Impact of MET inhibition on small-cell lung cancer cells showing aberrant activation of the hepatocyte growth factor/MET pathway

Hirokazu Taniguchi,1,2 Tadaaki Yamada,1 Shinji Takeuchi,1 Sachiko Arai,1 Koji Fukuda,1 Shuichi Sakamoto,3 Manabu Kawada,3 Hiroyuki Yamaguchi,2 Hiroshi Mukae2 and Seiji Yano1

1Division of Medical Oncology, Cancer Research Institute, Kanazawa University, Kanazawa, Japan; 2Department of Respiratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 3Institute of Microbial Chemistry, Numazu, Japan

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Small-cell lung cancer (SCLC) accounts for approximately 15% of all lung cancers, and is characterized as extremely aggressive, often displaying rapid tumor growth and multiple organ metastases. In addition, the clinical outcome of SCLC patients is poor due to early relapse and acquired resistance to standard chemotherapy treatments. Hence, novel therapeutic strategies for the treatment of SCLC are urgently required. Accordingly, several molecular targeted therapies were evaluated in SCLC; however, they failed to improve the clinical outcome. The receptor tyrosine kinase MET is a receptor for hepatocyte growth factor (HGF), and aberrant activation of HGF/MET signaling is known as one of the crucial mechanisms enabling cancer progression and invasion. Here, we found that the HGF/MET signaling was aberrantly activated in chemoresistant or chemorelapsed SCLC cell lines (SBC-5, DMS273, and DMS273-G3H) by the secretion of HGF and/or MET copy number gain. A cell-based in vitro assay revealed that HGF/MET inhibition, induced either by MET inhibitors (crizotinib and golvatinib), or by siRNA-mediated knockdown of HGF or MET, constrained growth of chemoresistant SCLC cells through the inhibition of ERK and AKT signals. Furthermore, treatment with either crizotinib or golvatinib suppressed the systemic metastasis of SBC-5 cell tumors in natural killer cell-depleted SCID mice, predominantly through cell cycle arrest. These findings reveal the therapeutic potential of targeting the HGF/MET pathway for inhibition, to constrain tumor progression of SCLC cells showing aberrant activation of HGF/MET signaling. We suggest that it would be clinically valuable to further investigate HGF/MET-mediated signaling in SCLC cells.

Lung cancer is the leading cause of cancer-related deaths worldwide.1,2 Approximately 15% of lung cancer cases are classified as being small-cell lung cancer (SCLC), an extremely aggressive cancer type characterized by rapid tumor growth leading to multiple organ metastases, such as to the brain, bone, liver, and/or lymph nodes.3 In fact, approximately 70% of SCLC patients are categorized as suffering extensive disease with distant metastases at the time of diagnosis. For the past two decades, the standard treatment for these advanced SCLC patients has been combined chemotherapy, comprising treatment with cisplatin and either etoposide or irinotecan. The response rate for this therapy is approximately 70–80%; however, the median survival time is only 9–12 months, and the overall survival rate of SCLC patients with extensive disease at 5 years is only 5–10%, largely due to early relapse and acquired resistance.4567 Thus, novel therapeutic strategies for the treatment of SCLC are urgently required to improve patient clinical outcomes. Several molecularly targeted therapies have been evaluated and trialed; however, these failed to improve the clinical outcome for this disease.7

The receptor tyrosine kinase MET acts as the receptor for hepatocyte growth factor (HGF), such that MET activated by HGF-binding forms a homodimer, and transduces downstream signals to various pathways.8910 In several cancers, aberrant activation of HGF/MET signaling is reported as a crucial mechanism enabling cancer progression and invasion.10,11 Recently, a MET exon 14 skipping mutation was reported to be a novel driver mutation in non-small-cell lung cancer (NSCLC) patients.12 Similarly, MET-activating mutations and dysregulated autocrine/paracrine HGF/MET signaling have both been reported in SCLC patients; furthermore, MET phosphorylation predicts poor clinical outcome in SCLC.13,14,15 Recently, Canadas et al.16 reported that HGF stimulation in SCLC cells promotes epithelial–mesenchymal transition and chemoresistance, whereas treatment with a MET inhibitor restored chemosensitivity.17

Although activated MET is reported as a prognostic factor for poor clinical outcome in SCLC, the mechanisms underlying the deleterious effects of aberrant activation of the HGF/MET pathway in SCLC have not been clearly elucidated.16 We previously established a mouse model of multiple-organ SCLC metastasis, by utilizing natural killer (NK) cell-depleted SCID mice, to produce an animal model whose tumor distribution resembles that of advanced SCLC patients.18,19,20 The
aim of the present study was to evaluate the effect of inhibiting the HGF/MET pathway on tumor progression in SCLC with multi-organ metastasis, using this in vivo mouse xenograft model, as well as various cell-based in vitro assays.

**Materials and Methods**

**Cell cultures and reagents.** The human SCLC cell lines SBC-3 and SBC-5, were kindly provided by Dr. K. Kiura (Okayama University, Okayama, Japan), DMS237 and DMS273-G3H were obtained as reported previously. Briefly, DMS273-G3H was established using tumor cells from a bone metastasis after we had implanted DMS273 orthotopically into the left lung of nude mice. H196 and H1048 were purchased from ATCC (Manassas, VA, USA). DMS114 and DMS454 were purchased from the European Collection of Authenticated Cell Cultures (Porton Down, Hampshire, UK). All cells were maintained in RPMI-1640 medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (1 μg/ml) in a humidified, 5% CO2 incubator at 37°C. Cell cultures (Porton Down, Hampshire, UK). All cells were main-

**Antibodies and Western blot analysis.** Protein aliquots (25 μg) were separated by SDS-PAGE (Bio-Rad, Hercules, CA, USA) and transferred to PVDF membranes (Bio-Rad). The membranes were washed three times and then incubated with Blocking One solution (Nacalai Tesque, Kyoto, Japan) for 1 h at room temperature. The membranes were then incubated overnight at 4°C with primary antibodies against anti-MET (2SH2), anti-phospho-MET (pMET) (Tyr1234/1235), anti-protein kinase B (AKT), anti-phospho-AKT (Ser473), anti-cleaved poly(ADP-ribose) polymerase (PARP) (Asp214), anti-cleaved caspase-3 (Asp175) (1:1000; Cell Signaling Technology, Danvers, MA, USA), anti-human HGF (200 μg/ml), anti-human/mouse/rat ERK (Erk1/Erk2; 0.2 μg/ml), anti-phospho-Erk1/Erk2 (T202/ Y204; 0.1 μg/ml), GAPDH antibodies (1:1000; R&D Systems, Minneapolis, MN, USA), anti-cyclin A (H432), anti-cyclin B1 (GNS1), anti-cyclin D1 (A12), or anti-cyclin E (HE12) antibodies (1:200; Santa Cruz Biotechnology, Dallas, Texas, USA). The membranes were then washed three times and incubated for 1 h at room temperature with specific-species HRP-conjugated secondary antibodies. Immunoreactive bands were visualized with SuperSignal West Dura Extended Duration Substrate (an enhanced chemiluminescent substrate) (Pierce Biotechnology, Minneapolis, MN, USA), anti-cyclin A (H432), anti-cyclin B1 (GNS1), anti-cyclin D1 (A12), or anti-cyclin E (HE12) antibod-

**Knockdown of siRNA.** Duplexed Stealth RNAi against MET (RSS351362; Invitrogen, Carlsbad, CA, USA) and HGF (HSS179213; Invitrogen), and Silencer Select siRNA for Negative Control no.1 (Invitrogen), were transfected with Lipofectamine RNAiMAX (Invitrogen) in accordance with the manufacturer’s instructions.

**Cytokine production.** Cells (2 × 10^5) were cultured in RPMI-1640 medium with 10% FBS for 24 h, then washed with PBS and incubated for 48 h in 2 mL of the same medi-

**Fluorescent in situ hybridization.** The MET 7q31.2 chromoso-

**Cell apoptosis.** Cells (3 × 10^5) were seeded into each well of a 96-well, white-walled plate, incubated overnight, and treated with the indicated compounds or vehicle (DMSO) for 8 h. Cell-

**Tumor cell inoculation in mice with SCID.** Five-week-old male SCID mice were obtained from Clea Japan (Tokyo, Japan). Mice were pretreated with anti-mouse interleukin-2b antibody to deplete NK cells and thereby facilitate the formation of metastatic sites. Two days later, 1.2 × 10^6 SBC-5/EGFP-Eluc cells were introduced into mice by tail vein injection. After 10 days, mice were randomized (n = 6 per group) and drugs were given once daily by oral gavage. Tumor lumines-

**Luciferase expression and radiographic analyses with an IVIS imaging system.** After inoculation, the development of tumors was tracked in live mice by repeated non-invasive optical imaging of tumor-specific luciferase activity using the IVIS Lumina XR Imaging System (PerkinElmer, Alameda, CA, USA) as described. The intensity of the bioluminescence signal was analyzed using Living Image 4.0 software (PerkinElmer) by serially quantifying the peak photon flux in a selected region of interest within a given tumor. The intensity of the bioluminescence signal was corrected to take into account the total area of the region of interest, and the elapsed time for which biolumi-

**Histological analyses of tumors.** Formalin-fixed, paraffin-

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Proliferating cells were detected by incubating tissue sections with Ki-67 antibody (clone MIB-1; Dako, Glostrup, Denmark). Antigens were retrieved by microwaving tissue sections in 10 mM citrate buffer (pH 6.0). After incubation with a secondary antibody and treatment with the Vectastain ABC Kit (Vector Laboratories, Burlingame, CA, USA), peroxidase activity was visualized by a DAB reaction. The sections were counterstained with hematoxylin.

Quantification of immunohistochemistry results. The five areas containing the highest numbers of stained cells within each section were selected for histological quantification by light or fluorescence microscopy at 400-fold magnification.

Statistical analysis. Differences between groups were analyzed using one-way ANOVA. All statistical analyses were undertaken using GraphPad StatMate 4 (GraphPad Software, San Diego, CA, USA). P < 0.05 was considered statistically significant.

Results

Expression of HGF and MET in SCLC cell lines. To evaluate the effects of HGF/MET activation in SCLC, we first measured the expression of HGF with Western blot analysis, and the levels of both total MET and pMET proteins, in eight SCLC cell lines (Fig. 1a). Both HGF and pMET proteins were expressed in SBC-5, DMS273, and DMS273-G3H cells at varying levels, whereas DMS114 cells did not express either pMET or total MET proteins, despite high levels of HGF expression. To confirm HGF production, we measured the levels of HGF protein in the cell culture supernatant by ELISA assay. Hepatocyte growth factor was highly expressed in SBC-5, DMS114, DMS273, and DMS273-G3H cells, consistent with the results of the Western blot analysis (Fig. 1b). These results indicate that HGF/MET signaling is activated in some SCLC cell lines.

Growth and viability of SCLC cells effectively suppressed by MET inhibitors. To examine the effect of MET inhibitors on the viability of SCLC cells, we used crizotinib and golvatinib. Treatment with either crizotinib or golvatinib alone significantly inhibited both the viability and growth of SBC-5, DMS273, and DMS273-G3H cells, each of which was identified to show high levels of both HGF and pMET expression (Fig. 2). The IC50 values of crizotinib or golvatinib in these SCLC cell lines were as follows. SBC-5: crizotinib, 0.112 μM; golvatinib, 0.094 μM. DMS273: crizotinib, 0.208 μM; golvatinib, 0.613 μM. DMS273-G3H: crizotinib, 0.432 μM; golvatinib, 0.535 μM. In contrast, the MET inhibitors had only a minimal effect on the other SCLC cell lines (Fig. S1). In addition, exogenous HGF did not affect the cell viability of these cells without HGF expression, although Western blot analyses revealed that the phosphorylation of MET, AKT, and ERK was increased in the presence of HGF (Fig. S2). To investigate the downstream effects of the inhibitors, we examined the phosphorylation statuses of MET, AKT, and ERK proteins using Western blot analysis. In the presence of either crizotinib or golvatinib, the phosphorylation of MET, AKT, and ERK was markedly inhibited in SCLC cells (Fig. 2c). Together, these results clearly show that the MET inhibitors crizotinib and golvatinib were able to inhibit the viability and growth of SCLC cells showing high expression of both HGF and pMET by inhibition of both MAPK and phosphoinositide-3 kinase signaling.

To confirm whether the viability of the SCLC cells was mediated by the HGF/MET pathway, we next knocked down MET and HGF using specific siRNAs. The viability of all three SCLC cell lines was inhibited by knockdown of either MET or HGF gene expression, and this effect was most pronounced in the SBC-5 cell line (Fig. 3a). Western blot analysis revealed that the phosphorylation of AKT and ERK was inhibited by MET knockdown, whereas HGF knockdown inhibited ERK phosphorylation to a lesser degree than AKT phosphorylation (Fig. 3b). These results indicated that the viability of these SCLC cells was dependent on the HGF/MET pathway, and suggested that MET may be a promising molecular therapeutic target in SCLC cells that show aberrant activation of the HGF/MET pathway.

Small-cell lung cancer cells showing aberrant activation of the HGF/MET pathway show MET copy number gain. In general, MET activation is induced not only by HGF-mediated stimulation, but also by altered MET gene expression, for example, as caused by activating mutations or gene amplification. Thus, to identify underlying mechanisms for the aberrant activation of the HGF/MET pathway, we next investigated possible sources of abnormal MET gene expression in SCLC cells. We found that the MET gene copy number was significantly increased according to FISH analysis of SBC-5, DMS273, and DMS273-
G3H cells; however, remarkable copy number gain were not detected in DMS114 cells, which had the highest secretion of HGF in the eight cell lines (Table 1). In contrast, neither MET amplification (Table 1) nor previously reported SCLC-associated MET mutation (for example, E168D, R988C, T1010I, and R1166Q) (data not shown), was detected in the SCLC cell lines.

Fig. 2. MET inhibitors effectively suppress the growth of small-cell lung cancer cells showing aberrant activation of the hepatocyte growth factor (HGF)/MET pathway, through inhibition of ERK and protein kinase B (AKT) signals. (a) SBC-5, DMS273, and DMS273-G3H cells (1–2 × 10^5 per well) were incubated with various concentrations of either crizotinib or golvatinib for 72 h. Cell viability was determined by MTT assay. Data are shown as the mean ± SD of three independent experiments. (b) SBC-5, DMS273, and DMS273-G3H cells (1 × 10^4 per well) were incubated with 1 μM crizotinib or golvatinib, then harvested and counted daily. Data are shown as the mean ± SD of three independent experiments. (c) SBC-5, DMS273, and DMS273-G3H cells were treated with either 1 μM crizotinib or golvatinib for 2 h. Cell lysates were evaluated for protein expression by Western blot analysis. Three independent experiments were carried out, and a representative result is shown. p, phosphorylated. (d) SBC-5, DMS273, and DMS273-G3H cells (1 × 10^4 per well) were treated with 1 μM crizotinib or golvatinib every 72 h for 14 days. Cell plates were stained with crystal violet and imaged. Two independent experiments were carried out, and a representative plate is shown.

Fig. 3. Viability of small-cell lung cancer cells showing aberrant activation of hepatocyte growth factor (HGF)/MET is dependent on the HGF/MET pathway. SBC-5, DMS273, and DMS273-G3H cells were treated with siRNAs (si-) specific to either MET or HGF, or scrambled controls (SCR). (a) Cell viability was determined by MTT assay 72 h after siRNA treatment. Data are shown as the mean ± SD of three independent experiments. *P < 0.05 by Student’s t-test, si-SCR versus si-MET or si-HGF. (b) Cell lysates were evaluated for protein expression by Western blot analysis 48 h after siRNA treatment. Three independent experiments were carried out, and a representative result is shown. AKT, protein kinase B; p, phosphorylated.
These results suggest that the MET copy number gain, in addition to the secretion of HGF, may contribute to the aberrant activation of the HGF/MET pathway in SCLC cells. MET inhibitors promote cell cycle arrest of SCLC cells showing aberrant activation of the HGF/MET pathway. To further assess the underlying mechanism by which crizotinib and golvatinib inhibit MET activity, we investigated the effect of these compounds on cell cycle and apoptosis. Western blot analysis revealed that the level of cyclin A, a known key modulator of the S-G2 cell cycle phase, was remarkably decreased in SCLC cells by treatment with either crizotinib or golvatinib (Fig. 4a). Cyclin A was also decreased by knockdown of either MET or HGF using specific siRNAs in SBC-5 cells (Fig. S3). In contrast, MET inhibitor treatment did not affect the expression of cleaved PARP or cleaved caspase-3, and furthermore did not inhibit caspase-3/7 activity, all of which are associated with apoptosis (Fig. 4). These results suggest that crizotinib and golvatinib inhibit cell viability predominantly through cell cycle arrest in the S-G2 phase, but do not induce apoptosis.

MET inhibitors significantly inhibited tumor progression of SBC-5 cells in NK cell-depleted SCID mice. We next examined the efficacy of the MET inhibitors crizotinib and golvatinib on tumor progression using our previously established in vivo imaging model with SBC-5 EGFP-Eluc-transfected (SBC-5/EGFP-Eluc) cells. Bioimun fluorescent signals were detected in the SBC-5/EGFP-Eluc cells, and observed to increase in a time-dependent manner, consistent with the time-course of metastasis formation. Having confirmed that EGFP-Eluc expression had no effect on the sensitivity of the SBC-5 cells to either crizotinib or golvatinib in an in vitro assay (Fig. S4), the SBC-5/EGFP-Eluc cells were injected i.v. into NK-cell-depleted SCID mice. We initiated treatment with 100 mg/kg crizotinib or 50 mg/kg golvatinib prior to detection of bioluminescent signals of systemic metastasis. The results of our analysis show that the expected increase in systemic metastasis was significantly inhibited by daily oral treatment with 100 mg/kg crizotinib or 50 mg/kg golvatinib compared to the control group (Fig. 5a,b). Furthermore, the phosphorylation of MET, ERK, and AKT, as well as the expression of cyclin A, was inhibited by treatment with either crizotinib or golvatinib, consistent with the results of our in vitro analyses (Fig. 5c). None of the groups of mice showed significant weight loss (Fig. S5a). We next assayed cell proliferation and apoptosis by immunohistochemical staining of liver tumors. Crizotinib and golvatinib treatment significantly reduced the number of proliferating cells in liver tumors as compared to controls (Fig. 5d, e); in contrast, the expression of cleaved PARP and cleaved caspase 3 were not altered by MET inhibitor treatment (Fig. S5b,c). These results show that, in the mouse model used here, the MET inhibitors crizotinib and golvatinib were able to

| Cell line | MET : CEP7 ratio | Gene copy number | Positive cells (%) |
|-----------|-----------------|-----------------|------------------|
| SBC-5     | 1.3             | <2              | 0                |
|           |                 | 3-4             | 20               |
|           |                 | 5               | 80               |
| DMS273    | 1.0             | <2              | 5                |
|           |                 | 3-4             | 65               |
|           |                 | 5               | 30               |
| DMS273-G3H| 1.0             | <2              | 3                |
|           |                 | 3-4             | 8                |
|           |                 | 5               | 89               |
| DMS-114   | 0.8             | <2              | 12               |
|           |                 | 3-4             | 82               |
|           |                 | 5               | 6                |

MET copy number determined by FISH.

Fig. 4. MET inhibitors induce cell cycle arrest, but not apoptosis, in small-cell lung cancer cells. (a) SBC-5, DMS273, and DMS273-G3H cells were treated with 1 µM crizotinib or golvatinib for 72 h. Cell lysates were then evaluated for protein expression by Western blot analysis. Three independent experiments were carried out, and a representative result is shown. PARP, poly(ADP-ribose) polymerase. (b) SBC-5, DMS273, and DMS273-G3H cells were treated with 1 µM crizotinib or golvatinib for 8 h, and the activity of caspase-3/7 was then measured using a Caspase-Glo3/7 assay kit. Error bars represent mean ± SD.
constrain the progression of systemic metastasis by SCLC cells by cell cycle arrest, but not apoptosis.

Discussion
In the present study, we showed that the aberrant activation of the HGF/MET pathway mediates the growth and survival of SCLC cells showing HGF secretion and/or MET copy number gain. Furthermore, the MET inhibitors crizotinib and golvatinib were able to inhibit cell growth and prevent systemic metastasis of SCLC cells in vivo, predominantly by cell cycle arrest. These findings indicate that inhibition of HGF/MET signaling may constrain tumor progression of SCLC cells showing aberrant activation of the HGF/MET pathway.

It is well known that aberrant activation of the HGF/MET pathway promotes cancer progression, invasion, and metastasis. We and other researchers have reported that the alternative bypass signaling by HGF/MET (e.g., by HGF overexpression or MET amplification) induces resistance to both epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) in EGFR-mutated NSCLC cells, and anaplastic lymphoma kinase (ALK)-TKIs in ALK-rearranged NSCLC cells. In addition, we recently reported that MET copy number gain induced EGFR-TKI gefitinib resistance in leptomeningeal carcinomatosis of EGFR-mutant lung cancer cells. Thus, HGF/MET pathway activation in lung cancer is associated not only with malignant transformation, but also with drug resistance that enables cell survival. Furthermore, it has been shown that HGF/MET is the pivotal pathway disrupted in the pathophysiology of SCLC tumors. Ozasa et al. reported that the MET activation caused by an increase of MET gene loci promotes resistance to cytotoxic anticancer drugs in SCLC cells. Sakamoto et al. showed that treatment with MET inhibitors constrained the formation of distant metastases in SCLC cells showing high HGF production in an orthotopic model. Maulik et al. reported that HGF stimulation resulted in phosphorylation of multiple cytoskeletal proteins such as p125FAK or PYK2 in H69 SCLC cells. Wang et al. showed that siRNA could inhibit c-MET expression and to show activation of pMET by cell cycle arrest, but not apoptosis.

**Fig. 5.** MET inhibitors significantly inhibit tumor progression of SBC-5 small-cell lung cancer cells in an in vivo multi-organ metastasis mouse model. (a) EGFP-Eluc-transfected SBC-5 (SBC-5/EGFP-Eluc) cells were injected i.v. into natural killer cell-depleted SCID mice. At 10 days after inoculation, the mice were randomized into vehicle (control), crizotinib (100 mg/kg), or golvatinib (50 mg/kg) treatment groups (n = 6 per group), and began treatment once daily by oral gavage. Luminescence was evaluated twice weekly. Bars indicate standard error. *P < 0.05 by Mann-Whitney test, versus control group. (b) Representative images of mice showing merged bioluminescence and photograph. (c) Liver tumors were resected from mice 3–4 h after treatment on day 35. Relative levels of proteins observed in each tumor were determined by Western blot analysis. AKT, protein kinase B; HGF, hepatocyte growth factor; p, phosphorylated. (d) Representative images of liver tumors immunohistochemically stained with antibodies to human Ki-67. Bar = 50 μm. (e) Quantification of proliferating cells in liver tumors, determined as the observed percentage of Ki-67-positive cells. The data shown represent the mean of five areas ± SD. *P < 0.05 by Student’s t-test, versus control group.
gain. Notably, each of these SCLC cell lines were derived from tumors of chemoresistant or chemorelapsed SCLC patients. This suggests that the therapeutic targeting of HGF/MET signaling may be effective in treating SCLC even in patients showing resistance to conventional cytotoxic anticancer drugs.

To determine the best subsequent treatment option, lung cancer patients who show resistance to chemotherapy or targeted therapy often undergo rebiopsy. This procedure may provide an opportunity to identify underlying sources of drug resistance, such as histological or genetic changes. For instance, EGFR-T790M mutations are often observed in tumors from EGFR-mutation NSCLC patients that are rebiopsied after acquiring resistance to first-generation EGFR-TKIs. For resistant or relapsed SCLC patients, it may be beneficial to diagnose recurrent tumors by rebiopsy, to ascertain whether they show aberrant activation of the HGF/MET pathway. Identification of the genetic mechanisms underlying acquired drug resistance may lead to early intervention treatment with MET inhibitors, potentially prolonging the duration before tumor recurrence. Thus, our findings suggest that it may be beneficial, even in SCLC patients who are resistant to cytotoxic chemotherapy, to re-evaluate the overexpression of HGF and/or the phosphorylation of MET proteins, so as to identify those patients who are likely to respond positively to treatment with MET inhibitors.

A major impediment to this process is the difficulty of identifying tumors that show aberrant activation of the HGF/MET pathway within the clinical setting. It may be critical to detect not only increased levels of MET phosphorylation, but also to identify MET copy number gain, MET gene activating mutations, and MET gene amplifications. In addition, having identified these genetic changes, the most effective therapeutic strategy for each patient needs to be determined, for example, designing combinations of chemotherapy with MET inhibitor, sequential treatment regimes, maintenance or therapy. In this study, crizotinib did not show antitumor effects after systemic sequential treatment regimes, maintenance or therapy. In this study, crizotinib did not show antitumor effects after systemic

In the present study, we showed that treatment with MET inhibitors suppressed the spread of systemic metastasis induced by SCLC cells in NK cell-depleted SCID mice, predominantly through cell cycle arrest, but not apoptosis. Interestingly, we also showed that siRNA-induced knockdown of MET reduced the expression of HGF proteins, suggesting that HGF-induced autocrine systems may be dependent on MET activation. However, we could not clarify the exact mechanisms. Elucidation of the mechanisms underlying the reduction in HGF protein expression induced by MET knockdown will require further investigation.

Crizotinib is an ATP-competitive, small-molecule inhibitor of MET kinase that is already approved for the treatment of patients with ALK fusion-positive lung cancer in several countries including Japan, and for NSCLC patients with ALK fusion or ROS1 fusions in the USA. Golvatibin is a dual MET and vascular endothelial growth factor receptor-2 TKI under clinical development. It is clinically valuable to assess the impacts of these compounds on the inhibition of systemic metastatic tumors in mouse xenograft models, as has been shown here.

In summary, we showed in the present study that HGF/MET signaling was aberrantly activated in chemoresistant or chemorelapsed SCLC cell lines (SBC-5, DMS273, and DMS273-G3H) by the secretion of HGF and/or MET copy number gain. The MET inhibitors exerted antitumor effects on these SCLC cell lines in both cell-based in vitro assays and in vivo xenograft models. These findings indicate the therapeutic potential of inhibiting HGF/MET signaling to constrain tumor progression in SCLC cells showing aberrant activation of the HGF/MET pathway. Therefore, we suggest that it would be clinically valuable to further investigate HGF/MET-mediated signaling in SCLC cells.

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