Original Article

Value of c-Met for Predicting Progression of Precancerous Gastric Lesions in Rural Chinese Population

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ABSTRACT

Objective: To investigate the value of c-Met in predicting progression of precancerous gastric lesions.

Methods: A population-based study was conducted to detect the overexpression of c-Met by immunohistochemical analysis in 124 subjects with precancerous gastric lesions. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated for the association of c-Met overexpression with the risk of advanced gastric lesions.

Results: The positive rates of c-Met were 55.7% in intestinal metaplasia (IM) and 64.8% in dysplasia (DYS), respectively. Stratified analysis indicated that the proportion of c-Met overexpression was 71.4% for IM progressive group, significantly higher than that for IM persistent group (40.0%, $P<0.05$). Compared to the IM persistent group, unconditional logistic regression showed that OR of c-Met overexpression for the IM progressive group was 7.416 (95% CI: 2.084–26.398).

Conclusion: c-Met plays an important role in gastric carcinogenesis. Detection of c-Met is of value in predicting progression of precancerous gastric lesions from IM to DYS.

Key words: c-Met; Intestinal metaplasia; Dysplasia; Follow-up

INTRODUCTION

Two main histological types of gastric carcinoma have been identified: intestinal and diffuse types. The former, which is the most common in populations at high risk, is preceded by a precancerous stage, characterized by the following sequential steps: superficial gastritis (SG), atrophic gastritis, intestinal metaplasia (IM), dysplasia (DYS), and gastric carcinoma (GC)[1-5]. Our preliminary results from a prospective screening study in a high-risk population of China have provided new evidence to support this concept[6-8]. Further study is needed to elucidate molecular and genetic alterations underlying the prevalence and progression of precancerous gastric lesions.

c-Met receptor tyrosine kinase (RTK), a cell surface receptor for hepatocyte growth factor (HGF), plays an important role in the pathogenesis of a variety of malignancies[9-11]. Multiple mechanisms that confer oncogenic potential on c-Met have been identified. These include autocrine/paracrine stimulation, c-Met overexpression, genomic amplification, translocation, point mutation and alternative splicing[12-15]. Recent studies have shown that tumor cells displaying *MET* amplification, which results in receptor overexpression and ligand-independent activation, occur frequently in gastric cancer[16-18]. Our previous study suggested that the overexpression of c-Met may be an early event in gastric carcinogenesis[19]. So the aim of the present study was to assess the relationship between c-Met overexpression and evolution of precancerous lesions.

MATERIALS AND METHODS

Follow-up Population and Specimen

In 1989, we conducted an endoscopic screening survey for GC in Linqu County, a rural area in which residents had a high risk of GC, in Shandong Province,

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China. Details are described in an earlier report[6]. The endoscopic biopsy specimens were taken from seven standard sites in the stomach. The presence or absence of IM, DYS, carcinoma, and other pathological changes was recorded for each biopsy, and a global diagnosis of each case represented the most advanced lesion. The subsequent endoscopic examinations were performed respectively in 1994 and 1999 to determine the progression of precancerous gastric lesions that had been observed at baseline.

A total of 124 cases selected in our study were divided into two groups as follows: progressive group which changed to a more advanced lesions (Group A), and persistent group which showed no histological changes during 5-year follow-up period (Group B). Group A was subdivided into two groups: (1) progression from IM to DYS (n=35); and (2) progression from DYS to GC (n=27). Group B involved two groups: (1) persistence in IM (n=35); and (2) persistence in DYS (n=27). The rule for the slide selection specified the same sites to be sampled during two times of biopsy. For example, when a case's global diagnosis was DYS in 1994, we reviewed the corresponding site's lesion of 1989 survey; if it was IM, this case was included into the group that progressed from IM to DYS.

Immunohistochemistry

Slides were deparaffinized in xylene and rehydrated in graded alcohol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min. Microwave antigen retrieval was performed in citrate buffer (0.01 mol/L, pH 6.0) for 15 min. Then the slides were incubated with 0.3% bovine serum albumin (BSA) solution for 30 min to reduce background nonspecific staining. Primary antibodies of c-Met (1:30, clone 8F11, Novocastra, USA) were applied at 4°C overnight. The subsequent reaction was performed using an ELIVISION™ immunohistochemical staining kit (Maxim, USA) according to the recommended procedure. Finally, the slides were incubated with 3,3'-diaminobenzidin and counterstained with hematoxylin. Section known to express high levels of c-Met was included as positive controls, while negative control slide omitted the primary antibody.

Evaluation of Immunohistochemistry

If cells with strong cytoplasmic staining exceeded 30% of the counted cells, the case was considered to be positive[20].

Statistical Analysis

The Pearson's χ^2 test was used to examine the differences among the different histological patterns in age, sex, *Helicobacter pylori* infection, smoking, and drinking, and to explore the association between c-Met overexpression and all of those variables. We utilized unconditional logistic regression model to calculate the ORs and 95% confidence intervals (CIs) for the association of c-Met overexpression with the risk of advanced gastric lesions, adjusting for age, sex, *Helicobacter pylori* infection, smoking and drinking status. All statistical analyses were carried out using Statistical Analysis System (SAS, version 9.1; SAS Institute, Cary, NC, USA). All statistical tests were two tailed, and the significance level was set at P<0.05.

RESULTS

The frequency distributions of age, sex, *helicobacter pylori* infection, smoking and drinking status in subjects with precancerous gastric lesions were presented in Table 1 and Table 2. There were no

Table 1. Clinicopathological findings of IM progressive and persistent groups

Patients characteristics	Progression from IM to DYS, n=35 [n (%)]	Persistence in IM, n=35 [n (%)]	P
Age (years)			
<50	15 (42.86)	21 (60.00)	0.1513
≥50	20 (57.14)	14 (40.00)	
Sex			
Male	17 (48.57)	18 (51.43)	0.8111
Female	18 (51.43)	17 (48.57)	
Helicobacter pylori infection			
Positive	6 (17.14)	7 (20.00)	0.7586
Negative	29 (82.86)	28 (80.00)	
Smoking			
Yes	15 (42.86)	21 (60.00)	0.1513
No	20 (57.14)	14 (40.00)	
Drinking			
Yes	18 (51.43)	21 (60.00)	0.4704
No	17 (48.57)	14 (40.00)	

Table 2. Clinicopathological findings of DYS progressive and persistent groups

Patients characteristics	Progression from DYS to GC *n*=27 [*n* (%)]	Persistence in DYS *n*=27 [*n* (%)]	*P*
Age (years)			
<50	11 (40.74)	5 (18.52)	0.0738
≥50	16 (59.26)	22 (81.48)	
Sex			
Male	6 (22.22)	6 (22.22)	–
Female	21 (77.78)	21 (77.78)	
Helicobacter pylori infection			
Positive	9 (33.33)	11 (40.74)	0.5730
Negative	18 (66.67)	16 (59.26)	
Smoking			
Yes	13 (48.15)	16 (59.26)	0.4129
No	14 (51.85)	11 (40.74)	
Drinking			
Yes	4 (14.81)	12 (44.44)	0.0171
No	23 (85.19)	15 (55.56)	

Table 3. Expression of c-Met in IM progressive and persistent groups

Groups	Positive (*n*, %)	Negative (*n*, %)	*P*	OR* (95% CI)
Persistence in IM (*n*=35)	14 (40.0)	21 (60.0)		
Progression from IM to DYS (*n*=35)	25 (71.4)	10 (28.6)	0.0020	7.416 (2.084–26.398)

*Unconditional logistic regression, adjusted for age, sex, Helicobacter pylori infection, drinking and smoking status.

Table 4. Expression of c-Met in DYS progressive and persistent groups

Groups	Positive (*n*, %)	Negative (*n*, %)	*P*	OR* (95% CI)
Persistence in DYS (*n*=27)	17 (63.0)	10 (37.0)		
Progression from DYS to GC (*n*=27)	18 (66.7)	9 (34.3)	0.2344	2.310 (0.581–9.181)

*Unconditional logistic regression, adjusted for age, sex, Helicobacter pylori infection, drinking and smoking status.

significant differences in age, sex, *Helicobacter pylori* infection, smoking and drinking status between progressive and persistent groups.

We first assessed the relationships between c-Met overexpression and all of the variables, and found that c-Met overexpression was not associated with age, sex, *Helicobacter pylori* infection, smoking and drinking status (P>0.05, respectively) (data not shown).

We further evaluated the association between c-Met overexpression and severity of gastric lesions. The positive rates of c-Met were 55.7% in IM and 64.8% in DYS, respectively. Stratified analysis indicated that the proportion of c-Met overexpression was 71.4% for IM progressive group, significantly higher than that for IM persistent group (40.0%, P<0.05). Adjusted for age, sex, *Helicobacter pylori* infection, drinking and smoking status, unconditional logistic regression showed that compared to IM persistent group, the OR of c-Met overexpression for IM progressive group was 7.416 (95% CI: 2.084–26.398) (Figure 1, Table 3). On the other hand, the proportion of c-Met overexpression was 66.7% for DYS progressive group, slightly higher than that for DYS persistent group (63.0%, P>0.05). As shown in Table 4, compared to DYS persistent group, the OR of c-Met overexpression for DYS progressive group was 2.310 (95% CI: 0.581–9.181) (Figure 2).

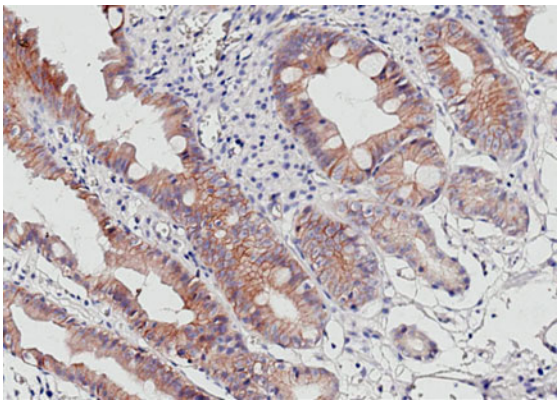

Figure 1. c-Met overexpression in gastric IM progressive to DYS.

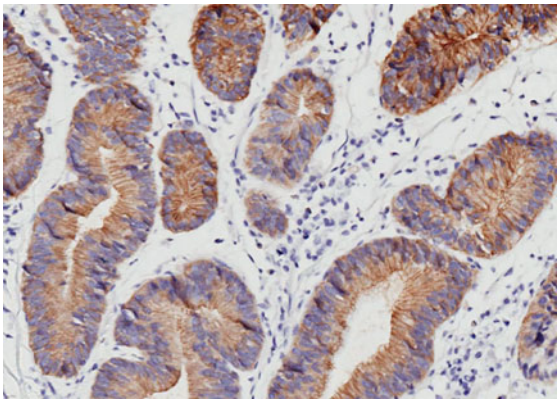

Figure 2. c-Met overexpression in gastric DYS progressive to GC.

DISCUSSION

In an area with exceptionally high rates of GC, we investigated c-Met overexpression among 124 subjects with precancerous gastric lesions and its association with evolution of the lesions. Our finding that the positive rates of c-Met were increased gradually from IM to DYS, suggesting that c-Met might play an important role in the process of gastric carcinogenesis.

The *MET* protooncogene is a member of the protein tyrosine kinase growth factor receptor gene family. Activation of *MET* oncogene was shown to involve a chromosome translocation event, giving rise to rearranged *TPR-MET* gene[21, 22]. Previous studies have shown that *TPR-MET* RNA was detected in all stages of gastric carcinogenesis, from early SG through end-stage Ca. The expression of *TPR-MET* gene at the early stage of SG suggests a possible functional role of this oncogene during the initial stages of gastric carcinogenesis.

In the present study, we found the proportion of c-Met overexpression was 71.4% for IM progressive group, significantly higher than that for IM persistent group (40.0%, $P<0.05$). Compared to IM persistent group, the OR of c-Met overexpression for IM progressive group was 7.416. Our finding provided the new evidence that c-Met might contribute to the progression of gastric IM to DYS.

The human microbial pathogen *Helicobacter pylori* can induce chronic gastritis. One of the early morphological changes observed in the pathogenesis is inflammation followed by atrophy or IM. Growing evidences from recent studies have shown that the *Helicobacter pylori* effector protein, cytotoxin associated gene A (CagA), intracellularly modulates the RTK c-Met[23]. Binding of the natural ligand HGF to c-Met stimulates mitogenesis and morphogenesis in epithelial cells[24]. Abnormal c-Met signaling is strongly related to tumorigenesis. Therefore, c-Met may be overexpressed in the early stage in gastric tumorigenesis as a consequence of the inflammatory response[25].

It was shown that the proportion of c-Met overexpression was 66.7% for DYS progressive group, slightly higher than that for DYS persistent group (63.0%, $P>0.05$). Although no apparent correlation was observed between the overexpression of c-Met and the evolution of DYS, the OR of c-Met overexpression for DYS progressive group was markedly elevated. The reason may be that the number of subjects in our study was relatively small.

In conclusion, our population-based study provided strong evidence that detection of c-Met is of value in predicting progression of precancerous gastric lesions from IM to DYS.

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