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Clinical Significance of *MET* Gene Copy Number in Patients with Curatively Resected Gastric Cancer

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The present study analyzed the prognostic impact of *MET* gene copy number in patients with curatively resected gastric cancer who received a combination regimen of cisplatin and S-1. The *MET* gene copy number was analyzed by use of quantitative real-time polymerase chain reaction. From January 2006 to July 2010, 70 tumor samples from 74 patients enrolled in a pilot study were analyzed. According to a cutoff *MET* gene copy number of ≥2 copies, a high *MET* gene copy number was observed in 38 patients (54.3%). The characteristics of the 2 groups divided according to *MET* gene copy number were similar. With a median follow-up duration of 26.4 months (range, 2.6-73.2 months), the estimated 3-year relapse-free survival and overall survival rates were 54.3% and 77.4%, respectively. No significant association was observed between the *MET* gene copy number and survival in a multivariate analysis. The *MET* gene copy number investigated in this study was not found to be associated with prognosis in patients with curatively resected gastric cancer.

Key Words: *MET; Stomach neoplasms; Chemotherapy, Adjuvant; Prognosis*

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INTRODUCTION

Although the survival rate for advanced gastric cancer is steadily improving, the prognosis remains very poor.[1] Gastric cancer is a particularly heterogeneous disease that is implicated in various gene-environment interactions resulting in the activation of several molecular pathways.[2] *MET* is a proto-oncogene located on chromosome 7 (7q31) and encodes a tyrosine kinase membrane receptor for its physiologic ligand hepatocyte growth factor (HGF).[3] The intracellular signaling cascades activated by the MET pathway include the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MEK) pathways. Activation of these signals leads to alterations in the transcription of various cellular genes and cross-signaling pathways such as the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor.[4] Thus, MET and HGF play an essential role in tumor-related metastasis and angiogenesis as well as in the cellular proliferation and survival of tumors.[5] Clinical studies have also shown that the alteration of MET or its family is associated with prognosis for various solid tumors.[6-10]

Several recent reports have indicated that MET may be a prognostic marker and important target for cancer treatment using co-signal networks in the case of gastric cancer.[11] For example, the expression of MET has been shown to be correlated with a poor prognosis for gastric cancer.[12,13] Toiyama et al. also reported that increased MET and HGF expression was significantly associated with poor prognosis and predicted peritoneal dissemination.[14] In addition, the *MET* gene copy number appears to influence the survival of patients with gastric cancer.[15,16] Consequently, given these results, MET seems to play an important role in tumor growth and spread, thereby affecting the prognosis of gastric cancer.

Notwithstanding, relatively few published studies have investigated the *MET* gene copy number and its relationship to the clinical outcomes of resected gastric cancer. Accordingly, the present study analyzed the *MET* gene copy number and its impact on the survival of patients with

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curatively resected gastric cancer.

MATERIALS AND METHODS

1. Patients

Tissues were obtained from patients who participated in a pilot study of a combination regimen of cisplatin and S-1. The inclusion criteria and results of that study have been reported previously.[17] In brief, the study included patients with histologically confirmed adenocarcinoma of the stomach who underwent curative surgery and received adjuvant chemotherapy with cisplatin and S-1. The pathologic staging was assessed according to TMN classifications from the 6th edition of the American Joint Committee on Cancer (AJCC). This pilot study was approved by the institutional review board (IRB) at Kyungpook National University Hospital (KNUH).

2. Analysis of *MET* gene copy number

Written informed consent for quantitative polymerase chain reaction (qPCR) was received from the patients, and the study was approved by the IRB at KNUH. The DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples by use of a QuickExtract^{TM} FFPE DNA extraction kit (Epicentre Biotechnologies). This involved adding 100 μL of the QuickExtract FFPE DNA extraction solution to the paraffin-embedded tissue sections in a microcentrifuge tube; the samples were then incubated at 56°C for 1.5 hours and 98°C for 2 minutes. The DNA concentration was measured by using a DNA Quantitation Kit, Fluorescence Assay (Sigma), with the fluorescent dye bisBenzimide H33258 (Hoechst 33258). The genomic qPCR was performed by using a LightCycler480 real-time PCR instrument (Roche, Basel, Switzerland). The thermal cycling conditions consisted of one cycle at 95°C for 10 minutes and 40 cycles at 95°C for 15 s and 60°C for 1 minute. The PCR reactions were performed in a total volume of 10 μL. At the end of the PCR, the samples were analyzed by using reported methods.[16] A high copy number was defined as more than 1.99 copies, which was calculated by using the median of the *MET* gene copy number.[18]

3. Statistical analysis

The descriptive statistics are reported as the proportion and median. The baseline characteristics were compared by using a chi-square test and Fisher's exact test. Overall survival (OS) was defined as the time from the date of surgery to death from any cause. Relapse-free survival (RFS) was defined as the time from the date of surgery to relapse or death from any cause. Follow-up duration was defined as the time from the date of surgery to patients' last visits or events. OS and RFS were analyzed by using a Kaplan-Meier test and were compared by using log-rank tests. Cox's proportional hazard regression model was used for the survival analyses. The analyses were adjusted for age, sex, tumor size, and stage. The hazard ratio (HR) and 95% confidence interval (CI) were also estimated. A cutoff p value of 0.05 was adopted for all statistical analyses. All analyses were performed by using the Statistical Package for the Social Sciences, version 14 (SPSS Inc., Chicago, IL, USA).

RESULTS

1. Patient characteristics

This study analyzed 70 tumor tissues samples from 74 patients enrolled in the pilot study. The patient character-

TABLE 1. Patient and tumor characteristics

	Total (n=70)
Age (Years)	56 (22-71)
Sex	
Male	43
Female	27
ECOG performance	
0	37
1	33
Pathologic stage	
II	22
IIIA	15
IIIB	13
IV (M0)	20
T stage	
T1	1
T2	27
T3	39
T4	3
N stage	
N0	9
N1	21
N2	20
N3	20
Bormann type	
I	0
II	13
III	37
IV	16
Mixed	1
Unknown	3
Lauren classification	
Intestinal	14
Diffuse	53
Mixed	3
Histology	
Adenocarcinoma, well differentiated	0
Adenocarcinoma, moderate differentiated	13
Adenocarcinoma, poorly differentiated	47
Adenocarcinoma, signet ring cell type	5
Adenocarcinoma, mucinous type	5
Tumor size (cm)	6.0 (2-19)
Gastrectomy type	
Subtotal	38
Total	32
***MET* amplification status**	
High gene copy number (>1.99)	38
Low gene copy number (≤ 1.99)	32

istics are shown in Table 1. The median patient age was 56 years (range, 22-71 years), and 43 patients were male. The stages after surgical resection were as follows: stage II (n=22, 31.4%), stage IIIA (n=15, 21.4%), stage IIIB (n=13, 18.6%), and stage IV (n=20, 28.6%). The predominant histology was poorly differentiated adenocarcinoma. Most patients were classified as diffuse type by the Lauren classification. Among the 70 patients, 30 relapses (42.9%) were documented and 17 patients (24.3%) died (Table 2). The most common site of relapse was the peritoneum (50.0%). With a median follow-up duration of 26.4 months (range, 2.6-73.2 months), the median RFS time was 39.9 months (95% CI: 14.7-65.2), whereas OS time cannot yet be calculated. The estimated 3-year RFS and OS rates were 54.3% and 77.4%, respectively.

2. Relationship between *MET* gene copy number and clinicopathologic factors

When using the cutoff *MET* gene copy number of ≥2 copies, 38 patients (54.3%) were in the high *MET* gene copy number group, and 32 patients (45.7%) were in the low *MET* gene copy number group. The relationships between the *MET* gene copy number and the clinicopathologic factors are shown in Table 3. No significant correlations were observed between the *MET* gene copy number and the clinicopathologic features.

3. Survival analysis

In the univariate and multivariate analyses including age, sex, tumor size, and stage, no significant association was observed between the *MET* gene copy number and survival (Table 4 and Fig. 1). Stage, tumor size, and age were found to be independent prognostic factors of survival for the patients with resected gastric cancer.

DISCUSSION

When investigating the MET gene copy number and its impact in patients with surgically resected gastric cancer, no significant association was observed between the *MET*

TABLE 2. Survival results

	Total (n=70)	High *MET* gene copy number (n=38)	Low *MET* gene copy number (n=32)
Relapse	30	16	14
Site of relapse			
Local	0	0	0
Peritoneum	15	9	6
Ovary	5	2	3
Liver	2	1	1
Bone	1	1	0
Distant lymph node	1	0	1
Combined	6	3	3
Death	17	10	7
Cause of death			
Disease related	15	8	7
Other causes	2	2	0

TABLE 3. Relationship between *MET* gene copy number and clinicopathologic features

	High *MET* gene copy number (n=38)	Low *MET* gene copy number (n=32)	p value
Age (Years)	55 (22-71)	60 (30-71)	0.317
Sex			0.746
Male	24	13	
Female	14	19	
ECOG performance			0.683
0	20	17	
1	18	15	
Pathologic stage			0.230
II	8	14	
IIIA	10	5	
IIIB	8	5	
IV (M0)	12	8	
T stage			0.680
T1	0	1	
T2	14	13	
T3	22	17	
T4	2	1	
N stage			0.213
N0	2	7	
N1	13	8	
N2	12	8	
N3	11	9	
Bormann type			0.115
I	0	0	
II	12	1	
III	19	18	
IV	6	10	
Mixed	1	0	
Unknown	0	3	
Lauren classification			0.565
Intestinal	9	5	
Diffuse	28	25	
Mixed	1	2	
Histology			0.919
Adenocarcinoma, well differentiated	0	0	
Adenocarcinoma, moderate differentiated	7	6	
Adenocarcinoma, poorly differentiated	26	21	
Adenocarcinoma, signet ring cell type	2	3	
Adenocarcinoma, mucinous type	3	2	
Tumor size (cm)	6.0 (2-19)	5.0 (2-14)	0.412
Gastrectomy type			0.481
Subtotal	21	17	
Total	17	15	

ECOG: Eastern Cooperative Oncology Group.

TABLE 4. Multivariate survival analysis

		No (%)	RFS			OS		
			HR	95% CI	p value	HR	95% CI	p value
Age	57≥	15 (21.4)	0.008	0.001-0.148	<0.001	0.036	0.007-0.184	<0.001
Sex	Male	43 (61.4)	0.592	0.203-1.926	0.337	0.810	0.372-1.765	0.596
Stage	III/IV	48 (68.6)	0.202	0.044-0.932	0.040	0.226	0.076-0.668	0.007
Tumor size	≤5.5 cm	36 (51.4)	3.713	2.206-5.492	0.009	7.149	2.130-23.999	0.001
MET gene copy number	Low	32 (45.7)	1.540	0.531-4.468	0.427	2.367	1.005-5.575	0.069

RFS: relapse-free survival, OS: overall survival, HR: hazard ratio, CI: confidence interval.

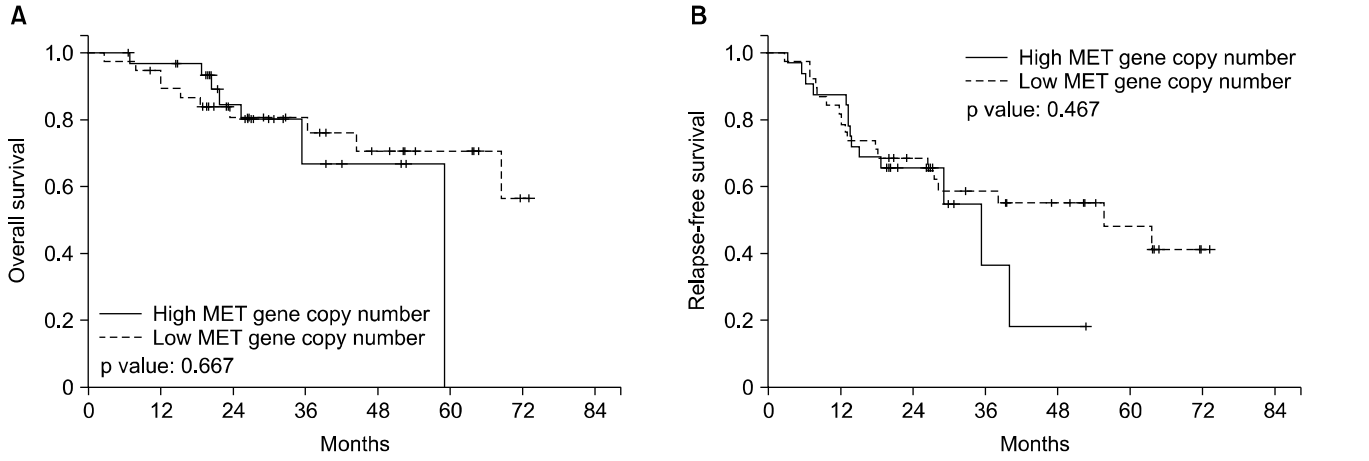

FIG. 1. Survival curves according to the *MET* gene copy number: (A) overall survival and (B) relapse-free survival.

gene copy number and patient survival.

Activation of the *MET* pathway is mainly accompanied by amplification of the *MET* gene, which leads to subsequent protein overexpression and kinase activation.[2] Thus, alteration of the *MET* gene can be detected by overexpression, gene mutations, amplification, or rearrangements.[19] In general, the frequency of overexpression is high (40-80%) and amplification is present in 5% to 10% of gastric cancer.[20,21] In contrast, 38 patients (54.3%) were in the high *MET* gene copy number group (median, 2.0; range, 1.14-6.27). As such, there may have been different sensitivities and specificities between the methods used to detect the *MET* gene copy number.

Clinical studies have already demonstrated that activation of the MET pathway produces a higher grade and worse survival outcome for solid tumors including gastric cancer.[5-10] For example, overexpression of MET has been connected to the risk and progression of gastric cancer.[13] In a study by Graziano et al. that evaluated the effects of *MET* gene copy number and sequencing for HGF on survival in 230 patients with gastric cancer, the survival outcome was worse among the patients with five or more copies.[16] Recently, Lee et al. also showed that an increased *MET* gene copy number was associated with poorer survival.[15] Furthermore, MET activation has been more specifically associated with liver and peritoneal metastases,[12,14] whereas activation of the *MET* gene has been strongly linked to uncontrolled cell proliferation, oncogenesis, and aggressive cellular invasiveness, leading to poor survival outcome.

In the present study, the prognostic impact of the *MET* gene copy number in gastric cancer patients was not statistically significant. There are several reasons for this finding. One reason is that the sample size was too small to make conclusions about the prognostic significance of *MET* gene copy number. Second, although qPCR is considered a standard method for MET testing, various concordance rates have been reported between fluorescence in situ hybridization and qPCR.[22] Plus, the methodology of interpreting copy number and the cutoff values will need to be standardized. Third, the present findings need to be confirmed to determine whether the adjuvant chemotherapy may have contributed to the survival outcomes.

In conclusion, the *MET* gene copy number investigated in this study was not found to be a prognostic marker for patients with curatively resected gastric cancer. However, further investigation is needed to clarify the role of the *MET* gene as a biomarker and new target in gastric cancer.

CONFLICT OF INTEREST STATEMENT

None declared.

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