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Cancer Biology and Signal Transduction

Molecular Cancer Therapeutics

MEK Inhibitor for Gastric Cancer with *MEK1* Gene Mutations

Shunsuke Sogabe[1,2], Yosuke Togashi[1], Hiroaki Kato[1,2], Akihiro Kogita[1,2], Takuro Mizukami[1], Yoichi Sakamoto[1], Eri Banno[1], Masato Terashima[1], Hidetoshi Hayashi[1], Marco A. de Velasco[1], Kazuko Sakai[1], Yoshihiko Fujita[1], Shuta Tomida[1], Takushi Yasuda[2], Yoshifumi Takeyama[2], Kiyotaka Okuno[2], and Kazuto Nishio[1]

Abstract

The prognosis for patients with unresectable advanced or recurrent gastric cancer remains poor. The identification of additional oncogenes with influences similar to those of epidermal growth factor receptor gene mutations, upon which the growth of cancer cells is dependent, is needed. In this study, we evaluated sensitivity to MEK inhibitors (GSK1120212 and PD0325901) in several gastric cancer cell lines *in vitro* and found three poorly differentiated gastric cancer cell lines that were hypersensitive to the inhibitors. The sequence analyses in these three cell lines revealed that one cell line had a novel *MEK1* mutation, while the other two had previously reported *KRAS* and *MEK1* mutations, respectively; the gene statuses of the other resistant cell lines were all wild-type. Experiments using *MEK1* expression vectors demonstrated that the *MEK1* mutations induced the phosphorylation of ERK1/2 and had a transforming potential, enhancing the tumorigenicity. The MEK inhibitor dramatically reduced the phosphorylation of ERK1/2 and induced apoptosis in the cell lines with *MEK1* mutations. *In vivo*, tumor growth was also dramatically decreased by an inhibitor. One of the 46 gastric cancer clinical samples that were examined had a *MEK1* mutation; this tumor had a poorly differentiated histology. Considering the addiction of cancer cells to active *MEK1* mutations for proliferation, gastric cancer with such oncogenic *MEK1* mutations might be suitable for targeted therapy with MEK inhibitors. *Mol Cancer Ther; 13(12); 3098–106. ©2014 AACR.*

Introduction

Gastric cancer is the third most common cause of death from malignant disease in men (fifth in women) worldwide (1). The prognosis of patients with unresectable advanced or recurrent gastric cancer remains poor, with a median survival time of less than 1 year in individuals receiving conventional therapy (2–5). The combination of trastuzumab, an antibody targeting human epidermal growth factor receptor (EGFR) type 2 (HER2), with chemotherapy has yielded a survival benefit for patients with HER2-positive gastric or gastro-esophageal junction cancer (3); however, HER2-positive tumors only account for 7% to 17% of all gastric cancers (6–9). Fibroblast growth factor receptor 2 gene (*FGFR2*) or MET gene (*MET*) amplification has been also regarded as a potential target in gastric cancer, but the frequency is expected to be very low (10–12). Therefore, the identification of additional oncogenes with effects similar to those of *EGFR* mutations or anaplastic lymphoma kinase (*ALK*) gene rearrangements and upon which cancer cells are dependent is needed (13).

The mitogen-activated protein kinase (MAPK) pathway includes RAS, RAF, MEK, and ERK. Constitutive activation of this pathway can lead to uncontrolled cell growth and survival, ultimately resulting in oncogenic transformation and progression (14). Reflecting the central role of the MAPK pathway in cell proliferation, activated mutants of RAS family members (HRAS, KRAS, and NRAS) are among the oncoproteins most frequently detected in human malignancies (15). The discovery of mutations of the *BRAF* gene in melanoma has further reinforced the substantial contribution of the MAPK pathway to carcinogenesis (16). However, very limited information is available about somatic *MEK1* mutations in human malignancies (17). In this study, we tested the effects of MEK inhibitors in several gastric cancer cell lines *in vitro* and found three cell lines that were hypersensitive to the inhibitors; one of these cell lines had a novel *MEK1* S72G mutation. Furthermore, the role of these mutations, a xenograft study using a MEK inhibitor, and the *MEK1* gene statuses of clinical samples of gastric cancer were investigated.

[1]Department of Genome Biology, Kinki University Faculty of Medicine, Osaka, Japan. [2]Department of Surgery, Kinki University Faculty of Medicine, Osaka, Japan.

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

S. Sogabe and Y. Togashi contributed equally to this article.

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

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Materials and Methods

Cell cultures and reagents

The HEK293 cell line (human embryonic kidney cell line) and the NIH-3T3 cell line (mouse fibroblast cell line) were maintained in DMEM medium (Nissui Pharmaceutical) supplemented with 10% FBS (GIBCO BRL) in a humidified atmosphere of 5% CO_2 at 37°C. All the gastric cancer cell lines used in this study were maintained in RPMI-1640 medium (Sigma-Aldrich), except for IM95 (DMEM; Nissui Pharmaceutical), supplemented with 10% FBS in a humidified atmosphere of 5% CO_2 at 37°C. The IM95 and OCUM-1 cell lines were obtained from the Japanese Collection of Research Bioresources, while the other cell lines were provided by the National Cancer Center Research Institute (Tokyo, Japan) in 2006. The OCUM-1, Okajima, SNU-16, and HEK293 cell lines were analyzed using a short tandem repeat (STR) method in July 2014, and the OCUM-1, SNU-16, and HEK293 cell lines were authenticated. The database did not include the STR pattern of the Okajima cell line, but the pattern did not match any of the other cell lines. GSK1120212 and PD0325901 (MEK inhibitors) were purchased from Selleck Chemicals and Wako, respectively (Fig. 1A).

Growth inhibition assay *in vitro*

The growth-inhibitory effects of GSK1120212 and PD0325901 were examined using the MTT assay (Sigma-Aldrich), as described previously (18). The experiment was performed in triplicate.

Antibody

Rabbit antibodies specific for MEK1/2, ERK1/2, phospho-ERK1/2, caspase-3, cleaved caspase-3, PARP, cleaved PARP, and β-actin were obtained from Cell Signaling Technology.

Western blot analysis

A Western blot analysis was performed as described previously (18). Briefly, subconfluent cells were washed with cold phosphate-buffered saline (PBS) and harvested with Lysis A buffer containing 1% Triton X-100, 20 mmol/L Tris–HCl (pH 7.0), 5 mmol/L EDTA, 50 mmol/L sodium chloride, 10 mmol/L sodium pyrophosphate, 50 mmol/L sodium fluoride, 1 mmol/L sodium orthovanadate, and a protease inhibitor mix, Complete (Roche Diagnostics). Whole-cell lyses were separated using SDS-PAGE and were blotted onto a polyvinylidene fluoride membrane. After blocking with 3% bovine serum albumin in a TBS buffer (pH 8.0) with 0.1% Tween-20, the membrane was probed with the primary antibody. After rinsing twice with TBS buffer, the membrane was incubated with a horseradish peroxidase–conjugated secondary antibody and washed, followed by visualization using an enhanced chemiluminescence

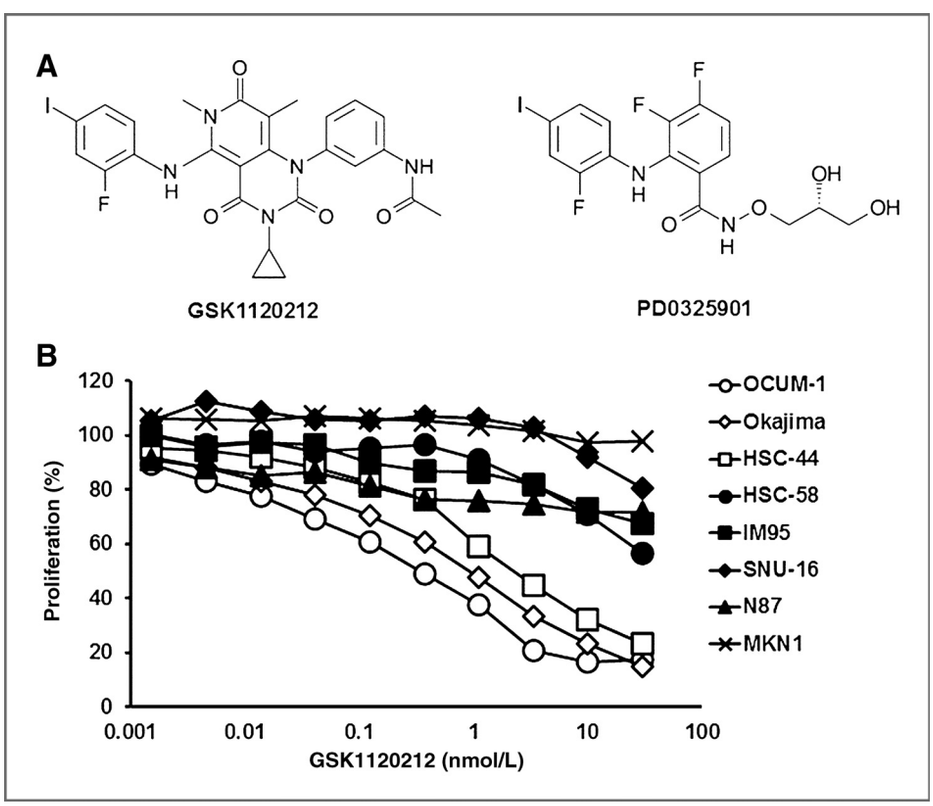

Figure 1. Structures of MEK inhibitors and sensitivities of several gastric cancer cell lines to these inhibitors. A, structures of GSK1120212 and PD0325901. B, sensitivities of several gastric cancer cell lines to MEK inhibitors. To examine the sensitivities of MEK inhibitors, we used the MTT assay. The experiment was performed in triplicate. The OCUM-1, Okajima, and HSC-44 cell lines were hypersensitive to the inhibitor. All of these cell lines had a poorly differentiated histology. Line, mean of independent triplicate experiments.

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(ECL) detection system and LAS-4000 (GE Healthcare). When the phosphorylation levels of ERK1/2 and apoptosis-related molecules were examined after GSK1120212 exposure, the samples were collected 3 and 48 hours after stimulation, respectively.

Mutational analysis for *KRAS*, *BRAF*, and *MEK1* genes

Genomic DNA samples from gastric cancer cell lines were screened for *KRAS* mutations (exon 2), *BRAF* mutations (exons 2, 4, 11, 12, and 15), and *MEK1* mutations (exons 2–11). The PCR reactions were performed using TaKaRa ExTaq (TaKaRa). The primers are summarized in Supplementary Table S1. The PCR products were then directly sequenced using the BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems). To confirm the results, the PCR amplification was repeated, and the PCR products were subcloned using TOPO TA Cloning kits (Invitrogen).

Plasmid construction and transfectants

PcDNA-MEK1 with myc-tag vector was obtained from Addgene. The *MEK1* exon point mutation Q56P or G72S was amplified using the PrimeSTAR Mutagenesis Basal Kit (TaKaRa) and primers (Supplementary Table S1). Vectors were transfected into HEK293 cells or NIH-3T3 cells using FuGENE6 transfection reagent (Promega). Hygromycin selection (100 μg/mL) was performed on days 2 to 8 after transfection, and the cells were then cultured in normal medium. The vectors and stable transfectant cell lines were designated as PcDNA-mock, PcDNA-MEK1 WT, PcDNA-MEK1 Q56P, PcDNA-MEK1 S72G, HEK293-mock, HEK293-MEK1 WT, HEK293-MEK1 Q56P, HEK293-MEK1 S72G, 3T3-mock, 3T3-MEK1 WT, 3T3-MEK1 Q56P, and 3T3-MEK1 S72G.

Focus formation assay

The transfectant NIH-3T3 cell lines were then cultured for 2 to 3 weeks in DMEM medium supplemented with 5% FBS. The focus formations were counted and photographed using a light microscope. The experiment was performed in triplicate.

Annexin V binding apoptosis analysis

The Annexin V binding apoptosis analyses were performed as described previously (19). Briefly, the cells were exposed to GSK1120212 (1 nmol/L) for 48 hours, and the binding of Annexin V and propidium iodide (PI) to the cells was then measured using the FITC Annexin V Apoptosis Detection Kit I (BD Biosciences), according to the manufacturer's instructions. The cells were stained with FITC Annexin V and PI at room temperature for 15 minutes and were analyzed using a flow cytometer and CellQuest software (BD Biosciences). The experiment was performed in triplicate.

Xenograft studies

Nude mice (6-week-old females; CLEA Japan) and NOD/SCID mice (6-week-old females; CLEA Japan) 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 Animals Experiments, Kinki University (Osaka, Japan). The ethical procedures followed met the requirements of the United Kingdom Coordinating Committee on Cancer Research guidelines. To evaluate tumorigenicity, a suspension of 1×10^6 NIH-3T3 transfectant cells (in 100 μL of PBS) was subcutaneously inoculated into the right flank of each nude mouse ($n = 5$), and tumor formation was examined after 2 weeks based on a previous report (17). To evaluate the effects of GSK1120212, a suspension of 1×10^7 cells (in 50 μL of PBS) with 50 μL of Matrigel (Okajima cell line) or 5×10^6 cells (in 50 μL of PBS) with 50 μL of Matrigel (SNU-16 cell line) was subcutaneously inoculated into the right flank of each NOD/SCID mouse ($n = 5$); treatment was then initiated when the tumors in each group achieved an average volume of approximately 150 mm^3. In the treatment groups, GSK1120212 (0.5 or 1.0 mg/kg) was administered by oral gavage daily for 7 days based on the results of a previous study (20); the control animals received 0.5% methylcellulose as a vehicle. The tumor volume was calculated as the length $\times$ width^2 $\times$ 0.5. The tumor formation and volume were assessed every 2 to 3 days. This method has been previously described (21).

Patients

Patients with advanced gastric cancer who underwent surgical resection at Kinki University Hospital (Osaka, Japan) between April 2009 and March 2012 were enrolled. This study was retrospectively performed and was approved by the Institutional Review Board of the Kinki University Faculty of Medicine (Osaka, Japan).

Isolation of genomic DNA

Genomic DNA samples were extracted from surgical specimens preserved as formalin-fixed paraffin-embedded (FFPE) tissue using the QIAamp DNA Micro Kit (Qiagen), according to the manufacturer's instructions as described previously (10). Macrodissection of the FFPE samples was performed to select a cancer region, which was marked by a pathologist after deparaffinization. The DNA concentration was determined using NanoDrop2000 (Thermo Fisher Scientific).

Statistical analysis

Continuous variables were analyzed using the Student *t* test, and the results were expressed as the average and standard deviation (SD). The statistical analyses were two-tailed and were performed using Microsoft Excel (Microsoft). A *P* value of less than 0.05 was considered statistically significant.

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Results

OCUM-1, Okajima, and HSC-44 cell lines were hypersensitive to MEK inhibitors, and these cell lines had MEK1 mutations or a KRAS mutation

To examine the sensitivities of several gastric cancer cell lines to MEK inhibitors, we used the MTT assay (Fig. 1B). The 50% inhibitory concentrations (IC_{50}) of the two MEK inhibitors (GSK1120212 and PD0325901) are summarized in Table 1. The OCUM-1, Okajima, and HSC-44 cell lines were hypersensitive to both MEK inhibitors; all three of these cell lines exhibited a poorly differentiated histology.

Next, to search for *KRAS*, *BRAF*, and *MEK1* mutations, which were associated with sensitivity to MEK inhibitors, we sequenced these genes using direct sequencing. The *MEK1* Q56P and S72G mutations were found in the OCUM-1 and Okajima cell lines, respectively, and a *KRAS* G12V mutation was also found in the HSC-44 cell line (Fig. 2A). In contrast, all cell lines that were not sensitive to MEK inhibitors did not have any mutations. The *MEK1* Q56P mutation in the OCUM-1 cell line and the *KRAS* G12V mutation in the HSC-44 cell line have been previously reported (17), whereas the *MEK1* S72G mutation in the Okajima cell line is a novel mutation. This novel mutation was confirmed using the TOPO TA Cloning Kit (Fig. 2B).

MEK1 Q56P and S72G mutations increased the phosphorylation level of ERK1/2, had transformational abilities, and enhanced tumorigenicity

To address the role of the *MEK1* mutations, MEK1-overexpressed HEK293 and NIH-3T3 cell lines were created using each *MEK1* expression vector (wild-type, Q56P, or S72G). ERK1/2 was phosphorylated in the HEK293-MEK1 Q56P, HEK293-MEK1 S72G, 3T3-MEK1-Q56P, and 3T3-MEK1-S72G cell lines, compared with the controls (Fig. 3A and B). We then investigated the transformational abilities and tumorigenicities of the *MEK1* mutations using a focus formation assay and a tumorigenicity assay with the NIH-3T3 cell lines and nude mice; the results showed that the *MEK1* Q56P and S72G mutations had transformational abilities and enhanced the tumorigenicity, compared with the controls. Foci or tumors were not formed in the controls (mock and wild-type; Fig. 3C and D). These findings suggest that both the *MEK1* Q56P mutation and the novel *MEK1* S72G mutation have transformational abilities and enhance tumorigenicity by activating the MAPK pathway.

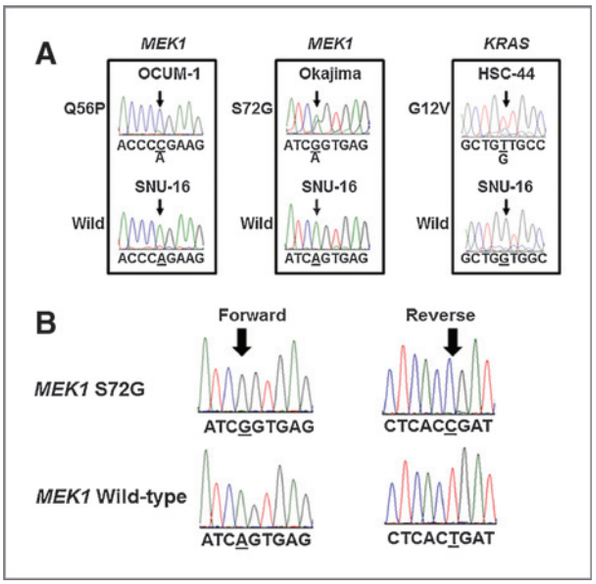

Figure 2. *MEK1* and *KRAS* mutations in each cell line. To search for *KRAS*, *BRAF*, and *MEK1* mutations, which were associated with the sensitivity to MEK inhibitors, we sequenced these genes using direct sequencing. A, *MEK1* Q56P mutation in the OCUM-1 cell line, *MEK1* S72G mutation in the Okajima cell line, and *KRAS* G12V mutation in the HSC-44 cell line. *MEK1* gene exon 2 sequencing revealed a *MEK1* Q56P mutation (A > C) in the OCUM-1 cell line and a *MEK1* S72G mutation (A > G) in the Okajima cell line. *KRAS* gene exon 2 sequencing revealed *KRAS* G12V (G > T) in the HSC-44 cell line. All the cell lines that were not sensitive to MEK inhibitors did not have any mutations. We used the SNU-16 cell line as a wild-type control. B, *MEK1* gene sequence of the Okajima cell line after the insertion of a TOPO cloning vector. To confirm the results, the PCR amplification was repeated and the PCR products were subcloned using TOPO TA Cloning kits. The *MEK1* S72G–mutant allele and wild-type allele were both confirmed.

Reduction in the phosphorylation level of ERK1/2 and induction of apoptosis in response to GSK1120212 in the OCUM-1 and Okajima cell lines

Next, we examined the phosphorylation levels of ERK1/2 after GSK1120212 exposure (0, 1, 3, 10, and 30 nmol/L) in each gastric cancer cell line. Three hours of exposure to GSK1120212 induced a significant decrease in the phosphorylation levels of ERK1/2 in the hypersensitive cell lines (OCUM-1 and Okajima), compared with the level in a nonsensitive cell line (SNU-16; Fig. 4A). We then analyzed the Annexin V binding apoptosis of the cell lines after exposure to GSK1120212 using a flow cytometer. The number of apoptotic cells in the OCUM-1 and Okajima cell lines, but not the SNU-16 cell line, increased greatly after

Table 1. IC_{50} of MEK inhibitors

	OCUM-1	Okajima	HSC-44	HSC-58	IM95	SNU-16	N87	MKN1
GSK (nmol/L)	0.33	0.92	2.23	43.6	1,485	8,140	>10,000	>10,000
PD (nmol/L)	3.4	33	38	453	6,570	>10,000	>10,000	>10,000

Abbreviations: GSK, GSK1120212; PD, PD0325901.

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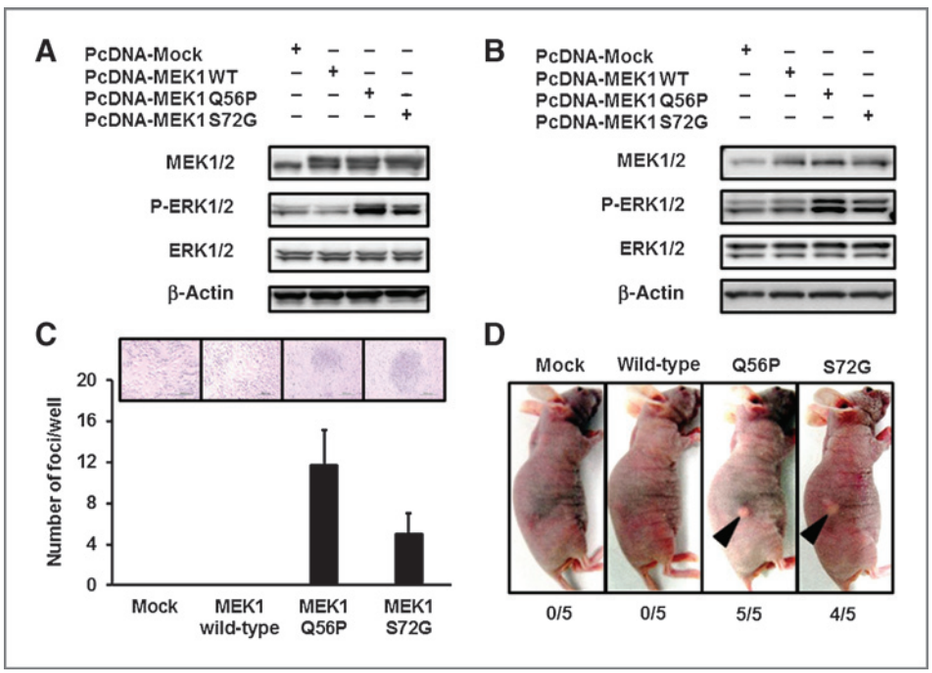

Figure 3. Transformational ability of each *MEK1* mutation. A, MEK1 expression and phosphorylation of ERK1/2 in transfectant HEK293 cell lines. To address the role of the *MEK1* mutations, expression vectors and *MEK1*-overexpressed HEK293 cell lines were created. Each *MEK1* gene was equally introduced into the cell lines, and ERK1/2 was phosphorylated in the HEK293-MEK1 Q56P and HEK293-MEK1 S72G cell lines, compared with HEK293-mock or HEK293-MEK1 WT. β-Actin was used as an internal control. B, MEK1 expression and phosphorylation of ERK1/2 in transfectant NIH-3T3 cell lines. The expressions of MEK1 in the transfectant cell lines were confirmed using Western blot analyses. Similar to the HEK293 cell lines, ERK1/2 was phosphorylated in the 3T3-MEK1 Q56P and 3T3-MEK1 S72G cell lines. β-Actin was used as an internal control. C, transformational ability. To investigate the transformational abilities of these mutations, we used a focus formation assay to examine NIH-3T3 cell lines. Transfectant NIH-3T3 cell lines were cultured for 2 to 3 weeks and photographed. Both *MEK1* mutations had transformational abilities (mock, 0; wild-type, 0; Q56P, 11.7 ± 3.5; S72G, 5 ± 2). The controls (mock and wild-type) did not exhibit the formation of any foci. Columns, mean of independent triplicate experiments; error bars, SD; scale bar, 20 μm. D, tumorigenicity. To investigate the tumorigenicities of these mutations, we used a tumorigenicity assay to examine NIH-3T3 cell lines and nude mice. Transfectant NIH-3T3 cells (1×10^6) were injected subcutaneously into the right flank of nude mice; tumor formation was then examined 2 weeks after injection based on the results of a previous report (17). Both *MEK1* mutations enhanced the tumorigenicity (mock, 0/5; wild-type, 0/5; Q56P, 5/5; S72G, 4/5). The controls (mock and wild-type) did not exhibit the formation of any tumors.

GSK1120212 exposure (1 nmol/L; Fig. 4B). Western blot analyses for apoptosis-related molecules revealed that 48 hours of exposure to the reagent also greatly increased the levels of cleaved PARP and cleaved caspase-3 in the OCUM-1 and Okajima cell lines, compared with the SNU-16 cell line (Fig. 4C).

In vivo efficacy of GSK1120212 in the Okajima cell line

To perform a xenograft study, we used the Okajima (*MEK1* Q72S) and the SNU-16 cell line (*MEK1* wild-type). To evaluate the effects of GSK1120212, a suspension of 1×10^7 cells (in 50 μL PBS) with 50 μL of Matrigel (Okajima cell line) or 5×10^6 cells (in 50 μL PBS) with 50 μL of Matrigel (SNU-16 cell line) was subcutaneously inoculated into the right flank of each NOD/SCID mouse ($n = 5$). In the treatment groups, GSK1120212 (0.5 or 1.0 mg/kg) was administered by oral gavage daily for 7 days; the control animals received 0.5% methylcellulose as a vehicle. The tumors from the Okajima cell line were dramatically reduced by treatment with GSK1120212 [vehicle: 179.86 ± 44.88 mm^3 vs. GSK1120212 (0.5 mg/kg): 89.4 ± 22.84 mm^3; *, $P = 0.0039$ or vs. GSK1120212 (1.0 mg/kg): 27.04 ± 26.7 mm^3; *, $P = 0.00018$; GSK1120212 (0.5 mg/kg) vs. GSK1120212 (1.0 mg/kg); *, $P = 0.0041$; Fig. 5A and B]. The phosphorylation of ERK1/2 in the tumors was inhibited by GSK1120212 (Fig. 5B). In contrast, the tumors from the SNU-16 cell line were not reduced by the drug [vehicle: 335.62 ± 131.36 mm^3 vs. GSK1120212 (0.5 mg/kg): 346.5 ± 182.31 mm^3; $P = 0.92$, or vs. GSK1120212 (1.0 mg/kg): 307.68 ± 106.03; $P = 0.72$; GSK1120212 (0.5 mg/kg) vs. GSK1120212 (1.0 mg/kg); $P = 0.69$; Fig. 5A].

Clinicopathologic features of patients with MEK1-mutated gastric cancer

A total of 46 patients with advanced gastric cancer participated in this study. We evaluated the patient characteristics according to their *MEK1* gene status. The isolated genomic DNA samples were directly sequenced. The clinical features of all the patients are summarized in Supplementary Table S2. One of the patients had gastric cancer with a *MEK1* Q56P mutation; this patient was a 64-year-old male whose gastric cancer had been diagnosed as

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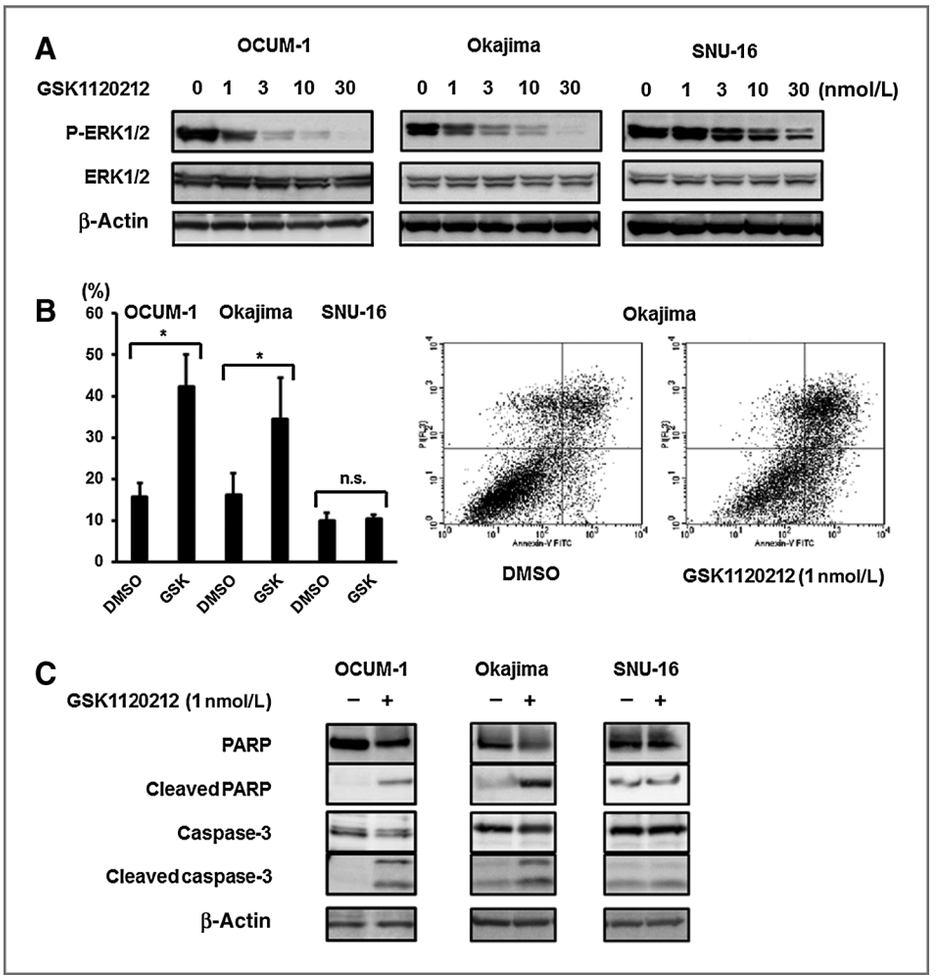

Figure 4. Phosphorylation level of ERK1/2 and apoptosis after GSK1120212 exposure. A, phosphorylation levels of ERK1/2 after GSK1120212 exposure. When the phosphorylation levels were examined after GSK1120212 exposure (0, 1, 3, 10, and 30 nmol/L), the samples were collected 3 hours after stimulation. GSK1120212 induced a significant decrease in the phosphorylation levels of ERK1/2 in the hypersensitive cell lines (OCUM-1 and Okajima), compared with that in the nonsensitive cell line (SNU-16). β-Actin was used as an internal control. B, Annexin V binding apoptosis analyses. The cells were exposed to GSK1120212 (1 nmol/L) for 48 hours and were then harvested and stained with FITC Annexin V and PI. The cells were analyzed using a flow cytometer. The experiment was performed in triplicate, and DMSO was used as a control. The number of apoptotic cells in the OCUM-1 and Okajima cell lines increased greatly after GSK1120212 exposure (DMSO: 15.7% ± 3.3% vs. GSK1120212: 42.3% ± 8.0%; *, $P = 0.017$, and DMSO: 16.2% ± 5.1% vs. GSK1120212: 34.4% ± 10.0%; *, $P = 0.025$, respectively), but not in the SNU-16 cell line (DMSO: 9.9% ± 2.0% vs. GSK1120212: 10.4% ± 1.1%; $P = 0.54$). Columns, mean of independent triplicate experiments; error bars, SD; GSK, GSK1120212; *, $P < 0.05$; n.s., not significant. C, Western blot analyses for apoptosis-related molecules. When apoptosis-related molecules were examined after GSK1120212 exposure (1 nmol/L), the samples were collected 48 hours after the stimulation. GSK1120212 greatly increased the expression of cleaved PARP and cleaved caspase-3 in the OCUM-1 and Okajima cell lines, compared with the SNU-16 cell line. β-Actin was used as an internal control.

a poorly differentiated scirrhous adenocarcinoma stage IV located in corpus.

Discussion

The Cancer Genome Atlas (TCGA) dataset for gastric cancer has shown that the gastric cancer populations with nonsynonymous *KRAS*, *BRAF*, or *MEK1* mutations were relatively small (28 of 289, 24 of 289, and 7 of 289, respectively), and *MEK1* Q56P or S72G mutations have not been identified in the TCGA dataset. In this study, we identified *MEK1* mutations in poorly differentiated gastric cancer cell lines that were hypersensitive to MEK inhibitors and showed that these mutations have transformational abilities and that the growth of the cancer cells is dependent on these mutations. Specifically, the *MEK1* S72Q mutation in the Okajima cell line is a novel activating mutation, whereas the *MEK1* Q56P mutation in the OCUM-1 cell line has been previously reported by Choi and colleagues (17). In addition, a *MEK1* Q56P mutation was identified in a clinical sample of a poorly differentiated gastric cancer; to the best of our knowledge, this is the first study in which a clinical sample of a *MEK1* Q56P-mutated gastric cancer has been identified.

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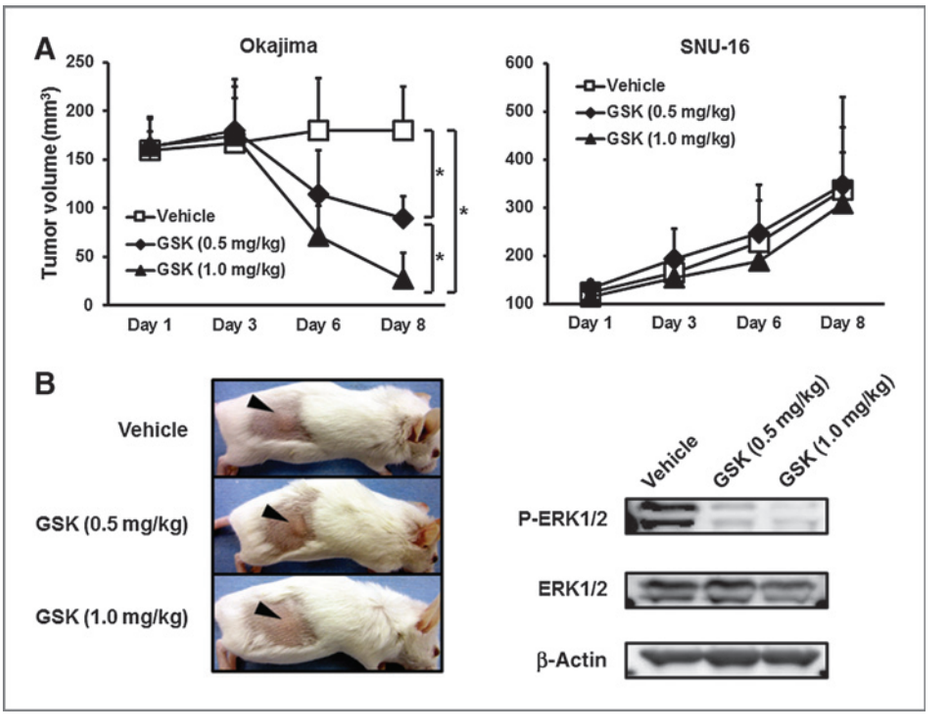

Figure 5. Xenograft study. We used the Okajima (*MEK1* Q72S) and the SNU-16 (*MEK1* wild-type) cell lines in the xenograft study. A suspension of 1×10^7 cells (in 50 μL of PBS) with 50 μL of Matrigel (Okajima cell line) or 5×10^6 cells (in 50 μL of PBS) with 50 μL of Matrigel was subcutaneously inoculated into the right flank of each NOD/SCID mouse ($n = 5$). In the treatment groups, GSK1120212 (0.5 or 1.0 mg/kg) was administered by oral gavage daily for 7 days; the control animals received 0.5% methylcellulose as a vehicle. A, tumor volumes of Okajima and SNU-16 cell line. The tumors from the Okajima cell line dramatically decreased in size after GSK1120212 exposure [vehicle: 179.86 ± 44.88 mm^3 vs. GSK1120212 (0.5 mg/kg): 89.4 ± 22.84 mm^3; *, $P = 0.0039$, or vs. GSK1120212 (1.0 mg/kg): 27.04 ± 26.7; *, $P = 0.00018$; GSK1120212 (0.5 mg/kg) vs. GSK1120212 (1.0 mg/kg), *, $P = 0.0041$]. Because the tumor growth of the Okajima cell line was very slow, only a small change was observed in the vehicle group. In contrast, the tumors from the SNU-16 cell line did not decrease in size after drug exposure [vehicle: 335.62 ± 131.36 mm^3 vs. GSK1120212 (0.5 mg/kg): 346.5 ± 182.31 mm^3; $P = 0.92$ or vs. GSK1120212 (1.0 mg/kg): 307.68 ± 106.03; $P = 0.72$; GSK1120212 (0.5 mg/kg) vs. GSK1120212 (1.0 mg/kg); $P = 0.69$]. Lines, mean of 5 mice; error bars, SD; GSK, GSK1120212; *, $P<0.05$. B, photographs of tumors in the Okajima cell line and Western blot analyses of Okajima *in vivo* samples. The tumors from the Okajima cell line decreased in size in a dose-dependent manner. The phosphorylation of ERK1/2 was inhibited by GSK1120212. β-Actin was used as an internal control. GSK, GSK1120212.

GSK1120212 is an inhibitor of MEK1/2 that exhibits a high potency, selectivity, and long-circulating half-life (22). The results of a phase III study have demonstrated that GSK1120212 is associated with a significant improvement in progression-free survival and overall survival, compared with chemotherapy, in patients with V600E or V600K *BRAF*-mutated advanced melanoma (23). Several studies have shown that *KRAS* and/or *BRAF* mutations are associated with the sensitivity to MEK inhibitors in melanoma, thyroid cancer, colon cancer, and ovarian cancer (23–27). However, very limited information is available about the somatic *MEK1* mutations in human malignancies. Similar to our present study, Choi and colleagues (17) have reported that the *MEK1* Q56P mutation identified in the OCUM-1 cell line has a transformational ability, and somatic mutations in the *MEK1* gene have been reported in several other cancers including lung cancer, ovarian cancer, colon cancer, and melanoma (28–32). Our present study demonstrated that both the *MEK1* G56P and the novel *MEK1* S72G mutation in poorly differentiated gastric cancer cell lines that were hypersensitive to MEK inhibitors have transformational abilities and that the growth of the cancer cells was dependent on these mutations. In both *in vitro* and *in vivo* studies, the gastric cancer cell lines with *MEK1* mutations dramatically responded to the MEK inhibitor. Therefore, MEK inhibitors can be effective for patients with *MEK1* mutations in a manner similar to the effect of EGFR-tyrosine kinase inhibitors in patients with *EGFR* mutations and the effect of ALK inhibitors in patients with *ALK* rearrangements (33–36). Then, not only *KRAS* and *BRAF* mutations, but also *MEK1* mutations should be recognized as predictive biomarkers for the efficacy of MEK inhibitors.

In general, patients with a poorly differentiated gastric cancer histology have a poor prognosis and their treatment is challenging (37). *FGFR2* or *MET* amplification seems to be predominant in poorly differentiated gastric cancer (10–12). Similarly, in this study, the two gastric cancer cell lines with *MEK1* mutations had a poorly differentiated histology, and the one gastric cancer clinical

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sample with a *MEK1* mutation was a poorly differentiated adenocarcinoma. Choi and colleagues (17) previously reported that 1 of 86 gastric cancer samples had a *MEK1* mutation and that the sample was a poorly differentiated adenocarcinoma (well-differentiated, 0 of 40 and poorly differentiated, 1 of 46). Despite the relatively small number of samples, these results suggest that gastric cancer with *MEK1* mutations might be likely to have a poorly differentiated adenocarcinoma histology, similar to that resulting from *FGFR2* or *MET* amplification, and treatment with a MEK inhibitor might be a promising option for such patients with gastric cancer. To confirm these findings, larger studies are needed.

In conclusion, we have identified *MEK1* mutations in poorly differentiated gastric cancer cell lines and a poorly differentiated gastric cancer clinical sample and have shown that the mutations have transformational abilities and that the growth of the cancer cells is dependent on these mutations. In particular, the MEK1 S72Q mutation in the Okajima cell line is a novel activating mutation. Our results warrant strong consideration in the development of MEK inhibitors for the treatment of gastric cancer with *MEK1* mutations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Sogabe, Y. Togashi, H. Kato, E. Banno, Y. Takeyama, K. Nishio
Development of methodology: S. Sogabe, H. Kato, Y. Takeyama
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Sogabe, Y. Togashi, H. Kato, A. Kogita, T. Yasuda
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Sogabe, Y. Togashi, H. Kato, S. Tomida, T. Yasuda
Writing, review, and/or revision of the manuscript: S. Sogabe, Y. Togashi, H. Hayashi, M.A. de Velasco, K. Nishio
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Mizukami, Y. Sakamoto, M. Terashima, K. Sakai, Y. Fujita
Study supervision: K. Okuno, K. Nishio

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Molecular Cancer Therapeutics

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Shunsuke Sogabe, Yosuke Togashi, Hiroaki Kato, et al.

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