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Original Article

The clinical significance and expression of TGF-β1 and CD13 in primary lesion and metastasis of gastric cancer

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Abstract: Objective: To investigate the changes of expressions of transforming growth factor (TGF-β1) and tumor stem cell marker (CD13) in primary lesion and metastasis of gastric cancer (GC), and then explore its clinical significance. Methods: The radical resection of GC was performed for 50 GC patients with lymph node metastasis and 38 GC patients without lymph node metastasis. During the operation, the adjacent mucosas at the cutting edge, the primary lesion of GC and the metastatic lymph node were collected (the adjacent mucosas samples were divided into MA group which with lymph node metastasis and NMA group which without lymph node metastasis; the primary lesion samples were divided into MP group which with lymph node metastasis and NMP group which without lymph node metastasis; the metastatic lymph node samples were classified as M group). The blood samples were collected to take the serum and the peripheral blood mononuclear cells (PBMCs) were then seperated. At the same time, plasma was collected from 20 healthy volunteers and the PBMC was separated (H group). The expressions of TGF-β1 and CD13 in each sample were detected by the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) or S-P immunohistochemical method. The expressions of TGF-β1 in plasma were detected by enzyme-linked immunosorbent assay (ELISA). Then, the relevance of TGF-β1 and CD13 expressions and its relationship with the clinical parameters of GC were analyzed. Results: TGF-β1 and CD13 were expressed in both primary tumor of GC and its metastatic lymph nodes: compared with MA group, the levels of TGF-β1 and CD13 mRNA in MP group and M group were obviously increased ($P<0.01$), and there was no significant difference between MP group and M group ($P>0.05$); compared with NMP group, the levels of TGF-β1 and CD13 mRNA in the MP group were increased ($P<0.05$) while there was no distinct difference between the MA group and the NMA group ($P>0.05$); compared with H group, the levels of plasma TGF-β1 in both patients with lymph node metastasis and patients without lymph node metastasis were increased ($P<0.05$), and the content of TGF-β1 in plasma in MP group was significantly higher than that in the NMP group ($P<0.01$). The expressions of TGF-β1 mRNA and CD13 mRNA in the peripheral blood mononuclear cells (PBMC) of the three groups also showed the similar results. The immunohistochemical results of different GC tissues were consistent with the results of RT-qPCR. The expressions of TGF-β1 and CD13 in the primary lesion had significant correlations ($P<0.01$), and they were related to the tumor size, lymph node metastasis, degree of tumor differentiation and clinical stage ($P<0.05$), but was not associated with the gender and age ($P>0.05$). Conclusion: TGF-β1 and CD13 were closely related to the metastasis of GC, and might be a potential target for the treatment of GC.

Keywords: GC, primary lesion, metastasis, TGF-β1, CD13

Introduction

As a common alimentary tract malignant tumor, GC is one of the malignant tumors with a high incidence in China [1]. The specificity of early GC is unapparent, and its diagnostic accuracy is poor. Meanwhile, the survival rate of GC patients with lymph node metastasis is still not very high, though there are many therapies [2], so the treatment effect and the prognosis of GC is an important clinical problem that needs to be solved.

A large number of cytokines are involved in the invasion and metastasis of tumor. TGF-β1 is an important cytokine in tumor formation and development and it has a variety of biological functions. The main functions of TGF-β1 include promoting the chemotaxis of inflammatory cells, facilitating cell proliferation, differentia-

tion and migration, affecting the formation of blood vessels and the degradation and synthesis of extracellular matrix, etc. [3]. Additionally, TGF-β1 has different biological functions towards different kinds of cells. It can restrain the proliferation of normal cells and promote their differentiation. For cancer cells, however, TGF-β1 can promote the formation of blood vessel in the tumor microenvironment, and then promote their growth and metastasis [4, 5].

At present, the cancer stem cell hypothesis is used to explain the heterogeneity and metastasis of tumor, and it holds that cancer stem cells are the source of tumorigenesis and have relevance with the metastasis and tolerance of cancer cells in the later stage [6]. CD13, also known as aminopeptidase N (APN), a new type of cancer stem cell surface marker, is a kind of proteinase which can promote the formation of blood vessels and regulate the invasion and metastasis of cancer cells. Previous studies have shown that CD13 is highly expressed in breast cancer, prostate cancer and other tumors [7, 8], and also expressed in tumor vascular endothelial cells and all kinds of cells that can constitute the tumor microenvironment [9]. In addition, it has been reported that CD13 inhibitors and CD13 antibody could induce the cell apoptosis, and still have effects on drug-resistant tumor cells [10].

TGF-β1 in GC cells can raise the tumor stem cell surface marker CD44, thereby promoting the invasion and metastasis of tumor [11]. However, in regard to CD13, which is another tumor stem cell surface marker, there are few reports about whether its expression can be affected by the expression of TGF-β1 and what's their relevance in the primary tumor, and whether there are differences between its expressions in primary lesion and metastasis of tumors. Therefore, in this study, the real-time polymerase chain reaction (RT-PCR) and immunohistochemical method were applied to detect the peripheral blood mononuclear cells (PBMCs), normal tissues adjacent to the tumor and expressions of TGF-β1, CD13 mRNA and protein in the primary lesion of GC and the corresponding lymph node metastases, and the enzyme-linked immunosorbent assay (ELISA) was employed to detect the concentration of TGF-β1 in serum, which aimed to compare the changes of expressions of TGF-β1 and CD13 in primary lesion and metastasis of GC and analyze the relationship between their expressions and the clinical characteristics of GC. Moreover, the role of TGF-β1 and APN/CD13 in the development and metastasis of GC were further illustrated, thereby providing potential therapeutic targets for GC.

Materials and methods

Materials

Patients: This clinical study was approved by the East Branch of Qingdao Municipal Hospital Ethics Committee and tissue samples were obtained with the consent of each patient. Eighty-eight samples of GC tissues were collected via the histopathologic examination at the General Surgery Department of East Branch of Qingdao Municipal Hospital from November 2015 to November 2016. The clinical data were complete and detailed. And all the patients had been diagnosed without preoperative radiotherapy, chemotherapy, immunotherapy or other underlying diseases in vital organs. Among these patients, 50 patients had lymph node metastasis and 38 patients had not. The corresponding non-tumor normal tissues, the primary lesion of GC and the metastatic lymph nodes were collected during the operation (the corresponding non-tumor normal tissues samples were divided into MA group which with lymph node metastasis and NMA group which without lymph node metastasis; the primary lesion samples were divided into MP group which with lymph node metastasis and NMP group which without lymph node metastasis; the metastatic lymph nodes samples were named as M group). The blood samples were collected before operation, the peripheral blood mononuclear cells (PBMC) were then separated, and the plasma was finally collected for later use. At the same time, the blood samples from 20 healthy volunteers were collected (H group), the PBMC were separated and the plasma was collected for later use. The clinical stage of GC referred to the standard of Japanese clinical stage of GC.

Main reagents: TRIzol® Reagent (15596-026, Invitrogen, California, United States), Ficoll-Paque PLUS (17-1440-02, GE Healthcare, Sweden), reverse transcriptase PrimeScriptTM RT Master Mix (RR036A, TaKaRa, Japan), QPCR enzyme SYBR® Premix Ex TaqTM II (RR820A,

TaKaRa, Japan), S-P kit (S-P 9001, Zhongshanjinqiao Biotechology Company, Beijing), rabbit anti-human TGF-β1 monoclonal antibody (Bioss Biotechology Company, Beijing), rabbit anti-human CD13 monoclonal antibody (Epitomics, United States), goat anti-rabbit IgG polyclonal antibody (Epitomics, United States), TGF-β1 ELISA kit (Jinmei Biotechology Company, Shenzhen).

Methods

Separation of peripheral blood mononuclear cells (PBMC): 10 ml of anticoagulated blood was mixed adequately with the equivalent volume of RPMI medium. 10 ml of Ficoll were added into a 15 ml centrifuge tube and the mixture was then added to the Ficoll surface slowly along the tube wall. The interface should be kept definite and it was then centrifuged at 400 g for 30 min. After that, the intermediate film layer was aspirated into a new 15 ml centrifuge tube and 2 times volume of PBS were added. It was then centrifuged at 1500 rpm for 10 min and the supernatants were removed and 1 ml of Trzol were added to extract RNA.

Detection by fluorescence quantitative PCR instrument: The total RNAs of corresponding adjacent normal tissues, primary lesion of GC, metastatic lymph nodes and PBMC were extracted by the Trizol method, and the complementary DNA (cDNA) was obtained using a Takara reverse transcription kit. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was taken as internal control; the ABI 7500 fluorescence quantitative PCR instrument (Applied Biosystems, USA) was employed as the detection device. All the primers were synthesized by Shanghai Shengong Company. The specific primers (5'->3') were as follows: TGF-β1, forward primer: GACTTCAGCCTGGACAACGA, reverse primer: GTTCAGCAGGACCCACTCAT; CD13, forward primer: GCCCATCACATCCATCAGAG, reverse primer: GTTCAGCAGGACCCACTCAT; GAPDH, forward primer: GACAGTCAGCCGCATCTTCT, reverse primer: AAATGAGCCCCAGCCTTCTC. The amplification conditions were as follows: initial denaturization for 30 s at 95°C, then 40 cycles of 5 s at 95°C, and 30 s at 60°C. The Ct values of each sample were calculated, and the relative quantitation was calculated by comparing the Ct values of internal reference genes and applying the $2^{-\Delta\Delta CT}$ method.

S-P immunohistochemical method: Samples in each group were embedded with paraffin and cut into 4-um-thick sections. The conventional dewaxing was performed after roasting at 60°C for 2 h in a constant-temperature incubator. The TGF-β1 antibody was diluted 1:100 and the CD13 antibody was diluted 1:200. The second antibody was goat anti-rabbit IgG polyclonal antibody. The immunohistochemistry was performed according to the kit instructions, and the samples were counterstained with hematoxylin after with diaminobenzidine (DAB).

Result criterion: TGF-β1 was mainly expressed in the cytoplasm of GC tissues and occasionally in the nucleus. CD13 was mainly expressed in the cell membrane and partly in the cytoplasm. The semi-quantitative integration method was applied to judge the positive result [12]. Staining intensity was graded as follows: 0 (no staining), 1 (light yellow), 2 (yellow brown) and 3 (brown). 10 views with high magnification (*400) were randomly selected from each slice, and 100 cells were counted in each field of view to calculate the percentage of positive cells (the positive rates of cells were 0, <10%, 11-50%, 51-80% and >80% accordingly and their grades were 0, 1, 2, 3, 4 points respectively). If the cross product of integral of the above staining intensity and positive cell rate was less than or equal to 3, it was labeled as negative; if the cross product was greater than 3, it was labeled as positive.

ELISA detection of TGF-β1 in plasma: 10 ml blood containing Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant was collected from all the patients in each group. Then the plasma was taken after being centrifuged at 1600 g for 10 min. The plasma level of TGF-β1 was detected by the ELISA kit, and the operation was performed strictly according to the kit instruction.

Statistical analysis

The softwares of SPSS18.0 and GraphPad Prism 6 were used for statistical analysis. The measurement data was presented as mean ± standard deviation, and comparisons between two groups were analyzed by using the t test; comparison among more than two groups were analyzed using one-way ANOVA. The enumeration data was presented as case number and percentage, and analyzed using the χ^2 test and

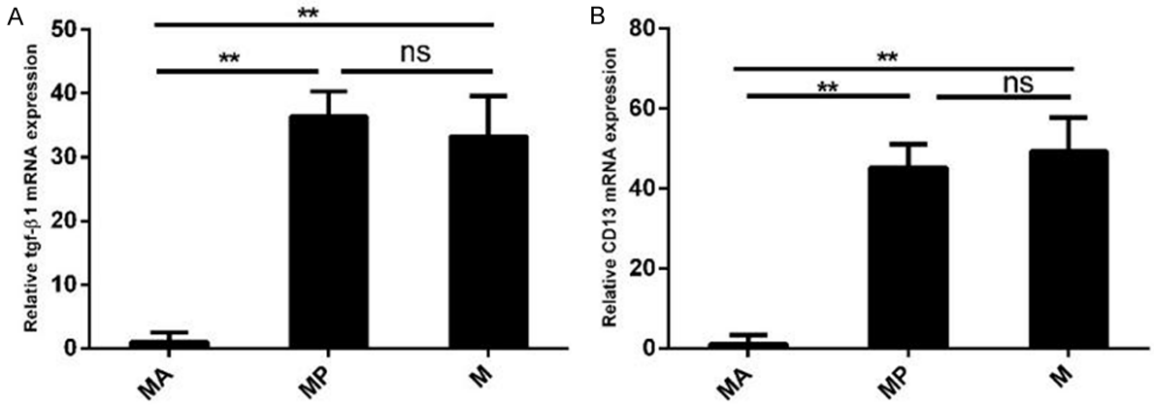

Figure 1. TGF-β1 and CD13 mRNA expression levels in different tissues of GC patients with lymph node metastases. A: TGF-β1 mRNA expressions in different groups; B: CD13 mRNA expressions in different groups (**P<0.01).

Table 1. The expressions of TGF-β1 and CD13 in different tissues of GC patients with lymph node metastases

Group	n	TGF-β1		CD13	
		- (%)	+ (%)	- (%)	+ (%)
MA	50	48 (96.0)	2 (4.0)	50 (100.0)	0 (0.0)
MP	50	12 (24.0)	38 (76.0)**	9 (18.0)	41 (82.0)**
M	50	12 (24.0)	38 (76.0)&&	14 (28.0)	36 (72.0)&&

Note: Compared with MA group, **P<0.01, &&P<0.01.

Spearman's correlation test. P<0.05 indicated that the difference had statistical significance.

Results

High expressions of TGF-β1 mRNA and CD13 mRNA in the primary lesion with lymph node metastasis and its metastatic lymph nodes

Fifty biological samples from patients with lymph node metastasis were detected by the real-time polymerase chain reaction (RT-PCR) and the results were as follows: TGF-β1 and CD13 were expressed in both MP group and M group; compared with MA group, the levels of TGF-β1 and CD13 mRNA in both MP group and M group were increased (P<0.01, **Figure 1**); and there was no significant differences between TGF-β1 and CD13 mRNA in MP group and M group (P>0.05, **Figure 1**). The immunohistochemical results in **Table 1** shows as follows: TGF-β1 was positively expressed in 38 cases (76%) in both MP group and M group, and TGF-β1 was hardly expressed in MA group (P<0.01). CD13 was positively expressed in 41 cases (82%) in MP group and 36 cases (72%) in M group, but CD13 was not expressed in MA group (P<0.01).

Differential expressions of TGF-β1 mRNA and CD13 mRNA in primary lesions with/without lymph node metastasis and their corresponding adjacent normal tissues with/without lymph node metastasis

The expression levels of TGF-β1 mRNA and CD13 mRNA in primary lesion samples with lymph node metastasis (n=50, MP), primary lesion samples without lymph node metastasis (n=38, NMP), corresponding adjacent normal tissue samples with lymph node metastasis (n=50, MA) and corresponding adjacent normal tissue samples without lymph node metastasis (n=38, NMA) were detected by the the real-time polymerase chain reaction (RT-PCR). Compared with NMA group and MA group, the expression levels of TGF-β1 and CD13 mRNA in NMP group and MP group were increased (P<0.01, **Figure 2**). And there was no statistical differences in TGF-β1 or CD13 mRNA expressions between MA and NMA group (P>0.05). However, the expression levels of TGF-β1 and CD13 mRNA in MP group were higher than that in NMP group (P<0.05, **Figure 2**).

The positive rates of TGF-β1 and CD13 expressions in NMP group and MP group were further compared with that in NMA group and MA group. **Table 2** shows that the positive rates of TGF-β1 and CD13 expressions were high in both NMP group and MP group. TGF-β1 was found positively expressed in 18 cases (47.3%) in NMP group and 38 cases (76.0%) in MP

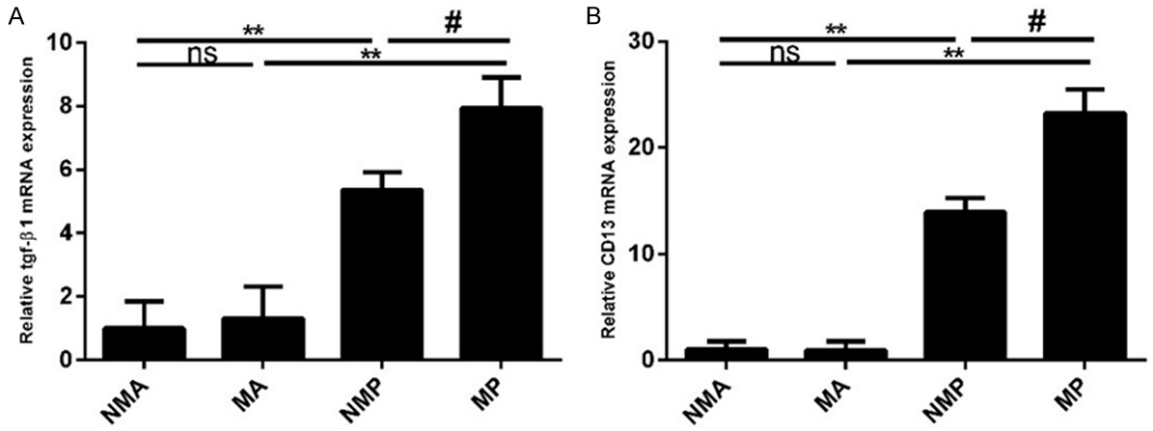

Figure 2. TGF-β1 and CD13 mRNA in primary lesions of both gastric cancer patients with or without lymph node metastases. A: TGF-β1 mRNA in different groups; B: CD13 mRNA in different groups (**P<0.01, #P<0.05).

Table 2. The expressions of TGF-β1 and CD13 in primary lesions with/without lymph node metastasis and their corresponding adjacent normal tissues with/without lymph node metastasis

Group	n	TGF-β1		CD13	
		- (%)	+ (%)	- (%)	+ (%)
NMA	38	37 (97.4)	1 (2.6)	36 (94.7)	2 (5.3)
MA	50	48 (96.0)	2 (4.0)	50 (100.0)	0 (0.0)
NMP	38	20 (52.6)	18 (47.3)&&	14 (36.8)	24 (63.2)&&
MP	50	12 (24.0)	38 (76.0)**,##	9 (18.0)	41 (82.0)**,#

Note: Compared with MA group, **P<0.01; compared with NMA group, &&P<0.01; compared with NMP group, ##P<0.01, #P<0.05.

group, which showed significant differences (P<0.01). And CD13 was found positively expressed in 24 cases (63.2%) in NMP group and 41 cases (82.0%) in MP group, which also showed distinct differences (P<0.05). However, the TGF-β1 and CD13 were hardly expressed in the MA group or NMA group.

The TGF-β1 expressions in blood samples of patients with GC in the two groups were significantly higher than that in healthy volunteers, and TGF-β1 mRNA and CD13 mRNA expressions in PBMC were also different between the three groups

The blood samples from 88 patients with GC and 20 healthy volunteers were detected by ELISA and the results were as follows: compared with the H group, the plasma levels of TGF-β1 in both GC patients with lymph node metastasis and without lymph node metastasis were increased (P<0.05, **Figure 3A**), and the plasma level of TGF-β1 in MP group was significantly higher than that in NMP group (P<0.01, **Figure 3A**). And after the detection via RT-PCR, we found that there was differences between the expressions of TGF-β1 mRNA and CD13 mRNA in PBMC: the expressions of TGF-β1 mRNA and CD13 mRNA in PBMC in GC patients were higher than that in healthy volunteers (P<0.05, **Figure 3B**, **3C**), and the expression levels of TGF-β1 mRNA and CD13 mRNA in patients with metastasis was substantially higher than that without metastasis (P<0.01, **Figure 3B**, **3C**).

Relationship between expressions of TGF-β1 and CD13 in primary lesion

The immunohistochemical results in **Tables 3** and **4** are shown as follows: the TGF-β1 expressions and CD13 expressions in 38 cases of primary lesion without lymph node metastasis were significantly correlated (r_s=0.506, χ^2=9.731, P=0.002); and the TGF-β1 expressions and CD13 expressions in 50 cases of primary lesion with lymph node metastasis were also significantly correlated (r_s=0.468, χ^2=10.954, P=0.001).

TGF-β1 and CD13 were correlated with the clinical stage, degree of tumor differentiation and lymph node metastasis

The immunohistochemical examination and statistical analysis had showed that TGF-β1 and CD13 were associated with the tumor size, lymph node metastasis, degree of tumor differentiation and clinical stage (P<0.05), but were not related to gender and age (P>0.05). Information is detailed in **Table 5**.

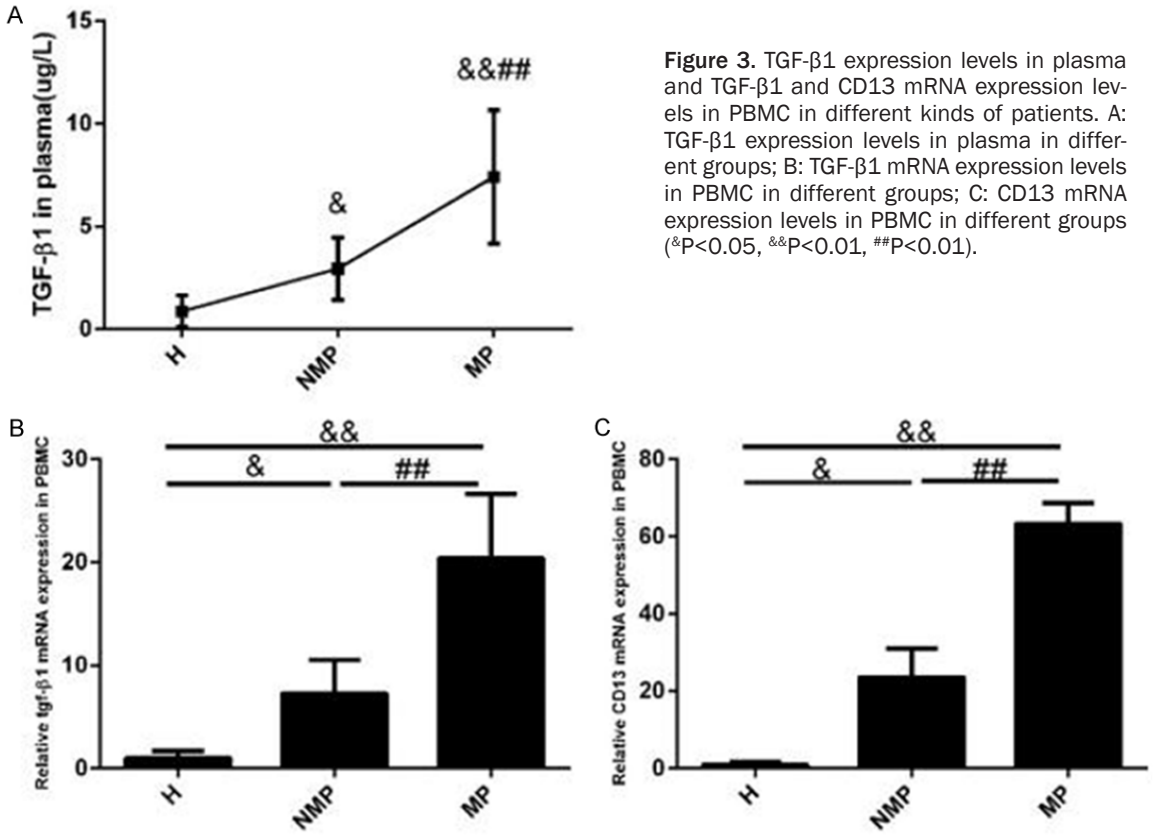

Figure 3. TGF-β1 expression levels in plasma and TGF-β1 and CD13 mRNA expression levels in PBMC in different kinds of patients. A: TGF-β1 expression levels in plasma in different groups; B: TGF-β1 mRNA expression levels in PBMC in different groups; C: CD13 mRNA expression levels in PBMC in different groups ($^{\&}P<0.05$, $^{\&\&}P<0.01$, $^{\#\#}P<0.01$).

Table 3. The relationship between TGF-β1 expressions and CD13 expressions in primary lesions of GC without lymph node metastases

TGF-β1	CD13		χ^2	*P* value	r_s
	-	+			
-	12	8	9.731	0.002	0.506
+	2	16			

Table 4. The relationship between TGF-β1 expressions and CD13 expressions in primary lesions of GC with lymph node metastases

TGF-β1	CD13		χ^2	*P* value	r_s
	-	+			
-	6	6	10.954	0.001	0.468
+	3	35			

Discussion

The invasion, infiltration and metastasis of tumor are important factors which can affect the prognosis of patients [13]. And the occurrence may be related to the increase of cancer stem cells and the expressions of metastasis promoting factors. As a cytokine with multiple functions, TGF-β1 can promote the formation of new tumor vessels and thereby playing an important role in promoting tumor growth and metastasis [4, 5]. CD13 also plays an important role in the physiological and pathological conditions. The previous researches have showed that CD13 is expressed in normal epithelial cells, myeloid cells and fibroblasts, and has relations with the inflammatory signals [14]. Other studies also found that [15, 16] CD13 was highly expressed in solid tumors such as liver cancer, small-cell lung cancer and ovarian cancer. What's more, CD13, as a marker of tumor stem cells, is a kind of enzyme related to the degradation of extracellular matrix proteins. Additionally, the expression of CD13 in tumor cell membrane can enhance the mobility of tumor cells and accelerate the proliferation and metastasis of cancer cells [17]. At the same time, some studies showed that TGF-β1 in GC can up-regulate the tumor stem cell marker CD44, thereby promoting the tumor invasion and metastasis [11]. However, there were few studies about the correlation between the tumor stem cell marker CD13 and TGF-β1 in pri-

Table 5. The relationship between the expressions of TGF-β1, CD13 in primary lesions of all kinds of GC patients and the clinical parameters

Clinical parameters	n	TGF-β1			CD13		
		+ (%)	χ^2	*P* value	+ (%)	χ^2	*P* value
Gender							
Male	55	36 (65.5)	0.210	0.6471	40 (72.7)	0.098	0.3132
Female	33	20 (60.6)			25 (75.8)		
Age(y)							
≤60	37	22 (59.5)	0.481	0.4878	27 (73.0)	0.026	0.1620
>60	51	34 (66.7)			38 (74.5)		
Tumor size(cm)							
<5	39	20 (51.3)	4.620	0.0316	23 (59.0)	8.043	0.0046
>5	49	36 (73.5)			42 (85.7)		
Lymph Nodes Metastasis							
Y	50	38 (76.0)	7.649	0.0057	41 (82.0)	3.971	0.0463
N	38	18 (47.4)			24 (63.2)		
Differentiation							
High and Moderate	36	15 (41.7)	12.710	0.0004	22 (61.1)	5.132	0.0235
Low	52	41 (78.8)			43 (82.7)		
TNM							
I+II	48	25 (52.1)	6.091	0.0136	30 (62.5)	7.064	0.0079
III+IV	40	31 (77.5)			35 (87.5)		

mary lesion of GC and the differences of their expressions in primary lesion and metastasis of GC. In this study, the expressions of CD13 and TGF-β1 in different tissue samples of GC and the levels of TGF-β1 in plasma were detected and the expression levels of CD13 and TGF-β1 mRNA were tested by RT-qPCR, which used to compare with the clinical data of patients. In this way, the correlations between tumor stem cell marker CD13 and TGF-β1 in primary lesion of GC were demonstrated.

There was significant differences between CD13 and TGF-β1 mRNA levels which were found in the adjacent mucosa, primary foci and metastatic lymph nodes of 50 GC patients with lymph node metastasis (P<0.01, **Figure 1**), and CD13 and TGF-β1 were highly expressed in the primary lesion and lymph node metastasis, but the expressions showed no statistical differences (P>0.05, **Figure 1**), which indicated that both CD13 and TGF-β1 could promote the tumor metastasis and formation. Next, we compared and analyzed the expressions of CD13 and TGF-β1 in primary lesion of GC. Among them, 38 cases of GC had not lymph node metastasis and 50 cases of GC had lymph node metastasis. All the expression were found to be higher than that in the corresponding adjacent mucosa tissues (P<0.01, **Figure 2**), but the expressions of primary lesion with lymph node metastasis were significantly higher than that without metastasis (P<0.05, **Figure 2**), which indicated that CD13 and TGF-β1 may be related to the metastasis of GC cells. The expression levels of TGF-β1 in plasma in 88 GC patients were substantially higher than that in 20 healthy volunteers (P<0.05, **Figure 3**). Among them, the expressions levels of TGF-β1 in plasma in patients with lymph node metastasis were higher than that inpatients without metastasis (P<0.01, **Figure 3**), and the expressions of CD13 and TGF-β1 mRNA in PBMC in healthy volunteers and patients with GC showed the similar trends. The expression trends of TGF-β1 in plasma, TGF-β1 mRNA in PBMC were consistent with the expression trend of TGF-β1 mRNA in the GC primary lesion, which suggested that the level of TGF-β1 in blood reflected the gene expression in primary lesion of GC, and TGF-β1 played an important role in the invasion and lymphatic metastasis of GC. Other studies had showed that the TGF-β1 was associated with the tumor staging in colorectal cancer serum and tissue samples [18], and the levels of TGF-β1 in GC tissues

were correlated with tumor types and stages [19], which have similarities to the results of this study. All these above suggested that TGF-β1 had a significant correlation with the tumor angiogenesis and metastasis.

CD13 also known as aminopeptidase N, is currently regarded as the cancer stem cell surface markers [20] and TGF-β1 in GC can up-regulate the tumor stem cell surface marker CD44, thereby promoting the tumor invasion and metastasis [11]. Therefore, in this study, we investigated the relations between CD13, which is also a kind of tumor stem cell surface markers, and TGF-β1 in GC. Meanwhile, this study analyzed the correlation between the expressions of CD13 and TGF-β1 in GC patients with lymph node metastasis and without lymph node metastasis, and finally found that there was a positive correlation between CD13 and TGF-β1 ($P=0.002$, $P=0.001$).

We also found that the expression levels of CD13 and TGF-β1 in primary lesion of GC had no relation with the age and gender of patients ($P>0.05$), but had correlation with tumor size, lymph node metastasis, degree of tumor differentiation and clinical analysis. This finding showed that CD13 and TGF-β1 were related to the degree of malignancy and invasive ability of GC cells, which was roughly the same as the research conclusions about CD13 in GC proposed by Kawamura et al [21]. We also found that the positive rate of TGF-β1 and CD13 were 51.3% and 59% respectively when the tumor size was smaller than 5 cm. And the positive rate of TGF-β1 and CD13 were 73.5% and 85.7% respectively when the tumor size was greater than 5 cm. The above result suggested that CD13 and TGF-β1 played a vital role in tumor proliferation. Other previous studies have shown that CD13 inhibitors can interfere with the G1/G0 phase of tumor stem cells and inhibit the growth of tumor cells [20]. And the generation of TGF-β1 plays an important role in promoting the proliferation, invasion and metastasis of tumor cell [21]. These results are consistent with our study. This study found that the positive rates of TGF-β1 and CD13 in patients with lymph node metastasis were 76% and 86% respectively, significantly higher than the expressions in primary lesions of patients without lymph node metastasis (47.3% and 63.2%, $P<0.01$ and $P<0.05$). The above result suggested that TGF-β1 and CD13 played an essential role in the tumor metastasis. In addition, TNM staging system is an important index to judge the progress of GC, design the treatment plan and evaluate the prognosis. In this study, TGF-β1 and CD13 positive rates in the clinical stage III+IV were 77.5% and 87.5% respectively and were substantially higher than the TGF-β1 and CD13 positive rates (52.1%, 62.5%) in the clinical stage I+II, showing the functions of TGF-β1 and CD13 in the tumor infiltration, which was consistent with the function of CD13 as the index affecting the prognosis in hepatocarcinoma [22].

In this study, the number of samples was limited, so we should increase the number of samples and combine the basic research to further explore the relevance between TGF-β1 and CD13 and their influences on behaviors of cancer cells. Therefore, as far as this research, we found that TGF-β1 and CD13 may play an important role in the tumor cell proliferation, metastasis and infiltration. And it was helpful to understand the disease progress and prognosis of patients in GC from the expressions of CD13 and TGF-β1 in plasma or tissues samples, thereby providing the optimal treatment plan for patients. In conclusion, this study has potential to provide a new direction for the targeted therapy of tumor in the future since CD13 and TGF-β1 may become new targets of new anticancer drug development.

Disclosure of conflict of interest

None.

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