Research Article

Cancer Epidemiology, Biomarkers & Prevention

MET Expression and Amplification in Patients with Localized Gastric Cancer

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Abstract

Background: MET, the receptor for hepatocyte growth factor, has been proposed as a therapeutic target in gastric cancer. This study assessed the incidence of *MET* expression and gene amplification in tumors of Western patients with gastric cancer.

Methods: Tumor specimens from patients enrolled on a preoperative chemotherapy study (NCI 5700) were examined for the presence of *MET* gene amplification by FISH, MET mRNA expression by quantitative PCR, *MET* overexpression by immunohistochemistry (IHC), and for evidence of MET pathway activation by phospho-MET (p-MET) IHC.

Results: Although high levels of MET protein and mRNA were commonly encountered (in 63% and 50% of resected tumor specimens, respectively), none of these tumors had *MET* gene amplification by FISH, and only 6.6% had evidence of MET tyrosine kinase activity by p-MET IHC.

Conclusions: In this cohort of patients with localized gastric cancer, the presence of high MET protein and RNA expression does not correlate with *MET* gene amplification or pathway activation, as evidenced by the absence of amplification by FISH and negative p-MET IHC analysis.

Impact: This article shows a lack of *MET* amplification and pathway activation in a cohort of 38 patients with localized gastric cancer, suggesting that MET-driven gastric cancers are relatively rare in Western patients. *Cancer Epidemiol Biomarkers Prev; 20(5); 1021–27. ©2011 AACR.*

Introduction

Despite a worldwide prevalence of nearly one million new cases annually (1), drug development in gastric cancer has lagged and the prognosis for patients with gastric cancer remains poor. Conventional therapy for metastatic gastric cancer remains palliative, with a median survival for metastatic disease of less than one year (2–4). New therapeutic targets for gastric cancer are needed.

Preclinical data suggest that the hepatocyte growth factor (HGF)/MET pathway may represent a therapeutic target for gastric adenocarcinoma (5). The *MET* proto-oncogene, located on the 7q31 locus, encodes the receptor tyrosine kinase MET, also known as the MET or HGF receptor (6, 7). The binding of HGF to its receptor, MET, results in C-terminus receptor tyrosine phosphorylation and receptor activation. MET receptor targets include activation of the phosphoinositide 3-kinase-Akt/protein kinase B (PI3K-Akt), mitogen-activated protein kinase (MAPK), and phospholipase Cγ pathways, all of which suppress apoptosis, promote tumor cell survival, gene transcription, angiogenesis, cellular proliferation, migration, mitosis, and differentiation (8). In tumors, *MET* oncogene dependence occurs when the MET tyrosine kinase becomes constitutively active, resulting in gain of function due to *MET* mutation [found in hereditary papillary renal carcinomas (9) and lung cancers (10)] or *MET* amplification [reported in gastric, esophageal (11), and lung cancers (12)]. *MET* mutations are exceedingly rare in gastric cancer (13–16).

Earlier reports describe *MET* amplification in approximately 20% of gastric tumors (17–21) and MET protein overexpression [assessed by immunohistochemistry (IHC)] in approximately 50% of advanced gastric cancers (22–24). *MET* amplification and overexpression may herald aggressive tumor biology and worse clinical outcome (8, 17, 22, 24). MET protein overexpression correlates with increased depth of tumor invasion and increased metastatic potential (23, 24). On the basis of evidence that *MET* dysregulation contributes to the growth and progression of gastric adenocarcinoma and that *MET* inhibition may be an attractive new target for the treatment of gastric

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adenocarcinoma, this study was conducted to determine the incidence of *MET* expression and amplification in gastric cancer in a uniform population of U.S. patients with locally advanced gastric adenocarcinoma.

Materials and Methods

Patients and tissue samples

The Memorial Sloan-Kettering Institutional Review Board approved the study. All patients provided written informed consent for participation on National Cancer Institute–sponsored protocol of a preoperative chemotherapy study (NCI 5700) between June 2003 and November 2005. Thirty-eight patients provided tissue for MET assessment. Patient clinical characteristics included sex, age, histology, and pathologic stage.

FISH

FISH was conducted on formalin-fixed, paraffin-embedded tissues. DNA probes for MET (bacterial artificial chromosome clone RPC11-163C9; Invitrogen Life Technologies) and the centromere of chromosome 7 were directly labeled via nick translation with SpectrumRed (MET) and SpectrumGreen (centromere of chromosome 7) fluorophores, respectively. Slides were prepared by using standard cytogenetic techniques. The slides were denatured in 70% formamide/2× SSC for 5 minutes at 72°C and dehydrated in 70%, 85%, and 100% ethanol. The slides were then hybridized in 50% formamide, 2× SSC, Cot-1 DNA, and 50 ng of each probe at 37°C in a humid chamber overnight. After washing in 2× SSC/0.3% NP-40 at 72°C for 2 minutes, the slides were air-dried, counterstained with 0.2 μmol/L 4′,6-diamidino-2-phenylindole (DAPI), and cover slipped. The signals were visualized with a Nikon Eclipse fluorescence microscope containing SpectrumRed (*MET* locus), SpectrumGreen (centromere), and DAPI filters (Nikon Instruments). A total of 200 interphase cells were analyzed from each sample. The Metasystem software (Digital Scientific) was used for capturing the images.

Quantitative PCR for analysis of MET RNA amplification

Quantitative PCR for analysis of MET genomic amplification primers and probes for MET and 18s rRNA were obtained from Applied Biosystems. Primer and probe sequences for MET were (5′-3′): F-GGAGCCAAAGT-CCTTTCATCTGTAA, RGCAATGGATGATCTGGGAA-ATAAGAAGAAT, and FAM-CCGGTTCATCAACTTC. Reactions were done in triplicate under standard thermocycling conditions by using 10 ng of genomic DNA, primers at 900 nmol/L, and probes at 250 nmol/L. Levels of expression of MET mRNA are reported as relative copies that are normalized against 18S rRNA expression (25).

IHC for MET and phospho-MET protein expression

All specimens were fixed in 10% phosphate-buffered formalin and embedded in paraffin. For each case, all available hematoxylin and eosin–stained sections were reviewed, and a representative tissue block was selected for additional studies. Lauren classification was used to classify tumors according to histologic type. Standard ABC peroxidase techniques were used for IHC that was carried out on 4-μm paraffin sections of formalin-fixed, paraffin-embedded resected gastric cancer specimens. The following antibodies were used: anti-MET (C-12) from Santa Cruz Biotechnology and phospho-MET [(p-MET) Y1234/Y1235] from Cell Signaling Technology. A pathologist coded *MET* and p-MET expression as the percentage of positive tumor cells (scale 0%–100%) with staining intensity from 0 to 3+. Positive IHC expression is defined as 25% or more staining with intensity 2 or 3+. The reference pathologist (L.T.) reviewed all IHC MET and p-MET stains.

Statistical analysis

MET mRNA (PCR) and protein (IHC) expression was correlated with histology, tumor location, and treatment response by using the Fischer exact and Wilcoxon rank-sum tests.

Results

Table 1 shows clinical characteristics of the patients. The patient cohort consists predominantly of middle to distal stomach tumors (71%), with a similar number of patients with Lauren's diffuse (38%) and intestinal tumors (40%). Fifty-seven percent of the patients had

Table 1. Clinical characteristics of patients on study ($N = 38$)

Age at diagnosis, median (range)	59 (35–77)
Sex	
Women	15
Men	23
Median KPS	90%
Tumor location	
GEJ and proximal	9
Mid to distal stomach	29
Lauren classification	
Diffuse	15
Intestinal	17
Mixed	6
AJCC (5th edition 1998) stage at surgery	
0[a]	1
I–II	17
IIIA/IIIB	14
IV (occult peritoneal disease)	7

Abbreviations: KPS, Karnofsky performance status; AJCC, American Joint Committee on Cancer.
[a]One cases with no residual tumor at surgery.

locally advanced stage III or IV (occult peritoneal disease) tumors.

FISH for *MET* amplification

FISH analysis of *MET* was successful in all 38 (100%) tumor specimens. Table 2 presents the fractions of cells with *MET*/CEP7 signal in each tumor specimen. The presence of more than 2 gene-specific signals (red) accompanied by the same number of chromosome 7 centromere-specific signals (green) was regarded as indication of polysomy of chromosome 7 (CEP7; Fig. 1). Eleven (33%) tumors were polysomic for chromosome 7 and displayed equal numbers of copies of *MET* and CEP7 (range, 3–8 copies). Nine of these 11

Table 2. Analysis of the resected tumors for *MET* copy number (FISH), mRNA (PCR), MET protein expression (IHC), and phosphorylation status (p-MET IHC)

FISH copy ratio of *MET*/CEP17 ($n = 38$)	Relative MET mRNA expression (median 5.5, $n = 14$[a])	IHC	
		MET ($n = 38$)	p-MET ($n = 30$)
3 to 5/3 to 5		2+	0
2/2	9.9	1+	Luminal
2/2		1+	1+
2/2		2+	
2/2	14.4	1+	
2/2	11.4	2–3+	1+
2/2		1+	0
2/2	1.9	2+	0
2/2	3.1	2+ (10%)	Luminal
3/3		0	
2/2		2+	0
2/2		2+	1+
3 to 8/3 to 8	3.3	2+	2+
3 to 4/3 to 4 in 5% cells	34.2	2–3+	0
2/2		1+	1+
2/2	52.4	2+	0
2/2	2.3	2+	
2/2		2+	
3 to 4/3 to 4	5.2	2+	
2/2		2+	1+
2/2	14.4	2+	
3 to 6/3 to 6		2+	0
2/2	5.9	2+	0
2/2	2.1	1+	0
2/2	4.2	1+	0
3 to 4/3 to 4		2+	0
2/2		2+	1+ focal
3 to 5/3 to 5		1+	0
2/2		1+	
3 to 4/3 to 4 in 3% cells		2+	Luminal
2/2		1+	2+
2/2		2+	Luminal
3 to 5/3 to 5		2+	Luminal
2/2		2+	0
2/2		1+	1+
2/2		2+	1+ focal
2/2		1+	0
3 to 5/3 to 5		2+	0

[a]Includes only surgical specimens.

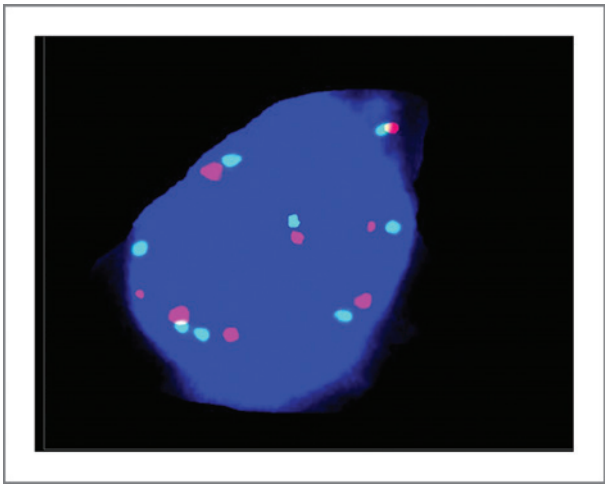

Figure 1. Representative interphase FISH analysis of a gastric tumor sample without *MET* amplification. The *MET* signal in red is associated with 8 individual copies of chromosome 7 centromere in green (polyploidy).

tumors had high MET IHC. *MET* amplification (defined as *MET*/CEP7 ratio > 2) was not identified in this sample set.

Table 2 summarizes individual tumor MET and p-MET IHC, MET mRNA PCR, and FISH results.

Quantitative PCR analysis for MET mRNA

Quantitaive PCR was done in 15 tumor specimens and matched normal gastric mucosa. Relative MET mRNA expression was significantly higher for tumor than for normal (9.9 vs. 3.0, $P = 0.008$), and this was due to high relative MET mRNA expression in Lauren's intestinal histology versus normal ($P = 0.02$; Table 3).

Immunohistochemical analysis for *MET* and p-MET expression

Resected tumor specimens of 38 patients were examined by IHC for MET, and 30 specimens were tested by IHC for p-MET. Table 4 summarizes the tumor characteristics of patients in MET IHC–positive and -negative groups. Positive MET staining by IHC was associated with Lauren intestinal histology ($P = 0.006$). Five of 7 (71%) cases with high MET mRNA expression (above median) were noted to have high MET IHC (Fig. 2). Two of 30 (6.6%) gastric cancer specimens revealed positive staining for p-MET (Table 2, Fig. 3). Of these, one specimen was MET IHC positive without an increase in MET mRNA expression (Table 3).

The result summary is presented in Table 5. Although MET IHC positivity was relatively common, increased MET phosphorylation was rare and *MET* amplification was not seen.

Discussion

MET amplification is believed to occur frequently in gastric cancer (5, 26). Previous studies noted *MET* amplification in up to 50% of gastric cancer cell lines (5, 17) and up to 20% of patients' gastric tumor samples (17–21). However, in this evaluation, we identified *MET* gene amplification in none of 38 locally advanced gastric adenocarcinomas that comprised the study set. We did observe 30% of gastric tumors with multiple *MET* gene copy numbers as a result of polysomy 7. It is known that breast tumors with an increased *HER2* gene copy number as a result of polysomy 17 behave as HER2-negative tumors (27). This phenomenon, therefore, suggests that gastric cancers with *MET* polysomy are unlikely to be *MET* driven.

Table 3. Relative MET mRNA expression by tumor histology

n = 15[a]	Median	*P*
Tumor vs. normal	9.9 vs. 3.0	0.008
Lauren intestinal vs. normal	20 vs. 3.0	0.002
Lauren diffuse vs. normal	5.2 vs. 3.0	NS
Lauren mixed vs. normal	3.8 vs. 3.0	NS

[a]Includes analysis of endoscopic biopsies.

The presence of *MET* amplification in prior studies (mostly from Japan) could possibly be linked to differences in tumor biology between Asian and Western patients. Distal stomach tumors are more common in Japan and have a favorable prognosis compared with proximal stomach and gastroesophageal junction (GEJ) tumors, which are more common in U.S. patients (5-year overall survival rates of approximately 60% vs. 20%; refs. 2, 4, 28–34). A recent study compared survival following resection of 2,357 Korean and U.S. patients. Even when evaluated by multivariate analysis, correcting for validated prognostic factors (35), Korean gastric cancer patients had improved survival over U.S. patients, suggesting that differences in tumor biology cannot be excluded (36). The distinct tumor biology of gastric cancer subtypes (37–40) and specific host genetic variations among ethnic groups (30, 41–43) might contribute to the difference in survival; although treatment approaches and mass screening programs in Japan also add to survival variability (44).

Another possible explanation for the discrepancy of our study with previous reports is that prior studies were conducted by using the Southern blot technique, which overestimated the incidence of *MET* amplification because it could not discriminate polysomy 7 from *MET* amplification. FISH methodology is technically more standardized and less affected by tissue variables, and it has replaced Southern blot in modern clinical diagnostic molecular pathology. In 1998, Hara and colleagues used FISH to examine 154 primary gastric tumors from Japanese patients and found that 6 (4%) tumors were *MET* amplified (45). It is likely that *MET* amplification does occur in gastric cancer but at a rate

Table 4. MET protein expression by IHC by tumor histology, location, and stage

$n = 38$	MET positive* ($n = 24$)	MET negative ($n = 14$)	P
Lauren classification, *n* (%)			
Diffuse or mixed	9 (28)	12 (86)	0.006
Intestinal	15 (63)	2 (14)	
Location, *n* (%)			
GEJ or proximal	8 (33)	4 (28)	NS
Mid to distal stomach	16 (66)	10 (71)	
Stage at surgery, *n* (%)			
I–II	10 (41)	6 (43)	NS
III–IV	14 (58)	8 (57)	

[a]MET positive defined as MET IHC intensity of ≥2 in ≥25% of tumor cells.

substantially lower than that commonly reported in the literature.

Smolen and colleagues have shown that gastric cancer cell lines with high-level amplification of *MET* are extraordinarily susceptible to the selective MET tyrosine kinase inhibitor (TKI) PHA-665752. Treatment with MET TKI resulted in massive apoptosis in 5 of 5 *MET*-amplified (FISH) gastric cancer cell lines and none of the 12 *MET*-negative cell lines (5). With such a dramatic benefit in preclinical models of MET-driven gastric cancer, the success of MET-targeted therapy (31) in gastric cancer will depend on correctly selecting the patient population whose tumors depend on MET for growth and development.

In our analysis, MET protein and mRNA expression were commonly encountered (in 63% and 50% of resected tumor specimens, respectively) and are concordant with reported literature (23, 24, 46). The significance of the increase in *MET* expression on a transcriptional level is unclear, especially when considering the absence of *MET*

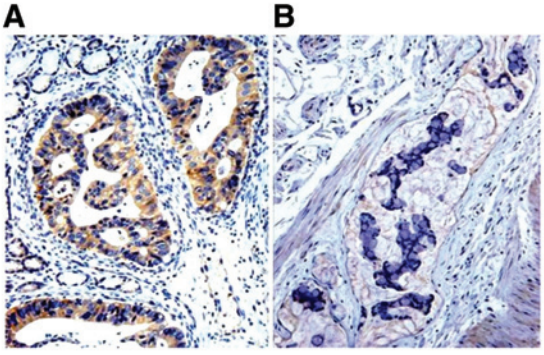

Figure 2. MET protein expression in gastric carcinoma by IHC. Positive MET immunoreactivity was identified in a moderately differentiated intestinal-type adenocarcinoma with cytoplasmic staining pattern (A). In contrast, MET reactivity was not observed in a poorly differentiated mucinous adenocarcinoma with signet ring cell features. Original magnification × 200.

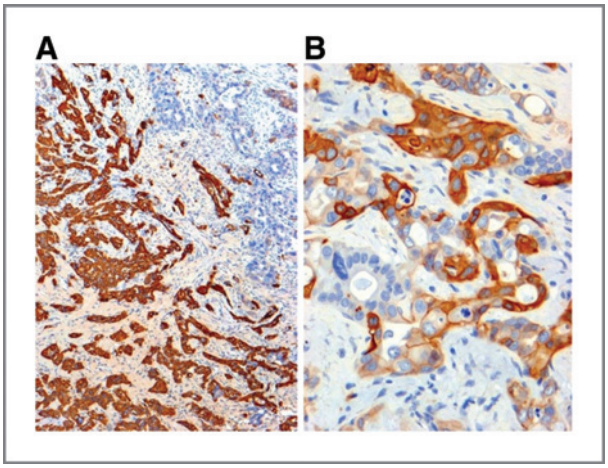

Figure 3. p-MET protein expression in gastric carcinoma by IHC. Positive p-MET immunoreactivity was shown in a portion of a moderately to poorly differentiated adenocarcinoma (A; bottom left) and was negative in other areas of the same tumor (top right). The staining pattern was membranous as well as cytoplasmic, although the immunoreactivity was not seen in all the cells (B). Original magnification × 100 (A) and × 200 (B).

amplification or MET tyrosine kinase activity. In breast cancer, multiple copies of chromosome 17 (location of the *HER2* gene) has been associated with increased HER2 oncoprotein staining by IHC, without *HER2* gene amplification (47).

In the absence of *MET* amplification, *MET* activation in gastric cancer might be related to deregulation of microRNA that is related to the *MET* gene as an alternative

Table 5. Results summary

	Positive	Negative
MET amplification by FISH ($n = 38$)	0	38
IHC		
MET ($n = 38$)	24	14
p-MET ($n = 30$)	2	28

pathway that is associated with aggressiveness of the gastric tumors. MicroRNAs are a class of small, noncoding RNAs that regulate gene expression, and they are increasingly implicated in the pathogenesis of cancer (48). Migliore and colleagues have shown that miR-34b, miR-34c, and mir-199a can decrease *MET* expression on the protein and RNA level and impair MET-mediated invasive growth in a gastric cell line that has *MET* amplification (49). Unique microRNA signatures are associated with different histologic subtypes, pattern of progression, and prognosis in gastric cancer (50). It is possible that certain microRNAs, the nature of which remains to be investigated, can increase MET protein expression (in the absence of *MET* gene amplification).

Our study shows the lack of *MET* amplification and pathway activation in Western patients with gastric cancer. In a separate study of unselected advanced gastric cancer patients, single-agent MET TKI (GSK1363089) failed to show antitumor activity (51). We conclude that MET-driven gastric cancers are rare in the Western population. Future studies of MET inhibitors will, therefore, require better patient selection and trial design.

Disclosure of Potential Conflicts of Interest

M.A. Shah obtained a commercial research grant from GlaxoSmith Klein. Y.Y. Janjigian is a consultant/advisory board member of Roche/Genentech and obtained a commercial research grant from Boehringer-Ingelheim.

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