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Impact of *MET* amplification on gastric cancer: Possible roles as a novel prognostic marker and a potential therapeutic target

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Abstract. Identification of critical genes which play pivotal roles in controlling tumor growth and survival will establish the basis for developing therapeutic targets. With the aim of establishing personalized medicine for treatment of solid tumors, we focused on *MET* amplification in gastric cancer patients, given the extreme sensitivity to c-Met inhibitor in *MET* amplified gastric cancer cell lines. We tested *MET* amplification and activation of c-Met in various gastric cancer cell lines and tissue samples from 482 gastric cancer patients who underwent curative surgery. Gastric cancer cell lines with *MET* amplification by quantitative real-time PCR (qPCR) and FISH predicted sensitivity to PHA-665,752, a selective c-Met kinase inhibitor. Of the 472 patients who had DNA sample available for qPCR analysis, 100 patients (21.2%) had a *MET* copy number greater than 4.0 copies and demonstrated poorer survival following curative surgery with statistical significance (5-year OS; 50.0 vs. 59.1%; *MET* amplification (+) vs. *MET* amplification (-); P=0.0134). These results suggest that the increased *MET* copy number measured by qPCR plays an important role in determining prognosis in gastric cancer patients. However, the predictive role of *MET* amplification for treatment response should be further explored in upcoming clinical trials.

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Introduction

Gastric cancer is the leading cause of cancer death worldwide with the incidence of 18.9/100,000 per year (1). The incidence of gastric cancer was estimated to be 934,000 cases, with 56% of the new cases occurring in East Asia (2). Gastric cancer accounts for 20.8% of all cancers in Korea according to the Central Tumor Registry data for 2002 (3). Although overall survival of gastric cancer has been enhanced owing to the application of national fiberoptic esophagogastroduodenoscopy (EGD) screening program in adults aged over 40 years in Korea, a large proportion of patients are still diagnosed at metastatic stage. The median survival time following cytotoxic chemotherapy is still less than 1 year and thus, metastatic gastric cancer remains a therapeutic challenge for medical oncologists. The role of molecularly targeted therapy has not been adequately explored in gastric cancer when compared to other common solid tumors such as non-small cell lung cancer, breast or colorectal cancer.

The *MET* oncogene encodes the receptor tyrosine kinase (RTK) for hepatocyte growth factor (HGF) and controls genetic programs leading to cell growth, invasion and protection from apoptosis (4). Although the definitive role of *MET* oncogene is yet to be determined in carcinogenesis of gastric cancer, overexpression and amplification of *c-Met* has been demonstrated in gastric cancer cell lines (5-9). In addition, approximately 10-20% of gastric cancer tissues and up to 40% of the scirrhous histological subtype were shown to harbor increased *MET* gene copy numbers (6,10,11). Importantly, PHA-665,752, a selective c-Met kinase inhibitor showed significant reduction of established tumor mass in mouse xenografts with GTL16, a gastric cancer cell line with >10-fold *MET* amplification (5,12). Another pivotal study showed that gastric cancer cells with *MET* amplification were extremely sensitive to PHA-665,752 and implicated a potential role of c-Met protein in developing theranostics in gastric cancer (13). Due to limited number of patients in previous studies,

the predictive capacity of c-Met protein overexpression or *MET* amplification for survival in gastric cancer needs to be determined.

We undertook this study to assess the impact of c-Met overexpression, c-Met activation, and *MET* amplification on survival of gastric cancer patients.

Materials and methods

Patients and tissues. We have previously reported the outcome of 544 stage II–IV (M0) gastric cancer patients, who received adjuvant chemoradiation therapy after curative surgery (14). Of these patients and additional 23 stage IB patients who were included in our previous study, formalin-fixed paraffin-embedded primary tumor tissues were available from 482 patients. The postoperative adjuvant treatment adopted was the same as that used for the INT-0116 (SWOG-9008) trial and the results were previously reported (15). All patients provided written informed consent according to the institutional guideline and the study was approved by the Institutional Review Board. The clinical and pathological features of the patients are shown in Table I. The median age was 54 (range 23-70) years. By Lauren classification, 29.9% of patients had intestinal type. All patients received D2 or greater lymph node dissection and 34.3% of patients had stage IB or II. After a median follow-up duration of 110.7 months (84.9-155.4 months), a 5-year overall survival (OS) rate was 57.7% and a 5-year disease-free survival (DFS) rate was 56.4%.

Table I. Patient characteristics.

Characteristics	N=482 (%)
Age (year) median, range	54, 23-70
≤60	357 (74.1)
>60	125 (25.9)
Sex	
Male	324 (67.2)
Female	158 (32.8)
Type of gastrectomy	
Subtotal gastrectomy	205 (42.5)
Total gastrectomy	277 (57.5)
Location of tumor	
Distal 1/3	230 (47.7)
Middle 1/3	210 (43.6)
Proximal 1/3	34 (7.1)
Diffuse	8 (1.7)
Histology	
Well differentiated tubular adenocarcinoma	48 (9.9)
Moderately differentiated tubular adenocarcinoma	87 (18.0)
Poorly differentiated tubular adenocarcinoma	255 (52.9)
Signet ring cell carcinoma	73 (15.1)
Mucinous adenocarcinoma	11 (2.3)
Papillary adenocarcinoma	4 (0.8)
Adenosquamous carcinoma	1 (0.2)
Hepatoid adenocarcinoma	1 (0.2)
Others	2 (0.4)
Lauren classification	
Intestinal	144 (29.9)
Diffuse	336 (69.7)
Indeterminate	2 (0.4)
T stage	
T1	21 (4.4)
T2	243 (50.4)
T3	196 (40.7)
T4	22 (4.6)
N stage	
N0	30 (6.2)
N1	221 (45.9)
N2	138 (28.6)
N3	93 (19.3)
AJCC stage	
IB	21 (4.4)
II	144 (29.9)
III	209 (43.4)
IV (M0)	108 (22.4)

Cell culture and cell growth assay. Human gastric carcinoma cells, MKN-1, MKN-45, MKN-74, N87 SNU-1, SNU-5, SNU-484, SNU-638 and SNU-668 cells were purchased from the Korean Cell Line Bank (Seoul, Korea). YCC-2, YCC-3 and YCC-7 were kindly provided by Dr Hyun Cheol Chung (Yonsei Cancer Center, Seoul, Korea). YCC-2, YCC-3 and YCC-7 were maintained in DMEM (Gibco-BRL, Carlsbad, CA) supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, 100 units/ml streptomycin, and 2 mM glutamine; the others were cultured in RPMI-1640 medium (Gibco-BRL) supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, 100 units/ml streptomycin, and 2 mM glutamine. All cells were incubated in a humidified atmosphere contained 5% CO_2 at 37˚C.

Growth and inhibition of growth were assessed by the 5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) assay according to previously established methods (16). PHA-665,752 was purchased from Tocris Bioscience (Ellisville, MO). Stock solution of 10 mM was prepared in DMSO and stored at -20˚C.

Antibodies and Western blotting. Cells grown under the previously specified conditions were lysed in the following lysis buffer composition: 20 mM/l Tris (pH 7.4), 150 mM/l NaCl, 1% NP40, 10% glycerol, 1 mM/l EDTA, 1 mM/l EGTA, 5 mM/l sodium pyrophosphate, 50 mM/l NaF, 10 nM/l β-glycerophosphate, 1 mM/l sodium vanadate, 0.5 mM/l DTT, 4 μg/ml leupeptin, 4 μg/ml pepstatin, 4 μg/ml apoprotein, and 1 mM/l phenylmethylsulfonyl fluoride. Lysates were centrifuged at 16,000 × g for 5 min at 4˚C. The supernatant was used for subsequent procedures. Western blot analyses were performed according to the antibody manufacturer's recommendations. Antibody binding was detected using an enhanced chemiluminescence system (Perkin-Elmer, Waltham, MA).

Anti-Met and anti-phospho-Met antibodies were from Cell Signaling Technology (Beverly, MA). Anti-phosphotyrosine (4G10) antibody was from Upstate Biotechnology (Lake Placid, NY).

Quantitative real-time PCR (qPCR). The *MET* and *MTHFR* (endogenous control) levels were evaluated using the following primers and methods previously published (17). *MET*-sense: 5'-CCA TCC AGT GTC TCC AGA AGT G-3'; *MET*-anti-sense: 5'-TTC CCA GTG ATA ACC AGT GTG TAG-3'; *MTHFR*-sense: 5'-CCA TCT TCC TGC TGC TGT AAC TG-3'; *MTHFR*-anti-sense: 5'-GCC TTC TCT GCC AAC TGT CC-3'. Genomic DNA (20 ng) was amplified for 40 cycles (15 sec 95˚C, 60 sec 60˚C) in a ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA), using the QuantiTect SYBR-Green PCR kit (Qiagen, Valencia, CA) and 400 nM primers. For all patients, triplicate cycle time (C_T) values were averaged. Fold changes were calculated using the equation $2^{-\Delta\Delta CT}$, where $\Delta\Delta CT = [C_T(MET)$ sample - $C_T(MTHFR)$sample] - [C_T(MET)reference DNA - C_T(MTHFR)reference DNA]. *MET* amounts were interpolated from the standard curves and normalized to *MTHFR* amounts. Normal DNAs from non-malignant gastric tissues and normal blood lymphocytes of healthy volunteers were used as reference DNAs for each PCR reaction. We also performed qPCR analysis using TaqMan primers which are directed at exon 2 of *MET* and have been previously described (13,16). The results of the relative copy number for MET by two different methods were not different in the gastric cancer cell line and gastric cancer tissues (data not shown). We also carried out corresponding fluorescent *in situ* hybridization (FISH) on these specimens.

Immunohistochemistry. Tissue microarray (TMA) blocks were sectioned with 4 μm thickness. Immunohistochemical study was performed using the streptavidin-biotin complex method and TechMate™ 1000 automated staining system (DakoChemmate, Glostrup, Denmark). Primary antibodies used and working dilutions employed were as follows; c-Met (24H2, Cell Signaling Technology; 1:100) phospho-Met (pY1349, Cell Signaling Technology; 1:100). Deparaffinized sections were treated with 3% hydrogen peroxide in methanol for 10 min to inhibit endogenous peroxidase. Sections were immersed in 0.01 M citrate buffer (pH 6.0) and heated in a pressure cooker for 30 min. Sections were then incubated with primary antibody for 50 min at room temperature. Each section was treated sequentially with biotinylated secondary antibody (anti-mouse immunoglobulin) and streptavidin-peroxidase complex (DakoChemmate). 3,3'-diaminobenzidine tetrahydrochloride was used as a chromogen, and then Mayer's hematoxylin counterstain was applied. Negative controls (isotype-matched irrelevant antibody) were run simultaneously. The results of staining were evaluated by two independent pathologists (S.J.W. and K.M.K.), who were blinded to the clinical data and the difference in interpretation was resolved by consensual agreement. Total Met-staining cells exhibited a combined membranous and cytoplasmic pattern, whereas phosphorylated Met-staining cells showed nuclear staining in addition to the combined membranous and cytoplasmic pattern. For assessment of the positivity of immunostaining for each section, the staining intensity was graded on the following scale: 0, no staining; 1, weak staining; 2, moderate staining; and 3, intense staining, and nuclear staining of phospho-Met in >5% of tumor cells was regarded as positive. Positive cells were counted by monitoring at least 1,000 cancer cells from more than five high power fields where positive cells were present at a relatively uniform density.

FISH. FISH was performed according to the established protocol using a D7S522 probe and chromosome 7 centromere probe (CEP7) purchased from Vysis (Des Plaines, IL) (16). Four micron (4 μm) tumor sections generated from TMA blocks were pretreated by deparaffinizing in xylene and dehydrating in ethanol. The sections were immersed in Tris-base and EDTA (TE), washed in phosphate-buffered saline (PBS), and then treated with Digest-All (Zymed, San Francisco, CA). Sections were then fixed with formalin and dehydrated in ethanol. After co-denaturation of the tissue and the probe mixture (D7S522 and CEP7) at 70˚C for 3 min, the sections were hybridized at 37˚C for 48-72 h, washed with sodium citrate and Tween-20 containing buffers and counterstained with DAPI. One hundred cells from each TMA core were analyzed and the number of D7S522 and CEP7 signals determined.

Statistical analyses. Disease-free survival (DFS) was defined as the time from surgery to the first relapse of cancer, or death of any cause. Overall survival (OS) was calculated from the date of surgery to the date of death. OS and DFS were calculated using the Kaplan-Meier method. Correlation analyses were performed using the two sided χ^2 test or Fisher's exact test. Differences in disease-free and overall survival were compared using log-rank tests and Cox proportional hazard analysis. P-value <0.05 was considered statistically significant.

Results

MET amplification and constitutive activation in human gastric cancer cells. Immunoblotting analysis showed heterogeneous phosphotyrosine and various levels of c-Met expression in a panel of gastric cancer cell lines. Immunoblotting using phospho-Met antibodies against Y1349 showed constitutive phosphorylation of the receptor in cells with *MET* amplification, whereas cells without amplification had low levels of c-Met phosphorylation (Fig. 1A). Analysis of the gastric cancer cell lines using qPCR identified the increased *MET* gene copy number which predicted sensitivity to PHA-665,752 (Fig. 1B). We also confirmed *MET* amplification in SNU-5, SNU-638 and MKN-45 by FISH.

Correlation between MET amplification and clinical variables. Of the 472 patients who had DNA sample available for qPCR analysis, 100 (21.2%) of the patients had MET copy number >4.0. Of the 100 patients who had amplified *MET* gene, 84 patients had 4.0-6.0 copies, 10 patients 6.0-8.0 copies, and 6 patients had 8.0-16 copies. The clinical features between the patients with MET amplification and those without amplification were not significantly different

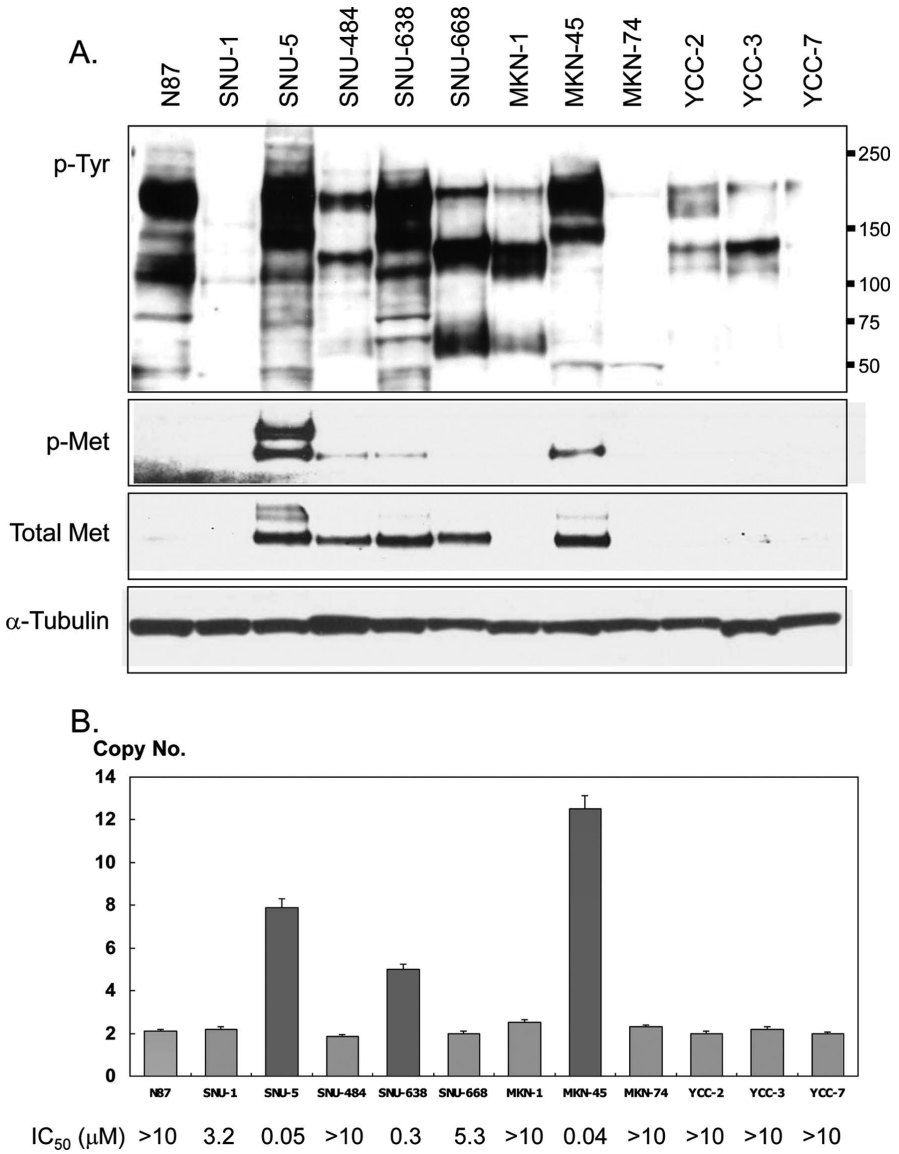

Figure 1. Immunoblot analysis showing heterogeneous phosphotyrosine and various levels of c-Met expression in a panel of gastric cancer cell lines (A). Analysis of the gastric cancer cell lines by using qPCR identified that increased *MET* gene copy number predicted sensitivity to PHA-665,752, a selective c-Met kinase inhibitor (B).

(Table II). There was a trend toward more advanced N stage in patients with *MET* amplification, but there was no statistical significance (P=0.060).

Correlation between MET amplification, c-Met protein expression and c-Met activation. In order to determine the most influential and important factors reflective of c-Met status, we tested *MET* amplification along with c-Met protein expression and c-Met activation which were evaluated by immunohistochemical staining against c-Met protein and phospho-Met (pY1349), respectively. In total, 452 specimens were interpretable for c-Met protein expression and activated phosphorylated Met expression. The representative immunohistochemical staining for activated c-Met is shown in Fig. 2A-F. Of the 103 tumor samples with c-Met activation identified by staining against phospho-Met (pY1349), 30 (29.1%) had *MET* amplification (≥4 copies) (P=0.026, Table III). In contrast, a concordance rate between c-Met protein expression and *MET* amplification was only 17.9% (32 of 179 samples) (Table III). Of the 472 samples evaluated, FISH analysis was successfully performed in 309 tissue samples (Fig. 2G). Thirty-one patients (10.0%) exhibited FISH positivity for *MET* gene and the concordance rate between *MET* amplification assessed by qPCR and FISH was only 58.1%; 18 of 31 FISH(+) patients had *MET* amplification (data not shown).

Impact of MET amplification on recurrence and survival. We performed survival analyses according to the c-Met status (Fig. 3). Gastric cancer patients with *MET* amplification had a significantly shorter disease-free survival following curative surgery (5-year DFS; 49.0 vs. 57.7%; *MET* amplification (+) vs. *MET* amplification (-); P=0.0216). Moreover, gastric cancer patients with *MET* amplification demonstrated poorer survival following curative surgery with statistical significance (5-year OS; 50.0 vs. 59.1%; *MET* amplification (+) vs.

Table II. Clinical features and *MET* amplification.

		MET amplification		
	No. of cases (N=472) (%)	*MET* copy No. ≥4.0 (N=100) (%)	*MET* copy No. <4.0 (N=372) (%)	P-value
Age				
≤60	349 (73.9)	81 (81.0)	268 (72.0)	0.070
>60	123 (26.1)	19 (19.0)	104 (28.0)	
Lauren classification				
Intestinal	142 (30.2)	28 (28.0)	114 (30.6)	0.658
Diffuse	328 (69.8)	72 (72.0)	256 (68.8)	
Sex				
Male	316 (66.9)	63 (63.0)	253 (68.0)	0.344
Female	156 (33.1)	37 (37.0)	119 (32.0)	
Tumor infiltration				
T1/T2	260 (55.1)	49 (49.0)	211 (56.7)	0.168
T3/T4	212 (44.9)	51 (51.0)	161 (43.3)	
AJCC stage				
IB-II	164 (34.7)	32 (32.0)	132 (35.5)	0.516
IIA-IV	308 (65.3)	68 (68.0)	240 (46.5)	
Lymph node metastasis				
N0/N1	247 (52.3)	44 (44.0)	203 (54.6)	0.060
N2/N3	225 (47.7)	56 (56.0)	169 (45.4)	
Histological grade (adenocarcinoma only)				
Well differentiated/ moderately differentiated	133 (34.8)	27 (33.3)	106 (35.2)	0.752
Poorly differentiated	249 (65.2)	54 (66.7)	195 (64.8)	

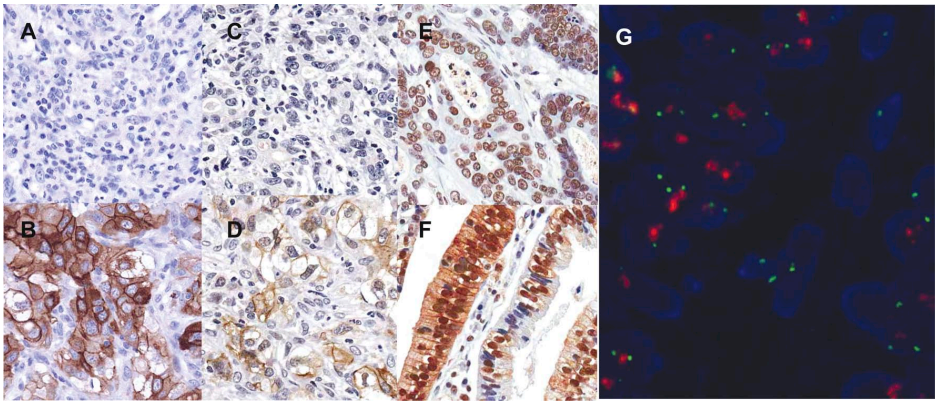

Figure 2. Immunohistochemical detection of the total Met (B, membranous and cytoplasmic staining) and phospho-Met [(D), membranous and cytoplasmic staining; (E), nuclear staining; (F), membranous, cytoplasmic and nuclear staining] on TMA. Negative control is seen in (A and C) (X 200). MET amplification is detected in a gastric cancer tissue (G). Dual-color FISH [CEP7 (green)/D7S522 (red)] was performed on TMA from a gastric cancer patient.

MET amplification (-); P=0.0134, Fig. 3A). Using backward stepwise Cox proportional hazards regression modeling, following variables were tested: age (≤60 vs. >60), Lauren classification, stage (IB/II vs. III/IVM0), *MET* amplification, c-Met FISH, and c-Met protein activation. For overall survival in all patients, *MET* amplification [P=0.022, hazard ratio (HR) = 1.601, 95% CI, 1.078, 2.380), c-Met protein activation (P=0.013, (HR) = 2.173, 95% CI, 1.098, 4.301] and advanced stage [P=<0.0001, hazard ratio (HR) = 2.871, 95% CI, 1.905, 4.327] predicted poor survival with statistical significance at multivariate level.

In total, 30 patients had *MET* amplification with c-Met protein activation while 283 patients did not harbor *MET* amplification and no c-Met protein activation in the series.

Table III. Correlation between *MET* amplification, c-Met protein expression and c-Met activation.

	c-Met protein expression			c-Met activation		
	Positive (N=179)	Negative (N=273)	P-value	Positive (N=103)	Negative (N=349)	P-value
MET amplification						
≥4 copies	32 (17.9)	64 (23.4)	0.157	30 (29.1)	66 (18.9)	0.026
<4 copies	147 (82.1)	209 (76.6)		73 (70.9)	283 (81.1)	

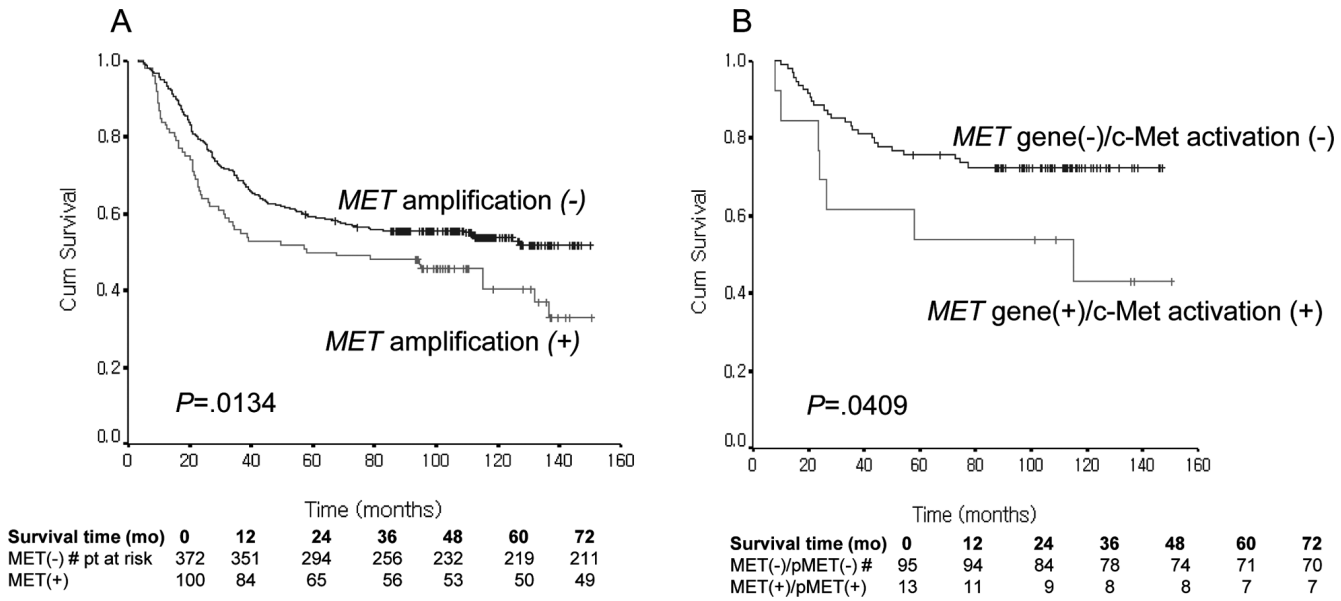

Figure 3. Overall survival curves according to the *MET* amplification (A) and c-Met activation status (B).

In subgroup analyses, there was a trend toward poorer prognosis in the subset of patients with *MET* amplification and c-Met protein activation (n=30) when compared with those without *MET* amplification or c-Met protein activation (n=283) (5-year OS; 50.0 vs. 57.9%, respectively; P=0.1317). In localized disease (stage I/II), however, concurrent detection *MET* amplification with c-Met protein activation significantly predicted worse survival when compared with those without the two variables with statistical significance (5-year OS; 53.8 vs. 75.9%, respectively; P=0.0409, Fig. 3B).

Discussion

Recently, gastric cancer cells with high-level stable chromosomal amplification of the growth factor receptor *MET* were shown to be extraordinarily susceptible to the c-Met selective inhibitor PHA-665752 (13). Identification of critical genes which play pivotal role in controlling tumor growth and survival will establish the basis for developing therapeutic targets. The most successful example of such identification of critical genes leading to overall improvement in treatment outcome is amplification of a locus on chromosome 17q which targets the *HER2* growth factor receptor and the use of trastuzumab in *HER2* amplified breast cancer. To establish personalized medicine for the treatment of solid tumors, we focused on *MET* amplification in gastric cancer patients given the extreme sensitivity to c-Met inhibitor in *MET* amplified gastric cancer cell lines (13).

We performed qPCR to determine the status of *MET* amplification in DNA samples from curatively resected gastric cancer tissues. Of the 472 patients who had DNA sample available for qPCR analysis, 100 (21.2%) of the patients had *MET* amplification. Of note, gastric cancer patients with *MET* amplification had a significantly shorter disease-free survival (5-year DFS; 49.0 vs. 57.7%; *MET* gene amplification (+) vs. *MET* amplification (-); P=0.0216) and poorer survival following curative surgery with statistical significance (5-year OS; 50.0 vs. 59.1%; *MET* gene amplification (+) vs. *MET* gene amplification (-); P=0.0134, Fig. 3).

Given the fact that *MET* amplification may not always lead to c-Met activation, we performed parallel analyses on c-Met activation and total c-Met protein expression using immunohistochemical staining against phosphorylated c-Met (pY1349) and c-Met protein, respectively. Approximately 30% of the tumor samples with *MET* amplification concordantly showed c-Met protein activation while 18% demonstrated concomitant c-Met protein expression. Moreover, about one third (66 of 181, 36.5%) of the c-Met (+) tumor samples were

associated with c-Met protein activation (data not shown). It has been reported that some cell lines with *HER2* amplification (JIMT-1 breast cancer cell line) or activating EGFR mutation (11-18 lung cancer cell line) without protein expression showed resistance to specific targeted agents (18,19). However, there are no *in vitro* models reported in regards to *MET* amplification without protein expression. Therefore, the clinical implication of this phenomenon should be further investigated. Nevertheless, the *MET* amplification status, not the activation status of c-Met protein was significantly influential on disease-free survival or overall survival in gastric cancer patients. In addition, the activation status of c-Met protein did not considerably influence survival (5-year OS; 56.7 vs. 59.6%; activated c-Met protein (+) vs. (-); P=0.7512). The form of c-Met status that has clinical implication as predictive factor for treatment response to c-Met inhibitor needs to be further investigated in several ongoing early phase trials using the c-Met inhibitor. Interestingly, gastric cancer patients with *MET* amplification with concomitant c-Met protein activation demonstrated the worst outcome in subgroup analyses, especially in localized gastric cancer (Fig. 3B). c-Met inhibitors have shown promising results as anti-cancer therapy in phase I trials (20). In addition, an interim analysis of phase II study on 18 evaluable gastric cancer patients demonstrated that c-Met inhibitor (GSK089) was feasible with manageable toxicities and 8% *MET* amplification rate (21). We plan to investigate whether treatment with c-Met inhibitor will actually confer survival benefit in this particular subset of patients.

Because a large-scaled analysis on c-Met status has not been performed in gastric cancer tissues or DNA samples, the cut-off value for *MET* amplification or c-Met protein expression and/or activation has not been standardized yet. Based on our experience, the FISH analysis was less feasible when compared with qPCR in assessing the *MET* amplification status. Of the 472 samples, only 309 samples yielded adequate FISH results for interpretation and of those, only 31 patients (10%) demonstrated *MET* amplification by FISH. The relatively low levels of concordance between *MET* amplification by qPCR and the FISH results might be due to tumor heterogeneity. TMA blocks were made by taking 2-mm sized core biopsies from individual formalin fixed paraffin-embedded (FFPE) gastric cancer tissues whereas genomic DNA for qPCR was prepared from FFPE sections following dissection of tumor to obtain sufficient tumor cell content. To test the tumor heterogeneity, therefore we repeated FISH analysis in selected cases with available tissue specimens.

Considerable attention has been focused on the role of *MET* amplification in tumorigenesis and in resistance mechanism to tyrosine kinase inhibitor since Engelman *et al* reported that *MET* amplification induced resistance to gefitinib in a gefitinib-sensitive lung cancer cell line and a c-Met tyrosine kinase inhibitor (PHA-665,752) restored gefitinib sensitivity (16). Since the molecular targeted therapy has been less extensively studied in gastric cancer as compared to non-small cell lung cancer, the role of *MET* amplification in such context needs to be defined in gastric cancer as well.

Taken together, we showed for the first time that *MET* is amplified in both cell lines and tumor tissues from gastric cancer patients. Importantly, *MET* amplification measured by qPCR was associated with shorter DFS and poorer OS but not c-Met protein overexpression or c-Met protein activation. Thus, *MET* amplification should be performed in addition to immunohistochemical studies for c-Met overexpression and c-Met activation (phosphorylated c-Met protein) as correlative analyses in clinical trials incorporating c-Met inhibitors. These studies may uncover a predictive role of *MET* amplification for treatment response, which should be also explored further as a novel therapeutic target in clinical trials.

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