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Original Article

Immunostaining by dual tumor tissue paraffin blocks increases the sensitivity of c-Met detection in gastric cancer

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Abstract: Background: Gastric cancer (GC) is the second most frequent cause of cancer deaths worldwide. c-Met, a receptor tyrosine kinase, transduces signals from extracellular growth factors. c-Met-targeted therapeutics hold a great potential in treating gastric and related cancers, and a precise evaluation of c-Met expression is a prerequisite for subsequent treatment. Methods: We compared the sensitivity between one and two paraffin blocks in evaluating c-Met expression in GC subjects by immunohistochemistry (IHC). A total of 365 GC patients were divided into cohort 1 (n = 206) for the one tumor tissue paraffin block test and cohort 2 (n = 159) for the dual tumor tissue paraffin block test. In the dual blocks group, we investigated the results from two different paraffin blocks, then we used the higher one as the final score. Results: Inconsistent c-Met expression in the dual paraffin blocks group occurred in 29 (18.2%) cases. The pooled data in cohort 1 and cohort 2 indicate that when using results from dual paraffin blocks, the c-Met positive (3+) rate of GC testing could be promoted. Conclusion: In GC, using dual tumor tissue paraffin blocks instead of one tumor tissue paraffin block is an efficient, economical and practical method of minimizing the false-negative rate of c-Met status assessment by IHC.

Keywords: Gastric cancer, c-Met, dual paraffin blocks, immunohistochemistry

Introduction

Gastric cancer (GC) is a common type of cancer and the second most frequent cause of cancer deaths worldwide [1]. This cancer continues to have a poor prognosis and few efficacious therapeutic options, particularly in its advanced stages, although mortality rates for GC are decreasing [2]. Because cancer in its advanced stages has such a poor prognosis, new treatment strategies were urgently needed, especially targeted therapy. Adenocarcinoma is the predominant histological type of GC (95% of tumors), and the sub-types are the intestinal, diffuse, and mixed types, based on the Laurén classification. However, intra-tumoral heterogeneity should be taken into account. For example, the intra-tumoral heterogeneity of Her2/neu protein expression within tumors has been observed by many pathologists [3, 4]. Also, the intra-tumoral heterogeneity in GC is much more significant than it is in breast cancer.

The oncogene c-Met is one of the most studied factors of cancer-associated receptors and pathways. c-Met is a tyrosine kinase, a cell-surface receptor for the hepatocyte growth factor (HGF, also known as scatter factor) involved in regulating cell proliferation, apoptosis and migration [5, 6]. In the defined stages of embryogenesis and organogenesis, c-Met activity can be detected [7]. The abnormal amplification of the c-Met gene and/or the overexpression of the c-Met protein have been verified in many human carcinomas, including gastric, breast, colorectal, liver and renal cancer [8]. There are effective methods available now for the detection of c-Met protein over-expression and gene amplification, which include immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and others. A significant correlation has been found between the c-Met IHC score and c-Met FISH results [9]. c-Met elevations have been shown to be associated with poor clinical outcomes [8, 10, 11]. A

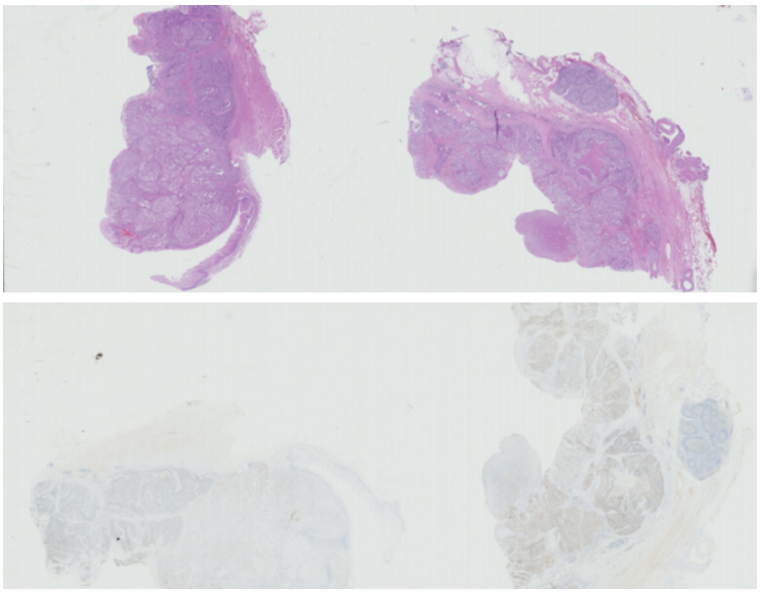

Figure 1. The entire view of one slide using two paraffin blocks assessing c-Met expression by IHC. The c-Met expression levels were 1+ and 2+.

series of new drugs for the various targets of the c-Met pathways have been developed. The HGF/SF monoclonal antibody is one of the most promising inhibitors that works towards c-Met pathways [12]. Rilotumumab combined with chemotherapy is a promising strategy for late c-Met-positive GC [13]. SAR125844 inhibits tumors in a dose-dependent way in GC models in which c-Met is amplified in [14]. Simm530 inhibits c-Met phosphorylation in a dose-dependent way and can also inhibit tumor growth in c-Met-driven GC xenografts [15]. Luteolin can also inhibit tumor growth in patient-derived human tumor xenograft models of c-Met-amplified GC [16].

At present, the heterogeneity of c-Met expression in GC has also been observed [9]. Usually, because there is no standard guideline for the c-Met IHC assay, pathologists detect c-Met expression with one paraffin block of GC tissue for practical and economic reasons. Assessing specimens from two paraffin blocks of the same patient could reduce the false-negative rate of c-Met assessment due to intra-tumoral heterogeneity. In our laboratory, two specimens are fit onto a single slide for c-Met IHC (**Figure 1**). In this study, we assessed the reliability of using two paraffin blocks from the same patient to estimate the c-Met expression level by comparing the positive rate between one cohort using one paraffin block and one cohort using two tumor tissue paraffin blocks.

Materials and methods

Patients and clinicopathological information collection

Between December 2013 and May 2014, a total of 365 patients who underwent curative surgery at the Zhongshan Hospital for primary gastric adenocarcinoma and who had no preoperative treatment were included in this study. Those patients were divided into a cohort using one tumor tissue paraffin block (cohort 1, n = 206) and a cohort using two tumor tissue paraffin blocks (cohort 2, n = 159) when evaluating c-Met expression status by IHC. All patients provided prior written informed consent and this study protocol received ethics board approval at the Zhongshan hospital, Fudan University (No. B2013-057). The following patient characteristics were collected after the diagnosis of GC was confirmed by histology in all cases: age, gender, histologic type (adenocarcinoma only based on World Health Organization [WHO] criteria), Laurén classification, tumor site (proximal and distal), histological grade, microscopic tumor extension, lymphatic or venous invasion, lymph node metastasis, and pTNM stage (according to seventh edition of the UICC guidelines).

H&E and IHC staining

The H&E staining was performed as before [17]. The sections were stained with hematoxylin and eosin, then reviewed by two pathologists to confirm the diagnosis of GC. Trained pathologists selected paraffin blocks from each case for the following IHC. The IHC assays were performed using the c-Met rabbit monoclonal antibody (Cell Signaling Technology, Clone number: EP1454Y) with the iView DAB Detection Kit (Ventana) on a BenchMark XT automated staining system (Ventana). The IHC assay was performed as before [17].

Evaluation of c-Met expression

The c-Met status was assessed by two independent observers. If there was any discrepan-

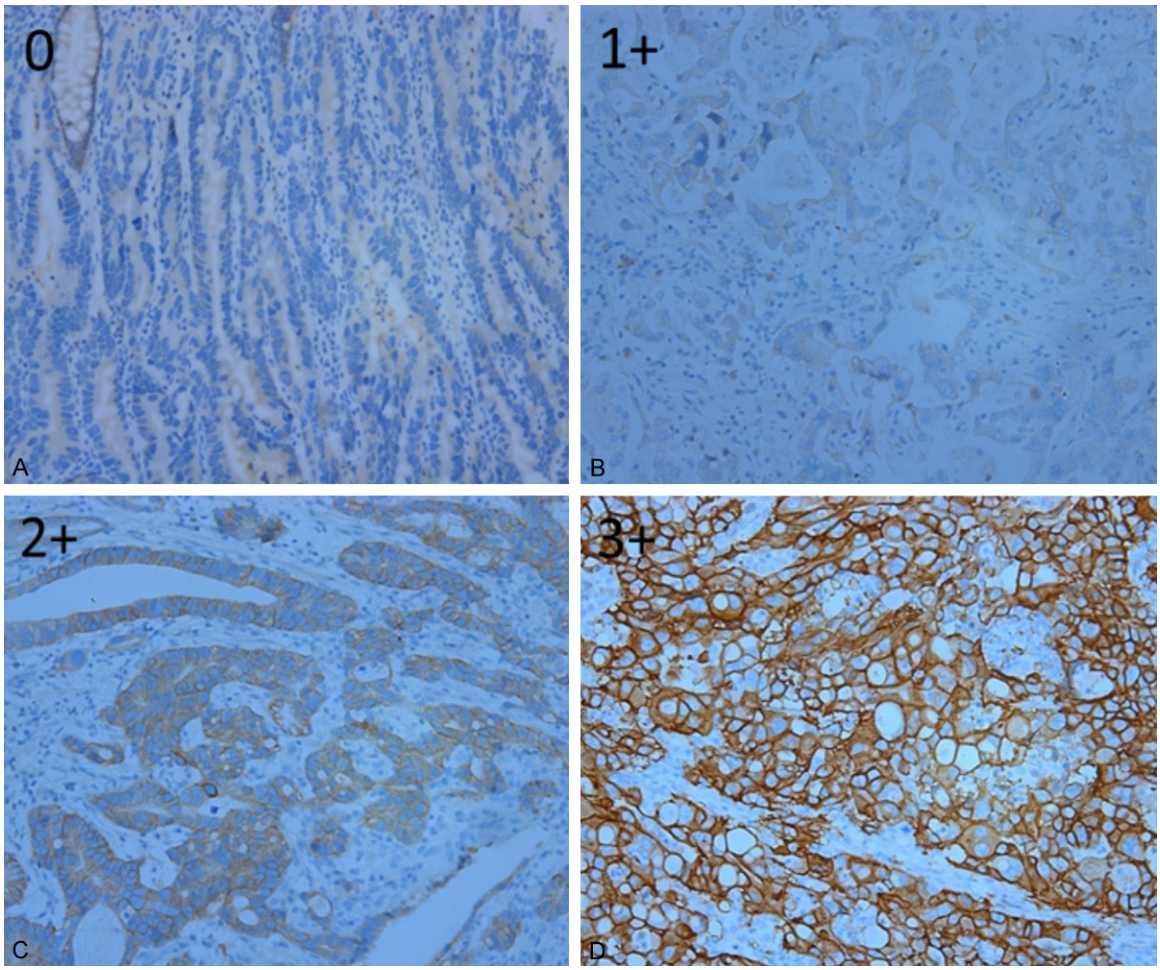

Figure 2. An example of the c-Met expression pattern detected by IHC. (A) c-Met expression was completely negative (IHC 0). (B) c-Met expression was immunostained at level 1+. (C) c-Met expression was immunostained at level 2+. (D) c-Met expression was immunostained at level 3+. Original magnifications ×20 (A-D).

cy, the c-Met status was confirmed by a three observer-based discussion. All of the observers were blinded with regard to the clinicopathological characteristics of the patients. In the present study, the evaluation of the immunostaining results was performed by examining the stain intensity and stain area (double scoring system) according to a published method with a previous modification [18]. Briefly, the extent of positivity was scored as 0 when no positive cells were observed; 1 when the percentage of positive cells was < 35%; 2 when the percentage of positive cells was 35-75%; and 3 when the percentage of positive cells was > 75%. The intensity was scored as 0 when no positive cells were identified; 1, when weak staining cells were identified; 2, when moderate staining cells were identified; and 3, when strong staining positive cells were identified. Multiplying the extent by intensity gave the following immunohistochemical staining grades as 0, 1, 2, 3, 4, 6, and 9. For statistical analyses, grade 0 was considered as no staining, grades 1 and 2, as weak staining (1+), grades 3 and 4, as moderate staining (2+), and grades 6 and 9, as strong staining (3+) (**Figure 2**). We classified c-Met overexpression 3+ staining as c-Met-positive (3+). When classified c-Met expression status in cohort 2, we combined the results from two different paraffin blocks and chose the higher one as the final c-Met score.

Statistical analysis

A χ^2 test was used for the univariate analysis; cross-tabulations with qualitative variables were analyzed with the Pearson χ^2 test. A *P*-value < 0.05 was recorded as statistically sig-

Table 1. Clinicopathological features of the GC patients

	Cohort1			Cohort2		
	Clinical features	c-Met 3+, n (%)	*P* value	Clinical features	c-Met 3+ n (%)	*P* value
Patients (n)	206	39		159	29	
Age (years)						
Mean ± SD	60.8 ± 10.6			61.7 ± 10.2		
Median	61			61		
Gender, n (%)						
Men	149 (72.3)	31 (79.5)		127 (79.8)	28 (84.8)	
Women	57 (27.7)	8 (20.5)	0.323	32 (20.2)	5 (15.2)	0.626
Laurén phenotype, n (%)			0.092			0.431
Intestinal	79 (38.4)	19 (48.7)		77 (48.4)	19 (57.6)	
Diffuse	54 (26.2)	5 (12.8)		34 (21.4)	5 (15.2)	
mixed	73 (35.4)	15 (38.5)		48 (30.2)	9 (27.3)	
Intestinal vs Diffuse			0.029*			0.395
Intestinal vs mixed			0.605			0.669
Diffuse vs mixed			0.081			0.424
Diffuse vs Intestinal and mixed			0.035*			0.327
Localization, n (%)						
Proximal	62 (30.1)	14 (35.9)		66 (41.5)	16 (48.5)	
Distal	144 (69.9)	25 (64.1)	0.38	93 (58.5)	17 (51.5)	0.361
pT-category, n (%)						
pT1a	34 (16.5)	7 (17.9)		19 (11.9)	1 (3.1)	
pT1b	31 (15.0)	8 (20.5)		19 (11.9)	8 (24.2)	
pT2	22 (10.7)	4 (10.3)		34 (21.4)	8 (24.2)	
pT3	49 (23.8)	11 (28.2)		35 (22.1)	8 (24.2)	
pT4a	68 (33.0)	9 (23.1)		49 (30.8)	7 (21.2)	
pT4b	2 (1.0)	0 (0)	0.65	3 (1.9)	1 (3.1)	0.057
pN-category, n (%)						
pN0	90 (43.7)	21 (53.8)		65 (40.8)	15 (45.5)	
pN1	27 (13.1)	4 (10.3)		26 (16.4)	7 (21.2)	
pN2	38 (18.4)	7 (17.9)		33 (20.8)	5 (15.2)	
pN3a	33 (16.0)	6 (15.4)		14 (8.8)	2 (6.1)	
pN3b	18 (8.8)	1 (2.6)	0.283	21 (13.2)	4 (12.2)	0.766
Lymphatic or venous invasion, n (%)						
pL/V0	122 (59.2)	22 (56.4)		94 (59.1)	21 (63.6)	
pL/V1	84 (40.8)	17 (43.6)		65 (40.9)	12 (36.4)	
UICCstage(7th edition), n (%)						
IA	50 (24.3)	12 (30.8)		27 (16.9)	7 (21.2)	
IB	10 (4.9)	2 (5.1)		20 (12.6)	3 (9.1)	
IIA	10 (4.9)	3 (7.7)		12 (7.6)	3 (9.1)	
IIB	15 (7.3)	1 (2.6)		9 (5.7)	2 (6.1)	
IIIA	8 (3.9)	0 (0)		15 (9.4)	4 (12.1)	
IIIB	14 (6.8)	3 (7.7)		6 (3.8)	1 (3.0)	
IIC	13 (6.3)	1 (2.6)		6 (3.8)	1 (3.0)	
IVIV	86 (41.6)	17 (43.6)		64 (40.2)	12 (36.4)	
Resected lymph nodes						
Mean ± SD	33.3 ± 13.7	34.3 ± 12.5		32.6 ± 13.9	30.2 ± 17.7	
Median, n	31	32		29	26	
Positive lymph node						
Mean ± SD	8.8 ± 9.0	7.1 ± 6.4		8.5 ± 8.7	8.7 ± 10.2	
Median, n	6	5		5	4	
Positive lymph node ration						
Mean ± SD	0.27 ± 0.23	0.24 ± 0.11		0.23 ± 0.26	0.34 ± 0.21	
Median, n	0.12	0.16		0.13	0.14	

*P < 0.05.

Table 2. Comparison of c-Met protein expression status between the single-block group and the dual-block group

	c-Met immunostaining						
	Cohort 1	*P* value	Cohort 2				
			The first paraffin block	*P* value	The second paraffin block	*P* value	Combination
0, n (%)	22 (10.7)		12 (7.6)		14 (8.9)		13 (8.2)
1+, n (%)	91 (44.2)		76 (47.8)		74 (46.5)		66 (41.5)
2+, n (%)	54 (26.2)		42 (26.4)		44 (27.7)		47 (29.6)
3+, n (%)	39 (18.9)	0.380	29 (18.2)	0.336	27 (16.9)	0.237	33 (20.8)
Total	206		159		159		159

Table 3. Consistency and inconsistency of c-Met expression pattern in the dual-block group

	Consistency, n (%)
Consistent	131 (82.4)
Inconsistent	28 (17.6)
0 v.s. 1+	2 (0.6)
0 v.s. 2+	0 (0)
0 v.s. 3+	0 (0)
1+ v.s. 2+	17 (10.7)
1+ v.s. 3+	1 (0.6)
2+ v.s. 3+	9 (5.7)
Total	159

nificant. All analyses were performed using the statistical package SPSS version 19.0 (SPSS, Inc., an IBM Company, Chicago, IL, USA). No adjustments were made.

Results

Characteristics of GC patients

A total of 365 GC patient surgical samples were obtained from primary tumors. Clinicopathological features of the GC patients are presented in **Table 1**. We enrolled 206 patients in cohort 1 (single block group) and 159 patients in cohort 2 (dual blocks group). Of the patients in cohort 1, the age of the patients ranged from 31 to 86 years with a median of 61 years of age. 149 (72.3%) patients were male and 57 (27.7%) were female. An intestinal type GC was found in 79 (38.4%), a diffuse type in 54 (26.2%), and a mixed type in 73 (35.4%) patients according to the Laurén classification. Venous or lymphatic invasion was found in 84 (40.8%) patients. 127 (61.6%) were graded as poorly cohesive and 79 (38.4%) were non-poorly cohesive based on cell cohesive status (**Table 1**).

In the dual blocks group, the age of the patients ranged from 34 to 84 years, with a median of 61 years of age. 127 (79.8%) patients were male and 32 (20.2%) were female. 77 (48.4%) patients had intestinal type GC, 34 (21.4%) patients had the diffuse type, and 48 (30.2%) patients had the mixed type according to the Laurén classification. 82 of the patients (51.6%) were poorly cohesive and 77 (48.4%) were non-poorly cohesive. Venous or lymphatic invasion was found in 65 (40.9%) patients (**Table 1**).

c-Met protein expression status by IHC

In the one block cohort, GC tumor tissues which c-Met scored as 0 for IHC were 22 (10.7%), tumor tissues which c-Met scored as 1+, 2+ and 3+ were 91 (44.2%), 54 (26.2%), and 39 (18.9%), respectively (**Table 2**). In the first paraffin block group of cohort 2, tumor tissues which c-Met scored as 0, 1+, 2+ and 3+ for IHC were 12 (7.6%), 76 (47.8%), 42 (26.4%) and 29 (18.2%), respectively (**Table 2**). In the second paraffin block group of cohort 2, the c-Met immunostaining scored as 0, 1+, 2+ and 3+ are 14 (8.9%), 74 (46.5%), 44 (27.7%), and 27 (16.9%), respectively (**Table 2**).

We found it noteworthy that, in cohort 2, the c-Met expression status of one paraffin block varied from its partner in some GC specimens. Consistent c-Met expression in dual paraffin blocks presented in 131 (82.4%) cases; however, an inconsistent c-Met expression in the dual paraffin blocks occurred in 28 (17.6%) cases in cohort 2 (**Table 3**).

Promoted c-Met-positive (3+) rate by using dual paraffin blocks

We compared the results of c-Met immunostaining between the two cohorts. The combined data in cohort 2 of c-Met immunostaining for

1+, 2+ and 3+ were 66 (41.5%), 47 (29.6%) and 33 (20.8%), respectively (**Table 2**). The pooled data from 206 cases in cohort 1 and 159 cases in cohort 2 shown in **Table 2** indicate that when using dual paraffin blocks, the c-Met-positive (3+) rate could be promoted in GC. The c-Met-positive (3+) rate of cohort 2 is 20.8% compared to 18.9% in cohort 1 ($P > 0.05$) (**Table 2**). In the dual blocks cohort, the c-Met-positive (3+) rate was increased from 18.2% and 16.9%, when evaluating only one paraffin block, to 20.8% ($P > 0.05$).

Correlation between c-Met expression and Laurén classification

A total of 39 (18.9%) GCs were classified as c-Met-positive (3+) in cohort 1 as were 33 (20.8%) GCs in cohort 2 (**Table 1**). The relationship of the c-Met expression status with the Laurén classification parameters is shown in **Table 1**. In cohort 1, c-Met-positive (3+) GC was significantly associated with intestinal type ($P = 0.029$) and non-diffuse type ($P = 0.035$) GC, but there were no such significant associations in cohort 2. Moreover, no statistically significant differences in the c-Met-positive (3+) rates by gender, lymphatic or venous invasion were found for either cohort ($P > 0.05$) (**Table 1**).

Discussion

The HGF-c-Met axis is frequently dysregulated in cancer by c-Met gene amplification, translocation, and mutation, or by the overexpression of c-Met or HGF. c-Met overexpression has been reported in a number of human primary tumors, including gastric, breast, colorectal, liver, and renal cancer. c-Met plays an important role in tumor development, metastasis and prognosis.

As the only receptor of HGF, the activation and overexpression of c-Met kinase results in the activation of downstream signaling, such as the RAS, PICK3, Beta-catenin and NOTCH pathways. The RAS pathway mediates scattering and proliferation signals which lead to branch morphogenesis [19]. PICK3 pathways activate either directly or downstream through the RAS pathway [20]. The Beta-catenin pathway participates in the transcriptional regulation of numerous genes while the NOTCH pathway activates through the Delta ligand [21, 22], leading to changes in gene expression and cell behavior, such as increased proliferation, survival, motility, invasiveness, and the stimulation of angiogenesis.

Although palliative chemotherapy has been shown to prolong survival and improve quality of life, the survival of advanced gastric cancer (AGC) patients remains poor. With the advent of targeted therapy, many molecular targeted agents have been evaluated in clinical studies. Trastuzumab, an anti-HER2 monoclonal antibody, has shown activity against HER2-positive AGC and became the first targeted agent approved for AGC [23].

Hence, it has been proposed that targeting the c-Met receptor by novel biological agents will inhibit cancer progression at the molecular level. Several different strategies are being explored to reach this goal, including the development of competitors of MET/HGF, such as NK4, Tivantinib [24, 25], monoclonal antibodies directed against HGF and c-Met, such as AMG-102 (rilotumumab), 0A5D5 (onartuzumab) [26, 27], and small-molecule tyrosine kinase inhibitors directed against c-Met, such as INC-280, EMD 1214063, GSK1363089 [28-30]. c-Met inhibitors that are currently in clinical trials include Cabozantinib (XL184) and Foretinib, which was approved by the U.S. FDA in November 2012 for the treatment of medullary thyroid cancer [31].

The evidence supporting intra-tumoral heterogeneity in GC is accumulating. For example, the heterogeneity of HER2 gene amplification within a tumor is considered to be an important potential cause of treatment failure by molecular analysis-based targeted therapy [32-34]. A heterogeneous Her2/neu immunostaining has a major impact on the Her2/neu status classified in a specimen [35]. However, in GC, c-Met heterogeneity has not been researched extensively. To clarify this issue, we collected 206 cases using one tumor tissue paraffin block of primary GCs (cohort 1) and 159 cases using dual tumor tissue paraffin block (cohort 2) and performed c-Met analysis by IHC.

Four sections of GC were recommended following the guidelines for the handling of surgical specimens in Rosai and Ackerman's *Surgical Pathology* (10th Edition). In routine diagnosis, it

is not cost-effective and practical to evaluate the c-Met IHC status of all the four sampled paraffin blocks. However, picking two paraffin blocks for evaluation on one slide is an economical, practical and efficient method. The two selected tissue paraffin blocks can be put on one slide to save on the cost of extra IHC reagents [17].

In this study, we promoted the c-Met-positive (3+) rate from 18.9% to 20.8% in GC (**Table 2**). Although this result did not reach significance, it is still meaningful because the false negative rate was minimized. A recent study reported that c-Met immunohistochemistry reflects well on gene amplification, and it could be a primary screening test for patient selection for anti-c-Met target therapy [36]. Every year, over 900 patients with resectable GC undergo curative surgery at Zhongshan Hospital, so our results would imply an addition of over 17 patients with c-Met IHC 3+ who could be a potential target therapy receiver.

The clinicopathological characteristics of the patients in both cohorts are generally balanced (**Table 1**). It has been reported that Her2/neu overexpression was more common in intestinal-type GCs compared with diffuse-type GCs [35, 37]. In this study, we found that samples with c-Met-positive (3+) were significantly associated with intestinal type GC ($P = 0.029$) in one paraffin block group (**Table 1**), indicating a potential underlying correlation between Her2/neu overexpression and c-Met amplification.

Conclusions

In routine diagnosis, using dual tumor tissue paraffin blocks in assessing c-Met expression status is an efficient, economical and practical method for the c-Met evaluation of GC. It can be executed without great additional effort but gives a more accurate c-Met evaluation result. A better c-Met positive rate provides beneficial effects for GC patients.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.

[3] Albarracin C, Edgerton ME, Gilcrease MZ, Huo L, Krishnamurthy S, Middleton LP, Resetkova E, Sahin AA, Sneige N, Fraser WS, Wu Y. Is it too soon to start reporting HER2 genetic heterogeneity? Arch Pathol Lab Med 2010; 134: 162-163; author reply 163.

[4] Seol H, Lee HJ, Choi Y, Lee HE, Kim YJ, Kim JH, Kang E, Kim SW, Park SY. Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance. Mod Pathol 2012; 25: 938-948.

[5] Park WS, Oh RR, Kim YS, Park JY, Shin MS, Lee HK, Lee SH, Yoo NJ, Lee JY. Absence of mutations in the kinase domain of the Met gene and frequent expression of Met and HGF/SF protein in primary gastric carcinomas. APMIS 2000; 108: 195-200.

[6] Xu Y, Peng Z, Li Z, Lu M, Gao J, Li Y, Shen L. Expression and clinical significance of c-Met in advanced esophageal squamous cell carcinoma. BMC Cancer 2015; 15: 6.

[7] Lee JH, Han SU, Cho H, Jennings B, Gerrard B, Dean M, Schmidt L, Zbar B, Vande Woude GF. A novel germ line juxtamembrane Met mutation in human gastric cancer. Oncogene 2000; 19: 4947-4953.

[8] Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nat Rev Drug Discov 2008; 7: 504-516.

[9] Ye P, Zhang M, Fan S, Zhang T, Fu H, Su X, Gavine PR, Liu Q, Yin X. Intra-tumoral heterogeneity of HER2, FGFR2, cMET and ATM in gas-

tric cancer: optimizing personalized healthcare through innovative pathological and statistical analysis. PLoS One 2015; 10: e0143207.
[10] Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol 2003; 4: 915-925.
[11] Stella GM, Benvenuti S, Comoglio PM. Targeting the MET oncogene in cancer and metastases. Expert Opin Investig Drugs 2010; 19: 1381-1394.
[12] Gordon MS, Sweeney CS, Mendelson DS, Eckhardt SG, Anderson A, Beaupre DM, Branstetter D, Burgess TL, Coxon A, Deng H, Kaplan-Lefko P, Leitch IM, Oliner KS, Yan L, Zhu M, Gore L. Safety, pharmacokinetics, and pharmacodynamics of AMG 102, a fully human hepatocyte growth factor-neutralizing monoclonal antibody, in a first-in-human study of patients with advanced solid tumors. Clin Cancer Res 2010; 16: 699-710.
[13] Doshi S, Gisleskog PO, Zhang Y, Zhu M, Oliner KS, Loh E, Perez Ruixo JJ. Rilotumumab exposure-response relationship in patients with advanced or metastatic gastric cancer. Clin Cancer Res 2015; 21: 2453-2461.
[14] Egile C, Kenigsberg M, Delaisi C, Begassat F, Do-Vale V, Mestadier J, Bonche F, Benard T, Nicolas JP, Valence S, Lefranc C, Francesconi E, Castell C, Lefebvre AM, Nemecek C, Calvet L, Goulaouic H. The selective intravenous inhibitor of the MET tyrosine kinase SAR125844 inhibits tumor growth in MET-amplified cancer. Mol Cancer Ther 2015; 14: 384-394.
[15] Wang Y, Zhan Z, Jiang X, Peng X, Shen Y, Chen F, Ji Y, Liu W, Shi Y, Duan W, Ding J, Ai J, Geng M. Simm530, a novel and highly selective c-Met inhibitor, blocks c-Met-stimulated signaling and neoplastic activities. Oncotarget 2016; 7: 38091-38104.
[16] Lu J, Li G, He K, Jiang W, Xu C, Li Z, Wang H, Wang W, Teng X, Teng L. Luteolin exerts a marked antitumor effect in cMet-overexpressing patient-derived tumor xenograft models of gastric cancer. J Transl Med 2015; 13: 42.
[17] Ge X, Wang H, Zeng H, Jin X, Sujie A, Xu C, Liu Y, Huang J, Ji Y, Tan Y, Liu T, Hou Y, Qin J, Sun Y, Qin X. Clinical significance of assessing Her2/neu expression in gastric cancer with dual tumor tissue paraffin blocks. Hum Pathol 2015; 46: 850-857.
[18] Xie LQ, Bian LJ, Li Z, Li Y, Li ZX, Li B. Altered expression of E-cadherin by hepatocyte growth factor and effect on the prognosis of nasopharyngeal carcinoma. Ann Surg Oncol 2010; 17: 1927-1936.
[19] O'Brien LE, Tang K, Kats ES, Schutz-Geschwender A, Lipschutz JH, Mostov KE. ERK and MMPs sequentially regulate distinct stages of epithelial tubule development. Dev Cell 2004; 7: 21-32.
[20] Graziani A, Gramaglia D, Cantley LC, Comoglio PM. The tyrosine-phosphorylated hepatocyte growth factor/scatter factor receptor associates with phosphatidylinositol 3-kinase. J Biol Chem 1991; 266: 22087-22090.
[21] Monga SP, Mars WM, Pediaditakis P, Bell A, Mule K, Bowen WC, Wang X, Zarnegar R, Michalopoulos GK. Hepatocyte growth factor induces Wnt-independent nuclear translocation of beta-catenin after Met-beta-catenin dissociation in hepatocytes. Cancer Res 2002; 62: 2064-2071.
[22] Abounader R, Reznik T, Colantuoni C, Martinez-Murillo F, Rosen EM, Laterra J. Regulation of c-Met-dependent gene expression by PTEN. Oncogene 2004; 23: 9173-9182.
[23] Okines AF, Cunningham D. Trastuzumab in gastric cancer. Eur J Cancer 2010; 46: 1949-1959.
[24] Matsumoto K, Nakamura T. NK4 gene therapy targeting HGF-Met and angiogenesis. Front Biosci 2008; 13: 1943-1951.
[25] Kang YK, Muro K, Ryu MH, Yasui H, Nishina T, Ryoo BY, Kamiya Y, Akinaga S, Boku N. A phase II trial of a selective c-Met inhibitor tivantinib (ARQ 197) monotherapy as a second- or third-line therapy in the patients with metastatic gastric cancer. Invest New Drugs 2014; 32: 355-361.
[26] Burgess TL, Sun J, Meyer S, Tsuruda TS, Elliott G, Chen Q, Haniu M, Barron WF, Juan T, Zhang K, Coxon A, Kendall RL. Biochemical characterization of AMG 102: a neutralizing, fully human monoclonal antibody to human and nonhuman primate hepatocyte growth factor. Mol Cancer Ther 2010; 9: 400-409.
[27] Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007; 316: 1039-1043.
[28] Blumenschein GR Jr, Mills GB, Gonzalez-Angulo AM. Targeting the hepatocyte growth factor-cMET axis in cancer therapy. J Clin Oncol 2012; 30: 3287-3296.
[29] Wang L, Ai J, Shen Y, Zhang H, Peng X, Huang M, Zhang A, Ding J, Geng M. SOMCL-863, a novel, selective and orally bioavailable small-molecule c-Met inhibitor, exhibits antitumor activity both in vitro and in vivo. Cancer Lett 2014; 351: 143-150.
[30] Zhang HT, Wang L, Ai J, Chen Y, He CX, Ji YC, Huang M, Yang JY, Zhang A, Ding J, Geng MY. SOMG-833, a novel selective c-MET inhibitor,

blocks c-MET-dependent neoplastic effects and exerts antitumor activity. J Pharmacol Exp Ther 2014; 350: 36-45.
[31] Colombo JR, Wein RO. Cabozantinib for progressive metastatic medullary thyroid cancer: a review. Ther Clin Risk Manag 2014; 10: 395-404.
[32] Bozzetti C, Negri FV, Lagrasta CA, Crafa P, Bassano C, Tamagnini I, Gardini G, Nizzoli R, Leonardi F, Gasparro D, Camisa R, Cavalli S, Silini EM, Ardizzoni A. Comparison of HER2 status in primary and paired metastatic sites of gastric carcinoma. Br J Cancer 2011; 104: 1372-1376.
[33] Kim MA, Lee HJ, Yang HK, Bang YJ, Kim WH. Heterogeneous amplification of ERBB2 in primary lesions is responsible for the discordant ERBB2 status of primary and metastatic lesions in gastric carcinoma. Histopathology 2011; 59: 822-831.
[34] Tafe LJ, Janjigian YY, Zaidinski M, Hedvat CV, Hameed MR, Tang LH, Hicks JB, Shah MA, Barbashina V. Human epidermal growth factor receptor 2 testing in gastroesophageal cancer: correlation between immunohistochemistry and fluorescence in situ hybridization. Arch Pathol Lab Med 2011; 135: 1460-1465.
[35] Warneke VS, Behrens HM, Boger C, Becker T, Lordick F, Ebert MP, Rocken C. Her2/neu testing in gastric cancer: evaluating the risk of sampling errors. Ann Oncol 2013; 24: 725-733.
[36] Lee HE, Kim MA, Lee HS, Jung EJ, Yang HK, Lee BL, Bang YJ, Kim WH. MET in gastric carcinomas: comparison between protein expression and gene copy number and impact on clinical outcome. Br J Cancer 2012; 107: 325-333.
[37] Tanner M, Hollmen M, Junttila TT, Kapanen AI, Tommola S, Soini Y, Helin H, Salo J, Joensuu H, Sihvo E, Elenius K, Isola J. Amplification of HER-2 in gastric carcinoma: association with Topoisomerase IIalpha gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. Ann Oncol 2005; 16: 273-278.