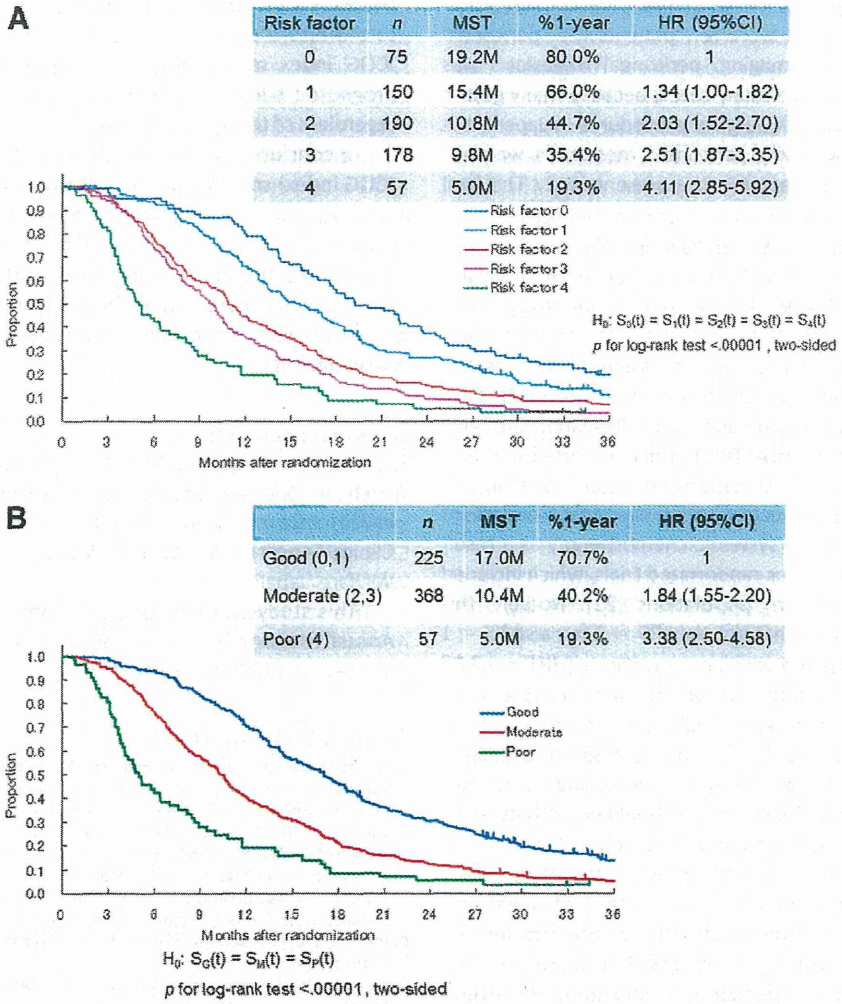

Figure 4. Survival curves according to the Japan Clinical Oncology Group prognostic index. **(A):** Survival according to the number of risk factors, from 0 to 4. **(B):** Survival was divided into three groups, good (0, 1), moderate (2, 3), and poor (4). Risk factors consist of performance status ≥1, number of metastatic sites ≥2, no prior gastrectomy, and elevated alkaline phosphatase. Good (0,1), low risk (0 or 1 risk factors); moderate (2,3), moderate risk (2 or 3 risk factors); poor (4), high risk (4 risk factors).

Abbreviations: %1-year, 1-year survival; CI, confidence interval; HR, hazard ratio; MST, median survival time.

study focused only on patients eligible for a specific clinical trial. In JCOG9912, for example, there were very few patients with PS = 2, and those with severe peritoneal metastasis were excluded. Indeed, the cutoff value of PS was set at PS = 1 in the present study, but was set at PS = 2 in the Korean studies. Thus, the patient population, such as patients enrolled in a clinical trial and patients in clinical practice, may have some influence on the prognostic factors.

Chau et al. [14] proposed the RMH prognostic index based on clinical trial data. When we applied the RMH index to our data, about three-quarters (74%) of the patients were classified into the moderate-risk group, and only 5% of the patients were classified into the poor-risk group. Whereas the criteria for the poor-risk group in the JCOG index covered more patients (9%) than the RMH index did (5%) in the present study, the survival of the poor-risk group in the JCOG index was worse than that in the RMH index, even although the overall survival was much better in the present study than that of the subjects of the RMH index [14]. In contrast, although the good-risk group in the JCOG index included more patients (35%) than the RMH index did (20%) in the present study, the survival of the good-risk group in the JCOG index was better than that of the RMH index. Furthermore, the impact on the survival difference was smaller by the RMH index than that observed after application of the JCOG index. These results suggest that the JCOG index may be a better indicator for survival than the RMH index on the points of proportion of the three risk groups and differences in survival.

Except for PS and ALP, the factors used in the JCOG index were substantially different from those used in the RMH index. This may be because of the following three reasons. First, there may be differences in the disease entities, because the studies used to formulate the RMH index included patients with esophageal cancer (27.3% vs. 0% in our study) and those with locally advanced disease (22.2% vs. 0% in our study). Actually, few patients with gastric cancer have locally advanced disease, whereas some patients with esophageal cancer have. Second, there may be differences in severity of peritoneal metastasis. There are two types of peritoneal metastasis: one, such as ascites, is associated with a poor prognosis and can be diagnosed by imaging, and the other can be diagnosed only at laparotomy

with small tumor burden, which has a small impact on survival. In JCOG9912, although there were few patients with peritoneal metastasis detected by imaging, peritoneal metastasis was diagnosed at laparotomy in many cases, because many gastric cancer patients go through surgical procedures in Japan. It is considered that this is why peritoneal metastasis was not adopted as a prognostic factor in the present study. The final reason is that there seemed to be some differences in PS between the RMH index and the JCOG index. The cutoff value for the RMH index was PS ≥2 as a risk factor of survival, whereas the cutoff value for PS was ≥1 in our study. This difference may have resulted from the difference in the proportion of patients with PS ≥2 between these studies (23.4% in the RMH studies vs. 1% in our study). Recently, the Global Advanced/Adjuvant Stomach Tumor Research through International Collaboration (GASTRIC) project reported that PS ≥1, disease status, number of metastatic organs, location of metastasis, and prior gastrectomy were prognostic factors for AGC patients treated with systemic chemotherapy as a result of meta-analyses of previous randomized trials, which included both Eastern and Western populations [21]. Notably, this GASTRIC study identified that not only PS = 2 but also PS = 1 were significantly associated with poor prognosis (HRs = 2.17 and 1.36, respectively), which showed the same trend as the present study. In recent phase III trials of gastric cancer, the proportion of patients with PS = 2 has decreased, because patient selection criteria have become more stringent. It can be proposed that the cutoff value for PS should be set between one and two for prognostic analysis in future clinical trials.

The JCOG index proposed in the present study has some limitations. First, whereas number of metastatic sites was an important prognostic factor, metastatic sites were designated by each investigator, and radiological images showing metastatic sites were not reviewed independently for this study. However, because metastatic sites were reported prospectively by checking the list of common metastatic sites in the case report form, the variability is relatively small. Second, it was not validated on other cohorts, especially those including Western patients. Therefore, we plan to validate this JCOG index using the data from other phase III trials. Third, the condition of the subjects in the present study was much better than those often encountered in clinical practice, such as those having good PS and fewer peritoneal metastases. Therefore, the JCOG index may not be applicable to the general patient population in clinical practice. Recently, however, oral fluoropyrimidines, such as capecitabine and S-1, have been replacing the continuous infusion of fluorouracil, and global trials of first-line chemotherapies for AGC have been based on the use of oral agents. Thus, future trials may also tend to exclude patients with severe peritoneal metastasis, which often impairs oral intake, and it is anticipated that exclusion of patients with severe peritoneal metastasis will lead to enrollment of good conditioned patients to the future clinical trials. Therefore, it is expected that this JCOG index may be useful for adjusting and/or balancing prognostic backgrounds even of patients in good condition regardless of their region of origin.

In conclusion, we propose a novel prognostic index (the JCOG index) consisting of four risk factors (PS ≥1, number of metastatic sites ≥2, no prior gastrectomy, and elevated ALP), which classified patients into three risk groups. Although further validation of this index using other trials for AGC is required, it is expected that the JCOG index will be useful in future clinical trials and studies investigating treatment options in AGC patients.

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Disclosures

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Clinical impact of c-MET expression and genetic mutational status in colorectal cancer patients after liver resection

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Key words

Colorectal cancer, c-MET expression, genetic mutational status, hepatic metastasectomy, relapse-free survival

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c-MET is implicated in the pathogenesis and growth of a wide variety of human malignancies, including colorectal cancer (CRC). The aim of the present study was to clarify the association between c-MET expression and tumor recurrence in CRC patients after curative liver resection, and to evaluate concordance in c-MET expression and various mutations of *KRAS*, *BRAF* and *PIK3CA* between primary CRC and paired liver metastases. A cohort of patients was tested for c-MET immunoreactivity (i.e. immunohistochemistry [IHC]) and *KRAS*, *BRAF* and *PIK3CA* mutations. Analyses were performed both on primary tumors and paired liver metastases, and the association between IHC and mutations results were assessed. A total of 108 patients were eligible. A total of 53% of patients underwent simultaneous resection of primary tumors and metastases, and the others underwent metachronous resection. Levels of concordance between primary tumors and metastases were 65.7%, 87.7%, 100% and 95.2% for c-MET, *KRAS*, *BRAF* and *PIK3CA*, respectively. High levels of c-MET expression (c-MET-high) in the primary tumors were observed in 52% of patients. Relapse-free survival was significantly shorter for patients with c-MET-high primary tumors (9.7 months) than for those with c-MET-low primary tumors (21.1 months) ($P = 0.013$). These results suggest that a high level of genetic concordance in *KRAS*, *BRAF* and *PIK3CA* between primary tumors and liver metastases, and c-MET-high in the primary tumors were associated with shorter relapse-free survival after hepatic metastasectomy.

The *MET* proto-oncogene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF).[1,2] HGF binds to c-MET receptor, which subsequently undergoes phosphorylation on intracellular tyrosine residues leading to the activation of downstream signaling. Signaling through the HGF/c-MET pathway results in tumor growth, angiogenesis and the development of invasive phenotypes in several types of malignancy, including colorectal cancer (CRC).[3,4]

The frequency of expression of c-MET protein in CRC as detected by immunohistochemistry (IHC) has been reported to be between 59.4% and 81.1%; it is associated with advanced tumor stages and poor clinical outcomes.[5–8] Similar to c-MET protein expression, c-MET gene amplification is linked to disease metastases.[9,10] The HGF/c-MET pathway is also well-known to be associated with liver regeneration and the development of normal organs, such as the placenta, muscle and the central nervous system.[2,11] The performance of hepatectomy for the treatment of liver metastases triggers the process of hepatic regeneration, in which numerous cells and molecules mediate multiple molecular pathways. Ample growth factors, which contribute to neoplastic development, such as HGF, are also present during liver regeneration. However, the presence of micrometastases and their association with tumor recurrence, as well as the responsible regenerative factors that support neoplastic progression remain only partly understood.

Despite increasing evidence for a role of c-MET in CRC metastases, few studies have, to our knowledge, compared c-MET expression in primary CRC and distant metastases, and they have obtained conflicting results.[5,12] Furthermore, the significance of performing genomic testing for somatic mutations in *KRAS*, *BRAF* and *PIK3CA* is recognized in molecular target therapy,[13–16] but material from metastatic tumors is not always included in the testing. Therefore, it is important to investigate the concordance of results from primary tumors and paired liver metastases.

The aim of the present study was to evaluate the association between c-MET expression and tumor recurrence in CRC patients after liver resection and to assess the concordance between primary CRC and paired liver metastases in the expression of c-MET and various mutations of *KRAS*, *BRAF* and *PIK3CA*.

Materials and Methods

Patients. Between January 2004 and December 2009, patients from our institution were included in this study if all liver metastases of CRC were technically resectable with

curative intent (i.e. with a tumor-free margin). A series of 108 consecutive patients were identified. Data for these patients were evaluated preoperatively with a baseline medical history and physical examination; serum laboratory tests, including liver function tests, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) level were carried out, as well as contrast-enhanced computed tomography (CT) of the chest and abdomen.

The width of the resection margin was assessed by the pathologist and defined as the shortest distance from the edge of the liver metastases to the transection line. In cases of multiple liver metastases, the closest margin was recorded as the final margin.

After liver resection, the patients were followed up at regular intervals, by serum CEA and CA 19-9 levels, and patients underwent follow-up examinations to identify possible tumor recurrence. Examination methods included CT, MRI and abdominal ultrasonography. Although recurrence could be diagnosed by clinical, radiological or pathological methods, the main evaluation technique was radiological (e.g. computed tomography and ultrasonography). The present study was approved by the institutional review board of the National Cancer Center.

Immunohistochemistry and polymerase chain reaction. We used formalin-fixed paraffin-embedded tissue samples for IHC and gene analysis. For IHC, the Bench-Mark XT automated slide processing system (Ventana, Tucson, AZ, USA) was used according to the manufacturer's protocol. In brief, after the tissue sections were deparaffinized using EZ Prep (Ventana), heat-induced epitope retrieval with CC1 (Ventana) was performed, and the slides were incubated with primary antibodies against c-MET (CONFIRM Anti-Total c-MET, clone SP44 [Ventana]). Immunoreactions were detected using the *ultra*View DAB Universal Detection Kit followed by counterstaining with Hematoxylin II (Ventana) and Bluing Reagent (Ventana).

Two independent observers without prior knowledge of the clinicopathological data scored the IHC findings; MET protein expression levels were scored dependent on the staining intensity, as previously described: 0, negative; 1, weak; 2, moderate; and 3, strong.[6] We defined scores 0 and 1 as c-MET-low, and scores 2 and 3 as c-MET-high.

Genomic DNA was extracted after microdissection at the laboratory of SRL (Hamura, Japan). Exon 2 of the *KRAS* gene, exon 15 of the *BRAF* gene, and exon 9 and exon 20 of the *PIK3CA* gene were amplified by PCR. The PCR products were visualized using agarose gel electrophoresis with ethidium bromide staining. The PCR DNA fragments were extracted from the agarose gel and directly sequenced using an ABI 3130 Genetic Analyzer (Life Technologies Japan, Tokyo, Japan) according to the manufacturer's instructions.

Statistics. Differences between categorical variables were assessed using Fisher's exact tests and the Mann–Whitney test. Relapse-free survival (RFS) was defined as the time from hepatectomy until detection of relapse or last disease assessment. Deaths of patients who died without evidence of a recurrence were treated as events. Patients who were lost to follow up were treated as censored observations. Median RFS was calculated using the Kaplan–Meier method, and survival curves were compared using the log-rank test. For univariate and multivariate analyses, the Cox proportional hazards regression model was used. Agreement between the test result of primary tumors and liver metastases was measured by the Kappa coefficient. All calculations except for the Kappa coefficients were performed using SPSS version 17 (SPSS, Chicago, IL, USA). The Kappa coefficients and the confidence intervals were calculated using SAS version 9.3 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics. Patient characteristics are listed in Table 1. There were 65 men and 43 women, with a median age of 63 years. The primary tumors were located in the colon

Table 1. Patient characteristics at diagnosis

Clinical feature	Number of cases (%)	c-MET IHC in primary tumor		*P*
		c-MET-low	c-MET-high	
Total *N*	108	52	56	
Sex				
Male	65 (60.2)	33	32	0.56
Female	43 (39.8)	19	24	
Age, years				
Median (range)		63 (22-86)	63 (26-84)	0.46
Primary tumor location				
Colon	69 (63.9)	33	36	1
Rectum	39 (36.1)	19	20	
Primary tumor				
Node-negative	32 (29.6)	18	14	0.30
Node-positive	76 (70.4)	34	42	
Liver metastases				
Metachronous	51 (47.2)	21	30	0.18
Synchronous	57 (52.8)	31	26	
Liver metastases				
H1 (number ≤4 and size ≤5 cm)	96 (88.9)	47	49	0.87
H2 (other)	10 (9.3)	4	6	
H3 (number >5 and size >5 cm)	2 (1.8)	1	1	
CEA				
<5	66 (61.1)	29	37	0.33
≥5	42 (38.9)	23	19	
CA19-9				
<37	32 (29.6)	11	21	0.09
≥37	76 (70.4)	41	35	
Histology				
Well differentiated	52 (48.1)	29	23	0.28
Moderately differentiated	50 (46.3)	20	30	
Poorly differentiated	6 (5.6)	3	3	
KRAS mutation type				
Wild type	69 (63.9)	35	34	0.77
Codon 12 mutation	32 (29.6)	14	18	
Codon 13 mutation	7 (6.5)	3	4	
BRAF mutation type				
Wild type	105 (99)	51	54	1
V600E mutation	1 (1)	0	1	
PIK3CA mutation type				
Wild type	94 (88.9)	47	47	0.74
Exon 9 mutation	10 (11.1)	4	6	

CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; H1, the number of tumors was 4 or less, and tumors were 5 cm or less in greatest dimension; H2, other than H1 and H3; H3, the number of tumors was more than 5, and tumors were more than 5 cm in greatest dimension.

in 69 patients (63.9%) and in the rectum in 39 patients (36.1%). Liver metastases were diagnosed synchronously in 57 patients (52.8%). In the remaining 51 patients, liver metastases developed after a mean interval of 18.3 months (range 6.5–69.7 months) from colorectal cancer resection. Among patients with metachronous resection, 14 patients received adjuvant chemotherapy after the resection of the primary tumors.

Sites of recurrence after hepatectomy. Among all patients, 75 (69.4%) patients developed a recurrence after hepatectomy. The most frequent sites of recurrence were the liver only (53.3%), lung only (21.3%), liver and lung (8.0%) and para-aortic/caval lymph nodes (8.0%).

Concordance in the expression of c-MET and mutations between primary tumors and paired metastases. *c-MET.* c-MET expression was assessed by IHC in primary tumors and liver metastases expression in all 108 specimens. c-MET staining intensity in the primary tumors was 3 in 7 cases (6%), 2 in 49 cases (45.8%), 1 in 51 cases (47.2%) and negative in 1 case (0.9%). c-MET staining intensity in the liver metastases was 3 in 4 cases (3.7%), 2 in 55 cases (51.9%), 1 in 45 cases (41.7%) and negative in four cases (3.7%) (Fig. 1).

For paired metastases, the c-MET status was found to be unchanged in 71 cases (39 cases confirmed c-MET-high and 32 cases confirmed c-MET-low). A change in c-MET status was observed in 37 cases (34.3%): 20 patients (18.5%) changed from low to high and 17 patients (15.7%) changed from high to low (concordance, 65.7%; $\kappa = 0.313$; 95%CI, 0.133–0.491). Among patients who received adjuvant chemotherapy after the resection of the primary tumors, a change was observed in two cases (2/14): one patient changed from low to high and another changed from high to low.

KRAS. *KRAS* mutational status was tested in 108 cases. A total of 39 patients (36.1%) had a *KRAS* mutation in the primary tumors; 15 of those patients had a G12V mutation, nine patients a G12D, 7 patients a G13D, four patients a G12S, two patients a G12C, one patient a G12A and one patient a G12R. A change in KRAS gene mutational status was observed in 13 cases (12.3%): five patients (4.7%) changed from wild type to codon 12 mutations, six patients (5.7%) changed from codon 12 mutations to wild type and two patients (1.9%) changed from codon 13 mutations to wild type (concordance, 87.7%; $\kappa = 0.747$; 95% CI, 0.617–0.876).

BRAF. *BRAF* mutational status was tested in 106 cases. Two pairs were excluded from analysis because of the low amount of available tumor tissue in the available samples. Of 106 cases, one patient had a V600E mutation (0.9%). There was no discordance between primary tumors and liver metastases (concordance, 100%).

PIK3CA. The status of *PIK3CA* mutational status was analyzed in 104 cases. Four pairs were excluded from analysis because of the low amount of available tumor tissue in the samples. A total of 10 patients (9.6%) had a *PIK3CA* exon nine mutation in the primary tumors; five of those patients had an E545K mutation, three patients an E542K mutation, one patient an E542Q mutation and one patient an E542G mutation. A change in *PIK3CA* exon 9 mutational status was observed in five cases (4.8%): four patients (3.8%) changed from wild type to exon 9 mutation, and one patient (1.0%) changed from exon 9 mutation to wild type (concordance, 95.2%; $\kappa = 0.756$; 95% CI, 0.552–0.960). In contrast, exon 20 mutation was not identified in any of the cases.

c-MET expression levels and relapse-free survival. The median RFS among patients with c-MET-high primary tumors (9.7 months) was significantly shorter than the median RFS among those with c-MET-low primary tumors (21.1 months) ($P = 0.013$; Fig. 2). However, the median RFS among patients with c-MET-high expression in liver metastases (9.1 months) was not significantly shorter than the median RFS among those with c-MET-low liver metastases (14.5 months) ($P = 0.147$; Fig. 3).

Multivariate analyses demonstrated that primary tumors with c-MET-high (hazards ratio [HR], 1.628; 95% confidence interval [95% CI], 1.011–2.620 for c-MET-high *vs* c-MET-low), hepatic resection for synchronous disease (HR, 2.410; 95% CI, 1.497–3.881 for synchronous *vs* metachronous resection), liver metastases H3 (HR, 5.090; 95% CI, 1.162–22.293 for H3 *vs*

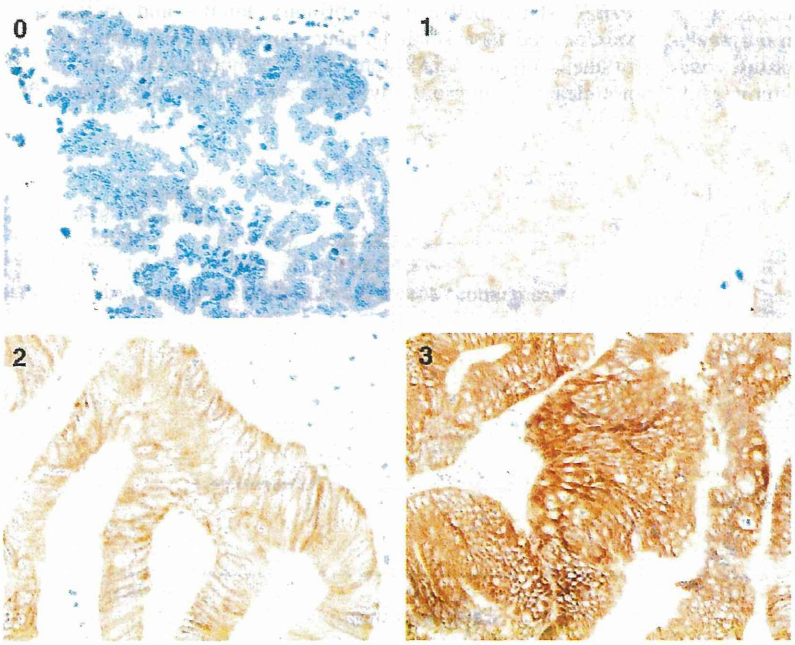

Fig. 1. Representative images of c-MET expression: 3, strongly positive immunostaining; 2, medium positive immunostaining; 1, negative staining with focally very weak immunoreactivity; 0, no membranous reactivity or only interstitial or cytoplasmic reactivity in any tumor cell.

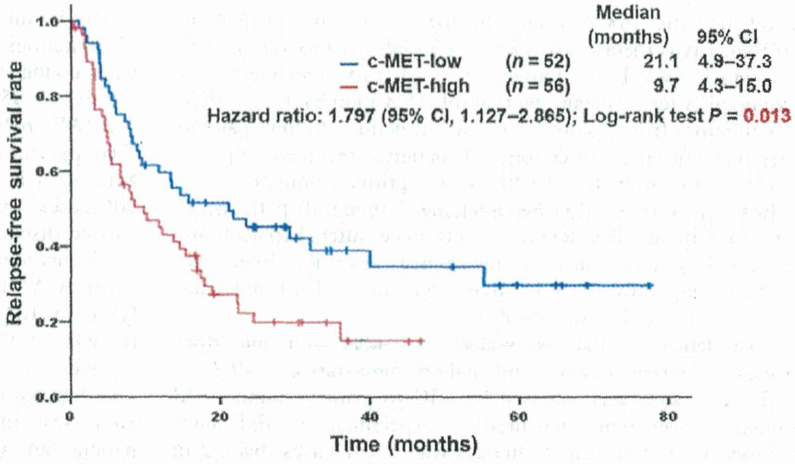

Fig. 2. Relapse-free survival curve calculated using the Kaplan–Meier method for groups classified according to the c-MET expression level in primary tumors.

H1) and *KRAS* mutation (HR, 1.852; 95% CI, 1.145–2.996 for mutation *vs* wild) were associated with worse RFS (Table 2).

Discussion

In the present study, we observed a change in the expression of c-MET from primary tumors to paired liver metastases in 37 of the 108 evaluated CRC patients (overall disagreement, 34.3%). Of the 37 patients, 20 (18.5%) changed from c-MET-low to c-MET-high, while 17 (15.7%) changed from c-MET-high to c-MET-low. With respect to the mutations of *KRAS*, *BRAF* and *PIK3CA*, the mutational status of the matched pairs was comparatively highly concordant (≥87.7% concordance). In addition, a high expression of c-MET in primary tumors was associated with worse RFS for patients who had undergone curative hepatectomy.

Some previous studies have analyzed c-MET protein expression in primary CRC and metastases. Two studies showed that c-MET protein expression tended to be decreased in distant metastases compared to their corresponding primary tumors.[5,17] In contrast, Voutsina *et al.*[18] observed that c-MET expression tended to be increased in distant metastases compared to their corresponding primary tumors. In our analysis, we found that c-MET expression in liver metastases was slightly increased compared to that in primary tumors. In breast cancer, neoadjuvant chemotherapy seems to affect the status of receptors such as ER, PR and HER2.[19,20] In our study, of the 14 patients who received adjuvant chemotherapy after the resection of the primary tumors, 2 (14.3%) altered the status of c-MET expression. Chemotherapy may be able to kill sensitive cells and leave behind the more resistant clones. However, our small numbers of individual c-MET expression concordance provided only limited insight.

We also assessed the concordance of genetic mutation status between primary tumors and paired metastases. Genetic testing of patients with CRC for somatic mutations in *KRAS* is usually used clinically to help make decisions about therapy in the metastatic setting. There is also emerging evidence that mutations in *BRAF* and *PIK3CA* are associated with resistance to epidermal growth factor receptor–targeted agents.[13–15] A high genetic mutational concordance in *KRAS*, *BRAF* and *PIK3CA* have been found in the literature.[21–23] In our study, the concordance in genetic mutational status was almost equal to that in the reported findings. The implication of these results is that both tissue of primary tumors and liver metastases may be used for testing of these mutations. A discordant *KRAS* and *PIK3CA* status between the primary tumors and metastases was observed in a small number of patients. The mechanism of the discordant *KRAS* and *PIK3CA* mutational status is as not clear.[24–26] In our study, there was no case of discordance

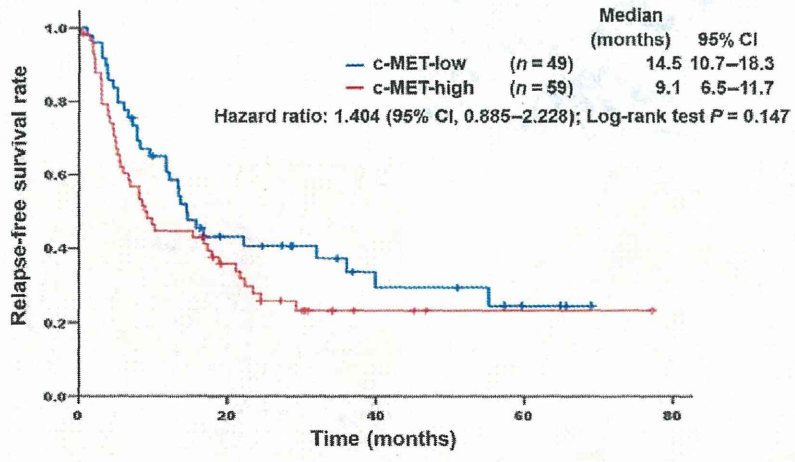

Fig. 3. Relapse-free survival curve calculated using the Kaplan–Meier method for groups classified according to the c-MET expression level in liver metastases.

Table 2. Univariate and mulitvariaite Cox regression analyses for RFS

Parameter	RFS Hazard ratio (95% CI)	*P*
Univariate Cox regression analysis		
Sex		
Male	1 (reference)	
Female	1.482 (0.939–2.340)	0.91
Age (years)		
≤63	1 (reference)	
>63	0.901 (0.572–1.420)	0.65
Primary tumor location		
Colon	1 (reference)	
Rectum	1.264 (0.793–2.106)	0.32
Primary tumor		
Node-negative	1 (reference)	
Node-positive	1.344	0.25
Liver metastases		
Metachronous	1 (reference)	
Synchronous	2.363 (1.494–3.739)	0.0002
Liver metastases		
H1 (number ≤4 and size ≤5 cm)	1 (reference)	
H2 (other)	1.069 (0.488–2.341)	0.87
H3 (number ≥5 and size >5 cm)	5.187 (1.233–21.812)	0.02
Histology		
Well + moderately	1 (reference)	
Poorly	2.126 (0.77–5.864)	0.15
Expression of c-MET in primary tumor		
c-MET-low	1 (reference)	
c-MET-high	1.797 (1.127–2.865)	0.01
Expression of c-MET in liver metastases		
c-MET-low	1 (reference)	
c-MET-high	1.404 (0.885–2.228)	0.15
CEA		
<5	1 (reference)	
≥5	0.876 (0.549–1.395)	0.58
CA19-9		
<37	1 (reference)	
≥37	1.272 (0.778–2.082)	0.34
KRAS status		
Wild	1 (reference)	
Mutation	1.627 (1.020–2.596)	0.04
BRAF status		
Wild	1 (reference)	
V600E mutaiton	1.274 (0.513–3.164)	0.60
PIK3CA status		
Wild	1 (reference)	
Exon 9 mutation	0.853 (0.409–1.779)	0.67
Multivariate Cox-regression analysis		
Liver metastases		
Metachronous	1 (reference)	
Synchronous	2.404 (1.486–3.889)	0.0004
Liver metastases		
H1 (number ≤4 and size ≤5 cm)	1 (reference)	
H2 (other)	0.860 (0.388–1.905)	0.79
H3 (number ≥5 and size >5 cm)	5.090 (1.162–22.293)	0.03
Expression of c-MET in primary tumor		
c-MET-low	1 (reference)	
c-MET-high	1.645 (1.014–2.668)	0.04
KRAS status		
Wild	1 (reference)	
Mutation	1.906 (1.163–3.123)	0.01
BRAF status		
Wild	1 (reference)	
V600E mutation	0.933 (0.359–2.426)	0.89
PIK3CA status		
Wild	1 (reference)	
Exon 9 mutation	0.729 (0.342–1.551)	0.41

RFS, relapse-free survival.

between the genetic mutational status before and after adjuvant chemotherapy. Therefore, the discordant results may be related to heterogeneity within primary tumors, or the development of mutations during the process of metastases.

Liver metastases affect approximately 30% of patients with CRC and determine its prognosis. Subgroups with advanced age, comorbid disease and synchronous hepatic and colon resection may have higher procedure-related mortality and worse long-term outcomes.[27] In our analysis, a high c-MET expression in the primary tumors, but not in the liver metastases, was associated with shorter RFS. To our knowledge, this report is the first to investigate the correlation between c-MET expression status in primary tumors with that in liver metastases, and RFS in such a population. Therefore, the reason for this discordance is uncertain. High tumor recurrence rates following hepatectomy in patients with colorectal liver metastases have been linked to the upregulation of growth factors required for liver regeneration. A recent preclinical study suggested that upregulation of c-MET after hepatectomoy stimulates growth of liver metastases.[28] Another study showed that increased expression of c-MET was observed when tumor cells escape from the primary tumors and start circulating in the bloodstream.[29] The increased plasma levels of HGF after liver resection may stimulate the growth of circulating CRC cells derived from primary tumors, which would result in increased expression of c-MET through activation of the HGF/c-MET pathway. This pathway includes the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase/AKT pathways, STAT3, RAC1 and the NF-κB pathway. At metastatic sites, CRC cells may change their biology, possibly due to different signals from the new microenvironment, and may be insignificantly affected by HGF. However, our study is limited by its insufficient sample size, so further research is needed to clarify this clinical question.

Our study showed that patients with *KRAS* mutations had a negative prognostic effect in recurrence of CRC after metastasectomy. However, the prognostic impact of *KRAS* status in patients with CRC is controversial. Thus, some studies demonstrate that *KRAS* mutations seem not to correlate with the prognosis of patients with CRC.[30] Moreover, no prognostic effect has been found in studies investigating the influence of *KRAS* mutations in patients undergoing liver resection.[31] By contrast, the RASCAL II study, which is so far the largest study to examine the impact of a mutation in *KRAS* on the outcome of patients with CRC, revealed that patients with *KRAS* mutations had a statistically poor outcome in terms of the risk of recurrence and death.[32] *KRAS* mutations, in particular, the presence of a codon 12 glycine to valine mutation, influenced progression. In our study, we could not analyze this effect because a G12V mutation was present in only 15

patients, and there were fewer other mutations. Further investigation is necessary on the relationship between *KRAS* status and prognosis.

In conclusion, the current study demonstrated that a high level of genetic concordance in *KRAS*, *BRAF* and *PIK3CA* between primary tumors and liver metastases, and high expression of c-MET in primary tumors increased the risk for tumor recurrence after hepatic metastasectomy.

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Disclosure Statement

The authors have no conflict of interest to declare.

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Clinicopathological features and prognostic roles of KRAS, BRAF, PIK3CA and NRAS mutations in advanced gastric cancer

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Abstract

Background: RAS-RAF-MEK-ERK and PI3K-AKT pathways form a significant cascade for potential molecular target therapy in advanced cancer. The clinical significance of mutations in these genes in advanced gastric cancer (AGC) is uncertain.

Methods: We collected formalin-fixed, paraffin-embedded and fresh frozen tumor samples from AGC patients and analyzed the *KRAS, NRAS, BRAF* and *PIK3CA* mutations by direct-sequencing. We retrospectively investigated the clinicopathological features of these mutations in AGC patients, and selected patients with metastatic gastric cancer.

Results: Among 167 AGC patients, mutations of *KRAS* codons 12/13 ($N = 8/164$, 4.9%), *PIK3CA* ($N = 9/163$, 5.5%), and *NRAS* codon 12/13($N = 3/159$, 1.9%) were detected. Comparison of the clinicopathological features of the mutated *KRAS, PIK3CA, NRAS* genes with an all-wild type of these genes showed that the frequency of the intestinal type was significantly higher in patients whose tumor tissue contained *KRAS* mutations ($P = 0.014$). Among 125 patients with metastatic gastric cancer, patients with *NRAS* codon 12/13 mutations in their tumors had shorter overall survival compared with *NRAS* wild-type patients (MST: 14.7 vs 8.8 months, $P = 0.011$). By multivariate analyses, *NRAS* codon 12/13 mutation was an indicator for poor prognosis in patients with metastatic gastric cancer (adjusted HR 5.607, 95% CI: 1.637-19.203).

Conclusions: Our study indicated that mutations of *KRAS, PIK3CA* and *NRAS* were rare in AGC. *NRAS* mutations were likely to associate with poor prognosis in metastatic state of AGC patients, but further validation of other research is required.

Background

Gastric cancer is the second leading cause of cancer death worldwide with approximately 989,600 new cases and 738,000 deaths per year, accounting for about 8 percent of new cancers [1]. The highest incidence rates are in Eastern Asia, the Andean regions of South America, and Eastern Europe, while the lowest rates are in North America, Northern Europe, and most countries in Africa and South Eastern Asia.

Owing to development of systemic chemotherapy, the survival time for advanced gastric cancer (AGC) has been improved during the past decade. A fluoropyrimidine and platinum regimen is a standard first-line chemotherapy in HER2-negative metastatic gastric cancer (mGC) patients, and trastuzumab added to XP is a standard chemotherapy in HER2-positive mGC patients in Japan [2-5]. Although some AGC patients obtained clinical benefit of systemic chemotherapy, most of the patients did not attain a clinically satisfactory outcome. Novel treatment of mGC with more effective and less toxic chemotherapy regimens was required.

Phase III trials of molecular therapy with mTOR inhibitor, anti-VEGF antibody, anti-EGFR antibodies were reported in AGC or gastro-esophageal cancer, but these drugs could not be demonstrated to have significant efficacy [6,7]. Recently, ramcirumab, anti-VEGFR target monoclonal antibody, was

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