr levels of the VEGFR2$^+$CD45^{dim} PBLs were significantly inhibited by BIBF 1120 treatment, but the difference was relatively small (Fig. 4B and C). On the other hand, the percentage of VEGFR2$^+$pTyr$^+$ PBLs was markedly decreased by BIBF 1120 administration (Cont: 1.8% ± 1.1%, B50: 0.34% ± 0.21%, B100: 0.37% ± 0.29%; Fig. 5A and B). These findings raise the possibility that evaluating the VEGFR2$^+$CD45^{dim} PBLs by flow cytometry as a surrogate tissue may contribute to the proof of concept of VEGFR2-targeting drugs or the monitoring of drug effects *in vivo*. Thus, VEGFR2$^+$pTyr$^+$ PBLs might be a useful pharmacodynamic biomarker of VEGFR2 TKIs in early clinical trials.

Discussion

HCC is one of the most hypervascular tumors, and vascular embolization has been used as a therapeutic strategy. A recent study showed that sorafenib exhibits

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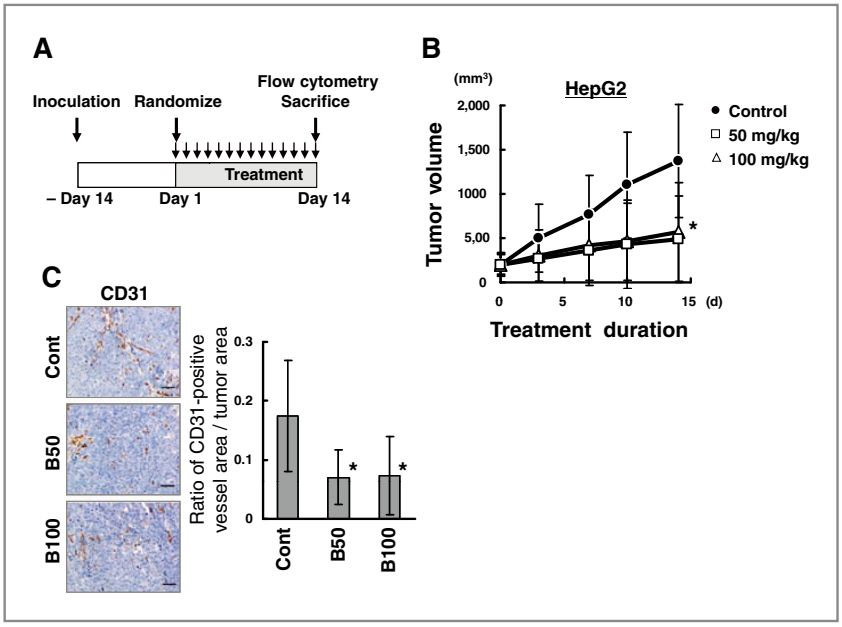

Figure 3. BIBF 1120 exhibited the antitumor and antiangiogenic effects against HCC *in vivo*. A, schema of the BIBF 1120 treatment schedules. Mice were inoculated with HepG2 cells for 14 days. The mice were then randomized into 3 groups ($n = 6$ in each group) and treated with BIBF 1120 (50 mg/kg/d, p.o.), BIBF 1120 (100 mg/kg/d, p.o.), or the vehicle control (p.o.) for 14 days. On day14, the mice were euthanized; blood was collected for the following biomarker study, and tumor specimens were collected for immunohistochemistry. B, inhibition of tumor growth by BIBF 1120 treatment. The tumor volume was assessed every 2 to 3 days ($n = 6$ in each group). Bars, SD. *, $P < 0.05$. C, inhibition of tumor angiogenesis by BIBF 1120 treatment was evaluated using the CD31 staining of tumor samples. Representative data are shown. MVD was quantified by measuring the number of CD31-positive endothelial cells in the tumors. Ten random fields per tumor sample at a magnification of ×200 were captured and saved for computer-assisted image analysis using the ImageJ software package. The *y*-axis represents the ratio of the CD31-positive vessel area/tumor area. Scale bar, 100 μm. Cont, tumor sample treated with vehicle control. B50 and B100, tumor sample treated with BIBF 1120 (50 mg/kg/d, 100 mg/kg/d, p.o.); *, $P < 0.05$.

clinical benefits in patients with advanced HCC (2, 3). This encouraging result suggests that molecular targeting drugs might be active against HCC, especially those that block VEGFR signaling. Our data showed that BIBF 1120 inhibited tumor growth and angiogenesis in HCCs *in vivo*, suggesting that BIBF 1120 may be an active and promising drug against HCC.

BIBF 1120 has a potent inhibitory effect on VEGFRs, similar to that of sorafenib and sunitinib, and it also has activities against FGFRs and Src (refs. 15, 23, 24; Supplementary Table S2). Recent evidence has shown that Src expression is elevated and active in HCC and that Src may play a key role in supporting HCC progression (25); furthermore, HBx increased the activation of the androgen receptor through c-Src kinase, which acts as a major switch in the activation of HCC (26). We conducted a Western blot analysis to detect the inhibitory effect of BIBF 1120 on Src activity, using HUVECs and HepG2, Huh7, HLE, and HLF cells (Supplementary Fig. S2). The inhibitory effect of BIBF 1120 on p-Src was observed in HUVECs and HLE and HepG2 cells, suggesting that BIBF 1120 actually has an inhibitory effect on Src. This effect may benefit HCC therapy in a manner independent of its antiangiogenic effect, although this topic needs to be further investigated. Similarly, we showed an inhibitory effect of BIBF 1120 on p-FGFR2 by using FGFR2-amplified gastric cancer cell lines (Supplementary Fig. S3). Brivanib (BMS-540215), a dual inhibitor of VEGFR and FGFR, is currently in development for the treatment of HCC and colon carcinoma, and preclinical studies have shown that FGFR signaling in HCC cells seems to be a promising therapeutic target (27, 28). These results suggest that the effect of BIBF 1120 on FGFR may contribute the antitumor effect, although further investigation is needed.

Numerous candidate biomarkers of angiogenesis have been identified, but the use of these markers for diagnosis, prognosis, and treatment monitoring remains investigational and of uncertain utility (4). Among them, biomarkers for detecting the blockade of VEGFR signaling have received particular attention because of the intimate involvement of this mechanism in drug activity of VEGFR TKIs. We have shown that VEGF-induced $VEGFR2^{+}pTyr^{+}$ PBLs in peripheral blood samples were markedly decreased by BIBF 1120 treatment *in vivo*. This analysis was done using only peripheral blood collection, VEGF stimulation, and analysis of 2-color flow cytometry; thus, this method is feasible

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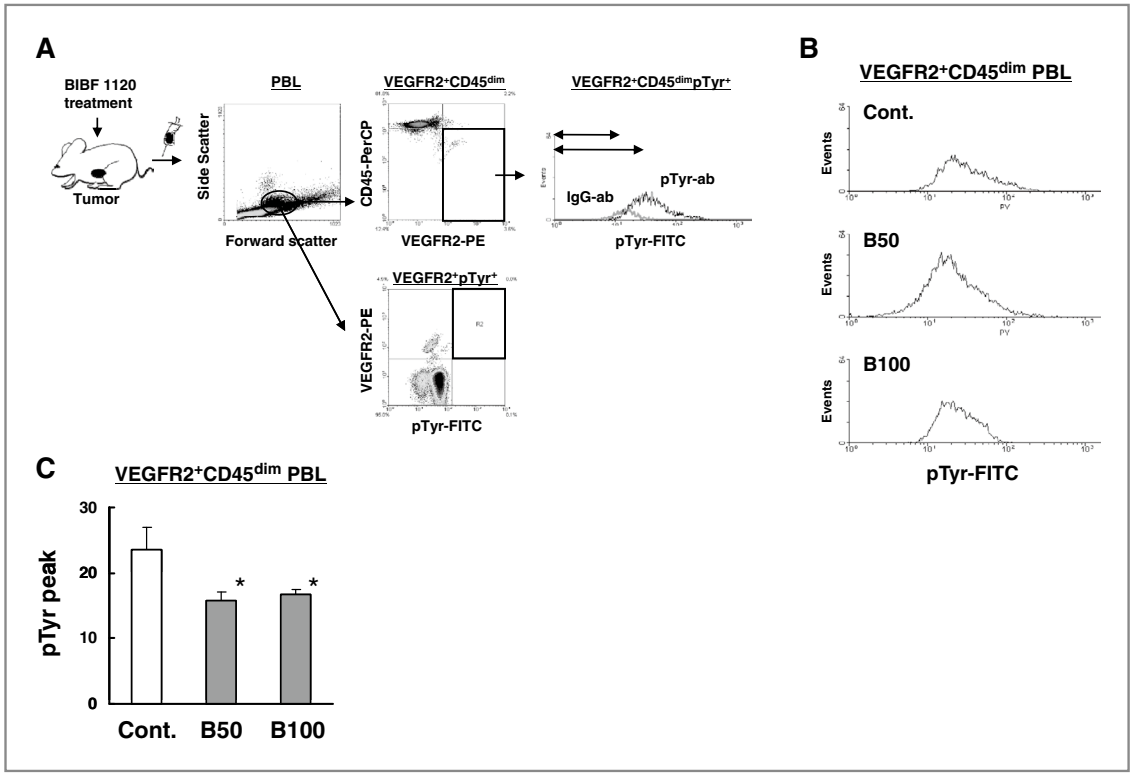

Figure 4. Evaluation of VEGFR2$^+$CD45^{dim} PBLs as a biomarker *in vivo*. A, schema of treatment schedules of BIBF 1120 and detection methods. Peripheral blood samples obtained from BIBF 1120–treated mice were stimulated with 20 ng/mL of VEGF for 30 minutes. The cells were fixed, permeabilized, and reacted with the following antibodies: anti-mouse CD45-PerCP, anti-mouse Flk-1-PE, and anti-pTyr-FITC (fluorescein isothiocyanate). Two methods, the tyrosine phosphorylation levels of VEGFR2$^+$CD45^{dim} PBLs and the percentage of VEGFR2$^+$pTyr$^+$ PBLs, were examined. B and C, BIBF 1120 significantly inhibited the pTyr levels of VEGFR2$^+$CD45^{dim} PBLs *in vivo*. Cont, blood sample from vehicle control. B50 and B100, blood samples from BIBF 1120 (50 mg/kg/d, 100 mg/kg/d; p.o.) treatment groups; bars, SD. *, $P < 0.05$.

and specific to VEGF signaling. Our method may contribute to the proof of concept for VEGFR2 TKIs and may help to determine the biological optimal dose, especially in phase I clinical trials.

Phase II studies of BIBF 1120 against lung cancer and ovarian cancer have been completed and phase I/II study of BIBF 1120 is currently evaluated in HCC (NCT 01004003). Two large phase III clinical trials against lung cancer

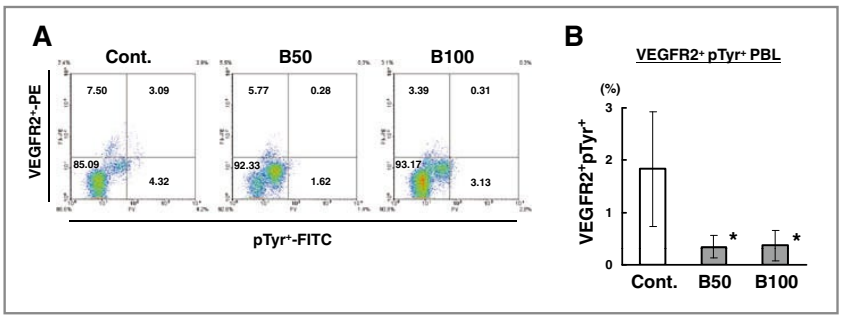

Figure 5. VEGFR2$^+$pTyr$^+$ PBLs can be used as a pharmacodynamic biomarker *in vivo*. A, the percentage of VEGFR2$^+$pTyr$^+$ PBLs obtained from BIBF 1120–treated mice. The numeral data indicate the percentage (%) in each quadrant. Representative data are shown. B, BIBF 1120 significantly inhibited the percentage of VEGFR2$^+$pTyr$^+$ PBLs. Cont, blood samples from vehicle control group ($n = 6$, not treated with drug). B50 and B100, blood samples from BIBF 1120 treatment groups ($n = 6$, 50 mg/kg/d; $n = 6$, 100 mg/kg/d; p.o.); bars, SD. *, $P < 0.05$.

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(LUME-Lung 1: docetaxel ± BIBF 1120; LUME-Lung 2: pemetrexed ± BIBF 1120) and 1 against ovarian cancer (LUME-Ovar 1: carboplatin/paclitaxel ± BIBF 1120) are now underway. We have shown that BIBF 1120 exhibited antiangiogenic and antitumor activity against HCC *in vivo*. These results may provide the scientific rationale for introducing BIBF 1120 as a treatment of HCC in the future. In addition, our approach of evaluating $VEGFR2^{+}pTyr^{+}$ PBLs in VEGFR TKI might be applicable to future phase I trials. We plan to use this method in clinical settings.

In conclusion, BIBF 1120 clearly inhibited VEGFR2 signaling in endothelial cells and exhibited relatively mild growth inhibitory effects on 4 HCC cell lines (IC_{50} values: 2–5 μmol/L) *in vitro*. BIBF 1120 exhibited potent antitumor and antiangiogenic activities against HCC *in vivo*, and the antitumor effect did not fail or show signs of weakening during the long-term administration period. In addition, $VEGFR2^{+}pTyr^{+}$ PBLs were found to be a noninvasive pharmacodynamic biomarker in a murine model.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgment

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

The cancer stem cell marker CD133 is a predictor of the effectiveness of S1+ pegylated interferon α-2b therapy against advanced hepatocellular carcinoma

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Abstract
Background Combination therapy with the oral fluoropyrimidine anticancer drug S1 and interferon is reportedly effective for the treatment of advanced hepatocellular carcinoma (HCC), but selection criteria for this therapy have not been clarified. In this study, we attempted to identify factors predicting the effectiveness of this combination therapy.
Methods Pathological specimens of HCC were collected before treatment from 31 patients with advanced HCC who underwent S1+ pegylated-interferon (PEG-IFN) α-2b therapy between January 2007 and January 2009. In these pathological specimens, the expression levels of CD133, thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and interferon-receptor 2 (IFNR2) proteins were determined by Western blot assay. The presence or absence of p53 gene mutations was determined by direct sequencing. The relationships between these protein expression levels and the response rate (RR), progression-free survival (PFS), and overall survival (OS) were evaluated.
Results The CD133 protein expression level was significantly lower in the responder group than in the nonresponder group. Comparing the PFS and OS between high- and low-level CD133 expression groups ($n = 13$ and 18, respectively) revealed that both parameters were significantly prolonged in the latter group. The expression levels of TS, DPD, and IFNR2 protein and the presence of p53 gene mutations did not correlate with the RR.
Conclusions CD133 was identified as a predictor of the therapeutic effect of S1+ PEG-IFN α-2b therapy against advanced HCC.

Keywords 5-Fluorouracil · Pegylated interferon · CD133 · Cancer stem cell · Hepatocellular carcinoma

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Abbreviations

5FU	5-Fluorouracil
DPD	Dihydropyrimidine dehydrogenase
HCC	Hepatocellular carcinoma
IFNR2	Interferon-receptor 2
NR	Nonresponder
OS	Overall survival
PD	Progressive disease
PEG-IFN	Pegylated interferon
PFS	Progression-free survival
PR	Partial response
RR	Response rate

SD Stable disease
TS Thymidylate synthase

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in Asia, including Japan [1, 2], and its prevalence has recently been increasing globally [3]. In most patients, the background of HCC is chronic hepatitis or liver cirrhosis due to hepatitis B or hepatitis C infection. Recently, HCC has increasingly been detected at relatively early stages due to the periodic follow-up of chronic liver disease patients and the development of diagnostic imaging modalities. There have been significant improvements in the treatment of patients with early HCC, and the therapeutic results have been markedly improved by site-specific treatments such as transcatheter arterial chemoembolization, percutaneous ethanol injection therapy, microwave coagulation therapy, and radiofrequency ablation, as well as hepatectomy [4–6].

However, when existing HCC is cured radically, new cancers develop due to the underlying chronic liver disorders. Treatments must then be performed alone or in combination each time a new cancer appears. Repeated treatment often leads to portal vein tumor thrombosis or distant metastasis, making standard treatments difficult to perform. Recently, the treatment efficacy and safety of the molecular targeted drug sorafenib (Nexavar; Bayer HealthCare Pharmaceuticals–Onyx Pharmaceuticals, Leverkusen, Germany) have been reported and the results placed it as a first-line drug [7]. Sorafenib is a small molecule that inhibits tumor-cell proliferation and tumor angiogenesis and increases the rate of apoptosis in a wide range of tumor models. It acts by inhibiting the serine–threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3 and platelet-derived growth factor receptor β (PDGFR-β). Llovet et al. [7] reported that sorafenib prolonged median survival and the time to progression by nearly 3 months in about 300 patients with advanced HCC. Although different types of molecular targeted drugs have been under development, there is no treatment option at present for the patients who fail to respond to sorafenib. Thus, second-line treatment for advanced HCC needs to be established.

The oral fluoropyrimidine anticancer drug, S1, includes the dihydropyrimidine dehydrogenase (DPD) inhibitor 5-chloro-2,4-dihydroxypyridine as a component, and this component is expected to exhibit a marked anticancer effect by preventing 5-fluorouracil (5FU) degradation [8–10]. Combination therapy with S-1 and interferon (IFN) has recently been attempted in patients with advanced HCC, and relatively satisfactory results have been reported [11–14]. However, the response rate for this therapy is limited, and the outcome deteriorates in patients resistant to it. Therefore, if the effectiveness of this therapy can be predicted in advance, unnecessary adverse effects can be avoided, and other treatments may be attempted.

We have noted some candidate proteins which may have a possible relationship with the effect of this therapy. The cancer stem-cell marker CD133 [15–18] can reportedly resist anticancer drugs through an intrinsic drug resistance mechanism [19, 20]. Thymidylate synthase (TS) and DPD are enzymes involved in 5FU metabolism, and many reports have suggested their relationship with the therapeutic effects of 5FU in lung [21] and colon cancers [22]. Furthermore, interferon-receptor 2 (IFNR2) is reported to be the most important of the receptors through which IFN acts directly on HCC [23, 24]. Apoptosis is the primary mechanism of the anticancer effect of anticancer drugs, and p53 is closely involved in apoptosis [25–27].

In the present study, we sought to identify factors predicting the effectiveness of S1+ pegylated-interferon (PEG-IFN) α-2b therapy. HCC tissue samples were collected before the therapy was started, the expression levels of CD133, TS, DPD, and IFNR2 proteins were determined by Western blot analysis, and the presence or absence of p53 gene mutations was examined by direct sequencing. We found that the expression level of CD133 was significantly correlated with the therapeutic effect. Thus, measurement of the CD133 expression level before treatment may facilitate prediction of the therapeutic effect and the avoidance of unnecessary adverse effects.

Subjects, materials, and methods

Patients

Between January 2007 and January 2009, a total of 31 patients with refractory HCC that could not be controlled by standard therapeutic modalities (transcatheter arterial chemoembolization, percutaneous ethanol injection therapy, microwave coagulation therapy, radiofrequency ablation, and hepatectomy) underwent S-1 and PEG-IFN α-2b combination therapy. Patient characteristics are shown in Table 1, with more details shown in Table 2. All pathological specimens of HCC were collected by needle biopsy.

Eligibility criteria

Eligibility criteria for the combination therapy included: (1) advanced HCC that was uncontrollable with standard treatment, or HCC with distant metastasis; (2) age <80 years; (3) an Eastern Cooperative Oncology Group performance status of 0 or 1; (4) Child–Pugh grade A; (5) encephalopathy

Table 1 Characteristics of patients treated with combination therapy of S1 and PEG-IFN α-2b

Characteristics	Number of patients
Total	31
Gender	
Male	28
Female	3
Age (years)	
Median	66
Range	30–80
Cause of disease	
HBV	10
HCV	15
Non-HBV, non-HCV	6
Child–Pugh stage	
A	31
BCLC stage	
C (advanced)	31
ECOG performance status	
0	28
1	3

PEG-IFN pegylated interferon, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *BCLC* Barcelona Clinic Liver Cancer Group, *ECOG* Eastern Cooperative Oncology Group

degree 0; (6) leukocyte count >3,000 cells/mm [3]; hemoglobin level >10 g/dl and platelet count >80,000 cells/mm^3; and (7) serum creatinine <1.5 mg/dl, serum aspartate aminotransferase <200 IU/l, serum alanine aminotransferase <200 IU/l, and serum total bilirubin level <3.0 mg/dl. The diagnosis of HCC was made based on the hematoxylin–eosin staining of histopathological specimens in all patients.

Treatment regimen

After the obtaining of informed consent, 31 patients were treated with S-1 (TS1; Taiho Pharmaceutical, Tokyo, Japan) and PEG-IFN α-2b (Pegintron; Schering-Plough, Kenilworth, NJ, USA) combination therapy. S-1 was given orally at a daily dose of 80–120 mg (depending on the body surface area: <1.25 m^2: 80 mg, >1.25 to <1.5 m^2: 100 mg, <1.5 m^2: 120 mg), divided into two equal doses, from days 1 to 28. PEG-IFN α-2b was given subcutaneously at a dose of 50 μg on days 1, 8, 15, and 22. One course consisted of consecutive administration for 28 days followed by a 2-week drug-free interval. The Medical Ethics Committee of Kinki University of Medicine approved the study.

Assessment of response

Responses of HCC patients to the combination therapy were assessed by contrast-enhanced computed tomography after each course. The response was defined according to the Response Evaluation Criteria in Solid Tumours (RECIST). A partial response (PR) was defined as a minimum 30% decrease in the sum of the longest diameters of the target lesions, with the baseline sum of the longest diameters of these lesions as the reference. Progressive disease (PD) was defined as a minimum 20% increase in the sum of the longest diameters of the target lesions. Stable disease (SD) was defined as meeting neither PR nor PD criteria. When the response achieved regarding intrahepatic HCC was different from that for extrahepatic HCC, the poorer one was determined as the achieved response.

Assessment of toxicity

Blood cell counts and biochemical profiles were performed at least once every week. Adverse reactions were assessed using the National Cancer Institute-Common Toxicity Criteria (NCI-CTC, version 3).

Western blot analysis

To prepare tissue lysate, HCC tissue was homogenized with CelLytic-MT Mammalian Tissue Lysis/Extraction reagent (Sigma-Aldrich, St. Louis, MO, USA) along with 2% sodium dodecyl sulfate (SDS) and the protease inhibitor, Complete^{TM} (Roche Diagnostics, Mannheim, Germany), and centrifuged. Equal protein amounts (8 μg) of tissue lysates were electrophoresed through a reducing SDS polyacrylamide gel and electroblotted onto a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked and incubated with polyclonal IgG for TS (1/500; Taiho Pharmaceutical, Tokyo, Japan), DPD (1/3,000; Taiho Pharmaceutical), IFN-α/βR (1/500; Otsuka Pharmaceutical., Tokyo, Japan), CD133 (1/1,000; Cell Signaling Technology, Danvers, MA, USA), and β-actin (1/2,000; Sigma-Aldrich) in Can Get Signal® immunostain solution (TOYOBO, Osaka, Japan). For CD133 detection, lysis of the human colon cancer cells WiDr and DLD1 (positive and negative controls, respectively) was examined. Protein levels were detected using horseradish peroxidase (HRP)-linked secondary antibodies and the ECL-plus System (GE Healthcare, Buckinghamshire, UK).

To evaluate the signal intensity, the obtained Western blot image data were quantified using Image J software (NIH, Bethesda, MD, USA).

Immunohistochemistry

We performed immunohistochemical analysis of paraffin-embedded sections of HCC. Immunohistochemical staining was carried out with antibodies raised against CD133 (1:100), and visualized using the Dako LSAB System-HRP

Table 2 Detailed characteristics and outcomes of patients treated with combination therapy of S1 and PEG-IFN α-2b

Patient no.	Age (years)	Gender	PS	HBs-Ag	HCV-Ab	Type of intrahepatic tumor	Vascular invasion	Metastasis	Tumor grade	Prior treatment	AFP (ng/ml)		DCP (mAU/ml)		Response	Outcome
											Before	After	Before	After		
1	80	M	0	−	−	Nodular	Absence	LN	Poor[a]	OP, TACE	3	3	16	17	PR	21.2 M dead
2	70	M	0	−	−	Nodular	Presence	–	Poor	OP	6	35	5,535	60,413	PD	1.7 M dead
3	66	M	0	−	−	Nodular	Absence	Lung	Moderate[b]	OP, RFA	243	65	74	21	PR	25.4 M alive
4	61	M	0	+	−	Nodular	Absence	LN	Moderate	TACE	474	22	31	13	PR	8.1 M dead
5	62	M	0	+	−	Nodular	Absence	Lung, adrenal	Moderate	OP, HAIC	3,268	1,122	18	21	PD	1.9 M dead
6	67	M	0	+	−	Nodular	Absence	Lung	Moderate	OP, TACE	7,964	8,643	3,124	6,676	PD	1.3 M dead
7	60	M	0	−	+	Nodular	Presence	–	Poor	OP	11	13	19	37	SD	16.7 M alive
8	74	M	1	−	+	Nodular	Absence	–	Poor	OP, TACE	2,242	490	1,147	2,356	PD	3.0 M alive
9	59	F	0	+	−	Nodular	Presence	Lung	Moderate	HAIC	867	536	1,300	413	PR	7.2 M alive
10	70	M	0	−	−	Nodular	Presence	–	Moderate	–	345	26	61,319	1,024	PR	9.2 M alive
11	74	M	0	−	+	Diffuse	Presence	Adrenal	Moderate	HAIC	2,246	1,352	13,303	11,167	PR	6.6 M dead
12	60	M	0	−	+	Diffuse	Presence	–	Moderate	–	51	48	13,007	8,523	PD	3.6 M dead
13	61	M	0	+	−	Nodular	Presence	–	Moderate	–	8	9	476	209	SD	3.8 M alive
14	75	M	0	−	+	Nodular	Absence	LN	Poor	TACE	2,476	11,722	3,711	5,452	NE	0.6 M dead
15	61	M	0	+	−	Nodular	Absence	–	Poor	TACE	251	175	1,470	2,795	PD	3.8 M alive
16	30	M	0	+	−	Nodular	Presence	–	Moderate	–	114,852	79,361	195	183	PR	5.2 M alive
17	80	M	1	−	+	Diffuse	Absence	Lung, LN, bone	Moderate	–	70	106	116,140	306,800	PD	3.2 M dead
18	78	M	1	−	+	Nodular	Presence	Lung	Moderate	–	13,544	10,192	3,401	4,805	PD	2.8 M dead
19	55	M	0	−	+	Nodular	Presence	Lung, LN	Moderate	–	470	468	62,938	31,608	SD	2.0 M dead
20	35	M	0	−	+	Nodular	Presence	–	Moderate	–	91	285	44,951	58,921	PD	2.3 M dead
21	63	M	0	−	+	Nodular	Absence	–	Moderate	OP	1,140	160	412	96	PR	10.2 M alive
22	66	M	0	−	+	Nodular	Absence	–	Poor	TACE	651	872	3,231	5,890	PD	3.1 M dead
23	67	M	0	−	+	Nodular	Absence	Lung	Poor	OP	73	86	16	24	PD	3.1 M dead
24	57	M	0	−	+	Diffuse	Presence	Lung	Moderate	–	154	43	3,891	123	PR	9.5 M alive
25	65	M	0	−	+	Nodular	Absence	Lung	Moderate	OP, TACE	2,118	4,860	45,359	67,894	PD	1.9 M dead
26	51	M	0	+	−	Nodular	Absence	–	Moderate	–	1,111	3,890	240	651	PD	1.9 M dead
27	72	M	0	+	−	Nodular	Presence	Lung	Moderate	TACE	652	612	1,131	980	PD	5.6 M dead
28	74	M	0	+	−	Nodular	Presence	Lung, LN	Moderate	OP, TACE	658	890	2,418	3,168	PD	2.0 M dead
29	75	F	0	−	+	Nodular	Presence	–	Moderate	HAIC	1,153	2,789	11,456	21,998	PD	5.3 M dead
30	68	F	0	−	−	Nodular	Presence	–	Moderate	–	7	7	231	164	PR	16.2 M alive
31	58	M	0	−	−	Nodular	Presence	Lung, adrenal	Poor	TACE	86	58	14,661	11,990	SD	2.0 M alive

PS performance status, *HBs-Ag* hepatitis B surface antigen, *HCV-Ab* anti-hepatitis C virus antibody, *LN* lymph node, *TACE* transcatheter arterial chemoembolization, *RFA* radiofrequency ablation, *HAIC* hepatic arterial infusion chemotherapy using implanted port system, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxyprothrombin, *OP* operation, *M* months, *PR* partial response, *PD* progressive disease, *SD* stable disease

[a] Poorly differentiated

[b] Moderately differentiated

(Dako, Carpinteria, CA, USA). Sections were counterstained with hematoxylin.

Determination of p53 sequence

Total RNA extracted from HCC tissue using TRIZOL (Invitrogen, Carlsbad, CA, USA) was reverse-transcribed employing the Takara RNA PCR kit (AMV) Ver.3 (Takara, Tokyo, Japan). p53 was amplified using the forward primer 5′-GAGCCGCAGTCAGATCCTA-3′ and the reverse primer 5′-CAGTCTGAGTCAGGCCCTTC-3′, and nested polymerase chain reaction (PCR) was performed using the primers 5′-CCCCTCTGAGTCAGGAAACA-3′ and 5′-TTATGGCGGGAGGTAGACTG-3′. The PCR product was purified and sequenced using BigDye terminator version 3.1 cycle sequencing on an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Differences between groups were examined for significance using the Mann–Whitney *U*-test and Fishers exact test where appropriate. Multivariate analysis was performed by using a logistic regression model. Cumulative survival and progression-free survival (PFS) curves were constructed using the Kaplan–Meier method and compared using the log-rank test. All the analyses described above were performed using the SPSS program (version 11.5; SPSS, Chicago, IL, USA).

Results

Response

Complete and partial responses were achieved in 0 (0%) and 10 (32.3%) of the 31 patients, respectively. The overall response rate was 32.3%. Stable disease (SD) was noted in 3 patients (9.7%), and the disease control rate (complete response + partial response + SD) was 41.9%. Progressive disease (PD) was noted in 17 patients (54.8%). One patient was excluded from the assessment of response, because the patient died of HCC rupture 17 days after the start of treatment and the computed tomography could not be performed.

Progression-free survival rate and survival assessment

The median PFS was 1.6 months (95% confidence interval [CI] 1.5–1.7 months). The cumulative PFS rates at 6, 12, and 18 months were 38, 19, and 9%, respectively.

All enrolled patients were also included in a survival assessment. Twelve patients were still alive at the end of the observation period (median 8.2 months, range 2–25.4 months), while 19 patients had died. The causes of death were tumor progression ($n = 18$) and infectious lung disease ($n = 1$). The median survival time was 5.3 months (95% CI 1.7–9.0 months). The cumulative survival rates at 6, 12, 18, and 24 months were 44, 35, 35, and 17%, respectively.

Relationship of CD133, TS, DPD, and IFNR2 protein expression levels in HCC with the anticancer effect of the combined therapy

The expression levels of CD133, TS, DPD, and IFNR2 proteins, which were candidate predictive factors for the therapeutic effect, were studied by Western blotting in all specimens. Figure 1 shows the results in five samples from the PR group and four samples from the PD group. The expression level of CD133 was low in the PR group but high in the PD group. However, no marked differences were noted in the expression levels of TS, DPD, or IFNR2 between the two groups.

Next, the relationships of the CD133, TS, DPD, and IFNR2 protein expression levels with the antitumor effect were evaluated. To compare the protein expression levels among specimens, the relative expression level was calculated by dividing the intensity of each signal on Western blotting by the signal intensity of actin, which is an internal control. The CD133 protein expression level was significantly lower in the responder group (median 0.05) than in the nonresponder group (median 0.58) group ($p = 0.005$) (Fig. 2a). In contrast, the TS, DPD, and IFNR2 protein expression levels showed no significant differences between the responder and nonresponder groups (Fig. 2b–d).

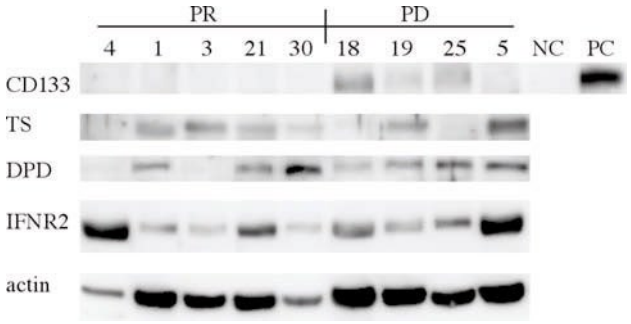

Fig. 1 Expression of CD133, thymidylate synthase (*TS*), dihydropyrimidine dehydrogenase (*DPD*), and interferon receptor 2 (*IFNR2*) as possible factors predicting the therapeutic effect in hepatocellular carcinoma (HCC). Results of Western blotting in typical cases in the partial response (*PR*) and progressive disease (*PD*) groups. For CD133, negative (DLD1) and positive (WiDr) controls were used

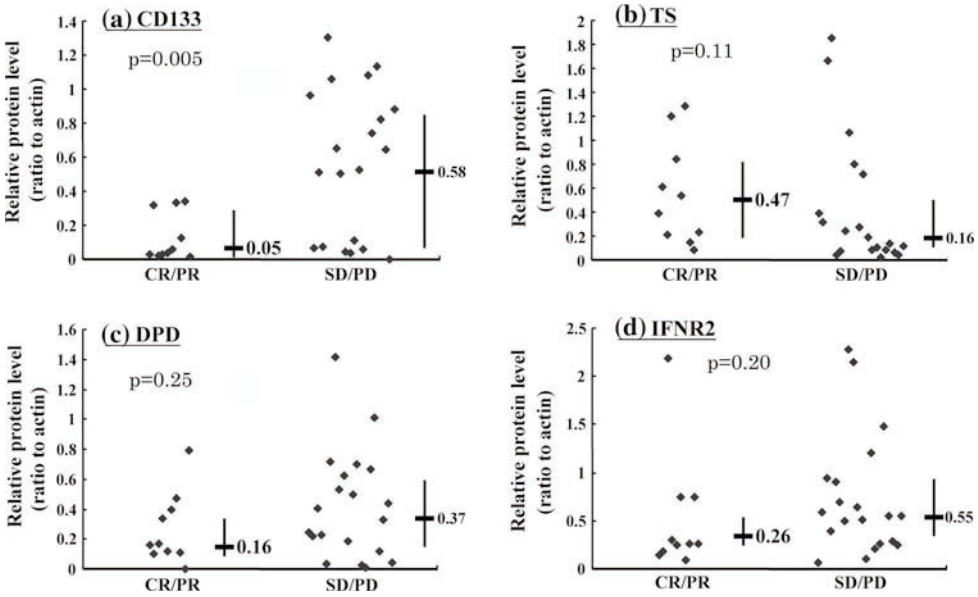

Fig. 2 Relationships between the expression levels of CD133 (**a**), TS (**b**), DPD (**c**), and IFNR2 (**d**) and the anticancer effect. *Vertical lines* on the right of these figures represent the quartile positions, and *horizontal lines* indicate the medians. The numbers of subjects with complete response (*CR*)/PR and stable disease (*SD*)/PD were ten and twenty, respectively

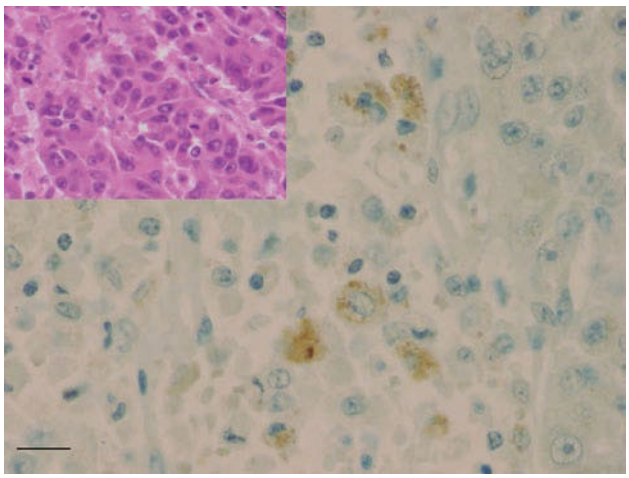

Fig. 3 Immunohistochemistry of CD133 in HCC tissue. This typical sample was intensely positive for CD133. Detection was facilitated using diaminobenzidine (DAB), which shows brown staining when positive. Magnification ×400. *Inset* image is H&E-stained specimen (magnification ×200)

The expression of CD133 was also studied in liver cancer tissues of all specimens using immunohistochemistry. Figure 3 presents specimens that showed intense staining for CD133. The presence or absence of an immunohistological signal was correlated with the CD133 protein expression level determined by Western blotting.

Comparison of the patient background and candidate factors predicting treatment effects with the final outcome (results of univariate analysis and multivariate analysis with a logistic regression model)

Univariate analysis showed a significantly higher CD133 level ($p < 0.01$) in the nonresponder (NR) than the responder group and a slightly higher TS level ($p = 0.11$) in the responder group (Table 3). Using CD133 and TS showing $p < 0.15$ on univariate analysis, multivariate analysis with a logistic regression model was performed. This analysis revealed that only CD133 was a significant factor (odds ratio 0.076, 95% CI 0.007–0.88, $p = 0.039$) (Table 4). Thus, irrespective of the TS level, CD133 was identified as an independent factor predicting the treatment effects.

Relationships of the CD133 protein expression level with the anticancer effect, PFS, and OS

The positive predictive value for nonresponders was 100% in patients whose CD133 expression level exceeded 0.4. Thus, we classified patients into high- and low-CD133 expression groups with a cutoff level of 0.4 (Fig. 2a). Thirteen patients were classified into the high-CD133 expression group and 18 patients into the low-CD133 expression group.

The relationship between the CD133 protein expression level and PFS is shown in Fig. 4. The median PFS was 1.6 months (95% CI 1.5–1.6 months) in the high-CD133 expression group and 7.2 months (95% CI 2–12.3 months) in the low-CD133 expression group. The log-rank test using the Kaplan–Meier method showed that the PFS was significantly prolonged in the low-level compared to the high-level CD133 expression group ($p = 0.036$).

The relationship between the CD133 protein expression level and overall survival (OS) is shown in Fig. 5. The median survival time was 3.1 months (95% CI 1.4–4.8 months) in the high-CD133 expression group, but 8.1 months (95% CI 0–19.3 months) in the low-CD133 expression group. The log-rank test using the Kaplan–Meier

Table 3 Comparison of patient characteristics according to the anticancer effect of the therapy

Variable	Responders (PR) ($n = 10$)	Nonresponders (SD + PD) ($n = 20$)	p value
Age (years)	64.5 (30–80)	65.5 (35–80)	0.85
Sex, no. (%)			0.25
Male	8 (80)	19 (95)	
Female	2 (20)	1 (5)	
Cause of disease, no. (%)			0.21
Hepatitis B	3 (30)	7 (35)	
Hepatitis C	3 (30)	11 (55)	
Non-B, non-C	4 (40)	2 (10)	
ECOG performance status, no. (%)			0.53
0	10 (100)	17 (85)	
1	0 (0)	3 (15)	
Tumor grade, no. (%)			0.21
Moderate	9 (90)	13 (65)	
Poor	1 (10)	7 (35)	
Vascular invasion, no. (%)	6 (60)	11 (55)	1
AFP (ng/ml), median (range)	409.5 (3–114,852)	560.5 (6–13,544)	1
PIVKA II (mAU/ml), median (range)	321.5 (16–61,319)	3,177.5 (16–116,140)	0.17
Previous therapy (last), no. (%)			0.83
OP	2 (20)	4 (20)	
TACE	2 (20)	7 (35)	
HAIC	2 (20)	2 (10)	
None	4 (40)	7 (35)	
CD133, median (range)	0.05 (0.01–0.34)	0.58 (0–1.30)	**<0.01**
IFNR2, median (range)	0.26 (0.09–2.19)	0.55 (0.06–2.27)	0.2
TS, median (range)	0.47 (0.08–1.29)	0.16 (0.03–1.85)	**0.11**
DPD, median (range)	0.16 (0–0.79)	0.37 (0–1.42)	0.25
p53 mutation, no. (%)	1 (10)	2 (10)	1

Values in bold are statistically significant

PR partial response, *SD* stable disease, *PD* progressive disease, *ECOG* Eastern Cooperative Oncology Group, *AFP* alpha-fetoprotein, *TACE* transcatheter arterial chemoembolization, *HAIC* hepatic arterial infusion chemotherapy using implanted port system, *IFNR2* interferon-receptor 2, *TS* thymidylate synthase, *DPD* dihydropyrimidine dehydrogenase, *PIVKA II* protein induced by vitamin K antagonist II

Table 4 Multivariate analysis with a logistic regression model

	Odds ratio	95% CI	p value
CD133 > 0.34	0.076	0.007–0.88	0.039
TS > 0.2	1.643	0.189–14.29	0.653

Cutoff value for each factor was determined by receiver operating characteristic curve (ROC) analysis

CI confidence interval

method showed that, in the low-level CD133 expression group, the OS was significantly prolonged compared to that in the high-level group ($p = 0.022$).

Relationship between p53 mutations and the anticancer effect

Mutant p53 was observed in 3 of the 31 patients. The response rate was 32.1% in the wild-type and 33.3% in the mutant specimens, with no significant difference. The disease control rate was 39.3% in the wild-type and 66.7% in the mutant specimens, with no significant difference.

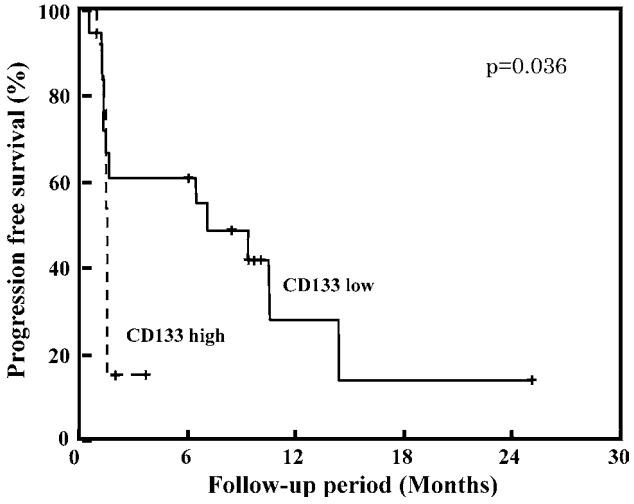

Fig. 4 Progression-free survival of patients who received combination therapy with S-1 and pegylated interferon (PEG-IFN) α-2b, stratified according to the CD133 expression level. Patients were divided into high- and low-CD133 expression groups, with a cutoff value of 0.4 (Fig. 2a). Thirteen and 18 patients belonged to the high- and low-expression groups, respectively

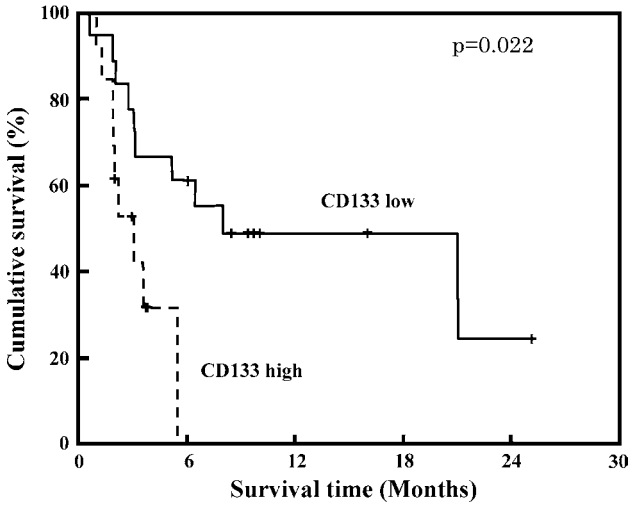

Fig. 5 Kaplan–Meier curve of overall survival in patients treated with combination therapy of S-1 and PEG-IFN α-2b, stratified according to the CD133 expression level

Toxicity

NCI-CTC grade 3 leukocytopenia, neutropenia, anemia, and thrombocytopenia were observed in 2 (6%), 2 (6%), 1 (3%), and 3 (10%) of the 31 patients, respectively. Grade 3 anorexia, stomatitis, rash, and fatigue were observed in 1 (3%), 1 (3%), 1 (3%), and 2 (6%) of the 31 patients, respectively. All adverse effects were alleviated when the treatment was discontinued, leading to no cases of mortality.

Discussion

Here we sought to identify factors predicting the therapeutic effect of S1+ PEG-IFN α-2b therapy in patients with advanced HCC. We collected pathological samples of HCC from all registered patients and studied proteins considered to be related to the therapeutic effect. The expression level of CD133 was significantly correlated with the therapeutic effect, but the expression levels of TS, DPD, and IFNR2, and the presence or absence of p53 mutations were not.

CD133 is a glycoprotein with five transmembrane regions and is a known blood stem-cell marker [28]. It also reportedly acts as a leukemia [15], brain [16], and colon cancer [17, 18] stem-cell marker. The characteristics of cancer stem cells include an ability to proliferate (self-replication capacity) and to differentiate into several cell types with different functions (multidifferentiation capacity), as well as a tumorigenic capacity, which was verified as tumor reproducibility in an experiment involving tumor implantation in an animal model [29–31]. In HCC, CD133-positive cells reportedly possess each of these cancer stem-cell characteristics. In 2007, Ma et al. [32] showed that 65–95% of cells in multiple HCC cell lines were CD133-positive, and Suetsugu et al. [33] reported that the HCC cell line Huh7 expressed CD133. In addition, Song et al. [34], who evaluated 60 patients with HCC, reported both significantly longer postoperative recurrence-free survival and total survival periods in a group with a low compared to that with a high CD133 level. There have been a few such reports on CD133 as a marker of postoperative recurrence.

In the present study, the CD133 protein expression level was significantly lower in the responder group than that in the nonresponder group. HCC showing high-level CD133 expression was resistant to the combination therapy used in this study. Several studies have suggested that the most cancer stem cells exist in the G_0 phase, and a reduced cell cycle velocity is involved in the drug resistance of cancer stem cells [35]. Furthermore, these cells are resistant to reactive oxygen-induced DNA damage because of their high radical-scavenging activities [36]. Furthermore, the drug resistance mechanism of cancer stem cells may also involve ATP binding cassette (ABC) transporters [16, 37]. The anti-apoptotic factors Akt/PKB and Bcl-2 are also activated in CD133-positive liver cancer cells by 5FU administration [38]. Because the anticancer effect of S1+ PEG-IFN α-2b therapy is primarily derived from apoptosis, the activation of Akt/PKB and Bcl-2 is considered to be directly related to the resistance to this therapy.

TS is a rate-regulating enzyme involved in the synthesis of deoxythymidine monophosphate, which is indispensable for DNA synthesis. Therefore, the anticancer effect of 5FU decreases when the TS expression level in the tumor is high, because the drug cannot sufficiently inhibit the enzyme [39]. In the present study, the TS expression level did not correlate with the therapeutic effect. Oie et al. [40] reported that TS expression was suppressed by IFN administration in all the HCC-derived cell lines they examined. Although we did not evaluate the TS expression level after IFN administration, the absence of a correlation between the TS expression level and the therapeutic effect may have been due to the inhibition of TS by IFN.

DPD is a 5FU-degrading enzyme present primarily in the liver. 5FU efficiency increases with low DPD expression in tumor cells [41]. In our study, no correlation between the DPD expression level and therapeutic effect was noted. This was an expected result, because S1 contains a DPD inhibitor.

IFNR, and particularly IFNR2, is considered to be the most important IFN-binding unit for IFN activity. IFNR2 is reportedly expressed in 61–77% of HCCs [42, 43], and its anticancer effect increases with its level of expression. In our study, IFNR2 expression did not correlate with the therapeutic effect. When multiple HCC cell lines were

treated with IFN-α in vitro, a relationship between IFNR2 expression and the anticancer effect was demonstrated [23]. Therefore, IFNR2 is undoubtedly important in the direct anticancer effect of IFN. However, indirect anticancer effects of IFN, such as the activation of natural killer cells and cytotoxic lymphocytes, must also be considered in regard to in vivo treatment [44–46]. Such indirect actions may be primarily responsible for the anticancer effects of IFN in some patients. Regardless of these findings, the IFNR2 expression level is not considered useful for the prediction of the therapeutic effect.

p53 is a typical tumor suppressor gene that may arrest the cell cycle and induce DNA repair or promote apoptosis, depending on the degree of DNA damage [47]. However, the mutation of p53 at some sites causes loss of its original function, allowing the initiation of tumor growth and acceleration of tumor proliferation [48]. In our study, no correlation was noted between the presence or absence of p53 mutations and the therapeutic effect. This may have been because there were only three patients with mutant p53, or because the mutations occurred at sites that do not affect p53 function.

In conclusion, S1+ PEG-IFN α-2b therapy can be a second-line treatment option for patients with advanced HCC, especially in those that show low CD133 expression. Further evaluation with a prospective randomized trial is necessary to confirm the results of the present study.

Acknowledgments The protocol of this study was approved by the Medical Committee of Kinki University of Medicine.

Conflict of interest None.

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Estimation of malignant potential of GI stromal tumors by contrast-enhanced harmonic EUS (with videos)

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Background: Contrast-enhanced harmonic EUS (CEH-EUS) is a new sonographic technique that uses US contrast agents and depicts intratumoral vessels in real time.

Objective: To evaluate whether assessment of tumor vascularity by CEH-EUS can predict the preoperative malignancy risk of GI stromal tumors (GISTs).

Design: Prospective study to observe GIST vascularity.

Setting: Kinki University School of Medicine.

Patients: Between June 2007 and September 2009, 76 consecutive patients suspected of having subepithelial lesions underwent CEH-EUS.

Intervention: CEH-EUS was performed by using a prototype echoendoscope in an extended pure harmonic detection mode.

Main Outcome Measurements: Resected GIST specimens in 29 patients who underwent surgical resection were divided into high-grade (n = 16) and low-grade (n = 13) malignancy groups based on mitotic activity. The abilities of EUS-guided FNA and CEH-EUS to diagnose the malignant potential were compared. The sensitivities with which contrast-enhanced multidetector CT, power-Doppler EUS, and CEH-EUS detected intratumoral vessels in high-grade malignancy GISTs also were compared.

Results: CEH-EUS identified irregular vessels and thereby predicted GIST malignancies with a sensitivity, specificity, and accuracy of 100%, 63%, and 83%, respectively. Diagnosis of high-grade malignancy GISTs by EUS-guided FNA had a sensitivity, specificity, and accuracy of 63%, 92%, and 81%, respectively. Contrast-enhanced multidetector CT, power-Doppler EUS, and CEH-EUS detected intratumoral vessels in high-grade malignancy GISTs with sensitivities of 31%, 63%, and 100%, respectively ($P < .05$).

Limitations: A single center was involved in this study.

Conclusions: CEH-EUS successfully visualized intratumoral vessels and may play an important role in predicting the malignancy risk of GISTs. (Gastrointest Endosc 2011;73:227-37.)

GI stromal cell tumors (GISTs) are the most common mesenchymal neoplasms of the GI tract.[1] The clinical presentation of GISTs can range from asymptomatic to symptomatic; asymptomatic GISTs are often detected incidentally during routine endoscopic screening. Although 10% to 30% of GISTs are clinically malignant, all

Abbreviations: CE-CT, contrast-enhanced multidetector CT; CEH-EUS, contrast-enhanced harmonic EUS; CEH-US, contrast-enhanced harmonic US; EUS-FNA, EUS-guided FNA; GIST, GI stromal tumor; HPFs, high-power fields; PD-EUS, power-Doppler EUS.

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GISTs are known to have some degree of malignant potential. However, diagnostic tools or histological tests that reliably estimate the malignancy risk of GISTs preoperatively are not available.[2-10] Transabdominal contrast-enhanced Doppler US and contrast-enhanced harmonic US (CEH-US) have been used recently to preoperatively assess GIST vascularity.[11-13] When measured by CEH-US by using the first-generation US contrast agent, Levovist (Schering AG, Berlin, Germany), GIST vascularity correlated better than histology (including mitotic count and KIT mutation) with a clinical diagnosis of malignancy.[11] Therefore, tumor vascularity may be predictive of GIST malignancy.

Most echoendoscope transducers have a limited frequency bandwidth that produces insufficient acoustic power for contrast-harmonic imaging with Levovist. However, second-generation US contrast agents, such as SonoVue (Bracco Imaging, Milan, Italy) and Sonazoid (Daiichi-Sankyo, Tokyo, Japan) produce harmonic signals at lower acoustic powers that are suitable for EUS imaging. CEH-EUS technology recently developed uses a broad-band transducer and a second-generation US contrast agent that permits contrast-enhanced harmonic imaging with lower acoustic power.[13-15] In this study, the microvessels and parenchymal flow of subepithelial lesions were assessed by CEH-EUS, and the relationship between GIST vascularity and malignant potential was evaluated.

Take-home Message

- Contrast-enhanced harmonic EUS (CEH-EUS) with second-generation US contrast agents was significantly more sensitive than contrast-enhanced multidetector CT in detecting intratumoral vessels in high-grade malignancy GISTs. CEH-EUS analysis showed that all high-grade malignancy GISTs had an abundance of vessels, whereas low-grade malignancy GISTs were less likely to exhibit high vascularity.

PATIENTS AND METHODS

Patients

Between June 2007 and August 2009, 76 consecutive patients with suspected subepithelial GI lesions, based on screening with US, CT, or upper GI endoscopy, underwent EUS, power-Doppler EUS (PD-EUS), CEH-EUS, EUS-guided FNA (EUS-FNA), and CE-multidetector CT (CE-CT). Patients with varices (n = 2) that were initially suspected to be subepithelial tumors (ie, large, bulging gastric varices), lipomas (ie, characteristic benign lesions on EUS that looked like homogeneously hyperechoic lesions; n = 4), extrinsic compressions (n = 2), or cysts (n = 10) were excluded from the study. The study was approved by the Institutional Review Board of Kinki University School of Medicine. All patients provided informed consent.

Fundamental B-mode, PD-EUS, and CEH-EUS

Before undergoing PD-EUS and CEH-EUS, all patients underwent fundamental B-mode EUS to determine the tumor size, presence of lobulations, echodensity, and the existence of echogenic foci and cystic spaces. All EUS examinations were performed by 2 experienced endosonographers using an Olympus GF-UE260P (Olympus Medical Systems Co, Ltd, Tokyo, Japan). US image analyses were performed by ALOKA Prosound α-10 (ALOKA Co Ltd, Tokyo, Japan).

For CEH-EUS, the extended pure harmonic detection mode was used, which combines the filtered fundamental and second harmonic component frequencies with a transmitting frequency of 4.7 MHz (Fig. 1). After a subepithelial tumor was detected by fundamental B-mode EUS, the setting was changed to power-Doppler mode to assess whether an intratumoral vessel was detected, after which the setting was changed to the extended pure harmonic detection mode. A bolus infusion of contrast agent (15 μL/kg Sonazoid) was administered, and the vascular structures were assessed in real time by examining continuous 0- to 15-second (vessel image) and 40- to 60-second (perfusion image) images. All clips were stored in the hard disk of the scanner for off-line investigation.

EUS-FNA

EUS-FNA was performed on sedated patients by using a convex array echoendoscope (GF-UC240P-AL5 and GF-UCT240P-AL5; Olympus, Tokyo, Japan) and a 19-, 22-, or 25-gauge needle. A maximum of 5 passes was made to obtain sufficient material. Material was then subjected to hematoxylin-eosin and immunohistochemical staining.

CE-CT

The CE-CT images were peer reviewed independently by 2 readers who were blinded to the EUS and pathological findings. The reviewers determined whether intramural vascular enhancement could be detected in the subepithelial lesions.

Histological diagnosis of lesions and determination of GIST malignancy

The GISTs were defined as subepithelial tumors composed of spindle cells that stained positive for c-kit and CD34. The criterion standard for evaluating GIST malignancy was histological analysis of hematoxylin-eosin–stained resected specimens for number of mitotic figures. GISTs in the resected specimens were divided into 2 groups on the basis of mitotic activity: high-grade malignancy had a mitotic activity of $\geq$10 figures per 50 high-power fields (HPFs), whereas low-grade malignancy had a

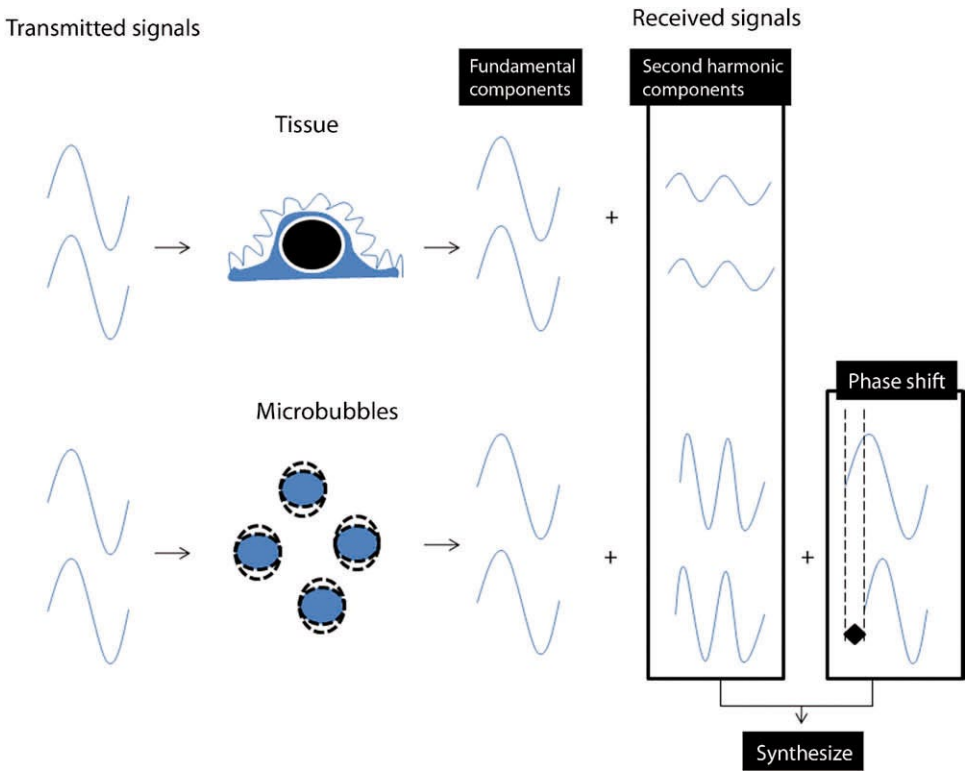

Figure 1. Principle of extended pure harmonic detection (ExPHD) mode. The microbubbles produce stronger second harmonic signals as well as a greater phase shift than does the tissue. Contrast harmonic imaging based on the ExPHD mode selectively depicts signals from microbubbles by synthesizing the phase shift signals with the second harmonic components.

mitotic activity of <10 figures per 50 HPFs. Because counting the mitotic figures of EUS-FNA specimens per 50 HPFs was difficult, low-grade and high-grade malignancy GISTs were differentiated by the existence of 1 mitotic figure per 5 HPFs for the EUS-FNA specimens.[16]

Statistical analyses

The sensitivities with which CE-CT, PD-EUS, and CEH-EUS correctly classified GISTs measuring ≤3 cm or >3 cm as high-grade malignancy GISTs (presence of intratumoral vessels) were calculated and compared by using the χ^2 test. The CEH-EUS brightness of the lesions was analyzed, and luminance histograms were generated by using Adobe Photoshop CS4. Unimodal and bimodal brightness distribution patterns were considered to indicate homogeneous and heterogeneous lesions, respectively. The χ^2 test data were used to compare the frequencies with which EUS or CEH-EUS detected features (the percentage of GISTs with >3 cm diameter, lobulations, echodensity, existence of echogenic foci and cystic spaces, irregular vessels, and necrotic center) in low-grade and high-grade malignancy GISTs. *P* values of <.05 were considered significant.

RESULTS

Final diagnoses based on histological findings were intramural abscess (n = 1), leiomyoma (n = 6), schwannoma (n = 1), GIST (n = 35), inflammatory fibroid polyp (n = 3), and ectopic pancreas (n = 12). The histology of the 29 surgically resected GISTs revealed 16 high-grade and 13 low-grade malignancy GISTs. Mean (± standard deviation) diameters of 2.9 ± 2.1 cm (range 1.1-5 cm), and 3.2 ± 2.5 cm (range 2-12.1 cm) were obtained for low-grade malignancy GISTs, and high-grade malignancy GISTs, respectively. Only 1 of the low-grade malignancy GISTs was symptomatic (7.6%); the other 12 were discovered incidentally during routine screening. Five of the high-grade malignancy GISTs (31%) were symptomatic; the remaining 11 were discovered incidentally (Table 1).

Ability of EUS-FNA to diagnose GIST malignancy relative to histological analysis of resected specimens

All 29 patients who underwent surgery also underwent EUS-FNA, but adequate specimens were obtained in only 21 cases (72.4%). The results for these 21 specimens were compared with those of the resected specimens. Based on the mitotic activity of the surgical specimens, 8 of 21 high-grade malignancy GISTs and 13 of 21 low-grade malignancy GISTs were obtained (Table 2). Based on the presurgical EUS-FNA specimens, 3 of 8 high-grade malignancy GISTs had been diagnosed as low-grade, and 1 of 13 low-grade malignancy GISTs had been diagnosed as high-grade (Table 2). High-grade malignancy diagnosis by

TABLE 1. Clinical characteristics of 29 patients with GISTs

	Low-grade malignancy GIST (n = 13)	High-grade malignancy GIST (n = 16)
Age, y, mean	61.7	64.5
Sex, female, %	53.8	62.5
Symptoms, no. (%)	1 (7.7)	5 (31.3)
Abdominal pain	0 (0)	3 (18.7)
Anemia	0 (0)	1 (6.3)
Bleeding	1 (7.7)	1 (6.3)
Tumor size, mm, mean ± SD (range)	29 ± 21 (11-50)	32 ± 25 (20-121)
Tumor size, no. (%)		
≤3 cm	7 (54)	4 (25)
>3 cm	6 (42)	12 (75)
Tumor location, no. (%)		
Fundus	3 (23)	1 (6)
Body	7 (54)	6 (38)
Pylorus	1 (7)	4 (25)
Duodenum	2 (16)	5 (31))

GIST, GI stromal tumor; *SD*, standard deviation.

TABLE 2. Ability of histological analysis of EUS-FNA specimens to correctly determine the degree of GI stromal tumor malignancy

	Resected specimens		
EUS-FNA specimens	Low-grade malignancy	High-grade malignancy	Total
Low-grade malignancy	12	3	15
High-grade malignancy	1	5	6

EUS-FNA, EUS-guided FNA.

EUS-FNA had a sensitivity, specificity, and diagnostic accuracy of 92%, 62%, and 81%, respectively. The average number of EUS-FNA passes was 2.8 (range 1-5). One case of bleeding was associated with EUS-FNA. It was treated with endoscopic clipping without requiring blood transfusion.

CEH-EUS image pattern of GISTs

Subepithelial tumor vessel images were categorized as displaying a regular vessel pattern (fine vessels flowing in the tumor), an irregular vessel pattern (large, irregular vessels flowing from the periphery to the center of the tumor), or no vessels. Perfusion images were categorized as having a homogeneous, heterogeneous, or no enhancement pattern. The heterogeneous pattern was because of irregularly branching vessels and avascular spots in the lesion. The histogram distribution pattern of the perfusion images indicated that the homogeneous and heterogeneous patterns were unimodal and bimodal, respectively (Fig. 2). Thus, the histogram distribution patterns were related closely to the perfusion phase CEH-EUS images. All GISTs with regular vessels displayed homogeneous enhancement on perfusion images, whereas all GISTs with irregular vessels displayed heterogeneous enhancement on perfusion images. Thus, the image patterns of the GISTs were classified into 2 types (Fig. 3): type I, regular vessels on the vessel image and homogeneous enhancement on the perfusion image; and type II, irregular vessels on the vessel image and heterogeneous enhancement on the perfusion image.

All type I tumors were low-grade malignancy GISTs (n = 8) (Fig. 4; Video 1, available online at www.giejournal.org). Of the 21 type II tumors, 5 were low-grade malignancy GISTS, and 16 were high-grade malignancy GISTs (Table 3). All high-grade malignancy GISTs were classified as type II (Table 3; Figs. 5 and 6; Video 2, available online at www.giejournal.org). Type II determined GIST malignancy with a sensitivity, specificity, and accuracy of 100%, 63%, and 83%, respectively. All benign spindle cell neoplasms including 6 leiomyomas and 1 schwannoma without low-grade malignancy GISTs were classified as type I.

Comparison of high-grade and low-grade malignancy GISTs in terms of EUS and CEH-EUS

Figure 7 shows a comparison of EUS and CEH-EUS features between low-grade and high-grade malignancy GISTs. Twelve of 16 patients with high-grade malignancy GISTs (75%) and 6 of 13 patients with low-grade malignancy GISTs (46%) had diameter of >3 cm. There was no significant difference in tumor size between low-grade and high-grade malignancy GISTs. According to EUS, lobulation and a heterogeneous appearance were more common among the high-grade (lobular, 10/16; heterogeneous, 11/16 GISTs) than among the low-grade malignancy GISTs (lobulation, 2/13; heterogeneous, 2/13 GISTs) (Figs. 5 and 6). Lobulation was found in 0 of 7 (0%) and 1 of 4 (25%) low-grade and high-grade malignancy GISTS of ≤3 cm, respectively, and in 2 of 6 (33%) and 9 of 12 (75%) low-grade and high-grade malignancy GISTs of >3 cm, respectively. Heterogeneous appearance was found in 0 of 7 (0%) and 0 of 4 (0%) low-grade and high-grade malignancy GISTs of ≤3 cm, respectively, and in 2 of 6 (33%) and 11 of 12 (100%) low-grade and high-grade malignancy GISTs of >3 cm, respectively.

According to CEH-EUS, the irregular vessels pattern was more common in high-grade than in low-grade malignancy GISTs. Irregular vessels were found in all 16 high-grade but only 5 of 13 low-grade malignancy GISTs (Figs. 5 and 6). Irregular vessels were found in 2

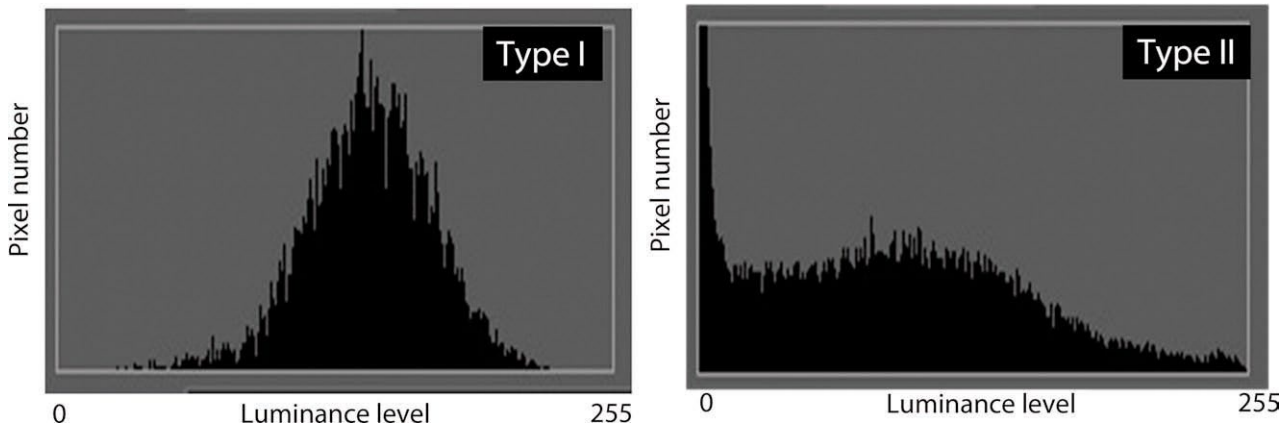

Figure 2. Histogram of the 2 types of tumors classified by CEH-EUS. The histogram distribution patterns of types I and II were unimodal and bimodal, respectively.

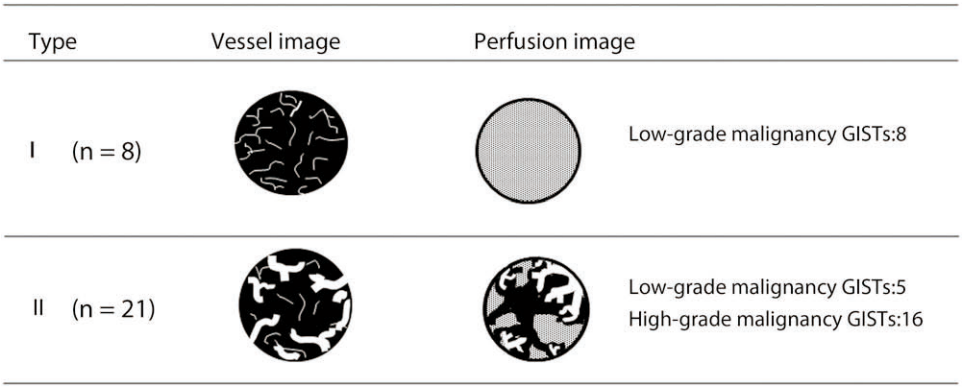

Figure 3. Classification of vascular patterns of GISTs. Type I is characterized by regular vessels on the vessel image and homogeneous enhancement on the perfusion image. Type II is characterized by irregular vessels flowing from the periphery to the center of the tumor on the vessel image and heterogeneous enhancement on the perfusion image.

of 7 (29%) and 4 of 4 (100%) low-grade and high-grade malignancy GISTs of ≤3 cm, respectively, and in 3 of 6 (50%) and 12 of 12 (100%) low-grade and high-grade malignancy GISTs of >3 cm, respectively. A centrally necrotic appearance was found in 0 of 13 and 7 of 16 low-grade and high-grade malignancy GISTs, respectively ($P < .05$) (Fig. 6).

Sensitivity with which CE-CT, PD-EUS, and CEH-EUS detected intratumoral vessels in high-grade malignancy GISTs

CEH-EUS detected intratumoral vessels in all high-grade malignancy GISTs. The sensitivity of CEH-EUS for detecting intratumoral vessels (100%) was significantly higher than that for CE-CT (31%) and PD-EUS (63%) (Table 4). Sensitivities with which CEH-EUS, PD-EUS, and CE-CT detected intratumoral vessels in smaller (≤3 cm) high-grade malignancy GISTs were 100%, 25%, and 0% respectively, whereas those values in larger (>3 cm) high-grade malignancy GISTs were 100%, 75%, and 42%, respectively (Table 4).

DISCUSSION

In the present study, we assessed the malignant potential of GISTs by using CEH-EUS to observe tumor microvessels. The CEH-EUS vessel patterns and perfusion images allowed the GISTs to be classified into 2 types; the type with regular vessels on vessel images and homogeneous enhancement on perfusion images (type I) and the type with irregular vessels on vessel images and heterogeneous enhancement on perfusion images (type II). There was a good relationship between the CEH-EUS images in the perfusion phase and the histogram distribution patterns. Thus, the classification system appeared to be reliable. All high-grade malignancy GISTs were classified as type II on the basis of irregular vessels, whereas 8 of 13 low-grade malignancy GISTs were classified as type I. Our previous study using transabdominal CEH-US demonstrated that assessment of intratumoral vessels is useful for estimating the malignant potential of GISTs.[11] However, some small, submucosal tumors may be difficult to detect by CEH-US because of inter-

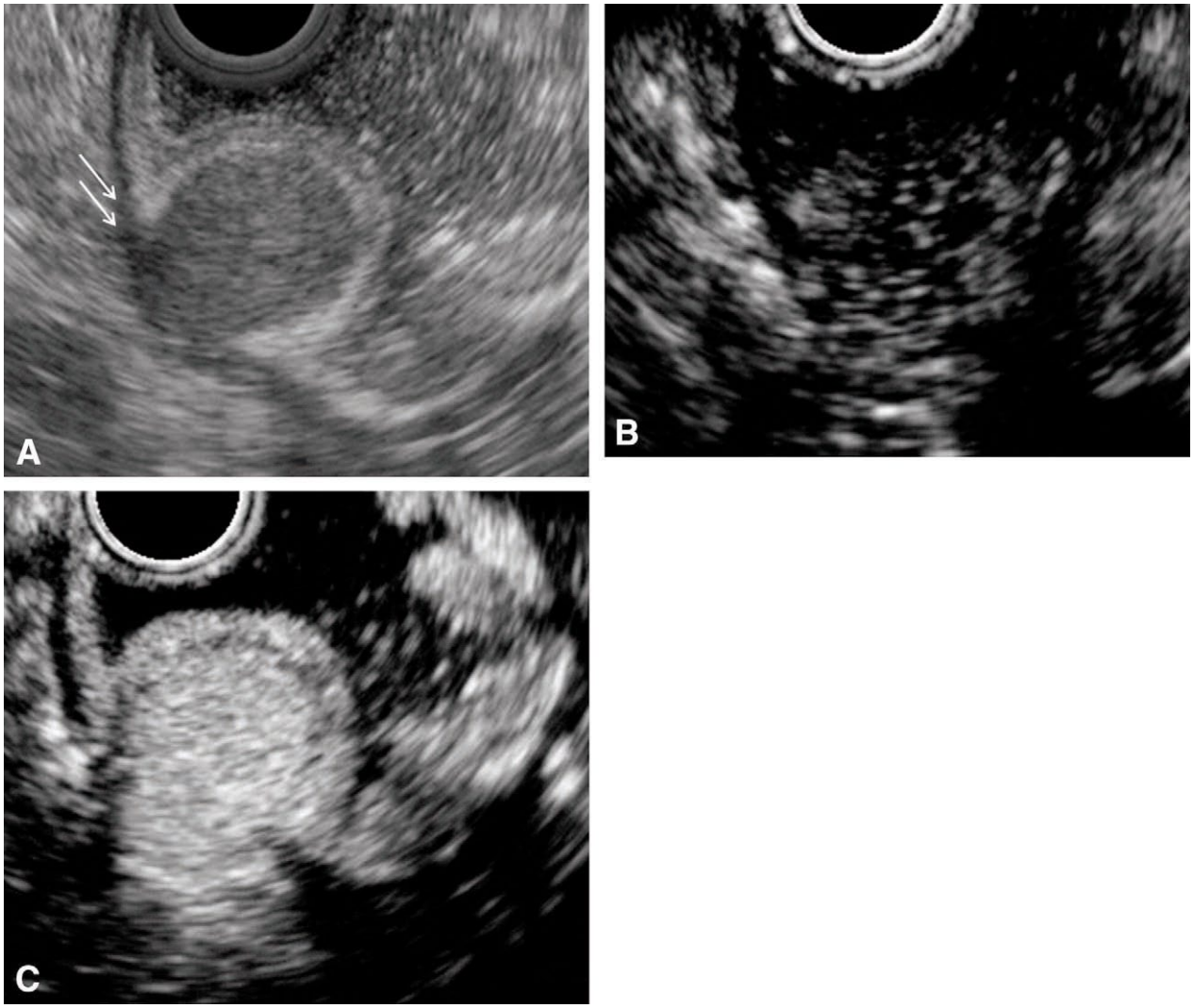

Figure 4. Typical type I lesion: low-grade malignancy GIST of the stomach in a 71-year-old man. **A,** EUS reveals homogenous, round to oval–shaped, hyperechoic foci (*arrows*) in the fourth wall layer of a 16-mm-diameter gastric subepithelial tumor. **B,** Vessel image of CEH-EUS showing fine intratumoral vessels. **C,** Perfusion image of CEH-EUS showing the homogeneous enhancement of the tumor 50 seconds after Sonazoid (Daiichi-Sankyo, Tokyo, Japan) injection.

TABLE 3. Ability of CEH-EUS to correctly determine the degree of GI stromal tumor malignancy

	Resected specimens		
CEH-EUS	Low-grade malignancy	High-grade malignancy	Total
Type I	8	0	8
Type II	5	16	21

CEH-EUS, Contrast-enhanced harmonic EUS.

vening abdominal fat or GI gas. In the present study, all 4 of the small, malignant lesions were estimated by CEH-EUS to be highly malignant. Thus, CEH-EUS may be more suitable than CEH-US for evaluating the vascularity (and therefore the malignant potential) of small, submucosal tumors.

With regard to assessment of intratumoral vessels for malignant GISTs by PD-EUS and CT, Sâftoiu et al[17] assessed the endoscopic management of submucosal tumors of the upper GI tract, with emphasis on the usefulness of color and PD-EUS. Intratumoral vessels were observed in 5 of 6 (83.3%) malignant GISTs by PD-EUS.[17] On the other hand, CE-CT cannot readily characterize GISTs by vascularity.[3-4] In the present study, the number of cases in which PD-EUS and CE-CT detected intratumoral vessels increased in parallel with the tumor size, suggesting that intratumoral vessels with greater diameter and faster flow develop according to tumor growth. Because color and power-Doppler imaging techniques process the vessel flow for detecting the vessel signal, a certain flow rate is

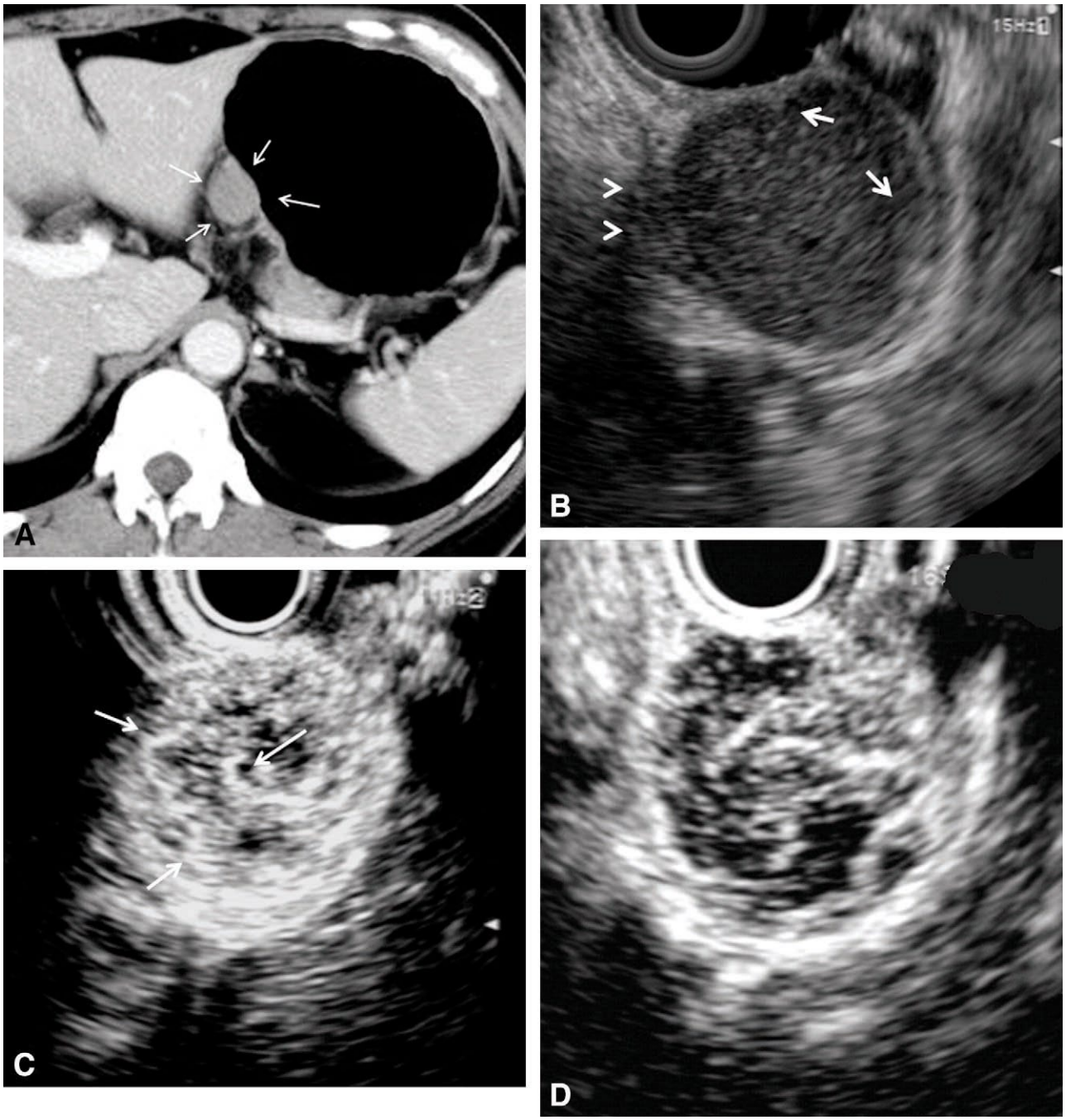

Figure 5. Typical type II tumor lesion: high-grade malignancy GIST of the stomach in a 41-year-old man. **A,** CE-CT showing homogeneous enhancement of a 24-mm-diameter tumor (*arrows*). Intratumoral vessels are not seen. **B,** EUS showing the homogenous appearance, incomplete tumor lobulation (*arrows*), and local invasion (*arrowheads*) of a 22-mm-diameter gastric tumor. **C,** Vessel image of CEH-EUS showing irregular vessels flowing from the periphery to the center of the tumor (*arrows*). **D,** Perfusion of CEH-EUS showing heterogeneous appearance within the tumor.

needed in order to identify the vessels. On the other hand, contrast-harmonic imaging directly depicts microbubbles in the vessels with any flow rate, particularly in the slow-flow vessels that Doppler imaging fails to identify.[18-20] Therefore, this technique can be used to visualize parenchymal staining and sensitively characterize tumor vascularity in various organs.[13-14,21] In the present study, CEH-EUS could detect intratumoral vessels in all high-grade malignancy GISTs more sensitively than PD-EUS and CE-CT. The superiority of CEH-EUS to the other modalities was remarkable for small GISTs. These results suggest that CEH-EUS is more sensitive for detecting intratumoral vessels with slow flow and superior to PD-EUS and CE-CT with regard to evaluation of small GISTs.

EUS is widely used to diagnose submucosal tumors because it is superior to other modalities in terms of spatial

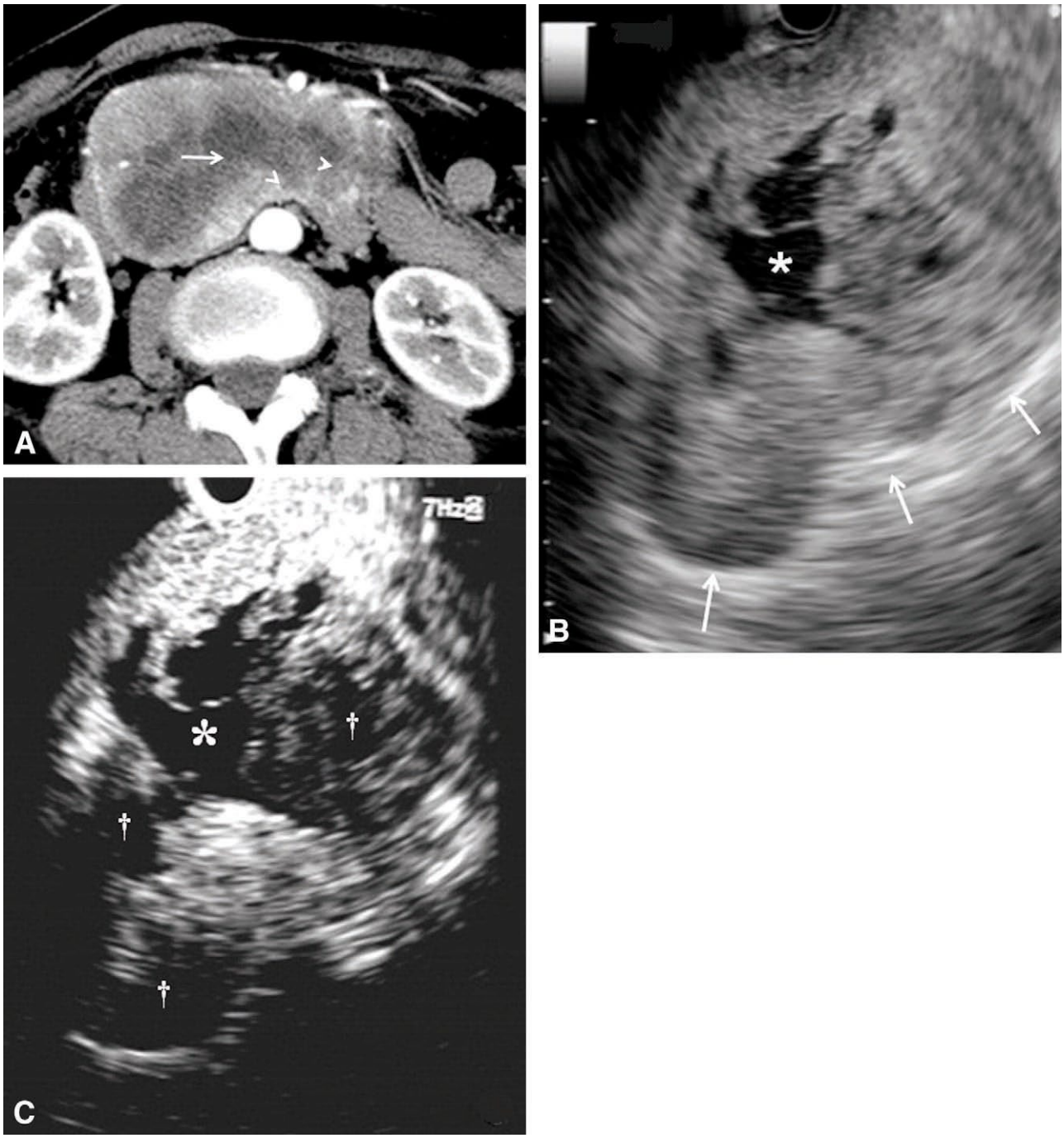

Figure 6. Typical type II tumor lesion: high-grade malignancy GIST of the stomach in a 67-year-old man. **A,** CE-CT showing heterogeneous enhancement, central necrosis, and irregular vessels within a 62-mm-diameter tumor. **B,** EUS showing a gastric subepithelial tumor with a central anechoic area (*cystic area: **) , heterogeneous appearance, and surface lobulations (*arrows*). **C,** Perfusion image of CEH-EUS showing a heterogeneous, strongly stained tumor. Parts of the tumor appear to lack vascularity (*necrotic areas: †*).

resolution.[9,22-25] Certain EUS features are also predictive of malignant GISTs, including a large diameter (>3-5 cm), irregular extraluminal border, and the presence of echogenic foci and cystic spaces.[2,7-8,26] In our series, there was no significant difference in tumor size between low-grade and high-grade malignancy GISTs, although more high-grade than low-grade malignancy GISTs tended to be >3 cm, suggesting that tumor size is not closely correlated with malignant potential. With regard to the present EUS findings, the high-grade malignancy GISTs were more likely than low-grade malignancy GISTs to display lobulations and to be heterogeneous. Most GISTs with lobulations and heterogeneous appearance were large and showed strong mitotic activity on pathological examina-

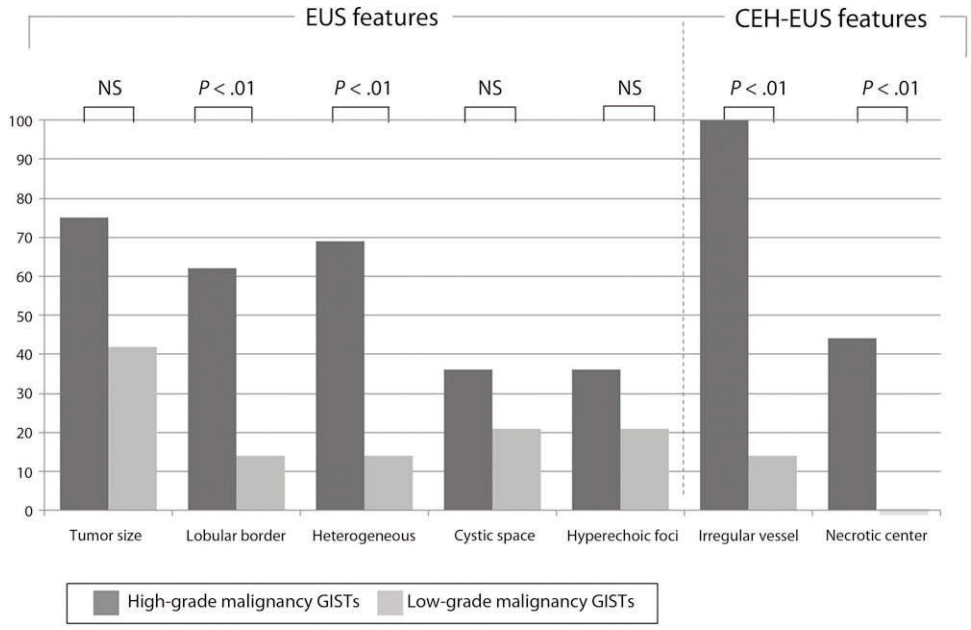

Figure 7. Comparison of EUS and CEH-EUS features between low-grade and high-grade malignancy GISTs. EUS detected lobulations and a heterogeneous appearance more frequently in high- than in low-grade malignancy GISTs. CEH-EUS detected irregular vessels and necrotic center appearances more frequently in high- than in low-grade malignancy GISTs.

TABLE 4. Comparison of sensitivity for detecting intratumoral vessels in high-grade malignancy GISTs among CE-CT, PD-EUS, and CEH-EUS

	Tumor size, no. (%)		
	≤3 cm	>3 cm	All GISTs
CE-CT	0/4 (0)	5/12 (42)	5/16 (31)
PD-EUS	1/4 (25)	9/12 (75)	10/16 (63)
CEH-EUS	*†4/4 (100)	*12/12 (100)	*†16/16 (100)

GISTs, GI stromal tumors; *CE-CT,* contrast-enhanced multidetector CT; *PD-EUS,* power-Doppler EUS; *CEH-EUS,* contrast-enhanced harmonic EUS.
*$P < .05$ vs CE-CT.
†$P < .05$ vs PD-EUS.

tion. Four of the high-grade malignancy GISTs were small (≤3 cm) and were highly vascularized according to CEH-EUS. Even small GISTs have malignant potential, and these small GISTs could be detected by CEH-EUS in this study. All high-grade malignancy GISTs were found to be highly vascular by CEH-EUS, especially for small GISTs without lobulation or heterogeneous appearance. Irregular vessels observed by CEH-EUS were more sensitive than lobulations and heterogeneous appearance observed by ordinary EUS for evaluation of high-grade malignancy GISTs. On the other hand, among low-grade malignancy GISTs, 6 were larger than 3 cm. Three of the 6 (50%) displayed a type I pattern by CEH-EUS. These results suggest that classification of vascular patterns by CEH-EUS is complementary for the tumor size and the other EUS features in evaluation of the malignant potential of GISTs. Central necrosis may be a feature suggestive of malignant GISTs. Burkill et al[27] reported that CE-CT showed central necrosis in 24 of 36 (67%) malignant GISTs. In the present study using CEH-EUS, 7 malignant GISTs displayed necrotic (avascular solid) areas, supporting that GISTs with necrotic areas were more likely to be malignant. This feature evaluated by CEH-EUS also may reveal an additional characteristic to predict the malignancy risk.

EUS-FNA is a widely accepted modality for diagnosing tumors,[26,28-32] including subepithelial tumors and GISTs.[32-38] The presence of mitoses in EUS-FNA specimens of GISTs appears to be associated with malignancy.[19] However, EUS-FNA is associated with several limitations. Only 78% to 86% of EUS-FNA procedures return adequate specimens, and the repeated needle passes needed to obtain sufficient tissue can result in complications.[33-41] Indeed, in the present study, sufficient material was obtained from only 21 of 29 GIST patients. Two cases of tumor seeding after percutaneous biopsy for malignant GIST were reported recently,[42,43] suggesting that EUS-FNA may be associated with an increased peritoneal seeding risk. Thus, if high-risk patients can be identified without EUS-FNA by using CEH-EUS instead, the complications and expense associated with the management of GISTs would be reduced. However, CEH-EUS could not distinguish other spindle cell neoplasms (leiomyomas or schwannomas) from GISTs, because all benign spindle cell neoplasms were classified as type I, indicating that EUS-FNA is necessary for histological differentiation of spindle cell neoplasms.

One potential limitation of this study is that the degree of malignancy was based on the mitotic index, which provides only an estimate of the clinical behavior of GISTs.[11] The presence of metastasis or invasion to other sites should be taken into consideration, because those malignant findings are observed in some GISTs with a low mitotic index. Further prospective studies should establish whether the presence of intratumoral vessels is predictive of the malignancy risk of GISTs by evaluation of the clinical behavior in addition to the histological diagnosis.

In conclusion, CEH-EUS can be used to successfully visualize the intratumoral microvascularity, which is an important factor in determining the malignancy risk of GISTs, especially for small lesions. Further research with more patients observed over a longer follow-up period is required to confirm the superiority of CEH-EUS in diagnosing GIST malignancy.

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Original Research

Optimal Scanning Protocol of Arterial Dominant Phase for Hypervascular Hepatocellular Carcinoma with Gadolinium-Ethoxybenzyl-Diethylenetriamine Pentaacetic Acid-Enhanced MR

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Purpose: To investigate optimal delay time of hepatic arterial phase in Gadoxetate-enhanced MR for detecting hypervascular hepatocellular carcinoma (HCC).

Materials and Methods: Forty-five patients with 85 hypervascular HCCs and 9 patients with 16 hypervascular HCCs underwent Gadoxetate- and Gd-DTPA-enhanced MR at 1.5 Tesla (T) system, respectively. All HCCs were analyzed 10–38 s after injection using a time-resolved dynamic MR sequence with keyhole data sampling. Seven sequential phase images (1 phase = 4 s) were obtained during a single breath hold of 28 s. Time–intensity curves of the abdominal aorta, liver parenchyma, and HCC were obtained, then aortic contrast arrival time, time of peak HCC enhancement, duration time of HCC and aortic enhancement, and time delay from aortic contrast arrival to peak enhancement of HCC were measured.

Results: Aortic contrast arrival time was 15.1 ± 2.9 s, time of peak HCC enhancement 29.9 ± 4.6 s, duration time of HCC enhancement 17.4 ± 6.4 s postinjection of Gadoxetate. Duration of aortic enhancement (23.6 ± 3.5 s) of Gadoxetate-enhanced MR was significantly less than that of Gd-DTPA-enhanced MR (26.3 ± 2.8 s) ($P < 0.0059$).

Conclusion: Peak enhancement time of HCC on Gadoxetate-enhanced MR imaging occurred at 14.6 ± 4.6 s after aortic contrast arrival.

Key Words: MRI; Gadoxetate; hepatocellular carcinoma; bolus tracking; Gd-DTPA

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IN MR EXAMINATION of patients with chronic liver disorders, dynamic MR imaging using conventional extracellular contrast agent, such as gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), is essential for detection and characterization of focal liver lesions because it enables hemodynamic analysis of the lesion (1–3). Enhanced MR imaging with tissue-specific contrast agent, such as superparamagnetic iron oxide, is also useful for detection and characterization of focal liver lesions because it provides functional evaluation of the reticuloendothelial system of the lesion (4,5). Gadoxetate (Gadolinium ethoxybenzyl diethylenetriamine; Gd-EOB-DTPA), which enables combined dynamic and hepatocyte-specific imaging in the one examination, has recently become clinically available (6,7). Gadoxetate behaves as an extracellular contrast agent in the early phase after intravenous injection and as a hepatocyte-specific agent in the hepatobiliary phase (8).

In detecting hypervascular hepatocellular carcinoma (HCC), the hepatic arterial phase (HAP) in dynamic imaging is important because moderately–poorly differentiated (and a part of well-differentiated) HCCs tend to occur as hypervascular nodules (9,10). Generally, liver MR imaging of HCC requires bolus injection and a bolus-tracking technique (11), although test injection technique and fixed arterial phase are also used. The total amount and concentration of Gadoxetate (0.025 mmol/kg) used clinically is smaller than that of Gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) (0.1 mmol/kg); however, to the best of our knowledge, optimal delay time of HAP for Gadoxetate has not been reported, and thus requires investigation.

The purpose of our study was to investigate the enhancement patterns in Gadoxetate-enhanced MR

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imaging in patients with hypervascular HCC and to determine the optimal scan delay time for HAP.

MATERIALS AND METHODS

Patient Population

This study followed the principles of the Declaration of Helsinki. Informed consent was obtained from all patients who underwent contrast-enhanced MR imaging with Gd-DTPA or Gadoxetate between September 2007 and October 2009 (September 2007 to January 2008; Gd-DTPA, February 2008 to October 2009; Gadoxetate). Institutional Review Board approval was obtained, although MR imaging in this study was acquired during routine clinical examination of liver tumors.

Definition of Hypervascular HCC

All examinations (both Gadoxetate and Gd-DTPA enhanced MR imaging) were reviewed by two radiologists with more than 10 years experience in liver MR imaging. Any interpretation discrepancies were resolved by consensus with the participation of a third radiologist. The reviewers were told that the patients had chronic hepatitis or cirrhosis, and treatment history was available. The 70-s, 5-min, and 20-min post-gadoxetate injections were performed, whereas, 70 s for the portal-venous phase and 5 min for the equilibrium phase were obtained in the patients with Gd-DTPA. Each arterial phase was evaluated for the presence of early enhancing hypervascular HCCs on dynamic MR imaging. Hypervascularity is defined as higher intense than surrounding liver parenchyma at the arterial phase. On Gadoxetate enhanced MR imaging, lower uptake of Gadoxetate than surrounding liver parenchyma at the hepatobiliary phase (20 min after injection) is regarded as HCC. Washout at the equilibrium phase (5 min after injection) is defined as HCC on Gd-DTPA enhanced MR imaging. On Gadoxetate enhanced MR imaging, less than 2-cm HCCs were homogeneously enhanced at arterial phases. Twenty-five of 85 HCCs showed more than 2 cm in size, 10 of 25 HCCs (>2 cm) had homogeneously arterial enhancement, whereas 15 of 25 HCCs had heterogeneous arterial enhancement. Thus, the 15 HCCs with heterogeneous arterial enhancement were measured at the intratumoral hyper-enhancing lesions. All of 16 HCCs were homogeneously enhanced at arterial phase in Gd-DTPA enhanced MR imaging.

Gadoxetate-Enhanced MRI

Of the 52 consecutive patients who underwent Gadoxetate-enhanced MR imaging for detection of liver tumor, 7 were excluded from the study because of marked artifacts on the arterial phase of dynamic MR imaging of the liver due to insufficient breath holding. A final total of 45 patients (33 men, 12 women; mean age, 68 years; age range, 45–82 years) with 85 untreated hypervascular HCCs in liver cirrhosis or chronic hepatitis were included in the study. Eighty-five HCCs were diagnosed using computed tomography (CT) and/or contrast-enhanced ultrasonography (US), based on the radiologic criteria of hypervascularity of HCC, contrast washout at the portal and/or equilibrium phase, response to transarterial chemoembolization (TACE), and interval progression in size. Follow-up CT or US imaging data were used to confirm the diagnosis of HCC if necessary. T2-weighted images were used to differentiate HCC from liver hemangioma. In six cases, a diagnosis of HCC was established histologically by percutaneous liver biopsy. Mean tumor size of hypervascular HCC was 17.2 ± 11.0 mm (range; 6–57 mm).

The treatment histories of our patients were as follows: TACE in 5 patients, radiofrequency ablation (RFA) in 4 patients, both TACE and RFA in 21 patients, hepatic intra-arterial chemotherapy in 1 patient, and hepatic resection in 3 patients; 11 patients had no treatment. These procedures were performed more than 2 months after treatment. As stated above, only untreated HCCs were evaluated (Table 1). TACE was performed super-selectively (and RFA performed focally), and these treatments were performed more than 2 months before the imaging obtained in the present study. The patient population consisted of Child-Pugh grade A in 32 patients and Child-Pugh grade B in 13 patients; no patients were Child-Pugh grade C. Causes of liver dysfunction were hepatitis B (n = 6), hepatitis C (n = 29), both hepatitis B and C (n = 2), neither hepatitis B nor C (n = 8). Mean patient body weight was 63 ± 10.4 kg (range; 44–86 kg).

Gd-DTPA Enhanced MRI

Of the 23 consecutive patients who underwent Gd-DTPA enhanced MR imaging for detection of liver tumor, 4 were excluded from the study because of marked artifacts on the arterial phase of dynamic liver MR imaging due to insufficient breath holding. We studied 19 patients (13 men, 6 women; mean age, 70 years; age range, 58–76 years) with suspected liver tumor in liver cirrhosis or chronic hepatitis who underwent dynamic MR imaging with Gd-DTPA. Nine of 19 patients had 16 hypervascular HCCs. Mean tumor size was 17.3 ± 5.5 mm (12 ± 32 mm).

Patient population, who underwent Gd-DTPA enhanced MRI, consisted of Child-Pugh grade A in 13 patients, Child-Pugh grade B in 4 patients, and 2 patients were Child-Pugh grade C. Causes of liver dysfunction were hepatitis B (n = 4), hepatitis C (n = 13), neither hepatitis B nor C (n = 2). Mean patient body weight was 56 ± 12.5 kg (range; 35–80 kg). Nine of 19 patients had hypervascular HCC. The treatment histories of nine patients with HCC were as follows: TACE in 1 patient, RFA in 1 patient, both TACE and RFA in 4 patients, and hepatic resection in 2 patients; 1 patient had no treatment. These procedures were performed more than 3 months after treatment. As stated above, only untreated HCCs were evaluated in our study (Table 1). TACE was performed super-selectively (and RFA performed focally), and these

Table 1
Data of Patient Population in Our Study

Parameters	Gadoxetate-enhanced MRI	Gd-DTPA-enhanced MRI
No. of patients	45	19
Sex	Male (33 pts) female (12 pts)	Male (13 pts) female (6 pts)
Age	Mean 68 (range 45 - 82) years	Mean 70 (range 58 - 76) years
Weight	63 ± 10.4 (range 44 - 86) kg	56 ± 12.5 (range 35 - 80) kg
Treatment history	TACE (5 pts), RFA (4 pts), TACE and RFA (21 pts) Hepatic intra-arterial chemotherapy (1 pt), Hepatic resection (3 pts), no treatment history (11 pts)	TACE (1 pt), RFA (1 pt), TACE and RFA (4 pts) Hepatic resection (2 pts), no treatment history (1 pt)
Hepatitis	B (6 pts), C (29 pts), B and C (2 pts), neither B nor C (8 pts)	B (4 pts), C (13 pts), neither B nor C (2 pts)
Elevation of tumor maker	PIVKA-II (27 pts), AFP (27 pts) PIVKA-II or AFP (38 pts)	PIVKA-II (6 pts), AFP (7 pts) PIVKA-II or AFP (8 pts)
Child–Pugh grade	A (32 pts), B (13 pts), C (none)	A (13 pts), B (4 pts), C (2 pts)
Differentiation (number)	Poor (1), moderately (5)	Poor (1), moderately (3)
HCC size	Mean 17.2 ± 11.0 (range 6 - 57) mm	Mean 17.3 ± 5.5 (range 12 - 32) mm
HCC location (number)	S1 (1), S2 (12), S3 (5), S4 (15), S5 (12), S6 (14), S7 (8), S8 (18)	S1 (1), S2 (1), S3 (1), S4 (1), S5 (1), S6 (4), S7 (2), S8 (5)

*TACE = transarterial chemo-embolization; RFA = radiofrequency ablation; PIVKA-II = protein induced by Vitamin K absence or antagonists-II; AFP = alpha-fetoprotein; HCC = hepatocellular carcinoma; pts = patients.

treatments were performed more than 3 months before the imaging obtained in the present study.

MR Imaging Technique

All patients underwent time-resolved dynamic MR imaging (both Gadoxetate and Gd-DTPA) of the entire liver using a 1.5 Tesla (T) clinical MR scanner (Gyroscan Intera Nova; Philips Medical Systems, Best, the Netherlands) equipped with a high-performance gradient system; a commercially available four-element phased-array body coil with improved signal detection was used for parallel imaging. For dynamic MR imaging of HAP, we used a 3-dimensional Fourier transformation (3DFT) gradient echo sequence with keyhole data sampling (four-dimensional high-resolution isotropic volume examination [4D THRIVE] sequence with fat saturation) (12,13); we obtained parallel imaging with Sensitivity Encoding (SENSE) and an MR scanner equipped with a gradient system (maximal gradient amplitude = 30 mT, slew rate = 150 T/m/s).

A full reference 3D data set without contrast was obtained initially, during a single breath hold. The patients were then administered intravenous injection of 0.025 mmol/mL/kg of Gadoxetate by power injector via the antecubital vein at a rate of 2 mL/s, followed by normal saline at a rate of 2 mL/s (14). Dynamic MR imaging with 0.1 mmol/mL/kg of Gd-DTPA was performed using the same sequence, parameters. Total injected volume (Gadoxetate + normal saline or Gd-DTPA + normal saline) was 40 mL in our protocol.

Imaging protocol of our study was as follows: Dynamic MR imaging was then performed at seven sequential phases (4 s/phase) from 10 to 38 s after initiation of the injection of Gadoxetate and Gd-DTPA, during a single breathhold. The center time of each imaging phase was used for numerical analysis. Therefore, the first, second, third, fourth, fifth, sixth, and seventh phases were obtained at 12, 16, 20, 24, 28, 32, and 36 s, respectively, after initiation of the injection of Gadoxetate and Gd-DTPA. In addition, for the diagnosis of HCC, portal venous phase and hepatobiliary phase imaging were obtained at approximately 70 s and 20 min, respectively, after initiation of the injection of Gadoxetate. For Gd-DTPA enhanced MRI, portal venous phase and equilibrium phase were used at approximately 70 s and 180 s. The acquisition parameters of 4D THRIVE were as follows: TR = 3.9 ms, TE = 1.9 ms, flip angle = 15°, matrix size = 256 × 256, slice thickness = 2.5 mm, field of view = 340 mm, SENSE factor = 2. Eighty slices were obtained at each arterial phase.

In the keyhole technique, a single acquisition of peripheral *k*-space corresponding to the reference scan is acquired, after which a predefined portion of the central region of ky–kz space is repeatedly acquired. In reconstruction, the central, dynamic part of *k*-space is combined with the static periphery of *k*-space that was acquired during the reference scan. The size of the central region can be configured: the smaller the size, the higher the temporal resolution. By adopting a 40% keyhole rate, dynamic MR images were acquired every 4 s.

Image Interpretation

Analysis by Time–Intensity Curve

The arterial enhancement patterns of HCC were evaluated using time–intensity curve (TIC) analysis. Dynamic MR imaging (both Gadoxetate and Gd-DTPA) sets were sent to a clinical workstation (ViewForum; Philips Medical Systems, Tokyo, Japan). Using a multi-window frame setting on this system, a region of interest (ROI) was placed on the abdominal aorta at the level of celiac artery. After HCC and liver parenchymal signal were measured at the same slice, the

Table 2
Comparison Between Gadoxetate and Gd-DTPA-Enhanced MR for Aortic and HCC Enhancement Analyzed by Time-Intensity Curves

	Gadoxetate	Gd-DTPA	*P* value
1) Aortic contrast arrival time	15.1 ± 2.9 s	17.6 ± 3.0 s	<0.00013
2) Time of peak aortic enhancement	21.0 ± 4.9 s	22.4 ± 5.6 s	NS
3) Duration time of aortic enhancement	23.6 ± 3.5 s	26.3 ± 2.8 s	<0.0059
4) Aortic enhancement (Aorta / muscle ratio)	5.6 ± 1.7	8.1 ± 1.7	<0.000013
5) Beginning time of HCC enhancement	20.8 ± 6.1 s	22.5 ± 4.1 s	NS
6) Duration time of HCC enhancement	17.4 ± 6.4 s	17.5 ± 4.1 s	NS
7) Time of peak HCC enhancement	29.9 ± 4.6 s	31.8 ± 3.7 s	NS
8) Time delay from aortic contrast arrival to peak enhancement of HCC	14.6 ± 4.6 s	14.5 ± 3.8 s	NS

*HCC = hepatocellular carcinoma; NS = not significant; Gadoxetate = Gadolinium ethoxybenzyl diethylenetriamine (Gd-EOB-DTPA); Gd-DTPA = Gadolinium-diethylenetriamine pentaacetic acid; aorta/muscle ratio; signal of aorta/signal of latissimus dorsi muscle ratio.

enhancement values were calculated using the ratio of the signal of HCC and liver parenchyma to the signal of latissimus dorsi muscle. TIC was created using commercially available Excel software (Microsoft Excel 2007). Thus, hemodynamic change of HCC through seven arterial phases was quantitatively analyzed. We calculated the following parameters by evaluating all phases of the dynamic series: (i) Arrival time of contrast agent at the abdominal aorta (aortic contrast arrival time), (ii) Time of peak aortic enhancement, (iii) Duration time of aortic enhancement (Aorta/muscle ratio 1.5), (iv) Aortic enhancement (Aorta/muscle ratio), (v) Beginning time of HCC enhancement (up 10% of HCC enhancement, in comparison to surrounding liver parenchyma), (vi) Duration time of HCC enhancement, (vii) Time of peak HCC enhancement, and (viii) Time delay from aortic contrast arrival to peak enhancement of HCC. The center time of each imaging phase was used for numerical analysis. Aortic enhancement was defined as aorta/muscle ratio. Duration of aortic enhancement was defined as the time with aorta/muscle ratio greater than 1.5 for both contrast agents.

Statistical Analysis

Comparison between Gadoxetate and Gd-DTPA enhanced MRI was made in all parameters analyzed by TIC curve. Mann-Whitney's U test was used for statistical analysis; A *P* value <0.05 was considered significant.

RESULTS

Analysis by Time–Intensity Curve

Aortic Enhancement

The eight analyzed parameters are listed in Table 2. Aortic contrast arrival time and duration time of aortic enhancement of Gadoxetate were significantly shorter than those of Gd-DTPA. Aortic enhancement of Gadoxetate was lower than that of Gd-DTPA.

Aortic contrast arrival time was variable. Eighteen of 45 patients with Gadoxetate-enhanced MR and 3 of 9 patients with Gd-DTPA had already contrast arrival to the aorta at first phase. Others (27 of 45 patients with Gadoxetate-enhanced MR and 6 of 9 patients with Gd-DTPA) showed dynamic inflow of the aorta since second phase.

HCC Enhancement

One case of typical hypervascular HCC is shown in Figures 1 and 2. Time of peak HCC enhancement of Gadoxetate showed a relatively wide range, from the third to seventh phases (29.9 ± 4.6 s; Table 2). 33 of 85 HCCs showed at 24 s (4 of 7 phases) in beginning time of HCC (Fig. 3), and 14, 29, 35, and 7 of 85 HCCs showed enhancement duration of 8, 12, 16, and 20 s in duration time of HCC, respectively. Twenty-nine of 85 HCCs showed peak enhancement of Gadoxetate at the sixth phase (32 s postinjection; Fig. 4).

In 18 HCCs, the enhancement continued to increase at the seventh phase (36 s) and peak HCC enhancement was expected to occur after the seventh phase in Gadoxetate enhanced MRI. Furthermore, in 41 HCCs, persistent enhancement remained evident at the seventh phase (Fig. 5).

In 8 of 16 HCCs in Gd-DTPA enhanced MRI, the HCC enhancement continued to increase at the seventh phase. And peak HCC enhancement was seen at forth phase in Gd-DTPA enhanced MRI.

Of interest, HCC enhancement was not affected according to different volume of contrast agent (Gadoxetate is half the volume of Gd-DTPA).

The time delay from aortic contrast arrival to peak enhancement of HCC was 14.6 ± 4.6 s (Table 2), which enables optimal HCC enhancement to be obtained when using bolus tracking in Gadoxetate-enhanced MRI.

Other parameters, such as aortic contrast arrival time, time of peak aortic enhancement, beginning time of HCC enhancement, HCC enhancement in the arterial phase, time of peak HCC enhancement, were shown as no significant difference between Gadoxetate and Gd-DTPA in Table 2.

Time intensity curve of HCC/muscle ratio was obtained (Fig. 6), and 12 to 24 s from aortic contrast arrival showed higher enhancement of HCC in Gadoxetate-enhanced MRI.

Twenty-six of 45 patients (53 of 85 HCCs) had TACE, and 19 of 45 patients (32 of 85 HCCs) did not have TACE. Time delay from aortic contrast arrival to peak enhancement of HCC with TACE (14.3 ± 4.9 s) is as same as that of HCC without TACE (15.0 ± 3.9 s) (Table 3). Other 5 parameters, such as aortic contrast arrival time, time of peak aortic enhancement, beginning time of HCC enhancement, HCC enhancement in

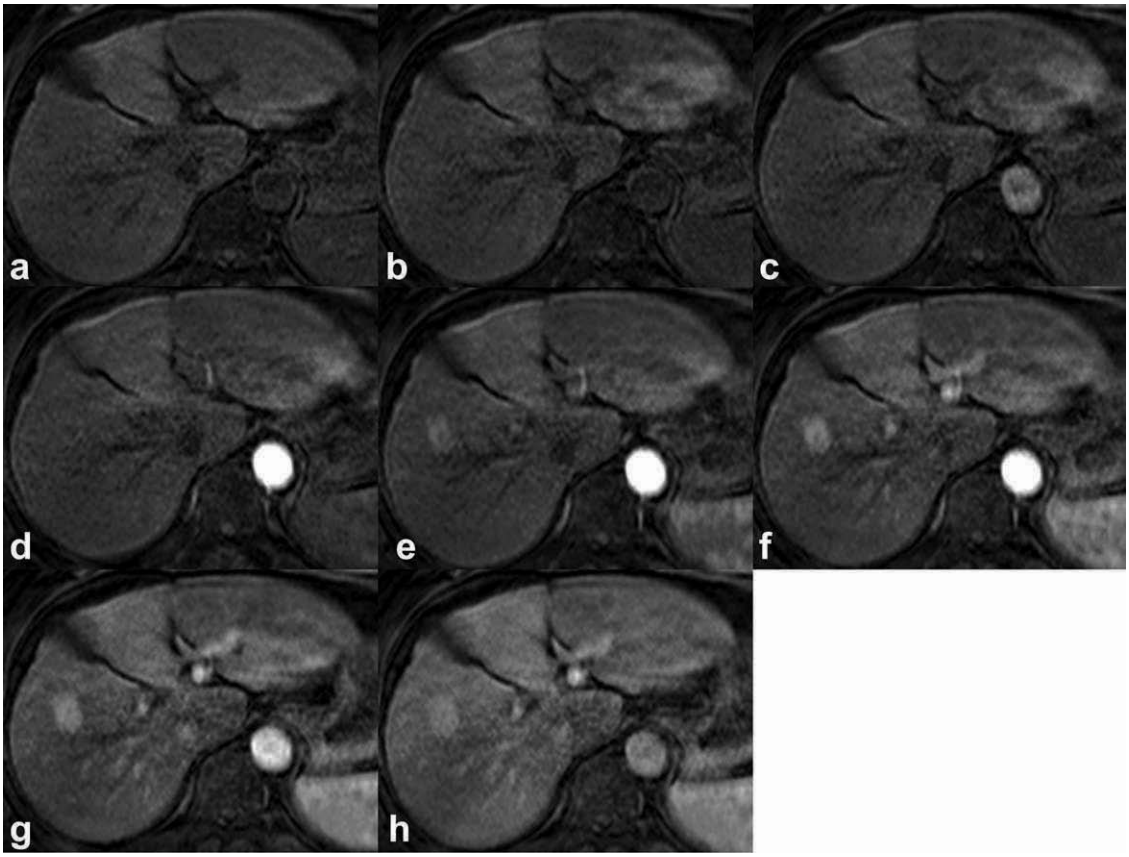

Figure 1. A 68-year-old man with a hypervascular HCC. First- to seventh-phase images obtained during dynamic MR imaging with Gadoxetate (gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid; Gd-EOB-DTPA). Aortic enhancement is not seen in the first (**a**) or second (**b**) phases. Aortic enhancement is seen in the third phase (**c**). Aortic peak enhancement is seen in the fourth phase (**d**). HCC enhancement occurs in the fifth phase (**e**). Peak HCC enhancement appears in the sixth phase (**f**). HCC enhancement continues in the seventh phase (**g**), and liver parenchyma shows a gradual increase in enhancement; thus, this phase shows decreased liver-to-HCC contrast (**h**). In this patient, the duration of HCC enhancement is from the fifth to seventh phases. Aortic enhancement shows a rapid decrease, because of the small amount of contrast agent. Artifact caused by stomach motion is apparent in the parenchyma of the hepatic left lobe as a high-intensity area in the second (b) and third phases (c).

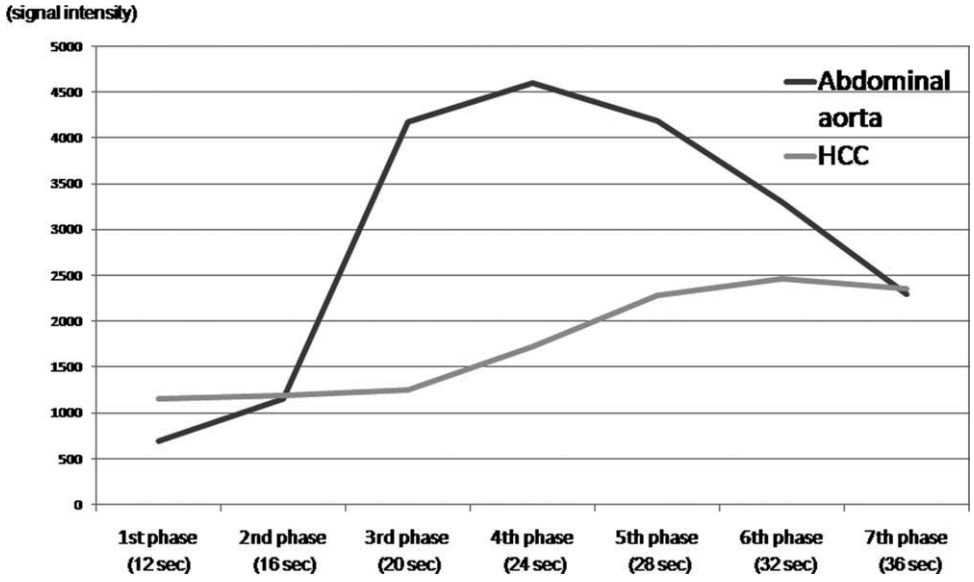

Figure 2. Time–intensity curve in the same patient as that shown in Figure 1. Aortic contrast arrival occurs at 16 s (second phase), while aortic peak enhancement occurs at 24 s (fourth phase). HCC enhancement occurs at 24 s, and peak HCC enhancement time is 32 s.

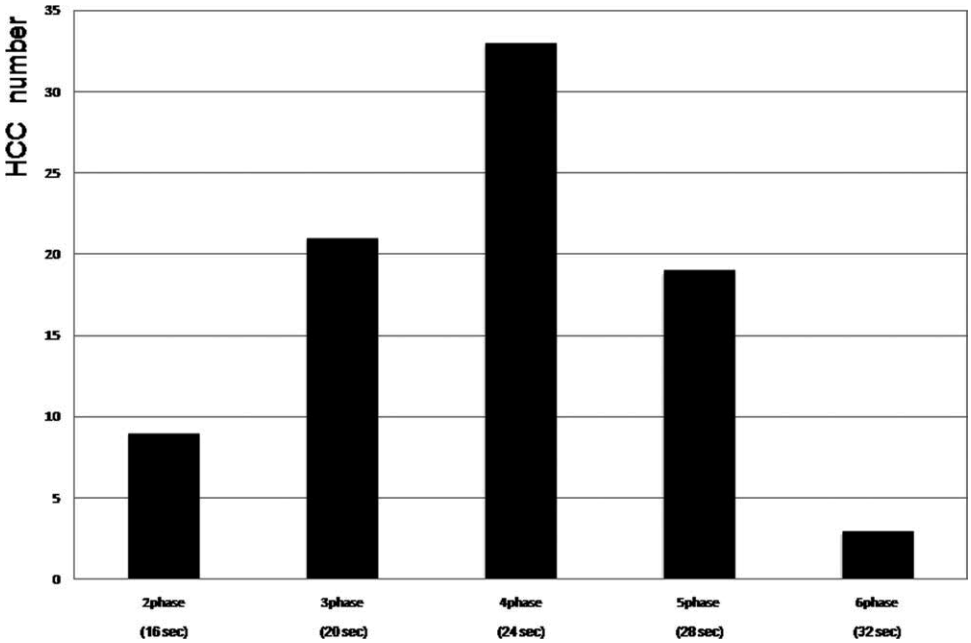

Figure 3. Beginning time of HCC enhancement in Gadoxetate-enhanced MRI. HCC enhancement began in the second phase in 9 nodules, third phase in 21 nodules, fourth phase in 33 nodules, fifth phase in 19 nodules, and sixth phase in 3 nodules.

the arterial phase, and time of peak HCC enhancement, were shown in Table 3. Only beginning time of HCC enhancement of patient with TACE (19.3 ± 5.4) was significantly earlier than that of patient without TACE (23.1 ± 6.5) ($P < 0.0071$).

DISCUSSION

Dynamic imaging such as CEUS, dynamic CT and MR imaging, are known as important modalities in the Association for the Study of Liver Diseases (AASLD) guideline (15). It is essential to detect hypervascularity on several imagings (tumor size of 1–2 cm; required 2 dynamic imaging modalities, whereas, tumor size greater than 2cm; required 1 dynamic imaging modality). In the present study, we investigated the optimal time delay after aortic contrast arrival for beginning HAP imaging, for the detection of HCC. Our most important finding was that the time delay from aortic contrast arrival of Gadoxetate to peak enhancement of HCC was 14.6 ± 4.6 s, as shown in Table 2. Therefore, to obtain optimal contrast between HCC and liver, the *k*-space center data sampling of MR imaging should be performed at approximately 14–15 s after aortic contrast arrival. Duration of HCC enhancement with Gadoxetate in the arterial phase was 17.4 ± 6.4 s. There was maximal variability of approximately 12–13 s (standard deviation × 2) in our patients. The mean duration (17.4 s) of HCC enhancement after Gadoxetate injection by visual analysis exceeded the standard deviation × 2. Thus, scanning time during the possible breathholding time should be optimized for the detection of arterial enhancement of HCCs.

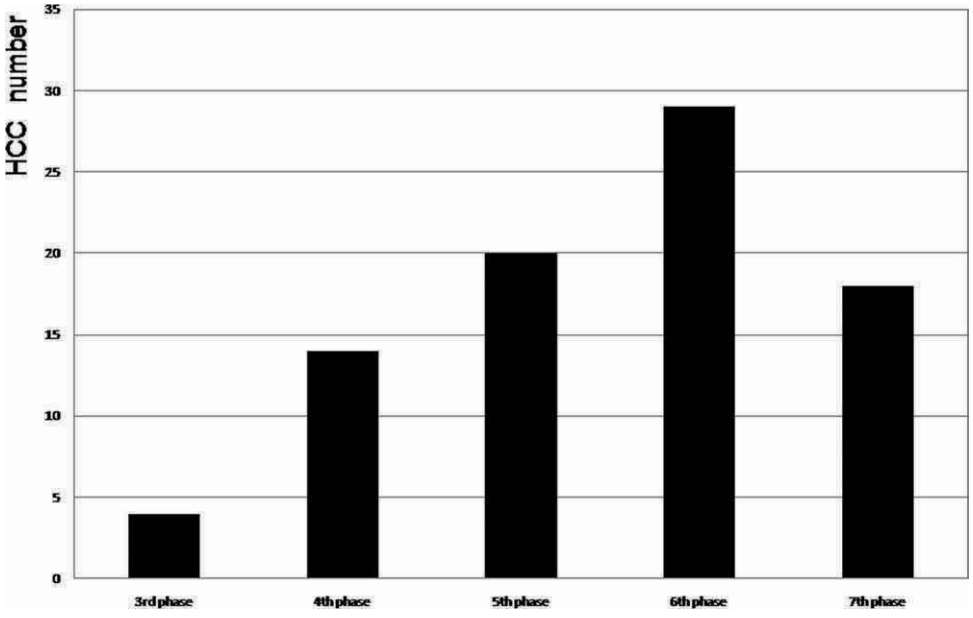

Figure 4. Time of peak HCC enhancement in Gadoxetate-enhanced MRI. Peak HCC enhancement is obtained between 20 and 36 s. Peak enhancement of 29 HCCs is obtained at 32 s.

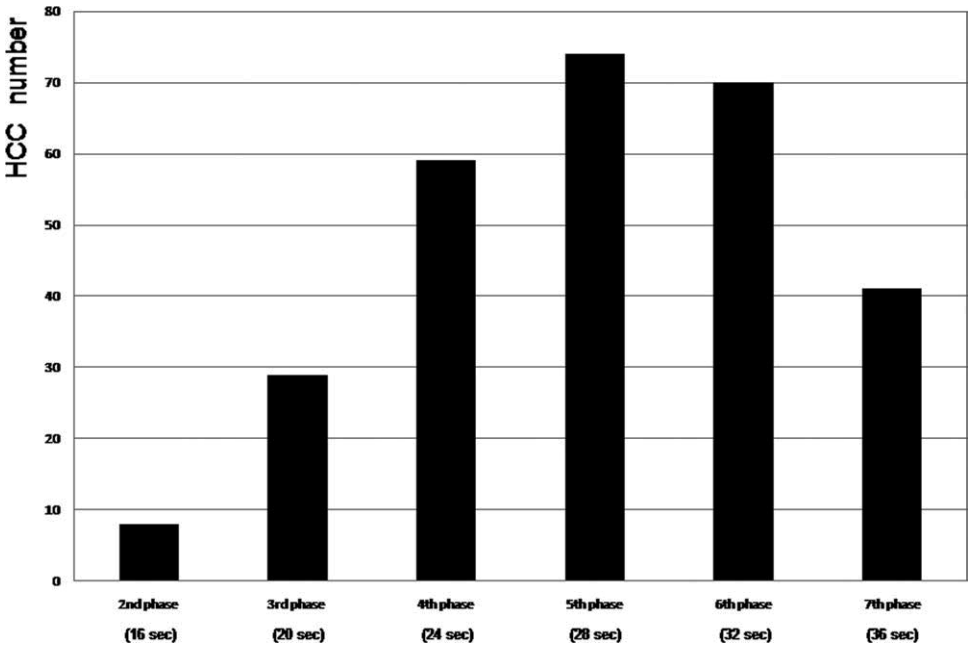

Figure 5. Number of enhancing HCCs in Gadoxetate-enhanced MRI. Enhancement is seen in most HCCs at 28 and 32 s. At 38 s (at the seventh phase), persistent enhancement remains in 41 HCCs.

We compared Gadoxetate-enhanced MR imaging with Gd-DTPA enhanced MR imaging with regard to the duration and peak intensity of aortic enhancement. The results suggest that aortic enhancement of Gd-DTPA is longer-lasting than that of Gadoxetate, and peak aortic enhancement of Gadoxetate was significantly lower than that of Gd-DTPA ($P < 0.000013$); this occurred because different injection volumes of contrast agent were used (0.2 mL/kg for Gd-DTPA versus 0.1 mL/kg for Gadoxetate), and the concentration of clinically used Gd in Gadoxetate (0.025 mmol/kg) is smaller than that in Gd-DTPA (0.1 mmol/kg). Therefore, optimal imaging timing of HAP is crucial. But, duration time of HCC enhancement of Gadoxetate was similar to that of Gd-DTPA.

Aortic contrast arrival time of Gadoxetate showed a wide range (15.1 ± 2.9 s). If the bolus tracking technique is used to indicate the starting point for HAP on dynamic MR imaging, it may be possible to overcome the problem of the varying time delays in aortic arrival, and thus improve the detection of hypervascular HCC. Aortic contrast arrival time was significantly different between Gadoxetate and Gd-DTPA ($P < 0.00013$). It is speculated that individual difference, such as cardiac function, may cause this difference.

Gadoxetate for liver MR imaging is provided commercially in 5- or 10-mL syringes. For example, a patient with body weight of 90 kg needs 9 mL of Gadoxetate, which leaves only 1 mL of contrast agent for the test bolus injection. In addition, contrast agent from the test bolus may affect liver intensity at image acquisition. Accordingly, a test bolus may be inappropriate for the detection of hypervascular HCC. Our study found that the time delay from aortic contrast

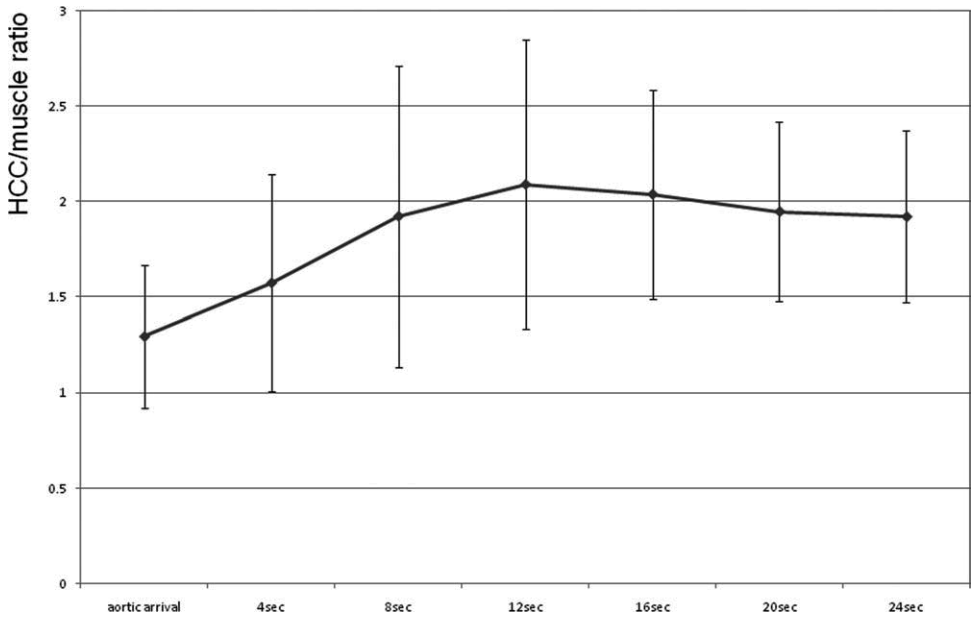

Figure 6. Time–intensity curve of HCC/muscle ratio from aortic contrast arrival in Gadoxetate-enhanced MRI. HCC shows higher and plateau enhancement at 12–28 s after aortic contrast arrival.

Table 3
Patients With or Without TACE Analyzed by Time–Intensity Curves After Gadoxetate Injection

Parameters	Patient with TACE	Patient without TACE	*P* value
Aortic contrast arrival time	14.8 ± 2.7 s	15.6 ± 3.2 s	NS
Time of peak aortic enhancement	20.5 ± 5.1 s	21.7 ± 4.7 s	NS
Beginning time of HCC enhancement	19.3 ± 5.4 s	23.1 ± 6.5 s	<0.0071
Duration time of HCC enhancement	18.4 ± 5.8 s	15.8 ± 7.2 s	NS
Time of peak HCC enhancement	29.1 ± 5.2 s	31.3 ± 3.1 s	NS
Time delay from aortic contrast arrival to peak enhancement of HCC	14.3 ± 4.9 s	15.0 ± 3.9 s	NS

*NS = not significant; TACE = transarterial chemo-embolization; HCC = hepatocellular carcinoma.

arrival to peak enhancement of HCC was 14.6 ± 4.6 s. Therefore, HCC can be imaged at peak enhancement by determining the aortic contrast arrival time using bolus tracking, confirming that the *k*-space center data sampling of MR imaging should be performed at peak enhancement time of HCC.

Sultana et al (16) stated that peak tumor–liver contrast during HAP occurred at 18 s after triggering (>100 HU), using 40 detector-row CT; this is longer than the time delay from aortic arrival of Gadoxetate found in the present study (14.6 ± 4.6 s). This discrepancy arose because a greater volume of contrast agent is used in CT than in MRI.

In CT scanning, the iodine dose is adjusted according to patient weight (17), and the patient is exposed to radiation. In contrast, MR imaging delivers no radiation exposure, and the image contrast is higher than that of CT; optimal imaging can be obtained when the time of *k*-space center filling corresponds to the time of peak tumor enhancement.

There are some limitations of the present study. First, although we evaluated only untreated HCCs, 30 of the 45 patients had a previous history of TACE, RFA, or TACE + RFA on Gadoxetate enhanced MRI. Untreated HCCs may have been affected by the previous embolization of the hepatic artery. From our result, the comparison between patients with TACE and those without TACE showed only beginning time of HCC enhancement significant difference ($P < 0.0071$), as shown in Table 3. But, both duration time of HCC enhancement and time of peak HCC enhancement between patients with TACE and without TACE did not show significant difference.

Second, the 4D THRIVE sequence with ultrafast acquisition ability has lower spatial resolution than conventional 3DFT-T1 weighted imaging such as THRIVE; however, the 4D image quality of THRIVE was sufficient to evaluate regional enhancement patterns.

Third, the scan time of each phase was 4 s; therefore, evaluation of HCC hemodynamics was not obtained in real time, as in angiography. In the present study, 4D THRIVE was used for both time–intensity analysis and actual diagnosis of the dynamic scans; therefore, we needed a balanced approach between time resolution and spatial resolution. Moreover, we think this sequence enables evaluation of regional enhancement patterns.

Fourth, in the seventh (last) phase at 38 s, TIC analysis revealed that persistent enhancement remained in 41 HCCs of Gadoxetate enhanced MRI. Thus, the scanning period for HAP used in the present study (seven phases) may be a little insufficient to obtain the endpoint of enhancement of HCCs, because of a limitation of breathhold keeping. In addition, initial aortic arrival may be insufficient at the first phase, because there were patients, who had very fast aortic contrast arrival. However, we could evaluate the usual arterial phase period by our protocol and believed to see the tendency of enhancement pattern.

In summary, bolus tracking system can help to obtain the peak enhancement of HCC on dynamic MR imaging with Gadoxetate. For optimal MR imaging of HAP, the *k*-space center data sampling should be performed at 14.6 ± 4.6 s after aortic contrast arrival.

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Review Article

Management of hepatitis B: Consensus of the Japan Society of Hepatology 2009

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Recently, much progress has been made in the field of hepatitis B, such as natural history of the disease in relation to the amount of hepatitis B virus (HBV) DNA, genotypes of HBV influencing the natural course and treatment effects, mutations of HBV influencing the severity of the disease and development of hepatocellular carcinoma, and antiviral treatment such as nucleos(t)ide analogues and pegylated interferon. To make the consensus for the diagnosis, management and treatment of hepatitis B, a meeting was held during 45th annual meeting of Japan Society of Hepatology (JSH) in June 2009. In the meeting, recommendations and informative statements were discussed on the following subjects: (i) natural history of HBV infection; (ii) clinical implication of HBV genotypes; (iii) HBV mutations and their potential impact on pathogenesis of HBV infection; (iv) indications for antiviral treatment of chronic hepatitis B; (v) nucleos(t)ide analogues for chronic hepatitis B; and (vi) interferon therapy for chronic hepatitis B. The presenters reviewed the data on these subjects and proposed the consensus statements and recommendations. These statements were discussed among the organizers and presenters, and were approved by the participants of the meeting. In the current report, the relevant data were reviewed and the 12 consensus statements and nine recommendations on chronic hepatitis B were described.

Key words: genotype, hepatitis B virus, interferon, mutation, natural history, nucleotide analogue

Hepatitis B virus (HBV) is one of the most distributed viruses which infect humankind. More than 3 billion people, one half of the world's population, have been exposed to HBV during their life.[1] Acute infection in adults is self-limited in general whereas infection during early childhood will develop into persistent chronic infection in most individuals.[2] More than 400 million people worldwide are chronically infected with HBV and are at risk of developing life-threatening complications including liver cirrhosis and hepatocellular carcinoma (HCC).[1] HBV is a major public health problem worldwide especially in East Asia and Africa. In Japan, approximately 1.5 million people are infected with HBV and it is one of the major causes of HCC and chronic hepatic failure. Other complications of HBV infection include fulminant hepatitis and acute liver failure.

The consensus meeting for diagnosis, management and treatment for hepatitis B was held during the 45th annual meeting of the Japan Society of Hepatology (JSH) in June 2009 (Congress President: M Kudo), where the recommendations and informative statements were discussed. Although the JSH consensus meeting of hepatitis B had been held four times so far, recommendations were hitherto published only in Japanese and this is the first report in English. Established

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information for pathogenesis and contributing factors for disease progression which was agreed by the organizers and presenters are shown as "consensus statements", and clinically useful consensus are shown as "recommendations". The quality of recommendations or informative statements are required to show a "level" (assessing strength or certainty) of evidence and "grading" of recommendations or assessment according to a standard reporting system of clinical guidelines.[3]

NATURAL HISTORY OF HBV INFECTION

AN EVALUATION OF studies on the natural history of HBV infection was done using the scoring system proposed by MacMahon *et al.*[4] in the present analysis because scoring systems for treatment studies cannot always be applied directly to those using natural history. The proposed scoring system consists of levels 1 (1a, 1b), 2 (2a, 2b, 2c), and 3. Level 1a is defined as a population-based longitudinal cohort study with a hepatitis B surface antigen (HBsAg) negative comparison group. Level 1b is identical to level 1a, but with no comparison group. Level 2a is defined as a clinic-based longitudinal cohort study, level 2b is a population-based or clinic-based cohort nested case–control study, and level 2c is a cross-sectional clinic-based study. Level 3 is defined as an observation study case series.

The natural history of chronic HBV infection can be classified into several phases based on levels of alanine aminotransferase (ALT), hepatitis B e-antigen (HBeAg) status, amounts of HBV DNA, and estimated immunological states.[4–9] A representative classification of these phases is shown in Table 1. In the immune tolerance phase, HBeAg is positive, serum levels of ALT are normal, histological activities of hepatitis are absent or minimal, and levels of HBV DNA are elevated. The immune tolerance phase is thought to occur most frequently in individuals who are infected through perinatal transmission, and this phase usually lasts until adolescence or young adulthood.[10–12]

The chronic hepatitis B phase is characterized by elevated ALT and HBV DNA levels. In this phase, the host's immune system recognizes HBV as being foreign and initiates an immune response that results in hepatitis. In cases who are HBeAg positive, active hepatitis can be prolonged and may result in cirrhosis. However, chronic hepatitis B eventually transitions into an inactive phase with a loss of HBeAg positivity in the majority of patients. Seroconversion to anti-HBe and the fall of serum HBV DNA to low levels result in the disappearance of disease activity, despite persisting HBsAg and low levels of HBV DNA.[13–16] Seroconversion rates range 7–16% per year according to reports with higher evidence levels (levels 1b, 2a).[16–19] Factors associated with seroconversion are age (level 1b),[20] ALT levels (level 1b), occurrence of acute exacerbation of hepatitis (level 1b),[19,21] and genotype (level 2c).[22,23]

The seroconversion of HBeAg results in the transition from hepatitis phase to inactive carrier phase, which is generally thought to be a benign course for HBV carrier, but sometimes hepatitis can be reactivated spontaneously.[24] Patients experiencing reactivation undergo another transition, with increases in HBV DNA and ALT levels and disease activity without reappearance of HBeAg.[24] This phase is referred to as HBeAg negative chronic hepatitis B. Occasional severe hepatitis B flare-ups with middle range HBV DNA levels (3–8 log copies/mL) occur in this phase.[8,25] HBeAg negative chronic hepatitis B is caused by mutant strains of HBV unable to produce HBeAg,[25,26] and tends to develop into cirrhosis and complicate HCC more than HBeAg positive chronic hepatitis B.[27–30]

Table 1 Phases in the natural history of HBV carriers (modified from [4])

Phase	Hepatitis	Blood			Liver
		DNA	HBeAg	HBsAg	cccDNA
Immune tolerance	–	8–11	+	+	+
HBeAg positive Chronic hepatitis	Usually Persistent	6–10	+	+	+
HBeAg negative Chronic hepatitis	Often Fluctuating	3–8	–	+	+
Inactive carrier	–	<4	–	+	+
Recovery	–	–	–	–	+

HBV DNA: log copies/mL. cccDNA, covalently close circular DNA; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

Many factors that are associated with the development of HCC have been reported so far. Higher age (level 1a), male sex (level 1a), presence of cirrhosis (level 2a) and familial cluster of carriers (level 2c) are reported as host factors.[31,32] Viral factors include high viral load (level 1b),[33–36] existence of pre-core and core promoter mutations (level 2a), genotype C and high ALT levels (level 1b). High viral load should be considered as a factor in patients over 35–40 years of age. Co-infection with hepatitis C virus, hepatitis D virus or HIV (level 2a), drinking habit (level 2c) and exposure to aflatoxin (level 2c) are reported as social and environmental factors.[37–39] Other lifestyle-related factors, such as smoking habit, obesity and complications from diabetes mellitus, have been documented as well.

Consensus 1
In patients with chronic hepatitis B, seroconversion of HBeAg usually results in the transition from hepatitis phase to inactive carrier phase, which generally has low HBV replication and normal ALT levels. However, reactivation of chronic hepatitis can spontaneously occur without the reappearance of HBeAg. At this point, active hepatitis continues and the risk of complicating cirrhosis and HCC is high in patients with HBeAg negative chronic hepatitis B. (Level 1b.)

In the inactive carrier phase, HBV replication is continuously suppressed as a result of predominantly host immunological pressure against HBV. Patients in the inactive carrier phase generally have a benign course because active hepatitis subsides and the risk of HCC decreases.[19,20,24,40] However, regular follow up is required because reactivation of HBV sometimes occurs spontaneously or as a result of immunosuppressive therapy.[19,24]

Hepatitis B surface antigen is known to fall to undetectable levels in some inactive carriers. This HBsAg negative phase, referred to as the recovery phase, has no hepatitis and a low risk of HCC. Still, caregivers must be aware that patients who are old or cirrhotic have a relatively higher risk of HCC.[41,42] Disappearance of HBsAg in the recovery phase does not indicate complete eradication of HBV because the HBV genome remains as covalently close circular DNA (cccDNA) in the nucleus of hepatocytes.

Consensus 2
2-1 HBV can not be completely eradicated using any currently existing treatment measures. (Level 2a.)
2-2 Patients in the inactive carrier or recovery phase have a benign clinical course. However, regular follow up of such patients is required because reactivation of hepatitis B and ensuing HCC can occur. (Level 1b, 2a.)

Clinicians have to consider two types of hepatitis B reactivation: one during the inactive carrier phase and the other in the recovery phase.[4] Both types of reactivation have been attributed with increasing incidence to strong immunosuppressive therapies. De novo hepatitis B, a reactivation of hepatitis B in the recovery phase, tends to develop into fulminant hepatitis, which has a very high mortality rate.[43–46] Thus, establishment of effective measures to prevent reactivation of hepatitis B is necessary.

Consensus 3
3-1 Reactivation of hepatitis B can occur during the inactive carrier or recovery phases and stems mainly from strong immunosuppressive treatment courses. (Level 2a.)
3-2 Recent advances in medical care have increased the use of immunosuppressive agents and thus the incidence of hepatitis B reactivation. (Level 2a.)
3-3 Reactivation of hepatitis B tends to develop into fulminant hepatitis. (Level 2a.)

Recommendation 1
In addition to the loss or seroconversion of HBeAg, a substantial decrease in HBV viral load and subsequent disappearance of hepatitis are the primary targets in the treatment of patients with chronic hepatitis B. (Level 1b.)

Recommendation 2
The main goals of HBV carrier treatment are patients in the inactive carrier and recovery phases. However, caregivers should be aware that reactivation of hepatitis B and complication of HCC can occur even in these benign phases. (Level 1b.)

Recommendation 3
Reactivation of hepatitis B due to immunosuppressive therapy tends to develop into severe hepatitis, thus requiring the establishment of effective preventative measures. (Level 2a.)

CLINICAL IMPLICATION OF HBV GENOTYPES

DISTINCT CLINICAL AND/OR virological characteristics of the HBV infection have been reported in different geographical parts of the world and are increasingly associated with host factors, environmental factors and the genetic diversity of the infecting virus.[47] HBV is classified into at least eight genotypes (A–H) based on an intergroup divergence of 8% or more in the complete nucleotide sequence and a number of subgenotypes (Aa/A1, Ae/A2, Bj/B1, Ba/B2, Cs/C1, Ce/C2, D1, D2, and so forth) that are currently known to have distinctive association with ethnic and/or geographical distribution.[48]

Association between HBV genotype and clinical manifestation

Acute hepatitis

The universal vaccination program against HBV has significantly reduced the number of new infection cases in most countries with levels of endemicity estimated from intermediate to high.[49] However, efficiency of universal vaccination in countries with a low level of endemicity still remains controversial. Japan is one of the countries with a low level of endemicity and mainly vertical (mother to baby) transmission route.[50] In Japan, HBV vaccination in combination with HBV immunoglobulin treatment is the only recommended measure for infants born to HBsAg positive mothers. Studies in Japan indicated genotype C (subgenotype Ce/C2) to be the major type in the country and genotype B (subgenotype Bj/B1) is the second distributed. Surveillance studies have shown a recent trend toward increase in number of acute hepatitis B infection among young adults mainly through sexual contacts.[51,52] Although most cases are associated with genotype C infection, there is a continuous trend toward increase in prevalence of genotype A among acute hepatitis cases.[51,53–56] Patients infected with genotype C have been known to be rarely associated with development of chronic persistence after acute infection in immune competent adults in Japan (1%) in contrast to the higher rates of those infected with genotype A (6–23%).[53,54] A recent multicenter study in Japan indicated a trend among chronic hepatitis B patients toward increase in prevalence of genotype A (from 1.7% in 2002 to 3.5% in 2006), whereas other genotypes remained stable at their prevalence during the same period.[57] The shift in genotype prevalence with the increase of genotype A among chronically infected carriers can be explained by higher risk of genotype A to develop persistence. This is consistent with higher rates of chronic persistence after acute infection in adults in European countries where genotype A is prevalent (10%).[48,58] This is also consistent with results of *in vitro* and *in vivo* comparisons of different genotype strains showing different dynamics of replication: slow for genotype A and rapid by genotype C.[59,60] The surveillance study indicated that all patients treated with lamivudine (LVD) recovered from acute hepatitis, whereas none of the three patients who developed a chronic outcome had received antiviral treatment during their acute phase of infection, indicating that LVD might be able to prevent the chronic outcome.[54] Cumulatively, these data indicate the clinical importance of routine genotyping for acute hepatitis B patients.

Fulminant hepatitis

One of the most serious complications of acute HBV infection is fulminant hepatitis. In Japan, the annual number of fulminant hepatitis reported was approximately 400 cases, with approximately half of these caused by HBV infection. Despite its rather low incidence, fulminant hepatitis is a national problem because the mortality rate is extremely high.[61] It is important to understand factors predisposing for development of fulminant hepatitis. Viral factors associated with the development of fulminant hepatitis are mutations in the core promoter (T1762/A1764)[62] and the pre-core region (A1896).[54,63,64] However, these findings were not consistent with studies in Europe and the USA.[65–67] A large-scale cross-sectional study in Japan revealed association between genotype B (subgenotype Bj/B1) infection and development of fulminant hepatitis; on the other hand, no cases of fulminant hepatitis were registered among those infected with genotype A (subgenotype Ae/A2).[54] Differences in genotypes circulating in Asia and Europe/USA may indicate that distinct viral factors are playing roles in manifestation of infection by different genotype.

Chronic hepatitis

Chronic HBV infection is the most common cause of HCC in Asia.[68] Efficient surveillance and early diagnosis of development of this life complication requires risk stratification of chronic hepatitis B patients. Older age, male sex and liver cirrhosis are well recognized factors associated with increased risk of HCC.[69,70] In addition, recent large-scale population-based and clinical case–control studies carried out in Asia, have shown that infecting virus factors associated with a high risk of HCC, include HBV DNA levels,[71,72] HBV basal core promoter mutations,[35] genotype C (vs B),[22,36,73,74] and sub-

genotype Ce/C2.[71,75] There are data indicating that genotype C infection associated with a higher viral load than genotype B.[76] Association of genotype F with HCC was found to be higher than that of genotype C in Alaskan natives.[77,78] Unfortunately, there are few prospective studies examining other HBV genotypes for association with adverse outcomes. Genotype A (subgenotype Aa/A1) was found in association with development of HCC in young adults in South Africa.[79,80] However, very high rates of detection of subgenotype Aa/A1 among asymptomatic carriers suggest contribution of environmental factors (aflatoxin contained in food) for the development of HCC. In comparison with Aa/A1, HCC associated with Ae/A2 is found primarily in older individuals. In addition, the rate of complications, including HCC, for those infected with subgenotype Ae/A2 appears to be less than that found in those infected with genotype D, C or F1.[77,81] A prospective study in Spain showed that genotype A (presumably Ae/A2) infection was associated with a significantly higher cumulative rate of sustained biochemical remission, HBV DNA and HBsAg clearance in patients with chronic HBV infection than genotype D infection.[81]

Consensus 4

4-1 Recently, there is an increase of HBV genotype A proportion among acute hepatitis B infection cases in Japan. (Level 3.)

4-2 HBV genotype A acute infection has a tendency to evolve in chronic hepatitis compared to genotype B/C. (Level 3.)

4-3 Antiviral therapy of acute infection might be efficient in prevention of chronic carrier stage. (Level 3.)

4-4 Genotype C compared with genotype B is associated with higher risk of outcome in HCC in chronic carriers. (Level 2a, grade B.)

4-5 Genotype A compared with genotype D and F in chronic carriers is associated with better prognosis in terms of spontaneous ALT normalization and DNA clearance. (Level 2a, grade B.)

HBV MUTATIONS AND THEIR POTENTIAL IMPACT ON PATHOGENESIS OF HBV INFECTION

THE HBV GENOME consists of double-stranded DNA, 3200 bp in length. HBV replicates through reverse transcription of a RNA intermediate, the pregenome RNA, different from all known mammalian DNA viruses. HBV infection is characterized by high levels of virus production, however, the HBV reverse transcriptase is an error-prone enzyme lacking proof-reading capacity, resulting in a large number of nucleotide substitutions during replication. The misincorporation rate has been estimated to be of the order of 10^{10} incorrect nucleotide incorporations per day. As a result, HBV has a quasispecies distribution in infected patients.

Naturally occurring mutations identified in the HBV genome are more prevalent in patients with chronic hepatitis than in HBeAg positive asymptomatic carriers. Among them, several specific mutations have been shown to be associated with the pathogenesis of HBV infection.

HBeAg seroconversion

A HBV strain harboring stop codon mutation in the precore region was first reported in anti-HBe positive patients with chronic hepatitis.[25] The precore region located upstream of the core region is involved in the production and secretion of HBeAg protein. HBeAg is secreted into blood after removal of N-terminal 19 amino acids (a.a.) and C-terminal 34 a.a. from HBeAg precursor protein composed of precore and core regions. Nucleotide substitution of G to A at nt 1896 confers stop codon (TAG) mutation from tryptophan (TGG) at codon 28 in the precore region, resulting in a failure to produce HBeAg protein.[82–84] Although controversial, 10 genotypes have been identified tentatively so far[85] and genotypes affect the occurrence of stop codon mutation in the precore region. The stop codon mutation in the precore region (G1896A) is rarely encountered in HBV genomes of genotype A, some of genotype C and F, because they possess C at position 1858 that makes a pair with G at position 1896 in the stem-loop structure of the *cis*-encapsidation signal.[86]

The HBV core promoter regions located upstream of core region are involved in the transcription of precore mRNA and pregenomic RNA. Nucleotide substitution of A to T at nt 1762 combined with substitution of G to A at nt 1764 in the core promoter region give rise to a reduced transcription of precore mRNA and increased level of viral DNA, resulting in a decreased production of HBeAg protein and enhanced viral replication.[87–89]

Consensus 5

Nucleotide substitution G1896A confers stop codon mutation in the precore region. Nucleotide substitution A1762T combined with substitution G1764A in

the core promoter region give rise to a reduced transcription of precore mRNA. These nucleotide changes in combination with a reduction of HBeAg caused by suppressed replication of HBV are closely associated with HBeAg seroconversion. (Level 2b, grade B.)

Association between HBV mutations and clinical manifestation

Fulminant hepatitis

Precore and core promoter mutations are very frequent in patients with fulminant hepatitis from Asia[62,63,90] and the Middle East.[64] However, these mutations were not detected in those from Western countries.[65,67,91,92] This difference could be attributable to the difference of genotype prevalence, frequent genotype Ae and rare Bj in Western countries.[86] The patients infected with the former genotype rarely have precore mutant virus, while the latter frequently have the mutant virus. Stop codon mutation in the precore region is inhibited in genotype A because of C at position 1858 that makes a pair with G at position 1896 in the stem-loop structure of the *cis*-encapsidation signal.[93]

Ozasa *et al.* analyzed the difference of host and viral factors between 40 patients with fulminant hepatitis B and 256 with acute self-limited hepatitis B in a multicenter cross-sectional study,[54] and showed that precore stop codon mutation of G1896A and genotype Bj are associated with fulminant hepatitis in Japan. They also reported the marked enhancement of viral replication by introducing either G1896A or A1762T/G1764A mutation into the Bj clone in *in vitro* transfection study. Because this type of HBV mutant is found not only in patients with fulminant hepatitis but also in asymptomatic HBV carriers,[94] the interaction between the virus and the host's immune response might influence the outcome of HBV infection.

In addition to the mutants mentioned above, pre-S2 defective virus or HBV defective in secretion because of surface gene mutations are reported in patients with fulminant hepatitis. These mutant viruses showed a characteristic feature of virus retention in hepatocytes and misassembly with high replication capacity.[95–97]

HCC development

Evidence has been accumulating over the past decade that the risk of developing cirrhosis and HCC is influenced by the patient's viral status, such as genotype, viral load and genomic mutations. Naturally occurring mutations have been identified in the structural and non-structural genes as well as the regulatory elements of the virus, and these mutations are more prevalent in patients with chronic hepatitis than in HBeAg positive asymptomatic carriers.[98]

A double mutation, A1762T/G1764A in the basal core promoter region has been found in patients with advanced liver disease and HCC. Several case–control studies,[30,35,99–102] retrospective cohort studies[103,104] and one prospective cohort study[105] confirmed this finding, while some conflicting results were also reported in the case–control studies[106,107] and one prospective study.[108]

The role of deletions in the pre-S region of the HBV genome has been shown to be associated with the development of progressive liver diseases including HCC. Several case–control studies confirmed this finding.[27,107–110] A further mapping study of the pre-S region showed that all the deletion regions encompassed T- and B-cell epitopes and most of them lost one or more functional sites including the polymerized human serum albumin-binding site.[109] Deletion of these functional sites may cause intracellular retention of HBV envelope proteins and viral particles and contribute to more progressive liver damage and HCC development.

In addition to these common mutations, several other mutations, C1653T in the enhancer II region, T1753C/A/G in the core promoter region, and G1317A/T1341C/A/G in enhancer I region, have been reported to be associated with the development of HCC in some case–control studies.[30,107,111]

Consensus 6
There is some evidence that emergence of HBV genomic mutations arising during the course of chronic infection influence the outcome of chronic liver disease. Among them, core promoter mutations A1762T/G1764A might have a potential for developing progressive liver disease and HCC. (Level 2a, grade B.)

HBsAg escape mutant

The HBsAg mutant was first described in a child born to a HBsAg positive mother who developed acute hepatitis B in spite of vaccination and passive immunization against HBV.[112] This viral strain contained a substitution of glycine to arginine at position 145 (sG145R) and was able to escape the immune surveillance, resulting in an infection despite the presence of anti-HBs antibodies, vaccine escape mutant. Similar mutants have been detected all over the world.[113–115]

Patients after liver transplantation for HBV-related chronic liver disease who had received anti-HBs antibodies to prevent re-infection of the graft showed an "immune escape mutant".[116–118] Furthermore, "diagnosis escape mutants" have also been described because HBsAg detection assays are based on anti-HBs antibodies.[119] The emergence of these variants may contribute to occult HBsAg negative HBV infection.[120]

The HBV genome is organized in such a way that the envelope gene is overlapped by the polymerase gene; therefore, HBV with changes in the polymerase gene associated with resistance to the nucleos(t)ide analog which are described in detail in section 5 may have consequent changes in the envelope gene. A triple mutant causing LVD resistance (rtV173L + rtL180M + rtM204V), which have an enhanced replication capacity compared with rtL180M + rtM204V alone, causes two amino acid changes in the overlapping surface gene (sE164D + sI195M). This mutant reduces anti-HBs binding to levels seen only with the vaccine escape mutant sG145R.[121] Some patients treated with LVD showed seroclearance of HBsAg with detectable circulating HBV DNA. An sP120A mutation was associated with HBsAg seroconversion in these patients and this mutation produces a reduced anti-HBs binding which causes the failure to detect HBsAg.[122]

Consensus 7
Amino acid substitutions, deletions or insertions across the "a" determinant of HBsAg, such as a substitution sG145R, give rise to vaccine and immunoglobulin escape mutant. (Level 4, grade C.)

INDICATIONS FOR ANTIVIRAL TREATMENT OF CHRONIC HEPATITIS B

ONCE THE LIVER is persistently infected with HBV, it is difficult to eradicate the virus. It is reported that the natural clearance rate of HBsAg in asymptomatic HBsAg carriers is approximately 1–2% per year.[123] Therefore, the first goal in treating chronic hepatitis B is to prevent patients from progression to cirrhosis and occurrence of HCC.

When the initiation of antiviral therapy for chronic hepatitis B is considered, it is very important to estimate the fibrosis stage of each patient. If possible, a liver biopsy should be performed in order to obtain sufficient information to determine the extent of hepatic fibrosis. When the fibrosis stage of patients with chronic hepatitis B is moderate to severe, or when the patients have cirrhotic liver, the administration of antiviral therapy should be considered. When inflammatory activity is high and the fibrosis seems to be progressive, the introduction of antiviral therapy should also be considered.

In order to prevent the occurrence of hepatic fibrosis and HCC, virological factors as well as biochemical factors are important. A long-term follow-up study of untreated HBsAg positive individuals in Taiwan in which the cumulative incidence of HCC and cirrhosis were studied for 13 years revealed that high baseline HBV DNA was associated with increased risk of HCC and cirrhosis. Incidence rate of HCC in patients whose viral load of HBV DNA was less than 300 copies/mL was 1.3%, whereas in patients whose viral load was more than 1 000 000 copies/mL the incidence rate was 14.9%.[33] Moreover, incidence of cirrhosis in patients whose viral load was less than 300 copies/mL was 4.5%, whereas it was 36.2% in patients whose viral load was more than 1 000 000 copies/mL.[124] Therefore, the introduction of antiviral therapy should be considered based on biochemical and virological findings.

As mentioned above, although high viral load of HBV DNA is one of the strong risk factors in predicting poor prognosis of HBV carriers, low HBV DNA level does not rule out risk in Asian patients. Among HBeAg positive patients, HBV DNA levels of less than 10^5 copies/mL predicted better histological outcome; however, 14.3% of patients still had established fibrosis.[125] The liver biopsy is also very useful for such cases.

Recommendation 4
- 4-1 Introduction of antiviral therapy should be considered on the biochemical and virological findings. (Level 2a, grade B.)
- 4-2 Antiviral therapy should be considered for patients with low virus load but progressed hepatic fibrosis. (Level 2a, grade B.)
- 4-3 Liver biopsy finding (if available) should be useful to determine the introduction of antiviral therapy. (Level 2a, grade B.)

On the other hand, when patients with HBV have obscure or mild fibrosis, a close observation without any medication could be considered for them. Once antiviral therapy with a nucleos(t)ide analogue is started, it is very difficult to stop. Therefore, for patients who are in an inactive carrier state and whose fibrosis stage is relatively mild, a coarse observation without any treatment could be a useful choice to treat the patients.

Young patients with chronic hepatitis B, especially those who are HBeAg positive, often face the flare-up of hepatitis. Because such patients are likely to achieve spontaneous HBe seroconversion and go into an inactive carrier state, unnecessary antiviral therapy should be avoided for them. A coarse observation without any medications should be considered for young patients or those with mild fibrosis.

Recommendation 5
Indication of antiviral therapy for chronic hepatitis B: Observation without therapy should be considered for young patients or those with mild fibrosis. (Level 3, grade B.)

NUCLEOS(T)IDE ANALOGUES FOR CHRONIC HEPATITIS B

AS STATED ABOVE, the goal of antiviral therapy in patients with chronic hepatitis B is to prevent cirrhosis and HCC. Maintaining persistent suppression of HBV replication reduces the development of cirrhosis and HCC. In the last decade, there has been a major advance in the treatment of chronic hepatitis B with nucleos(t)ide analogues such as LVD, adefovir (ADV), entecavir (ETV), telbivudine and tenofovir.[126–132] In treatment by nucleos(t)ide analogues for chronic hepatitis B in Japan, LVD, ADV and ETV are mainly used at present. Nucleos(t)ide analogues are potent inhibitors of the polymerase/reverse transcriptase and are easy to administrate p.o. to chronic hepatitis B patients because of low adverse effects and strong efficacy to suppress HBV replication. Thus, nucleotide analogue therapy could rescue liner decompensation, reduce fibrosis progression and prevent the development of HCC.[133–136] On the other hand, there are major disadvantages including requirement of prolonged or even indefinite therapy for most patients and the high incidence of antiviral resistance. Disadvantages of nucleos(t)ide analogues include the development of antiviral resistance.[137–140] Drug-resistant viruses emerge during the treatment and could be associated with flare-up of hepatitis. Due to no proof of reading activity of HBV polymerase, the spontaneous substitution rate of HBV genome is high in the natural course of the disease. Through the selection of pre-existing resistant variants and gradual accumulation of new a.a. substitutions, the mutations exhibiting the best replication capacity in the presence of the drug are selected under the circumstance of antiviral pressure. The level of intrinsic resistance and the replicative fitness determine the mutant spread and hence the annual incidence of drug resistance.

LVD

Lamivudine was the first nucleoside analogue licensed for the treatment of chronic HBV infection in Japan in 1999. LVD was given at a dose of 100 mg daily and has excellent safety and tolerability.[141–143]

Liaw *et al.* reported that continuous treatment with LVD delays the clinical progression of chronic hepatitis B with advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and risk of HCC (level 1b).[134] Matsumoto *et al.* also showed that LVD therapy effectively reduces the incidence of HCC in Japanese patients with chronic hepatitis B.[144] Thus, it is generally considered that control of viral load using nucleos(t)ide analogues is effective to prevent complicating HCC in patients with active chronic hepatitis B.

Consensus 8
The control of viral load using nucleos(t)ide analogues reduces the risk of complicating HCC in patients with chronic hepatitis B. (Level 1b, grade B.)

Lamivudine resistance is characterized by the mutation of the highly conserved tyrosine, methionine, aspartate, aspartate (YMDD) nucleotide-binding motif in the catalytic domain of the enzyme. YMDD to YIDD (rtM204I) or YVDD (rtM204V) mutations are associated with LVD resistance.[142,145,146] These resistant mutants appear to replicate less efficiently than the wild-type virus *in vitro*, however, additional mutations such as rtV173L and rtL180M can restore partially the replication capacity *in vitro*.[147,148] LVD resistance occurred in approximately 20% of patients after 1 year, which increased to approximately 70% after 5 years (Fig. 1).

A meta-analysis, which included Asian patients and North American/European patients, indicated that HBV subtype ayw (genotype D) appears to respond significantly better to LVD treatment than does HBV subtype adw (genotype A). Insufficient suppression of the adw subtype during the early phase of treatment may lead to the high incidence of LVD resistance in HBV subtype adw.[149] In a study comparing the virological outcome among infections with HBV genotypes A, B and C, patients infected with genotype A had the lowest rate of HBV DNA clearance than those with genotype B or C, and had the highest incidence of resistant mutations.[150]

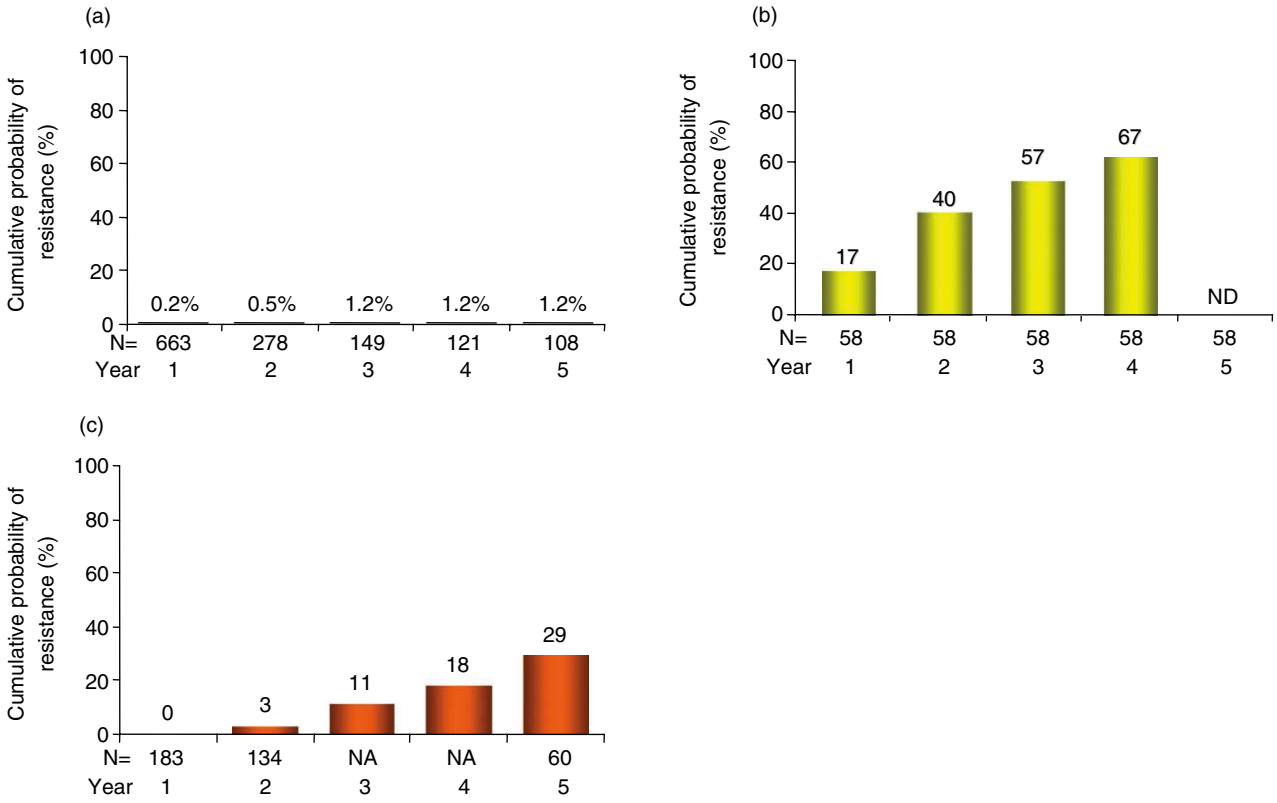

Figure 1 Cumulative probability of resistance after the initiation of entecavir (ETV), lamivudine (LVD) and adefovir (ADV) for patients with hepatitis B e-antigen. (a) Cumulative probability of resistance after the initiation of ETV.[159] (b) Cumulative probability of resistance after the initiation of LVD.[138] (c) Cumulative probability of resistance after the initiation of ADV.[153]

Lamivudine or hepatitis B immunoglobulin (HBIg) treatment induced vaccine/HBIg-escape mutations sP120T and sG145R in combination with LVD-resistance mutations. These mutations are associated with rtT128N and rtW153Q in the polymerase protein and have been found to partially restore the *in vitro* replicative capacity of LVD-resistant HBV.[121]

Another LVD resistant mutation, rtA181T, concomitantly generates a stop codon in the surface antigen (sW172stop), resulting in impaired secretion of HBsAg.[151] Neither the adefovir associated resistance mutation rtN236T nor the tenofovir associated resistance mutation rtA194T causes changes in the envelop protein.

ADV

Adefovir dipivoxil is a prodrug of ADV and has structural similarity to the natural substrate, dATP. Several studies have also been conducted using ADV.[128,152–154] In HBeAg positive patients, treatment with ADV for 1 year resulted in HBeAg seroconversion in 12%, serum HBV DNA in less than 10^3 copies/mL in 21% and normalization of ALT in approximately 48% of patients.[127] The rate of HBeAg seroconversion increased to 29% after 2 years and 43% after 3 years of treatment. In HBeAg negative patients, serum HBV DNA of less than 10^3 copies/mL and normalization of ALT were observed in 51% and 72%, respectively, after 1 year of ADV.[154] After 5 years of therapy, the serum HBV DNA were less than 10^3 copies/mL in 67% of patients, and ALT level normalized in 69%. The reported incidence of ADV resistance is 0% after 1 year, 3% after 2 years and 29% after 5 years of antiviral therapy (Fig. 1).[154] The primary mutations associated with ADV resistance are rtN236T and rtI233V in the D domain and rtA181V in the B domain of HBV polymerase. In comparison with more than 100-fold decrease in sensitivity to LVD associated with the two primary mutations, the rtN236T mutation confers only a 5–10-fold decrease in sensitivity to ADV *in vitro*,[155] which may explain the delayed emergence of this mutant.

In LVD-resistant patients treated with ADV monotherapy, the rate of antiviral resistance was 6–18% after

1 year and 21–38% after 2 years.[156,157] Switching therapy from LVD to ADV may enhance the acquisition of another mutation and induce replication of HBV DNA.[158–160] On the other hand, combination therapy of LVD and ADV effectively suppressed viral replication and maintained high efficacy in LVD-resistant patients with chronic HBV infection.

ETV

Entecavir is a guanine analogue and Chang *et al*. have reported that ETV is effective in reducing the serum level of HBV DNA compared with LVD in HBeAg positive patients (Table 2).[159] The cumulative proportion of patients with undetectable HBV DNA (<300 copies/mL) increased to 81% after 1 year of therapy and 93% after 5 years of therapy.[160] After 1 year of treatment with ETV, the serum ALT level was normalized in approximately 70% of patients, and increased to 90% of patients after 5 years. Lai *et al*. have reported that ETV is more efficacious in HBeAg negative patients compared with LVD (Table 2).[161] ETV is the most potent of the currently available anti-HBV drugs because it affects multiple functions of the polymerase, including priming, reverse transcription and DNA elongation.[162]

Entecavir was licensed for the treatment of chronic hepatitis B in Japan in 2006. In nucleos(t)ide-naive patients, ETV is given at dose of 0.5 mg/day.

The rate of ETV resistance was extremely low in nucleoside-naive patients.[160,163,164] The incidence of ETV resistance in nucleos(t)ide analogue-naive patients was reported to be 1.2% at 3 years (Fig. 1).[160,163,164] HBeAg loss was observed in 8% of these patients. The response to ETV was lower in LVD-resistant patients than in nucleos(t)ide analogue-naive patients. In LVD-resistant patients, 20% of patients had undetectable HBV DNA levels after 48 weeks of ETV therapy, and the resistance rate to ETV was 26% at 3 years. Patients with HBeAg at the initiation of ETV had a resistance rate to ETV of 36% at 3 years. On the other hand, patients without HBeAg at the initiation of ETV did not have resistance to ETV at 3 years (Fig. 2).[160,165] In LVD-resistant patients, the risk of the development of resistance to ETV is much higher than those without LVD resistance.[160,165]

The resistance to ETV is principally associated with the mutations rtM250V, rtI169T or rtS202I in addition to the primary LVD resistance mutations rtM204V + rtL180M. The need for multiple mutations to induce ETV resistance suggests a higher genetic barrier to resistance and explains the low rate of resistance to ETV in nucleos(t)ide analogue-naive patients.

Table 2 Efficacy of nucleoside analogues for chronic hepatitis B

	Subject: HBeAg positive patients[159]						
	n	Change of HBV DNA (log copies/mL)		Negativity of HBV DNA of <300 copies/mL		Normalization of ALT	SC
ETV 0.5 mg	354	−6.9	$P < 0.001$	67%	$P < 0.001$	68%	$P < 0.05$	21%	$P = 0.33$
LVD 100 mg	355	−5.4		36%		60%		18%	
	Subject: HBeAg negative patients[161]						
	n	Change of HBV DNA (log copies/mL)		Negativity of HBV DNA of <300 copies/mL		Normalization of ALT	
ETV 0.5 mg	325	−5.0	$P < 0.001$	90%	$P = 0.001$	78%	$P < 0.05$		
LVD 100 mg	323	−4.5		72%		71%			

ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; LVD, lamivudine; SC, seroconversion; VR, virological response.

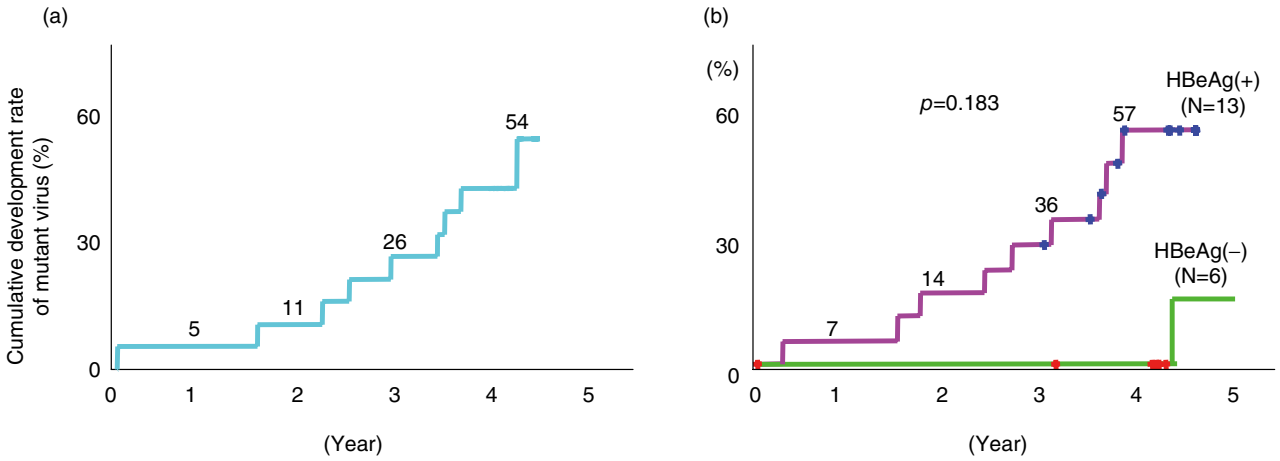

Figure 2 Cumulative development rate of mutant virus after the initiation of entecavir monotherapy in hepatitis B patients with resistance after the administration of lamivudine monotherapy.[164] (a) Cumulative development rate of mutant virus in all patients. (b) Cumulative development rate of mutant virus based on the difference of hepatitis B patients with positive hepatitis B e-antigen (HBeAg) and hepatitis B patients with negative HBeAg.

Consensus 9
Drug-resistant virus with specific mutations in the polymerase/reverse transcriptase gene emerges during nucleos(t)ide analogue therapy in chronic hepatitis B patients. The rtM204V/I and rtL180M mutations are associated with LVD resistance, the rtN236T and rtI233V or rtA181V with ADV resistance, and the rtM250V or rtT184G or rtS202I combined with rtM204V + rtL180M with ETV resistance. (Level 4, grade C.)

Recommendation 6
When patients with chronic hepatitis B are treated with nucleos(t)ide analogues, ETV should be given as the first-line drug because of its high efficacy and low emergence of viral resistant mutant. (Level 1b, grade A.)

Recommendation 7
The combination therapy of LVD and ADV is an effective treatment for LVD-resistant patients. (Level 1b, grade B.)

INTERFERON THERAPY FOR CHRONIC HEPATITIS B

INTERFERON (IFN) WAS the first antiviral treatment approved for chronic HBV infection. IFN-α and -β have a predominantly antiviral effect but also have an immunomodulatory effect and antiproliferative effect which is in contrast to direct antiviral agents such as nucleos(t)ide analogues. The duration of treatment is defined (usually 24–48 weeks) in IFN therapy. This finite duration of therapy is an advantage over direct antiviral agents which are usually given indefinitely. The long-term outcome of therapy is more precisely described in IFN compared to LVD due to its longer history of clinical usage.

Selection of patients

Factors associated with favorable response to IFN therapy are vigorously studied (Table 3). For HBeAg positive patients, high pretreatment ALT levels,[166] high grade of necroinflammation on liver histology and low serum HBV DNA level have consistently been shown to be predictive of favorable response.[167] Other predictive factors include female sex,[166] younger age,[168,169] and HBV genotype A versus D or B versus C.[169,170] Patients fulfilling these predictors are the best candidates for IFN treatment. For HBeAg negative patients, there is no consistent predictor of response. Adverse events such as severe infection or exacerbations of liver disease were common when IFN was given for decompensated cirrhosis. Thus, patients with decompensated cirrhosis should not be treated with IFN due to a risk of precipitating hepatic failure and fatal complications.[171,172]

Table 3 Predictive factors for response to interferon therapy

Predictive factors	HBeAg positive	HBeAg negative
Race	No correlation	No correlation
Age	No correlation or Younger	No correlation or Younger
Sex	No correlation or Female	No correlation or Female
ALT	Higher level	No correlation or Higher level
Activity	Higher grade	No correlation
Fibrosis	Conflicting	No correlation
HBV DNA titer	Lower titer	No correlation or lower titer
Genotype	A > D, B > C	A > D, B > C
Precore	Conflicting	No correlation
Core promoter	mutant	

ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus.

Recommendation 8
Younger age, high ALT levels, low HBV load, genotype A or B and high inflammatory activity in liver biopsy are predictive of good response to IFN. IFN therapy should be considered in patients fulfilling these predictors. (Level 2a, 2b, grade B.)

Recommendation 9
Interferon should be avoided for patients with decompensated cirrhosis. (Level 4, grade D.)

Standard IFN therapy in HBeAg positive chronic hepatitis B

A meta-analysis of 16 randomized controlled studies have shown that treatment with IFN-α for 16–24 weeks versus an untreated control is associated with higher rate of HBeAg loss (33% vs 12%), HBeAg seroconversion (difference of 18%), undetectable HBV DNA by hybridization or branched chain assay (37% vs 17%), HBsAg loss (7.8% vs 1.8%) and ALT normalization (difference of 23%) (Table 4).[173] A controlled trial has shown that extending therapy for up to 32 weeks in patients who remained HBeAg positive at the end of 16 weeks of therapy improved the rate of HBeAg seroconversion.[174] The durability of HBeAg seroconversion is more than 80%, and even delayed seroconversion could occur in 10–15% of patients 1–2 years after completion of therapy.[175–177] The loss of HBsAg is reported to occur in 12–65% of patients who cleared HBeAg.[175,178] However, this is a rare event in Asian patients.[176,177]

Consensus statement 10
10-1 In HBeAg positive patients, treatment with IFN versus untreated control is associated with higher rate of HBeAg loss, HBeAg seroconversion, undetectable HBV DNA, HBsAg loss and ALT normalization. Extension of therapy improves the rate of HBeAg seroconversion. (Level 1a,1b.)
10-2 Durability of HBeAg seroconversion is more than 80%. The loss of HBsAg is rare in Asian patients. (Level 1b.)

Standard IFN therapy in HBeAg negative chronic hepatitis B

Although the rate of response at the end of therapy is 60–90%, the durability of long-term response is less

Table 4 Standard interferon therapy for HBeAg positive chronic hepatitis B. Meta-analysis of 16 randomized controlled trials

	Interferon		Control	*P*-value
Loss of HBV DNA	37%		17%	0.0001
Loss of HBeAg	33%		12%	0.0001
Loss of HBsAg	7.8%		1.8%	0.001
Seroconversion		Difference of 18%		0.002
ALT normalization		Difference of 23%		0.0001

ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus.

than 50%.[179,180] Longer duration of therapy is associated with improved durability of response: 10–15% with 4–6 months of therapy, 22–30% with 6–12 months of therapy and 30% with 24 months of therapy.[181–184]

Consensus statement 11
- 11-1 Durability of response is less than 50% in HBeAg negative patients. (Level 1b.)
- 11-2 Longer duration of therapy (>48 weeks) is associated with improved durability of response. (Level 2b.)

Pegylated IFN (PEG IFN)

Twenty four weeks of PEG IFN-α-2a monotherapy had higher rate of combined response (loss of HBeAg, suppression of HBV DNA <500 000 copies/mL and ALT normalization) compared to standard IFN-α-2a.[185] Another study with 24 weeks of PEG IFN-α-2b monotherapy also showed a higher rate of HBeAg loss and HBV DNA suppression compared to standard IFN-α-2b.[169]

Controlled studies comparing the 48 weeks of PEG IFN-α-2a and LVD in HBeAg positive and negative patients revealed that PEG IFN had a higher rate of sustained response.[170,171] Seroconversion of HBeAg (32% vs 19%), ALT normalization (41% vs 28% in HBeAg positives and 59% vs 44% in HBeAg negatives), HBV DNA suppression (HBV DNA <10 000 copies/mL, 32% vs 22% in HBeAg positives; HBV DNA <20 000 copies/mL, 43% vs 29% in HBeAg negatives) and negative HBV DNA (14% vs 5% in HBeAg positives and 19% vs 7% in HBeAg negatives) were more frequent in PEG IFN treated patients.

Differences were reported in outcome of the antiviral treatment of patients infected with different genotypes; genotype B is associated with a higher rate of antiviral response to IFN treatment than HBV genotype C among Asian patients with HBeAg positive chronic hepatitis B.[169,186,187] In multicenter trials comparing combination therapy of PEG IFN-α-2b and LVD versus PEG IFN-α-2b alone, it was shown that treatment with PEG IFN-α-2b is the best therapy to achieve HBsAg clearance in patients with genotype A compared with D.[188,189]

Combination or sequential therapy

Combination of two antiviral agents with different mechanisms of action seems a logical approach to improve efficacy. In fact, simultaneous combination of LVD and PEG IFN has a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone. However, there is no difference in treatment response between the simultaneous combination of LVD and IFN or PEG IFN compared to IFN or PEG IFN alone (Table 5).[132,133,170]

There are several clinical trials of sequential therapy with LVD followed by IFN.[190–194] Common to all studies is that the sequential therapy had no advantage over IFN alone. Some studies have shown the suggestive evidence that sequential therapy had a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone (Table 5).[190–194] However, because the study protocols and their results are variable, a conclusive result could not be drawn.

Table 5 Sequential therapy of lamivudine and interferon

		BR	SC	VR	LVD-R
Manesis *et al.* 2006	Sequential	39%	NA	28%	
($n = 36$)[190]	IFN	22%	NA	19%	
Shi *et al.* 2006	Sequential	53%	NA	14%	0%
($n = 162$)[191]	LVD	36%	NA	18%	23%
Yurdaydin *et al.* 2005	Sequential	51%	NA	54%	24%
($n = 78$)[193]	LVD	41%	NA	59%	53%
Sarin *et al.* 2005	Sequential	40%	40%	40%	15%
($n = 75$)[194]	LVD	14%	11%	16%	8%
Schalm *et al.* 2000	Sequential	50%	36%	55%	0%
($n = 226$)[192]	IFN	50%	22%	49%	0%
	LVD	63%	19%	63%	31%

BR, biochemical response; IFN, interferon; LVD, lamivudine; LVD-R, lamivudine resistant mutation; NA, not applicable because hepatitis B e-antigen patients are studied; SC, seroconversion; VR, virological response.

Long-term outcome

The end-point of antiviral therapy is to prevent liver cirrhosis and HCC. Meta-analysis of five studies including 935 patients revealed that IFN treatment significantly decreased the incidence of cirrhosis with the combined risk ratio of 0.65 (95% confidence interval [CI] = 0.47–0.91).[195] Meta-analysis of 11 studies including 2082 patients revealed that IFN treatment significantly decreased the incidence of HCC with the combined risk ratio of 0.59 (95% CI = 0.43–0.81).[195] These results suggest that IFN prevents progression of liver disease to liver cirrhosis or delays the development of HCC, as long as it is within 4–7 years of follow up which is the length of follow up in these studies. Sustained response to IFN therapy was associated with increased survival.[175,181,196,197] To further elucidate the impact of IFN on the natural course of chronic hepatitis B, studies with larger populations followed for longer periods may be needed.

Consensus statement 12

12-1 IFN therapy prevents progression to cirrhosis or the development of HCC. (Level 1a.)

12-2 IFN therapy is associated with improved survival. (Level 1b.)

Adverse effects

The most frequent adverse effects are flu-like symptoms, fatigue, myelosuppression and dermal reaction at the injection site. Others include alopecia, depression and thyroid dysfunction. Less frequent but severe adverse events include interstitial pneumonitis, exacerbation of underlying autoimmune disorders, cerebral vascular events and flare of hepatitis.

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Estimation of Liver Function Using T1 Mapping on Gd-EOB-DTPA-Enhanced Magnetic Resonance Imaging

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Objectives: To investigate the ability of T1 mapping of liver on gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging for the estimation of liver function.

Materials and Methods: Local institutional review board approved this study. Ninety-one patients (64 men, 27 women; mean age, 67.4 years) were classified into 4 groups as follows: normal liver function (NLF), n = 16; chronic hepatitis (CH), n = 38; liver cirrhosis with Child-Pugh A (LCA), n = 20; and liver cirrhosis with Child-Pugh B (LCB), n = 17. Look-Locker sequences (single slice multiphase imaging using gradient-echo sequence with inversion recovery pulse) were obtained before and at 3, 8, 13, and 18 minutes after Gd-EOB-DTPA administration. T1 mapping of liver parenchyma was calculated from the Look-Locker sequence. T1 relaxation time of liver and reduction rate of T1 relaxation time between pre- and postcontrast enhancement were measured. The Bonferroni *t* test was used for comparisons between the 4 groups.

Results: Precontrast T1 relaxation times were significantly longer for LCA and LCB than for NLF, and that of LCB was longer than that of chronic hepatitis ($P < 0.05$). Postcontrast T1 relaxation times were significantly longer for LCB than for other groups at all time points. Those of LCA were longer than those of NLF at all time points. Reduction rates were significantly lower for LCB than for the other groups at ≥8 minutes.

Conclusions: Evaluation of hepatic uptake of Gd-EOB-DTPA using T1 mapping of liver parenchyma can help estimate liver function.

Key Words: Gd-EOB-DTPA, liver function, T1 mapping

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Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) has recently come into use in routine clinical practice as a magnetic resonance (MR) contrast agent for T1-weighted images in many countries. After intravenous injection, Gd-EOB-DTPA is cleared from the body by 2 routes of elimination: receptor-specific uptake in hepatocytes with subsequent biliary excretion; and glomerular filtration in the kidney with subsequent urinary excretion. The compound was completely eliminated in urine and feces in almost equal amounts (43.1%–53.2% and 41.6%–51.2%, respectively).[1] With hepatobiliary elimination, Gd-EOB-DTPA is gradually taken up by hepatocytes and eventually excreted via the biliary pathway without any change to the chemical structure. When the hepatobiliary or renal eliminating function is severely insufficient, the remaining elimination pathway may compensate for the impaired pathway.[2]

Gd-EOB-DTPA is used to evaluate focal liver lesions, such as hepatocellular carcinoma or liver metastasis on T1-weighted imaging.[3–8] However, as signal intensity of the liver parenchyma after Gd-EOB-DTPA administration depends on uptake by hepatocytes and bile excretion, Gd-EOB-DTPA is expected to be useful in imaging to evaluate liver function, as well as to evaluate focal liver lesions.[9–14]

Since Gd-EOB-DTPA has a T1-shortening effect, measurement of the T1 relaxation time of liver parenchyma before and after Gd-EOB-DTPA administration allows quantitative evaluation of Gd-EOB-DTPA uptake by the liver parenchyma, indicating liver function.

The purpose of this study was to investigate whether Gd-EOB-DTPA-enhanced MR imaging can evaluate liver function using the T1 relaxation time of liver parenchyma.

MATERIALS AND METHODS

Patients

This study was a prospective study following the principles of the Declaration of Helsinki. The ethics committee of our institution approved all study protocols, and informed consent was obtained from all patients who underwent Gd-EOB-DTPA-enhanced MR imaging.

Consecutive patients, for whom hepatologists or hepatic surgeons needed Gd-EOB-DTPA-enhanced MR imaging to examine suspicious focal liver lesions from March 2009 to October 2009 in our institute, were included in this study. Patients, who had contraindications to MR imaging (eg, pacemaker, claustrophobia, allergy to contrast media, or renal dysfunction), were excluded. A total of 109 patients underwent Gd-EOB-DTPA-enhanced MR imaging. Moreover, patients were excluded from this study if they had undergone segmental resection of the liver, hemi-hepatectomy, biliary surgery, transarterial chemoembolization, radiofrequency ablation, percutaneous ethanol injections, or chemotherapy ≤2 months before MR imaging, or if they showed biliary dilatation or portal vein thrombosis. As a result, 18 patients were excluded for the reasons mentioned above, and 91 patients (64 men, 27 women; mean age, 67.4 years; range, 46–86 years) were selected for evaluation in this study.

Patients with suspected focal liver lesions in normal liver parenchyma were classified into a normal liver function (NLF) group (n = 16). Patients with chronic hepatitis (CH) were classified into a CH group (n = 38). Patients with liver cirrhosis (n = 37) were classified into 2 groups according to Child-Pugh classification: a liver cirrhosis with Child-Pugh A (LCA) group (n = 20); and a liver cirrhosis with Child-Pugh B (LCB) group (n = 17). No patients showed liver cirrhosis with Child-Pugh C (Table 1).

The NLF group comprised patients with suspected focal liver lesions (eg, metastases) in normal liver parenchyma. Gd-EOB-DTPA-enhanced MR imaging of these patients revealed no or few focal lesions

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in the liver. We judged that focal lesions did not influence liver function in these cases, because of a lack of obstruction of the main bile ducts and blood vessels, and the patients showed no abnormal hepatobiliary data on blood examination. Liver biopsy was used in 13 patients to confirm CH (n = 4) or liver cirrhosis (n = 9). CH and liver cirrhosis in 62 patients without liver biopsy were confirmed mainly by imaging findings using MR imaging, computed tomography, and ultrasonography (irregularity of the liver surface, marginal dullness, atrophy of the liver right lobe, swelling of the left lobe, splenomegaly, and development of collateral veins), with referring blood examinations (platelet counts, aspartate aminotransferase, alanine aminotransferase, and prothrombin time).[15–19] Causes of CH or liver cirrhosis were hepatitis C (n = 44), hepatitis B (n = 15), alcohol abuse (n = 5), autoimmune hepatitis (n = 1), and unknown (n = 10).

MR Imaging

All study cases were performed on a clinically available 3-T system (Achieva; Philips Medical System, Netherlands). For signal reception in all examinations, a 6-channel phased-array surface coil was used and covered the whole liver. For all patients, Look-Locker sequences (single slice multiphase imaging using gradient-echo sequence with inversion recovery pulse: repetition time, 12 milliseconds; echo time, 1.7 milliseconds; flip angle, 7°; field of view, 420 × 285 mm; matrix, 112 × 66, 256 zip; thickness, 10 mm; acquisition time, 1 phase = 145 ms, 31 phases; acceleration factor, 2) were obtained before and at 3, 8, 13, and 18 minutes after Gd-EOB-DTPA administration.[20] The sequence was obtained as only 1 axial slice at the level of the porta hepatis.

TABLE 1. Patient Characteristics

Group	NLF (n = 16)	CH (n = 38)	LCA (n = 20)	LCB (n = 17)
Age (yr)	59.1 ± 9.8	69.2 ± 9.1	70.7 ± 6.2	67.1 ± 8.0
Body weight (kg)	58.2 ± 14.3	59.7 ± 9.2	65.1 ± 11.9	60.7 ± 7.6
Albumin (mg/dL)	4.24 ± 0.25	4.14 ± 0.51	3.76 ± 0.43	3.08 ± 0.32
Total bilirubin (mg/dL)	0.70 ± 0.27	0.77 ± 0.28	0.77 ± 0.25	1.57 ± 0.87
AST (IU/L)	20.5 ± 8.7	42.9 ± 30.3	49.5 ± 21.3	59.1 ± 24.1
ALT (IU/L)	25.7 ± 16.8	36.3 ± 22.0	44.8 ± 29.3	38.6 ± 21.3
Prothrombin time (INR)	0.97 ± 0.04	1.05 ± 0.07	1.10 ± 0.06	1.27 ± 0.11
Platelet count ($\times 10^4/\mu$L)	25.0 ± 7.0	14.8 ± 6.2	11.5 ± 5.7	7.3 ± 3.0

Values indicate mean ± 1 standard deviation.

NLF indicates normal liver function; CH, chronic hepatitis; LCA, liver cirrhosis with Child-Pugh A; LCB, liver cirrhosis with Child-Pugh B; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio.

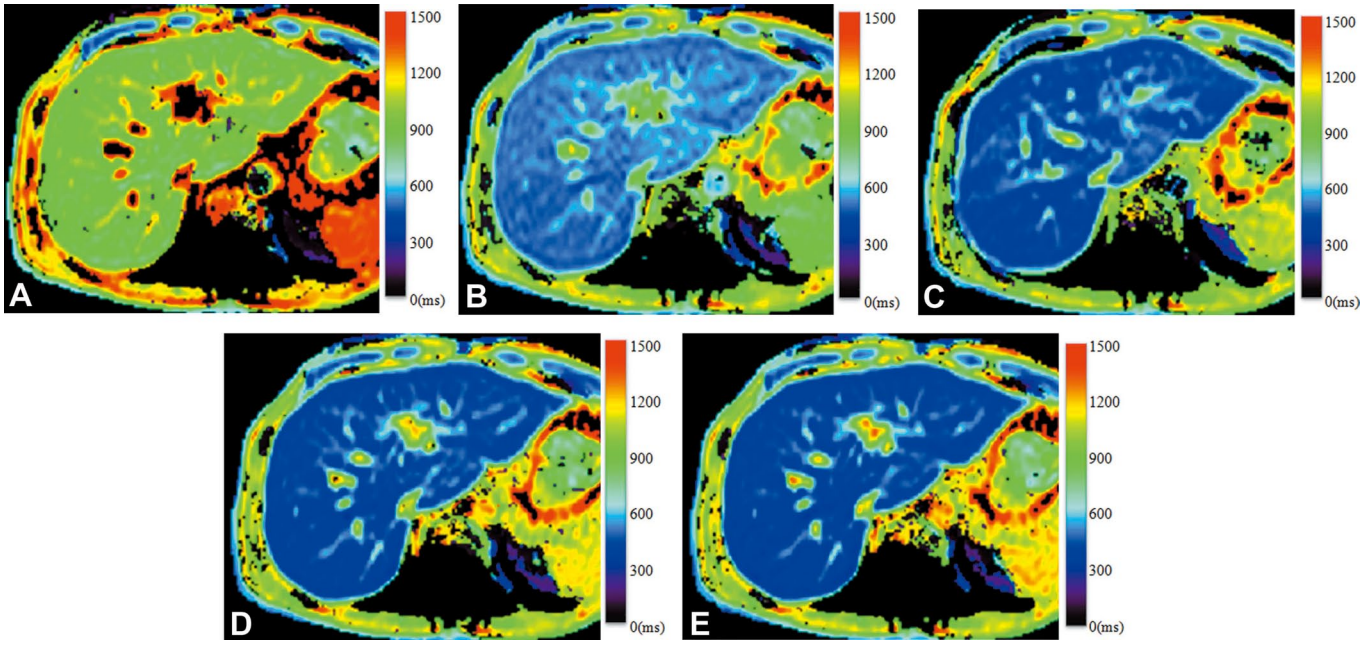

FIGURE 1. A 67-year-old man classified into the normal liver function group with suspected focal liver lesion. Albumin, 3.8 mg/dL; total bilirubin, 0.3 mg/dL; aspartate aminotransferase, 9 IU/L; alanine aminotransferase, 8 IU/L; prothrombin time (international normalized ratio), 0.96; platelet count, 29.6 × $10^4/\mu$L. A–E, T1 mapping images calculated from a Look-Locker sequence (TR, 12 milliseconds; TE, 1.7 milliseconds; flip angle, 7°) were obtained before Gd-EOB-DTPA administration (A) and at 3 (B), 8 (C), 13 (D), and 18 (E) minutes after administration. Precontrast T1 relaxation time was 853.9 milliseconds and postcontrast T1 relaxation times and reduction rates of T1 relaxation time at 3, 8, 13, and 18 minutes were as follows: 458.1, 375.7, 357.6, and 348.8 milliseconds; 46.4%, 56.0%, 58.1%, and 59.2%, respectively. We can perceive differences in T1 relaxation times as color distributions on T1 mapping.

Gd-EOB-DTPA (Primovist; Bayer Schering Pharma AG, Berlin, Germany) was used as a hepatocytic contrast agent. All patients received 0.025 mmol/kg body weight of Gd-EOB-DTPA administered at 2 mL/s through an intravenous line placed in a cubital or cephalic vein and flushed with 35 mL of 0.9% saline at the same speed.

Image Analysis

The Philips Research Integrated Development Environment (PRIDE) T1 fitting tool (Philips Healthcare, Best, Netherlands) was employed for measurement of T1 relaxation time using data from the Look-Locker sequence.[20,21] PRIDE (The Philips Research Integrated Development Environment) software can depict T1 relaxation time on a pixel-by-pixel basis in a color distribution map (T1 mapping) (Figs. 1, 2).

For T1 relaxation time assessment of the liver, a region of interest (ROI) with a range of 75 to 125 pixels was drawn manually in the liver on T1 mapping images obtained before and at 3, 8, 13, and 18 minutes after Gd-EOB-DTPA administration. Liver T1 relaxation time was evaluated using a pixel-wise technique. Five ROIs were sparsely placed in both lobes of liver parenchyma without focal hepatic lesions, major branches of the portal or hepatic veins, or imaging artifacts. No patients in this study showed inhomogeneous distribution of contrast uptake between the both lobes on visual observation. For reproducible ROIs before and after Gd-EOB-DTPA administration, every effort was made to place ROIs at the same positions in the liver of each patient. Mean T1 relaxation time for the 5 ROIs were considered as the representative T1 relaxation time for the liver. In addition, the reduction rate of T1 relaxation time between pre- and postcontrast enhancement at each time was calculated using the following definition:

$$\text{Reduction rate (\%)} = [(T1_{pre} - T1_{post})/T1_{pre}] \times 100$$

where $T1_{pre}$ is the T1 relaxation time before Gd-EOB-DTPA administration and $T1_{post}$ is the T1 relaxation time after Gd-EOB-DTPA administration.

Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences software (version 11.0; SPSS, Chicago, IL). Data are expressed as mean ± standard deviation (SD). The Bonferroni *t* test was used to compare the 4 groups for T1 relaxation time of the liver before and after Gd-EOB-DTPA administration and reduction rate of T1 relaxation time in the liver. We used an adjusted *P* value which represented the original *P* value multiplied by total number of comparison, and an adjusted *P* value less than 0.05 was considered to indicate a significant difference in all statistical tests. Since this study was an initial experience, no power analysis was performed to determine sample sizes.

RESULTS

T1 Relaxation Time of the Liver

Precontrast T1 relaxation time of the liver showed significant differences between NLF and liver cirrhosis groups LCA and LCB

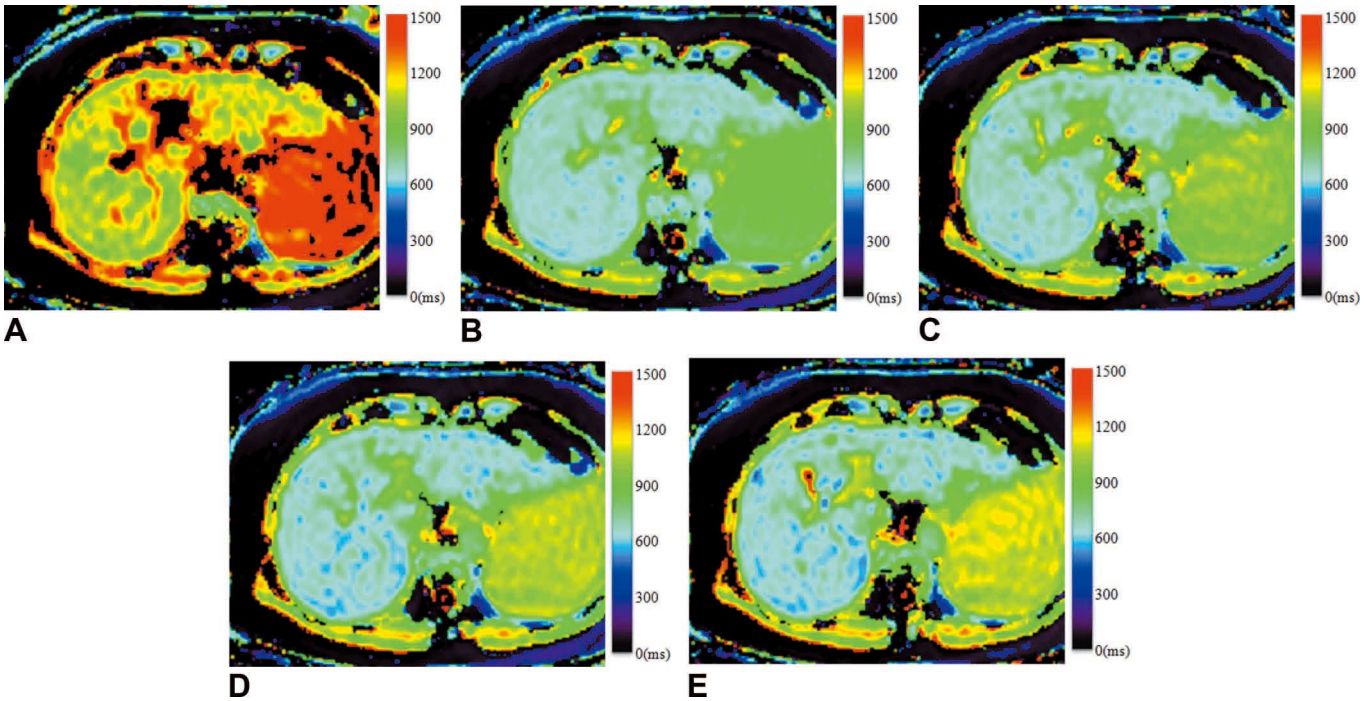

FIGURE 2. A 63-year-old woman classified into liver cirrhosis with Child-Pugh B and suspected hepatocellular carcinoma. Albumin, 2.8 mg/dL; total bilirubin, 1.8 mg/dL; aspartate aminotransferase, 64 IU/L; alanine aminotransferase, 24 IU/L; prothrombin time (international normalized ratio), 1.45; platelet count, $5.2 \times 10^4/\mu L$. A–E, T1 mapping images calculated from a Look-Locker sequence (TR, 12 milliseconds; TE, 1.7 milliseconds; flip angle, 7°) were obtained before Gd-EOB-DTPA administration (A) and at 3 (B), 8 (C), 13 (D), and 18 (E) minutes after administration. Precontrast T1 relaxation time was 1073.4 milliseconds and postcontrast T1 relaxation times and reduction rates of T1 relaxation time at 3, 8, 13, and 18 minutes were as follows: 632.9, 630.7, 608.6, and 615.2 milliseconds; 41.0%, 41.2%, 43.3%, and 42.7%, respectively. On T1 mapping, prolonged pre- and postcontrast T1 relaxation times showed as obvious differences in color distribution by comparisons with those of normal liver function such as in the case depicted in Figure 1. Color in liver parenchyma could be visually confirmed to show barely any change on postcontrast T1 mapping.

TABLE 2. T1 Relaxation Time (Milliseconds) of the Liver Before and After Gd-EOB-DTPA Administration

		Postcontrast			
	Precontrast	3 min	8 min	13 min	18 min
Group					
NLF	836.4 ± 68.5	456.9 ± 50.6	398.2 ± 49.1	364.2 ± 44.4	344.4 ± 46.8
CH	873.2 ± 90.2	482.7 ± 70.9	449.2 ± 86.1	422.8 ± 87.5	405.7 ± 87.4
LCA	925.9 ± 75.8	518.0 ± 69.3	494.7 ± 87.9	465.7 ± 95.4	443.4 ± 107.2
LCB	978.2 ± 117.2	594.5 ± 63.7	613.9 ± 75.3	603.4 ± 83.5	597.5 ± 88.7
			Adjusted *P*		
Compared groups					
NLF CH	1.000	1.000	0.204	0.119	0.125
NLF LCA	0.023	0.043	0.003	0.003	0.006
NLF LCB	<0.001	<0.001	<0.001	<0.001	<0.001
CH LCA	0.218	0.340	0.244	0.383	0.723
CH LCB	0.001	<0.001	<0.001	<0.001	<0.001
LCA LCB	0.485	0.004	<0.001	<0.001	<0.001

Values indicate mean ± 1 standard deviation.

The Bonferroni *t* test was used to compare the 4 groups, and adjusted *P* values represented the original *P* value multiplied by 6, as the total comparison number.

Gd-EOB-DTPA indicates gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid; NLF, normal liver function; CH, chronic hepatitis; LCA, liver cirrhosis with Child-Pugh A; LCB, liver cirrhosis with Child-Pugh B.

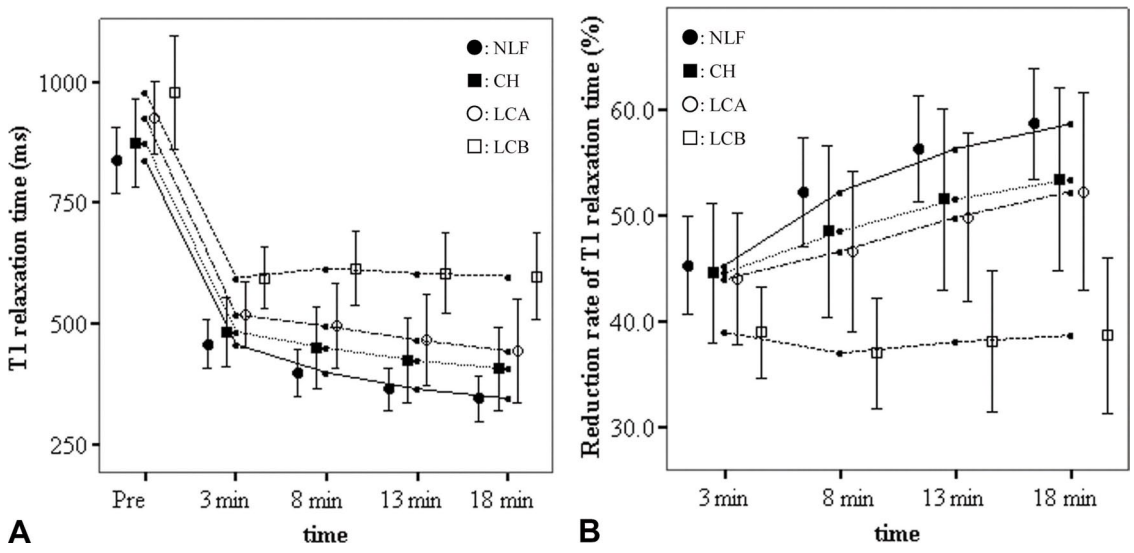

FIGURE 3. Line graphs of T1 relaxation time (A) and reduction rate of T1 relaxation time of the liver (B). Data represent mean ± 1 standard deviation. The 4 groups are represented as follows: ●, NLF; ■, CH; ○, LCA; □, LCB. LCB showed a significant difference from the other groups at ≥8 minutes in each graph.

(NLF-LCA, $P = 0.023$; NLF-LCB, $P < 0.001$), and between CH and LCB ($P = 0.001$). T1 relaxation times of liver cirrhosis groups LCA and LCB were significantly prolonged in comparison with that of NLF, and that of LCB was significantly prolonged in comparison with that of CH (Table 2, Figs. 1A, 2A, 3A).

In terms of postcontrast T1 relaxation time, T1 relaxation times were significantly prolonged for LCB in comparison with other groups at 3, 8, 13, and 18 minutes (NLF-LCB, $P < 0.001$ at all time points; CH-LCB, $P < 0.001$ at all time points; LCA-LCB, $P = 0.004$ at 3 minutes, $P < 0.001$ at ≥8 minutes), and T1 relaxation times were significantly prolonged for LCA in comparison with those for NLF at 3, 8, 13, and 18 minutes ($P =$ 0.043, 0.003, 0.003, and 0.006, respectively) (Table 2, Figs. 1B–E, 2B–E, 3A).

Also, sensitivities, specificities, and accuracies at various T1 relaxation times were calculated for distinguishing LCB from other groups (Table 3). Cut-off value of T1 relaxation time to distinguish LCB from other groups with best accuracy was 520≤ ms (sensitivity, 88.2%; specificity, 89.2%; accuracy; 89.0%).

Reduction Rate of T1 Relaxation Time of the Liver

At 3 minutes after Gd-EOB-DTPA administration, reduction rates of T1 relaxation time were significantly lower for LCB than for NLF or CH (NLF-LCB, $P = 0.014$; CH-LCB, $P = 0.008$), and at

TABLE 3. Various Cut-off Values and MR Performance to Distinguish LCB From Other Groups: T1 Relaxation Time (Milliseconds) of the Liver After Gd-EOB-DTPA Administration

T1 Relaxation Time (ms)	8 min			13 min			18 min		
	Sens	Spec	Accu	Sens	Spec	Accu	Sens	Spec	Accu
480≤	94.1	71.6	75.8	94.1	75.7	79.1	94.1	82.4	84.6
500≤	94.1	75.7	79.1	94.1	81.1	83.5	94.1	85.1	86.8
520≤	94.1	78.4	81.3	88.2	86.5	86.8	88.2	89.2	89.0
540≤	88.2	83.8	84.6	76.5	87.8	85.7	76.5	90.5	87.9

Values of Sens, Spec, and Accu indicate percentage (%).

Gd-EOB-DTPA indicates gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid; MR, magnetic resonance; LCB, liver cirrhosis with Child-Pugh B; Sens, sensitivity; Spec, specificity; Accu, accuracy.

TABLE 4. Reduction Rate (%) of T1 Relaxation Time of the Liver

	Postcontrast			
	3 min	8 min	13 min	18 min
Group				
NLF	45.3 ± 4.6	52.3 ± 5.2	56.3 ± 5.0	58.7 ± 5.2
CH	44.6 ± 6.6	48.5 ± 8.1	51.6 ± 8.5	53.5 ± 8.9
LCA	44.0 ± 6.2	46.6 ± 7.6	49.9 ± 8.0	52.3 ± 9.4
LCB	39.0 ± 4.3	37.0 ± 5.2	38.1 ± 6.7	38.7 ± 7.4
	Adjusted *P*			
Compared groups				
NLF CH	1.000	0.074	0.220	0.205
NLF LCA	1.000	0.118	0.075	0.125
NLF LCB	0.014	<0.001	<0.001	<0.001
CH LCA	1.000	1.000	1.000	1.000
CH LCB	0.008	<0.001	<0.001	<0.001
LCA LCB	0.061	0.001	<0.001	<0.001

Values indicate mean ± 1 standard deviation.

The Bonferroni *t* test was used to compare the 4 groups, and adjusted *P* values represented the original *P* value multiplied by 6, as the total comparison number.

NLF indicates normal liver function; CH, chronic hepatitis; LCA, liver cirrhosis with Child-Pugh A; LCB, liver cirrhosis with Child-Pugh B.

≥8 minutes, rates were significantly lower for LCB than for all other groups (NLF-LCB, $P < 0.001$ at ≥8 minutes; CH-LCB, $P < 0.001$ at ≥8 minutes; LCA-LCB, $P = 0.001$ at 8 minutes, $P < 0.001$ at ≥13 minutes) (Table 4, Fig. 3B). NLF, CH, and LCA showed no significant differences (Table 4, Fig. 3B).

Also, sensitivities, specificities, and accuracies at various reduction rates were calculated for distinguishing LCB from other groups (Table 5). Cut-off value of reduction rate to distinguish LCB from other groups with best accuracy was 43% or 45% (sensitivity, specificity, accuracy; 64.7%, 89.2%, 84.6%; 82.3%, 85.1%, 84.6%, respectively).

DISCUSSION

Gd-EOB-DTPA is a contrast medium with T1-shortening effects, and some researchers have assessed liver function using signal intensity on T1-weighted imaging with Gd-EOB-DTPA.[9–14] To the best of our knowledge, there has been no report to evaluate liver function by T1 relaxation time with Gd-EOB-DTPA. The signal intensity of MR imaging is not an absolute value unlike CT value. It can depend on the gain of radiofrequency amplifier, and it can vary considerably each time. Therefore, quantitative comparison of signal intensity in each image before and after contrast enhancement does not show any straightforward relationships. So, we cannot directly compare signal intensities alone using ROIs in hepatic parenchyma. Conversely, T1 relaxation time is an absolute value, and is unaffected by these different factors, so T1 relaxation time as measured by the Look-Locker sequence is considered to be a very useful method for comparing pre- and postcontrast enhancement.[21,22]

TABLE 5. Various Cut-off Values and MR Performance to Distinguish LCB From Other Groups: Reduction Rate (%) of T1 Relaxation Time Between Pre-and Postcontrast

Reduction Rate (%)	8 min			13 min			18 min		
	Sens	Spec	Accu	Sens	Spec	Accu	Sens	Spec	Accu
≤43	94.1	75.7	79.1	70.6	85.1	82.4	64.7	89.2	84.6
≤45	100	71.6	76.9	94.1	81.1	83.5	82.3	85.1	84.6
≤47	100	64.9	71.4	100	79.7	83.5	94.1	81.1	83.5

Values of Sens, Spec, and Accu indicate percentage (%).

MR indicates magnetic resonance; LCB, liver cirrhosis with Child-Pugh B; Sens, sensitivity; Spec, specificity; Accu, accuracy.

Precontrast T1 relaxation times in liver cirrhosis groups LCA and LCB were prolonged compared with NLF. Prolonged T1 relaxation time in liver cirrhosis has been reported previously, and prolonged T1 relaxation time without contrast media may suggest liver cirrhosis.[23–25] However, no correlation was found between the degree of fibrosis and T1 relaxation time, and prolonged T1 relaxation time is not specific to liver cirrhosis.[26–28] T1 relaxation time without contrast media thus does not seem useful to estimate liver function.

Postcontrast T1 relaxation times were significantly longer for LCB than for other groups at any time point. Also, times for LCA were longer than those for NLF. Postcontrast T1 relaxation time may enable us to distinguish liver cirrhosis from normal liver and to evaluate degree of liver cirrhosis, given the significant difference between LCA and LCB. However, shortening of the postcontrast T1 relaxation time for NLF and prolonged postcontrast T1 relaxation time in LCA and LCB liver cirrhosis may be affected by precontrast T1 relaxation time. We therefore calculated the reduction rate of T1 relaxation time to evaluate the degree of Gd-EOB-DTPA uptake in liver parenchyma. Reduction rate of T1 relaxation times were significantly lower in LCB than in other groups.

Organic anion-transporting polypeptide 1 (OATP1) is known to mediate uptake of Gd-EOB-DTPA by hepatocytes and multidrug resistance protein 2 mediates biliary excretion of Gd-EOB-DTPA.[29,30] In addition, expression of OATP1 and multidrug resistance protein 2 is reportedly decreased in hepatitis and cirrhosis.[31–34] Reduced reduction rates of T1 relaxation time in LCB may be explained by hypofunction of OATP1 and fewer normal functioning hepatocytes in liver cirrhosis.

At 3 minutes after Gd-EOB-DTPA administration, no significant difference in reduction rate of T1 relaxation time was seen between LCA and LCB. This was caused by insufficient hepatocytic uptake of Gd-EOB-DTPA and comparative persistence of Gd-EOB-DTPA in blood plasma at a few minutes after Gd-EOB-DTPA administration. Later time points such as 18 minutes were more appropriate to evaluate liver function, because accuracy of T1 relaxation time and reduction rate at 18 minutes to distinguish LCB from other groups was higher than the other time points. CH was not significantly distinguishable from NLF or LCA by T1 relaxation time or reduction rates of T1 relaxation time, although longer T1

relaxation times and lower reduction rates were seen in order of LCA, CH, and NLF.

In our study, LCB was strikingly distinguishable from other groups with very significant differences in postcontrast T1 relaxation time and reduction rate of T1 relaxation time ($P < 0.001$ each). By using cut-off values of 520 milliseconds for T1 relaxation time or 43% to 45% for reduction rate of T1 relaxation time at 18 minutes, our T1 mapping technique yielded high accuracy for distinguishing LCB from other groups (89.0% or 84.6%, respectively). Child-Pugh classification has been reported as a significant prognostic factor after therapies of partial hepatic resection, radiofrequency ablation, percutaneous ethanol injection therapy and transarterial chemoembolization, and the survival rate after these therapies is shorter for Child-Pugh B patients than for Child-Pugh A patients.[35–39] Child-Pugh classification is one of the most generally used parameters to evaluate reserve liver function. Also, the indocyanine green clearance (ICG) test is an important parameter to evaluate liver function, as with the Child-Pugh classification, and is used to estimate the safe limits of the hepatic resection rate.[40–42] We were sorry that we were not able to inspect the ICG test for the patients. In past articles, the ICG test showed the most significant correlation with Gd-EOB-DTPA dynamic state of any single parameter (serum albumin, serum total bilirubin, prothrombin time, etc).[12,14,43] Evaluation of hepatic uptake of Gd-EOB-DTPA, which showed a significant correlation to Child-Pugh classification and ICG test, may be applicable to estimate liver function. Imaging modalities such as T1 mapping used in this study enable the evaluation of not only whole liver function, but also regional liver function, and may be applied to estimate safe segmental hepatic resection volume and evaluate liver function before and after focal radiofrequency ablation, percutaneous ethanol injection therapy, or transarterial chemoembolization. When liver function is inhomogeneous, Child-Pugh classification or ICG test may be occasionally inadequate for estimating residual liver function after a partial hepatectomy. On the other hand, T1 mapping can be more accurate for estimation of residual liver function, because the technique can evaluate regional liver function.

We used 3-T system in this study. Because T1 relaxation times are reportedly longer and T1 shortening effects of contrast material are more prominent at 3-T compared with 1.5-T, 3-T system is more useful for this study. However, the values reported in this study could be somewhat different at 1.5 T.[44]

The present study has some limitations. First, our group classification may not have precisely reflected histologic hepatic damage, given the lack of histologic proof for the majority of patients in our study. There may be some patients assigned to a wrong group, in particular when we classified the patient in CH or LCA, though the imaging findings were reliable for differential diagnosis of CH and LCA.[17–19] Indeed, Child-Pugh scores were not different between CH and LCA groups in our study. This might cause no significant differences in both T1 relaxation time and reduction rate of 1 relaxation time between CH and LCA. However, our study revealed that T1 mapping could distinguish LCB from these groups. Second, we compared hepatic uptake of Gd-EOB-DTPA with only Child-Pugh classification. We did not use other tests for evaluating liver function, such as ICG test. Further studies were required. Third, we obtained images until 18 minutes after Gd-EOB-DTPA administration, so signal changes in MR imaging were not determined after 18 minutes. However, Reimer et al found no further increase in liver enhancement at 45 minutes after Gd-EOB-DTPA administration in comparison to that at 20 minutes.[45] With daily clinical use, the ability to evaluate imaging within around 20 minutes after Gd-EOB-DTPA administration appears desirable, and we obtained significant differences among groups classified according to Child-Pugh classification within 18 minutes after Gd-EOB-DTPA.

In conclusion, T1 mapping calculated with the Look-Locker sequence appears very useful for measuring signal intensity in liver parenchyma, and evaluation of hepatic uptake of Gd-EOB-DTPA using T1 mapping can help estimate liver function.

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Preface

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The prognosis of patients with pancreatic cancer is extremely poor. The incidence and mortality of patients with pancreatic cancer are almost equal, and the number of patients dying from pancreatic cancer has recently been increasing. Difficulties in the detection and identification of pancreatic cancer are closely related to the poor prognosis of the disease. Many attempts to improve the detection of pancreatic carcinoma at an early stage have been taken. For effective surveillance, the risk factors for pancreatic cancer development have to be determined. Various foods, coffee, cigarette smoking and the intake of animal protein have been associated with an increased risk of pancreatic cancer. Numerous reports have described the relationships between chronic pancreatitis, pancreatic lithiasis and pancreatic cancer. The incidence of pancreatic cancer was found to be generally increased in diabetics, and cohort studies have suggested a close relationship between pancreatic cancer and diabetes mellitus, although the underlying mechanisms remain unclear. More carcinomas might be detected at an early stage if asymptomatic patients with specific risk factors underwent preventive imaging examinations.

The identification of reliable biomarkers for pancreatic cancer in serum and pancreatic juice is extremely important. CEA, CA19-9, DUPAN-2, SPAN-1 and elastase-1 are well-established tumor markers of pancreatic cancer. Other serum tumor markers, e.g. mesothelin [1], synuclein-γ [2] and MIC1 (macrophage inhibitor cytokine 1) [3], have recently been detected, and increases in their expression were consistently observed in tumor tissues. This issue of *Pancreatology* moreover discusses the role of S100P and hENT1 (human equilibrative nucleoside transporter 1), as well as CEA, CA19-9, and DUPAN-2 in pancreatic cancer [4]. As mentioned above, diabetes mellitus, chronic pancreatitis and cystic lesions in the pancreas are risk factors for pancreatic cancer. In particular, invasive ductal cancers sometimes occur in patients with intraductal papillary mucinous neoplasms [5].

Additionally, technical innovations in imaging techniques are crucial for the detection of pancreatic cancers. Transabdominal ultrasonography is frequently used in the first-line surveillance of pancreatic cancers. Recent advances in contrast harmonic ultrasonography have contributed to improvements in the identification and characterization of pancreatic cancers [6]. Contrast-enhanced endoscopic ultrasonography (EUS), which is performed by attaching a probe at the tip of the ultrasound endoscope used for contrast-enhanced harmonic ultrasonography, has also turned out to be useful for pancreatic cancer [7]. Technical innovations such as contrast harmonic imaging in EUS have further improved the characterization of pancreatic masses, particularly of

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small tumors that are only depicted by EUS [8]. EUS is superior to all other imaging modalities for the detection of small pancreatic cancers [9–12].

Besides, EUS-guided fine needle aspiration (EUS-FNA) plays an important role in the pathological diagnosis of pancreatic tumors [10]. EUS-FNA is more sensitive in the diagnosis of cancers than any other method, reaching a sensitivity of >90%. Furthermore, EUS-FNA can be used for therapeutic purposes. Since the celiac ganglion located adjacent to the stomach can be easily punctured under EUS guidance, EUS-guided neurolysis is useful for pain relief in patients with pancreatic cancer [13]. EUS-guided puncture can also be employed for biliary drainage in difficult cases by establishing transpapillary drainage [14, 15]. For the treatment of patients requiring biliary tract drainage for malignant biliary tract stenosis, various methods have been developed to puncture the gallbladder or bile duct via the stomach or duodenum under EUS guidance [16], and the patients' quality of life has markedly improved following the introduction of these techniques.

Another unique approach is EUS elastography, which may help to accurately discriminate between malignant and benign nodules [17]. Presently, however, the specificity of this approach is unsatisfactory, and further improvements in equipments and techniques are anticipated. Although not directly related to pancreatic cancer, angiotensin-II and reactive oxygen species seem to be involved in the development of pancreatic fibrosis and hence pancreatic cancer, and this is therefore a topic that deserves attention [18].

This special issue entitled 'Pancreatic Cancer: Hot Topics in 2011' compiles a selection of articles that describe the hot topics that pancreatologists will encounter in 2011. We hope that readers in this field will find this special issue both educational and informative.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

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Involvement of Angiotensin II and Reactive Oxygen Species in Pancreatic Fibrosis

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Key Words
Angiotensin II · Angiotensin receptor blocker · AT1R · α-Smooth muscle actin · Pancreatic fibrosis · Reactive oxygen species · Wistar Bonn/Kobori rat

Abstract
Background: Pancreatic cancers often develop in the context of pancreatic fibrosis caused by chronic pancreas inflammation, which also results in the accumulation of reactive oxygen species (ROS), pancreatic parenchymal cell death, and stellate cell activation. Angiotensin II, which is converted from angiotensin I by the angiotensin-converting enzyme (ACE), stimulates ROS production via NADPH oxidase. In stellate cells, angiotensin II activates the stress-activated protein kinase p38. However, the molecular mechanism by which angiotensin II regulates pancreatic inflammation and fibrosis remains to be determined. ***Methods:*** Wistar Bonn/Kobori (WBN/Kob) rats spontaneously develop chronic pancreatic inflammation. To examine whether blockade of the renin-angiotensin system affects the development of pancreatic fibrosis, WBN/Kob rats were given angiotensin II type 1 receptor (AT1R) blocker or ACE inhibitor (ACEI). Next, we assessed the role of angiotensin II and its possible downstream target p38α in stellate cell activation using primary stellate cells. ***Results:*** Treatment with AT1R blocker and ACEI prevented the development of chronic pancreatitis and fibrosis. In stellate cells, angiotensin II upregulated the expression of angiotensin II receptors, α-smooth muscle actin (SMA) and transforming growth factor-β. In addition, p38α was found to be essential to collagen type I production and α-SMA expression. ROS accumulation is enhanced in chronic pancreatic inflammation, which increases the risk of pancreatic cancer. ***Conclusions:*** Inhibition of the angiotensin II signaling pathway might be a promising strategy to prevent pancreatic fibrogenesis and subsequent carcinogenesis.

Introduction

Pancreatic cancer is a highly lethal disease with the worst prognosis of all the major malignancies. It is difficult to predict, detect and diagnose, and is resistant to all current treatments except early surgery. Long-standing chronic pancreatitis is a substantial risk factor for the development of pancreatic cancer. A multicenter study of more than 2,000 patients with chronic pancreatitis showed a 26-fold increase in the risk of developing pancreatic cancer, which increased linearly with time,

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amounting to 4% in patients with chronic pancreatitis diagnosed 20 years ago [1]. Chronic pancreatitis is an irreversible progressive disease characterized by destruction of exocrine parenchyma and subsequent fibrosis. The stellate cell is the major cell type involved in fibrosis. Repeated episodes of acute pancreatitis can lead to increasing residual damage to the gland and chronic inflammation, eventually resulting in stellate cell activation and pancreatic fibrosis [2]. However, the molecular mechanism by which inflammation accelerates carcinogenesis in the pancreas remains to be determined.

The renin-angiotensin system (RAS) is traditionally considered an endocrine system regulating blood pressure and body fluid homeostasis [3]. Angiotensin I is converted into angiotensin II through removal of two C-terminal residues by the angiotensin-converting enzyme (ACE). Angiotensin II is the physiologically active mediator of RAS. The biological roles of angiotensin II are mediated by high-affinity membrane-bound receptors, which are classified into two subtypes: angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R) [4]. Angiotensin II interaction with AT1R stimulates the production of reactive oxygen species (ROS) via the mediation of the NADPH oxidase system [5, 6]. In stellate cells, angiotensin II activates p38 mitogen-activated protein kinase (MAPK) [7]. RAS components are intrinsically present in the pancreas and their levels are enhanced during pancreatic inflammation [8]. There are several controversial reports regarding the effects of RAS inhibition in pancreatitis. Ulmasov et al. [9] reported that angiotensin II signaling did not play a role in the development of cerulein-induced chronic pancreatitis. In a study by Tsang et al. [10] in rats, ramiprilat, an ACE inhibitor (ACEI), enhanced acute pancreatitis. Blockade of AT1R by losartan ameliorated pancreatic injury induced by cerulein, whereas blockade of AT2R by PD123319 did not result in any beneficial effect [11]. We now show that AT1R blocker and ACEI prevent the development of pancreatic fibrosis. AT1R blocker, but not ACEI, reverses established pancreatic fibrosis. Furthermore, angiotensin II activates stellate cells most probably via the p38α signaling pathway.

Materials and Methods

Animals

All animal procedures were performed in accordance with the guidelines on the care and use of animals in research of Kinki University. Four-week-old male Wistar Bonn/Kobori (WBN/Kob) rats were purchased from Japan SLC Inc. (Shizuoka, Japan). Age-matched healthy Wistar rats were used as controls. Rats were fed a special breeding diet (MB-3; Funabashi Farm, Chiba, Japan) throughout the experimental period [12]. The ACEI perindopril and the AT1R blocker candesartan were supplied by Daiichi Pharmaceutical Co. (Tokyo, Japan) and Takeda Pharmaceutical Co. (Tokyo, Japan), respectively. Rats were orogastrically challenged with either perindopril or candesartan once daily at a dose of 8 mg/kg, a dose which is clinically comparable to the one used by Yoshiji et al. [13]. $p38\alpha^{F/F}$ mice have previously been described by Nishida et al. [14]. $p38\alpha^{\Delta L+H}$ mice were generated by treating $p38\alpha^{F/F}$*:Mx1-Cre* mice with poly(IC) as previously described [15]. They were housed in cages at a temperature of 23 ± 3°C and a relative humidity of 50 ± 20%, with a 12-hour light/dark cycle.

Biochemical and Immunochemical Analyses

Immunoblotting, immunohistochemistry, and RNA isolation have already been described [15]. Anti-α-smooth muscle actin (α-SMA; Sigma-Aldrich, St. Louis, Mo., USA) was used as antibody. ROS accumulation in the pancreas was assessed by the OxyBlot Protein Oxidation Detection Kit (Millipore, Billerica, Mass., USA). TaqMan reverse transcription reagents were used as described in the manual of the ABI Prism 7700 Sequence Detection System (PE Applied Biosystems, Foster City, Calif., USA). Sense and antisense primers for AT1aR were 5′-GCACACTGGCAATGTAATGC-3′ and 5′-GTTGAACAGAACAAGTG ACC-3′, respectively. Sense and antisense primers for AT1bR were 5′-GCCTGCAAGTGAAGTGATTT-3′ and 5′-TTTAACAGTGGCTTTGC TCC-3′, respectively. Sense and antisense primers for AT2R were 5′-CAAGACTTGGTCACG GGT-3′ and 5′-CTGGCTGTGGCTGACTT-3′, respectively. Sense and antisense primers for GAPDH were 5′-TCCCTCAAGATTGTCAGCAA-3′ and 5′-AGATCCACAACGGATACATT-3′, respectively. The sequences of the primers amplifying collagen type I α_1, α-SMA and transforming growth factor (TGF)-β have been described previously [16]. 7s-collagen and procollagen III N-terminal peptide (PIIIP) in the serum were measured as described by Yoshiji et al. [13].

Isolation and Culture of Stellate Cells

Rat pancreatic stellate cells were prepared from pancreatic tissues of male Wistar rats weighing 200–250 g as described previously using the Nycodenz solution (Nycomed Pharma, Oslo, Norway) after perfusion with 0.03% collagenase P [17]. The cells were resuspended in Ham's F-12 containing 10% fetal bovine serum (ICN Biomedicals, Aurora, Ohio, USA), penicillin sodium, and streptomycin sulfate. Cell purity was confirmed at ≥90% by detecting vitamin A autofluorescence. Stellate cells were cultured in the medium with or without angiotensin II (1 or 10 μM; Bachem, Bubendorf, Switzerland) and angiotensin II receptor blocker (0.1 or 1 μM), following culture in serum-free medium for 24 h. Hepatic stellate cells were isolated from $p38\alpha^{\Delta L+H}$ mice and control $p38\alpha^{F/F}$ mice, and cultured as described previously [18].

Statistical Analysis

Data are presented as means ± SEM. Differences were analyzed by Student's t test. p values <0.05 were considered significant.

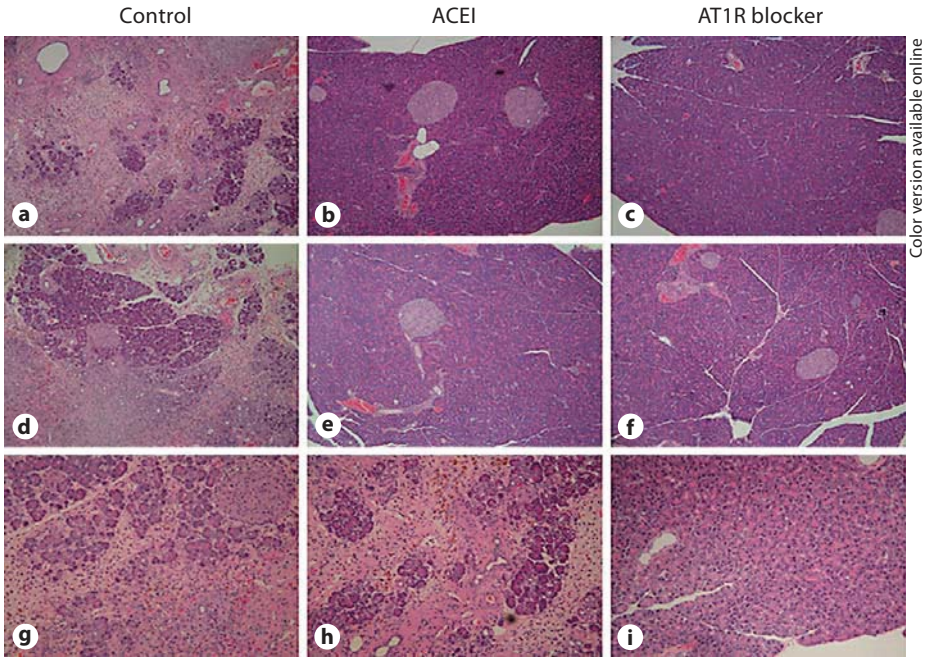

Fig. 1. AT1R inhibition prevented the development of chronic pancreatitis and pancreatic fibrosis. **a–f** WBN/Kob rats were orogastrically challenged with AT1R blocker or ACEI starting at 4 weeks of age. Rats were sacrificed at 12 (**a–c**) and 16 weeks of age (**d–f**), and sections of the pancreas were examined using HE. **g–i** Treatment with AT1R blocker or ACEI was started at 12 weeks of age, and rats were sacrificed at 16 weeks of age. Representative slides are shown.

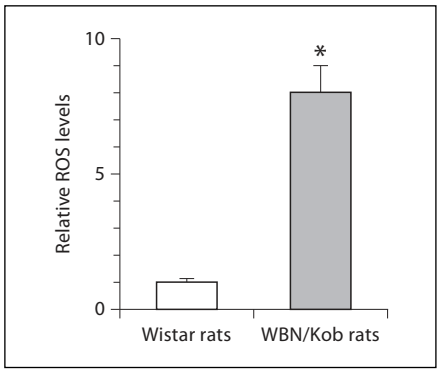

Fig. 2. ROS accumulation was enhanced in pancreatic inflammation and fibrosis compared to normal pancreas. ROS accumulation was assessed in the pancreas of 16-week-old WBN/Kob rats (n = 4) and Wistar rats (n = 3) by immunoblotting (OxyBlot) and quantified using NIH image software. Results are means ± SEM. * $p < 0.05$ versus normal pancreas.

Results

Inhibition of AT1R Prevented the Development of Chronic Pancreatitis and Pancreatic Fibrosis

WBN/Kob rats spontaneously develop chronic pancreatitis and pancreatic fibrosis. Pathohistologically, at 12 weeks of age, inflammatory cell infiltration, hemorrhage, hemosiderin deposition and fibrosis around the pancreatic ducts or blood vessels were observed (fig. 1a). To assess the role of RAS in chronic pancreatitis and pancreatic fibrosis, WBN/Kob rats were orogastrically challenged with AT1R blocker or ACEI once daily starting at 4 weeks of age. AT1R blocker and ACEI significantly suppressed the development of chronic pancreatitis and pancreatic fibrosis (fig. 1a–f). When treatment with AT1R blocker or ACEI was started at 12 weeks of age, AT1R blocker significantly reversed already established pancreatic inflammation and fibrosis (fig. 1i). In contrast, ACEI did not reverse pancreatic fibrosis (fig. 1h). Thus, angiotensin II is critical for pancreatic fibrosis. A causal link between oxidative stress and fibrosis was proposed [19]. Indeed, ROS accumulation was enhanced in chronic pancreatitis and fibrosis in comparison with the normal pancreas of Wistar rats used as controls (fig. 2).

Inhibition of RAS Downregulates α-SMA and TGF-β Expression in the Pancreas

Pancreatic fibrosis is a common consequence of chronic pancreas injury [2]. Stellate cells which undergo a transition from a quiescent to an activated state after tissue injury play an important part in the pathogenesis of pancreatic fibrosis [20, 21]. Stellate cell activation includes an increased proliferation rate, a phenotypic transition to a myofibroblast-like, α-SMA-positive cell, and a dramatic increase in the synthesis of extracellular matrix proteins.

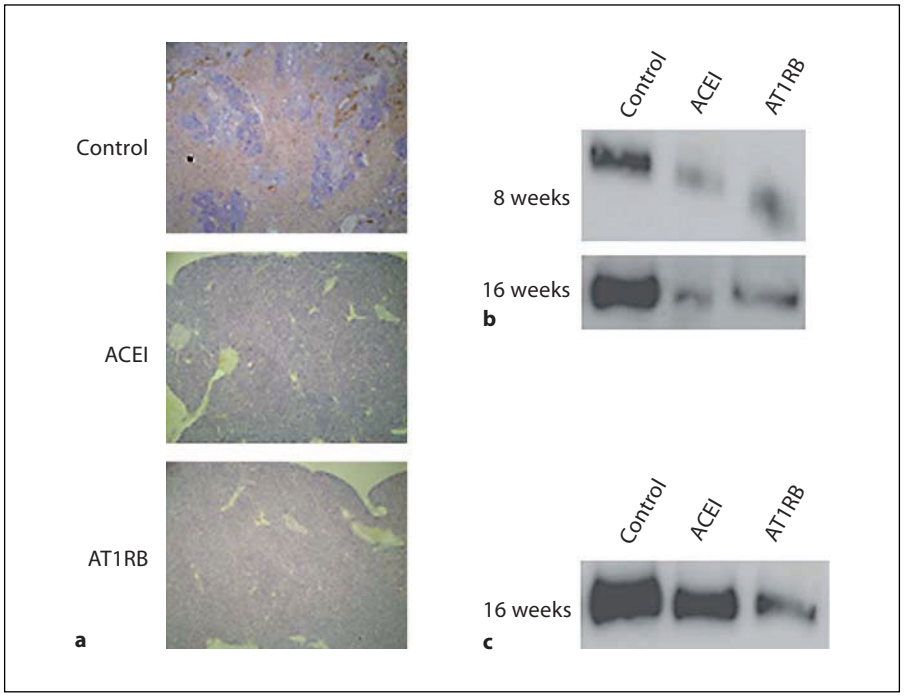

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Fig. 3. RAS inhibition downregulated α-SMA expression in the pancreas. **a** WBN/Kob rats were treated with AT1R blocker (AT1RB) or ACEI starting at 4 weeks of age. Rats were sacrificed at 16 weeks of age. Sections of the pancreas were examined by immunohistological staining with α-SMA-specific antibody. **b** WBN/Kob rats were treated with AT1RB or ACEI starting at 4 weeks of age. Rats were sacrificed at the indicated times. Lysates of the pancreas were gel separated and immunoblotted with α-SMA antibody. **c** Treatment with AT1RB or ACEI was started at 12 weeks of age, and rats were sacrificed at 16 weeks of age. Lysates of the pancreas were gel separated and immunoblotted with α-SMA antibody.

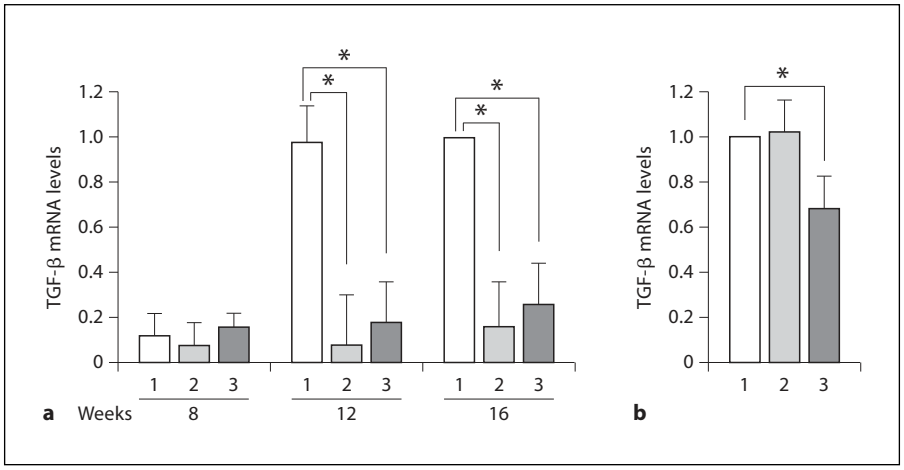

Fig. 4. RAS inhibition downregulated TGF-β expression in the pancreas. **a** WBN/Kob rats were treated with AT1R blocker or ACEI starting at 4 weeks of age. **b** Treatment with AT1R blocker or ACEI was started at 12 weeks of age, and rats were sacrificed at 16 weeks of age. **a**, **b** Pancreas RNAs were extracted and levels of TGF-β mRNAs were determined by real-time quantitative PCR. 1 = Untreated rats; 2 = ACEI-treated rats; 3 = AT1R blocker-treated rats. Results are means ± SEM. * $p < 0.05$.

At the age of 16 weeks, we observed inflammation, stellate cell activation, and formation of fibrotic septa assessed by immunohistochemistry with a specific antibody against α-SMA (fig. 3a). There were lower numbers of α-SMA-positive cells in AT1R blocker- or ACEI-treated rats compared to control rats (fig. 3a). Western blot analysis also showed marked reduction in α-SMA expression following treatment with AT1R blocker or ACEI (fig. 3b). When treatment with AT1R blocker or ACEI was started at 12 weeks of age, AT1R blocker, but not ACEI, decreased α-SMA expression (fig. 3c).

TGF-β is a potent pro-fibrotic factor and many in vitro studies using cell systems have implicated its functional role in the pathogenesis of pancreatic fibrosis [22]. Inhibition of RAS significantly decreased expression of mRNA for TGF-β (fig. 4a). AT1R blocker, but not ACEI, reversed the established pancreatic fibrosis (fig. 1g–i). Correspondingly, AT1R blocker significantly downregulated TGF-β mRNA, whereas ACEI produced minor changes in the reduction of TGF-β mRNA levels (fig. 4b). The serum levels of 7s-collagen and PIIIP were not affected by RAS inhibition (data not shown).

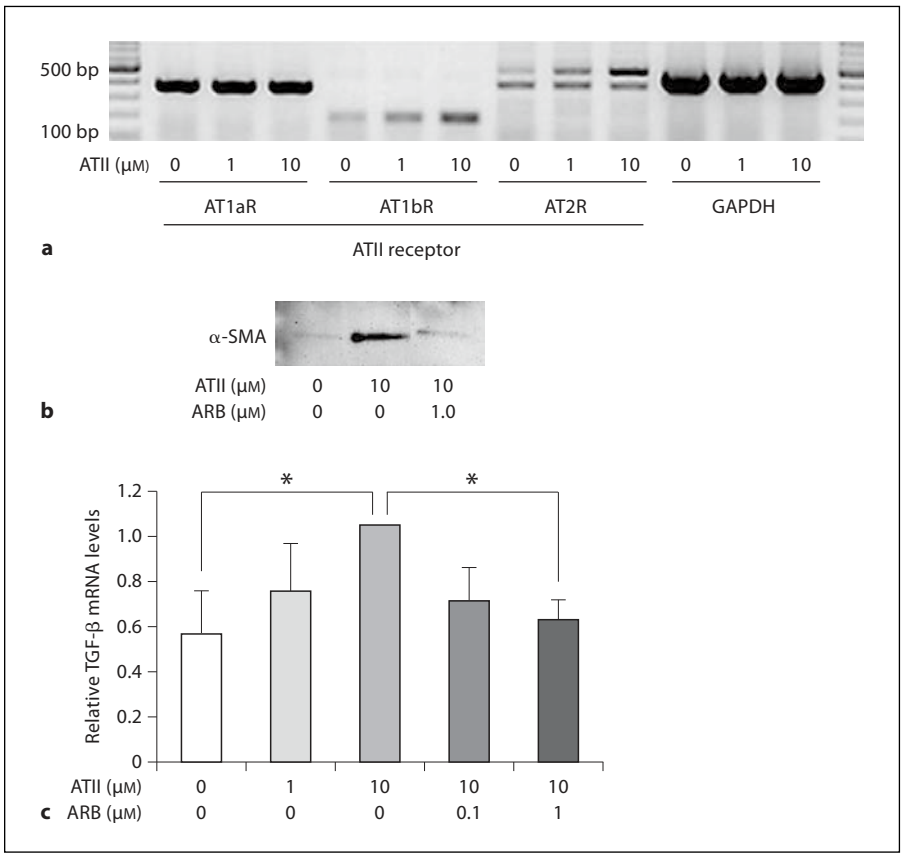

Fig. 5. Angiotensin II upregulated the expression of angiotensin II receptors, α-SMA, and TGF-β in stellate cells. Stellate cells were cultured in the medium with or without angiotensin II (ATII; 1 or 10 μM) and AT1R blocker (ARB; 0.1 or 1 μM), following culture in serum-free medium for 24 h. **a** RNAs were extracted and levels of ATII receptor mRNAs were determined by PCR. **b** Lysates were gel separated and immunoblotted with α-SMA antibody. **c** RNAs were extracted and levels of TGF-β mRNAs were determined by real-time quantitative PCR. Results are means ± SEM. * $p < 0.05$.

Angiotensin II Upregulates Expression of Angiotensin II Receptors, α-SMA and TGF-β in Stellate Cells

A local RAS has been described in the pancreas, and pancreatic stellate cells were identified as a potential target of angiotensin II action [8]. We investigated the effects of angiotensin II on angiotensin II receptors, α-SMA and TGF-β expression in pancreatic stellate cells isolated from Wistar rats. Activated stellate cells expressed AT1aR, AT1bR and AT2R. Addition of angiotensin II led to an elevation in AT1bR and AT2R but not AT1aR expression (fig. 5a). Angiotensin II stimulated stellate cell transdifferentiation and activation, as assessed by α-SMA and TGF-β expression (fig. 5b, c). Activated stellate cells are the major collagen type I-producing cells during fibrogenesis [23]. In pancreatic stellate cells, p38 MAPK is activated by angiotensin II [7]. To assess the role of p38α, the major p38 MAPK isoform, in stellate cell activation, hepatic stellate cells were isolated from livers of $p38\alpha^{\Delta L+H}$ mice. Stellate cells in $p38\alpha^{\Delta L+H}$ mice were deficient in p38α (data not shown). p38α-deficient stellate cells produced less collagen type I α_1 and α-SMA compared to control stellate cells (fig. 6).

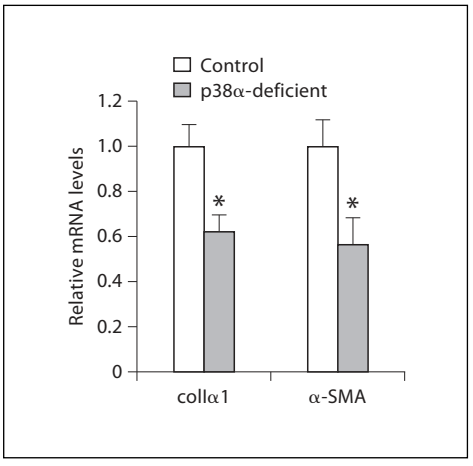

Fig. 6. p38α plays an important role in the production of collagen type I α_1 and α-SMA in hepatic stellate cells. RNAs were extracted and levels of mRNAs were determined by real-time quantitative PCR. colIα_1 = Collagen type I α_1. Results are means ± SEM. * $p < 0.05$.

Discussion

The main bioactive peptide of RAS is angiotensin II, which is produced from its hepatic precursor angiotensinogen. The main angiotensin II receptors are G protein-coupled receptors designated as AT1R and AT2R. The balance of AT1R and AT2R expression is an important pathological factor [24]. Angiotensin II upregulates AT1R and AT2R expression in stellate cells, suggesting that angiotensin II establishes a positive feedback loop in RAS. The majority of the pathophysiological functions of angiotensin II are mediated through AT1R [11]. In contrast to AT1R signaling, AT2R signaling modulates protective antifibrogenic effects [25]. Correspondingly, we found that AT1R blocker reverses pancreatic fibrosis, whereas ACEI, which blocks both AT1R and AT2R, does not ameliorate established fibrosis. Thus, AT1R and AT2R have distinct effects on pancreatic fibrogenesis.

Oxidative stress is thought to play a major role in the pathogenesis of pancreatic fibrosis [19] and cancer development [26, 27], exerting many effects, including alterations in gene expression [28], enhanced cell death and proliferation, and induction of higher DNA mutation rates [29], as well as genomic instability [27]. Epidemiological studies have demonstrated an association between pancreatic fibrosis and cancer [1]. Indeed, we found enhanced ROS accumulation in pancreatic fibrosis compared to normal pancreatic tissue, which increases the risk of pancreatic cancer. Given that angiotensin II regulates ROS production [5, 6], angiotensin II-mediated oxidative stress would promote pancreatic fibrogenesis. However, the exact impact of oxidative stress and antioxidant responses on chronic inflammation and subsequent pancreatic cancer development needs to be further elucidated.

MAPK and stress-activated protein kinase (SAPK) play a pivotal role in the transduction of extracellular signals to the nucleus, thereby modulating numerous cellular responses, including cell survival, proliferation, differentiation, and metabolism [30]. One of the SAPKs, p38α, the major p38 MAPK isoform, is activated in response to inflammation and oxidative stress and in turn controls the expression of several cytokines, inflammatory mediators, survival genes, and antioxidants [15, 31]. As ubiquitous p38α ablation in all cells results in mid-gestational lethality mainly due to placental insufficiency [32], we used a conditional *p38α* 'floxed' *($p38\alpha^{F/F}$)* strain [14] to generate $p38\alpha^{\Delta L+H}$ mice [15], whose hepatic stellate cells as well as hepatocytes and Kupffer cells are deficient in p38α (data not shown). In the present study, we observed that angiotensin II upregulates the expression of α-SMA and that p38α is essential to α-SMA expression in stellate cells. Angiotensin II is known to activate p38 MAPK [7]. Taken together, angiotensin II stimulates stellate cell activation, as assessed by α-SMA expression, most probably through the p38 signaling pathway. Enhanced ROS accumulation is associated with increased inflammation and pancreatic fibrogenesis, which would eventually contribute to the development of pancreatic cancer.

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Disclosure Statement

The authors declare that they have no financial conflict of interest.

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Endoscopic Ultrasonography and Contrast-Enhanced Endoscopic Ultrasonography

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Key Words
Contrast-enhanced harmonic imaging · Doppler imaging · Endoscopic ultrasonography · Microvessels · Parenchymal perfusion · Small pancreatic cancer · Ultrasound contrast agent

Abstract
Endoscopic ultrasonography (EUS) is superior to all other imaging modalities in detecting small pancreatic cancers. However, its ability to characterize hypoechoic pancreatic masses is limited: most carcinomas, neuroendocrine tumors, and inflammatory pseudotumors are simply depicted as hypoechoic masses. Contrast enhancement helps EUS to characterize such hypoechoic masses. Intravenous ultrasound (US) agents increase the signal from the blood and, thus, act as amplifiers and improve visualization of blood flow in small vessels using Doppler US. Contrast-enhanced Doppler EUS can differentiate small pancreatic carcinomas that cannot be detected by other imaging modalities. The development of second-generation US contrast agents and an EUS system with a broad-band transducer enabled the visualization of microvessels and the parenchymal perfusion in the pancreas. This contrast-enhanced harmonic EUS has shown that most pancreatic cancers exhibit hypovascular heterogeneous enhancement with irregular network-like microvessels. Moreover, it can diagnose pancreatic cancers with a high sensitivity (89–92%).

Utility of Endoscopic Ultrasonography in Pancreatic Diseases

Ultrasonography (US) and endoscopy were firstly combined in 1980 when DiMagno et al. [1] published the first report of a gastroscope equipped with an ultrasonic probe. In the same year, Strohm et al. [2] reported their experience with ultrasonic fiberendoscopy of the abdomen in 18 patients with known biliary, pancreatic, and hepatic disorders or postoperative anatomic changes. The pancreas could be studied in 50% of the patients, and the lesions in patients with known pancreatic malignancies were visualized. Since those early days, endoscopic US (EUS) using high ultrasonic frequencies has played an important role in the diagnosis of pancreatobiliary diseases. Its advantage over other imaging modalities is its ability to obtain high-resolution images of intra- and paramural structures and organs such as the pancreas after placing the ultrasonic transducer at the tip of the

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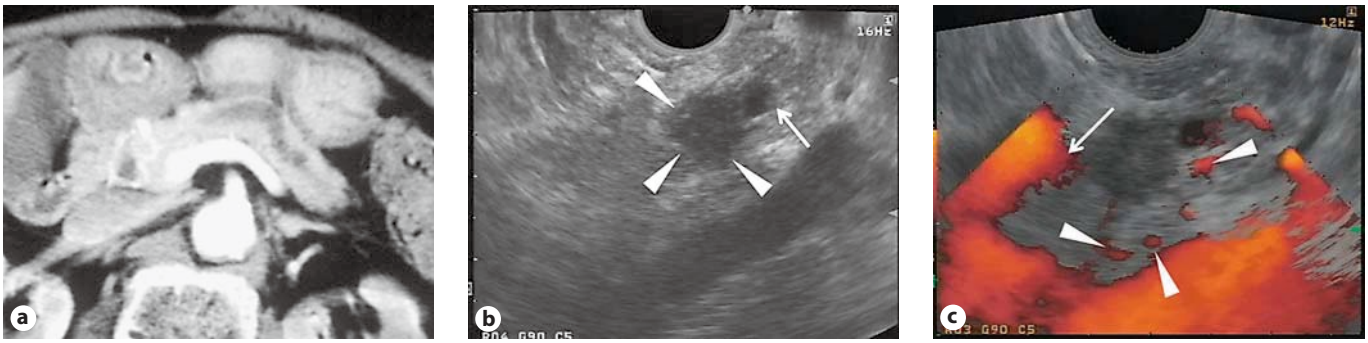

Fig. 1. Typical MDCT, EUS, and contrast-enhanced power Doppler EUS images of a small ductal carcinoma. **a** The MDCT image shows the dilated duct in the body and tail of the pancreas, but fails to depict a nodule. **b** The conventional EUS image shows a hypoechoic nodule (arrowheads) 11 mm in diameter. The arrow indicates the dilated duct. **c** The contrast-enhanced power Doppler EUS image shows the absence of vessels in the nodule, whereas several vessels are observed in the surrounding tissue (arrowheads). A blooming artifact is observed at the large vessel (arrow).

instrument into the duodenum and stomach. Several groups have shown that EUS is a highly sensitive method for the diagnosis of pancreatic tumors (fig. 1) [3–11] since it can detect a pancreatic mass with a sensitivity of ≥90%. Since EUS is also able to visualize the parapancreatic vessels, it can be used in the local staging of pancreatic and periampullary carcinomas [12–17]. However, EUS is limited in its ability to characterize pancreatic masses. Like most carcinomas, endocrine tumors and inflammatory pseudotumors caused by focal pancreatitis are simply depicted as hypoechoic masses. To characterize such hypoechoic masses, contrast enhancement is needed.

Intravenous US Contrast Agents

US contrast agents are microbubbles of gas covered by a shell of biocompatible material such as a protein, lipid or polymer [18, 19]. The diameter of contrast agents (3–10 μm) is smaller than that of red blood cells. When exposed to the high acoustic power of US beams, the microbubbles are disrupted, which releases a large amount of acoustic energy that is rich in harmonic components. At low acoustic power, specific pulse sequences that induce the microbubbles to resonate are applied for real-time imaging, producing harmonic frequencies that can be received selectively. Early US contrast agents such as Levovist and Albunex consist largely of room air microbubbles that are encapsulated in or stabilized by microspheres of albumin, lipids, or polymers. Higher acoustic power is needed to produce harmonic signals from these first-generation US contrast agents. Until recently, a contrast-enhanced harmonic imaging technique was not available for EUS because the transducer of the echoendoscope was too small to produce enough acoustic power for contrast-enhanced harmonic imaging using the first-generation US contrast agents. However, the use of gases such as perfluorocarbons and sulfur hexafluoride, which are poorly soluble and diffusible, have been found to markedly improve microbubble persistence in the peripheral circulation. This has led to the second-generation US contrast agents SonoVue, Definity and Sonazoid, which produce harmonic signals at lower acoustic power and are therefore suitable for EUS imaging.

Contrast-Enhanced EUS Using Fundamental B-Mode EUS

The first report of contrast-enhanced EUS where carbon dioxide microbubbles were employed showed that this technique is a sensitive and accurate tool for differentiating pancreatic ductal carcinomas from chronic pancreatitis and pancreatic endocrine tumors [20]. However, it requires an angiographic technique that is relatively invasive because the carbon dioxide microbubbles must be selectively infused into the celiac artery or superior mesenteric artery. The development of intravenous US contrast agents has made EUS examinations more convenient. The recently developed sonographic contrast agents, which are infused intravenously, are well tolerated

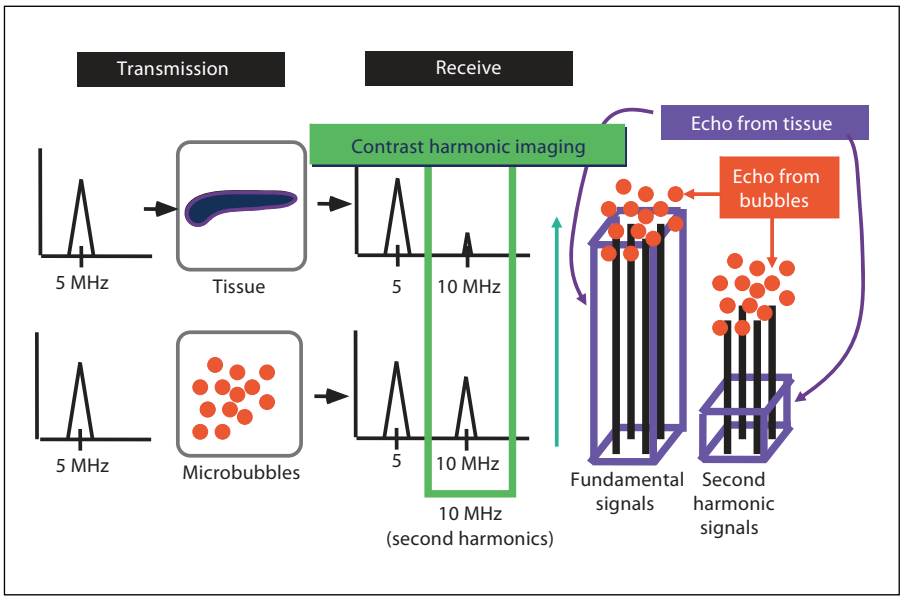

Fig. 2. Principle of contrast-enhanced harmonic imaging. The signals from the microbubbles are selectively depicted by filtering the second harmonic components.

and have a fairly good safety profile [18, 19]. Moreover, a preliminary study using the US contrast agent Albunex showed that the enhancement of normal pancreatic parenchyma in contrast-enhanced EUS after intravenous infusion of the contrast agent was superior to that of ductal adenocarcinomas [21]. However, the echo intensity of the tissue was too strong to selectively evaluate the signals emanating from the contrast agent.

Contrast-Enhanced Power and Color Doppler EUS

The intravenous US contrast agents increase the signal from the blood and act as an amplifier to improve the detectability of blood flow in small vessels by Doppler US. Contrast-enhanced power Doppler EUS is significantly more sensitive and accurate than power Doppler EUS in detecting the relatively hypovascular ductal adenocarcinomas of the pancreas (this tumor hypovascularity is observed in 85–92% of patients with ductal adenocarcinoma of the pancreas) [22–26] (fig. 1). For example, a study comparing the efficacy of contrast-enhanced power Doppler EUS with that of Doppler EUS and contrast-enhanced multidetector-row CT (MDCT) in the differential diagnosis of small pancreatic tumors revealed that these three techniques differentiated ductal carcinomas from other tumors in pancreatic masses <2 cm in diameter with sensitivities of 83, 11 and 50%, respectively [26]. This study also showed that contrast-enhanced Doppler EUS diagnosed small pancreatic carcinomas significantly better than MDCT. Thus, contrast-enhanced EUS can differentiate small pancreatic carcinomas that cannot be detected by other imaging modalities (fig. 1). However, Doppler US suffers from several limitations: blooming artifacts can appear, spatial resolution is poor, and Doppler US has a low sensitivity to detect slow flow and high sensitivity to motion artifacts. Blooming means that a blood vessel appears wider when the power Doppler mode is used compared to when fundamental B-mode imaging is performed (fig. 1). The term 'motion artifact' refers to the situation where the signal intensity of flowing blood is much lower than that of tissue movement.

Contrast-Enhanced Harmonic EUS

Contrast-enhanced harmonic imaging is a new technology that visualizes the microcirculation and parenchymal perfusion by selectively depicting the signals emanating from the US contrast agents while simultaneously filtering the signals originating from tissue (fig. 2). This is possible because when the tissue and the microbubbles receive transmitted US waves, both produce harmonic components that are integer multiples of the fundamental frequency, but the harmonic content from the microbubbles is higher than that from the tissues (fig. 2). Selective depiction of the second harmonic component visual-

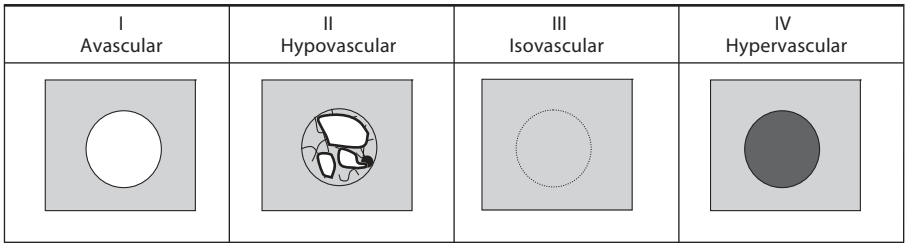

Fig. 3. Vascular patterns that can be depicted by contrast-enhanced harmonic EUS. Solid pancreatic lesions can be categorized into 4 patterns, namely avascular, hypovascular, isovascular and hypervascular.

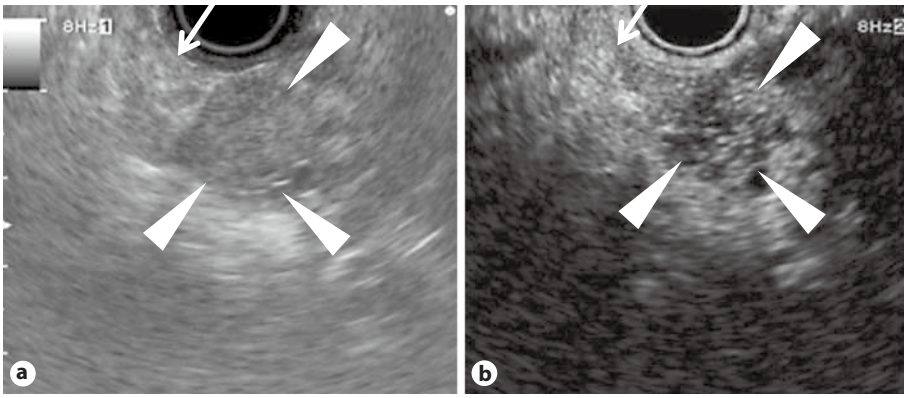

Fig. 4. Typical contrast-enhanced harmonic EUS image of a ductal carcinoma. The conventional EUS (**a**) shows a slightly hypoechoic lesion (arrowheads) in the tail of the pancreas, although the margins of the lesion are indistinct. Arrow indicates surrounding pancreatic tissue. Contrast-enhanced harmonic EUS (**b**) reveals the lesion as a hypovascular heterogeneous nodule (arrowheads) with a clear margin, while the surrounding tissue exhibits homogeneous enhancement (arrow).

izes the signals from the microbubbles more strongly than those from the tissue. This technology can detect signals from microbubbles in vessels with very slow flow without Doppler-related artifacts and has been used to characterize tumor vascularity in the liver, pancreas, gallbladder, and gastrointestinal tract during transabdominal US [18, 27–29].

Dietrich et al. [30] firstly reported the use of contrast-enhanced, low-mechanical index, real-time EUS using adapted dynamic contrast harmonic wideband pulsed inversion software. They could identify the celiac trunk, the common hepatic artery, the splenic artery, and the portal vein and its branches, as well as collaterals in patients with portal vein thrombosis. However, parenchymal enhancement was only observed in patients who did not have underlying liver disease.

The development of a new prototype echoendoscope with a broadband transducer and a specific mode for contrast-enhanced harmonic EUS has enabled us to obtain parenchymal perfusion images of the digestive tract [31–34]. The mode that is specific for contrast-enhanced harmonic imaging (ExPHD mode) strongly depicts signals from the contrast agents while filtering the signals from the tissue by synthesizing the phase shift signals with the second harmonic components. The vascular structure of some digestive diseases can also be observed using this new contrast-enhanced harmonic imaging EUS system. In contrast, contrast-enhanced Doppler EUS cannot provide such parenchymal perfusion images and images of branching vessels; instead, blooming artifacts of large vessels are observed. The new EUS system also depicts pancreatic adenocarcinomas as hypovascular nodules that mostly have irregular network-like vessels (fig. 3, 4), whereas all cystic lesions are depicted as avascular lesions.

Recently, two groups reported that this new contrast-enhanced harmonic EUS system allowed them to diagnose pancreatic carcinomas with a high sensitivity (89 and 92%) in 35 and 90 patients, respectively [35, 36]. The vast majority of the diagnosed pancreatic carcinomas exhibited hypovascularity that was characterized by a heterogeneous distribution and lower density of vessels relative to the surrounding pancreatic tissue (fig. 3, 4). Interestingly, Fusaroli et al. [36] reported that contrast-enhanced harmonic EUS allowed them to detect small lesions in 7 patients who had uncertain standard EUS findings because of biliary stents or chronic pancreatitis. This suggests that, compared with fundamental B-mode

imaging, contrast-enhanced harmonic imaging improves the depiction of some pancreatic tumors. Similarly, when Imazu et al. [37] compared the efficacy of conventional EUS and contrast-enhanced harmonic EUS in terms of preoperative T staging of pancreatobiliary tumors, they found that using contrast-enhanced harmonic EUS, T staging was correct in 24 of 26 pancreatobiliary tumors, 6 of which were misdiagnosed by conventional EUS. In particular, contrast-enhanced harmonic EUS depicts the wall of the portal vein more clearly, which means that it is superior in diagnosing portal vein invasion by pancreatic and bile duct cancers.

Conclusions

Technical innovations in EUS imaging have advanced the diagnostic accuracy of pancreatic cancers. Initial EUS procedures enabled the detection of small cancers in the pancreas. Subsequent EUS procedures with contrast enhancing agents further improved the efficacy of EUS to characterize pancreatic lesions. The most recent improvement was to add contrast-enhanced harmonic imaging, which allows the visualization of microvessels and parenchymal perfusion in the pancreas.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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Endoscopic Ultrasonography-Guided Biliary Drainage: Evaluation of a Choledochoduodenostomy Technique

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Key Words

Choledochoduodenostomy · Endoscopic ultrasonography · Endoscopic retrograde biliary drainage · Percutaneous transhepatic biliary drainage

Abstract

Background: Endoscopic ultrasonography (EUS)-guided choledochoduodenostomy (CDS) is as an alternative to percutaneous transhepatic biliary drainage (PTBD) in patients with biliary obstruction when endoscopic retrograde biliary drainage (ERBD) is unsuccessful. ***Purpose:*** We reviewed our experience and technique in patients undergoing EUS-CDS. ***Patients:*** Over a 2-year period to December 2008, 15 patients with unsuccessful ERBD underwent EUS-CDS. ***Methods:*** EUS-guided needle puncture was performed to access the bile duct from the duodenal bulb. After cholangiography, a guidewire was inserted through the needle and directed to the hepatic hilum. The punctured fistula was then dilated with a biliary dilator and a plastic stent was inserted. ***Results:*** The technical success rate of EUS-CDS was 93% (14/15 patients); 1 patient underwent an EUS-guided rendezvous approach because the choledochoduodenal fistula could not be dilated. Decompression of the bile duct was achieved in all patients. Complications included cholangitis in 4 patients, self-limiting local peritonitis in 2 and distal stent migration in 1 patient. The median follow-up time was 125 days and the median duration of stent patency was 99 days. ***Conclusion:*** EUS-CDS may be effective for patients following unsuccessful ERBD and offers an attractive alternative to PTBD.

Introduction

Endoscopic retrograde biliary drainage (ERBD) is the first choice for biliary decompression, and the success rate of this technique ranges from 90 to 95% [1]. However, ERBD may fail in patients with anatomic variations due to prior surgery, periampullary diverticula, tortuous ducts, impacted stones, or tumor infiltration. For patients with unsuccessful ERBD, next-step options include repeated ERBD [2], percutaneous transhepatic biliary drainage (PTBD) [3, 4], and surgical intervention [5].

Recent technical advancements have broadened the clinical applications of endoscopic ultrasonography (EUS)-guided fine needle aspiration in gastrointestinal diseases. Interventional EUS procedures, including ethanol injection for celiac plexus neurolysis [6], gene therapy,

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Table 1. Characteristics of the study patients undergoing EUS-CDS

Patient No.	Age years	Sex	Diagnosis	Device used for puncture	Access method	First plastic stent	Complication	Re-intervention	Follow-up period, days	End point
1	64	F	lymph node metastasis	NK	direct	pigtail	self-limiting peritonitis	–	248	dead
2	61	M	pancreatic carcinoma	NK	direct	straight	–	–	12	dead
3	83	M	pancreatic carcinoma	NK	direct	straight	cholangitis	+	142	dead
4	36	F	malignant lymphoma	NK	direct	straight	distal migration	–	619	alive
5	67	F	pancreatic carcinoma	NK	direct	straight	self-limiting peritonitis	–	125	dead
6	63	F	pancreatic carcinoma	NK	direct	straight	cholangitis	+	119	dead
7	55	F	pancreatic carcinoma	NK	direct	straight	–	–	66	dead
8	76	M	pancreatic carcinoma	FN	rendezvous	straight	–	–	201	dead
9	73	F	lymph node metastasis	NK	direct	straight	cholangitis	+	106	dead
10	87	F	pancreatic carcinoma	NK	direct	straight	–	–	142	dead
11	72	F	pancreatic metastasis	FN	direct	straight	–	–	28	dead
12	59	M	lymph node metastasis	FN	direct	straight	–	–	29	dead
13	73	M	pancreatic metastasis	FN	direct	straight	cholangitis	+	235	alive
14	62	F	pancreatic carcinoma	FN	direct	straight	–	–	125	dead
15	70	M	pancreatic carcinoma	FN	direct	straight	–	–	60	dead

NK = Needle knife; FN = fine needle.

and immunotherapy for pancreatic cancer [7], pancreatic pseudocyst drainage [8], and pancreaticogastrostomy [9], have already been reported. EUS-guided drainage of an obstructed biliary system has also been described [10]. Therefore, in this study, we state our experience with 15 patients who underwent EUS-guided choledochoduodenostomy (CDS) at our institution and review the past reports on this technique.

Patients and Methods

Patients

Between June 2006 and December 2008, 15 patients (6 males and 9 females; median age 67 years) with unsuccessful ERBD underwent EUS-CDS and were included in this study. The major papilla was not reached because of duodenal infiltration in 6 patients, and biliary cannulation was aborted in 9 patients (table 1). Two of the 15 patients showed minimal or moderate perihepatic ascites, a relative contraindication to PTBD.

Informed consent was obtained from all patients. Our institutional review board waived formal review and approved the procedure, deeming the technique to be an extension of existing procedures. All procedures were performed by three dedicated pancreatobiliary endoscopists.

Technique

ERBD was initially attempted in all patients using conventional techniques with either a JF260V or a TJF260V duodenoscope (Olympus Medical Systems, Tokyo, Japan). When ERBD was unsuccessful, EUS was performed with a GF-UCT240 or a GF-UC240P linear-array echoendoscope (Olympus Medical Systems). When dilation of the extrahepatic bile duct was visualized at the duodenal bulb, color Doppler ultrasound (US) was used to confirm the absence of the regional vasculature. EUS-guided puncture of the extrahepatic bile duct was carried out with a 19-gauge fine needle (Echo-Tip; Cook, Winston-Salem, N.C., USA) or a needle knife (Zimmon; Cook; fig. 1a). To confirm successful biliary access, a contrast medium was injected under fluoroscopy for cholangiography (fig. 1b). A 0.035-inch guidewire (Revowave; Olympus Medical Systems) was introduced through the EUS needle and orientated vertically toward the hepatic hilum (fig. 1c). Next, 6-, 7- and 9-french tapered biliary dilator catheters (Sohendra; Cook) were inserted and removed (in this order) over the guidewire to dilate the tract. Finally, a 7-french straight stent (Flexima; Boston Scientific, Natick, Mass., USA) was advanced through the CDS incision to the extrahepatic bile duct (fig. 1d, e).

Follow-Up

Biochemical parameters and a simple abdominal X-ray were performed after 2 days, 1 week, and monthly for 3 months after the procedure, and thereafter every 3 months. Abdominal US or computed tomography was performed every 3 months.

Results

The technical success rate of EUS-CDS was 93% (14/15 patients), and all 15 patients had successful EUS-assisted cholangiography. In 14 patients, the stents could be placed through the choledochoduodenal fistula. In 1 patient, we could not dilate the choledochoduodenal fistula because of sclerosing cholangitis. Therefore, in this patient, we placed stents across the major papilla with the rendezvous technique. Fine needles were required in 6 of 14 patients for biliary access. The median duration of the procedure was 37 min (range 25–43 min).

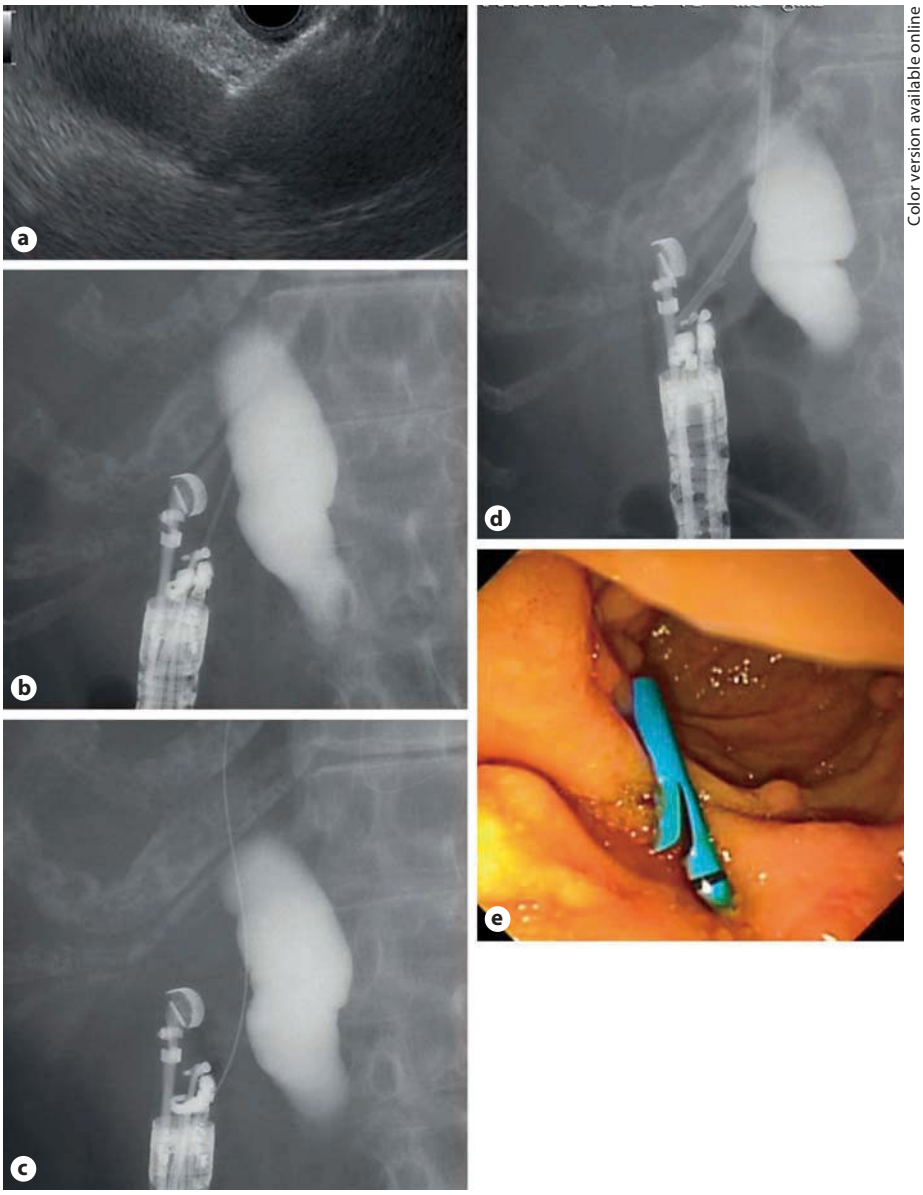

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Fig. 1. Technique of EUS-CDS. **a** Endoscopic US image of the fine needle inserted into the common hepatic duct. **b** Fluoroscopic image obtained by endoscopic US during cholangiography through the fine needle. **c** Fluoroscopic image during insertion of the guidewire through the puncture. **d** Fluoroscopic image during insertion of a stent through the puncture. **e** Endoscopic view of the plastic stent inserted from the duodenal bulb into the common hepatic duct.

Decompression of the bile duct was achieved in all patients with a success rate of 100% (14/14). After stent placement under EUS guidance, the median bilirubin level decreased significantly from 6.6 to 1.6 IU/ml ($p = 0.0004$).

The median follow-up time was 125 days (range 12–619 days); 13 patients died because of primary cancer growth. Two patients (14%) showed self-limiting peritonitis with mild abdominal discomfort and were managed conservatively, with spontaneous recovery within 1 week in both patients.

The median duration of stent patency was 99 days (12–248 days). All 4 patients with retrograde cholangitis underwent stent exchange via duodenoscopy. One patient who was treated with chemotherapy for malignant lymphoma showed distal stent migration, although this stent passed spontaneously without becoming trapped into the bowel. In this patient, the abdominal lymphoma decreased following chemotherapy in the absence of jaundice.

Table 2. Summary of published reports on EUS-CDS

Study	n	Device used for puncture n	Device used for dilation	Treatment success %	Initial stent n	Complications n
Giovannini et al. [10]	1	NK	dilator	100	10-F PS	none
Burmester et al. [17]	2	NK	not performed	100	8.5-F PS	bile peritonitis (1)
Puspok et al. [18]	5	NK	balloon/not performed	100	7–10-F PS	none
Kahaleh et al. [19]	1	FN	not described	100	10-mm SEMS	pneumoperitoneum (1)
Ang et al. [20]	2	NK	dilator	100	7-F PS	pneumoperitoneum (1)
Yamao et al. [21, 22]	5	NK	dilator	100	7–8.5-F PS	pneumoperitoneum (1)
Fujita et al. [24]	1	NK	dilator	100	7-F PS	none
Tarantino et al. [23]	4	FN	balloon	100	PS	none
Itoi et al. [25]	4	NK (2)/FN (2)	dilator	100	7-F PS (in 3 patients), NBD (1)	bile peritonitis (1)
Park et al. [27]	5	FN	dilator	100	10-mm CSEMS	none
Hanada et al. [26]	4	FN	dilator	100	6–7-F PS	none

n = Number of patients; NK = needle knife; FN = fine needle; PS = plastic stent; SEMS = self-expanding metallic stent; CSEMS = covered self-expanding metallic stent; NBD = nasobiliary drainage.

Discussion

Biliary obstruction is preferentially managed by ERBD [11]. However, ERBD may be unsuccessful because of tumor extension [12] or prior surgery [13]. Alternatives to unsuccessful ERBD include PTBD and surgery. PTBD has a complication rate of up to 32%, including fistula formation, cholangitis, peritonitis, empyema, hematoma, and liver abscesses [14, 15]. Surgery offers long-term patency but is also associated with increased morbidity and mortality [16]. EUS-CDS is a relatively new technique, permitting therapeutic biliary procedures when ERBD is unsuccessful.

To date, 11 studies have assessed the role of EUS-CDS (table 2) [10, 17–27]. According to these studies, EUS-CDS has been performed in 34 cases, including 33 patients with abdominal malignancies (22 pancreatic cancers, 6 papilla of Vater cancers, 2 bile duct cancers, 1 pancreatic lymphoma, 1 hepatoma, and 1 gastric cancer) and in 1 patient with bile duct stones. Overall, 18 needle knives and 15 fine needles were used for puncture. Except for 6 patients with a self-expanding metallic stent and 1 with nasobiliary drainage, 7- to 10-french plastic stents were used for placement. Once the stents were placed, all patients showed biliary decompression. The complication rate was 14% (5/34). Two patients developed focal bile peritonitis and 3 patients developed pneumoperitoneum, but none of the adverse events were fatal. A needle knife was used in 4 of these 5 patients, but there was no significant difference between the use of a needle knife or a fine needle in terms of adverse events.

In this study, we reviewed our experience with EUS-CDS. Biliary decompression was accomplished in all patients after prior failure of ERBD. However, self-limiting peritonitis occurred in 2 patients and cholangitis in 4 patients, although neither fatal adverse events nor major complications occurred.

The advantage of EUS-CDS over PTBD is the ability to puncture the biliary tree with minimal vascular injury using real-time color Doppler US imaging without external drainage. A limitation of EUS is more restricted access to the right hepatic biliary system, but in our opinion, EUS-CDS is a more useful treatment approach than ERBD. Currently, the procedure entails a high degree of complexity and its use should be limited to facilities with extensive experience in EUS and ERBD.

EUS-CDS may replace PTBD at tertiary care centers. Multicenter studies comparing EUS-CDS with PTBD are necessary to demonstrate the utility and indications of both techniques and better evaluate the risks of complications with both techniques.

Disclosure Statement

All authors report that they have no disclosures relevant to this publication.

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Endoscopic Ultrasound-Guided Neurolysis in Pancreatic Cancer

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Key Words
Abdominal pain · Celiac plexus neurolysis · Endoscopic ultrasound · EUS-guided broad plexus neurolysis · EUS-guided celiac ganglia neurolysis · Pancreatic cancer

Abstract
Abdominal pain in patients with pancreatic cancer is a common symptom that is often difficult to manage. Opioids are frequently used in an attempt to mitigate pain; however, side effects may develop. Celiac plexus neurolysis (CPN) affords effective pain control in patients with pancreatic cancer and is not associated with opioid side effects. Endoscopic ultrasound (EUS)-guided CPN has demonstrated safety and efficacy due to real-time imaging and anterior access to the celiac plexus from the posterior gastric wall, thereby avoiding complications related to the puncture of spinal nerves, arteries and the diaphragm, and is now practiced widely. Furthermore, two new techniques of EUS-guided neurolysis for abdominal pain management in pancreatic cancer patients have recently been developed. The first technique is EUS-guided celiac ganglia neurolysis (EUS-CGN) in which EUS facilitates CGN by enabling direct injection into the individual celiac ganglion, and the second technique is EUS-guided broad plexus neurolysis (EUS-BPN) which extends over the superior mesenteric artery. This review provides evidence for the efficacy of EUS-CPN. Particular attention is paid to the two new techniques of EUS-guided neurolysis, EUS-CGN and EUS-BPN.

Introduction

Pancreatic cancer commonly induces severe pain. In a large prospective study by Brescia et al. [1], 44% of patients with pancreatic cancer suffered from severe pain. Although only 30–50% of patients with pancreatic cancer reported moderate to severe pain at the time of diagnosis, more than 80% of patients with advanced cancer experienced severe pain [2, 3]. The cause of pain in patients with pancreatic cancer is not well understood. One theory attributes the severe pain to neuropathy, with damage to intrapancreatic nerves and tumor invasion into the extrapancreatic nerves leading to abdominal pain [4]. Others hypothesize that the pain may be due to the proximity of critical structures, e.g. the stomach, duodenum, liver, and transverse colon, as well as destruction of pancreatic tissue, which may cause ischemia, inflammation, and pain [5]. Pain management in patients with advanced pancre-

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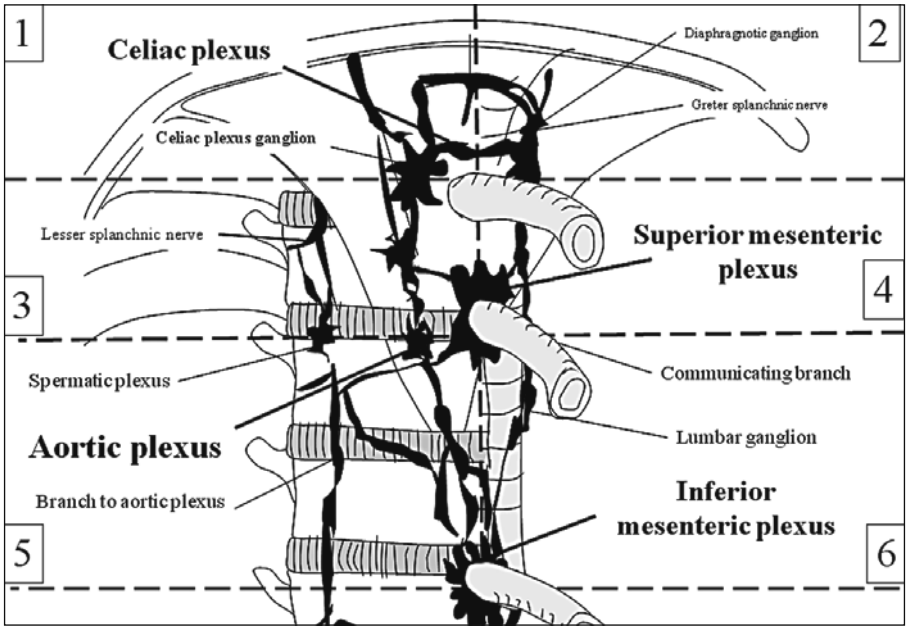


Fig. 1. Anatomy of abdominal plexuses. Figure reprinted from Sakamoto et al. [10].

atic cancer is very important to improve their quality of life. Recommendations for pain management from the World Health Organization include an analgesic ladder, with medication titration progressing from non-steroidal anti-inflammatory agents (NSAIDs) to opioids [6]. Although opioids are very effective in relieving pain, they are associated with dry mouth, constipation, nausea, vomiting, drowsiness, and impaired immune function [7].

Celiac plexus neurolysis (CPN) affords effective pain control in patients with pancreatic cancer and is not associated with opioid side effects. Pain impulses originating from all the abdominal and most pelvic viscera are carried by visceral nerve fibers that pass through the celiac plexus (CP) and splanchnic nerves. Interruption of nociceptive input at the level of either the CP or the splanchnic nerves therefore is a potentially effective means of visceral pain control [8]. Since its initial description by Kappis [9] in 1914, CPN is most commonly used as a palliative treatment in patients with pain due to pancreatic cancer. Recently, interventional endoscopic ultrasound (EUS) has been reported as a new approach for neurolysis in pancreatic cancer patients with pain, either as EUS-guided CPN (EUS-CPN), as EUS-guided celiac ganglia neurolysis (EUS-CGN) or as EUS-guided broad plexus neurolysis (EUS-BPN) over the superior mesenteric artery (SMA).

Anatomy

The CP is adjacent to the aorta and extends down from the origin of the celiac artery (CA) to the origin of the SMA. The CP contains 1–5 ganglia. The dominant ones are found on the right or left side, and their level, in relation to the celiac trunk, is variable. The upper inferior mesenteric plexus (IMP), the abdominal aortic plexus, and the lumbar ganglion are situated on the lateral and anterior aspects of the aorta between the origins of the SMA and the inferior mesenteric artery. The IMP surrounds the inferior mesenteric artery and is mainly derived from the aortic plexus. The abdominal aortic plexus has connections to both the CP and the IMP. It is formed by branches that are derived on either side from the CP and ganglia, and it receives filaments from some of the lumbar ganglia. There are various similar plexuses in the upper and middle abdominal cavity, and they are composed of a network of nerve fibers that originate from both the sympathetic and parasympathetic nervous systems. These plexuses also receive parasympathetic fibers from the vagus nerve (fig. 1) [10]. The autonomic nerves of the CP supply the liver, pancreas, gallbladder, stomach, spleen, kidneys, and ascending colon. Hence, CPN provides pain relief for visceral pain caused by cancer in the upper abdomen.

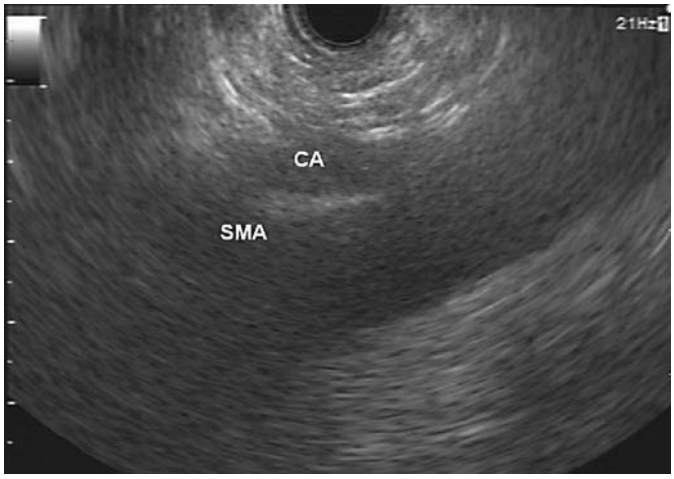


Fig. 2. EUS imaging from the lesser curve of the stomach demonstrates a longitudinal view of the aorta at the level of the CA and SMA.

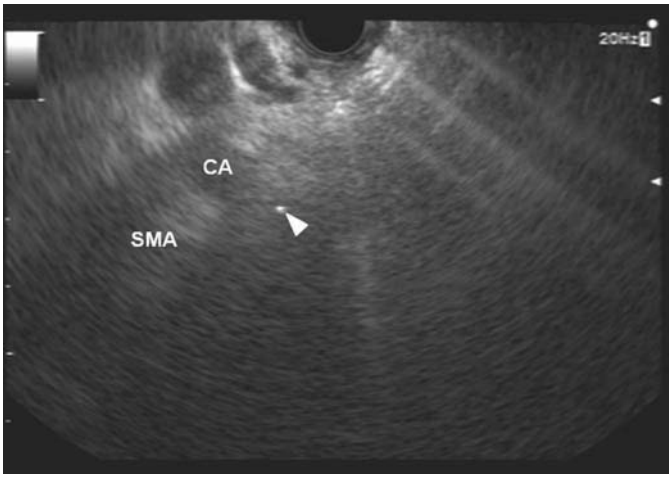


Fig. 3. The needle is inserted adjacent and anterior to the lateral aspect of the aorta at the level of the celiac trunk. The arrowhead indicates the needle tip.

EUS-Guided Celiac Plexus Neurolysis

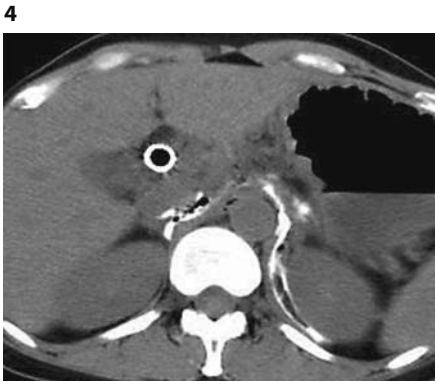
Traditionally, CPN has been performed by the percutaneous, posterior approach with computed tomography (CT) or ultrasound (US) guidance, for example, using fluoroscopy and a transcrural approach with CT-guided techniques. However, serious complications occur in 1% of patients, including paraplegia as a result of the needle entering a spinal artery or piercing the dura mater, and pneumothorax as a result of piercing the diaphragm [11–16]. These complications have led to the development of an anterior approach under the guidance of transcutaneous US, CT or EUS [17–20]. However, they can be avoided through the use of EUS, which allows direct access to the CP and real-time imaging of the celiac space for CPN as well as fine needle aspiration for diagnostic purposes and tumor staging [21, 22]. Thus, since it was first reported by Wiersema and Wiersema [23] in 1996, EUS-CPN is now a widely practiced alternative approach.

EUS-CPN Techniques

When using a curvilinear array echoendoscope, the region of the CP is visualized from the lesser curve of the stomach by following the aorta to the origin of the main CA and is traced, by using counterclockwise rotation, to its bifurcation into the splenic and hepatic arteries, with Doppler US control if needed (fig. 2). 22- or 19-gauge EUS-fine needle aspiration needles are used. The tip of the needle is placed slightly anterior to the origin of the CA. Aspiration is performed to ensure that vascular puncture has not occurred. Bupivacaine is injected first, followed by alcohol. Injection of the entire solution into the area cephalad of the celiac trunk can be performed, or the echoendoscope may be rotated to one side of the CA and only half of the solution is injected (fig. 3). The other half is then injected on the opposite side of the CA origin [24–28]. Patients should be observed for 2–4 h, with careful monitoring of pulse, blood pressure, temperature, and pain score.

Efficacy of EUS-CPN for Abdominal Pain in Pancreatic Cancer

In 1996, Wiersema and Wiersema [23] studied EUS-CPN in 30 patients with pain due to an intra-abdominal malignancy (25/30 with pancreatic cancer). Pain improvement at 2, 4, 8 and 12 weeks after CPN was noted in 79–88% of patients. In 2001, Gunaratnam et al. [24] reported 58 patients with unresectable pancreatic cancer who underwent EUS-CPN. The patients were followed for 6 months. CPN reduced pain in 78% patients; however, there was no significant alteration in narcotic usage. Only minor complications were seen, consisting of transient pain, diarrhea, and hypotension [23, 24]. When performing EUS-CPN, the injection site and surrounding



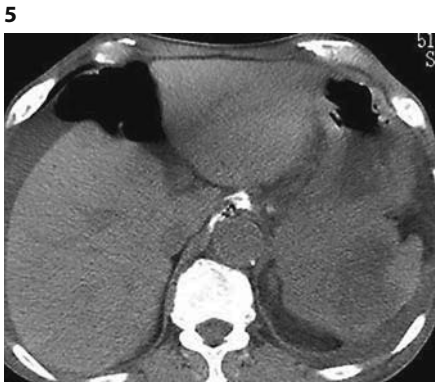


Fig. 4. Patients were classified into the bilateral injection group when the contrast medium was seen in the bilateral anterocrural spread.

Fig. 5. Patients were classified into the unilateral injection group when the contrast medium was seen in the unilateral anterocrural spread.

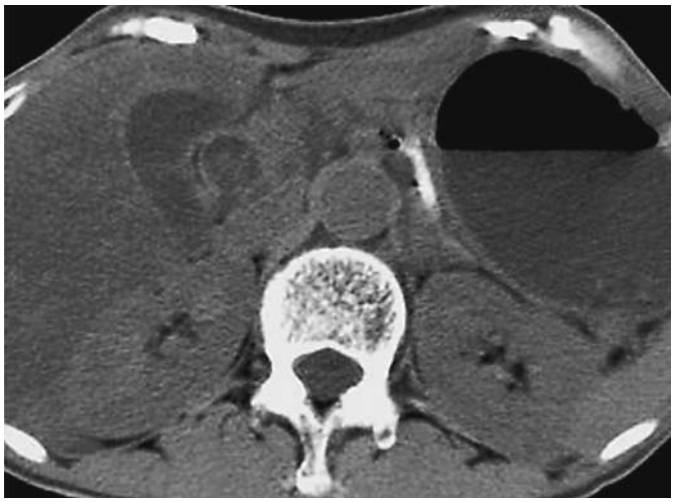


Fig. 6. Patients were classified into the inappropriate injection group when the contrast medium was seen besides the anterocrural spread.

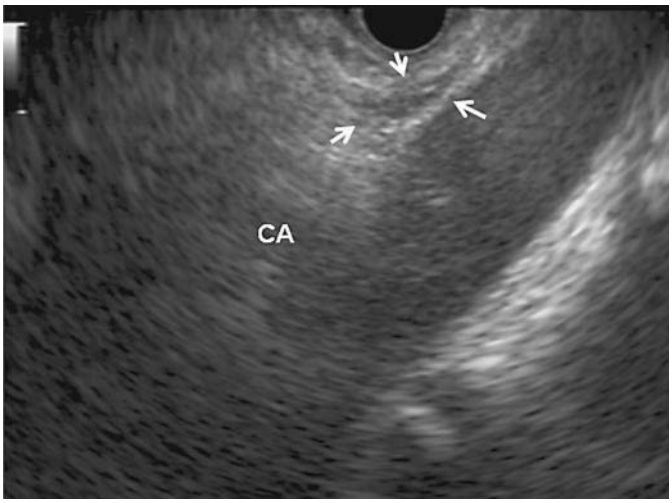


Fig. 7. EUS images of the celiac ganglion (arrows) from the lesser curve of the stomach.

region cannot be precisely assessed from the EUS image. To overcome this difficulty, we co-injected ethanol and contrast medium during EUS-CPN and immediately confirmed the injection site by abdominal CT, which allowed us to assess the relationship between the accuracy of injection and pain relief [20]. In our study, images of the distribution of injected solutions were classified into three groups. Injection solution dispersed in the unilateral and bilateral anterocrural space was defined as unilateral injection or bilateral injection, respectively (fig. 4, 5), while injection solution located outside the anterocrural space was defined as an inappropriate injection (fig. 6). Pain improvement score in the bilateral injection and unilateral injection groups was significantly superior to the changes observed in the inappropriate injection group. Overall, EUS-CPN was effective in 61.5% of patients; however, by performing an additional EUS-CPN in the inappropriate injection group, the response rate was increased to 84.6%. Therefore, we recommend the use of contrast medium in EUS-CPN procedures in order to confirm the injection site and improve the response rate to pain relief.

Complications

Transient diarrhea, transient hypotension and transient pain have been reported in 44, 38 and 9%, respectively, of patients undergoing EUS-CPN [14, 24–29]. The American Society of Gastrointestinal Endoscopy (ASGE)

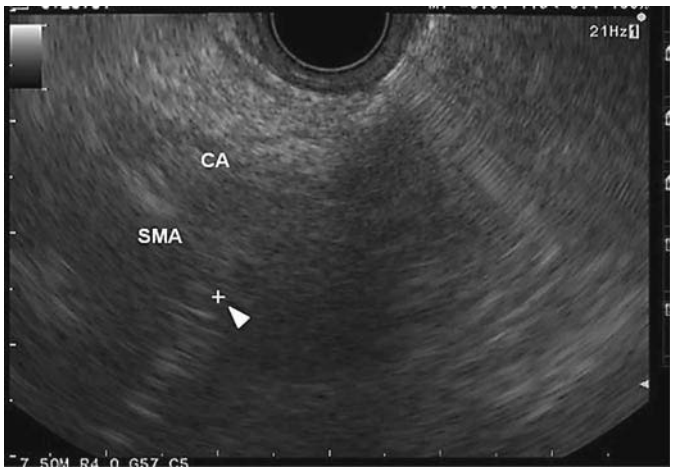


Fig. 8. A 25-gauge needle is inserted adjacent and anterior to the lateral aspect of the aorta over the level of SMA trunk (arrowhead).

reviewed the complications associated with EUS-CPN in a clinical guideline published in 2005 [30]. The perforation rate with EUS-CPN was 0.03–0.07%. Based on limited data, the EUS approach probably has a rate of perforation similar to that of standard esophagogastroduodenoscopy (0.03%). The risk of infection is reported to be similar to that of standard esophagogastroduodenoscopy, which ranges from 0 to 8%; there have also been reports of retroperitoneal abscesses. The risk of pancreatitis is 0–2%. Mild intraluminal hemorrhage occurs in 1.3–4% of cases, and severe hemorrhage is infrequently reported [31]. Sahai et al. [28] reported that bilateral EUS-CPN using a 19-gauge needle led on one occasion (1.6%) to a serious bleeding complication caused by trauma to the left adrenal artery.

EUS-Guided Neurolysis: Two New Techniques with Applications in Pancreatic Cancer

EUS-CGN Technique

Recently, it has been reported that celiac ganglia can be identified by EUS, where they have a characteristic appearance and location. Typically, they are small and hypoechoic and are either multilobulated or composed of confluent small spheres with hypoechoic bands [25, 26, 31] (fig. 7). In 2008, Levy et al. [32] offered an initial evaluation of the efficacy and safety of EUS-CGN for both pancreatic cancer patients and chronic pancreatitis patients. In their study, they demonstrated that, in order to perform CGN, EUS made it possible to inject directly into the individual celiac ganglia [31]. EUS-CGN was performed in 17 patients with pancreatic cancer, which resulted in 94% of patients reporting improvement of pain scores. Among these patients, narcotic use increased in 2 patients, remained unchanged in 13, and decreased in 3. There were no severe complications. Thus, EUS-CGN may be a new and effective method for pain relief in pancreatic cancer. However, this report was based on a retrospective study; this technique has not been studied in the setting of a controlled trial.

EUS-BPN over the SMA

As previously described, although EUS-CPN is effective, it is not beneficial for some patients with extended abdominal cancer. It is possible that nociceptive impulses from the abdominal viscera cannot be intercepted by standard EUS-CPN in cases where a cancer has expanded extensively within the abdominal cavity to beyond the reach of the CP. We suggested EUS-BPN over the SMA using a 25-gauge needle in order to relieve abdominal pain in pancreatic cancer. In 2010, we reported results from a study comparing the effectiveness of EUS-CPN and EUS-BPN in managing abdominal pain in pancreatic cancer [10] and found that the EUS-BPN procedure was more effective, especially in cases where the cancer had expanded extensively within the abdominal cavity beyond the distribution of the CP, and without incurring serious complications. Moreover, it seemed that BPN over the SMA may provide superior analgesia. However, this was also a retrospective study; the procedure has not yet been studied in controlled trials in comparison with EUS-CPN and EUS-CGN. Therefore, it is necessary to carry out a randomized prospective controlled trial comparing these 3 procedures.

EUS-BPN Technique

Under endoscopic visualization, the endoscope was advanced to the gastroesophageal junction; once it had entered the stomach, the endoscope tip was deflected upward so that the US probe came into contact with the gastric wall. Subsequently, the endoscope was torqued so that the aorta was identified in an elongated cross-section and was then advanced until the celiac trunk could be seen to branch anteriorly and inferiorly from the aorta. At a point 1–2 cm inferior to the celiac trunk, the SMA branch of the aorta could be seen in a similar fashion. These vascular landmarks were confirmed by color Doppler imaging. At the level of the SMA, the probe was rotated clockwise toward the patient's left

side until the SMA origin could no longer be visualized but the aorta could still be seen. A 25-gauge needle was placed under direct EUS visualization adjacent and anterior to the lateral aspect of the aorta over the level of the SMA trunk (fig. 8). An aspiration test was then performed. Lidocaine (3 ml) was injected first, followed by 10 ml alcohol. The same process was performed on the opposite side of the aorta (with counter-clockwise rotation).

Conclusion

EUS-CPN is a valuable tool for pain management in patients with pancreatic cancer. Although sustained pain reduction can be achieved by EUS-CPN, it is considered as adjunct to opioid use and adjuvant therapy in patients with pancreatic cancer. However, there have not yet been enough prospective, large, and randomized studies to confirm its efficacy. The EUS 2008 working group of the ASGE proposed that numerous case reports and several prospective case series should be used to evaluate the efficacy and safety of EUS-CPN for pain relief in patients with malignancy. However, they also recommended that the following studies should be conducted: (1) a randomized comparison of EUS-CPN versus sham therapy using well-defined quality-of-life measures with long-term follow-up; (2) early (onset of pain) versus late (opiate-toxic or resistant pain) implementation of EUS-CPN for pancreatic cancer; and (3) single versus scheduled multiple injections EUS-CPN. Therefore, a large cohort of patients and randomized studies are required to obtain more evidence on the efficacy of EUS-CPN techniques.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

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Review Article

Radiofrequency Ablation of Hepatocellular Carcinoma: A Literature Review

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Radiofrequency ablation (RFA) of liver cancers can be performed safely using percutaneous, laparoscopic, or open surgical techniques, and much of the impetus for the use of RFA has come from cohort series that have provided an evidence base for this technique. Here, we give an overview of the current status of radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC), including its physical properties, to assess the characteristics that make this technique applicable in clinical practice. We review the technical development of probe design and summarize current indications and outcomes of reported clinical use. An accurate evaluation of treatment response is very important to secure successful RFA therapy since a sufficient safety margin (at least 0.5 cm) can prevent local tumor recurrences. We also provide a profile of side effects and information on the integration of this technique into the general management of patients with HCC. To minimize complications of RFA, physicians should be familiar with each feature of complication. Appropriate management of complications is essential for successful RFA treatment. Moreover, adjuvant therapy, such as molecular targeted therapies following curative therapy, is expected to further improve survival after RFA.

1. Introduction

Hepatic resection forms part of the conventional treatment for patients with hepatocellular carcinoma (HCC); however, the majority of primary liver cancers are not suitable for curative resection at the time of diagnosis. Difficulties of surgical resection may be related to size, site, and number of tumors, vascular and extrahepatic involvement as well as liver function of the patient [1–4]. There is a need to develop a simple and effective technique for the treatment of unresectable tumors within the liver. Therefore, local ablative techniques (percutaneous ethanol injection (PEI), microwave coagulation therapy (MCT), and radiofrequency ablation (RFA)) have emerged in clinical practice to expand the pool of patients considered for liver-directed therapies [5–8]. Especially, RFA is not associated with some of the side effects of other ablative techniques [9]. Thus, RFA is currently performed widely due to the ease of use, safety, reasonable cost, and applicability to minimally invasive techniques [10].

This paper reviews the evidence supporting the use of RFA for HCC.

2. Background

2.1. Localized Application of Radiofrequency Energy. RFA is a localized thermal treatment technique designed to induce tumor destruction by heating the tumor tissue to temperatures that exceed 60°C [11]. The alternating current of radiofrequency waves passing down from an uninsulated electrode tip into the surrounding tissues generates changes in the direction of ions and creates ionic agitation and frictional heating. This tissue heating then drives extracellular and intracellular water out of the tissue, resulting in tissue destruction by coagulative necrosis [12, 13]. When tumor cells are heated above 45–50°C, intracellular proteins are denatured and cell membranes are destroyed through dissolution and melting of lipid bilayers. As a result, successful ablations usually increase the temperature of the ablated tissue to above 60°C.

Percutaneous RFA under local anesthesia was feasible, although intraoperative RFA under general anesthesia was also performed to prevent severe pain and discomfort during the procedure.

2.2. RFA Electrodes and Generators. Three types of RF electrodes are currently available commercially: two brands of retractable needle electrodes (model 70 and model 90 Starburst XL needles, RITA Medical Systems, Mountain View, CA; LeVeen needle electrode, Boston Scientific, Boston, MA) and an internally cooled electrode (Cool-Tip RF electrode; Radionics, Burlington, MA) [14].

The needle electrodes of RITA consist of a 14-gauge insulated outer needle that houses nine retractable curved electrodes of various lengths. When the electrodes are extended, the device assumes the approximate configuration of a Christmas tree. Nine of the electrodes are hollow and contain thermocouples in their tips in order to measure the temperature of adjacent tissue. The alternating electric current generator comes in a 250 W model at 460 kHz (Model 1500X RF Generator, RITA Medical Systems). The ablation algorithm is based on the temperature at the tips of the electrodes. After the ablation cycle is completed, a temperature reading from the extended electrodes in excess of 50°C at 1 min is considered to indicate satisfactory ablation.

Another RFA device (LeVeen Needle Electrode; Radiotherapeutics) has retractable curved electrodes and an insulated 17-gauge outer needle that houses 10 solid retractable curved electrodes that, when deployed, assume the configuration of an umbrella. The electrodes are manufactured in different lengths (2 to 4.0 cm umbrella diameter). The alternating electric current generator is 200 W operated at 480 kHz (RF 3000; Boston Scientific). The ablation algorithm is based on tissue impedance, and ablation is considered successful if the device impedes out.

The third RFA device (Cool-Tip radiofrequency electrode; Radionics) has an insulated hollow 17-gauge needle with an exposed needle tip of variable length (2 or 3 cm). The tip of the needle contains a thermocouple to record the temperature of adjacent tissue. The shaft of the needle has two internal channels that allow the needle to be perfused with chilled water. In an attempt to further increase the size of the ablation area, the manufacturer placed three of the cooled needles in a parallel triangular cluster with a common hub. The generator has a peak power output of 200 W and is operated at 480 kHz (CC-1; Radionics). The ablation algorithm is based on tissue impedance, and ablation is considered successful if the device impedes out. As a result, successful ablations usually increase the temperature of the ablated tissue to above 60°C.

2.3. Treatment Algorithm in Japan and the West. RFA is basically recommended for HCC nodules with a maximum diameter of 3 cm in patients with not more than three tumors who are contraindicated for surgery, although the typical treatment algorithms in Japan, North America, and Europe are each slightly different [35].

One of the major treatment algorithms in Japan is the "consensus-based clinical practice manual for HCC" [14, 36] edited by the Japan Society of Hepatology (JSH). This consensus recommends (1) hepatectomy for a single tumor regardless of tumor size, but local treatment may be selected for a tumor 2 cm or smaller in Child-Pugh B patients; (2) hepatectomy or local treatment when there are 2 or 3 tumors and the tumor size is within 3 cm; (3) liver transplantation for Child-Pugh C patients with 3 or fewer tumors 3 cm or smaller or a single tumor with a tumor size within 5 cm (Milan Criteria); (4) RFA combined with transcatheter arterial chemoembolization (TACE) is recommended for tumors more than 3 cm in diameter. RFA is also recommended for 4 or more nodules where applicable.

In Europe and North America, the algorithm established by the American Association of the Study of the Liver Disease (AASLD) [37] recommends local treatment for 3 or fewer 3 cm or smaller early-stage HCCs and 2-cm or smaller very-early-stage HCCs with complications, such as portal hypertension.

2.4. Assessment of Technical Effectiveness. The assessment of the therapeutic effect of RFA is very important. The technical effectiveness of ablation is commonly assessed by findings on contrast-enhanced CT or MRI. A tumor was considered to have been successfully ablated when there were no longer any enhanced regions within the entire tumor during the arterial phase and at least a 0.5 cm margin of apparently normal hepatic tissue surrounding the tumor during the portal phase [38–40]. Failure to establish a sufficient ablative safety margin was shown to be an independently significant risk factor for local tumor progression on multivariate analysis [41]. Part of the tumor was diagnosed as remaining viable when images of the ablated area showed nodular peripheral enhancement [42].

Basically, the local recurrence rate following a single RFA treatment depends on how strictly the therapeutic effect is assessed. In cases of HCC in which local curative therapy was achieved by securing a safety margin, the 4-year survival rate was relatively high, at 66%–82% (results in Japan) [35, 43].

3. Clinical Outcomes

3.1. Percutaneous Approach

3.1.1. Survival: Comparison with Those after Resection. A randomized control trial (RCT) has shown that RFA achieved survival rates similar to those achieved by resection (Table 1) [15]. Chen et al. conducted RCT on 180 patients with a solitary HCC $\leq$5 cm indicated to receive either percutaneous RFA or surgical resection [15]. This study showed that percutaneous RFA achieved the same overall and disease-free survival rates as surgical resection for patients with small solitary HCC. The 1- and 4-year overall survival rates after percutaneous RFA and surgery were 95.8%, 67.9% and 93.3%, 64.0%, respectively. The corresponding disease-free survival rates were 85.9%, 46.4% and 86.6%, 51.6%, respectively. Recently, Huang et al. reported an RCT trial in which the 1-, 3-, and 5-year overall survival rates for the RFA group and the RES group were 86.96%, 69.57%, 54.78% and 98.26%, 92.17%, 75.65%, respectively. Overall survival and recurrence-free survival were significantly higher in the

Table 1: Survivals: RFA versus hepatic resection for HCC.

Author/Year	Study type	*n* (RFA/resection)	Mean tumor size (cm) (RFA/resection)	Overall survival (%) (RFA versus resection)	*P*
Chen et al. [15], 2006	RCT	90/90	ND/ND	65.9 versus 64.0 (4-year)	NS
Huang et al. [16], 2010	RCT	115/115	ND/ND	54.78 versus 75.65 (5-year)	.001
Vivarelli et al. [17], 2004	Retrospective	79/79	ND/ND	33 versus 65 (3-year)	.002
Montorsi et al. [18], 2005	Prospective	58/40	ND/ND	30 versus 53 (4-year)	.018
Ogihara et al. [19], 2005	Retrospective	40/47	4.6/7.4	39 versus 31 (5-year)	.79
Wakai et al. [20], 2006	Retrospective	64/85	ND/ND	30 versus 53 (10-year)	.012
Guglielmi et al. [21], 2008	Retrospective	23/33	ND/ND	45 versus 55 (5-year)	.7
Abu-Hilal et al. [22], 2008	Retrospective	34/34	3.0/3.8	57 versus 56 (5-year)	.3
Hiraoka et al. [23], 2008	Retrospective	105/59	ND/ND	59.3 versus 59.4 (5-year)	NS
Ueno et al. [24], 2009	Retrospective	123/110	2.0/2.7	63 versus 80 (5-year)	.06
Takayama et al. [25], 2009	Retrospective	1315/1235	1.6/1.8	95 versus 94 (2-year)	.28

HCC: hepatocellular carcinoma; ND: not described; NS: not significant; RFA: radiofrequency ablation.

Table 2: Local tumor progression rates after RFA for HCC.

Author	Year	*n*	Tumor size (mean, cm)	Follow-up period (mean, months)	Local tumor progression rate (%)
Rossi et al. [26]	1996	41	2.3	22.6	5.0
Buscarimi et al. [27]	2001	60	ND	26.8	14
Choi et al. [28]	2004	53	2.1	23	21
Lu et al. [29]	2005	87	2.5	12.7	5.8
Shiina et al. [30]	2005	118	ND	34.8	1.7
Solmi et al. [31]	2006	63	2.8	32.3	41
Hänsler et al. [32]	2007	21	4.2	ND	21
Waki et al. [33]	2010	88	ND	36	4.8
Li et al. [34]	2010	117	2.4	21	9.4

HCC: hepatocellular carcinoma; ND: not described; RFA: radiofrequency ablation.

surgical resection group than in the RFA group ($P = .001$, $P = .017$). However, percutaneous RFA can be expected to have an advantage over liver resection in providing a better short-term postoperative result because local ablative therapy is a less invasive procedure [16–25].

3.1.2. Local Controllability (Local Tumor Progression). The local recurrence rate after RFA for HCC ranged from 1.7% to 41% [26–34] (Table 2). Local tumor progression is related to incomplete tumor ablation. It is often difficult to obtain a specific safety margin in three dimensions all around a large tumor. Some researchers reported that the most important factor associated with failure of local tumor control could be tumor size [8, 36–38]. In Table 2, local tumor progression did not necessarily depend on the tumor size; however, recurrence could occur even after a sufficient margin had been ensured. It is considered that local recurrence appears to arise from residual cancer after RFA while recurrence from a microsatellite or by microvascular invasion other than the main nodule may also appear as a late local recurrence. The local tumor progression rate can differ markedly depending on whether or not a 5 mm circumferential safety margin has been secured. Nishijima et al. categorized the presence of no margin, a partially lacking margin, margin narrower than 5 mm, and complete margin wider than 5 mm as R0, R1, R2, and R3 on the assessment of the therapeutic effect of RFA, respectively, and found significant differences in the local recurrence rate between R0 and R1 and between R2 and R3. The local recurrence rate significantly differed between patients with and without a sufficient safety margin [44].

Therefore, ensuring a safety margin in RFA is important for not only the simultaneous treatment of microsatellite lesions, but also to ensure sufficient tumor ablation on the assumption of a partial volume effect-associated limitation on evaluation of the therapeutic effect by imaging.

3.1.3. Advances of Techniques: Large HCC. Tumor size is an important factor influencing the local recurrence rate after RFA [45]. To increase the size of the coagulation zone in RFA, physicians have tried using vascular occlusion during RFA because vascular occlusion reduces heat dispersion. It was shown in the consensus meeting "HCC Treatment" at the 45th Annual Meeting of the JSH in Kobe in 2009 [46] that about 90% of physicians performing RFA employ lipiodol TACE-preceded RFA for 3 cm or larger HCCs. Lipiodol TACE-preceded RFA is relatively curative and can be readily performed for the following reasons: (1) lipiodol regurgitates into the portal branches via the peribiliary venous plexus, causing a transient state of liver infarction, which reduces the cooling effect, expanding the ablative area, and resulting in (2) coagulation of satellite lesions [43]. Peng et al. reported a series of 120 patients with HCC, and the 1-, 3-, 5-year overall survival rates for TACE-preceded RFA and RFA groups were 93%, 75%, 50%, and 89%, 64%, 42%, respectively ($P = .045$) [47]. Yamakado et al. reported that the survival rates of large HCC cases treated with resection and lipiodol TACE-preceded RFA were almost equivalent [48]. TACE combined with RFA therapy might improve the overall survival status for patients with large HCCs (Table 3) [47, 49–52].

3.1.4. Advanced Techniques: Tumors Abutting the Diaphragm and Gastrointestinal Tract. Ultrasound- (US-) guided procedures are necessary but limited for tumors located under the diaphragm. However, saline solution injection into the pleural cavity can separate the lung and liver on B-mode US, that is, artificial pleural effusion acts as an acoustic window. There are reports on the feasibility and safety of RFA with artificially induced pleural effusion for HCC located in the right subphrenic region [53–56]. In a series of 24 patients with HCC located in the hepatic dome, 200–1100 mL of 5% glucose solution was infused intrathoracically to separate the lung and liver, thus, complete tumor necrosis in a single session was achieved in 96.4% [56].

Artificial preparation of a space between the intestine and nodule by infusing normal saline or 5% glucose (artificial ascites method) for treatment has recently become possible [57, 58]. These techniques markedly expanded the indication for RFA. Laparoscopic resection or laparotomic RFA had to be inevitably performed in patients with HCC nodules <2.0 cm in diameter before the introduction of artificial ascites, but more than 90% of cases are now treatable by the "artificial ascites method".

3.1.5. Advanced Techniques: Cases That Are Unclear on B-Mode US. Multiple RFA sessions for HCCs were frequently required because of HCC nodules that are unclear on B-mode US. Under CT fluoroscopy using either CT arteriography or iodized oil injection, we can target and puncture hepatic malignancies using a percutaneous ethanol injection needle. Real-time CT fluoroscopy is useful to guide the needle puncture and to monitor ethanol injection in small hepatic malignancies [67]. Another merit is that the efficacy of treatment can be evaluated using contrast enhanced CT immediately after treatment.

Contrast enhanced harmonic US imaging is able to evaluate small hypervascular HCCs even when B-mode US cannot adequately characterize the tumors [68–72]. The microbubbles of these contrast agents provide stable nonlinear oscillation in a low-power acoustic field because of the hard shells of these bubbles, producing great detail in the harmonic signals in real time [71–73]. It has been reported that contrast harmonic sonography-guided RFA is an efficient approach for guiding further ablation of hepatic malignancies that are not clearly demarcated by B-mode US [74–78].

Virtual CT sonography using magnetic navigation (Real-time Virtual Sonography (RVS); HITACHI Medico, Tokyo, Japan) provides cross sectional images of CT volume data corresponding to the angle of the transducer in the magnetic field in real-time. This imaging technique displays a real-time synchronized multiplanar CT image in precisely the same slice of the US plane. Thus, RVS can be used for real-time needle insertion guidance, especially for nodules demonstrated on CT, but not on US [79, 80].

3.2. Laparoscopic/Open Surgical Approach. The use of a laparoscopic or open approach allows repeated placement of RFA electrodes at multiple sites to ablate larger tumors [59–66] (Table 4). Moreover, a hand-assisted technique can be applied safely and effectively to laparoscopic liver surgery and offers the advantages of intraoperative US, which provides better resolution of the number and location of liver tumors. The postoperative recovery of patients was shorter compared with that after an open surgical approach. Ishiko et al. reported that the surgical procedures consisted of 5 RFA sessions for tumors in the caudate lobe with hand-assisted laparoscopic surgery (HALS) and a postoperative CT scan demonstrated sufficient ablation in all patients and there was no surgical mortality [63]. The HALS approach has several advantages; it facilitates and expedites the procedure, reduces the stress factor on the surgeon, greatly improves exposure, and facilitates immediate and efficient control of bleeding vessels with the internal hand. However, the local treatment failure rate of the laparoscopic approach was higher in patients with HCC nodules situated deep within the liver and measuring 4 cm or more in diameter [81]. Great difficulty can be encountered during treatment of lesions in contact with the diaphragm.

Although more invasive, open RFA can be performed more easily, and the puncture course of RF needle can be more widely selected than that during laparoscopic approach. Some have reported that patients undergoing radical open RFA demonstrated few ablation site recurrences even though the nodules measured more than 4 cm in diameter and/or there were more than three nodules [59, 62, 65].

3.3. Complications. A recent review indicated that complication rates for percutaneous, laparoscopic, and open RFA of hepatic tumors in 3670 patients were 7.2%, 9.5%, and

Table 3: Survivals: RFA combined with TACE versus RFA alone for HCC.

Author/year	*n* (TACE+RFA/RFA)	Tumor size (mean, cm) (TACE+RFA/RFA)	Overall survival (%) (TACE+RFA/RFA)	*P*
Kitamoto et al. [49]/2003	10/16	3.9/3.4	ND	
Wang et al. [50]/2007	43/40	ND	68.3/57.6 (1-year)	<.05
Shibata et al. [51]/2009	46/43	ND	84.8/84.5 (3-year)	.515
Morimoto et al. [52]/2010	19/18	3.6/3.7	93/80 (3-year)	.369
Peng et al. [47]/2010	120/120	ND	50/42 (5-year)	.045

HCC: hepatocellular carcinoma; ND: not described; RFA: radiofrequency ablation, TACE: trans catheter arterial chemoembolization.

Table 4: Laparoscopic/open RFA for liver malignancies: local tumor progressions and survivals.

Author/year	Arms	*n*	Tumor size (mean, cm)	Follow-up period (mean, months)	Local tumor progression	Survival (%)
Topal et al. [59]/2003	LS/open	9/9	3.8/3.5	12.2	1/9, 0/9	ND
Berber et al. [60]/2005	LS	66	4.1	25.3	ND	38% (3-year)
Hildebrand et al. [61]/2007	LS	14	ND	23.2	1/14	ND
Minami et al. [62]/2007	open	30	3.2	18.9	1/30	71.6% (3-year)
Ishiko et al. [63]/2008	HALS	5	ND	32.2	1/5	ND
Ballem et al. [64]/2008	LS	104	3.5	23	ND	21% (3-year)
Tanaka et al. [65]/2009	open	26	ND	ND	1/26	ND
Salama et al. [66]/2010	LS	72	ND	14.3	2/72	ND

HALS: hand-assisted laparoscopic surgery; LS: laparoscopy; ND: not described; RFA: radiofrequency ablation.

9.9%, respectively [82]. Overall, the frequency of major complications of percutaneous RFA ranged from 0.6%–8.9%, which was higher than that of PEI, but generally less than that of MCT [43]. Complications of percutaneous RFA reported in 2320 patients treated at 41 different hospitals in Italy indicate that the mortality rate was 0.3% with an overall complication rate of 7.1% [83, 84]. The authors described major complications (2.4% incidence) including death, hemorrhage, RFA needle-track seeding, RFA lesion abscess, perforation of gastrointestinal viscus, liver failure, biloma, biliary stricture, portal vein thrombosis, and hemothorax or pneumothorax requiring drainage, and minor complications (4.7% incidence) including pain, fever, and asymptomatic pleural effusion. Although Llovet et al. [85] reported that dissemination along puncture route was observed in 12.5% of their patients, dissemination might not occur at such a high frequency. This complication was almost absent in many reports from Japan [43].

Theoretically, a tumor that is contiguous to a large vessel is more likely to have some viable tumor cells following local thermal therapy because there is a significant tissue cooling effect caused by blood circulation of normal body temperature. Thus, the effort to thoroughly ablate the lesion with a safety margin under such conditions increases the total number of electrode insertions, and this may increase the risk of complications. Some investigators have suggested that tumor location is closely related to the risk of major complications. Central tumors close to the hepatic hilum were reported to be unsuitable for percutaneous RFA because of the risk of injuring adjacent bile ducts [7]. Moreover, peripheral tumors adjacent to extrahepatic organs were also suggested to be unsuitable because of the risk of heat injuries, such as intestinal perforation and pleural effusion [84, 86]. However, Teratani et al. reported that there was no difference in early complication rates according to tumor location [87]. The effort to achieve thorough ablation increased the total number of electrode insertions, and this may have led to an increase in complications.

Not only elevating the survival rate and reducing the incidence of local recurrence but also avoiding complications as much as possible are major tasks. To minimize complications of RFA, knowledge of risk factors and prevention methods is required. In addition, because early and accurate diagnosis is necessary for the appropriate management of complications, physicians should be familiar with all features of complication.

4. Future Perspective

Currently, a multicenter randomized controlled study (prospective randomized study of surgery or RFA for early HCC: SURF Trial) is underway in Japan, involving patients with 3 or fewer tumors 3 cm or smaller for which both hepatectomy and RFA are applicable [88], and a large global study is currently underway (the Sorafenib as Adjuvant Treatment in the Prevention of Recurrence of HepatocellularCarcinoma

(STORM) trial), looking at the efficacy of sorafenib therapy after potentially curative treatment with liver resection or RFA.

5. Conclusion

Here, we have assessed the role of RFA in the overall therapeutic strategy for patients with HCC and highlighted deficiencies in current knowledge. We intend to strive for a balanced discussion between the tendency to overemphasize the potential advantages of RFA and the tendency to understate a potentially useful treatment. Percutaneous RFA can achieve the same overall and disease-free survival rates as surgical resection for patients with small HCC, while causing few side effects. Percutaneous RFA combined with TACE will make the treatment of larger tumors a clinically viable treatment alternative. The use of a laparoscopic or open approach allows repeated placement of RFA electrodes at multiple sites to ablate larger tumors. In addition, an accurate evaluation of treatment response is very important to secure successful RFA therapy since a sufficient safety margin (at least 0.5 cm) can prevent local tumor recurrence. Adjuvant therapy, such as molecular targeted therapies following curative therapy, is expected to further improve survival after RFA.

Authors' Contributions

Y. Minami drafted the paper and wrote the final version of the paper. M. Kudo reviewed and approved the final version of the paper.

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Original article

Recurrence-free survival more than 10 years after liver resection for hepatocellular carcinoma

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Background: **High recurrence rates after liver resection with curative intent for hepatocellular carcinoma (HCC) remain a problem. The characterization of long-term survivors without recurrence after liver resection may help improve the therapeutic strategy for HCC.**

Methods: **A nationwide Japanese database was used to analyse 20 811 patients with HCC who underwent liver resection with curative intent.**

Results: **The 10-year recurrence-free survival rate after liver resection for HCC with curative intent was 22·4 per cent. Some 281 patients were recurrence-free after more than 10 years. The HCCs measured less than 5 cm in 83·2 per cent, a single lesion was present in 91·7 per cent, and a simple nodular macroscopic appearance was found in 73·3 per cent of these patients; histologically, most HCCs showed no vascular invasion or intrahepatic metastases. Multivariable analysis revealed tumour differentiation as the strongest predictor of death from recurrent HCC within 5 years.**

Conclusion: **Long-term recurrence-free survival is possible after liver resection for HCC, particularly in patients with a single lesion measuring less than 5 cm with a simple nodular appearance and low tumour marker levels.**

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Introduction

Hepatocellular carcinoma (HCC) is a common malignancy in Japan, and often develops in virus-infected cirrhotic liver[1]. The high incidence of recurrence following treatment renders it difficult to cure this disease completely. On the other hand, long-term survival has been reported even beyond 10 years, with or without recurrence, after potentially curative liver resection[2–4]. However, there have been few reports regarding recurrence-free survival (RFS) for more than 10 years after liver resection with curative intent for HCC[5].

The Liver Cancer Study Group of Japan (LCSGJ) has conducted a nationwide survey of patients with primary liver carcinoma since 1969 to evaluate the clinicopathological characteristics and outcomes of these patients[6]. The large-scale registration system of the LCSGJ was used here to evaluate the characteristics of patients who survived without recurrence for at least 10 years after curative liver resection. These patients were compared with patients who died from recurrent HCC within 5 years in order to gain insight into the demography and biological behaviour of HCCs. In addition, such data might be important in determining follow-up strategies, and encouraging patients to undergo treatment, including surgical resection.

Methods

A nationwide follow-up survey of all patients with primary HCC was conducted by the LCSGJ. All patients with

primary malignant liver tumours diagnosed by imaging, preoperative clinical data, and/or histopathological studies at approximately 800 institutions in Japan were registered and followed prospectively every 2 years.

At the time of this analysis, the LCSGJ database contained 142 900 patients diagnosed with a liver tumour and 130 748 patients ultimately diagnosed with HCC. The present study enrolled 20 811 patients with HCC who had undergone liver resection with curative intent before 1993, and were registered in the JCSGJ database between 1988 and 2003 (from the 10th to the 17th surveillance). The indications for hepatic resection and operative procedures were based on both anatomical location of the tumour and liver function. Follow-up ended on 31 December 2003.

Patients who survived more than 10 years without recurrence of HCC and those who died from recurrent HCC within 5 years of liver resection were identified. Patients were further examined according to the degree of background liver damage, as advocated by the JCSGJ as an alternative to the Child–Pugh score (*Table 1*)[7]. The serological presence of hepatitis B antigen was considered evidence of hepatitis B infection, and that of hepatitis C antibody as an indicator of hepatitis C infection. Hepatic resections were classified according to the terminology of the Liver Cancer Study Group of Japan[7]. The macroscopic appearance of HCC was classified into six types: type 1 (simple nodular type), type 2 (simple nodular type with extranodular growth), type 3 (confluent multinodular type), type 4 (multinodular type), type 5 (others, including infiltrative, mass and diffuse types) and unknown[6,8]. Serum levels of α-fetoprotein (AFP) and des-γ-carboxyprothrombin (DCP) were measured as tumour markers. Microscopic portal vein invasion was defined as the presence of tumour emboli within the portal vein. Intrahepatic metastasis was classified into four groups: 0 (no intrahepatic metastasis), 1 (intrahepatic metastasis to the segment in which the main tumour is located), 2 (intrahepatic metastases to two segments), 3 (intrahepatic metastases of the three or four segments). Non-cancerous liver was classified microscopically as normal, or as having chronic hepatitis, fibrosis or cirrhosis.

Hepatic recurrence of HCC was diagnosed at each centre by ultrasonography and/or dynamic computed tomography. Distant metastases were diagnosed by computed tomography (lung) and scintigraphy (bone)[9].

Table 1 Degree of liver damage according to the Liver Cancer Study Group of Japan

	Degree of liver damage		
	A	B	C
Ascites	None	Controllable	Uncontrollable
Serum bilirubin (mg/dl)	> 2·0	2·0–3·0	< 3·0
Serum albumin (g/dl)	> 3·5	3·0–3·5	< 3·0
ICG-R15 (%)	< 15	15–40	> 40
Prothrombin activity (%)	> 80	50–80	< 50

The degree of liver damage was classified as grades A, B and C based on the highest grade containing at least two of five items. Then, if two or more items scoring the same grade occur in the three grades, the higher grade is adopted as the degree of liver damage. ICG-R15, indocyanine green retention rate at 15 min.

Statistical analysis

Continuous data were expressed as mean(s.d.) and analysed by means of Student's *t* test. The χ^2 test was used to analyse the distribution of nominal variables, and the Wilcoxon rank sum test for analysis of ordered categorical variables. RFS curves were generated by the Kaplan–Meier method. A multivariable logistic regression model was used to investigate odds ratios. $P < 0{\cdot}050$ was considered statistically significant.

Results

Stratification according to the time of recurrence identified 281 patients who survived more than 10 years without recurrence of HCC (10-year RFS group), whereas 918 patients died from recurrent HCC within 5 years of liver resection. Median follow-up was 11·2 and 0·9 years respectively. The RFS rate at 10 years was 22·4 per cent after liver resection with curative intent (*Fig. 1*). Clinical

No. at risk	4977	3399	2253	1423	572	39
Cumulative recurrences	0	543	1047	1349	1533	1704
Cumulative deaths without recurrence	0	471	812	1110	1275	1339

Fig. 1 Recurrence-free survival after liver resection with curative intent for hepatocellular carcinoma

Table 2 Comparison of clinical data between recurrence-free survivors at 10 years and patients who died from recurrent hepatocellular carcinoma within 5 years

	10-year RFS ($n = 281$)	Died within 5 years ($n = 918$)	P§
Age (years)*	57·5(9·4)	60·8(8·5)†	< 0·001¶
Sex ratio (M : F)	219 : 62	755 : 162‡	0·115
Liver damage grade			< 0·001
A	212 (79·1)	553 (65·1)	
B	52 (19·4)	257 (30·3)	
C	4 (1·5)	39 (4·6)	
Unknown	13	69	
HBsAg-positive	82 of 255 (32·2)	179 of 812 (22·0)	< 0·001
HCV Ab-positive	103 of 198 (52·0)	356 of 474 (75·1)	< 0·001
AFP (ng/ml)			< 0·001#
< 20	140 (50·9)	272 (30·8)	
≥ 20 to < 400	73 (26·5)	345 (39·1)	
≥ 400 to < 1000	15 (5·5)	79 (9·0)	
≥ 1000	47 (17·1)	186 (21·1)	
Unknown	6	36	
DCP (mAU/ml)			< 0·001#
< 40	118 (69·4)	222 (50·5)	
≥ 40 to < 500	16 (9·4)	83 (18·9)	
≥ 500 to < 1000	36 (21·2)	135 (30·7)	
≥ 1000	0 (0)	0 (0)	
Unknown	111	478	
Operative method			0·270
> 1 segment	135 (48·2)	410 (44·9)	
Subsegment	71 (25·4)	216 (23·6)	
< 1 subsegment	74 (26·4)	288 (31·5)	
Unknown	1	4	

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.). Data missing for †six and ‡one patients. RFS, recurrence-free survival; HBsAg, hepatitis B surface antigen; HCV Ab, hepatitis C antibody; AFP, α-fetoprotein; DCP, des-γ-carboxyprothrombin. §χ^2 test, except ¶Student's t test and #Wilcoxon rank sum test.

Table 3 Comparison of histopathological data between recurrence-free survivors at 10 years and patients who died from recurrent hepatocellular carcinoma within 5 years

	10 year RFS ($n = 281$)	Died within 5 years ($n = 918$)	P*
Maximum tumour size (cm)			0·009
< 2	91 (32·5)	198 (21·7)	
2–5	142 (50·7)	480 (52·6)	
> 5	47 (16·8)	234 (25·7)	
Unknown	1	6	
No. of tumours			< 0·001
1	253 (91·7)	675 (74·1)	
2	20 (7·2)	145 (15·9)	
≥ 3	3 (1·1)	91 (10·0)	
Unknown	5	7	
Macroscopic type			< 0·001
1	198 (73·3)	521 (60·2)	
2	32 (11·9)	174 (20·1)	
3	28 (10·4)	69 (8·0)	
4	6 (2·2)	66 (7·6)	
5	6 (2·2)	35 (4·0)	
Unknown	11	53	
Tumour differentiation			< 0·001
Well	52 (24·0)	95 (13·7)	
Moderate	133 (61·3)	427 (61·4)	
Poor	31 (14·3)	167 (24·0)	
Unclassified	1 (0·5)	6 (0·9)	
Unknown	64	223	
Vascular invasion			0·281
Yes	4 (1·4)	23 (2·6)	
No	272 (98·6)	875 (97·4)	
Unknown	5	20	
Intrahepatic metastases			< 0·001
0	258 (92·5)	673 (75·3)	
1	15 (5·4)	154 (17·2)	
2	6 (2·2)	62 (6·9)	
3	0 (0)	5 (0·6)	
Unknown	2	24	< 0·001
Non-cancerous liver			
Normal	35 (14·4)	50 (6·6)	
Chronic hepatitis/fibrosis	105 (43·2)	189 (25·1)	
Cirrhosis	103 (42·4)	514 (68·3)	
Unknown	38	165	

Values in parentheses are percentages. RFS, recurrence-free survival. *χ^2 test.

and histopathological characteristics of the two groups are compared in *Tables 2* and *3* respectively.

In the 10-year RFS group, at the time of liver resection the background liver damage was grade A in 79·1 per cent, grade B in 19·4 per cent and grade C in 1·5 per cent. Some 32·2 per cent of these patients were positive for hepatitis B virus antigens, whereas 52·0 per cent were positive for hepatitis C virus antibody. Serum levels of AFP and DCP were normal in 50·9 and 69·4 per cent of patients respectively. Surgical procedures comprised resection of less than a subsegment in 26·4 per cent, subsegmentectomy in 25·4 per cent and resection of more than one segment in 48·2 per cent of patients.

The maximum size of HCC at resection was less than 5 cm in 83·2 per cent of patients in the 10-year RFS group. Some 91·7 per cent of these patients had a single HCC at resection. HCCs in this group were of the single nodular type in 73·3 per cent, and 61·3 per cent were moderately differentiated; most showed no vascular invasion (98·6 per cent) or intrahepatic metastases (92·5 per cent). The non-cancerous tissue was cirrhotic in 46·5 per cent.

Comparison of the characteristics of patients who survived for at least 10 years without disease recurrence and those who died from recurrent HCC within 5 years revealed significant differences in age, degree of liver damage, positivity for hepatitis B antigen and hepatitis C antibody, serum levels of AFP and serum levels of DCP

(*Table 2*). Indeed, the 10-year survivors were younger, less frequently positive for hepatitis C and more frequently positive for hepatitis B. Levels of tumour markers (AFP, DCP) were lower in this group, whereas HCCs were smaller and fewer in number. There were also statistically significant differences in macroscopic appearance, tumour differentiation, intrahepatic metastasis and non-cancerous liver histology.

Table 4 Multivariable logistic regression analysis for death from recurrent hepatocellular carcinoma within 5 years

	Odds ratio	*P*
Age (years)		
≥ 60	1·00	
< 60	1·67 (1·06, 2·61)	0·026
Maximum tumour size (cm)		
< 2	1·00	
2–5	1·10 (0·63, 1·93)	0·728
> 5	2·56 (1·16, 5·65)	0·020
No. of tumours		
1	1·00	
≥ 2	1·99 (0·85, 4·62)	0·111
Macroscopic type		
1	1·00	
2	1·44 (0·75, 2·75)	0·270
3	0·76 (0·36, 1·62)	0·473
4	1·31 (0·36, 4·78)	0·687
5	1·68 (0·50, 5·67)	0·405
Tumour differentiation		
Well	1·00	
Moderate	1·59 (0·86, 2·92)	0·138
Poor	3·33 (1·46, 7·60)	0·004
Unclassified	1·01 (0·08, 12·67)	0·995
Vascular invasion		
No	1·00	
Yes	1·21 (0·25, 5·74)	0·813
Intrahepatic metastasis		
No	1·00	
Yes	2·34 (1·02, 5·37)	0·046
Non-cancerous liver		
Normal	1·00	
Chronic hepatitis/fibrosis	0·71 (0·30, 1·72)	0·450
Cirrhosis	2·25 (0·93, 5·40)	0·071
Liver damage grade		
A	1·00	
B or C	1·58 (0·96, 2·62)	0·075
AFP (units/l)		
< 20	1·00	
≥ 20 to < 400	1·96 (1·19, 3·25)	0·009
≥ 400 to < 1000	2·88 (1·19, 6·94)	0·019
≥ 1000	1·63 (0·86, 3·08)	0·134
DCP (units/l)		
< 40	1·00	
≥ 40 to < 500	2·73 (1·28, 5·41)	0·004
≥ 500 to < 1000	0·90 (0·39, 2·08)	0·804
≥ 1000	1·42 (0·76, 2·68)	0·273

Values in parentheses are 95 per cent confidence intervals. AFP, α-fetoprotein; DCP, des-γ-carboxyprothrombin.

Multivariable analysis revealed that tumour differentiation had the highest odds ratio related to death from recurrent HCC within 5 years, followed by raised levels of AFP and DCP (*Table 4*). When both the size and number of HCCs were categorized, the frequency of single HCC was significantly higher for any diameter of HCC in the 10-year RFS group than in patients who died from recurrent HCC within 5 years (data not shown).

Among patients whose levels of AFP (400–1000 units/l) and DCP (500–1000 units/l) were moderately raised, those with a single HCC had a lower risk of death from recurrent HCC than those with multiple tumours (data not shown). The number of HCCs yielded a higher odds ratio than the diameter of HCC in this specific group.

Discussion

The present study characterized tumour and patient factors among patients who survived without recurrence for 10 years after liver resection with curative intent for HCC. Although the characteristics of 10-year survivors after liver resection have already been investigated, there are few reports on 10-year RFS[2–5,10]. The present research was conducted as a nationwide large-scale comprehensive study of long-term recurrence-free survivors of HCC following liver resection in Japan.

In the present study, patients in the 10-year RFS group were younger with less background liver damage than patients who died from recurrent HCC within 5 years after liver resection. This was probably because there was less inflammatory change resulting from hepatitis C infection in the 10-year RFS group. The importance of underlying liver disease has been noted previously with regard to the degree of liver fibrosis and cirrhosis[10]. Underlying liver disease has more impact on patient survival than tumour factors[11]. Although two extreme HCC groups were compared in the present study (long-term RFS and short-term relapse), the present findings are of importance in determining possible factors associated with long-term RFS after curative liver resection.

Failure to detect latent intrahepatic HCC before surgery has no prognostic impact on the outcome or recurrence of HCC after liver transplantation[12,13]. The explanted diseased liver may show early HCCs that could not be detected before surgery, which can therefore appear as multicentric HCC on later examination. In the present study, patients in the 10-year RFS group had better liver function, despite a higher rate of positivity for hepatitis B surface antigen. Although the inflammatory activity in the resected liver was not investigated here, it was likely to have been lower in the remnant liver of the long-term survivors.

Tumour markers such as AFP or DCP have been reported to predict the early recurrence of HCC, even in the absence of microvascular invasion in the resected specimen[14,15]. The documentation of microvascular invasion depends on the slice width of the resected specimen and the number of slices investigated. Therefore, early recurrence can occur despite the absence of documented microvascular invasion. However, AFP or DCP levels are raised in nearly 60 per cent of patients with HCC, reflecting the biological behaviour of malignant tumours. The present data indicate that patients with no increase in AFP and DCP levels before surgery have a higher chance of survival without recurrence. In multivariable analysis, both tumour markers were independently associated with death due to recurrence after liver resection with curative intent. Furthermore, patients with a single HCC who had moderately raised AFP and DCP levels still had the prospect of surviving for longer after liver resection than those with high levels of tumour markers.

Considering the number and size of HCCs, a considerable percentage of patients in the 10-year RFS group had a single HCC (91·7 per cent) at the time of liver resection. Even with a raised AFP or DPC level, the risk of early death from recurrent HCC increased when there was more than one lesion. In other words, if a single HCC is found, a patient has an increased chance of surviving for longer after liver resection with curative intent.

Macroscopic HCC appearance was valuable for predicting 10-year RFS after curative liver resection, as shown previously[8]. HCCs of a contiguous multinodular type with clustering of small and contiguous nodules, and simple nodular types with extranodular growth carry a worse prognosis, most likely owing to microvascular invasion. In line with this, patients with these macroscopic types of HCC had a lower chance of long-term survival after liver resection in the present series.

The authors' group previously reported that anatomical resection has therapeutic value for treating patients with HCCs of 2–5 cm in diameter[16]. However, in the present study, this benefit of curative resection was not confirmed, even for HCCs with a diameter of 2–5 cm. This may have been because two extreme patient groups were compared. For example, even for HCC of 2–5 cm in size, the macroscopic appearance, vascular invasion, inflammatory status and fibrosis in the tumour-bearing liver may have been largely different between the two groups.

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THE ROLE OF EUS FOR DIAGNOSIS OF PANCREATIC MALIGNANCIES

NEW TECHNIQUES AND FUTURE PERSPECTIVE OF EUS FOR THE DIFFERENTIAL DIAGNOSIS OF PANCREATIC MALIGNANCIES: CONTRAST HARMONIC IMAGING

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Although endoscopic ultrasonography (EUS) has the advantage over other imaging methods in that it is possible to obtain high resolution images of the pancreas, it is limited in its ability to characterize pancreatic masses. Contrast-enhanced power Doppler ultrasonography suffers from several limitations such as blooming artifacts, poor spatial resolution, low sensitivity to slow flow and high sensitivity to motion artifacts. Recently, EUS system specific for contrast harmonic imaging has been developed. The use of this EUS system enabled us to observe images of microcirculation and parenchymal perfusion without Doppler-related artifacts in the pancreas. Contrast-enhanced harmonic EUS could diagnose pancreatic carcinomas as hypovascular masses with a high sensitivity (89–96%) and specificity (64–88%). Contrast-enhanced harmonic EUS also discriminates mural nodules from mucous clots in the intraductal papillary mucinous neoplasms.

Key words: contrast-enhanced harmonic EUS, EUS, pancreatic carcinoma, ultrasound contrast agent, vascularity.

HISTORY OF CONTRST-ENHANCED ENDOSCOPIC ULTRASONOGRAPHY

Endoscopic ultrasonography (EUS) has the advantage over other imaging method in that it is possible to obtain high resolution images of the pancreas, which is a highly sensitive method for the diagnosis of pancreatic tumors.[1–9] A hypoechoic mass with irregular contour or heterogeneous region are typical features of pancreatic carcinomas on EUS. However, in some cases, it is still difficult to diagnose pancreatic lesions by EUS. Evaluation of vascularity using contrast agents is one of candidates to improve the ability to characterize pancreatic lesions depicted by EUS.

In the first report of contrast-enhanced EUS, carbon dioxide microbubbles were used as the ultrasound contrast. The contrast-enhanced EUS using this carbon dioxide gas depicts ductal carcinomas as hypovasculsr masses whereas it depicts chronic pancreatitis and neuroendocrine tumors as isovascular and hypervascular masses, respectively.[10] It required an angiographic technique that was relatively invasive because the carbon dioxide microbubbles must be selectively infused into the celiac artery or superior mesenteric artery. A preliminary study using the intravenous ultrasound contrast agent, Albunex® (Shionogi, Osaka, Japan), air-filled microbubbles stabilized with a thin shell of human albumin, showed that ductal adenocarcinomas were less enhanced than the normal pancreatic parenchyma in contrast-enhanced EUS after the intravenous infusion of the contrast.[11] However, by the echoendoscope using fundamental B mode, the echo intensity of the tissue was too strong to selectively evaluate the signals from the contrast.

Technical advances in electric echoendoscopes equipped with color and power Doppler mode enabled us to depict vessels with fast flow as colored structure. There were several reports on the utility of contrast-enhanced EUS with the Doppler mode for diagnosing lesions in esophagus, stomach, gallbladder, pancreas and lymph nodes.[12] Particularly, power Doppler EUS with or without contrast agents depicted most adenocarcinomas in the pancreas as a hypovascular tumor. Such tumor hypovascularity is observed in 85–92% of patients with ductal adenocarcinoma of the pancreas.[13–17] Nevertheless, there were several limitations in contrast-enhanced Doppler ultrasonography such as blooming artifacts, the poor spatial resolution, low sensitivity to slow flow and high sensitivity to motion artifacts.[18,19]

Contrast harmonic imaging detects signals from microbubbles and filters signals originating from tissue, by selectively detecting the harmonic components.[18,19] This technology can detect signals from microbubbles in vessels with very slow flow without Doppler-related artifacts and is used to characterize tumor vascularity in liver, pancreas, gallbladder and the gastrointestinal tract during transabdominal ultrasonography.[20–23] Until recently, a contrast harmonic imaging technique was not available for EUS because the transducer of the echoendoscope was too small to produce enough acoustic power for contrast harmonic imaging using the first generation ultrasound contrast agents like Levovist® (Schering AG, Berlin, Germany). Second generation ultrasound contrast agents like SonoVue® (Bracco Imaging, Milan, Italy) and Sonazoid® (Daiichi-Sankyo, Tokyo, Japan; GE Healthcare, Milwaukee, WI, USA) produce harmonic signals at lower acoustic powers,[18,19] and are therefore suitable for EUS imaging at low acoustic powers.

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PRINCIPLE OF CONTRAST HARMONIC IMAGING

Ultrasound contrast agents are microbubbles of gas covered by a shell of biocompatible material such as a lipid.[18,19] When exposed to the ultrasound beams, the microbubbles are disrupted or resonated, which releases a large amount of harmonic signals.[18,19] When the tissue and the microbubbles receive transmitted ultrasound waves, both produce harmonic components that are integer multiples of the fundamental frequency, but the harmonic content from the microbubbles is higher than that from the tissues (Fig. 1). Selective depiction of the second harmonic component visualizes the signals from the microbubbles more strongly than those from the tissue (Fig. 1). In addition, signals from the contrast agents vary greatly in phase with no relation to its motion when ultrasound pulses are transmitted multiple times successively. However, signals are hardly changed between multiple transmissions in the part without the contrast agent. The extended pure harmonic detection mode equipped with the EUS system specific for contrast harmonic imaging receives not only the second harmonic components but also signals produced by the relative phase shifts of the received signals by transmitting and receiving ultrasound beams multiple times.[24,25] This processing is capable of enhancing the imaging of signals from contrast agents.[24,25]

CONTRAST-ENHANCED HARMONIC EUS IN PANCREATIC DISEASES

The use of contrast-enhanced harmonic EUS (CH-EUS) using second generation contrast agents is first reported by Dietrich *et al.*,[26] who reported that arterial and portal venous phases were achieved by identifying enhancement of large vessels such as celiac artery and portal vein. We also developed another EUS system equipped with an echoendoscope with a broad-band transducer and an imaging mode (extended pure harmonic detection mode) specifically for CH-EUS.[24,25] The echoendoscope has a broad-band transducer that can produce and detect harmonic signals from second generation contrast agents. These echoendoscopes equipped with a specific mode for CH-EUS have enabled us to obtain images of microcirculation and parenchymal perfusion in the digestive organs.[24–31] The vascular structure of some digestive diseases can be also observed by this new CH imaging EUS system.

Pancreatic solid lesions are characterized on the basis of CH-EUS-determined vascular patterns, namely, hypovascular, isovascular or hypervascular patterns (Figs 2–4).[25,30,31] The vast majority of the pancreatic carcinomas were characterized as having a hypovascular pattern (Fig. 2), whereas CH-EUS depicts most neuroendocrine tumors as a hypervascular pattern (Fig. 4). Most benign lesions are depicted

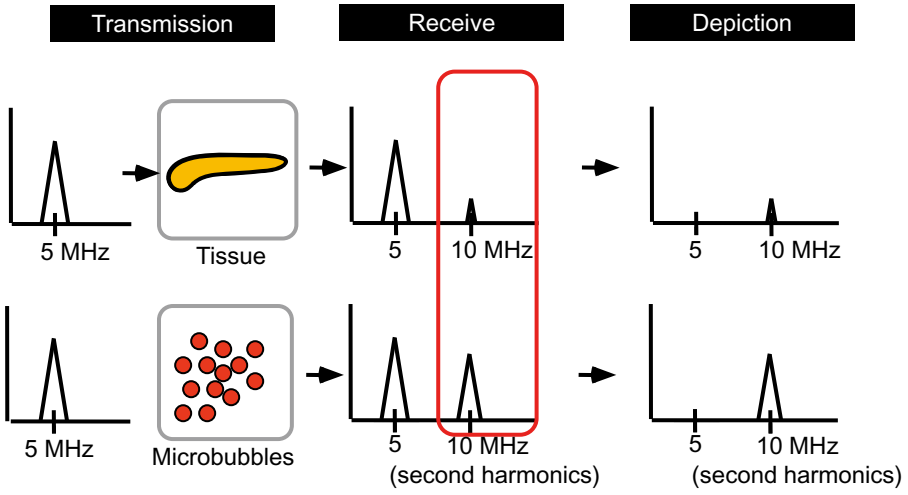

Fig. 1. Principle of contrast harmonic imaging. By selective depiction of the second harmonic component, the signals from the microbubbles are depicted more strongly than those from the tissue.

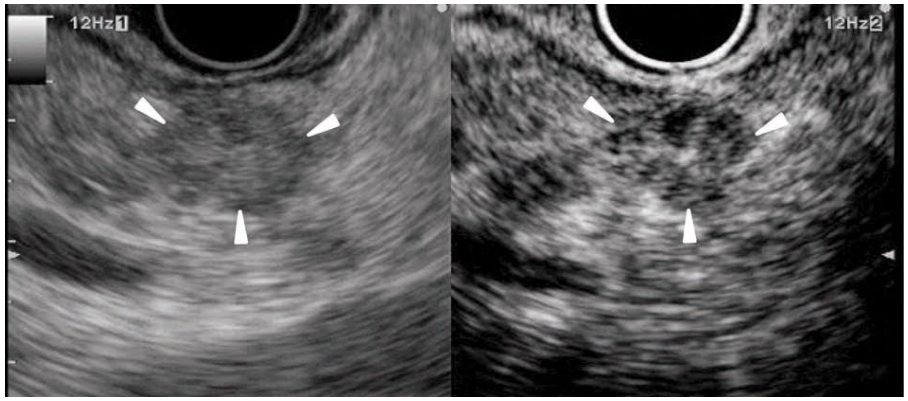

Fig. 2. Typical example of a ductal carcinoma. A hypoechoic lesion of 18 mm in diameter (arrowheads) is shown at the pancreas tail by the conventional mode (left). By the contrast harmonic imaging mode (right), heterogeneous enhancement is observed in the lesion (arrowheads) although the lesions was hypovascular.

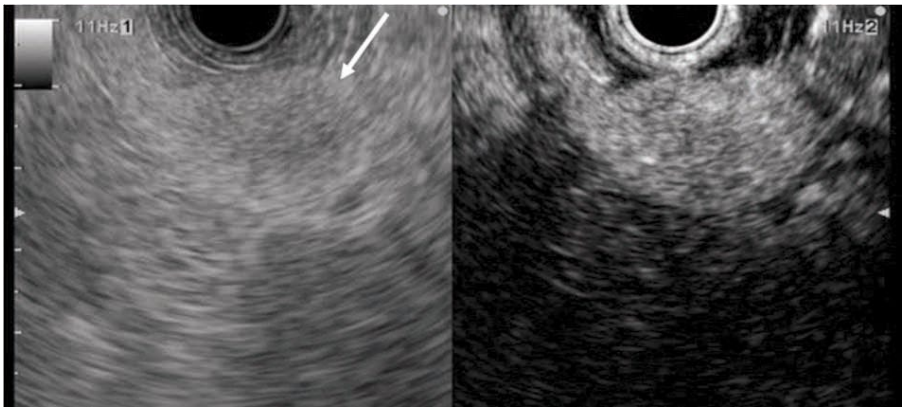

Fig. 3. Typical example of an inflammatory pseudotumor. A hypoechoic lesion of 19 mm in diameter (arrow) is shown at the pancreas tail by the conventional mode (left). Homogeneous enhancement without any clear margin is observed in the lesion by the contrast harmonic imaging mode (right).

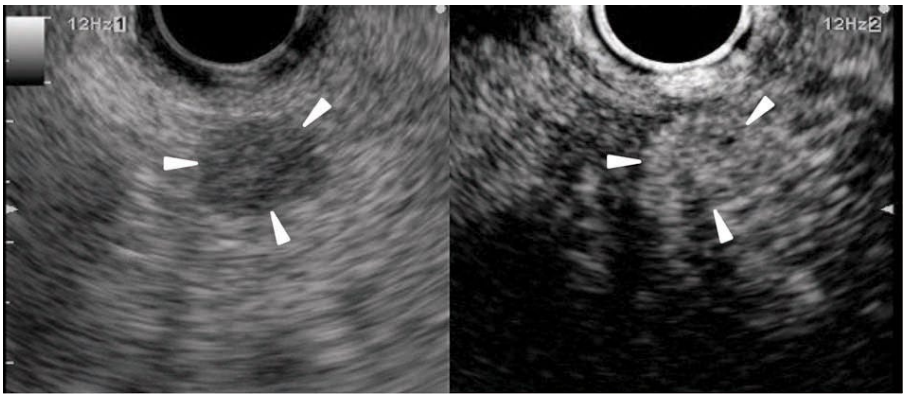

Fig. 4. Typical example of a neuroendocrine tumor. A hypoechoic lesion of 11 mm in diameter (arrowheads) is shown at the pancreas head by the conventional mode (left). The contrast harmonic imaging mode (right) shows the lesion is hypervascular.

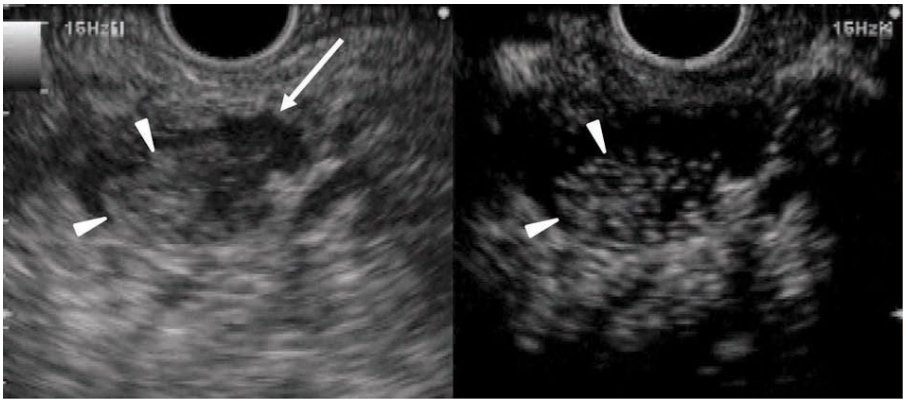

Fig. 5. Typical example of an intraductal papillary mucinous neoplasm. The conventional mode (left) shows an echogenic structure (arrowheads) in a cystic lesion (arrow) at the pancreas head. The contrast harmonic imaging mode (right) depicts vessels in the echogenic structure, showing that the structure is a mural nodule.

as an isovascular pattern (Fig. 3). Recently, two different groups reported that CH-EUS could diagnose pancreatic carcinomas as hypovascular masses with a high sensitivity (89% and 96%) and a specificity (88% and 64%) in 35 and 90 patients, respectively.[30,31] Interestingly, Fusaroli *et al*. reported that CH-EUS improves the depiction of pancreatic tumors, compared with conventional EUS. In their report, the outline of the tumor was unclear in seven patients with a pancreatic carcinoma, although subabnormalities such as stenosis of the bile duct and swelling of the pancreas are observed on fundamental B-mode imaging. CH-EUS allowed the detection of these uncertain lesions.[31] EUS is useful for evaluating cystic lesions in the pancreas, particularly intraductal papillary mucinous neoplasms (IPMN), because of its superior spatial resolution compared with other imaging method.[32–35] Diagnosis of IPMN by EUS depends largely on whether a mural nodules are detected.[32–35] However, it is sometimes difficult to discriminate sludge or mucous clots from mural nodules by conventional EUS. CH-EUS excludes avascular regions (mucous clots), whereas it depicts the mural nodules with enhancement (Fig. 5).[25] Therefore, CH-EUS might improve the ability of EUS in depicting mural nodules that are an important feature of IPMN.

FUTURE PERSPECTIVE OF CONTRAST-ENHANCED HARMONIC EUS

So far, the technique of contrast-enhanced EUS has been used for evaluation of vascularity. However, recent studies showed this technique can be applied for molecular imaging in the future.[36,37] The feasibility of new technologies using contrast-enhanced ultrasound with microbubbles targeted to vascular endothelial growth factor receptor type 2 are currently being tested. Moreover, the development of contrast-enhanced EUS will be beneficial for ultrasound-assisted drug-delivery applications in pancreatic tumors.[12]

CONCLUSION

Although EUS is a highly sensitive diagnostic method for the detection of small lesions in the pancreas, it is sometimes difficult to characterize such lesions with conventional EUS. The EUS system specific for CH imaging enabled us to observe microcirculation and parenchymal perfusion in the pancreas. CH-EUS might be useful for the characterization of pancreatic lesions detected by conventional EUS, particularly small ones which other imaging method fails to detect.

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Molecular Targeted Therapy for Hepatocellular Carcinoma: Bench to Bedside

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Key Words
Hepatocellular carcinoma · Molecular targeted therapy · Signal transduction · Sorafenib

Abstract
According to the International Agency for Research on Cancer, approximately 670,000 new cases of hepatocellular carcinoma (HCC) developed in 2005, making it the fifth most common cancer and third most common cause of cancer-related death worldwide. HCC is a complex and heterogeneous tumor with several genomic alterations. There is evidence of aberrant activation of several signaling cascades such as EGFR, Ras/Raf/MEK, PI3K/mTOR, HGF/MET, Wnt, Hedgehog and apoptotic signaling pathway. Recently a multikinase inhibitor, sorafenib, has shown survival benefits in patients with advanced HCC. It has been proposed that signaling pathway disruption in cancer can be grouped in six function capabilities, some of which need to be altered for cancer development: self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis and tumor invasion and metastases. The aim is to integrate these concepts into the molecular pathogenesis of HCC. It has also been proposed that there are common disturbances universal to all liver cancers on top of the more specific mechanisms. Based on this basic research, a molecular targeted agent has recently been developed. There have been no effective chemotherapeutic agents for advanced HCC. Sorafenib, an oral multikinase inhibitor, has set a milestone in the management of HCC in that it is the first agent to significantly improve the overall survival in patients with advanced HCC in a double-blind, placebo-controlled, phase III study. Clinical trials testing new agents for first- and second-line agents, as well as in combination with existing treatment options such as transarterial chemoembolization or arterial infusion chemotherapy, are ongoing. The results of these trials are therefore eagerly awaited.

Introduction

The 7th Japan-Korea Liver Symposium, the main theme of which was 'Molecular Targeted Therapy for Hepatocellular Carcinoma: Bench to Bedside', was held in Kyoto, Japan, on July 17–18, 2010, to focus on and discuss current topics of basic science and clinical application of

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molecular targeted agents. The symposium was full of eye-opening lectures by world-leading scientists, followed by extensive discussion. This special issue of *Digestive Diseases* selects the most important articles presented at this congress.

Molecular Pathogenesis

Kim and Lee's group [1] described the possibility of TGFBR3 polymorphisms and its haplotypes might be associated with HBV clearance and age of HCC occurrence.

Molecular alterations such as the p53 mutation, p16 gene silencing, and AKT signaling activation are found in the late stage of HCC progression. The overexpression of some marker molecules is observed at the early stage. Transforming growth factor-β (TGF-β), a potent inhibitor of cell proliferation, is frequently overexpressed in HCC, although the role of TGF-β signaling during HCC development remains controversial [2]. Authors previously reported that HCC cells show TGF-β receptor-dependent growth inhibition in response to TGF-β. Also, reduced TFG-β receptor II in HCC correlates with intrahepatic metastasis and shorter time to recurrence, suggesting a role of TGF-β signaling in tumor suppression. In contrast, TGF-β overexpression in HCC is known to correlate with malignant potential, suggesting a role in tumor promotion. Enhanced formation of stroma is a feature of advanced HCC, and TGF-β also promotes the proliferation of stromal fibroblasts. The microenvironment produced via tumor-stromal interactions may be the key to the modulation of the dual roles of TGF-β signaling in HCC progression [2].

Signaling Pathways and HCC

As in other cancers, the molecular mechanisms involved in the development and progression of HCC are complex. It has been shown that, after HBV/HCV infection and alcohol or aflatoxin B_1 exposure, genetic and epigenetic changes occur, including oncogene activation and tumor-suppressor gene inactivation due to inflammation-induced increase in hepatocyte turnover and oxidative stress-induced DNA damage. Through apoptosis and cell proliferation, these changes lead to the multistep development and progression of a hyperplastic to dysplastic nodule, early HCC, and advanced HCC. A number of studies have reported changes in gene expression, chromosomal amplification, mutations, deletions and copy number alterations (gain/loss), somatic mutations, CpG hypermethylation, and DNA hypomethylation, as well as molecular abnormalities, which can constitute therapeutic targets [3–7].

The binding of growth factors to their receptor proteins activates protein-phosphorylating enzymes, thus activating a cascade of proliferative signaling pathways to transmit proliferative signals into the nucleus. Growth factors, such as epidermal growth factor (EGF), TGF-α/-β, insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF), also function in liver regeneration after injury, while fibroblast growth factor and the platelet-derived growth factor (PDGF) family are involved in liver fibrosis and HCC growth [6, 7]. The receptors for these growth factors are broadly classified into G-protein-coupled receptors and protein kinases. On ligand binding, these receptors activate their downstream intracellular molecules in a cascade fashion. Many of the growth factor receptors and oncogenes have tyrosine kinase activity, and the tyrosine kinases are classified into transmembrane receptor tyrosine kinases such as the EGFR and VEGFR, and cytoplasmic non-receptor tyrosine kinases such as Abl and Src. On the other hand, Raf, MAP kinase/ERK kinase (MEK) and mammalian target of rapamycin (mTOR) are serine/threonine kinases.

In general, the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt/mTOR, c-MET, IGF, Wnt-β-catenin and Hedgehog signaling pathways, and the VEGFR and PDGFR signaling cascades show altered activity in HCC, and agents targeting these pathways are under development. Many molecular targeted agents are now under development and the target signaling pathways and growth factors are outlined below.

Sorafenib for HCC as a Stepping Stone

Disease stabilization with sorafenib lasts a few months, possibly due to the development of resistance, and thus the survival advantage was modest, even in patients with preserved liver function. Furthermore, there is currently no biomarker for monitoring the response or resistance to sorafenib. Currently, various kinds of molecularly targeted agents have been developed and are being evaluated in clinical trials. There are several steps required to improve the outcome from sorafenib therapy. First, a reliable predictive and prognostic biomarker is urgently needed. A compelling indication of sorafenib treatment

for HCC needs more clinical studies and consensus. The actual benefits of sorafenib to patients with advanced liver dysfunction should be clarified and a more effective strategy for targeted therapy needs to be developed, for example, using a combination of targeted agents acting on different pathways or different levels of a key pathway. Finally, sorafenib could be used with other treatment modalities, such as local ablation or transarterial chemoembolization (TACE), to synergize efficacy. Based on the successful introduction of sorafenib, future studies should focus on plans to further improve the outcome of HCC patients by overcoming resistance and maximizing the efficacy of molecularly targeted therapy.

Des-γ-Carboxy Prothrombin as a Promising Biomarker of Sorafenib to HCC

Murata et al. [8–10] reported that hypoxia induces des-γ-carboxyprothrombin (DCP). They explained this phenomenon as follows. The fine filamentous actin network, which plays a crucial role in clathrin-mediated endocytosis of vitamin K, is disrupted in DCP-producing cells because of hypoxia. It is considered that this offers one explanation for the elevated serum DCP level in patients with HCC, for which sorafenib is effective. In this issue, Ueshima et al. [11] reported that TTP in patients with a rapid increase in DCP within 2 weeks after starting sorafenib was significantly longer than that in patients with no increase in DCP. The CT findings for HCC with rapid DCP elevation tended to include reduced vascularity or presence of necrosis. This indicates that hypoxia was responsible for the change in DCP production. Accordingly, DCP may offer a surrogate marker for hypoxia.

Sorafenib induces hypoxia in HCC by inhibiting angiogenesis. TACE exposes the HCC to hypoxia, as does sorafenib, but this change is very rapid and most of the tumor cells become necrotic. It is thought that not enough DCP is produced after TACE. On the other hand, sorafenib induces tissue hypoxia relatively slowly and many viable HCC cells are exposed to hypoxia. During sustained hypoxia, the tumor cells gradually die and the serum level of DCP subsequently decreases.

During molecular targeted HCC therapy using sorafenib, Ueshima et al. [11] found that the rapid increase in DCP after starting sorafenib does not indicate tumor progression, but rather indicates HCC tissue hypoxia. Therefore, DCP may be a useful predictive marker for the duration of tumor suppression.

Branched-Chain Amino Acid Granules and HCC Incidence

Protein/energy malnutrition is commonly observed in patients with cirrhosis, and is represented by a decreased serum albumin level and skeletal muscle volume, and a decline in the non-protein respiratory quotient [12]. A decreased blood concentration of branched-chain amino acid (BCAA), caused by enhanced uptake and consumption of BCAA by skeletal muscle for ammonia metabolism and energy generation, is another manifestation of protein/energy malnutrition in patients with cirrhosis and is associated with disorders of protein synthesis and liver regeneration, and hyperammonemia [13]. Therefore, BCAA supplementation is a rational treatment for patients with cirrhosis.

Two large randomized controlled trials recently demonstrated that oral BCAA supplementation decreased the frequency of complications of cirrhosis and improved event-free survival in patients with decompensated cirrhosis [14, 15]. Based on these findings, oral BCAA supplementation is now recommended in Japanese guidelines as part of the treatment of HCV-related cirrhosis [16]. Similar to the reports mentioned above, Hayaishi et al. [17] showed that the event-free survival of patients with Child-Pugh A cirrhosis was better among patients given oral BCAA supplementation than in those without, although the difference did not reach statistical significance. BCAA supplementation has also been reported to be useful as an adjuvant nutritional therapy following hepatectomy and TACE, showing reduced risk of complications and better maintenance of liver function [18–20]. According to the earlier reports, BCAA seems to reduce the incidence of complications of cirrhosis by enhancing ammonia detoxification, upregulating protein synthesis and downregulating proteolysis, enhancing liver regeneration, and improving immune function [21–24].

Consensus-Based Clinical Practice Manual for HCC Proposed by the Japan Society for HCC of Hepatology

Following the publication by the European Society of Study of the Liver (EASL) in 2001 [25], the American Association for the Study of Liver Disease (AASLD) published the Clinical Practice Guidelines of hepatocellular carcinoma (HCC) in *Hepatology* in November 2005 [26] and updated in 2010 [27].

In Japan, the original Evidence-Based Clinical Practice Guidelines of HCC were published in 2005 [28] and updated in 2009 [29], disclosed on the website of the Japan Society of Hepatology (JSH) (www.jsh.or.jp/), and then widely used for liver cancer treatment in Japan. An excerpted version has also been published in an English journal by Makuuchi and Kokudo's group [29–31]. These guidelines were prepared after critical evaluations based on about 100 reports with a high evidence level in each field selected from 7,118 reports on HCC published between 1966 and 2002. In the 2009 revised version, 2,950 articles were reviewed and 532 were incorporated into the new version. Since the guidelines were prepared based as much as possible on highly evidenced data, some points may slightly deviate from actual practices related to HCC routinely performed based on the experience and consensus of HCC experts in Japan.

Considering this situation, the JSH summarized HCC treatment as performed in Japan with the consensus opinions of many experts, even though clear evidence was not available, and published a simple manual in 2007 [32, 33] and updated in 2010 [34, 35]. This was an experience- or consensus-based manual based on evidence-based guideline in respect to the evidence level, and summarized the consensus of expert opinions, widely reflecting the actual state of HCC treatment in Japan.

The manual was prepared in accordance with the Evidence-Based Clinical Practice Guidelines reported by Makuuchi and Kokudo [30], Kokudo et al. [31] and Arii et al. [36], and thus contains no conflict with those guidelines. Points that slightly differ are a more detailed explanation of liver cancer treatments based on expert opinions, and a summary of the consensus by the expert panel. Although it may seem unusual that two different guidelines are available and followed in Japan, they both have different roles and are not contradictory.

The report in this issue introduces the revised version of Consensus-Based Clinical Practice Manual of HCC published by the JSH in 2010, and focuses on prevention, surveillance, pathology, diagnosis, staging, and treatment. This constitutes a 'practice manual' summarized by the expert panel of the JSH, and is different from the Clinical Practice Guidelines. The contents of this report may be considered as the current state of the most advanced HCC treatment practices in Japan.

Disclosure Statement

The author has no conflict of interest to declare.

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Signaling Pathway and Molecular-Targeted Therapy for Hepatocellular Carcinoma

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Key Words
Hepatocellular carcinoma · Molecular-targeted agent · Sorafenib · Sunitinib · Brivanib · Complete remission

Abstract
In recent years, molecular-targeted agents have been used clinically to treat various malignant tumors. In May 2009, sorafenib (Nexavar®) was approved in Japan for 'unresectable hepatocellular carcinoma (HCC)', and was the first molecular-targeted agent for use in HCC. To date, sorafenib is the only molecular-targeted agent whose survival benefit has been demonstrated in two global phase III randomized controlled trials, and has now been approved worldwide. Phase III clinical trials of other molecular-targeted agents comparing them with sorafenib as first-line treatment agents are now ongoing. Those agents target the vascular endothelial growth factor, platelet-derived growth factor receptors, as well as target the epidermal growth factor receptor, insulin-like growth factor receptor and mammalian target of rapamycin, in addition to other molecules targeting other components of the signal transduction pathways. This review outlines the main pathways involved in the development and progression of HCC and the agents that target these pathways. Finally, current status and future perspective will also be discussed.

Introduction

The advances in molecular cell biology over the last decade have clarified the mechanisms involved in cancer growth, invasion and metastasis, and enabled the development of molecular-targeted agents, best represented by trastuzumab for breast cancer, imatinib and rituximab for hematopoietic tumors, and gefitinib and erlotinib for lung cancer. These molecular-targeted agents are broadly classified into two categories; drugs targeting cancer cell-specific molecules, and non-specific molecular-targeted drugs for molecular biological abnormalities induced in the host stroma or blood vessels by the presence of cancer. Examples of the former approach include trastuzumab, which targets HER2, the expression of which is a poor prognostic factor for breast cancer; rituximab, which is used to treat B-cell lymphoma, targets CD20 expressed on normal and neoplastic mature B cells; while imatinib binds to the ATP-binding site of Bcr-abl, a protein that causes chronic myelogenous leukemia. However, no critical target molecules responsible for treatment response have been identified in hepatocellular carcinoma (HCC).

In recent years, clinical trials have been conducted for many agents that act on growth factor receptors (such as epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR)) and intra-

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cellular signaling pathways. In addition, multikinase inhibitors, including sorafenib, have emerged and evaluated. Clinical trials are now ongoing to compare drugs with the same mechanism of action and to test the combined efficacy and relative merits of these drugs with existing drugs for many cancers. Since the main treatment option for metastatic, advanced-stage cancers, such as breast and colorectal cancer, is systemic chemotherapy, clinical trials are ongoing to investigate how to combine molecular-targeted agents with standard therapies based on the results of long-term, large-scale clinical trials, and to identify which molecular-targeted agents should be used as initial or second-line therapy.

However, for HCC, background liver damage limits the indication for systemic chemotherapy and no anticancer drugs were found to be effective in large-scale randomized controlled trial except sorafenib. Now that the usefulness of sorafenib has been demonstrated in two large-scale randomized clinical trials, the development of new drugs that are effective for poor prognostic advanced HCC, who are resistant to a standard of care agent, sorafenib.

Signaling Pathways and Molecular-Targeted Agents in HCC

As in other cancers, the molecular mechanisms involved in the development and progression of HCC are complex. It has been shown that, after HBV/HCV infection and alcohol or aflatoxin B1 exposure, genetic and epigenetic changes occur, including oncogene activation and tumor-suppressor gene inactivation due to inflammation-induced increase in hepatocyte turnover and oxidative stress-induced DNA damage. Through apoptosis and cell proliferation, these changes lead to the multistep development and progression of a hyperplastic to dysplastic nodule, early HCC, and advanced HCC. A number of studies have reported changes in gene expression, chromosomal amplification, mutations, deletions and copy number alterations (gain/loss), somatic mutations, CpG hypermethylation, and DNA hypomethylation, as well as molecular abnormalities, which can constitute therapeutic targets [1–5].

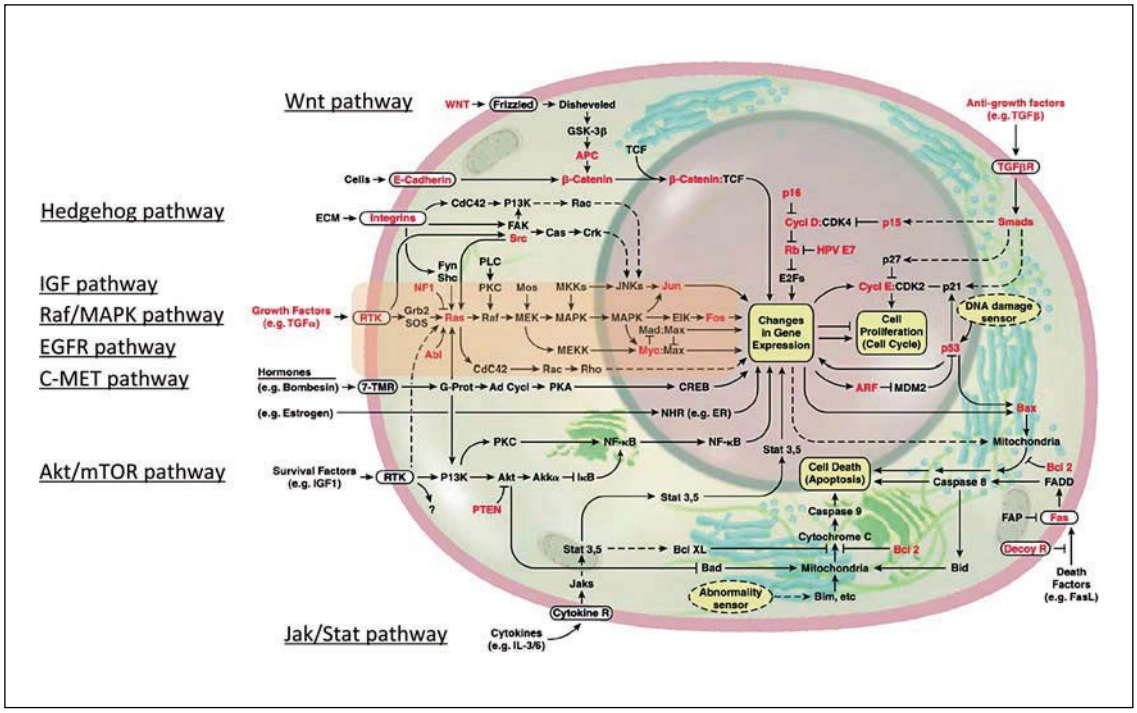
The binding of growth factors to their receptor proteins activates protein-phosphorylating enzymes, thus activating a cascade of proliferative signaling pathways to transmit proliferative signals into the nucleus. Growth factors, such as epidermal growth factor (EGF), transforming growth factor (TGF)-α/-β, insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF), also function in liver regeneration after injury, while fibroblast growth factor (FGF) and the platelet-derived growth factor (PDGF) family are involved in liver fibrosis and HCC growth [6–8]. The receptors for these growth factors are broadly classified into G-protein-coupled receptors and protein kinases. On ligand binding, these receptors activate their downstream intracellular molecules in a cascade fashion. Many of the growth factor receptors and oncogenes have tyrosine kinase activity, and the tyrosine kinases are classified into transmembrane receptor tyrosine kinases such as the EGFR and VEGFR, and cytoplasmic non-receptor tyrosine kinases such as Abl and Src. On the other hand, Raf, MAP kinase/ERK kinase (MEK) and mammalian target of rapamycin (mTOR) are serine/threonine kinases.

In general, the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt/mTOR, c-MET, IGF, Wnt-β-catenin and Hedgehog signaling pathways, and the VEGFR and PDGFR signaling cascades show altered activity in HCC, and agents targeting these pathways are under development (fig. 1, 2; table 1) [9–11]. Many molecular-targeted agents are now under development and the target signaling pathways and growth factors are outlined below.

MAPK Pathway (Ras/Raf/MEK/ERK)

The MAPK intracellular signaling pathway, which is mainly involved in cell growth and survival, and regulates cell differentiation, is upregulated in cancer cells. Therefore, this pathway has been extensively studied as a therapeutic target. The MAPK pathway is a common downstream pathway for the EGFR, PDGFR and VEGFR, and is universally used for signal transduction downstream of cytokine receptors, integrin complexes and G-protein receptors to Ras. The MAPK pathway also plays an important role in HCC in that its activation is reportedly involved in HCC growth and survival [5]. The downstream extracellular signaling-regulated kinase (ERK) is activated by two upstream protein kinases, which are coupled to growth factor receptors by Ras proteins. Ras, which is activated by ligand binding, activates Raf serine/threonine kinases and MEK (MAP kinase/ERK kinase), while MEK phosphorylates and activates ERK, which phosphorylates proteins involved in cell growth, apoptosis resistance, extracellular matrix production and angiogenesis [12–15].

Raf and Ras Inhibitors. Raf and Ras are proto-oncogenes. In particular, K-ras mutations are commonly observed in many cancers, including pancreatic and colorec-



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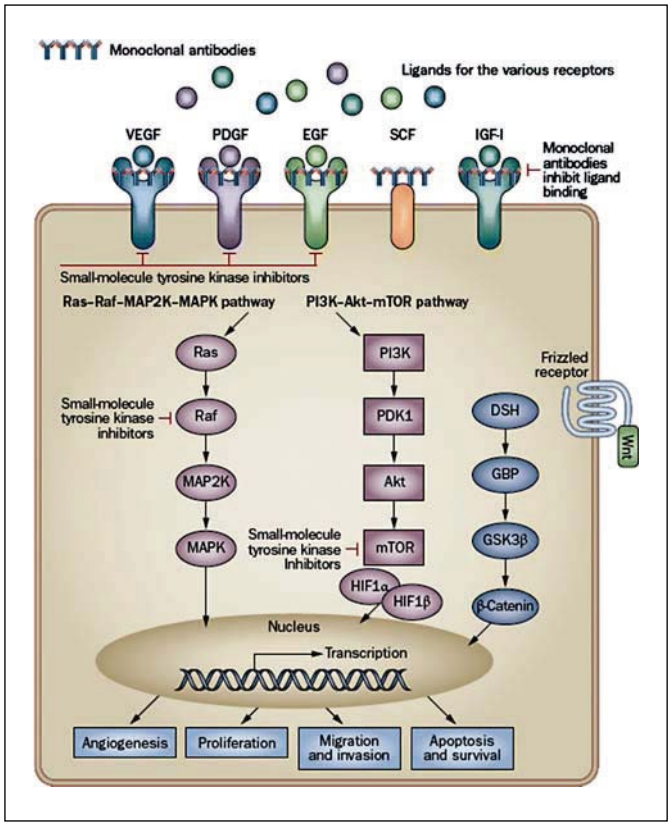

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Fig. 1. Signal transduction in solid cancer cells including HCC. Some of the genes known to be functionally altered are highlighted in red. These signaling pathways including growth factor pathway, Wnt pathway, Hedgehog pathway, Akt/mTOR pathway, and Jak/Stat pathway can be a molecular target for treatment of HCC [cited and modified from 10, with permission].

Fig. 2. Signaling pathways and molecular-targeted agents. Monoclonal antibodies (VEGFR: bevacizumab, EGFR: cetuximab), tyrosine kinase inhibitors (VEGFR: sorafenib, brivanib, linifanib, axitinib, EGFR: erlotinib, lapatinib), serine/threonine kinase inhibitors (Raf: sorafenib, mTOR: rapamycin and everolimus, PIK: KL-755) [cited and modified from 11, with permission].

Table 1. Molecular-targeted agents for HCC: targets and development status in Japan as of March 2011

Agents	Targets (angiogenesis)			Targets (proliferation)				Positioning	Development status
	VEGFR	PDGFR	FGF	EGFR	Raf	mTOR	TRAIL-R2 DR5		
Sorafenib	●	●			●			1st line	Approved
Sunitinib	●	●						1st line	PIII terminated
E7080	●	●	●					1st/2nd line	PII ongoing
Tigatuzumab (CS1008)							●	1st line	PI/II ongoing
Linifanib (ABT-869)	●	●						(Sorafenib combination) 1st line	PIII ongoing
Brivanib	●		●					1st line, 2nd line, TACE adjuvant	PIII ongoing
TSU-68	●	●						TACE combination	PIII ongoing
Ramucirumab	●							2nd line	PIII ongoing
Everolimus (RAD001)						●		2nd line	PIII ongoing
Axitinib	●	●						2nd line	PIII ongoing

tal cancers. One study reported that 30% of HCCs have Ras mutations [16]. To our knowledge, no agents targeting Ras are planned to enter clinical trials at the present. However, because the binding of Ras protein to the cell membrane and its functional activation require farnesylation, several farnesyltransferase inhibitors are being tested for Ras-related tumors. In addition, vaccine therapy for mutant Ras proteins is currently being tested for solid cancers, including HCC. The Raf family consists of three isoforms, A-Raf, B-Raf and C-Raf/Raf-1. Genetic abnormalities, such as point mutations and gene rearrangements, have been reported in various cancers [17]; however, in HCC, *ras/raf* mutations are rare, and no *k-ras* or *b-raf* mutations have been detected [18]. On the other hand, wild-type Raf-1 was reported to be hyperactivated in many cancers, including HCC [19–21]. Sorafenib inhibits Raf, and has multiple characteristics in that it exhibits strong inhibitory activity against Raf-1 (C-Raf) kinase, B-Raf (wild-type B-Raf and mutant V600E B-Raf) serine/threonine kinase, the pro-angiogenic receptor tyrosine kinases VEGFR, PDGFR and FGFR1, and tyrosine kinases such as c-kit, Flt-3 and RET, which are involved in tumor progression and overall prognosis [22].

MEK. The MEK family consists of MEK1 and MEK2 proteins, which specifically phosphorylate tyrosine and threonine residues, and phosphorylates downstream Erk1 and Erk2 [23]. In an immunohistochemical study, MEK1/2 overexpression, ERK1/2 overexpression, and ERK1/2 phosphorylation were observed in 100% (46/46), 91% (42/46) and 69% (32/46) of HCCs, respectively, and the in vitro treatment of HepG2 and Hep3B cells with MEK1/2 inhibitors inhibited cell growth and upregulated apoptosis [14]. The MEK inhibitors CI-1040, PD0325901, AZD6244 and RDEA119/BAY869766 have been tested in several cancers including solid tumors such as HCC. Recently, results of phase I of AS703026 and E6201 studies against solid tumors were reported in ASCO2010. A phase II study of AZD6244 (selumetinib, ARRY-142866) and a phase I/II study of RDEA119/BAY869766 in combination with sorafenib are being conducted.

PI3K/Akt/mTOR Pathway

The PI3K/Akt/mTOR pathway also plays an important role in cell growth, survival regulation, metabolism and antiapoptosis. The membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) is phosphorylated by phosphatidylinositol 3-kinase (PI3K) into phosphatidylinositol 3,4,5-triphosphate (PIP_3), which binds to and activates the serine/threonine kinase Akt. The tumor-suppressor gene product PTEN (phosphatase and tensin homolog deleted on chromosome) is antagonistic to PI3K activity. PTEN is a lipid phosphatase that dephosphorylates inositol phosphates such as PIP_3. The inactivation of PTEN through gene deletion increases PIP_3 levels, and activates Akt, which inhibits apoptosis, leading to the development of tumors. The serine/threonine kinase mTOR is an important mediator in the PI3K/Akt pathway that binds intracellularly to a protein called raptor or rictor, and exists

as two different complexes, complex 1 and 2 (mTORC1 and mTORC2). mTORC2 (mTOR-rictor) activates Akt, while mTORC1 (mTOR-raptor) is activated downstream of Akt; thus, both molecules regulate protein synthesis [24].

A study of 528 HCC samples showed that the expression of pAkt, PTEN, p27 and S6 ribosomal protein (pS6) was a poor prognostic factor for survival [25]. A tissue microarray analysis of HCC samples revealed that the loss of PTEN and overexpression of pAkt and p-mTOR were correlated with tumor grade, intrahepatic metastasis, vascular invasion, TNM stage, Ki-67 labeling index and matrix metalloproteinase (MMP)-2 and -9 upregulation. Meanwhile, PTEN mRNA expression in the cancerous tissue was downregulated, compared with that in the non-cancerous tissue. The levels of PTEN, MMP-2, and MMP-9 mRNA expression were correlated with tumor stage and metastasis, and the levels of PTEN and MMP-9 mRNA expression were inversely correlated [26]. In an extensive analysis of 314 HCC samples in terms of mutation analysis, DNA copy number changes, mRNA levels and immunostaining, Villanueva et al. [27] found that activation of the IGF pathway, upregulation of EGF, dysregulation of PTEN, and aberrant mTOR signaling were present in half of the samples, and that inhibiting mTOR activity with everolimus was effective in improved survival and suppression of recurrence.

The PI3K inhibitor RG7321 and the Akt inhibitor perifosine target the PI3K/Akt/mTOR pathway and are in early stages of clinical development, while the mTOR inhibitors everolimus (RAD001), sirolimus (Rapamune) and temsirolimus (CCI-779) are at more advanced stages of development. Everolimus is used to treat sorafenib-intolerant patients or for patients showing disease progression after sorafenib administration. A phase III study to compare everolimus and a placebo (EVOLVE-1: Advanced Hepatocellular Carcinoma after Disease Progression or Intolerance to Sorafenib EverOlimus for LiVer cancer Evaluation) and a phase I/randomized phase II study (sorafenib + everolimus vs. sorafenib alone) to test the efficacy and tolerance of sorafenib in combination with everolimus are underway. Since mTOR inhibitors exhibit cytostatic and antiangiogenic effects, they are expected to be effective in combination with other angiogenesis inhibitors such as bevacizumab, and may be appropriate for administration after transarterial chemoembolization (TACE). Furthermore, since the mTOR pathway is stimulated by factors such as EGFR, PDGFR and TGF-α, and is closely related to other signaling pathways including the Ras/Raf/MEK/ERK pathway, they are likely to show promising efficacy when used in combination with other growth factor inhibitors [28].

VEGF/VEGFR, PDGFR, FGFR

Angiogenesis is an important event not only for HCC but also for cancer growth and metastasis, and occurs due to complex alterations involving promoting factors such as VEGF, angiopoietin and FGF, and inhibitory factors including thrombospondin (TSP) and angiostatin, as well as the surrounding tissue. The VEGF family consists of VEGF-A, -B, -C, -D, and -E, and placental growth factor (PIGF). The VEGFR family comprises VEGFR-1 (flt-1), VEGFR-2 (flk-1/KDR) and VEGFR-3 (flt-4). VEGF-A binds to VEGFR-1 and -2 and is involved in angiogenesis and the maintenance of mature blood vessels, while VEGF-C and -D mainly bind to VEGFR-3, and are involved in lymphangiogenesis [29, 30]. VEGF isoforms such as $VFGF_{121}$ and $VEGF_{165}$ have been identified, and isoform subtypes also exist, such as $EGF_{166}b$. Thus, it is clear that these growth factors do not exhibit angiogenesis-promoting effects alone, and they have attracted attention as new therapeutic targets [31].

HCC typically exhibits active angiogenesis. During the progression from early to well, and to moderately differentiated HCC, angiogenesis increases and cancer cells acquire the ability to invade vessels and metastasize. Scientific and clinical studies have revealed that, during the progression from hepatitis to cirrhosis, angiogenesis and disruption of the vascular architecture are linked to the progression of HCC, and contribute to increased hepatic vascular resistance and portal hypertension, and decreased hepatocyte perfusion [32]. In addition, a meta-analysis has demonstrated that VEGF expression is a prognostic factor in HCC [33].

Phase II studies have been started to test the usefulness of bevacizumab (Avastin®), which directly targets VEGF, in TACE-treated HCC, and the use of bevacizumab in combination with erlotinib (Tarceva®), an EGFR tyrosine kinase inhibitor.

Sunitinib (Sutent®) is a multikinase inhibitor that inhibits tyrosine kinases such as VEGFR-1, -2, -3, PDGFR-α, -β and c-kit. A phase II study of sunitinib in 37 advanced HCC patients showed that the median progression-free survival (PFS) and median overall survival (OS) were 3.7 and 8 months, respectively. In that study, adverse events included grade 3/4 thrombocytopenia in 37.8% of patients, neutropenia in 24.3%, asthenia in 13.5%, and hand-foot syndrome in 10.8% [34]. Since sunitinib has a lower IC_{50} for each target than sorafenib, it is expected to exhibit greater antitumor activity. However, this factor

may be responsible for the higher incidence of adverse events with sunitinib. The main evaluation item in the above phase II trial was the response rate which did not reach the expected value, leading to the conclusion that it was a negative study [35]. In that study, sunitinib was administered at 50 mg/day for 4 weeks followed by 2 weeks of rest per cycle [34], whereas Zhu et al. [35] used a dosing schedule of 37.5 mg/day for 4 weeks followed by 2 weeks of rest per cycle, and reported that the median PFS and OS were 3.9 and 9.8 months, respectively. An ongoing global cooperative phase III controlled clinical trial to compare sorafenib and sunitinib head to head and to seek approval for first-line indications for advanced HCC adopted a sunitinib dosing schedule of 37.5 mg/day. However, in a 'reflection and reaction' regarding the above trial results, Forner et al. [36] casted doubt on whether the drugs at this dose could maintain tolerance and ensure efficacy. Consequently, the trial was terminated in March 2010 because of the recommendation by data monitoring committee (DMC) based on interim analysis, showing relatively high toxicity and no superior efficacy to sorafenib.

Brivanib is a kinase inhibitor that selectively inhibits VEGFR-1, -2 and -3, and FGFR-1, -2 and -3. As for sunitinib, an international global phase III clinical trial to compare brivanib and sorafenib head to head and to seek approval for first-line therapy for advanced HCC has already been started, and the results are eagerly awaited. Japanese centers are participating in this clinical trial. Since brivanib targets FGF and VEGF, and is associated with relatively mild adverse effects, a second-line study of brivanib in sorafenib-ineffective and -intolerant patients and a trial to evaluate the use of brivanib in combination with TACE are underway. Depending on the results of these trials, indications for use in HCC may be obtained; therefore, positive results are eagerly anticipated. The results have been reported for a phase II study of brivanib in 55 patients (cohort A) who had not received systemic therapy for curatively unresectable HCC and 46 patients (cohort B) previously treated with angiogenesis inhibitors such as sorafenib or thalidomide [37]. The median time to progression (TTP) and OS were 2.8 and 10 months, respectively, in cohort A versus 1.4 and 9.8 months, respectively, in cohort B. Adverse events included fatigue (51.5%), diarrhea (41.6%), hypertension (42.6%), anorexia (41.6%), and nausea/vomiting (40.6%/30.7%) in total. Thus, these results demonstrated the efficacy of brivanib as a second-line treatment. The results of three phase III clinical trials, BRISK-PS (sorafenib failure or sorafenib-intolerant patients; brivanib + best supportive care (BSC) vs. placebo + BSC), BRISK-FL (advanced HCC; brivanib vs. sorafenib) and BRISK-TA (patients with unresectable HCC, brivanib vs. placebo as post-TACE adjuvant therapy) are awaited. Japanese centers participated in all three trials.

In a Japanese phase I/II trial of TSU-68, an oral molecular inhibitor of VEGFR, PDGFR and FGFR, to test its safety and efficacy in 35 HCC patients, the response rate was 5.6% (CR, PR, SD, PD and NE in 1, 2, 15, 16 and 1 patients, respectively), and the disease control rate was 51.4% [38]. The global phase III trial of TACE in combination with TSU-68 has just started in January 2011.

In addition, several phase I/II trials are being conducted to assess kinase inhibitors such as linifanib (ABT-869) and cediranib (AZD2171), which inhibit VEGFR, PDGFR, CSF-1R (cFms), Kit and Flt3. Furthermore, a phase III global study of axitinib, which is currently being tested in renal cell carcinoma, has also been started as a second-line agent in 2011.

EGF/EGFR

EGFR is a member of the human epidermal growth factor receptor (HER) family that includes EGFR (erbB1), HER2/neu (erbB3) and HER4 (erb4). All members of this family, except HER3, have an intracellular tyrosine kinase domain, and the binding of a ligand to its extracellular domain triggers signal transduction through the above-described MAPK and PI3K/Akt/mTOR pathways. Thus, these receptors are involved in cell growth, differentiation, survival and adhesion [39]. EGFR overexpression has been reported in many cancers, and in HCC. For example, Buckley et al. [40] reported that EGFR, detected by immunohistochemical analysis, was overexpressed in 50 (66%) of 76 HCCs, and that fluorescence in situ hybridization (FISH) showed extra EGFR gene copies in 17 (45%) of 38 HCCs.

EGFR-targeting drugs include anti-EGFR antibodies, such as cetuximab and panitumumab, and small-molecule inhibitors of EGFR tyrosine kinases such as gefitinib, etc., and have been used widely for the treatment of several cancers other than HCC. Unfortunately, except for phase II trial data, there are little clinical data on the efficacy of these drugs for the treatment of HCC.

Similar to gefitinib (Iressa®), erlotinib (Tarceva®) is an oral EGFR tyrosine kinase inhibitor. Philip et al. [41] and Thomas et al. [42] have reported the results of phase II studies of erlotinib in HCC; the median OSs in their studies were 13 and 10.7 months, respectively. A phase III clinical study (SEARCH study: Sorafenib and Erlotinib, A Randomized Trial Protocol for the Treatment of Pa-

tients with Hepatocellular Carcinoma) for sorafenib in combination with erlotinib versus sorafenib plus placebo is ongoing. Since erlotinib is associated with a high incidence of skin rash, dry skin, and gastrointestinal toxicity, such as diarrhea, the results of the SEARCH study should be evaluated to assess whether this combination therapy can be used in clinical settings. Thomas et al. [43] conducted a phase II clinical study of erlotinib in combination with bevacizumab in 40 advanced HCC patients, and reported promising results; the median PFS and OS were 9 and 15.7 months, respectively. However, they noted frequent treatment-related grade 3/4 toxicities, including fatigue (20%), hypertension (15%), gastrointestinal bleeding (12.5%), wound infection (5%), diarrhea (10%), elevated transaminase levels (10%) and thrombocytopenia (10%), which necessitates further evaluation for drug tolerance. Although a clinical study of erlotinib in combination with bevacizumab (OPTIMOX-3 study) was also conducted in colorectal cancer patients, no tolerance was observed, which led to a change in the protocol [44, 45].

After the introduction of a number of molecular-targeted drugs, strategies for the inhibition of similar or different signaling pathways (vertical or horizontal inhibition) with several drugs have been proposed. However, the combined use of molecular-targeted agents has remained largely unsuccessful, including panitumumab in combination with bevacizumab for the treatment of colorectal cancer [46]. Similarly, the results of sorafenib in combination with bevacizumab (vertical inhibition) have been reported [47]. Although some therapeutic responses were obtained, the combination therapy resulted in greater toxicity [47], suggesting the need for detailed evaluation of the dosing regimen.

Lapatinib (Tykerb®) is a dual inhibitor of EGFR and HER-2/neu, and inhibits tumor growth by downregulating MAPK, AKT and p70S6 kinase [48]. In Japan, lapatinib is indicated for the treatment of breast cancer. In a phase II clinical trial of lapatinib in 26 patients with unresectable advanced HCC, the median PFS and OS were 1.9 and 12.6 months, respectively, and adverse events included diarrhea (73%), nausea (54%) and skin rash (42%) [49].

Cetuximab (Erbitux®) is a human/mouse chimeric monoclonal antibody consisting of the variable region of a mouse anti-human EGFR monoclonal antibody and the human IgG1 constant region. Cetuximab inhibits the binding of endogenous EGFR ligands, such as EGF and TGF-α, to EGFR. In a phase II clinical trial of cetuximab in 30 patients with unresectable or metastatic HCC, the median PFS and OS were 1.4 and 9.6 months, respectively, and treatment-related toxicities included grade 3 hypomagnesemia (3.3%) and grade 1/2 acne-like rash (83.3%), which was observed for the duration of anti-EGFR therapy in that study [50].

The EGFR offers a very interesting therapeutic target. As described above, the use of erlotinib in combination with sorafenib is still in the research stage. However, based on the results of phase II studies, the efficacy of cetuximab or lapatinib as a monotherapy seems to be limited, and the results of further studies evaluating their efficacy in sorafenib-refractory or -intolerant patients are awaited with interest.

HGF/c-Met Pathway

Since the hepatocyte growth factor (HGF)/Met pathway is involved in tumor growth, invasion and angiogenesis in a wide range of neoplasms, HGF and Met have recently attracted attention as a therapeutic target. HGF is a heterodimer consisting of α- and β-chains bound together by a disulfate bond. The α-chain contains four kringle domains, and the β-chain contains a serine protease-like domain. Met is a receptor tyrosine kinase for the HGF ligand, and contains a semaphorin-like domain. HGF or Met overexpression and Met gene mutations and duplications have been reported in various cancers, and abnormalities due to HGF/Met pathway activation have also been noted [51]. These abnormalities activate the downstream signaling cascade, leading to epithelial-mesenchymal transition and increased proliferative, migratory, invasive and metastatic potentials of cancer cells [51].

HGF/c-MET-targeted drugs, including kinase inhibitors, HGF inhibitors and decoy c-Met receptor molecules are being developed. Of particular interest is ARQ-197, a c-Met receptor tyrosine kinase inhibitor, which as a non-ATP-competitive molecule that binds near the ATP-binding site. A randomized phase II study of ARQ-197 versus placebo is ongoing in patients with unresectable HCC after systemic therapy failure. In addition, the results of a phase I study of ARQ-197 in combination with sorafenib were reported in ASCO 2010 (abstr. No. 3024).

IGF/IGFR

The IGF/IGFR system is involved in cell growth and the chemotherapeutic response. The ligands IGF-I and -II bind to their receptors IGF-1R and IGF-2R, and are involved in DNA synthesis and cell growth. Abnormalities in IGF and IGF-1R or their overexpression have been reported in various cancers, including HCC. Their associations with disease stage, metastasis and survival [52] and

the functions of IGF and IGFR in HCC [53] have been reported.

IGF-targeting drugs are currently being developed, and mainly including anti-IGF-1R antibodies, such as BIIB022, AVE1642 and cixutumumab (IMC-A12). A phase II study of cixutumumab, a phase Ib/II study of sorafenib versus sorafenib plus BIIB022, and phase I/II studies of AVE1642 as monotherapy or in combination with sorafenib or erlotinib are ongoing.

Sorafenib: Trial Results and Clinical Experience

Clinical Results for Sorafenib in HCC

As described above, sorafenib is a multikinase inhibitor of tumor growth and angiogenesis, and exhibits a strong inhibitory effect on C- and B-Raf serine/threonine kinases (comprising the Raf/MEK/ERK pathway), VEGFR and PDGFR tyrosine kinases, and Flt-3 and c-kit [22]. To date, sorafenib is the only molecular-targeted agent approved for the treatment of HCC based on the results of two large-scale clinical trials, namely the SHARP (Sorafenib HCC Assessment Randomized Protocol) study [54] and the Asia-Pacific study [55]. The median OSs for the sorafenib group in the SHARP and Asia-Pacific studies were 10.7 months (vs. 7.9 months for the placebo group, $p < 0.001$; HR 0.69) and 6.5 months (vs. 4.2 months for the placebo group, $p = 0.014$; HR 0.68), respectively, indicating that sorafenib prolongs survival by approximately 30%. These data should compel HCC specialists to challenge their preconception that systemic anticancer drug therapy is not effective for HCC.

Current Status regarding the Use of Sorafenib in Japan

Sorafenib was approved in Japan in May 2009. A survey has confirmed that, at the time of writing (May 2011), over 10,000 patients have been prescribed sorafenib. Across several centers, 100 Japanese patients have achieved CR or near CR (superresponded PR), which was not observed in the SHARP or Asia-Pacific trials. This suggests that some Japanese patients may be very sensitive to sorafenib [56]. The reason for this or predictive biomarkers is now actively under investigation.

On the other hand, it has been reported that hand-foot syndrome occurs early after sorafenib administration [57] more often than was noted in the SHARP and Asia-Pacific studies, and the drug is often discontinued because of the adverse effects in many patients [57]. As demonstrated in the SHARP and Asia-Pacific studies, sorafenib is only used to achieve stable disease; therefore, it is important to improve drug efficacy by extending the period of administration for as long as possible. Therefore, it is no exaggeration to say that, in the case of sorafenib, the 'successful management of side effects' is equal to 'successful treatment'. According to the 'post-TACE phase III clinical study' [57] performed in Japan and Korea, it is strongly speculated that physicians who are unaccustomed to prescribing molecular-targeted agents and who fail to see marked efficacy, as induced by conventional chemotherapeutic agents, often do not understand the properties of this drug, and they (as well as the patients) do not fully comprehend therapeutic efficacy. Moreover, they feel too anxious about side effects

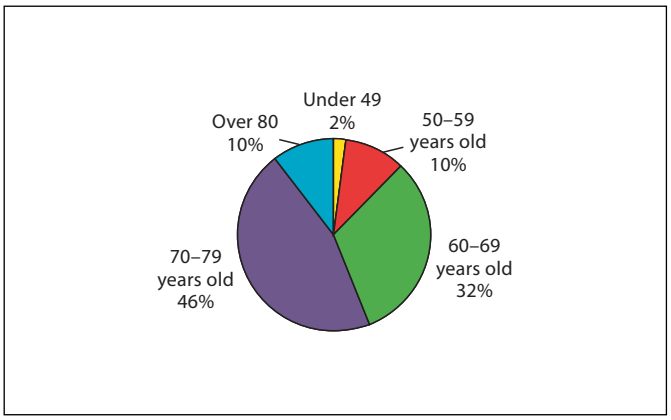

Fig. 3. Age distribution of patients treated with sorafenib.

Table 2. Patients' background treated with sorafenib (n = 113; data from Kinki University Hospital)

Age	70.1 (31–90)	
Gender	M	80 (70.8%)
	F	33 (29.2%)
Etiology	HBV	23 (20.4%)
	HCV	60 (53.1%)
	NBNC	30 (26.5%)
Child-Pugh score	5	64 (56.6%)
	6	33 (29.2%)
	7	16 (14.2%)
Child-Pugh grade	A	97 (85.8%)
	B	16 (14.2%)
Stage	III	51 (45.1%)
	IVA	28 (24.8%)
	IVB	34 (30.1%)

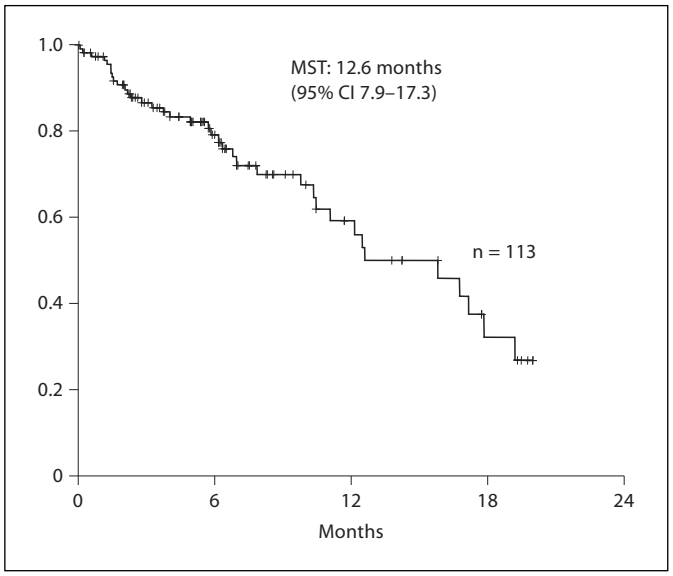

Fig. 4. Overall survival in patients treated with sorafenib.

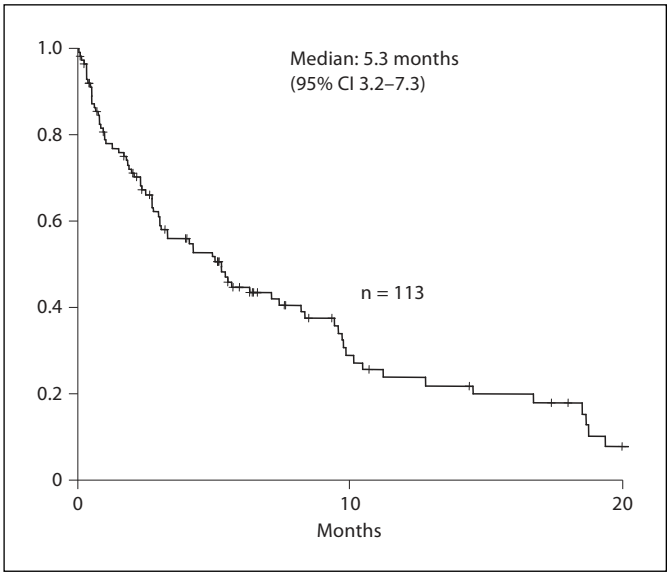

Fig. 5. Treatment duration in patients treated with sorafenib.

that have not been encountered before. These circumstances may result in treatment discontinuation in many patients. Clearly, greater awareness among physicians for therapeutic efficacy and approaches to manage adverse effects is needed to improve treatment outcomes.

Experience of Sorafenib Use at Our Institute

Since the approval of sorafenib on May 20, 2009, we have treated approximately 150 patients with sorafenib during 20 months, but few have discontinued therapy due to adverse effects or patient refusal to continue. Of these 113 patients, 2 achieved CR [56]. These 2 CR patients, in whom pulmonary, adrenal metastases and intrahepatic lesions all disappeared, survived free of recurrence for more than 3 and 2 years, respectively at the time of writing (May 2011), i.e., they are still alive at the present. In other patients who apparently achieved SD, the tumor marker levels reached a plateau after sorafenib administration, when their levels were rising rapidly before sorafenib administration. Even if hepatic lesions do not show a clear tendency to undergo necrosis or regression on CT images, three tumor markers (AFP, PIVKA-II and AFP-L3) are widely considered to serve as surrogate markers. In fact, there is very little data on serum tumor markers, except for AFP, outside Japan. Nevertheless, Japanese researchers have demonstrated the value of changes in these markers and the antitumor efficacy of sorafenib [56].

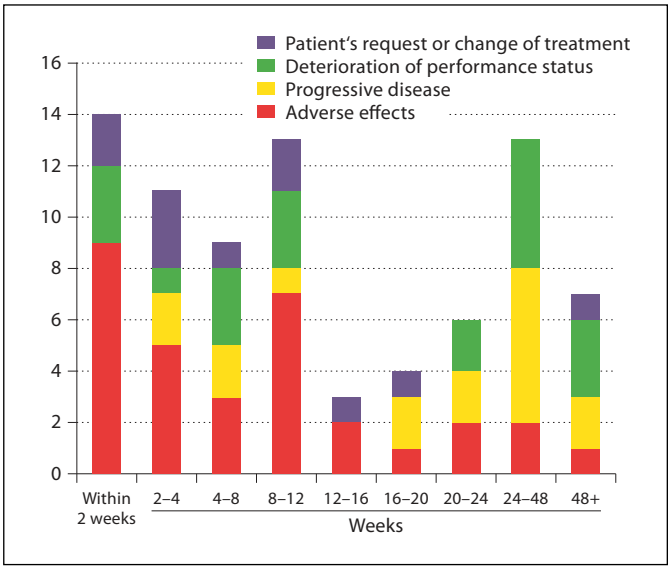

Fig. 6. Causes of discontinuation of sorafenib. Only 17 of 113 (15%) cases were terminated due to PD. Most of discontinuation of sorafenib due to adverse effects was within 12 weeks.

Interestingly, it was previously demonstrated that the levels of PIVKA-II or DCP tend to be increased by inducing hypoxia [58]. Therefore, PIVKA-II or DCP may be a good predictive marker for evaluating the hypoxic response to antiangiogenic therapy for HCC [59].

Of 113 patients, 85.8% was Child-Pugh A stage and 53.1% of patients were HCV-related HCC (table 2). A total of 56% of patients were over 70 years of age (fig. 3). The initial status of patients treated with sorafenib is listed in table 3. Median survival time (MST) was 12.6 months (fig. 4) and median treatment duration was 5.3 months (fig. 5). The causes of discontinuation are listed in figure 6. Only 10% of the 113 patients showed PD by RECIST. However, since the speed with which the patient develops progressive disease may slow down due to tumor growth inhibition, it is very difficult to determine when to discontinue treatment because of tumor refraction. Important issues for future studies include: (1) to identify biomarkers that can predict therapeutic responses, including CR or PR, in patient groups; (2) to evaluate the role of tumor markers in the determination of therapeutic responses; (3) to establish response evaluation criteria that can determine the therapeutic responses to molecular-targeted agents, and (4) to develop effective second-line therapies after sorafenib failure (fig. 2, 7) [11, 60].

According to the consensus-based treatment algorithm by the Japan Society of Hepatology (fig. 7) [60], updated in 2010 [61], sorafenib is indicated for the treatment of patients with Child-Pugh A HCC with extrahepatic metastasis, vascular invasion or refractoriness to TACE or arterial infusion chemotherapy.

In addition to the pharmaceutical-sponsored clinical trials of linifanib and brivanib as first- and second-line therapy in sorafenib-refractory patients, investigator-initiated trials (IIT) of sorafenib in combination with hepatic arterial infusion chemotherapy (SILIUS trial: trial No. NCT01214343), pharmaceutical and IIT trials of sorafenib in combination with TACE (SPACE, TACICS (trial No. NCT 01217034) and BRISK-TA trials), and a trial to test the inhibitory effect of sorafenib on tumor recurrence after curative treatment (STORM trial) are ongoing, and the results of these trials are eagerly awaited (fig. 7) [60].

The working hypotheses in these studies can be deduced by extrapolating the MST and hazard ratios in OS calculated in a subanalysis of the SHARP study (table 4). The results obtained suggest that starting treatment with molecular-targeted drugs at an earlier tumor stage in combination with standard treatment options such as resection, ablation, TACE or hepatic arterial infusion chemotherapy can improve the prognosis of HCC. Thus, sorafenib has the potential to induce a paradigm shift in the treatment of HCC. For example, in a subanalysis of the SHARP trial, the hazard ratios for OS and MST ratio in intermediate-stage HCC without vascular invasion or extrahepatic spread were 0.52 and 1.50, respectively (table 4). This suggests that survival of early-stage HCC and intermediate-stage HCC may be prolonged from 5 to 7.5–10 years by using sorafenib in an adjuvant setting after curative treatment, and from 3 to 4.5–6 years by using sorafenib in combination with TACE (fig. 8) [60].

Table 3. Initial status of patients treated with sorafenib

Refractory to TACE	36
Impossible of TACE due to:	
AP shunt	2
Stenosis of artery	5
Macrovascular invasion	4
Multiple nodules at first diagnosis	3
Portal vein invasion at first diagnosis	8
Hepatic vein invasion at first diagnosis	1
Extrahepatic spread	28
Refractory to HAIC	4
Refractory to standard treatment	2
Candidate of clinical trials: SILIUS (phase I)	12
Patient's request	6
Bile duct invasion	1
Others	1

Table 4. Subanalysis data of the SHARP study (data from M. Sherman et al., ASCO 2008)

	Advanced HCC	
	with vascular invasion and extrahepatic spread	without vascular invasion or extrahepatic spread
Hazard ratio	0.77	0.52
95% CI	0.60–0.99	0.32–0.85
Median OS (MST)		
Sorafenib	8.9 months (n = 209)	14.5 months (n = 90)
95% CI	7.6–10.3 months	14.0 months (N/E)
Placebo	6.7 months (n = 212)	10.2 months (n = 91)
95% CI	5.2–8.0 months	8.6–15.5 months

Summary and Future Prospects

Several clinical trials [34, 35, 37, 41, 42, 50, 62–66] of the molecular-targeted agents are ongoing. Angiogenesis-inhibiting drugs, particularly sorafenib, have been evaluated for HCC, and drugs targeting EGFR and mTOR are being developed. The results (numerical values) of phase II clinical trials show no marked differences in the

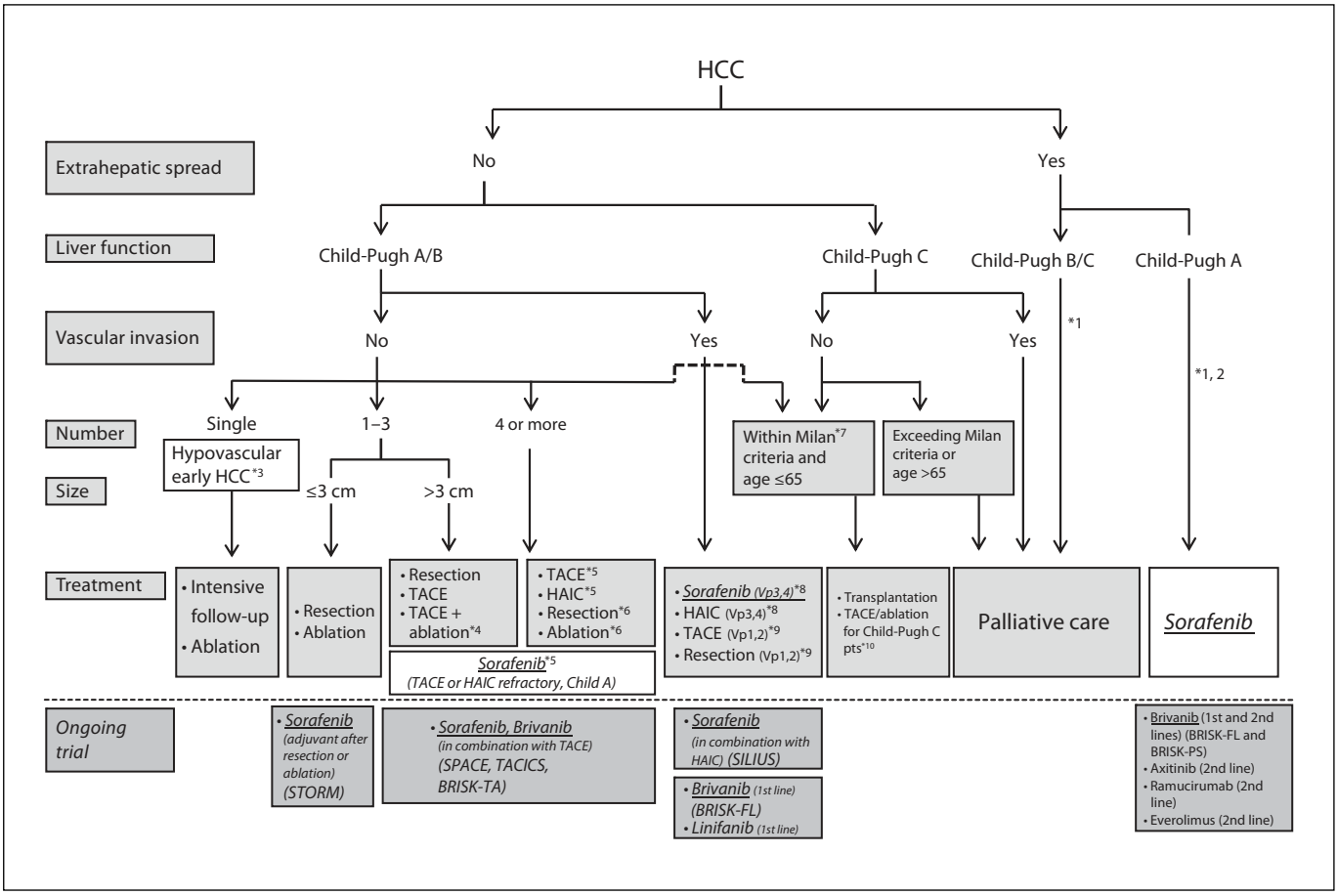

Fig. 7. Consensus-based Treatment Algorithm for Hepatocellular Carcinoma proposed by Japan Society of Hepatology (JSH) revised in 2010 [cited and modified from 60, with permission]. Footnotes: *1 = Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not considered as a prognostic factor in Child-Pugh class A/B patients. *2 = Sorafenib is the first choice of treatment in this setting as a standard of care. *3 = Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (1) when the nodule is diagnosed pathologically as early HCC, (2) when the nodules show decreased uptake on Gd-EOB-MRI, or (3) when the nodules show decreased portal flow by CTAP, since these nodules frequently progress to advanced HCC. *4 = Even for HCC nodules exceeding 3 cm in diameter, TACE in combination with ablation is frequently performed when resection is not indicated. *5 = Transcatheter arterial chemoembolization (TACE) is the first choice of treatment in this setting. Hepatic arterial infusion chemotherapy (HAIC) using an implanted port is also recommended for TACE-refractory patients. The regimen for this treatment is usually low-dose FP (5FU + CDDP) or intra-arterial 5FU infusion combined with systemic interferon therapy. Sorafenib is also recommended for TACE- or HAIC-refractory patients with Child-Pugh class A liver function. *6 = Resection is sometimes performed when more than 4 nodules are detected. Ablation is sometimes performed in combination with TACE. *7 = Milan criteria: tumor size ≤3 cm and tumor number ≤3, or solitary tumor ≤5 cm. Even when liver function is good (Child-Pugh A/B), transplantation is sometimes considered for frequently recurring HCC patients. *8 = Sorafenib and HAIC are recommended for HCC patients with major portal invasion such as Vp3 (portal invasion in the 1st portal branch) or Vp4 (portal invasion in the main portal trunk). *9 = Resection and TACE are frequently performed when portal invasion is minor, such as Vp1 (portal invasion in the 3rd or more peripheral portal branch) or Vp2 (portal invasion in the 2nd portal branch). *10 = Local ablation therapy or subsegmental TACE is performed even for Child-Pugh C patients when transplantation is not indicated when there is no hepatic encephalopathy, no uncontrollable ascites, and a low bilirubin level (<3.0 mg/dl). However, it is regarded as an experimental treatment because there is no evidence of a survival benefit in Child-Pugh C patients. A prospective study is necessary to clarify this issue.

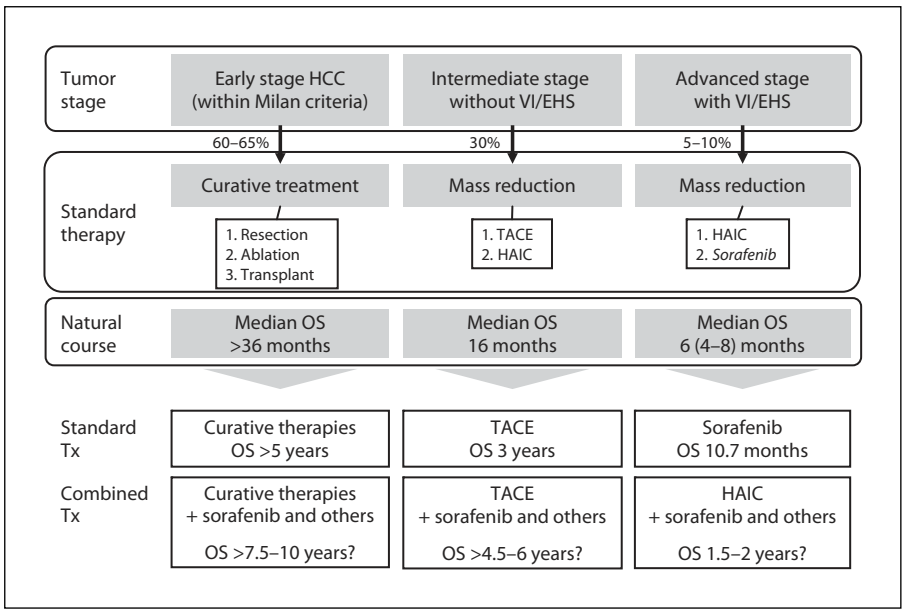

Fig. 8. Outcomes of standard treatment modalities and expected future outcomes of combination therapy with molecular-targeted agents. By combining molecular-targeted agents with resection or ablation, life expectancy is expected to be prolonged to 7.5–10 years. In addition, for intermediate-stage HCC, the prognosis is expected to be improved to 4.5–6 years by combination with TACE. OS = Overall survival.

Table 5. Ongoing clinical trials (PIII)

First line
Comparison study between sorafenib and single agent (head to head):
Sunitinib → endpoint did not meet!
Brivanib
Linifanib
Combination with sorafenib and another agent:
DXR, erlotinib (SEARCH), everolimus, CS-1008, etc.
Second line
Sorafenib failure: brivanib, everolimus (RAD001), ramucirumab, axitinib, S-1, etc.
Combination with standard therapy
Adjuvant setting after surgery or RFA: *STORM*
Combination with TACE: *SPACE, BRISK-TA, TACTICS, ECOG1208*
Combination with HAIC: *SILIUS*

TACTICS Phase II study = Transcatheter Arterial Chemoembolization Therapy In Combination with Sorafenib (ClinicalTrials.gov ID: NCT01217034); SILIUS = Randomized Controlled Trial Comparing Efficacy of Sorafenib versus Sorafenib In combination with Low-dose cisplatin/fluorouracil hepatic arterial InfUSion chemotherapy in Patients with Advanced Hepatocellular Carcinoma And Exploratory Study of Biomarker Predicting Its Efficacy (ClinicalTrials.gov ID: NCT01214343); HAIC = hepatic arterial infusion chemotherapy.

therapeutic efficacy evaluated by TTP or PFS. However, phase II studies may be subject to patient selection bias and cannot be compared with the results of other trials. Thus, when determining the therapeutic efficacy of drugs, we should review the efficacy of the respective drugs, and consider where the theoretical target molecules are present and what combinations of drugs have a theoretical rationale, and thus evaluate options for monotherapy and combination therapy based on the efficacy and safety data obtained from phase III clinical trials.

Molecular-targeted agents that have been introduced into clinical use in recent years are approved for the treatment of specific cancer and are then frequently used to treat other cancers. Although not discussed here, studies to identify predictors of efficacy (i.e., biomarkers) for angiogenesis inhibitors and EGFR tyrosine kinase inhibitors, and factors involved in drug resistance, are making steady progress, and the associated therapeutic strategies are undergoing major changes. Therefore, even in the treatment of HCC, it is necessary for HCC specialists to expand their knowledge of and techniques for applying existing treatment modalities (resection, ablation, TACE, arterial infusion chemotherapy) to physically remove, destroy or necrotize the tumor, and to better understand clinical oncology, particularly the role and mechanisms of action of molecular-targeted agents. We are entering an era in which physicians treating HCC should pay close attention to the development of therapeutic agents not only for HCC but also for other cancers, and be aware of

the use of molecular-targeted agents for treating cancers in clinical and basic research settings, and understand approaches to limit or control adverse effects associated with these drugs.

Although sorafenib was recently approved, many issues remain to be addressed, including (1) how to determine and define refractoriness, and (2) whether to continue TACE or hepatic arterial infusion chemotherapy (a de facto standard in Japan) in patients with TACE-refractory tumors or portal tumor thrombi before starting sorafenib therapy. For oncology, in particular, the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan has approved several drugs based on results from global clinical trials and from Japanese phase I study data alone. We strongly recommend that, based on the molecular-targeted agents currently under development, clinical studies (including IITs) should be conducted aggressively, and therapeutic strategies should be devised to resolve the limitations of currently used therapeutic approaches and to improve the therapeutic outcomes (table 5).

The introduction of sorafenib to treat HCC in 2007 in Western countries and in 2009 in Japan was undoubtedly the *real* beginning of a paradigm shift of HCC treatment, representing a significant breakthrough for HCC treatment not previously experienced for this unique tumor.

Disclosure Statement

The author has no conflict of interest to declare.

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mTOR Inhibitor for the Treatment of Hepatocellular Carcinoma

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Key Words
Hepatocellular carcinoma · mTOR inhibitor · RAD001 · Evelorimus

Abstract
Mammalian target of rapamycin (mTOR) plays a central role in the regulation of cellular growth, proliferation, and survival via a cytoplasmic serine/threonine kinase. mTOR also works as a nutrition sensor to monitor cellular metabolism. mTOR is located downstream in the PI3K/Akt pathway, in which Akt and the tuberous sclerosis complex (TSC) 1/2 are involved, to form a signal transduction pathway. New anticancer agents that target mTOR in the PI3K/Akt pathway of the signal transduction pathways involved in cell proliferation control have recently been developed and are already commercially available. A phase III clinical trial of mTOR inhibitor for hepatocellular carcinoma (HCC) is now ongoing worldwide to expand indications. RAD001 is a signal-transduction inhibitor (STI) that targets mTOR (more specifically, mTORC1). mTORC1 signaling is intricately regulated by mitogens, growth factors, energy, and nutrients. mTORC1 is a regulator essential for general protein synthesis, located downstream of the PI3K/AKT/mTOR pathway, which is dysregulated in most human cancers. Inhibiting mTOR with molecules, such as RAD001, generates additive effects that accompany upstream and downstream target inhibition; alternatively, upstream receptor inhibition is compensated for by inhibiting the downstream pathway, even if some resistance develops against receptor inhibition regardless of initial or acquired resistance. In conclusion, RAD001 is a potential targeted agent for HCC and therefore final results of a phase III study are awaited.

Introduction

With advances in the molecular biology of tumors, new anticancer agents, such as molecular targeted agents, have been developed. Traditional anticancer agents exhibit nonselective cytotoxic activity, while molecular targeted agents target molecules associated with the proliferation, angiogenesis, apoptosis, metastasis, etc., of cancer cells. Also, new anticancer agents that target mammalian target of rapamycin (mTOR) in the PI3K/Akt pathway of the signal transduction pathways involved in cell proliferation control have recently been developed and are already commercially available. A phase III clinical study of mTOR inhibitor for hepatocellular carcinoma (HCC) is ongoing to expand indications.

PI3K/Akt Pathway and mTOR

mTOR plays a central role in the regulation of cellular growth, proliferation, and survival via a cytoplasmic serine/threonine kinase. mTOR also works as a nutrition sensor to monitor cellular metabolism. mTOR is located downstream in the PI3K/Akt pathway, in which Akt and

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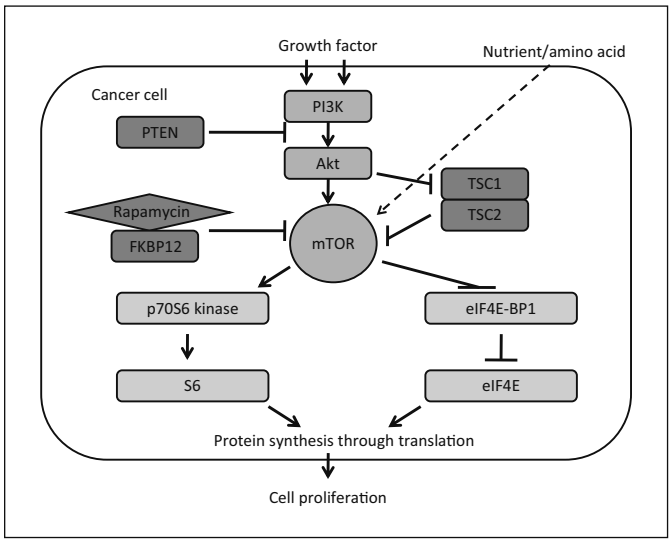


Fig. 1. PI3K/Akt/mTOR signaling pathway.

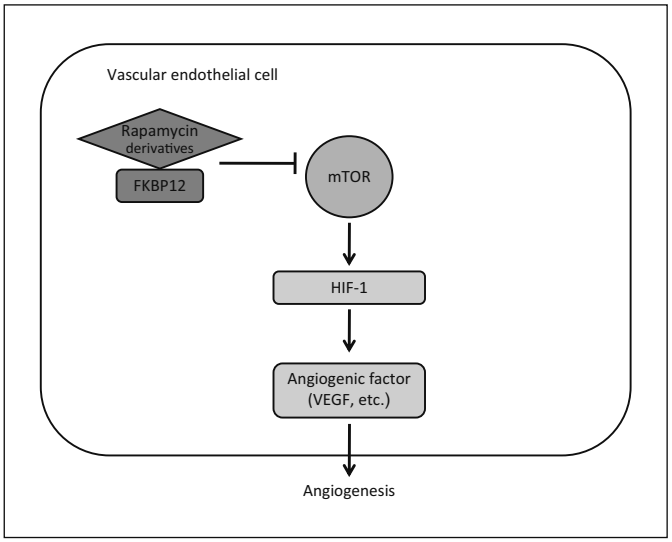


Fig. 2. mTOR/HIF-1/VEGF signaling pathway.

the tuberous sclerosis complex (TSC) 1/2 are involved, to form a signal transduction pathway [1, 2]. The PI3K/Akt pathway is involved in protein synthesis and translation via mTOR, while acting on cell-cycle progression and antiapoptosis via signal transduction to play an important role in cell proliferation. mTOR is a serine/threonine kinase (289 kDa) activated by Akt or Rheb. Activated Akt suppresses TSC, a tumor suppressor gene. Then, TSC suppresses Rheb [3, 4]. Reportedly, this pathway depends on various growth factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF), as well as the nutritional and oxygen state, etc., and is activated in various cancers [5, 6]. mTOR causes cell proliferation through the following steps (fig. 1): mTOR activates p70S6 kinase that efficiently translates mRNA into a protein by phosphorylating the S6 of 40S ribosomal protein [7], and mTOR phosphorylates eukaryotic initiation factor (eIF)-4 E-binding protein (eIF4E-BP) 1 that binds to eIF4E, a translation initiation factor, to release eIF4E bound to 4E-BP1 and initiate the translation of proteins required for shifting from G_1 to S phases of the cell cycle [8]. p70S6k is a serine/threonine kinase that phosphorylates S6 protein, a component of the ribosomal 40S subunit, and has cell proliferation and antiapoptotic effects when activated [9] (fig. 1).

p70S6k is also considered to be a positive regulator of hypoxia-inducible factor (HIF)-1, a potential master switch for the gene expression of factors that play an important role in angiogenesis (e.g. VEGF) [10] (fig. 2).

mTOR as a Molecular Target of Cancer

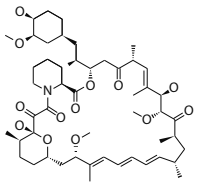
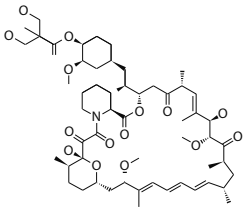
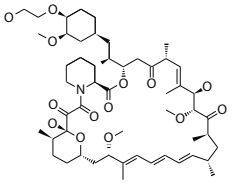
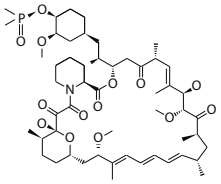
Sirolimus (rapamycin), an mTOR inhibitor, is an agent clinically developed as an antifungal or immunosuppressive drug. Recently, its anticancer effects have attracted attention. Basic and clinical research on rapamycin derivatives, temsirolimus (Torisel™), RAD001 (Afinitor®), and deforolimus (AP23573, MK8669), has been conducted. In addition, an inhibitor (PI-103) for both PI3K and mTOR factors has been clinically developed.

The mTOR signaling pathway is enhanced in various cancers. Increased eIF4E in colorectal cancer and activated Akt and p70S6 kinases in neuroblastoma and thyroid cancer have been reported. Reportedly, cells showing the deletion or mutation of tumor suppressor genes (e.g. PTEN and p53) are highly susceptible to mTOR inhibitors.

The macrolide antibiotic rapamycin, an mTOR inhibitor, binds to FKBP12 (FK-506 binding protein) to form a rapamycin/FKBP12 complex. This complex binds to mTOR to arrest the cell cycle at the G_1 phase and exert antitumor effects through apoptosis induction [7, 11, 12].

Furthermore, inhibitory effects of rapamycin on tumor angiogenesis have recently been demonstrated. Such inhibitory effects on tumor angiogenesis include decreased VEGF production and apoptosis induction in neovascular endothelial cells that are stimulated by VEGF induced in the PI3K/Akt pathway [13–16].

Table 1. mTOR inhibitor

Drug	Chemical structure	Development phase	Administration route
Rapamycin (sirolimus)		phase I	oral
CCI-779 (temsirolimus)		phase III	oral, injection
RAD001 (everolimus)		phase III	oral
AP23573		phase I/II	oral, injection

At present, a clinical study is ongoing on a new mTOR-targeting anticancer agent, a rapamycin derivative that acts as an mTOR inhibitor, to develop a second-line agent against HCC (tables 1, 2).

Mechanism of Action and Characteristics as an Anticancer Agent

Inhibition of Tumor Cell Proliferation

The PI3K/Akt/mTOR signaling pathway is enhanced in various cancers, suggesting mTOR dependence of tumor cell proliferation caused by increased protein synthesis [10]. mTOR targeting is expected to be applied to therapy. Rapamycin and its derivative bind to FKBP12 to form a complex, and further bind to mTOR to inhibit the pathway downstream of mTOR to exert cytostatic antitumor effects by arresting the cell cycle at the G_1 phase [11] (fig. 1).

Inhibition of Tumor Angiogenesis

Inhibitory effects on angiogenesis have attracted attention as additional anticancer effects of mTOR inhibitor. Rapamycin inhibits the activation of HIF-1, a factor involved in angiogenesis, as previously described [17]. In addition, rapamycin strongly inhibits VEGF production and the proliferation of vascular endothelial cells caused by VEGF [13].

Preclinical and Clinical Studies of RAD001 in the Various Cancers

RAD001 (everolimus), a rapamycin derivative, is an mTOR inhibitor that can be administered orally. Reportedly, RAD001 dose-dependently inhibits cell proliferation and angiogenesis both in vivo and in vitro. Currently, clinical studies of various phases are ongoing employing monotherapy or combined therapy with cytocidal

Table 2. Results of mTOR inhibitor in clinical trials

mTOR inhibitor	Subject	Trial phase	Treatment line	Dose-associated drug	Dose/ schedule	Results
Temsirolimus	advanced HCC	randomized phase II	1st, 2nd	25 vs. 75 vs. 250 mg	i.v./weekly	RR 7% (CR 1 case), PFS 5.8 months
	advanced RCC	phase III	1st	25 vs. IFN-α vs. IFN-α + T 25 mg	i.v./weekly	median OS: IFN-α 7.3 months, T 10.9 months, IFN-α + T 8.4 months
	recurrent glioblastoma	phase II	1st, 2nd	250 mg	i.v./weekly	PFS, 6 months, 7.9%
	advanced breast cancer	randomized phase II	2nd line	75 vs. 250 mg	i.v./weekly	RR 9.2%, TTP 12 weeks
	malignant lymphoma	phase II	2nd	250 mg	i.v./weekly	RR 38%, TTP 6.5 months
RAD001	metastatic RCC	phase II	2nd, 3rd	10 mg	oral/daily	RR 29%
	advanced RCC	phase III	2nd, 3rd	placebo vs. 10 mg	oral/daily	placebo 1.9 months, E 4 months
	metastatic gastric cancer	phase II	2nd, 3rd	10 mg	oral/daily	DCR (CR+PR+SD 8 weeks) 59.4%
	advanced HCC	phase I/II		10 mg	oral/daily	RR 30%, RFS 3.8 months, OS 8.4 months
Deforolimus	advanced sarcoma	phase II	1st	12.5 mg	i.v./5 days/2 weeks	CBR (CR+PR+SD 16 weeks) 28%
	relapsed hematologic malignancy	phase II	2nd	12.5 mg	i.v./5 days/2 weeks	RR 10%, SD 40%

CBR = Clinical benefit response; CR = complete response; DCR = disease control rate; E = everolimus; IFN = interferon; OS = overall survival; PFS = progression-free survival; PR = partial response; RCC = renal cell cancer; RR = response rate; SD = stable disease; T = temsirolimus.

antineoplastic and molecular targeted agents in various cancers, such as neuroendocrine tumor, breast cancer [18], stomach cancer [19], lung cancer, malignant lymphoma, and advanced gastrointestinal stromal tumor, and mainly in renal cell cancer [20, 21] (table 2).

In a phase II study of first- and second-line therapies for metastatic renal cancer (10 mg, daily administration), RAD001 was effective in 12 of 41 patients (29%) [20]. Based on these results, a randomized double-blind phase III clinical study of RAD001 (10 mg, daily administration) and placebo was conducted on an international scale, including Japan, in patients with advanced renal cell cancer, in whom the standard therapy with sorafenib tosilate or sunitinib alone or both was ineffective. RAD001 significantly prolonged progression-free survival, a primary endpoint, from 1.9 to 4 months, and reduced the risk of cancer progression by 70% [21]. The rate of adverse drug reactions that led to withdrawal was as low as 6%.

A randomized phase II clinical study was conducted in postmenopausal patients with ER-positive breast cancer using response rates to letrozole and RAD001 (10 mg, daily administration) and letrozole and placebo as primary endpoints; the response rate was significantly higher in the letrozole and RAD001 group than in the letrozole and placebo group (47 vs. 58%, respectively, p = 0.035) [18].

In a phase I clinical study conducted in Japan, responses were noted in patients with advanced/recurrent stomach cancer, in whom all other chemotherapies were ineffective. Thus, a phase II clinical study was conducted employing monotherapy with RAD001 (10 mg, daily administration) in patients with metastatic stomach cancer, in whom pretreatment was ineffective. This therapy was desirable because DCR, a primary endpoint, was favorable (59.4%) and progression-free survival was as long as 84 days, although as many as half of the patients underwent a third therapy [19].

Major adverse events of RAD001, reported in the phase I study involving once-weekly and daily single administration for advanced cancer, include rash, stomatitis, fatigue, nausea, anorexia, diarrhea, vomiting, hyperlipemia, hyperglycemia, and thrombocytopenia. Noninfective pneumonitis as a characteristic toxicity requires caution on using RAD001.

In the phase I study involving the once-weekly administration of RAD001, PK/PD is also examined using p70S6 kinase as a biomarker. The major toxicities include anorexia, general malaise, eruption, stomatitis, headache, hyperlipemia, and gastrointestinal disorder. PR was noted in patients with non-small cell lung cancer. RAD001 inhibited the p70S6 kinase activity of peripheral blood mononuclear cells for 3–5 days in doses of 5 and 10 mg, and inhibited the activity for at least 7 days in 7 of 8 patients at a dose of 20 mg. The phase II study (10 mg, daily administration) was conducted in 25 patients with metastatic renal cancer, with PR being 33%.

RAD001 is a selective inhibitor of mTOR, which specifically targets the mTOR-raptor signal transduction complex (mTORC1). RAD001 interacts with FKBP12, an intracellular receptor protein, with high affinity to exert its activity. The FKBP12/RAD001 complex binds to mTORC1 to inhibit signal transduction. The effects on mTORC1 signal transduction are generated by regulating the phosphorylation of downstream effectors. The most characteristic downstream effectors include S6 ribosomal protein kinase (S6K1), a translational regulator, and 4E-binding protein (4E-BP), a eukaryotic growth factor. Inhibiting mTORC1 suppresses the functions of S6K1 and 4E-BP1, and has effects on the translation of mRNAs that encode major proteins involved in cell cycle regulation, glycolysis, and adaptation to low-oxygen conditions. Consequently, tumor proliferation and HIFs (e.g. HIF-1 transcription factor) are inhibited. Furthermore, the expression of factors (e.g. VEGF), involved in the promotion of tumor angiogenesis, is suppressed by the inhibition of HIFs. RAD001 strongly inhibits the growth and proliferation of tumor cells, endothelial cells, fibroblasts, and vascular smooth muscle cells. RAD001 was demonstrated to control tumor proliferation, glycolysis, and angiogenesis in solid tumors in vivo, thereby inhibiting tumor proliferation through two independent mechanisms, i.e. direct antitumor activity and inhibition of the tumor stroma. This is consistent with the finding that mTORC1 plays a central role in the control system.

RAD001 is a signal-transduction inhibitor (STI) that targets mTOR (more specifically, mTORC1). mTORC1 signaling is intricately regulated by mitogens, growth factors, energy, and nutrients. mTORC1 is a regulator essential for general protein synthesis, located downstream of the PI3K/AKT/mTOR pathway. The PI3K/AKT/mTOR pathway is dysregulated in most human cancers [22]. Inhibiting mTORC1 with RAD001 in vivo was demonstrated to suppress the proliferation of solid tumors, glycolysis, and angiogenesis through both direct antitumor effects and inhibition of the tumor stroma. This is consistent with the known function of mTORC1. RAD001 inhibits the proliferation of human umbilical vein endothelial cells (HUVEC), induced by VEGF, in vitro, and inhibited the angiogenesis caused by VEGF in a chamber-implanted mouse model and in orthotopic melanoma-transplanted and xenograft mouse models [23–26].

Many nonclinical studies have demonstrated that this pathway plays a role in tumor proliferation. In a gain-of-function model, kinases, such as AKT, were constitutively activated, and the same malignant tumors as those that arise in patients with highly activated kinases almost certainly developed. RAD001 markedly inhibits the growth of various human tumor cell lines in vitro and suppress tumor growth in vivo in xenograft, allograft, or orthotopic graft animal models. RAD001 has inhibitory effects on cell proliferation in nanomolar quantities, and, thus, is therapeutically applicable in the doses used in clinical studies. In addition, the inhibitory effects of RAD001 on vascular endothelium proliferation and angiogenesis have been demonstrated.

The following can be considered: inhibiting mTOR with molecules, such as RAD001, generates additive effects that accompany upstream and downstream target inhibition; alternatively, upstream receptor inhibition is compensated for by inhibiting the downstream pathway, even if some resistance develops against receptor inhibition (regardless of initial or acquired resistance). The results demonstrate that RAD001 can be used with other anticancer agents (e.g. paclitaxel, doxorubicin, cisplatin, carboplatin, gemcitabine, imatinib, EGFR/VEGF STI, and letrozole) and radiotherapy. Furthermore, a combination of RAD001 and a cytolytic drug is also effective. The beneficial interaction observed with these combinations was facilitated by inhibiting the involvement of mTOR in the cell survival-associated mechanism (anti-apoptotic mechanism that protects cells from the effects of the cytolytic drug).

mTOR as a Target in the Treatment of HCC

Besides the finding that mTOR plays a key role in cell biology, it was also demonstrated that mTOR and S6K are overexpressed in 15–41% of HCCs, and mTOR inhibitors have antitumor effects in various HCC cell lines and animal models [27–30]. Activation of mTOR is correlated with the development of HCC and recurrence after the excision of early HCC. Regulating this specific intracellular pathway (Ras-Raf pathway) with RAD001 is potentially more effective in suppressing sorafenib-resistant tumors.

A phase I/II trial conducted by Zhu et al. [31] revealed a median RFS of 3.8 months, median TTP of 3.9 months, and median OS of 8.4 months in 25 patients without a few severe adverse effects. RAD001 responded well to the sorafenib failure patients too (median RFS 3.4 months, median OS 7.9 months).

Currently, a phase III clinical trial as a second line to sorafenib is in progress globally in patients who are unresponsive and intolerant to sorafenib. The results of this trial are eagerly awaited to give patients with advanced HCC, who failed to respond sorafenib, additional hope for prolonged survival.

Conclusions

mTOR inhibitor RAD001 is expected to be a potential targeted agent for HCC in patients where sorafenib treatment failed as well as in sorafenib-naive patients.

Disclosure Statement

The author has no conflict of interest to declare.

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Future Treatment Option for Hepatocellular Carcinoma: A Focus on Brivanib

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Key Words
Hepatocellular carcinoma · Molecular targeted therapy · Brivanib · Sorafenib

Abstract
Hepatocellular carcinoma (HCC), one of the most common cancers worldwide, is particularly prevalent in the Asia-Pacific region. Guidelines on the treatment of HCC in Japan come from both consensus-based and evidence-based treatment algorithms. However, patients with extensive liver damage and/or more advanced disease (major vascular invasion and/or extrahepatic spread) are currently ineligible for any treatment. Recent knowledge of hepatocarcinogenesis has led to the targeting of new pathways, particularly the angiogenic pathway, with a specific focus on the vascular endothelial growth factor receptor (VEGFR). Apparently the most studied systemic antiangiogenic agent for HCC is sorafenib. An updated version of the aforementioned treatment algorithms recommends sorafenib therapy for advanced HCC patients with Child-Pugh A liver function and extrahepatic spread or major vascular invasion. Moreover, sorafenib is recommended for use in HCC patients who are refractory or intolerant to transarterial chemoembolization (TACE) with well-preserved liver function (Child-Pugh A). However, one of the unresolved issues is anti-VEGF resistance. It is speculated that novel antiangiogenic agents that combine inhibition of other pathways such as fibroblast growth factor receptor signaling in addition to VEGFR signaling might provide a potential mechanism to overcome anti-VEGF resistance in HCC. Brivanib inhibits both VEGF and fibroblast growth factor receptor signaling. To further investigate the benefits of brivanib for advanced HCC, a broad-spectrum, global, phase III development plan, the Brivanib studies in HCC patients at RISK (BRISK) clinical program, has been initiated. Clinical benefits seen with brivanib in the first-line setting, and following the failure of sorafenib therapy, highlight the potential to improve the clinical course of patients with advanced HCC, and this agent may provide a novel therapeutic option for the growing population of patients for whom no other treatment choice exists.

Introduction

Hepatocellular carcinoma (HCC), one of the most common cancers worldwide, is particularly prevalent in the Asia-Pacific region, with more than two thirds of global cases occurring in Asia-Pacific countries [1, 2]. In Japan, HCC is now the third leading cause of cancer death among males and females, and is responsible for the death of more than 33,000 Japanese citizens every year [3]. Throughout the Asia-Pacific region, the most important etiologic factors related to HCC are hepatitis B virus (HBV) and hepatitis C virus (HCV). Among Japanese

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HCC patients, the primary etiology is HCV, with approximately 70–80% chronically infected with HCV and only a small proportion with HBV (<16%) [4, 5].

Treatment Algorithm and Unmet Medical Needs

Guidance on the treatment of HCC in Japan comes from both consensus-based and evidence-based treatment algorithms [6, 7]. As nationwide HCC screening programs are common in Japan, most patients present in the early stages of the disease and are eligible for potentially 'curative' treatments, such as surgical resection or local ablation (radiofrequency ablation or percutaneous ethanol injection) [6, 7]. If resection or ablation is contraindicated, or if the disease has progressed, then transarterial chemoembolization (TACE) or hepatic infusion chemotherapy may be recommended [6, 7]; however, patients with extensive liver damage and/or more advanced disease (major vascular invasion and/or extrahepatic spread) are currently ineligible for these treatments [6]. As such, there remains a significant unmet medical need for patients with advanced HCC in Japan.

Present Status of Molecular Targeted Therapy

Recent knowledge of hepatocarcinogenesis has led to the targeting of new pathways, particularly the angiogenic pathway, with a specific focus on the vascular endothelial growth factor receptor (VEGFR). Indeed, agents that inhibit angiogenesis via blockade of the VEGFR have seen some success in the treatment of HCC. Moreover, recent research data suggest the potential for an additional synergistic role for antiangiogenic agents whereby they might be used following TACE therapy to increase response rates [8]. Apparently the most studied systemic antiangiogenic agent for HCC is sorafenib. This is an oral multikinase inhibitor that targets the tyrosine kinase activity of VEGFRs 1, 2, and 3, as well as platelet-derived growth factor receptor-β, and has recently demonstrated some efficacy over placebo in Child-Pugh A patients with advanced HCC [9]. Similar results have been observed in a study of sorafenib for patients with advanced HCC conducted in the Asia-Pacific region [10], and a recent phase 1 study has indicated favorable safety/tolerability and promising antitumor activity in a Japanese population [11]. On the basis of these results, sorafenib has been approved in Japan for the treatment of advanced HCC since May 2009.

Indication of Sorafenib in Treatment Algorithm

There are, however, unresolved issues regarding the optimal use of sorafenib for HCC. To date, survival benefits in clinical trials have been modest, and a relatively high incidence of hand-foot syndrome (all-grade events reported in ~20–45% of patients) [9, 10] and an increased risk of bleeding events have been reported in the international literature [12]. In Japan, primarily due to the design of the pivotal trials and the available data in HCC patients, sorafenib use has been strictly regulated and limited to patients with Child-Pugh A cirrhosis who are not candidates for resection, ablation, or TACE. Moreover, post-marketing surveillance of sorafenib in Japan has raised safety concerns regarding interstitial pneumonia, hepatic coma, and hepatic failure, which has led to revision of the Japanese package insert. Updated version of the aforementioned treatment algorithms recommend sorafenib therapy for advanced HCC patients with Child-Pugh A liver function and extrahepatic spread or major vascular invasion. Moreover, sorafenib is recommended for use in HCC patients who are refractory or intolerant to TACE with well-preserved liver function (Child-Pugh A) (for details, see Kudo, fig. 7, p. 299) [13–15].

Anti-VEGF Resistance

Recent studies suggest that tumor progression following treatment with antiangiogenic agents that target the VEGF signaling pathway alone may result from either evasive or intrinsic resistance [16]. Furthermore, there is strong evidence to support the hypothesis that evasive resistance to anti-VEGF blockade is associated with reactivation of tumor angiogenesis by alternative signaling pathways, one such mechanism of resistance being activation of the fibroblast growth factor (FGF) signaling pathway [17, 18]. Basic FGF (FGF2) is a potent angiogenic factor. Indeed, expression of FGF2 enhances growth, invasion, and angiogenesis of many tumor types [19, 20]. Moreover, recent evidence has shown that FGF is overexpressed and activated in HCC and that high FGF2 levels may predict for a poor clinical outcome among patients with HCC [20].

Importance of FGF Signaling

Considering the proposed importance of FGF signaling in HCC angiogenesis, it is clear that novel antiangiogenic agents that combine inhibition of FGF receptor sig-

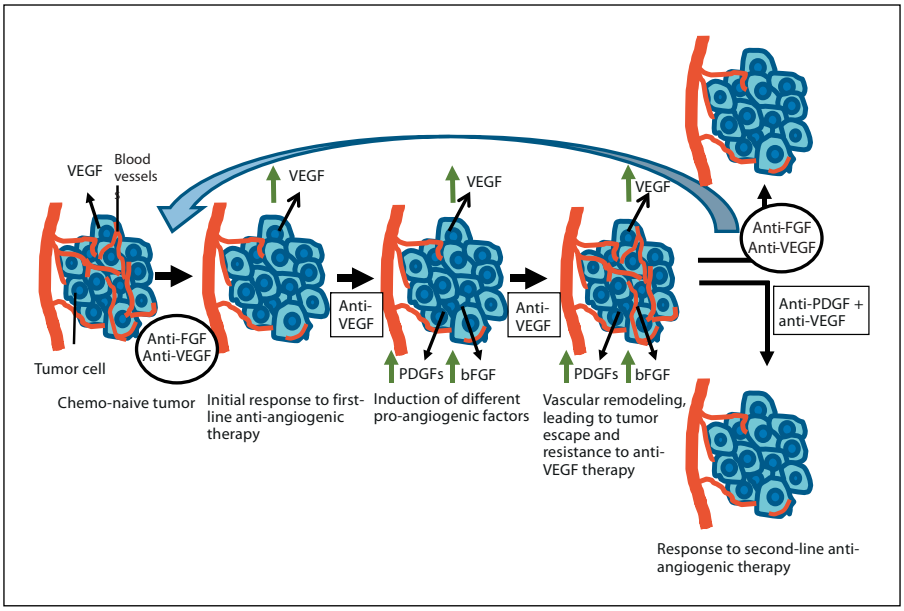

Fig. 1. Targeting angiogenesis: mechanisms of resistance. Brivanib may be effective for the failure or resistance of first-line antiangiogenic therapy for VEGF.

naling with inhibition of VEGFR signaling might provide a potential mechanism to overcome anti-VEGF resistance in HCC (fig. 1). With this in mind, it is worthwhile considering the potential future impact of brivanib on the treatment of advanced HCC. Brivanib, a small-molecule tyrosine kinase inhibitor, is the first oral selective dual inhibitor of FGF and VEGF signaling. In multiple preclinical models of human xenograft tumors, including patient-derived HCC xenografts, brivanib has shown potent antitumor activity and no overt toxicity when dosed orally [21, 22]. Furthermore, brivanib has demonstrated promising antitumor activity and acceptable tolerability in a phase 2, open-label study in patients with unresectable locally advanced or metastatic HCC [23, 24]. Crucially, within this trial, brivanib showed activity both as first-line therapy (overall survival: 10 months) or as second-line therapy in patients who had failed prior antiangiogenic treatment, primarily with sorafenib (overall survival 9.5 months) [24]. Of note, the incidence of all-grade hand-foot syndrome was only 8% in this study.

Phase I and II Data of Brivanib

Additional retrospective studies and subanalyses have also confirmed that brivanib is effective in patients from the Asia-Pacific region. In a subanalysis performed to evaluate the effects of brivanib among Asian versus non-Asian patients enrolled in the aforementioned phase II study, median overall survival was 10.6 months among Asian patients treated with first-line brivanib (versus 5.7 months in non-Asian patients) and 9.8 months among Asian patients receiving brivanib as second-line therapy (versus 9.4 months in non-Asian patients) [25]. Another subanalysis, this time including only patients who received first-line brivanib therapy in the phase 2 study, indicated that overall tolerability was similar or slightly better in the Asian population versus non-Asian patients [26]. A further subanalysis comparing 125 Asian and non-Asian patients enrolled in separate phase I and II studies [23, 27] confirmed that exposures in these patient subpopulations were similar following brivanib doses of 800 mg daily [28]. Finally, a phase 1 study of brivanib in Japanese patients with advanced or metastatic solid tumors, including HCC, has shown manageable tolerability and a similar safety profile at the same 800-mg once-daily dose as used in Caucasian patients [29]. Moreover, the study provided evidence of antitumor activity in this uniquely Japanese population, with 8 of 13 patients (62%) showing stable disease.

Design of Phase III Global Study

To further investigate the benefits of brivanib for advanced HCC, a broad-spectrum, global, phase III development plan, the Brivanib studies in HCC patients at

RISK (BRISK) clinical program, has been initiated. The global BRISK program will enroll patients from countries in Africa, Asia (including Japan), Australasia, Europe, and North, South, and Central America, and will include investigations of brivanib in a variety of clinically relevant settings, including first-line head to head with sorafenib, second-line post-sorafenib, and TACE adjuvant settings. In addition, it is noteworthy that the BRISK study of brivanib as adjuvant treatment to TACE therapy is being led by Japanese investigators and is one of the first global registration programs to be led from Japan.

Conclusion

HCC continues to be a major healthcare burden in Japan. Although it is detected in the early stages in most Japanese patients and treated accordingly, there remains a population of patients with advanced HCC who have limited therapeutic choices. With the recent approval of the antiangiogenic agent sorafenib, options for these patients have improved, but clinical studies to date suggest only a modest survival benefit with sorafenib and there is potential for safety/tolerability issues and the development of resistance to the anti-VEGF blockade. Clinical benefits seen with brivanib in the first-line setting, and following the failure of sorafenib therapy, highlight the potential to improve the clinical course of patients with advanced HCC, and this agent may provide a novel therapeutic option for the growing population of patients for whom no other treatment choice exists.

Disclosure Statement

The author has no conflict of interest to declare.

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Des-γ-Carboxyprothrombin May Be a Promising Biomarker to Determine the Therapeutic Efficacy of Sorafenib for Hepatocellular Carcinoma

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Key Words

Des-γ-carboxyprothrombin · Protein-induced vitamin K absence II · Hepatocellular carcinoma · Antiangiogenic therapy · Hypoxia · Sorafenib

Abstract

Objective: The purpose of this study was to evaluate the role of des-γ-carboxyprothrombin (DCP) as a marker for the efficacy of sorafenib therapy for hepatocellular carcinoma (HCC). ***Methods:*** Patients with advanced HCC treated with sorafenib were retrospectively evaluated, focusing on DCP levels and clinical characteristics. ***Results:*** 50 patients with advanced HCC were treated with sorafenib alone. In 25 of these patients, the serum levels of DCP were evaluated twice (pretreatment and within 2 weeks after starting therapy). The time to progression was significantly longer in patients in whom the DCP level at 2 weeks after starting sorafenib was ≥2-fold higher than the pretreatment levels, as compared with patients without an increase in DCP ($p = 0.0296$). ***Conclusions:*** The serum level of DCP is a surrogate marker for tissue hypoxia and can be a predictive marker to assess the tumor response to sorafenib therapy.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in Japan and is the fifth most common cancer worldwide [1, 2]. It is well known that HCC is less sensitive to chemotherapeutic agents than other tumors. Furthermore, because of pancytopenia and poor hepatic preservation caused by underlying hepatic cirrhosis, systemic chemotherapy is unsuitable for patients with HCC. Thus, locoregional therapies such as hepatic resection, radiofrequency ablation and transcatheter chemoembolization (TACE) have been developed and are widely used. However, more effective systemic chemotherapy is necessary for patients who are refractory to locoregional therapy or who progress to advanced stage cancer with extrahepatic spread and/or vascular invasion.

Sorafenib (Nexavar®; Bayer HealthCare Pharmaceuticals-Onyx Pharmaceuticals) is a small molecule that inhibits tumor proliferation and angiogenesis. It inhibits serine-threonine kinase Raf-1, a member of the RAF/MEK/ERK signaling pathway, and several receptor tyrosine kinases involved in neovascularization and tumor progression, including vascular endothelial growth

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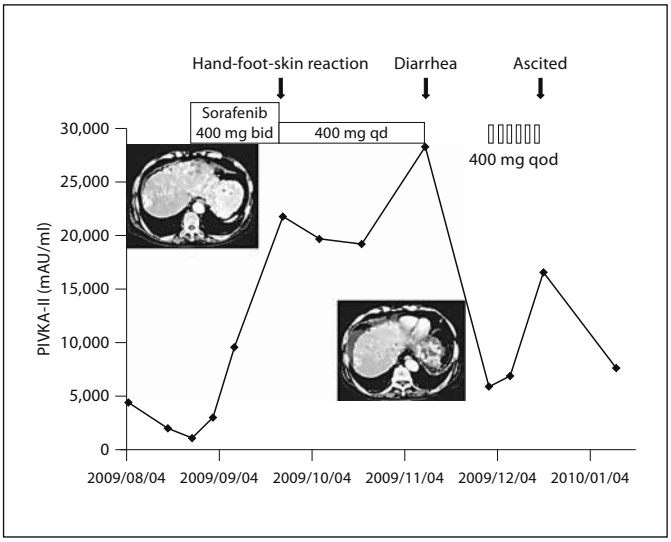

Fig. 1. Discrepancies between the changes in serum DCP levels and clinical findings for a 76-year-old man with advanced HCC. The serum DCP level rapidly increased after starting sorafenib but decreased after reducing or discontinuation of sorafenib therapy. CT showed that the HCC decreased and became necrotic, despite increased DCP levels.

factor receptor (VEGFR)-2, VEGFR-3, platelet-derived growth factor receptor-β (PDGFRβ), Flt-3 and c-KIT [3–7]. Sorafenib-based chemotherapy is used for HCC because HCC is a hypervascular tumor that expresses VEGF and the VEGFR [8], and the survival of tumor cell depends on its vascularity. A meta-analysis has shown that tissue and serum VEGF levels are prognostic factors in HCC [9].

The SHARP study, a phase III randomized trial for advanced HCC, revealed that sorafenib prolonged the overall survival time to progression (TTP) [10]. Based on such findings, sorafenib is now used worldwide. On the other hand, tumor shrinkage was infrequently observed in the SHARP study because the partial response in that study was only 2%. However, stable disease was achieved in 76% of patients, and the overall disease control rate was 78%. The tumoristatic effect of sorafenib contributes to prolongation of overall survival.

Cases of complete response or partial response have frequently been observed since sorafenib was approved in Japan in May 2009. It is thought that the Japanese race has some genetic characteristics that improve the efficacy of sorafenib therapy.

Dynamic computed tomography (CT) and dynamic magnetic resonance imaging (MRI) are often used to evaluate the antitumor effect in the treatment of HCC. However, since the introduction of molecular-targeted therapy, tumor necrosis without tumor regression is often observed. Tumor necrosis is not always induced by a cytotoxic antitumor agent. It was recently suggested that the Response Evaluation Criteria for Solid Tumors (RECIST) [11, 12] and the World Health Organization Criteria [13] are unsuitable for the evaluation of the anticancer effects of molecular-targeted therapy, which inhibits angiogenesis. To overcome the limitations of these criteria, a modified RECIST [14] for HCC was proposed, and evaluates the size of the viable tumor tissue. The validity of the modified RECIST or RECICLE [15] has been discussed, but their utility is still not established. Tumor markers have also been used to evaluate the antitumor effects of therapy. α-Fetoprotein (AFP), the lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), and des-γ-carboxyprothrombin (DCP) are the most widely used tumor markers and are well established for the diagnosis and follow-up of HCC [16–18]. These tumor markers indicate the activity of HCC and are useful for patient follow-up [19]. In particular, tumor markers can be used to examine the efficacy of antiangiogenic molecular-targeted agents.

At our institute, we have experienced discrepancies between changes in DCP levels and clinical findings during sorafenib therapy (fig. 1). Therefore, we retrospectively evaluated the associations between changes in DCP levels and clinical findings.

Patients and Methods

Sixty-two consecutive patients with advanced HCC treated with sorafenib at Kinki University Hospital between May 2009 and April 2010 were included in this study. The criteria for sorafenib therapy were as follows: (1) patients with HCC refractory to TACE or the presence of major vascular invasion or extrahepatic spread; (2) ECOG Performance Status Score of 0 or 1, and (3) Child-Pugh score of ≤7 (Child-Pugh A and some B patients). Patients with laboratory values meeting the following criteria were also eligible for sorafenib: (a) hemoglobin ≥8.5 g/dl, (b) neutrophil count >1,500/mm^3, (c) platelet count >50,000/mm^3, (d) total bilirubin <3 mg/dl, (e) ALT and AST <5 times the institutional upper limits of normal, and (f) serum creatinine <1.5 times the institutional upper limits of normal.

Patients continuously received 400 mg of oral sorafenib (two 200-mg tablets) twice daily. If adverse effects were observed, the sorafenib dose was reduced according to the treatment guidelines.

Tumor response was evaluated by RECIST version 1.1. TTP was constructed by the Kaplan-Meier method and was compared using the log-rank test. Statistical analysis was conducted using SPSS version 11.5.1J for Windows.

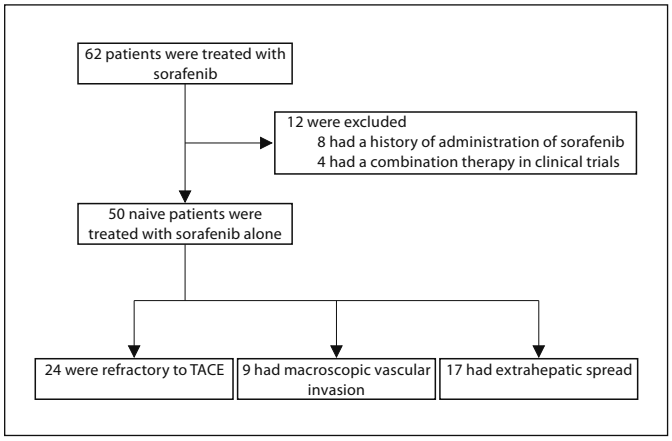

Fig. 2. Patient disposition.

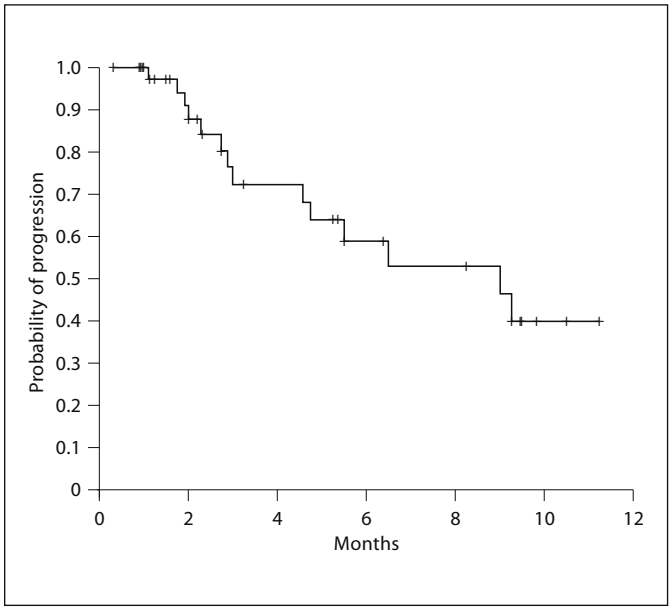

Fig. 3. TTP in 50 patients with HCC treated with sorafenib.

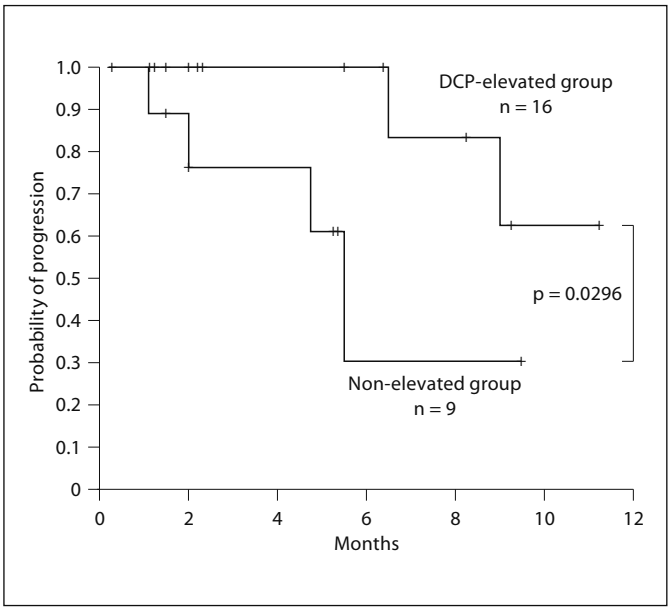

Fig. 4. Comparison of TTP between patients with (elevated) and patients without (non-elevated) an increase in DCP by ≥2-fold at 2 weeks after starting sorafenib therapy compared with pretreatment.

Results

Eight of 62 patients were excluded because they had already been treated with sorafenib before it was approved in Japan. Four patients were excluded because they participated in other clinical trials. Thus, 50 patients were evaluated in this study: 24 were refractory to TACE, 9 had major vascular invasion, and 17 had extrahepatic spread (fig. 2).

The mean duration of treatment was 5.2 months (95% confidence interval (CI) 4.1–6.8 months). The mean dose of sorafenib was 480.0 mg daily, overall survival was 9.5 months (95% CI 8.1–10.8 months), and TTP was 9.0 months (95% CI 4.75–13.25) (fig. 3).

In 25 of the patients treated with sorafenib, the serum levels of DCP were evaluated twice, i.e. before and within 2 weeks after starting treatment. The TTP in the patients in whom the DCP level at 2 weeks was ≥2-fold greater than the pretreatment level was significantly longer than that in patients without elevated DCP (i.e. <2 times the pretreatment levels) (p = 0.0296) (fig. 4). There were no statistically significant differences in other clinical characteristics between the two groups of patients (table 1).

Discussion

Sorafenib shows the significant activity against several receptor tyrosine kinases including VEGFR-2, VEGFR-3, PDGFRβ, Flt-3, and c-KIT, and inhibits angiogenesis. Antiangiogenic activity plays a very important role in HCC therapy because HCC is a typical hyper-

Table 1. Characteristics of patients according to change in DCP

Characteristics	Elevated group (n = 16)	Non-elevated group (n = 9)	p value[1]
Age	73.06 ± 6.03	68.22 ± 6.40	0.065
Male/female	12/4	7/2	0.876
Serum albumin, g/dl	3.44 ± 0.56	3.64 ± 0.32	0.329
Serum bilirubin, mg/dl	0.93 ± 0.51	0.91 ± 0.36	0.803
Prothrombin time, %	86.55 ± 17.65	84.18 ± 10.12	0.978
Child-Pugh score (5/6/7/8)	6/7/2/1	6/3/0/0	0.413
Platelet count	15.90 ± 7.00	16.21 ± 11.18	0.598
Etiology, NBNC/HBV/HCV	4/3/9	4/1/4	0.593
Stage, III/IV	10/6	6/3	0.835
MVI, with/without	3/13	2/7	0.835
EHS, with/without	3/13	4/5	0.170
AFP, ng/ml	977.0 (2–35,014)	304.5 (6–721,260)	0.728
DCP, mAU/ml	2,327.5 (41–34,170)	2,088.0 (46–357,580)	0.846

Elevated: DCP increased by ≥2-fold at 2 weeks compared with pretreatment; non-elevated: DCP increased by <2-fold at 2 weeks compared with pretreatment.

MVI = Macroscopic vascular invasion; EHS = extrahepatic spread.

[1] All values were non-significant.

vascular tumor. In other words, HCC induces angiogenesis to maintain adequate blood supply.

Liebman et al. [20] were the first to report the utility of DCP as a tumor marker for the diagnosis of HCC. DCP, also known as PIVKA-II (proteins induced by vitamin K absence or antagonist-II), is an abnormal prothrombin induced by vitamin K deficiency. Vitamin K-dependent coagulation factors such as prothrombin are synthesized in hepatocytes and contain γ-carboxy-glutamic acid (Gla residues), which can bind calcium. Normal prothrombin contains 10 Gla residues at the amino terminal. However, in the vitamin K-deficient state the Gla residues at the amino terminal are not fully γ-carboxylated. This incomplete prothrombin is known as DCP and is functionally inactive.

It is unclear why DCP is elevated in patients with HCC. Several reports have proposed mechanisms for DCP production, which include: (1) vitamin K deficiency [21]; (2) decreased activity of γ-glutamyl carboxylase in the HCC tissue because of a point mutation in the γ-glutamyl carboxylase gene [22, 23]; (3) abnormal vitamin K metabolism [24]; (4) overexpression of the prothrombin precursor in HCC cells [25, 26], and (5) abnormal uptake of vitamin K into HCC cells. We have focused on the abnormal uptake of vitamin K into the HCC cells. Murata et al. [27–29] reported that hypoxia induces DCP. They explained this phenomenon as follows. The fine filamentous actin network, which plays a crucial role in clathrin-mediated endocytosis of vitamin K, is disrupted in DCP-producing cells because of hypoxia. It is considered that this offers one explanation for the elevated serum DCP level in patients with HCC, for which sorafenib is effective. In this issue, we found that HCC patients with a rapid increase in DCP within 2 weeks after starting sorafenib had a significantly better outcome than patients with no increase in DCP [30]. The CT findings for HCC with rapid DCP elevation tended to include reduced vascularity or presence of necrosis. This indicates that hypoxia was responsible for the change in DCP production. Accordingly, DCP may offer a surrogate marker for hypoxia.

Sorafenib induces hypoxia in HCC by inhibiting angiogenesis. TACE exposes the HCC to hypoxia, as does sorafenib, but this change is very rapid and most of the tumor cells become necrotic. It is thought that not enough DCP is produced after TACE. On the other hand, sorafenib induces tissue hypoxia relatively slowly and many viable HCC cells are exposed to hypoxia. During sustained hypoxia, the tumor cells gradually die and the serum level of DCP subsequently decreases.

During molecular-targeted HCC therapy using sorafenib, we found that the rapid increase in DCP after starting sorafenib does not indicate tumor progression, but rather indicates HCC tissue hypoxia. Therefore, DCP may be a useful predictive marker for the duration of tu-

mor suppression. To our knowledge, this is the first report to show that DCP could be a good biomarker to predict the therapeutic efficacy of sorafenib in HCC.

In conclusion, the serum level of DCP during sorafenib treatment may be a promising biomarker for the therapeutic efficacy of sorafenib therapy for HCC.

Disclosure Statement

The authors have no conflict of interest to declare.

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Oral Branched-Chain Amino Acid Granules Reduce the Incidence of Hepatocellular Carcinoma and Improve Event-Free Survival in Patients with Liver Cirrhosis

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Key Words
Branched-chain amino acids · Liver cirrhosis · Hepatocellular carcinoma · Event-free survival

Abstract
Background: It has been reported that branched-chain amino acid (BCAA) supplementation can improve nutritional status and prevent liver-related complications in patients with decompensated cirrhosis. We investigated the effects of oral BCAA supplementation on the incidence of hepatocellular carcinoma (HCC) and liver-related events in patients with compensated and decompensated cirrhosis. ***Methods:*** We enrolled 211 patients with cirrhosis including 152 patients with Child-Pugh A cirrhosis, but no history of HCC. Of these, 56 received oral administration of 12 g/day BCAA for ≥6 months (BCAA group), and 155 were followed-up without BCAA treatment (control group). The HCC occurrence and event-free survival rates were compared between the two groups. We used a propensity score analysis to overcome selection bias of this retrospective analysis. ***Results:*** The HCC occurrence rate was significantly lower and event-free survival rate was significantly higher in the BCAA group than in the control group. Multivariate analyses showed BCAA supplementation was significantly associated with reduced incidence of HCC (hazard ratio (HR) 0.416, 95% confidence interval (CI) 0.216–0.800, p = 0.0085). BCAA supplementation also reduced the incidence of liver-related events in patients with Child-Pugh A cirrhosis, although the difference did not reach statistical significance (HR 0.585, 95% CI 0.336–1.017, p = 0.0575). ***Conclusions:*** Oral BCAA supplementation is associated with reduced incidence of HCC in patients with cirrhosis and seems to prevent liver-related events in patients with Child-Pugh A cirrhosis.

Introduction

Protein malnutrition is a major problem among patients with cirrhosis, characterized by a decreased blood concentration of branched-chain amino acids (BCAA) (i.e., valine, leucine, and isoleucine) and an increased concentration of aromatic amino acids. BCAA deficiency is caused by enhanced uptake and consumption of BCAA by skeletal muscle for ammonia metabolism and energy generation, despite adequate daily food intake [1, 2]. BCAA supplementation has been reported to improve the nutritional status and prevent liver-related complications in patients with decompensated cirrhosis [3–5].

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Table 1. Comparison of baseline characteristics of patients

	BCAA group n = 56	Non-BCAA group n = 155	p value
Male/female ratio	23/33	92/63	0.0198
Age, years (mean ± SD)	62.5 ± 10.3	63.0 ± 11.3	0.7917
Median (range)	63.7 (43–91)	64.9 (29–84)	
Body weight, kg (mean ± SD)	59.6 ± 11.3	59.4 ± 12.2	0.9483
Median (range)	59.0 (37.1–85.0)	60.0 (34.0–101.5)	
Baseline BMI (mean ± SD)	24.1 ± 4.1	23.1 ± 3.7	0.1272
Median (range)	24.4 (16.0–34.6)	22.9 (15.1–36.1)	
Cause of liver cirrhosis HCV/HBV/nonB-nonC	34/9/15	86/14/55	0.5320
History of IFN treatment (+/–)	5/36	18/82	0.4617
Concurrent diabetes (+/–)	13/43	30/125	0.5640
Total bilirubin level, mg/dl (mean ± SD)	1.4 ± 1.0	1.3 ± 1.4	0.4150
Median (range)	1.1 (0.5–5.2)	1.0 (0.2–14.6)	
Serum albumin level, g/dl (mean ± SD)	3.4 ± 0.6	3.7 ± 0.5	<0.0001
Median (range)	3.4 (2.2–4.9)	3.7 ± 0.5	
Platelet count, $\times 10^4/mm^3$ (mean ± SD)	9.0 ± 1.9	9.1 ± 2.0	0.7054
Median (range)	9.5 (4.5–11.9)	9.2 (3.9–11.9)	
Child-Pugh grade A/B/C	29/20/7	123/29/3	<0.0001
Serum ALT level, IU/l (mean ± SD)	54.6 ± 40.4	67.6 ± 53.5	0.0636
Median (range)	43.0 (9–185)	49.0 (2–307)	
AFP (<20/≥20 ng/ml)	15/19	28/77	0.0863
PIVKA-II (<40/≥40 mAU/ml)	6/26	12/76	0.5647
Fasting blood sugar, mg/dl (mean ± SD)	132.1 ± 76.	124.9 ± 63.8	0.5321
Median (range)	105.0 (64–510)	105.0 (60–508)	

SD = Standard deviation; BMI = body mass index; IFN = interferon; ALT = alanine aminotransferase; AFP = α-fetoprotein.

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. In Japan, HCC is the third most common cause of death from cancer in males and the fifth in females. Hepatitis C virus (HCV)-related HCC accounts for 70% of all HCC cases [6]. Advanced fibrosis is one of the predisposing factors for liver carcinogenesis in patients with HCV infection [7, 8]. Among several treatment approaches intended to reduce the incidence of HCC in patients with advanced HCV infection, interferon (IFN) has been endorsed as the standard treatment of care [9–11]. However, the virological response to IFN is significantly reduced in patients with cirrhosis and infection with HCV genotype 1 [11], and recent studies showed that maintenance IFN treatment did not reduce the incidence of HCC in patients who failed prior IFN therapy [12, 13]. Recently, several in vivo studies have shown novel effects of BCAA on the suppression of carcinogenesis [14–18]. In the present study, we investigated the effects of oral BCAA supplementation on the incidence of HCC and event-free survival in patients with cirrhosis.

Patients and Methods

Patients

We retrospectively analyzed 211 patients who were diagnosed with cirrhosis but not HCC between April 1998 and July 2008 at Kinki University Hospital. The study protocol was approved by the institutional ethics review board of Kinki University. Cirrhosis was diagnosed based on the following criteria: collateral vessels, splenomegaly or ascites on imaging finding, and a platelet count of <120,000/μl. Patients with a history of HCC were excluded from this study. Patients were classified into either the BCAA group (n = 56) as those who received oral administration of 12 g/day BCAA (LIVACT Granules; Ajinomoto Co., Inc., Tokyo, Japan) for more than 6 months or a control group (n = 155) (patients who did not receive BCAA supplementation). The baseline characteristics of patients in both groups are shown in table 1.

Follow-Up and Diagnosis of HCC

Ultrasonography and serum liver function tests and measurement of tumor markers (α-fetoprotein (AFP) and des-γ-carboxyprothrombin (DCP)) were performed every 3 months. Dynamic computed tomography (CT) was performed every year.

The patients were diagnosed with HCC based on histological or reliable clinical criteria as follows: typical imaging findings and increased HCC-related tumor markers, such as serum AFP and

Table 2. Factors associated with receiving BCAA supplementation

Variables	Odds ratio	95% confidence interval		
		lower limit	upper limit	p value
Female	0.4200	0.2040	0.8640	0.0184
HCV-positive	2.2150	0.9740	5.0370	0.0578
HBV-positive	4.6010	1.4470	14.6310	0.0097
Concurrent diabetes	2.0820	0.8880	4.8830	0.0918
Serum albumin level	0.5040	0.2330	1.0890	0.0812
Child-Pugh grade	2.5060	1.1630	5.4000	0.0190

DCP levels. Typical imaging findings for HCC include a high-density mass in the arterial phase and a low-density mass in the portal phase on dynamic CT or magnetic resonance imaging (MRI). In case they did not show typical imaging findings or no increases in tumor markers, a biopsy was performed to confirm HCC diagnosis.

The incidence of HCC development was compared between the two groups. In addition, the incidence of complications of cirrhosis that needed hospitalization, such as hepatic encephalopathy, uncontrollable ascites, rupture of esophageal or gastric varices, development of HCC and infection, was compared between the two groups.

Statistical Analysis

Data are expressed as means or medians ± SD. The χ^2 test was used to assess the differences in patient distribution. Normally distributed variables were compared using Student's t test and non-normally distributed variables were compared using the Mann-Whitney U test between the two groups. Differences in the incidence of HCC were assessed by comparing the HCC occurrence rate, which was defined as the time between study entry and HCC diagnosis. Event-free survival was defined as the time between study entry and the occurrence of the liver-related events described above. The HCC occurrence rate and the event-free survival rate were estimated by the Kaplan-Meier method and differences between the two groups were analyzed by the log-rank test. Patients were censored at the end of follow-up, or when they died of causes other than liver diseases. The Cox proportional hazard model was used for multivariate analyses of factors associated with the incidence of HCC and event-free survival. $p < 0.05$ was considered statistically significant. All statistical analyses were performed with SAS software version 9.1.3 (SAS Institute, Cary, N.C., USA).

Propensity Score Analysis

Because this study was conducted retrospectively, there were significant differences between the two groups in the distribution of sex, serum albumin level and Child-Pugh grade, as shown in table 1. This result indicates the physicians more commonly prescribed BCAA granules for patients with more deteriorated liver function. Thus, we used propensity score analysis to eliminate bias toward use of BCAA. The propensity score is the probability that a patient with specific factors will receive treatment. Briefly, the results obtained by retrospective studies using a propensity score are assumed to be similar to those obtained in prospective randomized trials. A predictive model to calculate the propensity score was constructed using factors that were associated with the use of BCAA. Based on a logistic-regression analysis using all of the available variables, six factors (sex, HCV infection, hepatitis B virus (HBV) infection, diabetes, serum albumin value, and Child-Pugh grade) were identified by a stepwise selection method and were included in the model (table 2). A propensity score was assigned to each patient.

Results

Patient Characteristics Adjusted by Propensity Score

Table 3 shows the adjusted characteristics of patients in each group according to the propensity score quartile. The differences in sex, serum albumin level, and Child-Pugh grade between the BCAA and the control group disappeared after adjustment, while differences in body weight and body mass index (BMI) appeared. Body weight and BMI were both greater in the BCAA group than in the control group.

Effects of BCAA on the Incidence of HCC

Figure 1a shows the cumulative HCC occurrence curves in both groups. The cumulative HCC occurrence rates at 3, 5 and 10 years were 90, 78 and 64%, respectively, in the BCAA group, versus 69, 59 and 38% in the control group. There was a significant difference in the incidence of HCC between the two groups ($p = 0.0038$).

Effects of BCAA on the Incidence of HCC in HCV-Related Cirrhosis

Figure 1b shows the cumulative HCC occurrence curves in patients with HCV-related cirrhosis in both groups. The cumulative HCC occurrence rates at 3, 5 and 10 years were 91, 72 and 58%, respectively, in the BCAA group, and 56, 45 and 21% in the control group. There was a significant difference in the incidence of HCC between the two groups ($p = 0.001$).

Effects of BCAA on Event-Free Survival in Patients with Child-Pugh A Cirrhosis

Figure 2 shows the cumulative event-free survival curves of patients with Child-Pugh A cirrhosis in both groups. The cumulative HCC-free survival rates at 3, 5 and 10 years were 93, 60 and 28% in the BCAA group, and 71, 53 and 13% in the control group. There was a significant difference in the event-free survival rates between the two groups ($p = 0.0408$).

Table 3. Comparison of patient characteristics stratified by propensity score quartile

Variables	Group	Lowest 25%	2nd 25%	3rd 25%	Highest 25%	p value
Propensity score	BCAA	2 (3.7%)	8 (15.7%)	19 (35.8%)	27 (50.9%)	<0.0001
	non-BCAA	52 (96.3%)	43 (84.3%)	34 (64.2%)	26 (49.1%)	
Male/female	BCAA	2 (100.0%)	6 (75.0%)	5 (26.3%)	10 (37.0%)	0.6304
	non-BCAA	49 (94.2%)	23 (53.5%)	12 (35.3%)	8 (30.8%)	
Age, years	BCAA	57.0 ± 8.7	59.1 ± 8.5	65.8 ± 11.8	61.6 ± 9.6	0.8855
	non-BCAA	62.8 ± 12.2	63.8 ± 11.9	63.0 ± 10.2	62.1 ± 10.	
Body weight, kg	BCAA	74.8 ± 14.4	59.8 ± 14.4	55.0 ± 10.7	61.6 ± 9.4	0.0045
	non-BCAA	65.0 ± 8.	58.0 ± 11.0	58.4 ± 14.8	52.9 ± 12.2	
Baseline BMI	BCAA	26.3 ± 6.4	23.1 ± 4.0	22.9 ± 3.4	25.1 ± 4.4	0.0054
	non-BCAA	23.9 ± 3.2.	22.6 ± 3.0	23.4 ± 4.6	22.2 ± 4.2	
Cause of liver cirrhosis	BCAA	2 (100.0%)	3 (37.5%)	3 (15.8%)	7 (25.9%)	0.8096
	non-BCAA	27 (51.9%)	14 (32.6%)	8 (23.5%)	6 (23.1%)	
History of IFN treatment	BCAA	0 (0%)	3 (60.0%)	14 (87.5%)	19 (95.0%)	1.0000
	non-BCAA	20 (80.0%)	24 (82.8%)	19 (73.1%)	19 (95.0%)	
Concurrent diabetes	BCAA	1 (50.0%)	5 (62.5%)	16 (84.2%)	21 (77.8%)	0.1222
	non-BCAA	44 (84.6%)	39 (90.7%)	27 (79.4%)	15 (57.7%)	
Total bilirubin level, mg/dl	BCAA	0.8 ± 0.4	0.9 ± 0.3	1.2 ± 0.6	1.7 ± 1.2	0.8361
	non-BCAA	1.1 ± 0.4	1.3 ± 2.1	1.1 ± 0.5	1.7 ± 1.9	
Serum albumin level, g/dl	BCAA	4.3 ± 0.9	4.0 ± 0.5	3.6 ± 0.3	3.0 ± 0.5	0.3005
	non-BCAA	4.1 ± 0.4	3.8 ± 0.5	3.7 ± 0.4	3.1 ± 0.5	
Platelet count, $\times 10^4/mm^3$	BCAA	9.7 ± 0.1	9.0 ± 1.7	9.3 ± 1.8	8.7 ± 2.0	0.7114
	non-BCAA	9.3 ± 1.9	9.1 ± 2.1	9.2 ± 1.8	8.5 ± 2.2	
Child-Pugh grade	BCAA	2 (100.0%)	6 (75.0%)	17 (89.5%)	4 (14.8%)	0.2830
	non-BCAA	51 (98.1%)	40 (93.0%)	25 (73.5%)	7 (26.9%)	
Serum ALT level, IU/l	BCAA	24.0 ± 18.4	89.3 ± 55.2	59.2 ± 37.4	43.4 ± 32.7	0.2280
	non-BCAA	65.4 ± 45.7	74.5 ± 62.0	68.0 ± 45.1	59.9 ± 64.8	
AFP, ng/ml	BCAA	2 (100.0%)	5 (83.3%)	2 (18.2%)	10 (66.7%)	0.4320
	non-BCAA	28 (75.7%)	24 (77.4%)	16 (80.0%)	9 (52.9%)	
PIVKA-II, mAU/ml	BCAA	2 (100.0%)	6 (85.7%)	8 (88.9%)	10 (71.4%)	0.5914
	non-BCAA	26 (81.3%)	25 (96.2%)	13 (86.7%)	12 (80.0%)	
Fasting blood sugar, mg/dl	BCAA	104.5 ± 17.7	151.9 ± 52.6	107.4 ± 21.3	146.5 ± 101.9	0.2644
	non-BCAA	124.3 ± 58.2	128.4 ± 75.1	120.7 ± 50.6	126.0 ± 74.1	

BMI = Body mass index; IFN = interferon; ALT = alanine aminotransferase; AFP = α-fetoprotein.

Results of Multivariate Analyses

Table 4 shows a summary of the results of multivariate analyses using the Cox proportional hazard model. BCAA supplementation was a significant contributing factor to reduce the incidence of HCC in all patients (hazard ratio (HR) 0.416, 95% confidence interval (CI) 0.216–0.800, p = 0.0085) and in patients with HCV-related cirrhosis (HR 0.373, 95% CI 0.182–0.764, p = 0.0071). The effect of BCAA on the suppression of HCC development was slightly greater in patients with HCV-related cirrhosis than among all patients. In addition, BCAA supplementation seemed to reduce the incidence of liver-related events in patients with Child-Pugh A cirrhosis, although this did not reach statistical significance (HR 0.585, 95%

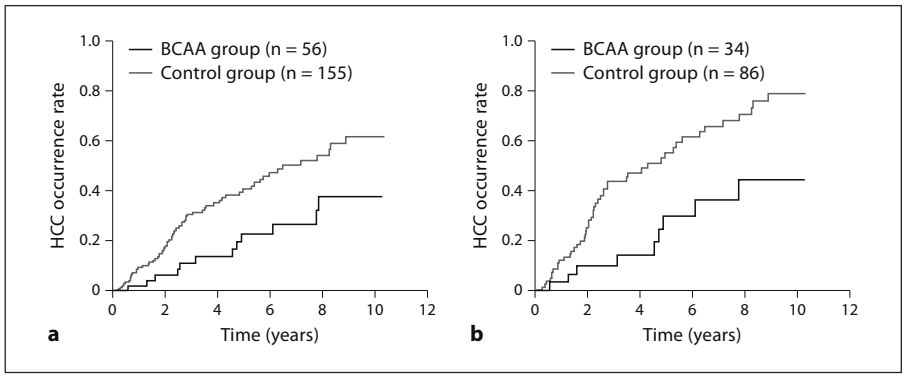

Fig. 1. a Rate of HCC occurrence. The HCC occurrence rate was significantly lower in the BCAA group than in the control group (p = 0.0038; log-rank test). **b** Rate of HCC occurrence in patients with HCV-related cirrhosis. The HCC occurrence rate was significantly lower in the BCAA group than in the control group (p = 0.001; log-rank test).

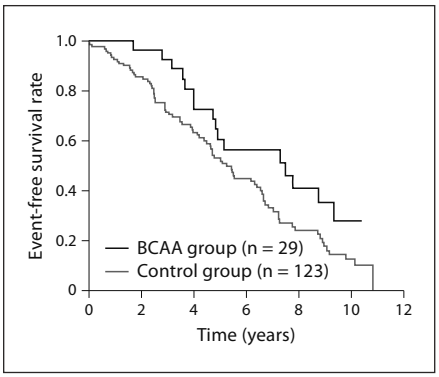

Fig. 2. Event-free survival rate in patients with Child-Pugh A cirrhosis. The event-free survival rate was significantly higher in the BCAA group than in the control group (p = 0.0408; log-rank test).

CI 0.336–1.017, p = 0.0575). The age of patients was another significant factor associated with the HCC development and the incidence of liver-related events among patients with Child-Pugh A cirrhosis.

Table 4. Summary of multivariate analyses

Variables	HR	95% CI	p value
Incidence of HCC (all cases)			
BCAA supplementation	0.416	0.216–0.800	0.0085
Propensity score	1.774	0.397–7.920	0.4526
Male sex	1.325	0.793–2.215	0.283
Age (continuous)	1.038	1.014–1.063	0.0017
Incidence of HCC (HCV)			
BCAA supplementation	0.373	0.182–0.764	0.0071
Propensity score	1.326	0.218–8.054	0.7591
Male sex	1.353	0.752–2.436	0.3133
Age (continuous)	1.032	1.000–1.064	0.0468
Event-free survival (Child-Pugh A)			
BCAA supplementation	0.585	0.336–1.017	0.0575
Propensity score	3.616	0.158–82.517	0.4206
Male sex	1.598	0.916–2.786	0.0988
Age (continuous)	1.034	1.012–1.056	0.0026

Discussion

Protein/energy malnutrition is commonly observed in patients with cirrhosis, and is represented by a decreased serum albumin level and skeletal muscle volume, and a decline in the non-protein respiratory quotient [2]. A decreased blood concentration of BCAA, caused by enhanced uptake and consumption of BCAA by skeletal muscle for ammonia metabolism and energy generation, is another manifestation of protein/energy malnutrition in patients with cirrhosis and is associated with disorders of protein synthesis and liver regeneration, and hyperammonemia [1]. Therefore, BCAA supplementation is a rational treatment for patients with cirrhosis.

Two large randomized controlled trials recently demonstrated that oral BCAA supplementation decreased the frequency of complications of cirrhosis and improved event-free survival in patients with decompensated cirrhosis [3, 4]. Based on these findings, oral BCAA supplementation is now recommended in Japanese guidelines as part of the treatment of HCV-related cirrhosis [19]. Similar to the reports mentioned above, the present study showed that the event-free survival of patients with Child-Pugh A cirrhosis was better among patients given oral BCAA supplementation than in those without, although the difference did not reach statistical significance. BCAA supplementation has also been reported to be useful as an adjuvant nutritional therapy following hepatectomy and transarterial chemoembolization, showing reduced risk of complications and better maintenance of liver function [20–22]. According to the earlier reports, BCAA seems to reduce the incidence of complications of cirrhosis by enhancing ammonia detoxification, upregulating protein synthesis and downregulating proteolysis, enhancing liver regeneration, and improving immune function [23–26].

In the present study, we did not find any beneficial effects of BCAA on the prevention of liver-related events in patients with decompensated cirrhosis, i.e. Child-Pugh B and C (data not shown). However, the relatively small number of patients classified as Child-Pugh B or C (59 of 211 patients) may account for this result. However, two other explanations seem possible. First, protein synthesis and liver regeneration, principal mechanisms involved in the improvements in liver function achieved by BCAA supplementation, are significantly aggravated in patients with decompensated cirrhosis [27]. Therefore, the benefits of therapy in terms of improvements in liver function might be limited in these patients. Second, because the liver-related events were more frequent in patients with decompensated cirrhosis than in those with compensated cirrhosis, and because BCAA supplementation does not exhibit a rapid response, the liver-related events might have occurred before the therapy induced sufficient effects in the present study. Further prospective studies are needed to address this issue.

The incidence of HCC was significantly lower in the BCAA group than in the control group (HR 0.416, 95% CI 0.216–0.800, p = 0.0085). Kobayashi et al. [28] reported that oral BCAA supplementation tended to suppress HCC development in patients with cirrhosis whose serum albumin level was <4 g/dl. Similarly, Muto et al. [29] reported that oral BCAA supplementation reduced the incidence of HCC in overweight patients (BMI $\geq$25 kg/m^2) with decompensated cirrhosis (HR 0.30, 95% CI 0.12–0.78). Based on these results, we assume that BCAA supplementation has another beneficial effect, suppressing liver carcinogenesis, in patients with cirrhosis. Although additional prospective trials are needed to confirm this novel effect of BCAA, this view is supported by several experimental and clinical studies that investigated the mechanisms underlying the anticarcinogenic effects of BCAA. According to these studies, the improvements in insulin resistance [16–18], antiangiogenesis via inhibition of vascular endothelial growth factor [15], and reduction of oxidative stress [30] that are induced by BCAA play roles in the suppression of liver carcinogenesis.

Insulin resistance, which is often observed in patients with chronic liver disease, is associated with the development of HCC [31, 32]. Kawaguchi et al. [33] demonstrated that BCAA supplementation improved insulin resistance in patients with chronic liver disease. Several in vivo studies revealed that the mechanisms underlying this effect include enhanced glucose uptake by skeletal muscle, adipocytes and hepatocytes [34–36]. These results prompted us to consider that the improvements in insulin resistance induced by BCAA supplementation play an important role in suppressing liver carcinogenesis. Although we failed to show an association between insulin resistance and liver carcinogenesis in the present study because of limited data regarding the serum insulin level, this view is supported by the finding that the effect of BCAA on the suppression of HCC development was greater among patients with HCV-related cirrhosis, which is more closely associated with insulin resistance than HBV-related cirrhosis [37].

Because this study was performed in a retrospective manner, we used propensity score analysis to reduce the effect of selection bias in the indication of BCAA supplementation. Although the differences in patient characteristics at baseline disappeared after adjustment by the propensity score, differences in body weight and BMI were found; body weight and BMI were greater in the BCAA group than in the control group. However, we believe that this does not affect the results of our study because greater body weight and BMI in patients with cirrhosis are unfavorable factors associated with an increased risk of disease progression and HCC development [32, 38].

In conclusion, we found that oral BCAA supplementation in patients with cirrhosis is associated with a reduced incidence of HCC. Oral BCAA supplementation also seems to be effective in the prevention of liver-related complications in patients with Child-Pugh A cirrhosis. Although these findings remain to be validated in prospective trials, these results advocate the necessity of BCAA supplementation in the management of patients with cirrhosis, even when liver function is compensated.

Disclosure Statement

The authors have no conflict of interest to declare.

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Management of Hepatocellular Carcinoma in Japan: Consensus-Based Clinical Practice Guidelines Proposed by the Japan Society of Hepatology (JSH) 2010 Updated Version

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Key Words
Clinical practice guidelines, evidence-based · Clinical practice manual, consensus-based · Hepatocellular carcinoma, prevention · Hepatocellular carcinoma, staging · Hepatocellular carcinoma, surveillance · Hepatocellular carcinoma, diagnostic algorithm · Hepatocellular carcinoma, treatment algorithm

Abstract
Hepatocellular carcinoma (HCC) is one of the leading causes of cancer death not only in Japan but also worldwide. Clinical practice guidelines for HCC were first published in 2001 by the European Society of Study of the Liver (EASL) followed by the American Association for the Study of Liver Disease (AASLD) published in 2005 and updated in 2010. However, these guidelines have proven to be somewhat unsuitable for Japanese patients. In 2005, supported by the Japanese Ministry of Health, Labour and Welfare, evidence-based clinical practice guidelines for HCC were compiled in Japan. In 2009, a revised version of evidence-based guidelines was published. Based on both 'evidence-based' guidelines and the consensus of an expert panel on HCC, the Japan Society of Hepatology (JSH) published the Consensus-Based Clinical Practice Manual in 2007 and updated in 2010. In this article, the 2010 updated version of this manual, especially issues on prevention, surveillance, pathology, diagnosis, staging, and treatment algorithm are summarized.

Introduction

Following the publication by the European Society of Study of the Liver (EASL) in 2001 [1], the American Association for the Study of Liver Disease (AASLD) published the Clinical Practice Guidelines of hepatocellular carcinoma (HCC) in *Hepatology* in November 2005 [2] and updated in 2010 [3].

In Japan, the original Evidence-Based Clinical Practice Guidelines of HCC were published in 2005 [4] and updated in 2009 [5], disclosed on the website of the Japan Society of Hepatology (JSH) [www.jsh.or.jp/], and then widely used for liver cancer treatment in Japan. An ex-

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Table 1. JSH expert panel on Consensus-Based Clinical Practice Manual of the HCC, 2010 revised version (alphabetical order)

Hepatologists	
Norio Hayashi	Kansai Rosai Hospital
Naoki Hiramatsu	Osaka University
Takafumi Ichida	Juntendo University, Shizuoka
Akio Ido	Kagoshima University
Kenji Ikeda	Toranomon Hospital
Tatsuo Inoue	Kinki University
Takao Iwasaki	Tohoku University
Namiki Izumi	Musashino Red Cross Hospital
Shuichi Kaneko	Kanazawa University
Akinori Kasahara	Osaka University
Kazuhiko Koike	Tokyo University
Masatoshi Kudo	Kinki University
Takashi Kumada	Ogaki Municipal Hospital
Yasushi Matsuzaki	Tokyo Medical University
Masahito Minami	Kyoto Prefectural University of Medicine
Yasunori Minami	Kinki University
Takeshi Okanoue	Saiseikai Suita Hospital
Masao Omata	Yamanashi Prefectural Hospital
Yukio Osaki	Osaka Red Cross Hospital
Shuichiro Shiina	Tokyo University
Masatoshi Tanaka	Kurume University Medical Center
Hidenori Toyoda	Ogaki Municipal Hospital
Yoshihide Ueda	Kyoto University
Tatsuya Yamashita	Kanazawa University
Hepato-Biliary-Pancreatic and Transplant Surgeons	
Shigeki Arii	Tokyo Medical and Dental University
Hiroto Egawa	Murakami Memorial Hospital Asahi University
Takumi Fukumoto	Kobe University
Kiyoshi Hasegawa	Tokyo University
Toshimi Kaido	Kyoto University
Seiji Kawasaki	Juntendo University
Norihiro Kokudo	Tokyo University
Yonson Ku	Kobe University
Masatoshi Makuuchi	Japanese Red Cross Medical Center
Morito Monden	Osaka University
Hiroaki Nagano	Osaka University
Tadatoshi Takayama	Nihon University
Ryosuke Tateishi	Tokyo University
Shinji Uemoto	Kyoto University
Shintaro Yamasaki	Nihon University
Pathologists	
Masamichi Kojiro	Kurume University
Osamu Nakashima	Kurume University
Michiie Sakamoto	Keio University
Radiologists	
Osamu Matsui	Kanazawa University
Takamichi Murakami	Kinki University
Kenichi Takayasu	National Cancer Center Hospital
Medical Statistician	
Kenichi Yoshimura	Translation Research Center, Kyoto University

cerpted version has also been published in an English journal by Makuuchi and Kokudo et al. [5–7]. These guidelines were prepared after critical evaluations based on about 100 reports with a high evidence level in each field selected from 7,118 reports on HCC published between 1966 and 2002. In the 2009 revised version, 2,950 articles were reviewed and 532 articles were incorporated into the new version. Since the guidelines were prepared based as much as possible on highly evidenced data, some points may slightly deviate from actual practices related to HCC routinely performed based on the experience and consensus of HCC experts in Japan.

Considering this situation, the JSH summarized HCC treatment as performed in Japan with the consensus opinions of many experts, even though clear evidence was not available, and published a simple manual in 2007 [8] and updated in 2010 [9]. This was an experience- or consensus-based manual based on evidence-based guidelines with respect to the evidence level, and summarized the consensus of expert opinions – widely reflecting the actual state of HCC treatment in Japan.

The manual was prepared in accordance with the Evidence-Based Clinical Practice Guidelines reported by Makuuchi and Kokudo et al. [5–7], and thus contains no conflict with those guidelines. Points that slightly differ are a more detailed explanation of liver cancer treatments based on expert opinions, and a summary of the consensus by the expert panel [10]. Although it may seem unusual that two different guidelines are available and followed in Japan, both have different roles and are not contradictory.

This report introduces the revised version of Consensus-Based Clinical Practice Manual of HCC published by the JSH in 2010, and focuses on prevention, surveillance, pathology, diagnosis, staging, and treatment algorithm. This constitutes a 'practice manual' summarized by the expert panel of the JSH (table 1), and is different from the Clinical Practice Guidelines. The contents of this report may be considered as the current state of the most advanced HCC treatment practices in Japan.

Prevention

Antiviral Therapy

Hepatitis B Virus-Related HCC

Preventive therapy for HCC should be indicated for these patients. In Japan, HBe antigen-positive chronic hepatitis B patients with an ALT level of ≥31 IU/l and an HBV DNA level of ≥5 log IU/ml, HBe antigen-negative

chronic hepatitis B patients with an HBV DNA level of ≥4 log IU/ml, and liver cirrhosis patients with an HBV DNA level of ≥3 log IU/ml are recommended for antiviral therapy.

Previously, a randomized controlled trial (RCT) examined the inhibitory effects of interferon (IFN) therapy on carcinogenesis in patients with chronic hepatitis B. In 1999, Lin et al. [11] randomly divided 101 HBe antigen-positive patients with type B chronic liver disease into three groups: placebo (n = 31), placebo + IFN (n = 34), and prednisolone + IFN (n = 36) groups, and continued follow-up, with a mean follow-up of 8.4 (1.1–11.5) years. HCC was detected in 1 of 67 patients treated with IFN and in 4 of 34 patients receiving a placebo. They reported that carcinogenesis was significantly inhibited in the IFN-treated groups (p = 0.013). However, when investigating only chronic hepatitis patients, excluding 12 with liver cirrhosis, there were no significant differences in the incidence of HCC between the IFN-treated and non-IFN-treated groups.

On the other hand, the incidence of HCC was compared between 233 IFN-treated and 233 untreated patients in a case-control study involving 466 HBe antigen-positive patients with type B chronic liver disease. In the IFN-treated group, carcinogenesis was significantly inhibited (p = 0.011) [12].

Camma et al. [13] conducted a meta-analysis involving seven articles, and examined whether IFN therapy reduces the risk of compensatory liver cirrhosis B-derived carcinogenesis. IFN therapy decreased the absolute risk of liver carcinogenesis by 6.4%. However, the values markedly differed among the studies. A study involving groups in Europe with slight differences reported that there were no differences.

Prevention of Chronic Hepatitis/Liver Cirrhosis B-Derived Liver Carcinogenesis with Nucleoside Analogues

Two RCTs investigated the effectiveness of nucleoside analogue on preventing liver carcinogenesis in patients with chronic hepatitis/liver cirrhosis B. One of these involved 651 patients with marked hepatitis B-related fibrosis or compensatory liver cirrhosis. During the follow-up period (32.4 months), HCC was noted in 17 (3.9%) of 436 patients treated with lamivudine and in 16 (7.4%) of 215 patients treated with a placebo. In the former, carcinogenesis was significantly inhibited [14]. The other trial involving 222 patients with liver cirrhosis B compared lamivudine-treated and additionally adefovir-treated groups with a non-treated group, and reported that HCC incidence was significantly inhibited in the former two groups (p = 0.003) [15]. Furthermore, a non-randomized, comparative study also indicated that lamivudine and additional adefovir treatments significantly inhibited carcinogenesis compared to control group [16]. Thus, antiviral therapy with nucleoside analogues is useful for preventing HCC in patients with chronic hepatitis B or compensatory liver cirrhosis B.

Hepatitis C Virus-Related HCC

Primary Prevention of Chronic Hepatitis C-Derived Liver Carcinogenesis with IFN

The risk of HCC in patients in whom IFN therapy achieved sustained viral response (SVR) was one-fifth of that in untreated patients. In non-SVR group it was significantly inhibited to one-fourth to one-half in comparison with patients with ALT normalization at the end of IFN therapy and biochemical responders (BR) with ALT normalization for ≥6 months after the completion of such therapy [17]. A meta-analysis involving 4,614 patients examined the relationship between the presence or absence of IFN therapy in patients with type C chronic liver disease, including those with liver cirrhosis, and the incidence of HCC indicated that IFN therapy decreased the risk of HCC by 13%. The effects were more marked in BR [18]. These results suggest that IFN therapy inhibits the development of HCC in comparison with untreated patients, and that not only SVR but also BR are related to the prevention of HCC. Furthermore, a retrospective cohort study regarding the inhibitory effects of combination therapy with IFN and ribavirin on HCC in patients with chronic hepatitis C showed that the risk of HCC development was significantly lower in responders to this combination therapy [19]. Based on these findings, it is recommended that antiviral therapy with IFN be performed to prevent HCC incidence in patients with chronic hepatitis C. The primary goal of IFN treatment is virus eradication (SVR). When it is impossible, the liver function should be normalized as much as possible (BR).

Recently long-term follow-up of the HALT-C study confirmed this observation [20].

Two RCTs investigated the effectiveness of IFN therapy for liver cirrhosis C on preventing liver carcinogenesis. Of these, one reported that there was no difference in the incidence of HCC between IFN-treated and non-treated groups. However, the other study indicated that IFN therapy inhibited the development of HCC. Seven non-randomized, comparative studies, in which a non-IFN-treated group was set as a control group, have been

published. In six of these, IFN therapy inhibited the development of HCC in patients with liver cirrhosis C. Two meta-analyses also affirmed the preventive effects of IFN therapy on the development of HCC in patients with liver cirrhosis C. These effects were marked in patients who achieved SVR. Previously, one study examined the inhibitory effects of combination therapy with IFN and ribavirin on HCC in liver cirrhosis C patients, and reported that, in the combination therapy group, the development of HCC was inhibited in comparison with the non-treated group.

Anti-Inflammation Therapy

Glycyrrhizin Preparations

The intravenous administration of glycyrrhizin for chronic hepatitis/liver cirrhosis is commonly performed to improve the transaminase level. No RCT has investigated whether glycyrrhizin preparations inhibit liver carcinogenesis. However, a retrospective cohort study reported that the intravenous administration of glycyrrhizin preparations for chronic hepatitis C decreased the risk of liver carcinogenesis [21]. It is recommended that glycyrrhizin be intravenously administered for prevention of HCC development in patients with chronic hepatitis C when IFN therapy is not effective or indicated.

Ursodeoxycholic Acid

When administering ursodeoxycholic acid (UDCA) to patients with chronic hepatitis C, cytotoxic bile acid may be substituted for UDCA, protecting the hepatocyte membrane. Furthermore, a study suggested that the immunity-regulating and apoptosis-inhibiting actions of UDCA are involved in the protection of the hepatic cell membrane.

To date, no study has reported the preventive effects of long-term UDCA administration on liver carcinogenesis. However, UDCA administration at 600–900 mg/day improved the serum ALT level [22].

Phlebotomy Therapy

Phlebotomy therapy decreases the serum ALT level, suggesting the usefulness of phlebotomy for the treatment of chronic hepatitis C. Kato et al. [23] reported that long-term iron chelation significantly inhibited the development of HCC. In the future, a large-scale comparative study should be conducted.

Consensus Statements

1 Among patients with type B chronic liver disease, the incidence of HCC is high in those with a high HBV DNA level.
2 Nucleoside analogues are useful for preventing HCC in patients with chronic hepatitis B or compensatory liver cirrhosis B.
3 Among patients with chronic hepatitis C, the incidence of HCC is higher in those with marked fibrosis or liver cirrhosis.
4 It is recommended that antiviral therapy with IFN be performed to prevent HCC in patients with chronic hepatitis C. Firstly, virus elimination is important. When it is impossible, the liver function must be normalized.

Surveillance of Hepatocellular Carcinoma

Definition of the Population at High Risk for HCC

Persistent infections with hepatitis B and C viruses (HBV and HCV, respectively) are the highest risk factors for liver carcinogenesis. The carcinogenesis risk for HBV carriers is about 200 times higher than that for non-carriers, and the risk is higher in patients with type C liver cirrhosis than in those with hepatitis B-related cirrhosis. The HCV-associated risk is about 5 times higher than that associated with HBV. The characteristics of HCV-associated carcinogenesis are carcinogenesis in the F4 step in which liver cirrhosis is completed in most cases, and its occurrence in many cases at 60 years of age or older. The yearly carcinogenesis rate of cirrhosis type C is 7–8% in Japan, which is higher than that in Europe or North America; it might be that the mean age of carriers is closely involved. Liver cirrhosis induced by various causes, even though HBV and HCV are negative, is a risk for liver carcinogenesis. Since carcinogenesis occurs in some cases of liver cirrhosis associated with non-alcoholic steatohepatitis (NASH), alcoholic liver disease, primary biliary cirrhosis (PBC), and autoimmune hepatitis (AIH), the course of the disease should be followed paying close attention to carcinogenesis as in cases of viral liver cirrhosis. Alcohol increases the risk of chronic hepatitis B- and C-associated liver carcinogenesis.

Based on the above, patients with chronic hepatitis B and C and non-viral liver cirrhosis are defined as high-risk populations for HCC in both the Consensus-Based Clinical Practice Manual and Evidence-Based Practice Guidelines. Patients with liver cirrhosis types B and C are defined as a super-high-risk population (table 2). Risk factors other than hepatitis virus or liver cirrhosis are also proposed (table 3).

Table 2. Definition of populations at high risk for HCC

A. Super-high-risk population
1. Hepatitis B-related liver cirrhosis
2. Hepatitis C-related liver cirrhosis
B. High-risk population
1. Chronic hepatitis B
2. Chronic hepatitis C
3. Liver cirrhosis (causes other than HBV or HCV)

Table 3. Risk factors other than hepatitis virus infection or liver cirrhosis

Older age
Male gender
Diabetes mellitus
High body mass index (BMI)
High AST
High ALT
Low platelet count (PLT)
Heavy alcohol drinker
High viral load (HBV carrier)

Table 4. Surveillance protocol for early detection of HCC

1. Super-high-risk patients
Every 3–4 months
Ultrasound examination
AFP/PIVKA-II/AFP-L3 measurements
Every 6–12 months
Dynamic CT or dynamic MRI/EOB-MRI
2. High-risk patients
Every 6 months
Ultrasound examination
AFP/PIVKA-II/AFP-L3 measurements

EOB-MRI = Ethoxybenzyl-MRI.

Surveillance Protocol for Early Detection of HCC

For HCC screening, the HCC detection sensitivity of ultrasonography (US) is higher than that of α-fetoprotein (AFP) measurement, but specificities are not markedly different. For liver cirrhosis, a combination of the two methods has been reported to increase detection frequency compared to detection by US or AFP measurements alone.

No clear evidence is available to determine the optimum interval for periodic screening, but HCCs detected in periodic screenings by AFP, a protein induced by vitamin K absence or antagonist-II (PIVKA-II), and AFP lectin fraction (AFP-L3) measurement, and US are solitary and small in many cases, as compared to those detected in symptomatic patients. Thus, the Evidence-Based Clinical Guidelines [4, 5] proposed performing US and tumor marker measurements every 3–4 months in the super-high-risk population and every 6 months in high-risk populations. Based on HCC doubling times, these intervals appear appropriate (table 4). At present, AFP, PIVKA-II, and AFP-L3 are covered under the Japanese national health insurance as HCC tumor markers. Measurement of two or more tumor markers increases the sensitivity, while minimizing the specificity reduction, for small liver cancer, but alternate measurements of the AFP and PIVKA-II combination or the AFP and AFP-L3 combination is proposed according to the coverage under the current Japanese health insurance. For cases with a very rough background liver parenchyma because of cirrhosis and obesity with difficulty for US evaluation, periodic imaging screening by dynamic CT (multidetector-row CT (MDCT)) or dynamic MRI/EOB-MRI (ethoxybenzyl-MRI) every 6–12 months is proposed [9] (table 4), which is identical to the protocol in the Evidence-Based Clinical Practice Guidelines.

Consensus Statements

5 Patients at high risk for developing HCC should be entered into surveillance programs. The high-risk population and risk factors are identified in tables 2 and 3.
6 Surveillance for HCC should be performed using both US and tumor markers.
7 In Japan, three tumor markers (AFP, PIVKA-II, AFP-L3) are covered by the national health insurance in clinical settings for HCC surveillance.
8 Patients should be screened at 3- to 6-month intervals based on their risk of developing HCC.
9 The surveillance interval needs to be shortened for patients at higher risk for HCC, as described in table 4.

Pathology of Hepatocellular Carcinoma

For the diagnosis and treatment of HCC, it is important to understand the pathology of HCC growth/progression pattern. Clinicians should know the entity of early HCC and the association between pathological features of liver cancer growth/progression and malignancy.

The liver does not have any epithelial structure, different from the digestive tract; therefore, it is impossible to

evaluate the invasive stage of HCC based on the grade of infiltration. In addition, simultaneous/metachronal multicentric development is relatively frequent, making the definition of early HCC difficult. However, several studies showed that the pathological morphology and biological malignancy grade of HCC changed with an increase in the tumor diameter, suggesting the presence of lesions corresponding to early cancer of other organs [24, 25].

Definition of Early HCC with Respect to Pathological Morphology

According to the 'General Rule of Clinical and Pathological of Primary Liver Cancer', HCC is macroscopically classified into five types: vaguely nodular with indistinct margin-, simple nodular-, simple nodular type with extratumor growth-, and multinodular confluent type [26]. In addition, macroscopic findings of small HCCs are classified into two types: simple nodular and vaguely nodular type with indistinct margin. Histologically, most simple nodular type lesions are composed of moderately differentiated carcinoma, whereas vaguely nodular type with indistinct margin consist of well-differentiated carcinoma without severe atypia. In addition to findings such as small cells with an increase in the N/C ratio, an increase in the cell density, 2- to 3-thin layer arrangement, and a small pseudo-glandular structure, these lesions include the several original portal areas. At the boundary of the tumor, cancer cells proliferate to replace the normal hepatocellular cords in the non-cancerous region; therefore, macroscopically, the tumor border becomes unclear. Nodules with indistinct margin, which reflect the earliest change of hepatocarcinogenesis that can be clinically diagnosed, are defined as 'early HCC'. In patients with early HCC, vascular invasion is very exceptional, and there is no intrahepatic metastasis [24]. It is often difficult to differentiate early HCC from high-grade dysplastic nodules. However, the presence or absence of the infiltration of cancer cells in the portal area involved (stromal invasion) [27, 28] should be evaluated for differentiation.

Vascular Structure of Early HCC

It is well known that advanced HCC is completely supplied by arteries. However, early HCC is supplied by the portal venous flow at various levels, i.e. early HCC is supplied by both portal and arteries. However, the number of portal regions in cancer tissue accounts for approximately 25% of that in the non-cancerous region. In addition, arterial tumor vessels are undeveloped; portal and arterial blood may be decreased. On the other hand, arterial tumor vessels develop with an increase in the tumor diameter. However, tumors measuring approximately 10 mm in diameter show insufficient development, and vascularization of the tumor stroma, that is, the capillarization, is also insufficient. Therefore, early HCC does not show hypervascularity on angiography or contrast-enhanced CT.

Fatty Change of Early HCC

Although early, small liver cancer is often visualized as a hyperechoic nodule on US, most lesions reflect the fatty change of the nodule. The fatty change of HCC was the most frequent (approx. 40%) in lesions measuring 10–15 mm in tumor diameter, and the incidence decreases with an increase in the diameter and a reduction in the grade of differentiation. Based on this, fatty change is regarded as a morphological characteristic of early HCC. As previously described, with respect to the pathogenesis of such fatty change, portal blood flow and arterial blood flow may reduce via a decrease in the portal area in lesions measuring 10–15 mm in tumor diameter, and cancer may transiently show ischemia due to the insufficient development of arterial tumor vessels, causing fatty change [29].

Diagnostic Imaging of Early HCC

As many lesions of early HCC are hypovascular, they are difficult to demonstrate on CT through a hemodynamic basis; the correct diagnosis rate is not high. Recently, diagnostic imaging of intrahepatic nodular lesions by contrast-enhanced MRI with Gd-EOB-DTPA has been introduced. For Gd-EOB-MRI to be used to evaluate the hepatocellular function, lesions with a decreased intense at the hepatocyte phase are regarded as HCC. The CT diagnosis rate (including CTHA and CTAP) when lesions with a decrease in portal blood flow were regarded as HCC was approximately 60–70%, whereas the diagnosis rate of HCC by EOB-MRI is approximately 90% [30]; MRI may improve the diagnostic accuracy of early HCC. However, the presence of HCC with isointense and dysplastic nodule with low intense on hepatocyte phase of Gd-EOB-MRI has been indicated.

Macroscopic Classification of HCC and Malignancy Grade

The association between macroscopic findings and malignancy grade depends on the grade of tissue differentiation. When investigating resected specimens of HCC

Table 5. Pathology of small HCC: relationship between macroscopic classification, histological differentiation and tumor size (all resected cases, nodule diameter ≤3 cm) [cited from 9, with permission]

	n (%)	Well	Well + mod.	Mod.	Mod .+ poor	Tumor size, mm
SNIM	22	19 (86.4)	3 (13.6)	0	0	13.6 ± 5.4
SN	123	6 (4.9)	24 (19.5)	92 (74.8)	1 (0.8)	22.8 ± 5.6
SNEG	45	0	5 (11.1)	40 (88.9)	0	23.1 ± 5.4
CM	19	0	6 (31.6)	11 (57.9)	2 (10.5)	23.9 ± 5.3

SNIM = Small nodule with indistinct margins; SN = simple nodular type; SNEG = simple nodular type with extranodular growth; CM = confluent multinodular type. Percent values are shown in parentheses.

Table 6. Pathology of small HCC: macroscopic classification and microscopic findings (all resected cases) [cited from 9, with permission]

	fc	fc-inf	sf	vp	vv	im
SNIM	0	0	2 (9.1)	0	0	0
SN	90 (73.2)	79 (64.2)	65 (52.8)	23 (18.7)	3 (2.4)	5 (4.1)
SNEG	38 (84.4)	35 (77.8)	35 (77.8)	20 (44.4)	2 (4.4)	12 (26.7)
CM	1 (5.3)	1 (5.3)	14 (73.7)	12 (63.2)	3 (15.8)	5 (26.3)

fc = Capsular formation; fc-inf = capsular infiltration; sf = septum formatin; vp = portal vein invasion; vv = hepatic vein invasion; im = intrahepatic metastasis; SNIM = small nodule with indistinct margins; SN = simple nodular type; SNEG = simple nodular type with extranodular growth; CM = confluent multinodular type.
Percent values are shown in parentheses.

measuring ≤3 cm, approximately 85% of vaguely nodular type with indistinct margin lesions (early HCC) consisted of uniform, well-differentiated cancer tissue. The remaining 15% contained an area consisting of moderately differentiated HCC tissue, in which dedifferentiation was noted, showing unclear/clear 'nodule-in-nodule lesion' (table 5). In vaguely nodular type lesions, intrahepatic metastasis and portal tumor invasion are extremely rare. The mean tumor diameter is approximately ≤15 mm, and these lesions are significantly smaller than other macroscopic types of nodular lesions. Approximately 75% of simple nodular type lesions are classified as moderately differentiated HCC. Histologically, portal invasion is observed in 20%, and intrahepatic metastasis in 4%, suggesting advanced HCC. Simple nodular type with extratumor growth and multinodular confluent type lesions suggest advanced HCC. Most lesions consist of moderately to poorly differentiated HCC tissues. Portal invasion and intrahepatic metastasis are more frequently seen than in simple nodular type lesions (table 6). The number of intrahepatic metastatic foci and distance from the primary nodular are greater than in simple nodular type lesions [30] (table 7). In other words, lesions with high-level biological malignancy may be macroscopically evaluated as simple nodular type with extratumor growth or multinodular confluent type lesions. Therefore, curative treatment to avoid intrahepatic metastasis and recurrence must be kept in mind in these lesions in comparison with vaguely nodular type and simple nodular type HCCs.

Table 7. Pathology of small HCC: distance between main nodule and intrahepatic metastasis [cited from 9, with permission]

	n (%)	Distance, mm			
		≤2	2.1–5	5.1–10.0	>10.1
SN	9	6 (66.7)	1 (11.1)	0 (0.0)	2 (22.2)
SNEG	75	23 (30.7)	12 (16.0)	17 (22.7)	23 (30.7)
CM	65	27 (41.5)	19 (29.2)	13 (20.2)	6 (9.2)
Total	149	56 (37.6)	32 (21.5)	30 (20.1)	31 (20.8)

SN = Simple nodular type; SNEG = simple nodular type with extranodular growth; CM = confluent multinodular type.
Percent values are shown in parentheses.

Table 8. Pathology of small HCC: rate of portal venous invasion/intrahepatic metastasis and size of nodule (all resected specimens) [cited from 9, with permission]

	Nodule size, cm				
	0–1	1.1–2.0	2.1–3.0	3.1–5.0	5.1–10.0
PVI	0	28.3%	33.3%	49%	58.5%
IM	0	6.7%	17.1%	29.6%	43.9%

PVI = Portal venous invasion; IM = intrahepatic metastasis.

Differentiation and Malignancy Grade of HCC

Most early HCC lesions appear as well-differentiated lesions. Macroscopically, they are detected as nodules with an unclear border. However, the tumor diameter increases with dedifferentiation. Moderately to poorly differentiated HCCs, contained in the well-differentiated cancer tissue after dedifferentiation, are more malignant than the peripheral well-differentiated HCC tissue, showing expansive growth, completely replacing the well-differentiated cancer tissue, and leading to classical HCC with clear margin. When examining small HCC with 'nodule-in-nodule', p53 overexpression is detected in approximately 40% of moderately to poorly differentiated cancer tissues in the internal area. In addition, a Ki-67 labeling index, which reflects the proliferative capacity, indicates that the malignancy is advanced when the peripheral well-differentiated HCC (early HCC) shows 'nodule-in-nodule' pattern. This is consistent with the finding that an increase in the tumor diameter was accelerated with the appearance of 'nodule-in-nodule' during clinical follow-up. 'Nodule-in-nodule' type HCC is recognized as being in the dedifferentiation process from early to advanced HCC; clinical management similar to advanced HCC is necessary.

In 'nodule-in-nodule' type HCC, there is a marked difference in vascularity between the marginal well-differentiated and internal moderately to poorly differentiated HCC tissues. On contrast-enhanced US or CT, the marginal well-differentiated cancer tissue is visualized as a hypovascular area, because the development of arterial tumor vessels and the capillarization is insufficient. However, moderately to poorly differentiated cancer tissues in the internal area are visualized as hypervascular area due to sufficient neovascular development. Briefly, the vascular structure of liver cancer is closely correlated with the grade of differentiation. The malignancy of early liver cancer may be predicted to some degree based on hemodynamic findings.

Nodule Size and Malignancy Grade of HCC

The size of HCC is associated with the macroscopic morphology, grade of histological differentiation, and intrahepatic metastasis/portal invasion rates. Most vaguely nodular type lesions measure ≤ 2 cm, and lesions measuring ≥ 3 cm are rare. However, simple nodular type with extratumor growth and multinodular confluent type lesions become more frequent with an increase in the tumor diameter. Concerning the grade of histological differentiation, the proportion of lesions consisting of uniform, well-differentiated cancer tissue markedly decreases when the tumor diameter exceeds 2 cm. Most lesions consist of moderately to poorly differentiated HCC tissues. The portal invasion/intrahepatic metastasis rates also increase in proportion to the tumor diameter (table 8). Usually, there is a correlation between an increase in the tumor diameter and malignancy grade. However, exceptionally, large, well-differentiated, slowly expanding HCC is present [32].

Consensus Statements

10 Vaguely nodular type HCCs, which are composed of very well-differentiated HCC, are defined as 'early hepatocellular carcinoma'.
11 Early HCC does not show hypervascularity on angiography or dynamic CT/MRI.
12 Fatty change and stromal invasion are regarded as the morphological characteristics of early nodular HCC.
13 In simple nodular type with extranodular growth and multinodular confluent type HCCs, intrahepatic metastasis and recurrence are more frequent than in lesions with vaguely nodular type and simple nodular type HCCs. This must be kept in mind on the curative treatment.
14 'Nodule-in-nodule' findings of very well-differentiated carcinoma (early HCC) reflect higher malignancy grade than early HCC.

Diagnosis of Hepatocellular Carcinoma

Diagnostic Criteria

The diagnosis of HCC is determined by three factors: the background chronic liver disorder, tumor markers, and imaging diagnosis. When the liver has hepatitis B- and C-related cirrhosis, tumor marker levels are increased, and typical imaging findings are detected, HCC can be definitely diagnosed. Typical imaging findings are hypervascularity in the arterial phase and washout in the portal equilibrium phase on dynamic CT or dynamic MRI. Hypervascularity on CTHA and a perfusion defect on CTAP also leads to a diagnosis of typical HCC. How-

ever, HCC cannot be definitely diagnosed based on a combination of tumor markers and chronic liver disorder alone, or on the elevation of tumor markers alone. Moreover, hypervascular nodules in the arterial dominant phase without washout in the portal equilibrium phase are not typical and more precise investigations are necessary. Hypovascular nodules in the arterial dominant phase also require further examination. Cases meeting all A, B, and C criteria in table 9 are definitely diagnosable as HCC in Japan. Cases not accompanied by the typical imaging findings are diagnosed and treated by the examinations detailed in figures 1 and 2.

Table 9. Diagnostic criteria of typical HCC[1]

A. Background liver disease (one positive factor)
Hepatitis B-related liver disease
Hepatitis C-related liver disease
Liver cirrhosis
B. Tumor markers (at least one positive study)
AFP ≥200 ng/ml associated with a rising trend over time
PIVKA-II (≥40 mAU/ml) with a rising trend over time
AFP-L3 (>15%)
C. Typical imaging findings (one positive study)[2]
Arterial phase hypervascularity with portal-venous phase washout on dynamic CT, dynamic MRI or CEUS
Hypervascularity on CTHA with perfusion defect on CTAP

[1] A+B+C, A+C, B+C, C: HCC confirmed A+B, B: HCC highly suspicious, thus, dynamic CT/MRI is required.

[2] Nodules with atypical imaging study, namely, hypervascularity without portal/venous washout or hypovascular nodule on arterial phase should undergo further study (as shown in fig. 1 and 2).

Multistep Development of HCCs and Abnormal Blood Flow

Many cases of HCCs originate from HBV and HCV infections via multistep development. Premalignant lesions and early HCCs are mainly fed by a portal venous flow in contrast to overt HCCs, which are supplied by an arterial flow. Thus, there may be no objection to indicating a hypervascular HCC for treatment.

However, how to diagnose a typical HCC is the most problematic issue when the hemodynamic pattern is not typical for a HCC. Although the Guidelines for Evidence-Based Clinical Practice for the Treatment of Liver Cancer [4, 5] do not suggest any detailed imaging diagnostic criteria for atypical nodules, a more detailed algorithm referring to these issues has been proposed in the new practice manual (fig. 1).

Diagnostic Modalities of HCC

Dynamic CT

Dynamic CT using MDCT acquires images within several seconds during a single respiratory pause if the slice thickness is about 5 mm and is superior for detection of hypervascular HCC. On dynamic CT of the liver, 100–120 ml of contrast medium is rapidly infused within 30 s. The arterial dominant phase is generally acquired at around 30–45 s following the initiation of the injection of contrast medium [33]. A characteristic of HCC is a corona-shaped enhancement in the late arterial phase or portal venous dominant phase [34]. The vascular and extracellular contrast medium concentrations reach equilibrium about 200 s after infusion; scanning at this time point is called the equilibrium or parenchymal phase. In a typical HCC, attenuation decreases in the equilibrium phase.

In MDCT, time resolution is high and acquisitions of the arterial, portal, and equilibrium phases have markedly increased the ability to qualitatively diagnose tumorous lesions. The diagnosis of a typical hypervascular HCC is easy because the lesion is detected as a high attenuation area in the arterial phase, corona enhancement is noted in the late arterial or the portal phase, and this becomes a low attenuation area in the equilibrium phase. However, the frequency of obtaining typical findings varies depending on CT equipment and acquisition conditions. Diagnostic accuracy of hypervascular HCCs by CT has been reported to be 68–91% [35–37].

Contrast-Enhanced US

Sonazoid-enhanced US are classified into the two phases: the vascular and Kupffer phases (fig. 3).

Vascular Phase

The sensitivity of contrast-enhanced US with Sonazoid to detect intranodular blood flow in liver tumors is extremely high. The sensitivity of 4-phase imaging (plain, arterial, portal, and equilibrium phases) by MDCT, as a gold standard, for detecting intranodular blood flow is similar to or superior to that of MDCT. In addition, in most patients in whom there is no intranodular blood flow in the arterial phase on contrast-en-

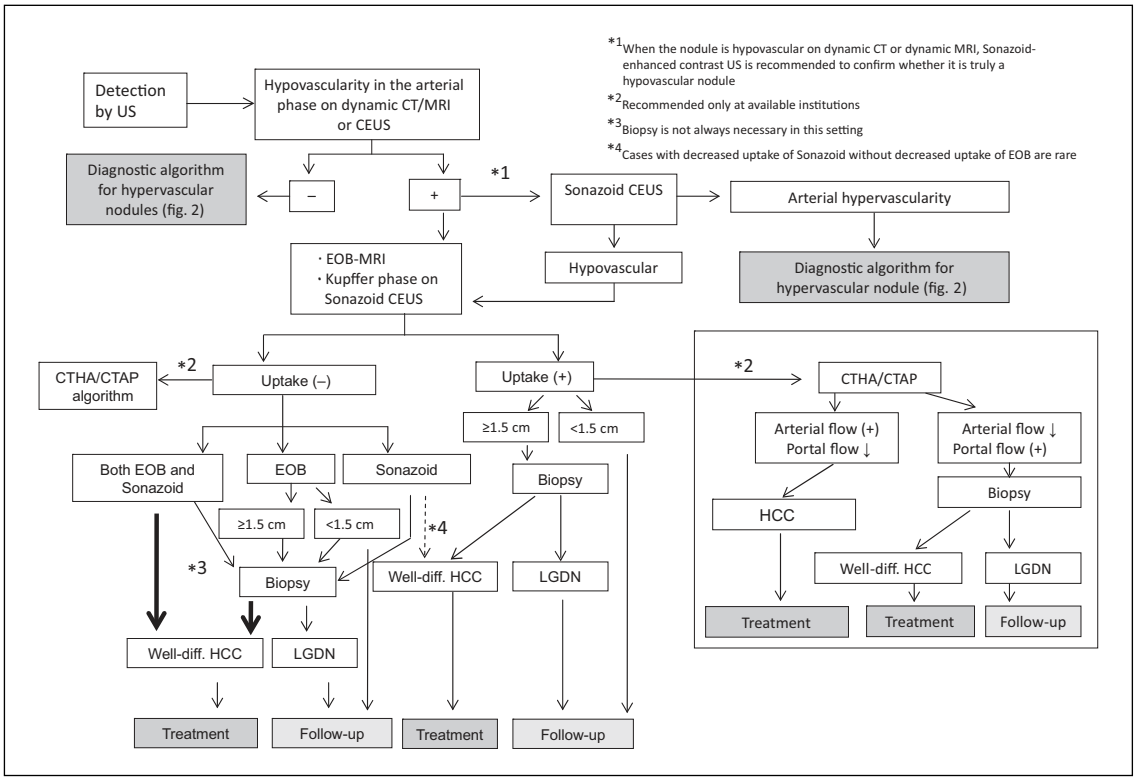

Fig. 1. Diagnostic and treatment algorithms for hypovascular liver nodules (JSH 2010) [cited from 9, with permission].

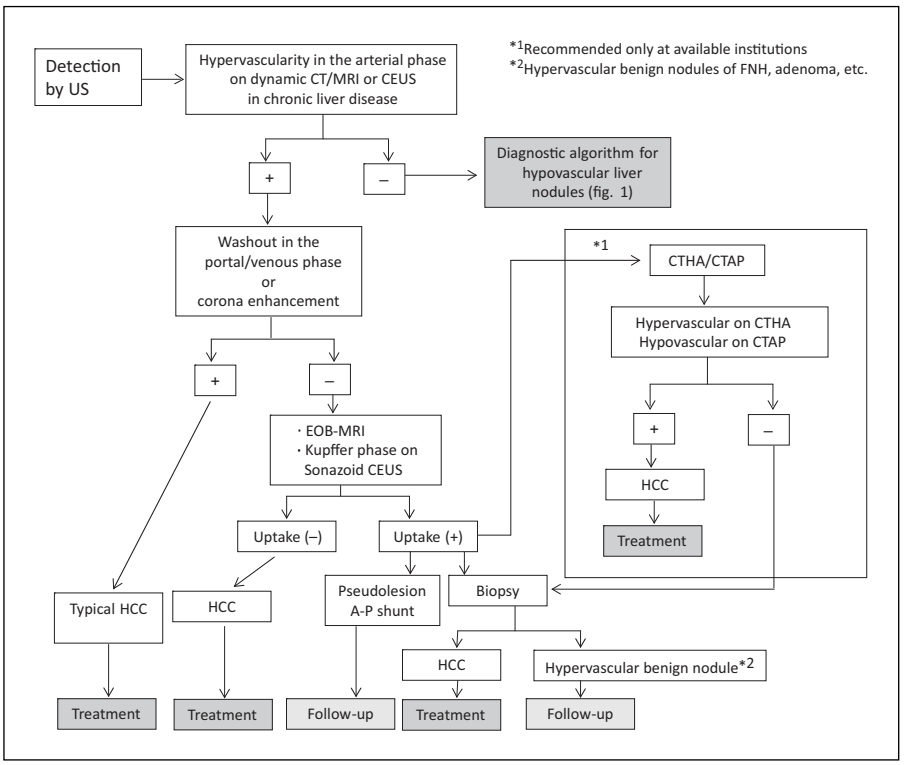

Fig. 2. Diagnostic and treatment algorithms for hypervascular liver nodules (JSH 2010) [cited from 9, with permission].

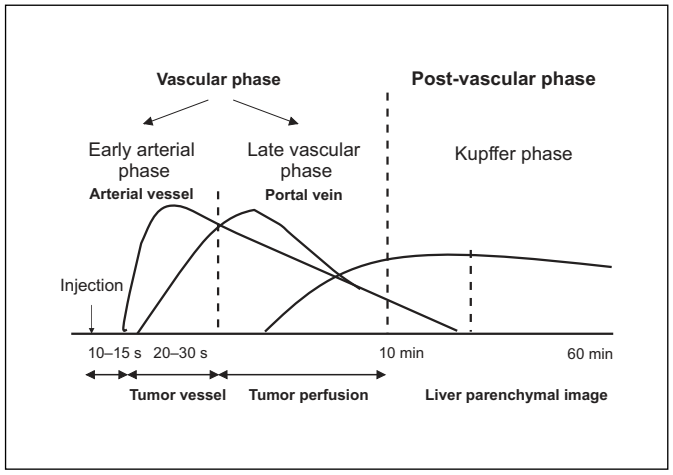

Fig. 3. Phases of contrast-enhanced US with Sonazoid. There are two phases: vascular and post-vascular phase.

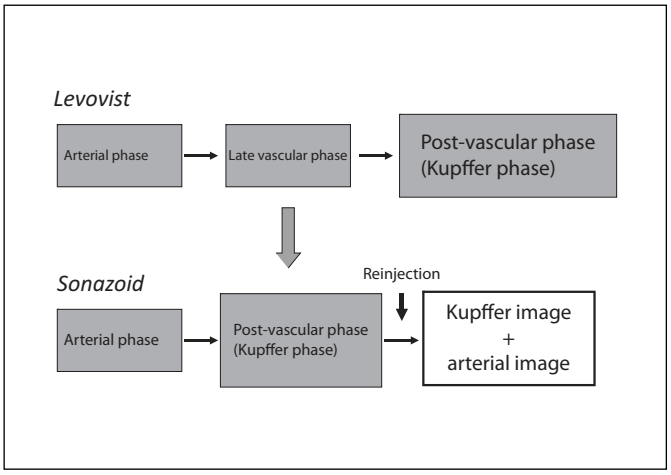

Fig. 4. Basic concept of defect reperfusion imaging: Reinjection at the Kupffer phase plays an important role.

hanced US, contrast-enhanced CT does not show any enhancing area in the arterial phase. However, in some patients without intranodular blood flow on dynamic CT, contrast-enhanced US reveals arterial blood flow. Briefly, contrast-enhanced US may be more sensitive than CT for the detection of intranodular arterial blood flow [38]. Basically, contrast-enhanced US should be performed as a precise examination tool of intranodular blood flow in nodules detected on B-mode US. When using Sonazoid, the Kupffer phase is very stable, differing from that with Levovist, SonoVue or Definity. Therefore, initially, entire liver scanning should be conducted in the Kupffer phase, and, additionally, Sonazoid should be intravenously injected into Kupffer defect sites (defect reperfusion imaging), which facilitates cancer detection to making a definitive diagnosis. In the future, contrast-enhanced US may be applied for screening [39, 40] and staging, which have been considered to be impossible using CEUS with blood pool agents, such as SonoVue or Definity.

Kupffer Phase

The Kupffer phase of Sonazoid is very important.

(1) Most lesions of moderately or well-differentiated hypervascular HCC in which arterial blood flow is abundant show a decrease in Sonazoid uptake or defect in the Kupffer phase.

(2) Among precancerous or early HCC lesions, Sonazoid uptake in the Kupffer phase is similar to that of the surrounding liver parenchyma in which arterial blood flow is reduced, with the influx of portal blood flow is preserved.

(3) For the differential diagnosis between a precancerous lesion, dysplastic nodule (DN), and early well-differentiated HCC (early HCC), the two lesions show iso uptake in the Kupffer phase in many cases; therefore, differentiation is difficult. However, hypovascular nodules in which Kupffer uptake is reduced in the Kupffer phase can be diagnosed as early HCC.

Significance of Defect-Reperfusion Imaging (Double Injection CEUS)

Recently, a new procedure (defect-reperfusion imaging) was developed, in which stable Kupffer images of Sonazoid and real-time blood flow imaging are applied, facilitating the accurate local diagnosis and treatment of typical liver cancer that shows arterial enhancement with venous wash on CT, washout in the late phase, and is not visualized as on B-mode US (fig. 4) [39, 41].

Sonazoid is intravenously injected. Presence or absence of Kupffer defect is evaluated in the Kupffer phase ≥10 min after confirming an arterial enhancement area in the vascular phase. In addition, an entire liver scan is necessary to detect a defect site in the Kupffer phase. If a Kupffer defect is found in the liver, Sonazoid is additionally administered whether or not arterial blood flows in the defect site is assessed (defect reinjection test). Regarding the presence of arterial vascularity in the defect area, typical liver cancer can be diagnosed at a rate of approx-

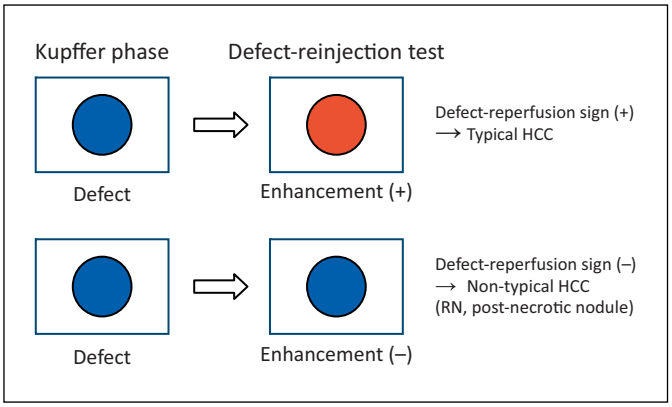

Fig. 5. Value of defect reperfusion imaging in B-mode undetectable nodule. Diagnostic ability of B-mode undetectable HCC by this technique using Sonazoid-enhanced US is 100%.

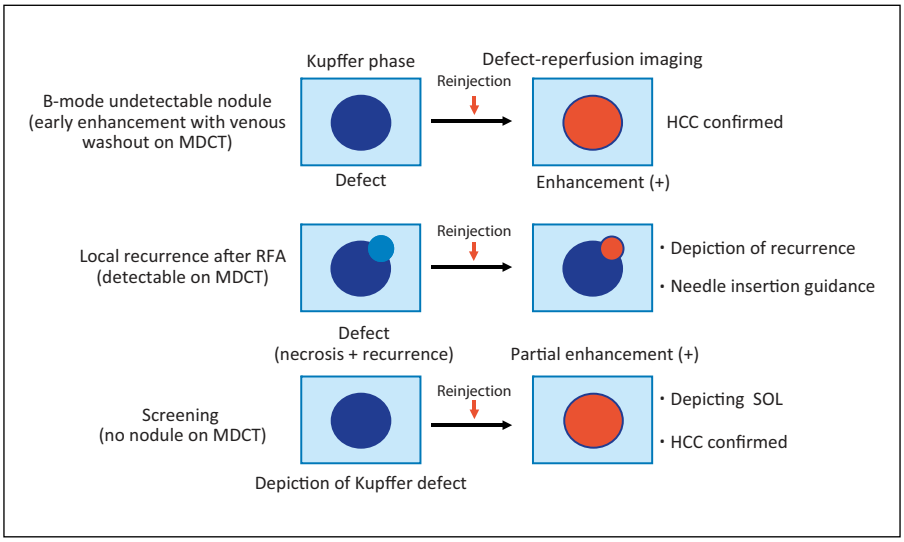

Fig. 6. Defect reperfusion image is useful for confirming B-mode undetectable nodule, local recurrence after RFA and surveillance of HCC in cirrhotic patients.

imately 100% (fig. 5). In addition, concerning nodules that cannot be visualized on B-mode US, it is possible to detect clear defects in the Kupffer phase, although diagnosis is impossible in the vascular phase since the detection of arterial blood flow is impossible to detect. The additional intravenous injection of Sonazoid in this area facilitates the detection of arterial vascularity in the defect site. In this area, Sonazoid contrast-guided radiofrequency thermal ablation (RFA) therapy becomes possible. In this procedure, initially, nodules that cannot be detected on B-mode US are detected as defects in the Kupffer phase. Subsequently, the additional intravenous injection of Sonazoid is performed, and whether or not nodules with defects involve arterial blood flow is evaluated. This procedure may be a breakthrough for diagnostic imaging. This contrast enhancement procedure does not require any specific device or analysis. Concerning typical CT findings, arterial enhancement with venous washout, defects are initially detected in the Kupffer phase, and, subsequently, arterial perfusion in the detect site is confirmed (fig. 5).

The introduction of such an idea facilitates the identification of nodules that show hypervascular typical features on CT, and are unclear on B-mode US, at a probability of approximately 100%. If there are nodules without arterial enhancement by reinjection for defects, they may differ from nodules detected on CT (fig. 6). Therefore, clinically, this procedure is breakthrough as a treatment aid of HCC [42]. In addition, this defect reperfusion imaging method can be applied for various purposes such as HCC screening in liver cirrhosis with rough liver parenchyma, the identification of local tumor progression sites after treatment. In addition, in cases of evaluation of the treatment response after RFA or transcatheter arterial chemoembolization (TACE) and contrast-enhanced guided needle placement it may be also very useful (fig. 6).

Dynamic MRI

The consistency rate of histopathology excised liver after liver transplantation with various imaging findings has been reported, with the sensitivity of dynamic MRI shown to be the highest [43]. In another report, CT, abdominal US, and MRI were compared with regard to evaluations of nodular recurrence after transarterial chemoembolization mixed with lipiodol; again, MRI was the superior modality [44]. However, detectability varies among MRI equipment models, and MRI cannot be readily used in some facilities. Use of MDCT has spread rapidly in Japan, and its usefulness is well established. It may be better to diagnose hypervascular HCC using dynamic MRI or MDCT depending on the conditions found in the institution, but the X-ray exposure problem arising with frequent MDCT images should be kept in mind.

Gd-EOB-MRI

Gd-EOB-MRI Is Superior to SPIO-MRI

A new hepatocyte-specific contrast agent for MRI, Gd-EOB-DTPA (gadoxetate sodium), became commercially available in Japan in January 2008. This contrast agent is taken up by hepatocytes and excreted from the kidney and from the liver through the bile duct. As a result, liver parenchyma is intensely enhanced showing definite hyperintensity in the hepatobiliary phase ≥20 min after intravenous injection based on T_1-weighted images, in addition to the diagnosis based on blood supply. Nodules without liver parenchymal cells, such as liver cancer, are visualized as hypointense. This diagnostic imaging method is simple for hepatologists in addition to radiologists specialized in MRI in comparison with SPIO as a black liver agent (black coloration of the entire liver on T_2-weighted images in which the spatial resolution is poor). Gd-EOB-DTPA-enhanced MRI is a breakthrough for diagnostic imaging of the liver and HCC.

Mechanism of Gd-EOB-DTPA Uptake and Findings with Respect to the Grade of Differentiation of HCC

Approximately 50% of the dose of Gd-EOB-DTPA is taken up by hepatocytes and excreted in bile. The remainder is excreted from the kidney. Hepatocellular uptake may be related to passive diffusion mediated by organic anion transporter 1 (OATP1) in the hepatocellular membrane in rat [45]. Furthermore, ATP-dependent active transport related to multi-drug resistance-associated protein 2 (MRP2) may be involved in excretion from hepatocytes into the capillary bile duct [46].

A recent study reported that Gd-EOB-DTPA uptake in humans depended on OATP1B3 (synonymous with OATP8) [47, 48] among various kinds of human OATP families. Based on these reports, concerning the mechanism via which Gd-EOB-DTPA is taken up in the hepatobiliary phase even when well- or moderately differentiated liver cancer is hypervascular HCC, OATP1B3 expression may be detected in some lesions, and visualized as an iso or high signal intensity in the hepatobiliary phase. According to the study, there was no statistically significant association between OATP1B3 expression and the bile-producing capacity (green hepatoma)/grade of differentiation [47, 48]. It is now considered that the expression of OATP1B3 may be decreased in accordance with the elevation of the grade of malignancy of the hepatocellular nodules. According to the recent immunohistochemical study, low-grade dysplastic nodule showed the same or increased OATP1B3 expression relative to the surrounding liver. On the other hand, around half of high-grade dysplastic nodules, around 80% of early HCCs, around 90% of well- and moderately differentiated HCCs and almost all of poorly differentiated HCCs demonstrated decreased expression [Matsui O., pers. commun., submitted]. Therefore, the majority of HCCs may be detected as hypointense nodule in the hepatobiliary phase when liver function is well preserved. However, in approximately 5–10% of patients with hypervascular, well- or moderately differentiated liver cancer, there was definite expression of OATP1B3 and no reduction in the signal intensity in the hepatobiliary phase, probably due to genetic alteration [47, 48].

Actually, in clinical practice, in some nodules detected on US, histopathological investigation with biopsy specimens leads to a diagnosis of well-differentiated liver cancer despite the absence of signal-intensity reduction in the hepatobiliary phase; well-differentiated liver cancer in which OATP1B3 expression is maintained may be present.

Another issue is whether signal-intensity reduction is absent in all dysplastic nodules in the hepatobiliary phase. Concerning this issue, biopsy/pathological findings of nodules with signal-intensity reduction in the hepatobiliary phase suggest dysplastic nodules in some cases. However, a study involving resected specimens (not biopsy materials) reported that all dysplastic nodules showed an iso signal intensity in the hepatobiliary phase. Therefore, there may be few dysplastic nodules with such a decrease in OATP1B3 expression according to pathologists specialized in the liver pathology of early HCC (table 10).

In our experience on the study of resected specimens, dysplastic nodules with a low signal intensity are extremely rare [49]. Unless stromal invasion is detected in the sample despite a pitfall of biopsy diagnosis, sam-

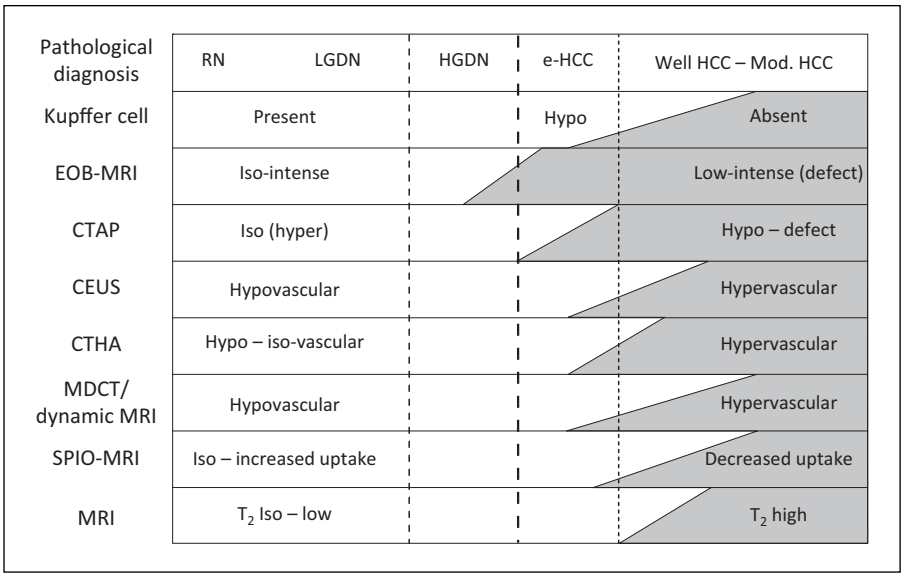

Fig. 7. Imaging findings of hepatocellular nodules in cirrhotic liver [cited from 47, with permission]. Hepatocyte phase of EOB-MRI is the most sensitive technique to detect initial change of multistep hepatocarcinogenesis.

pling errors, or similar cellular/structural atypia, under a biopsy diagnosis of dysplastic nodules, it is sometimes difficult for specialists of liver pathology to make a diagnosis of early HCC. Therefore, biopsy-based pathological diagnosis is limited, and lesions may be underestimated.

Differentiation of Early HCC from Dysplastic Nodules Using Gd-EOB-DTPA-MRI

As described in the above section, blood flow findings are hypovascular in many cases when investigating resected specimens of early HCC. Even when employing CTHA/CTAP, diagnosis of early HCC is difficult. In some patients with early liver cancer, there is a slight decrease in portal blood flow on CTAP. However, CTAP shows iso-perfusion in many patients. Most patients with a reduction in signal intensity in the hepatobiliary phase are diagnosed with early HCC based on resected specimens by specialists of liver pathology [49]. In most patients without a reduction in the signal intensity in the hepatobiliary phase, resected specimens suggest dysplastic nodules. Considering this, functional diagnosis with Gd-EOB-DTPA MRI may facilitate the more sensitive evaluation of initial changes of multistep hepatocarcinogenesis than hemodynamic assessment, SPIO-MRI, Kupffer cell function diagnosis using the Kupffer phase on Sonazoid contrast-enhanced US, or portal blood flow assessment to differentiate early HCC from dysplastic nodules (fig. 7). However the following two issues remain: (1) whether there are dysplastic nodules with a reduction in the signal intensity in the hepatobiliary phase, and (2) whether there are nodules without a reduction in the signal intensity in the hepatobiliary phase in which pathological findings lead to a diagnosis of early HCC. With respect to the two issues, data have now accumulated throughout Japan. However, a consensus has not been reached. To overcome these issues, hypovascular nodules with uptake on SPIO-MRI or in the Kupffer

Table 10. Relationship between the expression of OATP1B3 and findings in the hepatocyte phase

Uptake transporter (OATP1B3 (OATP8))	Hepatocyte phase imaging of EOB-MRI
Dysplastic nodule	
+	Iso-high intensity
– (rare)	Low intensity
Early HCC	
+ (rare)	Iso-intensity
–	Low intensity
Well- to mod. diff. HCC	
+ (5–10%)	Iso-high intensity/iso-high intensity
– (90–95%)	Low intensity
Poorly diff. HCC	
–	Low intensity

OATP = Organic anion transporter polypeptides.

phase of Sonazoid US can be differentiated from hepatocellular nodules. However, in nodules with a reduction in the signal intensity in the hepatobiliary phase of Gd-EOB-DTPA, biopsy findings should be obtained, and the natural course of these nodules must be followed up. It may be important to analyze the grade of malignant potential in a large number of patients.

Angiography

Diagnosis of HCC by angiography including DSA is 69.0%, lower than that (86.9%) of helical CT [50]. Also, detectability more markedly decreases than does CT sensitivity when the tumor size is small. In Japan, very few institutions perform angiography alone for diagnosis, and many facilities routinely perform CT during hepatic arteriography (CTHA) and CT during arterial portography (CTAP) in combination. The rate of detectability of liver cancer by angio-CT is the highest, but specificity is low, and some reports, mainly from Europe and USA, have questioned its diagnostic value. However, observation of corona-like intense staining around liver cancer in the second phase of CTHA (acquisition of images during contrast medium infusion is designated as the first phase, and acquisition after completion of infusion as the second) enables differentiation from pseudo-hypervascular lesions, thus increasing the specificity. The HCC diagnostic ability of arterial injection CT may be highest when second-phase CTHA images are acquired [34]. Sensitivity and specificity of hypervascular HCC diagnoses are highest when both CTHA and CTAP are performed. Since CTHA/CTAP detects nodules <1 cm in diameter, differentiation from pseudo lesions, such as arterial-portal (A-P) shunt or focal nodular hyperplasia (FNH), is necessary.

Diagnostic and Treatment Algorithm of Hypervascular Hepatocellular Carcinoma

For surveillance, abdominal US is a first-line test in many hospitals. As a subsequent examination, MDCT is the most commonly conducted, therefore it was decided as a first-line test in the surveillance algorithm both in the AASLD or JSH guidelines. As dynamic MRI is performed as a first-choice procedure in some hospitals, it should be regarded as similar to MDCT. When the lesion is enhanced in the early arterial phase, a diagnosis of HCC may be made based on washout in the equilibrium phase (table 5). However, FNH and A-P shunt must be ruled out. The number of hepatocytes and Kupffer cells was examined on Gd-EOB-MRI or in the Kupffer phase of Sonazoid contrast-enhanced US. If a defect is confirmed at a low signal intensity in the hepatobiliary phase of Gd-EOB-MRI or in the Kupffer phase of Sonazoid-enhanced US, a diagnosis of HCC can be made (fig. 2).

When low density is noted in the equilibrium phase without an enhancement in the early arterial phase on MDCT, the lesion can be evaluated as hypervascular nodule. CTHA, which is more sensitive, must be performed to examine whether or not it is hypervascular HCC. In institutions where CTHA cannot be conducted, it is necessary to confirm blood flow in the early phase of CO_2 angiography or contrast-enhanced US, or evaluate whether the lesion is hypervascular in the early arterial phase on dynamic MRI.

When abdominal angiography reveals hypovascular tumors, CTHA should be performed if possible, because the sensitivity of the former for the diagnosis of HCC is low. If impossible, CO_2 angiography with high-level spatial resolution should be simultaneously conducted to evaluate the number of hypervascular tumors.

For angiography or CTHA, the possibility of pseudotumors such as A-P shunt must be ruled out. In this case, a diagnosis of hypervascular HCC can be made if there is a decrease in the number of Kupffer cells in the hepatobiliary phase of Gd-EOB-MRI or in the Kupffer phase of Sonazoid-enhanced US. The HCC-diagnosing capacity of Gd-EOB-MRI is similar to that of CTHA/CTAP, therefore these invasive tests may be omitted.

Consensus Statements

15 Typical HCC can be diagnosed by imaging regardless of the size detected if a typical vascular pattern is obtained on dynamic CT, dynamic MRI, CEUS or a combination of CTHA and CTAP. Different from Western guidelines, only one dynamic study showing the typical pattern is sufficient to diagnose HCC even if nodule is <2 cm. The typical imaging pattern includes hypervascularity in the arterial phase and washes out in the portal venous phase.

16 Nodular lesions showing an atypical imaging pattern, such as iso- or hypovascular in the arterial phase or arterial hypervascularity alone without portal venous washout, should undergo the examinations shown in figures 1 and 2. EOB-MRI and Sonazoid-enhanced US play an important role.

17 Elevated AFP (≥20 ng/ml) and PIVKA-II (≥40 mAU/ml) with a rising trend over time and a positive AFP-L3 value (>10%) are highly suggestive of the presence of typical HCC even if US fails to depict an apparent nodule in the liver.

Diagnostic and Treatment Algorithm for Hypovascular Nodules

Among liver cirrhosis-related nodular lesions, hypovascular nodules include low-grade dysplastic nodules (LGDN), high-grade dysplastic nodules (HGDN), which are pathologically regarded as precancerous lesions, early HCC, and nodule-in-nodule type HCC [11, 24, 51, 52]. In this section, the current consensus of diagnosis and treatment algorithms with respect to the treatment of such nodules, especially findings important for the diagnosis and treatment of hypovascular nodules detected on imaging, will be described.

Diagnostic Algorithms for Hypovascular Hepatocellular Nodules (fig. 1)

There has been no diagnostic imaging method to accurately differentiate precancerous lesions such as LGDN and HGDN from early HCC. However, since Gd-EOB-MRI was introduced in 2008, such a situation is rapidly changing.

Among current diagnostic imaging procedures, the following modalities may facilitate the most sensitive tool to detect the initial change of hepatocarcinogenesis: (1) EOB-MRI [49], (2) CTAP [53, 54], (3) contrast-enhanced US [38, 41, 55], (4) CTHA, and (5) MDCT/dynamic MRI, SPIO-MRI (fig. 7). Usually, precancerous lesions show EOB uptake. However, most early liver cancer lesions show a low signal intensity in the hepatobiliary phase of EOB-MRI [49]. Therefore, EOB-MRI may facilitate the earliest, most sensitive assessment of the initial features of early HCC. As the second-most sensitive method, CTAP facilitates the evaluation of initial changes related to hepatocarcinogenesis. However, a partially increased area in intranodular arterial blood flow detected on CTHA or contrast-enhanced US, in which portal blood flow is maintained in the outer area in about two-thirds of early liver cancer nodules, reflects an advanced state of the carcinogenic process. Briefly, hypervascular foci visualized in the nodule (nodule-in-nodule or entirely hypervascular) may biologically reflect small advanced cancer even if histological findings suggest well-differentiated HCC. In some cases, satellite nodules or microvascular invasion is observed at the periphery of the nodule.

According to a study, EOB-MRI shows a decrease in uptake in the hepatobiliary phase in HGDN lesions, therefore a consensus regarding differentiation between benign and malignant tumors has not been reached. Briefly, hypovascular tumors with a decrease in uptake may be basically regarded as early HCC, however DN cannot be ruled out.

Although MDCT and dynamic MRI are relatively sensitive for the detection of arterial blood flow, some hypervascular nodules detected on CTHA or contrast-enhanced US are not visualized as arterial staining, which depends on the timing of imaging, tumor site, and liver function. Hypervascular nodules on MDCT or dynamic MRI may show high intensity on T_2-weighted MRI images.

Based on this, EOB-MRI or Sonazoid contrast-enhanced US should be performed in hypovascular nodules demonstrated on MDCT or dynamic MRI. If there is a decrease in uptake on EOB-MRI or in the Kupffer phase of Sonazoid contrast-enhanced US, malignancy must be initially considered. Furthermore, portal blood flow is reduced on CTAP in some nodules in which EOB-MRI or Kupffer-phase Sonazoid contrast-enhanced US shows uptake, although such cases are rare. When CTHA/CTAP is not conducted, or in institutions in which it is impossible to perform these procedures, biopsy should be conducted in such nodules measuring >1.5 cm, because they may become hypervascular nodules, leading to typical liver cancer. When performing CTHA/CTAP, nodules with increased arterial blood flow or the reduction of portal blood flow are biologically regarded as malignant. In nodules in which arterial blood flow is insufficient in the presence of portal blood flow, biopsy is necessary.

The capability of contrast-enhanced US is dependent on US equipment. However, in institutions in which high-end machines are available, the combination of this procedure and MDCT improves the accuracy of arterial/portal blood flow assessment in comparison with a single method alone. Furthermore, the application of the Kupffer phase and hepatobiliary phase of EOB-MRI makes the prediction of malignancy more accurate.

Concerning nodules that are not visualized as hypervascular nodule on MDCT or dynamic MRI, when both EOB-MRI and Kupffer-phase Sonazoid contrast-enhanced US reveal a reduction in uptake, these nodules should be treated as HCC. In this case, biopsy is not always necessary.

When EOB-MRI shows a reduction in uptake and the nodule size exceeds 1.5 cm, biopsy should be performed if possible. When a diagnosis of typical well-differentiated HCC is made, treatment should be performed. Even when EOB-MRI and Kupffer-phase Sonazoid contrast-enhanced US reveal uptake, CTHA and CTAP may be performed in nodules measuring >1.5 cm. When there is

an increase in arterial blood flow or a decrease in portal blood flow, even though such a case is rare, nodules are regarded as malignant. However, in nodules in which arterial blood flow is low, and portal blood flow is preserved, follow-up may be continued when biopsy suggests benign nodules. However, when biopsy leads to a diagnosis of typical well-differentiated HCC, the treatment of early HCC may be considered.

When EOB-MRI shows uptake, nodules in which biopsy suggests a borderline lesion may be followed up. In conclusion, currently, EOB-MRI is the most useful tool for diagnosing early HCC in hypovascular hepatocellular nodes (fig. 1, 7).

Consensus Statements

18 It is essentially difficult to differentiate a histopathological diagnosis of early HCC from a dysplastic nodule by imaging.
19 Gd-EOB-MRI is the most sensitive tool for detection of any initial change of hepatocarcinogenesis, i.e. low intense mass on hepatocyte image of Gd-EOB-MRI. Therefore, it is recommended that Gd-EOB-MRI be performed as much as is possible.
20 Sonazoid-enhanced contrast US is more sensitive for detection of intranodular hypervascularity than MDCT (dynamic CT) or dynamic MRI. Therefore, to confirm true hypovascularity, Sonazoid-enhanced CEUS is recommended.
21 Decreased intranodular portal flow on CTAP suggests a high malignant potential of a nodule. Therefore, such nodules should be treated as malignant.
22 Nodules with a low uptake of EOB-MRI and a size >1.5 cm should be treated as malignant after confirmation by biopsy.
23 Nodules with hypovascularity and negative findings on EOB-MRI and Kupffer phase imaging of Sonazoid CEUS are likely to be benign. Thus, they can be followed up without treatment when the nodule size is <1.5 cm.
24 Biopsy-proven early HCC should be treated.

Staging for Hepatocellular Carcinoma

Classification of Staging Systems

TNM stages representing the degree of cancer spreading are clinically used for various cancers, not only for HCC. It is well recognized that for HCC stages of not only tumors but also the liver functional reserve are very important for deciding a treatment strategy and prognosis prediction. Thus, HCCs should be treated with an understanding of the importance of the liver cancer staging systems.

Table 11. TNM stage by the Liver Cancer Study Group of Japan [cited from 54, with permission]

Stage	T category	N category	M category
Stage I	T1	N0	M0
Stage II	T1	N0	M0
Stage III	T3	N0	M0
Stage IVA	T4	N0	M0
	T1, T2, T3, T4	N1	M0
Stage IVB	T1, T2, T3, T4	N0, N1	M1

Currently there are three staging systems for HCC: (1) TNM staging as tumor spreading staging, (2) liver function staging, and (3) systems integrating (1) and (2).

TNM Stage

For TNM staging, UICC or AJCC classification is used internationally, but these are thought inappropriate because the cut-off tumor size is set to 5 cm. Since portal microinvasion and intrahepatic metastasis occurs in 27 and 10% of tumors with a tumor size of ≥2 cm, respectively, a TNM staging classification setting the cut-off size to 2 cm is necessary. TNM stages should be specified by three factors: 2-cm tumor size, solitary or multiple lesions, and the presence or absence of vascular invasion, as used by the Liver Cancer Study Group of Japan (LCSGJ), may be the most appropriate for countries, including Japan, where the early detection of HCC is possible [26] (tables 11, 12).

Liver Damage Stage

There are two liver function staging systems: Child-Pugh staging that is used internationally for a long time, and the liver damage staging established by LCSGJ (table 13). Liver damage staging [26] established by LCSGJ is different from Child-Pugh staging in that the ICG retention rate at 15-min ($ICGR_{15}$) value is incorporated instead of hepatic encephalopathy, and specifications of the prothrombin time and albumin level are more strict. Also, Child-Pugh staging employs scoring in which grades are determined based on the total score, whereas a higher stage with consistency of two items is regarded as

Table 12. T category of TNM stage by the Liver Cancer Study Group of Japan [cited from 26, with permission]

Criteria	T1	T2	T3	T4
(1) Number of tumors: solitary	(2) All three criteria are fulfilled	(3) Two of the three criteria are fulfilled	(4) One of the three criteria is fulfilled	(5) None of the three criteria are fulfilled
(2) Tumor diameter: no more than 2 cm				
(3) No vascular or bile duct invasion: Vp0, Vv0, B0		 	 	

Table 13. Degree of liver damage by the Liver Cancer Study Group of Japan [cited from 26, with permission]

Clinical and laboratory findings	Grade[1]		
	A	B	C
Ascites	none	controllable	uncontrollable
Serum bilirubin, mg/dl	<2.0	2.0–3.0	>3.0
Serum albumin, g/dl	>3.5	3.0–3.5	<3.5
$ICGR_{15}$, %	<15	15–40	>40
Prothrombin activity, %	>80	50–80	<50

[1] Degree of liver damage is designated as class A, B, or C, based on the highest grade containing at least two findings.

Table 14. Definition of the Japan Integrated Staging Score

	Variable			
	0	1	2	3
Child-Pugh stage	A	B	C	
TNM stage[1]	I	II	III	IV

[1] By the Liver Cancer Study Group of Japan.

the grade in LCSGJ liver damage staging. Since LCSGJ liver damage staging was originally designed for cases indicated for hepatectomy, $ICGR_{15}$ is specified as an essential factor. By contrast, Child-Pugh staging was originally widely used for diagnosis of liver functional reserve in cirrhotic patients, including terminal liver cirrhosis cases such as those with hepatic encephalopathy or ascites. However, differential use of the two staging systems is probably what is important. In the surgical field, LCSGJ liver damage staging is used for consideration of hepatectomy, and Child-Pugh staging is widely used for consideration of liver transplantation. The two systems are differentially used corresponding to the clinical objectives.

Integrated Staging System

The third type of staging system actually sees several systems integrating the TNM and liver damage stages. Various staging systems such as: (1) Okuda stage [56], (2) BCLC stage [1], (3) CLIP score, (4) JIS score [58, 59] (table 14), and (5) Tokyo Score [61], have all seen long-time use. The JIS score utilizing both the LCSGJ TNM and Child-Pugh stages is considered the most useful for overall staging of HCC in Japan [59, 60]. The CLIP score has disadvantages: specification of the tumor spreading degree is rough, only AFP is used as a biological malignancy marker, and stratification ability is also poor in advanced cases (many cases cluster to a score of 0–2). By contrast, the JIS score is superior for stratification of scores. The original JIS score employs Child-Pugh staging, but the modified JIS score employing liver damage staging in-

stead of Child-Pugh staging is frequently used in the surgical field [62]. The modified JIS score may be useful for hepatectomy cases because LCSGJ liver damage is more strictly classified. The original and modified JIS scores may be differentially used in accord with the clinical objectives, as with Child-Pugh and liver damage staging. Recently, biomarker combined JIS scores, which better stratify HCC patients than conventional JIS scores [63].

Importance of Integrated Staging Systems

There are several reasons why integrated staging systems are clinically important:

(1) For HCC, TNM staging is insufficient for predicting the prognosis, and a staging system integrating TNM and liver function stages is necessary to accurately predict the prognosis.

(2) Prediction of an accurate prognosis for individual patients.

(3) Establishment of a common scale for selection of the optimum treatment for individual patients.

(4) Identification of the patient population to be treated with the most curative therapy.

(5) Identification of the patient population in which the prognosis is worsened by overtreatment.

(6) Establishment of a fairly common scale for the comparison of outcomes among treatment methods and institutions. Although simple comparisons among treatment methods are difficult, it is useful for comparisons of a modality (resection, local treatment, or TACE) with identical scores among institutions.

(7) Evaluation of therapeutic effects of new treatment methods, for example comparison of therapeutic effects of liver transplantation and a new drug in homogenous populations, i.e. comparison of therapeutic effects between current and novel treatment methods.

(8) A graph contrasting outcomes of transplantation of individual JIS scores to long-term outcomes of preexisting treatment methods of individual JIS scores is useful for deciding indication of liver transplantation and for obtaining informed consent from patients indicated for this treatment.

Current State and Future Perspectives

Globally, CLIP scores or BCLC stage are used in Europe and North America as staging systems. However, these have different bases: the BCLC stage is basically a treatment selection system for deciding on a therapeutic strategy, whereas the CLIP and JIS scores are prognostic prediction stagings. The CLIP score and BCLC stage are useful for use in European and North American systems that tend to detect only large HCCs, but the JIS score is most useful for countries, such as Japan, where many small liver cancers are detected. At present, the JIS score is appropriate for Japan, while CLIP or BCLC score suits Europe and North America.

Attention needs to be paid to the fact that the BCLC stage corresponds to the Japanese treatment algorithm, but is not a prognostic prediction staging system. For countries incapable of the early detection of HCC or developing countries with insufficient screening systems and diagnostic instruments, the CLIP score may provide good stratification as a prognostic prediction system. The JIS score may be used worldwide when surveillance systems for early detection of HCC become more common and early detection of HCC reaches the same level as found in Japan.

Summary

Various liver cancer staging systems have been proposed. However, for practical purposes the following conditions are essential for comprehensive discussions of all liver cancer cases: (1) the system should be simple, (2) no data lacking, (3) can be applied by anyone anywhere, (4) the system is easy to memorize, and (5) the system is superior for stratifying early, advanced, and terminal groups. Considering these conditions, the JIS score may be the most appropriate staging system for the overall distribution of liver cancer cases in Japan.

Consensus Statements

25 The TNM stage proposed by the Liver Cancer Study Group of Japan is ideal for use in countries like Japan, where many small HCCs <2 cm in diameter are found based on an established nationwide surveillance system. Similarly, the JIS score, biomarker combined JIS score or a modified JIS score appears the best prognostic staging system for use in countries where small HCCs can be detected.

26 BCLC staging proposed by AASLD is a treatment selection staging not a prognostic predictive staging. Therefore, comparisons between treatment selection staging (BCLC) and prognostic predictive staging (CLIP or JIS score) are inappropriate. This issue is really important and should be kept in mind.

Treatment Algorithm of Hepatocellular Carcinoma

Evidence-Based Guidelines in Japan

In a 2005 version of the guidelines, a treatment algorithm was prepared by the Makuuchi Group, Ministry of Health, Labour and Welfare. In 2009, a revision was published [5, 64]. Concretely, treatment is recommended in accordance with the severity of liver disease, number of tumors, and tumor diameter. Initially, it is described that resection or local ablation be performed in solitary tumor patients with liver damage grade A/B. However, local ablation should be selected only in liver damage grade B patients with tumors measuring ≤2 cm in tumor diameter. In liver damage grade A/B patients with 2 or 3 tumors measuring ≤3 cm in tumor diameter, resection or ablation should be conducted. Resection or TACE is selected in those with 2–3 tumors measuring >3 cm. TACE or arterial infusion chemotherapy is recommended in those with multiple (four or more) tumors. In liver damage grade C patients with three or less tumors measuring ≤3 cm, or a solitary tumor measuring ≤5 cm, liver transplantation is recommended if a donor is available. In those with four or more tumors, best supportive care should be performed (fig. 8).

When establishing the 2009 revision, only articles based on high-level evidence were selected from the literature published between 2002 and June 2007. Therefore, a high-level evidence-based molecule-targeting agent reported in 2008 [65] and 2009 [66], sorafenib, was not included. This is somewhat controversial. However, in the footnotes, it was stated that 'chemotherapy is selected in some patients with extrahepatic metastasis'. This was noted considering sorafenib. The other revision point is 'liver transplantation should be performed in patients aged 65 years or younger', which is described in the footnotes. This algorithm consists of evidence described in high-level quality articles. Low-level evidence-based articles are omitted; therefore, this algorithm can be objectively understood, but seems to be a bit conservative. In the future, a practical algorithm involving the new evidence created worldwide should be included in this algorithm.

Treatment Algorithm in the West

The treatment algorithms in Europe and North America were published in the *Journal of Hepatology* as the EASL consensus in 2001, and then as the AASLD Clinical Practice Guidelines in Hepatology in 2005 [2] followed by an updated version in 2010 [3]. Both these were prepared based on BCLC staging [1, 57]. The BCLC staging classification consists of stages 0 to D. Only palliative treatment is specified for stage D, while stage 0 is defined as a very early stage, specifying 2-cm or smaller solitary liver cancers with carcinoma in situ and corresponds to early HCC in Japan. These are solitary, and resection is desirable when portal pressure and the bilirubin levels are normal. When portal hypertension is present, other potentially curative treatments, such as liver transplantation and local treatment, are selected. For solitary HCC for three or fewer 3-cm lesions with mild portal hypertension, liver transplantation or local ablation is recommended. These are very strict criteria, and only stages 0 and A are indicated for curative treatments, i.e. resection, local ablation, and liver transplantation. The moderate stage B specifies multinodular lesions, and the advanced stage C specifies cases with portal invasion or extrahepatic spread. For stage B, TACE is selected. For stage C HCCs with vascular invasion and/or extrahepatic spread, sorafenib is a choice of treatment.

These selection criteria do not meet the current conditions performed in Japan. Application of these staging methods is difficult because many parameters and stage classifications (performance status, Child-Pugh, and portal hypertension) are used, and their application in Japan is inappropriate and so unlikely. However, BCLC is basically identical to the simplified evidence-based treatment algorithm established by Makuuchi's group, except for the application of liver transplantation.

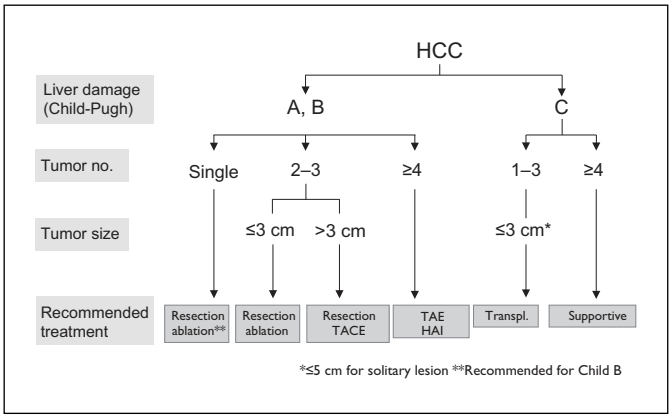

Fig. 8. Evidenced-based algorithm for HCC. Resection or TACE may be selected for liver damage A patients with vascular invasion. Chemotherapy may be selected for extrahepatic HCC. Liver transplantation is only for ≤65-year-olds [cited from 5, with permission].

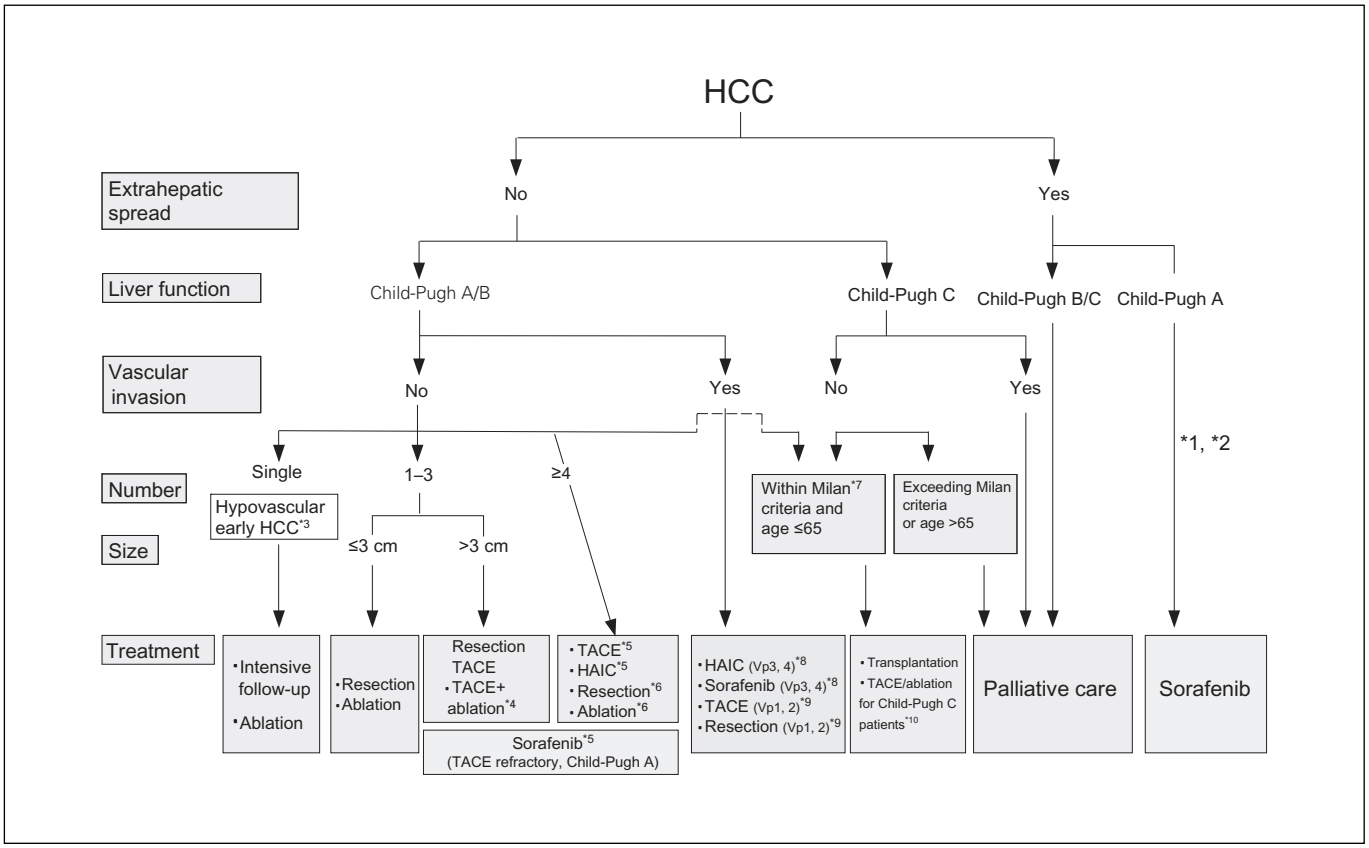

Fig. 9. Consensus-based treatment algorithm for HCC proposed by JSH revised in 2010. Footnotes: *1 = Treatment should be performed as if extrahepatic speed is negative, when extrahepatic spread is not regarded as a prognostic factor. *2 = Sorafenib is the first choice of treatment in this setting as a standard of care. *3 = Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (1) when the nodule is diagnosed pathologically as early HCC, (2) when the nodules show decreased uptake on Gd-EOB-MRI, or (3) when the nodules show decreased portal flow by CTAP, since these nodules are known to frequently progress to the typical advanced HCC. *4 = Even for HCC nodules exceeding 3 cm in diameter, combination therapy of TACE and ablation is frequently performed when resection is not indicated. *5 = TACE is the first choice of treatment in this setting. HAIC (hepatic arterial infusion chemotherapy) using an implanted port is also recommended for TACE refractory patients. The regimen for this treatment is usually low-dose FP (5-FU + CDDP) or intra-arterial 5-FU infusion combined with systemic IFN therapy. Sorafenib is also a treatment of choice for TACE refractory patients with Child-Pugh A liver function. *6 = Resection is sometimes performed even when numbers of nodules are over 4. Furthermore, ablation is sometimes performed in combination with TACE. *7 = Milan criteria: tumor size ≤3 cm and tumor number ≤3; or solitary tumor ≤5 cm. Even when liver function is good (Child-Pugh A/B), transplantation is sometimes considered for frequently recurring HCC patients. *8 = Sorafenib and HAIC are recommended for HCC patients with Vp3 (portal venous invasion at the first portal branch) or Vp4 (portal invasion at the main portal trunk). *9 = Resection and TACE is frequently performed when portal invasion is minimum such as Vp1 (portal invasion at the third or more peripheral portal branch) or Vp2 (portal invasion at the second portal branch). *10 = Local ablation therapy or subsegmental TACE is performed even for Child-Pugh C patients when transplantation is not indicated when there is no hepatic encephalopathy, no uncontrollable ascites, and a low bilirubin level (<3.0 mg/dl). However, it is regarded as an experimental treatment since there is no evidence of its survival benefit in Child-Pugh C patients. A prospective study is necessary to clarify this issue. Even in Child-Pugh A/B patients, transplantation is sometimes performed for relatively younger patients with frequently or early recurring HCC after curative treatments [cited from 10, 49, with permission].

A Consensus-Based Treatment Algorithm for HCC Proposed by the JSH

For HCC treatment, practice patterns markedly differ between Europe/USA and Japan. For this reason, a unique Japanese algorithm (JSH Consensus 2007) was proposed in 2007 [67]. Consequently, a revised draft was presented at the 45th Meeting of the Japanese Liver Society in 2009 (Congress Chair: Masatoshi Kudo), and an article was published in 2010 [10] (fig. 9). The consensus-based treatment algorithm recommended by this society consists of extrahepatic lesions, hepatic functional reserve, vascular invasion, number of tumors, and tumor diameter. Treatment is classified into curative treatment (resection, local ablation), TACE, arterial infusion chemotherapy, liver transplantation, and best supportive care. Basically, the contents are consistent with the evidence-based treatment algorithm established by the Makuuchi group. However, a consensus-based algorithm is not always based on evidence, but involves routinely employed treatment for which a consensus has been reached in Japan. For example, concerning the item of early HCC, local ablation is performed for the lesions in which biopsy diagnosis, CTHA/CTAP, or gadolinium-DTPA ethoxybenzyl (EOB)-MRI suggests malignancy. In evidence-based guidelines, hypovascular tumors are categorized as 'non-typical for HCC', reflecting lesions without an arterial enhancement. Evidence-based guidelines recommend that these lesions should be followed up. However, among hypovascular tumors, 'early liver cancer' definitively diagnosed based on CTAP, EOB-MRI, or biopsy findings, is known to frequently progress to typical HCC. Based on this fact, treatment is performed in many cases in a routine clinical setting; less invasive ablation therapy is performed rather than resection, which is more invasive. With respect to hypovascular lesions without malignant findings, intensive follow-up is recommended. For management, early hypovascular HCC should be separated from other types of hypervascular liver cancer.

Initially, resection or local ablation therapy should be performed to treat three or less tumors measuring $\leq$3 cm in diameter without extrahepatic lesions/vascular invasion in which the liver function is good. In this group, the prognosis of curative treatment may be favorable. In three or less lesions measuring >3 cm in diameter, resection or TACE is recommended. Curability may be improved by adding ablation therapy to previous transarterial treatment (TACE or lipiodol TACE). Secondly, TACE and arterial infusion chemotherapy are recommended to treat four or more lesions. However, arterial infusion chemotherapy is performed based on expert experience, but there is no solid evidence because there is no randomized controlled trial (RCT). The combination of local ablation therapy and TACE/arterial infusion chemotherapy for five to six or less lesions is beneficial in some cases. Furthermore, resection may be considered for such lesions if possible. In young Child-Pugh A/B hepatic functional reserve patients with early recurrence, liver transplantation is sometimes the choice of treatment when they meet the Milan criteria. In the presence of vascular invasion, resection is performed for patients with third or fourth branch of portal venous invasion if possible. In such patients, TACE can be a choice of treatment. In patients with main trunk or first branch of portal vein, arterial infusion chemotherapy, in addition to hepatic arterial infusion chemotherapy with implanted port, is a choice of treatment.

In Child-Pugh C hepatic functional reserve patients aged 65 years or younger, with an unfavorable liver function in the absence of vascular invasion, meeting the Milan criteria, liver transplantation is recommended. Furthermore, as test therapy, local ablation or subsegmental TACE is conducted in Child-Pugh C hepatic functional reserve patients without hepatic encephalopathy or refractory ascites, showing a bilirubin level of $\leq$3 mg.

However, there is no evidence regarding the survival benefits. In the future, a prospective clinical trial should be conducted. In Child-Pugh C hepatic functional reserve patients with vascular invasion or extrahepatic lesions, the best supportive care is basically selected. In this case, palliative radiotherapy to resolve pain is included. However, when extrahepatic lesions are not a prognostic factor, treatment in accordance with the standard treatment algorithm may improve the prognosis.

In Child-Pugh A hepatic functional reserve patients with extrahepatic lesions, sorafenib should be recommended as a first choice of treatment. This agent is recommended for patients with vascular invasion, especially patients with macrovascular invasion, in addition to arterial injection chemotherapy. In non-responders to TACE/arterial injection chemotherapy, sorafenib may become a treatment option when the hepatic functional reserve is preserved as Child-Pugh A.

The consensus-based treatment algorithm is not always based on scientific evidence. However, it is significant because a consensus has been reached among specialists belonging to the JSH, as demonstrated in BCLC, and therefore their own treatment algorithm is introduced. In the future, evidence-lacking parts must be re-

vised through a prospective study. The treatment algorithm for liver cancer reflects a primary concept for treatment strategies. Basically, it is important to perform individualized treatment in individual patients, considering various conditions.

Definition of TACE Failure

In Japan, repeated TACE is commonly performed for multiple nodules without major vascular invasion or extrahepatic spread in Child-Pugh A or B patients. Even though recurrence becomes very rapid, TACE has been repeatedly performed (sometimes over 10 times). The reason why this is that there was no further treatment option after TACE failure/refractory patients before sorafenib was introduced. Since hepatic arterial infusion chemotherapy is not effective for TACE failure patients, sorafenib is regarded as a first choice of treatment for TACE failure patients. Since up to now there was no clear definition of TACE failure JSH expert panel all agrees that the definition of TACE is mandatory to change the treatment strategy to sorafenib if TACE failure is confirmed.

In this regard, the definition of TACE failure has been proposed for the first time in the world as shown in table 15.

Consensus Statements

27 The following situation should be regarded as TACE failure or refractory:
(a) Intrahepatic lesion.
(i) More than two consecutive incomplete necrosis (depositions (<50%) of lipiodol) are seen by response evaluation CT within the treated tumors at the 4 weeks after adequately performed TACE.
(ii) More than two consecutive appearances of a new lesion (recurrence) are seen in the liver by response evaluation CT at the 4 weeks after adequately performed TACE.
(b) Appearance of vascular invasion.
(c) Appearance of extrahepatic spread – continuous elevation of tumor markers even though right after TACE.
(d) Tumor marker – continuous elevation of tumor markers even though right after TACE.
28 Since hepatic arterial infusion chemotherapy (HAIC) is not effective for TACE failure patients, molecular-targeted therapy is the first choice of treatment.

Table 15. Definition of TACE failure [cited and modified from 9, with permission]

Intrahepatic lesion
– More than two consecutive incomplete necrosis (depositions (<50%) of lipiodol) are seen by response evaluation CT within the treated tumors 4 weeks after adequately performed TACE
– More than two consecutive appearances of a new lesion (recurrence) are seen in the liver by response evaluation CT 4 weeks after adequately performed TACE
Appearance of vascular invasion
Appearance of extrahepatic spread
Tumor marker
– Continuous elevation of tumor markers even though right after TACE

Summary

Establishment of an original consensus-based Japanese treatment algorithm was necessary because the situation in Japan, including the availability of transplantation, is different from that found in Western countries. The algorithm established by the JSH is not necessarily based on scientific evidence; indeed consensus-based practices were combined with an evidence-based algorithm. Since it is equally hard to determine if the European or North American algorithm is always based on evidence, the newly established consensus-based treatment algorithm may be a valid guideline. Thus, a treatment algorithm widely agreed on and performed in Japan was presented. However, this algorithm should be revised step by step through prospective investigations of low-evidenced issues. The treatment algorithm for HCC presents the general concept for a therapeutic strategy. It is important to undertake accurate treatments after considering the various conditions found in individual cases.

Consensus Statements

29 The treatment algorithm proposed by the Consensus-Based Clinical Practice Guideline was established based on an evidence-based treatment algorithm and consensus among an expert panel of the JSH. More details are described in the treatment algorithm proposed by the Consensus-Based Clinical Practice Manual.
30 The treatment algorithm proposed by the AASLD is not suitable for application in Japan.
31 Definition of TACE failure is important as described earlier.

Conclusion

Management of HCC in Japan has been described by citing the recently published 'Clinical Practice Manual for HCC' authored by an expert panel of the JSH [9]. This is a consensus-based practice manual, not an evidence-based practice guideline. This manual was established after extensive consideration by combining evidence-based guidelines and the consensus opinions on HCCs of an expert panel. Therefore, no conflict exists between these two documents.

The consensus-based manual presented here includes much detail on the diagnosis and treatment of HCC. However, there are several issues that have no scientific evidence-based support in the diagnostic and treatment algorithm in this manual. In that sense, further extensive efforts involving prospective studies are essential to confirm the validity of this manual and consequently to improve the Evidence-Based Clinical Practice Guidelines for HCC.

Disclosure Statement

The authors have no conflict of interest to declare.

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Effect of Vitamin K2 on the Recurrence of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is characterized by frequent recurrence, even after curative treatment. Vitamin K2, which has been reported to reduce HCC development, may be effective in preventing HCC recurrence. Patients who underwent curative ablation or resection of HCC were randomly assigned to receive placebo, 45 mg/day, or 90 mg/day vitamin K2 in double-blind fashion. HCC recurrence was surveyed every 12 weeks with dynamic computed tomography/magnetic resonance imaging, with HCC-specific tumor markers monitored every 4 weeks. The primary aim was to confirm the superiority of active drug to placebo concerning disease-free survival (DFS), and the secondary aim was to evaluate dose-response relationship. Disease occurrence and death from any cause were treated as events. Hazard ratios (HRs) for disease occurrence and death were calculated using a Cox proportional hazards model. Enrollment was commenced in March 2004. DFS was assessed in 548 patients, including 181 in the placebo group, 182 in the 45-mg/day group, and 185 in the 90-mg/day group. Disease occurrence or death was diagnosed in 58, 52, and 76 patients in the respective groups. The second interim analysis indicated that vitamin K2 did not prevent disease occurrence or death, with an HR of 1.150 (95% confidence interval: 0.843-1.570, one-sided; $P = 0.811$) between the placebo and combined active-drug groups, and the study was discontinued in March 2007. Conclusion: Efficacy of vitamin K2 in suppressing HCC recurrence was not confirmed in this double-blind, randomized, placebo-controlled study. (HEPATOLOGY 2011;54:532-540)

Hepatocellular carcinoma (HCC) is the third-leading cause of cancer death worldwide, claiming 600,000 victims each year. Because of advances in diagnostics and therapeutics, HCC can now be curatively treated, if detected at an early stage. Nevertheless, the long-term prognosis of HCC is not satisfactory, mainly because of its very frequent recurrence, which may occur after a long interval from initial "curative" treatment. Most cases of HCC develop in the liver with cirrhosis or advanced fibrosis.[1-4] Even if HCC nodules have been completely resected or ablated, the remaining liver retains the potential for *de novo* carcinogenesis.[5-7] In addition, precancerous lesions and microscopic metastasis may already exist in the remaining liver.

Adjuvant chemotherapy would be considered for other solid malignancies with high risk of recurrence. However, this is difficult in the case of HCC because few conventional chemotherapeutic agents are effective and hepatotoxicity can be of critical significance, as liver function is often already impaired. A randomized trial was performed with uracil-tegafur as postoperative

Abbreviations: AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein lens culinaris agglutinin fraction-3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer Staging System; CI, confidence interval; CT, computed tomography; DCP, des-gamma-prothrombin; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HRs, hazard ratios; IDMC, independent data monitoring committee; MRI, magnetic resonance imaging; RR, risk ratio.

adjuvant therapy, but did not improve recurrence-free survival, and overall survival appeared to be worsened.[8] Safety is clearly a prerequisite to the use of adjuvant therapy agents for HCC. Recently, a randomized trial with peretinoin, a retinoid, in patients with previously treated HCC was conducted. Although recurrence-free survival was higher with high-dose peretinoin than with placebo, there was no statistically significant difference in the predefined primary analysis.

In 2004, Habu et al.[9] reported that the incidence of development of HCC was reduced among cirrhotic women assigned to receive oral vitamin K2 (45 mg/day), originally for the prevention of osteoporosis, compared to controls (risk ratio [RR]: 0.13; 95% confidence interval [CI]: 0.02-0.99) with a limited number of subjects. Des-gamma-carboxy prothrombin (DCP), an abnormal prothrombin produced in vitamin K deficiency, is not only an HCC-specific tumor marker, but also a predictor of portal venous tumor invasion.[10] A number of findings *in vitro* have indicated that vitamin K may play a role in controlling cell growth, including inhibition of growth of HCC cells.[11-15] Vitamin K2 (menatetrenone) reportedly induced differentiation of human myeloid leukemia cells, as well as apoptosis in immature blast cells.[16-18] Vitamin K2 has been widely used for osteoporosis, and its long-term safety has been confirmed.[19-22] Thus, vitamin K2 would be an ideal adjuvant agent, if it could reduce HCC recurrence by preventing *de novo* carcinogenesis or suppressing tumor growth.

In fact, a few small-sized, controlled trials enrolling 45-61 patients have been performed to assess the effects of vitamin K2 on HCC recurrence. Mizuta et al.[23] reported that vitamin K2 reduced HCC recurrence with a multivariate-adjusted RR of 0.27 (95% CI: 0.12-0.60) and, possibly, improved survival. A preventive effect on HCC recurrence was also suggested by Kakizaki et al.,[24] who found an adjusted RR of 0.45 (95% CI: 0.10-2.05) for recurrence, although they failed to observe survival benefits. Another study failed to detect a reduction of HCC recurrence.[25] Although these previous results were inconsistent, considering the urgent need for prevention of HCC recurrence, we judged that the effect of vitamin K2 on HCC recurrence deserved evaluation in a larger scale, randomized, controlled trial. The present study was, therefore, performed as a multicenter, placebo-controlled, double-blind trial enrolling 548 patients at 31 study sites in Japan.

Patients and Methods

Patients. Candidate participants were those who had received curative treatment, in the form of local ablation or surgery, for primary HCC or first intrahepatic recurrence. Diagnosis of HCC was based on

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histopathologic examination or typical findings on dynamic computed tomography/magnetic resonance imaging (CT/MRI) (i.e., hyperattenuation in the arterial phase with washout in a later phase[26]). Inclusion criteria were the following: 20 years of age or older; performance status (Eastern Cooperative Oncology Group; ECOG) 0-2; and compensated liver function (albumin, $\geq$2.8 g/dL; total bilirubin, $<$2.0 mg/dL; prothrombin time activity, $\geq$40%). Exclusion criteria included the following: previous systemic or hepatic arterial chemotherapy; extrahepatic metastasis; portal vein invasion; interferon treatment within the previous 2 years or a sustained virologic response; uncontrollable encephalopathy, ascites, or plural effusion; a history of gastrectomy or extensive resection of the digestive tract; malabsorption of lipophilic agents, including a history of cholecystectomy; comorbidity with severe cardiovascular, hematological, or renal disease; a history of cancer other than HCC within 5 years; administration of warfarin; administration of vitamin K preparations within the previous 6 months; pregnant or breast-feeding women, or women with childbearing potential or intention; and ongoing participation in other clinical studies.

Assignment. The study was conducted as a multicenter, three-armed, randomized, placebo-controlled, double-blind, comparative, clinical study. Patients who met all criteria were enrolled and randomly assigned in double-blind fashion to receive 45 or 90 mg/day of oral vitamin K2 or a placebo with dynamic allocation, based on the modified minimization method by the registration center, which randomly allocated each patient a randomized study-drug number in the order of registration with a preset computer algorithm, adjusting for balance within each study site and across total registration, considering factors that may affect HCC recurrence (i.e., primary or recurrent HCC, medical ablation or surgical resection, hepatitis C virus (HCV)-related or -unrelated disease, and concomitant administration of glycyrrhizic acid).[27] The investigators, study sponsor, and patients remained blinded to the allocated drug during the study. The protocol was approved by the ethics committee of each participating institution. Patients were well informed of the details of the study and agreed to participate with written informed consent. This trial was conducted in conformity with CONSORT statements and in accord with the Declaration of Helsinki and good clinical practice and is registered as NCT00165633 at Clinicaltrial.gov.

Vitamin K2/Placebo Administration. Each patient took one of the identical capsules (Eisai Co., Ltd., Tokyo, Japan), containing 15 or 30 mg of menatetrenone, vitamin K2 with four isoprenoids, or a placebo, according to group assignment, three times a day after each meal. Medications for chronic hepatitis, such as glycyrrhizic acid and ursodeoxycholic acid, were continued but could not be newly commenced. Antiviral therapies (i.e., interferon, ribavirin, and nucleos(t)ide analogues, such as lamivudine) could not be administered during the study. Vitamin K2/placebo administration was discontinued when recurrent HCC was detected.

Sample Size. The sample size was determined based on previous reports on HCC recurrence among patients who received vitamin K2 and those who did not. Although a previous study reported an adjusted HR of 0.27 (95% CI: 0.12-0.60),[23] the study was conducted in a small number of subjects and the 95% CI ranged widely. We considered 30% risk reduction clinically significant, and the 30% risk reduction was conservatively adopted. Median disease-free survival (DFS) was considered to be 2 years in the placebo group, and the HR in the combined active drug groups was assumed to be 0.67-0.70. Assuming that DFS function followed an exponential distribution, a total of 240-360 events were required to detect the effect of vitamin K2 on DFS, with a one-sided significance level of 2.5%, power of 90%, and an allocation ratio of 1:2 (placebo group:combined active drug groups). To observe the number of events during the follow-up of 3-3.5 years, 180 patients were required in each group (540 in total), assuming loss of information in 5% patients.

DFS. The primary endpoint was DFS, defined as the interval between randomization and either diagnosis of HCC recurrence (i.e., intrahepatic lesions adjacent to or distant from previously treated nodules, and extrahepatic metastasis), cancer other than HCC, or death from any cause. Patients who survived without HCC recurrence or cancer other than HCC at the end of the study were censored on the day of last CT/MRI examination showing no recurrence.

Assessment of Recurrence. HCC recurrence was surveyed every 12 weeks with dynamic CT/MRI, together with ultrasonography. HCC-specific tumor markers, including alpha-fetoprotein (AFP), AFP lens culinaris agglutinin fraction-3 (AFP-L3), and DCP, were monitored every 4 weeks, and dynamic CT/MRI was additionally performed when recurrence was suspected by an increase in tumor marker levels. HCC recurrence was diagnosed by hyperattenuation in the arterial phase and hypoattenuation in the portal venous or equilibrium phase of dynamic CT/MRI. Tumor biopsy was performed when findings on CT/

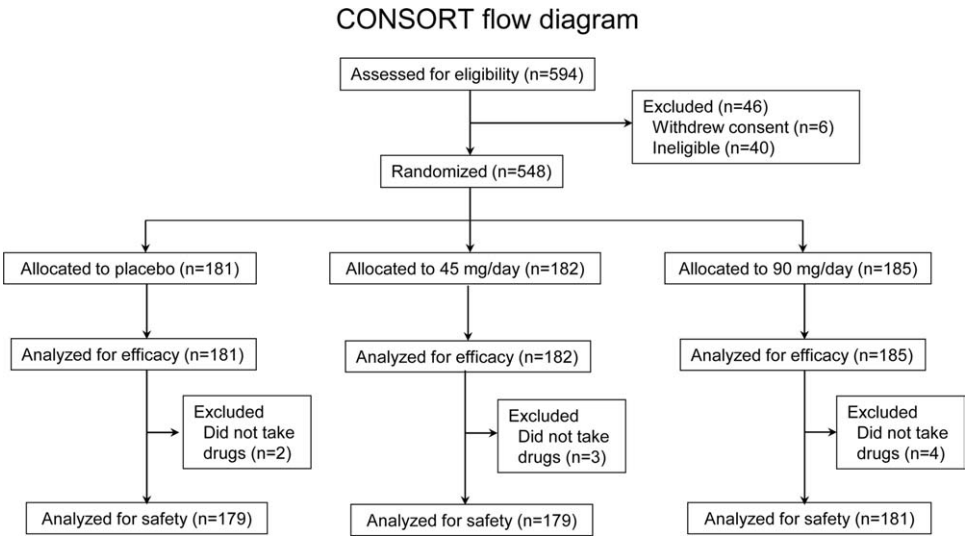


Fig. 1. CONSORT flow diagram.

MRI were equivocal. The presence of recurrence was finally judged by an independent review committee, which thoroughly reviewed the diagnostic imagings in blind fashion. The day of recurrence was defined at the time of first detection of recurrence.

Assessment of Safety. Safety was assessed at 4-week intervals by interview, physical examination, and laboratory tests. Adverse events were defined as any untoward or unintended events that occurred in a subject receiving a study drug. Serious adverse events were defined as those that resulted in death or required hospitalization. Adverse drug reactions were defined as adverse events possibly related to the study drug.

Statistical Analysis. The primary aim of this study was to confirm the superiority of active drug to placebo concerning DFS, and the secondary aim was to evaluate the dose-response relationship between the two active drug groups. DFS rate and median DFS were calculated using the Kaplan-Meier method. Superiority and dose-response relationship were evaluated by the log-rank test, using score statistics with contrast coefficients (−2, 1, and 1) and (0, −1, and 1), respectively, for placebo, 45-mg/day, and 90-mg/day groups. HRs were calculated using Cox's proportional hazards regression model. Adverse events and adverse drug reactions were tabulated based on groups and compared with placebo by Fisher's exact test.

Two interim analyses by the independent data monitoring committee (IDMC) were scheduled. The first was planned 1 year after the commencement of registration to assess safety. The second was planned when 160 events were recorded to assess significance of effect by the finding of $P < 0.005$ (one-sided) or futility. Alpha spending was, for this interim analysis, defined as 0.5% (one-sided), and the overall significance level of statistical tests for the primary aim was maintained at one-sided 2.5%, adjusted for multiplicity associated with interim analyses by the method of Lan and DeMets.[28] The rule for stopping for reasons of futility was defined as follows: The Bayesian predictive probability[29] of detecting a significant effect on observation of 360 events was less than 5%, or the number of events required to assure 50% conditional power exceeded 360. If the IDMC decided to continue the trial, the final required number of events (maximum, 360 events) was to be recalculated to assure 80% conditional power, with the overall significance level maintained for recalculation of the required number of events by Cui's method.[30]

Significance levels for homogeneity among the groups were two-sided 15%, and others were two-sided 5%.

Results

A total of 548 patients were enrolled at 31 study sites in Japan and randomly assigned between March 2004 and September 2005 (Fig. 1). Tumor biopsy was performed in 14 patients, whereas diagnosis was obtained radiologically in remaining patients.[26] Efficacy (i.e., DFS) was assessed among 548 patients (placebo group: 181; 45-mg/day group: 182; 90-mg/day group: 185). Safety was assessed among 539 patients, excluding nine patients who never took drugs. Two patients took drugs at a dose different from that allocated. They were included in the group of allocated dose in the efficacy analysis, but in the group of actually received dose in the safety analysis.

Table 1. Demographic Data

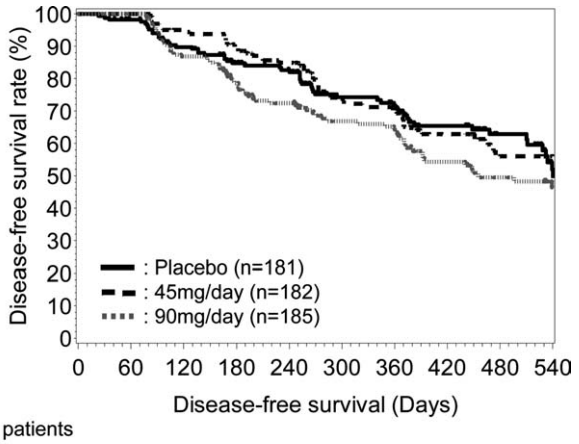
Parameter Category/mean ± SD	Placebo (n = 181)	45 mg/day (n = 182)	90 mg/day (n = 185)	Total (n = 548)	*P* Value
Gender (male/female]	108/73	117/65	117/68	342/206	0.635†
Age (y)	68.9 ± 8.1	68.2 ± 7.8	68.6 ± 7.7	68.6 ± 7.9	0.716‡
Primary or recurrence (primary/first recurrence)	144/37	144/38	144/41	432/116	0.915†
Medications given immediately before registration (local therapy/surgery)	174/7	173/9	180/5	527/21	0.534†
History of drinking (no/yes)	79/102	67/115	73/112	219/329	0.407†
Hepatitis (no/yes)	3/178	1/181	3/182	7/541	0.563†
Etiology§ (HBV/HCV/alcoholic/UK)	20/150/6/5	22/152/10/3	16/153/11/5	58/455/27/13	–
Concomitant administration of glycyrrhizic acid (no/yes)	101/80	99/83	101/84	301/247	0.958†
Liver cirrhosis (no/yes)	32/149	37/143	45/137	114/429	0.253†
Number of tumors	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.7	1.4 ± 0.7	0.953‡
(1/2/3 ≤)	127/39/15	129/40/13	131/37/17	387/116/45	–
Diameter of tumor (mm)	20.3 ± 7.6	20.4 ± 7.9	19.3 ± 7.2	20.0 ± 7.6	0.340‡
Stage¶ (I/II/III)	81/75/25	87/74/21	93/74/18	261/223/64	0.439‖
PS (ECOG) (0/1/2)	165/14/2	171/19/1	176/7/2	512/31/5	0.295‖
Child-Pugh class** (A/B)	154/27	163/19	160/25	477/71	0.430‖
BCLC staging system (0/A/B/C)	53/115/11/2	54/117/10/1	61/109/13/2	168/341/34/5	0.862‖
Albumin (g/dL)	3.81 ± 0.50	3.83 ± 0.40	3.85 ± 0.46	3.83 ± 0.46	0.631‡
Total bilirubin (mg/dL)	0.93 ± 0.36	0.91 ± 0.35	0.86 ± 0.35	0.90 ± 0.35	0.139‡,*
Active prothrombin (%)	79.4 ± 13.9	80.0 ± 13.7	81.1 ± 15.1	80.2 ± 14.3	0.512‡
Platelet count ($\times 10^4/\mu$L)	10.66 ± 4.38	10.72 ± 5.10	11.32 ± 5.69	10.90 ± 5.08	0.389‡
AST (IU/L)	61.7 ± 28.7	71.1 ± 50.0	59.6 ± 29.8	64.1 ± 37.7	0.008‡,*
ALT (IU/L)	55.9 ± 33.4	60.8 ± 46.3	53.6 ± 38.2	56.7 ± 39.7	0.211‡
DCP (mAU/mL)††	33.7 ± 71.5	184.1 ± 1,869.5	27.4 ± 26.0	81.9 ± 1082.7	0.295‡
(<40/40 ≤/UK)	155/25/1	165/17/0	163/19/3	483/61/4	–
AFP (ng/mL)††	38.79 ± 74.42	355.50 ± 4,212.33	30.71 ± 50.25	140.86 ± 2,423.86	0.346‡
(< 100/100 ≤/UK)	164/17/0	166/15/1	178/7/0	508/39/1	–
AFP-L3 (%)††,‡‡	4.09 ± 8.96	3.46 ± 6.99	4.75 ± 10.76	4.10 ± 9.06	0.399‡
(<15.0/15.0 ≤/UK)	174/6/1	173/5/4	171/13/1	518/24/6	–

*$P < 0.15$.
†χ^2 test.
‡One-way analysis of variance.
§Multiple complication.
¶The General Rules for the Clinical and Pathological Study of Primary Liver Cancer, November 2000 (4th ed.).
‖Kruskal-Wallis test.
**Classified in accord with the General Rules for the Clinical and Pathological Study of Primary Liver Cancer.
††Calculated, excluding unknown cases.
‡‡Calculated, assuming that values less than the lower limit of detection were 0.
AFP, alpha-fetoprotein; AFP-L3, alpha-fetoprotein lens culinaris agglutinin fraction-3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer Staging System; DCP, des-gamma-carboxy prothrombin; HBV, hepatitis B virus; HCV, hepatitis C virus; PS, performance status.

The first interim analysis was performed in June 2005, and no problem was found concerning safety. The second interim analysis, performed in November 2006, indicated that vitamin K2 did not prevent recurrence. The IDMC thus recommended discontinuation of the study. Data on efficacy shown in the current report were those presented at the second interim analysis, and data on safety were those obtained at termination of the study (March 2007).

Patients. Baseline characteristics of the 548 patients are summarized in Table 1. The study population was composed of 342 males (62.4%) and 206 females (37.6%), with a mean age of 68.6 years (range, 39-88). The majority (432 patients; 78.8%) were enrolled after treatment of primary HCC. Medical ablation was the dominant therapeutic modality for HCC (527 patients; 96.2%). The tumor nodule was solitary in the majority of patients (387 patients; 70.6%), and median diameter was 19 mm (range, 6-60). HCV infection (455 patients; 83.0%) and the presence of cirrhosis (429 patients; 79.0%) were both common. The majority of patients had liver function reserve in Child-Pugh class A (477 patients; 87.0%) and ECOG performance status of 0 (512 patients; 93.4%). Homogeneity was shown among the three groups for all baseline characteristics, including all stratification parameters, except total bilirubin and aspartate aminotransferase levels.

Events. During the study, HCC recurrence (i.e., intrahepatic lesions adjacent to or distant from previously treated nodules, and extrahepatic metastasis), cancer other than HCC, or death from any cause were detected in 58, 52, and 76 patients in the placebo,



No. of patients

Placebo	181	166	146	125	117	85	79	58	39	23
45mg/day	182	165	150	132	114	76	71	50	30	17
90mg/day	185	168	144	116	103	77	74	50	37	25

Fig. 2. Disease-free survival of placebo, 45-mg/day, and 90-mg/day groups.

45-mg/day, and 90-mg/day groups, respectively. Three patients developed cancer other than HCC. One patient in the placebo group developed malignant lymphoma, one patient in the 90-mg/day group developed colon cancer, and another developed lung cancer. In addition, four patients in the placebo group and one patient each in the 45-mg/day and 90-mg/day groups died without HCC recurrence. Causes of death were liver failure in four patients and acute myocardial infarction and pneumonia in one patient each. Death without HCC recurrence was treated as an event, along with HCC recurrence and development of cancer other than HCC, in DFS analysis.

Local recurrence, as defined by adjacency to a previously treated HCC nodule, is mainly the result of incomplete ablation and may have compromised the efficacy of the active drug. Whether or not recurrence was local was rigorously reviewed by the independent review committee, and HCC recurrence in 8, 6, and 11 patients in the placebo, 45-mg/day, and 90-mg/day groups, respectively, was judged to be local. Incidence of local recurrence did not differ among groups.

Intrahepatic recurrence not adjacent to previously treated nodules may have actually been the result of a small HCC not detected at the time of initial treatment. Although such a residual tumor cannot easily be distinguished from *de novo* carcinogenesis, recurrence resulting from residual tumor is thought to occur early after treatment. Incidences of recurrence within 180 days of HCC treatment were 25, 16, and 34 in the placebo, 45-mg/day, and 90-mg/day groups, respectively ($P = 0.029$ among the groups by log-rank test).

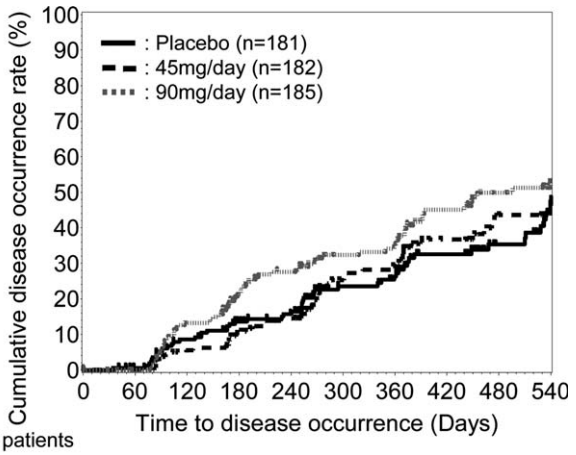
Extrahepatic metastasis also indicates the presence of surviving cancer cells. However, extrahepatic recurrence as the first manifestation of recurrence was rare in the present study and was found in only one patient each in the placebo and 90-mg/day groups.

DFS, Time to Disease Occurrence, and Overall Survival. Median DFS values were 540 and 541 days for the placebo and combined active-drug groups, respectively, as estimated by the Kaplan-Meier method. DFS rates were 69.8% (95% CI: 61.4%-76.7%) and 64.9% (58.8%-70.4%) at 1 year for placebo and combined active-drug groups, respectively. The difference in DFS was not statistically significant (HR: 1.150 [0.843-1.570]; one-sided; $P = 0.811$ by log-rank test).

The dose-response relationship was assessed between the 45-mg/day and 90-mg/day groups. Median DFS values were 560 days in the 45-mg/day group and 455 days in the 90-mg/day group (Fig. 2). DFS rates at 1 year were 68.3% (95% CI: 59.2%-75.8%) in the 45-mg/day group and 61.6% (53.0%-69.1%) in the 90-mg/day group. There was no trend toward dose-dependent increase in DFS (HR: 1.451 [1.018-2.067]; one-sided; $P = 0.982$ by log-rank test).

Analysis of DFS for per protocol population was performed among 510 patients, excluding 38 from 548 randomized patients because of major protocol violations. Similar results were obtained in the per protocol population in DFS analysis.

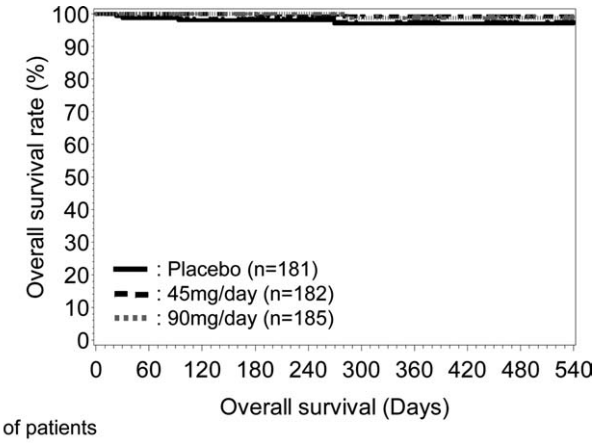
Median time to disease occurrence was 547, 560, and 496 days in the placebo, 45-mg/day, and 90-mg/day groups, respectively (Fig. 3). Cumulative disease occurrence rates at 1 year were 28.2% (95% CI:



No. of patients

Placebo	181	165	146	125	117	85	79	58	39	23
45mg/day	182	165	149	131	114	76	71	50	30	17
90mg/day	185	168	144	116	103	77	74	50	37	25

Fig. 3. Cumulative disease occurrence rate of placebo, 45-mg/day, and 90-mg/day groups.



No. of patients

Placebo	181	166	146	125	117	85	79	58	39	23
45mg/day	182	165	150	132	114	76	71	50	30	17
90mg/day	185	168	144	116	103	77	74	50	37	25

Fig. 4. Overall survival rate of placebo, 45-mg/day, and 90-mg/day groups.

21.4%-36.6%), 31.2% (23.7%-40.4%), and 37.7% (30.2%-46.3%), respectively.

Overall survival rates at 1 year were 97.2% (95% CI: 92.4%-99.0%), 99.2% (94.7%-99.9%), and 98.7% (91.4%-99.8%) in the placebo, 45-mg/day, and 90-mg/day groups, respectively (Fig. 4).

Subgroup Analyses. Enrollment was stratified by whether patients had been treated for primary HCC, medical ablation or surgical resection, HCV-related or -unrelated disease, and concomitant administration of glycyrrhizic acid. There was no significant difference in DFS between the placebo and combined active-drug groups in any stratification parameters (Table 2).

Safety. Safety was assessed among 539 patients. Incidences of adverse events were 88.3%, 88.3%, and 89.0% in the placebo, 45-mg/day, and 90-mg/day groups, respectively, and those of adverse drug reactions were 11.2%, 18.0%, and 15.5%, respectively (Table 3). There was no significant difference in the incidence of any adverse event or adverse drug reaction between the placebo and active-drug groups.

Discussion

In this study, we found no effect of vitamin K2 on the recurrence of HCC. Even the dose of 90 mg/day of vitamin K2, twice the recommended dose for osteoporosis, was not effective. In fact, recurrence was more frequent in the 90-mg/day than in the 45-mg/day group, though not to a statistically significant extent. There was a trend toward high AFP-L3 positivity at entry in the 90-mg/day group, including 13 patients positive for AFP-L3, compared to six and five patients in the placebo and 45-mg/day groups, respectively. AFP-L3 positivity may have indicated residual cancer cells, which may have been related to the increased incidence of recurrence. However, the results of analysis of recurrence remained similar when patients positive for AFP-L3 were excluded.

In this study, status after treatment of recurrent lesions versus naive was associated with an increased risk of recurrence (data not shown). Because this was characteristic of the original neoplasm, this was probably related not with *de novo* or secondary primary

Table 2. Subgroup Analyses of DFS by Stratification Parameter

Parameter Level	Treatment Group	N	HR	(95%CI)
Primary or recurrence HCC				
Primary	Placebo	144	1.000	
	Combined active drug	288	1.061	(0.742-1.519)
Recurrence	Placebo	37	1.000	
	Combined active drug	79	1.414	(0.751-2.664)
Medical ablation or surgical resection				
Medical ablation	Placebo	174	1.000	
	Combined active drug	353	1.152	(0.840-1.579)
Surgical resection	Placebo	7	1.000	
	Combined active drug	14	0.807	(0.113-5.745)
HCV-related disease				
Yes	Placebo	150	1.000	
	Combined active drug	305	1.214	(0.862-1.710)
No	Placebo	31	1.000	
	Combined active drug	62	0.837	(0.397-1.767)
Concomitant administration of glycyrrhizic acid				
Yes	Placebo	80	1.000	
	Combined active drug	167	1.360	(0.869-2.129)
No	Placebo	101	1.000	
	Combined active drug	200	0.958	(0.620-1.479)

DFS, disease-free survival; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio.

Table 3. Summary of Adverse Events (Safety Analysis Set)

			Incidence			
	Treatment Group	N	Case	%	(95% CI)	*P* Value*
Adverse event	Placebo	179	158	88.3	(82.6-92.6)	–
	45 mg/day	179	158	88.3	(82.6-92.6)	1.000
	90 mg/day	181	161	89.0	(83.5-93.1)	0.869
Adverse drug reaction†	Placebo	179	20	11.2	(7.0-16.7)	–
	45 mg/day	179	32	18.0	(12.6-24.3)	0.098
	90 mg/day	181	28	15.5	(10.5-21.6)	0.278
Serious adverse event	Placebo	179	52	29.1	(22.5-36.3)	–
	45 mg/day	179	40	22.4	(16.5-29.2)	0.183
	90 mg/day	181	48	26.5	(20.2-33.6)	0.638
Serious adverse drug reaction†	Placebo	179	1	0.6	(0.0-3.1)	–
	45 mg/day	179	3	1.7	(0.3-4.8)	0.622
	90 mg/day	181	2	1.1	(0.1-3.9)	1.000

*Comparison with placebo group by Fisher's exact test.

†Among adverse events, causal relationship of something other than "not related" to the study drug.

HCC, but with recurrence resulting from microscopic residual cancer or intrahepatic metastasis. On the other hand, other factors, such as alcohol consumption, low albumin concentration, and high total bilirubin concentration, were also associated with risk of recurrence (data not shown). These are also risk factors of primary HCC development among chronic hepatitis patients, and we consider them to indicate the risk of *de novo* carcinogenesis. In other words, we observed two types of HCC "recurrence," intrahepatic metastasis and *de novo* HCC, although it may be difficult to distinguish them in each case. Previous reports suggested the possibility that vitamin K may be effective against both types of HCC recurrence.[23] However, it is also possible that the effect of vitamin K on HCC recurrence is limited to either inhibition of tumor cell growth or reduction of *de novo* carcinogenesis. We performed subgroup analyses by stratifying patients, based on several tumor-related factors, and evaluated the effect of vitamin K on HCC recurrence in each stratum, but recurrence was decreased in none (data not shown).

Prevention of *de novo* hepatocarcinogenesis by vitamin K was first reported by Habu et al.[9] among cirrhotic women who took vitamin K2 to prevent osteoporosis. In the present study, HCC recurrence resulting from metachronous *de novo* carcinogenesis should have been reduced by vitamin K2. However, such an effect may have been obscured in the overall analysis because of the presence of recurrence resulting from intrahepatic metastases. In the subgroup analysis among patients with decreased platelet count, HCC recurrence was marginally reduced in the 45-mg/day group, compared to the placebo group (data not shown). However, no effect was observed with the dose of 90 mg/day. High-dose vitamin K is unlikely to induce hepatocarcinogenesis, because no carcinogenicity has been reported for this vitamin. However, the growth of HCC cells may be dependent on vitamin K. Vitamin K deficiency has been reported in HCC tissues,[31] but it is not known whether replacement of vitamin K facilitates or suppresses tumor growth *in vivo*. Caution is needed in the administration of high-dose vitamin K to HCC patients at high risk of intrahepatic metastasis. The estimated 30% risk reduction of recurrence was not confirmed, and the effect of vitamin K on recurrence, if any, might be observed only in carefully selected patients in a very large-scale trial. If effects of vitamin K2 on HCC prevention are to be further investigated, a preferable endpoint would be the suppression of primary HCC in patients with cirrhosis or advanced fibrosis using the dose of 45 mg/day.

Poon et al.[5] reported that intrahepatic recurrence were classified into early (<1 year) and late (>1 year) recurrences, which seemed to correspond to intrahepatic metastasis and be multicentric in origin, respectively. The present study was terminated approximately 1.5 years after the start of enrollment, according to the recommendation of IDMC. If we are to assume that vitamin K2 at 45 mg/day reduced *de novo* carcinogenesis, it may have been necessary to observe for recurrence for more than 2 years.

Conclusion

In conclusion, the efficacy of vitamin K2 in suppressing HCC recurrence was not confirmed in this double-blind, randomized, controlled study.

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Correlation between hyporesponsiveness to Toll-like receptor ligands and liver dysfunction in patients with chronic hepatitis C virus infection

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SUMMARY. Hepatitis C virus (HCV)-associated antigens, such as the core and nonstructural antigens, activate host innate immune systems via Toll-like receptors (TLRs). We previously showed that chronic exposure to the core antigen induces hyporesponsiveness to TLR ligands in antigen-presenting cells via activation of TLR2 and that stimulation with TLR ligands results in impaired IL-6 production by peripheral blood monocytes from HCV-infected patients. In the present study, peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic HCV or hepatitis B virus (HBV) infection were stimulated with TLR ligands to determine the production of IL-6 and IL-8 and to identify the clinical parameters associated with hyporesponsiveness to TLR ligands in patients with chronic HCV infection. The results showed that pro-inflammatory cytokine responses to TLR ligands were suppressed in PBMCs isolated from HCV-infected, but not HBV-infected, patients. The reduced cytokine responses to TLR ligands seen in HCV-infected patients correlated with platelet counts and serum prothrombin time levels. In contrast, there was no correlation between TLR-induced cytokine responses and serum levels of core antigen. Thus, hyporesponsiveness to TLR ligands in HCV-infected patients is correlated with liver dysfunction. In conclusion, both host factors and viral factors may be involved in the generation of hyporesponsiveness to TLR ligands in patients with chronic HCV infection.

Keywords: HCV core antigen, thrombocytopenia, toll-like receptor.

INTRODUCTION

Approximately 70% of patients with acute hepatitis C virus (HCV) infection do not clear the virus and go on to develop chronic infection [1]. Thus, HCV is a well-adapted human pathogen that causes persistent infection by avoiding the host immune system. HCV expresses several immunoregulatory viral proteins to evade host immune responses [2]. For example, the HCV nonstructural protein (NS) 3/4A protease blunts the innate antiviral type I IFN responses mediated via retinoic acid inducing gene-I and Toll-like receptor 3 (TLR3), both of which sense HCV RNA [3,4]. Thus, HCV NS 3/4A protease inhibits the production of type I IFNs necessary for host antiviral defence. Another HCV-associated protein with immunomodulatory properties is the HCV core antigen. Pattern recognition molecules, particularly TLRs, play a crucial role in host defence against pathogens by producing proinflammatory cytokines and antimicrobial peptides [5]. The HCV core antigen modulates TLR-mediated proinflammatory cytokine responses. Stimulation of antigen-presenting cells (APCs) with the HCV core antigen regulates innate immune responses through the activation of TLR2 [6–8]. We previously reported that chronic exposure to the HCV core antigen results in hyporesponsiveness to TLR ligands by human APCs via activation of TLR2 [8]. In addition, APCs isolated from HCV-infected patients show defective IL-6 production in response to TLR ligands, presumably because of chronic stimulation of TLR2 signalling by circulating HCV core antigen [8]. Although our previous data partially explain the molecular mechanisms by which patients with

Abbreviations: ALT, alanine aminotransferase; APC, antigen-presenting cell; BCAAs, branched-chain amino acids; ELISA, enzyme-linked immunosorbent assay; HBV, hepatitis B virus; HCV, hepatitis C virus; LPS, lipopolysaccharide; TLR, toll-like receptor; NS, nonstructural protein; PGN, peptidoglycan; PAM, Pam_3CSK4; PBMCs, peripheral blood mononuclear cells; PT, prothrombin time.

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chronic HCV infection are susceptible to bacterial infections [9], it remains uncertain whether the induction of hyporesponsiveness to TLR ligands is dependent on viral and/or host factors.

Patients in the advanced stages of chronic liver disease owing to viral infection or alcohol abuse exhibit increased susceptibility to microbial infections and are considered to be immunocompromised hosts [10]. Impaired activation of TLR signalling may also be involved in the susceptibility to infections in patients with liver cirrhosis. Riordan *et al.* [11] showed reduced production of TNF in response to microbial antigens by peripheral blood mononuclear cells (PBMCs) from liver cirrhosis patients. Thus, it is likely that suppression of TLR signalling pathways underlies the susceptibility to bacterial infection in patients with liver cirrhosis. However, it remains unknown whether blunted TLR activation is a common feature of APCs isolated from liver cirrhosis patients with chronic hepatitis B virus (HBV) or HCV infection. More importantly, the clinical parameters associated with suppression of TLR activation have not been identified. In this study, we examined the activation of TLR signalling using PBMCs from patients with chronic HBV or HCV infection. We found that PBMCs from patients with HCV, but not HBV, infection have defective proinflammatory cytokine responses to TLR ligands and that impaired TLR signalling in PBMCs from HCV-infected patients is strongly correlated with the severity of liver dysfunction.

MATERIALS AND METHODS

Patients and cells

Ethical permission for this study was granted by the review board of Kinki University. Healthy controls (n = 17), treatment-naïve patients with chronic HCV infection (n = 17) and treatment-naïve patients with chronic HBV infection (n = 10) were enrolled in the study after informed consent was obtained. Chronic HBV and HCV infection was confirmed by a positive result for serum HBsAg and serum anti-HCV antibodies, respectively. PBMCs (2×10^6/mL) isolated from each patient were stimulated with core antigen (5 μg/mL; Biodesign International, Saco, ME, USA), peptidoglycan (PGN, 10 μg/mL; Sigma-Aldrich, Saint Louis, MO), Pam_3CSK4 (PAM, 10 μg/mL; InvivoGen, San Diego, CA, USA) or lipopolysaccharide (LPS, 1 μg/mL; Sigma-Aldrich) as previously described [12,13]. Culture supernatants were collected 24 h after stimulation.

Virological assays

Serum levels of HCV core antigen were measured using an enzyme immunoassay as previously described [14]. Briefly, 100 μL of serum was mixed with 50 μL of a pretreatment solution containing 0.3% Triton X-100, 1.5% 3-[(3-cholamidopropyl) dimethylammonio]propanesulfonic acid and 15% sodium dodecyl sulphate. After incubation at 56 °C for 30 min, 100 μL of the pretreatment solution was added to wells coated with monoclonal antibodies against HCV core antigen (c11-3 and c11-7) and filled with 100 μL of reaction buffer (1% bovine serum albumin, 5 mM ethylenediaminetetraacetic acid, 0.1 M NaCl, 3% mouse serum, 0.3% Triton X-100, 0.1 M phosphate buffer, pH 7.2). The mixture was incubated for 90 min at room temperature and the wells washed with buffer. Alkaline phosphatase-conjugated monoclonal antibodies against the HCV core antigen (c11-10 and c11-14) were then added to the wells and incubated for 30 min at room temperature. After washing, CDP star (Tropix Inc., Bedford, MA, USA) was added and incubated for 15 min at room temperature. The relative chemiluminescence was measured and the concentration of HCV core antigen was read according to a standard curve generated using recombinant HCV core antigen. The concentration was expressed as femtomol per L (fmol/L), and the cut-off value was set at 20 fmol/L. Serum levels of HBV-DNA were measured using a transcription-mediated amplification assay (Chugai Diagnostics Science Co., Ltd., Tokyo, Japan), which has a quantitative range between 3.7 and 8.7 log genome equivalents/mL.

Measurement of clinical parameters

Platelet counts and haemoglobin levels were determined using an automated hematology analyzer (CELL-DYN Sapphire; Abbott Japan Co, Tokyo, Japan). Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase, total bilirubin and albumin were measured using an automated analyzer (Hitachi 7700; Hitachi Instruments Service Co, Tokyo, Japan). Prothrombin time (PT) was measured using an automated coagulometer (Coagrex 800; Sysmex Co., Kobe, Japan).

Enzyme-linked immunosorbent assays

Measurement of IL-6 and IL-8 was performed using enzyme-linked immunosorbent assay (ELISA) kits (BD Pharmingen, San Diego, CA, USA) as previously described [12,13,15].

Statistical analysis

Student's t-test was used to evaluate the significance of any differences between groups. Statistical analysis was performed using StatView v.4.5 (Abacus Concepts). A P value < 0.05 was regarded as statistically significant. Correlations between cytokine production and clinical parameters were analysed using the Spearmen rank correlation. Platelet counts and serum levels of PT, ALT, and core antigen were also analysed.

RESULTS

Impaired production of IL-6 and IL-8 in response to Toll-like receptor ligands by peripheral blood mononuclear cells isolated from patients with chronic hepatitis C virus infection

In our previous report [8], we showed that peripheral blood monocytes isolated from patients with chronic HCV infection exhibited defective production of IL-6 and IL-8 upon stimulation with TLR ligands, presumably because of chronic exposure to the TLR2 agonist, HCV core antigen. Thus, chronic activation of TLR2 by the HCV core antigen is associated with hyporesponsiveness to TLR ligands during chronic HCV infection. However, it remains unknown whether peripheral blood monocytes from patients with chronic HBV infection also show defective proinflammatory cytokine responses to TLR ligands. In this study, to address this issue, we stimulated PBMCs isolated from patients with HCV or HBV infection with TLR2 and TLR4 ligands. Table 1 lists the characteristics of the patients enrolled in the study. As shown in Fig. 1, PBMCs from patients with HCV infection showed reduced production of both IL-6 and IL-8 upon stimulation with TLR2 (PGN, PAM, core antigen) and TLR4 (LPS) ligands compared with healthy controls. In contrast, stimulation with TLR ligands induced comparable levels of IL-6 and IL-8 production by PBMCs from patients with chronic HBV infection and healthy controls. This suggests that hyporesponsiveness to TLR ligands is a specific feature of PBMCs in patients with chronic HCV infection.

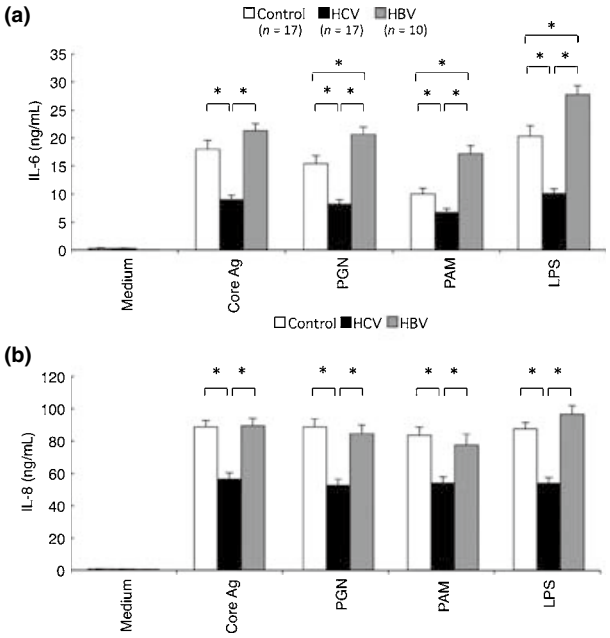

Fig. 1 Production of IL-6 and IL-8 by peripheral blood mononuclear cells isolated from patients with chronic viral hepatitis. PBMCs (2×10^6/mL) isolated from patients with hepatitis B virus or hepatitis C virus infection or healthy controls were stimulated with core antigen (Core-Ag, 5 μg/mL), PGN (10 μg/mL), Pam_3CSK4 (10 μg/mL) or lipopolysaccharide (1 μg/mL). Culture supernatants were collected 24 h after stimulation, and the levels of IL-6 (a) and IL-8 (b) were measured. Results are expressed as the means ± SE. *$P < 0.05$.

Table 1 Clinical characteristics of patients

	HBV (n = 10)	HCV (n = 17)	P value
Age (years)	56 ± 11 (34–65)	64 ± 12 (36–79)	0.021
Gender (male/female)	7/3	11/6	0.887
Child-pugh grade (A/B/C)	8/1/1	13/4/0	0.907
Laboratory data			
AST (IU/L)	60 ± 26 (26–115)	88 ± 40 (26–175)	0.053
ALT (IU/L)	75 ± 78 (18–250)	89 ± 54 (30–216)	0.132
γ-GTP (IU/L)	70 ± 75 (11–252)	111 ± 135 (13–556)	0.295
Total bilirubin (mg/dL)	1.3 ± 0.6 (0.6–2.5)	0.9 ± 0.4 (0.5–1.7)	0.066
Albumin (g/dL)	3.7 ± 0.6 (2.4–4.6)	3.6 ± 0.5 (2.3–4.2)	0.940
Prothrombin time (%)	76 ± 24 (30–108)	77 ± 9.5 (65–98)	0.692
Haemoglobin (g/dL)	13.5 ± 1.7 (10.2–16.6)	13.2 ± 1.7 (9.9–15.5)	0.808
Platelet count ($\times 10^4/\mu$L)	11.8 ± 6.7 (5.1–25)	12.8 ± 7.7 (4.5–29.3)	0.920
HCV Core-Ag (fmol/L)	N.D.	2766 ± 3118 (20–9500)	N.D.
HBV-DNA (LGE/mL)	5.9 ± 1.9 (3.7–8.5)	N.D.	N.D.

Data are expressed as the median ± standard deviation (range) or frequency. ND, not done; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase.

Impaired production of IL-6 in response to Toll-like receptor ligands by peripheral blood mononuclear cells isolated from patients with chronic hepatitis C virus infection does not correlate with serum levels of the core antigen

We next examined whether there was a correlation between the dose of core antigen and the production of proinflammatory cytokines, because stimulation of monocytes with core antigens leads to hyporesponsiveness to TLR ligands via induction of IRAK-M expression (one of the most potent negative regulators of TLR signalling) [8]. As shown in Fig. 2, there was no significant correlation between serum levels of the core antigen and TLR-induced IL-6 or IL-8 production, suggesting that the serum concentration of the core antigen is not primarily responsible for hyporesponsiveness to TLR ligands during chronic HCV infection. In addition, no correlation was found between patient age and the production of proinflammatory cytokines in patients with chronic HBV and HCV infection (data not shown).

Impaired production of IL-6 by peripheral blood mononuclear cells isolated from patients with chronic hepatitis C virus infection in response to Toll-like receptor ligands is associated with liver dysfunction

As TLR-induced proinflammatory cytokine responses are not associated with serum concentrations of the core antigen, we next determined the host factors that showed a strong correlation with hyporesponsiveness to TLR ligands. As shown in Fig. 3, no correlation was found between serum levels of ALT and TLR-induced IL-6 production. In contrast, reduced production of IL-6 by PBMCs from chronic HCV patients was associated with decreased platelet counts and prolonged PT (Figs 4 & 5). Because these parameters are very sensitive markers of liver function [10], the data suggest that liver dysfunction is associated with hyporesponsiveness to TLR ligands in patients with chronic HCV infection. No correlation was seen between platelet counts and TLR-induced IL-6 production in patients with chronic HBV infection (data not shown).

DISCUSSION

In this study, we examined proinflammatory cytokine responses to TLR ligands by PBMCs isolated from patients with chronic HBV or HCV infection. Surprisingly, reduced production of IL-6 and IL-8 in response to TLR ligands was observed in PBMCs from HCV-infected patients, but not those from HBV-infected patients. This finding suggests that HCV-associated immunomodulatory proteins play an important role in the generation of hyporesponsiveness to TLR ligands. In this regard, we previously reported that TLR2 activation of the HCV core antigen results in reduced pro-inflammatory cytokine responses to subsequent stimulation with TLR ligands in human APCs [8]. Given the fact that peripheral blood APCs from patients with chronic HCV infection are always exposed to circulating HCV core

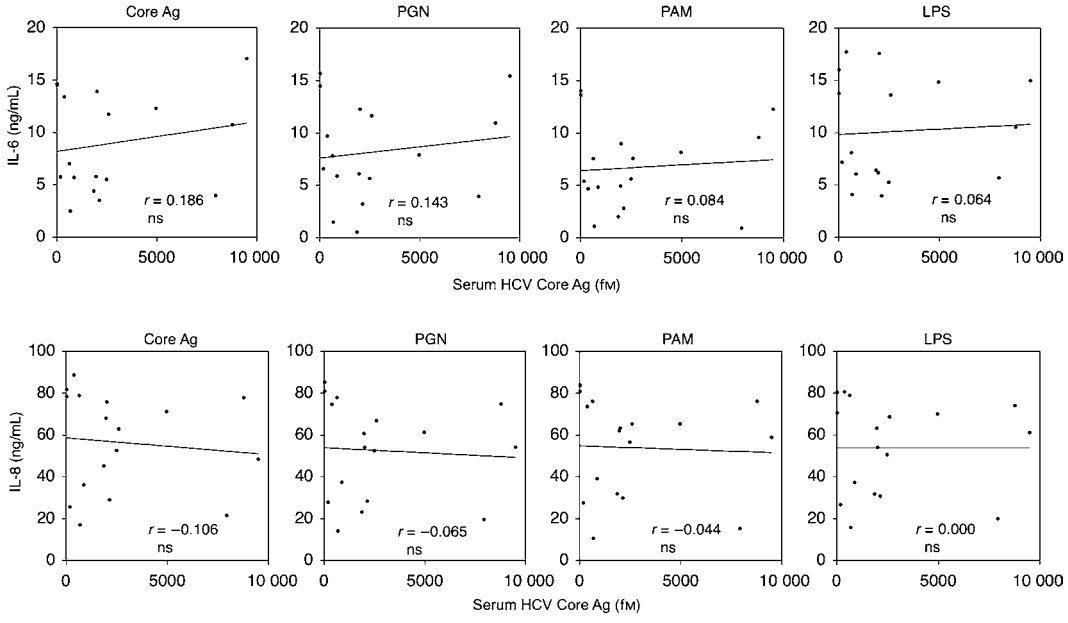

Fig. 2 Production of IL-6 and IL-8 by peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic HCV infection does not correlate with serum concentrations of hepatitis C virus (HCV) core antigen. Production of IL-6 and IL-8 by PBMCs in response to HCV core antigen and Toll-like receptor ligands was compared with the serum levels of HCV core antigen in each patient with chronic HCV infection. No correlation was seen between IL-6 and IL-8 production and serum concentrations of HCV core antigen.

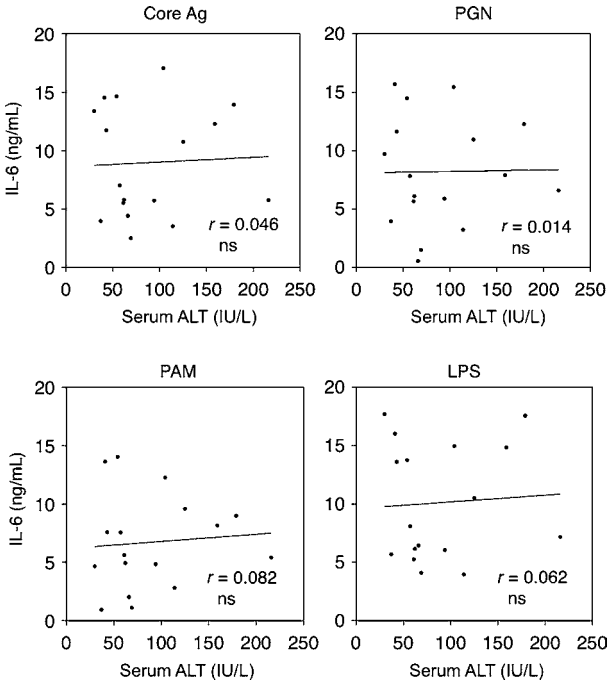

Fig. 3 Production of IL-6 by peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic HCV infection does not correlate with serum levels of ALT. Production of IL-6 by PBMCs in response to HCV core antigen and TLR ligands was compared with serum ALT levels in each patient with chronic HCV. No correlation was seen between IL-6 production and serum ALT levels.

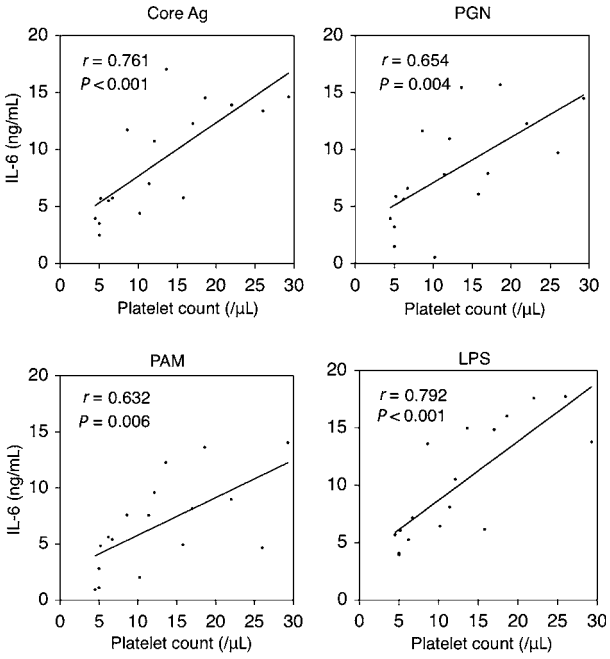

Fig. 4 Production of IL-6 by peripheral blood mononuclear cells isolated from patients with chronic hepatitis C virus (HCV) infection is correlated with thrombocytopenia. Production of IL-6 by PBMCs in response to HCV core antigen and Toll-like receptor ligands was compared with the platelet count in each patient with chronic HCV infection. IL-6 production correlated with platelet counts.

antigen, we speculate that chronic activation of TLR2 by the HCV core antigen induces hyporesponsiveness to TLR ligands. This idea partially explains the mechanisms underlying impaired TLR signalling specific to patients with HCV infection. However, there was no correlation between serum doses of HCV core antigen and TLR-induced cytokine production, suggesting that impaired production of IL-6 induced by TLRs cannot be explained by the dose of circulating HCV core antigen alone and that other viral proteins such as NS3/4A and host factors may be involved in the generation of hyporesponsiveness to TLR ligands. It should be noted that the role played by HCV core antigen-induced TLR2 activation in the generation of hyporesponsiveness to TLR ligands cannot be excluded by the negative correlation between core antigen concentrations and IL-6 production. Given the fact that peripheral blood APCs in patients with chronic HCV infection are constantly exposed to HCV core antigen for many years after the initial acute infection, the duration of the chronic infection may also be an important factor. Thus, both viral factors and host factors are associated with impaired TLR responses in patients with chronic HCV infection.

PBMCs from patients with chronic HBV infection showed comparable levels of IL-6 and IL-8 production with those from healthy controls. Thus, our data suggest that impaired production of TLR-induced cytokines is a specific immunological feature of chronic HCV infection. Although the mechanisms responsible for the different TLR responses observed during HCV and HBV infection are currently unknown, TLR stimulation by viral proteins may be involved. HCV-associated proteins, such as the core and NS3 antigens, are potent activators of TLRs [6–8], whereas HBV-associated proteins, such as the HBc and HBe antigens, fail to activate TLRs [16]. Therefore, it is possible that APCs from patients with chronic HCV infection are tolerant to TLR ligands as a result of constant exposure to the core and NS3 antigens. Taken together, these findings suggest that viral factors appear to play a role in the generation of hyporesponsiveness to TLR ligands in patients with chronic HCV infection.

Several lines of evidence suggest that chronic HCV infection causes impaired innate and adaptive immune responses. For example, adaptive T-helper type 1 and 17 responses are suppressed in patients with chronic HCV infection [17,18]. In addition, APCs isolated from patients with HCV infection exhibit defective proinflammatory cytokine responses upon stimulation with TLR ligands [19,20]. However, these previous studies did not address or determine the clinical parameters that are associated with impaired cytokine responses in

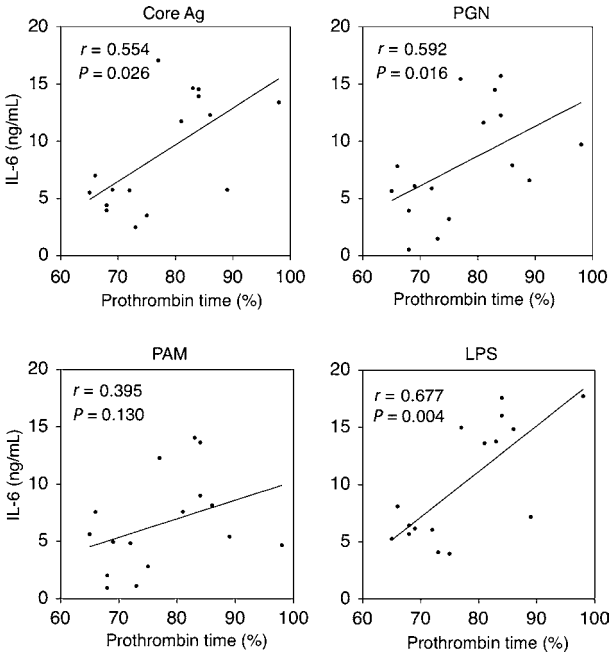

Fig. 5 Production of IL-6 by peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic hepatitis C virus (HCV) infection is correlated with prothrombin time (PT). Production of IL-6 by PBMCs in response to HCV core antigen and Toll-like receptor ligands was compared with the PT in each patient with chronic HCV infection. Production of IL-6 correlated with PT.

HCV infection. In this study, we clearly show that the reduction in TLR-induced IL-6 production correlates with the severity of liver dysfunction as assessed by platelet counts and PT in patients with chronic HCV infection. Because these parameters are sensitive markers for the evaluation of liver function, our data suggest that liver dysfunction may play a role in impaired TLR-induced cytokine responses in patients with chronic HCV infection. The mechanisms by which liver dysfunction predispose the host to defective TLR signalling are currently unknown. Kakazu *et al.* [21] showed that decreased levels of plasma branched-chain amino acids (BCAAs), which are the characteristic feature of severe liver dysfunction, impair APC function in patients with HCV. Furthermore, they also demonstrated that addition of BCAAs to the culture medium normalized IL-12 production by APCs isolated from cirrhotic patients. Therefore, reduced levels of BCAAs because of severe liver dysfunction, together with viral factors, contribute to an abnormal immune environment during chronic HCV infection in which APCs fail to produce TLR-induced host defence factors.

No correlation was found between serum levels of ALT and hyporesponsiveness to TLR ligands in patients with chronic HCV infection. In contrast, reduced production of IL-6 by PBMCs from chronic HCV patients was associated with decreased platelet counts and prolonged PT. As serum ALT is the most useful marker for assessing liver damage [22], our data suggest that liver dysfunction as assessed by PT rather than liver damage as assessed by ALT levels is associated with hyporesponsiveness to TLR ligands in chronic HCV infection.

In contrast to HCV infection, production of IL-6 did not correlate with thrombocytopenia in patients with chronic HBV infection. Thus, liver function does not seem to affect host innate immunity through TLRs in patients with chronic HBV infection. However, we need to be cautious in interpreting the data regarding HBV infection. Tejima *et al.* [23] reported that chronic HCV patients present with more severe levels of thrombocytopenia than chronic HBV patients, even in those with the same grade of splenomegaly and liver stiffness. They also found reduced levels of prothrombin activity in chronic HCV infection patients compared with chronic HBV infection patients [23]. Thus, virological aetiology may contribute to thrombocytopenia and reduced levels of serum PT in a specific manner, and it may not be accurate to directly compare the function of HBV- or HCV-infected livers using the clinical parameters chosen in the present study.

In conclusion, our results clearly show that PBMCs isolated from patients with chronic HCV infection have impaired proinflammatory cytokine responses to TLR ligands. In addition, there is a strong correlation between reduced proinflammatory responses to TLR ligands and the severity of liver dysfunction in these patients. Both viral and host factors contribute to the generation of impaired responses to TLR ligands during chronic HCV infection. Moreover, our results also suggest that platelet counts and PT can be used as markers to determine TLR responses in patients with chronic HCV infection. Future immunological studies using a large number of patients with chronic HBV or HCV infection at the same stage of liver fibrosis are required to identify the host defence responses mediated by TLRs in these patients.

ACKNOWLEDGEMENTS

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Original Paper

Endoscopic Characteristics of Colorectal Serrated Lesions

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ABSTRACT

Background/Aims: With the recent changes of pathological concepts, colorectal serrated lesions can be now divided into traditional serrated adenoma, typical hyperplastic polyp and sessile serrated polyp. The aim of this study is to clarify the endoscopic differences among these three groups.
Methodology: A total number of 362 serrated lesions larger than 5mm were evaluated. These were detected with ordinary view and observed also with magnifying chromoendoscopic view. The final pathologic diagnosis of the resected specimens was made blinded.
Results: There were significant differences between traditional serrated adenoma and sessile serrated polypconcerning location, configuration and color. In chromoendoscopy, most of sessile serrated polyps and typical hyperplastic polyps showed star-like pattern, in contrast with traditional serrated adenomas most of which had fern- or pinecone-like pattern. The differential diagnosis between traditional sessile polyp and the other two was possible with high accuracy. On the other hand, endoscopic distinction between sessile serrated polyp and typical hyperplastic polyp was not easy, except that the location and size were significantly different.
Conclusions: We can endoscopically differentiate between traditional serrated adenoma and sessile serrated polyp or typical hyperplastic polyp, but it is difficult to differentiate between the latter two.

KEY WORDS: Serrated adenoma, Hyperplastic polyp, Sessile serrated polyp, Colorectal serrated lesions, Chromoendoscopy, Pit pattern

ABBREVIATIONS: Traditional Serrated Adenomas (TSA), Typical Hyperplastic Polyp (THP), Sessile Serrated Polyp (SSP), Narrow Band Imaging (NBI)

INTRODUCTION

Now that we have various image enhancement modalities and magnification, endoscopic tissue characterization has become reality. Chromoendoscopy has been used to observe the structural patterns of the colorectal mucosa, or so-called "pit pattern" (1). It has been known to be useful not only for differentiating between neoplasia and non-neoplasia, but also for predicting the depth of cancer (2-5).

On the other hand, distinction between neoplasia and non-neoplasia may sometimes be difficult, as the concept of hyperplastic polyp has been changing recently. Longacre and Fenoglio-Preiser (6) first established the concept of serrated adenoma, which mimics hyperplastic polyp structurally but is still considered neoplastic cytologically, histochemically and molecular biologically. Jass *et al.* (7) and Torlakovic *et al.* (8) advocated that there is a group of lesions which closely resemble hyperplastic polyps but are genetically considered neoplastic and have a potential to evolve into carcinoma.

This group of lesion is now usually called sessile serrated polyp (SSP) (9). Serration is a characteristic common to serrated adenomas, hyperplastic polyps and SSPs; hence the comprehensive term colorectal serrated lesions. Endoscopic characteristics of each group of colorectal serrated lesions and differential diagnosis among them have been scarcely reported. The aim of this study is to clarify the endoscopic differences among these three groups.

METHODOLOGY

A total number of 11,253 colorectal localized lesions were resected in our institute from April 2001 to June 2009. Among them 673 (6.0%) were serrated lesions. In this study, serrated lesions more than 5mm in diameter were evaluated. The scopes used in this study were CF-H260AZI, CF-Q240ZI, and PCF-Q240ZI (Olympus, Tokyo). The insertion and withdrawal of the scope was done with ordinary view and magnifying observation was done only after a lesion was detected. Chromoendoscopy was performed with 0.2% indigocarmine and, in addition, 0.05% crystal violet as indicated (2). The pit patterns of serrated lesions were classified into pinecone-like, fern-like or star-like, according to our previous pilot study (**Figure 1**) (10). The pit pattern diagnosis was made by reviewing the digitally recorded pictures of magnifying chromoendoscopic images of the lesions. The review was done by the

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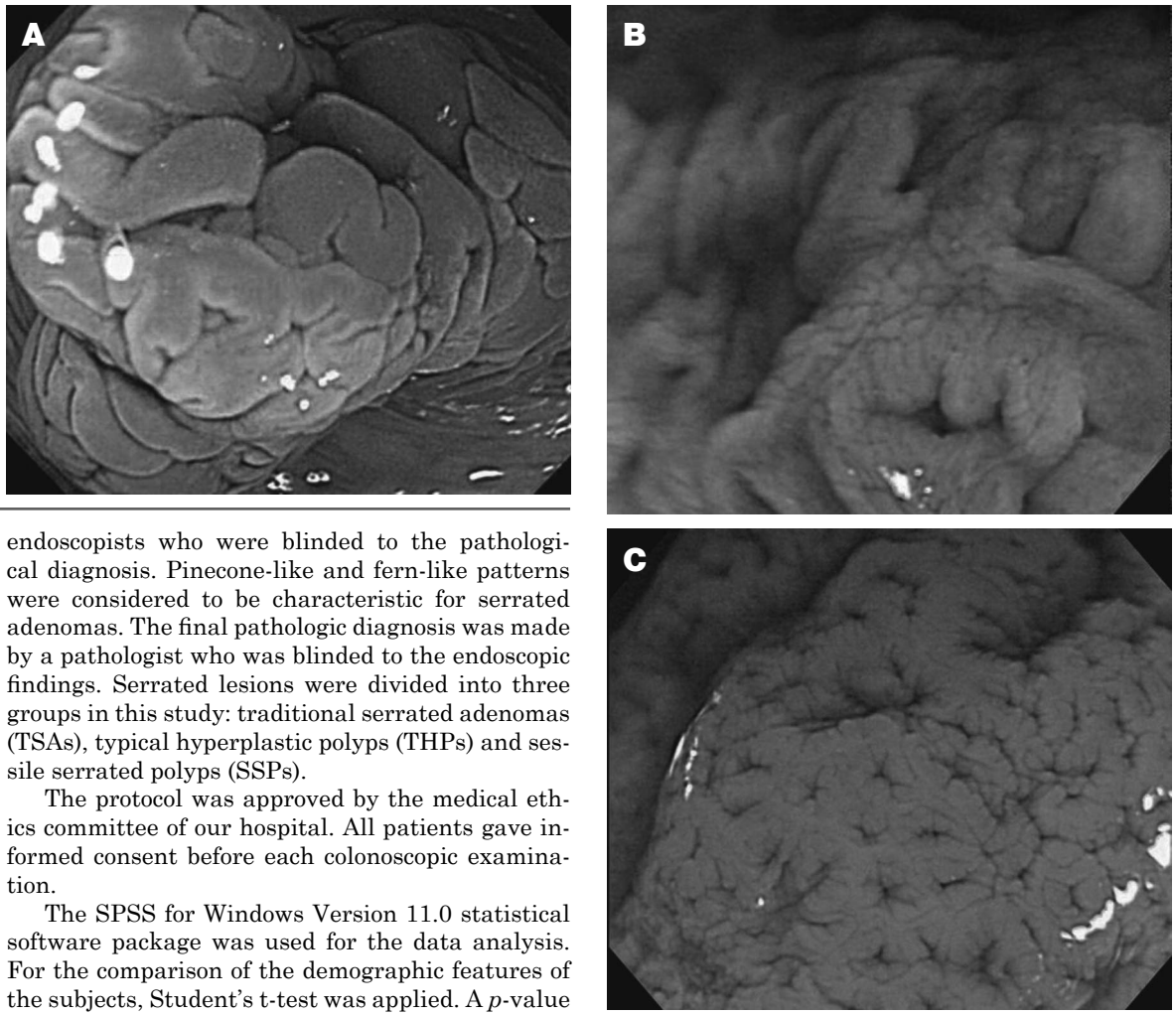

FIGURE 1 Pit patterns of colorectal serrated lesions.
A: Pinecone–like pattern.
B: Fern–like pattern.
C: Star–like pattern.

endoscopists who were blinded to the pathological diagnosis. Pinecone-like and fern-like patterns were considered to be characteristic for serrated adenomas. The final pathologic diagnosis was made by a pathologist who was blinded to the endoscopic findings. Serrated lesions were divided into three groups in this study: traditional serrated adenomas (TSAs), typical hyperplastic polyps (THPs) and sessile serrated polyps (SSPs).

The protocol was approved by the medical ethics committee of our hospital. All patients gave informed consent before each colonoscopic examination.

The SPSS for Windows Version 11.0 statistical software package was used for the data analysis. For the comparison of the demographic features of the subjects, Student's t-test was applied. A p-value of less than 0.05 was considered significant.

RESULTS

The final pathology was TSA in 232 lesions, THP in 95 and SSPs in 35. The average diameter of TSAs, SSPs and THPs was 12.0±9.0mm, 14.0±7.1mm and 7.9±3.6mm, respectively (**Table 1**). The difference of average size was significant between TSAs and THPs (p<0.001) and between SSPs and THPs (p<0.001). Traditional serrated adenomas had a preponderance of occurring in the distal colon (153 out of 232 (65.9%)), whereas SSPs were usually found in the proximal colon (29 out of 35 (82.9%)). There was a significant difference (p<0.0001) of location between TSAs and SSPs. As for the morphology of the lesions, the majority (156 out of 232 (67.2%)) of TSAs presented as protruded lesions, but SSPs were mainly flat (26 out of 35 (74.3%)). The difference of configuration was significant (p=0.0014). Sessile serrated polyps were usually normochromatic or slightly pale in color (28 out of 35 (80.0%)), while TSAs were more often (133 out of 232 (57.3%)) reddish. Again, the difference was significant (p<0.0001) between the two.

TABLE 1 Findings with Ordinary Endoscopic View

		Pathological diagnosis		
		TSA	**SSP**	**THP**
Number		232	35	95
Size (mm)	p<0.001			
			p<0.001	
		12.0±9.0	14.0±7.1	7.9±3.6
Location proximal : distal	p<0.0001	79 : 153	29 : 6	42 : 53
Gross appearance flat : protruded	p=0.0014	76 : 156	26 : 9	51 : 44
Color pale : reddish	p<0.0001	99 : 133	28 : 7	77 : 18

TSA: traditional serrated adenoma; SSP: sessile serrated polyp
THP: typicalhyperplastic polyp

The pit patterns of the serrated lesions seen with magnifying chromoendoscopy are listed in **Table 2**. Eighty-five out of 95 (89.5%) THPs and 26 out of 35 (74.3%) SSPs showed star-like pit pattern (**Figure 2**). The proportion of TSAs that presented with star-like pit pattern was only 11.6% (27 out of 232). The rest of the TSAs showed either fern-like pattern (113 out of 232 (48.7%)) (**Figure 3**) or pinecone-like pattern (92 out of 232 (39.7%)) (**Figure 4**). Conversely, 92 out of 93 lesions (98.9%) with pinecone pit pat-

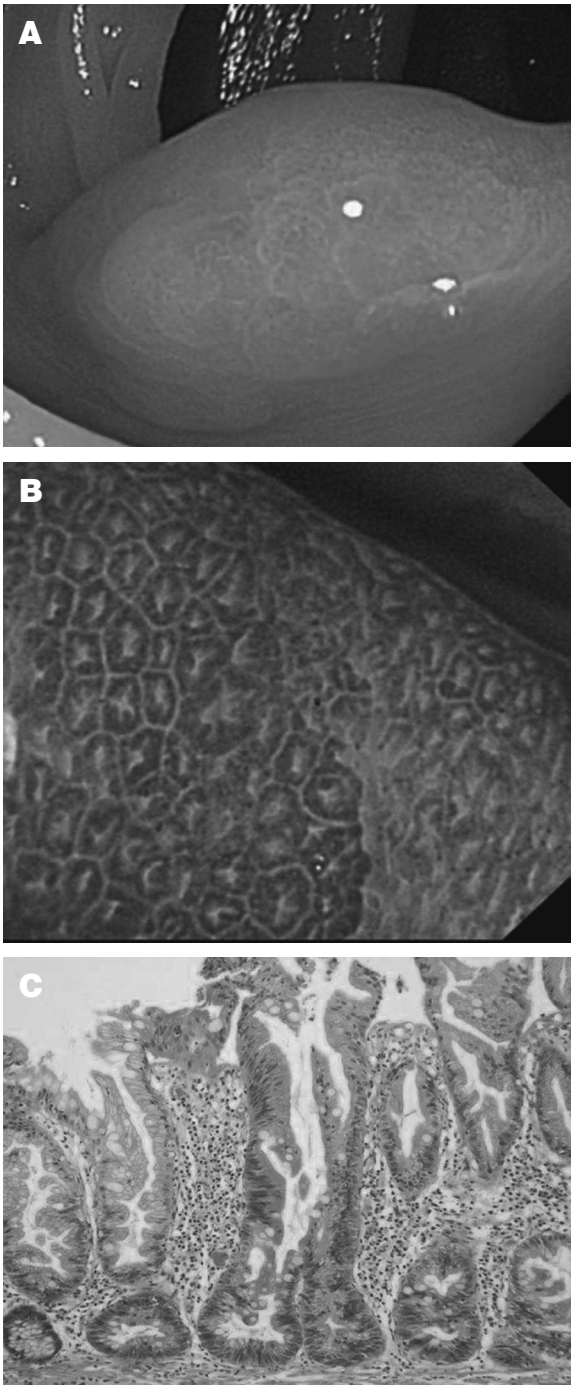

FIGURE 2 Typical case of sessile serrated polyp. **A:** A flat lesion was located over a fold in the ascending colon. **B:** Magnified view after crystal violet staining showed a star–like pit pattern. **C:** Histological view.

tern were TSAs. Most of the lesions with fern-like pit pattern (113 out of 131 (86.3%)) were also TSAs. However, those with star-like pattern encompassed not only THPs and SSPs but also TSAs.

According to the results of our previous pilot study we predicted that lesions with pinecone-like or fern-like pit pattern should be TSAs. The diagnostic properties of the pit pattern diagnosis were as follows: the sensitivity was 88.4%, the specificity was 85.4%, the positive predictive value was 91.5%,

TABLE 2 Pit Pattern with Magnifying Chromoendoscopy

Pit pattern	Pathological diagnosis			Total
	TSA n=232	SSP n=35	THP n=95	
pinecone-like	92	1	0	93
fern-like	113	8	10	131
star-like	27	26	85	138

TSA: traditional serrated adenoma; SSP: sessile serrated polyp
THP: typical hyperplastic polyp

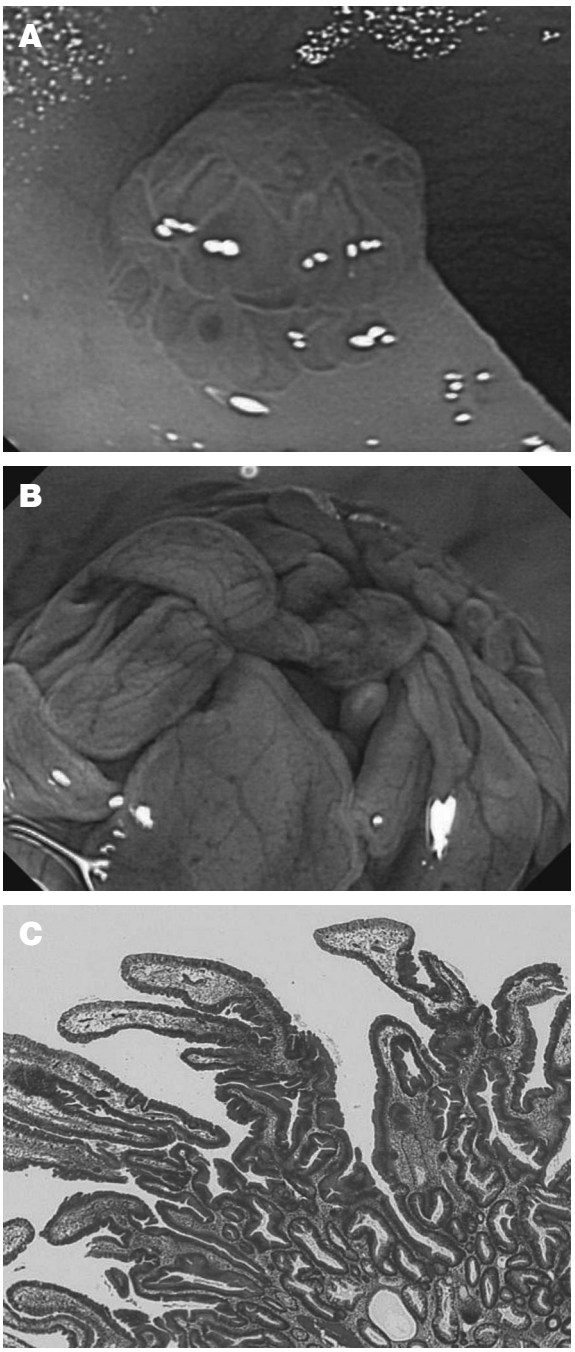

FIGURE 3 Typical case of traditional serrated adenoma (i). **A:** A protruded lesion was detected in the sigmoid colon with an ordinary view. **B:** Indigocarmine spraying and magnification showed a pinecone–like pit pattern. **C:** Histological view.

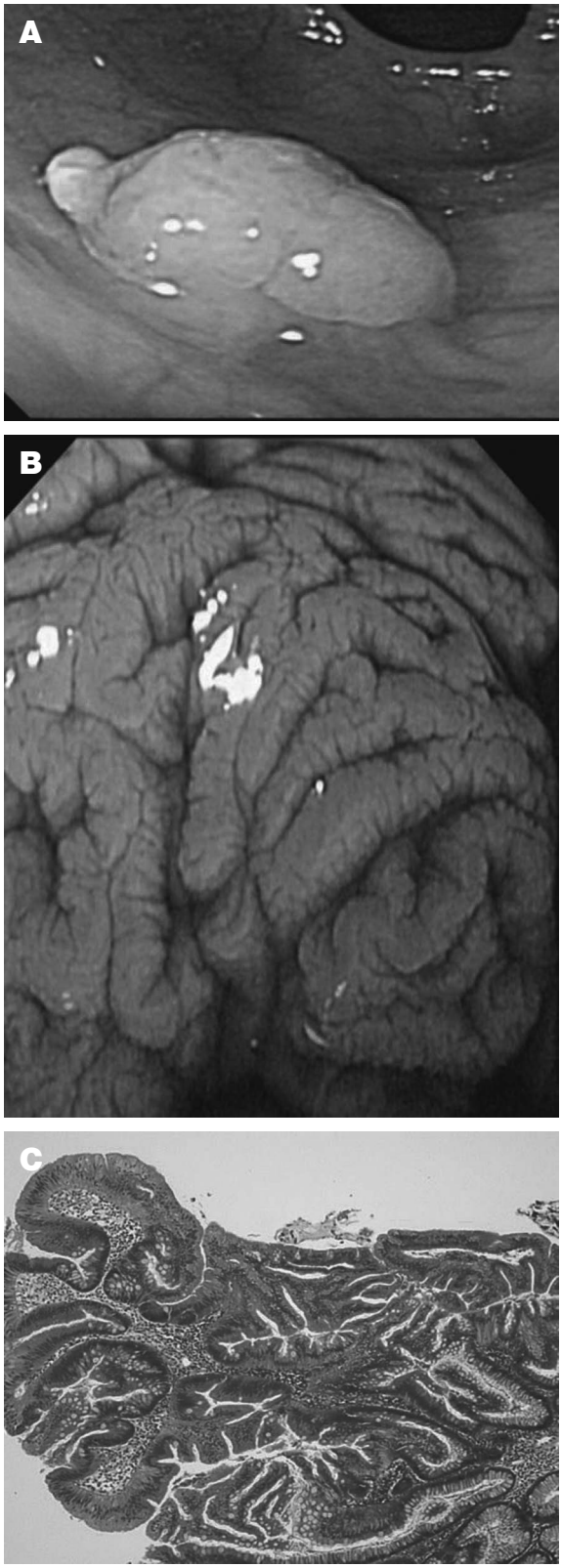

FIGURE 4 Typical case of traditional serrated adenoma (ii).
A: A protruded lesion was detected in the transverse colon with an ordinary view.
B: Indigocarmine spraying and magnification showed a fern–like pit pattern.
C: Histological view.

negative predictive value was 80.4%, and the overall accuracy was 87.3% (**Table 3**). On the other hand, it seemed that pit pattern analysis was not very useful for differentiating between THPs and SSPs.

TABLE 3 Diagnostic Properties of Pit Pattern Analysis in Colorectal Serrated Lesions

Pit pattern	Pathological diagnosis		Total
	TSA	THP or SSP	
pinecone-like or fern-like	205	19	224
star-like	27	111	138
Total	232	130	362

TSA: traditional serrated adenoma;
SSP: sessile serrated polyp
THP: typicalhyperplastic polyp
Sensitivity: 88.4%; Specificity: 85.4%;
Overall accuracy: 87.3%
Positive predictive value: 91.5%;
Negative predictive value: 80.4%

DISCUSSION

The reports by Longacre and Fenoglio-Preiser (6), Jass *et al.* (7) and Torlakovic *et al.* (8) have led to the new concepts of colorectal serrated lesions. A lot of pathologic and genetic studies suggest that serrated adenomas and sessile serrated polyps have potential for evolving into carcinoma, although they may resemble hyperplastic polyps at first sight. Although some of the serrated lesions may be potentially neoplastic, it would not be realistic to remove or biopsy all of them. It should be medically and economically beneficial if we could differentiate between the lesions with neoplastic potential and those without it.

Magnifying chromoendoscopy has been known to be useful for differentiating between tubular or villous adenomas and typical hyperplastic polyps (2-5). However, endoscopic characteristics of serrated adenomas and sessile serrated polyps have not been clarified enough.

Our results show that the average size of lesion was substantially bigger in TSAs and SSPs than in THPs. Traditional serrated adenomas had a preponderance of occurring in the distal colon, in contrast with SSPs (65.9% *vs.* 17.1%). The majority (67.2%) of TSAs presented as protruded lesions, but SSPs were mainly (74.3%) flat. Serrated adenomas were often (57.3%) reddish in color, but SSPs were usually (80.0%) normochromatic or slightly pale. In chromoendoscopy, 89.5% of THPs and 74.3% of SSPs showed star-like pattern, whereas 88.4% of TSAs showed fern- or pinecone-like pattern. In summary, a reddish and protruded lesion with pinecone- or fern-like pit pattern and can be endoscopically diagnosed as TSA. A proximally located, whitish flat lesion with star-like pit pattern is characteristic of SSP.

Thus we can differentiate between TSA and SSP or THP endoscopically, especially with magnifying chromoendoscopy. However, it is difficult to differentiate between SSP and THP except that the former is usually located in the proximal colon and is bigger in size.

Recently, a technique called narrow band imaging (NBI) has been developed (11), which changes

the optical filters of the current sequential lighting videoendoscopes to spectral narrow-band filters. It enables emphasizing the images of vascular and mucosal patterns without the use of dye. We (12), and other researchers (13-15), have reported that an NBI system is a promising tool for distinguishing between neoplastic and non-neoplastic lesions, as well as predicting the depth of cancer especially when combined with magnification. We believe that the combination of pit pattern and vascular pattern should be promising for the differential diagnosis among TSA, THP and SSP, but accumulation of more cases studied with NBI is necessary for the establishment the diagnostic criteria.

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Phase III study of sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma ☆

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ABSTRACT

Background: In Japan and South Korea, transarterial chemoembolisation (TACE) is an important locoregional treatment for patients with unresectable hepatocellular carcinoma (HCC). Sorafenib, a multikinase inhibitor, has been shown effective and safe in patients with advanced HCC. This phase III trial assessed the efficacy and safety of sorafenib in Japanese and Korean patients with unresectable HCC who responded to TACE.

Methods: Patients ($n = 458$) with unresectable HCC, Child-Pugh class A cirrhosis and $\geqslant 25\%$ tumour necrosis/shrinkage 1–3 months after 1 or 2 TACE sessions were randomised 1:1 to

☆ Results from this trial were presented at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium, Orlando, Florida, USA, 22–24 January 2010.

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Randomised
Controlled trial

sorafenib 400 mg bid or placebo and treated until progression/recurrence or unacceptable toxicity. Primary end-point was time to progression/recurrence (TTP). Secondary end-point was overall survival (OS).

Findings: Baseline characteristics in the two groups were similar; >50% of patients started sorafenib >9 weeks after TACE. Median TTP in the sorafenib and placebo groups was 5.4 and 3.7 months, respectively (hazard ratio (HR), 0.87; 95% confidence interval (CI), 0.70–1.09; $P = 0.252$). HR (sorafenib/placebo) for OS was 1.06 (95% CI, 0.69–1.64; $P = 0.790$). Median daily dose of sorafenib was 386 mg, with 73% of patients having dose reductions and 91% having dose interruptions. Median administration of sorafenib and placebo was 17.1 and 20.1 weeks, respectively. No unexpected adverse events were observed.

Interpretation: This trial, conducted prior to the reporting of registrational phase III trials, found that sorafenib did not significantly prolong TTP in patients who responded to TACE. This may have been due to delays in starting sorafenib after TACE and/or low daily sorafenib doses.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, the third most common cause of cancer deaths in men and the sixth most common in women.[1] It has been estimated that 650,000 people per year die from HCC, about three-quarters in East Asian countries.[2,3] Aetiologic factors vary by geographic region; ~70% of HCC patients in the Asia-Pacific (AP) region have chronic hepatitis B virus (HBV) infection, except in Japan, where ~75% of HCC patients have chronic hepatitis C virus (HCV) infection.[2,3]

Many patients with HCC are not diagnosed until the disease is unresectable, such that only non-curative treatment options are available.[4,5] The most frequent locoregional treatment for unresectable HCC is transarterial chemoembolisation (TACE), which concentrates chemotherapeutic agents at the tumour site while blocking the primary artery feeding the tumour.[6,7] Compared with symptomatic treatment alone, TACE has been found to enhance survival in patients with unresectable HCC.[8,9] A meta-analysis of seven randomised trials of arterial embolisation in 545 patients showed that chemoembolisation with cisplatin or doxorubicin showed a significant 2-year survival benefit compared with control, whereas embolisation alone showed no benefit.[10] A subsequent meta-analysis of randomised trials showed that TACE improves patient survival compared with untreated patients, but not when compared with patients treated with arterial embolisation alone.[11] Furthermore, no chemotherapeutic agent was found superior to any other, and there was no evidence that lipiodol had any benefit.[11]

Although TACE effectively delays HCC progression or prevents recurrence within 6 months, it is less effective over longer periods,[12] with 2-year survival rates of 24–63%.[13] Recent trials in Asian patients have found that 2-year overall survival (OS) rates following TACE with a suspension of a fine powder formulation of cisplatin in lipiodol, an emulsion of doxorubicin in lipiodol, and epirubicin-loaded superabsorbent polymer microspheres were 76%, 46% and 59%, respectively.[14,15] Although multiple courses of TACE may improve local tumour control,[11] it may also worsen liver function, both because TACE itself damages the hepatic arterial system[16] and because many patients have poor underlying liver function due to cirrhosis.[17] New and effective treatment strategies for patients with unresectable HCC are therefore needed, including the optimisation of TACE and its combination with other treatment modalities.

The high rate of HCC recurrence after TACE may be due to its enhancement of angiogenesis and upregulation of vascular endothelial growth factor (VEGF) expression, resulting in the formation of rich vascular beds in residual tumours.[18–20] Post-TACE treatment with systemic multikinase inhibitors that are both antiproliferative and antiangiogenic may therefore lengthen time to recurrence, improve survival, and target lesions distal to the TACE site.

Sorafenib is a multikinase inhibitor with antiangiogenic and antiproliferative properties, targeting multiple pathways.[21–23] Two large randomised phase III studies, the Sorafenib Health Assessment Randomised Protocol (SHARP)[24] and Sorafenib Asia-Pacific (AP)[25] trials, demonstrated that sorafenib significantly improves OS in patients with advanced HCC, leading to its approval for the treatment of HCC in more than 90 countries. To date, sorafenib remains the only available systemic therapy proven to extend survival in these patients.

In patients with unresectable HCC, sorafenib after TACE may prolong time to recurrence/progression and/or minimise loss of liver function associated with repeated courses of TACE. This double-blind, placebo-controlled, phase III trial, designed before the results of the SHARP and Sorafenib AP trials were reported, assessed the efficacy and safety of sorafenib in patients in Japan and South Korea with unresectable HCC who responded to TACE.

2. Patients and methods

We screened patients ⩾18 years of age with unresectable HCC and Child-Pugh A cirrhosis who sustained a response 1–3 months after TACE, defined using the then-prevailing criteria in Japan as ⩾25% tumour necrosis and/or shrinkage.[26,27] Additional inclusion criteria were life expectancy ⩾12 weeks; maximum target lesion size of 70 mm; ⩽10 target lesions; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1; and adequate bone marrow (absolute

neutrophil count $\geqslant 1000/mm^3$; platelet count $\geqslant 50 \times 10^9/L$; prothrombin time [PT] – international normalised ratio $\leqslant 2.3$ or PT $\leqslant 6$ s above control), liver (total bilirubin $\leqslant 3$ mg/dL; alanine aminotransferase and aspartate aminotransferase $\leqslant 5 \times$ upper limit of normal [ULN]), and renal (serum creatinine $\leqslant 1.5 \times$ ULN; amylase and lipase $\leqslant 2 \times$ ULN) function.

Patients were excluded if they had macroscopic vascular invasion, renal failure, history of cardiac disease, active clinically serious infection, history of human immunodeficiency virus infection, symptomatic metastatic brain or meningeal tumour, extrahepatic metastasis, seizure disorder requiring medication, prior use of systemic agents for advanced HCC (although prior use of interferon, retinoid and/or vitamin K_2 as adjuvant treatment after curative local treatment was allowed), use of hematopoietic growth factors within 3 weeks before start of study drug, concomitant treatment with cytokines after the last course of TACE, history of organ allograft, documented history of substance abuse, or were pregnant or breast-feeding.

All patients provided written informed consent. The study was approved by the appropriate ethics committees and institutional review boards at each centre, and complied with Good Clinical Practice Guidelines, the Declaration of Helsinki, and local laws and regulations. Ongoing safety and efficacy were assessed independently by the Data Monitoring Committee. This study was registered at Clinicaltrials.gov as trial number NCT00494299.

2.1. Procedures

TACE was performed by injecting gelatin foam plus lipiodol in all cases. The chemotherapeutic agents used concurrently were epirubicin, cisplatin, doxorubicin and mitomycin. Eligible patients were stratified by response to TACE (complete response [CR], defined as 100% tumour necrosis or shrinkage versus non-complete response [non-CR], defined as $\geqslant 25\%$ but <100% tumour necrosis or shrinkage),[26] by ECOG PS (0 versus 1), and by number of courses of TACE (one versus two). Patients were blindly randomised 1:1 to 400 mg (two 200-mg tablets) sorafenib (Bayer Schering Pharma; Leverkusen, Germany) or matching placebo twice daily.

Treatment interruptions and dose reductions (first 400 mg qd, then 400 mg qod) were allowed for drug-related toxicity. Patients were monitored for adverse events (AEs) using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0, except that the hand-foot skin reaction (HFSR) was classified and managed by a protocol-defined scale. Treatment continued until radiologic progression or recurrence of HCC, unacceptable toxicity associated with study drug, or withdrawal of consent.

The trial was divided into 28-day cycles. Patients were evaluated for safety and compliance every 2 weeks during cycles 1–3, and every 4 weeks thereafter. Tumours were evaluated, centrally at an image registration centre, $\leqslant 28$ days before the first dose of study drug and every 8 weeks thereafter, or when evaluating recurrence or progression. Throughout treatment, lesions were evaluated by dynamic computed tomography (CT), preferably by the same investigator or radiologist as at screening.

The primary study end-point was time to progression (TTP) by central review, defined as time to recurrence in patients with CR and TTP in those with non-CR at study entry. Progression was defined as a $\geqslant 25\%$ increase in tumour size or development of a new lesion. The secondary end-point was OS, defined as time from randomisation to death from any cause. Exploratory analyses included TTP by investigator assessment and subgroup analyses of TTP by central review, based on aetiology (HBV versus HCV), response to TACE (CR versus non-CR), number of lesions ($\leqslant 3$ versus >3), number of prior courses of TACE (1 versus 2), age (<65 versus $\geqslant 65$ years), sex, treatment lag ($\leqslant 9$ versus >9 weeks), country of enrolment (Japan versus South Korea), and ECOG PS (0 versus 1).

2.2. Statistical analysis

Patient sample size was estimated based on TTP. If 30% and 70% of patients achieved CR and non-CR, respectively, in response to TACE, the median TTP for the placebo group in the mixed population would be 5.7 months. Clinically meaningful improvement was defined as median TTP 50% higher in the sorafenib than in the placebo group. Assuming one formal interim and one final analysis performed using an O'Brien-Fleming-type alpha spending function with a two-sided alpha of 0.05, 318 events would be required to achieve a statistical power of 95%. Accrual of 372 patients (186 in each group) within 18 months would be expected to result in 318 events after 30 months; if 10% of patients were lost to follow-up, 414 patients would have to be randomised to observe 318 events.

Efficacy was assessed in the intention-to-treat (ITT) population, defined as all randomised patients. The safety population included all patients who received at least one dose of study medication. TTP and OS in the two treatment arms were calculated by the Kaplan–Meier method and compared by the log-rank test, as were subgroups stratified by response to TACE (CR versus non-CR), ECOG PS (0 versus 1) and number of prior courses of TACE (1 versus 2). Hazard ratios (HRs) for sorafenib versus placebo and 95% confidence intervals (CI) were estimated by Cox proportional hazards models.

2.3. Role of the funding source

The study sponsors were involved in the design of the study; the collection, analysis and interpretation of data; the writing of the report; and the decision to submit the paper for publication.

3. Results

3.1. Patients

From 27th April 2006 to 10th July 2009, 552 patients were screened at 69 centres in Japan and seven centres in South Korea. Of these, 458 patients (387 at 67 centres in Japan and 71 at six centres in South Korea) met the eligibility criteria and were randomised, 229 each to the sorafenib and placebo groups. All were included in the ITT analysis (Fig. 1), whereas

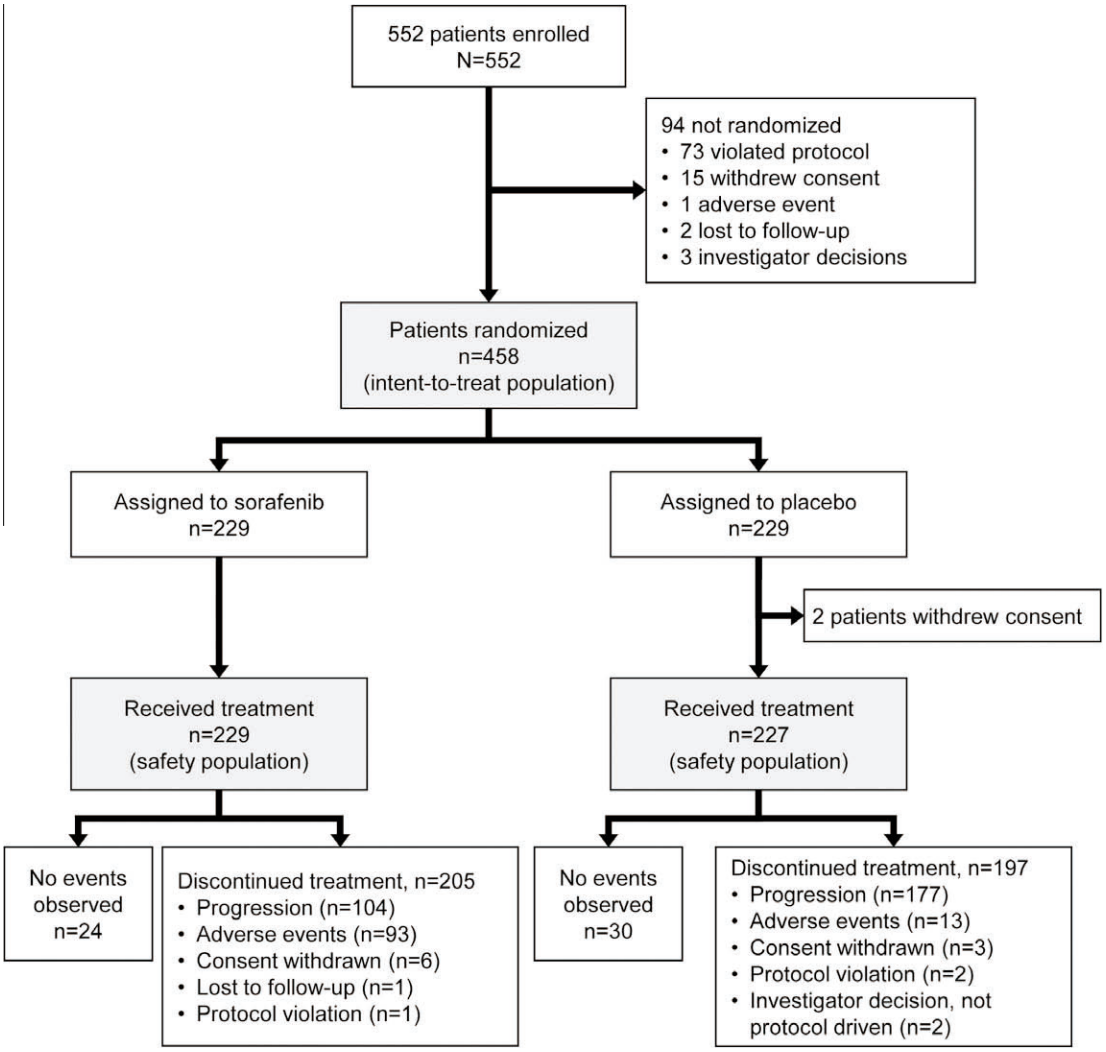

Fig. 1 – Enrolment and outcomes.

the 456 who received at least one dose of study drug were included in the safety analysis.

Demographic and baseline disease characteristics were similar in the sorafenib and placebo groups (Table 1). Of the 458 patients, 342 (74.7%) were male and 306 (66.8%) were ⩾65 years. Median age was 69 years (range, 29–86 years). At baseline, 403 patients (88.0%) had an ECOG PS of 0, 287 (62.7%) had HCV infection, and 336 (73.4%) had ⩽3 tumours. TACE consisted of gelatin foam plus lipiodol in all 458 patients, 60 for palliative intent and 398 for curative intent. Of these 458 patients, 355 received TACE monotherapy, including epirubicin ($n = 219$), cisplatin ($n = 89$), doxorubicin ($n = 49$) and mitomycin ($n = 1$); and 103 received combination treatments, including epirubicin + mitomycin ($n = 57$), cisplatin + epirubicin ($n = 16$), cisplatin + doxorubicin + mitomycin ($n = 13$), mitomycin + mitoxantrone ($n = 8$), doxorubicin + mitomycin ($n = 5$) and doxorubicin + iodixanol ($n = 4$). The median time from last TACE to randomisation was 9.3 weeks (range, 5.6–13.3 weeks), and the median time from initial diagnosis to study entry was 9.8 months (range, 1.6–144.3 months). Ten patients (2.2%) had received prior systemic anticancer therapy, consisting of prior adjuvant treatment with interferon, retinoid and/or vitamin K2 treatment after curative local treatment, and 219 (47.8%) had previously undergone some type of locoregional treatment, including radiofrequency ablation alone (10.7%), surgery alone (9.6%), percutaneous ethanol injection alone (5.9%), microwave coagulation therapy alone (0.2%) and other procedures (0.2%), with 21.2% having undergone multiple procedures (Table 1).

3.2. *Primary efficacy analysis*

By the cutoff date of 10th July 2009, 324 progression events (137 in the sorafenib and 187 in the placebo group) were confirmed by the Response Evaluation Committee. Median TTP by central review was 5.4 months (95% CI, 3.8–7.2 months) in the sorafenib group and 3.7 months (95% CI, 3.5–4.0 months) in the placebo group (HR [sorafenib/placebo], 0.87; 95% CI, 0.70–1.09; $P = 0.252$; Fig. 2). The 3-month progression-free rates in the sorafenib and placebo groups were 65.0% and 58.7%, respectively, and their 6-month progression-free rates were 45.7% and 33.5%, respectively.

Table 1 – Demographic and baseline characteristics of randomised patients (ITT population).

Variable	All patients			Japanese patients			Korean patients		
	Sorafenib + placebo (*n* = 458)	Sorafenib (*n* = 229)	Placebo (*n* = 229)	Sorafenib + placebo (*n* = 387)	Sorafenib (*n* = 196)	Placebo (*n* = 191)	Sorafenib + placebo (*n* = 71)	Sorafenib (*n* = 33)	Placebo (*n* = 38)
Median age (years)	69	69	70	71	70	71	60	61	59
Male (%)	74.7	76.0	73.4	72.9	74.0	71.7	84.5	87.9	81.6
ECOG PS[a] *(%)*									
0	88.0	87.8	88.2	91.5	91.3	91.6	69.0	66.7	71.1
1	12.0	12.2	11.8	8.5	8.7	8.4	31.0	33.3	28.9
Number of lesions (%)									
⩽3	73.4	72.9	73.8	70.8	69.9	71.7	87.3	90.9	84.2
>3	26.6	27.1	26.2	29.2	30.1	28.3	12.7	9.1	15.8
Aetiology (%)									
Alcohol	6.8	8.3	5.2	6.5	7.7	5.2	8.5	12.1	5.3
HBV	21.1	20.5	22.7	12.7	12.2	13.1	70.4	69.7	71.1
HCV	62.7	60.7	64.6	71.3	68.4	74.3	15.5	15.2	15.8
Other	5.9	7.0	4.8	7.0	8.2	5.8	0	0	0
Liver cirrhosis[b] (%)	68.3	69.4	67.2	66.7	67.3	66.0	77.5	81.8	73.7
Number of prior TACE[a] *(%)*									
1	64.4	64.2	64.6	66.7	66.3	67.0	52.1	51.5	52.6
2	35.6	35.8	35.4	33.3	33.7	33.0	47.9	48.5	47.4
Response to prior TACE[a,c] *(%)*									
CR	62.0	62.0	62.0	58.1	58.7	57.6	83.1	81.8	84.2
Non-CR	38.0	38.0	38.0	41.9	41.3	42.4	16.9	18.2	15.8
Prior local therapy (%)									
RFA	10.7	11.8	9.6	10.3	11.7	8.9	12.7	12.1	13.2
Surgery	9.6	7.0	12.2	10.3	8.2	12.6	5.6	0	10.5
PEI	5.9	4.8	7.0	6.5	5.1	7.9	2.8	3.0	2.6
MCT	0.2	0.4	0	0.3	0.5	0	0	0	0
Others	0.2	0.4	0	0	0	0	1.4	3.0	0
Multiple	21.2	20.5	21.8	24.0	23.0	25.1	5.6	6.1	5.3
Prior systemic therapy (%)	2.2	3.1	1.3	2.6	3.6	1.6	0	0	0

ITT = intention-to-treat; ECOG PS = Eastern Cooperative Oncology Group performance status; HBV = hepatitis B virus; HCV = hepatitis C virus; TACE = transarterial chemoembolisation; CR = complete response; non-CR = non-complete response; RFA = radiofrequency ablation; PEI = percutaneous ethanol injection; MCT = microwave coagulation therapy.

[a] Protocol-defined stratification factor.

[b] Clinically and/or histologically confirmed liver cirrhosis.

[c] Complete response was defined in the study protocol as 100% tumour shrinkage or necrosis.

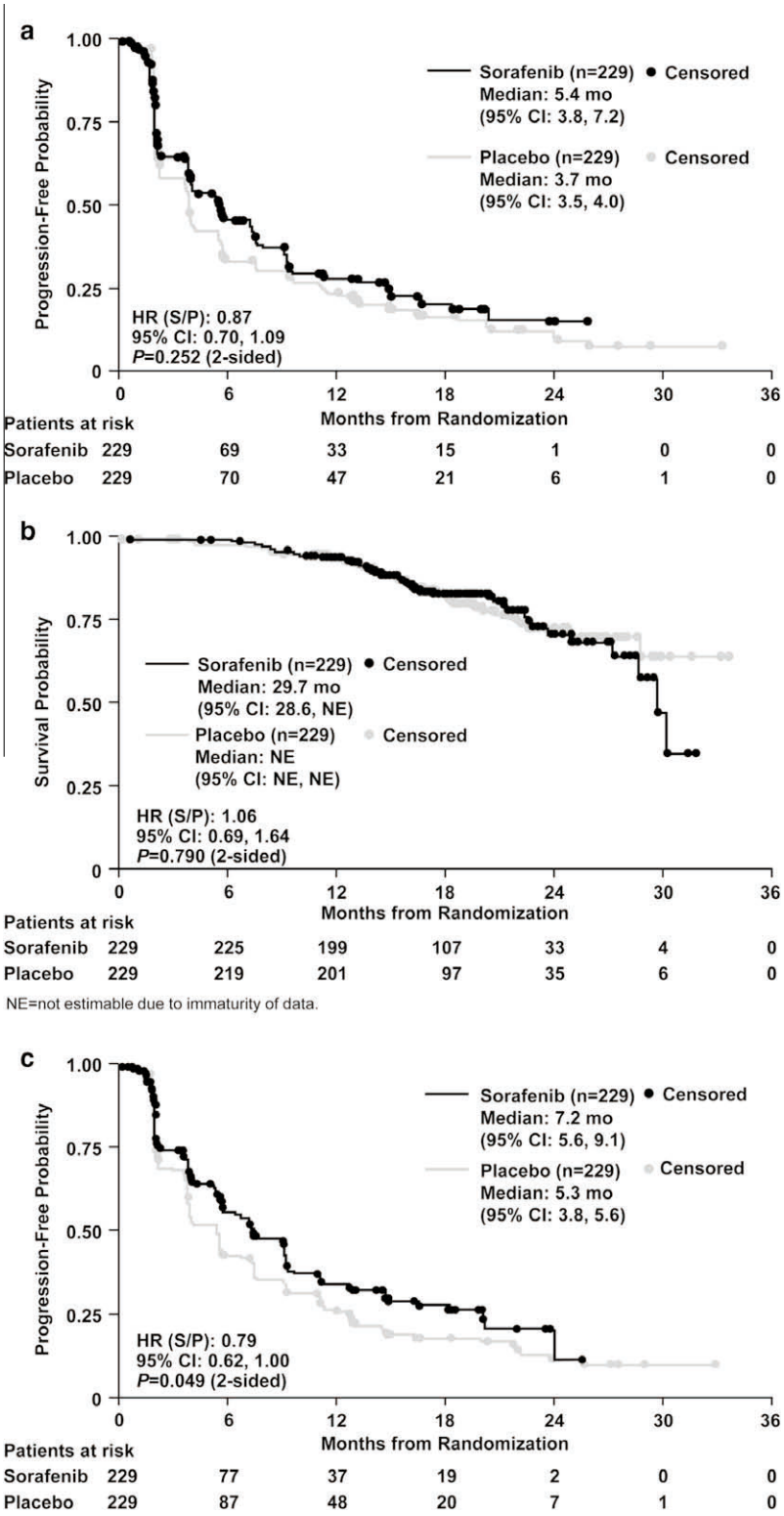

Fig. 2 – Kaplan–Meier analysis of time to progression (TTP) and overall survival (OS). (a) TTP by central review (primary intention-to-treat (ITT) analysis); (b) OS (secondary ITT analysis) and (c) TTP by investigator assessment (exploratory ITT analysis).

3.3. *Secondary efficacy analysis*

At the same cutoff date, there were 84 deaths, 43 in the sorafenib and 41 in the placebo group; the remaining patients were censored on that date. Median OS was 29.7 months in the sorafenib group (95% CI, 28.6 months – not yet reached) but had not yet been reached in the placebo group (HR [sorafenib/placebo], 1.06; 95% CI, 0.69–1.64; $P = 0.790$). The

Table 2 – Exploratory subgroup analyses of TTP by central review based on demographic, baseline and prognostic characteristics (ITT population; subgroups that included at least 10% of patients).

Variable	Subgroup	*n*	Number of events	Number of patients censored	Median TTP (95% confidence interval [CI]) (months)		Hazard ratio [HR] (95% CI) for Sorafenib/placebo
					Sorafenib	Placebo	
Aetiology	HBV	99	56	43	9.1 (5.6–20.3)	5.6 (3.7–10.9)	0.84 (0.49–1.44)
	HCV	287	217	70	5.3 (3.7–7.1)	3.6 (2.0–3.7)	0.81 (0.62–1.07)
Response to TACE	CR	284	179	105	7.4 (5.6–9.2)	5.3 (3.7–7.4)	0.84 (0.63–1.14)
	Non-CR	174	145	29	2.1 (1.8–3.9)	1.9 (1.8–3.6)	0.85 (0.61–1.18)
Number of lesions	⩽3	336	219	117	7.1 (5.3–7.8)	3.8 (3.7–5.5)	0.83 (0.64–1.09)
	>3	122	105	17	3.7 (2.0–5.3)	2.0 (1.9–3.7)	0.87 (0.59–1.29)
Number of prior TACE	1	295	212	83	5.4 (3.8–7.4)	3.7 (3.5–5.5)	0.91 (0.70–1.20)
	2	163	112	51	5.3 (3.7–7.8)	3.7 (2.1–3.8)	0.76 (0.52–1.11)
Age group	<65 years	152	90	62	9.1 (5.6–18.2)	3.7 (3.5–7.2)	0.68 (0.44–1.03)
	⩾65 years	306	234	72	3.8 (3.5–5.4)	3.7 (2.1–3.9)	0.99 (0.76–1.28)
Sex	Male	342	241	101	5.4 (3.8–7.4)	3.7 (3.5–5.3)	0.78 (0.60–1.00)
	Female	116	83	33	5.3 (3.6–7.4)	3.7 (2.1–5.3)	1.16 (0.75–1.79)
Treatment lag[a]	⩽9 weeks	205	150	55	5.5 (3.9–9.1)	3.7 (3.5–5.3)	0.74 (0.53–1.03)
	>9 weeks	253	174	79	5.1 (3.7–7.2)	3.7 (2.0–5.3)	0.95 (0.71–1.29)
Country of enrolment	Japan	387	289	98	3.9 (3.7–5.5)	3.7 (2.1–3.8)	0.94 (0.75–1.19)
	South Korea	71	35	36	NE[b] (9.0–NE)	5.5 (3.7–11.0)	0.38 (0.18–0.81)
ECOG PS	0	403	286	117	5.4 (3.8–7.2)	3.7 (3.6–5.3)	0.88 (0.69–1.11)
	1	55	38	17	5.4 (1.8–16.6)	3.5 (1.8–5.5)	0.78 (0.40–1.51)

[a] Treatment lag was defined as time from the most recent TACE to randomisation.
[b] NE = not estimable due to censored data.

1-year survival rates in the sorafenib and placebo groups were 94.6% and 94.1%, respectively, and their 2-year survival rates were 72.1% and 73.8%, respectively.

3.4. *Exploratory analyses*

At the cutoff date, investigators had reported 304 progression events, 120 in the sorafenib and 184 in the placebo group. Median TTP by investigator assessment in the sorafenib and placebo groups were 7.2 months (95% CI, 5.6–9.1 months) and 5.3 months (95% CI, 3.8–5.6 months), respectively (HR [sorafenib/placebo], 0.79; 95% CI, 0.62–1.00; $P = 0.049$). Their 3-month progression-free rates were 74.1% and 67.9%, respectively, and their 6-month progression-free rates were 54.9% and 41.4%, respectively.

Exploratory analyses of TTP by central review were performed in subgroups containing ⩾10% of patients, including by aetiology (HBV versus HCV), response to TACE (CR versus non-CR), number of lesions (⩽3 versus >3), number of prior courses of TACE (1 versus 2), age (<65 versus ⩾65 years), sex, treatment lag (⩽9 versus >9 weeks), ECOG PS (0 versus 1) and country of enrolment. These analyses were performed to provide descriptive information only; the study was not powered to compare subgroup response to treatment, and no adjustments were made for multiple comparisons. Median TTP and the HR for TTP (sorafenib/placebo) in each subgroup are shown in Table 2, and Forest plots of HRs for TTP are shown in Fig. 3. Most HRs favored sorafenib. Differences were observed, however, between Japanese and Korean patients. The HR for TTP was 0.94 (95% CI, 0.75–1.19) for Japanese patients and 0.38 (95% CI, 0.18–0.81) for Korean patients (Fig. 4). Median TTP in sorafenib-treated patients in the

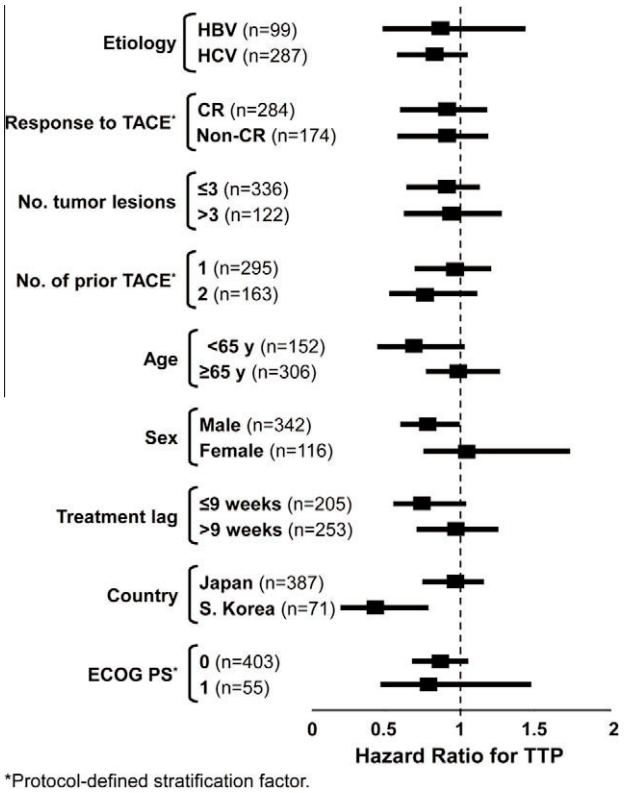

Fig. 3 – Subgroup analyses of TTP by central review (exploratory ITT analyses in subgroups that include at least 10% of patients): forest plot depicting hazard ratio (HR) for TTP (sorafenib over placebo) for each subgroup.

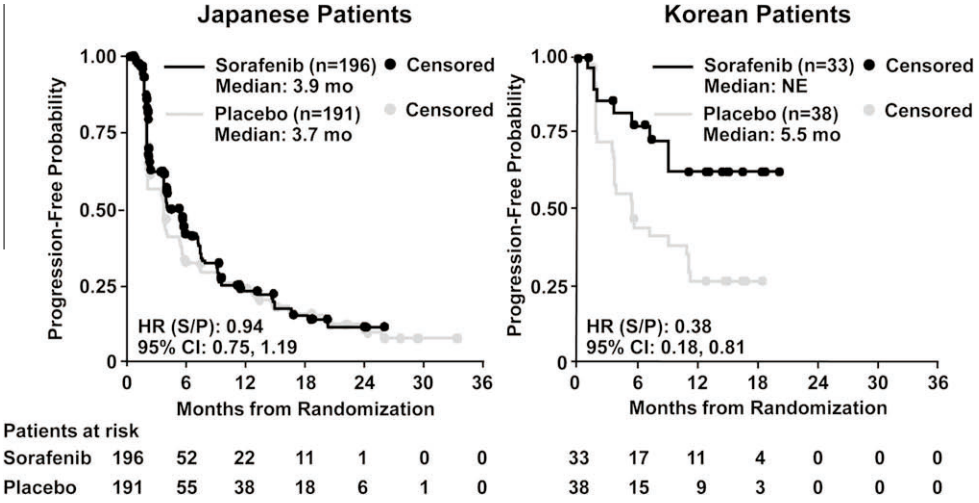

Fig. 4 – Kaplan–Meier analysis of TTP by central review, by country of enrolment (exploratory ITT analysis).

Korean subgroup could not be estimated since it was not attained by the study cutoff date.

3.5. *Safety*

The safety analysis included 229 sorafenib-treated and 227 placebo-treated patients; their incidence of drug-related AEs (DRAEs) were 100% and 61%, respectively. Most DRAEs were mild to moderate (Table 3), with the most frequent in the sorafenib and placebo groups being HFSR (82% versus 7%), elevated lipase (44% versus 8%), alopecia (41% versus 3%) and rash/desquamation (40% versus 11%). In the sorafenib group, 24% and 4% of patients experienced grades 3 and 4 elevated lipase, respectively, compared with 3% and <1%, respectively, in the placebo group. There was no radiographic or clinical evidence of pancreatitis in either group. The overall incidences of grade 3 HFSR (protocol-defined scale) in the sorafenib and placebo groups were 35% and 0%, respectively, and the overall incidence of serious DRAEs was 18% and 9%, respectively. There were no drug-related deaths.

The median durations of treatment in the sorafenib and placebo groups were 17.1 weeks (range, 1.0–112.1 weeks) and 20.1 weeks (range, 2.1–144.1 weeks), respectively (Table 4), and the median daily doses of sorafenib and placebo were 386.0 mg (range, 112.0–794.5 mg) and 785.8 mg (range, 276.1–810.3 mg), respectively. In the sorafenib group, 40 patients (17.5%) received >80% of the planned dose, compared with 206 (90.7%) in the placebo group. The most common reasons for discontinuing treatment in the sorafenib and placebo groups were disease progression (104/229 [45%] versus 177/229 [77%]) and adverse events (93/229 [41%] versus13/229 [6%]).

Doses were reduced in 166 of the 229 sorafenib-treated (72.5%) and in 33 of the 227 placebo-treated (14.5%) patients,

Table 3 – Treatment-emergent, drug-related adverse events occurring in ⩾20% of patients in either group.[a]

Adverse event	Sorafenib (n = 229) Grade (%)			Placebo (n = 227) Grade (%)		
	Any	3	4	Any	3	4
HFSR	82	35	–	7	0	–
Elevated lipase[b]	44	24	4	8	3	<1
Alopecia	41	–	–	3	–	–
Rash/desquamation	40	4	0	11	0	0
Other metabolic abnormality	32	8	1	4	2	<1
Diarrhoea	31	6	0	5	1	0
Hypertension	31	15	0	7	1	0
Hypophosphatemia	28	16	0	6	3	0
Thrombocytopenia	25	11	1	2	<1	0
Elevated AST	25	12	<1	5	3	0
Elevated ALT	21	8	<1	5	2	0
Elevated amylase	21	6	1	8	2	<1

HFSR = hand–foot skin reaction; AST = aspartate aminotransferase; ALT = alanine aminotransferase; NCI-CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

[a] Patients were monitored for adverse events using NCI-CTCAE v3.0, except for HFSR, which was classified according to a 3-grade, protocol-defined scale (grade 1, HFSR does not disrupt normal activities; grade 2, HFSR affects the activities of the patient; and grade 3, patient is unable to work or perform activities of daily living because of HFSR).

[b] There was no radiographic or clinical evidence of pancreatitis in either arm.

Table 4 – Summary of study drug administration.

Assessment	All patients		Japan		South Korea	
	Sorafenib (*n* = 229)	Placebo (*n* = 227)	Sorafenib (*n* = 196)	Placebo (*n* = 190)	Sorafenib (*n* = 33)	Placebo (*n* = 37)
Median duration of treatment (weeks)	17	20	16	20	31	33
Median daily dose (mg)	386	786	382	786	403	766
Patients with dose reduction (%)	73	14	71	11	82	32
Patients with dose interruption (%)	91	18	92	17	85	24
Patients with discontinuation (%)	90	87	93	88	70	78
Due to progression (%)	51	90	52	90	39	90
Due to adverse events (%)	45	7	44	7	57	3
HFSR	11	0	10	0	18	0
Thrombocytopenia	4	0	5	0	3	0
Hypophosphatemia	4	<1	4	1	3	0
Hypertension	4	0	5	0	0	0
Neutropenia	4	<1	4	1	0	0
Elevated AST	2	<1	2	1	3	0
Rash/desquamation	2	0	2	0	3	0
Elevated ALT	2	1	1	1	6	0
Diarrhoea	1	0	1	0	3	0
Other	11	4	19	3	18	3

HFSR = hand–foot skin reaction; AST = aspartate aminotransferase; and ALT = alanine aminotransferase.

due primarily to AEs (163 versus 27). Forest doses were interrupted temporarily in 208 of the 229 sorafenib-treated (90.8%) and 41 of the 227 placebo-treated (18.1%) patients, again due primarily to AEs (206 versus 38).

A total of 107 patients – 94 of the 229 (41.0%) in the sorafenib group and 13 of the 227 (5.7%) in the placebo group – permanently discontinued study drug due to AEs. The most common AEs leading to discontinuation of sorafenib were HFSR (11.4%), thrombocytopenia (4.4%), hypertension (3.9%), hypophosphatemia (3.9%) and neutropenia (3.5%); the most common AE leading to discontinuation of placebo was increased ALT (0.9%).

Death within 30 days of receiving study drug occurred in one patient (0.4%) in each group; neither was deemed drug-related.

4. Discussion

This phase III randomised, controlled trial, assessing the efficacy and safety of sorafenib after response to TACE in Japanese and Korean patients with unresectable HCC, employed a protocol consistent with the practice of TACE in these countries at that time.[28,29] Moreover, the protocol was designed before the combination or sequential use of TACE and sorafenib or their optimal timing had been adequately studied, and before the effect of TACE on susceptibility to sorafenib had been characterised. In this setting, sorafenib did not significantly prolong TTP or OS by central review in patients with unresectable HCC who responded to TACE. Exploratory secondary and subgroup analyses suggested, however, that post-TACE sorafenib had a positive impact on these patients. Median TTP by investigator review was approximately 2 months longer in the sorafenib than in the placebo group, and exploratory subgroup analyses suggested that TTP may have been affected by several factors, including age, number of prior TACE courses, treatment lag, treatment duration, total exposed dose and nationality.

Several factors may have contributed to these results. For example, unusually high percentages of sorafenib-treated patients required dose reductions (73%) and/or interruptions (91%), resulting in a much lower than planned median daily dose of sorafenib (386 mg). In comparison, 26% and 44% of sorafenib-treated patients in the SHARP trial, and 31% and 43% of those in the Sorafenib AP trial, required dose reductions and interruptions, respectively, due to AEs,[24,25] and median daily doses of sorafenib were higher in the SHARP (797 mg) and Sorafenib AP (795 mg) trials.

The better outcomes observed in Korean patients may have been due to their substantially longer median treatment duration (31 versus 16 weeks), resulting in a favourable HR in Koreans (0.38; 95% CI, 0.18–0.81). Moreover, the Korean and Japanese subgroups differed in baseline characteristics. Japanese patients were older and a higher percentage had $\geqslant 3$ lesions on enrolment. Moreover, Japanese patients were less likely to have received >1 TACE to achieve CR prior to sorafenib. Finally, these subgroups differed in principal aetiology of HCC, in that ~70% of Japanese patients had HCV and ~70% of Korean patients had HBV.

We found that the incidence of treatment-emergent adverse events in the sorafenib-treated patients in this trial was generally higher than that observed in previous trials of sorafenib in patients with HCC. We found that the rates of all grade HFSR, Grade 3 HFSR and discontinuation due to HFSR were higher in this trial than in the SHARP[24] and Sorafenib AP[25] trials. We also found that the rates of all grade alopecia; rash/desquamation; hypertension, including grade 3 hypertension; thrombocytopenia and elevated liver function enzymes were higher in this trial than in the two previous phase III trials of sorafenib in patients with HCC. These results were unexpected and may have been due to the combination of TACE with sorafenib treatment in this trial. These findings suggest that adjustments in sorafenib dose (e.g. starting at a lower dose after TACE) or the timing of sorafenib treatment with respect to TACE may be required for these two

modalities to be tolerated in combination and also have synergistic effects.

The timing of post-TACE sorafenib may also have contributed to the absence of a positive effect of sorafenib observed in this study. Local hypoxia resulting from TACE can induce angiogenesis[18] and enhance serum concentrations of VEGF,[19,20] suggesting that sorafenib may exert its greatest antiangiogenic effects when administered immediately after or even before TACE. Serum VEGF concentrations have also been found to correlate with impaired liver function, tumour size, tumour number, macroscopic vascular invasion,[30] and poor OS.[31] Of our sorafenib-treatment patients, 60% had a treatment lag >9 weeks prior to randomisation, due primarily to the need for central review of CT scans, and shorter lag time has been found associated with better outcomes.

Several ongoing phase II/III trials in patients with unresectable HCC may provide insight into the optimal combination treatment and the optimal timing of sorafenib relative to TACE. These include trials testing TACE with doxorubicin-eluting beads and sorafenib or placebo and alterations in timing of conventional TACE relative to sorafenib or placebo.[32–35]

5. Conclusion

Sorafenib did not significantly improve median TTP by central review in Japanese and Korean patients with unresectable HCC who responded to TACE, although exploratory analyses suggested that sorafenib may have clinical benefits in certain patient subsets, including males, patients <65 years of age, and those with a shorter treatment lag between TACE and sorafenib; and that longer treatment duration and greater total daily dose may be associated with clinical improvements. No new or unexpected AEs were observed. The results of these and other clinical investigations may help refine the use of sorafenib and TACE, and define their optimal combination, in patients with unresectable HCC.

Author contributions

Drs. Masatoshi Kudo and Kiwamu Okita were involved with the study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; and study supervision.

Drs. Kazuho Imanaka, Nobuyuki Chida, Kohei Nakachi, Won-Young Tak, Tadatoshi Takayama, Jung-Hwan Yoon, Takeshi Hori, Hiromitsu Kumada, Norio Hayashi, Shuichi Kaneko, Hirohito Tsubouchi, Dong Jin Suh, Junji Furuse, Takuji Okusaka, Katsuaki Tanaka and Osamu Matsui were involved with the acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; and study supervision.

Drs. Michihiko Wada, Iku Yamaguchi, Toshio Ohya and Gerold Meinhardt were involved with the study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; administrative and technical support; and study supervision.

Clinical trials

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Conflict of interest statement

Masatoshi Kudo received advisory and speaker fees and research and travel grants from Bayer. Won-Young Tak received advisory and speaker fees from Bayer, Junji Furuse received advisory fees from Bayer, Takuji Okusaka received advisory and speaker fees, research and travel grants from Bayer. Osamu Matsui received consulting and advisory fees and research grants from Bayer. Michihiko Wada, Iku Yamaguchi, Toshio Ohya and Gerold Meinhardt are employees of Bayer. Kiwamu Okita received consulting fees from Bayer. All other authors declared no conflicts of interest.

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SUPPLEMENT

Tissue Biomarkers as Predictors of Outcome and Selection of Transplant Candidates With Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is a common cause of cancer deaths worldwide, and its annual incidence is rising. Liver transplantation (LT) is an accepted curative treatment for patients with tumors satisfying the Milan criteria (a single tumor $\leq$ 5 cm in diameter or up to 3 tumors with individual diameters $\leq$ 3 cm and no macrovascular invasion). These criteria predict an overall 5-year survival rate of 70% after LT.[1] Since the introduction of the Milan criteria, subsequent studies have explored the expansion of transplant recipient selection to include individuals with tumors exceeding the Milan criteria.[2] A recent study demonstrated an acceptable overall 5-year survival rate (71.2%) for patients who underwent transplantation for tumors that were beyond the Milan criteria but satisfied the up-to-7 rule (7 is the sum of the size of the largest tumor in centimeters and the number of tumors) in the absence of microvascular invasion.[3] This approach is based on the best data available for understanding tumor behavior after LT, but it is still based on pathological data. The tumor size and the tumor number cannot be used to define subclasses of patients with better biology and better outcomes, so biomarkers are expected to be a major step forward in this setting during the next decade.

Numerous molecular pathways involved in the pathogenesis of HCC have been identified. These include activation pathways that are involved in angiogenesis [vascular endothelial growth factor (VEGF)], in cell proliferation and survival (epidermal growth factor, insulin-like growth factor, and hepatocyte growth factor/Met), and in cell differentiation and proliferation (Wnt/β-catenin and hedgehog signaling). The activation of

Abbreviations: AFP, alpha-fetoprotein; Ang2, angiopoietin 2; HCC, hepatocellular carcinoma; LT, liver transplantation; miRNA, microRNA; mRNA, messenger RNA; REMARK, Reporting Recommendations for Tumor Marker Prognostic Studies; VEGF, vascular endothelial growth factor.

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VEGF,[4] Serine/threonine protein kinase Akt (Bombyx mori) [AKT],[5] and met proto-oncogene (hepatocyte growth factor receptor) [MET][6] has been correlated with an aggressive phenotype and a poor prognosis after liver resection. Similarly, several gene signatures have been used to predict the outcomes of patients with HCC.[7] Gene expression profiling with formalin-fixed, paraffin-embedded tissue samples from HCC resection specimens has been described and validated for the prediction of survival outcomes for patients after resection for HCC.[8] This profiling technique offers the ability to perform retrospective studies with stored histological specimens. In addition, it potentially offers a practical clinical application through the ability to perform gene profiling with common formalin-fixed biopsy specimens rather than frozen tissue.

This article summarizes 3 areas in which molecular tissue biomarkers should be considered for the management of HCC in LT patients:

1. Role of tissue biomarkers in the diagnosis of HCC.
2. Role of biomarkers in the prediction of prognosis (ie, the use of gene signatures or tissue biomarkers to predict a patient's prognosis and thus aid in the extension of the Milan criteria for HCC).
3. Role of biomarkers in the prediction of the response to molecular-targeted therapies.

Serum markers such as alpha-fetoprotein (AFP), angiopoietin 2 (Ang2), and des-gamma-carboxyprothrombin are not analyzed here.

ROLE OF TISSUE BIOMARKERS IN THE DIAGNOSIS OF HCC

The diagnosis of HCC is based on pathological or noninvasive criteria.[9] The pathological differentiation of dysplastic nodules (particularly high-grade nodules) from very early HCC is sometimes difficult, especially with a cirrhotic background. Few studies have tested the accuracy of the molecular diagnosis of early HCC in this setting. For instance, gene signatures have allowed molecular demarcations between low-grade dysplastic nodules, high-grade dysplastic nodules, and early HCC in both Asian[10] and Western patients.[11] More specifically, a 3-gene signature (including glypican 3, lymphatic vessel endothelial hyaluronan receptor 1, and survivin) has been reported to distinguish early HCC (<2 cm) from dysplastic nodules with an accuracy of approximately 90%.[12] Nonetheless, this signature has not yet been externally validated. More recently, an immunohistochemistry study found the expression of glypican 3, heat shock protein 70, and glutamine synthetase to be useful in detecting well-differentiated HCC in biopsy samples,[13] and this is currently being considered for HCC management guidelines.[9]

ROLE OF BIOMARKERS IN THE PREDICTION OF PROGNOSIS

Patients who develop HCC with cirrhosis and undergo resection have a high rate of recurrence (approximately 70% at 5 years).[2,14] A molecular assessment of the prognosis could determine which patients with HCC would benefit from adjuvant therapy after resection or radio frequency ablation (2 curative treatments with a high risk of relapse). Moreover, it could be used to refine the group of patients who should undergo transplantation for HCC beyond the Milan criteria. Whether the risk of tumor seeding counterbalances the advantages of tissue-based molecular profiling is still an area of discussion. In a recent meta-analysis, the risk of tumor seeding after liver biopsy was 2.7% with a median time of 17 months between biopsy and seeding.[15] These data also include large tumors, so the risk of complications with small, early tumors is expected to be significantly lower and thus acceptable.

Biomarkers predicting a patient's prognosis or response to therapy are crucial in modern oncology. Novel prognostic biomarkers enabling tumor classification, disease state monitoring, or both could advance our efforts to realize the potential of personalized medicine in cancer.[16] Besides reports on AFP levels and outcomes,[17-19] recent studies have correlated various types of markers, such as gene expression, microRNAs (miRNAs), and methylation changes, with the survival of HCC patients; this topic has been reviewed elsewhere[20] (see Table 1). Five markers or signatures (epithelial cell adhesion molecule [EPCAM signature], which is a hepatic stem cell marker in tumor tissue[21,22]; the G3 proliferation subclass[23]; the expression status of the miR-26 miRNA precursor[24]; and 2 prognostic gene signatures in nontumor hepatic tissue[8,25]) have emerged as more consistent ones. Finally, both VEGF and Ang2 were shown to have independent prognostic value in a large cohort of patients with advanced HCC.[26] Although these results support the possibility of using these genetic and molecular markers as prognostic biomarkers for patients with HCC, they require external validation before they can be included in staging systems and/or incorporated into clinical management guidelines. The fractional allelic imbalance, which is used to measure chromosomal instability, has been associated with outcomes for patients with HCC and with recurrence after LT; this observation requires attention in future studies.[27,28] Similarly, data about $CD90^+$ circulating cells may lead to a tractable supply of tissue for molecular characterization, but this is still under investigation.[29]

In this era of limited organ availability, better predictors of HCC recurrence are needed for selecting appropriate LT candidates whose tumors exceed the Milan criteria. The identification of a subgroup of patients whose tumors are beyond the Milan criteria but who have a favorably low risk of recurrence after transplantation offers a potential cure to those who would otherwise be excluded according to current organ allocation policies. Whether any of the aforementioned biomarkers or gene signatures can be used to identify those patients with better biological profiles needs to be elucidated in molecular studies addressing this point. Only a small study has addressed this

TABLE 1. Main mRNA-Based, miRNA-Based, Epigenetic, and Structural Alterations Whose Prognostic Impact for HCC Patients Needs to Be Tested or Confirmed

Molecular Alteration	Clinical Significance	REMARK Recommendation	Current Status*
mRNA-based (gene signatures)†			
Poor survival signature	Poor survival	Okay	Lacks external validation
Epithelial cell adhesion molecule signature	Poor survival	Okay	Lacks external validation
Venous metastasis signature	Hepatic metastasis	Okay	Lacks external validation
Class A/hepatoblast signature	Poor survival	Okay	Lacks internal and external validation
G3 subclass	Poor survival	—	Lacks internal and external validation
AFP and Ang2	Poor survival	Okay (unclear cutoff)	Lacks external validation
miRNA-based			
Down-regulation of miR-26a	Poor survival	Okay	Lacks external validation
20-miRNA signature	Venous metastasis, overall survival	Okay	Lacks external validation
Down-regulation of miR-122	Poor survival	—	Lacks internal and external validation
Down-regulation of Drosophila melanogaster members	Early recurrence	—	Lacks internal and external validation
Up-regulation of miR-125a	Better survival	—	Lacks internal and external validation
19-miRNA signature	Poor survival	—	Lacks internal and external validation
Epigenetic			
Genome-wide hypomethylation	Tumor progression, survival	—	Lacks internal and external validation
Hypermethylation of E-cadherin or glutathione S-transferase π1	Poor survival	—	Lacks internal and external validation
Structural			
Fractional allelic imbalance/ chromosomal instability	Recurrence/survival	Okay	Lacks external validation

NOTE: Adapted with permission from *Clinical Cancer Research*.[20]
*In terms of clinical implementation.
†Molecular classifications (mRNA-based) with a prognostic impact have been thoroughly discussed elsewhere.[5,6,20]

question in a specific manner, and it found that chromosomal instability (measured with the fractional allelic imbalance) independently predicted which patients beyond the Milan criteria had a low risk of recurrence.[27] Similarly, preliminary reports describing surrogates of microvascular invasion (the main predictor of HCC recurrence after LT) require independent validation in the setting of transplantation.[30]

ROLE OF BIOMARKERS IN THE PREDICTION OF THE RESPONSE TO MOLECULAR-TARGETED THERAPIES

Biomarkers for treatment responses are still rarities in oncology; only a few have made their way into routine clinical use. Well-defined biomarkers are believed to characterize an oncogenic addiction loop (the proposed mechanism by which a tumor cell becomes largely reliant on a single activated oncogene[31]) and define particular tumor subtypes that respond to specific molecular-targeted therapies. Examples of oncogenic addiction include an amplification of human epidermal growth factor receptor 2 in patients with breast cancer responding to trastuzumab,[32] mutations in epidermal growth factor receptor that distinguish patients with non–small cell lung cancer responding to erlotinib,[33] and v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (c-KIT)–positive gastrointestinal stromal tumors responding to the multikinase inhibitor imatinib.[34] In addition, wild-type v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) has recently emerged as a marker of a response to cetuximab and panitumumab in patients with colorectal cancer, although the mechanism is entirely different and involves the downstream regulation of epidermal growth factor receptor signaling.[35] Moreover, a new step in personalized medicine has been achieved recently with the development of a specific inhibitor of mutated V600E v-raf murine

sarcoma viral oncogene homolog B1 (BRAF); this inhibitor has shown impressive clinical efficiency with few adverse events in a recent phase 2 study of melanoma.[36] In the future, therefore, mapping the genetic alterations of tumors before the treatment or after treatment failure will improve the clinical care of patients with cancer.[37]

The use of biomarkers for HCC is somewhat more complex because HCC is a very heterogeneous disease for which oncogenic addiction loops have yet to be characterized. Initial approaches for defining a molecular classification have not yet been linked to specific treatment responses.[38,39] So far, only 1 small molecule, sorafenib, has been shown to improve the survival of HCC patients.[40] Sorafenib is a multikinase inhibitor that targets a number of kinases; these kinases include VEGF receptors 2 and 3, platelet-derived growth factor receptor β, c-KIT, Ret proto-oncogene (RET), fms-related tyrosine kinase 3, and Raf kinase, efector of Ras (RAF).[41] Isolated reports have described the use of sorafenib in the adjuvant setting after LT. In a companion biomarker study of the pivotal Sorafenib HCC Assessment Randomized Protocol trial, 10 serum markers and 1 tissue marker were tested, but none of them succeeded in identifying subclasses of responders.[26] Nonetheless, the fast development of new biotherapies and the growing number of clinical trials for HCC are expected to lead to the use of the molecular features of tumors in defining types of treatment. In this setting, we have to reevaluate the utility of tumor biopsy for easy access to tissue and its frequency.

FUTURE PROSPECTS

Novel molecular data may change our approach to the diagnosis, staging, and prognosis of HCC in this decade. For prognosis assessments, recently reported prognostic gene signatures and miRNAs may be added to staging systems to complement clinical variables once they have been externally validated by independent studies. These advances in our understanding of HCC ultimately need to be transferred to clinical practice as daily tools for selecting management and treatment methods. Moreover, treatment response predictors will emerge along with novel drugs for the treatment of HCC. Positive results with sorafenib[40] have opened a new era in HCC research. Future trends in drug development will pivot on the accurate assessment of genetic traits associated with human diseases on an individual basis (ie, personalized medicine). For HCC, the identification of these singularities will allow maximization of the therapeutic response through the selection of the best drug for the ideal candidate.

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Tailor-Made Therapy for Viral Hepatitis: Recent Advances

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Key Words

Hepatitis C · Hepatitis B · Interleukin-28B · Inosine triphosphatase · Pegylated interferon ribavirin therapy · Double-filtration plasmapheresis

Abstract

Combination therapy of pegylated interferon-α with ribavirin (PEG-IFN/RBV) is a standard of care for chronic hepatitis C (CHC). The majority of CHC patients are infected with HCV genotype I. The recent discovery revealed by a genome-wide association study technology provides the important role of interleukin-28B (IL28B) and inosine triphosphatase (ITPA) in HCV infection. In addition, response to PEG-IFN/RBV therapy is correlated with insulin resistance, hepatic steatosis, and hepatic fibrosis in CHC patients. Double-filtration plasmapheresis together with IFN therapy has proved to be effective in the reduction of viral load during the early stage of treatment. In CHC patients, not only IL28B status, but also the treatment period of PEG-IFN/RBV is important. Even when new polymerase/protease inhibitors are introduced in the treatment of CHC, tailor-made treatment for CHC using IL28B, inosine triphosphatase testing or double-filtration plasmapheresis treatment prolonged treatment strategy is highly recommended. The relative etiologic role of prior hepatitis B virus infection in the development of non-B non-C hepatocellular carcinoma is also known in hepatitis B-endemic areas.

Introduction

The 8th Japan-Korea Liver Symposium, the main theme of which was 'Tailor-made therapy of viral hepatitis', was held in Kobe, Japan, on July 17, 2011, to focus on and discuss current topics in viral hepatitis. The symposium was full of enlightening lectures by world's leading scientists, followed by extensive discussions. This supplement issue of *Digestion* contains the most important articles presented at this meeting.

Insulin Resistance

Insulin resistance (IR) has been reported to be an independent predictor of treatment outcome in chronic hepatitis C (CHC) patients.

Associations among IR, steatosis and liver fibrosis have been observed in CHC patients [1–5]. IR has been suggested as the cause, more than a consequence, of hepatic steatosis and fibrosis in patients with HCV, particularly in those with genotype 1 infection [6]. The mechanisms of the more obvious and crucial influence of IR, more than steatosis and fibrosis, need further study. IR seems to be at least associated with body mass index and steatosis, but not with hepatic fibrosis [7].

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Double-Filtration Plasmapheresis

The use of double-filtration plasmapheresis (DFPP), approved in Japan in April 2008 for the retreatment of chronic CHC patients with genotype 1b and high viral loads, together with IFN administration has produced a substantial reduction in the viral load during the early stages of treatment and has effected a high sustained virological response (SVR) [8, 9], suggesting that this treatment is a new modality for difficult-to-treat CHC patients.

Recent reports have revealed factors associated with response to pegylated interferon-α with ribavirin (PEG-IFN/RBV) therapy such as single nucleotide polymorphisms (SNPs), as host genetic factors, located in interleukin-28B (IL28B; rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917, rs7248668, and rs12979860) on chromosome 19 [10–13]; amino acid (aa) substitutions in nonstructural protein 5a, especially those in the IFN/RBV resistance-determining region [14] and the IFN sensitivity-determining region (ISDR) [15], and the core regions of HCV [16], as viral genetic polymorphisms.

Kim et al. [17] reported that early viral dynamics with DFPP + IFN-β/RBV then PEG-IFN/RBV therapy is superior to the previous PEG-IFN/RBV combination therapy. There was a significant difference in viral reduction at 24 and 48 h, and 1, 2, 4, 8 and 12 weeks between non-viral response (NVR) and relapse patients. The rate of rapid viral response (RVR) and complete early viral response (cEVR) showed a significant difference between NVR and relapse patients: among the 20 patients, RVR was obtained in 75% (6/8) of relapse patients but in 0% (0/12) of NVR patients, and cEVR in 88% (7/8) of relapse patients but in only 8% (1/12) of NVR patients. On the basis of the above results, DFPP + IFN-β/RBV then PEG-IFN/RBV therapy is indicated more for relapse than for NVR patients. We could conclude that relapse patients would be better candidates than NVR patients [17].

IL28B and Inosine Triphosphatase

The recent discovery revealed by a genome-wide association study (GWAS) technology provides the unexpected role of *IL28B* and inosine triphosphatase *(ITPA)* in HCV infection. The former SNPs around the *IL28B* gene could improve the diagnostics on the prediction of spontaneous clearance and the response to anti-HCV treatment, suggesting that these findings could be strong evidence to enhance the development of a novel therapeutic strategy and the basic study of IFN-λs. Interestingly, the discovered *IL28B* SNPs revealed the enigma that the viral clearance rate was dependent on ethnic type. The latter functional SNP in *ITPA* locus was the most significant SNP associated with RBV-induced anemia as well as IFN-induced thrombocytopenia. Note that severe Hb decline, which is mainly found in *ITPA*-CC patients, was inversely correlated with platelet reduction, contributing to an association between severe anemia and relative reactive increase in platelet count.

The efficacy of triple therapy of telaprevir/PEG-IFN/RBV was high in the patients with genotype TT (rs8099917), who achieved SVR (84%), irrespective of substitution of core aa70. In the patients having genotype non-TT, those of Arg70 gained high SVR rate (50%), and SVR rate (12%) was the worst in patients who possessed both genotype non-TT and Gln70 (His70), suggesting genetic variation near the *IL28B* gene and aa substitution of the core region as predictors of SVR to a triple therapy in Japanese patients infected with HCV genotype 1b [18].

Genetic variants leading to ITPA deficiency, a condition not thought to be clinically important, protect against hemolytic anemia in CHC patients receiving RBV [19]. Results obtained in one GWAS study need to be evaluated in the context of different geographical and racial populations and independent cohorts. Tanaka confirmed that *ITPA* SNP (rs1127354) was a useful predictor of RBV-induced anemia in Japanese patients [20]. Excluding those with genotype 1b and high viral load, patients with *ITPA* minor variant A achieved significantly higher SVR rates than those with the major variant (CC; 96 vs. 70%, respectively, p = 0.0066) [20]. Because the typical PEG-IFN/RBV treatment period is shorter (24 weeks) in genotype 1 low viral load and genotype 2 patients than in genotype 1 high viral load (48 weeks) patients, early dose reduction in RBV may be more critical for the final outcome.

The recent discovery revealed by GWAS technology provides the unexpected role of *IL28B* and *ITPA* in HCV infection. These data may provide a valuable pharmacogenetic diagnostic tool for tailoring PEG-IFN/RBV dosing to minimize drug-induced adverse events and for further optimization of clinical anti-HCV chemotherapeutics.

Total PEG-IFN Dose, Core 70 Substitution and ISDR Substitution

Takita et al. [21] showed in their multivariate analysis that rs8099917 genotype and total PEG-IFN dose contribute to the successful outcome of PEG-IFN plus riba-

virin combination treatment for infection with HCV genotype 1. The study indicated the value of a combination of the rs8099917 genotype and core 70 substitutions for prediction of SVR. The patients with the rs8099917 genotype TT had high rates of SVR (67.9%). SVR was achieved by 30.7% of patients with the rs8099917 genotype non-TT and core 70 wild type. The SVR rate was worst in patients with rs8099917 genotype non-TT and core 70 mutant type. These results indicate the effects of both host and viral factors on IFN responsiveness. However, a combination of the IL28 genotype and ISDR substitutions for prediction of SVR was not useful.

Etiologic Role of Prior Hepatitis B Infection and Nonalcoholic Steatohepatitis on Hepatocellular Carcinoma Occurrence

A previous study on 1,145 Korean patients showed that the prevalence of cryptogenic hepatocellular carcinoma (HCC) was significantly increased during the last decade, and patients with cryptogenic HCC had a tendency to have risk factors for nonalcoholic fatty liver disease (NAFLD) such as DM, hypertension and obesity than those with virus or alcohol-related HCC [22]. It can be assumed that the increased proportion of risk factors for NAFLD may contribute to the development of cryptogenic HCC. However, there has been no study to compare the relative etiological role of prior HBV infection and NAFLD in the development of NBNC-HCC in HBV-endemic areas. Cho et al. [23] show in their study that the relative proportion of NAFLD-related HCC increased more than three times during the past 10 years, while that of prior HBV infection-related HCC decreased. A growing trend towards a rise in NAFLD-related HCC is expected in the near future, while the prevalence of new HBV infection has definitely decreased due to universal vaccination programs but that of NAFLD has increased recently [24].

Predictions of HBsAg Seroclearance

In chronic HBV infections, HBsAg seroclearance reportedly occurs at a rate of 0.50–2.26%. Several factors have been suggested to be associated with seroclearance including age and HBeAg negativity. However, there are few studies evaluating whether HBV DNA levels are an independent predictor of HBsAg seroclearance.

Old age, a sustained inactive phase, and low levels of HBV DNA were the independent predictors of HBsAg seroclearance [25]. The authors showed that HBsAg seroclearance occurred at a rate of 1.8% per year in HBeAg-negative chronic hepatitis B patients. In addition, multivariable analysis suggested that low HBV DNA levels, old age, and sustained inactive phase were independent predictors of HBsAg seroclearance, but use of antiviral agents was not. Therefore, spontaneous HBsAg seroclearance takes place in elderly patients with low serum HBV DNA levels (<2,000 IU/ml) who remain in a sustained inactive phase.

Non-B Non-C HCC

NBNC-HCC is associated with several etiologic factors such as alcoholic liver diseases (ALD) autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and NAFLD/nonalcoholic steatohepatitis. In addition, a variety of clinical factors are also involved in the development and progression of NBNC-HCC, including age, sex, alcohol consumption, and diabetes mellitus [26, 27]. There are only a few reports, however, on the clinical characteristics of NBNC-HCC, and the actual state of NBNC-HCC has not been fully elucidated [28–31].

Although the number of patients with NBNC-HCC has been increasing annually, many features of NBNC-HCC remain unknown. Based on the present study, the most common etiologic factor for NBNC-HCC was alcohol, and diabetes may be related to the occurrence of HCC in patients with non-alcohol-related liver disease. The comparison between groups revealed that non-ALD-HCC tended to be detected at a more advanced stage, whereas liver function in ALD-HCC was worse. Finally, the prognosis was equivalent between groups [32].

Conclusion

Tailor-made treatment for chronic hepatitis is a very important issue to be addressed even when new polymerase/protease inhibitors are available.

Disclosure Statement

The author has no conflict of interest to declare.

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Correlation between Insulin Resistance and Outcome of Pegylated Interferon and Ribavirin Therapy, Hepatic Steatosis, Hepatic Fibrosis in Chronic Hepatitis C-1b and High Viral Load

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Key Words

Insulin resistance · Hepatic fibrosis · Hepatic steatosis · Genotype 1b · High viral load · Pegylated interferon · Ribavirin · Sustained virological response

Abstract

Background/Aims: Insulin resistance (IR) has been reported to be an independent predictor of treatment outcome in chronic hepatitis C patients. ***Methods:*** We analyzed the relationship between IR and the outcome of pegylated interferon and ribavirin (PEG-IFN/RBV) therapy, taking into account host factors of body mass index and histological index, such as rate of fatty change and fibrosis. Japanese patients (n = 30; 19 men and 11 women; median age 60.0 ± 8.7 years) with chronic hepatitis C-1b with a high viral load were treated with PEG-IFN-α2b/RBV for 48 weeks. ***Results:*** Sustained virological response (SVR) was seen in 60% (18/30) and non-SVR in 40% (12/30). HOMA-IR (homeostasis model of assessment-insulin resistance index) at the start and at 24 weeks of treatment showed no statistical difference between SVR and non-SVR. Correlation was observed between HOMA-IR and body mass index (r = 0.45, p = 0.013). Among 20 patients, steatosis and fibrosis were assessed by biopsy. Correlation was observed between HOMA-IR and steatosis (r = 0.57, p = 0.0093), whereas no correlation was observed between HOMA-IR and fibrosis. ***Conclusion:*** A larger prospective study is needed to clarify the role of IR in the outcome of PEG-IFN/RBV combination therapy and hepatic fibrosis in Japanese patients.

Introduction

Chronic hepatitis C genotype 1b and a high viral load are known to be highly refractory to interferon (IFN) therapy. In Japan, such patients account for approximate-

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Table 1. Patient baseline characteristics

Age, years	60.0 ± 8.7
Males/females	19/11
Body weight, kg	60.2 ± 10.9
BMI	22.9 ± 3.8
HCV-RNA, log IU/ml	4.5 ± 1.7
AST, IU/l	63.4 ± 44.8
ALT, IU/l	71.3 ± 63.3
FBS, mg/dl	92.8 ± 16.4
γ-GTP, IU/l	84.1 ± 99.1
T-Cho, mg/dl	170.0 ± 39.6
TG, mg/dl	85.7 ± 39.4
HOMA-IR	2.1 ± 1.1

Data are shown as mean ± SD.

ly 70% of chronic hepatitis C cases, and various strategies have been investigated to improve treatment outcome. Currently, the first choice for refractory patients is a 48-week combined administration of pegylated IFN and ribavirin (PEG-IFN/RBV). Nonetheless, sustained virological response (SVR) is achieved in at most 50% of such patients [1, 2].

Host factors including HLA class I [3] and II [4], ethnicity [5] and body mass index (BMI) [6] also influence SVR [7]. Indeed, overweight [6] patients have shown characteristic resistance to combination therapy, and increased insulin resistance (IR) has been identified as an independent variable associated with a poor response [8]. Experimental and clinical studies have shown the role of hepatitis C virus (HCV) infection in the development of IR [9]. Patients with mild chronic hepatitis demonstrate a higher homeostasis model of assessment-insulin resistance index (HOMA-IR) than do healthy controls matched for age and BMI [8]. IR has also been implicated in the progression of fibrosis [10] and the development of steatosis [11, 12]. The latter finding is, however, observed mainly in European and American, including Caucasian and African, patients. The aim of this study was to analyze the relationship between IR and the outcome of PEG-IFN/RBV therapy, taking into account host factors of BMI and histological index, such as rate of fatty change and fibrosis, in Japanese patients with chronic hepatitis C-1b and high viral loads.

Patients and Methods

Patients

A total of 30 patients (19 men, 11 women; age 60.0 ± 8.7 years) seen at Kobe Asahi Hospital and diagnosed with chronic HCV-1b infection on the basis of the presence of anti-HCV antibodies and HCV-RNA, were enrolled in the study. The patients were treated with PEG-IFN-α2b (1.5 μg per kilogram body weight, once a week subcutaneously) and RBV (600–1,000 mg daily, per os) for 48 weeks, according to the standard treatment protocol for Japanese patients established by a hepatitis study group of the Ministry of Health, Labor and Welfare, Japan. The HCV genotype was determined according to the method of Okamoto et al. [13]. Informed consent in writing was obtained from each patient, and the study protocol conformed to the ethical guidelines approved by the Ethics Committee in Kobe Asahi Hospital. The baseline characteristics of 30 patients are listed in table 1.

Laboratory and Histological Tests

Serum samples were collected from the patients at intervals of 4 weeks before, during and after the treatment, and tested for HCV-RNA based on the COBAS TaqMan HCV test (Roche Diagnostics Corp., Basel, Switzerland). Fasting glucose and insulin were obtained at the start of and at 24 weeks of the treatment with PEG-IFN/RBV; IR was assessed by HOMA: [fasting insulin [μU/ml] × (fasting glucose [mg/dl]/18)]/22.5 [14, 15]; BMI was calculated as weight divided by height (kg/m^2).

Twenty biopsies were assessed for staging fibrosis and grading steatosis: fibrosis was staged on a scale from 0 to 4 according to the new classification by Desmet et al. [16]: with 0, no fibrosis; 1, mild fibrosis; 2, moderate fibrosis; 3, severe fibrosis, and 4, cirrhosis. Steatosis was graded on a scale from 0 to 4 according to the percentage of cells with fat: with 0, <5%; 1, 5–32%; 2, 33–65%; 3, ≥66%.

Statistical Analysis

Statistical differences in treatment responses according to patient baseline parameters of age, sex, body weight, BMI, HCV-RNA load, aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting blood sugar (FBS), γ-glutamyl transpeptidase (γ-GTP), total cholesterol (T-Cho) and triglyceride (TG) were determined by Fisher's exact test or the Mann-Whitney U test. Differences between the start of and at 24 weeks of therapy were assessed by the Wilcoxon signed rank test. Correlation between IR and the staging of fibrosis, the grading of steatosis and BMI was assessed by single regression analysis. Variables with a p value of <0.05 were considered statistically significant.

Results

Among the 30 patients, SVR was seen in 60% (18/30) and non-SVR in 40% (12/30). The baseline characteristics and the clinical responses are shown in table 2. Sex, body weight, BMI, HCV-RNA load, AST, ALT, FBS, γ-GTP, T-Cho and TG showed no significant difference between SVR and non-SVR, but age did ($p = 0.03$).

HOMA-IR at the start and at 24 weeks of treatment was 2.2 ± 1.0 and 2.5 ± 3.9 in SVR, and 2.0 ± 1.2 and 1.4 ± 0.5 in non-SVR, respectively, with no statistical difference between the two groups. BMI was ≥25 in 20% (6/30) and <25 in 80% (24/30) of patients, and correlation

Table 2. Baseline characteristics and the clinical response in SVR and non-SVR

	SVR (n = 18)	Non-SVR (n = 12)	p value
Age, years	57.1 ± 8.8	64.3 ± 6.6	0.03
Males/females	12/6	7/5	0.80
Body weight, kg	62.3 ± 12.0	57.0 ± 8.4	0.21
BMI	23.8 ± 4.0	21.8 ± 3.2	0.28
HCV-RNA, log IU/ml	4.7 ± 1.9	4.2 ± 1.4	0.85
AST, IU/l	65.2 ± 51.3	60.0 ± 34.7	0.82
ALT, IU/l	82.3 ± 76.0	54.8 ± 33.7	0.46
FBS, mg/dl	95.4 ± 20.0	88.8 ± 8.1	0.63
γ-GTP, IU/l	108.1 ± 121.9	48.3 ± 24.4	0.66
T-Cho, mg/dl	176.6 ± 44.0	160.3 ± 31.2	0.23
TG, mg/dl	88.1 ± 47.8	82.3 ± 23.2	0.85
HOMA-IR	2.2 ± 1.0	2.0 ± 1.2	0.53

Data are shown as mean ± SD.

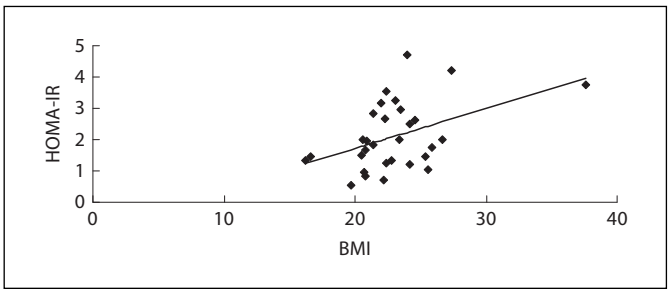

Fig. 1. Correlation observed between HOMA-IR and BMI (r = 0.45, p = 0.013).

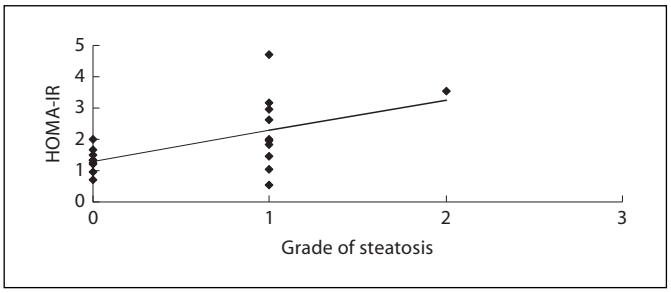

Fig. 2. Correlation observed between HOMA-IR and grade of steatosis (r = 0.57, p = 0.0093).

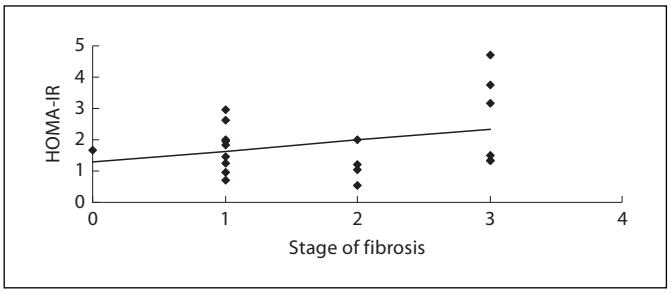

Fig. 3. No correlation was observed between HOMA-IR and stage of fibrosis (r = 0.32, p = 0.17).

was observed between HOMA-IR and BMI (r = 0.45, p = 0.013; fig. 1). Among 20 patients, steatosis assessed by histology revealed grade 0 in 45% (9/20), grade 1 in 50% (10/20), grade 2 in 5% (1/20), and grade 3 in 0% (0/20; fig. 2). Fibrosis was observed at stage F0 in 5% (1/20), F1 in 45% (9/20), F2 in 20% (4/20), F3 in 30% (6/20; fig. 3). Correlation was observed between HOMA-IR and steatosis (r = 0.57, p = 0.0093), whereas no correlation was observed between HOMA-IR and fibrosis (r = 0.32, p = 0.17).

Discussion

HCV genotype and HCV viral load remain the most important predictors of response to PEG-IFN/RBV combination therapy [17]. In contrast to HCV genotypes 2 and 3, which are significantly more susceptible to combination therapy with good outcome after standard or short-term treatment [18, 19], genotype 1 infection calls for developing more effective therapy and elucidating predictors of response conducive for optimizing individualized regimens. Therefore, the effect of host factors on the rate of SVR in anti-HCV therapy becomes a compelling concern for patients with unfavorable virological predictors.

IR, the stage of fibrosis and HCV genotype are independent predictors of response to anti-HCV therapy among Spanish patients [n = 159 (including genotype 1, n = 113); age, 41.7 years; BMI, 26.8]. In patients with genotype 1, a significantly lower SVR rate is observed in those with HOMA IR >2 than in those with HOMA-IR ≤2 (32.8%, 23/70 vs. 60.5%, 26/43, p = 0.007). The authors suggest that HOMA-IR might assist in further refining the prediction of antiviral response in genotype 1 patients [11]. In American, including African and Caucasian, patients with genotype 1 (n = 399; age, 47.7 years; BMI, 29.5), SVR rates of 49% for patients with HOMA-IR ≤2, and 36% for patients with HOMA-IR >2 have been

observed, the authors concluding that IR is independently associated with a low SVR rate [12]. HCV genotype 1b-infected Taiwanese patients (n = 150; age, 51.1 years; BMI, 23.5) with high IR demonstrated a lower SVR rate than those with low IR, suggesting the possible value of evaluating IR to predict response in HCV genotype 1b infection and a high pretreatment serum HCV-RNA level [20]. Noteworthy in that study is that the effect of HOMA-IR on response is observed in genotype 1b patients and particularly, for the first time, in those classified as 'difficult to treat' (genotype 1b infection and a high HCV-RNA level). Japanese patients with genotype 1b and a high viral load (n = 51; age, 57 years; BMI, 23.2) achieving SVR have lower HOMA-IR compared with non-SVR patients [21].

In the current study (BMI, 22.9), IR showed no difference between SVR and non-SVR patients, implying that the association of IR with response might be explained, in part, by ethnicity (Romero-Gomez, Spanish; Conjeevaram, American; the current study, Japanese), sample size (113, Spanish; 399, American; 150, Taiwanese, Dai's group; 51 Japanese, Mizuta, and 30 in the current study) and age (41.7, Spanish; 47.7, American; 51.1, Taiwanese, and 60.0 in the current study).

Since IR is a potentially modifiable factor, the response to the therapy might be improved by the modulation of insulin signaling and by improvements in IR and glucose control. The considerable potential for evaluating novel therapies and targets including insulin-sensitizing drugs for chronic hepatitis C patients deserves prospective investigation. Prospective studies for effective approaches resolving the IR issue before the initiation of combination therapy for chronic hepatitis C can significantly raise the SVR rate. HCV might induce IR irrespective of the severity of liver disease [8], and IR could be associated with severe hepatic fibrosis and might contribute to the progression of fibrosis in chronic HCV infection [8, 22, 23].

Around one to two thirds of liver biopsies from chronic hepatitis C patients show histological evidence of steatosis, which has been associated with being overweight, with hepatic fibrosis and TG levels [12, 24, 25]. Associations among IR, steatosis and liver fibrosis have been observed in chronic hepatitis C patients [25–29]. IR has been suggested as the cause, more than the consequence, of hepatic steatosis and fibrosis in patients with HCV, particularly in those with genotype 1 infection [30]. The mechanisms of the more obvious and crucial influence of IR, more than that of steatosis and fibrosis, need further study. In the current study, IR was found to be associated with BMI and steatosis, but not with hepatic fibrosis. The differences in the results regarding IR-associated hepatic fibrosis might also be explained by ethnic difference, sample size and age. Further large-scale study on Japanese patients is needed to clarify the role of IR in hepatic fibrosis.

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Disclosure Statement

None of the authors has any conflict of interest.

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Double-Filtration Plasmapheresis plus Interferon-β for HCV-1b Patients with Non-Sustained Virological Response to Previous Combination Therapy

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Key Words

Double-filtration plasmapheresis · Interferon-β · Ribavirin · Rapid virological response · Complete early virological response · Viral dynamics · Relapse · Null virological response

Abstract

Background and Aims: Double-filtration plasmapheresis (DFPP) together with interferon (IFN) administration produces a substantial reduction in the viral load during the early stages of treatment. ***Methods:*** Based on their responses to previous pegylated IFN and ribavirin (PEG-IFN/RBV) therapy, 20 patients were divided into null virological response (NVR; n = 12) and relapse (n = 8) groups. DFPP was used in combination with IFN-β/RBV with subsequent administration of PEG-IFN-α2a/RBV therapy (DFPP + IFN-β/RBV then PEG-IFN/RBV). Early viral dynamics was assessed, focusing especially on complete early virological response (cEVR) associated with sustained virological response. Additionally, the interleukin 28B gene, the IFN/RBV resistance-determining region, the IFN sensitivity-determining region and the core regions were analyzed. ***Results:*** Rapid virological response was achieved in 0% (0/12) of NVR and in 75% (6/8) of relapse patients, with a significant difference between the two groups (p = 0.001). Similarly, cEVR was achieved in 8% (1/12) of NVR and in 88% (7/8) of relapse patients, with a significant difference between the two groups

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(p = 0.037). By multivariate logistic regression analysis, interleukin-28B major was a significant determiner of cEVR (odds ratio = 24.19, p = 0.037). ***Conclusion:*** DFPP + IFN-β/RBV then PEG-IFN/RBV therapy is indicated more for relapse than for NVR patients.

Introduction

Currently, the therapy of first choice for patients refractory to chronic hepatitis C (CHC) treatment is the combined administration of pegylated interferon and ribavirin (PEG-IFN/RBV) for 48 weeks; however, the subsequent sustained virological response (SVR) is achieved in no more than 50% of the patients with genotype 1b and high viral loads [1]. To enhance this SVR rate, several trials have been undertaken, two of which are (1) re-treatment with combination therapy and (2) double-filtration plasmapheresis (DFPP). By the protocol-defined primary analysis of the former, the SVR rate has been 16% at the most, even for a 72-week induction group [2]. The use of DFPP (approved in Japan in April 2008 for the re-treatment of chronic CHC patients with genotype 1b and high viral loads) together with IFN administration has produced a substantial reduction in the viral load during the early stages of treatment, and has effected a high SVR [3, 4], suggesting that this treatment is a new modality for difficult-to-treat CHC patients.

Recent reports have revealed factors associated with response to PEG-IFN/RBV therapy: single nucleotide polymorphisms, as host genetic factors, located in interleukin (IL) 28B (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917, rs7248668, and rs12979860) on chromosome 19 [5–8]; amino acid (aa) substitutions in nonstructural protein 5a (NS5A), especially those in the IFN/RBV resistance-determining region (IRRDR) [9] and the IFN sensitivity-determining region (ISDR) [10], and the core regions of HCV [11], as viral genetic polymorphisms.

In this study, DFPP was used in combination with IFN-β/RBV with subsequent administration of PEG-IFN/RBV (DFPP + IFN-β/RBV then PEG-IFN/RBV) therapy to enhance the efficacy of the treatment of CHC patients whose hepatitis C virus (HCV) had not been eradicated by earlier PEG-IFN/RBV combination therapy; we also assessed early viral dynamics, focusing especially on complete early virological response (cEVR) associated with SVR. Additionally, IL28B, the core regions, ISDR and IRRDR were analyzed before treatment.

Patients and Methods

Patients

Twenty patients whose HCV had not been eradicated by previous PEG-IFN/RBV combination therapy carried out in several institutions between 2008 and 2010 were enrolled in this study. Based on the response to the previous treatment (PEG-IFN/RBV), the patients were divided into 2 groups: continuous viremia throughout the observation period (10 patients) or unknown outcome regarding viral dynamics (2 patients), referred to as the null virological response (NVR) group (n = 12), and transient disappearance of serum HCV RNA at a certain point in time with a subsequent rebound in viremia either before or after the end of the treatment, referred to as relapse group (n = 8). All the patients received DFPP + IFN-β/RBV then PEG-IFN/RBV therapy for a planned 48 weeks. Since none of the patients has completed the 48-week treatment, the results at 12 weeks are presented here, and the early viral dynamics are analyzed. Each patient gave written informed consent and agreed to receive the treatment; the study was approved by the review board of Kobe Asahi Hospital.

DFPP and Blood Collection

Blood collected from the peripheral vein for DFPP by a Plasmaflo™ OP-18W filter (Asahi Kasei Medical, Tokyo, Japan) was separated into plasma and cell components. The virus was then removed from the plasma by a second filter (Cascadeflo™ EC-50W; Asahi Kasei Medical) of an average pore of 30 nm. For each session, the final volume of treated plasma was 50 ml/kg, the number of sessions was 5 over 2 weeks, and the intervals between administrations of DFPP, based on the reduced plasma fibrinogen levels during DFPP, were decided by the physicians and as required by the patients.

IFN in Combination with DFPP

During and after DFPP, the patients received IFN-β (3 MU twice daily) and RBV for 4 weeks with subsequent administration of PEG-IFN-α2a (180 μg/per week) and RBV for a planned 48 weeks. The RBV dose in combination with IFN-β and PEG-IFN-α2a was 600–1,000 mg/day, per os and according to body weight.

Measurement of HCV RNA and Viral Dynamics

In the previous PEG-IFN/RBV therapy, HCV RNA levels had been measured before the start and at 4 weeks of treatment by real-time PCR (COBAS TaqMan HCV test; Roche, detection limit 1.2 log IU/ml), by HCV core antigen (IRM assay; Ortho Clinical Diagnostics), or by RT-PCR (Amplicor; Roche). Before the start of DFPP + IFN-β/RBV therapy, all the patients were confirmed to be HCV RNA positive with high transaminase levels, and with HCV RNA genotype 1b at levels exceeding 10^5 log IU/ml. Also, the patients were negative for hepatitis B surface antigen. In DFPP + IFN-β/RBV then PEG-IFN/RBV therapy, HCV RNA levels were measured by real-time PCR before the start, at 24 and 48 h, and 1, 2, 4, 8 and 12 weeks of treatment. The quantity of HCV RNA was converted to a log value at the start of the treatment (A) and at each virus measurement point (B); Δlog was then calculated as follows: Δlog = logA – logB = log (A/B). HCV RNA negative at 4 and 12 weeks of treatment was regarded as rapid virological response (RVR) and cEVR, respectively.

Table 1. Patient baseline characteristics

Group by previous treatment response	Case No.	Age years	Sex	HCV-RNA log IU/ml	AST IU/l	ALT IU/l	γ-GTP IU/l	Hemoglobin g/dl	Platelets $\times 10^4/\mu l$
NVR (n = 12)	1	43	F	6.2	38	25	50	8.2	14.3
	2	43	M	7.2	30	57	63	17.3	21.3
	3	67	F	7.6	82	60	69	13.0	9.7
	4	49	M	6.0	45	31	82	15.8	9.6
	5	52	F	5.8	14	10	11	10.2	19.4
	6	66	F	5.3	28	20	15	10.1	6.5
	7	47	F	6.8	51	73	161	14.2	16.7
	8	52	F	5.5	24	16	11	12.1	21.0
	9	64	M	6.7	35	41	24	15.8	15.0
	10	66	M	5.5	31	34	26	9.7	13.3
	11	55	M	6.3	62	60	151	13.1	7.9
	12	62	F	6.6	24	7	19	12.9	13.7
	Average	55.5		6.3	38.7	36.2	56.8	12.7	14.0
Relapse (n = 8)	13	61	F	6.5	25	20	26	10.7	6.7
	14	61	F	5.5	28	21	11	10.6	19.1
	15	65	F	6.4	48	71	23	11.0	15.0
	16	68	F	6.3	30	21	42	11.9	9.2
	17	67	M	3.2	17	10	16	9.6	20.7
	18	62	M	7.1	30	35	18	14.0	18.7
	19	43	M	5.6	26	37	20	15.1	30.5
	20	67	F	3.7	37	36	22	9.9	21.5
	Average	61.8		5.5	30.1	31.4	22.3	11.6	17.7
NVR vs. relapse	p value	0.15	0.85	0.33	0.33	0.82	0.18	0.35	0.26

Genetic Variation near the IL28B Gene

Genetic polymorphism, rs8099917 around the IL28B gene was determined by real-time PCR with the TaqMan assay [5]. Homozygosity for the major sequence (MA) was defined as having the IL28B major allele, whereas heterozygosity (HE) or homozygosity for the minor sequence (MI) was defined as having the IL28B minor allele.

Sequence Analysis of HCV NS5A and Core

Before the start of treatment, measurements were made of the HCV aa substitutions in nonstructural protein 5a (NS5A), including those in IRRDR and ISDR. The optimal cutoff number of mutations for predicting SVR has been estimated at 6 in IRRDR [9], but at 2 in ISDR [10]. Correlations between core regions (arginine at position 70, leucine at position 91) and treatment response have been estimated [11].

Statistical Analysis

Statistical differences in treatment responses according to patient baseline parameters of age, sex, HCV-RNA load, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP) and hemoglobin were assessed by Fisher's exact test or the Mann-Whitney U test. Differences between viral dynamics were assessed by the Wilcoxon signed rank test or the Mann-Whitney U test. The treatment responses according to NS5A, core mutations and the IL28B gene were evaluated using univariate and multivariate logistic regression analyses. Variables with a p value of <0.05 were considered statistically significant. The odds ratios and 95% confidence intervals were also calculated. All statistical analyses were carried out with EXCEL multivariate statistical analysis software version 6.0 (ESUMI Inc., Tokyo, Japan).

Results

Treatment Reponses and Viral Dynamics

The baseline characteristics of the patients and the laboratory data of age, sex, HCV-RNA, AST, ALT, γ-GTP, hemoglobin and platelets showed no significant difference between NVR and relapse patients (table 1).

In DFPP + IFN-β/RBV then PEG-IFN/RBV therapy, RVR was achieved in 0% (0/12) of NVR and in 75% (6/8) of relapse patients, with a significant difference between the two groups (p = 0.001). Similarly, cEVR was achieved in 8% (1/12) of NVR and in 88% (7/8) of relapse patients, with a significant difference between the two groups (p = 0.037).

DFPP + IFN-β/RBV then PEG-IFN/RBV therapy induced an average viral reduction of 3.24 log in the 20 pa-

Table 2. Viral dynamics of DFPP + IFN-β/RBV then PEG-IFN/RBV and previous PEG-IFN/RBV treatment

Group by previous treatment response	Case No.	DFPP + IFN-β/RBV then PEG-IFN/RBV therapy									Previous PEG-IFN/RBV therapy		
		HCV RNA								response	HCV RNA		response
		before treatment log IU/ml	log drop, log IU/ml								before treatment	log drop log IU/ml 4 weeks	
			24 h	48 h	1 week	2 weeks	4 weeks	8 weeks	12 weeks				
NVR (n = 12)	1	6.2	0.5	0.6	0.2	1.3	2.2	4.1	5.0		745 fmol/l	0.1	NVR
	2	7.2	0.6	1.0	1.4	2.5	2.9	4.1	6.0		426 kIU/ml	+0.1	NVR
	3	7.6	1.1	1.1	1.5	3.3	5.4	–	7.6	cEVR	–	–	–
	4	6.0	–0.1	0.6	1.5	2.0	3.4	–	4.8		6.9 log IU/ml	0.2	NVR
	5	5.8	0.4	0.7	1.0	1.6	–0.2	0.1	–0.3		6.3 log IU/ml	0.2	NVR
	6	5.3	0	0.5	0.8	1.2	1.3	0.8	0.1		11,500 fmol/l	0.8	NVR
	7	6.8	0.6	0.3	0.3	0.4	0.4	0.3	0.3		2,900 kIU/ml	0.3	NVR
	8	5.5	1.4	0.9	1.5	1.2	1.9	1.5	1.9		782 fmol/l	0.6	NVR
	9	6.7	1.1	0.8	0.4	0.8	0.8	–	0.9		–	–	–
	10	5.5	1.0	1.5	1.1	1.0	1.4	1.9	–		6.2 log IU/ml	0.7	NVR
	11	6.3	0.5	0.8	0.3	1.1	1.3	1.0	0.7		5.8 log IU/ml	+0.2	NVR
	12	6.6	1.5	1.8	1.3	2.6	2.7	–	3.2		–	–	NVR
	Average	6.3	0.7	0.9	0.9	1.6	2.0[a]	1.7	2.7		–	0.3[a]	
Relapse (n = 8)	13	6.5	1.2	2.1	3.4	5.0	4.8	5.3	5.3		8,450 fmol/l	2.6	PR
	14	5.5	1.3	2.6	2.6	4.3	5.5	5.5	–	RVR	9,700 fmol/l	2.7	PR
	15	6.4	1.5	2.4	3.8	5.2	6.4	6.4	6.4	RVR	6.5 fmol/l	2.8	PR
	16	6.3	0.9	1.5	2.4	3.7	5.0	6.3	6.3	cEVR	540 kIU/ml	2.7	PR
	17	3.2	2.0	2.0	2.0	2.0	3.2	3.2	3.2	RVR	6.4 log IU/ml	1.2	PR
	18	7.1	2.8	3.8	4.7	5.7	7.1	7.1	7.1	RVR	–	–	PR
	19	5.6	1.7	2.7	4.4	5.6	5.6	–	5.6	RVR	6.8 log IU/ml	2.5	PR
	20	3.7	2.5	3.7	3.7	3.7	3.7	–	3.7	RVR	629 fmol/l	1.2	PR
	Average	5.5	1.7	2.6	3.4	4.4	5.2[b]	5.6	5.4		–	2.2[b]	
Total (n = 20)	Average						3.24					1.14	
NVR vs. relapse	p value	0.334	0.003	0.0003	0.0002	0.001	0.001	0.005	0.037				

[a] p = 0.01; [b] p = 0.03.

tients at week 4, with a significant difference as compared with the previous PEG-IFN/RBV therapy (1.14 log): NVR 2.0 vs. 0.3 log, p = 0.01, and relapse 5.2 vs. 2.2 log, p = 0.03; also, the early viral dynamics between NVR and relapse patients showed an average viral reduction of 0.7 vs. 1.7 log at 24 h (p = 0.003); 0.9 vs. 2.6 log at 48 h (p = 0.0003); 0.9 vs. 3.4 log at 1 week (p = 0.0002); 1.6 vs. 4.4 log at 2 weeks (p = 0.001); 2.0 vs. 5.2 log at 4 weeks (p = 0.001); 1.7 vs. 5.6 log at 8 weeks (p = 0.005); 2.7 vs. 5.4 log at 12 weeks (p = 0.037), with a significant difference at each observation point (table 2).

Correlation between cEVR and IL28B, Core Regions, ISDR, IRRDR

In the NVR group, IL28B major (MA) was demonstrated by 17% (2/12) of patients, HCV core aa 70 wild by 67% (8/12), HCV core aa 91 wild by 50% (6/12), HCV isolates involving 2 or more mutations (ISDR ≥2) by 0% (0/12) and HCV isolates involving 6 or more mutations (IRRDR ≥6) by 30% (3/10). In the relapse group, IL28B major was demonstrated by 75% (6/8) of patients, HCV core aa 70 wild by 63% (5/8), HCV core aa 91 wild by 38% (3/8), ISDR ≥2 by 0% (0/8) and IRRDR ≥6 by 50% (4/8). Among 8 of the 20 cEVR patients, IL28B major was demonstrated by 75% (6/8), HCV core aa 70 wild by 62% (5/8), HCV core aa 91 wild by 38% (3/8), ISDR ≥2 by 0% (0/8) and IRRDR ≥6 by 38% (3/8; table 3). Only IL28B major showed a statistically significant difference between cEVR and non-cEVR by univariate analysis (p = 0.018; data not shown). Multivariate logistic regression analysis also identified IL28B major as a significant determiner of cEVR (odds ratio = 24.19, p = 0.037; table 4).

Table 3. Correlation between treatment responses and IL28B, core, ISDR, IRRDR

Case No.	Response to DFPP + IFN-β/ RBV then PEG-IFN/RBV	IL28B genotype	HCV core aa 70	HCV core aa 91	ISDR mutations	IRRDR mutations
NVR (n = 12)						
1		MI	wild	mutant	1	4
2		HE	wild	wild	0–1	–
3	cEVR	MA	wild	wild	0	3
4		MA	wild	wild	0	5
5		HE	wild	wild	1	–
6		HE	wild	wild	1	2
7		HE	mutant	mutant	1	7
8		HE	wild	mutant	1	4
9		HE	mutant	mutant	0	7
10		HE	wild	wild	0	3
11		HE	mutant	mutant	1	3
12		HE	mutant	mutant	1	7
Relapse (n = 8)						
13		MA	wild	wild	0	6
14	RVR, cEVR	MA	wild	mutant	1	6
15	RVR, cEVR	MA	mutant	mutant	1	5
16	cEVR	MA	wild	wild	0	8
17	RVR, cEVR	HE	mutant	wild	1	8
18	RVR, cEVR	MA	mutant	mutant	0	4
19	RVR, cEVR	MA	wild	mutant	1	3
20	RVR, cEVR	HE	wild	mutant	0	4

MA = Homozygosity for the major sequence; HE = heterozygosity; MI = homozygosity for the minor sequence.

Discussion

Granulocyte apheresis, plasma exchange and hemofiltration are modalities that have shown a reduction in HCV RNA in blood during the treatment of HCV-infected patients for cryoglobulinemia and vasculitis [3, 12–16]. The mechanisms of plasmapheresis have been described as related to the enhancement of the effects of IFN therapy by synergistically removing HCV from the blood [17]. Hemodialysis, hemofiltration and peritoneal dialysis given to chronic dialysis patients infected with HCV significantly lower HCV RNA levels in the blood [18]. Thus, the potential for effective IFN therapy combined with early physical removal of the virus is of particular interest.

The SVR rate is closely related to viral dynamics in the early stages of IFN therapy [19], and even to the combination therapy of IFN and DFPP [3]. In PEG-IFN/RBV therapy, reduction in the HCV RNA viral load by 4 weeks is considered essential, and a 2 log reduction is a prerequisite to achieving SVR [20]. Since a daily dose of IFN-β 6 MU is effective in reducing the HCV RNA load [21], all the patients in our study receiving a daily dose of IFN-β 6 MU (especially 3 MU twice daily) for 4 weeks demonstrated a reduction of ≥2 log at 4 weeks in the viral load of 65% (13/20) of patients.

In DFPP + PEG-IFN therapy, viral reduction after 4 weeks of treatment has been 2.43 ± 1.07 log IU/ml with overall SVR achieved in 70.8% (17/24) of patients [3], and 2.79 ± 1.85 log IU/ml with cEVR in 57.5% (104/181) [4]. The current study showed a viral reduction of 3.24 ± 1.00 log IU/ml with a cEVR rate of 45% (8/20 patients). Consequently, we expect to achieve high SVR rates at the end of the 48 weeks, as in the report [3].

The prerequisite for cEVR has been emphasized in predicting SVR and non-SVR in CHC patients undergoing IFN treatment; those who do not reach EVR fail to respond to further therapy. Treatment discontinued in patients not reaching EVR would reduce drug costs by more than 20%; consequently, early confirmation of viral reduction after initiating antiviral therapy for CHC is highly desirable [19]. Re-treatment with PEG-IFN-α2a/RBV of 107 relapsers to PEG-IFN/RBV therapy has achieved cEVR in 43% [22]; re-treatment with PEG-IFN-α2a/RBV of 469 relapse and NVR patients to PEG-IFN-α2b/RBV has achieved cEVR in only 13% [23]. Re-treatment with DFPP + PEG-IFN/RBV of 73 relapse and NVR patients to previous PEG-IFN/RBV therapy has achieved cEVR in 63.0% of relapsers and in 18.9% of NVR patients [4]. Therefore, it is expected that the addition of DFPP to PEG-IFN/RBV therapy will be effective in the re-treatment of relapse and NVR patients to previous PEG-IFN/RBV therapy.

In the current study, we clarified that early viral dynamics with DFPP + IFN-β/RBV then PEG-IFN/RBV therapy is superior to the previous PEG-IFN/RBV combination therapy. There was a significant difference in viral reduction at 24 and 48 h, and 1, 2, 4, 8 and 12 weeks between NVR and relapse patients. The rate of RVR and cEVR showed a significant difference between NVR and relapse patients: among the 20 patients, RVR was obtained in 75% (6/8) of relapse patients but in 0% (0/12) of NVR patients, and cEVR in 88% (7/8) of relapse patients but in only 8% (1/12) of NVR patients. On the basis of the above results, DFPP + IFN-β/RBV then PEG-IFN/RBV therapy is indicated more for relapse than for NVR patients. We could conclude that relapse patients would be better candidates than NVR patients.

Table 4. Multivariate analysis of factors associated with cEVR

Factor	Category	Patients	Odds ratio	95% CI	p value
IL28B genotype	major (MA)	75% (6/8)	24.19	1.22–479.58	0.037
	minor (HE+MI)	25% (2/8)			
HCV core aa 70	wild	62% (5/8)	0.82	0.03–19.43	0.903
	mutant	38% (3/8)			
HCV core aa 91	wild	38% (3/8)	3.18	0.12–87.4	0.494
	mutant	62% (5/8)			
ISDR mutations	≥2	0% (0/8)	0.83	0.05–14.2	0.898
	≤1	100% (8/8)			
IRRDR mutations	≥6	38% (3/8)	0.2	0.01–3.76	0.285
	≤5	62% (5/8)			

Recently, a new triple combination therapy comprising PEG-IFN-α, RBV, and a protease inhibitor such as telaprevir or boceprevir has been approved in the US for CHC patients with genotype 1 and high viral loads. Results have shown SVR was attained in 70% of naïve, 80% of relapse and 30% of NVR patients. The discontinuation rate due to adverse events such as anemia and skin eruption has not been low [24].

In this study, 80% of cEVR patients are expected to attain SVR, and despite the small number of subjects, the estimated SVR rate in our relapse patients is comparable to that of the new triple combination therapy. In view of the adverse events attending the triple combination therapy, however, DFPP could become an alternative for CHC patients with genotype 1b and high viral loads.

Among viral genetic polymorphisms such as HCV core, ISDR, IRRDR, and host genetic factor such as IL28B, only IL28B was defined as a determinant of cEVR by univariate and multivariate analyses.

Because of the small number of patients in the present study, further prospective study is needed to identify eligible candidates for DFPP + IFN-β/RBV then PEG-IFN/RBV therapy and independent predictive factors for cEVR and SVR.

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Disclosure Statement

None of the authors has any conflict of interest.

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Clinical Characteristics of Non-B Non-C Hepatocellular Carcinoma: A Single-Center Retrospective Study

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Key Words
Liver cancer · Non-B non-C hepatocellular carcinoma · Nonalcoholic steatohepatitis · Alcohol · Diabetes

Abstract
Background/Aims: To clarify risk factors and clinical features of both hepatitis B surface antigen and anti-HCV negative hepatocellular carcinoma (NBNC-HCC). ***Methods:*** HCC patients (n = 1,109) diagnosed at a single center were categorized based on the presence of serum hepatitis B surface antigen and HCVAb. Clinical characteristics of 127 NBNC-HCC patients were evaluated. ***Results:*** NBNC-HCC patients were stratified as those with alcoholic liver disease (ALD-HCC, n = 42) and alcohol-unrelated liver disease (non-ALD-HCC, n = 85). Compared with the ALD-HCC group, the non-ALD-HCC group had a higher prevalence of diabetes (p = 0.015), larger tumor size (p = 0.007), and higher tumor marker levels (p = 0.014). Liver function results were significantly worse in ALD-HCC than in non-ALD-HCC. Although the ALD-HCC group had a higher tendency toward recurrence than the non-ALD-HCC group, survival rates were similar between groups (p = 0.352). ***Conclusion:*** Alcohol consumption was the most common etiologic factor for NBNC-HCC, and diabetes may be related to the development of HCC in non-ALD-HCC patients. Non-ALD-HCC tended to be diagnosed at a more advanced stage, whereas liver function was worse, and tumor recurrence rate was higher in ALD-HCC patients. Further examination of the risk factors and establishment of a precise surveillance system are necessary for early diagnosis and the development of curative therapies for NBNC-HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related deaths worldwide [1, 2]. In Japan, chronic hepatitis C virus (HCV) infection is considered to be the most significant risk factor for the development of HCC, and the second most important factor is hepatitis B virus infection. Based on a report by the Liver Cancer Study Group of Japan, approximately 15% of HCC patients in Japan are hepatitis B surface antigen (HBsAg) positive (B-HCC) and 70% are anti-HCV (HCVAb) positive (C-HCC) [3]. Recent progress in the management of patients with viral hepatitis by specific antiviral therapy, including interferon and nucleotide

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analogues, however, has led to better prevention of cancer development and improved disease prognosis [4–7]. On the other hand, the number of patients that are both HBsAg- and HCVAb-negative HCC [non-B non-C HCC (NBNC-HCC)] has increased, and NBNC-HCC is reported to account for 12–20% of all HCC cases in Japan [3, 8].

NBNC-HCC is considered to be associated with several etiologic factors such as alcoholic liver injury, autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis, and nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH). In addition, a variety of clinical factors are also involved in the development and progression of NBNC-HCC, including age, sex, alcohol consumption, and diabetes mellitus [9, 10]. There are only a few reports, however, on the clinical characteristics of NBNC-HCC, and the actual state of NBNC-HCC has not been fully elucidated [3, 8, 11, 12].

Due to the lack of understanding of the clinical features of NBNC-HCC, neither early detection nor improvement in prognosis has been achieved in patients with NBNC-HCC. Therefore, it is important to understand the complex interactions of the risk factors and clinical features of NBNC-HCC. In this study, we aimed to clarify the clinical characteristics of NBNC-HCC and discuss the etiology of NBNC-HCC.

Methods

Patients

A total of 1,109 HCC patients diagnosed at Osaka Red Cross Hospital from April 1, 2004, to March 31, 2010, were enrolled in the study. The diagnosis of HCC was made based on the presence of both characteristic imaging findings and increases in serum tumor markers; the diagnostic criteria for HCC via imaging was based on previous reports of hyperattenuation at the arterial phase and hypoattenuation at the portal phase in the tumor, determined by dynamic computed tomography and/or magnetic resonance imaging [13]. In addition, increases in serum tumor markers such as α-fetoprotein, des-γ-carboxyprothrombin, or *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein, were also required for the diagnosis of HCC. In doubtful cases, the diagnosis was confirmed by pathologic findings based on liver biopsies obtained under ultrasound guidance.

Classification of HCC according to Etiology

The presence of serum HBsAg and HCVAb was determined in all patients using enzyme immunoassay kits (FUJIREBIO, Tokyo, Japan). Based on the presence of serum antigens/antibodies, the patients were categorized into four groups: B-HCC (HBsAg positive), C-HCC (HCVAb positive), BC-HCC (both HBsAg and HCVAb positive), and NBNC-HCC (both HBsAg and HCVAb negative). AIH was diagnosed based on the simplified diagnostic criteria proposed by the International Autoimmune Hepatitis Group [14], and PBC was diagnosed based on a PBC scoring system [15]. All NBNC-HCC patients, except those with AIH and PBC, were then further divided into two groups, the alcoholic liver disease group (ALD-HCC) and the nonalcoholic group (non-ALD-HCC group) depending on alcohol consumption based on the following criteria: patients whose daily alcohol consumption was over 80 g were included in the ALD-HCC group, and the remaining patients were included in the non-ALD-HCC group. We investigated the background characteristics between the two groups, including age, sex, diabetes, body mass index, hypertension, biochemical test results for liver function, Child-Pugh grade [16], tumor size, tumor number, portal invasion, TNM stage [17], and tumor markers at the time of diagnosis. The diagnosis of diabetes was based on the following criteria: random glucose >200 mg/dl or fasting glucose >126 mg/dl, or hemoglobin A1c >6.5% on two occasions. Hypertension was diagnosed when patients were pharmacologically treated for hypertension or if their arterial pressure was ≥140/90.

The treatment for HCC was performed based on a consensus-based treatment algorithm for HCC proposed by the Japanese Society of Hepatology [2]. Hepatectomy (surgery) or local ablation (radiofrequency ablation, RFA) was performed for 3 or fewer nodules, if the nodules were 3 cm or smaller with no extra-hepatic lesions, good liver function results, and no vascular invasion. Even if the number of nodules was 3 or fewer, if the tumor size exceeded 3 cm, hepatectomy or transcatheter arterial embolization (TACE) was selected. For cases with 4 or more lesions, TACE or transcatheter arterial infusion (TAI) was selected, and resection was considered.

We compared the overall survival rates and cumulative recurrence rates after initial radical treatment between the ALD-HCC and non-ALD-HCC groups. We also studied the factors contributing to recurrence after initial remission and survival for both ALD-HCC and non-ALD-HCC patients. Initial radical treatment (initial remission) was defined as follows: all of the HCC nodules (single/multiple) had disappeared following initial treatment, and no local recurrence or new tumors were detected on computed tomography within 6 months after the initial treatment.

Statistical Analysis

Results are expressed as the mean values with standard deviation or the number (percentage) of patients with each variable. Comparison of background characteristics between ALD-HCC and non-ALD-HCC patients was conducted using Fisher's exact test and Mann-Whitney U test. Overall survival rates and cumulative recurrence rates after initial remission were calculated using the Kaplan-Meier method and the differences between ALD-HCC and non-ALD-HCC patients were examined by log-rank test. The Cox proportional hazards model was used for multivariate analysis for factors that influenced survival and recurrence after the initial remission and performed separately for ALD-HCC and non-ALD-HCC. Statistical data analysis was performed using the SPSS program, version 18.0 (SPSS, Chicago, Ill., USA). All reported p values were two tailed, and statistical significance was set at $p < 0.05$.

Results

Patients

The 1,109 HCC patients in our study comprised 177 NBNC-HCC (16%), 127 B-HCC (11%), 783 C-HCC (71%), and 22 BC-HCC (2%) patients. Of the 177 NBNC-HCC patients, 8 (4 diagnosed with AIH and 4 diagnosed with PBC) were excluded from the study. In addition, 42 other patients were excluded for the following reasons: natural death (n = 15), transfer to other hospitals (n = 18), and missing data (n = 9). We examined the detailed characteristics of the remaining 127 patients. Based on alcohol consumption, 42 patients were included in the ALD-HCC group and the other 85 patients were included in the non-ALD-HCC group.

Clinical Characteristics of Patients with HCC: Comparison between ALD-HCC and Non-ALD-HCC Groups

The clinical features of the 127 NBNC-HCC patients were investigated and compared between the ALD-HCC and non-ALD-HCC groups (table 1). Mean age at the time of diagnosis of HCC was significantly higher in non-ALD-HCC patients than in the ALD-HCC group (71.4 vs. 66.4, respectively; $p < 0.001$). The proportion of men among non-ALD-HCC patients was significantly lower than that among ALD-HCC patients (78 vs. 93%, respectively; p = 0.034). The prevalence of diabetes in the non-ALD-HCC group was significantly higher than that in the ALD-HCC group (56 vs. 33%, respectively; p = 0.015). Aspartate aminotransferase, alanine aminotransferase, and total bilirubin were significantly higher in the ALD-HCC group than in the non-ALD-HCC group. In addition, albumin levels and prothrombin time were significantly lower in the ALD-HCC group than in the non-ALD-HCC group. Maximum tumor size in diameter (cm) at the time of diagnosis was significantly larger (5.22 vs. 4.20, respectively; p = 0.007), and the proportion of patients with α-fetoprotein levels greater than 100 ng/ml was significantly higher (32 vs. 12%, respectively; p = 0.014) in the non-ALD-HCC group than in the ALD-HCC group. The differences between groups in body mass index, hypertension, anti-HBc positivity, Child-Pugh grade, number of tumors, portal invasion, the TNM stage (I/II/III/IV), or des-γ-carboxyprothrombin were not significant.

Treatment

According to the consensus-based treatment algorithm for HCC proposed by the Japanese Society of Hepatology [2], initial treatment for NBNC-HCC patients was performed as follows: surgery (n = 7), RFA (n = 21), TACE/TAI (n = 12), and no treatment (n = 2) of the 42 ALD-HCC patients, and surgery (n = 32), RFA (n = 26), TACE/TAI (n = 26), and no treatment (n = 1) of the 85 non-ALD-HCC patients. The proportion of patients receiving surgery as the initial treatment was significantly higher in the non-ALD-HCC group than in the ALD-HCC group (38 vs. 17%, respectively; p = 0.016). The proportion of RFA was significantly lower in the non-ALD-HCC group than in the ALD-HCC group (31 vs. 50%, respectively; p = 0.033). In total, 22 of 42 ALD-HCC patients and 50 of 85 non-ALD-HCC patients received initial radical treatment, and there was no significant difference (52 vs. 59%, respectively; p = 0.491) between groups.

HCC Recurrence Rates after the Initial Radical Treatment

Of the 22 ALD-HCC patients with initial remission, recurrent HCC was detected in 14 patients during a median follow-up period of 28 months (range: 12–83 months). Of the 50 non-ALD-HCC patients with initial remission, recurrent HCC was detected in 19 patients during a median follow-up period of 23 months (range: 9–81 months). The 5-year cumulative recurrence rates in

Table 1. Clinical characteristics of NBNC-HCC

Group	Alcohol (n = 42)	Non-alcohol (n = 85)	p value
Age, years	66.4 ± 7.6	71.4 ± 9.1	<0.001
Males/females	39/3	66/19	0.034
DM +/–	14/28	48/37	0.015
BMI >25/≤25, %	41/59	41/59	NS
Hypertension +/–	13/29	35/50	NS
HBcAb +/–	24/18	37/48	NS
AST, IU/l	59.9 ± 35.5	50.9 ± 49.7	<0.001
ALT, IU/l	44.8 ± 33.3	35.9 ± 34.6	0.042
T-Bil, mg/dl	1.12 ± 0.65	0.92 ± 0.64	0.020
Albumin, g/dl	3.72 ± 0.54	3.92 ± 0.51	0.022
Prothrombin, %	81.4 ± 21.8	90.3 ± 20.3	0.037
Child-Pugh grade A/B/C	28/13/1	67/14/4	NS
Maximum tumor size, cm	4.20 ± 3.67	5.22 ± 3.88	0.007
Single/multiple tumors	21/21	50/35	NS
Portal invasion +/–	5/37	13/72	NS
TNM stage I/II/III/IV	10/16/11/5	6/43/23/13	NS
AFP >100/≤100, ng/ml	5/37	27/58	0.014
DCP >100/≤100, mAu/ml	26/16	58/27	NS

NS = Not significant; AFP = α-fetoprotein; DCP = des-γ-carboxyprothrombin; T-Bil = total bilirubin.

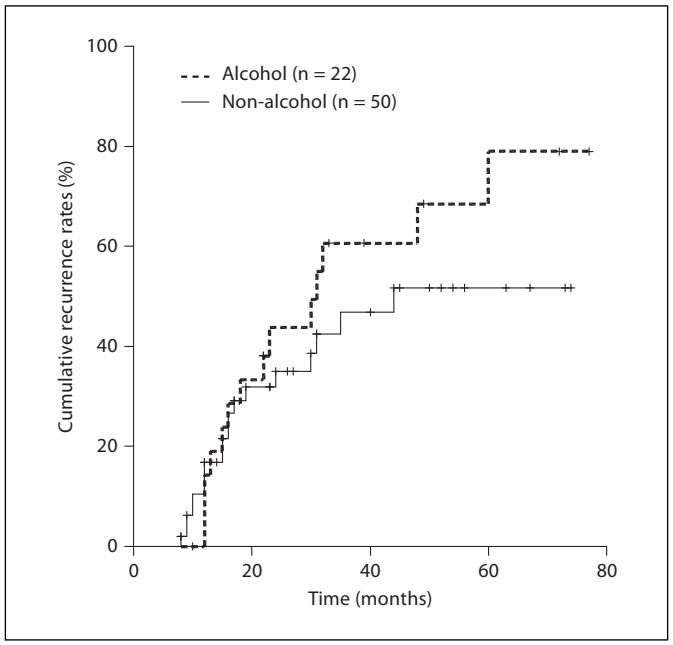

Fig. 1. Cumulative recurrence rates after initial remission. The 5-year cumulative recurrence rates in ALD-HCC and non-ALD-HCC patients were 69 and 52%, respectively. Although the ALD-HCC group was considered to have higher recurrence tendency than the non-ALD-HCC group, the difference was not statistically significant (p = 0.304, log-rank test).

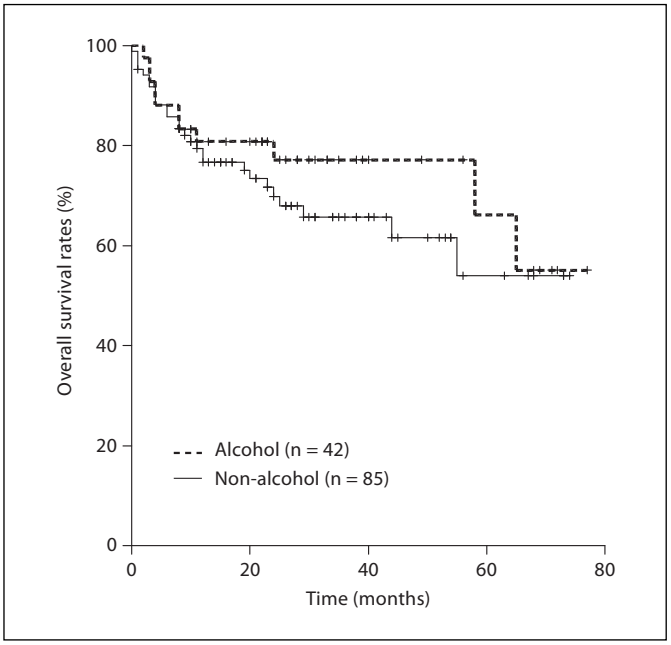

Fig. 2. Overall survival rates. The 5-year survival rates of ALD-HCC and non-ALD-HCC patients were 66 and 53%, respectively, and the difference between groups was not significant (p = 0.352, log-rank test).

ALD-HCC and non-ALD-HCC patients were 69 and 52%, respectively. Although the ALD-HCC group was considered to have higher recurrence tendency than the non-ALD-HCC group, the difference was not statistically significant (p = 0.304, fig. 1).

To identify the factors contributing to the cumulative recurrence of ALD-HCC and non-ALD-HCC, we performed univariate analysis for several factors, but we detected no significant factor responsible for recurrence after the initial radical treatment (data not shown).

Survival Rates

The 5-year survival rates of ALD-HCC and non-ALD-HCC patients were 66 and 53%, respectively, and the difference between groups was not significant (p = 0.352, fig. 2). To identify the factors contributing to the overall survival of ALD-HCC and non-ALD-HCC patients, the Cox proportional hazards model was performed for multivariate analysis for factors that were considered to significantly contribute to survival on univariate analysis (table 2). This analysis revealed that portal invasion of HCC was a significant factor for survival in ALD-HCC patients. On the other hand, a higher α-fetoprotein level (over 100 ng/ml) was a significant factor contributing to survival in non-ALD-HCC patients.

Discussion

The number of patients with hepatic virus-unrelated HCC, i.e. NBNC-HCC, has been increasing annually in Japan [3, 5, 8]. Indeed, the current retrospective cohort study confirmed that the number and proportion of NBNC patients has increased in Osaka Red Cross Hospital; the incidence of NBNC-HCC patients among all HCC patients ranged from 12.8% in 2004 to 22.6% in 2009 (data not shown). The clinical characteristics and prognosis of NBNC-HCC, however, have not been fully elucidated. Therefore, we studied in detail the clinical background and prognosis of a large number of NBNC-HCC patients in a single center, and compared the clinical characteristics between those with alcohol-related and those with alcohol-unrelated HCC.

Table 2. Factors associated with survival according to Cox proportional hazard analysis

Risk factors	Alcohol (n = 42)		Non-alcohol	
	hazard ratio (95% CI)	p	hazard ratio (95% CI)	p
Maximum tumor size >3 cm	NS	NS	NS	NS
Portal invasion	11.9 (0.90–157.4)	0.05	NS	NS
Tumor stage III/IV	NS	NS	NS	NS
AFP >100 ng/ml	NS	NS	4.63 (1.57–13.6)	0.005
DCP >100 mAU/ml	NS	NS	NS	NS

The findings of the present study demonstrated that diabetes was significantly more frequent in the non-ALD-HCC group than in the ALD-HCC group. An earlier large-scale epidemiologic study of 824,263 registered patients showed that among men with diabetes, the risk of HCC is doubled, and this increase in risk is independent of ALD, viral hepatitis, or demographic features [18]. Another population-based study of 8,244 patients reported that diabetes is associated with a 2- to 3-fold increase in the risk of HCC, regardless of the presence of other major HCC risk factors and that diabetes is an independent risk factor for HCC [19]. In Japan, the diagnosis of diabetes continues to increase. Indeed, between 1997 and 2007, people classified as 'strongly suspected of having diabetes' increased from approximately 6.9 to 8.9 million, and those classified as 'people for whom the possibility of diabetes cannot be precluded' increased from approximately 6.8 to 13.2 million [20]. This tendency may be associated with the increased incidence of NBNC-HCC patients.

The pathophysiology underlying the increased risk of HCC with diabetes is not certain. Diabetes is associated with NAFLD, including NASH with specific hepatic insulin resistance [21, 22]. Insulin resistance facilitates peripheral lipolysis and the accumulation of free fatty acids in the liver, thus leading to NAFLD. Hepatocellular injury, inflammation, and, eventually, hepatic fibrosis can result in the occurrence of HCC [18]. A recent study indicated that diabetes-related NAFLD/NASH with elevated liver enzymes is associated with a clinically significant risk of developing end-stage liver disease, including HCC [23]. The annual NAFLD cumulative incidence of HCC is reported to be 2.6% in patients with NASH cirrhosis [24]. Thus, in non-ALD-HCC patients, a higher prevalence of diabetes could contribute to accumulating liver damage as NASH, and eventually HCC.

The diagnosis of NASH is based on following pathologic findings: hepatic steatosis, hepatocellular ballooning, lobular inflammation, pericellular or perisinusoidal fibrosis, and Mallory body formation [25]. It is difficult to evaluate the role of hepatocyte fat deposition in the development of HCC; however, because the fat deposits in hepatocytes tend to disappear as liver fibrosis progresses; this phenomenon is referred to as burnout NASH [26]. Indeed, based on histological analysis of tissues from hepatectomy, it was difficult to confirm pathologic evidence of NASH. In 6 patients with non-ALD-HCC surgically treated, NASH-like pathologic findings, such as hepatic steatosis, hepatocellular ballooning, and Mallory body formation were identified, however, suggesting that some proportion of the non-ALD-HCC group had NASH-based HCC.

In the present study, tumor size was larger, and tumor marker levels were higher in the non-ALD-HCC group than in the ALD-HCC group. This is in part due to the lower opportunity of non-ALD-HCC patients to undergo annual surveillance for chronic liver disease, and the tendency to be diagnosed with definite HCC at a more advanced stage. Surveillance has not been established for patients with NBNC-HCC because the risk factors are not well understood, other than excessive alcohol drinking. As a result, cryptogenic HCC patients, especially those with non-ALD-HCC, are not detected until they reach an advanced stage [27]. Although the general consensus is that metabolic factors are related to the occurrence of HCC, we have not established a method for determining the high risk group. Among patients with metabolic factors, more detailed examination of the possible carcinogenic factors is necessary for earlier detection of HCC.

Liver function results were altogether significantly worse in ALD-HCC patients than in non-ALD-HCC patients. Because liver function in the ALD-HCC group was worse than that in the non-ALD-HCC group, those in the ALD-HCC group were thought to undergo more liver injury, and this cumulative injury may increase the occur-

rence of HCC. Therefore, poor liver function could be the major reason of higher recurrence rate after initial remission of ALD-HCC than non-ALD-HCC. As a result, ALD-HCC and non-ALD-HCC patients had a similar prognosis. The ALD-HCC group was superior to the non-ALD-HCC group in the tumor state, such as tumor size and tumor marker at the time of diagnosis, but inferior to the non-ALD-HCC group in liver function, leading to recurrence. These good and bad points counteract each other and result in an equivalent prognosis between groups.

In conclusion, although the number of patients with NBNC-HCC has been increasing annually, many features of NBNC-HCC remain unknown. Based on the present study, the most common etiologic factor for NBNC-HCC was alcohol, and diabetes may be related to the occurrence of HCC in patients with non-alcohol-related liver disease. The comparison between groups revealed that non-ALD-HCC tended to be detected at a more advanced stage, whereas liver function in ALD-HCC was worse. Finally, the prognosis was equivalent between groups. Further examination of the risk factors for NBNC-HCC and establishing a precise surveillance system are needed to diagnose HCC earlier and develop curative therapies.

Disclosure Statement

All authors disclose no conflicts.

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Association of Interleukin-28B and Hepatitis C Genotype 1 with a High Viral Load and Response to Pegylated Interferon plus Ribavirin Therapy

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Key Words
Chronic hepatitis C · Interleukin-28B · Pegylated interferon plus ribavirin therapy · Drug dose · Core aa70

Abstract
Background: Pegylated interferon (PEG-IFN) plus ribavirin therapy is the current standard treatment for chronic hepatitis C (CHC) genotype 1 with high viral load. A common genetic variation near the IL28B gene has been found to affect the response to PEG-IFN plus ribavirin therapy for CHC. The aims of this study were to analyze the association between the rs8099917 genotype and treatment response in a cohort study of CHC. ***Methods:*** This study evaluated clinical and laboratory parameters retrospectively in a cohort of 122 patients with chronic hepatitis C with genotype 1 and a high viral load who received PEG-IFN plus ribavirin therapy. We carried out univariate and multivariate statistical analyses of parameters and clinical responses. ***Results:*** Sixty-three of 122 patients (51.6%) had sustained virological responses (SVRs). Patients with the rs8099917 genotype TT achieved significantly higher SVR rates ($p < 0.01$). Univariate analysis revealed that SVRs were associated with BMI, fibrosis, albumin, total cholesterol, PEG-IFN dose, ribavirin dose and the rs8099917 genotype. Multivariate analysis revealed that the rs8099917 genotype (odds ratio 7.434, 95% CI 2.278–24.257, $p = 0.001$) and total PEG-IFN dose (odds ratio 7.162, 95% CI 1.565–18.15, $p = 0.007$) were significant factors. ***Conclusions:*** The rs8099917 genotype and total PEG-IFN dose were associated with SVR in patients with hepatitis C virus genotype 1.

Introduction

Infection with hepatitis C virus (HCV) is a global health problem, with an estimated 120–130 million carriers worldwide [1]. HCV is a causative agent of acute and chronic hepatitis, as well as liver cirrhosis and hepatocellular carcinoma [2–4]. Antiviral therapy with pegylated interferon (PEG-IFN) and ribavirin can be efficacious for patients with chronic hepatitis C and the prognosis of patients from whom HCV is successfully eradicated improves markedly [5]. However, this treatment is effective only in 50% of patients and has severe side effects, often requiring discontinuation or dose modification [6]. Variations in treatment response have been investigated and several contributory factors identified, including age, liver fibrosis, HCV genotype, HCV RNA levels and race.

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Among the viral factors, amino acid (aa) substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the IFN sensitivity-determining region (ISDR) of the NS5A protein have been shown to be associated with treatment outcome [7, 8].

Host factors also have been shown to be associated with the outcome of therapy, including age, sex, race, liver fibrosis and obesity [9, 10]. Recently, several highly correlated common single nucleotide polymorphisms on a linkage disequilibrium block in the vicinity of three IFN-γ genes on chromosome 19, encoding IFN-γ_1 *(IL29)*, -γ_2 *(IL28A)*, and -γ_3 *(IL28B)*, have been implicated in three genome-wide association studies in affecting the response to PEG-IFN/ribavirin among patients infected with HCV genotype 1 [11–13].

In this study, we investigated whether the rs8099917 polymorphisms are associated with susceptibility to HCV infection and with response to therapy with PEG-IFN and ribavirin in patients with chronic HCV infection.

Patients and Methods

Patients

This was a retrospective trial at Kinki University Hospital. A total of 122 patients with chronic hepatitis C with genotype 1 and a high viral load and who completed treatment with a combination of PEG-IFN-α2b and ribavirin between January 2005 and December 2008 were enrolled. The serum HCV RNA was measured before commencement of treatment and every month during the treatment by quantitative Amplicor HCV monitor assay (version 2.0, Roche Diagnostics; detection limit 500 IU/ml). When the quantitative assay showed undetectable levels of HCV RNA, a qualitative Amplicor HCV assay (version 2.0, Roche Diagnostics; detection limit 50 IU/ml) was used. Viral aa substitutions were determined for the ISDR in NS5A and substitutions at aa positions 70 and 91 in the core region, as described previously [7, 8, 14]. We genotyped each patient for the IL28B single nucleotide polymorphisms reported previously to be associated with treatment outcome, rs8099917 [12]. All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of Kinki University Hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

Treatment

All patients were treated with PEG-IFN-α2b (Peg-Intron; Schering-Plough, Kenilworth, N.J., USA) plus ribavirin (Rebetol; Schering-Plough) for 48 weeks. PEG-IFN-α2b was administered at 1.5 μg/kg subcutaneously each week. Ribavirin was administered orally at a dose of 600 mg/day to patients weighing <60 kg, 800 mg/day to those weighing 60–80 kg, 1,000 mg/day to those weighing 80–100 kg, and 1,200 mg/day to those weighing >100 kg. However, the patients who discontinued treatment because of side effects of previous IFN plus ribavirin therapy were administered 400 mg/day. The dose reduction and discontinuation of the combination treatment was determined according to standard protocols.

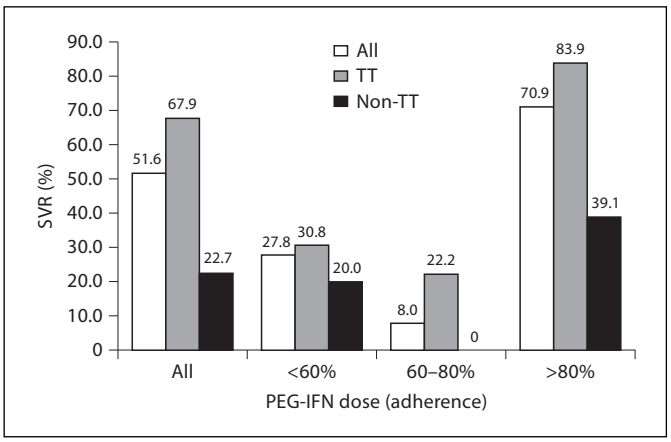

Fig. 1. Effect of IFN plus ribavirin therapy on patients with genotype 1 infection. SVR rates were analyzed according to the IL28B genotype, PEG-IFN dose and IL28 genotype.

Statistical Analysis

Data are expressed as median (range) values. Differences between groups were examined for significance using the Mann-Whitney U test and Fisher's exact test where appropriate. Multivariate analysis was performed using the logistic regression model. All the analyses described above were performed using the SPSS program (version 11.5; SPSS, Chicago, Ill., USA).

Results

Patient Characteristics

Baseline patient characteristics are shown in table 1. The median age on therapy was 61 (range 20–78) years. Sustained virological response (SVR) was achieved by 51.6% (63 of 122 patients). According to the genetic variation of rs8099917, SVR was achieved by 67.9% (53 of 78 patients) and 22.7% (10 of 44 patients) of patients with TT and non-TT, respectively (fig. 1). According to the substitution of core aa70, SVR was achieved by 46.1% (12 of 26 patients) and 21.0% (4 of 19 patients) of patients with wild type and mutant type, respectively.

Predictive Factors for SVR

As shown in table 2, univariate analysis revealed that SVR was associated with BMI, fibrosis, albumin, total cholesterol, total PEG-IFN dose, ribavirin dose and the rs8099917 genotype. Using BMI, fibrosis, albumin, total cholesterol, PEG-IFN dose, ribavirin dose and the rs8099917 genotype, multivariate analysis was performed using the logistic regression model. This analysis revealed that the rs8099917 genotype (odds ratio 7.434, 95% CI

Table 1. Baseline characteristics of the patients

	All (n = 122)	SVR (n = 63)	TR (n = 14)	NVR (n = 45)
Males/females	54/68	31/32	6/8	17/28
Age, years	61 (20–78)	62 (20–78)	60.5 (47–75)	59 (36–75)
BMI	22.9 (15.2–29.1)	22.2 (15.2–27.3)	24.55 (18.4–29.0)	23.5 (18.1–29.1)
Fibrosis (0–2/3–4)	66/18	36/4	9/2	21/12
Activity (0–1/2–3)	33/51	17/23	7/4	9/24
ISDR (0, 1/≥2)	30/18	9/9	4/2	17/7
aa70 (wild type/mutant)	26/19	12/4	5/1	9/14
aa91 (wild type/mutant)	29/19	12/4	4/2	13/13
WBC, /l	4.6×10^9 (1.4×10^9–8.1×10^9)	4.8×10^9 (2.1×10^9–7.8×10^9)	4.6×10^9 (3.0×10^9–6.0×10^9)	4.4×10^9 (1.4×10^9–8.1×10^9)
Hemoglobin, g/dl	13.6 (9.7–17.6)	13.7 (10.7–16.6)	13.75 (10.8–17.1)	13.2 (9.7–17.6)
Platelets, $\times 10^{-4}$/l	15.3×10^6 (4.0×10^6–39.8×10^6)	16.7×10^6 (4.0×10^6–39.8×10^6)	17.15×10^6 (6.8×10^6–27.1×10^6)	13.0×10^6 (5.5×10^6–30×10^6)
AST, IU/l	48 (17–146)	43 (18–138)	36 (17–123)	64.0 (20–146)
ALT, IU/l	49.5 (13–218)	39 (15–182)	42 (13–218)	57 (18–199)
γGTP, IU/l	32.5 (10–326)	30 (10–297)	29 (14–326)	43 (15–317)
Albumin, g/dl	4.2 (3.1–4.9)	4.3 (3.4–4.8)	4.2 (3.7–4.6)	4.0 (3.1–4.9)
Total cholesterol, mg/dl	168 (117–240)	176 (119–240)	167 (134–239)	160 (117–235)
Viral load	2,300 (103–40,000)	2,100 (110–40,000)	4,900 (500–25,000)	2,200 (103–22,000)
PEG-IFN-α2b, μg	80 (60–140)	80 (60–140)	90 (60–120)	80 (60–120)
PEG-IFN-α2b/kg/week, μg/kg/week	1.37 (0.31–1.95)	1.42 (0.31–1.95)	1.40 (0.71–1.59)	1.16 (0.51–1.66)
Ribavirin, mg	600 (400–800)	600 (400–800)	600 (400–800)	600 (400–800)
Ribavirin/kg/day, mg/kg/day	10.1 (2.85–15.59)	10.22 (4.21–15.59)	9.52 (4.48–12.9)	9.76 (2.85–12.94)
rs8099917 (TT/non-TT)	78/44	53/10	9/5	16/29

For categorical data, the number of patients in each category is shown. For continuous data, the median and range are displayed.

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; γGTP = γ-glutamyl transpeptidase; NVR = non-virological response; TR = transient response; WBC = white blood cells.

Table 2. Predictive factors for SVR using univariate analysis

	SVR (n = 63)	TR+NVR (n = 59)	p
Males/females	31/32	23/36	0.278
Age, years	62 (20–78)	59 (36–75)	0.216
BMI	22.2 (15.2–27.3)	23.7 (18.1–29.1)	0.005
Fibrosis (0–2/3–4)	36/4	30/14	0.149
Activity (0–1/2–3)	17/23	16/28	0.656
ISDR (0, 1/2≥)	9/9	21/9	0.222
aa70 (wild type/mutant)	12/4	14/15	0.118
aa91 (wild type/mutant)	12/4	17/15	0.212
WBC, /l	4.8×10^9 (2.1×10^9–7.8×10^9)	4.5×10^9 (1.4×10^9–8.1×10^9)	0.232
Hemoglobin, g/dl	13.7 (10.7–16.6)	13.4 (9.7–17.6)	0.418
Platelets, $\times 10^{-4}$/l	16.7×10^6 (4.0×10^6–39.8×10^6)	14.5×10^6 (5.5×10^6–30×10^6)	0.054
ALT, IU/l	39 (15–182)	54 (13–218)	0.055
γGTP, IU/l	30 (10–297)	34 (14–326)	0.072
Albumin, g/dl	4.3 (3.4–4.8)	4.1 (3.1–4.9)	0.018
Total cholesterol, mg/dl	176 (119–240)	161 (117–239)	0.018
Viral load	2,100 (110–40,000)	2,640 (103–25,000)	0.396
PEG-IFN-α2b, μg	80 (60–140)	80 (60–120)	0.184
PEG-IFN-α2b/kg/week, μg/kg/week	1.42 (0.31–1.95)	1.18 (0.51–1.66)	<0.001
Ribavirin, mg	600 (400–800)	600 (600–800)	0.196
Ribavirin/kg/day, mg/kg/day	10.22 (4.21–15.59)	9.76 (2.8–12.9)	0.014
rs8099917 (TT/non-TT)	53/10	25/34	<0.001

For categorical data, the number of patients in each category is shown. For continuous data, the median and range are displayed.

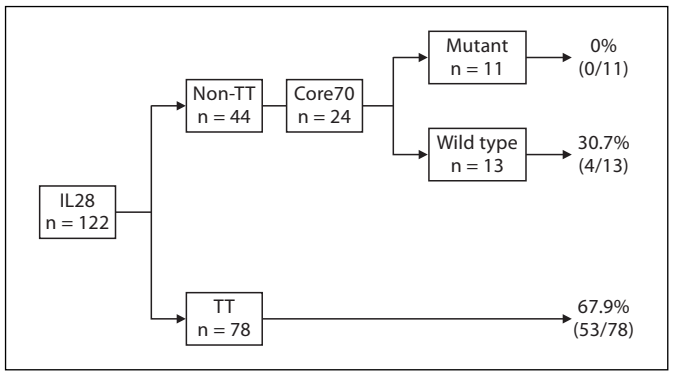

Fig. 2. Predicting SVR by core70 in combination with the IL28B genotype. SVR was achieved by 67.9% of patients with the rs8099917 genotype TT. Among the patients with the rs8099917 genotype TT, those with core70 wild type achieved a high rate of SVR (30.7%), and the SVR rate was worst in patients with core 70 mutant type (0%).

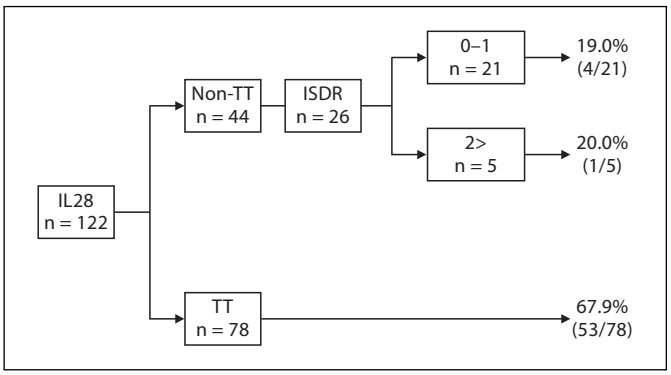

Fig. 3. Predicting SVR by ISDR in combination with the IL28B genotype. Among patients with the rs8099917 genotype TT, the SVR rate did not differ between ISDR wild type (20%) and ISDR mutant type (19.0%).

2.278–24.257, p = 0.001) and PEG-IFN dose (odds ratio 7.162, 95% CI 1.565–18.15, p = 0.007) were significant factors (table 3).

Relationship between PEG-IFN Dose and the rs8099917 Genotype

We evaluated the relationship between the cumulative PEG-IFN dose and the rs8099917 genotype (fig. 1). The cumulative PEG-IFN dose was classified as <60%, 60–80% and >80%. Among the patients with <60% of the PEG-IFN dose, SVR was achieved by 30.8% (4 of 13 patients), 20.0% (1 of 5 patients) and 27.8% (5 of 18 patients) of patients with genotype TT, non-TT and in total, respectively. Among the patients with 60–80% of PEG-IFN dose, SVR was achieved by 22.2% (2 of 9 patients), 0.0% (0 of 16 patients) and 8.0% (2 of 25 patients) of patients with genotype TT, non-TT and in total, respectively. Finally, among the patients with >80% of PEG-IFN dose, SVR was achieved by 83.9% (47 of 56 patients), 39.1% (9 of 23 patients) and 70.9% (56 of 79 patients) of patients with genotype TT, non-TT and in total, respectively.

Virological Response according to the rs8099917 Genotype, Core aa70 and ISDR

The SVR rates according to the rs8099917 genotype and core aa70 are shown in figure 2. Among patients with the rs8099917 genotype TT, SVR was achieved by 67.9% (53 of 78 patients). Among patients with the rs8099917 genotype non-TT, a marginally higher proportion of patients with core 70 wild type (30.7%) achieved SVR than did patients with core 70 mutant type (0%; p = 0.1323).

Table 3. Predictive factors for SVR using multivariate analysis (logistic regression model)

	Odds ratio	95% CI	p value
rs8099917 (TT/non-TT)	7.434	2.278–24.257	0.001
PEG-IFN dose (1.37 μg/kg/week)	7.162	1.565–18.15	0.007

The SVR rates according to the rs8099917 genotype and ISDR are shown in figure 3. Among patients with the rs8099917 genotype non-TT, the SVR rate did not differ between ISDR wild type (20%) and ISDR mutant type (19.0%; p = 1).

Discussion

In this study, we showed by multivariate analysis that the rs8099917 genotype and total PEG-IFN dose contribute to the successful outcome of PEG-IFN plus ribavirin combination treatment for infection with HCV genotype 1.

Several host and viral factors contribute to SVR in PEG-IFN plus ribavirin combination treatment of Japanese patients infected with HCV genotype 1 and who have high viral loads. The host factors include younger age, male gender, mild liver fibrosis, platelet count, LDL cholesterol values and γ-glutamyl transpeptidase values.

Recent reports have shown that the rs8099917 genotype was a pretreatment predictor of virological response to 48-week PEG-IFN plus ribavirin combination treatment for infection with HCV genotype 1 [11–13]. The present study revealed that the rs8099917 genotype was a significant factor for prediction of SVR (odds ratio 6.159, 95% CI 1.326–28.618, p = 0.02). The cumulative doses of PEG-IFN and ribavirin also were important factors that affected the treatment outcome [15–18]. This study revealed that the total dose of PEG-IFN was a significant factor for prediction for SVR (odds ratio 6.398, 95% CI 1.527–26.8, p = 0.011). For the rs8099917 genotype TT, SVR rates of patients given doses <60% and 60–80% of PEG-IFN were low (30.8 and 40%), and this was so even in those given >80% of PEG-IFN (71%). This result indicates that a dose of PEG-IFN above 80% is necessary for SVR in patients with the rs8099917 genotype TT. In addition, for the rs8099917 genotype non-TT, the SVR rates of patients given doses <60% and 60–80% of PEG-IFN were low (20 and 0%), and this was so even in those given >80% of PEG-IFN (40.0%). This result indicates that a dose of PEG-IFN above 80% is necessary for SVR in patients with the rs8099917 genotype non-TT as well.

A number of studies have reported a significant association between HCV core70/core91 substitutions and treatment outcome [8, 15, 16]. However, in this study, we found no such association but the numbers of patients who were tested for HCV core70/core91 mutations may have been too few. The ISDR located in the NS5A region was originally reported in 1996 by Enomoto et al. [14] and confirmed by several Asian studies [19–21]. This study did not find a significant association between the ISDR and SVR. In addition, controversial results have been reported from Western studies [22, 23].

This study indicated the value of a combination of the rs8099917 genotype and core70 substitutions for prediction of SVR. The patients with the rs8099917 genotype TT had high rates of SVR (67.9%). SVR was achieved by 30.7% of patients with the rs8099917 genotype non-TT and core 70 wild type. The SVR rate was worst in patients with the rs8099917 genotype non-TT and core 70 mutant type. These results indicate the effects of both host and viral factors on IFN responsiveness. However, a combination of the IL28 genotype and ISDR substitutions for prediction of SVR was not useful. Thus, in patients with the rs8099917 genotype non-TT, SVR rates did not differ between ISDR wild type (20%) and ISDR mutant type (19.0%).

In conclusion, the rs8099917 genotype and total PEG-IFN dose were associated with response to IFN in patients with HCV genotype 1. A combination of host and viral factors could improve prediction of the IFN response.

Disclosure Statement

The authors have no conflict of interest to declare.

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Introduction

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Hepatocellular Carcinoma in 2011 and Beyond: From the Pathogenesis to Molecular Targeted Therapy

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Key Words
Hepatocellular carcinoma · Pathogenesis · Signaling pathway · Locoregional therapy · Molecular targeted therapy

Abstract
Hepatocellular carcinoma is a malignant tumor responsible for approximately 600,000–700,000 deaths worldwide, and it is becoming more prevalent not only in Southeast Asia and Africa but also in Western countries; therefore, interest in hepatocellular carcinoma has mounted in recent years in the West, where little or no interest was evident 10–20 years ago.

Introduction

The second Asia-Pacific Primary Liver Cancer Expert Meeting (APPLE) was held on July 1–3, 2011, in Osaka, Japan. A total of 73 oversea guests, all globally recognized hepatocellular carcinoma (HCC) specialists, were invited to this symposium (table 1). Numerous topics were presented followed by extensive discussions with Japanese HCC specialists. This supplement issue focuses on these topics and especially on the Asian consensus on subjects from the pathogenesis and signaling pathway to molecular targeted therapy. I firmly believe that readers will gain a deeper insight into the latest progress and updated diagnosis and treatment of HCC.

Pathogenesis

Persistent infection with hepatitis C virus (HCV) is a major risk for the development of HCC. One of the characteristics of HCV infection is the unusual augmentation of oxidative stress, which is exacerbated by iron accumulation in the liver, as observed frequently in hepatitis C patients. Using a transgenic mouse model, in which HCC develops late in life after the preneoplastic steatosis stage, the core protein of HCV was shown to induce overproduction of reactive oxygen species (ROS) in the liver. In excessive generation of ROS, HCV affects the steady-state levels of a mitochondrial protein chaperone, i.e. prohibitin, leading to an impaired function of the mitochondrial respiratory chain with the overproduction of ROS.

Combination with the other activated pathway, including an alteration in the intracellular signaling cascade of MAP kinase, along with HCV-associated distur-

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Table 1. Invited speakers at the 2nd APPLE meeting (in alphabetical order)

Western speakers (n = 18)	Asian speakers (n = 20)	Japanese speakers (n = 21)
Jacques Belghiti (France)	Subrat Acharya (India)	Yasuaki Arai
Luigi Bolondi (Italy)	Oidov Baatarkhuu (Mongolia)	Yasuhiro Asahina
John F.P. Bridges (USA)	Ding-Shinn Chen (Taiwan)	Takafumi Ichida
Jordi Bruix (Spain)	Pei-Jer Chen (Taiwan)	Tomoaki Ichikawa
Adrian Michael Di Bisceglie (USA)	Ann-Lii Cheng (Taiwan)	Namiki Izumi
Richard S. Finn (USA)	Pierce K.H. Chow (Singapore)	Shuichi Kaneko
Peter R. Galle (Germany)	Kwang-Hyub Han (Korea)	Kazuhiko Koike
Jean-Francois H. Geschwind (USA)	Yun Hwan Joseph Kim (Korea)	Shigehiro Kokubu
Riccardo Lencioni (Italy)	Jeong Min Lee (Korea)	Norihiro Kokudo
Josep M. Llovet (Spain/USA)	Sung-Gyu Lee (Korea)	Masatoshi Kudo
Vincenzo Mazzaferro (Italy)	Laurentius A. Lesmana (Indonesia)	Masatoshi Makuuchi
Valerie Paradis (France)	Shi-Ming Lin (Taiwan)	Osamu Matsui
Lewis R. Roberts (USA)	Sheng-Nan Lu (Taiwan)	Kazuto Nishio
Riad Salem (USA)	Joong-Won Park (Korea)	Kiwamu Okita
Myron E. Schwartz (USA)	Young Nyun Park (Korea)	Takuji Okusaka
Morris Sherman (Canada)	Hunchul Rhim (Korea)	Masao Omata
Augusto Villanueva (Spain)	Hui-Chuan Sun (China)	Michiie Sakamoto
Andrew X. Zhu (USA)	Hee Jung Wang (Korea)	Shuichiro Shiina
	Sheng-Long Ye (China)	Kenichi Takayasu
	Jian Zhou (China)	Ryosuke Tateishi
		Kazuomi Ueshima

bances in lipid and glucose metabolism would lead to the unusual mode of hepatocarcinogenesis, i.e. very frequent and multicentric development of HCC, in persistent HCV infection [1].

Signaling Pathways in HCC

The capability of cells to receive and correctly respond to the microenvironment is basic for their homeostasis. Each cell acts as a complex system where multiple signaling pathways intertwine in parallel circuits. Within this context, a signaling pathway represents a series of chemical reactions ending up in changes in gene expression and cellular phenotype. Information on signaling pathways is crucial to understanding how genes are connected to each other and how they influence cellular functions and behavior in normal and diseased conditions [2–4].

Prevention

Prevention of Hepatitis B Virus-Related HCC

About 350 million people are chronic carriers of the hepatitis B virus (HBV) worldwide. The efficacy of universal immunization has been shown in many countries, with striking reductions in the prevalence of HBV carriage in children. A nationwide vaccination program against HBV launched in Taiwan [5, 6] has drastically reduced the HBsAg carrier rate in the younger populations [7]. More importantly, follow-up results from the Taiwan vaccination programs have shown that the incidence of HCC has been significantly reduced in children. The average annual incidence of HCC in children 6–14 years of age declined from 0.70/100,000 children between 1981 and 1986 to 0.57/100,000 between 1986 and 1990 and further to 0.36/100,000 between 1990 and 1994 ($p < 0.01$) [8, 9].

Secondary Prevention of HCC by Interferon Therapy

There was one randomized controlled trial (RCT) [10], which involved 101 Taiwanese men with chronic hepatitis B, 67 of whom received interferon (IFN) and 34 of whom received placebo. During 1.1–11.5 years after completion of therapy, the incidence of HCC in untreated patients was higher than that in IFN-treated patients (12 vs. 1.5%; $p = 0.043$). The cumulative incidence of HCC was also higher in untreated patients than in treated patients ($p = 0.013$).

A meta-analysis of randomized studies comparing IFN-treated patients versus untreated patients with HBV-

related cirrhosis showed that IFN seemingly decreased the rate of HCC [11].

Secondary Prevention of HCC by the Nucleoside Analog

To date, only one RCT suggests that lamivudine (LAM) treatment of chronic hepatitis B and advanced liver disease does reduce the incidence of HCC but with marginal significance (hazard ratio 0.49; 95% CI 0.25–0.99; $p = 0.047$) [12]. A multicenter retrospective study of 2,795 patients (657 treated with LAM and 2,138 not treated with LAM) was reported from Japan [13]. The cumulative HCC incidence was significantly lower in the LAM group ($p < 0.001$). These findings suggest that LAM effectively reduces the incidence of HCC in patients with chronic hepatitis B.

Prevention of HCV-Related HCC

Primary Prevention by Prevention of Viral Transmission

It is well known that HCV infection has become prevalent recently under artificial circumstances: mother-neonate transmission and sexual transmission of the virus are possible but not common. In many countries, new acquisition of HCV infection is decreasing due to growing concern about blood-transmitted infections, especially HIV, and this trend should be further encouraged considering the absence of effective vaccination against either HCV or HIV.

Secondary Prevention by Treatment of Chronic Hepatitis C

The effect of IFN therapy on HCC incidence in noncirrhotic patients has been evaluated in nonrandomized studies. All studies agree that the risk is reduced in patients who show sustained virologic response or persistent normalization of serum ALT levels [14–17]. Although documentation is rather scarce, the combination with ribavirin will produce a stronger effect on HCC prevention among overall treated patients [18].

Surveillance for Early Detection of HCC

Definition of the Population at High Risk for HCC

Liver cirrhosis induced by causes other than HBV and HCV is a risk for liver carcinogenesis [19]. Since carcinogenesis occurs in some cases of liver cirrhosis associated with nonalcoholic steatohepatitis, alcoholic liver disease, primary biliary cirrhosis, and autoimmune hepatitis, the course of the disease should be followed paying close attention to carcinogenesis, particularly in viral liver cirrhosis. Alcohol increases the risk of chronic hepatitis B- and C-associated liver carcinogenesis.

Based on the above mentioned information, patients with chronic hepatitis B and C and nonviral liver cirrhosis are defined as high-risk populations for HCC in both the Evidence-Based Practice Guidelines [20] proposed by the Japan Society of Hepatology (JSH) and the Consensus-Based Clinical Practice Manual [21] in Japan and the Practice Guideline published by the American Association of Study of the Liver (AASLD) [22]. Patients with liver cirrhosis types B and C are defined as a super high-risk population [20, 21].

Surveillance Protocol for Early Detection of HCC

No clear evidence is available to determine the optimal interval for periodic screening, but HCCs detected in periodic screening by AFP, a protein induced by vitamin K absence or antagonist-II (PIVKA-II), AFP lectin fraction (AFP-L3) measurement, and ultrasonography are solitary and small in many cases compared to those detected in symptomatic patients. Thus, the Japanese Evidence-Based Clinical Practice Guidelines [20] and the Consensus-Based Clinical Practice Manual [21] propose ultrasonography and tumor marker measurements every 3–4 months in the super high-risk population and every 6 months in high-risk populations.

Results of Early Detection of HCC in Japan

In Japan, approximately 65% of HCCs are detected in an early stage, for which curative treatment intervention is possible according to the nationwide survey in 198,000 patients [23]. This can be attributed to the establishment of a nationwide surveillance system across Japan.

Tumor Marker: Highly Sensitive AFP-L3

Five reported studies have analyzed the diagnostic significance of highly sensitive AFP-L3 (hs-AFP-L3) at low total AFP levels [24–29]. The sensitivity and specificity of hs-AFP-L3 at different cutoff levels in these five studies are summarized in table 1. The sensitivity of hs-AFP-L3 for HCC was approximately 25–50% in patients with total AFP levels below 20 ng/ml when the cutoff was fixed between 5 and 7%. The sensitivities of AFP-L3 measured by conventional methods in the serum samples of hs-AFP-L3 from two studies were 3.6 and 5.2% (cutoff of AFP-L3: 7%) [24, 26]. Thus, the sensitivity for HCC markedly in-

creased with the use of a newly developed, highly sensitive measurement method.

An important advantage of AFP-L3 is its high specificity for HCC. Therefore, attempts to increase the sensitivity of AFP-L3 for HCC should avoid a concomitant reduction in specificity. Based on the data from reported studies among patients with low total AFP levels, the specificity of hs-AFP-L3 for HCC was over 85% when the cutoff was fixed between 5 and 7%, except in one study. The original advantage of AFP-L3 produced by conventional methods, i.e. high specificity for HCC, appeared to be maintained in the case of hs-AFP-L3. The specificity was 54.0% when the cutoff was fixed at 5% in the study by Nouso et al. [27]. This was because the control group in their study included only patients with cirrhosis; patients with minute HCC that had not been detected by imaging examination might have been included in a control group.

Another reported advantage of AFP-L3 in the management of patients with HCC is its ability to indicate an advanced nature of HCC and to identify cases with poor prognoses. Previous studies have reported that HCC with high AFP-L3 levels demonstrate characteristics of advanced HCC by pathologic [30] and imaging findings [31]. Higher recurrence rates [32] and lower survival rates [33, 34] after treatment have also been reported in patients with increased AFP-L3 at diagnosis. We sought to determine whether these benefits of AFP-L3 persist with hs-AFP-L3.

hs-AFP-L3 increased the sensitivity of HCC at diagnosis, maintaining its high specificity and indicative value for poor prognoses. This biomarker can be used as a new tool in clinical practice for the management of patients with HCC. The utility of hs-AFP-L3 for the prediction of HCC development in high-risk patients under surveillance should be further investigated.

Imaging Diagnosis

Contrast-Enhanced Ultrasound with Sonazoid

Clinical Significance of Contrast-Enhanced Ultrasound

Sonazoid is a newly introduced second-generation ultrasound (US) contrast agent exclusively approved in Japan in 2007. The important characteristics of Sonazoid are that it facilitates real-time imaging in blood flow images at a low acoustic power and stable Kupffer phase imaging, tolerable for multiple scanning from 10 to 120 min after its injection [35]; this results in the invention of the breakthrough method, defect reperfusion imaging. Sonazoid-enhanced US with defect reperfusion imaging is an innovative technology that will greatly change the daily practices of HCC [36].

Development of Defect Reperfusion Imaging (Dual Phase Fusion Imaging)

We recently developed defect reperfusion imaging [37–39] using the properties of very stable Kupffer images and real-time fine blood flow images obtained with Sonazoid for typical HCC, which is depicted by CT but not by B mode scanning. This method is a breakthrough for accurate localization and treatment guidance [38]. Until recently, diagnosis in dynamic studies was usually based on the enhancement of patterns according to a time sequence or phase; however, by introducing the novel idea of dual phase imaging with the reinjection method, both Kupffer and arterial phase images are obtained at the same slice of ultrasound plane, which is really an innovative technique. Namely, this method is performed as follows: reinjection of Sonazoid is performed in areas that show defects in the postvascular phase [35, 37–39]. The introduction of this method has led to dramatic solutions for many limitations in the diagnosis and treatment of HCC, such as detection of small HCCs [40–42], evaluation of the treatment response [43], and needle insertion guidance [44]. The detection rate of small HCCs by Sonazoid-enhanced US is even more sensitive than that by MDCT [40].

MR Imaging Using a New Contrast Agent, Gadolinium-Diethylene-Triamine-Pentaacetic Acid, in the Diagnosis of Early HCC

A newly introduced contrast agent, gadolinium-diethylene-triamine-pentaacetic acid (Gd-EOB-DTPA), approved in 2008 in Japan, is a hepatocyte-specific MRI contrast medium with a different mechanism, utilizing both dynamic and Kupffer cell imaging. This new contrast medium is useful for the diagnosis of cases which would have been difficult using previous techniques such as dynamic MRI or SPIO-MRI [45].

In well-differentiated early HCC, some nodules may not be completely shown as a defective area on CTAP, but the Gd-EOB-DTPA uptake is apparently lower than that in the surrounding normal liver parenchyma, being imaged as a low-intensity nodule. Well-differentiated early HCC having Kupffer cells with enhanced SPIO uptake and receiving portal blood flow on CTAP has been difficult to characterize by SPIO-MRI or CTAP; however, it can be imaged clearly as a hypointense nodule using Gd-

EOB-DTPA MRI in many early HCC cases due to differences in biological characteristics, indicating that this contrast agent may lead to a breakthrough in the diagnosis of early HCC [46, 47], which has been clinically difficult and difficult even by pathological diagnosis when a biopsy sample is used. In other words, this technique may be the most sensitive tool in the detection of the phenotypic change of early hepatocarcinogenesis, much more sensitive than CTAP, CTHA, or SPIO-MRI [48].

Therefore, the diagnostic algorithm will be changed by introducing Gd-EOB-DTPA MRI in hypervascular and hypovascular liver nodules [49, 50].

Hepatic Arterial Infusion Chemotherapy for Advanced HCC

No effective anticancer drug for advanced liver cancer had been demonstrated before sorafenib was introduced [51]. 'Far advanced liver cancer' represents stage IVa liver cancer accompanied by vascular invasion and stage IVb liver cancer accompanied by distant metastasis, for which low-dose FP (5FU and *cis*-platinum) [52] therapy and hepatic arterial infusion of 5FU in combination with IFN treatment [53] have been established as effective treatment options in Japan. In fact, the response rate (CR+PR) reaches 45.9% according to the nationwide survey by the LCSGJ [23]. In addition, it is well established that the overall survival (OS) of the responders is definitely better than that of the nonresponder or best supportive care group. However, the intraarterial infusion procedure is complex because establishment of a reservoir port for arterial infusion is necessary; therefore, this technique is not performed in Western counties.

Hepatic intra-arterial infusion chemotherapy is not recommended in the AASLD guidelines [22]; therefore, although the response rate is high, the efficacy and especially the survival benefit of intra-arterial infusion chemotherapy and that using an intractable delivery port system should be confirmed by further randomized studies.

Treatment Algorithm

A Consensus-Based Treatment Algorithm for HCC Proposed by the JSH

For HCC treatment, practice patterns markedly differ between Europe/the USA, and Japan. For this reason, a unique Japanese algorithm (JSH Consensus 2007) was proposed in 2007 [21]. Consequently, a revised draft was presented at the 45th meeting of the Japanese Liver Society in 2009 (congress chair: Masatoshi Kudo), and an article was published in 2010 [54] and 2011 [55] (fig. 1). The consensus-based treatment algorithm recommended by this society consists of extrahepatic lesions, hepatic reserve, vascular invasion, number of tumors, and tumor diameter. Treatment is classified into curative treatment (resection, local ablation), transcatheter arterial embolization (TACE), arterial infusion chemotherapy, liver transplantation, and best supportive care. Basically, the contents are consistent with the evidence-based treatment algorithm established by the Makuuchi group. However, consensus-based algorithms are not always based on evidence but involve a routinely employed treatment for which a consensus has been reached in Japan. For example, concerning the item of early HCC, local ablation is performed for the lesions in which biopsy diagnosis, CTHA/CTAP, or gadolinium-DTPA ethoxybenzyl (EOB)-MRI suggests malignancy. In evidence-based guidelines, hypovascular tumors are categorized as 'nontypical for HCC', reflecting lesions without an arterial enhancement. Evidence-based guidelines recommend that these lesions be followed up. However, among hypovascular tumors, 'early liver cancer' definitively diagnosed based on CTAP, EOB-MRI, or biopsy findings is known to frequently progress to classical HCC. Based on this fact, treatment is performed in many cases in a routine clinical setting; noninvasive ablation therapy is performed rather than resection, which is more invasive. With respect to hypovascular lesions without malignant findings, intensive follow-up is recommended. For management, early hypovascular HCC should be separated from other types of hypervascular liver cancer.

Initially, resection or local ablation therapy should be performed to treat 3 or fewer tumors measuring 3 cm or less in diameter without extrahepatic lesions/vascular invasion in which the liver function is good. In this group, the prognosis of curative treatment may be favorable. In 3 or fewer lesions measuring more than 3 cm in diameter, resection or TACE is recommended. Curability may be improved by adding ablation therapy to previous transarterial treatment (TACE or lipiodol TACE). Secondly, TACE and arterial infusion chemotherapy are recommended to treat 4 or more lesions. However, arterial infusion chemotherapy is performed based on expert experience, but there is no solid evidence because there is no RCT. The combination of local ablation therapy and TACE/arterial infusion chemotherapy for 5 or 6 or fewer lesions is beneficial in some cases. Furthermore, resec-

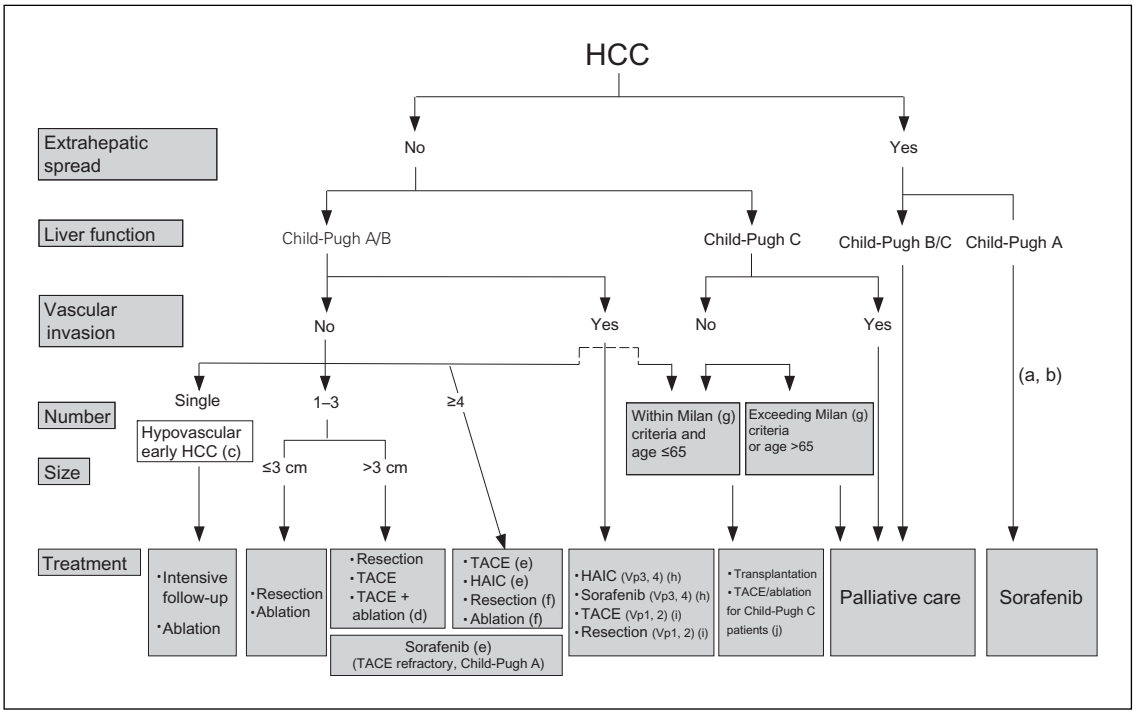

Fig. 1. Consensus-based treatment algorithm for HCC proposed by the JSH and revised in 2010. (a) = Treatment should be performed as if the extrahepatic spread is negative when extrahepatic spread is not regarded as a prognostic factor; (b) = sorafenib is the first choice of treatment in this setting as a standard of care; (c) = intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (1) when the nodule is diagnosed pathologically as early HCC, (2) when the nodules show decreased uptake on Gd-EOB-MRI, or (3) when the nodules show decreased portal flow by CTAP, since these nodules are known to frequently progress to the typical advanced HCC. (d) = Even for HCC nodules exceeding 3 cm in diameter, combination therapy of TACE and ablation is frequently performed when resection is not indicated; (e) = TACE is the first choice of treatment in this setting. HAIC using an implanted port is also recommended for TACE refractory patients. The regimen for this treatment is usually low-dose FP (5FU+CDDP) or intra-arterial 5FU infusion combined with systemic IFN therapy. Sorafenib is also a treatment of choice for TACE refractory patients with Child-Pugh A liver function. (f) = Resection is sometimes performed even when the number of nodules is greater than 4. Furthermore, ablation is sometimes performed in combination with TACE. (g) = Milan criteria: tumor size ≤3 cm and tumor number ≤3, or solitary tumor ≤5 cm. Even when the liver function is good (Child-Pugh A/B), transplantation is sometimes considered for frequently recurring HCC patients. (h) = Sorafenib and HAIC are recommended for HCC patients with Vp3 (portal invasion at the 1st portal branch) or Vp4 (portal invasion at the main portal branch); (i) = resection and TACE are frequently performed when the portal invasion is minimum, e.g. Vp1 (portal invasion at the 3rd or more peripheral portal branch) or Vp2 (portal invasion at the 2nd portal branch); (j) = local ablation therapy or subsegmental TACE is performed even for Child-Pugh C patients when transplantation is not indicated when there is no hepatic encephalopathy, no uncontrollable ascites, and a low bilirubin level (<3.0 mg/dl). However, it is regarded as an experimental treatment since there is no evidence of its survival benefit in Child-Pugh C patients. A prospective study is necessary to clarify this issue. Even in Child-Pugh A/B patients, transplantation is sometimes performed for relatively younger patients with frequently or early recurring HCC after curative treatments.

tion may be considered for such lesions if possible. In young Child-Pugh A/B hepatic reserve patients with early recurrence, liver transplantation is sometimes a choice of treatment when they meet the Milan criteria. In the presence of vascular invasion, resection is performed for patients with a 3rd or 4th branch of portal venous invasion if possible. In such patients, TACE can be a choice of treatment. In patients with a main track or 1st branch of portal vein, arterial infusion chemotherapy, in addition to hepatic arterial infusion chemotherapy with implanted port, is a choice of treatment.

In Child-Pugh C hepatic reserve patients aged 65 years or younger with an unfavorable liver function in the absence of vascular invasion who meet the Milan criteria, liver transplantation is recommended [56]. Furthermore, as test therapy, local ablation or subsegmental TACE is

conducted in Child-Pugh C hepatic reserve patients without hepatic encephalopathy or refractory ascites showing a bilirubin level of 3 mg or less. However, there is no evidence regarding the survival benefits. In the future, a prospective clinical study should be conducted. In Child-Pugh C hepatic reserve patients with vascular invasion or extrahepatic lesions, the best supportive treatment is basically selected. In this case, palliative radiotherapy to resolve pain is included. However, when extrahepatic lesions are not a prognostic factor, treatment in accordance with the standard treatment algorithm may improve the prognosis.

In Child-Pugh A hepatic reserve patients with extrahepatic lesions, sorafenib should be selected as a first choice of treatment [57]. This agent is recommended for patients with vascular invasion, especially patients with macrovascular invasion, in addition to arterial injection chemotherapy. In nonresponders to TACE/arterial injection chemotherapy, sorafenib may become a treatment option when the hepatic reserve is evaluated as Child-Pugh A.

The consensus-based treatment algorithm is not always based on scientific evidence. However, it is significant because a consensus has been reached among specialists belonging to the JSH, as demonstrated by the BCLC, and therefore an own treatment algorithm is introduced. In the future, evidence-lacking parts must be revised through a prospective study. The treatment algorithm for liver cancer reflects a primary concept for treatment strategies. Basically, it is important to perform individualized treatment in individual patients, considering various conditions [58].

Definition of TACE Failure

In Japan, repeated TACE is commonly performed for multiple nodules without major vascular invasion or extrahepatic spread in Child-Pugh A or B patients. Even though recurrence is very rapid, TACE has been repeatedly performed (sometimes over 10 times). The reason for this is that there was no next treatment modality after TACE failure/refractory patients before sorafenib was introduced. Since hepatic arterial infusion chemotherapy is not effective for TACE failure patients, sorafenib is regarded as a first choice of treatment for TACE failure patients. Up to now there had been no clear definition of TACE failure. The JSH expert panel agrees that the definition of TACE is mandatory to change the treatment strategy to sorafenib if TACE failure is confirmed [55].

In this regard, the definition of TACE failure has been proposed for the first time ever as shown in table 2.

Table 2. Definition of TACE failure

Intrahepatic lesion
– More than 2 consecutive incomplete depositions (<50%) of lipiodol detected by response evaluation CT within the treated tumors at 4 weeks after adequately performed TACE
– More than 2 consecutive appearances of new lesions (recurrence) detected in the liver by response evaluation CT at 4 weeks after adequately performed TACE
Appearance of vascular invasion
Appearance of extrahepatic spread
Tumor marker
Continuous elevation of tumor markers despite prior TACE

Molecular Targeted Therapy

Sorafenib, is a low-molecular-weight compound discovered by screening inhibitors of Raf kinase, an important molecule in the MAP kinase cascade located downstream of the growth factor receptor. Sorafenib exhibits strong inhibitory activity for not only the wild-type c-Raf and V600E mutant b-Raf but also the receptor tyrosine kinases involved in angiogenesis and cell growth, such as vascular endothelial growth factor receptor (VEGFR)-2, VEGFR-3, platelet-derived growth factor receptor (PDGFR), Fms-related tyrosine kinase-3 (Flt-3), and c-Kit.

The positive results of a phase III study for HCC (SHARP trial) [59] had a strong impact on the treatment strategy of HCC. This study was performed as a randomized double-blind placebo-controlled multicenter study initiated in March 2005. The subjects were advanced HCC patients at ECOG PS 0–2 with Child-Pugh A liver function without previous systemic chemotherapy. Regarding the study design, two groups, sorafenib (400 mg b.i.d.) and placebo treatment, were established, and the primary end point was OS. The secondary end point was time to progression.

Ongoing Clinical Trials with Molecular Targeted Agents

As stated earlier, the STORM trial using sorafenib as an adjuvant setting after curative treatment such as resection or ablation is ongoing as a global trial. In addition, the SPACE trial and TACTICS trial using sorafenib in combination with TACE are ongoing in Western counties and Japan, respectively. The SILIUS trial using sorafenib in combination with hepatic arterial infusion chemotherapy (HAIC) is under investigation in Japan. Further-

more, head-to-head trials of linifanib versus sorafenib and brivanib versus sorafenib for advanced HCC are ongoing globally. Finally, second-line trials of brivanib, RAD001, axitinib, and ramucimubab for sorafenib failure are also ongoing as global clinical trials [50, 60]. These trial results are expected to yield better outcomes for different stage of HCCs. If positive results are obtained by these clinical trials, the life expectancy in each stage is expected to be much prolonged based on theoretical calculations using hazard ratios of OS incorporated from the SHARP trial [50].

In Japan, although a phase III study in HCC patients following TACE was revealed to be a negative [61], an investigator-sponsored trial investigating the efficacy and tolerability of a combination of TACE with sorafenib is underway (TACTICS trial). In addition, a phase III trial for HCC of acyclic retionid, a vitamin A analog, after resection or RFA has been completed and was presented at the American Society of Clinical Oncology Meeting in 2010. However, the results did not meet the primary end point.

A global phase III trial of sorafenib as an adjuvant therapy after surgery or ablation is currently underway (STORM trial) and a global phase II trial of sorafenib as a maintenance therapy with a combination of TACE is also ongoing (SPACE trial). These results are expected to confirm its usefulness in daily clinical practice. In addition, use of sorafenib in combination with hepatic arterial infusion chemotherapy (SILIUS trial) is also ongoing in Japan. A paradigm shift in HCC treatment may be induced if positive results are obtained by these currently ongoing sorafenib trials.

Conclusion

Recent progress in HCC, including issues from pathogenesis to molecular targeted therapy for HCC, has been described in this article. It is strongly expected that this supplement issue will enhance the most up-to-date knowledge on HCC of the readers of *Oncology*.

Disclosure Statement

The author declares that he has no financial conflict of interest.

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Signaling Pathways Governing Tumor Angiogenesis

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Key Words
Stromal cell · Vascular endothelial growth factor · c-Jun N-terminal kinase

Abstract
Angiogenesis is regulated by the highly coordinated function of various proteins with pro- and antiangiogenic functions. Proangiogenic factors include vascular endothelial growth factor (VEGF), fibroblast growth factor, platelet-derived growth factor, insulin-like growth factor, transforming growth factor, angiopoietins, and several chemokines; antiangiogenic factors include thrombospondin-1, angiostatin, and endostatin. Matrix metalloproteinases display a dual role in vascular development. Notch signaling affects remodeling of the primary vascular network of uniformly sized vessels into functionally and morphologically distinct arteries, veins, and capillaries. Tumors, described as 'wounds that never heal', lose the appropriate balance among these factors. Although VEGF-targeted therapies are showing promise, new angiogenesis targets are needed to make additional gains. Here, we highlight recent advances in our understanding of the regulation of tumor angiogenesis and discuss the potential of molecular targeting as a new therapeutic approach.

Introduction

Angiogenesis, which is the process of new blood vessel growth from preexisting vessels, is imperative in malignant tumor growth. It is regulated by a balance of proangiogenic and angiostatic factors which, upon the switch of tumor cells to an angiogenic phenotype, leads to tumor growth and progression [1]. Since the enormous boost of angiogenesis research after the early 1990s, various angiogenesis regulators have been discovered. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) were identified as positive regulators of angiogenesis. Interferon-α, angiostatin, and endostatin are examples of the first generation of angiogenesis inhibitors, while compounds such as bevacizumab, sunitinib, and erlotinib are examples of current clinically used compounds [2].

Fundamental research as well as these clinical applications have demonstrated that tumor endothelial cells are considered a suitable target for cancer therapy as they play an essential role in angiogenesis. However, angiogenesis is now recognized as the product of evolving crosstalk between different cell types within the tumor and its stroma [3]. There is substantial evidence that the proinflammatory response at the tumor stroma could be rerouted in a tumor-promoting direction by stimulating angiogenesis and tissue remodeling [4]. In this review, we discuss the current literature regarding the molecular mechanisms by which tumor angiogenesis is regulated.

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Vascular Endothelial Growth Factor

VEGF and its receptors represent one of the best-validated signaling pathways in angiogenesis [5]. The VEGF family comprises 5 VEGF glycoproteins (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E) and placental growth factors 1 and 2, among which VEGF-A is the best characterized. VEGF-A and its receptor, VEGFR-2, are key targets of antiangiogenic agents. VEGF primarily targets endothelial cells and is secreted by cancer cells to mediate tumor angiogenesis [6]. VEGF is upregulated by hypoxia-inducible factor (HIF) 1α and platelet-derived growth factor (PDGF) B and can also be released from the extracellular matrix (ECM) by matrix metalloproteinase (MMP) 9 to initiate an angiogenic switch that promotes tumor growth. In hepatocellular carcinomas (HCCs), expression of VEGF is decreased by a disruption of c-Jun N-terminal kinase (JNK), a member of mitogen-activated protein kinase (MAPK) [7]. JNK may regulate VEGF transcription through AP-1, whereas the effect of JNK could be quite indirect and exerted through factors such as IL-1β and oxygen tension. In epithelial cancers, E-cadherin is downregulated by an HIF-1α-dependent mechanism via the transcription factor Snail, thus promoting epithelial-mesenchymal transition [8]. VEGF elicits epithelial-mesenchymal transition via an autocrine loop [9], suggesting that VEGF involves not only tumor angiogenesis but also the early dissemination of malignant cells outside the epithelial layer.

VEGFRs comprise an extracellular component and an intracellular domain with a consensus tyrosine kinase sequence. VEGFR-1 and VEGFR-2 were found to be predominantly expressed in endothelial cells and VEGFR-3 is primarily associated with lymphangiogenesis [10]. The VEGF family members each bind these receptors with different affinities. VEGFR-1 is a receptor for VEGF-B and placenta growth factor. During pathologic conditions such as tumorigenesis, it is a potent, positive regulator of angiogenesis. VEGFR-2 mediates most of the cellular effects of VEGF-A during angiogenesis, including microvascular permeability, endothelial cell proliferation, migration, and invasion [5]. VEGFR-3 binds with the highest affinities to VEGF-C and VEGF-D and drives lymphangiogenesis. Like VEGFR-2, VEGFR-3 signaling can contribute to angiogenesis in tumors, in which the receptor is expressed on tumor blood vessels as well as on lymphatics. Neuropilins 1 and 2 serve as coreceptors for VEGF by increasing the binding affinity of ligands to VEGFRs [11].

Targeting the tumor vasculature is a particularly attractive strategy because of the presumed genetic stability of endothelial cells [12]. Indeed, the current FDA approved antiangiogenic agents that inhibit the VEGF pathway. These agents include bevacizumab, a humanized anti-VEGF-A monoclonal antibody [13], and two small molecule inhibitors targeting VEGFR2, sorafenib and sunitinib [14, 15]. In combination with other anticancer agents, the addition of bevacizumab significantly increased the progression-free survival and the median overall survival in colorectal cancer and non-small cell lung carcinoma (NSCLC) [16, 17]. It has been shown that systemic administration of sorafenib, a tyrosine kinase inhibitor targeting the VEGF and ERK pathways, significantly extends the survival of patients with advanced stage HCC [18, 19]. However, antiangiogenic treatments targeting a single pathway such as VEGF-A rarely induce durable tumor responses, both in mice and in patients with cancer [20], and may also favor metastasis in selected tumor models [21, 22]. The mechanism for this acquired resistance is not well described but appears to be due in part to expansion or expression of redundant alterations in maturing vasculature [23] and epigenetic mechanisms [24]. Recently, tumor resistance or recurrence after antiangiogenic therapy was causally linked to the recruitment of bone marrow-derived myeloid cells [25]. Damaging the tumor vasculature indeed enhances tumor hypoxia, which in turn upregulates the expression of several myeloid cell chemoattractants that rouse the influx of myeloid cells to treated tumors [20]. Once recruited to the tumors, myeloid cells promote angiogenesis by releasing angiogenic and tissue-remodeling factors [26] and also stimulate tumor cell intravasation, dissemination, and metastasis [27, 28]. New antiangiogenesis targets need to be explored.

Fibroblast Growth Factor

The fibroblast growth factor (FGF) family includes 18 ligands. FGFs interact with 4 main receptors, i.e. FGFRs [29, 30]. The FGF ligands are among the earliest angiogenic factors reported and are involved in promoting the proliferation, migration, and differentiation of vascular endothelial cells [31, 32]. Overexpression of various FGF ligands in different types of tumors has been documented [30]. While the prototype members, FGF-1 and FGF-2, are devoid of a signal peptide and thus are poorly secreted [33], most members of the family have a signal peptide and are efficiently secreted [34]. FGFs have been reported to promote angiogenesis inde-

pendently of VEGF [29], and FGF-2 in particular has been shown to possess potent angiogenic activity [35]. FGFRs are often overexpressed in tumors, and mutations of the FGFR genes have been found in human cancers, making it particularly significant that FGFR activation in endothelial cell culture and animal models leads to angiogenesis [29, 30].

Platelet-Derived Growth Factor

The family of PDGFs consists of 5 members (PDGF-A, PDGF-B, PDGF-AB, PDGF-C, and PDGF-D), and there are 3 types of PDGF receptors (PDGFR-α, PDGFR-β, and PDGFR-αβ) [29]. PDGF signaling promotes cell migration, survival, and proliferation and regulates angiogenesis indirectly by inducing VEGF transcription and secretion [36]. PDGF is involved in vessel maturation and the recruitment of pericytes [37]. PDGF is expressed by endothelial cells and generally acts in a paracrine manner, recruiting PDGFR-expressing cells, particularly pericytes and smooth muscle cells, to the developing vessels [38]. Deletion of *PDGF* and *PDGFR* in mice results in hemorrhage and tissue edema in early embryos as a consequence of defective vascular development [37]. Mutations involving upregulation of PDGF and/or PDGFR have been described in human cancers [38], indicating a likely role for the PDGF pathway in carcinogenesis.

Transforming Growth Factor-β

Transforming growth factor-β (TGF-β) and the corresponding receptors are produced by nearly every cell type, although each of the 3 isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) demonstrates a different tissue expression pattern [39]. TGF-β participates in angiogenesis, cell regulation and differentiation, embryonic development, and wound healing and also has potent growth inhibition properties [39]. TGF-β receptors are classified as type I, II, or III. Type I and II receptors contain serine/threonine kinase domains in their intracellular protein regions, whereas type III does not possess kinase activity but is believed to participate in transferring TGF-β ligands to type II receptors. TGF-β ligands bind to and stimulate type II receptors that recruit, bind, and phosphorylate type I receptors, activating downstream signaling proteins known as SMADs. TGF-β is believed to have both proangiogenic and antiangiogenic properties, depending on the levels present. Low levels of TGF-β contribute to angiogenesis by upregulating angiogenic factors and proteases, whereas high doses of TGF-β stimulate basement membrane reformation, recruit smooth muscle cells, increase differentiation, and inhibit endothelial cell growth [40].

Angiopoietins and TIE Receptors

Angiopoietins (ANGs), important angiogenic molecules, have been identified as ligands for TIE2. ANG1 activates TIE2, leading to receptor autophosphorylation upon binding, and it mediates a range of effects such as tightening cell junctions to inhibit cell permeability and inflammation and activating endothelial cell migration and survival through the PI3K-AKT signaling pathway [41, 42]. ANG1 is a vasculogenic factor which is signaled through the endothelial and bone marrow cell-specific TIE2 receptor tyrosine kinase and promotes endothelial cell survival and vascular maturation by increasing endothelial cell-pericyte interaction [41, 42]. ANG2 is upregulated by hypoxia and may trigger angiogenesis via an autocrine loop in endothelial cells, which express TIE2. ANG2-TIE2 axis promotes angiogenesis in tumors by destabilizing the blood vessels and sensitizing endothelial cells to proliferation signals mediated by other proangiogenic factors such as VEGF [41, 42]. However, in the absence of VEGF, ANG2 promotes endothelial cell apoptosis and consequent blood regression [41–43]. Genetic or pharmacological targeting of ANG2 reduced tumor angiogenesis and delayed the growth of subcutaneous tumors to a variable extent in different studies [44–46]. The role of ANG2 in tumor angiogenesis and growth remains controversial and poorly defined. Expression of the ANG receptor TIE2 is not restricted to endothelial cells. TIE2 is weakly expressed by some circulating monocytes and is significantly upregulated upon their homing to tumors and differentiation into a subset of perivascular macrophages [27, 47]. These TIE2-expressing macrophages have features of M2-polarized tumor-associated macrophages (TAMs) [48], promote tumor angiogenesis [49], and are required for the formation of tumor blood vessels [26, 47]. Because tumor-infiltrating TAMs promote vascular regrowth following therapy-induced vascular damage [50], targeting these cells might increase the efficacy of antiangiogenic treatment by counteracting myeloid cell-mediated angiogenesis and resistance to therapy [20]. The ANG2-TIE2 pathway regulates the proangiogenic activity of TAM.

Delta/Jagged-Notch Signaling

Delta/Notch signaling mediates cell-cell communication and regulates cell fate determination as well as tumor angiogenesis. The family of Notch receptors consists of 4 members (Notch1–4) and there are 3 types of their transmembrane ligands Delta-like (Dll1, Dll3, and Dll4) and 2 members of Jagged (Jagged1 and Jagged2). Dll4 and Notch signaling regulate the cellular actions of VEGF [51]. Dll4 regulates excessive VEGF-induced vessel branching, allowing vessel formation to occur at a productive and efficient rate [52]. Inhibition of Dll4 leads to an increase in tumor vascular density. However, the vascular network is very poorly formed and essentially nonfunctional, and a significant decrease in tumor size was observed. In addition, the decrease in tumor size was noted even in tumor models that are resistant to VEGF blockade [53, 54]. Expression of Jagged1 is dependent on MAPK signaling. Overexpression of Jagged1 in head and neck squamous carcinoma cells leads to increased vascularization and tumor growth, suggesting that Jagged1 promotes angiogenesis [55]. Inhibition of specific components of the Notch signaling pathway, such as Dll4 or Jagged1, may prove to be effective for inhibiting functional angiogenesis in tumors.

Matrix Metalloproteinases

MMPs consist of a multigene family of zinc-dependent ECM-remodeling endopeptidases implicated in pathological processes such as carcinogenesis. MMPs are able to proteolytically process substrates in the extracellular milieu and, in so doing, promote tumor progression. In tumor vasculature, MMPs display a dual role because they can act as both positive and negative regulators of angiogenesis depending on the time point of expression during tumor angiogenesis and vasculogenesis as well as the availability of the substrates. The key players of the MMP family that participate in tumor angiogenesis are mainly MMP-2, MMP-9, and MMP-14 [56].

For tumor growth, it is necessary to eliminate the physical barriers by ECM degradation and subsequently to generate proangiogenic factors. Indeed, MMP-9 participates in the angiogenic switch because it increases the bioavailability of important factors in this process, such as VEGF and bFGF, by degradation of extracellular components, such as collagen type IV [57]. The angiogenic balance is tightly regulated by MMPs, which also downregulate blood vessel formation through the generation of degradation fragments that inhibit angiogenesis such as tumstatin, endostatin, and angiostatin, which are generated via cleavage of collagen types IV and XVII, plasminogen, and perlecan [58–60].

Chemokines

Structurally, chemokines are grouped into 4 families (designated CC, CXC, C, and CX_3C) based on the location of conserved cysteine residues near their amino terminus. In the CC subgroup the first two cysteine residues are adjacent, whereas in the CXC subgroup the first 2 cysteine residues are separated by a nonconserved amino acid, constituting the Cys-X-Cys or 'CXC' motif.

The CXC chemokines, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 (IL-8), promote the migration and proliferation of endothelial cells and are potent promoters of neovascularization. All of these CXC chemokine ligands in the mouse signal via the receptor, CXCR2, whereas in humans they signal through CXCR1 and CXCR2. CXCR2 is the primary receptor for angiogenesis in humans. In a syngeneic tumor model of lung cancer and renal cell carcinoma, *CXCR2* knockout mice had decreased tumor growth, increased necrosis, and decreased angiogenesis and metastases compared to wild-type mice [61, 62]. In colorectal cancer, in vivo tumor growth is induced by increased expression of CXCL1 [63]. CXCL1, CXCL2, and CXCL3 were shown to be highly expressed in patients with malignant melanoma [64]. A direct relationship was found between tissue levels of CXCL5 in surgical specimens of NSCLC and the extent of capillary density consistent with tumor angiogenesis [65]. The expression of CXCL8 (IL-8) in human prostate, lung, and gastric cancer cells is associated with tumorigenicity and neovascularization [66, 67]. Induction of CXCL8 (IL-8) preserved the angiogenic response in HIF-1α-deficient colon cancer cells, indicating that IL-8 mediates angiogenesis independently of VEGF signaling pathways [68]. In addition to the CXC chemokine family, 3 members of the CC chemokine family, CCL2 (MCP-1), CCL11, and CCL16, have also been implicated in neovascularization. Human endothelial cells express CCR2 and respond to MCP-1, resulting in angiogenesis and tumor progression [69].

CXCL4, the first chemokine shown to block angiogenesis [70], is a potent inhibitor of endothelial cell chemotaxis and proliferation and has been shown to inhibit the angiogenic effect of VEGF and bFGF [71]. CXCL9, CXCL10, and CXCL11 are also potent inhibitors of angio-

genesis. Their angiostatic effect appears to be mediated via CXCR3 [72], the expression of which is strongly induced by IL-2.

Conclusion

The complex molecular pathways that govern tumor angiogenesis are logical targets for pharmacological manipulation given the important role they play in the growth and development of cancers. Tumor cells are genetically unstable and biologically heterogeneous, which is considered the principal cause of the failure of systemic chemotherapies. It is believed that endothelial cells in tumor stroma are genetically stable and that these cells will not become drug resistant in response to antivascular therapy. However, recent studies showed that endothelial cells are aneuploid and that they express neoplastic markers [73]. Signals from different stromal cell types have been shown to modulate tumor growth and their responsiveness to therapies in a variety of models, raising the possibility that drugs interfering with these pathways could provide additional therapeutic strategies. Future research regarding the role of critical mediators altering tumor microenvironment involved in tumor angiogenesis may lead to novel therapeutic applications.

Disclosure Statement

The authors disclose no conflicts.

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Adriamycin Enhances Proteasome-Mediated Generation of the Proapoptotic Processed Form of MAGE-A4 in Hepatoma Cells

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Key Words
Adriamycin · MAGE-A4 · Proteasome · Apoptosis · Proteolysis

Abstract
Background: Melanoma antigen (MAGE)-A4 is processed to generate a C-terminal fragment with proapoptotic activity. Here we demonstrate that Adriamycin promotes generation of the processed MAGE-A4 by activating the proteasome. The proteasome is known to prevent accumulation of toxic proteins to maintain cellular homeostasis. ***Methods and Results:*** Treatment of hepatoma cells expressing MAGE-A4 with a sublethal dose of Adriamycin increased the MAGE-A4 processing and sensitized the cells to Adriamycin-induced apoptosis. The processing of MAGE-A4 was inhibited by the proteasome inhibitors MG115, MG132, lactacystin and epoxamicin. MAGE-A4 was coimmunoprecipitated with the S6 proteasomal ATPase, and present in the fractions containing the proteasome during glycerol gradient centrifugation. Consistent with the notion that the proteasome cleaves MAGE-A4, the 26S proteasome, ubiquitin, and cell lysates were necessary for efficient in vitro cleavage of MAGE-A4. ***Conclusions:*** The present study suggests that a low dose of Adriamycin increases the proteasome activity, which either maintains cellular homeostasis or leads to apoptosis depending, at least under the present conditions, on the expression of MAGE-A4.

Introduction

The proteasome degrades the majority of cellular proteins in eukaryotes. Its activity allows for surveillance by the immune system, controls the levels of various regulatory proteins, and prevents the accumulation of misfolded mutant and damaged proteins [1]. The proteasome is also implicated in the apoptotic process and endoproteolysis [2, 3]. It is composed of a cylindrical 20S, 670-kDa core particle and a 19S regulatory particle. The core particle is composed of 28 subunits, α and β in type, which are arranged in four stacked heptagonal rings [4]. The 19S regulatory particle, which is 900 kDa in size and is composed of about 17 subunits, binds to one or both ends of the core particle. Besides opening the core particle channel, the 19S cap recognizes and unfolds ubiquitin-conjugated proteins and thereby controls the access of the substrates into the cavity of the core particle [5]. The hexameric ring that contacts the heptameric α ring of the core

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particle is composed of six different ATPases, including S6 and S7 [6].

Adriamycin (doxorubicin) is an anthracycline anticancer agent used clinically. Although its cytotoxicity has been extensively studied [7, 8], the mechanism of action is not fully elucidated. Adriamycin has been reported to induce apoptosis by inhibiting proteasome activity [9]. Under different conditions, however, Adriamycin is associated with the proteasomal activation, leading to chemotherapeutic resistance [10].

The melanoma antigen (MAGE) gene family is composed of more than 25 genes in humans [11]. MAGE genes are highly expressed in various forms of cancers, but not in most healthy adult tissues except for the testis [12]. Because MAGE genes encode tumor-specific antigenic peptides presented by HLA class I molecules to CD8 T lymphocytes [13], they have been extensively studied as targets for cancer immunotherapy [14, 15]. We have previously identified a novel liver oncoprotein, gankyrin [16], and demonstrated that MAGE-A4 binds to gankyrin and suppresses its tumorigenic activity [17]. Recently, we have found that MAGE-A4 is cleaved between amino acid residues 213 (Glu) and 214 (Gly) to generate a proapoptotic C-terminal fragment [18]. In the present study, we analyzed the mechanism of the MAGE-A4 processing, and found that a low dose of Adriamycin increases the proteasome activities and promotes the proteasome-mediated processing of MAGE-A4.

Materials and Methods

Cell Culture and Plasmids

Human hepatoma HuH7 cells and embryonic kidney 293 cells, 293T cells, and monkey COS-7 cells were cultured and transfected with plasmid DNAs as described [18]. MAGE-A4 and S6 proteasomal ATPase cDNA tagged with HA or FLAG were cloned into eukaryotic expression vector pMkit-neo as previously described [18]. The cDNA for the PEST domain (amino acids 410–461) of mouse ornithine decarboxylase was isolated by PCR and cloned into pMkit-neo to express the green fluorescent protein (GFP)-PEST fusion protein. In some experiments, HuH7 cells and 293T cells were transfected with plasmids expressing MAGE-A4 and treated with the following chemicals: Adriamycin, etoposide, cisplatin, actinomycin D, cycloheximide, Z-VAD-FMK (Calbiochem, San Diego, Calif., USA), and proteasome inhibitors, MG115, MG132, lactacystin and epoxamicin (Peptide Institute Inc., Osaka, Japan).

Analyses of Protein Expression and Protein-Protein Interactions

Western blot analysis and immunoprecipitation were performed as described [18]. Antibodies used were mouse monoclonal anti-HA antibody (Roche, Basel, Switzerland), anti-FLAG antibody (Sigma, St. Louis, Mo., USA), anti-GFP antibody (Sigma), antibody to S7 proteasomal ATPase (Affiniti, Exeter, UK), anti-β-actin antibody (Chemicon International, Inc., Temecula, Calif., USA), rabbit polyclonal antibody to 20S proteasome α/β subunits (Affiniti), anti-gankyrin antibody (Santa Cruz Biotechnology, Santa Cruz, Calif., USA), and horseradish peroxidase-conjugated goat anti-mouse antibody (DAKO, Troy, Mich., USA) and anti-rabbit antibody (DAKO).

For glycerol gradient centrifugation and fractionation, cell lysates were obtained using a lysis buffer containing 20 mM Tris-HCl (pH 7.2), 1 mM EDTA, 1 mM NaN_3, 1 mM β-mercaptoethanol, 0.1% Nonidet P-40, and 10% glycerol. Centrifugation of the cell lysates in 10–40% glycerol gradients was performed as described [19].

Measurement of Cellular DNA Content

Cells were harvested with 4 mM EDTA/PBS, fixed in 70% ethanol and stained with 5 μg/ml propidium iodide (Sigma). The DNA content of the cells was analyzed by using a flow cytometer (EPICS XL; Beckmann Coulter, Tokyo, Japan) as described [18]. The bicistronic pIRES-hrGFP-1a vector (Stratagene, La Jolla, Calif., USA) was used to express human MAGE-A4 together with GFP. To selectively analyze the cells expressing transfected cDNA, only the fraction manifesting a high level of GFP fluorescence was analyzed.

Assay of Proteasome Activity

Proteasome activity was analyzed as described by Jana et al. [20]. Cells were treated with Adriamycin (0.4 or 1 μg/ml) for 6 h. They were then suspended in 100 μl of proteasome assay buffer (10 mM Tris, pH 7.4, 1 mM EDTA, 5 mM ATP, 5 mM dithiothreitol (DTT) and 20% (v/v) glycerol), lysed by sonication, and centrifuged at 15,000 *g* for 15 min at 4°C. The supernatant (25 μg) was incubated in the proteasome activity assay buffer (50 mM Tris, pH 7.4, 0.5 mM EDTA, and 50 μM of each proteasome substrate) for 2 h. The substrates Suc-Leu-Leu-Val-Tyr-MCA, Boc-Leu-Arg-Arg-MCA, and Z-Leu-Leu-Glu-MCA were used to determine chymotrypsin-like (Tyr or Phe at P1), trypsin-like (Arg or Lys at P1), and post-glutamyl peptidyl hydrolytic-like (Glu at P1) activity, respectively. The fluorescence intensity was measured at 380-nm excitation and 460-nm emissions by using a Wallac multilabel counter.

In vitro Proteolysis Assay

Ubiquitin-dependent proteolysis was analyzed in vitro essentially as described by Sun et al. [21]. Bacterially expressed MAGE-A4 tagged with FLAG at the carboxy terminus (0.5 μg) was added to a buffer containing the ATP/ADP regeneration system, 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT and 1/20 vol of rabbit reticulocyte lysate, and incubated at 37°C for 30 min in the presence or absence of 26S proteasome (Biomol, Plymouth Meeting, Pa., USA), cell lysates (10 μg) and ubiquitin (100 μM; Boston Biochem, Cambridge, Mass., USA).

Statistical Analysis

Values are expressed as the mean ± SD of triplicates. Significance of the differences was analyzed by an unpaired Student t test or repeated measures analysis of variance. $p < 0.05$ was considered to indicate statistical significance.

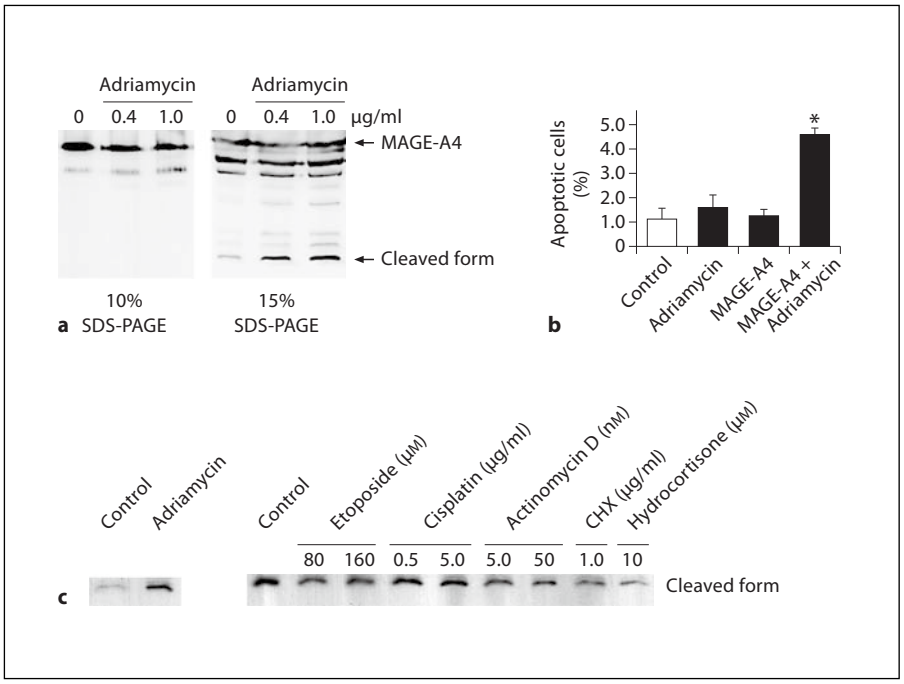

Fig. 1. Effects of Adriamycin on processing of MAGE-A4. **a** 293T cells were transfected with plasmids encoding FLAG-tagged MAGE-A4, and cultured for 30 h. Then, Adriamycin was added to the medium at indicated doses. 48 h after transfection, cell lysates were analyzed by 10 or 15% SDS-PAGE as indicated followed by Western blotting using an anti-FLAG antibody. **b** HuH7 cells were transfected with plasmids encoding GFP in combination with or without MAGE-A4, and cultured in the presence or absence (control) of Adriamycin (0.4 μg/ml) as indicated. 48 h after transfection, the percentage of cells with sub-G1 DNA content in GFP-expressing cells was analyzed by flow cytometry. Results are the mean ± SD of triplicates. * $p < 0.05$ vs. control. **c** HuH7 cells were transfected and analyzed as in **a** in the presence of indicated chemicals. The exposure time for the right panel is about 7 times longer than for the left panel. CHX = Cycloheximide.

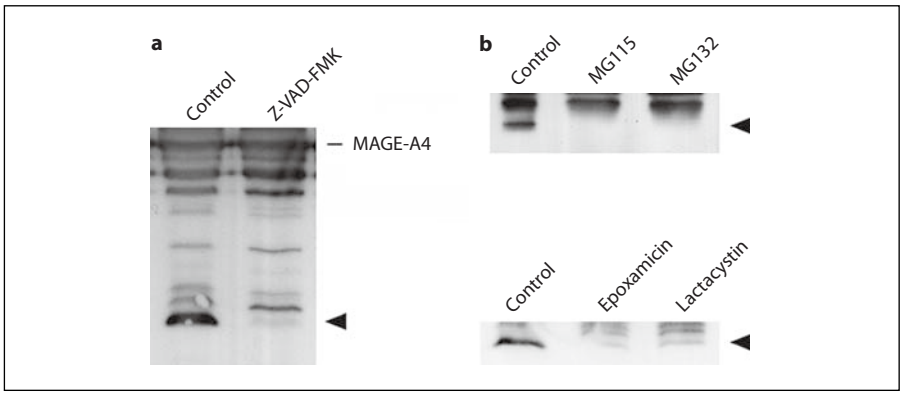

Fig. 2. Effects of proteasome inhibitors on the processing of MAGE-A4. 293T cells were transfected with plasmids encoding FLAG-tagged MAGE-A4 and cultured in the presence of Z-VAD-FMK (40 μM) (**a**), MG115 (30 μM), MG132 (30 μM), epoxamicin (4 μM), or lactacystin (50 μM) (**b**). 48 h after transfection, cell lysates were analyzed by Western blotting using an anti-FLAG antibody.

Results

Effects of Anticancer Agents on the Processing of MAGE-A4

MAGE-A4 is processed to generate the C-terminal fragment with proapoptotic activity [18]. As reported previously, the processing was augmented by a low dose of Adriamycin (fig. 1a). When HuH7 cells were treated with 0.4 μg/ml Adriamycin for 18 h, only a small increase in the number of apoptotic cells was observed (fig. 1b). When combined with the expression of MAGE-A4, apoptosis was significantly increased, while the expression of MAGE-A4 alone did not induce apoptosis (fig. 1b). These results suggest that a low dose of Adriamycin could induce apoptosis, at least partly, by increasing the processing of MAGE-A4. To determine whether the observed activity is specific to Adriamycin, we examined the effects of other anticancer agents on the processing. As shown in figure 1c, the processed form of MAGE-A4 was not increased by etoposide, cisplatin, actinomycin D, cycloheximide, or hydrocortisone under the present conditions.

Effects of Proteasome Inhibitors on the Processing of MAGE-A4

A broad-range caspase inhibitor suppresses the processing of MAGE-A4 (fig. 2a). Since there is no consensus amino acid motif known to be cleaved by caspases in the

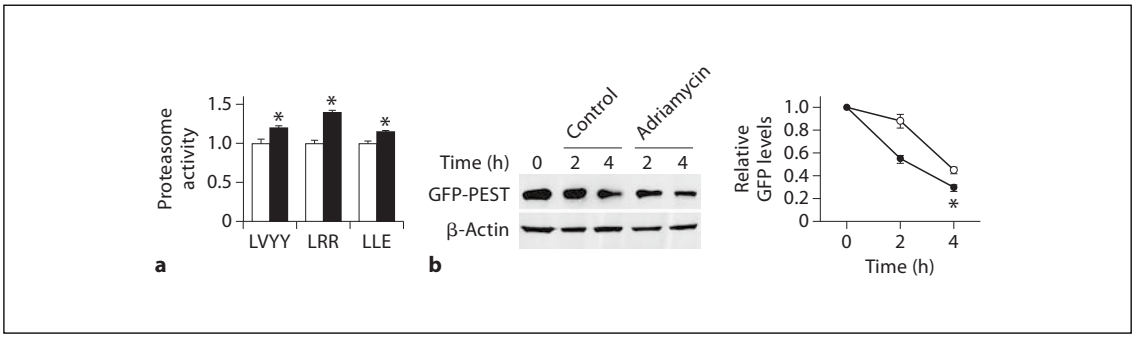

Fig. 3. Effects of Adriamycin on proteasome activities. **a** 293T cells were cultured in the absence (□) or presence (■) of Adriamycin (0.4 μg/ml) for 6 h, and proteasome activities of the cell lysates were measured using fluorogenic peptide substrates Suc-Leu-Leu-Val-Tyr-MCA (LVYY), Boc-Leu-Arg-Arg-MCA (LRR), and Z-Leu-Leu-Glu-MCA (LLE). Results are the mean ± SD of triplicates. * $p < 0.05$ vs. absence of Adriamycin. **b** Cells were transfected with plasmids encoding GFP chimeras containing the PEST domain of ornithine decarboxylase, and cultured in the presence of cycloheximide (10 μg/ml) in combination with (●) or without (control, ○) Adriamycin (1 μg/ml). Cell lysates were analyzed by Western blotting using an antibody against GFP or β-actin (left). Quantification of the band intensity is shown on the right. Results are the mean ± SD of triplicates. * $p < 0.05$ vs. control.

MAGE-A4 amino acid sequence [18], and caspase-1 inhibition is known to block the proteasomal activity as well [22], we suspected an involvement of the proteasome. As shown in figure 2b, all proteasome inhibitors examined including MG115, MG132, lactacystin and epoxamicin suppressed the processing of MAGE-A4, indicating that the proteasome is involved in the cleavage.

Effects of Adriamycin on Proteasome Activities

Since Adriamycin promoted the processing of MAGE-A4 (fig. 1) and the proteasome involvement in the processing was demonstrated (fig. 2), we assessed the effect of Adriamycin on the cellular proteasome activities. As shown in figure 3a, treatment of 293T cells with Adriamycin resulted in increased chymotrypsin-like, trypsin-like, and post-glutamyl peptidyl hydrolytic-like proteasome activities in cell lysates.

The PEST sequences are known to target proteins for destruction by the 26S proteasome [23]. When 293T cells expressing GFP-PEST fusion protein were treated with Adriamycin, the degradation of GFP-PEST was accelerated (fig. 3b). Taken together, these results indicate that Adriamycin increases activities of the proteasome in vivo.

Interaction between MAGE-A4 and the Proteasome

MAGE-A4 binds to gankyrin [17], which binds to S6 proteasomal ATPase [16]. Therefore, we assessed whether MAGE-A4 forms a complex with the proteasome. COS-7 cells were cotransfected with plasmids expressing HA-tagged S6 and FLAG-tagged MAGE-A4. When cell lysates were immunoprecipitated with an anti-FLAG antibody, HA-S6 was detected in the precipitates but not in the precipitates from cells cotransfected with parental FLAG vector and HA-S6 (fig. 4a, left). Reciprocally, MAGE-A4 was detected in the anti-HA immunoprecipitates from cells cotransfected with plasmids expressing HA-S6 and FLAG-MAGE-A4 (fig. 4a, right). Furthermore, during glycerol gradient centrifugation of lysates from 293 cells stably expressing FLAG-MAGE-A4, at least part of FLAG-MAGE-A4 was present in the fractions containing gankyrin and the proteasome (fig. 4b). Thus, MAGE-A4 most probably forms a complex with the proteasome and gankyrin in mammalian cells.

Ubiquitin Requirement for the Processing of MAGE-A4

We next examined whether ubiquitin is necessary for the processing of MAGE-A4 in vitro. As shown in figure 5, the purified 26S proteasome supplemented with HuH7 cell lysates cleaved the MAGE-A4 protein to generate the C-terminal fragment in the presence, but not absence, of ubiquitin (lanes 3 and 4). In the absence of cell lysates or the S26 proteasome, the amount of the cleaved form was markedly decreased (lanes 5 and 6), suggesting that both modification of ubiquitin and proteasome activity are necessary for the processing. These results are consistent with the notion that MAGE-A4 is processed by the proteasome to generate the C-terminal fragment.

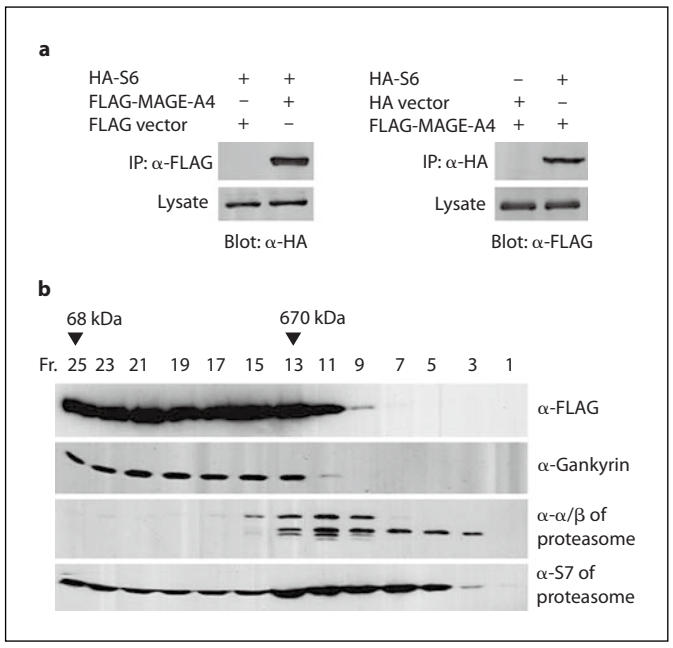

Fig. 4. Interaction between MAGE-A4 and the proteasome. **a** COS-7 cells were cotransfected with plasmids expressing HA-tagged S6 ATPase, HA alone, FLAG-tagged MAGE-A4 or FLAG alone as indicated. The lysates were prepared, immunoprecipitated with an antibody against FLAG or HA, and analyzed by Western blotting using the indicated antibodies. **b** After glycerol gradient centrifugation of lysates from 293 cells stably expressing FLAG-tagged MAGE-A4, fractions were analyzed by Western blotting using the indicated antibodies. Positions of the markers are shown at the top.

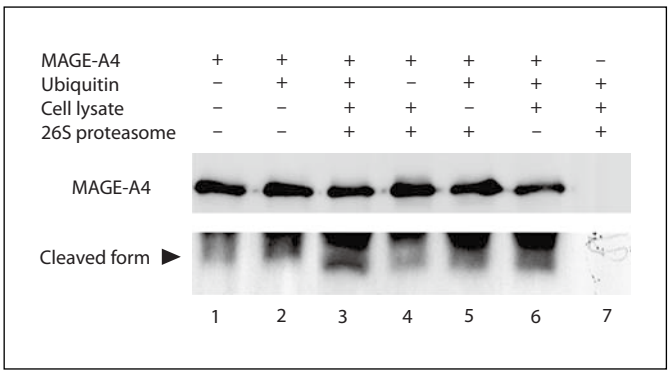

Fig. 5. In vitro proteolysis of MAGE-A4. FLAG-tagged MAGE-A4 was added to a buffer containing ATP/ADP regeneration system, 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT and 1/20 volume of rabbit reticulocyte lysates, and incubated at 37°C for 30 min in the presence or absence of 26S proteasome, HuH7 cell lysates and ubiquitin as indicated. Samples were analyzed by Western blotting using an anti-FLAG antibody.

Discussion

Cellular stress causes an imbalance of the cellular homeostasis, which in turn initiates a complex cascade of stress responses in an attempt to return the cell to its previous equilibrium. In eukaryotic cells, the large ATP-dependent proteolytic machine, the 26S proteasome, prevents the accumulation of nonfunctional, potentially toxic proteins to maintain cellular homeostasis in response to various stressors [4]. This process is considered to be of particular importance in protecting cells against harsh conditions and in a variety of diseases. Once damage exceeds the capacity of repair mechanisms, apoptosis or tissue damage is induced [24, 25]. During apoptosis proteasome is inactivated by caspases [21, 26]. Conversely, upregulation of the proteasome activity is paralleled by chemotherapeutic resistance in Adriamycin-treated leukemic cells [10]. Adriamycin is a potent anticancer agent. In the present study, we found that a low dose of Adriamycin increased the proteasome activities (fig. 3), suggesting that Adriamycin, at a sublethal dose, possibly helps maintain homeostasis of the cells by activating the proteasome. The mechanisms by which Adriamycin activates the proteasome are currently unknown.

MAGE-A4 specifically interacts with the liver oncoprotein gankyrin [16] and suppresses its tumorigenic activity [17]. The antitumorigenic activity of MAGE-A4 is at least partly attributable to the cleaved C-terminal fragment with proapoptotic activity [18]. Here we have demonstrated that the proteasome inhibitors suppress the processing of MAGE-A4 (fig. 2). Furthermore, the proteasome interacted with MAGE-A4 in vivo (fig. 4) and in combination with cell lysates and ubiquitin cleaved MAGE-A4 in vitro (fig. 5). The cell lysates probably provided a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-protein ligase (E3) necessary for ubiquitylation. Since the cleavage of MAGE-A4 occurs at the site following the amino acid residue 213 (Glu) [18] and the proteasome has post-glutamyl peptidyl hydrolytic-like activity, these data strongly suggest that MAGE-A4 is processed by the proteasome to generate the C-terminal fragment. Although a low dose of Adriamycine alone induced little apoptosis, a combination with MAGE-A4 expression significantly increased apoptosis (fig. 1b). Thus, MAGE-A4 sensitizes cells to Adriamycin-induced apoptosis, which is probably mediated by increased proteasomal generation of the proapoptotic MAGE-A4 fragment. Whether MAGE-A4 also sen-

sitizes cells to other anticancer agents remains to be determined.

In conclusion, the present study demonstrates that the proteasome is activated by a low dose of Adriamycin and that MAGE-A4 is processed by the proteasome to generate a proapoptotic C-terminal fragment. Thus, a sublethal dose of Adriamycin increases the proteasome activity, which either maintains cellular homeostasis or leads to apoptosis depending, at least under the present conditions, on the expression of MAGE-A4. These findings might shed some light on the mechanisms of action of Adriamycin and physiological function of MAGE-A4.

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Disclosure Statement

The authors disclose no conflicts.

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Adjuvant Therapy after Curative Treatment for Hepatocellular Carcinoma

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Key Words

Adjuvant therapy · Curative treatment · Hepatocellular carcinoma · Prevention of recurrence · Improvement of survival · Interferon molecular targeted agents

Abstract

It is widely accepted that hepatocellular carcinoma (HCC) has an annual recurrence rate of approximately 15–20% even after potentially curative treatment, with the 5-year recurrence rate reaching 80–90%. This recurrence rate is also known to be similar after various curative treatments including resection, percutaneous ethanol injection therapy, and radiofrequency ablation. Generally, in treating patients with HCC associated with hepatitis C or liver cirrhosis, aggressive efforts to prevent secondary carcinogenesis are necessary rather than simply observing the clinical course after treatment. Presently, a combination of peg-interferon and ribavirin is known to be highly effective in patients with difficult-to-treat hepatitis C with a high viral load and genotype I virus. Therefore, indications of these treatments must be considered to prevent secondary carcinogenesis in patients with hepatitis C. Recently, long-term follow-up of low-dose, long-term maintenance therapy using pegylated interferon-α2a for cirrhotic patients clearly showed a preventive effect on HCC occurrence and recurrence. Preventing secondary carcinogenesis by suppressing inflammation employing the same treatment as that against primary carcinogenesis is also important. The molecular targeted agent sorafenib markedly suppresses the serine/threonine kinases of Raf in the MAP kinase cascade and inhibits the tyrosine kinases of angiogenesis factor receptors such as vascular endothelial growth factor and platelet-derived growth factor receptors. It thus simultaneously prevents the proliferation of tumors and inhibits angiogenesis. A clinical trial to examine the recurrence-preventing effect of sorafenib by administration of it after curative treatment such as resection or ablation is in progress (STORM trial: http://clinicaltrials.gov.com, NCT00692770). Treatments to prevent recurrence (including intrahepatic metastasis and multicentric carcinogenesis) as well as early detection and early curative treatment are extremely important to improve the prognosis of patients with HCC. Thus, further research on this issue should be carried out, especially in relation to molecular targeted therapy.

Introduction

It is widely accepted that hepatocellular carcinoma (HCC) shows an annual recurrence rate of approximately 15–20% even after potentially curative treatment, with the 5-year recurrence rate reaching 80–90%. This recurrence rate is also known to be similar after various curative treatments including resection, percutaneous ethanol injection therapy, and radiofrequency ablation. Prevention of the recurrence rate is one of the unmet clinical needs in the treatment of HCC. In addition, of the recurrence rate

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of 15–20%, about 7% is considered to be due to multicentric carcinogenesis, and the remaining about 10–15% is though to be due to the regrowth of remaining microscopic intrahepatic metastatic foci. Therefore, treatments to prevent recurrence should be conducted with these mechanisms of recurrence in mind. Generally, HCC is considered to recur more frequently from remaining intrahepatic foci within 1–2 years of curative treatment and from multicentric carcinogenesis after 2 or more years.

Interferon Therapy for Viral Eradication in Patients after Curative Treatment for HCV-Related HCC

Generally, in treating patients with HCC associated with hepatitis C or liver cirrhosis, aggressive efforts to prevent secondary carcinogenesis are necessary rather than simple observation of the clinical course after treatment. If a diagnosis of chronic hepatitis C can be made clinically on the basis of the laboratory findings, pegylated interferon (IFN) ribavirin combination therapy to attempt eradication of the hepatitis C virus should be performed. Such patients did not used to be treated aggressively because the majority of them had genotype Ib and a high viral load, which made them 'difficult-to-treat patients'. Naturally, IFN therapy to eradicate the virus is easy after curative treatment for liver cancer in patients with a low viral load of genotype I virus. In the literature, complete viral eradication has been established to exhibit favorable effects on the subsequent lower recurrence and improved outcome [1]. Presently, a combination of peg-interferon and ribavirin is known to be highly effective in patients with difficult-to-treat hepatitis C with a high viral load and genotype I viral. Therefore, indications of these treatments must be considered to prevent secondary carcinogenesis in patients with hepatitis C.

A total of 8 prospective randomized controlled trials (RCTs) of IFN therapy after curative treatment for HCC have been reported despite differences in the type of IFN used and its dose or administration period [1–8]. Five were on HCV-related HCC [1–5], 2 on HBV- and HCV-related HCC [6, 7], and 1 on HBV-related HCC [8]. Of the 5 reports on HCV-related HCC, 4 were from Japan and 1 was from Europe. Four of these reports were about IFN therapy aimed toward viral eradication [1, 3–5], and the duration of IFN administration was 48–104 weeks. The sample size was the largest in a recent report by Mazzafero et al. [5] (n = 150), following a report by Shiratori et al. [4] (n = 74). Intention-to-treat analysis of the results of these studies led to the conclusion that the first recurrence cannot be reduced significantly by IFN therapy, but that an improvement in the outcome is clear in patients in whom the virus was successfully eradicated. However, in 4 reports on the long-term administration of IFN (36 months [2], 26 months [3], 26 months [1], and 24 months [6]), the therapy was clearly effective in decreasing the recurrence rate and improving the outcome.

Recently, long-term follow-up of low-dose, long-term maintenance therapy using pegylated IFN-α2a for cirrhotic patients clearly showed a preventive effect of HCC occurrence although those data are a subset analysis of the HALT-C Study [9].

Low-Dose, Long-Term IFN Therapy (IFN Maintenance Therapy) to Prevent Recurrence and Improve Survival after Curative Treatment

Recently, IFN has been intentionally administered at a low dose and over a long period to improve the ALT level and in expectation of its direct anticancer effect rather than to eliminate the virus. The RCTs reported by Ikeda et al. [2], Kubo et al. [1, 3], and Lin et al. [6] are considered to have been conducted based on this concept. Also, low-dose, long-term IFN therapy has also been reported in case-control studies involving subjects matched for age, platelet count, tumor factors, and hepatic functional reserve [10–15].

The direct anticancer effect of IFN has been demonstrated in vitro [16], and the idea of using IFN at a low dose and over a long period without interruption as a maintenance therapy staring immediately after curative treatment for HCC on the basis of its direct anticancer or carcinostatic effect to reduce the recurrence rate or delay of recurrence has also been proposed [10, 12]. The direct anticancer effect of IFN has been speculated to be derived from: (1) suppression of the cell cycle via IFN receptors, (2) induction of apoptosis via cell-mediated immunity, and (3) inhibition of angiogenesis (fig. 1). The IFN administration period is the longest, with a median duration of 4.7 years, and the sample size is largest (n = 127) in 'Maintenance Interferon Therapy' published in *Oncology* [10], in which the IFN therapy is continued for as long as possible, in principle. In this report, the results of the first low-dose, long-term maintenance IFN therapy using pegylated IFN are presented [10].

Of the case-control studies that have been reported, 5 are related to hepatitis C [11–13] and 2 are related to hepatitis B-related HCC [15, 17]. In all of these studies, the recurrence or survival rate was evaluated similarly to

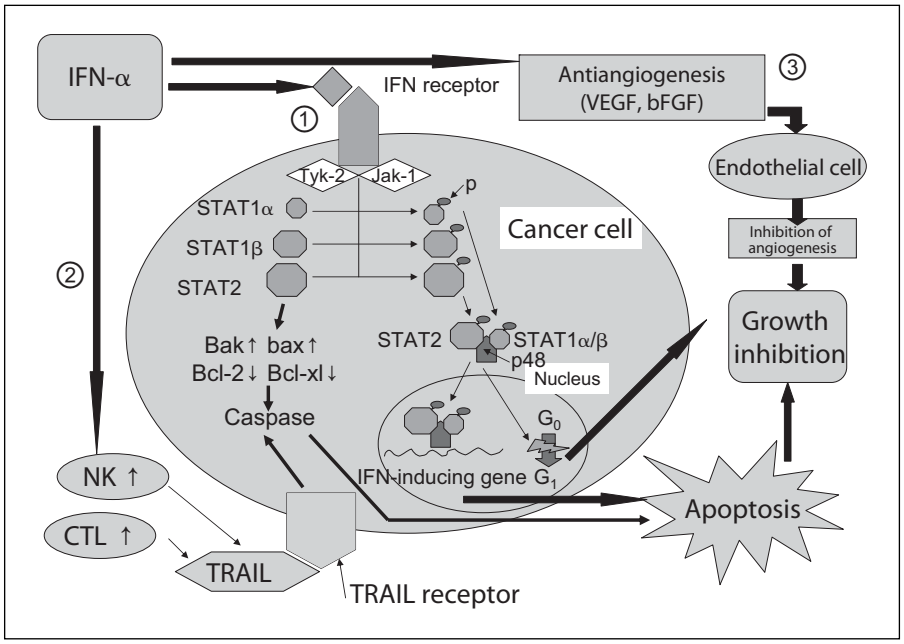

Fig. 1. Hypothesis of the direct antitumor growth inhibition effect of IFN.

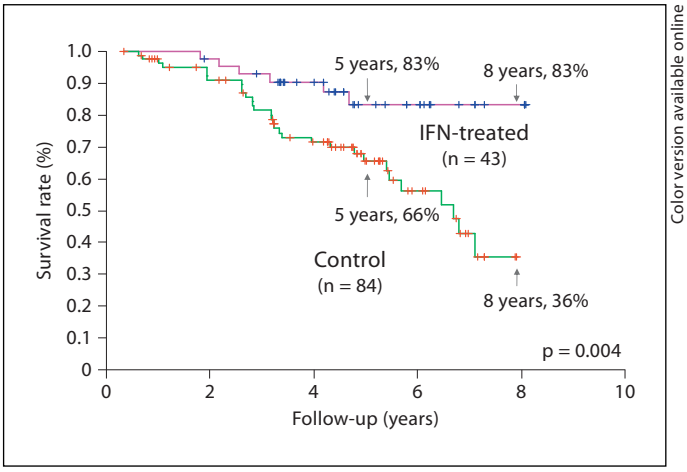

Fig. 2. Overall survival rates after RFA treatment. Kaplan-Meier analysis of cumulative survival rates after curative RFA in the IFN maintenance treatment and control groups. The survival of the maintenance IFN group was significantly better than that of the control group (p = 0.004). Multivariate analysis clearly showed that maintenance IFN therapy is an independent prognostic factor (cited from Kudo et al. [10] with permission).

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RCTs, and significant differences were found in the survival rate with a prolonged duration of IFN administration or the observation period (fig. 2). Improvements in the survival rate were observed in 2 studies with an administration period of 6 months [11, 13] and in 1 study with a median administration period of 4.7 years [10]. A significant difference in the recurrence rate (including the rates of the second and third recurrences) was noted in all 3 studies (fig. 3–5).

It has also recently been reported that recurrence is significantly suppressed, and survival is prolonged, in patients administered IFN for 2 years or longer [18]. Also, the recurrence rate of HBV-related HCC as well as the hazard ratio have been reported to be lower in an IFN-treated group than in a non-IFN-treated group [15]. IFN administration has also been reported to increase the overall survival rate [17]. These results suggest that IFN prevents recurrence by suppressing inflammation or by its direct anticancer effect not only in hepatitis C patients but also in patients with hepatitis B.

From these observations, IFN administration at a low dose and for as long as possible is considered to somewhat delay the first recurrence, prevent second and third recurrences, and, consequently, improve survival [4, 19]. According to Jeong et al. [14], during the follow-up period the Child-Pugh score showed no difference in the IFN-treated group but there was a significant worsening in the control group, and the percentage of patients who could not be candidates for cancer treatment because of the wide spread of cancer or worsening of the liver function was higher in the control than in the IFN-treated group.

To summarize these results, low-dose, long-term, or maintenance IFN therapy is considered to significantly

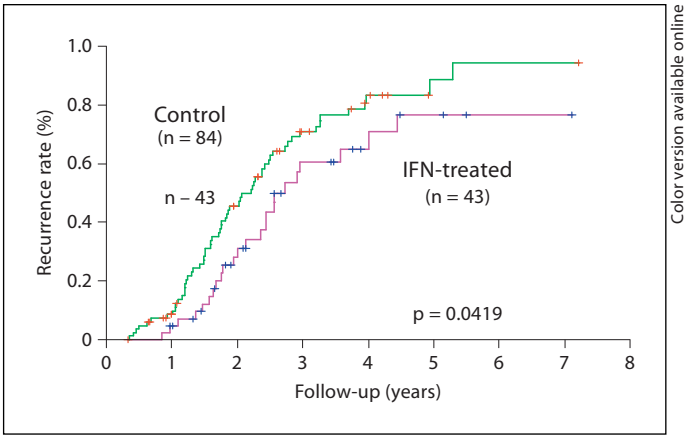

Fig. 3. Cumulative first recurrence rate of HCC. Kaplan-Meier analysis of the cumulative first recurrence rate after curative RFA treatment in the IFN maintenance and control groups. The first recurrence rate was significantly lower in the IFN maintenance group than in the control group (p = 0.04). However, multivariate analysis showed that IFN is not an independent factor (cited from Kudo et al. [10] with permission).

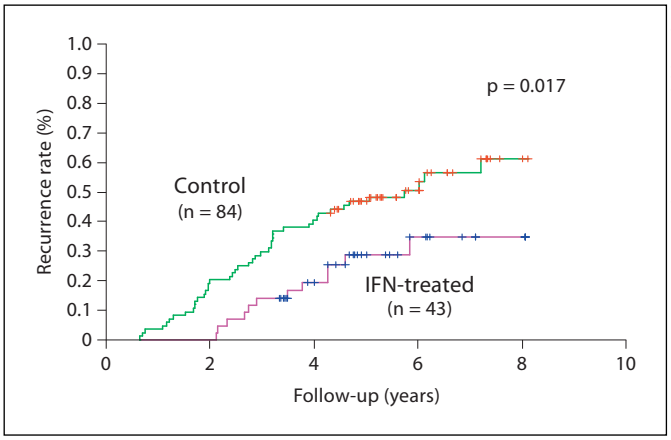

Fig. 4. Cumulative second recurrence rate of HCC. Kaplan-Meier analysis of the cumulative second recurrence rate after curative RFA treatment in the IFN maintenance and control groups. The second recurrence rate was significantly lower in the IFN maintenance group than in the control group (p = 0.017). Multivariate analysis also showed that IFN is a significant independent factor (cited from Kudo et al. [10] with permission).

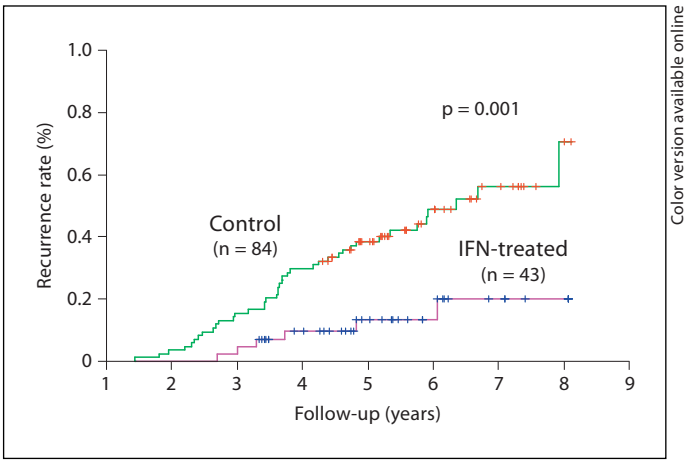

Fig. 5. Cumulative third recurrence rate of HCC. Kaplan-Meier analysis of the cumulative third recurrence rate after curative RFA treatment in the IFN maintenance and control groups. The third recurrence rate was significantly lower in the IFN maintenance group than in the control group (p = 0.001). Multivariate analysis also showed that IFN is a significant independent factor (cited from Kudo et al. [10] with permission).

prevent or delay the recurrence of HCC or secondary carcinogenesis by maintaining the liver function in an adequate state and by its direct anticancer effect, making it possible to detect a slight recurrence while the disease is still in its early stages and to improve the survival rate by making it possible to attempt curative treatment again. The decrease in the need to perform noncurative treatments such as transarterial chemoembolization which worsen the liver function and the liver function-preserving effect of IFN are also considered to contribute to the improvement of the survival rate [19].

Meta-Analyses of the Recurrence-Preventing or Survival-Prolonging Effect of IFN after Curative Treatment for HCC

Recently, 2 reviews [19, 20] and 6 meta-analyses [21–26] were published regarding the recurrence-preventing or survival-prolonging effect of IFN therapy after the resection or ablation of HCC. All of them suggested that IFN is effective in preventing recurrence or improving survival.

Anti-Inflammation Treatments to Prevent Recurrence

It is well known that carcinogenesis from chronic hepatitis or liver cirrhosis can be significantly prevented by lowering AST and ALT at low levels, i.e. suppression of inflammation. Preventing secondary carcinogenesis by suppressing inflammation employing the same treatment as that against primary carcinogenesis is also im-

portant. Since keeping the ALT level low with Strong NeoMinophagen C [27] and ursodeoxycholic acid [28] in addition to low-dose IFN therapy is generally considered to contribute to the prevention of carcinogenesis, it is recommended to conduct these anti-inflammation therapies as well.

Clinical Studies in Progress

The SHARP Study, carried out primarily in Western countries in 2007, showed that the molecular targeted agent sorafenib significantly prolonged the survival of patients with advanced HCC [29]. The Asia Pacific Study also showed similar results [30], and sorafenib is presently regarded as the standard of care for advanced HCC. Sorafenib markedly suppresses serine/threonine kinases of Raf in the MAP kinase cascade and inhibits tyrosine kinases of angiogenesis factor receptors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors. It thus simultaneously prevents the proliferation of tumors and inhibits angiogenesis. A clinical trial to examine the recurrence-preventing effect of sorafenib by administration of it after curative treatment such as resection or ablation is in progress (STORM trial: http://clinicaltrials.gov, NCT00692770). It is designed to validate the working hypothesis that sorafenib has a preventive effect against recurrence by suppressing the angiogenic switch in the progression of a premalignant tumor to a malignant tumor and also in intrahepatic metastatic lesions in which angiogenesis has been established via suppression of angiogenesis and proliferation. If the recurrence-preventing effect of sorafenib is demonstrated, the survival of patients after curative treatments is expected to be prolonged by several years [31, 32].

Other Recurrence-Preventing Drugs

Peretinoin [33, 34] and vitamin K may be effective for the prevention of secondary carcinogenesis and have an inhibitory effect on the formation of portal tumor thrombi [35]. Concerning vitamin K, a large-scale clinical trial was carried out, but it was discontinued as it was judged ineffective by a data monitoring committee at the interim analysis because no significant recurrence-preventing effect could be demonstrated. However, the results may have been different if the end point had been set as the overall survival rate or the time until the appearance of portal tumor thrombi.

Regarding peretinoin, a large-scale multicenter clinical trial was completed, and the results were reported in the annual meeting of American Society of Clinical Oncology (ASCO) in 2010 [36]. Unfortunately, a significant decrease in the recurrence rate, which was the primary end point, could not be demonstrated in the groups administered peretinoin at 300 and 600 mg compared with a placebo group. However, as a 600-mg dose of peretinoin was suggested to be effective compared with the placebo in combination with 300 mg, the positive results of future clinical trials are eagerly expected.

Conclusion

Treatments for the prevention of recurrence after curative treatments of HCC were reviewed. Treatments to prevent recurrence (including intrahepatic metastasis and multicentric carcinogenesis) as well as early detection and early curative treatment are extremely important to improve the prognosis in patients with HCC. In addition, even though HCC recurs, the recurrent lesion is often solitary and detected in a stage in which curative treatment is still possible in patients in whom recurrence-preventing treatment by IFN is continued, possibly because of the antiproliferative effect, antiangiogenic effect, and apoptosis-inducing effect of IFN [19]. Therefore, the prognosis will be markedly improved by such recurrence-preventing treatments as well as efforts for the early detection followed by treatment of recurrence. Finally, the results of clinical trials of adjuvant therapy using the molecular targeted agent sorafenib are also eagerly awaited.

Disclosure Statement

The author declares that he has no financial conflict of interest.

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Usefulness of Combination of Imaging Modalities in the Diagnosis of Hepatocellular Carcinoma Using Sonazoid®-Enhanced Ultrasound, Gadolinium Diethylene-Triamine-Pentaacetic Acid-Enhanced Magnetic Resonance Imaging, and Contrast-Enhanced Computed Tomography

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Key Words
Contrast-enhanced ultrasonography · Hepatocellular carcinoma · Sonazoid® · Imaging modalities · Combination diagnosis

Abstract
Objective: To clarify the diagnostic ability of combining imaging methods to diagnose hepatocellular carcinoma (HCC) using Sonazoid®-enhanced ultrasound (US), gadolinium diethylene-triamine-pentaacetic acid-enhanced (Gd-EOB-DTPA) magnetic resonance imaging (MRI), and contrast-enhanced computed tomography (CECT). ***Methods:*** A total of 32 patients who underwent surgical resection for HCC were studied. Sonazoid-enhanced US, Gd-EOB-DTPA MRI, CECT, and intraoperative contrast-enhanced ultrasonography were done for all patients. The definitive diagnosis of HCC in those patients was based on histopathological confirmation. ***Results:*** A total of 50 histologically proven HCCs were obtained from 32 patients; their mean (±SD) age was 68.3 years ± 8.1. The mean (±SD) nodule size was 2.6 cm ± 1.9. Twenty percent were well-differentiated HCC, 64% were moderately differentiated HCC, 10% were poorly differentiated HCC, 4% were combined HCC and CCC, and 2% were HCC with severe necrosis. The overall diagnostic sensitivity of CEUS, CECT, and Gd-EOB-DTPA MRI was 72, 74, and 86%, respectively; however, there was no significant difference between the three imaging modalities in diagnosing typical HCC ($p = 0.092$). When combining the diagnostic ability of the different imaging modalities, the diagnostic sensitivity of Sonazoid-enhanced US and Gd-EOB-DTPA MRI was 90%, while addition of Sonazoid-enhanced US to CECT and CECT to Gd-EOB-DTPA MRI had a sensitivity of 82 and 88%, respectively. There was no significant difference between the three imaging combinations ($p = 0.970$). ***Conclusion:*** Sonazoid-enhanced US and Gd-EOB-DTPA MRI can be confidently used in daily clinical practice for the management of HCC.

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Introduction

Hepatocellular carcinoma (HCC) is a common cause of cancer-related deaths and its incidence is increasing worldwide [1]. Considering the rising incidence of HCC, its detection at an early stage is an urgent clinical necessity not only in order to improve the disease outcome but also to provide a curative therapy for patients with HCC [2, 3]. The advancement of diagnostic imaging techniques has made a great leap in the diagnosis of HCC, particularly with the introduction of microbubble contrast agents and the development of contrast-enhanced ultrasound (CEUS) which represents a promising tool for the diagnosis of HCC [4]. Levovist is a first-generation contrast agent that is commercially available in many countries. Multiple real-time scanning is a major limitation due to the fragile properties of its microbubbles that can be easily collapsed under the acoustic pressure of ultrasound (US) [5].

Sonazoid® (Daiichi-Sankyo, Tokyo, Japan; GE Healthcare, Milwaukee, Wisc., USA), a lipid-stabilized suspension of perfluorobutane gas microbubbles, was exclusively approved in Japan in January 2007. It facilitates real-time blood flow images in addition to a stable Kupffer phase [6]. Sonazoid is a second-generation contrast agent that has many qualities that enable it to assume a prestigious position among its peers in terms of real-time good vascular imaging and very stable Kupffer phase images that may last up to 120 min after its injection [7, 8].

Defect reperfusion imaging is a novel innovative technique by which arterial blood flow can be demonstrated in nodules that show a defect in the Kupffer phase thanks to the stability of the Kupffer image of Sonazoid, which is unique to that contrast agent. This dual phase contrast US is a breakthrough in the diagnostic imaging of HCC [9, 10].

Several studies have reported the diagnostic sensitivity of the different imaging modalities for HCC [11]. The American Association for the Study of Liver Diseases (AASLD) proposed criteria for the noninvasive diagnosis of HCC recommending the use of a single dynamic imaging modality for nodules larger than 2 cm in diameter arising in cirrhotic liver; for nodules between 1 and 2 cm the diagnosis requires coincidental findings of two dynamic imaging techniques in order to reduce the rate of false positive results. These criteria confirm the fact that use of a combination of diagnostic imaging tools is essential for an accurate diagnosis of HCC [12].

In the present study we aim to clarify the diagnostic ability of combining imaging methods to diagnose HCC using CEUS, gadolinium diethylene-triamine-pentaacetic acid-enhanced (Gd-EOB-DTPA) magnetic resonance imaging (MRI), and contrast-enhanced computed tomography (CECT). To date, the role of combining the different imaging tools to diagnose HCC has not been reported.

Intraoperative US is the most accurate diagnostic technique for the detection and staging of HCC, and the use of contrast agents has an additional value in improving the diagnosis of HCC [13]. More importantly, the use of intraoperative US has overcome the limitations of transabdominal study due to anatomical problems related to the location of the tumor, such as near the diaphragm and the edges of the left and right lobes of the liver [14].

Patients and Methods

Patients

A total of 32 patients who underwent surgical resection for HCC at Kinki University Hospital between July 2008 and March 2010 were enrolled into this retrospective study. Sonazoid-enhanced US, CECT, Gd-EOB-DTPA-MRI, and intraoperative CEUS (IOCEUS) were performed for all patients. The definitive diagnosis of hypervascular HCC in those patients was based on histopathological confirmation after hepatic surgery. There were 6 lesions from 6 patients that underwent biopsy followed by intraoperative radiofrequency ablation; 3 of them (12%) were well-differentiated HCC and the remaining lesions (12%) were moderately differentiated HCC. The study protocol was approved by the Ethics Committee of Kinki University.

Imaging Techniques

CEUS Using Sonazoid

Sonazoid consists of perflubutane microbubbles with a median diameter of 2–3 μm. It was reconstituted with 2 ml sterile water for injection. The dose of the first injection was 0.010 ml encapsulated gas per kilogram of body weight. Sonazoid was injected as a bolus at a rate of 1 ml/s via a 22-gauge cannula placed in the antecubital vein, and flushed with 10 ml normal saline. B-mode US scans were obtained using a GE LOGIQ 7 (GE Medical Systems, Milwaukee, Wisc., USA) with a 4-MHz convex transducer or a 6.5-MHz transducer. The acoustic power of CEUS was set at the default value with a mechanical index of 0.2; the dynamic range was fixed at 60–65 dB. A single focus point was set 10 cm deep. The early vascular and portal phases were observed and after 15 min the target lesion was examined during the Kupffer phase or postvascular phase. After the first injection, the entire liver was scanned and if a defect was observed a second reinjection was performed. The images were stored in a computer.

Contrast-Enhanced Computed Tomography

A 64-channel Light Speed VCT Vision (GE Healthcare) was used with the following scanning parameters: auto mA, 120 kV; rotation time, 0.4 s; image thickness and image interval, 5.0 mm,

and helical pitch, 1.375. Scanning was performed during suspended full inspiration using a bolus trigger technique. A region of interest was drawn in the abdominal aorta at the celiac artery. A total dose of 600 mg/kg body weight of nonionic contrast material containing 300–370 mg/ml iodine was administrated intravenously with a dual-head injector (Dual Shot GX V; Nemoto Kyorindo, Tokyo, Japan) with a fixed duration of 30 s. The arterial phase scanning commenced manually 6 s after the attenuation value in the region of interest reached a plateau and the aortic curve was decreasing. The portal venous phase scan was started 30 s after the end of the arterial phase scan. Each entire liver scanning cephalad-caudal orientation was completed in 5 s.

Gadolinium-Ethoxybenzyl-Diethylenetriamine Pentaacetic Acid-Enhanced Magnetic Resonance Imaging

MRI was performed at 1.5 or 3 T using commercially available MRI systems, i.e. a Gyroscan Intera Nova (1.5 T) or an Achieva (3 T) series (Philips Medical Systems, Best, The Netherlands). All images were obtained on the axial plane. At first, unenhanced MRIs were obtained using a T_1-weighted gradient echo sequence (dual echoes; in-phase and out-of-phase) and Fat-suppressed respiratory-triggered T_2-weighted turbo spin-echo images.

Gd-EOB-DTPA (EOB-Primovist®; Bayer Schering Pharma, Osaka, Japan) was used as a hepatocyte contrast agent. All patients received 0.025 mmol/kg body weight of Gd-EOB-DTPA administered at 2 ml/s though an intravenous line placed in a cubital or cephalic vein and flushed with 35 ml of 0.9% saline at the same rate. Unenhanced scans were obtained using a T_1-weighted gradient echo sequence (dual echoes; in-phase and opposed-phase) and a T_1-weighted high-resolution sequence. After injection of Gd-EOB-DTPA, imaging in the arterial (22–35 s after injection), portal venous (70 s after injection), and hepatobiliary phases (20 min after injection) was obtained using a T_1-weighted high-resolution sequence in a single breath hold (18–20 s).

Intraoperative CEUS

All patients underwent IOCEUS at laparotomy. After hepatic mobilization, the extent of the disease was determined by bimanual palpation, and then IOCEUS was performed using Xario XG (Toshiba) with a PLT-705BTH probe. The dose of Sonazoid was 0.010 ml/kg body weight via an intravenous line followed by 10 ml saline flush. The acoustic power of the CEUS was set at the default value with a mechanical index of 0.2; the dynamic range was fixed at 60–65 dB. A single focus point was set 10 cm deep. The lesion was observed during the early vascular and Kupffer phases. After the first injection, the entire liver was scanned and if a defect was observed a second reinjection was performed. The images were stored in a computer.

Image Analysis

The CECT and Gd-EOB-DTPA MRI images were blindly reviewed by two radiologists who are well experienced in abdominal imaging. CEUS images were blindly reviewed by three hepatologists who have considerable experience with CEUS; another hepatologist who was trained in performing IOCEUS blindly reviewed the IOCEUS records of the patients. The reviewers were aware that the patients were at risk of developing HCC but they did not know any further information. In case of disagreement, the reviewers engaged in joint discussions until a consensus was reached.

Statistical Analysis

The results were expressed as numbers and percentages or means ± SD. A χ^2 test was used to compare the detection ability of the different imaging modalities; the sensitivity for detecting tumors was estimated. $p < 0.05$ was considered statistically significant. The statistical analysis was performed using SPSS statistical software (version 16; SPSS, Inc., Chicago, Ill., USA).

Diagnostic Criteria for HCC

The diagnosis of HCC is strongly dependant on the hemodynamic features of HCC; therefore, the diagnostic criteria for classical hypervascular HCC [11, 15] were as follows:

For Sonazoid-enhanced US:

(a) Hyperenhancement in the early vascular phase followed by a defect in the Kupffer phase.

(b) Defect in the Kupffer phase with a positive defect reperfusion imaging.

For CECT:

(a) Hyperattenuation in the arterial phase.

(b) Washout of contrast material in the portal venous or equilibrium phase.

For Gd-EOB-DTPA MRI:

(a) Hyperintensity in the arterial dominant phase.

(b) Washout of contrast material in the portal venous or low intensity area in the hepatobiliary phase of Gd-EOB-MRI images.

For IOCEUS:

(a) Hyperenhancement in the early vascular phase followed by a defect in the Kupffer phase.

(b) Defect in the Kupffer phase with positive defect reperfusion imaging.

If a lesion showed hypervascularity without washout in the portal venous or equilibrium phase of CECT, hypervascularity without washout in the portal venous phase, or a defect in the Kupffer phase of Sonazoid-enhanced US and hypervascularity without washout in the portal venous phase or low intensity area in the hepatobiliary phase of Gd-EOB-DTPA-MRI, this lesion was considered a atypical pattern of HCC.

Results

A total of 50 histologically proven HCCs were obtained from 32 patients (23 men and 9 women) with a mean (±SD) age of 68.3 years ± 8.1 (range 48–79). The mean (±SD) nodule size was 2.6 cm ± 1.9 (range 0.5–11); 50% of the patients HAD HCV related liver disease while 19% HAD HBV related liver disease. Non-B non-C liver disease accounted for 22% of our patients, combined HCV- and HBV-related liver disease was found in 6% and occult HBV was detected in 3%. The histological diagnoses were as follows: 25% of the lesions were well-differentiated HCC, 64% were moderately differentiated HCC, 10% were poorly differentiated HCC, 4% were combined

Table 1. Characteristics of 32 surgically treated patients and 50 nodules

Mean age ± SD (range), years	68.3 ± 8.1 (48–79)
Gender (males:females)	23:9
Mean nodule size ± SD (range), cm	2.6 ± 1.9 (0.5–11)
Underlying liver disease	
HCV	16 (50%)
HBV	6 (18.8%)
NBNC	7 (21.9%)
HCV and HBV	2 (6.2%)
Occult HBV	1 (3.1%)
Histological diagnosis	
Well-differentiated	10 (20%)
Moderately differentiated	32 (64%)
Poorly differentiated	5 (10%)
Combined HCC and CCC	2 (4%)
HCC with severe necrosis	1 (2%)

HCC and CCC, and 2% were HCC with severe necrosis (table 1).

Sonazoid-enhanced US could diagnose 36 lesions (72%), 3 lesions (6%) were diagnosed as atypical patterns, and 11 lesions (22%) were not detected. CECT provided the diagnosis of classical HCC in 37 lesions (74%), 4 lesions were detected as atypical patterns (8%), and 9 lesions were not detected (18%).

Gd-EOB-DTPA-MRI diagnosed 43 lesions (86%) as typical HCC. Two lesions were detected as atypical patterns (4%), while 5 lesions (10%) could not be detected (table 2). Therefore, the overall diagnostic sensitivity of CEUS, CECT, and Gd-EOB-DTPA-MRI was 72, 74, and 86%, respectively. However, there was no significant difference between the three imaging modalities in diagnosing typical HCC (p = 0.092).

When combining the diagnostic ability of the different imaging modalities, Sonazoid-enhanced US and Gd-EOB-DTPA MRI could diagnose 45 lesions (90%) while addition of Sonazoid-enhanced US to CECT and CECT to Gd-EOB-DTPA-MRI could detect 41 (82%) and 44 lesions (88%), respectively. there was no significant difference between the three imaging combinations (p = 0.970) (table 3).

IOCEUS could detect 46 lesions (92%) as typical HCC, and it could detect 10 (90%) of the 11 lesions (22%) that were not detected by abdominal Sonazoid-enhanced US. Four lesions (36.4%) were located in segment four, 3 lesions (27.3%) were in segment eight, 3 lesions (27.3%) were in segment six, and 1 lesion (9.1%) was present in segment five.

Table 2. Detection ability of HCC using Sonazoid-enhanced ultrasound (CEUS), CECT, and Gd-EOB-DTPA MRI

Imaging modality	All nodules (n = 50)		
	typical	atypical	not detected
CEUS	36 (72%)	3 (6%)	11 (22%)
CECT	37 (74%)	4 (8 %)	9 (18%)
Gd-EOB-DTPA MRI	43 (86%)	2 (4%)	5 (10%)

p = 0.092, a χ^2 test was used for comparisons between the different imaging modalities.

Table 3. Detection ability of HCC using a combination of the different imaging modalities

Imaging modality	All nodules (n = 50)		
	typical	atypical	not detected
CEUS + Gd-EOB-DTPA MRI	45 (90%)	3 (6%)	2 (4%)
CEUS + CECT	41 (82%)	2 (4%)	7 (14%)
CECT + Gd-EOB-DTPA MRI	44 (88%)	2 (4%)	4 (8%)

p = 0.970, a χ^2 test was used for comparisons between the combination of the different imaging modalities.

There were 10 HCC nodules (20%) that were detected as a defect during a Kupffer phase scan and they showed positive reperfusion upon the second injection of Sonazoid (positive reperfusion imaging).

Representative Cases

Case 1

A 65-year-old female with combined hepatitis B and C liver cirrhosis and Child grade A had the following tumor markers: AFP 16 ng/ml and PIVKA II 85 mAU/ml. B-mode US showed a 2.6-cm mixed echoic lesion, and Sonazoid-enhanced US revealed hypervascularity in the early vascular phase with a defect in the Kupffer phase (fig. 1a, b). Gd-EOB-DTPA MRI revealed hypervascularity in the arterial phase with a defect in the hepatobiliary phase (fig. 1c, d). CECT revealed hypervascularity in the arterial phase with a washout in the portal phase (fig. 1e, f).

Case 2

A 65-year-old male patient with combined hepatitis B and C liver cirrhosis and Child grade A had the following tumor markers: AFP 436 ng/ml and PIVKA II 306 mAU/

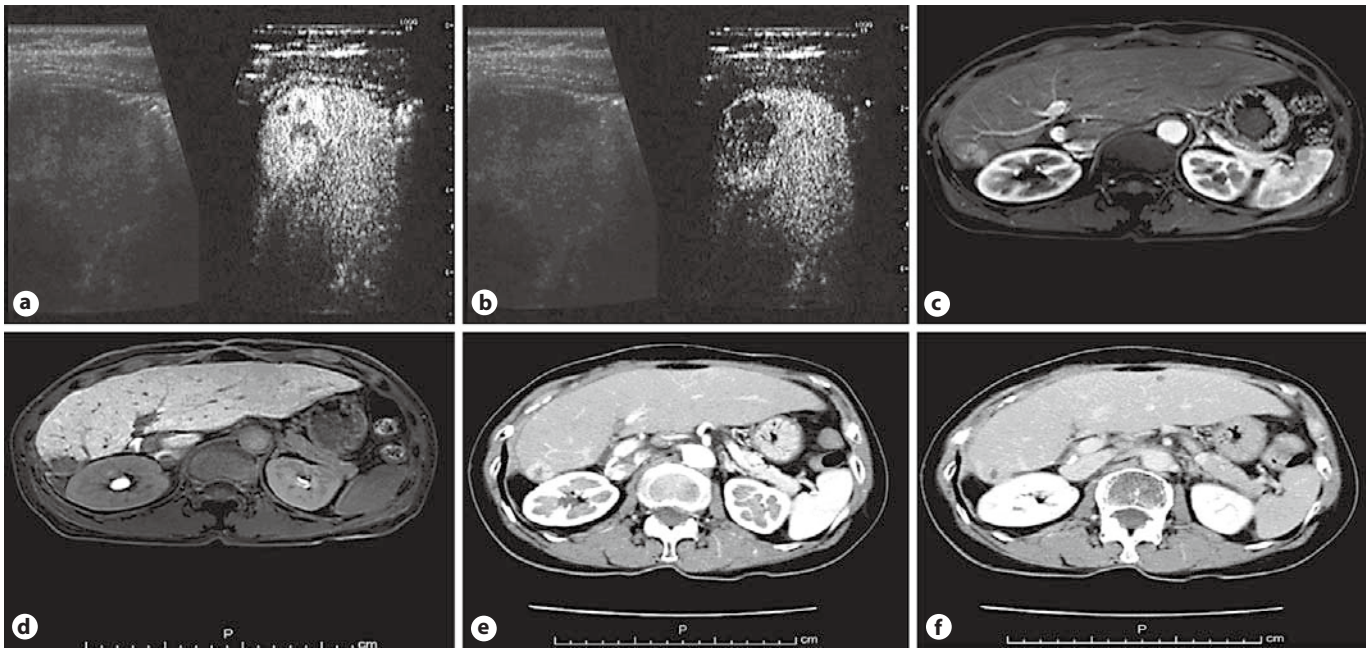

Fig. 1. Detection by Sonazoid-enhanced US, Gd-EOB-DTPA-MRI, and CECT. **a** Sonazoid-enhanced US revealed hypervascularity in the early vascular phase. **b** A defect in the Kupffer phase. **c** Gd-EOB-DTPA MRI revealed hypervascularity in the arterial phase. **d** A defect in the hepatobiliary phase. **e** CECT revealed hypervascularity in the arterial phase. **f** Washout in the portal phase.

ml. B-mode US showed a 2-cm low echoic lesion, and Sonazoid-enhanced US revealed hypervascularity in the early vascular phase with a defect in the Kupffer phase (fig. 2a, b). Gd-EOB-DTPA MRI could not reveal hypervascularity in the arterial phase but a defect in the hepatobiliary phase could be demonstrated (fig. 2c, d). CECT could not detect this lesion (fig. 2e, f).

Discussion

Hepatic carcinogenesis is a multistep process with the following sequence: regenerative nodule, low grade dysplastic nodule, high grade dysplastic nodule, early HCC, and finally overt HCC. The differentiation between high grade dysplastic nodules and early HCC remains an area of ambiguity; early detection and therefore curative treatment are very important [16]. The hemodynamic features of HCC are the conerstone of precise imaging diagnosis, with depiction of arterial hypervascularity being an important finding that requires therapeutic intervention [17, 18].

Our results indicated that the overall diagnostic sensitivity of Sonazoid-enhanced US, CECT, and Gd-EOB-DTPA-MRI was 72, 74, and 86%. Mita et al. [11] reported that Sonazoid-enhanced US had a sensitivity of 68% for diagnosing HCC while CECT and Gd-EOB-DTPA-MRI had a sensitivity of 53 and 77%, respectively. Although the figures of our study were similar to those of the previous one, Sonazoid-enhanced US had a better sensitivity than ours. This can be attributed to the design of the study being prospective while our study was retrospective. In addition most of these lesions were located near the dome and the periphery of the liver which are normal limitations of US. This can be proved by the results of IOCEUS which had a higher sensitivity of detection of HCC (92%), and 90% of the lesions not detected by transabdominal CEUS were diagnosed by IOCEUS. From these results, we can say that the detection ability of Sonazoid-enhanced US (transabdominal CEUS + IOCEUS) was good but the anatomical problem is a limitation of transabdominal CEUS. There was no significant difference between the three imaging modalities in diagnosing HCC; this was also found in the previous report.

The role of combination of the different diagnostic modalities has been clarified in our study; our results showed that the combination of Sonazoid-enhanced US

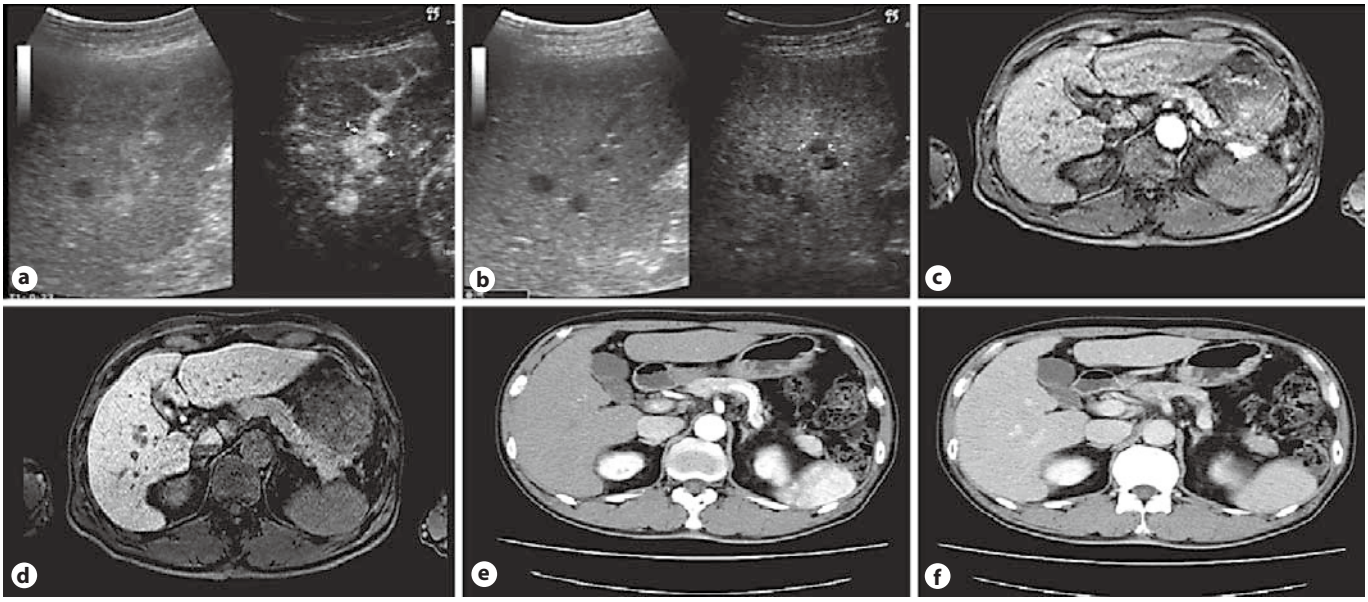

Fig. 2. Detection by Sonazoid-enhanced US and Gd-EOB-DTPA-MRI. **a** Sonazoid-enhanced US revealed hypervascularity in the early vascular phase. **b** A defect in the Kupffer phase. **c** No hypervascularity in the arterial phase of Gd-EOB-DTPA MRI. **d** A defect in the hepatobiliary phase. **e** CECT could not reveal hypervascularity in the arterial phase. **f** No washout in the portal phase.

and Gd-EOB-DTPA MRI had a high sensitivity of 90% for diagnosing HCC while the combination of Sonazoid-enhanced US and CECT had a sensitivity of 82% and the combination of CECT and Gd-EOB-DTPA-MRI had a sensitivity of 88%. Sonazoid-enhanced US could detect one lesion which failed to be detected by both CECT and Gd-EOB-DTPA MRI; only 2 cases that could not be detected by CECT were detected as typical HCC by both CEUS and Gd-EOB-MRI. Sonazoid-enhanced US and CECT could diagnose an HCC lesion which was not detected by Gd-EOB- MRI. In light of these results we can say that the combination of Sonazoid-enhanced US and Gd-EOB-DTPA MRI yields better results than Gd-EOB-DTPA MRI alone. Mita et al. [11] reported the combined sensitivity of Sonazoid-enhanced US and Gd-EOB-DTPA MRI to be 94.1%. The results obtained by this newly developed contrast agent were better than those obtained by Sonovue-enhanced US and CECT [19] or by Sonovue-enhanced US and gadolinium-enhanced MRI [20]. Although the combination of Sonazoid-enhanced US and Gd-EOB-DTPA MRI achieved the highest sensitivity percentage, there was no significant difference between these combinations of the different modalities in diagnosing HCC. Sonazoid-enhanced US has many advantages in terms of real-time scanning, i.e. the safety of Sonazoid, particularly in patients with renal dysfunction and iodine-allergic patients, in addition to no exposure to radiation unlike with other imaging methods [5, 21].

The method of Kudo et al. [9, 10] (defect reperfusion imaging or dual phase imaging) is an innovative technology that could diagnose B-mode ill defined lesions. With this method, the vascular and Kupffer phases can be combined in one real-time slice; for lesions that show a defect in the Kupffer phase, reperfusion of the defect with Sonazoid microbubbles confirms the malignant nature of the lesion. In our study, 20% (10/50) of the lesions were detected as HCC by this method, which can be achieved only with Sonazoid owing to its beautiful real-time vascular image and very stable Kupffer phase which are unique characteristics of that contrast agent.

Noninvasive diagnosis of HCC is based on the use of the different dynamic imaging modalities as proposed by the AASLD. The sensitivity of using a single modality is not sufficient for establishing the diagnosis of HCC. Moreover, the use of a combination of two techniques provides more accurate results [20]. Therefore, we tried to diagnose HCC using a combined method. The other purpose of using a combined method for diagnosis is that precise detection of the number of nodules results in

proper selection of the treatment methods, so we aimed to evaluate whether a combination diagnosis could yield better results than use of a single modality. In conclusion, Sonazoid-enhanced US and Gd-EOB-DTPA MRI can be confidently used in daily clinical practice for the management of HCC.

The shortcomings of our study are its retrospective nature, i.e. archived images being reviewed rather than real-time recordings, in addition to the relatively low number of cases. Another prospective study with an increased number of cases is therefore required.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

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Diagnostic Imaging of Hepatocellular Carcinoma: Recent Progress

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Key Words

Hepatocellular carcinoma · Contrast-enhanced ultrasound · Sonazoid · Defect reperfusion imaging · Double contrast ultrasound · Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging · Sonazoid contrast-enhanced ultrasound

Abstract

The diagnostic imaging of hepatocellular carcinoma (HCC) has recently undergone marked progress. The advent of the ultrasound (US) contrast agent Sonazoid, approved in January 2007, and magnetic resonance imaging (MRI) with the liver-specific MRI contrast agent gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA-MRI), approved in January 2008, are of particular significance. Sonazoid contrast-enhanced US (Sonazoid-CEUS) is useful not only for the diagnosis of HCC, but also for guiding treatment and assessing treatment response. Sonazoid-CEUS has proven to be particularly effective for screening and staging, which used to be considered impossible with CEUS, through the introduction of the newly developed diagnostic technique of defect reperfusion imaging. It is still not possible if other vascular agents such as SonoVue and Definity are used. In particular, Gd–EOB-DTPA-MRI has been suggested to be much more reliable in the differentiation of early HCC from precancerous dysplastic nodules than any other modalities such as multidetector raw computed tomography, dynamic MRI, and superparamagnetic iron oxide-MRI. A decrease in contrast uptake in the hepatocyte phase observed on EOB-MRI is strongly suggestive of cancer, and the absence of early staining in the arterial phase suggests early HCC. The differential diagnostic capacity of Gd-EOB-DTPA-MRI is considered to far exceed that of what were previously the most useful imaging techniques, computed tomography (CT) during hepatic arteriography or CT during arterial portography, and to be comparable to that of the pathological diagnosis by pathologists specialized in liver.

Introduction

Hepatocellular carcinoma (HCC) is the 6th most common cancer and 3rd most common cause of cancer death worldwide.

The diagnostic imaging of HCC has recently undergone marked progress. The advent of the second-generation ultrasound (US) contrast agent Sonazoid (manufactured and distributed by Daiichi Sankyo; provided by

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GE Healthcare), approved exclusively in Japan in January 2007, and magnetic resonance imaging (MRI) with the liver-specific MRI contrast agent gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA-MRI), approved in January 2008, are of particular significance. Sonazoid contrast-enhanced US (Sonazoid-CEUS) is useful not only for the diagnosis of HCC, but also for guiding treatment and assessing treatment response. Sonazoid-CEUS has proven to be particularly effective for screening and staging, which used to be considered impossible by CEUS, through the introduction of the newly developed diagnostic technique, defect reperfusion imaging [1–3]. In addition, Gd-EOB-DTPA-MRI has been suggested to be much more reliable than other modalities such as multidetector raw computed tomography (MDCT), dynamic MRI, and superparamagnetic iron oxide (SPIO)-MRI in areas in which conventional imaging techniques have been ineffective, i.e. the differentiation of early HCC from precancerous dysplastic nodules. The differential diagnostic capacity of Gd-EOB-DTPA-MRI is considered to far exceed that of conventional imaging techniques and be comparable to that of the pathological diagnosis. In this article, the latest advances in imaging techniques for HCC are reviewed.

Advances in US

B-Mode US

B-mode US possesses the advantages that it is noninvasive, can be performed at the bedside, and shows excellent temporal and spatial resolutions. It has therefore been used extensively as a screening method for high-risk patients with HCCs. Indeed, US is recommended as the initial screening modality in the evidence-based [4] and consensus-based [5] Clinical Practice Guidelines for HCC as proposed by the Japan Society of Hepatology (JSH), the practice guidelines on the management of HCC of the American Association for the Study of Liver Disease [6] and the practice guidelines of the European Association for the Study of the Liver [7]. However, the limitations of conventional B-mode US for the definitive diagnosis of HCC have declined considerably in the light of recent improvements in the temporal and spatial resolutions of computed tomography (CT) and MRI, and in MRI contrast agents. The role of US is thus currently limited, unless CEUS, as described below, is employed.

Table 1. Role of imaging in the management of HCC

Diagnosis
1 Screening (lesion detection)
2 Differential diagnosis (confirmation)
3 Diagnosis of malignant grade (dedifferentiated grade)
4 Staging
Treatment
5 Evaluation of treatment response after TACE or RFA
6 Treatment aid (treatment guidelines)
7 Identification of local recurrence or viable lesion
8 Detection of intrahepatic distant recurrence in entire liver

TACE = Transarterial chemoembolization.

Sonazoid-CEUS

Characteristics of Sonazoid-CEUS

Sonazoid is a second-generation US contrast agent that was first approved in Japan in January 2007, in advance of its approval in other countries. It is characterized by its ability to allow real-time blood-pool images to be obtained at a low acoustic pressure. More importantly, it is also taken up by Kupffer cells in the postvascular phase or Kupffer phase (starting 10 min postinjection) and provides extremely stable Kupffer images suitalble for repeated scanning from 10 to about 120 min after injection [2, 3, 8–11].

Table 1 shows the roles of imaging modalities in the diagnosis and treatment of HCC. Despite improvements in imaging modalities such as US, CT, and MR, until recently there have been many limitations associated with the diagnosis and treatment of HCC; this includes issues related to screening, staging, evaluation of therapeutic response, treatment guidance, identification of the site of local tumor progression after ablation, and diagnosis of intrahepatic distant recurrence after treatment (table 2). CEUS using Levovist was clinically useful in differential diagnosis [1, 12, 13], evaluation of the degree of malignancy [14], evaluation of the therapeutic response of transcatheter arterial embolization [15–17], and as a treatment guidance for radiofrequency ablation (RFA) [18, 19], but it is associated with limitations in the evaluation of treatment responses to RFA [20], screening, and staging [1].

Before the introduction of Sonazoid to the clinical use, the expectations of Sonazoid-CEUS were simply that it would 'be more effective and convenient for vascular imaging than Levovist. Imaging will be possible without

Table 2. Problems that should be solved in the management of HCC

Screening	Some tumors cannot be depicted by B-mode US due to coarse liver parenchyma
Staging	Difficult by B-mode or CEUS (using Levovist or SonoVue)
Evaluation of treatment response	CT is frequently used after RFA; difficult to determine the safety margin if Lipiodol is not injected
Localization of the local recurrence nodule after RFA	Difficult by CEUS Not possible by B-mode US alone
Treatment guidance	Difficult for the nodules which are not shown by B-mode US
Detection of recurrence	MDCT every 3 months (cost, X-ray exposure, etc.)

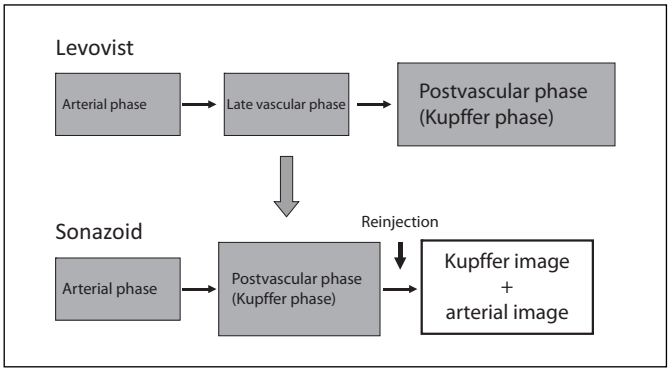

Fig. 1. Defect reperfusion imaging: basic concept. Defect reperfusion imaging of tumors that are unclear on B-mode US. Typical HCC shows arterial vascularity in the Kupffer defect after reinjection.

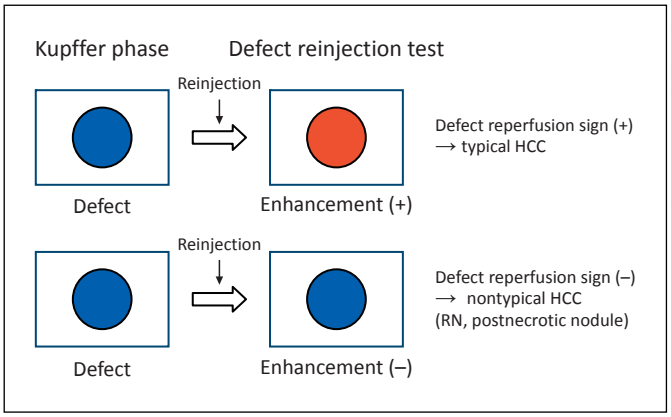

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Fig. 2. Value of defect reperfusion imaging in B-mode-undetectable nodules. Defect reperfusion imaging makes possible the confirmation of HCCs that are unclear on B-mode US but detectable on dynamic CT, local recurrence after RFA, nodules on screening, and a definitive diagnosis of HCC. RN = Regenerative nodule.

high-end equipment, leading to reducing the skill- or equipment-dependence and greater popularity of CEUS than at least in the Levovist era.' Regarding Kupffer imaging, several negative views were expressed, such as: 'It may provide very stable Kupffer imaging, but, considering that its major objective is staging of metastatic liver cancer, Kupffer imaging will not be used widely in Japan, and, in that sense, Sonazoid is unlikely to cause marked changes in the clinical practice concerning HCC.' However, these views have changed markedly over the approximately 3 years since its introduction on the market on January 10, 2007. Sonazoid-CEUS has emerged as an innovative breakthrough technology capable of revolutionizing the clinical approach to HCC through the development of an epoch-making technique of 'defect reperfusion imaging' [11] or 'double-contrast US' [10].

Defect reperfusion imaging is achieved by reinjecting Sonazoid into areas shown to be defects in the Kupffer phase [10, 11]. The introduction of this technique has resolved most of the limitations associated with the diagnosis and treatment of HCC, including those shown in table 2.

Development of Defect Reperfusion Imaging (Double-Contrast US)

We developed defect reperfusion imaging [2, 11] in February 2007, by applying Kupffer imaging, which is extremely stable and suitable for repeated scanning and real-time blood flow imaging. This represents a breakthrough technique in determining the sites of tumors and is a treatment aid for HCCs that are not distinctly delineated by B-mode US but show a typical vascular pattern on dynamic CT or MRI (fig. 1, 2) [2, 11].

CEUS is performed at a mechanical index value of 0.2. Sonazoid is intravenously injected at 0.01 ml/kg, early arterial enhancement can be evaluated in the vascular phase, and the presence or absence of defects was evaluated in the Kupffer phase from 10 min after injection. A probe is then applied to areas identified with defects in the Kupffer phase, after which an additional dose of Sonazoid (0.01 ml/kg) is injected, and the presence or absence of arterial blood flow into the defects can be evalu-

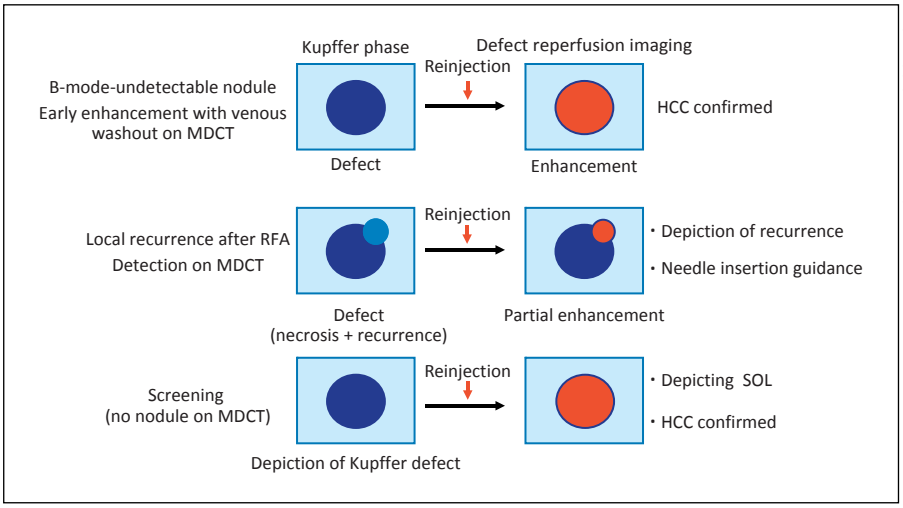

Fig. 3. Defect reperfusion imaging. In addition to B-mode-undetectable nodules, detection of area of recurrence followed by needle insertion guidance and screening is possible by defect reperfusion imaging. SOL = Space occupying lesion.

ated by defect reperfusion imaging of the stable Kupffer phase and the reinjection method [11]. By assuming that the defect reinjection sign was positive when arterial vascularity was noted inside a Kupffer defect, nearly 100% of typical HCCs could be diagnosed (fig. 1, 2). Moreover, when the tumor sites are not identified with B-mode US, vascular-phase CEUS is not useful at all. However, when clear defects are observed in the Kupffer phase, reinjection of Sonazoid into these defects facilitates the confirmation of HCC, leading to a precise treatment guidance at the RFA needle placement (fig. 3).

This method first uses stable Kupffer imaging 10 min postinjection without vascular imaging to detect tumors undetectable by B-mode US, and the reinjection of Sonazoid confirms whether or not these tumors receive an arterial supply (fig. 1–3) [2, 3]. A characteristic of this technique is that tumors exhibiting typical CT images, arterial enhancement with venous washout can be correctly confirmed as defects first in the Kupffer phase, and their arterial enhancement is then demonstrated by the reinjection of Sonazoid, requiring no special equipment or analytical procedures (fig. 1, 2). This novel technique allows the identification of tumors that present as typical CT images but are not identifiable on B-mode US with an almost 100% confidence. If a tumor is detected as a Kupffer defect, but shows no enhancement by the reinjection of Sonazoid, i.e., hypovascular nodule, it may be confirmed to be a different nodule from a hypervascular tumor, which was originally detected by CT (fig. 2). This technique is therefore expected to be a highly significant addition to the treatment guidance for HCC.

Defect reperfusion imaging also has a wide range of applications, allowing screening for HCC in cirrhotic livers with coarse hepatic parenchyma [11], diagnosis of malignant grade, identification of the site of local tumor progression after treatment [21, 22], and US-guided needle puncture [22–25] (fig. 3).

Impact of Defect Reperfusion Imaging on the Diagnosis and Treatment of HCC

Screening

To screen patients in the super-high-risk group (viral hepatitis B- or C-related liver cirrhosis), Sonazoid is injected intravenously in the outpatient clinic at 0.01 ml/kg. Patients then walk to the US department where they are examined by a US technologist, 10–60 min after the injection. Kupffer phase screening with Sonazoid-CEUS can be conducted simply as a routine examination, and the examiner can concentrate on depicting Kupffer defects in the enhanced normal liver parenchyma in the Kupffer phase, which is extremely simple. It can more easily pick up the abnormal Kuppfer defect as compared to B-mode US, which is much more difficult because of coarse liver parenchymal echo. If a defect is found, dual-phase fusion images, involving superimposed images of different phases (Kupffer and arterial phases), can be obtained by reinjecting Sonazoid, thus providing information on both the Kupffer function and arterial blood flow in one tomographic image (fig. 1). This dual-phase fusion imaging or double-contrast US allows HCC to be detected and definitively diagnosed with an accuracy of 100%. Sonazoid will also markedly improve the efficiency of HCC screening [10]. CEUS has conventionally been con-

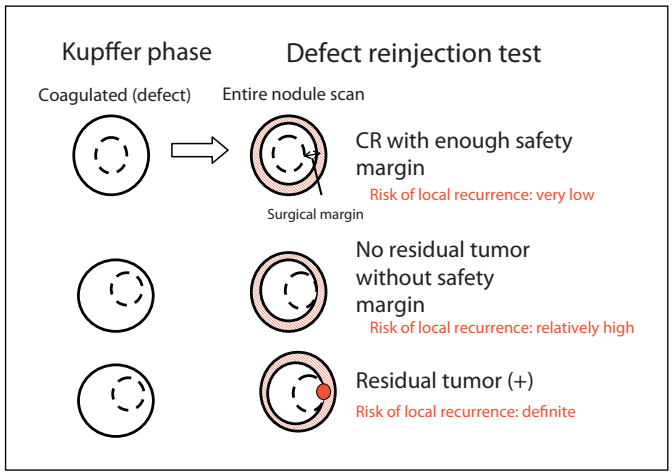

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Fig. 4. Value of defect reperfusion imaging (reinjection test) in the evaluation of treatment response by RFA. Evaluation of treatment response by defect reperfusion imaging with Sonazoid. CR = Complete response.

sidered as suitable for tumors already detected by B-mode US as in the case of SonoVue or Definity, rather than for screening or staging, but this concept has been completely changed by the introduction of defect-reperfusion imaging using Sonazoid. At present a prospective randomized study comparing which is the better modality for surveillance – B-mode US or Kupffer phase US – is ongoing (NCT trial No. 01214343).

Staging

The introduction of Sonazoid is also expected to result in breakthroughs in HCC staging. CT hepatic arteriography (CTHA) and CT arterial portography (CTAP) have previously been the most sensitive modalities for staging, but Sonazoid-CEUS allows the presence or absence of Kupffer defects unlike the main tumor to be evaluated by careful scanning of the entire liver in the Kupffer phase. If defects are detected, the possibility of intrahepatic metastases can be confirmed [2, 3], making accurate staging possible which is comparable to dynamic CT.

Evaluation of Therapeutic Response

The therapeutic response of HCC by RFA has usually been evaluated by CT [26]. However, the comparison with pretreatment CT images is necessary to determine the safety/ablative margin, and judgments based on CT images tend to be inaccurate when Lipiodol is not injected into the liver at the angiographic procedure. In addition, it is very difficult to evaluate the therapeutic effect using CEUS alone, because the margin of the original nodule becomes blurred after RFA [27]. However, defect reperfusion imaging allows the safety/ablative margin to be determined accurately, by delineating the treated nodule in the Kupffer phase, determining the coagulation area, and then after reinjection of Sonazoid scanning the entire tumor confirms whether the safety margin is large enough or not by assessing the distance of the arterial flow area and the ablated area (fig. 4). Further evaluation is needed to establish the percentage of patients in whom this procedure can be applied, i.e., how often the margin of the tumor can be correctly determined after RFA treatment using this procedure. The current situation is that evaluation CT can be omitted in about half of patients according to our experience [unpubl. data]. Sonazoid-CEUS is also more favorable than CT from the point of view of cost-effectiveness. In addition, Sonazoid-CEUS has several important advantages, including lower cost, reduced X-ray exposure, no allergy to the contrast medium, and applicability in patients with renal failure or bronchial asthma, in whom dynamic CT/MRI cannot be performed.

Treatment Guidance

Defect reperfusion imaging is most effective in the field of treatment guidance. Real-time virtual sonography [28, 29] or puncture under Levovist-enhanced US guidance [18, 19] used to be performed in tumors unidentifiable on B-mode US. However, real-time virtual sonography requires CT volume data and special equipment, and it is often difficult to examine exactly the same cross section. Levovist is technically difficult to use, in that the puncture must be performed during a very short period of time of the early vascular phase [18, 19], and it has thus failed to gain wide acceptance in this field.

In contrast, Sonazoid-CEUS makes it possible to accurately detect even the B-mode-unidentifiable nodules with the use of defect reperfusion imaging and then facilitates accurate needle placement after having confirmed their viability. After having confirmed the viability, needle placement can be performed during the long-lasting, stable Kupffer phase, allowing the operator more time for the procedure [21–25, 30].

US Imaging of Locally Recurrent Foci

Sonazoid-CEUS is also effective in determining the site of local tumor progression of previously ablated HCC. It has previously not been possible to guide the puncture needle or to determine the site of local tumor progression by B-mode US alone. This was because the previously co-

agulated tumor, surrounding liver tissue, and recurrent tumor (residual viable tumor) present complicated echogenicity, and it was not possible even for experienced operators to identify the recurring foci, which are clearly demonstrated as enhanced areas on CT, by B-mode US. Because of the presence of an infinite number of US planes and a different cross-sectional plane from CT [26], it is difficult to identify the viable tumor by referring to CT image. However, defect reperfusion imaging using Sonazoid now makes the detection of locally recurrent foci by CEUS possible, warranting its description as a revolutionary breakthrough.

Depiction of Recurrence after Local Treatment during Periodical Follow-Up

Both local recurrence and intrahepatic distant recurrence during follow-up after local ablation can be detected with 100% accuracy by defect reperfusion imaging, and Sonazoid-CEUS may, therefore, be substituted to some extent for 3-monthly CT examinations, leading to reduced cost and X-ray exposure [23]. On a practical level, it is recommended that CEUS and dynamic CT should be performed one after the other for a 3-month interval according to the Japanese practice guidelines [5]. However, this has to be confirmed by further studies.

Improvements on CT

MDCT has been a major improvement over single helical CT insofar that the collection of volume data for the entire liver within a period of less than 10 s has now become possible. This has been achieved by increasing the number of detector rows and improving the reconstruction algorithm. This technique makes it possible to resolve temporal and spatial data, whereas in the past it has always been necessary to do a traded off in order to render a semblance of clinically useful data in dynamic studies of the liver. Multiple scans have become possible during the arterial-dominant phase, which is time-dependent, and the spatial resolution has improved to allow reconstruction at a millimeter-order voxel level. However, the concern over X-ray exposure remains. CT is considered to have reached the stage of the 'optimal phase' and 'optimal spatial resolution'.

Regarding the diagnosis of hypervascular HCC, a study comparing the results of image reconstruction with collimation at 2.5, 5, and 7.5 mm reported no significant difference in the diagnostic accuracy for lesions of ≤5 mm [31]. From the viewpoint of hemodynamics, few HCCs ≤5 mm in diameter exhibit hypervascularity during the course of their growth, except for intrahepatic metastases, and collimation at 5 mm is therefore considered appropriate for screening [32].

Since the advent of helical CT, two- or three-phase dynamic studies consisting of artery-dominant and portal-dominant phases, with the equilibrium phase, have been essential for the detection and differential diagnosis of nodular lesions of the liver as recommended by the AASLD Guidelines [6] and JSH Guidelines [5]. The artery-dominant phase is extremely important for the diagnosis of hypervascular HCC, but tumor staining is observed for only 7–19 s, and it can be difficult to scan the entire liver in the optimal artery-dominant phase using conventional helical CT. MDCT makes it possible to scan the entire liver multiple times during the artery-dominant phase, and the possibility of detecting tumor staining during the optimal phase has increased. Studies have also reported on the usefulness of double-arterial CT, which scans the early and late arterial phases [33, 34], but subsequent studies by Ichikawa et al. [35], Laghi et al. [36], and Francis et al. [37] failed to show any significant differences compared with the late arterial phase alone.

Indeed, MDCT has clearly improved in the ability to detect the early staining of HCC compared with single-detector CT [38]. This improvement may depend on the properties of the target tumor, the degree of liver cirrhosis affecting the liver hemodynamics, the injection rate and dose of the contrast medium. If the time until the appearance of the contrast medium in the artery was measured using a test dose, imaging at the optimal timing is possible but the routine procedure needs to take into consideration the balance between the simplicity of the procedure, the X-ray exposure dose and diagnostic capacity.

Shortening of the scanning time and improvements in spatial resolution by the introduction of MDCT have markedly improved the quality of three-dimensional CT angiograms to a level nearly comparable to invasive digital subtraction angiography. However, further studies are needed to establish the optimal dose, concentration, and injection rate of the contrast medium, and the timing of scanning [39]. It is desirable to avoid invasive examinations in living liver transplant donors, and three-dimensional CT angiography by MDCT has made conventional angiography almost unnecessary [40].

Low-radiation-dose CT has been attempted as a measure to reduce radiation exposure, but the effectiveness of a noise reduction filter for preventing the deterioration of the image quality is unclear [41]. Further improvements

are needed to make the modality clinically relevant, but it represents a promising direction for the development of CT.

Improvements in MRI

Evaluation of Hemodynamics by Gd-DTPA-MRI

Gd-DTPA (Bayer Schering Pharma, Germany) is a conventional, nonspecific extracellular distribution-type contrast medium with kinetics similar to those of an iodine contrast medium and with a contrast-enhancing effect corresponding to the blood flow distribution. As noted above, dynamic multiphase studies have become possible as a result of the increased numbers of examination by MDCT, and the optimization of its conditions is based on its usefulness and radiation-exposure-free profile. The injection volume of an MRI contrast medium required to obtain a contrast-enhancing effect is much smaller than that of an iodine contrast medium, making it more appropriate for dynamic studies, and its usefulness has been recognized through the spread of high-speed scanning techniques such as gradient echo and fast spin echo. However, many masses ≤2 cm in diameter detected by this modality may include false lesions [42]. Ito et al. [43] performed a 6-phase dynamic study and reported the technique's usefulness for differentiating between HCC and false lesions, but the procedure is generally a bit too complicated for routine use.

Diagnosis Using Liver-Specific Contrast Agents

SPIO-MRI (Kupffer Cell Function)

The clinical use of SPIO-MRI with a liver-specific contrast medium has become available, and its usefulness has been reported by a number of authors [44, 45]. SPIO is taken up by the reticuloendothelial system (primarily Kupffer cells), thus producing a contrast-enhancing effect. There are usually no Kupffer cells in metastatic tumors, and SPIO therefore acts as a negative contrast medium for identifying these lesions. Its tumor-detecting ability is high compared to MDCT and has been reported to be comparable to that of CTAP [46–48]. The presence of Kupffer cells in primary tumors of the liver provides an index for discriminating between benign and malignant tumors and for evaluating the degree of differentiation [24, 43, 44]. However, because Kupffer cell function is reduced in patients with advanced liver cirrhosis, its diagnostic capacity for well-differentiated tumors containing residual Kupffer cells is not always satisfactory. Ferumoxide, the first SPIO approved for clinical use, must be intravenously infused over 30 min or longer and is thus inefficient despite its excellent usefulness [47–49]. Ferucarbotran (Resovist, Bayer Schering Pharma), which can be intravenously injected in a bolus, has been widely used until the time of EOB-MRI approval, resulting in marked shortening of the examination time.

There have also been reports of the simultaneous use of a contrast medium with a type of nonspecific extracellular fluid distribution and SPIO, making use of their respective characteristics for the qualitative diagnosis and detection of tumors. Bhartia et al. [50] performed double-contrast imaging in a dynamic study using a gadolinium preparation and SPIO in patients before liver transplantation, and reported satisfactory results in tumors ≥10 mm in diameter. Kim et al. [51] administered SPIO and a gadolinium preparation at different times, and evaluated their diagnostic accuracies; the diagnostic accuracy was better with SPIO in hypovascular tumors, but was better with Gd-DTPA in hypervascular tumors. They recommended their combined use to improve the overall detection capacity.

Gd-EOB-DTPA (Hepatocyte Function)

Gd-EOP-DTPA (Bayer Schering Pharma, Germany) has been available for clinical use in Japan since January 2008. It has an excellent ability to detect liver tumors and evaluate hepatocyte function in the hepatobiliary phase, as well as providing information on the nodular hemodynamics by dynamic imaging. It has now become the most frequently used contrast medium in liver MRI. Gd-EOB-DTPA has been used clinically in European countries for several years, and satisfactory results in detecting focal liver lesions have been reported by a multicenter collaborative study [52]. Gd-EOB-DTPA has a high T_1-relaxing effect and is used primarily in T_1-weighted imaging. It serves as an extracellular fluid contrast medium in the early phase after intravenous injection, and the T_1-shortening effect of Gd thus makes evaluation of the hepatic blood flow possible. Although the Gd concentration of Gd-EOB-DTPA is one quarter that of the extracellular fluid MR contrast medium, Gd-DTPA, it has a T_1-shortening effect of about half compared to Gd-DTPA in light of its T_1-relaxing effect. This enables the diagnosis of hypervascular HCC by optimizing dynamic scanning and providing high-quality arterial-phase images [53, 54].

Recent marked improvements in high-speed MRI have also been made regarding the diagnostic accuracy

based on blood flow changes, and its spatial resolution is approaching that of MDCT. High-spatial-resolution three-dimensional Fourier transform-T_1-weighted imaging, such as T_1 high-resolution isotropic volume examination (Philips, Germany) and liver acquisition with volume acceleration (GE Healthcare, Milwaukee, Wisc., USA) used for liver MRI, have become powerful tools to examine the arterial phase on dynamic MRI of the liver. Dynamic MRI of the liver using Gd-EOB-DTPA and three-dimensional Fourier transform-T_1-weighted imaging has a very high diagnostic ability. Dynamic imaging using Gd-EOB-DTPA is therefore useful for the diagnosis of hypervascular HCC [52, 53]. However, as the injection volume per body weight of Gd-EOB-DTPA is about half of Gd-DTPA, the duration of staining of the aorta and hypervascular HCC tends to be short, and optimization of the timing of scanning in the arterial phase during dynamic imaging is thus extremely important. The optimization of imaging in the arterial phase depends largely on the performance of the MRI system. It is necessary to adjust the protocol at each facility to capture the peak tumor staining at the best timing, taking into consideration the scanning time in the scanning sequence used and the design of K-space data collection.

Regarding the portal/venous phase, the washout observed in hypervascular HCC on CT and Gd-DTPA dynamic MRI is an important clue for the diagnosis of HCC, but caution is needed concerning the judgment of washout on Gd-EOB-DTPA. Because the contrast medium during imaging using Gd-EOB-DTPA is present in both the extracellular fluid and the hepatocytes during the equilibrium phase, the tumor-liver contrast during the equilibrium phase is derived from washout of the contrast medium from the tumor, and contrast enhancement of the surrounding liver tissue by the hepatocyte-specific contrast medium, i.e., the mechanism of contrast enhancement during the equilibrium phase differs between dynamic CT/MRI and Gd-EOB-DTPA-MRI. Time-intensity analysis has shown that Gd-EOB-DTPA is already taken up by hepatocytes about 1 min after administration, such that signals from the blood vessels and those from the liver parenchyma are mixed during the portal phase 60–80 s after administration.

Similarly, it must be understood that contrast enhancement observed 2–5 min after the administration of Gd-EOB-DTPA differs from that observed by the use of a conventional extracellular fluid contrast medium. In the late phase, Gd-EOB-DTPA is gradually taken up by hepatocytes via organic anion transporters (OATPAs) such as OATP1B3 [55] or OATP8 [56]. It begins to be taken up by hepatocytes about 1 min after administration, and the T_1-signal intensity of the liver increases serially thereafter. Tumor/liver contrast sufficient for the detection of liver tumors can be achieved in patients with no liver dysfunction about 20 min after the intravenous injection. This mechanism resembles that by which SPIO, another liver-specific MR contrast medium, enhances the contrast depending on the hepatic reticuloendothelial function. Thus, the most important aspect of Gd-EOB-DTPA MRI is that the detection of liver tumors and their differential diagnosis can be achieved via evaluation of both their blood flow characteristics and hepatocyte function.

As discussed above, Gd-EOB-DTPA also achieves arterial staining of hypervascular HCC due to its extracellular fluid T_1-shortening effect, but the staining of capsule-like structures is weak in the arterial phase of contrast-enhanced MRI using the usual dose of Gd-EOB-DTPA [53]. In contrast, in the hepatocyte phase, HCC with no normal hepatocytes fails to take up the contrast medium and is imaged as a low-signal area on T_1-weighted imaging [57, 58].

The frequent use of Gd-EOB-DTPA-MRI has drawn attention to the presence of HCCs showing no signal reduction in the hepatocyte phase, despite tumors being hypervascular. If OATP1B3 or OATP8 is preserved, EOB is taken up by cancer cells. However, hepatocytes cannot excrete it; because of possible impairment of excretory transporter polypeptide, they are demonstrated as iso- or high intense in the hepatocyte phase. This phenomenon might be related to retention of bile juice, which appears green by formalin fixation; such resected specimens have long been known as 'green hepatomas', though it is difficult to determine their overall frequency. However, knowledge of the presence of a green hepatoma (or bile-producing/slow-bile-excreting HCC) and its characteristic features on imaging is important. This assumption regarding green hepatomas is just a hypothesis. The true mechanism or relation to transporter expression must be clarified in future studies.

Gd-EOB-DTPA-MRI can clearly discriminate hypovascular HCCs from dysplastic nodules, which were difficult to differentiate by conventional CTHA or CTAP [59, 60] in the resected series only. However, false-positive and false-negative lesions have been reported by other studies using liver biopsy specimens [61]. There have also been sporadic reports demonstrating that tumors showing signal reduction in the hepatocyte phase of EOB-MRI included dysplastic nodules, and that tumors showing no signal reduction in the hepatocyte phase included early

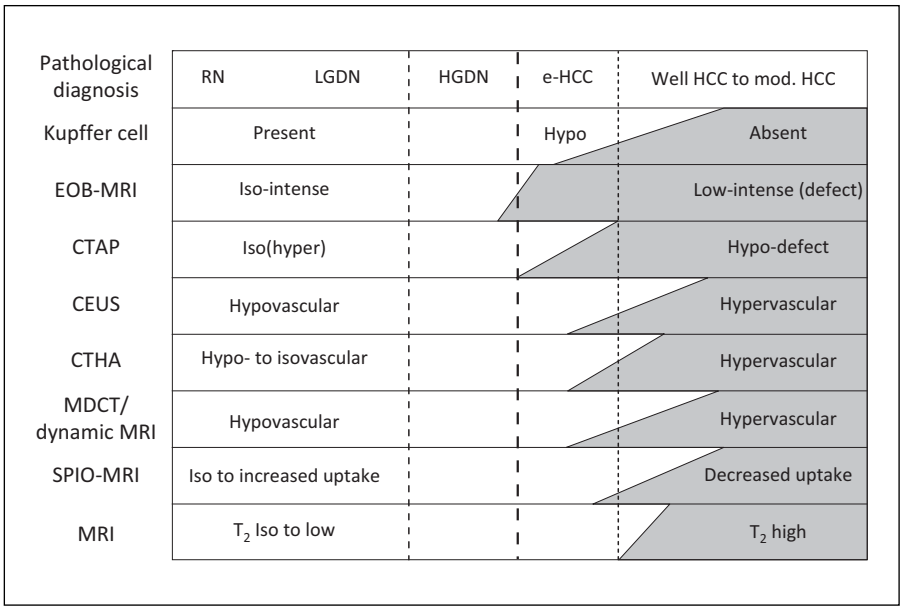

Fig. 5. Imaging findings of hepatocellular nodules in cirrhotic liver [cited from 59, with permission]. Imaging diagnosis of hepatocellular nodule associated with liver cirrrhosis. EOB-MRI is the most sensitive tool in the differentiation between early HCC and dysplastic nodules. Well = Well differentiated; mod. = moderately differentiated; RN = regenerative nodule; LGDN = low-grade dysplastic nodules; HGDN = high-grade dysplastic nodules; e-HCC = early HCC.

HCC (early well-differentiated HCC). In the light of the fact that the diagnosis of early HCC is impossible without the thorough examination of resected specimens, features such as stromal invasion cannot be diagnosed definitively without examination of the entire resected sample, the report by Ichikawa et al. [62] based exclusively on the examination of resected samples is highly reliable. This study involved the diagnosis of all samples by a specialist in liver pathology, that is an author of the liver pathology consensus paper [63], and suggested that Gd-EOB-MRI is the best imaging tool and may have a differential diagnostic ability comparable to that of a pathologist specializing in the liver (table 3; fig. 5).

However, nodules that show no signal reduction in the hepatocyte phase and are diagnosed as clearly well-differentiated HCC on the basis of histological examination of biopsy specimens may include those that have undergone pathological changes of carcinogenesis at a stage before signal reduction. Moreover, the possibility of the presence of exceptional cases of dysplastic nodules among the lesions showing signal reduction in the hepatocyte phase cannot be excluded. Importantly, the diagnosis and natural course of patients with hypovascular lesions that show signal reduction only in the hepatocyte phase should be studied extensively and the findings compared with those in resected specimens. Research on the natural course of hypovascular hepatocellular nodules with decreased intensity on hepatocyte phase of EOB-DTPA is strongly warranted.

Table 3. Accuracy of the differentiation of early HCC and premalignant lesions by hepatocyte phase Gd-EOB-DTPA-MRI for hypovascular hepatocytic nodules

Only resected specimens: n = 30	Pathological findings	
	e-HCC	DN or RN
Signal intensity in hepatobiliary phase with Gd-EOB-DTPA		
Low to slightly low (n = 24)	23	1
Iso to high (n = 6)	1	5

Accuracy: 93% (23 + 5/30). e-HCC = Early HCC; DN = dysplastic nodule; RN = regenerative nodule.

As discussed above, Gd-EOB-MRI is expected to approach a pathological examination in terms of its diagnostic accuracy of early HCC, but the decision of optimal timing of treatment remains an issue. Careful comparison with the natural course is, thus, also needed in this respect, particularly in nodules accompanied by typical hypervascular HCC in the different sites of the liver, rather than treating hypovascular nodules showing signal reduction in the hepatocyte phase without clear evidence of malignancy. Several observation studies suggest that the potential of malignant transformation of such nodules is high, and the doubling time and period of hypervascular change of hypovascular nodules showing low intensity on hepatocyte phase signal

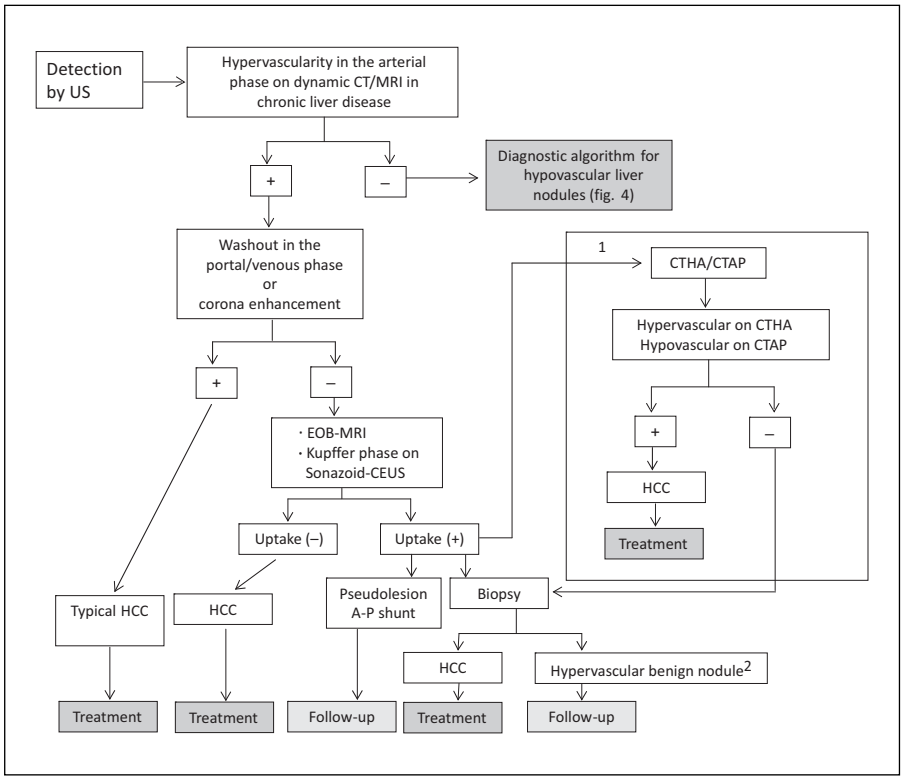

Fig. 6. Diagnostic and treatment algorithms for hypervascular liver nodules according to the consensus-based diagnostic and treatment guidelines established by the JSH 2010. 1 = Recommended only at appropriate institutions; 2 = hypervascular benign nodules of focal nodular hyperplasia, adenoma, etc.

compared with those showing no signal reduction. Further studies concerning the natural history are eagerly awaited.

Angiography

Angiography is no longer performed simply for the diagnosis of HCC, particularly hypovascular HCC, because improvements in CT, MRI, and US mean that clearly hypervascular HCC can be readily diagnosed using noninvasive modalities [5, 6]. Angiography is performed only when transarterial treatment is intended, i.e. interventional angiography with treatments such as hepatic arterial infusion chemotherapy or transarterial chemoembolization.

However, angiography is still performed in combination with CT, i.e. CTHA and CTAP for detailed evaluation of the blood flow of even hypervascular nodules, or to determine whether the blood supply of a hypovascular nodule is arterial- or portal-dominant. CTHA has an excellent diagnostic capacity for hypervascular HCC, but is associated with the problem of a high frequency of detecting pseudolesions caused by the overemphasis of the AP shunt. The detection of pseudolesions is also common with CTAP. However, these remain the ultimate techniques for the staging of patients with HCCs, and the discrimination between dysplastic nodules and early HCC is based on the blood supply pattern, but this is inferior to hepatocyte EOB-MRI.

It has recently been reported that the diagnostic capacity of a combination of noninvasive MDCT and SPIO-MRI was comparable to that of CTHA or CTAP [64], and therefore CTHA and CTAP may thus be gradually eliminated from the daily clinical practice even in Japan, where CTHA and CTAP used to be aggressively performed.

CTAP used to be regarded as the most sensitive modality for detecting the initial changes of malignant transformation in the multistep process of hepatocarcinogenesis from a dysplastic nodule to early HCC [65, 66]. However, changes in malignant transformation can now be more sensitively captured in the hepatocyte phase of Gd-EOB-DTPA-MRI, earlier than a decrease in the portal blood flow detected by CTAP, and the roles of CTHA and CTAP therefore will become limited in the future [5, 67].

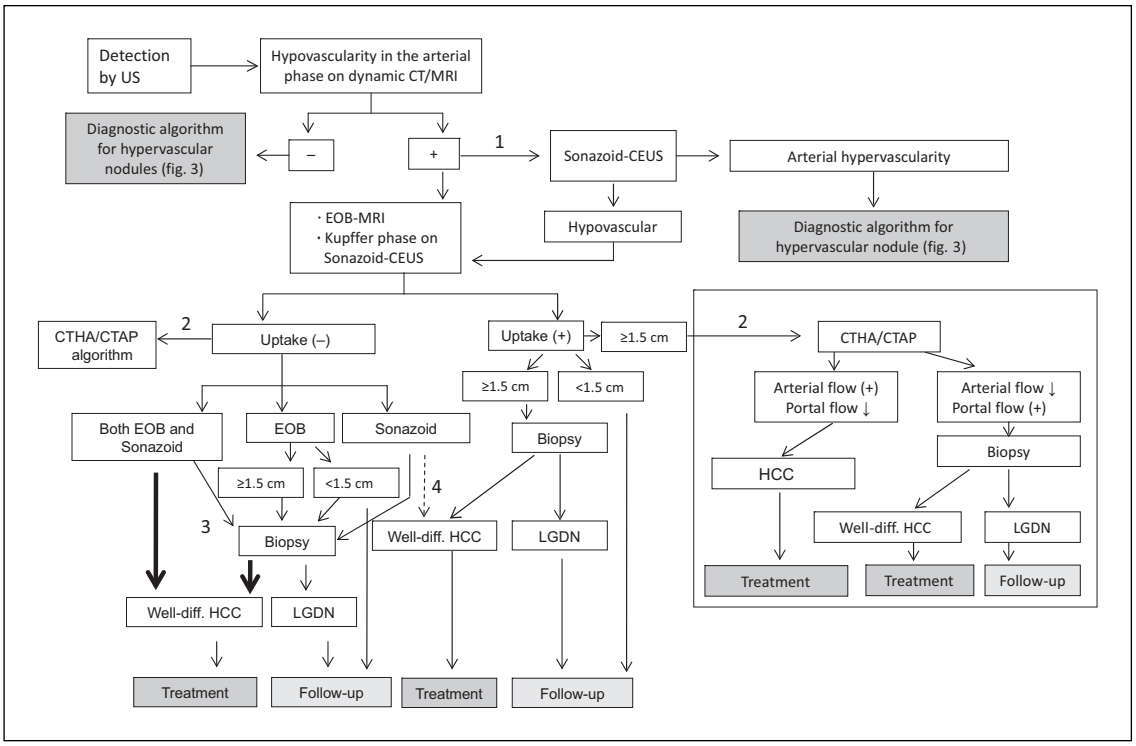

Fig. 7. Diagnostic and treatment algorithms for hypovascular liver nodules according to the consensus-based diagnostic and treatment guidelines established by the JSH 2010. 1 = When the nodule is hypovascular on dynamic CT or dynamic MRI, Sonazoid-CEUS is recommended to confirm whether it is truly a hypovascular nodule; 2 = recommended only at appropriate institutions; 3 = biopsy is not always necessary in this setting; 4 = the cases with decreased uptake of Sonazoid without decreased uptake of EOB are rare. Well-diff. = Well-differentiated; LGDN = low-grade dysplastic nodules.

Positron Emission Tomography

Positron emission tomography (PET) using fluorodeoxyglucose has gradually become commonly used for the staging and diagnosing of the malignant grade of HCC. However, the diagnostic usefulness of fluorodeoxyglucose-PET for HCC is not very high. PET is superior to other modalities regarding its ability to detect poorly differentiated HCC and it is useful to detect extrahepatic metastases. It is also valuable for assessing the treatment response after molecular targeted therapy for far-advanced HCC with extrahepatic spread.

Conclusion

Integrated medicine has already been initiated, advancing the diagnosis of liver tumors by performing all available imaging modalities including CEUS, CT, SPIO-MRI, and EOB-MRI, and comprehensively evaluating the findings. The present era requires modalities to be selected according to diagnostic efficiency. The JSH issued diagnostic algorithms for hypervascular and hypovascular liver cancers in 2007 [67] and revised them in 2010 [5] (fig. 6, 7). In 2007, EOB-MRI and Sonazoid-CEUS had not yet been clinically available. SPIO-MRI has currently been replaced by EOB-MRI, and Levovist-CEUS has been completely replaced by Sonazoid-CEUS in the updated diagnostic algorithms. Similarly, the role of CTHA and CTAP also needs to be reconsidered in the near future, as EOB-MRI now provides more reliable information regarding differentiation between early HCC and dysplastic nodules.

Disclosure Statement

The author declares that he has no financial conflict of interest.

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Complete Response of Advanced Hepatocellular Carcinoma with Multiple Lung Metastases Treated with Sorafenib: A Case Report

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Key Words
Hepatocellular carcinoma · Sorafenib · Hepatic necrosis · Lung metastasis · Complete response

Abstract
Sorafenib, an oral multikinase inhibitor, has demonstrated clinical efficacy in patients with advanced hepatocellular carcinoma (HCC). However, in the SHARP trial (Sorafenib HCC Assessment Randomized Protocol trial) and the Asia-Pacific trial (conducted in the Asia-Pacific region), no cases of complete response (CR) were reported. Thereafter, only a relatively small number of CR cases were reported worldwide for sorafenib therapy. We herein report a case of CR in a patient treated with sorafenib for 4 months. The patient had advanced HCC with multiple lung metastases, and there has been no recurrence after 8 months following cessation of administration. To our knowledge, this is the first time a female treated with sorafenib alone for HCC has had a CR.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer globally, the third most common cause of cancer-related death, and a major health problem [1]. Surgical and locoregional procedures [especially percutaneous radiofrequency ablation (RFA)] can be curative for early stage HCC. However, because no effective therapies for advanced HCC are available, HCC that is diagnosed at an advanced stage or with progression after locoregional procedures has a dismal prognosis [2]. Recently, two large phase III clinical trials, the SHARP trial (Sorafenib HCC Assessment Randomized Protocol trial) and the Asia-Pacific trial (conducted in the Asia-Pacific region), clearly demonstrated that sorafenib (Nexvar; Bayer Healthcare Pharmaceuticals), an oral multikinase inhibitor, is an active and effective therapy leading to a significant improvement in both progression-free survival and overall survival in patients with unresectable advanced HCC. However, there were no cases of complete response (CR) among the 449 patients treated with sorafenib in these trials [3, 4]. As far as we are aware, there have been few reports of patients achieving CR when treated with sorafenib alone [5–12].

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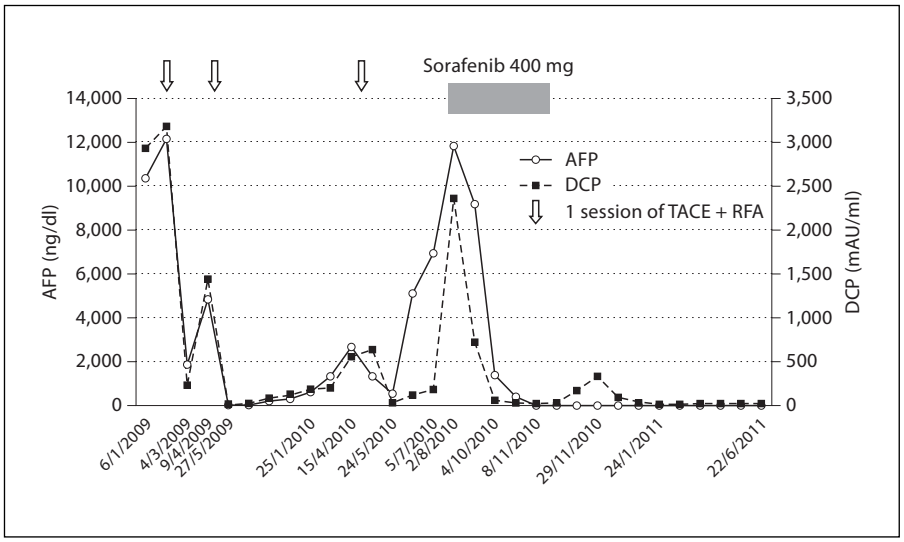

Fig. 1. Changes in AFP and DCP levels. The duration of treatment with sorafenib is indicated by the gray bar. The administration of sorafenib resulted in a dramatic reduction in serum AFP and DCP levels.

We herein report a case of CR in a patient treated with sorafenib for 4 months. The patient had advanced HCC with multiple lung metastases. There was no recurrence for 8 months after the cessation of administration, which was due to the development of hepatic failure as a serious adverse event of sorafenib.

Case Presentation

A 76-year-old Japanese female diagnosed with HCC and hepatitis C virus (HCV)-related liver cirrhosis, and who had previously received percutaneous ethanol injections in 2006, was referred to our department in January 2009 because of elevated α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) levels. She was 150 cm tall and weighed 53 kg. Based on her history of HCV, elevated tumor markers, and typical radiological findings for classical HCC in the right lobe of the liver on dynamic CT scan, we diagnosed recurrence of HCC without performing a biopsy. The tumor was solitary and 4.5 cm in diameter. The AFP and DCP levels were 10,341 ng/ml and 2,929 mAU/ml, respectively (fig. 1). We performed transcatheter arterial chemoembolization (TACE) for HCC followed by RFA in February 2009 and additional RFA for local tumor progression in April 2009 and April 2010. Subsequently, AFP and DCP levels decreased to 526 ng/ml and 32 mAU/ml, respectively. However, 3 months later, AFP increased to 5,127 ng/ml and DCP to 125 mAU/ml, and CT scan showed the presence of more than 60 HCC-derived lung metastases (fig. 2), even though the intrahepatic tumor was well controlled.

At that time, the significant laboratory test results of the patient were as follows: alanine aminotransferase (ALT) 90 IU/l, aspartate aminotransferase (AST) 131 IU/l, total bilirubin 1.2 mg/dl, albumin 3.0 g/dl, PT INR 1.16, AFP 6,952 ng/ml, and DCP 187 mAU/ml. The patient had no ascites or encephalopathy and had a Child-Pugh score of 6 (Child-Pugh class A) with an Eastern Cooperative Oncology Group (ECOG) performance status of 1. Therefore, oral sorafenib therapy was initiated at 400 mg, once daily (half the standard dosage), for multiple lung metastases of HCC from July 2010.

After 2 weeks, AFP increased to 11,800 ng/ml and DCP to 2,365 mAU/ml. After 3 weeks, the lung metastases were slightly enlarged on chest CT scan. However, after 3 months, both tumor markers markedly decreased (fig. 1) and the lung metastases had almost disappeared on chest CT scan (fig. 2). After 4 months, the lung metastases had completely disappeared and the patient achieved CR (fig. 2).

However, because of hepatic encephalopathy and deterioration of liver function from Child-Pugh class A to C 4 months after starting sorafenib, we were forced to cease sorafenib administration (fig. 3a). Four days following administration cessation, ALT and AST levels suddenly increased dramatically to 1,454 and 1,653 IU/l, respectively. CT scan revealed that multicentric hepatic necroses, not apparent 4 days earlier, had appeared suddenly in the right and left lobes of the liver (fig. 3b). Thereafter, the patient was managed conservatively and after

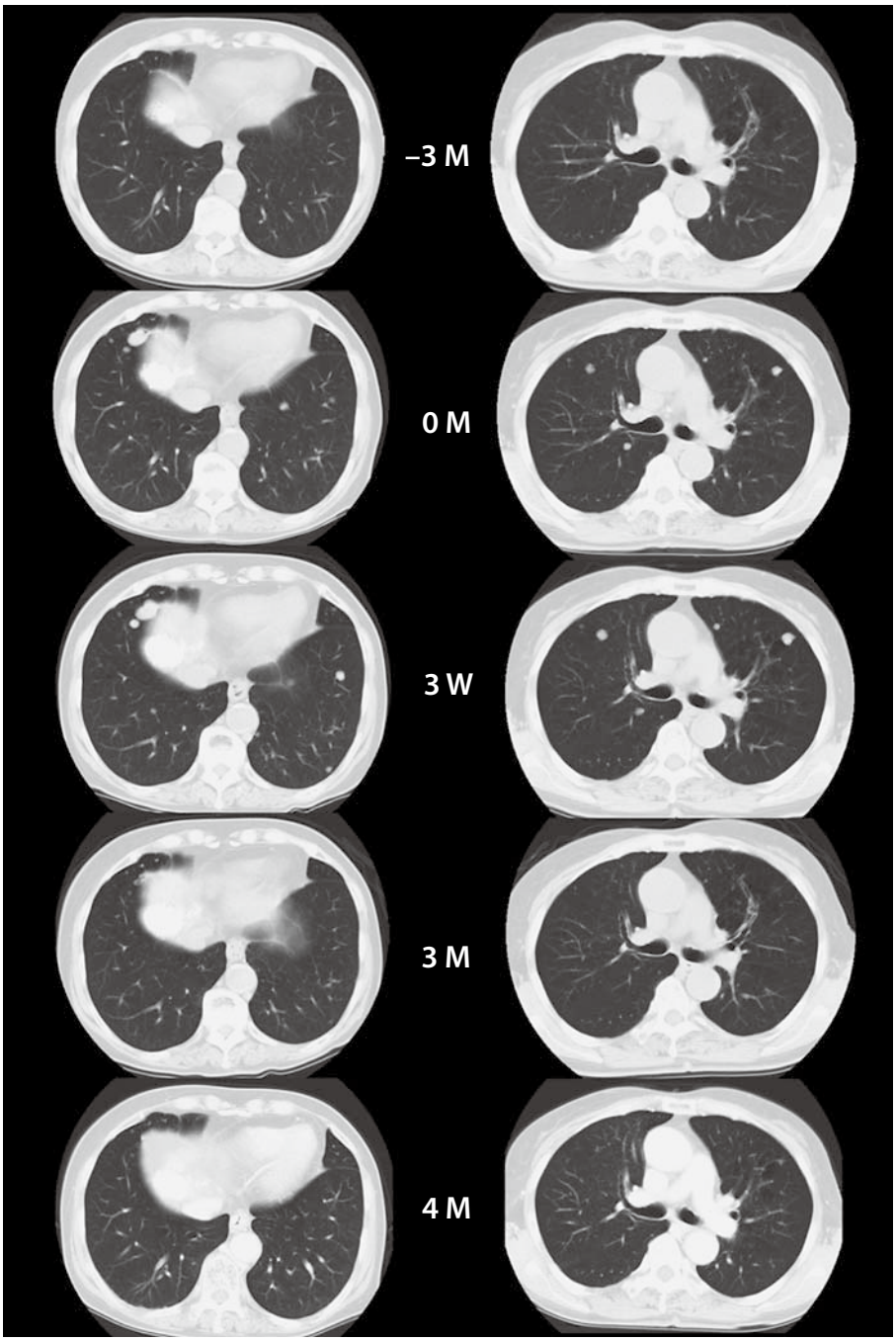

Fig. 2. Changes in chest CT scans. Follow-up CT scans show that multiple lung metastases were slightly enlarged after 3 weeks. After 3 months, they had almost disappeared. After 4 months, they had completely disappeared and the patient achieved radiological CR. The maximum diameter of lung metastases 3 weeks after commencement of therapy was 2 cm.

several weeks her liver function gradually improved. At the most recent follow-up, the patient remained in remission 8 months after the cessation of sorafenib therapy, without clinical or imaging evidence of disease recurrence. Both tumor markers were within the normal range; liver function improved to a Child-Pugh score of 7 (Child-Pugh class B) and her ECOG performance status was 1.

Discussion

Sorafenib is a multikinase inhibitor with reported activity against Raf-1, B-Raf, VEGFR2, PDGFR, and c-Kit receptors, among other receptor tyrosine kinases and serine threonine kinase [13]. It can reduce tumor progression in HCC patients because of its effect on tumor

proliferation and angiogenesis. Two large phase III clinical trials, the SHARP trial and Asia-Pacific trial, clearly demonstrated that sorafenib had efficacy in terms of overall survival in patients with unresectable advanced HCC [3, 4]. According to the SHARP trial, 299 patients had sorafenib therapy; of these, only 2% had a partial response and 71% had stable disease [according to the Response Evaluation Criteria in Solid Tumors (RECIST)]. No patient had a CR [3]. In the Asia-Pacific trial also, among the 150 patients treated with sorafenib, none showed a CR [4]. In a search of the literature, only 10 HCC patients worldwide were found to have achieved a CR with sorafenib (table 1) [5–12]. So et al. [5] first reported a CR in a patient with hemochromatosis and metastatic HCC treated with sorafenib. Wang et al. [7] reported a CR in a patient with HCV and HCC with portal vein tumor thrombosis treated with a reduced dose of sorafenib. Kudo et al. [8], Curtit et al. [11], and Irtan et al. [12] reported a complete histological response in a patient who underwent surgery after sorafenib therapy. All cases were male and most showed an acute decrease in AFP levels before CR was achieved [5–12]. It takes less than 6 months to achieve CR in most cases (table 1). Therefore, it should be possible to determine within 6 months whether sorafenib is effective in most HCC patients when they achieve a dramatic therapeutic response.

Our patient, who was treated with sorafenib alone, achieved CR. This case is especially unique and suggestive for five reasons. First, this is the first time a female patient treated with sorafenib alone for HCC has achieved CR. Second, the elevation of both tumor markers and the CT scan showed disease progression after several weeks of starting sorafenib, but subsequently CR was achieved after 4 months. A recent study has suggested that an early AFP response within the first 4 weeks is a surrogate marker predictive of progression-free survival and overall survival in HCC patients treated with antiangiogenic therapies like sorafenib [14]. However, our case suggests that an early decrease in AFP levels is not the only important factor involved in achieving CR. These findings suggest that 1 month may be insufficient to determine whether HCC patients respond to sorafenib. Third, this patient maintained a CR even after the cessation of sorafenib therapy. This indicates that the tumors completely disappeared due to sorafenib therapy alone. Fourth, this is the second case of CR in HCC patients in which the initial sorafenib dose was reduced to half the standard dosage. Our patient received 400 mg once daily due to her petite build. Even though our patient achieved

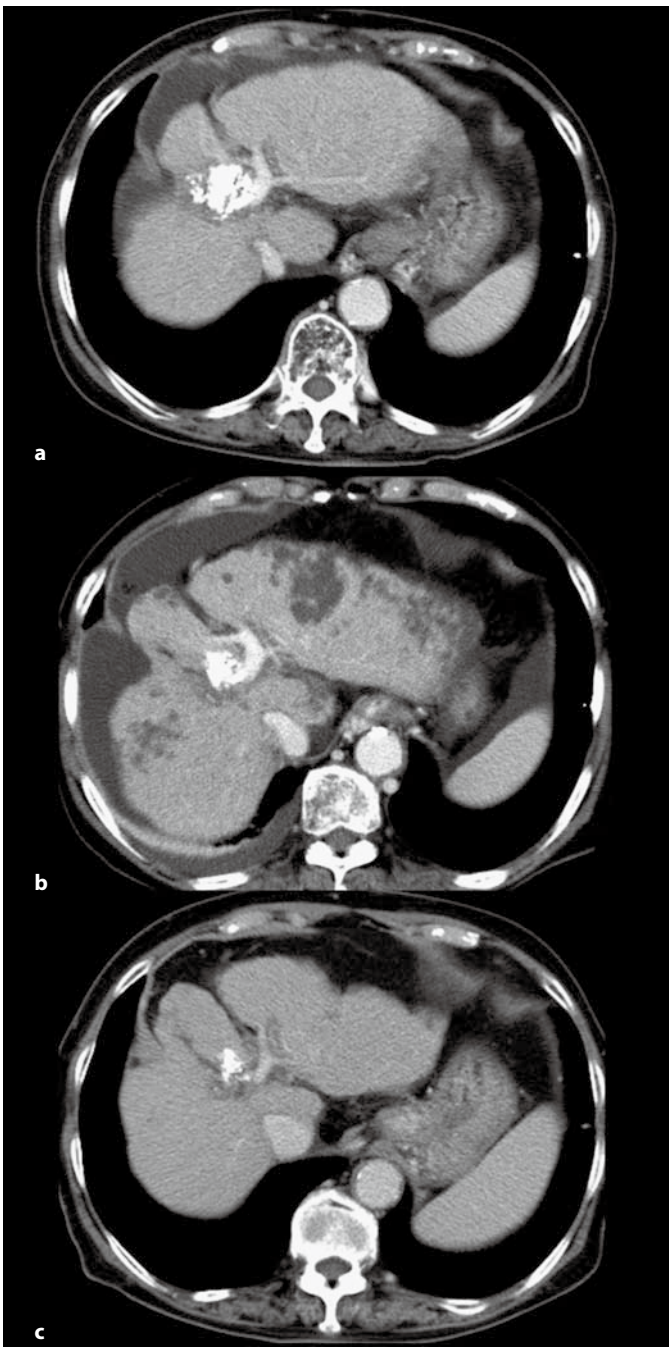

Fig. 3. Changes in abdominal CT scans after cessation of sorafenib therapy. **a** CT taken when sorafenib therapy was suspended because of hepatic encephalopathy and deterioration of liver function. **b** Four days after **a**, ALT and AST levels rose dramatically to 1,454 and 1,653 IU/l, and CT scan revealed the presence of multicentric hepatic necroses in the right and left lobes of the liver. **c** CT at the most recent follow-up revealed slight atrophy of the left lobe of the liver but no recurrence of HCC.

Table 1. CR in patients with HCC treated with sorafenib alone

Case No.	Characteristics[a]	Etiology	Metastasized to	Maximal diameter cm	Initial dose[b] mg	Time to CR months	Time to cessation	Published online	First author	Journal	Comments
1	78, M, USA, unknown	Hemochromatosis	Liver, lung	5.0	400	5	6 months	17/10/08	So [5]	Journal of Hematology & Oncology	First report
2	54, M, USA, Asian	HBV	Lung	4.1	400	18	None	1/9/09	Yeganeh [6]	American Journal of Transplantation	Posttransplant
3	74, M, USA, Caucasian	HCV	Liver (PVTT)	10	200	8	8 months	23/3/10	Wang [7]	Targeted Oncology	Low-dose
4	68, M, Japan, Asian	HBV	Liver, lung	–	400	2	None	8/7/10	Kudo [8]	Oncology	
5	68, M, Japan, Asian	HBV	Liver, lung, lymph node, adrenal gland	5.5	400	1	None				Histological CR
6	69, M, Greece, unknown	HBV + HIV	Liver, lymph node	–	400	6	None	31/8/10	Chelis [9]	Medical Oncology	HIV coinfection
7	84, M, Italy, unknown	HCV	Liver (PVTT)	6.0	400	6	None	17/1/11	Sacco [10]	BMC Gastroenterology	
8	56, M, France, unknown	HCV	Liver	15	400	6	Unknown	24/1/11	Curtit [11]	Journal of Clinical Oncology	Histological CR
9	59, M, France, unknown	Hemochromatosis	Liver (PVTT), lymph node, omentum	10	400	6	Unknown	14/3/11	Irtan [12]	Liver International	Histological CR
10	57, M, France, unknown	HBV	Liver (PVTT)	8	400	12	Unknown				Histological CR

Only 10 HCC patients worldwide have achieved a CR with sorafenib. All cases were male and most showed an acute decrease in terms of AFP levels before CR was achieved. It takes less than 6 months to achieve CR in most cases. PVTT = Portal vein tumor thrombosis.

[a] Presented as age (in years), sex, nationality, race. [b] Dose administered twice daily.

CR on half the usual sorafenib dose, it is unclear whether low-dose sorafenib therapy is as effective as the standard regimen (400 mg twice daily). More studies need to be conducted to clarify the effect of low-dose sorafenib therapy. Fifth, the patient had multicentric hepatic necroses in the right and left lobes of the liver after the cessation of sorafenib. It is assumed that nontumor liver tissues underwent necrosis because there was no evidence of HCC in the liver at that time. To our knowledge, there have been no previous reports of such adverse events in association with sorafenib, while this adverse event could have been caused by antiangiogenic therapies like sorafenib. Further investigation is required to confirm this finding.

In conclusion, we had a rare example of a dramatic therapeutic response in an HCC patient treated with sorafenib. Further studies are needed to elucidate the mechanisms of how CR is achieved at the molecular level and what the molecular biomarkers are in order to identify which patients are most likely to achieve CR. We believe that it is important to make such rare cases known and to search for a breakthrough therapy for advanced HCC.

Disclosure Statement

All authors have no conflict of interest to declare.

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Asian Consensus Workshop Report: Expert Consensus Guideline for the Management of Intermediate and Advanced Hepatocellular Carcinoma in Asia

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Key Words

Hepatocellular carcinoma · Epidemiology · Diagnosis · Treatment · Outcome · Survival · Staging system · Intermediate stage · Advanced stage

Abstract

Hepatocellular carcinoma (HCC) is a highly prevalent disease in many Asian countries, accounting for 80% of victims worldwide. Screening programs improve the detection of early HCC and have a positive impact on survival, but the majority of HCC patients in Asia still present with advanced stage disease. The treatment outcomes of HCC are affected by multiple variables, including liver function, performance status of the patient, and tumor stage. Therefore, it is not easy to apply a multidisciplinary therapeutic approach for optimal management. At present, limited numbers of HCC patients are eligible for curative therapies such as surgery or ablation in Asia. Therefore, most patients are eligible for only palliative treatments. For optimal management, the treatment choice is guided by staging systems and treatment guidelines. Numerous staging systems have been proposed and treatment guidelines vary by region. According to the Barcelona Clinic Liver Cancer (BCLC) guideline based on evidence from randomized clinical trials, only transarterial chemoembolization (TACE) is recommended for intermediate stage HCC and sorafenib for advanced stage HCC. However, treatment guidelines from Asian countries have adopted several other therapeutic modalities such as a surgical approach, hepatic arterial infusion chemotherapy, external radiation, and their combinations based on clinical experiences for intermediate and advanced stage HCC. Although TACE is the main therapeutic modality in the intermediate stage, overall therapeutic outcomes depend on the tumor size. In the advanced stage, the prognosis depends on the tumor status, e.g. major vessel invasion or extrahepatic spread.

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Thus, a new staging system representing prognoses suitable for Asian HCC patients and a corresponding optimal treatment algorithm should be further investigated using evidence-based data, which will finally bring about an Asian consensus for the management of intermediate and advanced stage HCC.

Introduction

Hepatocellular carcinoma (HCC) is endemic in Asia. It is expected that about 80% of new HCC cases worldwide will develop in Asia [1, 2]. HCC ranks as the first to fifth leading causes of death in many Asian countries. However, there is no consensus guideline to manage HCC in Asia. To build a bridge to a consensus on HCC management in Asia, the first Asia-Pacific Primary Liver Cancer Expert (APPLE 2010) meeting was held in Incheon, Korea, last year. Especially, the first consensus assembly in the APPLE meeting mainly focused on how to manage intermediate and advanced HCC in Asia.

The current standard of care for patients unsuitable for potentially curative therapy is locoregional therapy with transarterial chemoembolization (TACE). For patients with more advanced disease, sorafenib is the standard of care. Sorafenib is a targeted agent with proven survival benefits [3, 4]. Although other novel agents and therapeutic approaches are emerging, such as radioembolization and various targeted agents, further data based on randomized clinical trials (RCT) are needed.

This report is based on experts' reports from three Asian countries (Korea, Japan, and China) and a panel discussion, and it is divided into three topics: (1) definitions of intermediate and advanced HCC in staging systems, (2) treatment strategies for the intermediate stage in Asia, (3) and treatment strategies for advanced HCC in Asia.

Definitions of Intermediate and Advanced HCC in Staging Systems

The terminology of intermediate and advanced HCC comes from the Barcelona Clinic Liver Cancer (BCLC) staging system. The BCLC staging system is widely recognized and endorsed [5–7]. It includes variables linked to tumor stage and function, physical status, and cancer-related symptoms, and it combines each stage with a treatment algorithm. Patients with intermediate stage HCC are asymptomatic (PS score 0) with multinodular tumors but without vascular invasion or extrahepatic spread and are eligible for locoregional therapy such as TACE. Those with advanced stage HCC are either symptomatic (PS score 1–2) or have evidence of vascular invasion or extrahepatic spread; these patients are eligible for sorafenib. The BCLC system has been externally validated [7, 8] and is endorsed by both the American Association for the Study of Liver Diseases (AASLD) [9] and the European Association for the Study of the Liver (EASL) [10].

Although the BCLC staging classification is an emerging candidate for a standard classification in Western regions, validation across both Eastern and Western regions is required for global application because of the distinct differences between these patient populations and risk factors. Most Asian experts agreed that the BCLC staging system was not perfect to satisfy Asian experts in clinical practice. Early stage means that the disease can be controlled by curative treatment, but the advanced stage is hard to define in this system as advanced stages harbor so many disease categories. Therefore, it should be subcategorized for the application of each unique treatment for the future. Furthermore, the disease is a kind of continuous spectrum. Thus, it may not be easy to separate it into two stages, i.e. intermediate and advanced. The term 'advanced stage' which includes portal vein invasion and distant metastasis seems to harbor a wide spectrum of HCC, which indicates that the advanced HCC stage can be divided into two different groups: locally advanced with portal vein invasion and advanced with extrahepatic metastasis.

At present, most Asian countries have their own HCC staging systems. The Japanese Tumor-Node-Metastasis (TNM) staging system [11] is widely used in Japan and Korea (table 1). This staging system takes into account three criteria for the T stage, i.e. whether the tumor is solitary or multiple, the tumor size (≤2 cm or >2 cm),

Table 1. Japanese TNM staging system

Stage	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
IVa	T4	N0	M0
	T1–3	N1	M0
IVb	T1–4	N0–1	M1

Table 2. Chinese staging system

Stage	Tumor	Invasion	Lymph node involvement	Distant metastasis	Child-Pugh class
Ia	Solitary ≤3 cm	No	No	No	A
Ib	One or two ≤5 cm, one lobe	No	No	No	A
IIa	One or two ≤10 cm, one lobe or two ≤5 cm, two lobes	No	No	No	A
IIb	One or two >10 cm, one lobe or two >5 cm, two lobes	No	No	No	A
IIIa	Any	PVB/HV/BD	No	No	A
	Any	No	No	No	B
	Any	MPV/IVC	Yes or no	Yes or no	A or B
	Any	Yes or no	Yes	Yes or no	A or B
	Any	Yes or no	Yes or no	Yes	A or B
IIIb	Any	Yes or no	Yes or no	Yes or no	C

PVB = Portal vein branch; HV = hepatic vein; BD = bile duct; MPV = main portal vein; IVC = inferior vena cava.

and the presence of any vascular or bile duct invasion. Patients are thus classified as T1, T2, T3, or T4. For nodes and metastasis, it is similar to other TNM staging systems, based on the presence of lymph node or distant metastasis. Recently, the Japan Integrated Staging (JIS) system was developed by integrating Japanese TNM stages and Child-Pugh grades and was externally validated [12–14]. In contrast, the Chinese staging system considers the Child-Pugh class in addition to tumor factors (number, size, and one or both lobes), vessel invasion, lymph node involvement, and distant metastasis (table 2). As many Asian countries use their own staging systems, it is hard to communicate with reference to staging systems in the Asian-Pacific region. To provide Asian physicians with a common language on which to base treatment decisions and clinical research, it is necessary to have a consensus on the best applicable staging system.

In conclusion, Asian countries have their own staging systems with different constituent variables. Because the real situations vary among Asian countries, it seems not to be easy to reach a consensus on staging systems. The increasing need for a consensus on a new staging system which can upgrade the current staging system was expressed again at the first APPLE meeting. Continued efforts to improve our understanding of this complex disease will enable us to refine staging classifications and guide the optimal therapy according to different stages.

Treatment Strategies for Intermediate HCC

The current distribution of HCC based on the BCLC system spans mostly the intermediate or advanced stage in Asian countries, except Japan [2, 15]. The disease stage obviously affects the treatment modality. TACE is the most widely used locoregional treatment for patients with intermediate stage HCC, and it is considered the standard treatment option for patients with reasonable liver function with large (>5 cm) or multifocal tumors without major vessel invasion or extrahepatic spread. However, TACE cannot induce complete tumor necrosis especially in large tumors [16, 17]. Therefore, overall therapeutic outcomes might depend on the tumor size.

To improve locoregional therapies for patients with unresectable HCC, new liver-directed therapies have emerged. Preliminary results of the use of drug-eluting beads (DEBs) suggest that this approach is associated with a favorable toxicity profile and encouraging antitumor activity [18–20]. In a recent study comparing conventional TACE with DEB-TACE, the DEB-TACE group resulted in a better local response, fewer recurrences, and a longer time to progression compared to the conventional TACE group [21]. Radioembolization with yttrium-90 (^{90}Y)-embedded microspheres is a new method suggesting an effective treatment approach for patients with unresectable HCC [22].

The Japanese treatment guidelines, which covered a majority of early and intermediate stage HCC patients, were revised in 2009 (fig. 1) [23]. In addition, a consensus-

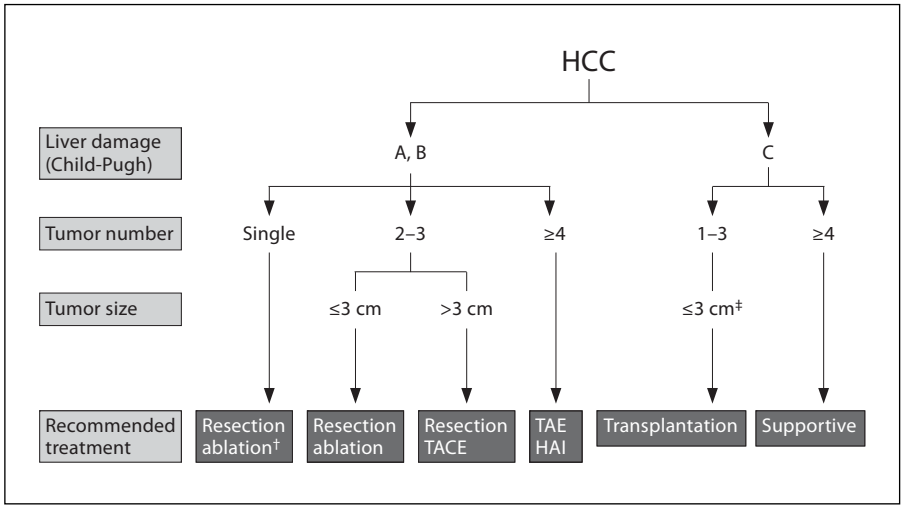

Fig. 1. EBM-based algorithm for HCC management (J-HCC guidelines 2009). Resection or transarterial chemoembolization may be selected for Child-Pugh A class patients with vascular invasion. Chemotherapy may be selected for extrahepatic HCC. Liver transplantation is only for patients ≤65 years of age. † Recommended for Child-Pugh B; ‡ <2 cm for solitary lesions. HAI = Hepatic arterial infusion.

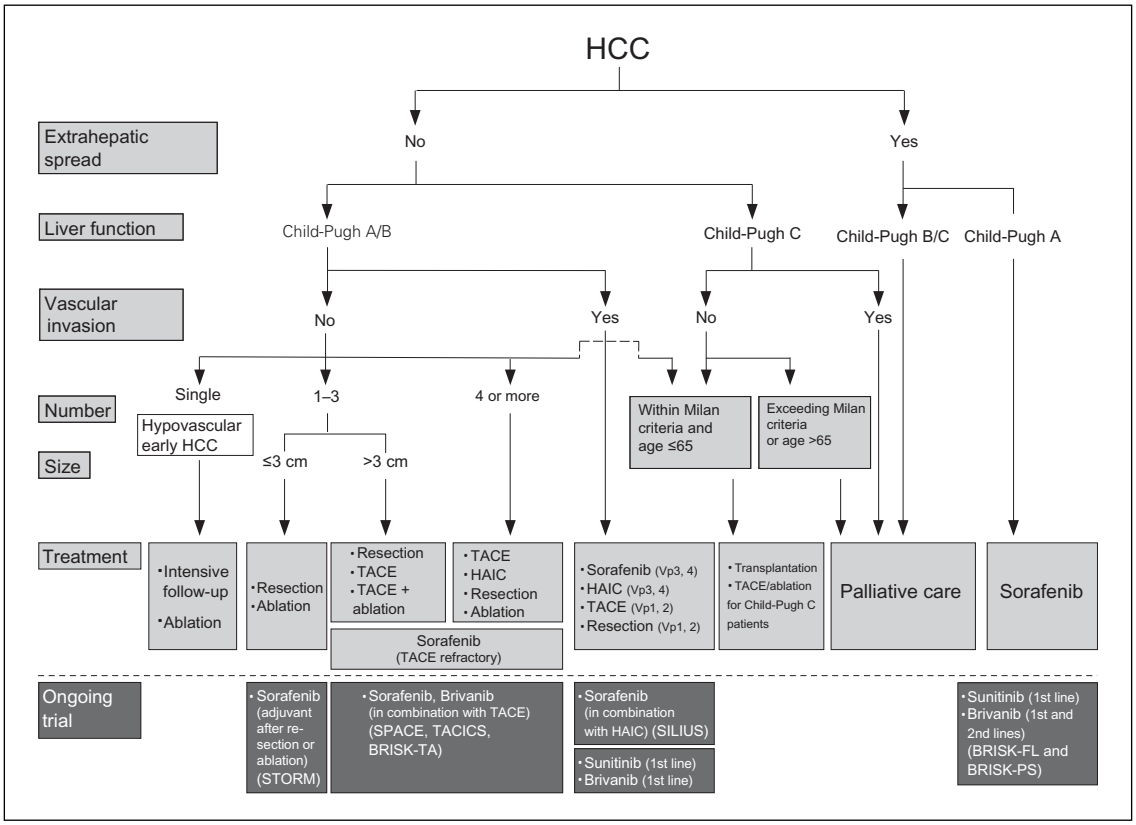

Fig. 2. Consensus-based treatment algorithm for HCC proposed by the Japan Society of Hepatology (JSH) in 2009 and revised in 2010.

based treatment algorithm for HCC was proposed (fig. 2) [24]. In this guideline, hepatic arterial infusion chemotherapy (HAIC) as well as TACE is recommended for the intermediate stage. Furthermore, Japanese treatment guidelines propose that liver transplantation can be performed for some patients with Child-Pugh C liver function. The Korean guidelines for the management of HCC were updated in 2009 (fig. 3) [25]. They regard tumor stage, Child-Pugh class, and performance status as the first aspects to consider when starting treatment. HCC

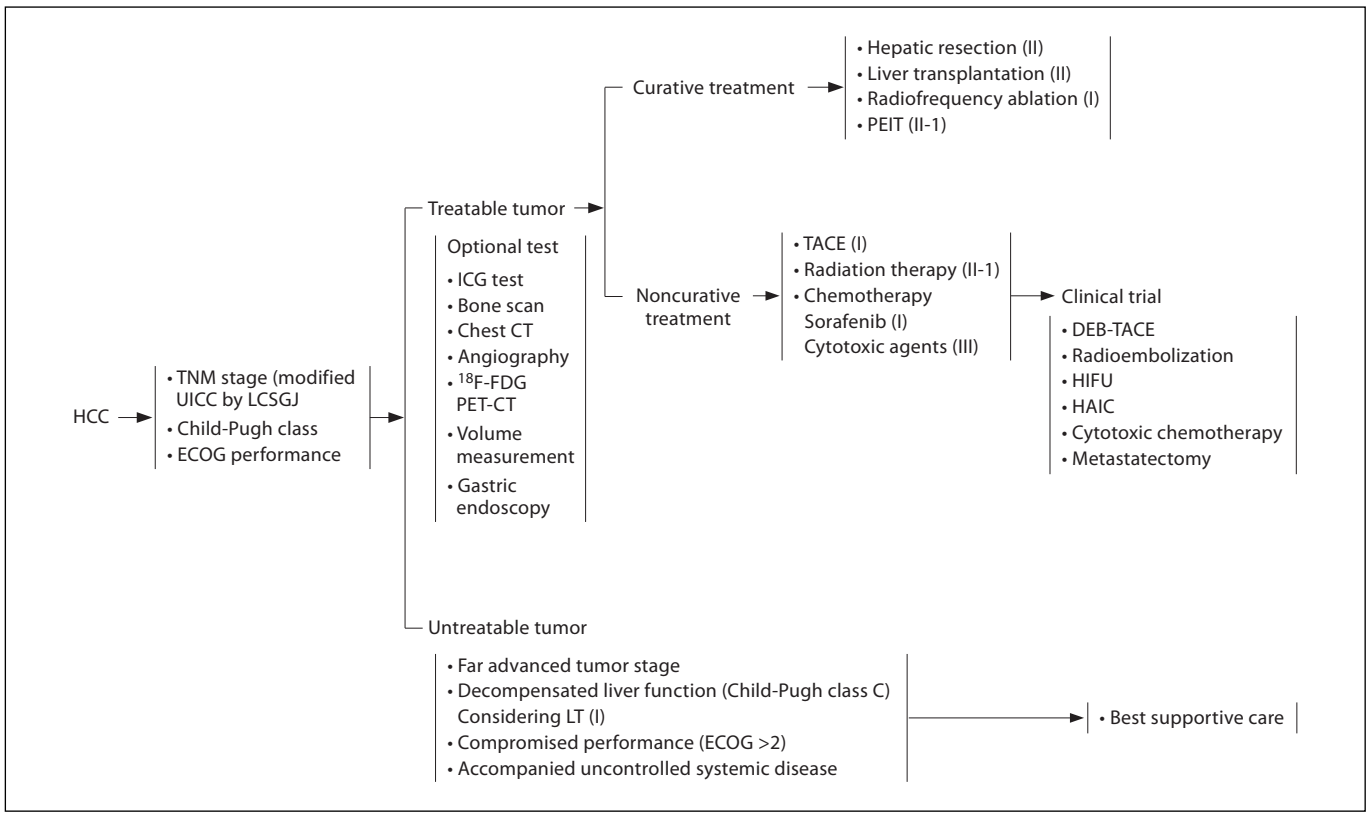

Fig. 3. Therapeutic algorithm for HCC in Korea.

was divided into treatable and nontreatable disease. For treatable HCC, curative or noncurative treatment can be performed. The algorithm of the Korean HCC treatment plan lists hepatic resection, liver transplantation, radiofrequency ablation, and ethanol injection as curative treatments. As the overall surgical mortality is very low, resection can be considered as an option even in intermediate stage HCC based on Asian experts' opinions. According to the treatment algorithm of China proposed by the Shanghai Fudan University Hospital (fig. 4), intermediate stage HCC can be treated with surgical resection, liver transplantation, and sorafenib as well as TACE.

Treatment Strategies for Advanced HCC

As sorafenib is the first targeted agent with survival benefits proven by two large-scale RCT, it is the standard of care for patients with advanced stage disease [3, 4]. However, sorafenib for advanced HCC is still not easy for Asian physicians to prescribe due to high costs [25–27]. For the management of advanced HCC, sorafenib has not yet been approved for reimbursement in most Asian countries due to a big burden on the national insurance budget. Therefore, most Asian experts on this panel agreed that the practical situation in Asian countries should be considered for the creation of practical guidelines. In addition, treatment for locoregional disease is quite different from treatment for systemic disease. Therefore, the advanced stage of HCC should be subdivided into a locally advanced stage and an extrahepatic advanced stage.

There are still many options aside from sorafenib for the locally advanced stage based on experts' opinions [23–30]. According to the treatment algorithm of Asia (Japan, Korea, and China), not only sorafenib but also many therapeutic options such as surgical resection, TACE, HAIC, and external radiation can be tried for the advanced stage. As extrahepatic disease is a systemic disease, sorafenib is the standard systemic therapy. However, in APASL guidelines, systemic chemotherapy can be an option in the practical setting [28].

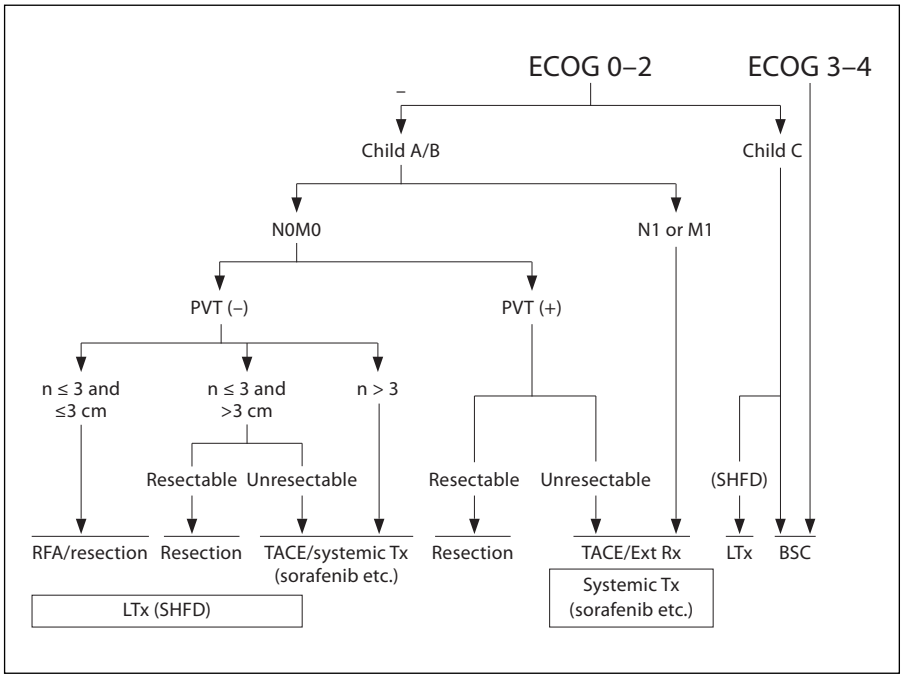

Fig. 4. Treatment algorithm for HCC at the Liver Cancer Institute of Fudan University. RFA = Radiofrequency ablation; Tx = therapy; Ext Rx = external radiation; LTx = liver transplantation.

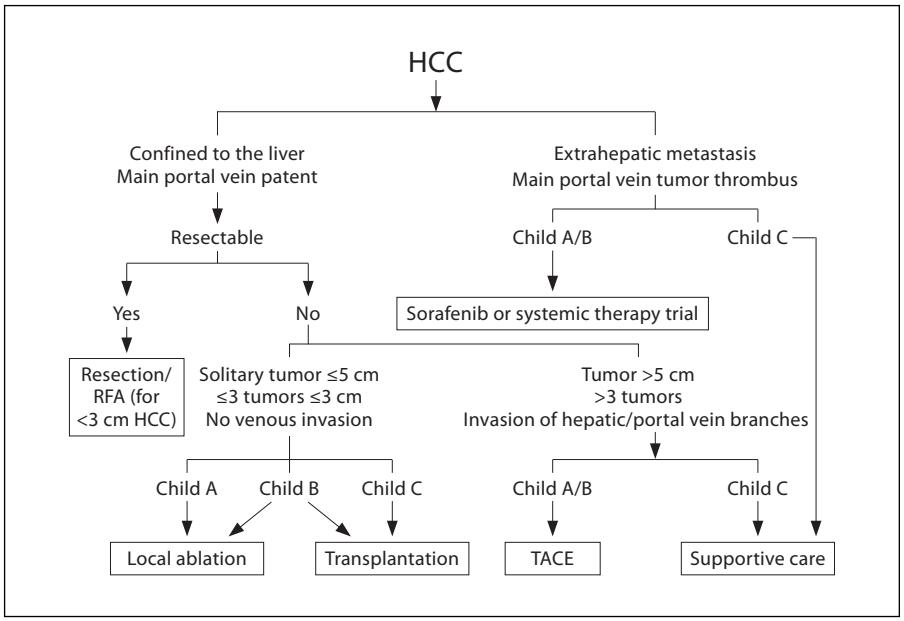

Fig. 5. APASL guideline on the treatment algorithm for HCC. RFA = Radiofrequency ablation.

In order to have a consensus guideline in the Asia-Pacific region, we need enough evidence-based data. Although, the guideline at present is not complete and does not cover various practical situations in Asia, we should try to enhance our practical guidelines in the near future by collecting more evidence based on RCT.

Conclusion

The majority of HCC patients in Asia still presents with intermediate and advanced stage HCC at diagnosis. In the first APPLE meeting, the significant different distribution of intermediate and advanced stage HCC was recognized among Asian countries. Furthermore, the significant differences in the treatment approach according to HCC stages were also identified. A consensus for

managing intermediate and advanced stage HCC cannot be easily drawn due to these disparities in clinical practices and guidelines among Asian countries. Thus, a new staging system suitable for Asian HCC patients and a corresponding optimal treatment algorithm should be further investigated using evidence-based data, which finally make way for an Asian consensus for the management of intermediate and advanced stage HCC.

Disclosure Statement

The authors declare that they have nothing to disclose.

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Recent Advances in the Management of Chronic Hepatitis B

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ABSTRACT

There are seven approved treatments for adults with chronic hepatitis B virus infection in the United States and European countries: interferon-α, pegylated interferon-α, lamivudine, adefovir dipivoxil, entecavir, telbivudine, and tenofovir disoproxil fumarate. At present, two new analogues, entecavir and tenofovir are recommended as the first line therapy by the guidelines of European Association for the Study of the Liver and American Association Study for the Liver Diseases. On the other hand, regarding interferon therapy, use of pegylated interferon-α is recommended as the first line therapy instead of standard interferon-α by both guidelines. Therefore, the main scientific interests and unmet medical needs for treatment of chronic hepatitis B have been narrowed down to long-term efficacy and safety of the two said analogues—entecavir and tenofovir—and combination therapy of pegylated interferon-α with the two analogues. To put it concretely, further studies are needed to assess (1) the long-term efficacy and safety and resistance to entecavir and tenofovir; (2) the efficacy of different durations (24 weeks to 2 years) and lower doses of pegylated interferon-α; (3) the role of combination therapy with two analogues to reduce resistance; and (4) the efficacy and safety of the two analogues with decompensated cirrhosis. Herein, we review the recent available data and results.

▸ *Implication for health policy/practice/research/medical education:*

Several therapeutic modalities exist for the treatment of chronic hepatitis B. Based on recent available data, this review endeavors to present the long-term efficacy and safety of these modalities especially for entecavir and tenofovir as well as pegylated interferon-α.

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1. Introduction

There are seven approved treatments for adults with chronic hepatitis B (CHB) in the United States and European countries: interferon (IFN) α, pegylated (PEG) IFN-α, lamivudine (LAM), adefovir dipivoxil (ADV), entecavir(ETV),telbivudine(TBV),andtenofovirdisoproxil fumarate (TDF). IFN-α and LAM have been approved for children with hepatitis B virus (HBV) infection. Two different treatment strategies are applicable in both HBeAg-positive and negative CHB patients: treatment with PEG IFN-α and long-term treatment with NUCs. There are several treatment options for patients, making rational choices for the first and second line treatment sometimes difficult. Although available randomized controlled trials show encouraging short-term results demonstrating a favorable effect of these agents on intermediate markers of the disease such as HBV DNA level, liver enzyme tests, and liver histology, limited rigorous evidence exists demonstrating the effect of these therapies on important long-term clinical outcomes, such as the development of hepatocellular carcinoma or a reduction in mortality rate. Questions therefore remain about which groups of patients benefit from therapy and at which point in the course of disease

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this therapy should be initiated.

Herein, we pooled the available data focusing on long-term efficacy and safety of two new analogues—entecavir and tenofovir—and combination therapy of PEG INF-α and the said two analogues. In the first section, we summarize recent findings based on the consensus of the guidelines of the European Association for the Study of the Liver (EASL) and the American Association Study for the Liver Diseases (AASLD) (1, 2). In section two, presentations at EASL and AASLD annual meetings in 2010 are reported.

2. Section I: Published Results Based on the Consensus of EASL and AASLD Guidelines

2.1. Assessment of Outcomes

Although various monitoring practices have been recommended, no clear evidence exists for an optimal approach. One proposed that the management algorithm used during therapy involves measuring HBV DNA and ALT levels every 12 weeks and HBeAg or anti-HBe levels every 24 weeks in patients who are HBeAg-positive. For patients who are HBeAg-positive and achieve a complete response (undetectable HBV DNA), seroconversion to anti-HBe may offer the opportunity to discontinue therapy after 6–12 months of "consolidation." During this period, regular monitoring of HBV DNA and HBeAg status should be done because relapse remains a possibility. HBsAg should be checked at 6-month intervals after HBe seroconversion if HBV DNA is undetectable. Quantitative HBsAg assay is still a research tool. HBeAg-negative patients should be similarly monitored for efficacy and safety through 48 weeks of treatment. A virological response with HBV DNA < 2000 IU/mL (approximately 10,000 copies/mL), i.e. 3.3 log10 IU/mL, is generally associated with remission of the liver disease. Undetectable HBV DNA in real-time PCR is the ideal desired of treatment sustained response with a high probability of HBsAg loss in the longer term. HBsAg should be checked at 6-month intervals if HBV DNA is undetectable. All patients treated with PEG IFN-α should be monitored for the known adverse effects of IFN. The balance of benefits and harms associated with screening for hepatocellular carcinoma is unknown and is an area for future research.

2.2. Antiviral Effect of NUCs

Table 1 summarizes the efficacy of NUCs treatment in a 48-week large randomized controlled trial with HBeAg-positive and -negative patients.

2.2.1. Lamivudine (LAM)

In large registration trials, both on HBeAg-positive and -negative patients with CHB and those with previous IFN failure, a daily dose of 100 mg of LAM was compared to 0.5 mg of ETV. LAM treatment for 48 weeks resulted in suppression of HBV DNA by an average of 5.4 log10 copies/mL in HBeAg-positive patients and 4.5 log10 copies/mL in HBeAg-negative patients. HBeAg seroconversion occurred in 18% of patients, rendered HBV DNA undetectable (<102 copies/mL) in 36% (HBeAg-positive) to 72% (HBeAg-negative) of patients (3, 4).

2.2.2. Adefovir (ADV)

In 48-week registration trials, CHB patients who were HBeAg-positive and -negative received 10 mg/day of ADV. ADV suppressed HBV DNA by 3.5 log10 copies/mL in HBeAg-positive patients and 3.9 log10 copies/mL in HBeAg-negative; HBV DNA decreased to an undetectable level (< 102 copies/mL) in only 21% of HBeAg-positive patients and to 51% of HBeAg-negative patients; suppression of HBV DNA was relatively slow; it was also less likely to induce HBeAg seroconversion (12%) (5, 6).

2.2.3. Entecavir (ETV)

A daily dose of 0.5 mg of ETV was found to be superior to 100 mg of LAM in terms of suppression of HBV DNA by 6.9 log10 copies/mL in HBeAg-positive patients and by 5.0 log10 copies/mL in HBeAg-negative patients.

Table 1. Summary of NUCs Treatment in Patients with HBeAg-Positive and -Negative Chronic Hepatitis B: 48 Weeks Post-Treatment Results

Treatment Group	HBeAg Status	HBV DNA Suppression (log10 copies/mL)	HBV DNA Undetectable, %	ALT Normalization %	HBeAg Serocon- Version %
LAM [a] (3, 4)	Positive	- 5.4	36	60	18
	Negative	- 4.5	72	71	—
ADV [a] (5, 6)	Positive	- 3.5	21	48	12
	Negative	- 3.9	51	72	—
ETV [a] (3, 4)	Positive	-6.9	67	68	21
	Negative	- 5.0	90	78	—
TBV [a] (7)	Positive	- 6.4	60	77	23
	Negative	- 5.2	88	74	—
TDF [a] (8)	Positive	- 4.5 (12w)	76	68	21
	Negative	- 3.0 (12w)	93	76	—

[a] Abbreviations: ADV, Adefovir; ETV, Entecavir; LAM, Lamivudine; TDF, Tenofovir; TVB, Telbivudine

Therapy with ETV was more likely to decrease HBV DNA to undetectable levels (< 102 log10 copies/mL) than LAM in 67% of HBeAg-positive patients and in 90% of HBeAg-negative of patients. Histological improvement was achieved in 72% of ETV-treated patients compared to 62% in LAM-treated patients (HBeAg positive); and in 70% of ETV-treated vs. 61% of LAM-treated patients (HBeAg-negative). The two drugs, however, did not differ in rates of HBeAg seroconversion—21% *vs.* 18%. Treatment effects were maintained with long-term ETV therapy, with HBeAg seroconversion rates increasing progressively to 31% at year two and 39% at year three. In addition, at the end of year two, HBsAg loss was recorded in 5% of ETV-treated and 2% of LAM-treated patients (9, 10). In the study of 96 weeks of treatment with 0.5 mg of ETV in naïve Japanese patients, resistance was reported in only 1.7% (11).

2.2.4. Telbivudine (TBV)

TBV is a potent L-nucleoside that is believed to cause chain termination and is highly potent against HBV in cell culture. TBV (600 mg/day) was superior to LAM (100 mg/day) in suppressing HBV DNA to undetectable levels of <102 copies/mL (60% *vs.* 40%; a reduction from 6.4 log10 to 5.5 log10 copies/mL), and in achieving histological improvement (65% *vs.* 56%) but not in normalization of ALT (77% *vs.* 75%) or serological responses (HBeAg seroconversion in 23% *vs.* 22%). In HBeAg-negative patients, TBV (600 mg/day) was superior to LAM (100 mg/day) in suppressing HBV DNA to undetectable levels (88% *vs.* 71%; reduction from 5.2 log10 to 4.4 log10 copies/mL) but not in achieving histological (67% *vs.* 66%) or normalization of ALT (74% *vs.* 79%) (7). These responses were well maintained during the second year of therapy, and HBeAg seroconversion increased to 30% by the end of year two (12).

2.2.5. Tenofovir (TDF)

In two 48-week randomized controlled trials, oral TDF (300 mg/day) was compared to ADV (10 mg/day) in treatment-naive patients with HBeAg-positive and -negative CHB. In HBeAg-positive patients, TDF reduced HBV DNA levels by 4.5 log10 IU/mL (12 weeks results) and suppressed HBV DNA to undetectable levels (<102 IU/mL) in 76% of patients vs. in only 13% in the ADV group. TDF and ADV treatments resulted in similar rates of histological benefit (74% *vs.* 68%) and HBeAg seroconversion (21% *vs.* 18%). An important finding was HBsAg loss in 3% of patients during the first 48 weeks of therapy in the TDF group (8). In the HBeAg-positive group, at the end of year two of continuous TDF treatment, HBeAg seroconversion increased to 27% and HBsAg loss increased to 6% (13). In HBeAg-negative patients, TDF reduced HBV DNA levels by 3.0 log10 IU/mL (12 weeks results) and suppressed HBV DNA to undetectable levels (< 102 IU/mL) in 93% of patients vs. in only 63% in the ADV group.

2.3. Antiviral Resistance to NUCs

Frequencies of antiviral resistance within five years of administration for the five NUCs are shown in *Table 2.* Although LAM has the most extensive safety record, its current use is limited by the high frequency of LAM resistance (24% in year one, and 70% in year four) (14), and the availability of more potent agents with superior efficacy and markedly improved resistance profiles.

ADV is more expensive than TDF, is less effective, and produces higher rates of resistance. Although resistance to ADV is slow to emerge, resistant variants increase progressively after the first year, reaching 29% in year five (16). The advantages of ADV are its limited resistance during first two years, the absence of cross-resistance with LAM and other L-nucleosides and, therefore, its value as treatment for LAM-resistant CHB (17, 18) and for hepatic decompensation associated with LAM resistance prior to and after liver transplantation (19). The high potency and excellent safety profile of ETV are complemented by its very high barrier to resistance in treatment-naive patients (< 1%) in year four. ETV and TDF are potent HBV inhibitors and they have a high barrier to resistance (3, 20, 21). Therefore, they can be confidently used as the

Table 2. Frequency of Antiviral Resistance to Nucleoside Analogs Treatment

Treatment Group	Treatment Duration, y	Antiviral-resistance, %
LAM [a] (14)	1	24
	2	42
	3	53
	4	70
ADV [a] (naive patients) (6)	1	0
	2	3
	3	11
	4	18
	5	29
ETV [a] (naive patients) (10)	4	<1
TBV [a] (naive patients) (7, 12)	1	2-5
	2	11-25
TDF [a] (15)	2	0

[a] Abbreviations: LAM, Lamivudine; ADV, Adefovir; ETV, Entecavir; TVB, Telbivudine; TDF, Tenofovir

first-line monotherapies. The role of monotherapy with ETV or TDF could be modified if higher rates of resistance become apparent with longer treatment duration. In a study of 96 weeks of treatment with TDF, no evidence of TDF resistance was found (15).

TBV is a potent inhibitor of HBV but, due to a low genetic barrier to resistance, a high incidence of resistance has been observed in patients with high baseline levels of replication and in those with detectable HBV DNA after 24 weeks of therapy. In a large registration trial, TBV was compared to LAM in HBeAg-positive and -negative patients. The frequency of antiviral resistance to TBV at one year was 5% of HBeAg-positive and in only 2% of HBeAg-negative patients (7).

Virological breakthrough in compliant patients is related to viral resistance. Resistance is associated with prior treatment with NUCs (i.e., LAM, ADV, TBV, emtricitabine) or, in treatment-naive patients, with high baseline HBV DNA levels, a slow decline in HBV DNA and partial virological response during treatment. Resistance should be identified as early as possible before clinical breakthrough (i.e. increased ALT) by means of HBV DNA monitoring; if possible, the pattern of resistance mutations should be identified to adapt therapeutic strategies. Indeed, clinical and virological studies have demonstrated the benefit of an early treatment adaptation—as soon as viral load increases (22, 23). In case of resistance, an appropriate rescue therapy should be initiated with the most effective antiviral effect and the minimal risk to induce multiple drug-resistant strains. Therefore, adding-on a second drug without cross-resistance is the only efficient strategy.

- LAM resistance: Add TDF (add ADV if TDF is not yet available).
- ADV resistance: It is recommended to switch to TDF if available and add a second drug without cross-resistance. If an N236T substitution is present, add LAM, ETV or TBV or switch to TDF plus emtricitabine. If an A181T/V substitution is present, add ETV (the safety of the TDF-ETV combination is unknown) or switch to TDF plus emtricitabine.
- TBV resistance: Add TDF (add ADV if TDF is not yet available). The long-term safety of these combinations is unknown.
- ETV resistance: Add TDF (the safety of this combination is unknown).
- TDF resistance: Resistance to TDF has not been described so far. It is recommended that genotyping and phenotyping be done by an expert laboratory to determine the cross-resistance profile. ETV, TBV, LAM or emtricitabine could be added (the safety of these combinations is unknown).

2.4. Long-term Therapy with NUCs

HBV DNA levels should be monitored at week 12 to ascertain virological response and then every 12 to 24 weeks. HBV DNA reduction to undetectable levels by real-time PCR (i.e. < 10–15 IU/mL) should ideally be achieved to avoid resistance. HBV DNA monitoring is thus crucial to detect treatment failure. In HBeAg-positive patients, HBeAg and subsequently anti-HBe antibodies once HBeAg is negative should be measured at intervals of 6 to 12 months. NUCs are cleared by the kidneys, and appropriate dose adjustments are recommended for patients with reduced creatinine clearance. Drug concentrations are comparable in patients with varying degrees of hepatic impairment but this has not been fully studied. Exacerbations of hepatitis B may occur and require more intensive monitoring (monthly in the first three months) in patients with cirrhosis. The onset of complications in these patients requires urgent management. Renal impairment has rarely been reported in patients with HIV infection receiving anti-HBV drugs, or in patients receiving nephrotoxic drugs and treated with TDF or ADV, thus, appropriate monitoring for nephrotoxicity and dose adjustments are necessary.

Long-term monitoring for carcinogenesis with ETV is ongoing. Myopathy has rarely been reported in CHB patients treated with TBV. Peripheral neuropathy has been observed in patients treated with PEG IFN and TBV and thus this combination should be avoided.

2.5. Treatment with PEG IFN-α

The main theoretical advantages of IFN-α (conventional or PEG) are the absence of resistance and the potential for immune-mediated containment of HBV infection with an opportunity to obtain a sustained virological response off-treatment and a chance of HBsAg loss in patients who achieve and maintain undetectable HBV DNA. Frequent side effects and subcutaneous injection are the main disadvantages of IFN-α treatment. IFN-α is contraindicated in patients with decompensated HBV-related cirrhosis or autoimmune disease and in those with uncontrolled severe depression or psychosis. Full information about the advantages, adverse events and inconveniences of PEG IFN-α vs. NUCs should be provided so the patient can participate in the decision. There has been a resurgence of interest in IFN therapy the past five years, largely based on results of large clinical trials demonstrating that PEG IFN has more potent antiviral activity than standard IFN-α and that in contrast to NUCs, it does not result in any antiviral resistance and can be given for a finite period rather than indefinitely. Therefore, when compared to the standard IFN α-2a in a dose of 4.5 million units three times weekly, PEG IFN in a dose of 180 μg once weekly for 12 months resulted in a greater decline in HBV DNA levels and a higher rate of HBeAg seroconversion (33% *vs.* 25%) (24). Three large multicenter trials of PEG IFN therapy have been published—two in HBeAg-positive (25, 26) and one in HBeAg-negative CHB patients (27). Each study included treatment arms in which PEG IFN was used alone or in combination with LAM. Two studies used PEG IFN α-2a and one used PEG IFN α-2b. The results 24 weeks after

treatment are shown in *Table 3*.

In a multinational European study, PEG IFN α-2b was given in a dose of 100 μg weekly for 32 weeks followed by 50 μg weekly until completion of 52 weeks of treatment with or without LAM (100 mg daily) in 266 patients who were HBeAg-positive (25). Seroconversion of HBeAg by six months after treatment occurred in similar proportions of patients receiving monotherapy as combination therapy (29% *vs.* 29%) as did loss of HBsAg (7% *vs.* 7%). Suppression of HBV DNA levels and normal ALT values or HBV DNA levels below 20,000 copies/mL was significantly higher with PEG IFN monotherapy (59% and 43%, respectively) than with 48 weeks of LAM monotherapy (44% and 29%, respectively). Again, the addition of LAM to PEG IFN did not appear to increase the response rates even though there was greater HBV DNA suppression in combination therapy. Furthermore, 3% (12/356) of patients who received PEG IFN but none of 181 patients who received LAM alone became HBsAg-negative.

Table 3. Summary of Combination Therapy of PEG INF and LAM in CHB Patients 24 Weeks Post-treatment

HBeAg Status	Treatment Arms	No.	HBV DNA Suppression, %	HBV DNA Undetectable, %	ALT Normalization, %	HBeAg Seroconversion, %
Positive (25)	PEG IFN 100 μg/wk × 32 wk□ 50 μg × 20 wk	136	27	7	32	29
	PEG IFN 100 μg/wk × 32 wk□ 50 μg × 20 wk + LAM	130	32	9	35	29
Positive (26)	PEG IFN 180 μg/wk × 48 wk	271	32	14	41	32
	PEG IFN 180 μg/wk + LAM 48 wk	271	34	14	39	27
	LAM for 48 wk	272	22	5	28	19
Negative (27)	PEG IFN 180 μg/wk × 48 wk	177	43	19	59	—
	PEG IFN 180 μg/wk + LAM 48 wk	179	44	20	60	—
	LAM for 48 wk	181	29	7	44	—

loss of HBeAg were greater on combination therapy than monotherapy, but relapse rates were higher in the group that received LAM so that sustained responses six months after stopping treatment were equivalent. A comparison group receiving LAM alone was not included. In a second larger multicenter trial, a total of 814 patients with HBeAg-positive CHB were given either PEG IFN α-2a alone (180 μg once weekly), LAM alone (100 mg daily), or the combination for 48 weeks (26). Again, HBV DNA suppression was greater in patients receiving combination therapy than in those receiving either PEG IFN or LAM monotherapy. However, rates of HBeAg seroconversion six months after discontinuation of therapy was greater with PEG IFN than LAM monotherapy (32% *vs.* 19%) and was no higher with combination therapy (27%). Loss of HBsAg occurred in 16 of 542 (3%) patients who received PEG IFN (alone or with LAM) but in none of 272 patients receiving LAM alone ($P = 0.004$).

Finally, in another large multicenter trial, patients with HBeAg-negative hepatitis B were treated with PEG IFN α-2a alone (180 μg once weekly), LAM alone (100 mg daily), or the combination for 48 weeks (27). Six months after stopping therapy, the percentage of patients with

These three studies showed that a one-year course of PEG IFN induced HBeAg seroconversion in about one-third of HBeAg-positive patients and induced a lasting biochemical and virological response in almost 40% of HBeAg-negative patients. Furthermore, therapy with PEG IFN led to loss of HBsAg in a small proportion of patients, an outcome not seen with a one-year course of LAM therapy. Adding LAM to PEG IFN did not increase the rate of sustained responses. These results suggested that a trial of one-year course of PEG IFN might be appropriate in selected patients with CHB, before embarking on long-term suppressive therapy with NUCs.

3. Section II. Topics of EASL and AASLD Presentations at Annual Meetings in 2010

3.1. Efficacy of NUCs

3.1.1. EASL Abstract 1009

Effectiveness of ETV for NUC-naive HBeAg-negative CHB patients in clinical practice: A two-year multicenter cohort study on 311 patients. Lampertico P.

3.1.1.1. Key Results

311 consecutive NUC-naive HBeAg-negative CHB patients, recruited in 17 Italian liver units, were treated with ETV 0.5 mg for 23 months (10, 28).

- 294 (94%) patients achieved a virological response (97% at week 48).
- Two patients had primary non-response at week 12, and three (1%) patients had a virological breakthrough. No ETV resistance in two patients and suboptimal compliance in one patient.
- Two (0.6%) patients cleared HBsAg, seroconverted to anti-HBs and stopped ETV.
- No ETV-related serious adverse events were reported.
- 19 (6%) patients had a partial virological response at week 48; 50% with HBV DNA > 1000 IU/mL. TDF + ETV inhibited HBV replication in the high viral load partial responders.

3.1.1.2. Comments

This cohort supports ongoing evidence that ETV was effective in this real life population of primarily HBeAg-negative patients, of whom almost 50% were with LC.

3.1.2. AASLD PO 391

Maintained long-term suppression of HBV replication in NUC-naive patients with CHB treated with ETV monotherapy in field practice: The Italian multicenter experience. Lampertico P, *et al.*

- Virological responses increased over time in both HBeAg-positive and -negative patients, with more than 90% achieving undetectable HBV DNA.
- HBV remained suppressed over time in the vast majority of patients; only 4% of patients showing a short lasting virological blip.
- Serological responses, i.e. HBeAg seroconversion and HBsAg loss, increased over time. Five patients stopped ETV successfully. And most patients developed a normal ALT level.
- Renal safety: Serum creatinine increased in few patients (< 1%)—an event not considered drug-related.
- Patient retention rates were 84%.

3.1.2.1. Comments

This reconfirms ETV monotherapy suppresses HBV replication in most NUC-naive patients in real practice up to 30 months, independently of serology and safety profile was consistent with registration studies.

3.1.3. AASLD PO 369

Effectiveness and safety of TDF in field practice: A multicenter European cohort study of 737 patients with CHB. Lampertico P, *et al.*

- Most NUC-naive patients achieved undetectable HBV DNA by PCR assay and developed a normal ALT level. Viral suppression was however significantly faster in patients with lower baseline viremia.
- Primary non-response at week 12 and partial virological response at week 48 occurred in 3% and 18% of the patients, respectively.
- Eight patients seroconverted to anti-HBe with an 18-month cumulative probability of 32% and two cleared HBsAg.
- In NUC-experienced patients, HBV DNA became undetectable in almost 74% of the patients, independently of treatment regimen (TDF *vs.* TDF + LAM).
- No major changes of renal function (glomerular and tubular) were observed over 18 months of treatment. Dose adjustments, hypophosphatemia and increased phosphate wasting occurred more frequently in NUC-experienced patients.

3.1.3.1. Conclusions

TDF suppressed HBV replication in most NUC-naive and NUC-experienced patients in field practice up to 18 months. The safety profile was favorable with few patients, mainly NUC-experienced, showing some degrees of renal dysfunction.

3.1.3.2. Comments

This adds another clinical data of TDF. Data on renal toxicity seem not to be consistent. Therefore, we will wait and see the accumulation of more data.

3.2. Resistance Data

3.2.1. AASLD PO 1365

No resistance to TDF was detected in following up to 192 weeks of treatment in patients mono-infected with CHB virus. Snow-Lampart A, *et al.* This evaluated NUC-naive, 176 HBeAg+ and 250 HBeAg– patients on TDF treatment up to four years. No resistance was detected in 348 patients at 192 weeks. Some doctors chose to add emtricitabine (FTC) 200 mg to TDF for seven patients from the 3rd year and five from the 4th year on, if patients were confirmed to be viremic at week 72 or beyond. They reported good tolerability of TDF.

3.2.1.1. Comments

TDF is shown to be free from resistance in this clinical trial setting. It needs to be examined in real practice usage. (cf. ETV has been presented to have 1.2% resistance rate in five years as shown in 2009 EASL guidelines).

3.3. Safety Evaluation of NUCs

3.3.1. Long-term Data

3.3.1.1. EASL Poster 1016

Low rates of nucleos(t)ide-associated adverse events in the long-term experience with ETV. Manns M, *et al.*

3.3.1.1.1. Key Results

Long-term safety data from the roll-over study ETV-901 are reviewed, focusing on adverse events (AEs) with a potential nucleos(t)ide association. Median exposure to ETV in ETV-901 was 184 weeks (almost 3.5 years) (*Table 4)*. Of the 1,051 patients in this analysis, 448 (46%) had prior ETV exposure in previous studies. Overall, the most common AEs (related and unrelated) were upper respiratory tract infection (27%), headache (20%) and nasopharyngitis (16%). Lactate increase or bicarbonate decrease occurred in six (< 1%) patients and no cases of lactic acidosis syndrome were reported. Rates of serious AEs, discontinuations due to AEs, liver disease progression and ALT flares were consistent with previous Phase III observations. AEs typically associated with NUCs use were reported infrequently by investigators. Study ETV-901 demonstrates that ETV is generally a well-tolerated treatment at a dose of 1.0 mg/day when used to treat a diverse population of CHB patients.

3.3.1.1.2. Comments

This roll-over study ETV-901 provides an opportunity to assess safety events in a large cohort of diverse CHB patients, 1051 patients who received long-term ETV (1.0 mg/day) therapy in the study over a median of 184 weeks. Study ETV-901 demonstrates that ETV is a generally well-tolerated long-term treatment.

3.3.2. Renal Data

3.3.2.1. EASL Abstract 1007

Risk of renal toxicity with TDF for CHB. Gish R, *et al.*

3.3.2.1.1. Key Results

84 patients on TDF (either monotherapy or in combination with another antiviral drug) were matched by age (± 5 years) and gender to 84 ETV monotherapy patients.

• TDF was shown to be well tolerated: Serum creatinine increases of 0.2 mg/dL were found to be common, whereas such an increase was rare for the TDF arm and less than ETV group (2%, P = 0.029) probably due to a significantly higher rate of dose adjustments.

• History of diabetes, and transplant, significantly increased the risk of renal injury in all CHB patients (P = 0.004, and 0.002, respectively).

Table 4. Adverse Event (AE) Results from 901 Studies (Mean of 184 Weeks of Treatment) (n=1051)

Adeverse Events (AEs)	Total, No.(%)
Any AEs	900 (86)
Serious AEs	169 (16)
Discontinuations due to AEs	14 (1)
Grade 3-4 AEs	203 (19)
Grade 3-4 AEs considered related to ETV	45 (4)
All deaths	27 (3)
Liver-related deaths	12 (1)
Non-liver-related deaths	15 (1)

3.3.2.1.2. Comments

This is a presentation with the risk of TDF with nephrotoxicity. The study, however suffers from some limitations including, retrospective analysis of data that may cause a selection bias for patients with renal problems to be given ETV as TDF was given as monotherapy or in combination therapy. Patients on ETV had longer duration of disease and comorbidities were not equally matched between the study arms. Furthermore, dose of TDF were often adjusted.

3.3.2.2. EASL Abstract 1010

OPTIB study: A multicenter prospective open label study on TDF for CHB patients with suboptimal response to ADV or ADV+LAM treatment. Levrero M, *et al.*

3.3.2.2.1. Key Results

Adults with HBV monoinfection and HBV DNA > 103 copies/mL after 48 weeks of ADV with or without LAM were enrolled and switched to TDF 300 mg daily with or without LAM.

• 91 patients were screened and 85 were enrolled. 13 (15%) patients were switched from ADV to TDF and 72 (85%) to TDF + LAM combination.

• The median duration of prior ADV therapy was 29.2 months.

• At 24 weeks of treatment, median HBV DNA fall from baseline was 2.02 log10 IU/mL and 62% had HBV DNA levels < 69 IU/mL and 49% HBV DNA levels < 12 IU/mL.

• At 48 weeks, 81% of patients had HBV DNA levels < 69 IU/mL and 65% had HBV DNA levels < 12 IU/mL.

• The proportion of patients reaching negativity through 48 weeks was not correlated with HBeAg status or the presence of ADV resistance mutations at the baseline.

• No clinically significant side effects related to TDF were reported.

3.3.2.2.2. Comments

Despite the concerned nucleotide cross-resistance profile, this study showed higher response rates than other presented data sets. This study implies that TDF can be used to salvage patients exposed to ADV and/or ADV + LAM.

3.3.2.3. EASL Abstract 1028

Renal safety and antiviral efficacy of TDF monotherapy in nucleos(t)ide analogue refractory patients with

hepatitis B virus (HBV) mono-infection. vanBommel F, *et al.*

3.3.2.3.1. Key Results

Data from all HBV monoinfected patients treated with TDF monotherapy in 19 European centers were retrospectively analyzed. Of 343 patients screened, 195 were found eligible for retrospective data analyses; 137 were HBeAg-positive. The mean ± SD HBV DNA level was 6.9 ± 1.5 (range 4-10) log10 copies/mL.

- After 48 months of TDF therapy a mean decrease of estimated glomerular filtration rate (eGFR) of 9% was observed.
- During the total observation period, 10 patients had a moderate decrease (20%–30%) in eGFR; six patients had a severe decrease (> 30%), however eGFR remained within normal values in most patients and did not decrease to < 50 mL/min in patients with initially normal eGFR values.
- TDF dosage did not need to be adjusted due to changes in creatinine.
- A model assessing the influence of age on the eGFR rates as determined by the MDRD formula confirmed a mild decrease in eGFR driven by increase in serum creatinine during the 48 months.
- A comparison of the mean eGFR rates in the TDF group and the control group showed no significant differences in eGFR decrease.

3.3.2.3.2. Comments

In this ongoing real world, independent cohort study evaluating TDF in refractory patients, it was shown that TDF is not associated with renal issues, though this study has excluded patients with higher risk of renal toxicity including, concomitant comorbidities—i.e. those with kidney disease, arterial hypertension, and/or diabetes.

3.3.2.4. AASLD PO 393

Prevalence of renal alterations indicative of proximal tubular damage (PTD) in patients with CHB virus infection during long-term therapy with TDF. vanBommel, *et al.*

3.3.2.4.1. Summary

In total, 24 of 61 (39%) patients showed at least one sign of PTD, which would be either renal phosphate loss (hypophosphatemia and/or TmPO4/GFR↓), glucosuria or increased α1-microglobulin/creatinine ratio.

3.3.2.4.2. Conclusion

This study confirms that long-term treatment with TDF does not lead to a significant decrease in eGFR in HBV-infected patients, regardless of age or risk factors for kidney dysfunction. However, signs of PTD were prevalent in 39% of patients after mean treatment duration of 29 months. As there were no samples from baseline available, there was no clear association between these alterations and the use of antiviral agents. Therefore, further follow-up data are needed to determine the role of TDF therapy in possible proximal tubular damage. More specific markers may help to further determine the drug's influence on renal function.

3.3.3. Bone Study

3.3.3.1. AASLD PO 414

High prevalence of reduced bone mineral density in patients with CHB under nucleos(t)ide analogues treatment. Vigano M, *et al.* Single center (Universita di Milano), cross-sectional study studied 319 patients with CHB receiving NUC over a one-year period. Dual X-ray absorptiometry (DEXA) of the lumbar spine (LS) and femoral neck (FN) revealed that two thirds of CHB patients undergoing NUC treatment had reduced bone mineral density (BMD), osteoporosis at either LS or FN was present in 19% and osteopenia in 49% of the patients. Multivariate analysis showed that female sex, older age and nucleotide (ADV and TDF) treatment were independently associated with a reduced BMD.

3.3.3.1.1. Comments

It is noteworthy that only nucleotides (ADV and TDF), not nucleosides (ETV and LAM), was associated with reduced BMD. Clinicians may need periodical screening of patients for osteoporosis.

3.3.4. PEG IFN for CHB

3.3.4.1. EASL Abstract 98

Extended (two years) treatment with PEG INF α-2a [40 kD] improves sustained response rates in genotype D patients with HBeAg-negative CHB. Lampertico P, *et al.*

3.3.4.1.1. Results

PEG IFN α-2a 180 μg/week was evaluated for HBeAg-negative patients with CHB (n = 128) for its duration (48 *vs.* 96 weeks) *(Table 5)*. Virologic response was superior with 96 weeks and notably HBsAg loss retention was observed in 10% of patients one year after the therapy of 96 weeks. These edges NUCs in efficacy in this study population, but it should be noted that different genotypes would respond differently and we need further studies in patients with various backgrounds.

3.3.4.1.2. Comments

It is still in the experimental stage but this deserves to be examined further, although long-term treatment poses cost and safety concerns and may limit the number of eligible patients for this therapy.

Table 5. Safety Profiles of Extended PEG IFN Therapy

Safety Outcome	48-Week PEG IFN [a] (n = 51)	96-Week PEG IFN (n = 52)
≥ 1 AE [a], %	82	77
≥ 1 serious AE, %	14	6
Need for dose reduction, %	31	19
Study withdrawal, %		
Due to AEs	16	12
Reasons other than safety	8	12
Death [b], No.	1	0

[a] Abbreviations: AE, adverse event; PEG IFN, peginterferon
[b] Patient died of hepatocellular carcinoma during follow-up

3.4. Efficacy and Safety of NUCs in Decompensated Cirrhosis

Decompensated cirrhosis is a serious complication of CHB. The five-year survival of patients with decompensated cirrhosis (14%) has been reported and is lower than that for patients with compensated cirrhosis (84%) (2). However, suppression of viral replication with antiviral therapy has been shown to result in clinical improvement and increased survival (2). There are limited data on safety and efficacy of NUC therapy in patients with CHB and decompensated cirrhosis. Summarized here are recently presented data including two randomized clinical trials (28, 29), and a cohort study on Korean patients pertaining to the use of ETV. in this patient population.

3.4.1. EASL Oral Abstract 7

Treatment of decompensated HBV-cirrhosis: results from a two-year randomized trial with telbivudine or lamivudine. Gane EJ, *et al. (Table 6)*. This study was to evaluate clinical and virological outcomes of TBV *vs.* LAM in 232 patients (mean CTP and MELD score TBV 8.1 and 14.7; LAM 8.5 and 15.5). At baseline, the median age was almost 50 years—65% Asian, almost 73% males, and approximately 57% HBeAg-negative. This RCT showed that both therapies were safe but with high rates of rebounds/virological breakthroughs. There was only a limited improvement in MELD score of 0.2 with TBV, and 1 with LAM.

3.4.1.1. Comments

In this large scale study with long-term follow-up, TBV was well tolerated, stabilized liver function and had comparable tolerability to LAM. Safety profiles were similar between treatment arms, however, both TBV and LAM showing almost 30% viral breakthrough. This result seems to reinforce the need to use potent antiviral treatment with low rates of resistance in this population with advanced disease, like ETV or TDF.

3.4.2. EASL Abstract 1011

Risk and predictors of mortality or hepatocellular carcinoma among ETV- or ADV-treated CHB patients with evidence of hepatic decompensation. Liaw Y, *et al.*

3.4.2.1. Key Results

This industry-sponsored study examined predictors of death and HCC in pooled data from ETV-treated and ADV-treated patients. The baseline predictors for death and HCC were examined in the 191 patients randomized to receiving either 1.0 mg/day ETV or 10 mg/day ADV for up to 96 weeks using univariate and multivariate Cox proportional hazard models with pooled data. Significant predictors of mortality in univariate analysis included serum creatinine level, MELD score, total bilirubin and albumin level. The multivariate analyses showed that a decreased hepatic function (increased bilirubin and decreased albumin level) is a significant predictor of mortality among CHB patients with decompensated liver disease treated with nucleos(t)ide analogues. Cumulative HCC rates were 12% and 20% among ETV-treated and ADV-treated patients, respectively. Cumulative death rates were 23% and 33% among ETV-treated and ADV-treated patients, respectively. HBV genotype B/C was the only predictor for development of HCC *(Table 7)*.

3.4.2.2 Comments

The ETV-048 subanalysis reinforces the importance of

Table 6. Outcomes in telbivudine (TBV) and lamivudine (LAM) Groups

2-Year Outcome (ITT Population)	TBV [a] (n = 114)	LAM [a] (n = 114)	*P* value
HBV DNA, % (< 300 copies/mL)	49	40	0.15
Viral breakthrough, % (HBV DNA > 1 log10 copies/mL above nadir)	28	37	0.16
Composite endpoint, %	34	24	0.004
CTP score improved or stabilized, %	75	74	NS [a]

[a] Abbreviations: LAM, lamivudine; NS, not significant; TBV, telbivudin

Table 7. Cumulative Efficacy and Safety in Both entecavir (ETV) and adefovir dipivoxil (ADV) Groups

	Week 48	
	ETV[a]	ADV[a]
Cumulative efficacy of NUCs		
HBV DNA change from baseline, (log10 copies/mL)	4.66	3.90
HBV DNA, No. (%) (< 300 copies/mL)	57 (100)	20 (91)
HBeAg loss, No.%	11 (54)	18 (51)
HBeAg seroconversion, No.%	6 (54)	10 (51)
HBsAg loss, No.%	5 (100)	0 (91)
CTP score improvement or no worsening, No.%	61 (100)	67 (91)
CTP score, No.% (≥ 2-point reduction)	35 (100)	27 (91)
MELD score change from baseline, Mean (SE) [a]	2.6 (0.62)	1.7 (0.50)
Cumulative safety of NUCs		
Any AE, No.%	91 (89)	86 (97)
Grade 3-4 AEs, No.%	55 (54)	42 (47)
Deaths, No.%	23 (23)	29 (33)
Serum Cr, No.%, (≥ 0.5 mg/dL increase)	17 (17)	21 (24)
HCC, No.%	12 (12)	18 (20)
Discontinuation due to AEs, No.%	7 (7)	5 (6)

[a] Abbreviations: ADV, adefovir dipivoxil; ETV, entecavir; SE, standard error

biologic risk factors (including baseline characteristics and inclusion criteria) as predictors associated with increased HCC and/or mortality in decompensated cirrhotic patients.

3.5. Cohort Study in Korean Patients

Shim JH *et al.* Efficacy of ETV in treatment-naive patients with HBV-related decompensated cirrhosis (30). This cohort study evaluated the effect of ETV monotherapy (0.5 mg QD for ≥ 12 months) on viral suppression and hepatic function in 70 consecutive treatment-naive patients with HBV-associated decompensated cirrhosis (defined as CTP ≥ 7 [class B and C]), or the presence of portal hypertension complications). Comparator group consists of compensated LC patients with HBV (n = 144). Virologic response in this decompensated group (n = 55) was also compared to compensated cirrhosis. 15 patients in the decompensated group received ETV < 12 months and therefore were not included in the comparative analysis with the compensated group. The baseline characteristics for decompensated and compensated groups were similar for gender ratio, HBV DNA levels (mean ± SD for total patients was 7.34 ± 1.43 log10 copies/mL; n = 199), and proportion HBeAg-positive (mean for total patients: 58.8%; n = 199).

However, in comparison to the compensated group, those with hepatic decompensation had a greater mean age (52.6 *vs.* 46.8 year, $P < 0.001$), lower mean ± SD serum ALT (101.9 ± 110.7 *vs.* 156.5 ± 160.5 IU/L, $P = 0.021$); and higher mean ± SD CTP (8.1 ± 1.7 *vs.* 5.3 ± 0.05) and MELD (11.5 ± 3.9 *vs.* 7.0 ± 1.5) scores ($P < 0.001$ for both).

Virologic, serologic and biochemical responses after 12 months of ETV therapy in the decompensated and compensated groups are presented in *Table 8*. Overall, at 12 months the rates for undetectable HBV DNA, HBeAg loss/seroconversion, and ALT normalization were not significantly different between the compensated and decompensated groups. In an intention-to-treat analysis of efficacy of all 70 patients with decompensated cirrhosis, the cumulative rates of HBV DNA undetectability and HBeAg loss at 12 months were 92.3% and 54.0%, respectively. For those patients with decompensated cirrhosis (n = 70), the cumulative incidence of HCC was 6.9% at month 24; four patients developed HCC during the follow-up. The cumulative incidence of mortality or OLT was 12.9% at month 12 and 17.0% at month 24. For 55 patients with decompensated liver function treated with ETV for ≥ 12 months, improvements from baseline in CTP score and its components (albumin, total bilirubin, prothrombin time) and MELD score were observed ($P < 0.05$ for all). CTP class A (score 5 or 6) was achieved in 65.5% (30) of the patients, and improvement in CTP (≥ 2 points reduction) was observed in 49% (27) of the patients after 12 months of treatment.

Table 8. One-year Results of Virologic and Biochemical Indices

One-year Results	Compensated (n = 144)	Decompensated (n = 55)	P value
Change in HBV DNA, (log10 copies/mL)	6.74 ± 1.88	6.82 ± 1.32	0.793
HBV DNA undetectable, No. (%), (< 300 copies/mL by PCR)	113/144 (78.5)	49/55 (89.1)	0.104
HBeAg seroconversion, No. (%)	22/90 (24.4)	6/27 (22.2)	0.812
HBeAg loss, No. (%)	37/90 (41.1)	13/27 (48.1)	0.517
ALT normalization, No. (%)	108/144 (75.0)	42/55 (76.4)	0.535

3.5.1. Comments

This cohort study supports the use of ETV as a first-line treatment option for NUC-naive patients with decompensated HBV cirrhosis. Further follow-up of similar studies are needed to identify the optimal treatment for these patients and those with LAM-resistant HBV cirrhosis.

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REVIEW

Review of dynamic contrast-enhanced ultrasound guidance in ablation therapy for hepatocellular carcinoma

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Abstract

Local ablative techniques-percutaneous ethanol injection, microwave coagulation therapy and radiofrequency ablation (RFA)-have been developed to treat unresectable hepatocellular carcinoma (HCC). The success rate of percutaneous ablation therapy for HCC depends on correct targeting of the tumor *via* an imaging technique. However, probe insertion often is not completely accurate for small HCC nodules, which are poorly defined on conventional B-mode ultrasound (US) alone. Thus, multiple sessions of ablation therapy are frequently required in difficult cases. By means of two breakthroughs in US technology, harmonic imaging and the development of second-generation contrast agents, dynamic contrast-enhanced harmonic US imaging with an intravenous contrast agent can depict tumor vascularity sensitively and accurately, and is able to evaluate small hypervascular HCCs even when B-mode US cannot adequately characterize the tumors. Therefore, dynamic contrast-enhanced US can facilitate RFA electrode placement in hypervascular HCC, which is poorly depicted by B-mode US. The use of dynamic contrast-enhanced US guidance in ablation therapy for liver cancer is an efficient approach. Here, we present an overview of the current status of dynamic contrast-enhanced US-guided ablation therapy, and summarize the current indications and outcomes of reported clinical use in comparison with that of other modalities.

Key words: Dynamic contrast-enhanced ultrasound; Hepatocellular carcinoma; Percutaneous ethanol injection; Radiofrequency ablation

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INTRODUCTION

Hepatic resection forms part of the conventional treatment for patients with primary liver cancers; however, the majority of hepatocellular carcinomas (HCCs) are not suitable for curative resection at the time of diagnosis. Difficulties of surgical resection may be related to size, site, and number of tumors, vascular and extrahepatic involvement as well as liver function of the patient[1-4]. There is a need to develop a simple and effective technique for treatment of unresectable HCCs; therefore, local ablative techniques [percutaneous ethanol injection (PEI), microwave coagulation therapy (MCT) and radiofrequency ablation (RFA)] have emerged in clinical practice to expand the pool of patients considered for

liver-directed therapies[5-8]. In particular, RFA is not associated with some of the side-effects of other ablative techniques[9]. Thus, RFA is currently more widely accepted due to the ease of use, safety, reasonable cost and applicability to minimally invasive techniques[10].

Percutaneous ablation therapy for HCC is widely performed under real-time sonographic guidance. The success rate of percutaneous RF ablation depends on correct targeting *via* an imaging technique. However, multiple sessions of ablation therapy are often required for small HCCs, which are poorly defined on conventional B-mode ultrasound (US) alone[11]. There are two particular situations in which B-mode US cannot adequately characterize the tumors[12]. The first is the presence of residual HCC nodules after ablation, because B-mode US findings cannot adequately differentiate between treated and viable residual tumor tissue. The second is the presence of naïve HCC nodules among many large regenerated nodules in cirrhotic liver. Color Doppler and power Doppler have increased the sensitivity of hepatic lesion detection compared to that using gray-scale US, but these modalities do not provide levels of sensitivity comparable to those of contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI)[13-16]. However, two breakthroughs in US technology, harmonic imaging and the development of second-generation contrast agents, have demonstrated the potential to dramatically broaden the scope of US diagnosis of hepatic tumors[14-16]. Dynamic contrast harmonic US can depict tumor vascularity sensitively and accurately, and is able to evaluate small hypervascular HCCs even when B-mode US cannot adequately characterize the tumors[17-21]. Therefore, contrast-enhanced harmonic US is expected to improve the detectability of HCC nodules, and decrease the number of sessions required for ablation of HCC in difficult cases[22,23].

This paper reviews the evidence for the use of dynamic contrast-enhanced US guidance in ablation of HCC, and illustrates the potential of the techniques for improving the targeting in percutaneous ablation therapy.

DIAGNOSIS AND TREATMENT OF HEPATOCELLULAR CARCINOMA

HCC can be diagnosed radiologically, without the need for biopsy if the typical imaging features are present[20,24,25]. This requires a contrast-enhanced study (dynamic CT or MRI). HCC enhances more intensely than the surrounding liver in the arterial phase, whereas the presence of 'washout' persists in the delayed phase. Tumor markers including alpha-fetoprotein and descarboxyprothrombin have been used for the diagnosis of HCC.

The management of HCC involves multiple disciplines including hepatology, surgery, diagnostic and interventional radiology, oncology, and pathology[20,25-27]. One has to consider several patient and tumor factors including the severity of underlying liver disease, tumor bulk, and associated comorbidities, as well as several practice-setting factors including availability and expertise in surgical resection, transplantation, and ablative therapies. RFA is basically recommended for HCC nodules with a maximum diameter of 3 cm in patients with not more than three tumors who are contraindicated for surgery.

DYNAMIC CONTRAST-ENHANCED ULTRASOUND

Contrast agents

Levovist (Schering, Berlin, Germany) is a first-generation US agent made of galactose[28]. A trace of palmitic acid is added as a surfactant to stabilize the resultant microbubbles. These bubbles have a weak encapsulating shell and are easily destroyed by US exposure. The contrast effect of Levovist is based on the destruction of microbubbles by high mechanical index (MI) pulses. In addition, Kupffer cells phagocytose Levovist microbubbles; therefore, liver parenchymal findings are obtained as Kupffer imaging in the postvascular phase at least 10 min after administration.

Sulfur hexafluoride microbubbles (SonoVue; Bracco SpA, Milan, Italy), perflutren lipid microbubbles (Definity; Bristol-Myers Squibb, North Billerica, MA), perflutren protein microbubbles (Optison; GE Healthcare, Buckinghamshire, United Kingdom), and perfluorocarbon microbubbles (Sonazoid; Daiichi-Sankyo, Tokyo, Japan) are second-generation contrast agents[29-33]. These microbubbles provide stable nonlinear oscillation in a low power acoustic field because of the hard shell of these bubbles, producing great detail in the harmonic signals in real-time. The only second-generation contrast agent that can be taken up by Kupffer cells in the liver is Sonazoid. Sonazoid microbubbles accumulate in the liver parenchyma over time[34,35].

Generally, few drug toxicities have been reported; these being pain at the point of injection, sense of heat and sense of cold. The incidence of complications was shown not to differ from historical controls (1.7%, P = 0.867 by Fisher's exact probability test)[36].

Imaging and procedure

In point of fact, there is a clinical need for high resolution and real-time imaging for dynamic contrast-enhanced US guidance in ablation therapy.

Using Levovist, real-time images of tumor enhancement by the simultaneous collapse of microbubbles caused by high mechanical index pulses can be obtained in the early vascular phase only[28]. The collapse of microbubbles in viable HCC lesions is seen as white flashes on the screen[12]. However, maintaining real-time US imaging for guidance reduces the enhancement period to approximately one minute after injection because Levovist microbubbles are easily disrupted (Figure 1). Therefore, great skill is required because the procedure time is too short to search for enhanced HCC nodules and insert the probe.

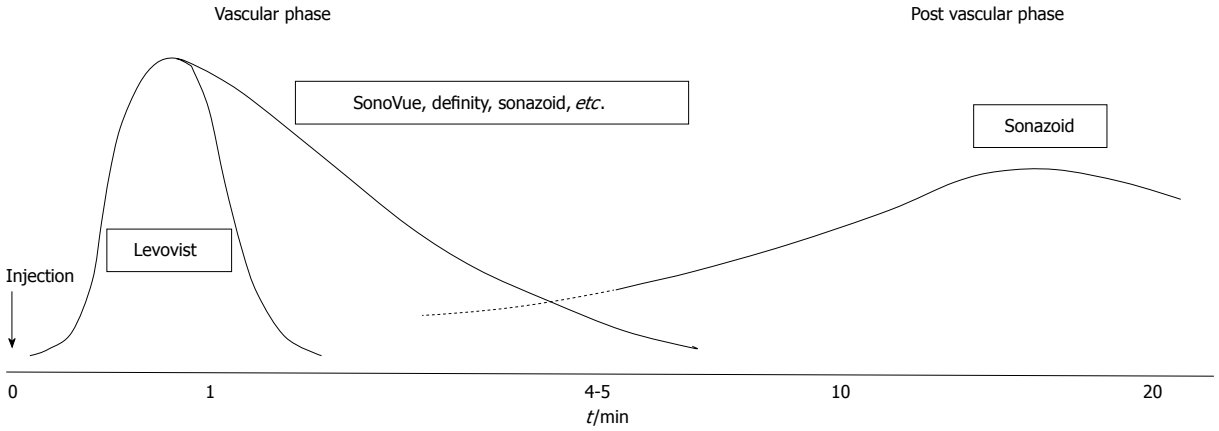

Figure 1 ***In vivo*** **kinetics of intravenous contrast ultrasound agents in the liver.**

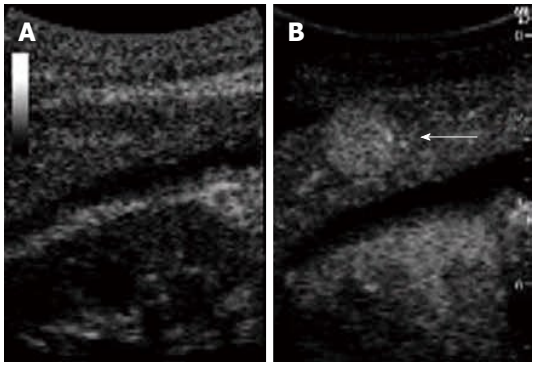

Figure 2 A 67-year-old man with a 1.5-cm hepatocellular carcinoma nodule located in segment 3 of the liver. A: B-mode ultrasound (US) cannot clearly depict the hepatocellular carcinoma (HCC) nodule; B: Contrast-enhanced US shows enhancement of HCC focus (arrow) in early vascular phase after administration of sonazoid.

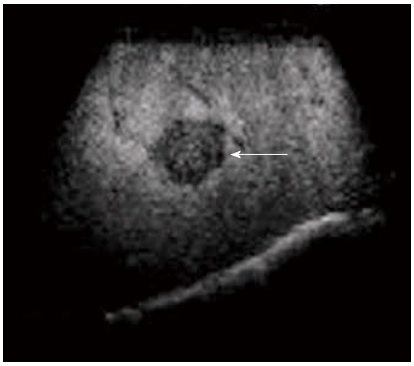

Figure 3 A 70-year-old man with a 2.0-cm hepatocellular carcinoma nodule located in segment 6 of the liver. Contrast-enhanced ultrasound using sonazoid shows the defect (arrow) imaging in post-vascular phase. The defect lesion can be targeted for insertion of a single needle by extending the time limitation.

Using second-generation contrast agents, real-time findings are better than those reported with Levovist[37] (Figure 1). Hypervascular HCC shows a short early arterial flushlike enhancement for less than 20 s, followed by homogeneous enhancement of the lesion in the late phase under low MI imaging (Figure 2). Needle insertion can be performed between the early arterial phase to late vascular phase in which maximum lesion conspicuity is observed[38]. In particular, HCCs have been visualized as defects in the liver parenchyma in the post-vascular phase only with Sonazoid use (Figure 1)[39-43]. Therefore, we can use these defect lesions as a target for insertion of a single needle (Figure 3). In patients who had previously undergone ablation for HCC, demonstration of viable nodules among all nodules detected in the post-vascular phase was achieved by injecting an additional new dose of Sonazoid in order to confirm tumor vascularity before needle insertion[44]. This defect-reperfusion US imaging is extremely useful in the depiction and confirmation of HCCs that are otherwise undetectable on US.

CLINICAL OUTCOMES

Treatment sessions and local tumor progression

Table 1 shows the treatment sessions of percutaneous ablation guided by contrast-enhanced US for HCC in published papers[12,36,45-49]. Numata *et al*[45] first reported that nine HCC nodules were successfully treated with percutaneous ablation therapy guided by intravenous contrast-enhanced US. These nine lesions were not detected on conventional US but were depicted on real-time contrast-enhanced harmonic gray-scale US with Levovist (incomplete local treatment, $n = 4$; small new lesion, $n = 5$). Since 2004, second-generation contrast agents of US have been used in percutaneous ablation guided by contrast-enhanced US. Particularly with Sonazoid use, complete tumor necrosis has been achieved in 94% with a single session of RF ablation[49]. In cases of HCC that are not clearly demarcated by B-mode US, dynamic contrast-enhanced sonography-guided RFA and are efficient approaches for guiding ablation.

Table 1 Treatment sessions of percutaneous ablation guided by contrast-enhanced ultrasound for hepatocellular carcinoma

Author[Ref.]	Year	Procedure	*n*	Contrast agent	Tumor size (mean, cm)	Treatment sessions (mean)
Numata *et al*[45]	2003	PEI, RFA	9	Levovist	1.4	ND
Minami *et al*[12]	2004	RFA	21	Levovist	1.7	1.05
Solbiati *et al*[46]	2004	RFA	51	SonoVue	ND	ND
Numata *et al*[47]	2008	RFA	15	Sonazoid	ND	1.04
Maruyama *et al*[48]	2009	PEI, RFA	42	Sonazoid	1.3	ND
Miyamoto *et al*[49]	2009	RFA	52	Sonazoid	ND	1.04
Minami *et al*[50]	2010	RFA	108	Sonazoid	1.7	1.1
Masuzaki *et al*[36]	2010	RFA	291	Sonazoid	1.6	1.33

HCC: Hepatocellular carcinoma; ND: Not described; PEI: Percutaneous ethanol injection; RFA: Radiofrequency ablation.

Table 2 Local tumor progression rates of percutaneous ablation guided by contrast-enhanced ultrasound for hepatocellular carcinoma

Author[Ref.]	Year	Procedure	*n*	Tumor size (mean, cm)	Follow-up (mean, mo)	Local tumor progression (%)
Maruyama *et al*[48]	2009	PEI, RFA	42	1.3	8.6	0
Minami *et al*[50]	2010	RFA	108	1.7	4.3	0
Masuzaki *et al*[36]	2010	RFA	291	1.6	ND	2.1
Miyamoto *et al*[51]	2010	RFA	17	1.6	11	12

HCC: Hepatocellular carcinoma; ND: Not described; PEI: Percutaneous ethanol injection; RFA: Radiofrequency ablation.

The local tumor progression rates after RFA have ranged from 0% to 12% during the follow-up period[47,36,49,50] (Table 2). The risk of local tumor progression increases with size, but the local tumor progression rates of small HCCs were markedly dependent on whether or not the center of the HCC nodule was penetrated by the RF needle.

Dynamic contrast-enhanced US guidance vs conventional B-mode US guidance

The effectiveness of contrast harmonic sonographic guidance for RFA of HCC was evaluated in comparison with conventional B-mode US guidance[12,36,51] (Table 3) (Level of evidence: grade B, level 2b). Dynamic contrast-enhanced US significantly helps in the placement of RFA electrodes in hypervascular HCCs that cannot be adequately depicted by B-mode sonography. In a randomized controlled study, the number of treatment sessions was significantly lower in the contrast harmonic US group (mean, 1.1 ± 0.2 *vs* 1.4 ± 0.6, $P = 0.037$)[51]. Treatment analysis showed that the complete ablation rate after a single treatment session was significantly higher in the contrast harmonic US group than in the B-mode US group (94.7% *vs* 65.0%, $P = 0.043$). Moreover, Masuzaki *et al*[36] reported in a large-scale study that the detectability of tumor nodules was 83.5% in conventional US and 93.2% in contrast-enhanced US ($P = 0.04$). The number of RFA sessions was 1.33 ± 0.45 with contrast-enhanced US as compared to 1.49 ± 0.76 in the historical controls ($P = 0.0019$). The number of RFA sessions required for complete ablation could be decreased in contrast-enhanced US-assisted RFA.

Few toxicities using US contrast agents have been reported, therefore the incidence of complications did not differ from that reported in patients treated by RFA alone[12,51].

Advances in techniques: Tumors abutting the diaphragm

HCC nodules abutting the diaphragm are difficult to depict because of ultrasound scatter due to pulmonary air. However, contrast-enhanced US through artificial pleural effusion can depict tumor vascularity in HCC. Thus, percutaneous RFA guided by contrast-enhanced US with artificial pleural effusion is an efficient approach[36,52]. Thirteen tumors were treated by contrast-enhanced US-guided RFA with artificial pleural effusion, and complete tumor necrosis was achieved in a single session in 12 lesions (92.3%)[52]. It took approximately 1 wk for pleural effusions to spontaneously resolve.

OTHER MODALITIES

Computed tomography guidance and computed tomography fluoroscopy

CT has high spatial resolution, good contrast, wide field of view, good reproducibility, and applicability to bony and air-filled structures. Potential advantages of CT guidance include confirmation of probe placement in relation to the tumor, improved visualization of the extent of ablation, and good correlation with actual lesion size[53-56] (grade C, level 3b). The use of a CT-guided method can be expected to reduce the rate of local tumor progression associated with percutaneous RFA. Laspas *et al*[53] reported that the ablation success rate was 87.3% (281/322 HCC nodules), and the survival rates at 1 year, 3 years and 5 years were 94.8%, 73.1% and 51.1%, respectively. Another merit is that the efficacy of treatment can be evaluated using contrast-enhanced CT immediately after treatment. Despite the advantages of CT, there are several limitations such as the increased time that is necessary for the procedure and exposure of the patient to ionizing radiation.

CT fluoroscopy guidance combines the high spatial resolution and good contrast resolution inherent in contrast-enhanced CT with the immediacy of fluoroscopic monitoring. Under CT fluoroscopy using either CT arteriography or iodized oil injection, we can target and puncture hepatic malignancies using a percutaneous ethanol injection needle. Real-time CT fluoroscopy is useful to guide the needle puncture and to monitor ethanol injection in small hepatic malignancies (grade C, level 3b). Takayasu *et al*[57] reported that the overall success rate in puncturing the lesions was 94.4% (17/18 sessions), the average number of punctures was 3.3, and this significantly decreased after the introduction of a puncture guide compared with freehand puncture ($P < 0.01$). However, the operator's hands are directly exposed to the beam of CT fluoroscopy, posing a potentially serious problem.

Magnetic resonance imaging guidance

MRI with its high soft tissue contrast can be advanta-

Table 3 Treatment sessions of radiofrequency ablation: Dynamic contrast-enhanced ultrasound guidance *vs* conventional B-mode ultrasound guidance

Author[Ref.]	Year	Study type	*n* (CEUS/B-mode)	Tumor size, (mean, cm) (CEUS/B-mode)	Mean treatment sessions (CEUS *vs* B-mode)	*P* value
Minami *et al*[12]	2004	Case control study	21/25	1.7/1.7	1.05 *vs* 2.0	0.002
Minami *et al*[53]	2007	RCT	19/20	1.2/1.3	1.1 *vs* 1.4	0.043
Masuzaki *et al*[36]	2010	Case control study	291/291	1.9/1.9	1.33 *vs* 1.49	0.0019

CEUS: Contrast-enhanced ultrasound; HCC: Hepatocellular carcinoma; RCT: Randomized controlled trial.

geous, and the capability of MRI for multiplanar imaging can be of value for needle placement and surveillance of the ablation procedure. Most of the current open MR scanners operate between 0.2 and 0.5 T, while clinical MR systems with a closed cylindrical design allow for significantly higher field strengths of up to 3.0 T or even more. While open MR systems allow for online monitoring of the puncture and easy replacement of the RF needle within a wide range, closed-bore MR systems improve lesion conspicuity and tumor delineation[58-63] (grade C, level 3b). Wu *et al*[59] reported that MRI and optical navigation system-guided ablation procedures were successfully performed on all 32 patients (36 tumor sites), and the 6- and 12-mo overall survival rates were 96.8% and 90.6%, respectively. Although MRI can be used to obtain reference images in ablation therapy, RF needle puncture is actually performed under sonographic guidance. Therefore, an MR-guided system can be used for ablation monitoring, but not for puncture guidance.

CO_2-enhanced ultrasound (ultrasound angiography)

CO_2-enhanced sonography is a sensitive means of detecting small HCC lesions. Kudo *et al*[64] reported that the detection rate of tumor hypervascularity on CO_2-enhanced sonography (86%) showed that it was more sensitive than digital subtraction arteriography (70%) and CT with iodized oil (82%). Imari *et al*[65] reported that CO_2-enhanced sonography is useful for the detection of hypervascular HCC and PEI treatment of lesions not detectable by conventional US. After direct intra-arterial injection of CO_2, enhancement of the tumor lasts approximately 10-60 min. This enhancement provides sufficient time to perform percutaneous ablation therapy (grade C, level 3b). Chen *et al*[66] reported that thirty-four (64.2%) of the 53 tumors showed complete necrosis after treatment, and the cumulative 1-, 2- and 3-year survival rates of patients who underwent CO_2-enhanced sonographically-guided percutaneous ethanol injection were 81%, 71% and 44%, respectively. However, nodules may become unclear because bubbles become trapped and accumulated in sinusoids with repeated injections of CO_2 microbubbles[67,68]. In addition, this method involves angiographic procedures that are invasive.

Virtual computed tomography sonography

Cross-sectional multiplanar reconstruction images from almost isovoxel volume data can be used for virtual sonographic visualization. This technique is available for patients with HCCs that became enhanced in the arterial phase of dynamic CT but were not well visualized with conventional B-mode US. Virtual CT sonography using magnetic navigation [real-time virtual sonography (RVS); HITACHI Medico, Tokyo, Japan] provides cross-sectional images of CT volume data corresponding to the angle of the transducer in the magnetic field in real-time[69-70]. RVS displays a real-time synchronized multiplanar CT image in precisely the same slice of the US plane. Thus, RVS can be used for real-time needle insertion guidance, especially for nodules demonstrated on CT, but not on US (grade C, level 3b). It has been reported that the technical success rate after a single treatment session was significantly higher in the virtual CT sonography group ($P = 0.017$)[71]. However, RVS has a weakness in that imaging gaps might be attributable to variations in the depth of breath-holding on CT and US examinations, as well as in the fact that the distance error between the magnetic sensor attached to the ultrasonic transducer and the magnetic generator becomes greater on intercostal US examination.

CONCLUSION

Percutaneous ablation therapy guided by dynamic contrast-enhanced US is an efficient approach for HCCs that are not clearly demarcated by B-mode US in both untreated and locally recurrent HCC cases. Moreover, second-generation microbubbles could facilitate dynamic contrast-enhanced US guidance of ablation therapy by extending the time limitation, simplifying the procedure, and improving detectability. RF ablation guided by second generation microbubble-enhanced US could become easier and be an efficient approach for hepatic malignancies that are not clearly depicted on B-mode US.

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Original Article

Demonstration of quality of care measurement using the Japanese liver cancer registry

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Aim: Despite advances in medical therapy, studies have reported gaps between current evidence and actual practice in many areas of medicine. Process-of-care quality indicators (QIs) are tools to measure the evidence–practice gap. This study aims to examine the feasibility of applying QIs for liver cancer care to the national registry database operated by the Liver Cancer Study Group of Japan.

Methods: Prior research developed a set of process-of-care QIs developed on the basis of the Japanese Clinical Practice Guidelines for hepatocellular carcinoma. Each QI describes target patients and care processes indicated for such patients. Among the 25 developed QIs, six appeared scorable using the information contained in the dataset from the 17th Nationwide Survey of Primary Liver Cancer.

Results: In total, 16 187 patients were eligible for the six QIs for 34 599 times, among which the indicated care was provided 83.9% times. The scores ranged from 64.4% (surgical therapy in patients with HCC 3–5 cm in diameter) to 91.1% (indocyanine green checkup before surgical resection). The information was generally available to determine eligibility (78.3%–100%) and pass/fail (91.9%–99.9%) for the QIs.

Conclusions: Applying QIs to the liver cancer registry, the quality of hepatocellular carcinoma care can be measured. In future, providing feedback regarding the results to the participating society may improve the quality of liver cancer care nationwide.

Key words: cancer registry, hepatocellular carcinoma, quality indicators, quality of health care.

INTRODUCTION

LIVER CANCER IS the third leading cause of cancer deaths worldwide.[1] Eastern countries generally exhibit higher incidences of liver cancer, but many Western countries have experienced a steady increase.[2,3] Liver cancer is prevalent in Japan, and it was the fourth leading cause of cancer deaths in 2009.[4] Despite recent advances in diagnostics and therapeutics, the 5-year survival rates based on population-based cancer registries remains relatively low at 23.1%.[5]

To improve survival, both medical therapeutic advances and their dissemination into clinical practice are necessary. To distribute current knowledge and facilitate clinical decision making for liver cancer treatment, the first evidence-based Clinical Practice

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Guidelines for Hepatocellular Carcinoma in Japan were published in 2005 with financial support from the Ministry of Health, Labor, and Welfare.[6,7] A follow-up survey of specialists and generalists involved in liver cancer care demonstrated successful outreach and acceptance of these guidelines among frontline practitioners of hepatocellular carcinoma (HCC) care.[8]

The next step in monitoring the effectiveness of the HCC clinical practice guidelines is the assessment of the quality of care. Although the quality of care can be assessed by structure, process, and outcome,[9] an evaluation of the process best fits the context of guideline implementation. Quality assessments that examine the processes of care compare the actual care provided to the patient against the pre-specified standards of care. Although standards may not exist for all aspects of care, examining how well the standards are incorporated into daily practice in those areas that do exist can reveal aspects of quality and create a basis for improvement. In addition, gaps in the process quality highlight areas for the guideline committee to focus on in the next round of revisions. Accordingly, we developed a set of process quality indicators (QIs) that describe standards for HCC patient care.[10,11] Although the QIs were designed to be implemented through the review of medical records, some QIs can be used on the Nationwide Survey of Primary Liver Cancer – the liver cancer registry operated by the Liver Cancer Study Group of Japan. This provided a unique opportunity to pilot test the QIs and examine certain aspects of the quality of care of some liver cancer patients in Japan.

METHODS

Development of the QIs

THE QIS WERE developed using Japanese HCC guidelines, adopting the RAND/University of California, Los Angeles appropriateness methods.[12] Details of processes and results were previously reported in Japanese.[11] Briefly, we first created candidate QIs based on the recommendations of the Japanese HCC guidelines and the medical literature. Each QI described the care standards defining target patients and indicated the care processes. From a literature review, we summarized the rationale for each candidate QI.

Next a nine-member multidisciplinary panel was convened that consisted of two hepatobiliary surgeons, three hepatologists, a gastrointestinal surgeon, a general internist, and two interventional radiologists. The panel members were nationally recognized clinicians from various practice settings, including the university and general hospital settings. The geographic distributions of the clinical practices were also taken into account.

The panel examined candidate QIs by following the modified Delphi process that consisted of two rounds of anonymous rating of the validity (scale of 1–9; 1 = definitely invalid, 9 = definitely valid) coupled with a face-to-face group discussion between rounds. During the process, the panel was allowed to modify the QIs. As per prior studies, QIs that had a median rating of 7 or higher and were rated 3 or lower by two or fewer panelists in the second ratings were accepted.[12,13]

Liver cancer registry database

The Liver Cancer Study Group of Japan operates the Nationwide Survey of Primary Liver Cancer in Japan, which is a cancer registry specifically for liver cancer.[14] Biannually, it collects 178 data items from the newly treated primary liver cancer patients and 46 items for following the previously registered patients. Participation is voluntary and is estimated to cover approximately 20% of all primary liver cancer patients in Japan.[15] We used data on patients receiving therapy for liver cancer at 645 participating institutions during 2002 and 2003. The data consisted of detailed clinical information and included the patients' baseline conditions, imaging findings, treatment modality, and pathological findings. Here, we have limited our analysis to HCC patients ≥20 years of age and have excluded patients who lacked age or diagnosis information.

Quality scores

The expert panel process resulted in 25 QIs,[11] which targeted a wide range of care processes including the pre-therapeutic evaluation, treatment choice, patient explanation of the treatment and results, and follow-ups. Of the 25 QIs, six could be scored using the information in the Liver Cancer Registry Database (Table 1). Patients were eligible for QIs if they met the criteria described in the denominator, and they were considered to have "passed" the QI if they received the care processes stated in the numerator. The quality score was calculated for each QI as the percentage of "passed" patients among those eligible. For example, the first QI in Table 1 was scored as the percentage of the patients whose alpha-phetoprotein (AFP) and protein indiced by vitamin K absence -2 (PIVKA-2) levels were measured before treatment (numerator) among those who were diagnosed with hepatocellular carcinoma (denominator). When necessary information was unavailable (i.e. either missing or coded as "unknown" in the dataset),

Table 1 Quality indicators (QIs) applied to the liver cancer registry, quality scores, and data completeness

Denominator (target patients)	Numerator (standard care processes)	*n*	Quality score (%)	Data availability (%)	
				Denominator	Numerator
Tumor marker before initiation of therapy					
Patients who were diagnosed with hepatocellular carcinoma (HCC)	AFP and PIVKA-2 levels were measured before treatment†	16 187	82.3%	100%	94.3%
ICG check-up before surgery					
Patients who underwent surgical resection of HCC for the first time	15-min ICG retention rate was measured before treatment†	4 802	91.1%	99.2%	94.6%
Local therapy for new HCC (≤3 cm)					
HCC patients with liver damage class A, having three or less tumors of 3 cm or smaller in diameter	Surgical resection or percutaneous local ablation therapy (PEI, MCT, or RFA) was performed.	3 934	76.9%	78.3%	99.5%
Surgical therapy for new HCC (3–5 cm)					
HCC patients with liver damage class A having a solitary tumor of 3–5 cm in diameter	Surgical resection was performed.	1 029	64.4%	78.3%	99.9%
TACE indication					
HCC patients with Stage IVa or earlier, Vp 0–2 and Child–Pugh class A or B, in whom surgery and percutaneous local ablation therapy were not possible (patients who did not receive surgery or percutaneous local ablation therapy within 3 months after diagnosis)	TACE was performed.	3 741	84.5%	82.0%	99.9%
Documentation of after surgical resection					
HCC patients who received surgical resection	Medical record (including pathological report) documented the degrees of vascular invasion‡ and tumor differentiation was postoperatively determined.	4 906	81.4%	99.5%	91.9%

†Timing of the measurement was uncertain because the date of the test was not present in the registry.
Whether surgical resection was the first-line therapy was unclear because the registry did not distinguish the first-line therapy from subsequent therapies. ‡Includes invasion to the portal vein (vp), hepatic vein (vv), hepatic artery (va), and bile duct (b).
HCC, hepatocellular carcinoma; ICG, indocyanine green; MCT, microwave coagulation therapy; PEI, percutaneous ethanol injection; RFA,radiofrequency ablation; TACE, transarterial chemoembolization.

we treated the patients as follows: if QI eligibility information (applicability to the denominator) was missing, we excluded the patients from the denominator; if the information needed to determine the "pass" or "fail" status (the numerator) was unavailable, we considered that the care was not provided, and thus, the patient was counted as "fail" on the QI.

To evaluate the feasibility of applying these QIs to the liver cancer registry, we examined the completeness of the data to determine patient eligibility (the proportion of patients having all data items necessary to examine the denominator criteria [i.e. target patients]) and pass/fail (the proportion of patients having all necessary data items among all eligible patients) for QIs. Because the analysis revealed that the liver damage classification of the Liver Cancer Study Group[16] was the most frequently missing information, we further evaluated the usability of Child–Pugh classification to substitute for the liver

damage classification by examining the agreement between the two classification systems among patients with both sets of data. Because the QIs that target treatment choice focused on patients with class A liver damage, we calculated the sensitivity and specificity of the Child–Pugh class A in predicting liver damage class A. All of the statistical analyses were performed using STATA 11.1 (College Station, TX, USA). The study protocol was approved by the institutional review board of the National Cancer Center of Japan.

RESULTS

Sample characteristics

IN TOTAL, 16 187 patients were included. Table 2 presents the sample characteristics. The mean age of patients was 67 years (71.6% male). Approximately 50% of patients had liver damage of class A and 50% had solitary tumors. Similar numbers of patients underwent surgery, percutaneous local ablation, and transcatheter arterial chemoembolization (TACE).

Table 2 Sample characteristics

	n (%)
Age, mean (SD)	67 (SD = 9.4)
Male *n* (%)	11 592 (71.6%)
Liver damage class	
A	8089 (50.0%)
B	4439 (27.4%)
C	1058 (6.5%)
Unknown/No response	2601 (16.1%)
Child–Pugh class	
A	10 585 (65.4%)
B	3444 (21.3%)
C	867 (5.4%)
Unknown/No response	1291 (8.0%)
Number of tumors	
1	8970 (55.4%)
2	2727 (16.9%)
3	1198 (7.4%)
>3	3733 (15.7%)
Unknown/No response	757 (4.9%)
Tumor diameter (cm), mean (SD)	4.1 (4.0)
Primary treatment modality	
No treatment	1238 (7.7%)
Surgical resection, transplantation	4895 (30.2%)
Percutaneous local ablation	4733 (29.2%)
TACE	4423 (27.3%)
Systemic chemotherapy	718 (4.4%)
Other treatment	110 (0.7%)
No answer	70 (0.4%)

SD, standard deviation; TACE, transarterial chemoembolization.

Table 3 Cross-tabulation of Child–Pugh and Liver damage classes

CP	LD				
	A	B	C	Unknown	Total
A	7729	1813	35	1008	10 585
B	131	2445	290	578	3 444
C	6	56	693	112	867
Unknown	223	125	40	903	1 291
Total	8089	4439	1058	2601	16 187

CP, Child–Pugh classification; LD, liver damage.

Quality scores

On average, quality indicators had 5767 patients applicable, and overall the indicated care processes were provided 83.9% of the time. Table 1 presents quality scores and data completeness for each QI. The score was lowest for the QI "Surgical therapy in patients with HCC 3–5 cm in diameter" (64.4%) and highest for the QI "Indocyanine green (ICG) checkup before surgical resection" (91.1%). Although the availability of data for denominators ranged from 78.3% to 100%, information for numerators was available for more than 90% of patients for all QIs. QIs that use liver damage classification, tumor number, and tumor size were least commonly available for the denominator (78.3%). Liver damage classification, tumor number, and tumor size were missing or unknown for 2601 (16%), 757 (4.7%), and 1134 patients (7.0%), respectively.

Distribution of liver damage and the Child–Pugh classification

Table 3 presents the analysis of the concordance between Child–Pugh and liver damage classifications. These two classification systems agreed in 82.3% of patients for whom sufficient data were available. Child–Pugh A could predict liver damage class A with 98.3% sensitivity and 65.3% specificity.

DISCUSSION

WE HAVE DEMONSTRATED that certain aspects of the quality of care for patients with liver cancer can be measured using the liver cancer registry operated by the Liver Cancer Study Group of Japan. To our

knowledge, this was the first study to measure the quality of care for HCC. Standardizing the care process is challenging given the complexity of HCC care, as a range of treatment modalities from surgical resection to percutaneous and transcatheter therapy exists. The choice of treatment is influenced not only by the cancer stage but also by the baseline liver function. The QIs in this study, developed by the consensus of clinical experts, examined the actual care provided against the standards of pretherapeutic evaluation, the collection of pertinent tumor information, and treatment choice. The quality scores were high for most of the QIs, but there was also room for improvement. Although not all of the QIs developed were used for this analysis, we believe that the identification of a focus for improvement is an important initial step.

The information available in the registry was sufficiently complete for quality measurements to be made. Although information required to determine eligibility for QIs was occasionally missing, the information required to assign each QI a "pass" or "fail" status was generally available, which indicated little ambiguity in the scoring of the eligible patients. Among the missing information, the liver damage classification was the most frequently missing, presumably due to the lack of the ICG test. Although the liver damage classification was used for the QIs that focused on treatment choice in accordance with the Japanese Clinical Practice Guidelines, alternative criteria would be necessary to review actual practices. The comparison of the Child–Pugh class and liver damage class, however, revealed that the former underestimated the liver damage. For example, the Child–Pugh class A includes patients with more severe disease and is broader than liver damage class A. This result was expected, as the prothrombin criteria threshold is lower for the Child–Pugh classification.[16] Furthermore, this is consistent with a previous report that reviewed the medical records of the HCC patients.[17] If the Child–Pugh classification is used in place of the liver damage classification for the patients whose liver damage classification data are missing, the QIs targeting patients with liver damage class A would also include a broader group of the patients with liver damage class B or C. Thus, caution should be exercised when using these liver function classifications interchangeably.

For other types of cancer, we have a predecessor on using the national database for quality measurements and feedback. In the National Cancer Database, the Commission on Cancer of the American College of Surgeons measured six QIs (three for breast cancer and three for colorectal cancer) and provided feedback regarding the scores of the individual participating facilities and the distribution of these scores among other facilities.[18] This program is now developing the Rapid Quality Reporting System, in which the facilities submit and update the information continuously and the quality of care is monitored in real time. Our study indicates that the same service is theoretically possible in Japan using the liver cancer registry.

Some limitations must be considered when interpreting the results of the current study. First, the QIs that examined the appropriate documentation of vascular invasion and tumor differentiation were scored based on the availability of data in the dataset rather than on the actual medical records. This may underestimate or overestimate the quality scores for these QIs. Underestimation occurs when physicians keep appropriate documentation but fail to enter that information into the dataset, and overestimation occurs when physicians enter the information into the dataset but fail to document it in the medical record. Accordingly, caution must be exercised while interpreting these scores. Second, quality assessment requires the consideration of exceptional cases. For example, in some cases where a QI indicated surgery, surgery may not be appropriate due to compromised cardiac or respiratory functions. As the database does not contain information on the reasons why surgery was not performed, it is possible that patients who were appropriately excluded from surgery may be labeled as having received poor quality care. Hence, the results of the measurements of quality from the database should be regarded as starting points for discussions of quality and not as the final conclusions about quality. Third, the fact that the facilities participated in the registry voluntarily must be taken into account, as they are motivated and likely to be more specialized than the average Japanese hospitals. Therefore, the quality scores from these facilities may be higher than those provided by typical hospitals in Japan. Fourth, the QIs were based on the clinical practice guidelines issued in 2005, [6] but our study was comprised of patients diagnosed in 2002 and 2003. Thus, the guidelines used may have already improved some of the aspects of care scored in this analysis, but our study has demonstrated that the Liver Cancer Registry Database can be a useful data source for analyzing quality of care. Finally, the timing of the evaluation and the start of treatment for each patient was uncertain. Although the QIs targeting pretherapeutic laboratory tests (tumor markers and ICG retention) require knowledge of whether these tests were performed before the treatment was initiated, the test dates were not available in the

registry. Thus, we assumed that the tests were performed before the start of the therapy and we therefore overestimated the quality scores.

Despite these limitations, we have demonstrated that the Liver Cancer Registry Database can be a tool for quality measurement. To date, cancer registries have primarily focused on clinical and epidemiological research, and the examination of the quality of care is a new area of research. Professional societies, however, have the responsibility to promote improved quality of patient care. Because the ultimate goal is to improve patient outcome, the role of these societies should not be limited to the discovery of new knowledge but should also include the monitoring of the extent to which the new knowledge is applied to patient care nationwide. This study serves as an initial step for the future growth of such activities.

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APPENDIX

The list of the quality indicators (QIs) approved by the expert panel

	Denominator (target patients)	Numerator (standard care processes)
Pre-treatment work-up		
1	Patients who were diagnosed with hepatocellular carcinoma (HCC)	AFP and PIVKA-2 levels were measured before treatment
2	HCC patients who underwent surgical resection, percutaneous local ablation therapy and transarterial chemoembolization (TACE) therapy	Dynamic CT/MRI study was performed before treatment
3	Patients who were diagnosed with HCC and received treatment	The medical records documented the clinical stage (TNM or TNM factors) and liver function level (the Child–Pugh class or the liver damage class)
4	Patients who underwent surgical resection of HCC for the first time	15-min ICG retention rate was measured before treatment
Treatment choice of local therapy		
5	HCC patients with liver damage class A, having three or less tumors of 3 cm or smaller in diameter	Surgical resection or percutaneous local ablation therapy (PEI, MCT, or RFA) was performed.
6	HCC patients with liver damage class A having a solitary tumor of 3–5 cm in diameter	Surgical resection was performed.
7	HCC patients with liver damage class A or B and three or fewer tumors smaller than 3 cm who had surgical resection or percutaneous local ablation therapy	The advantages and disadvantages of each therapy were explained and documented in the medical records
8	HCC patients with liver damage class C who underwent surgical resection, percutaneous local ablation therapy or TACE	The risks and benefits of the treatments received were explained and documented in the medical records
9	HCC patients receiving percutaneous ethanol injection (PEI) as the initial treatment	Medical records documented the reasons why RFA was not performed
10	HCC patients with Stage IVa or earlier, Vp 0–2 and Child–Pugh class A or B, in whom surgery and percutaneous local ablation therapy were not possible (patients who did not receive surgery or percutaneous local ablation therapy within 3 months after diagnosis)	TACE was performed.
11	Recurrent HCC patients with liver damage class A and a solitary tumor of 3–5 cm in diameter	Surgical resection was performed, or the medical record documented the reasons for not performing surgery
12	Recurrent HCC patients with liver damage class A and solitary tumor of 3 cm or smaller in diameter	Surgical resection or percutaneous local ablation therapy (PEI, MCT or RFA) is performed or the medical record documents the reasons for not performing these therapy
13	Recurrent HCC patients with liver damage class A and two or three tumors of 3 cm or smaller in diameter	Surgical resection, percutaneous local ablation therapy (PEI, MCT or RFA), or TACE was performed, or the medical record documented the reason for not performing these therapies.
14	HCC patients who received TACE	Lipiodol was used in the procedure
15	HCC patients with liver damage class C who satisfied Milan criteria	The option of liver transplantation was explained and documented
Documentation and explanation		
16	HCC patients who underwent surgical resection	Medical record (including pathological report) documented the degrees of vascular invasion and tumor differentiation was postoperatively determined.
17	HCC patients who underwent surgical resection	The medical record documented the physician's judgment on the postoperative risk of recurrence
18	HCC patients who underwent surgical resection	The pathological findings after surgery were explained to patients and were documented in the medical record

	Denominator (target patients)	Numerator (standard care processes)
Systemic therapy		
19	HCC patients who received systemic chemotherapy	Medical records documented the explanation to patients that surgical resection, percutaneous local ablation therapy or TACE could not be performed and that evidence for the efficacy of chemotherapy was lacking.
20	Patients who received treatment for HCC	Hormone therapy was avoided
Follow-up monitoring		
21	HCC patients who underwent surgical resection or percutaneous local ablation therapy	AFP and PIVKA-2 were monitored for at least 4-month intervals for 2 years after the curative treatment
22	HCC patients who received TACE	CT/MRI and tumor marker tests were performed within 2 months after TACE
23	HCC patients who received TACE	Image studies (contrast-enhanced CT/MRI, if not contraindicated) were performed at least every 3 months
24	HCC patients who received TACE	Tumor marker tests (AFP, PIVKA-2) were monitored at least every 3 months
25	HCC patients who received TACE and who showed elevated tumor marker levels, increases in the tumor size from diagnostic imaging or the appearance of new tumors with rich blood flow	TACE was repeated, or the medical record indicates the TACE was considered

AFP, Alpha-fetoprotein; CT, computed tomography; HCC, hepatocellular carcinoma; ICG, indocyanine green; MCT, microwave coagulation therapy; MRI, magnetic resonance imaging; PEI, percutaneous ethanol injection; PIVKA-2, protein induced by vitamin K absence-2; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Phenotype-dependent production of des-γ-carboxy prothrombin in hepatocellular carcinoma

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Abstract
Background Des-γ-carboxy prothrombin (DCP) is an established tumor marker for hepatocellular carcinoma (HCC), but the precise mechanism of its production remains unknown. We have recently demonstrated that cytoskeletal rearrangement during the phenotypic changes involved in epithelial mesenchymal transition (EMT) plays a crucial role in DCP production through the impairment of vitamin K uptake. However, DCP production in long-lasting severe hypoxic conditions with nutrient deprivation–such as transarterial embolization–remains unknown.
Methods We examined the effects of long-lasting hypoxia with nutrient deprivation, as well as the constitutive expression of hypoxia-inducible factor (HIF)-1α, on EMT status, DCP production, and protein synthesis in human hepatoma cell lines by enzyme-linked immunosorbent assay, immunofluorescent studies, and western blotting. Immunohistochemistry findings for DCP, anti-hepatocyte paraffin 1 (Hep Par 1), and vimentin were examined using human resected HCC samples.
Results Both severe hypoxia with nutrient deprivation and HIF-1α transfection led to the cessation of DCP production, by attenuating protein synthesis through the hypophosphorylation of mammalian target of rapamycin and its effector proteins, indicative of a further phenotypic shift involving impaired liver-specific protein synthesis. In immunohistochemistry, the distribution of DCP- and Hep Par 1-positive HCC cells was mostly similar and vimentin-positive HCC cells were frequently observed in the areas that were negative for Hep Par 1 and/or DCP.
Conclusions HCC cells produce DCP when they undergo mild phenotypic changes. However, when HCC cells adopt mesenchymal properties they lose their capacity for protein synthesis, and the production of DCP is attenuated. Building upon our previous works, it appears that DCP could be a unique tumor marker that reflects the stepwise phenotypic changes of HCC.

Keywords Tumor marker · Epithelial mesenchymal transition · O_2 conformance · Mammalian target of rapamycin pathway

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Introduction

Des-γ-carboxy prothrombin (DCP), also known as protein induced by vitamin K absence or antagonist factor-II (PIVKA-II), is an inactive form of prothrombin that is

increased in the sera of hepatocellular carcinoma (HCC) patients. Since the report by Liebman et al. [1] on DCP, this molecule has been recognized as a highly specific marker for HCC, and the majority of the case-controlled studies in this field have validated the utility of DCP for the diagnosis of HCC [2, 3]. Recent Japanese official guidelines recommend the measurement of α-fetoprotein (AFP), AFP-lectin fraction (AFP-L3), or DCP for the early detection of HCC [4]. The diagnostic accuracy of DCP and AFP-L3 was found to be superior to that of AFP [5, 6], and the serum DCP level was reported to be the most useful clinical parameter for indicating a predisposition for the development of portal venous invasion [7]. However, DCP is not used worldwide as a marker because DCP alone is not suitable for the early detection of HCC [8, 9]. Economic issues are also a factor. Monthly measurement of up to 2 different tumor markers in patients at high risk for HCC is covered by all forms of Japanese health insurance, but in most other countries such measurements are not covered by health insurance systems.

Robust tumor growth requires a local vascular network that supplies both oxygen and nutrients to tumor cells. For adaptation, hypoxic cells release angiogenic factors that stimulate neovascularization to meet their growing metabolic demands [10]. However, the neovascularization cannot generally catch up with rapidly proliferating tumor growth, and severe hypoxia leads to cell death, as indicated by the presence of central necrosis in tumors [11]. Epithelial mesenchymal transition (EMT) is critical for appropriate cell movement and organ formation in embryonic development, and this process is re-engaged in adults during wound healing, tissue regeneration, organ fibrosis, and cancer progression [11]. EMT leads to reduced cell–cell contacts and increased cell motility according to the microenvironment [12]. A key mediator of cellular responses to low oxygen is hypoxia-inducible factor (HIF)-1α, a heterodimeric transcription factor [13]. HIF-1α has been identified as a regulator of EMT in several cancer cell lines [14, 15], and over-expression of HIF-1α has been reported to induce EMT [16]. Clinically, hypoxia and HIF-1α are associated with tumor invasion, increased metastasis, and poor survival [5, 17].

During these phenotypic changes, cytoskeletal rearrangement, which is crucial for cells to leave the epithelium and allow them to migrate through the extracellular matrix, has been observed [18]. Because the cytoskeleton (filamentous actin) plays a major role in clathrin-mediated endocytosis, the uptake of vitamin K (a fat-soluble vitamin) is impaired in hepatoma cells with cytoskeletal rearrangement, resulting in DCP production during phenotypic changes induced by either chemicals or hypoxia, as we previously reported [19–21]. Importantly, our proposed mechanism of DCP production is supported by several clinical observations; such as the finding that the supplementary administration of vitamin K_2 analogues reduced serum DCP levels in HCC patients [22]. This observation could be explained by supplementary vitamin K_2-induced recovery of total vitamin K uptake into HCC cells through a functionally impaired actin-dependent or actin-independent pathway [20]. In addition, DCP-producing HCC cells represent cells that have gained migratory and/or invasive properties because of phenotypic changes [19–21]. In support of these results, several clinical studies have reported that high serum DCP in HCC patients is associated with a high risk of vascular invasion [7] and tumor recurrence [23].

However, not all cases of HCC can be explained by our proposed mechanism of DCP production. Here, we demonstrate a patient with non-alcoholic steatohepatitis (Fig. 1A), who was first diagnosed with HCC in 2001 (Fig. 1B-a) on the basis of typical ultrasonography (US), computed tomography (CT), and angiography findings. Three rounds of transarterial embolization (TAE) and radiofrequency ablation (RFA) normalized her serum DCP levels, which remained normal for 1 year. However, her serum DCP levels gradually increased because of the recurrence of HCCs (Fig. 1B-b). Each TAE lowered her serum DCP levels. After the last TAE, she rejected further treatment even though some uncontrollable HCCs still remained, and her serum DCP gradually increased thereafter. Serum AFP was normal throughout follow-up. Surprisingly, her serum DCP suddenly normalized after peaking (13,900 mA U/ml) in September 2005 (Fig. 1A). Enhanced CT in November 2005 revealed a hypovascular tumor in the caudate lobe (Fig. 1B-c). Serum DCP remained normal although the tumor continued to grow (Fig. 1B-d), and the patient ultimately died of liver failure. Postmortem pathological examination of the tumor revealed spindle-shaped cells that were strongly positive for vimentin, suggestive of sarcomatous changes from HCC. In addition, immunohistochemically, this tumor was completely negative for DCP and anti-hepatocyte paraffin 1 (Hep Par 1), whereas non-tumor tissues were clearly positive for Hep Par 1 and negative for vimentin (Fig. 1C).

Generally, prior to O_2 becoming metabolically limiting, hypoxia can initiate cellular energy conservation strategies for cell survival, thus decreasing ATP consumption [24]. When hypoxic conditions are long-lasting, energy depletion and hypoxia suppress mammalian target of rapamycin (mTOR) to reduce energy-consuming protein synthesis, allowing for cellular adaptation and survival [25, 26]. These findings and the findings in the present patient with repeated TAE led us to the hypothesis that DCP production in HCC cells might be impaired under conditions of long-lasting hypoxia, with or without nutritional deprivation, although mild hypoxia induces HCC to produce DCP [21].

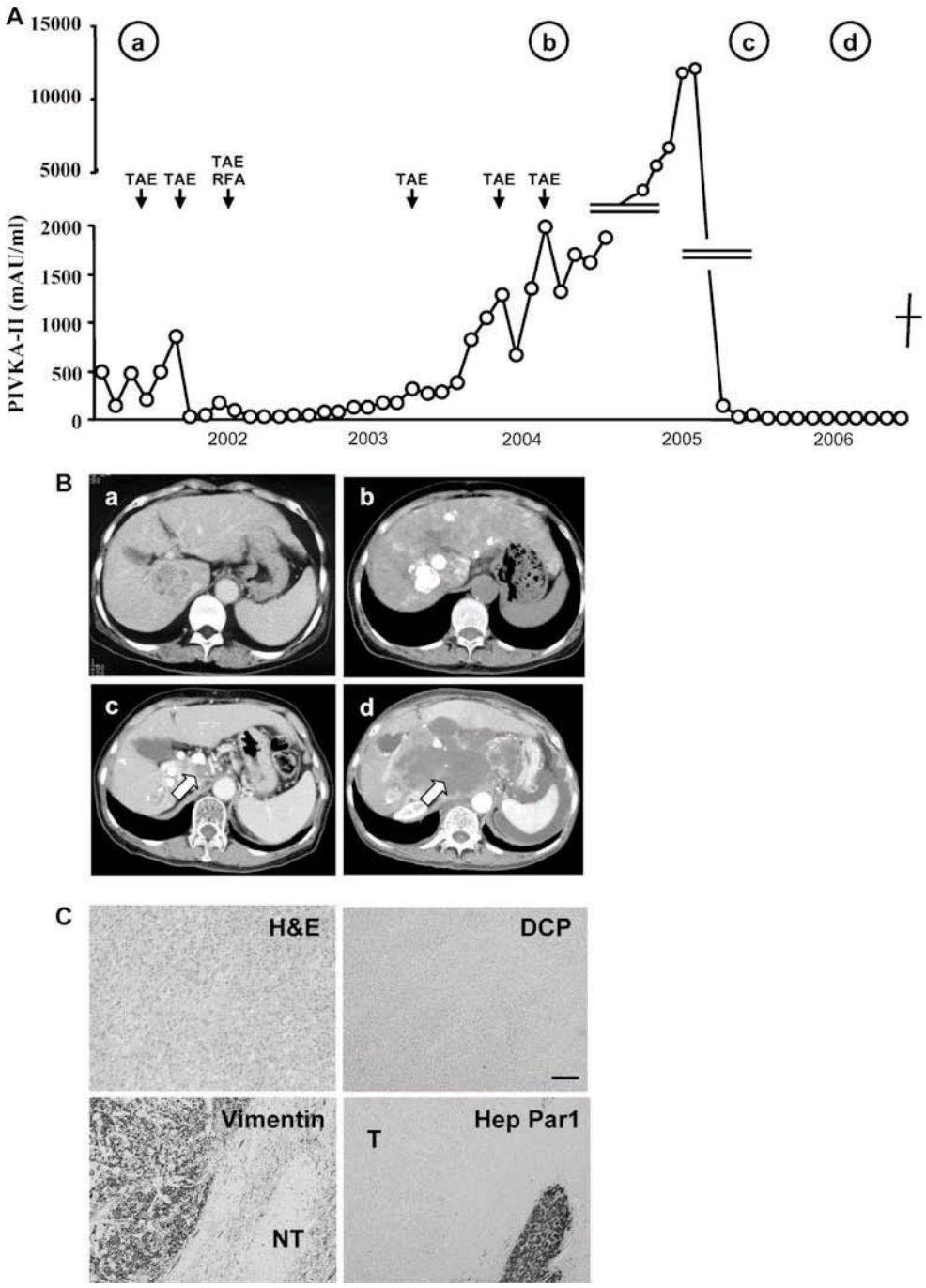

Fig. 1 Case of a hepatocellular carcinoma (HCC) patient with sudden reduction of serum des-γ-carboxy prothrombin (DCP). **a** Clinical course. *TAE* transarterial embolization, *RFA* radiofrequency ablation. *Symbols* (*a*, *b*, *c*, *d*) in the figure represent each time point of computed tomography (CT). **b** Sequential abdominal CT scans. *a* A single HCC was initially found in 2001, and TAE was successfully performed. *b* Multiple HCCs recurred. Each TAE session lowered serum DCP levels, but they never normalized. *c* Serum DCP levels were suddenly normalized after several sessions of TAE, even though poorly enhanced tumor development, beside the Lipiodol deposit, was clearly observed in the caudate lobe (*white arrows*). *d* Serum DCP levels were still within normal limits in spite of HCC progression. **c** Postmortem pathology. H&E staining showed a spindle-shaped palisade tumor with strong vimentin immunopositivity. The tumor was completely negative for DCP. The vimentin-positive tumor (*T*) was not stained with anti-hepatocyte paraffin 1 (Hep Par 1), whereas the non-tumor (*NT*) lesion without vimentin staining was clearly stained with Hep Par 1. Serial sections were used. *Bar* represents 100 μm. *PIVKA-II* vitamin K absence or antagonist factor-II

Methods

Human HCC samples

Liver samples were obtained at the autopsy of an HCC patient who had been treated at Iwaki Kyoritsu General Hospital. Surgically resected specimens were collected from 40 patients, between 2005 and 2007, at Jichi Medical University Hospital and Suzuka General Hospital, Japan. To use surgically resected samples, informed consents were obtained from the patients, and the ethics committees of all involved hospitals approved the protocol.

Immunohistochemistry

The specimens were immunohistochemically examined using an avidin–biotin complex (ABC) kit (DAKO Japan, Tokyo, Japan). Anti-vimentin (DAKO Japan), anti-PIVKA-II

(anti-DCP) (MU-3, a kind gift from Eisai Pharmaceutical, Tokyo, Japan), anti-hepatocyte paraffin 1 (Hep Par 1, DAKO Japan), and anti-phospho-mTOR (p-mTOR) (Assay Biotechnology, Sunnyvale, CA, USA) were used as primary antibodies. Sections were counterstained with hematoxylin. The existence of vimentin-positive cells was defined when more than 1% of cells showing positive staining for vimentin were observed in 1,000 HCC cells counted in the section.

Cell culture

Human hepatoma cell lines (HepG2, PLC/PRF/5, Huh7, SNU-423, HLE, and SKHep-1) were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) (10% DMEM) at 21% O_2. After growing to 80% confluence, HepG2 and PLC/PRF/5 cells were cultured in DMEM with or without FCS at 1% O_2 for indicated times. In other experiments, HepG2 and PLC/PRF/5 cells were serum-starved for 48 h in DMEM and incubated under normoxic (21% O_2, 5% CO_2) or hypoxic (1% O_2, 5% CO_2) conditions for 30 min before stimulation with 200 nM insulin (Sigma, St. Louis, MO, USA) for 60 min. Where indicated, cells were reoxygenated for 60 min. For the immunofluorescent study, cells were cultured in chamber slides (Nagle Nunc International, Rochester, NY, USA).

Western blot analysis

Cells were resolved using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. The membranes were incubated with anti-vimentin (Chemicon, Billerica, MA, USA), anti-E-cadherin (BD Transduction Laboratories, San Jose, CA, USA), or anti-human albumin (Sigma). Phosphorylated-mTOR, mTOR, phosphorylated 4E-BP1, 4E-BP1, and p70S6K antibodies were purchased from Cell Signaling (Denver, CO, USA). Phophorylated p70S6K antibody was purchased from R&D systems (Minneapolis, MN, USA). An antibody directed against β-actin (Abcam, Tokyo, Japan) was used as an internal control. The proteins were detected using an electro-chemiluminescence technique (Pierce Chemicals, Rockford, IL, USA).

Enzyme-linked immunosorbent assay for DCP

Cells were seeded at a density of 2×10^5 cells/well into 24-well plates. The next day, the medium was replaced with new 10% DMEM, and the cells were incubated at normoxic conditions for 24 h. In another experiment, after pre-incubation with 10% DMEM containing 10 nM rapamycin (Sigma) or dimethylsulfoxide (DMSO) at 21% O_2 for 48 h, the cells were cultured for another 24 h under normoxic or hypoxic conditions. The supernatant was subjected to an enzyme-linked immunosorbent assay (ELISA). The DCP concentration was determined using a PIVKA-II ELISA kit (Eisai Pharmaceuticals).

Immunofluorescent studies

After fixation with 4% paraformaldehyde, anti-E-cadherin (IBL, Fujioka, Japan), anti-vimentin (Chemicon) or anti-PIVKA-II was applied to cell monolayers. After washing, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-IgG (Sigma) or Alexa Fluor 568-conjugated anti-IgG (Invitrogen, Carlsbad, CA, USA). The cell nuclei were labeled with mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI) (Vector, Burlingame, CA, USA). Alexa Fluor 546-conjugated phalloidin (Invitrogen) was applied for 40 min to reveal filamentous actin. Images were obtained using the All-In-One Immunofluorescence Microscopy system (KEYENCE, Tokyo, Japan).

HIF-1α cDNA cloning and expression vector constructs

To obtain HIF-1α cDNA from HepG2 cells treated with 100 nM $CoCl_2$ (Sigma), the polymerase chain reaction (PCR) was performed with forward primer 5′-TATAG GTACCATGGAGGGCGCCGGCG-3′ and reverse primer 5′-GCGCGGTACCTCAGTTAACTT-3′. The full-length HIF-1α cDNA was cloned into pEGFP-C1 vector (Clontech, Mountain View, CA, USA)–which is fused with green fluorescent protein (GFP) at the N terminus–in the 5′ to 3′ direction or the reverse direction (control). The amplified fragments were confirmed by sequence analysis.

Stable transfectant of HIF-1α and control

PLC/PRF/5 cells were transfected with 2 μg of each construct. After growing for 3 weeks with 700 μg/ml of G-418 (Sigma), cells were re-plated at a lower density in 96-well plates until a single colony was formed. To obtain GFP-positive single clones, we selected cells by fluorescence activating sorting, and repeated the lower-density cloning step to ultimately isolate single clone cells, named HIF-PLC and control-PLC. HIF-PLC and control-PLC were maintained in 10% DMEM with 700 μg/ml of G-418 at 21% O_2.

Statistical analysis

Intergroup comparisons were performed using Student's *t*-test or Fisher's exact test. Values of $p < 0.05$ were considered to be statistically significant.

Results

Characterization of EMT and DCP production in human hepatoma cell lines

We examined E-cadherin and vimentin expression and albumin synthesis to determine the extent of EMT in each naïve hepatoma cell line. HepG2 cells were classified as epithelial on the basis of their expression of E-cadherin. SNU-423, HLE, and SKHep-1 cells were classified as mesenchymal on the basis of vimentin expression and a lack of E-cadherin. PLC/PRF/5 and Huh7 cells showed intermediate features (Fig. 2A). Interestingly, albumin synthesis was observed in cells with E-cadherin expression (HepG2, PLC/PRF/5, and Huh7 cells), whereas it was not observed in cells without E-cadherin expression, which had strong mesenchymal characteristics (SNU-423, HLE, and SKHep-1 cells). DCP production was also observed in the cell lines that demonstrated E-cadherin expression with albumin synthesis, whereas DCP production was not observed in cells with strong mesenchymal characteristics and which lacked albumin synthesis (Fig. 2B). These results suggest that hepatoma cells with marked phenotypic changes related to EMT lose liver-specific functions such as albumin synthesis. In addition, among the hepatoma cells with E-cadherin expression, higher DCP production was observed in cells with stronger expression of vimentin. Therefore, naïve hepatoma cells with an intermediate phenotype produced DCP, which is consistent with our previous reports [19–21].

Severe hypoxia with nutrient deprivation impairs both albumin synthesis and DCP production in hepatoma cells through the mTOR pathway

To induce a phenotypic shift, hypoxia alone was not sufficient, but too long a duration of hypoxic culture resulted in cell death. Therefore, we cultured cells under hypoxic conditions with nutrient deprivation for 48 h. Immunofluorescent labeling of DCP indicated that both HepG2 and PLC/PRF/5 cells produced DCP after exposure to hypoxic conditions (1% O_2) in 10% DMEM for 24 h (Fig. 2C), as previously reported [21]. However, DCP production was attenuated when the cells were cultured in more severe conditions (1% O_2 without FCS for 48 h) even though the cells were alive (data not shown). In addition, albumin synthesis in both HepG2 and PLC/PRF/5 cells was attenuated in a time-dependent manner (Fig. 2D).

Because long-lasting hypoxia suppresses the mTOR pathway to reduce energy-consuming protein synthesis [24], we examined the effect of such conditions on the mTOR pathway. Serum-starved HepG2 or PLC/PRF/5 cells were incubated under hypoxic or normoxic conditions for 30 min before 60-min insulin stimulation during which the indicated O_2 concentrations were maintained. Insulin stimulation led to the phosphorylation of mTOR and its effector proteins 4E-BP1 and p70S6K under normoxic conditions. After 30 min of hypoxia, insulin-stimulated phosphorylation of mTOR, 4E-BP1, and p70S6K was suppressed to below baseline levels in both cell lines, and recovered after 1 h of reoxygenation (Fig. 2E). Hypoxic conditions with nutrient deprivation were thus shown to impair liver-specific protein synthesis through the mTOR pathway. In addition, we confirmed an inhibitory effect of rapamycin on hypoxia-induced DCP production (Fig. 2F). Pre-incubation with rapamycin for 48 h was needed to block mTOR completely before incubation in normoxic or hypoxic conditions, whereas other experiments were done without pre-incubation. Therefore, the basic level of DCP production was higher under the conditions shown in Fig. 2F than under the conditions shown in Fig. 2B.

HIF-1α transfectant demonstrates EMT with attenuation of albumin synthesis and DCP production

To see the effect of hypoxia alone on phenotypic shift and protein synthesis, we developed PLC/PRF/5 cells constitutively expressing HIF-1α cDNA (HIF-PLC). A transfectant inserting reversed HIF-1α cDNA (control-PLC) was used as a control. These cells were maintained in 10% DMEM containing G-418 at 21% O_2. The HIF-PLC cells expressed HIF-1α in the nuclei, as indicated by GFP, whereas the control-PLC cells were GFP-positive in the cytoplasm but GFP-negative in the nuclei (Fig. 3A). These observations are consistent with a previous report that hypoxia results in the accumulation of HIF-1α in the nucleus [13]. Thus, HIF-PLC may mimic cells cultured in severe and long-lasting hypoxic conditions. Linear E-cadherin expression along cell membranes was clearly observed in the control-PLC, but was markedly reduced in the HIF-PLC (Fig. 3B). Fibrous vimentin expression was stronger in the HIF-PLC than in the control-PLC (Fig. 3C). These observations were confirmed by western blotting (Fig. 3D). Taken together, these findings indicate that HIF-PLC cells are similar to the phenotype of PLC/PRF/5 cells undergoing EMT. Interestingly, DCP production was significantly impaired in the HIF-PLC compared with that in the control-PLC ($p < 0.0001$) (Fig. 3E). Filamentous actin was similarly disrupted in both the HIF-PLC and the control-PLC cells (Fig. 3F), whereas fine fibrous actin was clearly

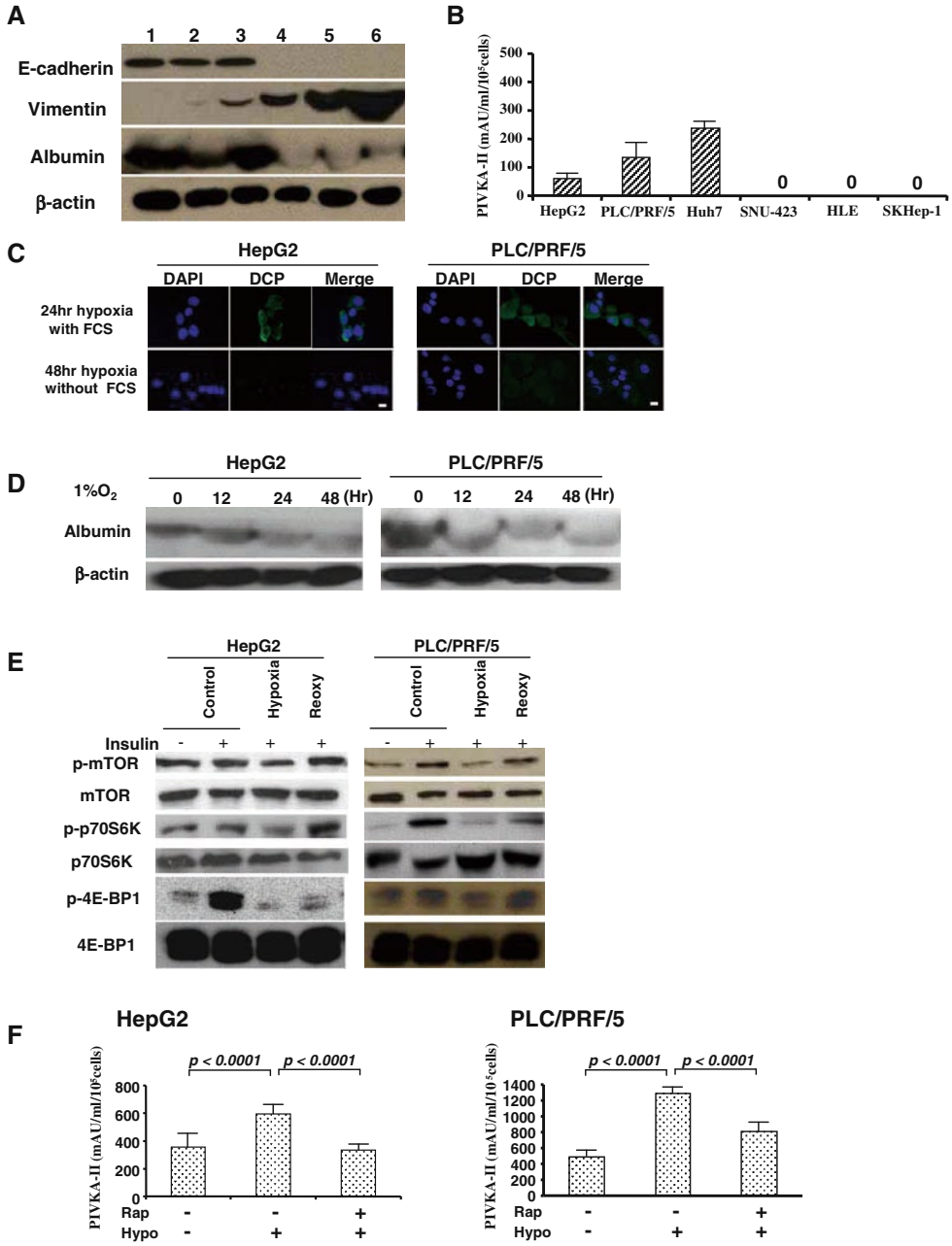

Fig. 2 Epithelial mesenchymal transition status and albumin and DCP production in naïve hepatoma cell lines. **a** Albumin production was observed in the cells with E-cadherin expression, whereas it was severely impaired in cells with strong vimentin expression (*1* HepG2, *2* PLC/PRF/5, *3* Huh7, *4* SNU-423, *5* HLE, *6* SKHep-1). A representative western blot is shown. **b** DCP production was observed only in naïve HepG2, PLC/PRF/5, and Huh7 cells. **c** Serum-starved HepG2 and PLC/PRF/5 cells stopped producing DCP when cultured in hypoxic conditions (1% O_2) for 48 h, whereas a 24-h hypoxic culture with standard medium induced DCP production (*green*). Nuclei are stained by DAPI (*blue*). The *bars* represent 20 μm. **d** Albumin production was impaired in a time-dependent manner in cells with hypoxia with nutrient deprivation. **e** Serum-starved HepG2 and PLC/PRF/5 cells were incubated at normoxia (21% O_2) or hypoxia (1% O_2) for 30 min before stimulation with 200 nM insulin for 1 h and reoxygenation for 1 h as indicated. **f** Rapamycin (*Rap*) attenuated hypoxia-induced DCP production. After pre-incubation with 10 nM rapamycin or dimethylsulfoxide (DMSO) at 21% O_2 for 48 h, the cells were incubated in each medium at 1% (*Hypo*) or 21% O_2 for another 24 h. Representative results of at least 3 independent experiments are shown. *FCS* fetal calf serum, *p-mTOR* phospho-mammalian target of rapamycin, *Reox*y reoxygenation

observed in naïve PLC/PRF/5 cells. Additionally, albumin production was severely impaired in the HIF-PLC cells in comparison with the control-PLC (Fig. 3D). Phosphorylation of mTOR, 4E-BP1, and p70S6K was also impaired in HIF-PLC compared with control-PLC (Fig. 3G).

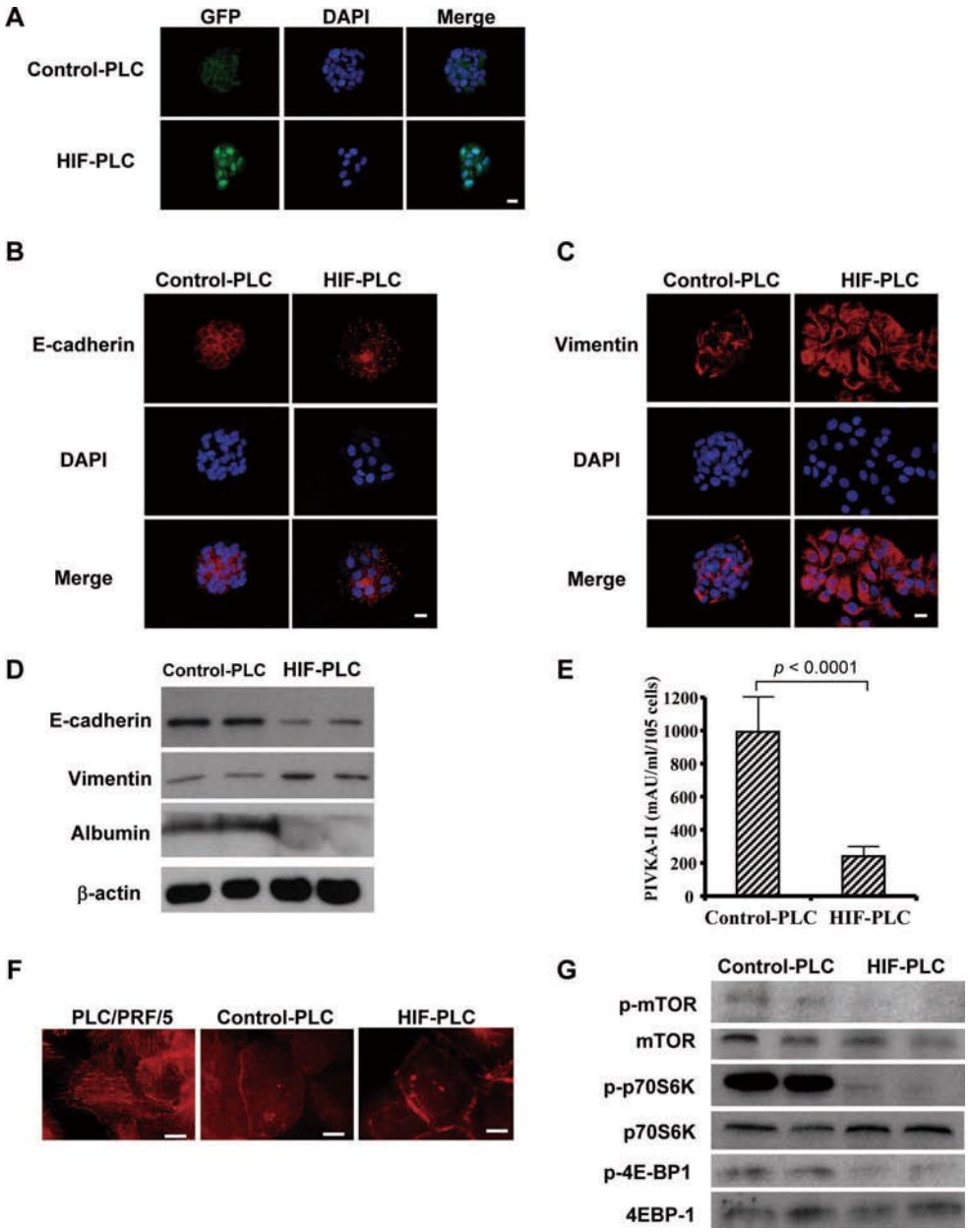

Fig. 3 Stable transfectants of hypoxia-inducible factor (*HIF*)-1α. **a** Nuclear localization of green fluorescent protein (*GFP*) was observed in HIF-PLC cells, whereas no nuclear GFP was observed in the control-PLC. In the HIF-PLC, E-cadherin expression (*red*) was down-regulated (**b**) and vimentin expression (*red*) was up-regulated (**c**), in comparison with the control-PLC. *Bars* represent 10 μm. **d** Western blotting showed E-cadherin down-regulation and vimentin up-regulation in the HIF-PLC, which confirmed the immunofluorescent studies. **e** DCP production was significantly impaired in the HIF-PLC compared to the control-PLC ($p < 0.0001$). **f** Filamentous actin in stable transfectants and naïve PLC/PRF/5 cells. Actin rearrangement was observed in both the control-PLC and the HIF-PLC, whereas fine actin filaments were clearly observed in naïve PLC/PRF/5 cells. *Bars* represent 10 μm. **g** Representative western blotting for total or phosphorylated mTOR, 4E-BP1, and p70S6K is shown. In the HIF-PLC, phosphorylation of these proteins was attenuated in comparison with the control-PLC

DCP and liver-specific protein (Hep Par 1) production is impaired in human HCC cells with vimentin expression

To confirm the association of mesenchymal changes with hepatocyte-specific protein synthesis and DCP production in HCC cells, we immunohistochemically examined vimentin, Hep Par 1, and DCP staining in serial sections of surgically resected human HCC samples. A total of 40 patients (29 males and 11 females) with surgically resected HCC were included in this study. The median age was 66 ± 11 years at the time of resection. The causes of chronic liver diseases were hepatitis B infection ($n = 8$), hepatitis C infection ($n = 23$), and cryptogenic cirrhosis ($n = 9$). No vimentin-positive HCC cells were observed in the sections of 18 cases. In the other 22 cases with vimentin-positive HCC cells, the percentages of vimentin-positive cells and the degrees of staining intensity varied, with uneven distribution. In well-differentiated HCCs, vimentin-positive HCC cells were rare (6.7%), whereas they were frequently observed in moderately and poorly differentiated HCCs (76.5 and 100%, respectively) (Fig. 4A). Vascular invasion was significantly more prevalent in patients that had vimentin-positive HCCs in comparison to those that had vimentin-negative HCCs (Fig. 4B, 54.5 vs. 16.7%, $p = 0.022$). The distribution of Hep Par 1 and DCP staining was uneven and mostly showed similar patterns (Fig. 4C, D). Interestingly, Hep

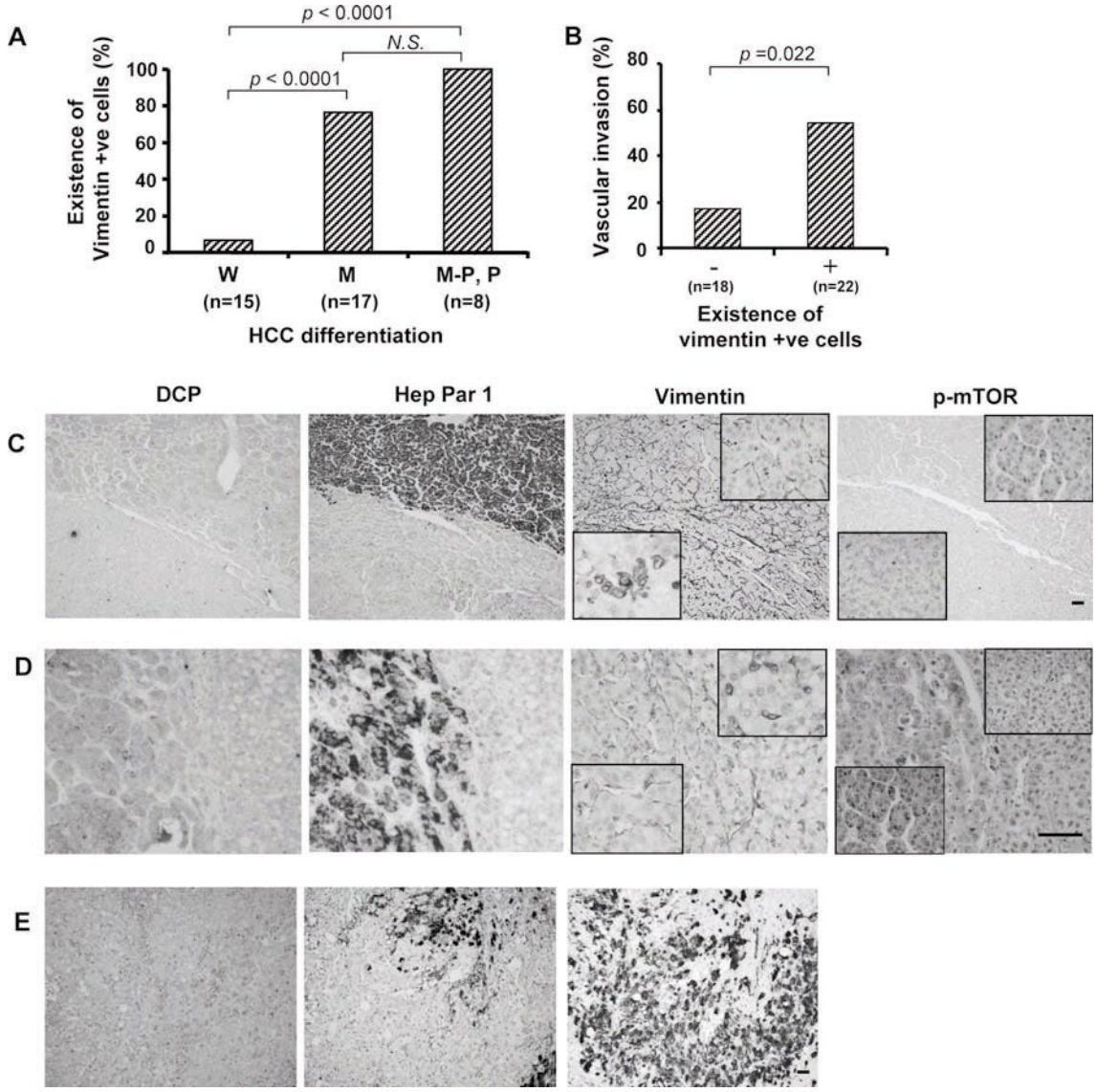

Fig. 4 Immunohistochemistry of vimentin and Hep Par 1 in surgically resected HCCs. **a** Vimentin immunopositivity was mainly observed in moderately or poorly differentiated HCCs. *W* well-, *M* moderately-, *M–P* moderately to poorly differentiated-, *P* poorly differentiated HCC. **b** A high rate of vascular invasion was observed in tumors having vimentin-positive HCC cells. **c–e** Typical staining of Hep Par 1, DCP, vimentin, and p-mTOR. HCC cells with strong vimentin immunoreactivity were negative or weakly immunoreactive for Hep Par 1, p-mTOR, and/or DCP. The distribution patterns of Hep Par 1, p-mTOR, and DCP staining were mostly similar. *Insets in* **c** and **d** show magnified images. Serial sections were used. *Bars* represent 50 μm

Par 1- and/or DCP-positive HCC cells were not observed around areas of strongly vimentin-positive HCCs (Fig. 4C–E). As indicated by the insets showing magnified images in Fig. 4 C, D, vimentin-positive HCC cells were frequently observed in Hep Par 1-negative areas, whereas vimentin-positive cells were mainly sinusoidal cells, not HCC cells, in Hep Par 1-positive areas. In addition, p-mTOR staining was strongly positive in Hep Par 1-positive areas, but negative or weakly positive in Hep Par 1-negative areas (Fig. 4C, D).

In some cases, Hep Par 1- and vimentin-positivity showed reciprocal images (Fig. 4E). These results were consistent with the case depicted in Fig. 1 and support our in vitro experimental results. DCP production was not always observed in Hep Par 1-positive cells (Fig. 4E). These HCC cells might not change their phenotypes.

Discussion

Many animals possess the ability to alter their metabolism in responses to changes in O_2 [27]. In particular, mTOR, a highly conserved serine/threonine kinase, and associated proteins integrate multiple environmental cues to regulate

translation in response to nutrient availability and stress [24, 25]. Hypoxia rapidly and reversibly triggers the hypophosphorylation of mTOR and its effectors, although the detailed mechanism through which hypoxia regulates the mTOR pathway remains unclear. We demonstrated that HepG2 and PLC/PRF/5 cells under conditions of long-lasting hypoxia (48 h) with nutrient deprivation (conditions which mimic rapid tumor growth without vascular compensation, or TAE) stopped producing both albumin and DCP. Hypophosphorylation of the mTOR pathway was observed after short hypoxic stimulation and phosphorylation recovered with reoxygenation, as seen in previous studies [24, 28]. In addition, hypoxia-induced DCP production was inhibited by rapamycin (Fig. 2F), supporting the results of western blotting. However, we cannot rule out the possibility that rapamycin inhibited EMT, resulting in the attenuation of DCP production, because mTOR may regulate EMT [29].

To confirm whether conditions of long-lasting hypoxia affect phenotypic shift or protein synthesis, we developed the stable HIF-1α transfectant (HIF-PLC), which showed further phenotypic changes, compared with the control-PLC cells and naïve PLC/PRF/5 cells. Filamentous actin was similarly disrupted in both the HIF-PLC and the control-PLC cells, perhaps because of G-418 maintenance (Fig. 3F). Therefore, these cells might be able to produce DCP, in terms of cytoskeletal rearrangement. However, protein synthesis through the mTOR pathway was impaired in the HIF-PLC cells. Constitutive HIF-1α expression, and therefore long-lasting severe hypoxia, leads to further phenotypic shift and attenuation of protein synthesis by means of regulatory mechanisms implicating the mTOR pathway, followed by decreased DCP production, which is consistent with the in vitro experimental results illustrated in Fig. 2.

The present patient with sudden reduction of DCP (Fig. 1) initially had DCP-producing HCCs. However, postmortem immunohistochemistry showed negative staining for both DCP and Hep Par 1. Hep Par 1 has been identified as carbamoyl phosphate synthetase 1 (CPS1), a relatively liver-specific, intra-mitochondrial rate-limiting enzyme in the urea cycle [30]. Immunoreactivity for Hep Par 1 has been reported to be absent in poorly differentiated HCC [31]. CPS1 could be a surrogate marker that reflects the function of normal hepatocytes or cancerous "well-differentiated" hepatocytes [30], and a lack of Hep Par 1 immunoreactivity may be indicative of a loss of normal hepatocyte protein synthesis. Therefore, after several sessions of TAE in the present patient, the HCC cells possibly changed their phenotype to one with mesenchymal properties that could not produce liver-specific proteins, resulting in the attenuation of DCP production. The reciprocal images of Hep Par 1- and vimentin-positive HCC cells in the immunohistochemistry of human HCCs may support this idea (Fig. 4C–E). In addition, p-mTOR staining was strongly positive in the Hep Par 1-positive area, but negative or weakly positive in Hep Par 1-negative and vimentin-positive areas (Fig. 4C, D), and these findings were consistent with our in vitro experiments showing that mesenchymal changes attenuated protein synthesis through the mTOR pathway. As markers of liver-specific protein synthesis, we used albumin for our in vitro experiments and Hep Par 1 for the in vivo experiments, because HepG2 cells do not express CPS1 [30]. We note that immunohistochemical staining for albumin may not have been clearly indicative of liver-specific protein synthesis because albumin is abundant in the serum and several other tissues in vivo.

For the early detection of HCC, US surveillance is superior to tumor markers [32], and DCP or AFP alone is not optimal for the detection of early HCCs [8, 9]. According to our proposed mechanism of DCP production by HCC cells, this evidence is understandable because small HCCs are generally at low risk of hypoxia or malnutrition; therefore, they are at low risk of phenotypic shift. High serum DCP in HCC patients may indicate the presence of HCC cells with these phenotypic changes and, therefore, an increased risk of vascular invasion or metastases, and such findings are consistent with several clinical studies [7, 23, 33]. Further, serum DCP elevation was observed in HCC patients with a favorable response to treatment with a multikinase inhibitor, sorafenib, and this DCP elevation is explained by hypoxic exposure of HCC cells due to the antiangiogenic effect of sorafenib [34]. Thus, most clinical evidence of DCP elevation can be explained by our proposed mechanism of DCP production. However, some contradictions still remain. HCCs do not always produce DCP when they show phenotypic changes. This issue needs to be addressed in the future.

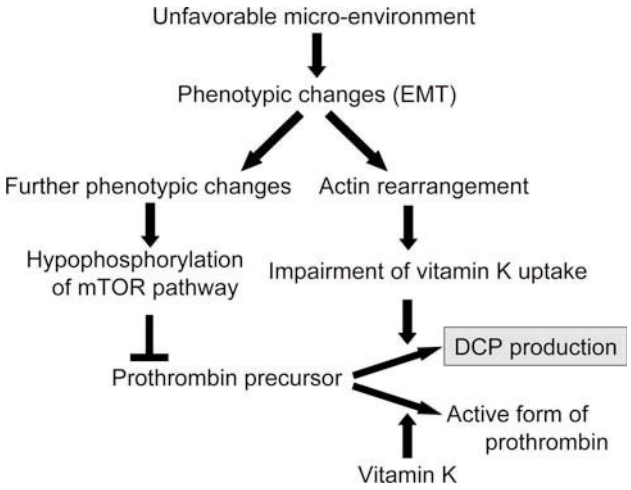

Fig. 5 Model proposing a mechanism of DCP production and reduction in HCC. *EMT* epithelial mesenchymal transition

In conclusion, HCC cells do not initially produce DCP when they synthesize liver-specific proteins and have sufficient vitamin K uptake. However, HCC cells produce DCP when vitamin K uptake is impaired by the cytoskeletal rearrangement that occurs during the phenotypic changes associated with EMT, a process which is induced by strong selective pressure under environmental stimuli that are unfavorable for cell survival [19–21]. More severe conditions induce HCC cells to undergo further phenotypic changes to adopt mesenchymal properties, leading to a reduction of liver-specific protein synthesis, including DCP, through hypophosphorylation of the mTOR pathway (Fig. 5). From this perspective, DCP is not merely an HCC tumor marker, but it is also a marker of HCC phenotypic status. In addition, DCP might be a unique tumor marker that will lead to an understanding of the ecology of HCC progression.

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Conflict of interest The authors declare that they have no conflict of interest.

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RESEARCH COMMUNICATION

Needs for Hepatocellular Carcinoma Control Policy in the Asia-Pacific Region

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Abstract

Background: Hepatocellular carcinoma (HCC) is particularly burdensome in the Asia-Pacific region, however, cross-country comparisons have been limited to somewhat unreliable epidemiological measures. We conducted a comparative needs assessment for HCC control policy to inform HCC control efforts in the Asia-Pacific region. The aims were to identify regional needs, to compare overall competence across the region, and to identify which needs were concordant across the region. Method: Using the self-explicated method, a stated-preference approach, clinical experts from Australia, China, Japan, Korea, Taiwan, and the United States valued ten previously identified dimensions of HCC control: clinical education; risk assessment; HBV strategy; HCV strategy; life-style risk factors; national statistics; funding for screening; funding for treatment; political awareness; and public awareness. Results were normalized and analyzed using Z-scores and ANOVA, with concordance of need across the region tested via the F-test. Results: Seventy-two respondents, equally drawn from the study sites, completed the survey (response rate: 36%). Respondents were hepatologists (39%), oncologists (21%), radiologists (17%), surgeons (17%), and other specialists (7%) who were involved in liver cancer control at local/regional (35%) national (44%) or international (21%) levels. In aggregate, the most significant needs were political awareness, public awareness, and life-style risk factors (all $p<0.001$). Significant differences in aggregate competence were observed across the region ($p<0.001$), with better than expected competence reported by respondents from Taiwan ($p<0.001$), Japan ($p=0.006$), and Korea (0.041), and close to expected competence reported by respondents from Australia, China, and USA (all $p>0.05$). There were differences in the extent of needs across the region ($p<0.05$) on all dimensions except funding for screening, clinical education and life style risk factors. Conclusions: As the first comparative needs assessment for HCC for the Asia-Pacific region, our results can inform national and cross-national priorities for intervention and facilitate the identification of best practices. Regional efforts to control HCC should adopt as objectives the needs for greater political and public awareness and improved management of lifestyle risk factors because these are the most significant needs, and are shared concerns across the region.

Keywords: Hepatocellular carcinoma (HCC) - liver cancer - needs assessment - public policy - economic evaluation

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Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of death from cancer (Ferlay et al., 2010). The Western Pacific region has the highest incidence and mortality rates from HCC in the world, at around 38 times higher than the Eastern Mediterranean, which is the region with the lowest reported rates (Ferlay et al., 2010). The wide variation in incidence, even among lower-income countries, suggests that the Asia-Pacific region has the potential to reduce the burden of disease significantly with improved policies aimed at controlling HCC.

Given the burden of disease from HCC in the Asia Pacific region, the Western Pacific Region Office (WPRO) of the World Health Organization (WHO) could take a leading role in HCC control efforts across the region. In its Manual for the Prevention and Control of Common Cancers (WHO, 1998) WPRO has made recommendations for liver cancer treatment and primary and secondary prevention through hepatitis B virus (HBV) immunization and screening of HBV carriers. Some individual jurisdictions have made progress in reducing the risk

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of HCC through HBV vaccination and other programs (Chang et al., 1997; Yoo, 2010; Zhou et al., 2009).

Although HBV is an important risk factor in the region, lifestyle risk factors such as alcohol, smoking, obesity, and diabetes are becoming increasingly important in the etiology of HCC, particularly with global increases in the prevalence of obesity (Nordenstedt et al., 2010). Hepatitis C virus (HCV) is also becoming increasingly important (Chen, 2007). The Asia-Pacific Working Party on Prevention of Hepatocellular Carcinoma recognized the need for broader efforts as well as interventions on HBV in its Consensus Statements for Asia-Pacific Countries, which were based on reviews of HCC epidemiology, HBV, HCV, other liver disease and surveillance (Farrell et al., 2010).

The Asia-Pacific Working Party's broader view of HCC prevention represents a significant advance, but its recommendations should be supplemented with information on current capabilities. Tools have been developed and used in the region for assessing needs and improving outcomes for individual cancer patients (Chen et al., 2009; Shim et al., 2010; Lee et al., 2010; Schlairet et al., 2010;.Li et al., 2011; Akechi et al., 2011). In contrast, no systematic analysis of needs for HCC control at a policy level is available to indicate what improvements are needed by individual countries, or whether needs are sufficiently similar across the region to warrant cross-national efforts.

The analysis of the gap between "what is" and "what should be" by identifying the level of existing competencies has been an influential model for educational needs assessment in continuing medical education over the last 30 years (Fox, 2011). In contrast to much of economics which favors positive approaches, this model is normative. A normative approach may be considered appropriate in the case of needs assessment because need is a subjective concept (Blaug, 1998), or at least a concept that is subject to imprecise measurement and conflicting definitions (Williams & Doessel, 2011). In the present study, we assess needs based on a subjective valuation of competence, an approach similar to that in the medical education needs assessment literature (Fox, 2011). Specifically, identified needs by assessing liver cancer clinicians' views of current competencies across ten dimensions that are important in any public policy effort to control HCC. The aims were to identify regional needs, to compare overall competence across the region, and to identify which needs are concordant across the region.

Materials and Methods

Sample selection

Clinical experts in liver cancer from Australia, China, Japan, Korea, Taiwan, and the United States of America (USA) were selected using a two-stage purposive quota sampling process. Initial selection was based on peer reviewed publications, presentations at major liver disease conferences, leadership roles in national societies/centers, government agencies or recommendations from HCC-related research and other advisory groups. In the second stage of selection, respondents were included if they were oncologists, surgeons, radiologists, other HCC and hepatobiliary specialists, hepatologists, pathologists, and other specialists who may be involved in HCC prevention, diagnosis, treatment, and care, or leaders of major medical institutions (including cancer and other liver disease centers). Respondents were excluded if they were not board certified, had been certified for less than one year, had practiced medicine for less than three years, or had lived or practiced in that jurisdiction for less than three years.

Questionnaire

Each participant ranked his or her country's competence on ten dimensions of HCC control. For example, respondents assigned 1 to the dimension on which their country was most competent, 2 for the next-ranked dimension and so on until 10 was assigned to the dimension on which the country was least competent. The dimensions were selected based on interviews with liver cancer clinicians involved in policy from eleven countries (Bridges et al., 2011a). For each dimension, participants then rated their country's competence using a five point Likert scale (1= poor, 5=excellent). Table 1 shows the dimensions as presented in the choice task along with abbreviated labels and more detailed descriptions of each dimension. Demographic data also were collected.

Data collection

A request for participation was mailed or emailed to potential respondents in English and the local language, if appropriate. Potential respondents who did not respond within two weeks were contacted again by telephone or email. Up to four reminders were sent before a potential respondent was coded as "no response". The survey was administered as a supervised one-to-one survey by telephone or in person between October 2010 and April 2011.

Data analysis

The study used the self-explicated method to conduct needs assessment. The self-explicated method is a relatively simple stated-preference method that allows assessment of both cardinal and ordinal aspects of respondents' preferences and is particularly appropriate for assessing many attributes (Green & Srinivasan, 1990). It was first used in marketing, but recent applications have demonstrated its potential for use in health research (Pavlova et al., 2003; Fraenkel et al., 2010; Bridges et al., 2011b).

For each respondent, ranking (R) and Likert rating (L) values for each dimension were multiplied to give a score between 1 and 50, where lower scores indicated poorer competence and greater needs. The product of the midpoint of the two scales, 16.5, was used as the expected mean score or benchmark. An observed mean score of 16.5 would suggest that respondents answered the ranking and rating scales at random, that they were indifferent, or that they considered competence to be average.

For each dimension, the deviations between observed and expected mean scores were normalized to create z-scores according to:

$$zj = Rj \times Lj - 16.5 / se(Rj,Lj)$$

where *Rj* is the mean ranking for site j, *Lj* is the mean rating for site j, and *se(Rj,Lj)* is the standard error of the product of the mean ranking and rating for site j.

P-values were calculated to assess whether observed mean scores were significantly different from the expected mean. To test for concordance of needs across the region analyses of variance (ANOVAs) and F-tests were conducted. Fisher's exact test was used to test for significant differences in respondent characteristics across the region. Data were analyzed using STATA 11.0 for Windows (StataCorp LP, College Station, TX).

Ethics

All participants were informed about the study and the potential risks and benefits of participation. Respondents participated voluntarily and were not reimbursed. The Johns Hopkins University, Bloomberg School of Public Health Institutional Review Board decided the study did not require human subjects consideration. Local experts were consulted to ensure compliance with any local ethics requirements.

Results

Seventy-two respondents, twelve from each site, completed the survey (response rate 36%). Recruitment results are shown in Figure 1. Respondents identified their main areas of involvement as HCC (67%), hepatitis (22%), transplantation (7%), and metastatic liver cancer (cancer of other organs that has metastasized to the liver) (4%). Thirty-nine percent identified as hepatologists, 21% as oncologists, 17% as radiologists, 17% as surgeons and the other 7% included immunologists, pathologists and researchers. Respondents identified as being involved in liver cancer control at a local/municipality (14%), regional/provincial (21%), national (44%), or international (21%) level. Respondent characteristics by site are shown in Table 1. F-tests for heterogeneity found no significant difference between respondents based on their main area of involvement (p=0.639), specialty (p=0.751), or level of involvement (p=0.502).

Aggregate competence across sites and dimensions was better than the expected benchmark (p<0.001). As shown in Figure 2, average competence across the six sites was significantly below the benchmark for political

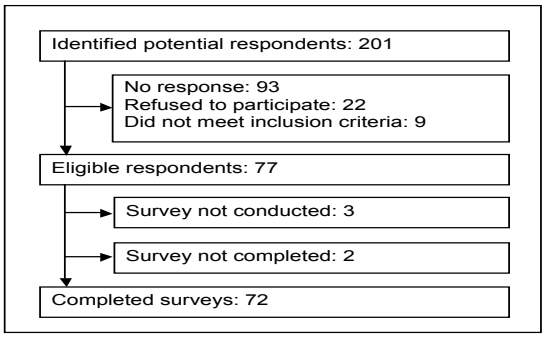

Figure 1. Recruitment Results

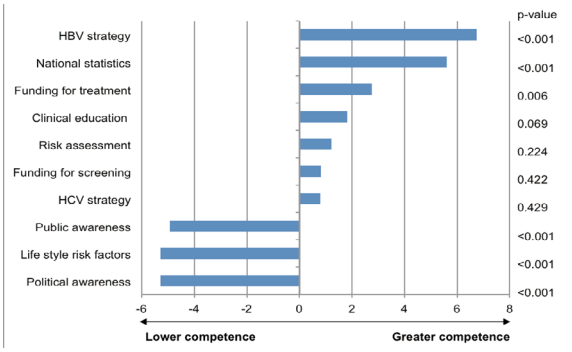

Figure 2. Aggregate Needs for 10 Dimensions of HCC Control

Table 1. Definition of the Dimensions of Liver Cancer Control

Label	Wording in choice task	Definition of dimension
Risk assessment	Early risk assessment	Appropriate assessment and risk stratification in primary care facilitating appropriate management, surveillance or referral to liver cancer specialists
Funding for screening	Funding/reimbursement of screening/detection	Adequate funding and infrastructure to promote and implement appropriate population screening and surveillance for prevention and early diagnosis
Political awareness	Political awareness and action	Heightened awareness, advocacy and political leadership to promote the necessary prevention, early detection, treatment and care for liver cancer
HCV strategy	Comprehensive HCV strategy	Appropriate strategies for prevention, screening, treatment and surveillance of patients either with or at risk of contracting hepatitis C
Public awareness	Public awareness and advocacy	Adequate funding, infrastructure, staffing and leadership to promote necessary public awareness and advocacy programs for liver cancer
Clinical education	Broad clinical education and awareness	Education for general practitioners and hepatologists on the importance of screening and early diagnosis and of the benefits of treating liver cancer
Funding for treatment	Funding/reimbursement of treatment	Appropriate payment for recommended treatments without barriers to access such as unaffordable copayments or delays in funding approval
Life style risk factors	Assessment and management of lifestyle risk factors	Effective programs for at-risk populations to prevent or manage lifestyle risk factors (including alcohol, obesity, diabetes, IV drug use and tobacco)
HBV strategy	A comprehensive HBV strategy	Appropriate strategies for vaccination, prevention, screening, treatment and surveillance for patients either with or at risk of contracting hepatitis B
National statistics	National statistics on liver disease and liver cancer	National programs to collect and maintain data on the incidence, prevalence and outcomes of patients with or at risk of liver cancer, including registries

Table 2. Respondent Characteristics

Characteristic	China	Korea	Japan	Taiwan	USA	Australia	Total	p-value
Main area of involvement								
Hepatitis %	8.3	16.7	8.3	25.0	25.0	50.0	22.2	0.099
HCC %	75.0	83.3	75.0	66.7	66.7	33.3	66.7	
Metastatic liver cancer %	16.7	0.0	0.0	0.0	8.3	0.0	4.2	
Transplantation %	0.0	0.0	16.7	8.3	0.0	16.7	6.9	
Involvement in liver cancer control								
Local/municipality %	8.3	0.0	16.7	25.0	8.3	25.0	13.9	<0.001
Regional/provincial %	0.0	66.7	0.0	8.3	0.0	50.0	20.8	
National %	66.7	25.0	66.7	41.7	41.7	25.0	44.4	
International %	25.0	8.3	16.7	25.0	50.0	0.0	20.8	
Major area of focus								
Hepatologist %	16.7	33.3	50.0	25.0	33.3	75.0	38.9	0.033
Oncologist %	66.7	16.7	8.3	25.0	8.3	0.0	20.8	
Radiologist %	0.0	16.7	16.7	16.7	33.3	16.7	16.7	
Surgeon %	8.3	25.0	25.0	25.0	16.7	0.0	16.7	
Other %	8.3	8.3	0.0	8.3	8.3	8.3	6.9	

Table 3. Deviations from Benchmark by Site and Dimension

Dimension	Normalized deviations from expected mean (z-scores)						Concordance
	Australia	China	Japan	Korea	Taiwan	USA	(p-values)
Risk assessment	-0.94	-0.24	3.45**	1.25	1.47	-6.45***	<0.001
Funding for screening	1.47	-0.48	0.59	0.50	-1.08	0.24	0.468
Political awareness	-12.1***	-0.18	-6.38***	-0.25	-0.94	-10.2***	0.002
HCV strategy	2.10*	-0.82	3.49***	-3.27***	1.67	-1.55	<0.001
Public awareness	-8.63***	-1.87	-5.41***	0.71	0.05	-7.35***	<0.001
Clinical education	-0.08	1.47	1.43	1.12	0.31	-0.30	0.483
Funding for treatment	1.37	-0.72	-0.13	2.40*	0.03	3.84***	0.001
Life style risk factors	-2.69**	-1.90	-4.46***	-3.40***	-0.45	-1.93	0.536
HBV strategy	-0.40	6.24***	2.29*	7.27***	8.53***	0.19	<0.001
National statistics	2.72**	-1.05	7.57***	-0.03	3.53***	3.49***	<0.001
N	12	12	12	12	12	12	

*(p<0.05); **(p<0.01); ***(p<0.001).

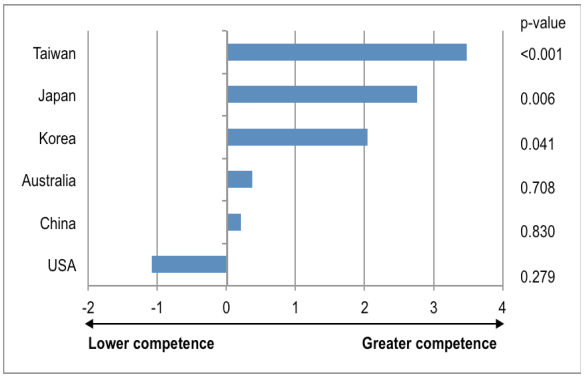

Figure 3. Aggregate Needs for Six Sites

awareness, public awareness, and lifestyle risk factors (all p<0.001). Aggregate competence was significantly above the benchmark for HBV strategy (p<0.001), national statistics (p<0.001) and funding for treatment (p=0.006).

Aggregate results by site are shown in Figure 3 and results by site and dimension are shown in Table 2. Taiwan had the highest aggregate score (p<0.001), the highest score on HBV strategy and the second highest for national statistics (both p<0.001). The second highest aggregate score was for Japan (p=0.006), which had the highest score for HCV strategy and national statistics (both p<0.001), positive scores also for risk assessment (p<0.001) and HBV strategy (p=0.022) but the lowest score for lifestyle risk factors (p<0.001) and scores significantly below the benchmark for political and public awareness (both p<0.001). Korea's aggregate score was also above the benchmark (p=0.041) with positive results for HBV strategy (p<0.001) and funding for treatment (p=0.016) but the lowest score for HCV strategy (p=0.001) and the second lowest score for lifestyle risk factors (p<0.001).

The other three sites had aggregate scores that did not differ from the benchmark average. Australia had positive scores for national statistics (p=0.006) and HCV strategy (p=0.036), but the lowest score of all sites for political and public awareness (both p<0.001) and negative results also for lifestyle risk factors (p=0.007). China scored above the benchmark for HBV strategy (p<0.001) and did not deviate from the benchmark for other dimensions. USA had the lowest aggregate score, although it did not deviate significantly from the benchmark (p=0.279), and the lowest score for risk assessment (p<0.001). It also had negative results for public and political awareness (both p<0.001) but had a positive score for national statistics (p<0.001) and the highest score for funding for treatment (p<0.001).

The last column in Table 2 shows the results of F-tests for concordance, with p-values below 0.05 indicating significant discordance. Overall there was significant discordance across sites (p=0.003), with Japan (p=0.006), Korea (p=0.041), and Taiwan (p<0.001) scoring significantly above the benchmark and the other sites having no significant deviation from the mean.

Concordance across sites was observed only for funding for screening (p=0.468), clinical education (p=0.483) and lifestyle risk factors (0.536). No site had results significantly above or below the benchmark for clinical education or funding for screening, but for lifestyle risk factors, Australia (p=0.007), Japan (p<0.001) and Korea (p<0.001) all had significant negative scores.

Discordance across sites in risk assessment (p<0.001) reflects extremes from significantly above the benchmark in Japan to significantly below in USA (both p<0.001) while other sites did not deviate from the benchmark. The discordance for HCV strategy also reflected a range from significant positive scores in Japan (p<0.001) and Australia (p=0.036) to a significant negative score in Korea (p=0.001). For political awareness and public awareness, the discordances (p=0.002 and p<0.001, respectively) resulted from Australia, Japan, and USA (all p<0.001) having large negative scores while the other sites did not deviate from the benchmark. Discordance on funding for treatment (p=0.001) reflected significant positive scores from Korea (p=0.016) and USA (p<0.001). For HBV strategy there was discordance (p<0.001) due to four sites, China (p<0.001), Japan (p=0.022), Korea (p<0.001), and Taiwan (p<0.001) having significant positive scores. The same pattern was observed for discordance on national statistics (p<0.001), but the sites with significant positive scores for this indicator were Australia (p=0.006), Japan (p<0.001), Taiwan (p<0.001), and USA (p<0.001).

Discussion

Based on ratings and rankings by clinical experts from each of six sites, this study found competence was poorer than expected, and therefore needs existed for improvements in lifestyle risk factor management and political and public awareness. In contrast, competence was better than expected on funding for treatment, HBV strategy, and national statistics, suggesting less need for improvement on these dimensions. The study also found significant discordance of needs across sites for most dimensions of HCC control. Respondents from Taiwan, Korea, and Japan gave the best aggregate scores, while scores for Australia, China, and USA were no different to the expected benchmark score.

For three dimensions (funding for screening, clinical education, and lifestyle risk factors) this study found concordance across sites. This suggests a similar level of need for improvement across sites in these areas, and that cross-national efforts may be beneficial. Cross-national efforts may be particularly welcome for managing lifestyle risk factors, because this was the only dimension for which the aggregate result was significantly below the benchmark and concordant across sites. Where results are discordant across sites, there may be less justification for cross-national efforts, but the results do suggest where decision makers may look for examples of best practice, such as to Japan for risk assessment of HCC.

The finding of positive or near-zero scores for some dimensions cannot be interpreted as meaning no improvement is necessary. Non-significant scores near zero simply mean no effect was observed, that is, respondents may be indifferent, may not know or may consider competence to be average or as expected. There may also be room for improvement when scores are significant and positive. It is possible (although very unlikely given the inclusion of ranking in the scores) for one dimension within any given site to have a perfect score of 50. The highest observed score was 40.6 (z-score of 8.53), for HBV strategy in Taiwan, suggesting there may be room for improvement even there. Similarly, despite a positive aggregate score, only 29 of the 60 site-by-dimension means were positive, suggesting room for improvement across the board.

The results are consistent with some evidence from policy implementation and epidemiological data. Korea's high score for HBV strategy is consistent with the fact that its HBV vaccination program has been credited with significantly reducing HBsAg seropositivity and HCC mortality rates (Yoo, 2010). A study of Korean cancer screening patients found that awareness of their infection status was only about 33% among HCV carriers and 75% among HBV carriers (Shin et al., 2009), which is also consistent with our finding that competence on HCV strategy was significantly below the benchmark. Similarly, our finding of competence above benchmark for HBV strategy in China is not surprising given its recent improvements in blood safety regulation and vaccination, including achievement of 90% coverage for neonatal vaccination (Zhou et al., 2009).

Taiwan had the highest score for HBV strategy, consistent with the fact that it has over 99% vaccine coverage among children in their first year of elementary school (Taiwan CDC, 2006) and it has observed significant reductions in HBsAg prevalence and HCC incidence in children since the vaccination program was introduced (Chang et al., 1997; Ni et al., 2001). As part of its J-HCC Guidelines, Japan uses a surveillance and diagnostic algorithm to track patients with HBV, HCV, and liver cirrhosis for testing every 3-6 months (Song et al., 2010). The successful implementation of this system is consistent with our finding that only Japan scored significantly above the benchmark on risk assessment.

The result that lifestyle risk factors are a significant need does not lead to an obvious conclusion because it may refer to several different concerns. The result may reflect expectations of the increasing importance of lifestyle risk factors in the etiology of HCC with increasing prevalence of obesity and decreasing HBV prevalence (Nordenstedt et al., 2010). Alternatively, it may reflect views that current efforts to reduce alcohol consumption, smoking, risky sexual behaviors, and/or drug injection practices are insufficient. Further research to separate these factors would be useful. In addition, the fact that no site had a positive score on this indicator suggests there may not be an example of best practice available such that research to investigate the effectiveness of policies to reduce lifestyle risk factors is a priority.

The use of the self-explicated method, a technique previously used mostly in marketing research (Green & Srinivasan, 1990), had several benefits for this study. The method is superior to Likert ratings alone because the addition of a ranking scale allows comparison across

different attributes or options. Similarly, the method is superior to a ranking scale alone because the resulting scores also can demonstrate magnitude. Conjoint analysis, another method of preference elicitation that produces results that are both cardinal and ordinal, is less appropriate for use in policy because a maximum of around seven attributes can be considered in one experiment. Many more attributes are often necessary for policy analysis, decision-making across jurisdictions, consultation, and planning for treatment services. Use of the self-explicated method in these contexts is a pragmatic way to add quantitative analysis to qualitative information from interviews, focus groups, surveys, and submissions.

One limitation of the study is the potential for bias from cross-cultural differences in reporting on Likert scales. Previous research has found differences among Chinese, Japanese, and Americans, and between Koreans and Americans in responses to Likert scales (Lee, Jones, Mineyama, & Zhang, 2002; Willis & Zahnd, 2007). Cross-cultural differences may have affected our results, but the addition of ranking scales reduced the impact of any bias in ratings.

Another potential limitation is the differences in HCC etiology and prevalence across the sites in the study. It is important to interpret results in the context of these international differences. For example, our finding of scores well below the benchmark for public and political awareness in Australia, Japan, and USA might be expected given that they have much lower HCC incidence than in China, Korea, and Taiwan (Ferlay et al., 2010). Similarly, sites with the highest scores for HBV strategy are also the sites where HBV contributes most to the epidemiology of HCC (Raza, Clifford, & Franceschi, 2007), suggesting either that the policy response is appropriate or that respondents considered competence on what is a major risk factor to be more important than competence on dimensions that may be perceived as less critical to HCC control.

A third limitation is the fact that the results may not generalize to other jurisdictions in the region. It may be appropriate to generalize for dimensions where results were concordant across sites, but there are likely to be differences in needs for dimensions that are discordant across our sample.

Clinicians who specialize in HCC and related areas were chosen as the respondents for this study because they are likely to be well-informed about the experience of HCC patients and about policy and technological developments in the field. However, the views of clinical experts alone should not be expected to provide sufficient evidence for decision-making. One model of needs assessment requires that information on needs be collected from epidemiological and demographic statistics, key informants and users or the public in order to inform goal-setting and program planning that is oriented to the needs of a particular community (Neuber, 1980). The reviews of epidemiology and intervention effectiveness conducted by the Asia-Pacific Working Party on Prevention of Hepatocellular Carcinoma (Farrell et al., 2010) provide the first part of this evidence set and our study contributes the second. A comprehensive assessment of needs therefore still requires consideration of the views of a broader population, including patients, members of the public, and other types of experts.

This study has found that significant needs exist for improvements in public and political awareness and lifestyle risk factors, but that other needs vary across six sites in the Asia-Pacific region. There is potential for the region to benefit from cross-national efforts to improve HCC control that focus on areas of concordant needs. Where competence differs across sites, there is also significant potential to learn from jurisdictions that have been relatively successful.

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EUS 2010 in Shanghai – Highlights and Scientific Abstracts[1]

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Education Around The World

EUS education in the Americas (A. Chak): Medical education in the United States begins after 12 years of high school and 4 years of college. Following four years of medical school, which results in the MD degree and three years of internal medicine residency physicians become eligible for specialty training, a fellowship in gastroenterology. Physicians who are board certified in internal medicine learn basic endoscopy during their three years of gastroenterology training and then become eligible to take board examinations in gastroenterology. To become an endosonographer most physicians who are board certified or eligible in gastroenterology obtain EUS training during an additional year of apprenticed training generally at an academic University hospital where such training is available.

To understand how graduate medical education is conducted and regulated in the United States you have to understand the multiple organizations that regulate education. The American Council for Graduate Medical Education or the ACGME accredits all training programs at different hospitals. Many of these hospitals are affiliated with universities and medical schools. The American Board of Internal Medicine or the ABIM tests individual physicians after they are trained and certifies that they have training in internal medicine or a specialty such as cardiology or gastroenterology. There is no separate board for EUS. The American Society for Gastrointestinal Endoscopy or the ASGE is a national society of gastrointestinal endoscopists who has the primary responsibility of educating post graduates and along with other sister GI societies developing guidelines and curricula for endoscopic training of fellows in training programs. The training of gastroenterologists is actually provided at individual independent academic hospitals. Most of these hospitals are either affiliated with a University or owned by a University.

The ACGME requires that GI training programs afford trainees the opportunity to attain competence in a core group of standard procedures, which include EGD, esophageal dilation, colonoscopy etc. Advanced procedures such as EUS are more complex and generally require an additional year of dedicated training. Not all trainees should pursue these procedures both due to variation in skill and manpower needs. Not all training programs should offer such training.

The components of education in EUS include an understanding of 3 dimensional anatomy, learning the principles of basic endoscopy, some understanding of the physics of ultrasonographic imaging, familiarity with other radiology techniques of abdominal imaging, learning the staging of GI cancers, and a rudimentary understanding of cytology. The ASGE has outlined the following endoscopic training objectives.

- Trainees need to learn when it is appropriate to recommend or not recommend EUS.
- Trainees need to learn safe and complete performance of the procedure. This includes training in the principles and safe use of sedation.
- Trainees need to identify and correctly interpret EUS findings.
- Trainees need to identify and minimize risk factors for complications.

A training program in endoscopic procedures should include the following components: a) Core Curriculum, b) Training director, c) Endoscopic trainers, and d) Defined training process. The training process will be different for standard

[1] The largest EUS international symposium sponsored by Olympus was held in November 2010 in Shanghai, China with over 1000 attendents. This congress reports briefly reviews the main topics and conclusions of most of the invited oral presentations; at the end the scientific abstracts are listed.

procedures, advanced procedures and new procedures. The ASGE has developed a series of Core Curricula for training directors that contain information on pre-procedural management, patient considerations, post-procedural management, basic techniques, therapeutic techniques, applications, and the goals of training. Curricula are available for EGD, colonoscopy, ERCP, and enteral nutrition through the ASGE website. The curriculum for EUS is currently being developed.

Training programs should designate a training director. This should generally be an expert in endoscopy who also has the aptitude of a teacher. The training director should regularly monitor each trainee in terms of their technical and cognitive skills in relation to their level of training and achievement of performance standards. He should incorporate endoscopic teaching resources such as textbooks, videos, CDs. The training methodology in the program should be periodically reviewed and updated and the training director should meet with the trainee periodically and review the evaluation forms from other trainers and give the trainee opportunity for feedback. In the past, the EUS training program director was also the one and only EUS trainer. But now many programs have more than one EUS trainer. Qualities that make someone a good trainer are: a) established endoscopic skills; b) ability to communicate, providing tactile as well as verbal instruction; c) academic programs must provide trainers protected time to participate in the training as well as regular evaluation processof trainees; and d) gastroenterologists doing research in endoscopy are especially valuable as trainers.

EUS training occurs in stages. The first step is for the trainee to learn basic anatomy and how to manipulate the scope. The trainee then moves on to diagnostic procedures, learns how to intubate the esophagus and recognizes the difference between normal and abnormal anatomy. In the next stage, the trainee learns how to perform the basic procedure without supervision and becomes more active in interventional procedures. Finally the trainee is able to perform all aspects of the procedures independently and is deemed competent.

Competency in endoscopic procedures needs to be assessed. The measurement can be divided into technical competency, i.e., endoscopic manipulation, and cognitive competency, i.e., recognizing the ultrasound image and understanding disease. Each individual acquires technical and cognitive skills at different rates. Cognitive skills, which include knowledge of indications and contraindications, interpretation of endoscopic findings, integration of findings into clinical management, recognition and management of procedure related complications, safe administration of sedation and analgesia, informed consent and ethics are tested by the ABIM. Technical competence needs to be assessed by individual programs. Methods for assessing technical competence require the trainees to maintain logbooks of their procedures (examples are available on the ASGE website); periodic subjective evaluation by the training director and endoscopic trainers; and an assessment that the trainee has met objective performance standards of benchmark numbers and performance criteria. The minimum number of procedures threshold is a convenient and widely used practice for assessing competency. These numbers are based on consensus expert opinion only. Learners progress at different rates and number of procedures does not guarantee proficiency. These threshold numbers are meant to simply be a threshold before trainees can be expected to have achieved competency. Most trainees require far greater numbers to achieve competency.

Trainees should be asked to keep a log of procedures they participate in and this log should periodically be monitored. Trainees can be evaluated subjectively using comprehensive skill evaluation forms l available to training directors on the ASGE web site. The results of these periodic evaluations should be discussed with the trainees with opportunity for feedback. Performance criteria for EUS include the ability to visualize the desired anatomical structure, accurately stage cancers using the TNM system, and obtain adequate yield when performing FNA

In summary, training in EUS must occur in a comprehensive program that integrates teaching of cognitive and technical skills. Despite obvious problems, threshold numbers for individual procedures remain an objective criterion for assessing technical competence. Education in EUS and assessment of competence is by necessity largely subjective. Rigorous monitoring of trainees helps ensure that patients receive the best outcome.

Europe (C. de Angelis with information provided by C. Boustière, J.-M. Godchaux, L. Palazzo and the CFED, G. Caletti, G. Bonanno and the Italian Endosonography Club (IEC), A. Ginès, E. Vasquez-Sequeiros and the Spanish AEG-EUS Interest Group, P. Deprez, V. Gillard, S. Seewald and A. Fritscher-Ravens, J. Meenan, N.Carroll, J. Penman and the UK EUS Users Group, E.D. Fedorov, Z.V. Galkova, S.Yu. Orlov, O. Malikova, T. Silina and the RASEUS, C. Kalayci, D. Oguz and the Turkish EUS Group of Interest, M. Polkowski, K. Markoglou, I. Scotiniotis and V. Nosek): One of the major problems in EUS diffusion in clinical practice has been a well structured and organized training of good quality endosonographers. Apart from some pioneer centers, EUS had difficulties to diffuse into the medical practice, with mostly use of only one instrument type, radial or linear; only recently of both scope types has become routine in major centers. This slow spread could be both a cause and a consequence of the relative lack of formal EUS education included in gastroenterology fellowship training in Europe. In the last 15 years however we witnessed an almost exponential growth in the number of EUS units and, to a lesser degree, in the number of centers performing FNA. This is in some contrast to the relative lack of formal EUS training and the low number of EUS training centers. Therefore, EUS clubs or groups have been formed in some European countries endosonographers grouped themselves in some sort of national associations or groups, named EUS Clubs or Groups of interest in EUS, in other countries there are some Groups of work or committees within the national endoscopic Societies, but in many other European countries. In 2002 France, Belgium and Italy started a new cooperation of their national EUS Clubs. In 2003 the Spanish EUS Group of interest joined in and an International EUS Working Group named European Group for Endoscopic Ultrasound (EGEUS) was founded. The EGEUS is an Association of National Clubs or Groups of Interest or Committees in the field of EUS and /or individual members dedicated to EUS. Main aims of the association are to develop and spread the knowledge of EUS and other associated endoscopic/ultrasonographic or gastroenterological techniques and to promote education and training of doctors and nurses in endosonography. Very recently the Russian Association for Specialists of EUS (RASEUS) and the Turkish EUS Group of Interest joined or are going to join the EGEUS, which today has nearly 800 members. The Group has also relationships and is starting cooperation with EUS Groups of Interest or EUS Committees outside Europe, like the EUS Working Group of India (EWGI) that emanates from the Gastrointestinal Endoscopy Society of India (SGEI). Data, information and opinions contained in the following presentation

were collected from many European experts (see the Acknowledgments section), from surveys and census conducted by some national EUS clubs and Groups of interest (French Digestive Endosonography Club, CFED, Italian Endosonography Club, IEC, Spanish AEG-EUS Interest Group, Belgian Group of digestive Endosonography, BDGES, Russian Association for Specialists of EUS, RASEUS and Turkish EUS Group of interest) and last but not least from meetings and Courses organized by the EGEUS.

EGEUS and EUS education: The European Group for Endoscopic Ultrasound (EGEUS) organizes an European meeting every two years (we had already Paris 2005, Turin 2007 and Barcelona 2009), the 4th EGEUS meeting will be held in Brussels in 2011. Each meeting has been attended by 250 – 350 participants. In these meetings participants can attend state of the heart assessments on all possible topics in EUS, can follow more practical workshops, can discuss with the experts in the Speaker's corner sessions or Breakfast with the experts. They have also the opportunity to present their scientific works and experiences as oral communication or video-communication in the Free papers sessions or as posters. During the congress all participants can see a lot of EUS images and videos in a rich Learning center and can test themselves against the EUS simulator. In 2008 (Castel San Pietro Terme, Imola) and 2010 (Villa Gualino, Turin) the EGEUS in collaboration with the Italian Endosonograghy Club (IEC) organized an International EUS live Course with more than 10 hours of live demonstrations and also theoretical lessons and video-sessions. On our website: www.egeus.org and the correlated www.eusclub.it you can find every kind of EUS images, videos, lectures of international experts, news about new and old EUS meetings and Courses and you can test yourself with the video- or the image-quiz of the month. Now we are working on the possibility of e-learning and CME on-line Courses or real-time live-demonstration via Internet. In the next future the EGEUS Board has the program to try to standardize a formal EUS educational program in Europe, will try to establish guidelines for education and some EGEUS certified EUS training centers to be proposed for a certified training.

EUS education in different European Countries: The issue of education in EUS is very differently dealt with in the various European countries. The most important topics are listed in the folllowing
France: EUS has a very good distribution on the territory, the national mean is of 1 EUS center × 250000 inhabitants, with obviously great geographical differences in distribution because of demographic conditions, with a case volume of about 65600 EUS examinations/year in 2008. The French EUS Club is a well established association of endosonographers strictly linked to the major gastroenterological and endoscopic scientific Societies, like the SFED and SNFGE. It has more than 300 members and organizes an annual EUS meeting with live demonstrations every two years. The CFED has organized an academic diploma of digestive EUS on the behalf of M. Barthet and 2 co-Directors (M. Giovannini e C. Boustière). It accepts 35 students every year that have to attend 3 sessions of 1 week of theoretical and practical training. The inscriptions for the next 2 years are already closed! Even if this educational program in France is probably one of the best formal academic training program for EUS in the world, learning EUS is still a problem also in France: first of all this interuniversity diploma is not mandatory in order to perform EUS and secondly less than a third of people practicing EUS were trained via this interuniversity qualification.

Italy: the Italian Endosonography Club (IEC) was born in 2002, after the breakup of the Committee first and then the Group of work for EUS of the Italian Society of Digestive Endoscopy (SIED). It works in close relationship with the SIED and other Italian Gastroenterological Societies, like AIGO and SIGE. It groups together more than 180 doctors who are practicing or are simply interested in EUS and EUS education and training are between its major commitments. In this respect the Italian EUS Club organizes an annual Joint Meeting with other gastroenterological societies during their national congress. A national EUS Course every two years (only theoretical the first two editions, Bertinoro I e II), with live demonstrations the last three (Monte del Re, Castel San Pietro Terme and Turin). Some centers organize practical Courses of 1 – 4 weeks for 1 – 3 fellows, with daily frequency in the procedure room. In cooperation with the IEC the University of Bologna started in 2003 the first University Master of 2nd level in Advanced Endoscopy. It lasts 1 year, at least 40 days of attendance in the endoscopic room are requested and a relevant part of the theoretical and practical activity deals with EUS. Since 2006 a yearly Master in Endosonography is held in Milan, Vita-Salute San Raffaele University. The IEC is planning to organize a new Master in EUS in cooperation with another Italian University. In 2008 the Italian Society of Digestive Endoscopy (SIED) and the Italian EUS Club (IEC) started an educational program in EUS, named National EUS training Course IEC/SIED, dedicated to doctors and nurses, with 2 – 5 different modules in distinct venues, with basic and advanced courses. There is an Italian website, that has also an English version: www.eusclub.it linked with the above-mentioned European website, www.egeus.org. On these websites you can find a lot of EUS images, videos, lectures of international experts, news about new and old EUS meetings and Courses and you can test yourself with the video or the image-quiz of the month. Again is ongoing the possibility of e-learning and CME on-line Courses or real-time live-demonstration via Internet.

Spain: also Spain witnessed an exponential increase in the number of EUS centers and EUS procedures performed in the last 15 years, from 2 centers in 1995 to 58 centers in 2008. Data from a survey in 50% of centers give us a very good news: 91% of endosonographers practicing EUS in Spanish centers had a specific training in EUS and only 9% are self-taught. They have a Group of interest in EUS, the Spanish AEG-EUS Interest Group, linked with the Spanish Association of Gastroenterology; it groups together more than 70 doctors. They organize every year three specific EUS Courses, few hands-on courses and one annual meeting of the EUS Group of interest. Audience is constantly increasing in the Spanish national courses.

Belgium: in Belgium there was one of the oldest EUS Club in Europe, the Belgian Club of digestive EUS (BDGES), that today has joined the French Club in the new Club Francophone of EUS (CFED): they have about 50 members, the EUS centers in Belgium are about 40, performing nearly 7000 EUS cases a year in 2008. They don't have a formal program of education in EUS, but they actively participate in the program of the CFED.

Germany: probably (data were supplied by manufacturers of EUS equipment and also by German experts, personal communications) there are more than 500 EUS centers in Germany, it means about 1 center/200000 inhabitants and they perform almost 200000 examinations a year. In Germany there was one of

the first national EUS Club, but as far as we know it seems to be no more active today. There is probably a regional EUS Club in the area of Berlin, but only locally active. An EUS Working Group is in the DEGUM (Deutsche Gesellschaft fur Ultraschall in der Medizin) Society, that is the German Society for Ultrasound in Medicine. At least 10 EUS training Courses a year were organized by Companies like Olympus or Pentax.

United Kingdom: in Great Britain nearly all healthcare is provided by the State and it is free. Training isssues and Endoscopy units are strictly controlled by national standards, all endoscopists must be assessed to complete training and must demonstrate ongoing "quality indicators" every six months. There are 66 EUS centers, in which 95 endosonographers are working, with an estimate number of about 15000 cases a year. British EUS users grouped themselves in the UK EUS User Group, that is a multidisciplinary society which aims to promote "education, best practice and innovation in endosonography and to forge links between clinicians with an interest in EUS both in the UK and overseas" (see www.eususers.com). They held an annual meeting in October/November each year. The UK EUS users Group prepared a document named Recommendations for training in Endosonography (EUS)" (N. Carroll and J. Penman). In this document they stated that "in the UK intake to EUS training may be from trainees in gastrointestinal medicine, gastrointestinal surgery, pancreatico-biliary surgery or radiology. All trainees should be competent in diagnostic and appropriate aspects of therapeutic upper gastrointestinal endoscopy before attempting to learn EUS and should have attended a JAG (Joint Advisory Group) approved or JAG compliant basic endoscopy course at a minimum. Gastroenterology trainees seeking to acquire skills in EUS must have completed at least 24 months of a standard gastrointestinal training scheme and have documented competence in routine endoscopic procedure. The UK EUS Users Group considers it desirable if not essential for trainees from other backgrounds to gain some preliminary experience in transabdominal ultrasound prior to EUS training. JAG guidelines were used as basis for EUS training in the UK. Trainees are expected to attend at least one session per week for a minimum of 6 months. With increasing use of linear EUS particularly linear EUS/FNA this period should ideally be for 1 year. A period of intensive training at a recognised centre of excellence in the UK or overseas should be considered to enhance the learning process. A log-book should be kept of all procedures undertaken and audit of performance against recognised standards from peer review literature is essential.
Trainees should attend the UK EUS Users Group meeting. International meetings are also recommended. Interventional techniques should only be commenced following adequate training in diagnostic EUS". In the same documents they give other statements about numbers of procedures required to achieve competency, citing also the ASGE Guidelines for credentialing and granting privileges for Endoscopic Ultrasound, GIE 2001, but also declaring that the numbers themselves are less relevant than the context, quality and outcome of each procedure. They give suggestions about training in EUS-FNA: "Interventional techniques should only be commenced following adequate training in diagnostic EUS. Trainees may commence EUS-FNA after 50 or so examinations" … "Animal and mechanical models and short courses may help in the development of initial EUS-FNA skills; however, these cannot replace supervised experience under the guidance of a recognised specialist in EUS-FNA". The aims of the UK EUS Users Group are to achieve standardisation of UK training and to promote methods of assessment of "best practice ". In this respect the Group will establish centers to be recognized by JAG for training, establish guidelines for training, set objectives and standardise across centers indications, performance, reporting and terminology and monitoring of trainees. Very ambitious goals, as you can see, but we don't know if till now they were partially or totally achieved.

Russia: Russian people had at the very beginning an EUS Committee in the Russian Endoscopy Society (RES) and training of the first nine endosonographers was obtained at leading EUS clinic in Japan, TKH, Sapporo and other 4 ones were trained in Europe. The issue of EUS training was then faced by opening educational Centers in Russia, in which Russian-Japanese EUS Hands-on trainings and EUS live-courses were held. Today there are five Centers: Moscow University Hospital №31, Irkutsk Regional Diagnostic Center, Leningrad Central Regional Hospital, Russian Oncological Center, Moscow, Yaroslavl Regional Clinical Oncological Hospital. In recent times the Russian Association for Specialists of EUS (RASEUS) was founded and it has a governing Board and about 60 members, it manages an EUS School and an EUS website, of which they are building also an english version. The idea, in such an immense country like Russia, is to develop mainly Internet education. They are dealing today with big problems: lack of certification of EUS by the Ministry of Health, no formal EUS training program at Endoscopy study courses and their aim is to include EUS into the state university program and postgraduate courses of Gastroenterology and Surgery. They are convinced that only training will promote the spread of EUS all over Russia. In the month of October 2010, the RASEUS entered the EGEUS as national Club member and will have its own representative in the EGEUS governing Board.

Turkey: EUS started in 2000, but became more available in the country after 2004, more than 90% of the centers are located in three major cities (Istanbul, Ankara and Izmir). The situation of training is disappointing, there are no formal training programs, most of Turkish endosonographers were trained by means of short visits (1–8 weeks) to North American and European sites, the educational program consists mainly in observation, rarely in hands-on courses and is mostly sponsored by companies manufacturing endoscopes. The need for a more formal training program recently brought Turkish endosonographers to set up an EUS Interest Group that emanates from the Turkish Society of GI Endoscopy and they now are going to apply to EGEUS national Clubs or Groups of Interest or EUS Committees membership in order to become member of the European Group for Endoscopic Ultrasound.

Other European Countries: EUS status and EUS education in other European countries is also disappointing, there are no Societies, Clubs, organizations or networks devoted to EUS and no structured training programs nor certification systems in Bulgaria, Czech Republic, Greece, Hungary, Poland, Switzerland, Serbia, Slovenia, Slovakia and so on.

In conclusion, EUS practice is expanding in Europe in many countries, but in some countries and in some proportion also in the best organized nations EUS is still self-taught. No condition or diploma or certification is required in order to perform EUS even where there is a formal university Diploma or Master in EUS. During fellowship, EUS learning and teaching is not efficient. Anyway

EUS is today considered as a major tool by gastroenterologists, surgeons and oncologists in most European countries and is now a widely accepted method for pre-therapeutic staging of GI malignancies and for diagnosis of benign and malignant biliopancreatic diseases. EUS-guided FNA and therapy is rapidly evolving and expanding, even if mainly in specialized centers. There is a strong need for organized training. Improvement of the training issue is mandatory in order to guarantee the future of EUS. One of the first priority for the EGEUS is to organize training and education in EUS by creating a teaching network, put together endosonographers, experiences, ideas from different people in order to support EUS development and training programs, favour communication and exchanges between different countries and realities.

Asian-Pacific Countries (K. Yasuda): According to the widespread of imaging diagnosis of EUS and EUS-FNA, education and training become important subjects. Nowadays, everybody knows the clinical significance of EUS imaging and interventional EUS. However, there are some difficulties for endoscopists and gastroenterologist to perform EUS study and EUS-FNA. Some are not familiar with the ultrasound imaging and some are not familiar to manipulate ultrasound endoscopes which have bigger diameter and two functions of endoscope and ultrasound.

In Asian-Pacific countries including Japan, the diffusion of EUS system and system models, such as the ratio of radial and linear models, mechanical radial and electronic radial, and ultrasound endoscopes and probes, are various. And the spreading of the systems is also various. Some countries have long history of EUS and some countries show the quick development of EUS studies, and some just start the use of EUS.

At first, when we started EUS in 1980 by limited institutes and clinicians, "learning by doing" in clinical cases was a general agreement to perform EUS. Since then, our circumstances were changed. We have to perform EUS after having the education, training and clinical experience under supervisor, as EUS is a new technique to the doctors who start the EUS study for the first time to perform safe and complete EUS examinations. Training centers for new techniques not only for EUS but also for new therapeutic endoscopy procedures are becoming common. Recently education and training courses are established in most countries in this area, though the number of training center and supervisors, who had learned EUS in Japan, US and Europe in the late 1990 s, is different in each country. They prepare many kinds of training systems not only in imaging EUS but also in EUS-FNA in limited centers.

At present, EUS training modalities are many such as 1, Monograph, Textbook, Printed paper, 2, Lecture by expert, 3, DVD, Video forum, 4, Hands-on training using animal models, phantoms and simulators, 5, Live case demonstration, 6, Hands-on training through live case, 7, Web based education, Internet conference.

Although the contents depend on the conference, each country has their own EUS workshop and endoscopy workshop including EUS for the education and training of EUS in Asian-Pacific region. Regular workshops are held in most countries as follow, Korea, China, Hong Kong, Taiwan, Malaysia, Singapore, Thailand, India, Australia, New Zealand and Japan with selected contents. They are so useful for spreading and learning basic techniques of EUS and EUS-FNA procedures to achieve the successful clinical results.

EUS Imaging and FNA – New Horizons and Old Problems

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Contrast Harmonic Imaging – General Overview (B Napoleon, MV Alvarez-Sanchez): In the last decade, the development of stabilized microbubbles contrast agents and the improvements of ultrasonic equipment, such as harmonic imaging, have enabled to display microbubbles enhancement in gray scale with optimal contrast and spatial resolution. Recent technological advances have made contrast harmonic technology firstly available for endoscopic ultrasound (EUS) in 2008. Thus, the evaluation of microcirculation is now feasible with EUS, prompting the evolution of contrast EUS from vascular imaging to imaging of perfused tissue. Although experience is still preliminary, several reports highlight contrast enhanced harmonic EUS (CEH-EUS) as a promising non-invasive method to characterize lesions. Most reported studies have dealt with the challenging issue of the characterization of solid pancreatic masses. Results from these studies show that a hypo-enhancing pattern is 86 % accurate (range: 82 – 89 %) and 88 % sensitive (range: 75 – 96 %) in diagnosing pancreatic adenocarcinoma, while hyper-enhancing pattern is a strong predictor of lesions other than adenocarcinoma (NPV of 93 %; range 89 – 98 %). Moreover, in one series, CEH-EUS exhibited better performance to diagnose adenocarcinoma when comparing with EUS-FNA, with sensitivity and NPV of 89 and 88 %, vs 72 and 77 % respectively. Even if histology remains the gold standard, the combination of CEH-EUS and EUS-FNA can not only render EUS more accurate, but also assist physicians in making decisions when EUS-FNA is inconclusive, and increase EUS-FNA yield by guiding the puncture with simultaneous imaging of vascularity. In this setting, one series found that more adequate samples can be obtained by CEH-EUS guided FNA by avoiding necrotic or fibrotic areas with no enhancement. Anecdotal reports on malignant pancreatic lesions, depicted by CEH-EUS in difficult cases (biliary stents or diffuse chronic pancreatitis), suggest that CEH-EUS can also improve detection ability of EUS. Prevalent lesions, other than solid masses, are pancreatic cysts. Brief experience on patients with intraductal papillary mucinous neoplasias has revealed that CEH-EUS can increase the ability to determine malignancy by enhancement of mural nodules or solid components. In addition to depiction and characterization of lesions, a meaningful indication of EUS in clinical practice is the cancer staging. Thereby, CEH-EUS has also been evaluated in preoperative staging of pancreaticobiliary neoplasias. The overall accuracy for T-staging was 92 % with clearer displaying of depth and vascular invasion. Also, one of most profitable application of CEH-EUS may be the assessment of lesions with unknown origin, especially when they cannot be accessed for EUS-FNA. Heterogeneous enhancement, as a sign of malignancy, has recently demonstrated excellent accuracy (98 %) to differentiate malignant from benign intra-abdominal lesions of unknown origin (mostly lymphadenopathies, stromal tumors and lymphomas).

Further studies in larger number of patients are required to confirm these encouraging findings, and other indications must be explored, such as biliary diseases, stromal tumors, lymphadenopathies, differentiation of mass-forming pancreatitis from pancreatic cancer, and portal hypertension. CEH-EUS has also opened exciting perspectives in other research areas: monitoring response to anticancer chemotherapy or to ethanol-induced pancreatic tissue ablation, anticancer therapies by ultrasound-triggered drug and gene delivery, and therapeutic adjuvant by contrast ultrasound induced apoptosis. Contrast harmonic imaging

is gaining popularity because its efficacy, simplicity and non-invasive nature, and many expectations are now placed on this technique. If confirmed in next future, contrast harmonic imaging will become a standard practice in EUS.

Contrast Harmonic Imaging – Experience with Sonazoid (M. Kitano): Contrast harmonic imaging has not been available until recently for EUS examination, because the transducer for current echoendoscopes is of a limited frequency bandwidth and is small to produce enough acoustic power for contrast harmonic imaging. Second generation ultrasound contrast agents produce harmonic signals at lower acoustic powers, and are therefore suitable for EUS imaging at low acoustic powers. The newly developed echoendoscope (GF-UCT260, OLYMPUS, Tokyo, Japan) has a broad-band transducer that can produce and detect harmonic signals from second generation contrast agents. Image analysis is performed using ALOKA ProSound α-10 (ALOKA, Tokyo, Japan). Compared to the other ultrasound contrast agents, Sonazoid enabled us to observe long-lasting perfusion imaging. In the pancreas, echo signals from the ultrasound contrast peaked at about 20 s after the infusion was commenced and parenchymal perfusion imaging was observed throughout the pancreas for at least 90 s. We evaluated the usefulness of CEH-EUS with Sonazoid for diagnosis of digestive diseases.

CEH-EUS in pancreatic diseases: Based on our experience on 668 consecutive patients with a suspected pancreatic disease, all lesions could be categorized into eight patterns. Most of the pancreatic carcinomas (194/204) had the hypovascular solid enhancement pattern. When the small lesions (≤2 cm) were considered, CH-EUS was significantly more sensitive in diagnosing pancreatic carcinoma than multidetector CT (MDCT) (91.2 % vs. 70.6 %). Similarly, the presence of a nodular enhancement in a cystic lesion was regarded as intraductal papillary mucinous neoplasms, and reached a high sensitivity. CH-EUS using Sonazoid more effectively depicted and characterized small pancreatic neoplasms of 2 cm or less than MDCT.

CEH-EUS for diagnosis of intra-abdominal lesions of undetermined origin: Our experience includes 43 patients, each with a lesion of undetermined origin, who underwent CEH-EUS. The lesions were categorized as having no, homogeneous, or heterogeneous enhancement. Almost all of the malignant lesions (26/27) exhibited heterogeneous enhancement. The malignant and benign lesion groups differed significantly in terms of homogeneous and heterogeneous enhancement. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy with which CEH-EUS differentiated malignant from benign lesions were 96.3 %, 100 %, 100 %, 94.1 % and 97.6 %, respectively. CEH-EUS depicted the microvasculature of intra-abdominal lesions of undetermined origin very clearly, and may be useful for characterizing such lesions.

CEH-EUS for estimation of malignant potential of GIST: 76 consecutive patients suspected of having a subepithelial lesion underwent CEH-EUS. We evaluated whether vascularity is related to the preoperative malignancy risk of gastrointestinal stromal tumors (GISTs). Resected GIST specimens in 29 patients who underwent surgical resection were divided into high- (n = 16) and low-grade (n = 13) malignancy groups based on mitotic activity. CEH-EUS identified irregular vessels and thereby predicted GIST malignancies with a sensitivity, specificity and accuracy of 100 %, 63 % and 83 %, respectively. The sensitivity of CEH-EUS (100 %) in detecting intramural vessels in high-grade malignancy GISTs was higher than contrast-enhanced CT (31 %) and power Doppler EUS (63 %). CEH-EUS may play an important role in predicting the malignancy risk of GISTs.

In conclusion, CH-EUS with Sonazoid enabled continuous real-time observation of real-time microcirculation and perfusion images of digestive organs. Depiction of detailed vascularity can be applied to identification and characterization of lesions in digestive organs and evaluation of their pathophysiological condition by vascularity.

EUS-FNA (R. Chen): Since the advent of endoscopic ultrasound (EUS) in the 1980 s, EUS has become an essential tool in diagnosing diverse pathology and staging tumors in but exclusive to gastrointestinal tract. By placing the ultrasound transducer in close proximity to the pathology in question provides detailed images of the lesion being assessed. However, EUS is in competition with other fast advancing technology such as CT, MRI and PET scan. Thus, one of the cornerstones of EUS in maintaining its widespread and crucial role is its ability to obtain cytological diagnosis by performing real time EUS guided fine needle aspiration (EUS-FNA). This is one of the main advantages of EUS over all other types of imaging modality.

From the initial description of EUS-FNA in the 1990 s, EUS-FNA has an important role in managing patients with pathologies (especially tumor staging) in multiple organs including esophagus, stomach, pancreas, lung and rectum nowadays. It has become part of the staging tool in cancers of gastrointestinal tract and lung cancer in many parts of the world. Various techniques have been described that may alter the yield of EUS-FNA including needle size, use of stylet, application of suction. Overall, EUS-FNA is highly accurate and has a yield of adequate cytology diagnosis of around 80 – 90 % regardless of the technique used.

EUS-FNA is also in general less invasive in most circumstances and is an ideal technique to sample small lesions and lymph nodes. It is safe in capable hands with a complication rate of less than 1 %. Complications are in general minor and serious complications such as perforation are rare.

Despite these advantages, there are challenges to EUS-FNA. Some of these limitations are: a low rate of inadequate cytology; inability to obtain cytology from lymph nodes with tumor in the trajectory path of needle aspiration; low yield of definite diagnosis in cystic lesions in pancreas. Further advancement in EUS-FNA technology may be able to resolve these issues.

In conclusion, EUS-FNA is a unique ability that sets EUS apart from other radiological technique. EUS-FNA has a high yield of diagnosis, is safe and minimally invasive and will continue to ensure that EUS has an important role in management of patients.

EUS-Guided FNA (Mohamad A. Eloubeidi): Endoscopic ultrasound (EUS) combines endoscopy and ultrasound to image the gut wall and surrounding structures. The use of curvilinear array scanning echoendoscope allows visualization of the needles parallel to the long axis of the endoscopes, thus permitting sampling of the target lesions under real time guidance by fine needle aspiration biopsy. Since its introduction in the early 1990's, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) emerged as a safe and accurate tool in tissue acquisition in patients with gastrointestinal, pancreatic and thoracic malignancies. It allows targeting of mural lesions otherwise not diagnosed by mucosal

biopsies, extra-intestinal organs, masses and peri-intestinal lymphadenopathy. Important premises are needed to achieve successful EUS-guided FMA:
Recognition of the lesion/structure that is being targeted for EUS-guided FNA b) lack of intervening vessel c) Apposition of the lesion to the needle pathway. d) Immobilization of the lesion to insure shearing of tissue and thus adequate cellularity.
Studies with large numbers of patients published to date suggest a high degree of accuracy in achieving tissue diagnosis. In one large study by this author, 540 patients underwent EUS-FNAs of 656 lesions. These included lymph nodes (LNs), solid pancreatic masses (SPMs), cystic pancreatic masses (CPM), mural, bile duct, gall bladder, liver, mediastinum/lung adrenal, spleen and kidney. The overall sensitivity, specificity, PPV, NPV and accuracy of EUS-FNA was 91.7%, 97.1%, 98.1%, 87.7%, and 93.8% (95% CI: 91.9–94.8) respectively; Out of the 540 patients (656 lesions), six patients 1.1% (95% CI: 0.4–2.4) were reported to have major complications. EUS-FNA related pancreatitis occurred in 1 out of 286 procedures 0.35% (95% CI, 0.01–1.93). One patient died immediately after the procedure (0.18%).
In addition, Rapid onsite interpretation (ROSE) of cytology specimens is a very important tool that helps triage the specimen and helps reduce the physician work load. In general, there is about a 15% increment yield when immediate feedback is obtained by ROSE. For instance, additional immunostains can be obtained for GIST, lymphoma, or other metastatic diseases to various organs. In case of pathologists unavailability, a new web-based system has been developed that could circumvent this problem.
Special Aspects of Cystic Pancreatic Lesions (H. Maguchi): Recently, with the rapid advancement in less invasive diagnostic modalities such as CT and MRI, pancreatic cystic lesions have been increasingly indentified. Pancreatic cystic lesions can be classified broadly into two groups: primary cystic neoplasms and non-neoplastic cyst.
Primary cystic neoplasms are generally comprised of serous cystic neoplasm (SCN), mucinous cystic neoplasm (MCN) and intraductal papillary mucinous neoplasm (IPMN). SCNs, which are called "microcystic adenoma", are composed histologically of innumerable tiny cysts, but sometimes containing macrocysts. SCNs are not generally considered at risk of malignant progression. In contrast, MCNs have a malignant potential even if they are histologically adenoma. The most characteristic histological finding in MCNs is the presence of a unique ovarian-type stroma (OTS) and typical MCN with OTS is rare in males. IPMNs are characterized by variable biological behavior and histologically classified as adenoma, borderline or carcinoma, last category including both non-invasive and invasive.
In 2006, the international consensus guidelines for the management of IPMN/MCN of the pancreas were published in the journal Pancreatology. The guidelines recommended surgical resection for all MCNs and MD-IPMNs. In addition, patients with BD-IPMNs who have symptoms, mural nodules (MNs), a dilated main pancreatic duct (MPD), or a cyst size > 30 mm were recommended to undergo surgery. However, it is still controversial as to whether these BD-IPMNs should be resected immediately. In addition, solid pancreatic tumors, such as solid-pseudo papillary neoplasm (SPN) and neuroendocrine tumor (NET), sometimes show the secondary cystic change.
How can EUS play an important role for the diagnosis and eventually contribute to determine the management strategy of these cystic pancreatic neoplasms? EUS is superior to the other diagnostic modalities in terms of spatial resolution. EUS provides real-time and high-resolution images of cystic pancreatic lesions in morphological details. EUS identifies multiple small anechoic lesions of SCNs, and "cysts in cyst" pattern of internal structure of MCNs. EUS is also the most useful device for identification of MNs in the dilated branch duct and MPD of IPMNs. The presence of MNs has been reported to be strongly suggestive of malignancy. However, multimodal diagnosis is still important, since EUS only can evaluate the lesions morphologically. It is believed that for accurate diagnosis of cystic pancreatic neoplasms, evaluation of vascularity, pancreatogram and surrounding parenchyma of the lesions by contrast-enhanced CT, MRI, and/or ERCP are required.

The Role of EUS in Autoimmune Pancreatitis (M. Levy): Endoscopic ultrasound (EUS) is a useful tool in the evaluation of autoimmune pancreatitis (AIP), because of the high resolution imaging and the ability to precisely guide tissue acquisition. AIP is part of a systemic fibro-inflammatory process that afflicts a variety of organs, most notably the bile duct and pancreas. Affected organs typically manifest a lymphoplasmacytic infiltrate containing IgG_4 positive cells and the disease is usually readily steroid responsive, in particular the inflammatory component. Mayo HISORt criteria consider various factors including histology, imaging, serology, other organ (non-pancreatic) involvement, and response to steroid therapy. The characteristic EUS finding is diffuse (sausage-shape) pancreatic enlargement with a hypoechoic, coarse, patchy, heterogeneous appearance. However, there may be significant overlap between the appearance of AIP and other pancreatic disorders. The pancreas may also appear hypoechoic with course, patchy and heterogeneous involvement, without diffuse enlargement. Patients may also have a diffusely enlarged pancreas that is not as hypoechoic as typically seen and the changes are not as course, patchy, and heterogeneous in nature. EUS may reveal an isolated or multiple mass lesions. And the presence of apparent vascular invasion may falsely suggest an unresectable carcinoma. Finally changes suggestive of usual or non-specific chronic pancreatitis may be present. Due to the absence of pathognomonic features on imaging studies, and because classic features are uncommon and may seen other disorders, and because of the diverse spectrum of features which limits the specificity, tissue sampling is generally necessary to firmly established the diagnosis. Large caliber biopsies may be taken with the EUS-guided trucut biopsy device. Classic histologic features include obliterative phlebitis representing intense lymphoplasmacytic infiltration surrounding venules with preservation of the arterioles. IgG_4 immunostaining of tissue samples identifies IgG_4 positive plasma cells and presence of moderate (11–30 cells/HPF) or severe (> 30 cells/HPF) infiltration is considered diagnostic. Finally, the following points should be emphasized: 1.) the diagnosis of autoimmune pancreatitis cannot rely on EUS imaging alone; 2.) trucut biopsy specimens are sufficient for diagnosis of autoimmune pancreatitis and fine needle aspiration is not; and 3.) a negative fine needle aspiration does not exclude pancreatic carcinoma and a negative trucut biopsy does not exclude autoimmune pancreatitis.

Interventional EUS Techniques

Interventions on Fluid Collections – A Combined West-East Approach (S. Seewald, T. L. Ang): Endoscopic transenteric drainage, especially with endoscopic ultrasound (EUS)-guidance, is now

regarded as the technique of choice for the management of symptomatic pancreatic fluid collections, due to a lower morbidity compared to surgery and percutaneous methods, and similar efficacy as surgery. Surgery remains important in the overall strategy and will have to be considered in the event of complications or unsuccessful endoscopic drainage.
Indications and criteria for drainage:

Indications for drainage: 1) Symptomatic pseudocysts, such as pain and mechanical obstruction of the gastric outlet or biliary system; 2) the pseudocyst is larger than 6 cm and continues to increase in size or does not resolve after 6 weeks, in order to avoid subsequent development of complications; 3) presence of infected collections such as pancreatic abscesses and infected necrosis.

Criteria for endoscopic drainage: 1) the presence of a well-defined mature wall; 2) for pseudocysts, a time frame of 4 – 6 weeks is needed; 3) the fluid collection must be accessible endoscopically, such as being located within 1 cm of the duodenal or gastric walls.
Pseudocysts may be adequately treated by transenteric drainage. Pancreatic necrosis will require more aggressive endoscopic necrosectomy. To prevent recurrent collections, pancreatic duct disruptions, fistulas and strictures require treatment.

Rationale for EUS-guidance

1. EUS-guided endoscopic drainage is less invasive than surgery, and does not require general anesthesia.
2. EUS-guided drainage can avoid local complications related to percutaneous drainage. Because the endoscope is just adjacent to the fluid collection, it can have direct access to the fluid cavity, unlike percutaneous drainage which traverses the abdominal wall. Complications such as bleeding, inadvertent puncture of adjacent viscera, secondary infection and prolonged period of drainage with resultant pancreatico-cutaneous fistula may be avoided.
3. Non-EUS-guided endoscopic drainage is a blind procedure and the presence of endoscopic bulging is a prerequisite. The fluid collection is punctured at the site of maximum endoscopic bulging. There is a potential risk of hemorrhage from interposed vessels during transmural drainage. With EUS-guidance, the fluid collection is visualized during the entire puncture process, and endoscopic bulging is not necessary. One may potentially decrease the bleeding rate by avoiding interposed blood vessels through the use of Doppler ultrasound.
4. Endoscopic ultrasound can also differentiate a pseudocyst from a cystic tumor, and ascertain the nature of a fluid collection and guide the drainage strategy; for instance, a pseudocyst may be treated by placing transmural stents, whereas a necrotic collection requires additional endoscopic debridement.

Technique of endoscopic drainage:

1. *Transenteric drainage:* Under EUS guidance, the cavity was punctured using a 19G EUS-FNA needle. A guidewire is inserted through the needle into the cavity under fluoroscopic guidance, forming at least two loops before withdrawing the needle. The puncture site is dilated using a balloon catheter to a diameter of 6 – 8 mm and a double pigtail transmural (8.5 to 10F size) stent is inserted for drainage. When there is a need to perform irrigation for treatment of sepsis or prevention of secondary infection, a nasocystic catheter is inserted. This was removed once sepsis had resolved. When multiple stents or a nasocystic catheter are required, repeated cannulation of the cavity, or double wire techniques can be used.
2. *Endoscopic necrosectomy:* In patients with infected walled off necrosis, or significant solid debris within an abscess collection, endoscopic necrosectomy is performed. This is not performed during the index endoscopy. The cavity is cannulated with a catheter and guidewire, and balloon dilatation (CRE™, Boston Scientific, Natick, MA, USA) of the enteric-cystostoma is performed. Stepwise dilatation is performed at 1 – 2 days intervals up to a diameter of 18 mm. A pediatric or standard gastroscope is then inserted into the cavity, and gentle debridement using saline lavage and aspiration, baskets, soft snares and retrieval nets is performed, until all necrotic debris are removed.

Results of endoscopic drainage:
High treatment success rates exceeding 91 % have been achieved for pseudocysts. Although the data on abscess drainage are more limited than pseudocyst drainage, similarly high treatment success rates ranging from 80 % to greater than 90 % have been reported. The results are poorer for infected walled-off pancreatic necrosis and may be as low as 25 % if there is no adjunctive endoscopic necrosectomy. With adjunctive endoscopic necrosectomy when feasible, the treatment success rates may be improved to 70 – 80 %.

Celiac plexus neurolysis: European Perspective (I. Penman): EUS-guided celiac plexus block (CPB) or neurolysis (CPN) is a simple, well-established technique. Using curved linear array endosonography, the celiac axis is traced from its origin from the aorta and with care celiac ganglia may be identified. After confirming landmarks and excluding intervening vessels, a long-acting local anaesthetic (0.25 % bupivicaine) is injected along with triamcinolone (for CPB) or dehydrated alcohol (for CPN). Injection is performed using a 22 g or 19 g needle just anterior and cephalad to the origin of the celiac artery. Patients must be monitored post-procedure for pain, hypotension, fever or leg weakness. Two recent meta-analyses report successful pain relief in 72 – 80 % of patients with malignancy and in 51 – 59 % of chronic pancreatitis patients. Minor complications are common (5 – 20 %) and include transient worsening of pain, mild fever and diarrhoea but major complications e.g. retroperitoneal haemorrhage or abscess are rare (0.5 – 1.0 %). Recent studies have tried to improve on these results especially in patients with chronic pancreatitis. Improved outcomes following bilateral injections have not been confirmed in all studies. Reports of identification and direct targeting of injections into celiac ganglia appear promising and case reports have shown the feasibility of extending injections over the region of the superior mesenteric artery. Finally, whether results would be better if CPB/CPN was performed earlier in the patient's illness or with repeated injections is unknown and worthy of further study.

EUS-guided celiac plexus neurolysis and celiac ganglia neurolysis – Japanese view (Ichiro Yasuda):
Summary
EUS-guided celiac-plexus neurolysis (EUS-CPN) is an effective therapeutic procedure for alleviating upper abdominal pain due to pancreatic cancer or chronic pancreatitis. Two different ap-

proaches are currently used when applying EUS-CPN. The "bilateral procedure" is reportedly more effective than the classical, "central procedure," whereas the latter is easier and potentially safer than the former. Moreover, it was recently established that celiac ganglia can be examined and visualized by EUS. Therefore, EUS-guided, direct celiac-ganglia neurolysis (EUS-CGN) has been introduced as a new promising method, and is expected to be more effective than EUS-CPN.

Celiac plexus

The celiac plexus surrounds the celiac axis (CA) and the superior mesenteric artery (SMA) as it originates from the anterior of the abdominal aorta. This plexus contains several ganglia and the interconnecting neural rami. It is responsible for transmitting pain sensations originating from the upper abdominal organs, including the pancreas, liver, gallbladder, stomach, and ascending and transverse colons.

History of celiac-plexus neurolysis

Celiac-plexus neurolysis (CPN) disrupts the transmission of pain signals from afferent nerves to the spinal cord via a neurolytic agent injected into the celiac plexus. It was initially described as an intraoperative procedure by Kappis in 1914. Since then, it has been performed under the guidance of radiographic, fluoroscopic, computed tomographic, or ultrasonographic imaging. Later, the endoscopic ultrasound-guided procedure (EUS-CPN) was introduced by Wiersema in 1996.

EUS-CPN

EUS-guided procedures have several advantages over other classical approaches. They are highly accurate, safe, and convenient if performed under real-time-imaging guidance and with Doppler assessment of the interposing vessels.

The primary indication for EUS-CPN is pain associated with pancreatic cancer. Pain is experienced by 30 – 60 % of pancreatic cancer patients in the early stages where the cancer is relatively limited. However, the occurrence and severity of pain increase with cancer progression. More than 80 % of patients in the advanced stages of pancreatic cancer experience pain. Therefore, pain control is a major challenge in the management of pancreatic cancer patients.

Another major indication of EUS-CPN is chronic pancreatitis. However, in this case, a steroid is usually injected instead of a neurolytic agent (e.g., absolute ethanol or phenol) to treat the associated pain. This procedure, EUS – celiac-plexus block (EUS-CPB), differs from EUS-CPN.

The EUS-CPN procedure

Two approaches are used when applying EUS-CPN. The classic approach, known as the central procedure, involves injection of a neurolytic agent at the base of the CA. In the second approach, the bilateral procedure, the neurolytic agent is injected bilaterally into the CA.

Central procedure

Initially, the abdominal aorta is visualized on the EUS images through the posterior wall of the upper gastric body, in the longitudinal plane. The aorta is then traced to locate the CA. Subsequently, a needle is introduced and advanced to the area just superior to the aortal origin of the CA. Absolute ethanol is then injected into this region until a resultant echogenic cloud appears and spreads sufficiently.

Bilateral procedure

In this approach, the aortal origin of the CA is first identified. Then, the echoendoscope is rotated clockwise until the CA and SMA are no longer visible. A needle is then advanced leftward, alongside the CA and SMA, up to an area just lateral to the aortal origin of the SMA. Absolute ethanol is injected into this region. Next, the needle is withdrawn and the echoendoscope is rotated counterclockwise until the CA and SMA are no longer visible. A second needle is then advanced to the right lateral base of the SMA, and absolute ethanol is injected once again.

Efficacy of EUS-CPN

In an initial evaluation by Wiersema, 79 – 88 % of patients showed a long-lasting improvement in their pain scores, whereas 82 – 91 % of patients required the same or less pain medication. Recently, Puli et al. published a meta-analysis and a systematic review . They collected data on pancreatic cancer from 8 different reports (N = 283), 4 of which were full-text articles and the remaining 4, abstracts. Nine reports (N = 376) on chronic pancreatitis were also reviewed. These included 3 full-text articles and 6 abstracts. Accordingly, 80.12 % (95 % CI = 74.44 – 85.22) of pancreatic cancer patients and 59.45 % (95 % CI = 54.51 – 64.30) of chronic pancreatitis patients treated by EUS-CPN showed pain alleviation. Of all the patients assessed in the above studies, complications were noted only in two, who suffered diarrhea and were subsequently treated with anti-diarrheal medications.

Recently, there have been 2 other studies reporting the complications associated with EUS-CPN and EUS-CPB. O'Toole et al. reported an overall complication rate of 1.6 % and 3.2 % in 189 EUS-CPB and 31 EUS-CPN patients, respectively. They reported 1 case of asymptomatic hypotension following EUS-CPN, 2 cases of severe, self-limiting post-procedural pain, and 1 case of retroperitoneal abscess following EUS-CPB. In another study, Sakamoto et al. studied 13 EUS-CPN-treated cases and reported 1 case of transient drunkenness, 2 cases of transient hypotension, and 1 case of transiently increased pain.

Puli et al. also compared the treatment efficacy between the 2 patient subgroups treated by the bilateral and unilateral procedures. The rate of pain relief was much higher in pancreatic cancer patients treated with the bilateral procedure (84.54 %; 95 % CI = 72.15 – 93.77) than in those treated by the central procedure (45.99 %; 95 % CI = 37.33 – 54.78). Sahai et al. assessed the short-term safety and efficacy of central and bilateral EUS-CPN/EUS-CPB in 160 patients (71 treated centrally, 89 treated bilaterally). The mean pain reduction score was 70.4 % in patients treated bilaterally versus 45.9 % in those treated centrally ($P = 0.0016$). A positive response (> 50 % reduction in pain score) was also significantly more frequent in the bilaterally treated group (77.5 %) than in the centrally treated group (50.7 %) ($P = 0.0005$). The only predictor of a positive response was the use of the bilateral procedure (odds ratio = 3.55; 95 % CI = 1.72 – 7.34). Only 1 complicated case was reported that – of patient under anticoagulant treatment who developed self-limiting retroperitoneal bleeding following laceration of the left adrenal artery after bilateral CPB. These results suggest that the bilateral procedure is more effective than the central procedure, but the former is potentially easier and safer than the latter approach.

Identification of celiac ganglia by EUS

Although EUS-CPN is a highly effective approach, it sometimes failed to alleviate pain. This was because the neurolytic agents were injected at the probable vicinity of the celiac ganglia, which

were difficult to locate by EUS-CPN. This limited the efficacy of EUS-CPN in relieving pain effectively.

However recently, Gerke et al. and Levy et al. reported that the celiac ganglia can be visualized precisely using EUS-guided procedures. Similarly, Gleeson et al. could identify celiac ganglia in 81% of their study patients (162/200 patients). We also investigated the frequency with which celiac ganglia were successful visualized. We thus identified the celiac ganglia in almost all of our patients (78/79 consecutive patients). Most frequently, the celiac ganglia are located to the left of the CA, between the aorta and the left adrenal gland, at the level between the CA and the left renal artery. They are also located cephalad to the CA in some cases. The ganglia appear as hypoechoic masses of various forms, including caterpillar-like and nodular, and often exhibit hypoechoic connections, representative of the adjoining neural rami.

EUS-guided celiac-ganglia neurolysis

More recently, a new procedure involving the direct puncture and injection of a neurolytic agent or a steroid into an individual celiac ganglion was introduced by Levy et al. The above procedure, called EUS-guided celiac-ganglia neurolysis (EUS-CGN) or block (EUS-CGB), may be safer and more efficacious than EUS-CPN, because it allows for precise delivery of neurolytic agents into an individual celiac ganglion. Indeed, initial evaluations showed a high rate of success with this procedure. Pain relief was achieved in 16 of 17 (94%) pancreatic cancer patients treated by EUS-CGN. in the case of chronic pancreatitis, 80% (4/5) of those who received alcohol injections reported pain relief, versus 38% (5/13) of those who received steroid injections. Thirteen (34%) patients experienced initial pain exacerbation but subsequently achieved an improved therapeutic response. Transient hypotension and diarrhea developed in 12 (33%) and 6 (17%) patients, respectively. However, the authors concluded that prospective trials are necessary to confirm the therapeutic efficacy of this method, because their study involved a small sample size.

The EUS-CGN procedure

Generally, 2 or 3 celiac ganglia (range, 1 – 6) can be visualized by EUS. After a celiac ganglion has been identified, a needle is advanced to puncture the ganglion, and absolute ethanol is injected. The needle tip is advanced toward the center of the ganglion in case the observed ganglion is relatively small (smaller than 8 mm within the axis of the needle plane). For any ganglion larger than 8 mm in the needle plane, the needle tip is advanced deeply within the ganglion. Thereafter, absolute ethanol is injected as the needle is slowly withdrawn. The injected ganglion becomes hyperechoic and difficult to visualize after the injection. To ensure effective blockage, this procedure is repeated until identification of all ganglia becomes difficult. The volume of injected ethanol is usually 2 ml for small ganglia and 3 – 5 ml for relatively large ganglia.

Conclusions

EUS-CPN is a safe and effective method for reducing pain due to pancreatic cancer. The bilateral procedure is more effective than the central procedure, although the latter is easier and possibly safer than the bilateral procedure. More recently, visualization of the celiac ganglia was confirmed by EUS. The initial evaluation of EUS-CGN suggested that it is more effective than EUS-CPN. However, further studies are necessary to confirm this possibility.

EUS-Guided Biliary Drainage – US Experience (K. Chang): For the Interventional endoscopist and endosonographer, EUS-guided biliary drainage is emerging as an important salvage technique for failed ERCP drainage. Although the success rate of deep cannulation is high (98%) with the use of wire guided cannulation and precut sphincterotomy techniques, it is still not perfect and some patients require percutaneous transhepatic biliary drainage (PTBD) or surgical intervention due to technical failure or altered anatomy. However, both PTBD and surgical intervention are associated with considerable morbidity and occasional mortality. EUS-guided approaches have the advantage of direct needle access into the intra and extra-hepatic biliary system from the stomach and duodenum. The ideal EUS approach would achieve the following criteria: 1) immediate biliary drainage 2) leak-free anastomosis 3) circumvents anatomic alterations and 4) creates a long-term device-free fistula. All current and future techniques and devices should be assessed against these criteria.

EUS-guided Rendez-vous (RV) technique for wire insertion: Since the first report of EUS-guided access to the bile duct in 1996, there have been a number of small case series describing the EUS-RV technique to facilitate guide wire insertion for re-attempt at therapeutic ERCP. EUS-RV can be divided into intrahepatic bile duct (IHBD) and extrahepatic bile duct (EHBD) approaches in terms of access to the biliary tree. The over-all success rate of EUS-RV from a recent small series was 80%. Unless there is altered anatomy necessitating the IHBD approach, we generally prefer the EHBD approach from the duodenum, if anatomically and technically possible, because the short distance from the punctured biliary site to the obstruction allows better maneuverability of the guide wire and the larger caliber of the biliary duct allows for easier targeting.

EUS-guided Hepatico-gastrostomy (EUS-HG) and Choledochoduodenostomy (EUS-CD) for Biliary Drainage (BD): Once a wire is passed by EUS approaches, definitive drainage of the obstructed biliary system (in situations of biliary stricture or altered anatomy) via EUS is appealing. This is accomplished by either the EUS-GH or EUS-CD routes with either plastic or metal stents. Although plastic stents have become the standard approach, stent malfunction owing to stent clogging after EUS-BD can be a problem, with a high rate of re-intervention. Of concern, has been the overall rate of procedure-related complications, such as pneumoperitoneum or bile leakage, which can be as high as 19% in extrahepatic approaches using plastic stents. For this reason, many experts advocate EUS-HG if using a plastic or partially covered stent. Most recently, fully covered metal stents have been used. At this meeting, the group of Park et al. has reported an abstract of a larger multi-center trial comparing the efficacy and safety of EUS-BD vs Percutaneous (PTBD) after failed ERCP among 111 patients (see abstract part of this report).

EUS-guided Compression Devices for Choledochoduodenostomy: For EUS-guided biliary drainage techniques, the still unresolved major concern is the complication of bile leak with subsequent bile peritonitis. Although it appears that biliary access with subsequent drainage is largely achievable, unless we can achieve a predictable leak-free anastomosis between the biliary system and the gut lumen, we still fall short of the ideal solution. To this end, various techniques have been explored, including compression balloons, compression magnets and compression coils. In this meeting, there is a canine survival report entitled "EUS-guided Choledocho-duodenostomy (ECD) for immediate and long-

term treatment of biliary obstruction using prototype PathCreator(TM) compression coil and twin-headed needle" (see our abstract in the abstract section). In 4 dogs, our ECD was accomplished using a prototype coil delivery device (19 g needle preloaded with stretched coil in the lumen, Olympus Medical Systems Corp). EUS-guided needle puncture into the dilated CBD was followed by deployment of 50 % of the coil into CBD, and remaining 50 % stayed within the duodenal bulb to hold CBD and duodenum walls tightly with its compressive force. A 15 g twin-headed needle was used to create a transmural hole through the center of the compressed coil for immediate biliary drainage. All animals survived per protocol without complications. Immediate drainage was successful in 3 /4 (determined by immediate decrease and subsequent normalization of serum bilirubin), with over-all drainage (normalization of bilirubin) successful in 4 /4. Creation of a chronic fistula between CBD and duodenum was achieved in all 4 dogs with no evidence of bile leak or perforation. Such devices may get us closer to the ideal solution.

EUS-Guided Biliary Drainage – Japanese Experience (K. Yamao): Endoscopic ultrasonography (EUS) is the combination of endoscopy and intraluminal ultrasonography. This allows imaging with a high frequency transducer from a short distance, to generate high resolution ultrasonographic images. EUS is now a widely accepted modality for the diagnosis of pancreatobiliary diseases. EUS-guided fine needle aspiration (EUS-FNA) using a curved linear array echoendoscope was initially described over 20 years ago, and since then many researchers have expanded its indications to sample diverse lesions, and also for a variety of therapeutic purposes. EUS-guided biliary drainage (EUS-BD) is one of the therapeutic procedures which have been developed using a curved linear array echoendoscope. Technically, EUS-BD includes rendezvous technique via transesophageal, transgastric and transduodenal route, and EUS-guided direct access methods including choledochoduodenostomy (EUS-CDS), and EUS-guided hepaticogastrostomy (EUS-HGS). Published data has demonstrated a high technical and treatment success rate, albeit with a comparatively high rate of non-fatal complications for EUS-CDS and EUS-HGS, and a comparatively low technical success rate with a low complication rate for the rendezvous technique. At present, these procedures are applicable as an alternative to surgery or percutaneous transhepatic biliary drainage (PTBD) for patients with obstructive jaundice, when endoscopic biliary drainage (EBD) has failed. However, these procedures should be performed in centers with extensive experience in linear EUS and therapeutic biliary ERCP. Large prospective studies to establish the standardization of EUS-BD procedures as well as controlled comparative trials between EUS-BD versus PTBD, rendezvous technique versus direct access technique (EUS-CDS and EUS-HGS), and EBD versus EUS-BD are needed in the near future.

EUS-Guided FNI: Fine Needle Injection & Fine Needle Imaging (K. Chang): EUS-guided Fine Needle Injection (FNI) is an important component of Interventional EUS. EUS-guided FNI includes injection of anesthetic agents for pain control (celiac ganglion neurolysis), placement of fiducial markers and radioactive seeds (brachytherapy), and delivery of anti-tumor agents. An exciting new technology is EUS-guided fine needle imaging, which allows real-time in-vivo imaging of cells and blood flow within cystic and solid lesions/organs through an FNA needle.

EUS-guided implantation (Brachytherapy and Fiducial Markers): EUS-guided brachytherapy has been reported in recurrent esophageal cancer and primary unresectable pancreatic cancer. Iodine (I^{125}) and palladium (Pd^{103}) radio-isotopes have been used. Potential indications will be discussed. EUS-guided fiducial markers will have increasing demand in the context of image guided radiation therapy (IGRT). Future development for fiducials will need to include: multi-marker deployment device, and different shape markers such as wires or coils.

EUS-guided fine-needle injection of antitumor agents: A number of preliminary clinical trials have been reported on EUS-guided FNI of anti-tumor agents into the pancreas. A single center survival analysis of 29 patients with locally advanced pancreatic cancer patients who underwent EUS-guided FNI of TNFerade (along with chemo/XRT) vs standard of care (chemo/XRT) showed a survival advantage with the addition of TNFerade (14.7 mo vs 11.1 mo; p = .02). EUS-guided FNI has also been applied to esophageal cancer. A most recent 5 year follow-up analysis of a study using TNFerade (along with chemo/XRT) among 24 patient with locally advanced esophageal cancer showed a median survival of 48 months. While these preliminary studies are encouraging, larger clinical trials are necessary to evaluate the treatment efficacy of this technique. At this point, the efficacy will largely depend on the therapeutic agent rather than refinement of the administration technique. That being said, future development of FNI needles specifically suited for administration of antitumor agents under EUS guidance is important. Such needle should be of sufficient caliber (especially if delivering live cells) and able to deliver drug in a uniform, sterile and diffuse fashion.

EUS-guided fine-needle imaging: Endoscopic probe-based Confocal Laser Endomicroscopy (pCLE) enables in-vivo real time imaging of cells and blood flow within the mucosal layer of the GI tract. Recently, a prototype high-resolution CLE probe has been developed (Cellvizio® Mauna Kea Technologies) which can be introduced through a 19 g FNA needle creating the possibility for needle-based Confocal Laser Endomicroscopy (EUS-guided nCLE). In this meeting, Shinoura et al reports on the feasibility and quality of images obtained using EUS-guided nCLE in solid organs (live porcine) such as the pancreas, liver, spleen, gallbladder and lymph node with correlation to normal histology (Shinoura et al, EUS 2010). Images were rated as fair to excellent and histological features could be identified in all nCLE images.

EUS-FNI for treatment of pancreatic cancer (A. Irisawa): Interventional EUS is widely performed for not only tissue diagnosis but also treatment in patients with abnormalities of various organs. EUS-guided fine needle injection (EUS-FNI) as the treatment has expanded the clinical utility of EUS. Anti-tumoral EUS-FNI, with its minimally invasive access for anti-tumoral agent delivery, is the most exciting field of interventional EUS. Several applications of EUS-FNI for anti-cancer efforts have included drug delivery into the tumors, such as ablation using ethanol, chemotherapy, gene therapy, and cytoimplantation. These procedures are divisible onto 3 categories based on the associated therapeutic mechanism, physicochemical therapy, molecular biological therapy, and immunological therapy. Since Chang et al. firstly reported EUS-FNI for advanced pancreatic cancer in 2000, various anti-tumoral agents have been injected directly into tumors. Recently, we performed EUS-FNI using dendritic cells (DCs) for cancer treatment as immunotherapy. Actually, DCs are potent antigen-presenting

cells for induction of primary T-cell-dependent immune response. When injected intratumorally, DCs acquire and process tumor antigens in situ, migrate to regional lymphoid organs, and initiate a strong tumor-specific immune response. Seven patients with metastatic disease and/or locally advanced pancreatic cancer who had previously been treated unsuccessfully with gemcitabine were undergone EUS-FNI of DCs into pancreatic cancer. The result was tolerable (median survival period was 9.9 months) nevertheless the objective patients were resistant to gemcitabine. Demonstrably, EUS-guided intervention has opened new and exciting clinical applications for the management of malignancies. For greater development in this field, it is anticipated that endosonographers, basic scientists, and engineers will collaborate fruitfully with much greater mutual effort.

EUS-Guided Implantation Therapy (Z. Jin): The values of EUS have been expanded from diagnosis to the treatment area. Because the resolution of EUS in local anatomy is better, and the puncture path has many advantages, EUS-guided implant treatment has been carried out and is demonstrated with broad application.

Radiotherapy Position: HK Kim et al reported the use of EUS-guided implantation of gold particles in abdominal radiation. The previous positioning of radiation required CT-guided percutaneous placement of some location marks. The process was often difficult because it was necessary through some abdominal organs, important ducts and vessels, etc. Under the guidance of EUS, some gold particles were implanted in the pancreas and liver tumors with 19-gauge fine needle. All cases were successful with no complications. EUS-guided implantation of gold particles is a more convenient and effective way of locating.

EUS-guided iodine 125I implantation in pancreatic cancer: The radioactive seeds recommended in brachytherapy are iodine-125, iridium-192 or palladium-103. Compared with the later two sources, iodine-125 has a longer half-time of 59.7 days, which is appropriate in targeting the rapidly growing tumor such as pancreatic cancer. Iridium-192 is always introduced in in brachytherapy for gynecological malignancies such as endometrial cancer, with a similar survival rate as the external beam radiotherapy. Palladium-103 has been widely accepted as a standard particle in brachytherapy for prostate and breast cancers. Iodine-125 source is Na125I, and the package is a titanium alloy tube sealed by laser. Each seed source is 4.5 mm in length and 0.8 mm in diameter, with a mean photon energy of 27 – 35 KeV gamma ray, an initial dose rate of 7cGy/h, and a mean radioactivity of 0.694 ± 0.021 mCi (25.6 MBq). As mostly concerned, the penetration distance in the human tissue for each seed is only 1.7 cm, which allows localizing the energy inside the tumor instead of irradiating the surrounding organs. For the same reason, the implanted seeds are harmless to the patient's relatives. The potential harm to the operators can be minimized by adequate shielding.

The most notable feature of radioactive seeds is the low dose rate. The radiation of low dose rate can maintain enough radiation dose in target while minimizing damage to surrounding normal tissue. About how to choose the right radioactive sources, some experts considered that Pd103 I125 is more suitable than others in pancreatic cancer through theoretical analysis. Currently, the most common radioactive seeds was 125I in clinic. When the doubling time of pancreatic cancer cell was short, it was difficult to reach the adequate treatment dose in a short time after implantation. The effects of treatment of radioactive particles may also be less than ideal. At this time, external beam radiotherapy or external beam radiotherapy combined brachytherapy should be chose.

Before EUS-guided radioactive seeds implantation could be applied in human tumor treatment, animal studies were necessary to confirm the safety and to simulate the protocol. Due to the resemblance of anatomy and physiology to human, pig becomes the most suitable animal to build up the model. Under EUS, the maximal diameter of the tumor is measured by real-time sector ultrasound and the relationship between the surrounding vasculature and the tumor is then identified. The puncture points should be determined by color Doppler technology to prevent the injury to the pancreatic duct or the vessels.

By now the only available two clinical trials on EUS-guided brachytherapy came from China including our group. The number of patients enrolled in these two studies was 15 and 22, respectively, with stage III or IV pancreatic cancer in a majority of cases. Estimated median survival time of 10.6 months (27 % of patients reached partial tumor response) with a mean 22 seeds load per patient. Procedure-related pancreatitis or pseudocyst was only found in three patients, which was considered mild and easily managed. With the combination of gemcitabine, our group further evaluated the clinical efficacy and safety of EUS-guided interstitial implantation of radioactive iodine-125 seeds in advanced pancreatic cancer. Although the novel technique did not significantly improved the overall survival rate, it showed an estimated median survival time of 9.0 months, with a partial remission rate of 13.6 % and an estimated one-year survival rate at 27.3 %. Moreover, the visual analogue scale pain score significantly dropped from 5.07 to 1.73 one week after brachytherapy and maintained for one month. Therefore, these two reports show promising preliminary data that pancreatic cancer can be treated safely with EUS-brachytherapy. Additional larger studies are needed to establish this as an acceptable option for inoperable pancreatic cancer.

Following the proved safety and feasibility in human, next upcoming randomized controlled trials with long-term follow-up are expected to evaluate the efficacy between single EUS-guided implantation and single standardized chemotherapy. It would also be of interest to compare the efficacy and tolerability between EUS-guided brachytherapy and conventional external beam radiation.

EUS-guided celiac ganglion radiation for pain relief: Partial pain relief can be achieved simultaneously when the radioactive seeds release the tumor-killed ray inside the tumor. It is the physician's duty to retrieve the patient from the unbearable pain which leads to the poor living condition accompanied within the rest of the survival life. Celiac plexus neurolysis (CPN) and celiac plexus block (CPB) have been considered the first-line adjuvant therapies for the treatment of pain in pancreatic cancer patients. However, CPN can only relieve the pain in a limited degree, lasts a short period, and the analgesic effect is inversely correlated with the extent of invasion of celiac ganglia. EUS-guided brachytherapy with the implantation of iodine-125 seeds beside the celiac ganglion seems to be another choice for the pain relief. In a recent animal study, compared with the control group, neuronal apoptosis in the ganglion was seen in both brachytherapy groups, and the intensity of necrosis increased with the radiation dose increasing. There were no significant complications during the experiment. This is the first preliminary evidence for the feasibility

and safety of EUS-guided celiac neuron brachytherapy. The new technique may introduce an alternative treatment for pain accompanied pancreatic diseases in human. However, iodine-125 seeds, which own a long period of decay and thus may lead to the maintenance of analgesia, cannot reach a rapid effect as CPN or CPB. More animal and clinical trials are needed to determine whether celiac ganglion radiation is superior to classic CPN, and whether the technique can be applied eventually in clinical practice.

We reported iodine-125 seeds under EUS guidance which were implanted beside the celiac ganglion in 15 patients with 0.7mCi (group 1) and 15 were treated with pharmacological therapy (group 2). Immediate and long-term efficacy, mean analgesic consumption, mortality and morbidity were evaluated. A mean number of four radioactive seeds per patient were implanted into the celiac ganglion. Immediately after the precedure, pain relief and analgesic consumption did not differ between two groups. Inversely ,three patients reported pain exacerbation .But 10 days latter, patients in group 1 reported significant pain relief compared with those in group 2. Mean analgesic consumption was lower in group 1. There were no deaths. No complications such as transient diarrhoea, hypotension or infection occured. Drug-related adverse effects were constipation (6 of 15 patients in group 1 versus 13 of 15 in group 2), nausea and/or vomiting (four of 15 patients in group 1 versus 12 of 15 in group 2).

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The efficacy of radioactive seeds implantation: EUS-guided radioactive seeds implantion has been found to be effective that may improve quality of life and survival in patients with advanced unresectable pancreatic cancer. The evaluation of it's effects remains a challenging clinical problem. Liu et al. reported the evaluation value of fuzzy classification of EUS texture features in radioactive seeds-implanting treatment in pancreatic cancer. The seeds treatment effect was evaluated through comparing the probability of cancer about before-treatment and after-treatment cases. 22 texture features are extracted according to the digital image processing algorithm. 216 EUS images are used as the train set and test set to perform several random experiments. Then the other 360 EUS images of 25 patients including before-treatment and after-treatment states are classified fuzzily according to the preceding best training results. Then the tumor's volumes' change, CA19 – 9's < variation, and variation of probability of cancer (both before-treatment and after-treatment) are comparing to patients' survival time (whether more than 3 months). 19 patients' variation of probability of cancer vary with the patients' survival time (whether more than 3 months), which is much higher than the rates of volumes' change (14 patients in 25) and CA19 – 9's variation (9 patients in 25).

Problems and future: The common radioactive seeds in clinic have the size limitation, which is approved by SFDA and FDA. This has resulted in the difficulties in implanting seeds in pancreatic head and uncinate process, where EUS endoscopy was bended over in site. The radioactive seeds is relatively difficult to go through the fine needle. However, in the pancreatic neck, body and tail lesions, the operation is relatively easy. The development of new radioactive particle with smaller size, even for radioactive liquid is a follow-up research of EUS-guided implantation.

Although the exact seed number needed in the therapy can be calculated by the three-dimension computer system, the excise model for EUS-guided implantation has not been set up yet. But under EUS guidance, the evenly arrangement of radioactive seeds in the three-dimensional space is very difficult or even impossible. Therefore, the development of a new treatment planning system based on ultrasound image is very important.

Mediastinum – EUS FNA

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EUS Staging of Lung Cancer (M. B. Wallace): Although the age-adjusted incidence of lung cancer has been decreasing in those countries where smoking cessation efforts have been successful, the disease has acquired epidemic proportions worldwide. In Asia the lung cancer burden will be increasing rapidly adding to the other public health challenges some nations in this region are already facing. The majority (up to 80 %) of new cases will be non-small cell lung cancer. Evidence-based treatment strategies for lung cancer require as a prerequisite, accurate staging with an apparently bewildering choice of non-invasive, minimally-invasive and invasive staging methods. Over the last decade endoscopic ultrasound guided FNA of mediastinal lymph nodes (EUS-FNA) has been established as a valuable adjunct to the diagnosis and staging of lung cancer with numerous publications attesting to that fact. More importantly, EUS-FNA combined with the recently developed endobronchial ultrasound guided FNA (EBUS-FNA) may allow complete minimally-invasive mediastinal staging at a lesser initial cost to patient and society than other traditionally employed methods. Furthermore, both methods combined can help avoid futile thoracotomies leading to further savings and a decrease in treatment related morbidity.This review will recent developments in the field, highlight current controversies and present an outlook for the future.

Value of endobronchial ultrasound-guided transbronchial needle aspiration in hilar and mediastinal lesions (G. Wang): Introduction: Endoscopic ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a new endoscopic technique which developed in recent years, the original endoscope enabled the doctors only observe the inside of trachea, bronchus, while EBUS-TBNA further extended to the outside of bronchial wall, it has a high value and some advantages in the diagnosis of intrathoracic lesions. The indications for EBUS-TBNA as follows: (1) diagnosis of lung cancer; (2) N-stage for lung cancer patients; (3) the diagnosis of unknown hilar and (or) mediastinal lymph nodes; (4)the diagnosis of mediastinal tumors. In 2003, Denmark Krasnik first reported this technique used for mediastinal and hilar lesions, then, the technology is widely used in foreign countries. In 2008, EBUS-TBNA was introduced to domestic, only until now, only several hospitals have carried out this technology. Our hospital began to develop this technology form September 2008, this report have summarized its clinical application and evaluated the role of EBUS TBNA in the diagnosis of intrathoracic lesions.

Methods: Including criteria Cases were enrolled in the study from September 2008 to September 2010. Including criteria as follows: (1) undiagnosed pulmonary lesions with hilar and/or mediastinal lymph enlargement by bronchoscope; undiagnosed central lung cancer without lymph node enlargement by bronchoscope. (2) staging of lung cancer which may change the treatment plan; (3) Lymph nodes outlined in patients with an undiagnosed primary lesion suspicious. (4) diagnosis of an undiagnosed mediastinal lesion. Exclusion criteria: Case with superficial lymph nodes enlargement can be diagnosed by biopsy of superficial lymph nodes. A total of 255 patients with intrathoracic lesions underwent EBUS TBNA, 150 men, 105 women, aged from 24 ~ 80 years, mean age 54 years. Including 134 intrapulmonary

tumors, 15 lymph node staging in lung cancer patients; 82 unknown hilar and/or mediastinal lymphadenopathies and 24 mediastinal tumors.
Endobronchial ultrasound: Endobronchial ultrasound (EBUS-TBNA) examinations were done with the linear scanner (EU-C2000, BF-UC260F-OL8, Olympus, Tokyo, Japan) and aspiration was done with a 22-G needle (NA-201SX-4022, Olympus, Tokyo, Japan). The scope is a flexible video bronchoscope integrated with a convex 7.5 MHz ultrasound transducer located at the distal end of the endoscope in front of a 30 degree oblique forward viewing fiber-optic lens (angle of view is 80 degree), that makes it possible to visualize mediastinal and hilar structures. The ultrasound picture is obtained by a waterinflated balloon to facilitate air-free contact with the bronchial wall. The size of lesions can be measured in two dimensions and vascular structures can be identified with Doppler mode. The endoscopy has a biopsy channel of 2 mm which makes it possible to introduce a needle into most peribronchial targets and aspirate material under real-time visual control.

Patients: Blood routine and Coagulation function should be examined within a week. Enhanced CT was done within one month. All patients signed informed consent. Patients were usually given 1~2 mg midazolam intravenous and were monitored for ECG, pulse oximetry and blood pressure without the presence of an anaesthesiologist. If Patients feel discomfort such as anxiety, nausea or resist during the produce, additional 1 mg midazolam could be given, but make sure the maximum dose does not exceed 5 mg.

Procedure: EBUS-TBNA was conducted with the transducer in direct contact with the wall of the trachea or bronchus. When a lesion was outlined, a power doppler examination was carried out immediately before the biopsy to prevent unintended puncture of vessels between the wall of the bronchi and the lesion. Then the needle was introduced via the biopsy channel of the endoscope. Under real-time ultrasonic guidance, the needle was placed in the lesion, and the stylet was then removed. Suction was applied with a syringe, and the needle was moved back and forth inside the lesion. The specimen was expelled into a tube containing cytolyt, precipitating for a moment, gathering the sediment specimen onto a filter paper, embed with plastic, then fixed in 10% formalin solution for histopathology. Un-sediment specimen left in the tube for cytology. The specimen form the last aspiration was expelled onto glass slides and smeared, fixed in 95% alcohol for cytological examination. No cytopathologist was present during the procedure. After EBUS TBNA, patients should stay in hospital for 24 hours without drinking or eating, using antibiotics and hemostatic for one day to avoid infection and bleeding, then follow up for 1 week to observe complications.

Statistics anylasis: Cytology or histopathological diagnosis proved to be positive lymph node puncture. The result of surgery, mediastinoscopy or cervical lymph node biopsy followed by EBUS-TBNA was considered as the final diagnosis; All the examinations mentioned above failed to confirm the diagnosis, the clinical diagnosis was as the final diagnosis but with at least 6 months follow-up. According to the following formula sensitivity, specificity, positive predictive value of EBUS-TBNA: sensitivity = a/(a+c), specificity = d/(d+b), positive predictive value = a/(a+b), negative predictive value = d/(d+c); a: true positive cases, b: false positive cases, c: true negative cases, d: false negative cases.
Results: 255 patients with a total of 341 EBUS-TBNA puncture station. Sampled lesion size: mean diameter of mediastinal or pulmonary in length was 4.06 (1.12 ~ 10.30) cm, short diameter at an average of 2.36 (1.1 ~ 9.00) cm; mean diameter of lymph node in length was 3.24 (1.00 ~ 7.32) cm, short diameter at an average of 2.19 (0.98 ~ 5.15) cm. Puncture site lesions: 264 were located in the mediastinum (including 24 mediastinal lesions and 240 mediastinal lymph nodes). 39 hilar, 38 outside airway. The mean number of sampled was 3 (1 ~ 5) needles per patient. The mean procedure time was 23.6 (21.0 ~ 35.5) min.
A total of 255 patients with intrathoracic lesions underwent EBUS-TBNA, 6 cases excluded as unsatisfied samples. 225 had positive results, 18 negative and 6 suspicious cancers. In 134 patients with pulmonary disease, EBUS-TBNA demonstrated 103 malignant tumors, 16 squamous cell carcinoma, 33 adenocarcinoma, 27 small cell carcinoma, 15 unclassified non-small cell carcinoma, 9 unclassified cancer, 1 malignant melanoma, 1 lymphoma, 1 sarcomatoid carcinoma, 14 benign diseases, 5 suspicious cancers, 10 negative case and 2 unsatisfied samples. In 15 lung cancer patients staged by EBUS-TBNA, 13 showed metastasis thus changing the original treatment plan, 2 showed no metastasis. In 82 cases with mediastinal and/or hilar lymphadenopathy, EBUS-TBNA demonstrated 38 malignant tumors, 6 squamous cell carcinoma, 5 adenocarcinoma, 9 small cell carcinoma, 8 unclassified non-small cell carcinoma, 10 unclassified cancer, 1 suspicious cancer, 33 benign diseases, 6 negative cases and 4 unsatisfied samples. In 24 mediastinal lesions, 18 malignant cases, 4 squamous cell carcinoma, 3 adenocarcinoma, 8 small cell carcinoma, 1 spindle cell tumors, 2 unclassified cancer, 6 benign cases. Six suspicious cancers were confirmed, one by CT-guided percutaneous transthoracic needle biopsy, one by mediastinoscopy and 16 by clinical follow-ups. In 2 lung cancer patients with EBUS-TBNA negative, 1 surgical sample showed lymph node metastasis and another no metastasis by PET-CT. 16 negative cases were diagnosed as benign by clinical follow-ups.
The diagnostic sensitivity, specificity, positive predictive value and negative predictive value of EBUS-TBNA were 93%, 100%, 100% and 5.5% respectively.
No complications occurred from EBUS-TBNA. Only some cases with bloody sputum without special treatment and usually go away 2 ~ 3 days later.

Discussion: EBUS-TBNA is conducted with the transducer in direct contact with the wall of the trachea or bronchus. It can be used for diagnosis for intrapulmonary, hilar or mediastinal lesions, as well as staging for lung cancer under real-time ultrasonic guidance. Our study included all the lesions above. EBUS-TBNA provide access to 2R, 2L, 3P, 4R, 4L, 7, 10R, 10L, 11R and 11L. It has reported that the sensitivity, specificity, positive predictive value of EBUS-TBNA is 86% ~ 100%, 92% ~ 100% and 11%~ 97%. In our study, the sensitivity, specificity, positive predictive value of EBUS-TBNA is 93%, 100% and 5.5% respectively.
The assessment of mediastinal lymph nodes and masses is important for both diagnosis purpose and (lung) cancer staging. The sensitivity and specificity of computed tomography (CT) and positron emission tomography (PET) were 57% ~ 82%(CT) and 84%~ 89% (PET), respectively. Compared with CT and PET-CT, EUBS-TBNA has certain advantages. Yasufuku has reported the sensitivity of CT, PET and EBUS-TBNA in the diagnosis of mediastinal and hilar lymph node metastasis were 76.9%, 80.0% and 92.3%, the

specificity was 55.3%, 70.1% and 100%, the accuracy were 60.8%, 72.5% and 98.0%. The sensitivity and specificity of EBUS-TBNA are significantly higher than that of CT and PET. Some lung cancer patients were proved to be lymph node metastasis while was not detected by CT and PET-CT, In addition, some patients may lose the surgery opportunity because of false-positive diagnosed by CT or PET-CT. In this study, in 15 lung cancer patients for N staging, 13 cases were determined with lymph node metastasis by EBUS-TBNA, thus changed the original treatment, so that patients benefit from it.

Mediastinoscopy has so far been the gold standardand. Toloza et al found in a review of 14 studies involving a total of 5687 lung cancer patients that standard cervical mediastinoscopy had a sensitivity of 0.81(range 0.67 ~ 0.92). However, mediastinoscopy requires thoracotomy under general anesthesia, more invisive, more complications, the cost is more expensive, and mediastinoscopy is difficult to show windows and the main pulmonary artery, the posterior mediastinum lesions, which limit it to be used widely. In contrast, EBUS-FNA is a safe, effective, relatively less invisive technique, it plays an import role in diagnosis and staging of lung cancer, unexplained mediastinal mass. No complications has so far not been reported associated with EBUS-TBNA. In this study, the average operating time of EBUS-TBNA was 23.6 min. No complications occurred, which also confirmed that EBUS-TBNA was simple and safe.

Currently, only 10% ~ 30% of clinical doctors use TBNA. Compared with the traditional transbronchial needle aspiration (TBNA), EBUS-TBNA and EUS-FNA are used widely, the main limitations for the use of TBNA is that TBNA can not be performed under real time, and its diagnostic accuracy varies greatly from 15% to 85%. In addition, many factors affect its sensitivity, such as the operator experience, the size and location of lesion, et al.

There are some limitations with EBUS-TBNA. First, NPV of EBUS-TBNA is low. At present, most researchers advocate for mediastinoscopy to be further confirmed when the result from EBUS-TBNA proved to be negative. In this study, 1 patient diagnosed with lung cancer by biopsy was proved to be without lymph node metastasis by EBUS-TBNA, while pathologically confirmed lymph node metastasis after surgery. Therefore, mediastinoscopy will help develop the best treatment plan for puncture negative cases. Secondly, results of EBUS-TBNA depends on experience of the pathologist and cytologist. In addition, whether cytologist on site is directly related to the puncture positive rate. In this study, EBUS-TBNA specimens of 6 cases were not satisfied and could not be analyzed. If a cytologist on-site makes sure satisfied specimen obtained, it will increase puncture success rate.

We conclude that EBUS-TBNA is a specific, sensitive, less invasive and safe diagnostic technique. EBUS-TBNA has play an important role in clinical application, it has attracted great interests to chest physicians and endoscopists. Except for diagnosis and staging, EBUS-TBNA can also be used for the treatment of mediastinal cysts. In addition, the combination of EBUS-TBNA and EUS-guided transesophageal fine needle aspiration biopsy could greatly improve the diagnosis accuracy of intrathoracic.lesions.

New Aspects Outside of Gastroenterology – EBUS TBNA ▼

European experience (F. Herth): A tissue diagnosis of mediastinal nodes is frequently needed for accurate lung cancer staging as well as the assessment of mediastinal masses. Non-invasive imaging techniques such as CT, MRI, PET and PET-CT provide some answers but no tissue diagnosis. TBNA has a high impact on patient management. Unfortunately, TBNA remains underused in current daily practice, mainly due to the lack of real-time needle visualization. The introduction of echo-endoscopes has overcome this problem. Endobronchial ultrasound-guided TBNA (EBUS-TBNA) allows real-time controlled tissue sampling of paratracheal, subcarinal and hilar lymph nodes. Mediastinal lymph nodes located adjacent to the esophagus can be assessed by transesophageal ultrasound-guided fine needle aspiration (EUS-FNA). Owing to the complementary reach of EBUS-TBNA and EUS-FNA in assessing different regions of the mediastinum, recent studies suggest that complete and accurate mediastinal staging can be achieved by the combination of both procedures.

It is expected that implementation of minimally invasive endoscopic methods of endobronchial ultrasound-guided transbronchial needle aspiration and transesophageal ultrasound-guided fine needle aspiration will reduce the need for surgical staging of lung cancer significantly.

Japanese experience (K. Yasufuku): Despite the advances in surgical treatment and multimodality treatment, lung cancer is still the leading cause of death from malignant disease worldwide. Accurate lymph node staging is important not only to determine the prognosis but also to decide the most suitable treatment plan. Non-invasive staging which mainly consists of conventional imaging methods alone is inaccurate and therefore tissue sampling is the preferred and most reliable. On the other hand, invasive staging offers tissue proof of the mediastinum. Mediastinoscopy is still the gold standard for mediastinal lymph node staging. However, it requires general anesthesia and the complications cannot be ignored.

Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) is a real-time procedure performed under local anesthesia using the convex probe endobronchial ultrasound. EBUS-TBNA can be used for (a) lymph node staging in lung cancer patients; (b) diagnosis of intrapulmonary tumors; (c) diagnosis of unknown hilar and/or mediastinal lymphadenopathy; and (d) diagnosis of mediastinal tumors. EBUS-TBNA is an excellent modality with high yield for minimally invasive lymph node staging. Its role in preoperative lymph node staging in lung cancer patients has been well described and is becoming the standard of care for selected patients with mediastinal adenopathy. Although the reported yield of EBUS-TBNA for lymph node staging is similar to mediastinoscopy, there is little evidence on the actual comparison of the two procedures. Since EBUS-TBNA is an endoscopic procedure, it can be repeated without difficulties and has been shown to be useful for re-staging of the mediastinum after induction chemotherapy. The yield is similar to that of a repeat mediastinoscopy.

There are some limitations in endoscopic staging by EBUS-TBNA. All of the mediastinal lymph nodes accessible by mediastinoscopy are accessible by EBUS-TBNA, with additional access to hilar lymph nodes. However, paraesophageal nodes (#8, #9) and aortic nodes (#5, #6) are not accessible with EBUS alone. Endoscopic ultrasound guided fine needle aspiration (EUS-FNA) has better access to the inferior and posterior lymph nodes. VATS is the preferred approach for sampling of aortic nodes and should be performed in selected patients.

Future EUS Technology

Future Perspectives – Company Perspective (K. Hirooka): OLYMPUS has been developing Endoscopic Ultrasound ("EUS") since 1978 as the leading company in the Endoscopy world and brought a number of innovative devices into the market, such as a full-360 degree electronic scanning radial echoendoscope (in addition to the mechanical scanning radial echoendoscope), high-quality curvilinear array echoendoscopes, with allowing physicians to perform Sampling EUS (EUS-Fine Needle Aspiration, "EUS-FNA") and Interventional EUS procedures, and a tiny curvilinear array echoendoscope for EndoBronchial Ultrasound guided TransBronchial Needle Aspiration ("EBUS-TBNA").
With devices developed for a wide variety of indications, EUS has expanded its role in Imaging Sampling, and Interventional EUS. We believe that one of the most important aspects for EUS is image quality, which will be consistently important not only in the Imaging EUS, but also in the Sampling EUS and Interventional EUS, and we will devote ourselves to further improvements.
This session will cover Olympus' activities on 1) the improvement of image quality, 2) a new device development in the sampling EUS and Interventional EUS, especially with a newly-developed echoendoscope, and 3) future technology developments. Olympus, as industry, strongly believes that it is essential to collaboratively work with endosonographers to expand EUS and we look forward to future opportunities.

Future Perspective – Clinical Perspective (M. Kida): Endoscopic ultrasonography (EUS) for gastroenterological diseases was first reported in 1980, then EUS has widened its applications rapidly. And Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was first described by Vilmann et al. in 1992. EUS-FNA is also used clinically for a broad range of indications widely. EUS-FNA has become an essential tool for diagnosis and treatment in clinical practice.
What is the optimal EUS? Concerning about EUS scope, now we have radial, convex, forward-viewing scope. These scope should become thinner and have shorter hard tip like standard endoscope. In San Francisco, we have developed Bi-Plain EUS scope, however it has longer hard tip which seems to be difficult to insert. Then we have developed Bi-Plain EUS scope using forward-viewing scope and have shortened hard tip part.
With reference to processor, recently contrast enhancement has made great advancement, however EU-ME1 has not covered contrast enhancement. 2nd generation of EU-ME1 should provide contrast enhancement. With the advancement of transducer, EUS should have good enough penetration and image quality.
With reference to needles, the optimal needle for EUS-FNA should be easy to puncture, could take samples perfectly, and should have no complications. 25-guage needle has been widly used in the clinical fields because of "puncturability" and no difference on sampling rate. Dr. Kaffes reported high sampling rate with his developed needle with side-port. And needle forceps has developed for EBUS. We have to improve and develop optimal needles for each purpuse.
With reference to devices, now days EUS-biliary drainage and EUS-pancreatic duct drainage etc. have been employed in the clinical practice. However each equipments have not developed its own purpose, we have to develop them which are suitable for own purpuse.
With reference to future direction of EUS, natural orifice translumenal endoscopic surgery (NOTES) will widen its indications in the near future. Maybe EUS-FNA technique will have some role on NOTES such as forward-viewing EUS scope and Endolifter etc.
With reference to combination with basic science, maybe gene analysis, gene therapy will be realized in the clinical practice. And regeneration medicine such as treatment of diabetes mellitus will be also realized in the near future.
In conclusion, EUS and EUS-FNA have promising future and we have to develop new things for the future by the help of advancement of basic science.

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Identifying and prioritizing strategies for comprehensive liver cancer control in Asia

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Abstract

Background: Liver cancer is both common and burdensome in Asia. Effective liver cancer control, however, is hindered by a complex etiology and a lack of coordination across clinical disciplines. We sought to identify strategies for inclusion in a comprehensive liver cancer control for Asia and to compare qualitative and quantitative methods for prioritization.

Methods: Qualitative interviews (N = 20) with international liver cancer experts were used to identify strategies using Interpretative Phenomenological Analysis and to formulate an initial prioritization through frequency analysis. Conjoint analysis, a quantitative stated-preference method, was then applied among Asian liver cancer experts (N = 20) who completed 12 choice tasks that divided these strategies into two mutually exclusive and exhaustive subsets. Respondents' preferred plan was the primary outcome in a choice model, estimated using ordinary least squares (OLS) and logistic regression. Priorities were then compared using Spearman's Rho.

Results: Eleven strategies were identified: *Access to treatments; Centers of excellence; Clinical education; Measuring social burden; Monitoring of at-risk populations; Multidisciplinary management; National guidelines; Public awareness; Research infrastructure; Risk-assessment and referral*; and *Transplantation infrastructure*. Qualitative frequency analysis indicated that *Risk-assessment and referral* (85%), *National guidelines* (80%) and *Monitoring of at-risk populations* (80%) received the highest priority, while conjoint analysis pointed to *Monitoring of at-risk populations* ($p < 0.001$), *Centers of excellence* ($p = 0.002$), and *Access to treatments* ($p = 0.004$) as priorities, while *Risk-assessment and referral* was the lowest priority ($p = 0.645$). We find moderate concordance between the qualitative and quantitative methods (rho = 0.20), albeit insignificant ($p = 0.554$), and a strong concordance between the OLS and logistic regressions (rho = 0.979; $p < 0.0001$).

Conclusions: Identified strategies can be conceptualized as the ABCs of comprehensive liver cancer control as they focus on *Antecedents*, *Better care* and *Connections* within a national strategy. Some concordance was found between the qualitative and quantitative methods (e.g. *Monitoring of at-risk populations*), but substantial differences were also identified (e.g. qualitative methods gave highest priority to risk-assessment and referral, but it was the lowest for the quantitative methods), which may be attributed to differences between the methods and study populations, and potential framing effects in choice tasks. Continued research will provide more generalizable estimates of priorities and account for variation across stakeholders and countries.

Background

Hepatocellular carcinoma (HCC), the predominant form of liver cancer, is the sixth most common cancer and the third most frequent cause of cancer-related death worldwide [1,2]. At least two thirds of the people who die each year from HCC live in the Asia-Pacific region [3]. The majority of patients with HCCs are diagnosed in the advanced stages of presentation due to the relative paucity of symptoms in the early stages [4]. Because of the multifocal and advanced stage of disease at time of diagnosis, potentially curative treatment for HCC is not feasible in 80% of patients [5].

Chronic liver disease is closely associated with HCC. In areas where hepatitis B virus (HBV) is endemic, the incidence of HCC is high. It has been estimated that

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about 75% of the world's chronic HBV carriers are in Asia [6]. However, the etiology of HCC in Japan is different as hepatitis C virus (HCV) is more prevalent than HBV. Ninety percent of the HCC in Japan is HCV related [5]. As stated in a recent report by the United States Institute of Medicine, both HBV and HCV can be prevented and controlled, which would reduce the incidence of HCC and liver disease [7].

The relative burden and complexity of liver cancer, especially in Asia, lends itself to a comprehensive cancer control plan. However, there is a paucity of data or experience to design such a policy response. While comprehensive cancer control plans regularly target lung, colorectal, breast and cervical cancer, such approaches have not been applied to liver cancer [8]. The WHO guidance for the development of national cancer programs offers some guidance for implementation [9]. The WHO conceptualizes its model around disease progression and is focused around six dimensions: prevention, early detection, diagnosis/treatment, pain relief/palliative care, cancer control research, and surveillance. One of the limitations of this approach is that it distinguishes between appropriate strategies that should be used in countries with low, middle and high levels of resources-a barrier to a common policy framework that would be appropriate for a pan-Asian response [10].

This paper reports the findings of a study aimed at identifying strategies appropriate for inclusion in a comprehensive liver cancer control plan and at assessing the relative priorities among these strategies. We also sought to compare the implied priorities in the qualitative data (i.e. via semi-quantification using frequency analysis) to those found using a quantitative stated-preference methodology (conjoint analysis)-with a particular focus on Asia.

Our research is of interest to those focused on liver cancer control, especially in Asia, for three reasons. First, beyond clinical guidelines, there is very little in the way of comparative research on liver cancer policy internationally. This paucity of data extends even to basic epidemiological data on HCC, which are fragmented and come from diverse populations, using different methodologies and from studies performed at different times [2]. Second, while Japan and Taiwan have demonstrated successful strategies to combat HCC, especially through HBV vaccination and control, there is an absence of models of best practice for comprehensive liver cancer control beyond HBV vaccination in most countries of Asia [3,11,12]. Third, there are few templates available for the development of comprehensive cancer control plans for liver cancer, and it is uncertain if general cancer-control frameworks, such as the one proposed by the WHO [9], are relevant for liver cancer control.

We also make an important methodological contribution that is relevant to a wider audience of policy makers and health services researchers. Specifically, we demonstrate that while qualitative methods are valuable in identifying strategies [13], semi-quantification methods such as frequency analysis [14] may be less desirable for prioritization [15]. We demonstrate this by comparing our frequency data with the results of a conjoint analysis-a qualitative stated-preference method [16] that is increasingly used to identify priorities for health care policy [17-19] and more broadly in health services research [20-22].

Methods

The study utilized both qualitative and quantitative research methods. First, in-depth, open-ended interviews were used to identify possible strategies and to explore possible priorities using frequency analysis, a common semi-quantification method [14]. Qualitative methods are an important method for identifying complex issues in health care, including priority setting [23,24], and are an important way to include clinical stakeholders in decision making processes [25-27], including the study of cancer care and coordination [28-30]. Second, quantitative stated-preference methods were used to focus more on the priorities for Asia and to compare the implied priorities based on the qualitative data.

All participants were informed about the study and its potential risks and benefits. Participation in the study was voluntary and respondents were not reimbursed for participation. The study was deemed exempt from human subjects consideration from the Johns Hopkins University, Bloomberg School of Public Health Institutional Review Board (IRB). All respondents were guaranteed anonymity and confidentially.

Identification (qualitative)

Respondents for the qualitative study were purposively sampled to constitute a geographically and professionally diverse sample of clinical experts in liver cancer and related disease [31]. Potential respondents were actively involved in clinical practice, academic medical centers and/or policy relating to the prevention, detection and/or management of liver cancer. Potential respondents were identified through published literature, medical societies and peer referral. Respondents were included if they were i) working in liver disease and liver cancer in their country; ii) involved in HCC clinical practice and policy; iii) active members in national and international liver associations and/or published extensively in peer review journals, and were excluded if they were not board certified or licensed to practice medicine in their countries and with at least three years of clinical experience or were unwilling or unable to complete the

interview within the period required for completion of all interviews.

It is clear that our focus on clinical expertise is restrictive, but we wanted to ground our results in those who actually implement liver cancer control. This said, other stakeholders, including patients, family members, nursing staff or community leaders, may have given important insights. While this certainly is a limitation in our study, we aimed at assessing complex clinical issues, and as such needed experts who were experienced with discussing national liver cancer policies.

Information about the study and an invitation to participate was sent to respondents via mail or email in English, and in the respondents' native language where necessary. If no response was received within two weeks, follow-up included a second email and/or telephone call.

Open-ended qualitative interviews were conducted via face-to-face interviews or, in a limited number of instances, as telephone interviews. Multiple interviewers were used so as to accommodate multiple languages, with many of the interviews completed by the study leaders (JB, BB), an important triangulation method.

After respondents were informed about the study and consented to participate, they were asked about their country's "*strategies to promote liver cancer prevention, treatment and research*" and then "*the main gaps in public policy*." Finally, respondents were asked "*if you had an opportunity to develop a comprehensive liver cancer control strategy, what elements would it cover?*"

Interviews were recorded, transcribed (translated where necessary) and systematically analyzed in conjunction with any field notes. Respondents were allowed and encouraged to discuss other factors via open-ended questioning, but conversations were facilitated through the use of a comprehensive *aide memoire* based on previous research [10]. While saturation of themes was achieved after 16 interviews, we completed 20 interviews to facilitate semi-quantification.

Analysis was guided by Interpretive Phenomenological Analysis (IPA) [32] in order to capture respondents' experiences, perceptions, practices, and processes associated with liver cancer control. Data were initially reviewed and coded by two researchers, including one who participated in data collection and one who did not. To ensure reliability, coding was compared and discussed with senior study members (JB and BB), and a final selection and appropriate labeling of identified themes was determined.

Triangulation methods included the use of multiple interviewers and analysts, geographical and professional heterogeneity of respondents, the comparison of transcripts with field notes, and comparison of results to the published literature via a targeted literature review. Content experts (MK, KO, K-HH and S-LY) were consulted to ensure the validity of interpretation and to resolve any ambiguity in the data. After this, the two researchers reviewed the data to identify representative quotes and to ensure the reliability of the coding. Finally, to ensure that this manuscript reported all relevant information, we utilized the RATS guidelines [33,34].

Prioritization (qualitative)

The use of numeration and/or semi-quantification in qualitative research remains controversial [35,36]. This said, such methods are frequently used in health care research [37-39], and are called for by the RATS guidelines [34]. Within the framework of IPA, numeration through an analysis of the frequency with which a theme is supported can be used as an indicator of its importance. As noted by Smith and colleagues [32]:

> *"...it makes sense to think of the frequency with which emergent themes appear as one (though not the only) indication of the relative importance and relevance..."*

To examine the potential relative importance of the identified strategies, we examined the frequency with which these strategies (and any sub-ordinate concepts) were discussed [40]. Rather than examine the frequency within a respondent, we report the percentage of the respondents making any reference to each of the indentified themes.

Prioritization (quantitative)

As a means of offering a more quantitative assessment of importance, we developed and implemented conjoint analysis to examine the importance that respondents placed on the identified strategies. While it would have been beneficial to draw such data from the same respondents who participated in the qualitative research, it was decided to recruit new respondents from a single geographic region.

Conjoint-analysis methods, and more specifically discrete choice experiments, are grounded in both mathematical psychology and economic theory [41,42]. They are based on the notion of the assessment of multiple stimuli (referred to as objects or attributes) that are combined to create vignettes or profiles that are presented to respondents in order to evoke an action, choice, or valuation [43]. While such methods are widely used in health care [20-22], they have more recently been applied to examine issues associated with liver cancer control [44-46].

Our approach to conjoint analysis is similar to that of Bridges et al. [19], where conjoint analysis cards are developed to present a number of attributes (or objects)

that do not vary across levels. Hence, for any given scenario in a conjoint analysis task, the attribute is either turned on or off [47]. Rather than identifying the best object in each profile, we present competing plans that represent mutually exclusive and exhaustive subsets of the 11 attributes identified in the qualitative section.

Our experimental design utilized a 2^11 main-effects orthogonal design from a catalogue of designs [48]. This design consisted of a 12 × 11 matrix, with each row representing a specific experiment and each column representing the 11 strategies identified from the qualitative method. Each cell in the matrix was either a 0 or 1 and in developing the pair tasks we interpreted 0 as implying that the strategy should be assigned to the left plan and 1 as assigning the strategy to the plan on the right. The properties of the design were rigorously tested and the results cards did constitute a balanced, orthogonal, and minimal (i.e. zero) overlap design [49]. An example of the conjoint analysis task is provided in Figure 1, where a respondent is asked to identify which of two national liver cancer control plans would have the most impact in their own country.

Potential respondents for the conjoint analysis were identified in China, Japan and South Korea by country experts (MK, KO, KH and SY) and the inclusion/exclusion criteria from our qualitative analysis were used, as were the recruitment procedures. Again, we did not recruit stakeholders other than clinicians, so our results may be biased towards their viewpoint. As the aim of this analysis was to compare the results of the frequency and conjoint analyses, we thought that it was appropriate to use a similar sample size (n = 20). While this is small for a conjoint analysis, it is similar to mixed methods preference studies found in the literature [13], and many commercial and legal applications of conjoint analysis methods have used similar sample sizes (especially when the focus is on the preferences of experts). Given this sample size, the results should not be interpreted as being widely generalizable, but comparable in scope to the qualitative research.

The quantitative survey instrument was administered to the respondents through a face-to-face interview or, where this was not possible, the survey was sent to the respondent and administered via a telephone interview. Respondents were guided through the questionnaire and answered the questions in the presence of the researcher. Respondents were asked to select the set of strategies they thought would be most important in a liver cancer control plan. No other answers or justifications were sought, and this process was repeated 12 times per participant. While some applications of conjoint analysis follow each task with an open or closed question regarding either strength of preference, ease of task or confidence in the answer [43], we did not include such questions so as to minimize the time burden on respondents. This said, notes were taken if respondents made any comments on the conjoint tasks.

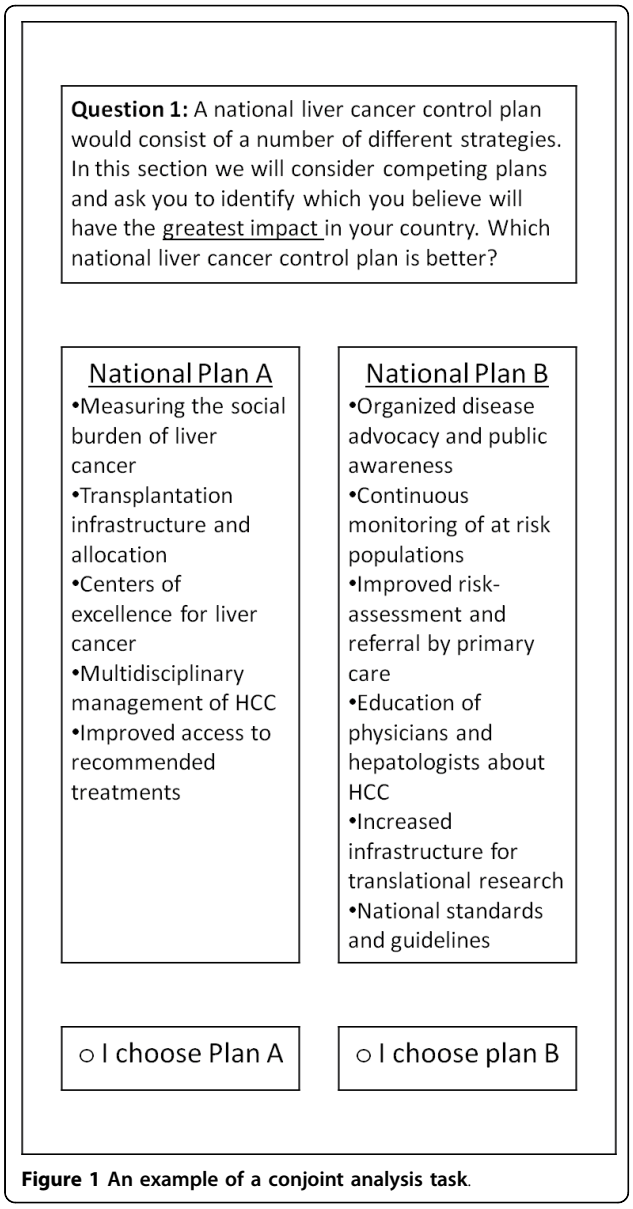

Figure 1 An example of a conjoint analysis task.

The primary outcome in the analysis was the liver cancer control plan selected by the participant for each task, which was coded as a zero if the left-hand-side was chosen and one if the plan on the right was chosen. An identical method was used to code the placement of the strategies on the left and right of the choice tasks. For the purposes of comparison, we utilized both a linear probability model (via ordinary least squares) and logistic regression to estimate choice models using SAS (Version 9.13, Cary, NC, USA), but substantive conclusions are draw from the latter. For both estimation methods, robust standard errors are estimated to account for

clustering of multiple choice tasks within each respondent [50,51]. Hypothesis testing was based on the null that respondents' choices were not affected by each strategy (i.e. the importance weight is zero). The natural alternative hypothesis was that the importance weights were positive (given that all factors were identified as having priorities), however, we allowed for strategies to have a negative sign (as was found in some previous research [52]), and utilized a two-tailed test.

To compare the implied priorities drawn from the qualitative and quantitative analyses, the estimated rank of the eleven strategies is presented graphically and in the results table. Further, the prioritization is compared between the qualitative and quantitative methods (and among the two quantitative estimation techniques) using the Spearman's Rho [53].

Strengths and weaknesses

This is the first paper to focus on the development of strategies for inclusion in a comprehensive liver cancer control program, and in doing so we demonstrate three important issues. First, there is a paucity of robust scientific research to inform the development of evidence-based cancer control plans. Second, preferences-based methods, both qualitative and quantitative, are valuable in identifying and prioritizing control strategies from the perspective of local stakeholders. Finally, such methods offer an important alternative to consensus methods that can be driven by "strong personalities", rather than generalizable data.

There are also several weaknesses in the research underpinning this paper. First, while it is clear that this is subjective research, it is somewhat unclear who the best subjects to recruit are. In some respects our respondents are too homogeneous (i.e. clinicians with a national or international profile), and we have omitted many important viewpoints (other clinical experts, policy makers/leaders and patients/advocates). On the other side, our respondents are heterogeneous, spanning many countries that may have different priorities, which may be biasing our results towards the null. Second, while this method is focused on the comparison of two methods (one qualitative, one quantitative), they have different samples-the former being more international to identify a range of possible strategies, the latter focusing on only three, albeit geographically close, countries. Finally, we have used a rather small sample size to illustrate conjoint analysis, and a much larger sample would be required to ensure generalizability of our results.

Given these weaknesses, there are several limitations in our research that must be addressed. First, while the study was primarily focused on the identification of possible strategies, this should not be considered as an exhaustive set. Second, while this study presents data on priorities, the primary purpose is to demonstrate the limitation of qualitative methods in identifying priorities and to illustrate the benefit of conjoint analysis, not to offer a definitive prioritization of strategies for Asia. Finally, although the data presented here are somewhat novel, more research is needed to see how priorities vary across countries and other stakeholders and to identify which priorities are common in Asia, and which are specific to individual countries in the region.

Results

Identification (qualitative)

Invitations to participate in the open-ended interviews were sent to 25 possible respondents, all of who met the eligibility criteria. One respondent refused to participate, and a further four consented, but a mutually agreeable time to schedule the interview could not be identified before the desired number of respondents was reached. Twenty interviews were conducted between February and June 2010 with experts based in eleven different countries (Australia, China, France, Germany, Italy, Japan, Spain, South Korea, Taiwan, Turkey and United States). The average duration of the interviews was 34 minutes (range 16-80 minutes).

Many respondents found the discussion of comprehensive liver cancer control a complicated task. As one respondent put it "*My gosh, that is a 40 hour discussion, it would cover many things*", and another cautioned at the end of a detailed discussion "*Those are some points* [but] *I am not being complete*." An example of the range of problems that need to be addressed by a comprehensive liver cancer control program was conveyed by one respondent:

> *"We will need to start with identifying the patients at-risk, we would then, after identifying those patients, need to come out with a surveillance strategy to monitor these patients regularly to minimize the chance that we overlooked the development of liver cancer in these patients, then we will need to have a general guideline on who should treat these patients meaning that they should be treated in specialized liver cancer centers that should be part of comprehensive cancer centers, and then we would need to have a study program using new drugs for the adjuvant treatment of those patients that have been treated and also palliative strategies to provide the best level of care for patients with incurable liver cancer."*

Based on these interviews, 11 possible strategies of a comprehensive liver cancer control plan emerged as key themes, including *Access to treatments; Centers of excellence; Clinical education; Measuring social burden;*

Monitoring of at-risk populations; Multidisciplinary management; National guidelines; Public awareness; Research infrastructure; Risk-assessment and referral; and *Transplantation infrastructure*. Rather than focus on the presentation of key quotes, we analyzed the data and worked with content experts (MK, KO, KH and SY) to elaborate a description of each of the 11 strategies (see table 1). This ensured that the findings constituted both a grounded and coherent interpretation of the data.

Prioritization (qualitative)

Initial prioritization was based on the frequency with which the 11 strategies were discussed by the 20 respondents (but not accounting for multiple references within a single interview). The frequency and rank ordering of priorities are presented in table 2. The frequency of discussion across the key themes varied between 20-85%.

The most discussed items were *Risk-assessment and referral* (85%), *National guidelines* (80%) and *Monitoring of at-risk populations* (80%) implying that they are potential priorities. *Research infrastructure* (20%), *Centers of excellence* (25%), *Measuring social burden* and *Transplantation infrastructure* (both 30%) were strategies that were discussed with the lowest frequency, implying a lower priority.

Prioritization (quantitative)

Invitations were sent to 42 potential respondents. Of these 23 (55%) consented to participate and 20 were eligible to participate. Field workers noted that after the first interviews in each country, which were all supervised by a senior investigator (JB or BB), respondents reported some difficulty with the choice tasks, mainly due to a lack of familiarity with conjoint analysis methods. Based on these concerns, all field workers discussed these difficulties, and strategies to overcome this problem were discussed. Here an example question, that was completed and explained, was added to ensure that all respondents were comfortable with the survey instrument and that all respondents were managed in a way that was consistent with these early interviews. This resolved the issue, with the remaining responders reporting no difficultly with the tasks.

Table 2 presents the importance weights (i.e. parameter estimates) from choice models estimated from the conjoint analysis data using both a linear probability model (estimated via ordinary least squares) and logistic regression. Robust standards errors, p-values (based on a two tailed test) and rankings of priorities are also shown. Statistical significance ($p < 0.05$) was achieved on six strategies for both methods, with both methods in agreement on the significance on the top five factors. Here *National statistics* was significant based on OLS ($p = 0.025$), but not based on the logistic model ($p = 0.056$). Likewise, *Clinical education* was significant when considering logistic estimation ($p = 0.019$), but not when using OLS ($p = 0.055$). Both methods identified *Measuring social burden* and *Risk-assessment and referral* as having negative importance weights, but neither aversion reach statistical significance. Overall, there was a very-high level of agreement between the two methods ($rho = 0.979$; $p < 0.0001$), so substantive findings are drawn only from the logistic estimation.

As seen in table 2 the highest priority as estimated using the conjoint analysis was *Monitoring of at-risk populations* ($p < 0.001$), followed by *Access to treatment* ($p = 0.004$), *Centers of excellence* ($p = 0.002$), *Multidisciplinary management* ($p = 0.004$), *Public awareness* ($p = 0.018$), *National guidelines* ($p = 0.056$) and *Clinical education* ($p = 0.019$).

Comparison of qualitative and quantitative priorities

When comparing the priorities from the conjoint analysis to the frequency analysis based on the qualitative data, there was some positive correlation ($rho = 0.20$), but this relationship was not significant ($p = 0.554$). When considering the priority given to individual attributes (see Figure 2), similar importance (as indicated by their rank) was given to *Monitoring of at-risk populations* (qual = 2/quant = 1), *Public awareness* (qual = 4/quant = 5), *Access to treatment* (qual = 5/quant = 2), *Clinical education* (qual = 6/quant = 7), *Multidisciplinary management* (qual = 7/quant = 4), *Transplantation infrastructure* (qual = 8/quant = 8), *Measuring social burden* (qual = 8/quant = 10), and *Research infrastructure* (qual = 11/quant = 9). Differences in priority between the two methods were found for *Risk-assessment and referral* (qual = 1/quant = 11) and *Centers of excellence* (qual = 10/quant = 2), and to a lesser extent *National guidelines* (qual = 2/quant = 6).

Discussion

When one considers the 11 strategies for comprehensive liver cancer control identified in this paper, we can see that they cover factors associated with facilitating, providing and integrating care into a single system. To facilitate the possible implementation of these strategies, one can conceptualize them into three categories: antecedents; better care; and connection. As seen in Figure 3, this can lead to a model that relates to the ABCs of comprehensive liver cancer control. Here *Antecedents* include *Clinical education*, *Measuring social burden* and *Public Awareness*, all factors that can motivate the adoption of comprehensive liver cancer control. Access to treatments, Monitoring of at-risk populations, Risk-assessment and referral and Transplantation infrastructure are all factors aimed at providing *Better care*, a vital component of any comprehensive cancer control plan.

Table 1 Strategies for comprehensive liver cancer control

Strategy	Description	Relevant quotes
Access to treatments	Appropriate coverage and reimbursement for necessary prevention, surveillance, treatment, pain relief and palliative services.	*"Creating access to treatment-screening is a waste of effort if you don't link it to care"* *"The national insurance system does not fully cover payment"* *"Patients ask for new treatments, however, they are not covered by insurance"* *"It is important to eradicate drug lag and make good medication available as soon as possible"*
Centers of excellence	Specialized liver cancer centers to provide coordinated surveillance, treatment and research within a national liver cancer program.	*"Transfer patients with a HCC diagnosis to a tertiary hospital to receive state-of-the-art treatment"* *"There is no organization that brings all liver cancer research together under one roof"* *"Build a large center, experienced with international techniques, with a large number of patients"* *"We need to continue to preach to establish centers of excellence with multidisciplinary efforts"*
Clinical education	Improve primary care provider's awareness of the benefits of screening and early treatment, and necessary skills in risk assessment.	*"Most of the educational resources need to go into educating healthcare professionals"* *"Increase awareness among general practitioner, most are not aware"* *"Education of general practitioners concerning the screening of HCC, and gastroenterologists too"* *"We need to focus on the general education for primary care physicians so they will become vigilant"*
Measuring social burden	Accurate measures of risk factors, cirrhosis, liver cancer, the societal costs of illness and the benefits of improving liver cancer care.	*"Research the epidemiology of liver cancer, I think that we underestimate liver cancer by 50%"* *"Prevalence, surveillance, burden of disease, effective and cost-effective strategies"* *"Know the epidemiological trend for non-alcohol fatty liver disease and its impact on HCC incidence"* *"We need to have some comparison about how many lives we can save if we improve"*
Monitoring of at-risk populations	National surveillance programs for at-risk patients through expert services to diagnose HCC in early stages and improve outcomes.	*"Get at-risk patients into adequate screening programs at appropriate intervals and tested by experts"* *"Of cause surveillance programs are important to prevent or to detect early HCC"* *"There should be a national surveillance program for liver cirrhosis"* *"Monitor high-risk patients so if they develop HCC they can be diagnosed at an early stage and treated"*
Multidisciplinary management	Diagnosis, treatment decisions and follow-up of all HCC patients through collaborative teams of all relevant specialists.	*"Follow-up of HCC patients should be in a multidisciplinary team of different specialists"* *"Collaboration among physicians, surgeons, radiologists and oncologists is very poor"* *"Create an appropriate interdisciplinary board where every single patient is evaluated by this team"* *"It is very important to appreciate that this disease is heterogeneous with regards to the etiology"*
National guidelines	National standards for diagnosis and guidelines for screening, surveillance, treatment and palliation related to liver cancer.	*"There are no national guidelines on how to deal with patients with liver cancer"* *"There should be a national treatment strategy recognized and outcomes captured"* *"There is a lack of standardization of clinical diagnosis and treatment"* *"Information exchange among world leaders to prepare a global standard for prevention and treatment"*

Table 1 Strategies for comprehensive liver cancer control *(Continued)*

Public awareness	Programs to improve public/political awareness about risk factors, surveillance, and survival benefits, and organized patient advocacy.	*"Greater public awareness of liver disease, risk factors and the fact that good treatments are available"* *"There is an absolute ignorance among the public and there is a clear need for education"* *"Patient groups are limited to popular types of cancer, but HCC is mainly the cancer of the poor"* *"Support experts to handle the details of patient advocacy so prevention and treatment could benefit"*
Research infrastructure	Funding, personnel, and facilities to conduct relevant basic, clinical and translational liver cancer research throughout the health system.	*"There is no specific program for HCC with public funding ... research infrastructure is always needed"* *"Train physicians who can lead clinical trials ... we also need research nurses"* *"Get thorough scientific research for HCC, genetics, biology, the pathways, it is very important"* *"There is an uneven distribution of research funding and the lack of grass-roots research funding"*
Risk-assessment and referral	Risk stratification conducted by primary care providers who refer patients to appropriate surveillance provided regularly by experts.	*"Identify at-risk patients, encourage them to be screened, and link them to appropriate care"* *"Primary doctors should not be treating viral hepatitis, they should be detecting it"* *"GPs don't consider it necessary and don't perform screening in patients with diagnosed cirrhosis"* *"We have very inefficient tools for identifying the high risk patients"*
Transplantation infrastructure	Improve awareness and capacity for organ donation, more capacity for transplantation, and alternatives to cadaveric transplantation	*"The situation cannot be altered without donors, but there is not much social infrastructure to support it"* *"It has been a major necessity to promote more cadaveric liver transplantation for more than decade"* *"The only shortcoming is transplantation, cadaveric transplantation is standard in other countries"* *"Real awareness of organ donation. There are some examples in the media, but still nothing happens".*

Representative quotes remain unidentified to ensure anonymity and confidentiality of respondents

Finally, a well functioning system must have its components well connected. In our model, *Centers of excellence*, *Multidisciplinary management*, *National guidelines* and *Research infrastructure* are important *Connections* of a comprehensive liver cancer control plan.

The strategies identified here parallel some of the strategies embedded in the WHO guidelines for general comprehensive cancer control with two exceptions [9]. First, strategies for pain relief/palliative care were not identified as an important cancer control strategy by our clinical respondents. This may have been different if more variety in the types of stakeholders were included in our sample (e.g. we had no nurses, patients or advocates), but there may be a lack of advocacy for liver cancer more generally [54]. This said, pain relief/palliative care can be seen as belonging to our *Access to treatment* strategy. Second, we do not differentiate strategies for implementation in low, middle and high income countries [10], nor did we examine such heterogeneity in priorities. These two differences highlight the need for further research to differentiate priorities across different stakeholders (including advocates, where they may exist) and across different countries in Asia and beyond.

While this paper identified certain priorities for implementation in an Asian comprehensive liver cancer control plan, it is important to compare these to current policies in Asia. Highest priority was given to *Monitoring of at-risk populations*, which has been shown to facilitate early diagnosis [55]. Such surveillance programs are related to surveillance in primary care [56], which may account for the low value given to Risk-

Table 2 Importance of strategies for liver cancer control

Strategy	Qualitative	Quantitative			
	Frequency (Rank)	OLS (SE)	P-Value (Rank)	Logit (SE)	P-Value (Rank)
Access to treatments	70%	0.192	0.010	1.146	0.004
	(5)	(0.07)	(3)	0.40	(2)
Centers of excellence	25%	0.192	0.001	1.079	0.002
	(10)	(0.05)	(2)	0.35	(3)
Clinical education	65%	0.108	0.055	0.625	0.019
	(6)	(0.05)	(8)	0.27	(7)
Measuring social burden	30%	-0.025	0.698	-0.185	0.613
	(8)	(0.06)	(11)	0.37	(10)
Monitoring of at-risk populations	80%	0.275	< .001	1.508	< 0.001
	(2)	(0.06)	(1)	0.41	(1)
Multidisciplinary management	35%	0.158	0.004	0.919	0.004
	(7)	(0.05)	(4)	0.32	(4)
National guidelines	80%	0.158	0.025	0.678	0.056
	(2)	(0.06)	(6)	0.36	(6)
Public awareness	75%	0.158	0.007	0.841	0.018
	(4)	(0.05)	(5)	0.36	(5)
Research infrastructure	20%	0.058	0.243	0.337	0.178
	(11)	(0.05)	(9)	0.25	(9)
Risk-assessment and referral	85%	-0.008	0.870	-0.135	0.645
	(1)	(0.05)	(10)	0.29	(11)
Transplantation infrastructure	30%	0.125	0.196	0.352	0.543
	(8)	(0.09)	(7)	0.58	(8)

Robust standard errors in parentheses.

assessment and referral (i.e. respondents found the former more beneficial to the latter). This said, risk stratification may be important to target surveillance strategies [57]. While *Centers of excellence* were only discussed by a minority of respondents in the qualitative interviews, this strategy was considered a priority in the conjoint analysis. Such specialized centers have been shown to be of value in early surveillance and improved outcomes for HCC in Japan [58].

Priority was also placed on *Access to treatment* by respondents in both the qualitative and quantitative portions of this study. While lack of robust financing systems is a major barrier in many Asian countries, barriers to access persist in those countries with national health insurance. Other barriers include a lack of reimbursement, high copayments, a lack of specialty centers, the availability of specialists and awareness of the disease among primary care physicians and the general public [59]. A shortage of organ donors and subsequent waiting lists also pose barriers to access to transplantation [60].

There are some omissions from our set of strategies. For example, in addition to the absence of pain management and palliative care, our study did not specifically characterize hepatitis control as a strategy. However, prevention (e.g. through HBV vaccination), treatment and control (which would include treatment of hepatitis associated with HCC) are within the descriptors for *Access to treatment*. While quality hepatitis control exists in many Asian countries [61,62], hepatitis control must be a priority in many countries not included in this study [63,64].

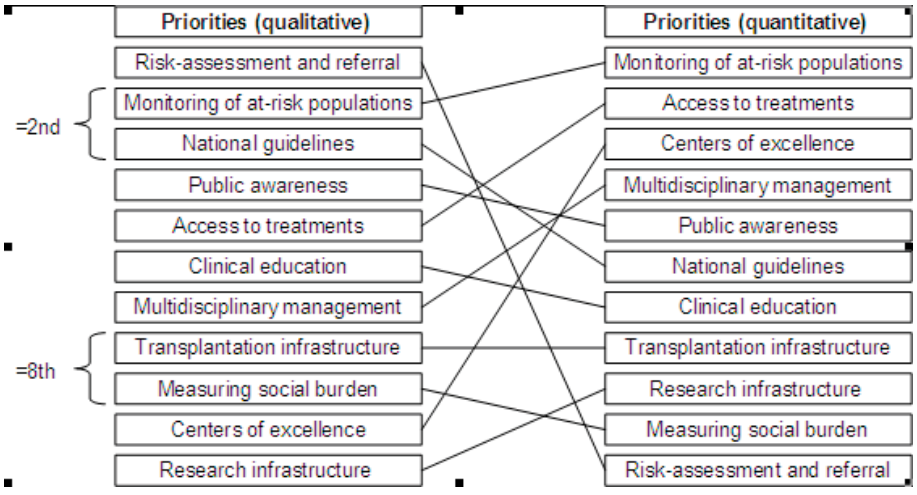

Figure 2 A comparison of priorities using qualitative and quantitative methods.

Figure 3 The ABCs of comprehensive live cancer control.

It is also important to consider some alternative interpretations of the data in this study. One interpretation is that valuation of some strategies in the qualitative analysis may be as a result of framing effects in the presentation of the choice tasks. For example, *Measuring social burden*, which received a negative value, was described as "Measuring the social burden of liver cancer" (see Figure 1). Here respondents may have found this label ambiguous, or as implying factors that were not implicit in the qualitative analysis. This label may have been better described with terms such as measuring incidence or prevalence, terms that are more familiar to the respondents.

Risk-assessment and referral received a negative valuation despite being the most frequently discussed strategy in the qualitative analysis. Here several factors may have contributed to this aversion. First, the factor was described in the conjoint tasks as "Improved risk-assessment and referral by primary care" (see Figure 1), and the "improved" may have unduly framed the factor (especially for countries that have good risk-assessment mechanisms) or made the factor ambiguous (especially for those who do not have such mechanisms). Second, it may have been more accurate to refer to this as "continuous surveillance" of at risk populations. Third, there was some confusion between "risk-assessment and referral"-mechanisms to stratify those at-risk of developing HCC and referring them to appropriate care-and "monitoring of at-risk populations"-mechanisms of surveillance for patients identified as being at-risk, preferably in specialty care. Finally, there may be heterogeneity in the valuation of this factor across the study countries, i.e. this may be a priority in some countries, but not in others, potentially because such services are already provided or because systems are not based upon primary care providers originating risk assessment and diagnosis.

While this study is motivated by a need for comprehensive liver cancer control in Asia [10], it also highlights a more general need for more quantitative research methods to guide priority setting in health care. The prioritization of limited resources across competing demands presents an "*economic challenge and a political puzzle*" [65], but is a vital element of systematic planning in public health [66]. While some health care planners utilize multiple evidence sources (both qualitative and quantitative) for the purposes of priority setting [67], stakeholder engagement in policy often is limited to nominal groups or consensus-based approaches (e.g. Delphi techniques) [68,69]. As such, this study makes a significant contribution towards demonstrating the value of conjoint analysis in prioritization of health care policy strategies and challenges the soundness of consensus-based approaches.

Conclusions

Identified strategies can be conceptualized as the ABCs of comprehensive liver cancer control as they focus on *Antecedents*, *Better care* and *Connections* within a national strategy. Some concordance was found between the qualitative and quantitative methods (e.g. *Monitoring of at-risk populations*), but substantial differences were also identified (e.g. qualitative methods gave highest priority to risk-assessment and referral, but it was the lowest for the quantitative methods), which may be attributed to differences between the methods and study populations, and potential framing effects in choice tasks. Continued research will provide more generalizable estimates of priorities and account for variation across stakeholders and countries.

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Authors' contributions

JB and BB conceptualized the study, designed the study instrument and made substantial contributions to the data interpretation and writing of the paper; MK, KO, K-HH and S-LY participated in the instrument design, recruitment and interpretation of the data; GG participated in data analysis and drafting of the final manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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手術数でわかる いい病院 2011

全国&地方別データブック

全国&地方 トップ3000病院

執刀医、治療医名リスト 4600人

がん、心臓病、脳疾患、放射線治療、人工関節、眼、耳の病気など

全総力を挙げた信頼のランキングデータ

12万5千件の手術数データを一挙掲載

全国5206医療機関の手術数総覧!

●現地ルポ

患者にやさしい全国「新築病院」

小倉記念病院、湘南鎌倉総合病院、淀川キリスト教病院

●密着ルポ

勇気を持って治療に臨む! 手術のための「あんしん手引」

虎の門病院泌尿器科

手術数データはこう読み解け!

惑わされない医療情報の正しい読み解き方

患者のための実用豆知識

謝礼、紹介状、医師の指名方法…

本誌は収益の一部を日本の対がん活動のために寄付します

朝日新聞出版

外科、内科にこだわらず、その人にあった治療の選択も大切

肝切除術とラジオ波のどちらが優れているか、かねて専門家の間で議論がされてきた。とくに、どちらも日本有数の症例数をもつ東京大学病院では、外科と内科がお互いの主張をぶつけ合っていた。

「以前から、両者を比較した臨床試験をしようという話があったのですが、本格的に外科と内科の話し合いが始まったのは、06年12月のことです。それから何度も会議を重ねて試験計画を練り、病院の倫理委員会の審査も受けて、ようやく09年4月にSURF-trialが始まりました」

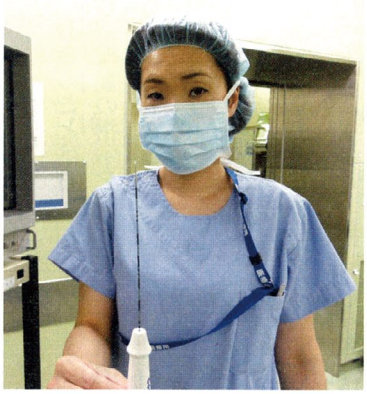
そう明かすのは、ラジオ波で世界でも最多の実績をもつ**東京大学病院**消化器内科講師の椎名秀一朗医師だ。議論だけでなく、実際に臨床試験をして白黒つけようという考えは一致していた。ただし、外科と内科で、考え方が微妙に異なるところもあったようだ。

「手術のほうが肝臓を大きく取り除くため、再発率が低くなります。しかし、ラジオ波では、7回、8回と再発しても、長生きしている患者さんはいくらでもいます。臨床試験では、先に無再発生存率（再発せずに生きている率）が出るので、その結果をもって『手術のほうがいい』と言われてしまうのが心配です。最終的にどちらが長生きしたか、全生存率で評価すべきです」（椎名医師）

最近では、若い人のC型肝炎ウイルスの感染率は下がり、肝がん患者の多くを60～70代が占めるようになった。それにつれて、ラジオ波が増え、手術を選択する人は減っているという。

「本来はどちらが優れているかで『標準治療』を決めるのではなく、それぞれの患者にどの治療がいいか『個別化治療』を検討すべきでしょう。いろいろな病院が参加するため、治療基準が病院により異なり、また、手術、ラジオ波とも、病院によって技術レベルに差があります。さらに、治療は時代とともに進化します。結果を評価する際にはそういった点を注意する必要があるでしょう」（同）

SURF-trialの参加施設のリストには、大学病院や基幹病院の名前が並ぶ。そのなかで、目を引くのが**明和病院**だ。甲子園球場に近い、兵庫県西宮市の地域密着型の病院で、ベッド数も349床と決して大きくない。


ラジオ波焼灼術で使われる電極針（明和病院で）

そんな病院が、ラジオ波で18位に入った。

院長の山中若樹医師は、同じ西宮市内の兵庫医科大学病院第一外科で肝胆膵（かんたんすい）外科を担当し、肝移植も手がけた経歴の持ち主。01年に同院の外科部長に就任してからも、肝がんの診療に取り組んだ。

「別に、病院の経営を考えて、肝臓の治療を売りにしようと思ったわけではありません。大学時代と同じように地道に努力していたら、自然に地域の医師から紹介される患者が増え、肝臓の治療を学びたい医師も集まってきたのです。日本肝胆膵外科学会の『高度技能医修練施設A』や兵庫県の『肝疾患専門医療機関』に指定されていますが、この規模の病院ではまずないでしょうね」（山中医師）

山中医師は外科医だが、ラジオ波の治療も自ら手がけてきた。外科、内科にこだわったり、一つの方法に固執したりせず、それぞれのいいところを生かせばいいというのが、山中医師の考えだ。

「切り取れるのならば、再発の少ない手術のほうがいいに決まっています。しかし、体力のない高齢者も多く、そういう人には手術できません。手術をしてほしいと紹介された人でも、無理しないほうがいいと判断してラジオ波にすることがあります。両方やっているからこそ、両方の利点・欠点がよ

針だけで腫瘍を焼ける治療法として定着

肝がんラジオ波焼灼術

ラジオ波焼灼術（以下、ラジオ波）が日本に導入されたのは1999年。それから10年以上たち、ラジオ波は肝がんの治療法として、すっかり定着した。

ラジオ波は、太さ1・5ミリの細長い電極針を肝臓に刺し、腫瘍を焼いてしまう治療法だ。多くはおなかを開けずに局所麻酔のみで、エコー（超音波）で肝臓の様子を見ながら進められる。また、開腹して肝臓に直接、電極針を刺す場合もある。

腫瘍が3個以内で、大きさは3センチ以下が、ラジオ波に適した条件とされる。この条件なら、切除できる患者もいるが、大きな傷が残る開腹手術に対して、ラジオ波なら全身麻酔もいらず、皮膚を2～3ミリ切るだけで治療できることから、こちらを選ぶ患者が増えた。

とくに、C型、B型肝炎ウイルスが原因の肝がん（肝細胞がん）は、肝臓自体が「がんの畑」のような状態になっており、何度も再発を繰り返すことが多い。それだけに、からだに大きな負担をかけないラジオ波の利点は大きい。定期検査で小さなうちに再発を見つけ、ラジオ波を繰り返すことで、長期生存する患者も多いという。ただし、経験の少ない施設で不十分にラジオ波治療を受けると、焼き残しによる局所再発も起こりうる。

外科医の多くが、「腫瘍の数が少なく、肝機能がいい場合や、ラジオ波では治療がむずかしい場合は、根治性の高い手術を受けたほうがいい」と主張する。これに対し、内科医は、「手術をしても5年間で70～80％の患者は再発する。ラジオ波は、再発が起こっても繰り返し治療でき、からだの負担も少ない。最終的な生存率を見れば、手術には負けない」と反論してきた。

そこで、2009年4月から、肝切除術とラジオ波の効果を比較する臨床試験（SURF-trial：85ページ参照）が、東京大学病院を中心とする全国96施設で始まった。結果が出るのはまだ5年、10年先だが、肝がんの治療選択の考え方が変わる可能性もはらんでいる。

その他、肝がんでは「ソラフェニブ（商品名・ネクサバール）」という、新しいタイプの抗がん剤の登場が話題だ。肝がんは抗がん剤の効きにくいがんの一つだが、ソラフェニブによって初めて、生存期間が延長するデータが示された。しかし、専門家の間ではまだ十分な評価を得ているとはいえず、今後の研究が待たれている。

肝がんには、放射線科で実施されることの多い「肝動脈塞栓術（血管を詰めて腫瘍の壊死を図る）」などの治療もある。それゆえ、肝がんは、内科、外科、放射線科など、各科の協力が不可欠と指摘する専門家もいる。肝がんを治療する病院を選ぶ際には、「総合力」の観点から見ることも大切だ。

くわかる。わたしは自分のことを、肝臓外科医というより、肝臓専門医だと思っています」

明和病院には、消化器内科医の春日井博志医師（春日井クリニック院長）が週に1回、非常勤で勤めている。07年に神戸市内で開業するまで、大阪府立成人病センター消化器内科に勤めていた春日井医師は、関西でラジオ波をいち早く始めた医師の一人だ。春日井医師は言う。

「明和病院は一流だと思います。週に1回、症例検討会が開かれており、内科医、外科医、放射線科医、病理医、みんなで議論して治療方針を決めています。外科だけ、内科だけだと、どうしても治療が偏りがちになるので、これはとても大事なことです」

ただ、同院では外科が中心にラジオ波を実施している。春日井医師はラジオ波のできる内科医を育てたいと、同院でその指導にあたってきた。大阪府立成人病センター時代は、年間約200例のラジオ波を手がけていた春日井医師だが、開業医になって、地域の肝がん患者を診るようになり、少し見方が変わったと話す。

東京大学病院
消化器内科講師
椎名秀一朗医師

明和病院
院長
山中若樹医師

春日井クリニック
院長
春日井博志医師

近畿大学病院
院長・消化器内科教授
工藤正俊医師

新しいタイプの抗がん剤も登場

「勤務医時代は、お金のことは考えずに薬を処方し、検査の指示を出していましたが、開業医になって初めて、患者さんが治療費をどれくらい払っているかわかるようになりました。いまは、できるだけ患者さんの負担が減る薬の出し方を工夫しています。仕事が終わってから来る人や、土曜日しか来られない人など、患者さんの都合に合わせて診察できるのも、開業医のいいところです」

患者の中には、10年以上もの長い付き合いになる人もいる。「開業してから3人の患者を自宅でみとらせてもらうことができた」と春日井医師は感慨深げに話す。こうした病院や医師が地域の医療を支えていることも忘れてはならないだろう。

ところで、09年5月、肝がん（切除不能な肝細胞がん）の治療薬として、分子標的薬と呼ばれる新しいタイプの抗がん剤「ソラフェニブ（ネクサバール）」が承認された。国際的な多施設共同の臨床試験で、生存期間の延長が確認され、画期的な成果といわれている。2位の**近畿大学病院**院長で消化器内科教授の工藤正俊医師は、この薬についてこう話す。

「肝がんは抗がん剤が効きにくく、あらゆる臨床試験で、腫瘍を縮小する効果はあっても、生存期間の延長には寄与しないという結果が出ていました。それだけに、ソラフェニブは、肝がん治療の選択肢を増やす薬として期待されています」

ただ、肝障害などの副作用による死亡例が複数報告されたこともあって、使用に慎重な医師が多いようだ。工藤医師は言う。

「日本で副作用が多かったのは、肝動脈塞栓術を繰り返して、肝機能が低下してから投与する例が多かったからです。肝機能が保てている時期に早く使えば、副作用はほとんど出ません」

現在、工藤医師が厚生労働省科学研究費幕内班の主任研究者となって、肝動脈塞栓術や肝動注化学療法などと併用した場合の効果を検証する臨床試験が進行中だ。まだデータが出るのは先の話だが、その結果次第では、今後、肝がんの標準治療に組み入れられる可能性もある。 **ライター・鳥集 徹**

◀表の見方

厚生労働省が届け出義務を課す「肝切除術等」が5例以上の医療機関を対象に調査し、肝がんに対するラジオ波焼灼術の治療数（2009年1年間）で並べた。治療数は1人の患者に実施した一連のラジオ波焼灼術を1回と数えるのべ患者数とした。

肝がんラジオ波焼灼術 全国データ

順位	病院名	治療数	所在地		常勤医数	主な医師名	
❶	東京大学病院	918	東京都	文京区本郷７－３－１ ☎03－3815－5411	11	椎名秀一朗	建石良介
❷	近畿大学病院	393	大阪府	大阪狭山市大野東377－２ ☎072－366－0221	11	工藤正俊	鄭　浩柄
❸	ＮＴＴ東日本関東病院	324	東京都	品川区東五反田５－９－22 ☎03－3448－6111	2	竹内　卓	寺谷卓馬
❹	武蔵野赤十字病院	303	東京都	武蔵野市境南町１－26－１ ☎0422－32－3111	11	泉　並木	朝比奈靖浩
❹	大阪赤十字病院	303	大阪府	大阪市天王寺区筆ケ崎町５－30 ☎06－6774－5111	12	大﨑往夫	木村　達
❻	済生会新潟第二病院	272	新潟県	新潟市西区寺地280－７ ☎025－233－6161	2	石川　達	窪田智之
❼	関東中央病院	265	東京都	世田谷区上用賀６－25－１ ☎03－3429－1171	1	小池幸宏	
❼	松山赤十字病院	265	愛媛県	松山市文京町１ ☎089－924－1111	8	上甲康二	大野芳敬
❾	虎の門病院	252	東京都	港区虎ノ門２－２－２ ☎03－3588－1111	7	池田健次	小林正宏
❿	和歌山県立医科大学病院	240	和歌山県	和歌山市紀三井寺811－１ ☎073－447－2300	7	玉井秀幸	上野昌樹
⓫	岐阜市民病院	225	岐阜県	岐阜市鹿島町７－１ ☎058－251－1101	4	西垣洋一	林　秀樹
⓬	岡山済生会総合病院	222	岡山県	岡山市北区伊福町１－17－18 ☎086－252－2211	3	大澤俊哉	藤岡真一
⓭	金沢大学病院	216	石川県	金沢市宝町13－１ ☎076－265－2000	4	山下竜也	荒井邦明
⓮	高知大学病院	208	高知県	南国市岡豊町小蓮185－１ ☎088－866－5811	6	高橋昌也	花﨑和弘
⓯	東邦大学医療センター大森病院	201	東京都	大田区大森西６－11－１ ☎03－3762－4151	6	飯田和成	和久井紀貴
⓰	三重大学病院	194	三重県	津市江戸橋２－174 ☎059－232－1111	3	——	——
⓱	岡山大学病院	189	岡山県	岡山市北区鹿田町２－５－１ ☎086－223－7151	9	中村進一郎	松田浩明
⓲	明和病院	176	兵庫県	西宮市上鳴尾町４－31 ☎0798－47－1767	8	相原　司	安井智明
⓳	埼玉医科大学病院	165	埼玉県	毛呂山町毛呂本郷38 ☎049－276－1111	10	濱岡和宏	渡邊一弘
⓳	横浜市立大学市民総合医療センター	165	神奈川県	横浜市南区浦舟町４－57 ☎045－261－5656	4	沼田和司	森本　学
㉑	札幌厚生病院	161	北海道	札幌市中央区北３条東８丁目５ ☎011－261－5331	7	大村卓味	桑田靖昭
㉒	手稲渓仁会病院	159	北海道	札幌市手稲区前田１条12丁目１－40 ☎011－681－8111	7	辻　邦彦	松居剛志
㉓	熊本大学病院	156	熊本県	熊本市本荘１－１－１ ☎096－344－2111	6	別府　透	田中基彦
㉔	徳島県立中央病院	150	徳島県	徳島市蔵本町１－10－３ ☎088－631－7151	3	柴田啓志	矢野充保
㉕	岡山市立市民病院	146	岡山県	岡山市北区天瀬６－10 ☎086－225－3171	4	狩山和也	湧田暁子
㉖	北里大学東病院	143	神奈川県	相模原市南区麻溝台２－１－１ ☎042－748－9111	4	日高　央	奥脇裕介
㉖	大垣市民病院	143	岐阜県	大垣市南頬町４－86 ☎0584－81－3341	4	熊田　卓	豊田秀徳
㉘	香川県立中央病院	141	香川県	高松市番町５－４－16 ☎087－835－2222	3	高口浩一	永野拓也
㉙	関西医科大学滝井病院	140	大阪府	守口市文園町10－15 ☎06－6992－1001	4	關　壽人	池田耕造
㉚	市立池田病院	139	大阪府	池田市城南３－１－18 ☎072－751－2881	7	今井康陽	井倉　技
㉛	大阪市立大学病院	137	大阪府	大阪市阿倍野区旭町１－５－７ ☎06－6645－2121	13	岩井秀司	小林佐和子
㉜	倉敷中央病院	135	岡山県	倉敷市美和１－１－１ ☎086－422－0210	5	利國信行	詫間義隆
㉝	千葉大学病院	134	千葉県	千葉市中央区亥鼻１－８－１ ☎043－222－7171	1	金井文彦	
㉞	三井記念病院	133	東京都	千代田区神田和泉町１ ☎03－3862－9111	1	大木隆正	
㉟	岩手医科大学病院	129	岩手県	盛岡市内丸19－１ ☎019－651－5111	1	黒田英克	
㊱	市立広島市民病院	124	広島県	広島市中区基町７－33 ☎082－221－2291	4	小林功幸	植松周二
㊲	北海道大学病院	121	北海道	札幌市北区北14条西５丁目 ☎011－716－1161	4	中馬　誠	中西　満
㊲	虎の門病院分院	121	神奈川県	川崎市高津区梶ケ谷１－３－１ ☎044－877－5111	2	小林正宏	保坂哲也
㊲	関西医科大学枚方病院	121	大阪府	枚方市新町２－３－１ ☎072－804－0101	2	池田広記	中橋佳嗣
㊵	東京女子医科大学病院	120	東京都	新宿区河田町８－１ ☎03－3353－8111	4	斎藤明子	片桐　聡

-introduction of Japanese sites-

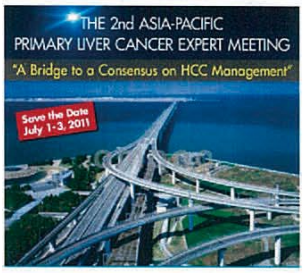
#1301 Kinki Univ. Hospital

Comments from Prof. Masatoshi Kudo

My hospital has participated in this clinical study since the safety cohort and has enrolled seven subjects so far. I think there will be no major problems in the concomitant therapy with CS-1008 and Sorafenib in terms of toxicity. The therapy will probably contribute to significant tumor suppression by the additive effect of both agents. I hope clinical development of CS-1008 will be successful in the near future so as to offer a new treatment option of HCC as an additional standard therapy.

It can be said that Japanese hepatologists, who have a lot of experience diagnosing and treating HCC, have the world's most advanced knowledge of pathological and imaging diagnosis of the disease. I believe Japan, which has the second largest number of patients with HCC in the world, can make a significant contribution to this CS-1008 study.

Lastly, we would like to express our sincere gratitude to investigators who submitted abstracts to The 2nd Asia Pacific Primary Liver Cancer Expert Meeting. We look forward to your active participation.



Our study fellows & facilities

「世界の肝がん治療を変える」パイオニア

名医はこの人

ブラックジャックを探せ

近畿大学医学部附属病院
大阪府大阪狭山市
工藤正俊主任教授（56）

近畿大学教授の工藤正俊医師は、日本の肝がん治療の進化の過程において、数多くの功績を残してきた。

現在では多くの医療機関が導入している「ラジオ波焼灼術」もそのひとつ。肝臓のがんのある部位に電極のついた針を刺し、そこからラジオ波という電流を患部に流してがん組織を死滅させる治療法で、効果と安全性に優れた治療法として、手術でのがん切除が困難なケースを対象に行われている。

工藤医師は日本におけるこの治療法の第一人者として、年間４００例に及ぶ症例を重ねてきた。自らが開発したさまざまな技術を駆使し、エコーでも見えないがんに対しても正確に、かつムダなくラジオ波を当てることを可能にした。

「新しい技術を思いつくのは、発想の転換なんですよ」と工藤医師は笑うが、そこで生まれた技術の数々が、多くの肝がん患者の命を救ってきたのも事実だ。

ラジオ波焼灼術後の患者に対してペグインターフェロンという抗ウイルス薬の少量投与法を確立し、従来肝がんでは難しかった「10年生存」を珍しくないものとした工藤医師。現在はラジオ波焼灼術後に分子標的治療薬を使う治療の臨床試験に取り組んでおり、彼をトップとする近大のチームは、この臨床試験で世界の中で5本の指に入る症例数を誇る。

他にも新薬の国際規模での治験の最高責任者として、また自らが組織した厚生労働省の研究班でも、さまざまな薬剤の組み合わせによる治療法の検証が進んでいる。

「世界の肝がん治療を変えていきたい」

そう語る工藤医師の研究に、世界中の肝がん治療医と患者の期待が集まっている。（長田昭二）

くどう・まさとし　１９５４年愛媛県生まれ。78年京都大学医学部卒業。神戸市立中央市民病院に勤務。87―89年米・カリフォルニア大学デイビス校客員教授。97年より近畿大学医学部に勤務し99年同附属病院消化器内科学主任教授。２００８年より同病院長兼務。近大医学部奈良病院教授、同堺病院教授、神戸市立中央市民病院顧問。医学博士。

病院の実力 大阪編36

肝臓がん

原因の9割「ウイルス」

取り残し少ない開腹手術

1年間に全国で3万人以上が命を落とす肝臓がん。患者の9割はC型またはB型の肝炎ウイルスが原因で、肝炎や肝硬変を発症している。肝臓がん予防の第一は、肝炎ウイルスの感染を防ぎ、感染していれば適切な治療を受けることだ。

読売新聞は5~6月、全国の主な医療機関約1400施設に対して、2009年の肝臓がん手術などの治療実績についてアンケートをした。

手術(肝切除)とラジオ波治療はいずれも、肝臓がんの標準的な治療法だ。

手術は、開腹してがんを直接見ながら切るので、取り残しの可能性が低い。ただ、体への負担が比較的大きく、特に体力に不安のある高齢者らには危険性も高まる。

ラジオ波治療は、体の外から肝臓に針を刺し、針先を電磁波で高温に熱してがんを焼き殺す。手術に比べて体への負担は小さい。超音波画像を見ながら行い、医師の技術が求められる。

肝臓がんの治療指針によると、がんの数が3個以内なら、手術かラジオ波のどちらかを選ぶ。ただし、がんが一つでも3㌢を超えたらラジオ波治療は対象外で、手術か、がんに栄養を運ぶ肝動脈をふさいで「兵糧攻め」にする肝動脈塞栓療法が勧められている。

また、大腸がんなどが肝臓に転移した場合にも、手術が行われている。表では「手術」とは別に、「転移性肝がんの手術」として件数を掲載した。

＊全国の調査結果は「くらし健康面」に掲載しています。

【修正】10月3日掲載の「大腸がん」の表で、耳原総合病院の申し出により、治療実績の数の「合計」を17から71に、「結腸がん」を8から40に、「直腸がん」を9から31に、「腹腔鏡手術」を31から38に修正します。

病院の実力「肝臓がん」

医療機関別2009年治療実績

(読売新聞調べ)

医療機関名	手術とラジオ波治療の合計(件)	手術(件)	ラジオ波(件)	転移性肝がんの手術(件)
近畿大	429	31	398	20
大阪赤十字	359	59	300	14
大阪市立大	281	126	155	21
市立池田	176	26	150	6
関西医大枚方	163	42	121	27
大阪大	155	53	102	16
府立成人病セ	139	35	104	23
国・大阪	133	26	107	29
大阪市立総合	122	57	65	16
市立岸和田市民	112	8	104	8
国・大阪南	111	25	86	7
大阪労災	107	32	75	21
済生会吹田	101	28	73	7
大阪医大	94	46	48	25
大阪警察	91	21	70	12
NTT西日本大阪	84	15	69	5
大阪市立十三市民	80	26	54	5
大阪厚生年金	80	8	72	10
箕面市立	75	5	70	8
東大阪市立総合	74	6	68	10
関西電力	81	10	71	7
市立豊中	69	12	57	21
JR大阪鉄道	68	16	52	7
岸和田徳洲会	66	12	54	5
住友	64	3	61	7
府立急性期・総合	62	6	56	5
済生会中津	61	8	53	10
市立堺	55	33	22	20
八尾徳洲会総合	47	16	31	6
ベルランド総合	39	10	29	15
高槻	34	2	32	3
東住吉森本	32	9	23	15
市立吹田市民	32	4	28	3
大阪回生	30	0	30	2
府中	29	17	12	3
石切生喜	28	17	11	9
淀川キリスト教	28	10	18	8
大手前	26	10	16	10
北野	19	19	—	9
八尾市立	19	19	0	9
PL	18	0	18	3
耳原総合	16	12	4	5
松下記念	15	10	5	6
南大阪	14	8	6	0
守口敬任会	13	9	4	11
近畿大堺	13	3	10	2
多根総合	13	2	11	0
藤井寺市民	12	5	7	1
市立泉佐野	11	8	3	6
市立貝塚	11	6	5	4
日生	10	4	6	4
永山	8	0	8	0
済生会茨木	7	4	3	4
済生会富田林	7	2	5	5
市立柏原	7	2	5	1
済生会千里	5	5	—	8
泉大津市立	5	3	2	6
大阪市立住吉市民	4	4	—	2
和泉市立	4	3	1	2
大阪船員保険	4	2	2	5
馬場記念	2	2	0	4
長吉総合	1	1	0	3
星ヶ丘厚生年金	1	1	0	2
若草第一	1	1	0	0
府立呼吸器・アレルギー	1	1	0	1

大阪府「国・」は国立病院機構、「セ」はセンター。「—」は無回答または不明。

主な医療機関の肝臓がん治療件数

①手術とラジオ波治療の合計(②と③の合計)
②手術③ラジオ波治療④転移性肝がんの手術

	①	②	③	④
北海道				
札幌厚生	219	61	158	17
手稲渓仁会	219	34	185	8
北海道大	202	72	130	25
市立旭川	79	0	79	3
帯広厚生	54	14	40	－
北海道消化器科	49	10	39	12
市立函館	46	13	33	3
青森				
県立中央	54	18	36	8
弘前大	53	22	31	22
岩手				
岩手医大	156	30	126	30
宮城				
仙台厚生	99	52	47	20
石巻市立	46	4	42	10
秋田				
秋田大	57	16	41	4
山形				
公立置賜総合	115	13	102	14
山形大	44	13	31	6
福島				
太田西ノ内	98	32	66	25
福島労災	48	9	39	9
茨城				
日立総合	91	13	78	22
県立中央	84	18	66	18
筑波大	74	29	45	14
東京医大茨城	58	11	47	3
栃木				
独協医大	100	76	24	39
自治医大	94	17	77	37
県立がんセ	50	26	24	15
群馬				
伊勢崎市民	81	2	79	8
済生会前橋	78	2	76	2
前橋赤十字	54	14	40	18
県立がんセ	46	25	21	7
埼玉				
埼玉医大	143	0	143	0
県立がんセ	95	45	50	48
さいたま赤十字	58	13	45	12
埼玉医大国際	50	48	2	30
埼玉医大総合	43	23	20	15
千葉				
千葉大	186	55	131	22
国立がん研究セ東	147	62	85	61
亀田総合	59	14	45	11
国・千葉医療セ	53	2	51	6
国保旭中央	50	23	27	16
県がんセ	45	30	15	13
成田赤十字	40	6	34	16
東京				
東京大	929	109	820	54
虎の門	349	88	261	35
武蔵野赤十字	257	3	254	13
ＮＴＴ東日本関東	236	5	231	9
東京女子医大	226	109	117	46
東京医大	150	12	138	14
関東中央	146	－	146	－
日大板橋	143	138	5	44
三井記念	141	8	133	2
国立がん研究セ中央	136	57	79	61
東京医科歯科大	130	69	61	24
都立駒込	125	24	101	37
日赤	118	66	52	54
癌研有明	97	55	42	107
昭和大	86	15	71	19
帝京大	85	16	69	14
慶応大	79	12	67	22
ＪＲ東京総合	77	2	75	3
日本医大	64	31	33	19
東邦大大橋	61	3	58	18
杏雲堂	60	0	60	0
杏林大	54	10	44	18
順天堂大練馬	54	4	50	9
順天堂大	52	52	0	41
東芝	51	0	51	6
国際医療福祉大三田	48	11	37	8
慈恵医大	44	41	3	11
町田市民	42	1	41	3
神奈川				
横浜市立大市民総合	171	11	160	13
北里大東	155	28	127	17
済生会横浜市南部	93	3	90	15
横浜市立大	88	24	64	36
聖マリアンナ医大	77	36	41	13
県立がんセ	76	37	39	17
東海大	55	23	32	11
平塚市民	52	16	36	3
済生会横浜市東部	43	9	34	23
新潟				
済生会新潟第二	183	5	178	13
県立新発田	56	8	48	9
富山				
県立中央	69	22	47	10
石川				
県立中央	54	3	51	12
福井				
福井大	37	13	24	10
山梨				
山梨大	139	48	91	12
県立中央	65	19	46	9
長野				
諏訪赤十字	68	38	30	18
佐久総合	62	25	37	21
岐阜				
岐阜市民	242	16	226	7
大垣市民	185	37	148	30
岐阜大	90	31	59	27
静岡				
県立総合	134	50	84	32
県立静岡がんセ	103	53	50	59
静岡市立清水	50	0	50	3
順天堂大静岡	48	6	42	5
磐田市立総合	47	21	26	11
愛知				
名古屋市立大	123	13	110	12
藤田保健衛生大	123	70	53	30
小牧市民	104	10	94	4
愛知医大	91	38	53	10
豊橋市民	71	29	42	22
名古屋共立	52	10	42	5
刈谷豊田総合	42	18	24	7
県がんセ中央	40	22	18	33
三重				
市立四日市	66	12	54	10
滋賀				
大津市民	33	7	26	5
京都				
京都大	189	75	114	16
国・京都	115	16	99	7
府立医大	108	30	78	10
京都桂	80	30	50	5
大阪				
近畿大	429	31	398	20
大阪赤十字	359	59	300	14
大阪市立大	281	126	155	21
市立池田	176	26	150	6
関西医大枚方	163	42	121	27
大阪大	155	53	102	16
府立成人病セ	139	35	104	23
国・大阪	133	26	107	29
大阪市立総合	122	57	65	16
市立岸和田市民	112	8	104	8
国・大阪南	111	25	86	7
大阪労災	107	32	75	21
済生会吹田	101	28	73	7
大阪医大	94	46	48	25
大阪警察	91	21	70	12
ＮＴＴ西日本大阪	84	15	69	5
関西電力	81	10	71	7
大阪市立十三市民	80	26	54	5
大阪厚生年金	80	8	72	10
箕面市立	75	5	70	8
東大阪市立総合	74	6	68	10
市立豊中	69	12	57	21
ＪＲ大阪鉄道	68	16	52	7
岸和田徳洲会	66	12	54	5
住友	64	3	61	7
府立急性期・総合	62	6	56	5
済生会中津	61	8	53	10
市立堺	55	33	22	20
八尾徳洲会総合	47	16	31	6
兵庫				
明和	264	88	176	20
姫路赤十字	230	68	162	11
兵庫医大	172	76	96	13
県立西宮	142	17	125	10
神戸市立中央市民	109	21	88	20
県立がんセ	104	59	45	24
西神戸	98	10	88	14
神戸大	93	68	25	20
神戸朝日※	91	0	91	0
神戸市立西市民	61	15	46	9
関西労災	53	19	34	13
奈良				
県立医大	103	25	78	14
天理よろづ相談所	100	29	71	3
大和高田市立	76	5	71	9
近畿大奈良	56	2	54	9
県立奈良	40	4	36	8
和歌山				
県立医大	276	58	218	15
済生会和歌山	70	5	65	2
鳥取				
鳥取大	104	22	82	6
島根				
県立中央	76	2	74	3
島根大	54	7	47	3
岡山				
岡山大	237	59	178	9
岡山済生会総合	227	58	169	26
倉敷中央	103	18	85	11
天和会松田	81	59	22	10
津山中央	71	19	52	7
倉敷成人病セ	59	2	57	3
川崎医大	42	12	30	－
広島				
中国労災	149	22	127	－
市立広島市民	144	43	101	15
広島大	142	88	54	19
ＪＡ尾道総合	97	21	76	6
国・呉	94	14	80	15
広島赤十字・原爆	93	78	15	6
福山市民	58	28	30	5
国・福山	55	18	37	7
県立広島	50	35	15	14
山口				
山口大	97	33	64	15
下関厚生	94	44	50	8
徳山中央	68	13	55	6
周東総合	42	6	36	3
徳島				
徳島大	218	58	160	17
県立中央	145	6	139	6
徳島市民	62	21	41	11
徳島赤十字	41	13	28	5
香川				
県立中央	148	28	120	14
香川大	118	20	98	5
ＫＫＲ高松	41	4	37	0
高松赤十字	40	10	30	21
愛媛				
松山赤十字	287	11	276	13
県立中央	151	34	117	10
国・四国がんセ	40	31	9	23
高知				
高知大	204	29	175	10
近森	69	3	66	2
高知医療セ	56	37	19	20
福岡				
飯塚	180	73	107	10
国・九州医療セ	169	29	140	12
福岡市民	130	47	83	8
福岡大	130	17	113	12
久留米大医療セ	115	15	100	20
久留米大	109	84	25	18
北九州市立医療セ	83	32	51	20
小倉記念	80	9	71	10
九州厚生年金	77	8	69	7
産業医大	70	36	34	5
福岡大筑紫	61	7	54	5
新古賀	53	25	28	0
戸畑共立	51	7	44	2
国・福岡東	49	19	30	1
浜の町	48	7	41	14
佐賀				
佐賀大	108	56	52	6
県立好生館	61	4	57	4
佐賀社会保険	50	3	47	2
長崎				
国・長崎医療セ	134	72	62	24
佐世保市立総合	129	104	25	6
長崎市立市民	108	6	102	3
長崎大	105	52	53	17
熊本				
ＮＴＴ西日本九州	45	15	30	5
大分				
大分大	105	17	88	12
大分赤十字	73	50	23	9
県立	73	15	58	4
市立中津市民	51	7	44	3
宮崎				
宮崎大	42	22	20	8
宮崎医療セ	41	5	36	0
鹿児島				
鹿児島厚生連	107	54	53	14
鹿児島大	100	53	47	13
鹿児島市立	82	25	57	5
南風	73	9	64	7
沖縄				
浦添総合	17	11	6	7

「国・」は独立行政法人国立病院機構、「セ」はセンター。「－」は無回答または不明。外科のみ、内科のみ回答の施設もある。
※神戸朝日病院は2009年4月～2010年3月実績

地域医療のカタチ

近畿大学医学部附属病院

工藤 正俊 病院長

南大阪の救急医療の向上と先進医療の提供・研究に尽力

南大阪唯一の大学病院として、専門性の高い先進医療の提供・研究、医師の教育・派遣など、医療全体の未来を支える役割を担う近畿大学医学部附属病院。特定機能病院にも認定される同院の工藤病院長にお話を伺った。

1.大阪府の指定を受け三次救急を担う救命救急センターを持つ近畿大学医学部附属病院。ドクターカーやドクターヘリで搬送される遠方からの患者も多い　2.院内では大学から寄付された絵画が飾られる　3.中核病院として1日平均2000人を超える外来患者が訪れる

地域における役割と現状

設立した3センターを中心に地域の救急医療を円滑に推進

特定機能病院に認定され、低出生体重児や心疾患・外科疾患新生児の治療を行う新生児集中治療室（ＮＩＣＵ部）や、一次・二次救急患者を受け入れる救急診療部（ＥＲ部）、三次救急に対応する救命救急センター、ＰＥＴ診断と再生医療を専門とした高度先端総合医療センターなどを併設し、最先端の医療を提供する近畿大学医学部附属病院。「救急で多い脳卒中、心筋梗塞などの心血管疾患、消化管出血、外傷の4つのうち、外傷を除く3つの疾患に対して、それぞれ脳卒中センター、心血管センター、吐下血に対して内視鏡で治療を行う光学治療センターを設立し、積極的な受け入れを行っています」と話してくれた工藤病院長。南大阪で受け入れ先の不足が問題となっている3疾患だから、地域のニーズに応えるように、救急の受け入れ数も増えているのだという。「地域の救急医療を円滑に行う為には、大学病院が行う三次救急はもちろん、一次・二次救急の整備が重要です。しかし、医療過疎が深刻な南大阪においては、当院もその役割を担っていかなくてはなりません。一次・二次救急に関してはＥＲ部で積極的に受け入れるほか、地域の救急医療向上の為に、救急ネットワーク会議などで情報提供を積極的に行っていく必要があります」。

専門性と地域医療連携

世界基準のがんの治験と高度医療の提供で地域に貢献

がん細胞の増殖を抑え、従来までは手のつけられなかった進行がんにも効果を発揮する『分子標的治療』をはじめとする先進的ながん治療への取組みを行っている近畿大学医学部附属病院。「当院は総合的にがん治療を行う『がんセンター』により、地域のがん患者に対して質の高い標準治療を提供できるほか、がん治療の臨床試験にも積極的に取組んでいます。治験の症例数は全国的に有数で、世界でも上位に入るほどグローバルな研究・開発を行っています」と工藤病院長。文部科学省が提唱する『がんプロフェッショナル養成プラン』において近畿エリアの基幹校となり、がん治療の専門医の育成に尽力。平成19年には『がん診療連携拠点病院』にも指定された。更に南大阪の医療の向上を目指す大学病院として、地域医療機関との連携は不可欠となる。「２００４年の研修医必修化問題により、南大阪から多くの大学病院が撤退しました。理想は当大学が地域医療機関に多くの医師を派遣する事で、地域全体として質の高い医療が提供できればと思いますが、現実的には難しい。ですので当院は、地域の中核病院として、連携パスなどの円滑な病診連携のもとで、患者さんに高度医療を提供していかなければなりません」。

南大阪の医療向上の為に

病院が行う取組みに加え患者さんの意識改革も必要

「在院日数が長期に渡り、他の病院でも同様の医療が受けられる患者さんに対して、受け入れ先を探す『退院調整ナース』を配置。他の病院では治療が困難な患者さんを少しでも受け入れられる体制を目指しています」。ＭＲＩやＣＴなど最先端の医療機器も導入し、高度医療の提供に万全な体制を取る工藤病院長は、患者さんの意識改革も必要だという。「皆さんの健康を広く診てくれる地域の開業医の先生は、専門ではない分野に関する新しい情報に必ずしも詳しくない場合もある。ですので、患者さん自身も御自分の病気についての知識を得る努力をし、医師まかせにせずに自覚を持って医療機関を利用してください」。

特定機能病院の認定や日本医療機能評価機構からの施設認定のほか、がん診療連携拠点病院、肝疾患診療連携拠点病院などに指定される近畿大学医学部附属病院

hospital data

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今週号の主な内容

座談会

新『消化器腫瘍 WHO分類』から考える 日本の病理戦略

2010年10月，10年ぶりに改訂された『消化器腫瘍 WHO分類（第4版）』（WHO Classification of Tumours of the Digestive System, 4th edition）がWHOより公表された。この新しい消化器病理のスタンダードの作成には，上部消化管，下部消化管，肝臓・胆道，膵臓の各分野のワーキンググループに日本からも病理医が参加し，日本発の疾患概念も取り入れられるなど国際標準化に大きく貢献した。ただ一方で，日本と欧米にはいくつかの病変で概念上のずれがあるのも事実だ。

本座談会では，2009年12月にフランス・リヨンで開催されたWHO分類最終コンセンサス会議（以下，リヨン会議）に出席した4人の病理医を迎え，改訂された新分類の最新情報と消化器病理の国際動向を議論。国際分類作成における日本の病理医の役割と戦略を展望する。

福嶋 敬宜氏＝司会
自治医科大学教授・病理学

下田 忠和氏
国立がん研究センター がん対策情報センター

中村 眞一氏
前慶應義塾大学教授
三菱化学メディエンス 病理・細胞診センター

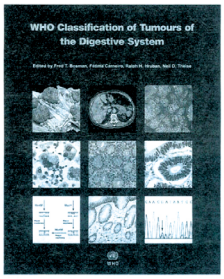
坂元 亨宇氏
慶應義塾大学医学部 教授・病理学

福嶋　2000年の第3版発行から10年，このたび『消化器腫瘍WHO分類（第4版）』が発行されました。

まず，ご担当領域ごとに今回の改訂のポイントをご説明ください。

焦点は「早期病変」の取り扱い

下田　私は上部消化管領域を担当しました。今回この領域では，「intraepithelial neoplasia」と分類される早期の腫瘍性病変が大きく取り上げられ，治療法まで踏み込んだことが最大の変更点です。また，わかりにくかった「神経内分泌腫瘍（neuroendocrine tumour）」の分類が膵臓も含めて明確になりました。以上の2点がポイントです。



●『消化器腫瘍 WHO分類（第4版）』

中村　私は下部消化管，大腸を中心に改訂に携わりました。この領域は第3版をほぼ踏襲したものとなりました。ただ上部消化管と同様，これまで炎症性腸疾患に関連した異形成という意味で用いられていた「dysplasia」という用語が，今版では炎症性腸疾患に限らず早期病変で使用可能となったため，何を具体的に示しているかわからず私自身戸惑っています。

下田　「dysplasia」の定義は今回新たな問題として浮かび上がりましたね。定義があいまいなため異形度分類や精度管理ができないとの議論から，今版では「intraepithelial neoplasia」が“消化管上皮性腫瘍診断のための国際コンセンサス分類（ウィーン分類）”[1]に準じて記載されました。しかし，診断名としての「dysplasia」が残されたことが問題になっていると考えられます。

福嶋　原因はそこですね。このほか下部消化管で新たな動きはありましたか。

中村　「serrated lesions」という分類が新設され，非常に珍しい「micropapillary carcinoma」や「serrated adenocarcinoma」といったタイプの腫瘍も取り入れられました。今回特にうれしかったのは，「粘膜内癌（intramucosal adenocarcinoma）」という用語が“日本では”という注釈付きながら認められたことです。

福嶋　そこは日本の主張が通った部分ですね。坂元先生，肝臓と胆道分野の解説をお願いします。

坂元　肝臓腫瘍の大半は肝細胞癌です。ですから他の臓器よりも分類はシンプルですが，肝細胞癌の早期癌と前癌病変に関するコンセンサスが明確に記載され，「early hepatocellular carcinoma」という用語が正式に認められたことは大きな進歩でした。これまで日本の概念や分類が国際的にはなかなか認められてこなかったなかで，これは極めて意義深かったと思います。

実は，このコンセプトは第3版にも掲載されていました。しかし前回の改訂では，疾患の遺伝子に関する知見が新たに追加された分，本来WHO分類が果たすべき用語や分類の標準化のための議論が十分になされず，あまり注目されなかった経緯がありました。第4版は，2009年のコンセンサス[2]を追認する形となったので，世界の肝臓病理のスタンダードになると思います。

また胆道では，消化管と同様に上皮内腫瘍という概念が取り入れられ，嚢胞性疾患なども含めて膵臓との類似性を意識した分類となり，より実情に合うように改訂されたことがポイントです。

福嶋　早期肝細胞癌のコンセプトは第3版でも採用されたのに，あまり引用されてきませんでしたね。

坂元　ええ。第3版とほぼ同時期に，早期癌の概念を否定した米国消化器病学会を中心とする「Working Party分類」が報告され，肝臓領域ではダブルスタンダードとなっていました。

こうした背景のなか，神代正道先生（久留米大名誉教授）らが中心になって国際会議で繰り返し議論し，結果的に10年近い年月を経て今回の合意形成へと至りました。病理医の議論を後押ししたのは，日本のエコーや造影CTによる画像診断です。欧米でも画像診断に対応して，臨床現場で日本と同じような病変の診断が必要となったことも影響し，今回につながったと思います。

福嶋　今回の改訂までには多くのステップがあったのですね。

私が担当した膵臓では，消化管・肝臓よりも改訂項目は少なかったと思いますが，日本から報告されていた「in-

（2面につづく）

（2面につづく）

座談会　新『消化器腫瘍 WHO 分類』から考える

（1面よりつづく）

traductal tubulopapillary neoplasm」という粘液を産生しない膵管内腫瘍の一群が，1つの分類として認められたことが大きなポイントです。これにより，行き場のなかったいくつかの症例がうまく収まるようになると思います。

また，IPMN（intraductal papillary mucinous neoplasma）やMCN（mucinous cystic neoplasma）の定義の変更もありました。膵臓では他の消化管と異なり，以前から上皮内癌や非浸潤癌の概念が認められていたのですが，今回は米国側の考えが強く反映されたためか消化管側に近寄り，浸潤がない場合は「IPMN with（＊）dysplasia（＊には mild，intermediate，high grade などの異形成の程度が入る）」として「carcinoma」を用いない形式となりました。MCNも同様です。このような消化管との用語統一の動きによって，むしろ病理学の進歩と逆行してしまったのではないかとも感じます。

「経験の有無」が日本と欧米を隔てる

福嶋　各領域の改訂ポイントを伺うと，「早期病変」の取り扱いが最もcontroversialな部分で，今後も重要となりそうですね。

下田　リヨン会議の前に行われた2009年9月の会議で，早期病変である粘膜内・上皮内腫瘍性病変の取り扱いや分類の議論がなされ，“浸潤がないものは癌と診断しない”と，特に米国側が強硬に主張しました。

その背景には欧米の病理医の早期病変の経験の少なさがあったのですが，欧州側は，日本の豊富な経験と病理学的解析に基づいたウィーン分類を無視するわけにいかないと反論してくれました。そこで私は，浸潤にかかわらず高度異形を示す上皮内病変は癌と記載すべきであると主張しました。その結果，ドラフトではそれが記載されていたものの米国側の賛同は最後まで得られず，高度異形上皮内腫瘍は日本での非浸潤性上皮内（粘膜内）癌と同じ，という記載にとどまりました。

この経緯を振り返ると，早期癌に対する「経験の有無」が日本と欧米の大きな差となり，この差が解決されない限りコンセンサスは得られないだろうと思います。

福嶋　その意味では，そういった病態があることを臨床側に気付かせるためにも分類の作成が必要です。分類が臨床側にも注目されれば，まわり回って病理医の理解につながる可能性もあると思います。

下田　その可能性は大いにあります。例を挙げると，バレット腺癌や潰瘍性大腸炎の癌では極めて異形の低い浸潤癌（低異形度上皮内腫瘍に相当）があることを米国の病理医も知るようになりました。そのような特殊な癌では浸潤が見られなくても既に癌として治療を行っています。

分子病理の取り入れも進む

福嶋　分子病理学の進展は，今回の改訂にも大きな影響を及ぼしていますね。

中村　分子病理の知見から，以前は遺伝性非ポリポーシス性大腸癌（HNPCC）と呼ばれていた疾患がリンチ症候群と名を変えました。これは，アムステルダム基準という臨床的な基準で診断されていたHNPCCの病因がミスマッチ修復遺伝子変異であることが明らかとなり，本疾患研究に功績のあったヘンリー・リンチにちなんだ病名が復活したことによります。

福嶋　分子病理を背景とした疾患概念となったわけですね。

中村　はい。このほかにも，以前は「hyperplastic polyposis」と呼ばれた疾患が「serrated polyposis」に変わるなど，より詳細に分類されてきています。

福嶋　肝臓では細胆管癌が分子病理の進歩から，stem cell feature を有する混合型肝癌に分類されましたね。

坂元　ええ。ただ肝臓では，大腸ほど分類ははっきりしていません。分子マーカーによる分類が本当に臨床的な意味を持つかは，さらなる検討が必要だと思います。

福嶋　一方，これまで腫瘍ではなくhyperplastic と考えられていた病変でも，分子異常により腫瘍性が疑われるものが出てきました。

中村　本当に腫瘍性病変かは論議の残る部分です。私個人としてはそのような見方に反対しています。

●下田忠和氏
1968年北大医学部卒。卒後同大大学院，国立がんセンター研究所，慈恵医大病理学教室を経て，94年国立がんセンター中央病院臨床検査部病理医長，2009年より現職。消化管病理研究に従事し食道癌，胃癌，大腸癌取扱規約作成に携わる。編著に『国立がんセンター大腸内視鏡診断アトラス』（医学書院），『外科病理学』（文光堂）など。UICC病理パネリスト。PCLジャパン病理細胞診センター特別顧問。

●中村眞一氏
1971年岡山大医学部卒。76年同大大学院医学研究科修了。浜松医大，高知医大，岡山大を経て，88年浜松医大病院病理部助教授。92年文部省在外研究員として英国St. Mark's病院に勤務。93年岩手医大教授。退職後，DPR株式会社を経て2011年4月より現職。日本病理学会評議員，日本消化器病学会評議員，胃癌取扱い規約委員。編著に『消化管病理標本の読み方（第2版）』（日本メディカルセンター）など。

坂元　私も中村先生と同意見です。「遺伝子変異があるから腫瘍性病変」といった議論が行われましたが，そういった判断を病理医が安易に行うことは反対です。

中村　遺伝子は盛んに討議され，大腸では組織学的なgradingと遺伝子学的な要素であるhigh level of microsatellite instabilityが同時に並べられた表[3]も掲載されました。しかし，これでは分類の趣旨がわからなくなってしまいます。

福嶋　種類の違うものを同じ土俵に置いているようなところは，確かに気になります。

坂元　それでも今回は，本当に臨床病理学的に意味のある分子病理知見を選んで載せるという流れがあり，前版よりは成熟してきたと感じています。

WHO 分類ができるまで

福嶋　本日ご出席の先生は私を含めリヨン会議の出席者です。下田先生は第3版に続いて2回目の参加ですが，出席者や編集担当者はどのような流れで選ばれるのでしょうか。

下田　編集にかかわる人選の詳細は私も知りませんが，WHOが各国から各臓器の専門家を抽出した後に，まず臓器ごとに責任編集者が決められます。その後さらに細かく，例えば各臓器で扁平上皮癌や腺癌などの分類ごとにそれぞれの執筆責任者が決まり，その責任者が数人の執筆者を指名します。

福嶋　出席者については，30人のうち11人が米国人で，アジアからは日本人4人のみでアンバランスな人選だと感じました。世界のスタンダードをめざすといっても，一部の人間の影響が大きいのだなと思いました。

私は初めての参加でしたが，一通り制作の流れを経験して最も驚いたのは，発行までの時間の短さです。十分なディスカッションのないまま流れでまとまってしまったと感じられる部分もありました。

下田　編集・執筆期間の短さは私も感じています。原稿執筆期間は3か月で，書き上げたらすぐ執筆責任者に送ります。そして web で原稿を公開し，内容を議論する web 会議を行いました。そこでの意見に基づいて執筆責任者が修正を行い，そのひと月後のリヨン会議で各領域の責任者が最終的なドラフトを作成しました。

リヨン会議では，最初の全体会議で決めた方針に従って臓器グループごとにドラフトの再点検をして仕上げていきました。そこで初めて掲載する写真も決まります。仕上がるとまた全体会議を開催し挙がった疑問点を議論する，ということの繰り返しが3日間行われました（写真）。

福嶋　膵臓の分類では，web 会議の段階で新しい疾患概念の追加や不適当なものを削除する作業が多少行われたものの，最初から枠組み自体は決まっていたと感じました。

坂元　新しい疾患概念の取り扱いは，シニアエディターと呼ばれる臓器ごとの責任者が決まった時点でかなり決まってきますね。

福嶋　中村先生は今回の会議で何か印象に残ることはありましたか。

中村　私は，今版に大腸の粘膜内癌を取り入れたいと思っていました。そこで，事前にその写真と日本の病理診断

●写真　リヨン会議のもよう
〈左〉膵臓グループの作業風景。
〈右〉全体会議では，30人が一堂に会し臓器横断的な概念などが話し合われた。

●坂元亨宇氏
1985年慶大医学部卒。89年同大大学院医学研究科修了。財団法人がん研究振興財団，国立がんセンター研究所病理部，同部長を経て2002年より現職。肝臓の早期癌，多段階発癌，分子診断に関する研究に従事。日本病理学会評議員，日本癌学会評議員，日本肝臓学会評議員，NEDO「がん超早期診断・治療機器の総合研究開発／病理画像等認識技術の研究開発」サブプロジェクトリーダーを務める。

●福嶋敬宜氏
1990年宮崎医大卒。国立がんセンター中央病院医員，米国ジョンズ・ホプキンス大研究員，東医大講師などを経て，06年東大大学院准教授。09年より現職。日本病理学会評議員，「Pathology International」常任編集委員。編著に『臨床に活かす病理診断学（第2版）――消化管・肝胆膵編』（医学書院，2011年4月発行予定），『その「がん宣告」を疑え』（講談社）など。

基準を米国人の編集責任者に送ったのですが，彼がその写真に感銘したようで掲載につなげることができました。

福嶋　実物を見せるのがいちばんですよね。しかし，そういったプロセスは会議ではほとんどありませんでした。

中村　ええ。概念の話ばかりで，症例を見ながらのディスカッションは最後の写真選びのときのみでした。

福嶋　思い返すと，早期病変の議論でも写真を見ながらディスカッションができればもう少し違った流れになったかも知れませんね。

下田　おそらくもっと早くまとまったでしょう。米国の病理医の頭の中には「浸潤のないものは癌ではない」という固定概念がありますから，言葉だけでは頑なに拒否してしまうわけです。ですから，もう少し実際の組織像と臨床所見（特に内視鏡）を見て，そこでコンセンサスを得た概念を載せる形にする必要がありますね。

福嶋　もう一つ，病理医のみが出席者であったことも気になります。臨床医がいないため，臨床的な視点が少し足りないのではないかとは感じられませんでしたか。

坂元　臨床的な特徴も分担執筆項目には含まれますが，ページ数を減らすという方針により，病理に関連するエッセンスのみとなってしまいました。

先に述べたコンセンサス[2]では，工藤正俊先生（近畿大教授）から画像で経過を追った症例と病理との対応などを繰り返し提示してもらいました。臨床からの視点はやはり説得力がありますし，臨床医が加わらないと本当に意味のあるものはできないと感じます。

福嶋　欧米側も，臨床医が参加すると変わってくるでしょう。ただ現状では，日本の病理医は臨床医の代弁を担うことも必要になりますね。

教科書としてのWHOブック

福嶋　WHO分類には，腫瘍分類のほか病理の教科書としても使用可能という特徴がありますが，その点についてはいかがですか。

坂元　WHO分類には，発展途上国も含め世界で使用可能という使命があるため，分子病理学的な解析が行えないような地域でも使える分類，言わば最先端と汎用性の両面を追求した内容とする狙いがあるようです。

この点において，肝臓領域で特に第3版との違いを感じたのは，細胞診が取り上げられたことです。肝臓の細胞診は日本ではほとんど行われませんが，組織診ができず吸引細胞診のみ実施可能な国もあることから記載されました。さらにリヨン会議では，アルゴリズムの作成に最も時間をかけました。経験の少ない方でもわかりやすい診断のアプローチを示すためです。このような取り組みはやはりWHOでないとできないので，今回の大きな成果ですね。

下田　WHO分類は，ブルーブックと呼ばれた初版と第2版では分類を記載するだけでしたが，第3版から分類だけではなく，その詳細な内容と診断に直接役立つ説明や写真を加えた現在の形式となり，アトラス的な要素も含まれてきました。

福嶋　多くの情報が記載され，WHO分類は病理医の部屋に必須の書籍になったと思います。

一方，臨床医は日本の「癌取扱い規約」などとの使い分けも必要ですよね。

下田　「WHO分類」と「癌取扱い規約」の内容は大きく異なります。臨床現場では癌取扱い規約を，海外の雑誌に論文を投稿するときはWHO分類などを使用すればよいと思いますが，それぞれどこが異なるかをきちんと頭に入れて，使い分けていく必要があります。

日本でも胃癌に関しては，UICC分類との整合性をできるだけとるようになってきています。ただ世界では，少なくとも胃ではWHOの癌組織型分類はあまり使われていません。したがって，世界のどの国でもWHO分類との乖離があるのが現状です。

福嶋　WHO分類は，論文に登場するすべての腫瘍の掲載をめざしたように，網羅性を重視しています。ですから，矛盾をはらむ可能性があることは認識しておく必要があります。

アジアでのコンセンサスが重要となる

福嶋　多くの動きがあった今回の改訂ですが，10年先にはおそらく次の改訂が待っています。日本のプレゼンスが反映されない部分もまだありましたが，次回改訂に向け日本の消化器医療にかかわる医療者は，どのような国際戦略をとればよいでしょうか。

下田　それは何よりも英語で論文を多く出すことです。今回，胃では新しい概念として胃型腺腫（幽門腺腺腫）が腺腫の1つとして記載されましたが，これは日本からの論文がきっかけです。

坂元　私も同じ意見です。WHO分類では，論文となっていない情報は絶対に載りません。論文は最低限の前提ですが，コンセンサス会議の場で急に新しい提案をしても受け入れられることはないため，日ごろから日本の概念や分類について，欧米の医師とface to faceで話し合うことが大事です。

福嶋　国際学会も増えてきているので，そういう場でのコミュニケーションが大切ということですね。

坂元　ええ。できれば臨床医や画像診断医も加わって，直接標本を見ながらディスカッションができるとよりよいですね。

また，アジアの診療レベル・画像診断レベルは急速に向上しています。欧米の臨床医や病理医と直接議論するのはさまざまな面で困難ですが，疾患が似ているアジアであれば日本の学会と同じ感覚でできるので，アジアの中での会議を頻回に行うことが有益だと思います。今後は中国も加わって，アジアでのコンセンサスを得ていくことが，欧米と議論する上でも大切なステップになると思います。

福嶋　いきなり米国と議論すると背景となる概念の違いからも困難が予想されるので，アジアからというのは実際的な提言ですね。

下田　アジアはますます重要になるでしょう。私は約20年前から，韓国の医師と交流を進めてきました。臨床医と病理医が一緒になった議論を通じて，韓国での消化管病理診断はいまや日本とほぼ同等となっています。

「若者よ，海外に出よ」

福嶋　国際的な仕事は今後より多く求められますが，将来を担う若手病理医にぜひアドバイスをお願いいたします。

中村　昨年ノーベル化学賞を受賞した根岸英一先生が「若者よ，海外に出よ」と受賞会見で述べましたが，外国に行くことは外国を見ることと同時に，振り返って日本を見るというもう1つの見方があるので，若手医師や研究者にはぜひ一度海外に出てほしいなと思っています。

坂元　私も同感です。若手のなかには外国の学会に演題を出したがらない人もいますが，ぜひ外国に出て行ってほしいと思います。また，日本の強みでもある「標本を丁寧に見る」ことを，ぜひ自信を持って行ってほしいです。

福嶋　私は米国に留学し，病理医の守備範囲の違いや診断基準の違いを肌で感じました。また外国の医療現場を実際に見ると，「日本のほうが優れている点も少なくない」という気付きにもなります。日本だけでなく外国の医療についても見識を深めることで，その延長線上にWHOを含めた国際的な活躍の場があるように思います。

最後に下田先生，お願いします。

下田　いちばん大切なことは，病理医であっても患者さんを治療するチームの一員であるという認識を持つことです。臨床医とも絶え間なくディスカッションを行い，その議論を基にまた標本を見る。そこには，必ず何か訴えかけてくるものがあるはずです。

そうした疑問を解決しようとする機運が出てくれば，研究をすればいい。そしてその成果を，外国に行って下手な英語でもいいのでどんどん発信していくことが大事です。

われわれは日本のことばかり主張する傾向がありますが，やはり相手を知った上でこちらの立場を強調することが大切で，そのためには外国へ行かなくては駄目です。このような活動によって理解が生まれ，必ず納得してもらえるようになると私は思っています。

福嶋　まさにおっしゃるとおりだと思います。

本日はWHO分類の改訂をテーマに，消化器病理の国際的戦略まで伺ってきました。良い点も不十分な点もまだたくさん含まれると思われるWHO分類ですが，腫瘍分類として国際的に最も影響力のあるものには違いありません。そう考えると，せっかく日本の消化器病理は世界の最先端を走っているわけですから，「認められない」とむくれるよりも海外の人たちに少しでも理解してもらうことを戦略的に考えるべきだと思います。そのためには，日本の臨床家や研究者だけでなく海外の研究者とのコミュニケーションも大事にしていかなければならないという強いメッセージがありました。

豊富な経験からのさまざまなお話，どうもありがとうございました。（了）

参考文献
1）Schlemper RJ, et al. The Vienna classification of gastrointestinal epithelial neoplasia. Gut. 2000; 47(2): 251-5.
2）International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology. 2009; 49(2): 658-64.
3）WHO Classification of Tumours of the Digestive System, 4th edition. IARC. p138. Table8.01.

第49回日本癌治療学会関連特集＝最前線の現場から

■ 肝がん・膵がん ■

●肝がん（肝細胞癌）　●肝がんの早期発見　●肝がんの治療アルゴリズム　●肝切除　●肝移植　●ラジオ波焼灼術　●TACE（Transcatheter arterial chemoembolization）　●動注化学療法　●全身化学療法　●肝細胞癌の今後の展望　●膵がん　●膵がんの診断　●膵がんの治療　●膵がんの今後の展望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1 肝がん（肝細胞癌）

原発性肝癌の約94％を占める肝細胞癌は，慢性ウイルス性肝炎や肝硬変を母地として発生する癌である。世界的にみれば，アジア，アフリカに多くみられる。わが国をはじめ欧米ではC型肝炎ウイルスがその主な原因であり，日本を除くアジア諸国およびアフリカではB型肝炎ウイルスが主な原因である。最近では，非アルコール性脂肪性肝炎（NASH）や糖尿病，肥満の患者からの発癌が増加してきており，問題となっている。

2 肝がんの早期発見

前述のように肝細胞癌は原因がはっきりとしているため，サーベイランスが行いやすい。肝癌診療ガイドライン2009[1]ではB型慢性肝炎，C型慢性肝炎，肝硬変のいずれかが存在すれば高危険群と位置づけている。なかでもB型肝硬変，C型肝硬変患者は超高危険群と位置づけられている。そしてこれに基づき肝細胞癌の早期発見のためにサーベイランスアルゴリズムが提唱されている（図1）。超高危険群に対しては，3～4カ月に1回の超音波検査と，AFP，PIVKA-Ⅱ，AFP-L3分画の測定を，高危険群に対しては6カ月に1回の超音波検査とAFP，PIVKA-Ⅱ，AFP-L3の測定が推奨されている。これらにより肝細胞癌の存在が疑われれば，dynamic CT/MRIを行い，確定診断を行う。最近では，ソナゾイド®（ペルフルブタン）を用いた造影超音波や肝特異性造影剤であるEOBを用いたMRIなどで詳細な質的診断が可能になっている。また非典型的な画像所見を呈する場合は超音波ガイド下の腫瘍生検を行い，確定診断を行う。

3 肝がんの治療アルゴリズム

前述の肝癌診療ガイドライン2009は，肝細胞癌に対するガイドラインとして初めて刊行された「科学的根拠に基づく肝癌診療ガイドライン2005年版」の改訂版である。このガイドラインには病態に応じた治療方法の選択基準として肝細胞癌治療アルゴリズムが推奨されている（図2）。これを基本としてより実臨床に即したものとして汎用されているアルゴリズムが日本肝臓学会推奨のコンセンサスに基づく肝癌治療アルゴリズム[2]である（図3）。実際の診療においては，これらの治療アルゴリズムに基づいて治療が選択される。基本的には肝機能，腫瘍数，腫瘍径に基づいて治療法が決定される。3cm，3個以下のものに対しては肝切除あるいは局所療法が行われるが，4個以上のものに関しては，TACEや動注療法が選択される。また脈管浸潤を有する場合，あるいは遠隔転移を有する場合は全身化学療法が選択される。また最初からChild-Pugh Cで肝機能不良の場合は基本的に緩和治療となるが，ミラノ基準内であれば肝移植が選択される。

4 肝切除

3cm，3個以下，あるいは大型肝癌に対して適応される。根治を目指すことができる治療方法であるが，侵襲が大きいことが問題であり，肝予備能が良好な症例に限られる。

3cm，3個以下の腫瘍条件では後述のラジオ波焼灼術との成績の優劣について結論が出ていないため，現在，SURF trialとして多施設共同の前向き比較試験が行われている。

5 肝移植

肝機能不良（Child-Pugh C）で，かつミラノ基準（3cm，3個以下，5cm以下単発）を満たす場合に選択される。肝細胞癌ならびに併存する肝疾患の両方を根治できる治療法であり，保険も適応されるが，ドナーの確保や移植肝への肝炎ウイルスの再感染や再発などが問題である。

6 ラジオ波焼灼術

3cm，3個以下，5cm以下単発の腫瘍条件に対して施行される。侵襲が少なく，安全性の高い治療法である。施行にあたっては超音波下に腫瘍を描出できることが必要であるが，描出困難な場合もあり，その場合は，造影超音波検査やRVS（Real time virtual sonography）を利用することで腫瘍の描出が可能となり，穿刺が可能になる。特に造影超音波検査でdefect像を描出しながらソナゾイド®を再投与することで再潅流（抜け染まり）を確認する方法（Defect Re-perfusion imaging）は，非常に有用な方法である[3]。

7 TACE（Transcatheter arterial chemoembolization）

4個以上の多発例に対して主に用いられる。栄養動脈の遮断による阻血効果と抗癌剤の徐放効果を狙った治療方法である。区域，亜区域レベルのTACEが標準的に行われる。マイクロカテーテルを用いて病巣の栄養動脈をできるだけ末梢まで選択し，抗癌剤とイオダイズドオイル（リピオドール®）を混合したものを注入後，多孔性ゼラチン粒を注入して，栄養動脈を塞栓する。抗癌剤として，ドキソルビシン，エピルビシン，マイトマイシンC，シスプラチンなどが用いられるが，どの抗癌剤を選択すべきかについては明確なエビデンスは存在しない。一般的に，腫瘍サイズが小さく，結節型のものに対して超選択的にTACEを行う場合は塞栓による壊血効果が，両葉多発例など片葉，あるいは全肝を対象にTACEを行う場合は抗癌剤の感受性が抗腫瘍効果に影響を与えるものと考えられる。

TACEは切除やRFAと違い，根治療法ではない。このため再発は必至である。3～4カ月ごとに定期的にTACE（いわゆるScheduled TACE）が施行されることも多いが，この方法は，抗腫瘍効果は認められるものの生命予後の向上には寄与しないという報告がある。繰り返しによる肝機能低下が原因であり，TACEが不応と判断される場合は，すみやかにソラフェニブによる全身化学療法に切り替える。多孔性ゼラチン粒を用いず，抗癌剤とイオダイズドオイル（リピオドール®）の混合液のみを注入する方法もあり，Chemolipiodolizationあるいは Lip-TAIと呼ばれている。この方法は比較的高頻度に用いられているが，TACEと違い，リピオドールがすぐにwash outされるのが問題である。また，リピオドールに混合する抗癌剤は水溶性であり，すぐに分離してしまう可能性がある。最近，リピオドールに親和性を持たせるべく，ミリスチン酸を側鎖として結合させたプラチナ製剤であるミリプラチン（ミリプラ®）が承認され，有効性が期待されている。

8 動注化学療法

動注化学療法は，主に脈管侵襲を伴う進行肝細胞癌に対する初回治療として行われている。TACEを繰り返し行ううちに門脈浸潤をきたした場合などにも選択される治療法である。動注化

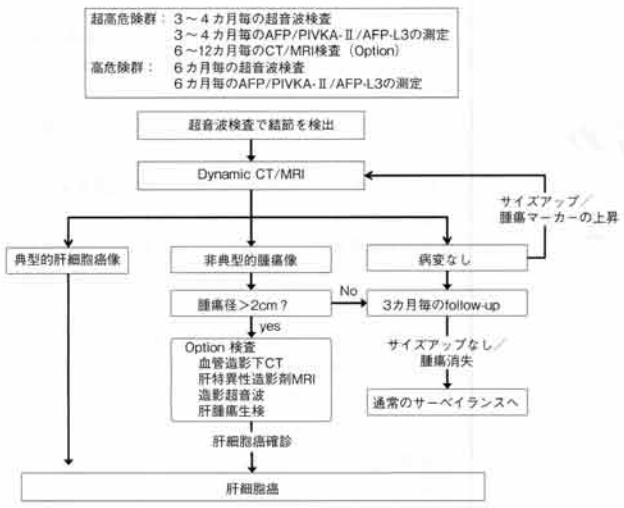

図1　肝細胞癌サーベイランスアルゴリズム・診断アルゴリズム[1]

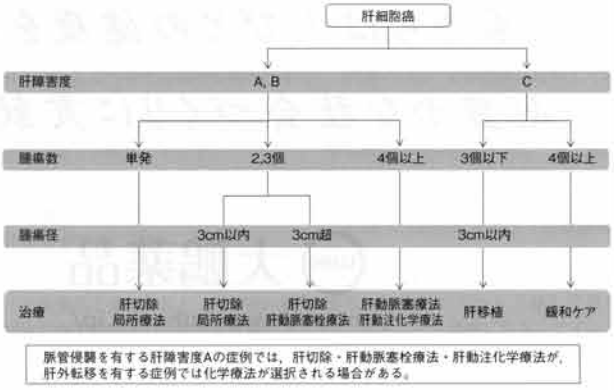

図2　「科学的根拠に基づく肝癌診療ガイドライン」の治療アルゴリズム[1]

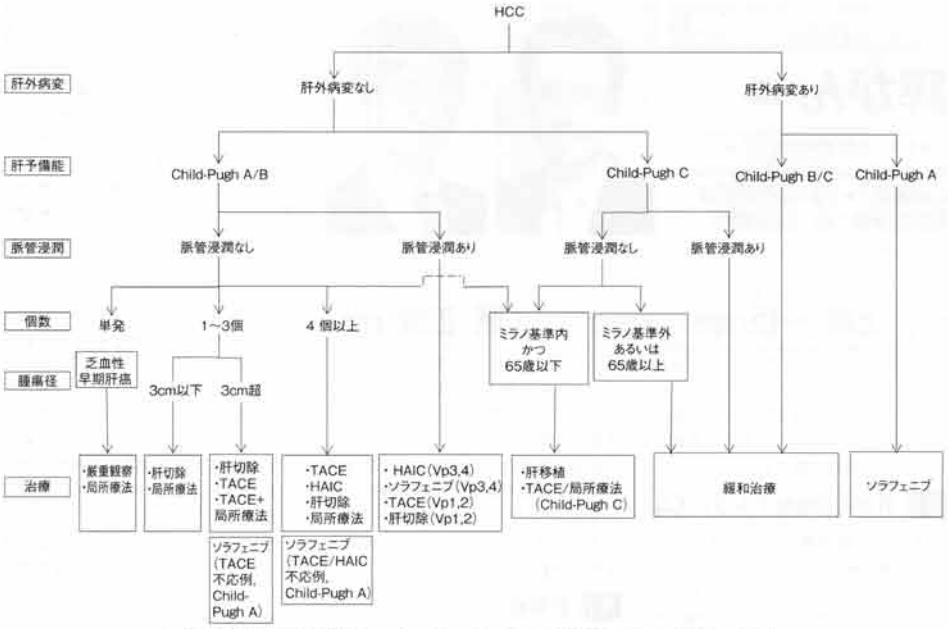

図3　日本肝臓学会提唱のコンセンサスに基づく肝細胞がん治療アルゴリズム2010[2]

学療法には，留置カテーテルを用いたいわゆるリザーバー肝動注化学療法と，その都度セルディンガー法でカテーテルを挿入し，ワンショット動注を行う方法の2つの方法がある。

腫瘍血管である肝動脈より直接抗癌剤を注入することにより，肝細胞癌組織での抗癌剤の濃度を高めることができ，また肝臓で薬剤が代謝されることにより全身臓器への薬剤の移行が減少する。すなわち少量の抗癌剤で最大の効果と最小の副作用というメリットがある点で全身化学療法と比べ有利である。

門脈浸潤とくにTACEの適応から外れるVp3，4症例に対してよく用いられているが，両葉多発症例やTACE困難な巨大な肝細胞癌症例に対しても選択される。遠隔転移をきたしていても，原発巣が予後を決定するような場合も相対的適応として行われることがある。

Low-doseFP療法は，最も広く行われている方法である。5-FUがキードラッグであり，その効果を増強させる目的(biochemical modulation)で少量の

ニュースレーダー

胆汁排泄型のDPP-4阻害剤「トラゼンタ錠」を発売

—日本ベーリンガーインゲルハイム㈱—
—日本イーライリリー㈱—

日本ベーリンガーインゲルハイムは9月15日，2型糖尿病治療剤「トラゼンタ錠」(一般名・リナグリプチン)を発売した。DPP-4(ジペプチジルペプチダーゼ-4)阻害剤としては4剤目となる。トラゼンタは胆汁排泄型なのが特徴。主に糞中に未変化体のまま排泄される。尿中排泄は少量(5%)である。1日1回経口投与する。現時点で他の糖尿病治療薬との併用療法の適応はない。

単剤療法において，腎機能の程度に応じて用量調節をする必要性は添付文書に記載がない。

同社と日本イーライリリーが共同で情報提供する。

臨床試験では，投与12週におけるHbA1cのベースラインからの平均変化量は，リナグリプチン群(n=159)が0.49%低下，プラセボ群(n=80)が0.39%上昇で，実薬群が有意に優れていた。

52週間にわたる長期投与でも効果が持続していた。

副作用は720例中86例(11.9%)に認められ，主なものは便秘(1.9%)，鼓腸(1.5%)，腹部膨満(1.0%)など。

◇

▷効能・効果＝2型糖尿病(ただし，食事療法・運動療法のみで十分な効果が得られない場合に限る。)
▷薬価＝5mg1錠　209.40円

シスプラチンを用いる。ほかにもIFN併用5FU動注化学療法(FU arterial infusion and interferon therapy: FAIT)などがある。5FUとインターフェロンの相乗効果を狙ったものであるが，インターフェロンは現時点で保険適応となっていないため注意が必要である。動注化学療法は，局所制御に優れた治療方法であるが，生命予後に寄与するかどうかについては明確なエビデンスがないため，これを証明するRCTとしてSILIUS trial(厚労科研 工藤班)が現在進行中である。

9 全身化学療法

遠隔転移や脈管浸潤を有する，あるいは標準治療が不応である場合に全身化学療法が選択される。現在のところ，有効性が証明された薬剤はソラフェニブのみである。ソラフェニブは，腫瘍増殖のシグナル伝達系であるMAPキナーゼカスケード(RAF-MEK-ERK)のRAF蛋白のセリンスレオニンキナーゼと血管新生のシグナル伝達系であるVEGFR，PDGFRの受容体型チロシンキナーゼを阻害する。他にも，Flt-3やc-Kitなど血管新生や細胞増殖にかかわる受容体型チロシンキナーゼを阻害し，multikinase inhibitorとも呼ばれる。Global Phase Ⅲ trial(SHARP試験)[6]により進行肝細胞癌患者の生命予後を改善することが明確に証明された。

ソラフェニブは腫瘍増殖を抑制することで生命予後を延ばす薬剤である。実際，先述のSHARP試験においてもresponse rateはわずか2％であったが，生命予後は有意に改善することが証明されている。ここが従来のresponse rateを期待した殺細胞性抗癌剤とは異なる部分である。またソラフェニブは，骨髄抑制が比較的少ないという特徴もあり，汎血球減少を合併している肝細胞癌患者に適していると考えられる。

治療効果判定は，4～6週毎に造影CTあるいは造影MRIにおいて行う。また腫瘍マーカーも定期的に測定する。効果がみられれば腫瘍の造影効果は減弱し，腫瘍内部が壊死に陥ることもある。またうまく虚血が得られておれば，腫瘍マーカーであるPIVKA-Ⅱが著明に上昇する現象もみられる[5]。PDでなければ投与を継続する。本剤はSDを長期狙う薬剤であるため，できるだけ長期間内服させることが薬効を発揮させるためには重要であるが，このためにはソラフェニブ特有の副作用である手足皮膚症候群，高血圧症，下痢などのコントロールが重要である。肝性脳症や腹水など，肝不全を呈することもあるため慎重な投与が必要である。

10 肝細胞癌の今後の展望

肝細胞癌に対して，分子標的薬であるソラフェニブが登場し，治療体系が大きく変化してきている。すなわちそれまでBSC(Best Supportive Care)とされてきた症例に対して，有効な治療が選択可能になったということである。しかし，ソラフェニブが登場したことで，すべてが解決されたわけではない。ソラフェニブの耐性をどう克服するか，また副作用により使用できない場合どうするかなど様々な問題点も存在する。これに対しては新規の分子標的薬の開発治験が進んでいる。また，より治療効果を高める工夫として，従来の治療方法との組み合わせ治療についても検討されている。根治療法後のアジュバントとしてのソラフェニブの有効性をみるSTORM治験や，TACEとの組み合わせ(TACTICS試験，ECOG1208試験など)，動注化学療法(Low-doseFP療法)との組み合わせ(SILIUS試験)などの臨床試験が進行している。分子標的薬と標準治療を組み合わせることにより，生命予後を大幅に延長できる可能性があり，今後その有効性が期待されている。

(上嶋一臣，工藤正俊)

11 膵がん

膵がんは増加傾向にあり，本邦では年間約2.6万人が死亡し癌死の第5番目となっている[6]。その殆どが切除不能な状態で診断され，切除可能例は20％前後にすぎない。即ち，その予後向上のためには，早期発見・診断による切除率の向上が必要不可欠である。また，大多数を占める切除不能症例に対する化学療法も重要な因子となる。

12 膵がんの診断

1．危険因子

膵がんの場合，肝癌と比較するとウィルス感染等の危険因子の絞り込みが難しい現状がある。その主症状は，黄疸，腹痛，背部痛，食欲不振，腹部膨満感等，多彩であるが，症状の出現した患者の多くは手術が困難な状態まで進行しており，症状が出現するまでに危険因子からいかに膵がんの診断までつなげていくかが重要である。危険因子として，膵癌の家族歴，糖尿病，慢性膵炎，遺伝性膵炎，膵管内乳頭粘液性腫瘍，喫煙が挙げられる[7]。糖尿病の初回発症や増悪は膵癌の存在を疑わせる所見である[8]。また，慢性膵炎や膵管内乳頭粘液性腫瘍は前癌病変となりうる疾患である。

2．拾い上げ

膵がんの診断は，拾い上げ，存在診断，確定診断，進行度診断の4段階に分かれる[7]。上記臨床症状あるいは危険因子を有する場合，腹部超音波検査，CEA，CA19-9，SPan-1，Dupan-2等の血清腫瘍マーカーの検査を行うことが勧められる。膵酵素である血清アミラーゼ，エラスターゼ1が高値である場合も膵炎以外に膵癌を念頭に入れるべき所見となる。危険因子や血液検査異常を持つ患者に対しては積極的に画像診断による精査を勧めることが早期発見につながると考えられる。検診における画像検査で偶然発見される場合もあるが，早期膵がんの中には，腫瘤像よりも膵管拡張，嚢胞性病変等の間接所見にて拾い上げられる場合があり，膵管拡張や膵嚢胞が超音波，CTで確認された場合には，経過観察ではなく，次の画像診断へと精査を進める[9]。

3．存在診断

存在診断には，超音波，造影CT，PET，MRI，ERCP，超音波内視鏡等が用いられる。膵がんは辺縁不整，乏血性腫瘤，膵管限局性狭窄等の画像的特徴を持つが，典型像を呈さずに診断に難渋することがあり，複数の画像診断を行うことが肝要である。各種画像検査の中で，超音波内視鏡は小病変の検出に優れており(**図4**)[10]，他の画像診断で何らかの間接所見が指摘された場合には，次に行うべき検査と考えられる。また，最近造影による血流評価も行えるようになり，その診断精度がさらに上昇している[11,12]。

4．確定診断

上記画像診断の技術革新により膵がんの診断精度は向上しているが，依然炎症性膵腫瘤あるいは炎症性限局性膵管狭窄との鑑別が困難な症例が存在する。このような良性膵病変との最終的な鑑別診断には，病理診断が重要である。大別すると超音波内視鏡下穿刺とERCPによるものがある。超音波内視鏡検査中に内視鏡先端部から出てくる穿刺針を用いて膵組織を採取する超音波内視鏡下穿刺は，欧米では1990年代より導入されている[13]が，本邦でも2010年4月より保険適応となっている。本法は穿刺針を超音波で観察しながら刺すことから血管などを避けることが可能で，他の方法と比較すると安全に行える検査である。一方，超音波内視鏡を含めたどの画像診断でも明らかな結節像が認められない膵管異常では，ERCPによる吸引・擦過細胞診を行う必要がある。診断に難渋する症例では，これらの内視鏡を用いた病理診断法が重要となり，どちらかあるいは併用により確定診断につなげていくことが必要である。

13 膵がんの治療

1．治療ストラテジー

治療は，外科切除，化学療法および化学放射線療法に大別されるが，根治を期待できるのは外科切除のみであるため，切除可能であるかどうかが第一に検討すべき事項である。Stage Ⅰ，Ⅱ，Ⅲは患者の全身状態が良ければ切除を検討する。Stage Ⅳaは切除可能であれば根治を目指した切除を行うことが勧められる(日本膵臓学会膵癌診療ガイドライン改訂委員会，科学的根拠に基づく膵癌診療ガイドライン，1-151，金原出版，東京)。Stage Ⅳaの場合には，腹腔動脈，上腸管膜動脈，総肝動脈等の動脈系大血管へ浸潤の有無が手術適応に最も関係し，動脈系への浸潤が認められた場合には，切除が困難なことが多い。一方，門脈浸潤例の場合には，門脈合併切除・再建が可能な症例では手術適応となる。3群のリンパ節転移や肝，肺への遠隔転移が認められるStage IVbには手術による根治は望めず，手術適応とはならない。Stage IVaの場合には，局所進行がんとして化学放射線療法を行うことがひとつの選択肢となる。

2．化学療法

進行膵がんに対して化学療法を行う際に，第一選択の抗癌剤として世界的に認められているのはデオキシシチジン誘導体のGemcitabineである。1990年代に北米で行われた第Ⅲ相試験では，5-fluorouracil (5-FU)を受けた患者群よりもGemcitabineを受けた患者群のほうが，症状緩和効果が高く，生存期間が優れていた(**表1**)[14]。本邦では，Gemcitabineは2001年4月より膵がんに対する保険適応が承認されたが，Gemcitabine登場以来，単独でGemcitabineに勝る抗癌剤は出現していなかった。数多くの臨床試験の結果，分子標的薬の1つであるErlotinib (Epidermal growth factor receptor tyrosine kinase阻害剤)がGemcitabineとの併用でGemcitabine単独に比べ生存期間延長が得られた唯一の抗腫瘍薬であることが報告されている[15](**表1**)。本邦でもGemcitabineとErlotinibの併用療法は本年7月に保険認可されているが，上記報告では生存期間中央値が約0.5カ月延長したのみであり，標準治療として使用するべきであるかどうかはコンセンサスが得られていない。一方，本邦では，経口5-FUプロドラッグおよびその代謝阻害薬の配合薬であるS-1が2006年に保険認可されるようになり，新しい治療選択肢が増えた。膵がんに対して本邦で初めて行われた大規模臨床試験であるGEST studyの結果が2011年ASCOで報告された[16]。本臨床研究では，切除不能進行膵がん患者834例を対象にGemcitabine単独，S-1単独およびGemcitabineとS-1の併用の3群に分け，その治療成績を比較検討した臨床研究である。本研究ではOverall survivalに関してはGemcitabie単独療法と比べた併用療法の優越

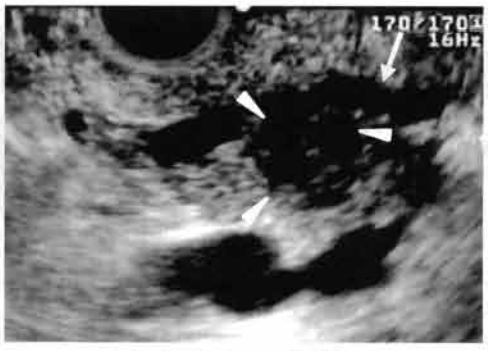

図4　膵頭部がんの超音波内視鏡像

十二指腸下降脚からの観察で膵頭部に最大径約1cmの辺縁不整の低エコー腫瘤(矢尻)が認められる。矢印：主膵管。

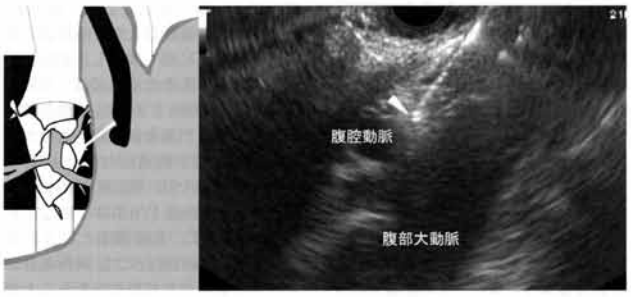

図5 超音波内視鏡ガイド下腹腔神経叢ブロック術
胃体部からの観察で腹部大動脈から腹腔動脈分岐部が明瞭に描出される。腹腔動脈周辺に超音波画像観察下で穿刺針先端部(矢尻)を進ませ，エタノールを注入する。

性は証明されなかったが，Gemcitabineに対するS-1の非劣性が証明された。即ち，S-1がGemcitabineと同様に膵がんの標準治療薬となることが証明された。多剤併用では，2011年FOLFIRINOX（Oxaliplatin+irinotecan+Leucovorin+5-FU）とGemcitabine単独のランダム化比較試験の結果が報告され，FOLFIRINOX治療がGemcitabine単独と比べ抗腫瘍効果と生存期間において有意に優れていた[17]（**表1**）。しかしながら，Grade3以上の有害事象がFOLFIRINOX群において有意に多く認められたことより，全ての切除不能膵がん患者に対して行うべき治療ではなく，対象を十分に検討する必要があるものと考えられる。

3．疼痛緩和治療

膵がんは進行するにつれてほとんどの症例が腹痛あるいは腰背部痛を訴えるようになる。鎮痛薬の投与を化学療法と並行して行うことが疼痛対策の基本となる。鎮痛薬は，原則として非オピオイド鎮痛薬をまず投与し，効果が不十分な場合はオピオイドを追加する。一方，腹腔神経叢ブロックは膵がんの疼痛に対する重要な選択肢となる。オピオイドが効きにくい場合あるいは副作用によりオピオイド継続が困難な場合が適応となる。特に超音波内視鏡ガイド下腹腔神経叢ブロック術は，穿刺経路が短い点，リアルタイムに穿刺針を観察しながら穿刺が可能という点で，安全かつ精度の高い治療として注目されている[18][19]。純エタノールを片側10mLを目標に腹腔動脈の両側に注入する（**図5**）。本治療により膵がん患者の約80%に疼痛緩和効果が得られる。

14 膵がんの今後の展望

上述のごとく，膵がんの予後は満足できる状況とは言い難い。予後向上のためにはいかに拾い上げを行っていくかということが最も重要な課題となっている。プライマリケアの段階で，血清マーカーの上昇，糖尿病，膵嚢胞，主膵管拡張など膵がんを疑う所見が認められた場合には次の精査を勧めることを啓発していくことにより，予後改善が期待される。また，膵がんに有効であると報告されている治療レジメンが少ないことから2nd line，3rd lineの治療に難渋していることも問題となっている。今後，Gemcitabine，S-1に匹敵する抗癌剤の登場が切望される。

（北野雅之，工藤正俊）

表1 進行膵がん化学療法に関する重要なランダム化比較試験[15]

Study design	Regimen	N	MST (Mo)	P value	Publication
GEM vs 5-FU	GEM	63	5.7	0.0025	Barris et al. JCO (1997)
	5-FU	63	4.4		
GEM vs ERL	GEM	284	5.9	0.038	Moore et al. JCO (2007)
	GEM+ERL	285	6.2		
GEM vs S-1 vs GEM+S-1	GEM	277	8.8		Ioka et al. ASCO (2011)
	S-1	280	9.7	Non-inferiority <0.001 (vs GEM)	
	GEM+S-1	277	10.1	0.15 (vs GEM)	
GEM vs FOLFIRINOX	GEM	171	6.8	<0.001	Conroy et al. NEJM (2011)
	FOLFIRINOX	171	11.1		

GEM: Gemcitabie, 5-FU: 5-Fluorouracil, ERL: Erlotinib
FOLFIRINOX: Oxaliplatin+Irinotecan+Leucovorin+5-FU

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UNITED EUROPEAN GASTROENTEROLOGY FEDERATION

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CONGRESS NEWS

UEGW STOCKHOLM 2011

CURRENT NEWS FROM THE 19TH UNITED EUROPEAN GASTROENTEROLOGY WEEK (UEGW) · STOCKHOLM 22-26 OCTOBER 2011

SCIENTIFIC PROGRAMME

Reaching out to East and West

UEGW offers several sessions in cooperation with the Asian-Pacific region and the USA

Be it an "East meets West" symposium on hepatocellular carcinoma, a Japanese/European joint endoscopy symposium or the "Best of" sessions from the American Gastroenterology Association presented at the recent Digestive Disease Week – UEGW has become a flourishing platform for scientific exchange between East and West.

The symposium **"When East meets West: Management of hepatocellular carcinoma"** will be held **today from 15.45 to 17.15 h in Hall A5.** "Hepatocellular carcinoma is a very active research field where novelties with impact in clinical practice are developed on a continuous basis. Years ago it was thought that this disease would have major differences according to geographic location, but several studies have shown that this is not the case. As a result, the exchange of information has become highly fruitful for both sides," says Professor Jordi Bruix from Spain, who will chair the session together with Professor Masatoshi Kudo from Japan.

On **Wednesday (8.30 to 10.30 h in Hall A12/13),** Professors Hisao Tajiri from Japan and Horst Neuhaus from Germany will chair the **Japanese/European joint endoscopy symposium.** It will focus on the state of the art in modern endoscopy. "The European Society of Gastrointestinal Endoscopy (ESGE) and the Japan Gastroenterological Endoscopy Society (JGES) have teamed up for their first joint symposium at UEGW," says Prof. Tajiri. "Don't miss this unique and exciting opportunity to learn about the latest developments in therapeutic endoscopy."

In addition, as a special service to the Japanese delegates attending, the *UEGW Congress News* will also be published in a Japanese language edition for the first time.

Of course, UEGW will also continue the tradition of hosting the **Best of AGA (American Gastroenterology Association) sessions from DDW (Digestive Disease Week). Tomorrow from 14.00 to 15.30 h in Hall A12/13,** delegates have the chance to get first-hand information about current research results from the recent DDW, which took place in Chicago. "Selected AGA experts will discuss data presented at DDW 2011 in oesophageal and upper GI disorders, inflammatory bowel disease, intestinal disorders, oncology and liver diseases," says coordinator and organiser Professor Peter Holt from the USA. In exchange, the next DDW will feature a "Best of UEGW" session.

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CURRENT NEWS FROM THE 19TH UNITED EUROPEAN GASTROENTEROLOGY WEEK (UEGW) · STOCKHOLM 22-26 OCTOBER 2011

SCIENTIFIC PROGRAMME

東洋と西洋の交流

UEGWはアジア・パシフィック地域および米国との合同セッションを開催

肝細胞がんに関する
"East meets West"シンポジウム、
日本と欧州の内視鏡合同シンポジウム、
米国消化器病学会（AGA）が提供する
"Best of Digestive Disease Week(DDW)"
セッションなど、
UEGWは東洋と西洋が交流する機会を
提供する。

"When East meets West: Management of hepatocellular carcinoma"と題するシンポジウムは、**24日（月）15:45～17:15にHall A5**にて開催される。「肝細胞がんは研究が盛んな領域で、実臨床でも有用性の高い新規治療法が次々に開発されている。数年前までは肝細胞がんには地域的に大きな違いがあると考えられていたが、最近の研究ではそれが否定された。その結果、東洋と西洋の情報交流が活発化した」とスペインのProfessor Jordi Bruixは語った。同氏は日本のProfessor Masatoshi Kudoとともに本シンポジウムの座長を務める。

26日（水）8:30～10:30には、**Hall A12/13**で日本のProfessor Hisao TajiriとドイツのProfessor Horst Neuhausが座長を務める**Japanese/European joint endoscopy symposium**が開催される。このシンポジウムでは内視鏡の最新技術にフォーカスする。Professor Tajiriは「欧州消化器内視鏡学会（ESGE）と日本消化器内視鏡学会（JGES）はUEGWにおける最初の合同シンポジウムを開催する」と語り、「内視鏡治療の最新の進歩を学ぶまたとない機会であるユニークかつエキサイティングなセッションを見逃してはならない」と続けた。また日本から参加された先生方には、特別サービスとしてUEGW Congress News日本語版が初めて刊行され、提供される。

もちろん例年通りにUEGWは**DDW（Digestive Disease Week）"Best of AGA（American Gastroenterology Association）"**セッションを、**25日（火）14:00～15:30にHall A12/13**で開催する。このセッションの参加者には、シカゴで開催された今年のDDWで論じられた最新情報の概要が提供される。本セッションのコーディネータおよびオーガナイザを務める米国のProfessor Peter Holtは「厳選されたAGAのエキスパートがDDW2011で発表された食道および上部消化管疾患、炎症性腸疾患、腸疾患、がん、肝疾患の演題について討論する」とその魅力を語った。交換イベントとして、次回のDDWでは"Best of UEGW"セッションが開催される予定である。

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名医に会える予約外来

医療ジャーナリスト
松井宏夫が選ぶ「ガン治療の名医50人」

日本最高レベルのガン治療を行えるのは誰か。ガン治療を専門とする医師への取材を基に、1990年に日本初の『名医名鑑』を上梓した医療ジャーナリストの松井宏夫氏が50人を選んだ。

病名	氏名	病院名	診療科名	特長
肺ガン	奥村 栄	癌研有明病院	呼吸器センター	"迅速かつ正確な診断と適切な治療"をモットーとし、実践している
	浅村尚生	国立がん研究センター中央病院	呼吸器外科	豊富な症例数があり皮膚切開が8cm程度の小開胸手術を実現
	永井完治	国立がんセンター東病院	呼吸器外科	肺ガン手術数全国第2位の約300例を行うスタッフを指導する
	鈴木健司	順天堂大学順天堂医院	呼吸器外科	難易度の高い肺ガン手術も確実に行う。手術は年間200例超
	池田徳彦	東京医科大学病院	呼吸器外科	肺ガンの診断・治療の総合力は高く、ロボット手術を2010年に開始
	河野 匡	虎の門病院	呼吸器センター	腹腔鏡手術の技術の高さは有名で、海外で指導も行っている
	坪井正博	神奈川県立がんセンター	呼吸器グループ	手術はアートと考え日々研鑽した技量は高く、患者中心の医療を貫く
	小池輝明	新潟県立がんセンター新潟病院	呼吸器外科	北陸で手術数は最多。部分的に切除する縮小手術にも積極的
	伊達洋至	京都大学医学部附属病院	呼吸器外科	妥協のない手術で知られる。肺ガンとともに肺移植も有名
	岡田守人	広島大学病院	呼吸器外科	開胸と胸腔鏡の利点を組み合わせたハイブリッド胸腔鏡手術が9割
胃ガン	多田正弘	埼玉県立がんセンター	消化器内科	内視鏡の治療、「粘膜切除術」を世界で初めて開発した
	山口俊晴	癌研有明病院	消化器センター	患者中心のチーム医療を進め、進行胃ガンに徹底した根治手術を行う
	北川雄光	慶應義塾大学病院	消化器外科	胃ガンの切除範囲を決めるセンチネルリンパ節生検のパイオニア
	矢作直久	慶應義塾大学病院	消化器内科	内視鏡治療用の電気メス、フレックスナイフの開発で世界的に有名
	後藤田卓志	国立国際医療研究センター病院	内視鏡室	内視鏡的粘膜下層剥離術を発展させ、さらに確立させてきた
	小野裕之	静岡県立静岡がんセンター	内視鏡科	内視鏡治療用の電気メス、ITナイフを開発。早期胃ガン治療の頂点
	宇山一朗	藤田保健衛生大学病院	消化器外科	腹腔鏡手術の名手。今は胃ガンなどのロボット手術の第一人者
	古河 洋	市立堺病院	外科	悪性度が高いスキルス胃ガン手術において世界ナンバーワン
	笹子三津留	兵庫医科大学病院	上部消化管外科	高度進行胃ガンに対する高難度の手術を体への負担を減らして行う
	北野正剛	大分大学医学部附属病院	消化管外科	胃ガンに対する腹腔鏡手術のパイオニアの1人として有名
肝臓ガン	川崎誠治	順天堂大学順天堂医院	肝胆膵外科	出血量と合併症の少ない手術で有名。生体肝移植も知られている
	山本雅一	東京女子医科大学	消化器病センター	肝臓ガンの年間手術症例数は220例。的確な手術で知られる
	椎名秀一朗	東京大学病院	消化器内科	内科的治療のラジオ波焼灼療法※の治療数は世界のトップ
	池田健次	虎の門病院	肝臓センター	肝臓ガンの内科的治療を的確に選択し、治療成績を上げる
	幕内雅敏	日本赤十字社医療センター	肝胆膵外科	切除位置を正確に把握する幕内式肝切除を開発。手術の腕は超一流
	高山忠利	日本大学医学部附属板橋病院	消化器外科	肝最深部の尾状葉肝臓ガンに対する高山術式を確立した
	泉 並木	武蔵野赤十字病院	消化器科	ラジオ波焼灼療法と分子標的治療薬など全分野で超一流
	大﨑征夫	大阪赤十字病院	消化器科	肝臓ガン治療の総件数は年間1200件以上。確実な治療に定評
	工藤正俊	近畿大学医学部附属病院	消化器内科	新しい造影超音波検査を開発し、肝臓ガンの早期発見に努める
	佐々木 洋	八尾市立病院	外科	肝切除1000例以上の経験から「安全な手術」「複合的治療」を推進
大腸ガン	大塚幸喜	岩手医科大学病院	外科	腹腔鏡手術を進行ガンに適応を広げ、良好な成績を報告している
	渡邊昌彦	北里大学病院	外科	大腸ガンと炎症性腸疾患に対する腹腔鏡手術に力を注ぐ
	長谷川博俊	慶應義塾大学病院	消化器外科	慶應の十八番の腹腔鏡手術を得意とし、技量は高く評価されている
	黒柳洋弥	虎の門病院	消化器外科	腹腔鏡手術で有名。直腸ガンに対しては肛門温存にこだわる
	工藤進英	昭和大学横浜市北部病院	消化器センター	拡大観察による早期大腸ガン内視鏡診断・治療では世界の第一人者
	平井 孝	愛知県がんセンター中央病院	消化器外科	直腸ガンの神経温存術・肛門温存術の確立。内視鏡手術TEMを導入
	趙 栄済	大津市民病院	消化器科	早期大腸ガンに超音波内視鏡を用いた浸潤度診断は卓越した成績
	岡島正純	広島大学病院	消化器外科	腹腔鏡手術のパイオニア。10年から大腸ガンにロボット手術導入
	田中信治	広島大学病院	内視鏡診療科	最先端の内視鏡診断と内視鏡治療のレベルの高さは知られている
	鶴田 修	久留米大学病院	消化器病センター	早期大腸ガンの発見と内視鏡治療のエキスパート
前立腺ガン	福井 巌	癌研有明病院	泌尿器科	根治手術の改良に力を入れ、手術の傷を少なく抑えるよう努める
	斉藤史郎	国立病院機構東京医療センター	泌尿器科	前立腺ガン小線源療法における日本の第一人者
	内田豊昭	東海大学八王子病院	泌尿器科	早期のガンを切らずに治すHIFU(高密度焦点式超音波療法)を開発
	木原和徳	東京医科歯科大学附属病院	泌尿器科	傷口が小さく体に優しいミニマム創内視鏡下手術を開発
	吉岡邦彦	東京医科大学病院	泌尿器科	前立腺ガンのロボット手術の日本のパイオニアで手術数は最多
	頴川 晋	東京慈恵会医科大学病院	泌尿器科	腹腔鏡手術が得意。放射線源を用いる小線源療法にも注力
	堀江重郎	帝京大学医学部附属病院	泌尿器科	HIFUに力を入れるとともにED(勃起不全)治療でも第一人者
	寺地敏郎	東海大学医学部付属病院	泌尿器科	前立腺ガンの腹腔鏡手術のパイオニアとして有名
	鳶巣賢一	静岡県立静岡がんセンター	泌尿器科	天皇陛下の前立腺ガンの術者として有名。個別に最適な治療を選択
	小川 修	京都大学医学部附属病院	泌尿器科	さまざまな治療法を併用して体に優しい治療を実践

※電極を挿入し、ラジオ波を流してガン細胞を焼く治療法

特別保存版
頼れる病院
2011→2012

Part 3
都道府県別 頼れる病院

前年度は診療報酬のプラス改定となり多くの病院の収支が改善した。それでもいまだ経営危機に瀕していたり、医師不足、看護師不足で医療崩壊に直面している病院は少なくない。あなたが住む地域の「頼れる病院」はどこなのか、都道府県別にランキングを作成した。

83ページから続く

M.K.

都道府県	順位	病院名	開設者	総病床数	得点合計	医療の機能								経営状態			
						❶診療科目数	❷医師数	❸専門医数	❹看護師配置	❺医療スタッフ	❻施設・設備	❼紹介率	❽災害拠点病院	❾病床利用率	❿平均在院日数	⓫人件費率	⓬経常収支比率
京都府	4	京都第一赤十字病院	日赤	745	84	3	9	8	15	3	4	4	2	8	10	8	10
	5	京都桂病院	民間	585	83	3	9	8	15	3	4	3	0	8	10	10	10
	6	京都医療センター	国立病院機構	600	82	3	9	5	15	3	5	4	0	8	10	10	10
	7	宇治徳洲会病院	民間	400	81	3	9	5	15	5	4	2	0	8	10	10	10
	8	綾部市立病院	自治体	206	79	3	9	5	15	3	4	2	0	8	10	10	10
	8	市立福知山市民病院	自治体	324	79	3	9	5	15	5	0	2	2	10	10	10	8
	10	府立医科大学附属病院	地方独法	1,065	78	3	9	8	15	1	5	3	0	8	8	8	10
	11	武田病院	民間	300	77	2	9	5	12	5	3	5	0	8	10	8	10
	12	武田総合病院	民間	500	75	3	9	5	12	4	4	2	0	10	8	8	10
	13	京都市立病院	地方独法	548	74	3	9	8	15	2	4	3	2	8	10	5	5
	14	京都民医連中央病院	民間	300	73	3	9	5	15	5	0	2	0	10	8	8	8
	15	舞鶴共済病院	共済組合	320	72	3	6	3	12	3	3	4	0	8	10	10	10
	16	公立山城病院	自治体	321	71	3	6	5	15	3	4	2	2	3	10	10	8
	17	京都南病院	民間	306	68	3	9	3	12	5	0	2	0	8	8	8	10
	17	府立与謝の海病院	自治体	295	68	3	6	5	15	2	3	4	2	8	10	5	5
	19	公立南丹病院	自治体	464	66	3	6	5	15	2	0	2	2	5	8	10	8
	19	済生会京都府病院	済生会	350	66	3	6	5	15	1	2	3	2	3	8	10	8
	19	舞鶴赤十字病院	日赤	198	66	2	6	3	12	5	0	2	0	10	8	8	10
	22	社会保険京都病院	社会保険	322	65	3	6	5	15	1	2	2	0	3	10	8	10
	23	洛西ニュータウン病院	民間	240	63	3	6	5	15	1	0	2	0	5	8	10	8
	24	亀岡市立病院	自治体	100	58	2	6	5	15	2	0	2	0	5	8	8	5
	24	京丹後市立弥栄病院	自治体	200	58	2	6	3	12	4	0	2	0	5	8	8	8
	26	市立舞鶴市民病院	自治体	198	23	1	3	3	6	0	0	2	0	3	3	1	1
大阪府	1	関西医科大学枚方病院	民間	744	95	3	15	8	15	2	5	5	2	10	10	10	10
	2	近畿大学病院	民間	941	92	3	15	8	15	2	5	4	2	8	10	10	10
	3	大阪大学病院	国立大学	1,076	91	3	15	10	15	2	5	5	2	8	8	10	8
	4	高槻病院	民間	477	90	3	12	8	15	5	3	4	0	10	10	10	10
	4	淀川キリスト教病院	民間	487	90	3	12	8	15	5	5	4	0	10	10	8	10
	6	北野病院	民間	707	89	3	12	8	15	2	5	4	0	10	10	10	10
	7	大阪警察病院	民間	580	88	3	12	8	15	2	3	3	2	10	10	10	10
	8	大阪医科大学病院	民間	921	86	3	15	8	15	3	4	4	2	8	8	8	8
	8	大阪赤十字病院	日赤	1,021	86	3	9	8	15	2	5	4	2	8	10	10	10
	8	府中病院	民間	380	86	3	9	5	15	5	4	5	0	10	10	10	10
	8	府立急性期・総合医療センター	地方独法	768	86	3	12	5	15	4	5	4	2	10	10	8	8
	12	岸和田徳洲会病院	民間	341	85	3	9	8	15	5	3	2	0	10	10	10	10
	12	大阪市立総合医療センター	自治体	1,063	85	3	12	10	15	2	5	3	2	8	10	10	5
	14	済生会中津病院	済生会	778	84	3	9	8	15	4	4	3	0	8	10	10	10
	14	大阪医療センター	国立病院機構	694	84	3	12	8	15	1	3	4	0	8	10	10	10
	14	大阪厚生年金病院	厚生年金	565	84	3	12	8	15	3	3	4	0	8	10	8	10
	17	大阪市立大学病院	地方独法	1,003	83	3	12	10	15	1	5	4	2	5	8	10	8
	18	済生会野江病院	済生会	382	82	3	9	8	15	4	4	3	0	8	10	10	8
	18	市立堺病院	自治体	493	82	3	9	10	15	2	4	4	2	8	10	10	5
	20	ベルランド総合病院	民間	522	81	3	9	5	15	3	3	5	0	8	10	10	10
	21	りんくう総合医療センター	地方独法	358	80	3	9	8	15	4	5	3	2	8	10	8	5

ANNOUNCEMENT

World Federation of Ultrasound in Medicine
and Biology (WFUMB) Asian Education,
in conjunction with
The 26th Annual Scientific Meeting of
Medical Ultrasonic Society of Thailand (MUST),

Innovative Practice in Ultrasound With Live Demonstration

16th – 18th February 2011
Bangkok, Thailand.

Faculty

• David Cosgrove	England
• Byung I Choi	WFUMB – Korea
• Masatoshi Kudo	WFUMB – Japan
• Seung H. KIM	WFUMB – Korea
• Ritsuko Kimata Pooh	Japan
• Hye-Sung Won	Korea
• Wilaiporn Bhothisuwan	AFSUMB – Thailand
• Panyu Panburana	MUST – Thailand
• Panruethai Trinavarat	MUST – Thailand
• Pramook Mutirangura	RCST – Thailand
• Suwimon Tangwiwat	RCAT – Thailand
• Supika Kritsaneepaiboon	RCRT – Thailand
• Lin Rachel	Singapore
• Jaeyoung Lee	Korea

Dear Colleague,

It is our great pleasure to invite you to attend The World Federation of Ultrasound in Medicine and Biology (WFUMB) Asian Education, in conjunction with The 26th Annual Scientific Meeting of Medical Ultrasonic Society of Thailand (MUST), which will be held in Bangkok, Thailand on the 16th-18th February 2011.

The scientific programme has been planned for the dedicated work and vast experience of our international lecturers, from WFUMB, AFSUMB, England, Australia, Thailand and more in the fields of advanced everyday practice and interesting topics. Live demonstrations, and quizzes at the end of each session will be the add-on highlight of the meeting. The answer with be discussed at the end of the meeting with prizes given to the participants who have got the highest marks. The meeting will be in a friendly atmosphere. You may try to practice with the materials we will provide for you. We are positive that you will gain additional knowledge and experience.

The meeting place is at The Convention Venue in The Royal Jubilee Building on New Petchburi Road, belongs to Thai Medical Association, and run by Thai Medical Consortium. We have 2 big buildings, one for academic functions and reception, another one for the offices of almost all royal colleges and medical societies, including MUST. There are over 300 car parking spaces. And it is near the fly-over express way.

We look forward to serving you in WFUMB Asian Education in conjunction with The 26th Annual Scientific Meeting of MUST in Bangkok, Thailand.

Please come!
Warm regards,

Wilaiporn Bhothisuwan,
President of MUST,

Byung Ihn Choi,
WFUMB

WFUMB - MUST February 16-18, 2011			
	Day 1: Feb 16, 2011	Day 2 : Feb 17, 2011	Day 3 : Feb 18, 2011
08.30 - 09.00	Prince PUSAN Honorary Lecture	PT1. US of pediatric chest	WB2. Automated breast ultrasound: a 6 month-practice and analysis.
09.00 - 09.30	DC1. Contrast Ultrasound	BIC3. High resolution US for the gallbladder	DC3. Elastography - an over view
09.30 - 10.00	MK1. Sonazoid enhanced US for the management of liver cancer	SHK1. Evaluation of renal masses with Doppler US.	JL1. Liver elastography
10.00 -10.30	Refreshment	Refreshment	Refreshment
10.30 -11.00	BIC1. Hepatic Nodules in Liver Cirrhosis: US findings	SHK2. Renal vascular diseases: Doppler US evaluation	SHK3. TRUS of the prostate and TRUS-guided biopsy
11.00 -11.30	BIC2. US for Diffuse liver disease	PM1. Practical duplex US in vascular surgery: lecture	MK3. Interventional US for pancreatic malignancy
11.30 -12.00	DC2. New modes on the Aplio system	PM2. Practical duplex US in vascular surgery: live demo	Answer the quiz, award presented closing remarks
12.00 -13.00	LUNCH	LUNCH	MUST General Assembly & LUNCH
13.00 -13.30	SK1. US in pediatric cystic kidney disease	RKP1. First Trimester Sonography	
13.30 -14.00	MK2. Diagnosis of Pancreatic Tumors by EUS-FNA and CE-EUS	HSW1. Clinical application of Fetal cardiac function test (MPI) in OB field	
14.00- 14.30	Definity Contrast enhanced US	RKP2. Neuroscan in Early and First Half of Pregnancy	
14.30 -15.00	Refreshment	Refreshment	
15.00 -15.30	ST1. US Guidance Regional Anaesthesia	HSW2. Clinical Usefulness of Volume Nuchal Translucency Using 3D US	
15.30 -16.00	WB 1. Interventional US: Miscellaneous Usage	LR1. WHC Ultrasound today and tomorrow	
16.00 -16.30	Live demo & Hand on US Guided Intervention	PP1.The Genetic Sonogram of Down Syndrome	

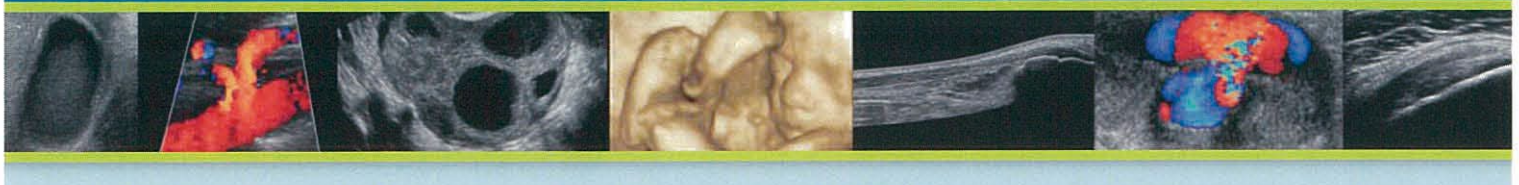
Lecturers initials:

DC = David Cosgrove
MK = Masatoshi Kudo
BIC = Byung I Choi
SK = Supika Kritsaneepaiboon
ST = Suwimon Tangwiwat
WB = Wilaiporn Bhothisuwan
PT = Panruethai Trinavarat
SHK = Seung H. KIM
PM = Pramook Mutirangura
RKP = Ritsuko Kimata Pooh
HSW = Hye-Sung Won
LR = Lin Rachel
PP = Panyu Panburana
JL = Jaeyoung Lee

AMERICAN INSTITUTE OF ULTRASOUND IN MEDICINE

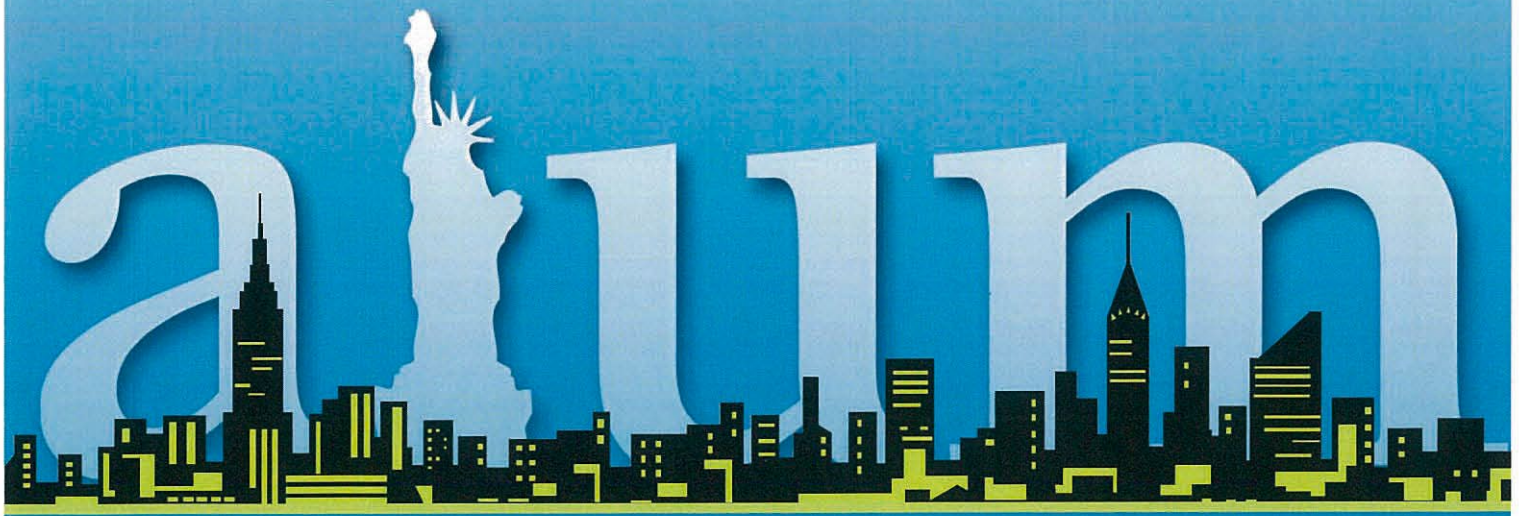
2011 Annual Convention and Preconvention Program

Earn more than 30 *AMA PRA Category 1 Credits*™ (accepted by the ARDMS) or ARRT Category A Credits over 4 days!



April 14–17, 2011 • New York Marriott Marquis Hotel • New York, New York

PRELIMINARY PROGRAM



ISTU
April 11–13

2011NYC
Confocal Meeting with the AIUM

AIUM
April 14–17

Convention

Friday, April 15, 2011

8:00 AM–9:00 AM

Just Images Sessions

CARDIO G&A **Abdominal Doppler Ultrasound: Interesting Cases**

Moderator: Vikram Dogra, MD

Speakers: George Berdejo, BA, RVT, Vikram Dogra, MD, and Marjorie Stein, MD

The objective of this course is to view and discuss interesting cases of abdominal Doppler ultrasound.

FE **Complex Anomalies**

Moderators: Leeber Cohen, MD, and Julia Drose, BA, RT, RDMS, RVT, RDCS

Speakers: Lisa Allen, BS, RDMS, RDCS, RVT, and Tracy Anton, BS, RDMS, RDCS

The objective of this session is to view and discuss cases of complex fetal cardiac anomalies that are initially not known and how to make the diagnosis.

NEURO MSK **Comprehensive Pediatric Neurosonology**

Moderator: Michael DiPietro, MD

Speakers: Dorothy Bulas, MD, Michael DiPietro, MD, and Vesna Kriss, MD

This session will present and discuss cases of the newborn spine as well as the newborn and fetal brain.

CEUS **Contrast-Enhanced Ultrasound: Clinical Applications**

Moderator: Michelle Robbin, MD

Speakers: Richard Barr, MD, PhD, David Cosgrove, MA, MSc, and Barry Goldberg, MD

The objective of this session is to make the general ultrasound community more aware of the advantages of using ultrasound contrast agents.

G&A **Sonography After 5 PM**

Moderator: Shweta Bhatt, MD

Speakers: Nira Beck-Razi, MD, Shweta Bhatt, MD, and Corrine Deurdulian, MD

This course will present difficult emergent abdominal and scrotal cases. These include renal colic, right upper quadrant pain, jaundice, scrotal pain, and trauma.

8:00 AM–9:00 AM

Basic Science Abstract Session

9:15 AM–11:30 AM

Opening/Awards Session

William J. Fry Memorial Lecture Award

Jacques Abramowicz, MD

Joseph H. Holmes Clinical and Basic Science Pioneer Awards

Clinical: Charles Kleinman, MD

Basic Science: J. Brian Fowlkes, PhD

Distinguished Sonographer Award

Julia Drose, BA, RT, RDMS, RVT, RDCS

Honorary Fellow Awards

Masatoshi Kudo, MD, PhD

Gianluigi Pilu, MD

11:30 AM–1:00 PM

Grand Opening Luncheon in the Exhibit Hall

After the Opening/Awards Session, the Grand Opening Luncheon will be held in the Exhibit Hall. Take the time to interact with colleagues as well as representatives from leading companies in the ultrasound industry. Lunch will be provided.

1:00 PM–2:00 PM

Film Panel Sessions

G&A **General Imaging Film Panel**

Chair: Sharlene Teefey, MD

OB **Obstetric Imaging Film Panel**

Chair: Diana Gray, MD

1:00 PM–2:00 PM

BSI G&A **Elastography: Principles, Methods, Applications, and Future Directions**

Moderator: James Miller, MS, PhD

Speaker: Jonathan Ophir, PhD

The objective of this presentation is to familiarize the audience with the basic principles of elastography. These will include discussions of the basics of mechanics, ultrasonic signal processing, and imaging methods that are involved in the generation of elastograms. Areas of clinical application and future directions of this technology will also be demonstrated and discussed.

2011 Opening General Session Schedule

Awards and Fry Memorial Lecture

Friday, April 15, 2011

9:15 am – 11:30 am

Broadway North/South 6th Floor

Opening General Session (9:15 am to 11:30 am)

- Awards and Fry Memorial Lecture (9:15am to 10:30am)
  - Welcome (9:15am 9:18am)
  - Joseph H. Holmes Clinical Pioneer Award (9:18am to 9:23am)
    - Charles Kleinman, MD
    - Presenter: Joshua Copel, MD
  - Joseph H. Holmes Basic Science Pioneer Award (9:23am to 9:28)
    - J. Brian Fowlkes, PhD
    - Presenter: Paul Carson, PhD
  - Distinguished Sonographer Award (9:28am to 9:330am)
    - Julia Drose, BS, RT, RDMS, RVT, RDCS
    - Presenter: Cindy Rapp, BS, RDMS
  - Honorary Fellow Award (9:33am to 9:38am)
    - Masatoshi Kudo, MD, PhD
    - Gianluigi Pilu, MD
    - Presenter: Harvey Nisenbaum, MD
  - Memorial Hall of Fame Announcements (9:38am to 9:43am)
    - Robert Bree, MD
    - Richard Jaffe, MD
    - Donna Kepple, MD
    - Presenter: Harvey Nisenbaum, MD
  - EER Grant Announcement (9:43am to 9:48am)
  - William J. Fry Memorial Lecture Award (9:48am to 10:25am)
    - Jacques Abramowicz, MD
    - Presenter: Ilan Timor, MD

- Opening Session (10:30am to 11:30am)
  - A Mock Trial: Is Ultrasound Safe? – Opening Statements

Grand Opening Luncheon in the Exhibit Hall (11:30am to 1:00pm)
Westside Ballroom 5th Floor

2011 AIUM Award Winners

Honorary Fellow Award

The Honorary Fellow Award bestows an honorary membership to those individuals who have contributed significantly to the field of ultrasound.

Masatoshi Kudo, MD, PhD

Dr Kudo received his undergraduate degree from Kyoto University in Japan in 1974. He continued on at the same university's School of Medicine and received his MD degree in 1978 and his PhD degree in 1986. In 1979, he joined the staff of the Kobe City General Hospital in the Division of Gastroenterology. While continuing his position at the hospital, in 1983 he began work as a research fellow in the Departments of Diagnostic Radiology and Nuclear Medicine at Kyoto University. He was promoted to assistant chief of the Division of Gastroenterology at Kobe City General Hospital in 1985. From 1987–1989, he was a visiting research scholar in gastroenterology and nuclear medicine at the University of California Davis Medical Center. He returned to his position as assistant and then associate chief at Kobe City General Hospital until 1997. At that time, he became an associate professor in the Second Department of Gastroenterology and Hepatology at Kinki University School of Medicine. He is currently a professor and the chair of the Department of Gastroenterology and Hepatology at Kinki University School of Medicine, as well as the president of Kinki University Medical Center.

Dr Kudo reviews for 34 international and 22 national journals. He is on the editorial boards of several journals, including *Ultrasound in Medicine and Biology*. His publications include 310 peer-reviewed articles, 3 English and 8 Japanese textbooks, and 24 English and 178 Japanese book chapters. He has been an invited lecturer for 198 international and 533 national events.

Dr Kudo has been involved in several international societies, including the World Federation for Ultrasound in Medicine and Biology and the Asian Federation of Ultrasound in Medicine and Biology, for both of which he is currently the president-elect. He is also a founding board and council member of the International Liver Cancer Association.

Throughout his career, Dr Kudo has been commended for his distinguished work. Some of the awards he has received include the Cancer Research Award from the Japan Cancer Center and the Cum Laude Award of the Radiological Society of North America.

Romanian Society of Ultrasound in Medicine and Biology (SRUMB)

SOCIETATEA ROMÂNĂ DE ULTRASONOGRAFIE ÎN MEDICINĂ ŞI BIOLOGIE

Honorary Diploma

It is awarded to MASATOSHI KUDO, JAPAN

for the outstanding merits and contributions brought to our society.

President of SRUMB	Honorary President SRUMB	General Secretary of SRUMB
Prof. Dr. Radu Badea	Prof. Dr. Ioan Sporea	Prof. Dr. Viorela Enăchescu

This diploma is awarded at the
14 th National Conference of the Romanian Society of Ultrasound in Medicine and Biology Ultrasound in Targu Mures, 10 -12 June 2011

Taiwan Association for the Study of the Liver

Awards this Certificate of Appreciation

to

Professor

Masatoshi Kudo

for participation in the

2011 Single Topic Conference
International Conference on Liver Cancer: From Bench to Bedside

June 25-26, 2011

Wan-Long Chuang

Wan-Long Chuang, MD., PhD.

President, Taiwan Association for the Study of the Liver (TASL)

企画・制作　朝日新聞社広告局

ウイルス肝炎と肝がんの理解のための
市民公開講座（肝がん撲滅運動）

参加者募集中

入場無料
定員
1,000人

肝臓病で命を失わないために

7月1日～3日にかけてハイアット リージェンシー大阪で開催されたアジア太平洋肝がん専門家会議（APPLE）を記念して、ウイルス肝炎と肝がんの検診と治療について、市民のみなさんに正しく最新の情報を発信する「市民公開講座」を開催いたします。

日時　8／21（日）
12：00開場
12：30開演
16：40終演（予定）

場所　大阪国際交流センター
（大阪市天王寺区上本町8-2-6）

学術講演


幕内 雅敏先生


工藤 正俊先生


泉 並木先生


國土 典宏先生

ゲスト 仁科 亜季子さん（女優）

にしな・あきこ／女優。映画やドラマなど多方面で活躍しながら、自身の経験をもとにしたがん治療に関する啓発活動や講演を行っている。

アクセス
●大阪市営地下鉄千日前・谷町線「谷町九丁目」駅もしくは谷町線「四天王寺前夕陽ケ丘」駅から徒歩500メートル
●近鉄大阪・難波線「大阪上本町」駅から徒歩400メートル

■プログラム

司会／幕内 雅敏先生（日本赤十字社医療センター病院長）
工藤 正俊先生（第2回アジア太平洋肝癌専門家会議会長、近畿大学医学部附属病院長・消化器内科学教授）
ごあいさつ／工藤 正俊先生（市民公開講座会長）

第1部 学術講演

講演1「ウイルス肝炎をどう治療するか？」
泉 並木先生（武蔵野赤十字病院副院長・消化器科部長）

講演2「肝がんを早く見つけて治療する」
工藤 正俊先生（近畿大学消化器内科学教授）

講演3「肝がんの治療ガイドラインと外科治療」
國土 典宏先生（東京大学肝胆膵外科学教授）

講演4「我が国の肝移植の現状」
幕内 雅敏先生（日本赤十字社医療センター病院長）

第2部 ゲスト講演

「元気な明日のために
～がんに負けない～」
仁科 亜季子さん（女優）

第3部 質疑応答とパネルディスカッション

パネリスト／工藤 正俊先生、幕内 雅敏先生、泉 並木先生、國土 典宏先生、仁科 亜季子さん
司会／中村 通子（朝日新聞編集委員）

応募方法

参加ご希望の方は、はがき、ファクスに、郵便番号・住所・氏名・年齢・性別・電話番号・参加人数（複数応募の場合は各人必要事項）を明記のうえ、下記までお送りください。車いすでご参加の場合は、その旨を明記してください。また、肝臓病に関するご質問がありましたら、質疑応答や講演の参考にさせていただきますので、ぜひお書きください。応募多数の場合は、厳正な抽選を行い、当選された方には聴講券を郵送しますので、当日忘れずに持参してください。

◆応募締め切り：8月10日（水）必着

申し込み
郵便はがき：〒530-8612　郵便事業株式会社　大阪支店　私書箱191号
朝日新聞社広告局「肝がんシンポ」係
FAX：06-6227-9597
朝日新聞社広告局「肝がんシンポ」係

問い合わせ
朝日新聞大阪本社広告局
TEL06-6201-8334（土・日・祝日除く10時～18時）

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主催：アジア太平洋肝がん専門家会議（APPLE）、日本肝臓学会、日本消化器病学会、日本肝癌研究会、大阪府、朝日新聞社
後援：厚生労働省（予定）、大阪府医師会、大阪府看護協会、日本肝がん臨床研究機構、近畿大学

肝臓病で命を失わないために

適切治療 見極め

武蔵野赤十字病院の泉並木副院長

——がんが小さいうちに見つかったときに、切除するのがいいのか、ラジオ波で焼くのがいいのか議論があるようです。治療方針はどう決まるのでしょう。

國土 実際、両方の治療を選択できる患者は非常に多いのです。そういう場合は、病院の中でも内科と外科の両方にかかっていただいて、それぞれの意見を聞いたうえで決めていただくことが多いと思います。

泉 内科医の僕らからみても、外科で手術した方がいいということもあります。内科医と外科医がきちんと相談して、どちらもできる患者には科学的な根拠を示すように努力していきたいと思っています。

工藤 毎週、外科と放射線科と消化器内科で会議をしています。さらに最近は電子カルテがありますので、外来の段階でも同じ画像を見ながら電話で相談することができます。

國土 患者さんの協力を得て、切除とラジオ波のどちらが真に有効なのかを科学的に調べる全国規模の研究（サーフトライアル）を実施しています。この研究が始まってからは、両方できる患者は外科と内科で相談するようになりました。確かに、患

近畿大の工藤正俊・消化器内科学教授

國土さん「外科と内科で相談可能」

東京大の國土典宏・肝胆膵外科学教授

者さんが治療を選ぶのが筋です。選びたい方はご自身で選んでいただければいい、この研究にご協力いただける方はぜひお願いしたいです。

——医者の当たりはずれで命が左右されるのでは困ります。

幕内 外科の手術では医者の腕の差はものすごくあります。当たりはずれで左右されるのは当たり前と思って、よく調べて選んだ方がいいと思います。

——でも、普通の人がブラックジャックみたいな先生を探すのは難しい。どう探せばいいのでしょう。

幕内 各病院にホームページがあります。具体的な数値が書いてあるのはダメです。自慢話だけ書いてあるのは信用できるんじゃないでしょうか。自慢話だけ書いてあるのはダメです。がん拠点病院では、様々な専門医やスタッフらみんなで話し合って、治療方針の決定が行われていますので、そういうところを選ぶのも方法でしょう。

國土 日本肝臓学会と、外科だと肝胆膵外科学会がありますが、ネットで検索してみてください。そのホームページに専門医や認定施設の名前が出ていますので、そういうところをご覧いただければいいと思います。

幕内さん「情報開示の病院調べて」

日本赤十字社医療センターの幕内雅敏院長

◇インターネットでもっと知るために
・国立国際医療研究センター肝炎情報センター　http://www.ncgm.go.jp/center/
・日本肝臓学会　http://www.jsh.or.jp/
・日本肝胆膵（かんたんすい）外科学会　http://www.jshbps.jp/
・医療情報サービスMinds　http://minds.jcqhc.or.jp/
※肝がんの診療ガイドラインや一般向け解説も公開

投薬 泉さん　専門家が果たす役割大

肝がんの9割以上がC型、B型肝炎のウイルスが原因。ウイルスを排除したり、増殖を抑えたりして、がんにならないようにすることが非常に大事です。

B型肝炎は、肝硬変が進んでいないと、がんになることが多い。厚生労働省の研究班ががんになりやすい因子を調べた結果、40歳以上でウイルス量が多く、血小板が少なくなっている場合、5年間で40％がんになっていることが分かりました。しかし、治療が進み、1日1回飲むだけでウイルスを抑える薬が使えるようになっています。

C型肝炎については、ペグインターフェロンという注射薬とリバビリンという飲み薬の二つを合わせて、治療効果が非常に上がったのが最近の特徴です。さらに、患者の遺伝子を調べると、治療が効くかどうかが分かるようになりました。

治りにくい遺伝子を持っている人は、プロテアーゼ阻害薬でもウイルスが消えない可能性が高い。むしろ薬が効かない耐性ウイルスができてしまうこともあります。次の新しい薬を待つという選択も考えられます。「いつ、どの薬で治療するのか」がとても大事。そのために、専門医の役割が大きいのです。

検査 工藤さん　がん発見率 欧米の2倍

肝がんの死者数は1975年ぐらいから急増しましたが、最近は若干の減少傾向にあります。しかし、今後20年近くは3万人台が続く可能性があるので、引き続き対策が必要です。

肝がんは、ウイルス肝炎から発がんすることが多いので、逆にがんになりやすい人の特定が可能。定期検査で早期発見できます。

日本は肝がんの治療成績が飛び抜けて世界一です。その大きな理由は、全国津々浦々できちんと検査が行われるようになり、早期発見率が高くなったこと。早期のステージで肝がんが見つかるのは、欧米ではよくても30％、日本では全国平均で60％以上です。

肝がんの内科的治療としては、ラジオ波を使って発生する熱で腫瘍（しゅよう）組織を焼く「ラジオ波治療」、がんに栄養を運ぶ動脈を詰めてしまう「動脈塞栓（そくせん）療法」、肝臓の動脈に抗がん剤を入れる「動注化学療法」などがあり、最近は、がん細胞が増殖するシグナルを伝わらないようにする「分子標的薬」が登場しています。

現在、分子標的薬とほかの療法を併用する試験も進行中です。臨床試験で効果が確認されれば、10年以上生きていけるようになることも夢ではありません。

出席者講演

切除 國土さん　生存率 世界的にも優秀

肝がんの外科治療には、根こそぎがんを取る切除と、ほかの肝臓と取り換える移植があります。

学会の診療ガイドラインでは、切除は肝機能が比較的良く、がんの数が3個以下のときに行うことになっています。がんの大きさに制限はありません。

肝がん研究会の全国調査のデータによると、最も新しい報告で、5年生存率は53％。世界的にみても非常に優秀な成績です。手術死亡率も0・7％と低く、海外と比べて格段に安全な手術ができるようになっています。日本のきめ細かい肝機能の評価と手術の技術の賜物だと思います。

残念ながら、再発が多いのが肝がんの特徴。手術から5年以内に8割が再発しています。でも、生存率は高い。再発しても、再治療して長生きしている患者さんが多いというのも、肝がんの特徴です。

長生きできるための一つのキーワードは肝機能だと思っています。肝炎の治療、それからお酒をのまないなど肝機能を悪くしないことが再発に備えるために大切です。

治療する側としては、肝臓を取りすぎないことが重要。外科医は肝臓や血管をできるだけ温存することを心がけています。

移植 幕内さん　効果は絶大 年齢に制限

肝移植は、肝機能が非常に落ちて肝不全の状態になった患者に対して、脳死体あるいは生体から、肝臓全体あるいは一部を移植する方法です。1960年代に欧米で始まりました。

効果は絶大です。特に劇症肝炎の患者は劇的。意識がなくなって、死にかかっている人に肝臓を移植すると、次の日にはパッチリと目が開く。神の奇跡を見る感じがします。

肝硬変の末期の患者さんも、肝機能は正常に回復します。

ただし、がんが進行していると、がん細胞が血中を回っているため、移植した肝臓にがんが再発するので注意が必要です。また肝がんは比較的高齢者にできやすいですが、移植後に使うステロイドによって自己免疫や骨粗鬆症などの副作用がもたらされます。移植によるメリットが少なくなるので、年齢を一応65歳までに制限せざるを得ません。

効果は絶大な治療法ですが、対象となる患者の中でほんの一部にしか行われていません。その理由の一つは、脳死からの臓器移植が日本では海外に比べて遅れていることがあります。厳しい条件があり年間10例ほどでしたが、臓器移植の改正で昨年からようやく増えてきました。

仁科さん「子宮頸がん同様、撲滅信じて」

女優の仁科亜季子さん

私が体験した子宮頸がんもウイルスによる感染症の一つです。その手術の輸血で感染し、C型肝炎にも感染してしまいました。

子宮と卵巣とリンパ節を取る大きな手術をしたのは38歳のとき。20年ほど経った今もなおリンパ浮腫などの後遺症を抱えています。

そういう経験から、一人でも多くの方に検診していただきたいと発信してきました。日本では子宮頸がんの検診率は低いまま。待ちに待った赤ちゃんが宿った子宮を摘出しないといけない悲しい思いをする女性が増えていると聞きます。

そんな中、2009年に画期的な子宮頸がん予防ワクチンが発売されました。中学の入学の女子にはお祝いに、国がワクチンをプレゼントしていただけたらと思います。それからボーイフレンドができたら必ず検診をしていただきたいと思います。

肝がんも撲滅できる可能性がとても大きいと分かりました。子宮頸がんも必ずや日本から、そして世界から撲滅できると私は信じています。できるはずです。

あらゆるがんは早期発見、早期治療が大切です。2人に1人がんになる時代ですが、ならない方が絶対にいいのです。これからも啓発活動に力を入れていきたいと思います。

早期発見が肝心

ウイルスで引き起こされる肝炎は、やがて肝がん（原発性肝細胞がん）になるおそれがある。市民公開講座「肝臓病で命を失わないために」が8月21日、大阪市天王寺区の大阪国際交流センターで開かれた。専門家らは、早期発見と適切な治療の大切さを訴えた。

（討論の司会は中村通子・朝日新聞編集委員）

パネルディスカッション

肝臓病について話すパネリストたち＝大阪市天王寺区、伊藤菜々子撮影

健康

幕内雅敏　日本赤十字社医療センター院長、東大名誉教授
國土典宏　東京大医学部肝胆膵外科学・人工臓器移植外科学教授
仁科亜季子さん　（女優）
工藤正俊　近畿大消化器内科学教授・医学部付属病院長
泉並木　武蔵野赤十字病院副院長・消化器科部長　＝写真左から

泉さん「性交渉で感染する新型警戒」

―感染防止にはウイルスという敵を知らなければなりません。感染ルートはどうなっていますか。

泉　注射針や輸血、血液製剤など医療行為で感染してしまうことは今はゼロです。C型肝炎の感染は、ピアスやタトゥー（入れ墨）などで起こっていますので気をつけていただきたい。B型肝炎は、20年前から母子感染の予防対策として赤ちゃんにワクチンを打って大成功したので、若い方の感染者はいません。一方で、欧米からの新しいB型肝炎が日本で流行し始めています。性交渉で感染します。これを防ごうと、国民にワクチンを打つことが来年度から始まります。

―日常生活で感染することはないですか。

泉　家族に感染者がいても通常の日常生活では感染しません。ウイルスは体の外に出てしまうとほとんど死んでしまいます。ただし血液がついたような歯ブラシやカミソリなどは一緒に使わないことが大切です。

―感染しても会員が肝がんへのルートをたどるわけでもないようですね。

仁科　私は子宮頸がんの手術での輸血でC型肝炎に感染してしまいました。手術後に症状も出て、ずっと感染者だと思っていました。治療していませんが、数年前に検査をすると、ウイルスがいないんじゃないかと言われました。どういうことでしょう。

工藤　抗体検査が陽性であったとしても全員にウイルスがいるとは限りません。自然治癒される方も2～3割ぐらいいると言われています。

―みなさんの関心が高い新薬ですが、治療法が変わっていくのでしょうか。

泉　大きく変わります。どんどん進歩しますから。ただ、現在の新薬で1回治療して治らなかったら耐性ウイルスが残り、次にどんないい薬が出ても効かなくなってしまうことがある。自分にどれくらい効果が出るのか、肝がんになるまでどれぐらいの期間があるかを調べて、いつ、どの薬を使うかを決めることが非常に大事になります。

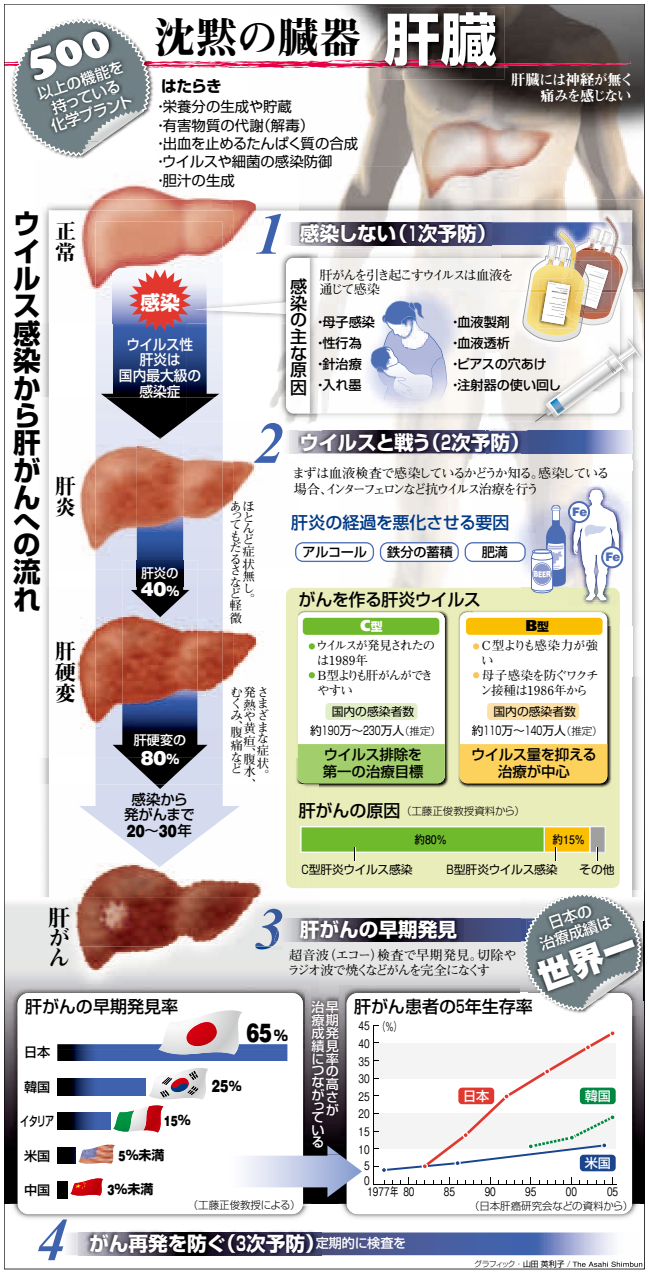
工藤　待てる場合もありますが、その間に発がんしてしまうケースもあります。基本的には時間がたてばたつほど、いい薬が出るのは間違いありませんが、年齢や肝臓の線維化の進展度合いなどによって、待てない人は早めに治療することも重要だと思います。

―がんの早期発見は重要ですが、自覚症状はあるんでしょうか。

工藤　患者の95％ぐらいは、がん自体の症状は感じないと思います。かなり進行したり、門脈という血管の中にがんが入ってしまったりした場合は症状が出ると思います。あるいは黄疸など悪化した肝硬変の症状は出るかもしれません。治療できる段階で発見するためには肝炎ウイルスを持っている人は定期検診が必須です。何カ月かに1度は、血液検査と画像検査で判定できる専門医のところに通う必要があると思います。

仁科　どんながんでも、がんにならない方がいい。早期発見のため検診を定期的に行っていただきたいと思います。

工藤さん「ウイルスある人、検診は必須」



主催　アジア太平洋肝がん専門家会議　大阪府　朝日新聞社
共催　日本肝臓学会　日本消化器病学会　日本肝癌研究会
後援　厚生労働省　大阪府医師会　大阪府看護協会
　　日本肝がん臨床研究機構　大阪対がん協会　近畿大学
協賛　MSD　ブリストル・マイヤーズ　バイエル薬品　味の素製薬

第1回

近畿消化器内視鏡ライブコース

1st Kinki Gastrointestinal Endoscopy Live Course

顧問	工藤正俊(近畿大学)
代表世話人	樫田博史(近畿大学)
会期	2011年11月27日(日) 10:00-16:00(予定)
場所	近畿大学医学部付属病院　円形講堂および光学治療センター 〒589-8511　大阪府大阪狭山市大野東377-2 Tel: 072-366-0221(代)
定員	300名(定員になり次第、締め切らせていただきます)
内容	上下部消化管、膵胆道における内視鏡処置のポイントを、 基礎から最新技術まで、ライブデモンストレーションでお示しいたします。 ■画像強調・拡大内視鏡検査 ■ESD/EMR ■EUS(消化管・胆膵) ■ERCP、EST、stenting　　その他予定
術者	豊永高史　先生(神戸大学) 八隅秀二郎　先生(北野病院) 樫田博史(近畿大学) 北野雅之(近畿大学) 松井繁長(近畿大学)
参加費 (昼食代込み)	医師:事前申し込み 5,000円(当日受付の場合7,000円) 研修医、コメディカル、その他:2,000円(当日2,000円) 学生、留学生:フリー ー事前申し込みは10月31日までー
お申し込み方法	お名前、御所属、連絡先住所・Fax番号・メールアドレスを明記の上、下記 連絡先にFaxまたはE-mailで参加費振込み口座をお問い合わせ下さい。 振込を確認させていただいた後、受理番号を発行させて頂きます。 当日は受付でその番号をお申し付けください。
お問い合わせ先	近畿大学医学部付属病院　消化器内科 近畿消化器内視鏡ライブコース事務局(松井繁長) E-mail:kin-live@med.kindai.ac.jp Fax:072-367-2880　　Tel:072-366-0221(内線3525)

同門会　名簿

名前	施設	卒業年度	出身大学
工藤　正俊	近畿大学医学部	昭和53年	京都大学
樫田　博史	近畿大学医学部	昭和58年	京都大学
汐見　幹夫	近畿大学医学部	昭和55年	近畿大学
北野　雅之	近畿大学医学部	平成　2年	鳥取大学
西田　直生志	近畿大学医学部	昭和60年	大阪医科大学
松井　繁長	近畿大学医学部	平成　3年	近畿大学
上嶋　一臣	近畿大学医学部	平成　7年	神戸大学
櫻井　俊治	近畿大学医学部	平成　7年	京都大学
南　康範	近畿大学医学部	平成　9年	近畿大学
萩原　智	近畿大学医学部	平成 10年	近畿大学
井上　達夫	近畿大学医学部	平成 11年	近畿大学
矢田　典久	近畿大学医学部	平成 11年	滋賀医科大学
坂本　洋城	近畿大学医学部	平成 12年	近畿大学
朝隈　豊	近畿大学医学部	平成 14年	近畿大学
北井　聡	近畿大学医学部	平成 14年	近畿大学
畑中　絹世	近畿大学医学部	平成 13年	川崎医科大学
川崎　正憲	近畿大学医学部	平成 15年	近畿大学
田北　雅弘	近畿大学医学部	平成 15年	近畿大学
永井　知行	近畿大学医学部	平成 16年	近畿大学
永田　嘉昭	近畿大学医学部	平成 16年	近畿大学
今井　元	近畿大学医学部	平成 17年	近畿大学
早石　宗右	近畿大学医学部	平成 18年	近畿大学
有住　忠晃	近畿大学医学部	平成 19年	近畿大学
鎌田　研	近畿大学医学部	平成 19年	近畿大学
峯　宏昌	近畿大学医学部	平成 19年	近畿大学
宮田　剛	近畿大学医学部	平成 19年	近畿大学
高山　政樹	近畿大学医学部	平成 19年	近畿大学
足立　哲平	近畿大学医学部	平成 21年	近畿大学
大本　俊介	近畿大学医学部	平成 21年	近畿大学
門阪　薫平	近畿大学医学部	平成 21年	近畿大学
工藤　可苗	近畿大学医学部	平成 12年	近畿大学
黒木　恵美(旧姓 石川)		平成 11年	近畿大学
岡田　無文	山本病院	平成 13年	近畿大学
柴田　千栄(旧姓 辰巳)		平成 15年	近畿大学
上田　泰輔		平成 15年	近畿大学
上硲　俊法	近畿大学医学部臨床検査学	昭和60年	近畿大学
前川　清	近畿大学医学部超音波室		
辻　直子	近畿大学医学部堺病院	昭和60年	京都府立医科大学
山本　典雄	近畿大学医学部堺病院		
奥村　直己	近畿大学医学部堺病院		
高場　雄久	近畿大学医学部堺病院		
梅原　康湖	近畿大学医学部堺病院	平成 12年	近畿大学
川崎　俊彦	近畿大学医学部奈良病院	昭和58年	京都大学
岸谷　譲	近畿大学医学部奈良病院	昭和 62年	近畿大学
宮部　欽生	近畿大学医学部奈良病院	平成 14年	近畿大学

豊澤　昌子	近畿大学医学部奈良病院	平成 12年	近畿大学
茂山　朋広	近畿大学医学部奈良病院	平成 17年	近畿大学
奥田　英之	近畿大学医学部奈良病院	平成19年	
木下　大輔	近畿大学医学部奈良病院	平成20年	
秦　康倫	近畿大学医学部奈良病院	平成21年	
水野　成人	近畿大学医学部奈良病院	昭和61年	京都府立医科大学
加藤　玲明	近畿大学医学部奈良病院	平成11年	近畿大学
宮本　容子(旧姓 北口)	近畿大学医学部奈良病院	平成12年	近畿大学
林　道友	近畿大学医学部奈良病院		
山本　俊夫(ご逝去)		昭和26年	京都大学
山本　健二	岡本クリニック		神戸大学
亀山　千晴	育和会記念病院	平成　7年	近畿大学
南野　達夫	なんの医院	昭和55年	近畿大学
中里　勝	上ヶ原病院		
鍋島　紀滋	天理よろづ相談所病院	昭和61年	京都大学
井上　良一	吉川病院内科	昭和43年	京都大学
由谷　逸朗	高石藤井病院	昭和62年	近畿大学
遠田　弘一		平成 7年	近畿大学
遠田　由紀			
谷池　聡子	和歌山串本病院	平成 7年	奈良県立医科大学
川端　一史	川端内科クリニック	平成元年	近畿大学
米田　円	米田内科	平成 元年	近畿大学
小川　力	高松日赤病院	平成11年	近畿大学
渡邉　和彦	結核予防会大阪府支部相談診療所	平成　3年	近畿大学
森村　正嗣	森村医院	平成　3年	帝京大学
中岡　良介	山本病院	平成 8年	近畿大学
冨田　崇文	冨田病院	平成 14年	近畿大学
西尾　健	南堺病院	平成 14年	近畿大学
仲谷　達也	仲谷・飯山クリニック	平成　3年	近畿大学
福永　豊和	北野病院	平成　4年	京都大学
福田　信宏	朝日大学村上記念病院	平成 10年	近畿大学
坂口　康浩	河崎内科病院	平成 11年	近畿大学
永島　美樹	桃坂クリニック	平成 12年	近畿大学
坂本　康明	坂本医院	平成 15年	近畿大学
市川　勉	市川クリニック	平成 12年	近畿大学
齊藤　佳寿(旧姓 野田)	庄内残目病院	平成 14年	近畿大学
高橋　俊介	市立堺病院	平成 14年	近畿大学
末冨　洋一郎	末冨放射線科医院	平成　8年	近畿大学
梅原　泰	辻腎太郎クリニック	平成 11年	近畿大学
鄭　浩柄	神戸市立医療センター中央市民病院	平成　8年	東京慈恵医科大学
小牧　孝充	富田林病院	平成　7年	近畿大学
鄭　扶美	近畿大学医学部　元秘書		
木村　由佳	近畿大学医学部　元秘書		
川辺　仁美	近畿大学医学部　元秘書		
西川　由佳	近畿大学医学部　元秘書		
二見　佳央里	近畿大学医学部　元秘書		

藤田　真紀	近畿大学医学部　教授秘書		
井上　真由美	近畿大学医学部　教授秘書		
村橋　亜季	近畿大学医学部　教授秘書		
弓削　公子	近畿大学医学部　教授秘書		
坂上　浩美	近畿大学医学部　教授秘書		
上田　由未子	近畿大学医学部　教授秘書		
小田　智裕子	近畿大学医学部　教授秘書		
胡桃　由佳	近畿大学医学部　医局秘書		
朝隈　智	近畿大学医学部　医局秘書		
林　直子	近畿大学医学部　医局秘書		
田村　利恵	近畿大学医学部　臨床研究補佐員（肝癌研究会）		
前原　なつみ	近畿大学医学部　臨床研究補佐員（肝癌研究会）		
小川　佳良子	近畿大学医学部　臨床研究補佐員（CRC）		
鏡　郁子	近畿大学医学部　基礎研究補佐員（実験助手）		

近畿大学消化器内科　同門会役員

会長　　工藤正俊

副会長　　北野雅之

幹事　　松井繁長

会計　　上嶋一臣

庶務　　西田直生志

同門会誌作製　秘書一同

近畿大学医学部消化器内科教室同門会会則

第一条　名称

本会は近畿大学医学部消化器内科教室同門会と称する。

第二条　目的

本会は会員相互の親睦及び教室の隆盛を図ることを目的とする。

第三条　会員

会員は消化器内科教室出身者、教室員及び同教室の発展に寄与するものをもって構成される。

第四条　役員

１．　本会の運営を円滑にするために幹事会を設ける。幹事会は代表幹事を長とし、代表幹事が指名する教室員をもって構成する。尚、幹事会は代表幹事が随時召集するものとする。その他、会計をおく。

２．　会長

①　会長は現職主任教授より選出される。

②　会長退任後は名誉会長となる。また、名誉会長は主任教授経験者からも選出できる。

３．　顧問

本会の発展に寄与したもので、幹事会が推戴する。

４．　役員の選出

①　幹事は役員より選出する。

②　代表幹事は医局長が兼任する。

５．　幹事の任期は２年とする。但し再任を妨げない。

第五条　会議

１．　総会は年１回の開催とする。

２．　幹事会において仮決議された条件の最終決定権は総会に委ねられる。

３．　決議は総会出席者の多数決により成立する。

第六条　会計

１．　本会の経費は会費をもって充てる。

２．　本会の会費は年額壱萬円とする。

３．　会計年度は４月１日から翌年３月 31 までとし、会計担当者は年１回会計報告を行う。

第七条

事務局は近畿大学医学部消化器内科教室内に置く。

第八条　会則の改正

会則の改正は幹事会の仮決議を経て総会で議決されるものとする。

附則　除名規定

本会の名誉を毀損したものや、その他本会に不適当と考えられるものは幹事会の動議により総会にて除名が議決される。

編集後記

2011 年版の annual report が完成しました。

消化器内科の秘書は、現在、くるみさん、藤田さん、朝隈さん、井上さん、田村さん、前原さん、林さん、村橋さん、小川さん、弓削さん、鏡さん、坂上さん、上田さん、小田さん（現在教授室 7 名、医局秘書 3 名、CRC 1 名、臨床研究補助 2 名、実験助手 1 名）での 14 人体制となっております。人数も増え、2011 年年報は早めの発刊が可能となりました。

引き続き、2012 年版も早い機会に発刊したいと思います。

年報業務に加えまして、その他業務におきましても、今後とも何卒宜しく御願い申し上げます。

平成 24 年 3 月 1 日

秘書一同