Study on the expression of c-Met in gastric cancer and its correlation with blood tumor markers and prognosis

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Abstract

Background: Studies have found that c-Met plays a critical role in the progression of solid tumors. This study aimed to investigate the expression of c-Met in gastric cancer (GC) and its correlation with blood tumor markers and prognosis. In order to provide a new idea and method for targeting c-Met in the treatment of GC.

Methods: Ninety-seven patients who underwent GC surgery in our hospital from December 2013 to September 2015 were included in this study. The tissue microchip was constructed by paraffin cutting, including 97 GC points and 83 para-cancer points. Then, it was used for c-Met immunohistochemical staining, followed by immunological H-score. The expression of c-Met was compared with clinicopathological features, blood tumor markers (AFP, CEA, CA199, CA153, CA125, CA50) and 5-year survival. Descriptive statistics, Pearson correlation test, Kaplan-Meyer survival curve and COX regression were used for statistical analysis.

Results: The high expression rate of c-Met was 64.95% (63/97) in GC tissues, and 28.92% (24/83) in para-cancer tissues. There were prominent statistical differences in the M-stage and clinicopathological stage between the low and high expression groups (P<0.05), the c-Met high expression group also had a higher M-stage and clinicopathological stage of GC. The correlation test between the c-Met H-score and CA125 was statistically significant (P=0.004), indicating a positive correlation. High c-Met expression correlated with poor overall survival (OS) for 5 years (HR=2.103, P=0.005). After the clinicopathological classification of patients, it was found that the high expression of c-Met in stage Ⅰ-Ⅱ patients was correlative with poor OS for 5 years (HR=2.486, P=0.026), while stage Ⅲ-Ⅳ patients had no statistical significance (P>0.05). Univariate and multivariate COX regression analysis showed that age, clinicopathological stage, c-Met high expression and preoperative serum AFP might be independent risk factors for survival 5 years after surgery.

Conclusion: This study found that the high expression of c-Met in GC was associated with poor 5-year OS in GC patients and was an independent risk factor for 5-year survival after GC surgery. The expression of c-Met in GC was positively correlated with preoperative serum CA125.

1. Introduction

Gastric cancer (GC) is one of the most common digestive system malignancies globally [1]. It is the fifth most common cancer and fourth most common cause of cancer death worldwide, with more than 1,000,000 new cases and 769,000 deaths due to GC in 2020[2]. Unfortunately, most patients are diagnosed in the middle and late stages, so the survival rate after surgery is meager, and some patients lose the opportunity for surgery. The five-year survival rate for GC is 31 percent in the US, 19 percent in the UK and 28 percent in China[3]. Despite using trastuzumab combined with routine chemotherapy in patients with positive HER-2, the survival prognosis is moderate [4]. Consequently, there is an urgent need for effective therapeutic methods to treat these patients and improve clinical outcomes.

C-mesenchymal epithelial transformation factor (c-Met) is involved in tumourgenesis of various cancers, including GC [5]. c-Met inhibitors have attracted much attention due to their antitumor activity in various solid tumors. In recent years, inhibitors and mAb of c-Met have not achieved significant efficacy in clinical studies of GC[6-9]. However, MET proto-oncogenes do participate in the progression of various solid tumors and mediate the proliferation and metastasis of various tumor cells[10]. Therefore, it is necessary to study further the expression of c-Met in GC and its role in tumor progression to provide the theoretical basis for optimising targeted c-Met therapy in gastric cancer to improve the survival of patients.

Studies have found that c-Met interacts with various molecules in tumor signaling pathways. For example, the signal intensity of c-Met and EGFR can interact with each other, and eventually the signaling pathway aggregates in the same downstream signaling medium, such as ERK/MAPK and PI3K/AKT [11]. Meghan Grojean et al.[12] Studied the use of c-Met/VEGFR2 inhibitor foretinib in GC xenograft tumor models, showing more potent anti-tumor activity. This finding highlights the antitumor effect of simultaneous inhibition of c-Met and VEGFR2 signaling in GC. In addition, RON (Recepteur d 'Origine Nantais) and c-Met are co-expressed in many types of cancer, and the interaction between c-Met and RON has been confirmed. In the absence of hepatocyte growth factor (HGF), the interaction between c-Met and RON receptor leads to phosphorylation of c-Met receptor[13], and promotes the metastasis of tumor stem cells by increasing tumor cell proliferation and inhibiting apoptosis[14]. Other researchers have found that the Y42 site of RhoA (cancer molecular) can be directly phosphorylated by c-Met and promote the proliferation and movement of GC cells [15]. This dynamic interaction of c-Met may also be the main reason why the efficacy of small molecule inhibitors and monoclonal antibodies alone in the treatment of GC is not apparent. Studies have also confirmed that combining c-Met with other targets can play a more robust anti-tumor activity in clinical studies on GC [16]. The study of c-Met expression in GC is helpful for the analysis of GC subtypes and conducive to the selection and optimization of targeted therapy methods for GC. Its expression in GC and its relationship with the prognosis of patients have always been a research hotspot [17-19]. However, data on c-Met in GC are still scarce, so understanding their characteristics is essential to improve treatment outcomes and survival in these patients.

Serum markers such as AFP, CEA, CA-199, CA-125, CA-153, CA-50 have been applied in cancer diagnosis and monitoring. Studies have found that the levels of some peripheral blood tumor markers are correlated with the prognosis of GC patients so that these tumor markers can be used as predictors of tumor progression in GC patients[20, 21]. Nonetheless, most of the studies mainly aimed to investigate the influence of preoperative and postoperative serum tumor marker levels on the clinical prognosis of patients[22-24]. There are few studies on the correlation between tumor markers and carcinogenic factors. If there is a correlation between them, peripheral blood tumor markers monitoring can better guide clinicians to use targeted therapy to develop personalized treatment strategies. At the same time, it will also bring more convenient gastric cancer monitoring services and more accurate medical treatment experience for patients.

Therefore, based on the expression of c-Met in GC tissues, the relationship between c-Met and clinical prognosis was researched in this study. Furthermore, the correlation between c-Met and blood tumor

markers (AFP, CEA, CA199, CA153, CA125, CA50) in GC progression of was discussed. Finally, the risk factors for postoperative survival of gastric cancer patients were examined. In order to provide a new idea and method for targeting c-Met in the treatment of GC.

2. Materials And Methods

2.1 Patients

This study included 97 patients who underwent GC surgery in our hospital from December 2013 to September 2015, and the included patients had complete clinicopathological data and 5-year postoperative follow-up records. Clinicopathological data included age, sex, operation date, operation method, tumor location, tumor size (maximum diameter), TNM stage, degree of tumor differentiation, Helicobacter pylori(H pylori) infection, HER2 expression in GC tissues, and preoperative peripheral blood tumor marker levels(AFP, CEA, CA-199, CA-125, CA-153, CA-50), which were obtained from medical records. The 7th edition of the AJCC TNM staging system was used for pathological TNM staging, and clinicopathological staging corresponding to TNM staging of GC was used (7th edition of NCCN, 2010).

There were 23 female patients (23.7%) and 74 male patients (76.3%) in the study. All patients underwent GC resection and lymph node dissection. All patients were diagnosed with gastric adenocarcinoma. All patients underwent elective surgery and received standard chemotherapy after surgery. The study was approved by the Ethics Committee of Lanzhou University Second Clinical Medical School and is consistent with the Declaration of Helsinki. The research group informed each patient of the significance of this study and signed a written consent form. Patients to be included in the study must meet the following inclusion criteria: (1) Therapeutic gastrectomy, (2) Postoperative pathological diagnosis was gastric adenocarcinoma, (3) Postoperative paraffin tissue specimens are available for study. Emergency surgery, concomitant other tumors and Palliative surgery for gastric cancer were excluded from the study.

2.2. Tissue chip construction

Firstly, HE stained sections were reviewed, representative tumor regions were selected, and target wax tissues were collected from the wax blocks. The automatic tissue chip instrument（Jinan Tangier Electronics Co., LTD., China） is used to drill the target paraffin blocks (diameter:1.5mm), and they were neatly arranged in another empty white wax block to make tissue chip wax blocks. Then, the tissue chip wax block was sliced (thickness: 4um), and then the slice was transferred to the slide to make the tissue chip. One tumor tissue and one paracancer tissue were taken from each case, and a total of 97 cases of gastric adenocarcinoma were included in this tissue chip, including 97 cancer points and 83 paracancer points.

2.3 Immunohistochemistry

c-Met rabbit monoclonal antibody (Cat: AB51067, Abcam, USA) was used as the primary antibody for immunohistochemistry. The tissue chips were heated in a 65℃ oven for 1 hour, then put into xylene for

dewaxing, and then put into graded ethanol for hydration. Subsequently, heat-induced antigen repair was performed with citric acid buffer and endogenous peroxidase was blocked by 3% hydrogen peroxide for 20 min. The slides were incubated overnight with primary antibody (the primary antibody concentration was 1:200 in the preliminary experiment) at 4 °C. The next day was incubated with 1:50 diluted goat anti-rabbit IgG secondary antibody at RT for 60 min. DAB color solution was added into the slices to make the color. Finally, the slices were restained with hematoxylin, then dehydrated and sealed.

2.4 Immunohistochemical scores

Tumor c-Met expression was evaluated according to the immunological histochemistry score (H-score) system, with H-score= staining intensity × staining area grade. The staining intensity was divided into 0 (no staining), 1+ (weak staining), 2+ (medium staining) and 3+ (strong staining). Staining area was classified as 0 (no cell staining), 1+ < 25%, 25%≤2+ < 50%, and 3+≥50%. In this study, H-score < 3 was defined as weak expression and H-score≥3 was defined as high expression. Immunohistochemical staining assessment was performed by two chief pathologists blinded to the clinicopathological diagnosis of the patient.

2.5 Statistical Analysis

SPSS 20.0 software was used for statistical analysis. N (%) was used to represent the counting data, and Mean(SD) represented the measurement data conforming to normal distribution. Chi-square test and T test were used to evaluate classified data and continuous data, respectively. Pearson correlation test evaluated the correlation between c-Met expression and blood tumor markers, and a two-sided test was used. The 5-year overall survival (OS) were compared using Kaplan-Meier curves and log-rank tests. OS is defined as counting from the date of surgery to the date of death from cause of death. Univariate and multivariate analyses were performed to analyze survival risk factors by COX regression. The hazard ratio (HR) and corresponding 95% confidence interval (CI) were calculated. All P<0.05 were considered statistically significant.

3. Results

3.1 Comparative analysis of c-Met expression in GC and paracancer tissues

c-Met was stained in all cell membranes, and some cells were stained in the inner membrane (**Figure 1**). The overexpression rate of GC tissues was 64.95% (63/97), and that of para-cancer tissues was 28.92% (24/83) (**Table 1**). The expression of c-Met in GC tissues was significantly higher than that in adjacent tissues (P<0.001) (**Figure 2A**), There were statistical differences in H-scroe between GC tissues and adjacent tissues in patients with high and low expression of c-Met (P=0.004, P=0.033) (**Figure 2B-C**). The mean H-score expression of c-Met in cancer tissues was higher than that in adjacent tissues in patients with high and low expression of c-Met.

Table 1. Comparative analysis of c-Met expression in GC and paracancer tissues

Varible	Tumor(97)	Adjacent(83)	t/X^2	*P-value*
Low expreesion group,n(%)	34(35.05)	59(71.08)	23.255	<0.001
High expreesion group, n(%)	63(64.95)	24(28.92)		
H-scroe of Low expreesion group, Mean(SD)	1.76(0.43)	1.54(0.50)	2.162	0.033
H-scroe of high expreesion group, Mean(SD)	5.97(2.24)	4.50(1.53)	2.958	0.004

3.2 Comparative analysis of c-Met low expression, high expression group and clinical baseline data

Comparison of clinical baseline data between c-Met low expression group and high expression group (age, sex, tumor size, tumor location, TNM stage, clinicopathological stage, degree of differentiation, positive expression of HER2 and H pylori)There was the apparent difference between M stage and clinicopathological stage (P<0.05) (**Table 2**). Compared with the low expression group, the high expression of c-Met was associated with a greater likelihood of tumor metastasis and higher clinicopathological stage.

Table 2 Comparative analysis of c-Met low expression, high expression group and clinical baseline data

Varible	c-Met expression [n(%)/ Mean(SD)]		t/X^2	*P-value*
	c-Met Low group(n=34)	c-Met High group (n=63)		
Age(years)	57.35(10.63)	59.95(9.61)	1.224	0.224
Sex				
Female	11(32.35)	12(19.05)	2.161	0.142
Male	23(67.65)	51(80.95)		
Tumor diameter□cm□	3.74(1.82)	4.11(1.75)	1.001	0.319
Tumor location				
Gastric body	5(14.71)	9(14.29)	0.554	0.758
Gastric antrum	25(73.53)	43(68.25)		
Gastric fundus	4(11.76)	11(17.46)		
T-Stage				
T (1,2)	13(38.24)	13(20.63)	3.487	0.062
T (3,4)	21(61.76)	50(79.37)		
N-Stage				
N-	11(32.35)	16(25.40)	0.532	0.466
N+	23(67.65)	47(74.60)		
M-Stage				
M-	33(97.06)	51(80.95)	4.936	0.022
M+	1(2.94)	12(19.05)		
clinicopathologic stage				
□-□	22(64.71)	25(39.68)	5.536	0.019
□-□	12(35.29)	38(60.32)		
Differentiated degree				
Poor differentiation	14(41.18)	40(63.49)	4.908	0.086
Moderate differentiation	17(50.00)	21(33.33)		
High differentiation	3(8.82)	2(3.17)		
HER2-IHC				
Missing	1(2.94)	1(1.59)		

Negative	31(91.18)	56(88.89)	0.365	0.546
Positive	2(5.88)	6(9.52)		
H pylori				
Missing	6(17.65)	17(26.98)		
Negative	14(41.18)	23(36.51)	0.001	1.000
Positive	14(41.18)	23(36.51)		

3.3 Correlation test between c-Met and blood tumor markers

Person test was performed on the H-score of c-Met in gastric cancer tissue and blood tumor markers(AFP, CEA, CA199, CA153, CA125 and CA50) related to the digestive system. The study found that the correlation test between c-Met and preoperative serum CA125 level of patients was statistically significant (*P*=0.004) (**Table 3**), showing a positive correlation. The expression of c-Met increased with the increase of CA125.

Table 3 Correlation test between C-MET and blood tumor markers

Varible	Total (n)	Value [Mean(SD)]	r	*P*-value
AFP(ng/ml)	78	43.96(118.05)	0.043	0765
c-MET(H-score)	78	5.70(2.21)		
CEA(ng/ml)	78	48.91(118.88)	0.020	0.890
c-MET(H-score)	78	5.70(2.21)		
CA199(u/ml)	78	51.87(115.62)	0.042	0.773
c-MET(H-score)	78	5.70(2.21)		
CA153(u/ml)	77	11.71(10.52)	0.131	0.370
c-MET(H-score)	77	5.73(2.22)		
CA125(u/ml)	77	13.66(34.33)	0.322	0.004
c-MET(H-score)	77	4.32(2.60)		
CA50(u/ml)	75	11.96(24.01)	0.052	0.658
c-MET(H-score)	75	4.37(2.61)		

3.4 5-year survival analysis of patients with high and low expression of c-Met

The 5-year OS curves of patients with high and low c-Met expression showed statistically significant differences (HR=2.103, *P*=0.005) (**Figure 3A**), the 5-year OS of the c-Met high expression group were

significantly worse than those of the low expression group. Patients were grouped according to different clinicopathological stages of the tumor, and then 5-year OS curves were constructed. The results showed statistically significant differences in stage Ⅱ-Ⅲ (HR=2.486, *P*=0.026) (**Figure 3B**), 5 years OS in c-Met high expression group were significantly lower than those in low expression group. There was no significant difference in 5-year OS between the two groups of patients in stage Ⅰ-Ⅱ (*P*>0.05) (**Figure 3C**).

3.5 Univariate COX regression analysis of 5-year survival

Univariate analysis of factors related to 5-year survival showed that age, tumor size, clinicopathological stage, c-Met expression and serum AFP level were correlated with 5-year survival risk (*P*<0.05) (**Table 4**). Patients with older age, larger tumor diameter, higher clinicopathologic staging, higher c-Met expression, and higher AFP levels were associated with greater risk of survival less than 5 years. Univariate analysis of other pathological related factors and 5-year survival showed no statistical significance (*P* > 0.05).

Table 4 Univariate COX regression analysis of 5-year survival

Variable	Univariable Survival Analysis		
	HR	95% CI	*P*-value
Age	1.030	1.004-1.057	0.024
Sex			
Male	1		
Female	1.068	0.616-1.852	0.815
Tumor diameter	1.271	1.109-1.457	0.001
Tumor location			
Gastric body	1		
Gastric antrum	0.504	0.202-1.258	0.142
Gastric fundus	1.329	0.170-10.386	0.786
Operation method			
Distal gastrectomy	1		
Proximal gastrectomy	0.960	0.484-1.902	0.906
Total gastrectomy	1.532	0.793-2.963	0.205
Clinicopathologic stage			
□-□	1		
□-□	3.190	1.913-5.317	<0.001
Differentiated degree			
Poor differentiation	1		
Moderate differentiation	2.213	0.683-7.169	0.185
High differentiation	1.459	0.438-4.865	0.539
c-MET expression	1.150	1.058-1.251	0.001
HER2-IHC			
Negative	1		
Positive	1.720	0.783-3.781	0.177
AFP	1.008	1.001-1.016	0.025
CEA	1.005	0.995-1.016	0.324
CA199	1.001	0.999-1.002	0.562

CA153	1.010	0.987-1.034	0.395
CA125	1.000	0.994-1.007	0.940
CA50	1.001	0.990-1.012	0.815
H pylori			
Negative	1		
Positive	0.836	0.485-1.442	0.520

3.6 Multivariate COX regression analysis of 5-year survival

Multivariate COX regression analysis was performed for the indexes with statistical significance in univariate analysis. The results showed that age, clinicopathological stage, high expression of c-Met and preoperative serum AFP may be independent risk factors for survival 5 years after surgery (P<0.01) (**Table 5**). Patients with older age, higher clinicopathological stage, higher c-Met expression and higher AFP levels were at greater risk of survival less than 5 years.

Table 5 Multivariate COX regression analysis of 5-year survival

5-Year Overall Survival Variable	B	S.E	Wals	*P*-value	HR	95% CI
Age	0.054	0.017	10.252	0.001	1.055	1.021-1.091
Clinicopathologic stage	1.139	0.309	13.630	<0.001	3.124	1.706-5.719
c-MET expression	0.192	0.055	12.298	<0.001	1.211	1.088-1.348
AFP	0.015	0.004	14.346	<0.001	1.015	1.007-1.023

4. Discussion

In this study, immunohistochemistry confirmed that the high expression rate of c-Met in GC tissues was significantly higher than that in adjacent tissues (64.95% vs 28.92%). Secondly, the study also found that the mean H-score expression of c-Met in patients with high and low expression of c-Met in cancer tissues was higher than that in adjacent tissues (P<0.05). It indicates that c-Met is highly expressed in most GCs and exerts a vital function. Many studies have also confirmed that c-Met promotes the development, proliferation, invasion, metastasis and angiogenesis of solid tumor cells through downstream signaling pathways, and even promotes chemotherapy resistance[25-28]. This study also found that the high expression of c-Met was associated with poor postoperative survival and was positively correlated with the preoperative blood CA125 level of patients, suggesting that c-Met is a promising molecule in the treatment of GC, which can be used for targeted therapy and also conducive to the monitoring of tumor progression.

C-Met-targeted therapy in GC mainly includes tyrosine kinase inhibitors, monoclonal antibodies and c-Met-targeted adoptive immunotherapy. Tyrosine kinase inhibitors and monoclonal antibodies have shown obvious antitumor activity in cell and xenograft tumor models [12, 29-32], while most tumors have not achieved prominent antitumor activity in clinical trials [7, 33]. Currently, only a few tumors have shown encouraging antitumor activity, especially in the treatment of NSCLC (non-small-cell lung cancer) [34, 35]. Furthermore, c-Met-targeted CAR-T cells have shown good antitumor activity in preclinical studies of GC[36, 37]. Since adoptive immunotherapy mainly relies on the particular expression of c-Met on the cell membrane of GC, it is not limited to the carcinogenic mechanism of c-Met. This study also found that c-Met expression was significantly increased in high-grade clinicopathological stages of GC. Therefore, the above indicated that targeted c-Met adoptive immunotherapy in the middle and late stages of GC might be a new direction for the treatment. Currently, two clinical studies on c-Met CAR-T cells in the treatment of liver cancer, GC and other solid tumors of the digestive system are being implemented in China (NCT03672305, NCT03638206) to evaluate the efficacy and safety of c-Met CAR T cells in solid tumors of the digestive system and expect to achieve good results.

Chuan Xie et al.[38] found that c-Met expression was significantly increased in GC specimens with H pylori infection, and in vitro experiments also confirmed that H pylori infection may activate the HGF/c-MET signaling pathway, which may be involved in the occurrence of GC. Secondly, Xiaojun Huang et al. [39] carried out an in-depth study and found that c-Met expression increased significantly in GC tissues with positive cytotoxin-related gene A (CagA) and H pylori infection. Meanwhile, it was also found that the activation of the c-Met signaling pathway was associated with inhibiting autophagy and promoting tumor cell invasion and metastasis in patients. However, our study found no significant increase in c-Met expression in the H pylori-positive group. H pylori infection is only one of the pathogenic causes of GC, and cancer progression and metastasis are a process of multiple oncogenes[40]. Studies have also confirmed that c-Met interacts with multiple molecules in promoting cancer progression[15, 41, 42]. The results of these studies may also be caused by the interaction between c-Met and downstream carcinogens of H pylori infection in the progression of GC. However, the occurrence and progression of GC caused by H pylori infection is also the result of the action of multiple oncogenes. Therefore, the correlation between H pylori infection and c-Met high expression requires more studies in the future to verify and explore its molecular mechanism.

As is known to all, AFP, CEA, CA199, CA153, CA125 and CA50 are common tumor markers of the digestive system. Exploring the correlation between these markers and c-Met may provide a new idea for optimizing c-Met targeting therapy strategies for GC. This study found that the correlation test between c-Met and serum CA125 level of patients was statistically significant, showing a positive correlation. Thus, it is possible to predict the expression of c-Met in tumors by detecting preoperative CA125 levels better to guide postoperative monitoring and prognosis assessment of patients. Recently, Can Hu [43] conducted a study on CA125 and its prognosis in GC patients undergoing neoadjuvant chemotherapy, and found that the level of CA125 before neoadjuvant chemotherapy was correlated with the prognosis of patients. The OS after chemotherapy decreased with the increase of CA125 levels. The study suggests that patients with serum CA125 normalization after neoadjuvant chemotherapy may benefit from survival. In addition,

Hongbo Zhou et al.[44] explored the relationship between serum CA19-9, CA125 levels and HER2 expression in patients with GC, and confirmed their correlation with the risk of recurrence and metastasis. GC patients with CA19-9, CA125 and HER2 positive had a significantly higher recurrence and metastasis than those with negative GC. There was also no correlation between serum CA19-9 and CA125 and HER2 positive expression. These studies confirmed the correlation between serum CA125 level and prognosis of GC patients and the possibility of the theory of correlation between c-Met and CA125. This finding is expected to help clinicians assess the role of c-Met in GC progression by monitoring peripheral blood CA125, and thus better guide clinicians to choose c-Met inhibitors or c-Met-CAR-T cell therapy. Therefore, it provides ideas for targeting c-Met in treating of GC and other solid tumors.

This study revealed that patients with high c-Met expression had a higher clinicopathological stage and a higher likelihood of tumor metastasis. High c-Met expression was also found to be associated with poor 5-year OS. In order to research the function of c-Met in different clinicopathological stages, subgroup analysis was also conducted according to different clinicopathological stages of the tumor. The results demonstrated that the high expression of c-Met in stage Ⅰ-Ⅱ was associated with poor 5-year OS, while there was no correlation in stage Ⅲ-Ⅳ patients. ZHANG Q and Ya'nan Yang et al.[17, 45] also found that the high expression of c-Met correlated with poor prognosis in GC patients, and the results were consistent with our study. However, their research for the patient was not for a more detailed analysis. Our study innovatively stratified patients according to clinicopathological stage. Results show that the c-Met at stage Ⅰ-Ⅱ tumor tissue plays a more critical role in promoting tumor proliferation and metastasis, while this effect in stage Ⅲ-Ⅳ perhaps be weakened by other molecular mechanisms of cancer. After all, the molecular mechanism and regulation of promoting tumor proliferation and metastasis in advanced cancer are more complex. These studies suggest that inhibitors and mAb of c-Met may achieve more significant benefits in patients with early-stage GC.

Univariate and multivariate COX regression analyses were performed to investigate further the risk factors associated with 5-year survival after surgery. The results revealed that age, clinicopathological stage, high expression of c-Met and preoperative serum AFP might be independent risk factors for survival 5 years. Tobias Jagomast et al.[19] studied the prognostic value of c-Met in patients undergoing radical gastrectomy in Canada. The results demonstrated that c-Met high expression was correlative with poor OS. Multivariate analysis showed that the co-expression of EGFR and c-Met was an independent risk factor for postoperative survival of GC. However, Marina Alessandra Pereira et al.[18] recently reported that c-Met was associated with postoperative survival, but not an independent risk factor for prognosis. These studies confirmed the role of c-Met in the progression of GC and demonstrated the important role of the interaction between EGFR and c-Met in GC progression. Therefore, this molecular interaction may account for the negative or positive results of c-Met being an independent risk factor for GC.

In addition, our study found that increased preoperative AFP may be an independent risk factor for postoperative survival of GC in our included population. Xiang Xu et al.[46] conducted a meta-analysis on the effect of serum AFP level on prognosis in patients with GC before treatment. Thirteen studies

involving 9,099 patients with GC was entered in the analysis. The results revealed that a high serum AFP level before treatment correlated with poor prognosis in GC patients. The above studies are consistent with our conclusions, suggesting that serum AFP level before treatment can act as a prognostic indicator of GC patients, and AFP can be used to assess the disease condition and prognosis of GC patients. However, AFP is a specific tumor marker of liver cancer, and its serum expression level in GC patients may be significantly lower than that in liver cancer patients. Therefore, more studies are needed to confirm whether AFP can be used as a specific tumor marker for GC to guide clinical practice.

This study also has some limitations. On the one hand, the sample size included in the study is limited, resulting in bias. Secondly, there is a lack of clinical data to monitor postoperative serum tumor markers in patients, leading to the failure of the correlation study between the above indicators and c-Met. However, the pathological data, clinical indicators and preoperative blood tumor markers of patients in this study were relatively complete. Therefore, the conclusion of this study is detailed and reliable.

5. Conclusions

In conclusion, this study describes the expression of c-Met in patients with GC and its correlation with prognosis. High expression of c-Met was associated with poor 5-year OS, especially in patients with clinicopathological stages □-□, and was an independent risk factor for postoperative survival in patients with GC. Meanwhile, the study found a positive correlation between the expression of c-Met in GC and the preoperative serum CA125 of patients. These findings have important clinical significance because they can guide the selection of patients with the appropriate pathological stage for the treatment of GC by targeting c-Met and better guide the postoperative monitoring and prognosis evaluation of patients with high c-Met expression by detecting the preoperative CA125 level of patients. It also confirms the importance of targeting c-Met therapy with its interacting molecular inhibitors in patients with advanced GC.

Abbreviations

c-Met: c-mesenchymal epithelial transformation factor

HGF: hepatocyte growth factor

GC: gastric cancer

H-score: histochemistry score

AFP: alpha-fetoprotein

CEA: Carcinoma Embryonic Antigen

RON: Recepteur d 'Origine Nantais

H pylori: Helicobacter pylori

OS: overall survival

HR: hazard ratio

CI: confidence interval

NSCLC: non-small-cell lung cancer

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Lanzhou University Second Clinical Medical School and is consistent with the Declaration of Helsinki. The consent and written certification of the patients were obtained during postoperative follow-up.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The four previously submitted projects provide research funds for the implementation of this research.

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Authors' contributions:

LY, TM, ZZ were responsible for the design of scientific research, ZZ, ML, WS, ZY, XY, YH, RZ, WG were responsible for the collection of specimens and patient-related pathological data, ZZ, ZY, XY were responsible for the examination of pathological tissues, ZZ, ML, WS were responsible for article writing and data processing, TM were responsible for the revision of the article, LY, ZY were responsible for the support of scientific research funds. All authors contributed to the article and approved the submitted version.

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Figures

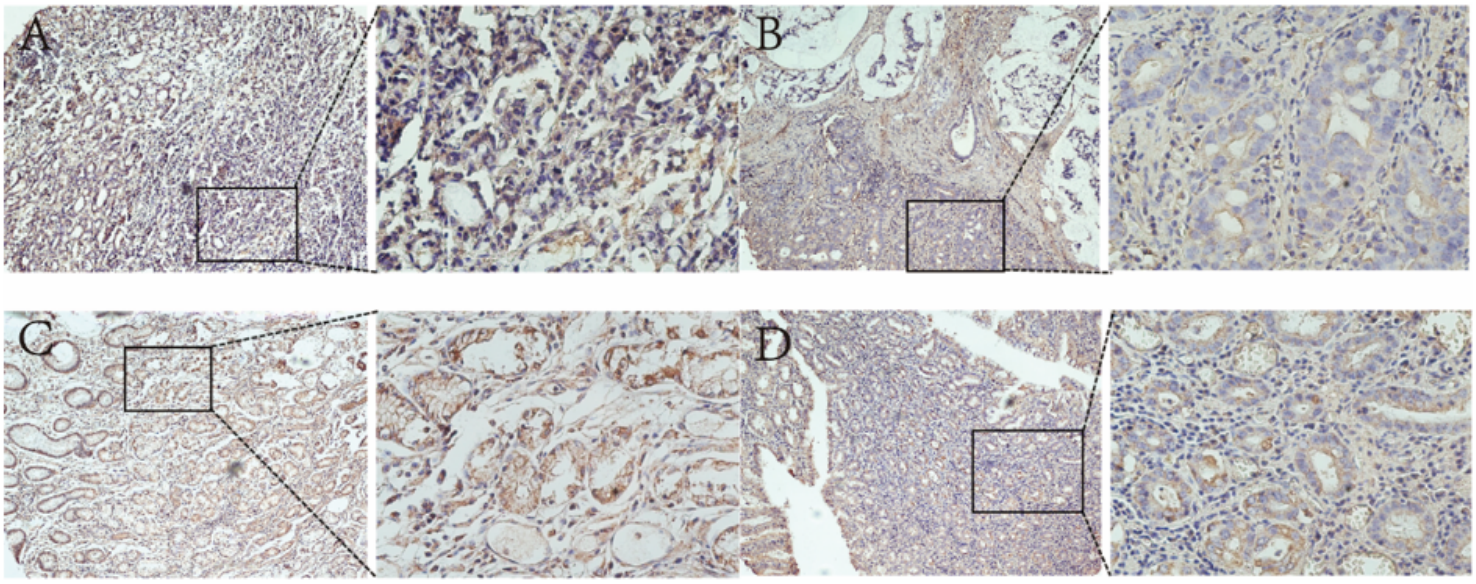

Figure 1

Representative immunohistochemical images of c-Met expression in GC and adjacent tissues. Figure A. High expression of c-Met in GC tissues, Figure B. Low expression of c-Met in GC tissues, Figure. C High

expression of c-Met in adjacent tissues, Figure D. Low expression of c-Met in adjacent tissues. The scale bars are 100um and 20um respectively.

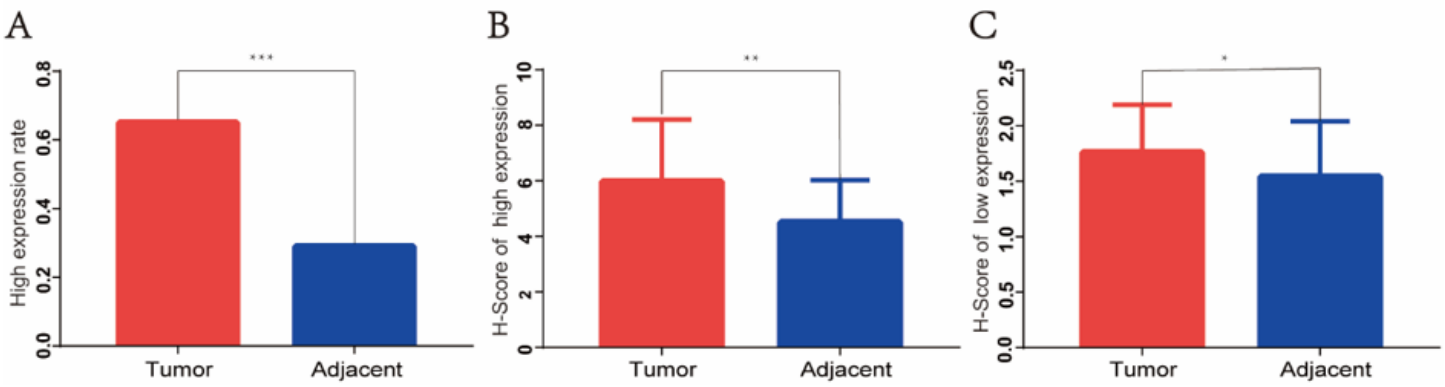

Figure 2

Comparison of c-Met high expression rate and H-score in cancer and adjacent tissues. Figure A. Comparison of c-Met high expression rate between GC tissues and adjacent tissues, Figure B. Comparison of H-score between GC tissues and adjacent tissues with c-Met high expression, Figure C. Comparison of H-score between GC tissues and adjacent tissues with c-Met low expression.

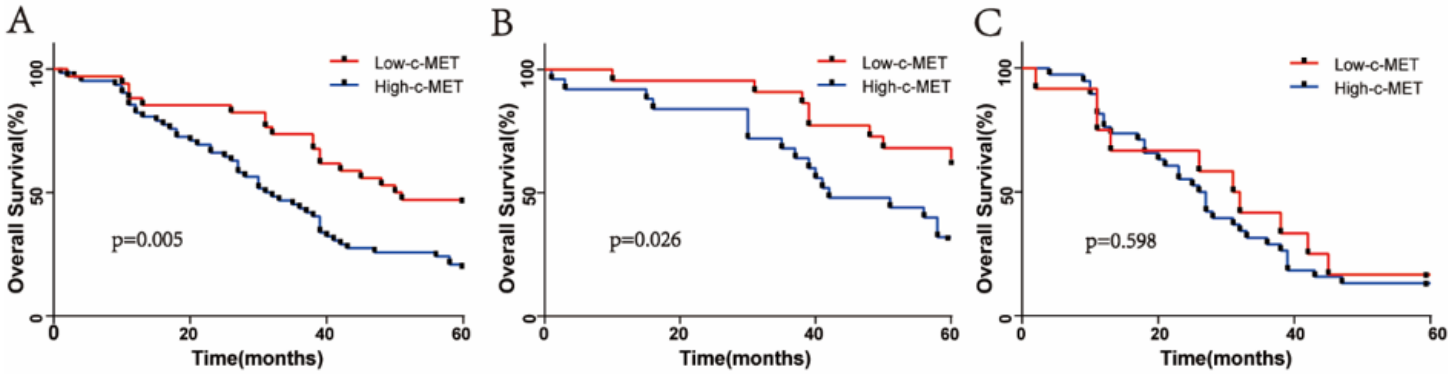

Figure 3

Kaplan-Meier surcival curves of log-rank test for 5-year OS. Figure A-C 5-year OS curves of patients with high and low expression of c-Met (totality, stage Ⅰ-Ⅱ patients and stage Ⅰ-Ⅱ patients).