GASTROENTEROLOGY

Overexpression of CD55 from Barrett’s esophagus is associated with esophageal adenocarcinoma risk


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Key words
brushing, magnified endoscopy, microarray, narrow band imaging.

Abstract

Background and Aim: Although several molecular biomarkers for esophageal adenocarcinoma (EAC) have been shown to be useful disease indicators, none has been established as a reliable indicator for risk of EAC or have progressed to routine use. The aim was to identify biomarkers of high risk for EAC in patients with Barrett’s esophagus (BE).

Methods: Following endoscopic observation by magnified endoscopy with narrow band imaging (ME-NBI), brushing was followed by obtaining biopsy samples from columnar-lined esophagus (CLE) and from EAC lesions of EAC patients, and from age- and sex-matched non-EAC controls with BE. Total RNA was extracted for microarray analysis using Affymetrix GeneChip Human Genome U133 plus 2.0 Array. Real-time-PCR analysis of identified candidate genes was used to confirm the results.

Results: Overall, 9 EAC patients and 50 patients with BE were studied. Seventy-nine candidate genes were identified by microarray analysis based on a proportional hazards model (P < 0.005). Six genes exhibited significantly differential expressions in both BE and cancer lesions of the EAC group compared to BE of the controls. In the brushing samples, median CD55 relative expression levels in cancer lesions were highest and decreased in BE of EAC group and BE of the controls, in that order (P < 0.001).

Conclusion: Over expression of CD55 in brushing samples taken from BE may be associated with the risk of EAC.

Introduction

The current definition of Barrett’s esophagus (BE) in North America is the endoscopic appearance of a columnar-lined esophagus (CLE) and a biopsy demonstrating specialized intestinal metaplasia (SIM), while BE is defined simply as CLE with or without SIM in the United Kingdom and Japan. SIM is recognized histologically by the presence of goblet cells, and is considered as the hallmark lesion of BE, because it is known to predispose to the development of dysplasia and adenocarcinoma regardless of the location in the esophagus.1 A number of endoscopic methods including chromoendoscopy have been tried for the optical detection of SIM in the esophagus and stomach.2–5 Magnified endoscopy with narrow band imaging (ME-NBI) has been introduced which negates the need for dye spray providing better details of the mucosa and vascular patterns of minute lesions, including early gastric adenocarcinoma and gastric intestinal metaplasia.6–7 Recent reports have indicated that ME-NBI was helpful in identifying SIM and dysplasia in BE, and several classification systems were developed for BE evaluation using ME-NBI.8,9 The tubular/villous pattern has been reported to be characteristic of the presence of SIM. We have recently shown that not only biopsy, but also endoscopic brushing in the tubular/villous mucosal pattern of BE visualized by ME-NBI is useful to detect SIM possessing intestinal phenotype such as MUC2 and CDX2.10 The possible problems of cytology and histology include sampling bias, whereas endoscopic brushing in the targeted columnar epithelium using ME-NBI seems to be able to reduce sampling bias and increase the detection rate for SIM and dysplasia in BE.

Esophageal adenocarcinoma (EAC) is now the most rapidly increasing type of cancer in Western countries11 and has a poor prognosis when detected at an advanced stage. In Japan, esophageal adenocarcinoma accounts for only 1% of all esophageal carcinomas, although the incidence has been gradually increasing.12 Over the past several years, research in BE has focused on identifying biomarkers for risk stratification to reduce the mortality and to minimize invasive and costly interventions.13 Several molecular biomarkers such as DNA content abnormalities (aneuploidy and tetraploidy) and loss of heterozygosity (LOH) of 17p (TP53 locus) and 9p (P16 locus) etc., have been shown to be useful disease indicators.13 However none has been established as a reliable indicator for risk of EAC or has progressed to routine use.13 The surveillance method commonly used in BE involves periodic upper gastrointestinal (GI) endoscopy with biopsies of
suspicious areas and random four-quadrant biopsies each 2 cm.1 This biopsy protocol is time-consuming, carries the risk of sampling error, and is hampered by low compliance.14 The data on endoscopic brushing for assessment of DNA ploidy by fluorescent in situ hybridization (FISH) has represented a valuable adjunct to conventional cytology to detect dysplasia or EAC.15 Alternatively, widespread sampling afforded by the nonendoscopic Cytosponge is the purpose of an ongoing trial.

In the present study, we performed a genome-wide search obtaining biopsy samples from the mucosa of BE visualized by ME-NBI of EAC patients and controls and from cancer lesions of EAC patients to identify the candidate genes as a risk marker of EAC. We also compared the candidate gene expressions in the brushing samples taken from BE of the EAC patients with those in the controls.

Subjects and methods

The subjects were EAC patients before endoscopic treatment for cancer and outpatients with BE, which maximum segment length of CLE was longer than 1 cm with or without SIM detected by previous endoscopy who were recommended prior to upper GI endoscopy following reflux esophagitis, screening of ulcers or cancer regardless of GI symptoms. The study was performed at Kawasaki Medical School Hospital in Japan and was approved by the Kawasaki Medical School Ethical Committee. Informed consent was obtained from each patient, and the patients were enrolled in the study between April 2010 and October 2012.

All subjects underwent upper GI endoscopy. Exclusion criteria were advanced stage of EAC, taking of non-steroidal anti-inflammation drugs (NSAIDs), and/or a history of gastrectomy. Patients were also excluded if they had gastric cancer or other malignant lesions, hemorrhagic diseases, cirrhosis, or renal failure. The patients whose lengths of CLE were less than 1 cm were excluded. Demographic data collected at study entry included age, sex, smoking habits, alcohol consumption, and drug treatments including the use of anti-secretory drugs. Drinking and smoking were defined as regular intake when consumption was more than 35 g of ethanol or five cigarettes per day, respectively.

Endoscopic examination. All endoscopies were performed with an endoscopic tri-modal imaging (ETMI) system using zoom endoscopy (GIF-Q240Z or GIF-H260Z, Olympus Inc., Tokyo, Japan). The light source (XCLV-260HP) contains two rotating RGB filters: one conventional for high-resolution white-light endoscopy (WLE) and an additional one for NBI in which the band-pass ranges are narrowed to wavelengths of 530–550 nm (green) and 390–445 nm (blue). Endoscopies were performed by experienced endoscopists using WLE and ME-NBI after patients had fasted for 12 h. Magnified observation of the lower esophagus using ME-NBI was performed to determine the mucosal patterns of BE which were classified into three patterns; small round, long oval, and tubular/villous.9,10 If the mucosal patterns tended to consist of two or three patterns, the tubular/villous patterns were preferentially classified and targeted because those were reported to be characteristics of the presence of goblet cells.9,16 In EAC patients, mucosal pattern of cancer lesions was irregular pattern.17,18 After observation by WLE and ME-NBI, brushing and biopsy samples were taken from the targeted BE surrounding cancer, from cancer lesions of EAC patients, and from the targeted BE of non-EAC patients.

At first, brushing samples were taken from half of the targeted columnar epithelium, and then, two specimens were taken from the remaining half of the targeted part as biopsy samples using endoscopic forceps (FB231K (A) Olympus). Biopsy samples were immediately frozen using liquid nitrogen and stored at −80 °C until use. To avoid the contamination, the brushing lesions were carefully observed by ME-NBI and taken using 5-mm small brushes (CELEBRITY™ Endoscopy; Boston Scientific, Boston, MA, USA).

Oligonucleotide microarray. A microarray analysis was performed using 15 biopsy samples which were taken from five EAC patients and five age- and sex-matched controls to identify candidate genes for EAC.

The microarray procedure was performed according to the Affymetrix protocols (Santa Clara, CA). In brief, the total RNA extracted from the biopsy samples was analyzed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) to determine the RNA integrity number (RIN), which is a measure of RNA degradation.12 The samples with RIN > 4.80 were chosen for microarray analysis. aRNA was synthesized and purified using the GeneChip 3'IVT Express kit (Affymetrix). Hybridization was performed using the Affymetrix GeneChip HG-U133 Plus 2.0 array for 16 h at 45 °C. The signal intensities were measured using a GeneChip Scanner3000 (Affymetrix) and converted to numerical data using GeneChip Command Console Software, ver. 1.1 (Affymetrix).

The microarray analysis was performed using the BRB Array Tools software ver. 3.6.1 (http://linus.nci.nih.gov/BRB-ArrayTools.html) developed by Dr. Richard Simon and Dr. Amy Peng. In brief, a log base 2 transformation was applied to the raw microarray data, and global normalization was used to calculate the median over the entire array. Genes were excluded if the percentage of data missing or filtered out exceeded 50%. Genes that passed the filtering criteria were then considered for further analysis. We computed a statistical significance level (P < 0.005) for each gene based on a univariate proportional hazards model.

RNA extraction and quantitative polymerase chain reaction. Total RNA was extracted from brushing and whole biopsy samples using the RNeasy Mini kit (Qiagen, Hilden, Germany). Then cDNA synthesis was performed using the SuperScriptIII First Strand Synthesis System (Invitrogen, Carlsbad, CA, US). Quantitative reverse transcription (RT)-PCR analysis of identified candidate genes and β-actin was performed using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) employing the TaqMan gene expression assay according to the manufacturer’s instructions (Applied Biosystems). The RT-PCR was performed with 20-ng RNA for both target genes and the endogenous control using the TaqMan one-step RT-PCR Master Mix Reagent kit (P/N 4309169). Each amplification reaction was performed in triplicate, and the average of the threshold cycles was used. The target amount was obtained by normalization to an endogenous reference (β-actin) and relative to a calibrator.
Immunofluorescence and immunohistochemistry. Two smears were made using each brushing the material. The fixed smear samples and cryostat section (4 μm) from frozen biopsy samples were permeabilized in 0.1% Triton X-100/ phosphate buffered saline (PBS). Nonspecific binding was prevented by immersion of samples in blocking solutions (PBS containing 0.1% Tween 20; PBST) for 1 h at room temperature. The samples were supplemented with primary antibodies against CD55 (dilution 1: 30, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or CDX2 antibody (1:500, BioGenex, San Ramon, CA, USA) overnight at 4 °C. After rinsing with PBST, samples were then supplemented with secondary antibodies conjugated with Alexa 488 (dilution 1:1000, Cell Signaling Technology, Beverly, MA, USA), for 1 h at room temperature. After rinsing with PBST, samples were stained by Propidium Iodide (PI), followed by rinsing and mounting, which were observed with laser scanning confocal microscope (DP73 digital camera, Olympus Inc., Tokyo, Japan).

The sections of EAC lesions treated by endoscopic submucosal dissection (ESD) were deparaffinized, and antigenic retrieval using the Ventana Discovery automated staining system with the DAB Map kit (Ventana, Tucson, AZ, USA). Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide for 10 min. After nonspecific binding was blocked, sections were incubated with CD55 antibody (1:50) for overnight at 4 °C. After washing in PBS, the sections were incubated with secondary antibody and counterstained using the Ventana Discovery automated staining system with the DAB Map kit (Ventana).

H. pylori diagnosis. H. pylori status was determined by the presence of serum H. pylori IgG antibodies with an enzyme-linked immunosorbent assay (ELISA) kit using the E plate test (Eiken Kagaku, Inc., Tokyo, Japan).

Statistical analyses. Values are expressed as the mean ± SD or the median with a 25–75% range, whichever was appropriate depending on whether the data were normally distributed. Mantel–Haenszel Chi square analysis and the unpaired t test were performed to measure differences in demographic and clinical characteristics. Statistical analyses for significant differences of parameters were performed using Kruskal–Wallis test for among the three groups. All pairwise multiple comparison procedures (Dunnett’s test) among the three groups were performed to isolate the group different from the others. The receiver operating characteristic (ROC) curve generated with expression levels of CD55 was obtained to establish the most suitable cut-off point. A two-sided P value of less than 0.05 was considered statistically significant. All statistical computations were performed using SPSS (SPSS Inc., Chicago, IL, USA).

Results

Array analysis. To identify the candidate genes from among over 54,000 transcripts, a microarray analysis was performed for a training set of 15 samples. A total of 11445 genes passed the filtering criteria and were further analyzed. Seventy-nine genes were identified based on a Cox proportional hazards model (P < 0.005). Among these genes, the genes exhibiting significantly differential expressions in both BE and cancer lesions of the EAC group compared to BE in the controls were nine genes (CD55 (Decay-accelerating factor; DAF), PIK3R1 (phosphoinositide-3-kinase, regulatory subunit), DSC2 (desmocollin 2), EGR3 (early growth response 3), EN1 (ectodermal-neral cortex 1), EPC2 (enhancer of polycomb homolog 2), HAS3 (hyaluronan synthase 3), PTPRR (protein tyrosine phosphate, receptor type, R), and COL16A1 (collagen, type XVI, alpha 1)).

Validation study. To confirm the array results, quantitative real-time PCR was used for the samples taken from the additional patients. The validation study group consisted of 9 EAC patients (seven men, two women, average age 70.9 years old) and 50 sex and age-matched patients (37 men, 13 women, average age 67.4 years old) with BE. Demographic and clinical characteristics of the study groups are shown in Table 1. The frequency of hiatus hernia and smoking in the EAC group tended to be higher compared to the controls; however, there was no significant difference in any clinical characteristics between the two groups. Histology of EAC was well differentiated adenocarcinoma in seven patients and moderately differentiated adenocarcinoma in the others. The mean tumor size was 17 mm (range, 5 to 34 mm). The tumor, node, metastasis (TNM) stages were stage I (n = 8) and stage IIb (n = 1).

Selected genes expression in biopsy samples. In the biopsy samples taken from BE and cancer lesions of the EAC patients and BE of the controls, the expression levels of 4 identified genes (CD55, EGR3, DSC2, and PTTPR) were significantly different among the three groups, but the others were not. CD55, EGR3, and EN1 were higher, and DSC2 and PTTPR were lower in the BE and cancer lesions of the EAC patients than those in the BE

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics of the patients</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EAC group (n = 9)</td>
</tr>
<tr>
<td>Age mean (SD)</td>
<td>70.9 (15.1)</td>
</tr>
<tr>
<td>Gender men (%)</td>
<td>7 (77.8%)</td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>24.8 (5.4)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>5 (55.6%)</td>
</tr>
<tr>
<td>Regular alcohol intake</td>
<td>5 (55.6%)</td>
</tr>
<tr>
<td>Anti-acid drug use</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>H. pylori-positive</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>H. pylori-negative</td>
<td>6 (66.7%)</td>
</tr>
<tr>
<td>GERD negative</td>
<td>6 (66.7%)</td>
</tr>
<tr>
<td>Grade A</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>Grade B</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>SSBE</td>
<td>6 (66.7%)</td>
</tr>
<tr>
<td>LSBE</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>Length of BE</td>
<td>1.9 (0.9)</td>
</tr>
<tr>
<td>Hiatus hernia</td>
<td>8 (88.9%)</td>
</tr>
</tbody>
</table>

EAC, esophageal adenocarcinoma; BMI, body mass index; GERD, gastroesophageal reflux disease; SSBE, short segment Barrett’s esophagus; LSBE, long segment Barrett’s esophagus.

*P Values calculated using the: t-test; *Mantel-Haenszel Chi square analysis.

*H. pylori-negative patients including 28 patients with history of H. pylori eradication.
Over expression of CD55 is the risk of EAC

T. Murao et al.

of the controls (Table 2). However, there was no significant difference in the gene expression by pairwise multiple comparison procedures among the three groups (Fig. 1).

**Selected gene expression in brushing samples.** In the brushing samples, the expression levels of six identified genes (CD55, EGR3, ENC1, HAS3, DSC2, and PTPRR) were significantly different among the three groups, but the others were not. CD55, EGR3, ENC1, and HAS3 were higher, and DSC2 and PTPRR were lower in the BE and cancer lesions of the EAC groups than those in the BE of the controls (Table 3). The median CD55 expression levels in cancer lesions (59.2×10⁻² vs 10.5×10⁻², P=0.030) and BE of the EAC group (44.1×10⁻² vs 10.5×10⁻², P=0.038) were higher than those in BE of the controls (Fig. 2). However, expressions of the other genes were not significantly different by pairwise multiple comparison procedures.

**CD55 and CDX2 immunohistochemistry.** The majority of cells taken by brushing and apical surfaces of SIM in the biopsy samples from the BE in the EAC groups expressed CD55 protein, and CD55 staining in the biopsy samples was detected at the nuclei of SIM cells which expressed CDX2 (Fig. 3). CD55-positive staining was observed in the surface of the cancer lesions most strongly and in the BE lesions surrounding the cancer lesions, while there was no CD55-positive staining lesion in the adjacent esophageal or gastric mucosa (Fig. 4).

**Determination of cut-off points of CD55 for screening for Barrett adenocarcinoma.** ROC curve analyses revealed CD55 mRNA relative expression levels in brushing samples differentiating BE in the EAC group from BE in the control group with the area under the ROC curve (AUC) of 0.837 (95% CI:0.641–0.837) (Fig. 5). The most suitable cut-off point of CD55 was 0.02, which enabled the estimation of the sensitivity and specificity for CD55 to be 87.5% and 74.0%, respectively (OR 19.9, 95% C.I. 2.2–177.8, P=0.007).

**Discussion**

This study represents the report of EAC associated genes identified by a genome-wide association survey using biopsy samples taken from BE in the patients with and without EAC. We identified the candidate genes associated with EAC and confirmed overexpression of CD55 not only in the cancer lesions, but also in the ME-NBI targeted BE of the EAC patients compared to the controls using the brushing samples.

CD55 is known as DAF and is a member of membrane-bound complement-regulatory proteins, and the function is regulating the complement system activity by accelerating the decay of the C3/C5-convertase of the classic as well as of the alternative pathway.20 Besides its function as a complement system regulator, CD55 appears to inhibit natural killer cells activation.21 It is also known as a receptor for certain viruses and microorganisms and as a ligand of the CD97 receptor.22 Physiologically, CD55 is expressed in cells exposing to the complement system and plays a role in protection of cells against complement attack, which is an innate mechanism in protection of the host against pathogens, including cancer,23 and its overexpression has been reported in

![Figure 1](image1.png)

**Figure 1** Comparison of CD55 expression levels in the biopsy samples among Barrett’s esophagus (BE) in the control group, BE in the esophageal adenocarcinoma (EAC) group and EAC. Horizontal bar = median; box = 25th–75th interquartile range; vertical lines = range of values. P values by Dunnett’s test.

from BE in the patients with and without EAC. We identified the candidate genes associated with EAC and confirmed overexpression of CD55 not only in the cancer lesions, but also in the ME-NBI targeted BE of the EAC patients compared to the controls using the brushing samples.

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**Table 2** Comparisons of mRNA levels of the identified candidate genes in the biopsy samples

<table>
<thead>
<tr>
<th>Gene</th>
<th>BE in the control group (x10⁻³)</th>
<th>BE in the EAC group (x10⁻³)</th>
<th>Cancer lesion in the EAC group (x10⁻³)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD55</td>
<td>3.63(2.06–6.59)</td>
<td>10.4(1.7–23.8)</td>
<td>23.9(4.9–40.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>2.31(0.76–3.88)</td>
<td>2.79(1.86–4.07)</td>
<td>1.37(1.18–2.16)</td>
<td>0.23</td>
</tr>
<tr>
<td>DSC2</td>
<td>41.8(23.0–112)</td>
<td>31.6(36.9–91.6)</td>
<td>13.8(7.0–28.8)</td>
<td>0.022</td>
</tr>
<tr>
<td>EGR3</td>
<td>0.10(0.04–0.32)</td>
<td>0.33(0.16–1.15)</td>
<td>0.66(0.48–8.55)</td>
<td>0.001</td>
</tr>
<tr>
<td>ENC1</td>
<td>0.15(0.08–0.30)</td>
<td>0.24(0.17–0.82)</td>
<td>0.30(0.16–0.72)</td>
<td>0.067</td>
</tr>
<tr>
<td>EPC2</td>
<td>2.30(1.63–3.54)</td>
<td>2.71(12.14–6.46)</td>
<td>2.22(1.71–3.48)</td>
<td>0.635</td>
</tr>
<tr>
<td>HAS3</td>
<td>1.57(0.67–3.54)</td>
<td>2.20(1.14–8.14)</td>
<td>3.04(1.03–10.1)</td>
<td>0.111</td>
</tr>
<tr>
<td>PTPRR</td>
<td>0.83(0.51–1.30)</td>
<td>0.44(0.36–0.89)</td>
<td>0.16(0.07–0.42)</td>
<td>0.001</td>
</tr>
<tr>
<td>COL1A1</td>
<td>1.23(0.66–1.94)</td>
<td>1.65(0.77–3.72)</td>
<td>1.25(0.56–3.98)</td>
<td>0.673</td>
</tr>
</tbody>
</table>


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Table 3 Comparisons of mRNA levels of the identified candidate genes in the brushing samples

<table>
<thead>
<tr>
<th>Gene</th>
<th>BE in the control group Median (25%–75%) (×10⁻³)</th>
<th>BE in the EAC group Median (25%–75%) (×10⁻³)</th>
<th>Cancer lesions in the EAC group Median (25%–75%) (×10⁻³)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD55</td>
<td>10.5(5.34–21.2)</td>
<td>45.8(28.5–57.2)</td>
<td>60.4(39.0–135)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PK3R1</td>
<td>0.84(0.34–1.57)</td>
<td>1.00(0.53–1.35)</td>
<td>0.81(0.64–1.83)</td>
<td>0.688</td>
</tr>
<tr>
<td>DSC2</td>
<td>39.4(19.3–51.1)</td>
<td>18.8(12.2–27.0)</td>
<td>19.1(9.24–20.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>EGR3</td>
<td>0.45(0.16–0.75)</td>
<td>5.68(1.29–9.30)</td>
<td>4.62(2.28–17.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>ENC1</td>
<td>0.01(0.01–0.05)</td>
<td>0.110(0.04–0.18)</td>
<td>0.25(0.08–0.35)</td>
<td>0.01</td>
</tr>
<tr>
<td>EPC2</td>
<td>0.65(0.29–1.11)</td>
<td>1.270(0.54–1.34)</td>
<td>1.84(0.84–2.37)</td>
<td>0.054</td>
</tr>
<tr>
<td>HAS3</td>
<td>0.88(0.43–1.91)</td>
<td>6.14(4.74–9.41)</td>
<td>4.42(1.60–13.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>PTPRR</td>
<td>1.60(0.78–3.29)</td>
<td>1.92(1.07–2.24)</td>
<td>0.26(0.23–0.61)</td>
<td>0.002</td>
</tr>
<tr>
<td>COL16A1</td>
<td>0.38(0.13–0.87)</td>
<td>0.61(0.21–1.22)</td>
<td>0.33(0.23–6.80)</td>
<td>0.75</td>
</tr>
</tbody>
</table>


various kinds of malignant tumors and is associated with poor prognosis in various malignant tumors including gastric cancer and rectal cancer.24–28

Cytology, which is a safer method than biopsy and is minimally invasive, as well as histology, can be complementary to each other in the evaluation of patients with BE,29–31 although cytology alone has been shown to be a poor diagnostic tool for dysplasia32 and has no generally accepted role in establishing the diagnosis of BE. However we previously confirmed that endoscopic brushing in the tubular/villous mucosal pattern of BE visualized by ME-NBI seems to be useful to detect the intestinal phenotype such as SIM,3 and CDX2 in BE, and is less invasive and better than targeted biopsy using ME-NBI. The long oval and/or tubular/villous patterns were visualized in 98% of the subjects with short segment Barrett esophagus (SSBE), and MUC2 expression was detected in 95% of the brushing samples.10 Moreover, Lin et al.33 also reported the utility of brushing cytology in BE, indicating that significantly more loss of heterozygosity (LOH) were detected by brushing than in the biopsy.

CD55 was stained in the majority of cells taken from the targeted BE by brushing and expressed in apical surfaces of SIM expressing CDX2 in the biopsy samples. In the previous report by Hiraoka et al.,34 CD55 was strongly stained on the apical surfaces of SIM, but not on SIM-negative columnar epithelium suggesting that CD55 is a surface marker for SIM. However, CD55 expression in BE of EAC patients has not been reported. We found that CD55 mRNA expression levels were significantly higher especially in brushing samples taken from EAC and BE surrounding the cancer than in BE of the controls. The reason of the difference between the two groups in CD55 mRNA expression levels of the obtained brushing or biopsy specimens from the tubular/villous pattern of BE visualized by ME-NBI, which was characteristic of the presence of SIM, may be because the presence of intestinal metaplasia was multifocal and the degree of intestinal metaplasia was different even in mucosa with tubular/villous pattern of BE. CD55 mRNA expression levels in cancer lesions were also higher than in the BE of the controls suggesting that BE in the EAC group may have not only more intensive intestinal phenotype, but also malignant potential compared to the BE in the controls. However there was no significant difference in the gene expression using the biopsy samples between the two groups by pairwise multiple comparison procedures. The reason for the significant difference of CD55 mRNA expression levels in the brushing samples, but not in biopsy samples, may be because brushing cytology can sample a greater area than biopsy. Moreover, the expression of the CD55 was relatively limited to the surface of epithelium.

The major limitation of the present study is a small number of patients with EAC. In Japan, the prevalence of long segment Barrett esophagus (LSBE) has been estimated to be in the range of 0.2–0.4%, and esophageal adenocarcinoma accounts for only 1% of all esophageal carcinomas.12,35 It is difficult to collect samples from the patients with EAC. Therefore, RNA samples of EAC with high quality for microarray analysis could not be constantly available. Our choice for p-value cut-off of 0.005, which may not be stringent enough to prevent false discovery, was partly due to such quantitative and qualitative limitation. The subsequent validation study using real-time PCR method, however, enabled

Figure 2 Comparison of CD55 expression levels in the brushing samples among Barrett’s esophagus (BE) in the control group, BE in the esophageal adenocarcinoma (EAC) group and EAC. Horizontal bar = median; box = 25th–75th interquartile range; vertical lines = range of values. P Values by Dunnett’s test.
us to identify CD55 as a candidate marker gene, although higher sample sizes should be necessary in the future to exclude the risk of false discovery and to further confirm these results. Another limitation is that the present study was a case control study, and further prospective cohort studies incorporating a large number of patients should be done to confirm the association between the identified genes and EAC. Moreover, the CD55 protein levels need to be quantified to confirm the differences among the three groups. There is the possibility of contamination of dysplastic mucosa in BE samples surrounding EAC. We pathologically confirmed that there was no high grade dysplasia in surrounding cancer lesions by reviewing all resected materials.

In conclusion, over expression of CD55 in brushing samples taken from BE may be associated with the risk of EAC.
Figure 5  Receiver operating characteristic (ROC) curve analysis of
CD55 displayed as sensitivity (y-axis) against 1-specificity (x-axis).

Acknowledgement

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