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Clinical Significance of Transcription Factor 7 (TCF7) as a Prognostic Factor in Gastric Cancer

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Transcription factor 7 (TCF7) plays an essential role in Wnt signaling by interacting with β-catenin. Emerging evidence demonstrates that overexpression of TCF7 promotes progression or correlates with poor progression in several types of cancers, but the functions of TCF7 in gastric cancer (GC) have not been revealed.

Material/Methods: A total of 168 patients with GC who underwent radical surgeries were collected and regarded as the test cohort. The expression of TCF7 in the 168 patients was detected with immunohistochemistry. Moreover, the mRNA levels of TCF7 in 11 pairs of GC and adjacent tissues were detected with quantitative real-time PCR (qRT-PCR). The correlations between TCF7 and the clinicopathological factors were evaluated with the chi-square test, and the prognostic value of TCF7 in GC was investigated with univariate analysis and multivariate analysis.

Results: The mRNA levels of TCF7 in GC tissues were significantly higher than in corresponding tumor adjacent tissues. The patients of low TCF7 expression and high TCF7 expression accounted for 76.79% (129/168) and 23.21% (39/168), respectively. In our experiments, TCF7 was significantly associated with positive lymphatic invasion (P=0.022) and metastasis (P<0.001). The high expression of TCF7 was correlated with low survival rates (P=0.012) and was confirmed as an independent prognostic factor (HR=1.92, 95%CI =1.06–3.47, P=0.031) of GC in multivariate analysis.

Conclusions: TCF7 expression is correlated with metastasis and is an independent prognostic factor of GC. TCF7 detection of GC could help stratify the patients with high risk and guide precise treatment.

MeSH Keywords: Neoplasm Metastasis • Prognosis • Stomach Neoplasms • Transcription Factor 7-Like 1 Protein

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Background

Gastric cancer (GC) is the fourth most common cancer and is the third leading cause of cancer mortality in the world [1]. In developing countries, gastric cancer threatens people's health more seriously compared with developed countries. In China, gastric cancer is the second leading cause of cancer death [2]. Radical surgical resection is the only curative method for gastric cancer, but most patients lose the opportunity because of early metastasis. Despite improvements in surgical equipment, chemical regimens, and targeted therapy, the overall survival rates of gastric cancer are still very dismal. The 5-year survival rate is approximately 20% in many countries [3]. Therefore, new predictive or prognostic biomarkers are in urgent need because of insufficient information to guide precise treatment and predict clinical outcomes of GC.

The TCF/lymphoid enhancer-binding factor (LEF) family consists of 4 members, mainly functioning as an effector of Wnt signaling [4]. Transcription factor 7 (TCF7, also known as T cell factor 1) belongs to the TCF family and has the molecular features of the TCF family as a high-mobility group DNA-binding domain and a β-catenin-binding domain. The former domain can recognize DNA and switch transcription activities in response to Wnt signaling, while the latter domain can interact with nuclear β-catenin [5]. As a transcriptional activator, much evidence demonstrates that overexpression of TCF7 promotes progression or correlates with poor prognosis in several types of cancers. Moreover, the downregulation of TCF7 by some miRNAs can suppress tumor progression or carcinogenesis [6–8]. Ectopic activation of Wnt signaling is critical in progression of gastric cancer [9], but the expression and clinical significance in gastric cancer has never been explored.

Here, we detected the expression of TCF7 in 168 patients with GC and compared TCF7 expression in 11 pairs of GC tissues and adjacent tissues. Moreover, we evaluated the clinical significance of TCF7 by analyzing the correlation between TCF7 and clinicopathological factors and survival rates.

Material and Methods

Patients and follow-ups

In Yidu Central Hospital, a total of 245 patients underwent radical surgery for GC from 2012 to 2015. A total of 168 patients were selected into the validation cohort with the following inclusion criteria: (1) the pathological diagnosis was gastric adenocarcinoma, (2) available follow-up information, and (3) no other malignancies. Moreover, a total of 11 pairs of GC tissues and adjacent tissues was collected in 2016 during surgery and immediately preserved in liquid nitrogen for mRNA extraction. The study was approved by the Ethics Committee of Linyi Central Hospital and Tiantai People's Hospital. All the specimens were obtained after obtaining the signed approval of patients. The tumor TNM stage was determined based on the guidelines of 7th American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC).

Immunohistochemistry and evaluation

The expression of TCF7 in GC tissues was visualized by immunohistochemistry (IHC) with streptavidin peroxidase complex method according to a previous report [10]. After deparaffinization and rehydration of the formalin-fixed and paraffin-embedded slides in graded ethanol, the optimal antigen retrieval was achieved by solid citrate buffer (pH=6.0). H_2O_2 at 3% was used to block the endogenous peroxidase activity and 5% fetal bovine serum was applied to block unspecific antigen binding. Specimens were incubated in the primary antibody (Cat. No. #2203, Cell Signaling Technology, MA, USA) at 1: 100 at 4°C overnight, and then in secondary antibody (Beyotime Biotechnology, Shanghai, China) at room temperature for 1 h. Finally, 3'-diaminobenzidine substrate was added for TCF7 visualization.

TCF7 expression was semi-quantified by the IHC score evaluated by 2 independent pathologists unaware of the clinical data, according to previous reports [11,12]. There are 2 aspects of the IHC score: the score for staining intensity and the score for positive cell percentage. Scores for staining intensity were defined as: score 0 was negative staining, score 1 was weak staining, score 2 was moderate staining, and score 3 was strong staining. Scores for positive cell percentage were classified as: 1 meant <25% of positively stained cells, 2 meant 25–50% of positive cells, 3 meant 50%–75% positive cells, and 4 meant more than 75% of positive cells. The final score was the product of the score (staining intensity) multiplied by score (positive cell percentage), which ranged from 0 to 12. The cut-off of IHC score was set as the point with the highest specificity plus sensitivity in the receiver operating characteristic curve (ROC) curve.

RNA extraction and quantitative real-time PCR

The mRNA of 11 pairs of GCs and corresponding adjacent tissues were extracted using TRIzol (Invitrogen, Carlsbad, USA) according to the manual. RNA purity was detected by absorbance ratio of 260 nm/280 nm. Complementary DNA (cDNA) synthesis was performed using a qPCR-RT-Kit (Toyobo Co., Osaka, Japan). The quantitative PCR of TCF7 was realized by SYBR Green Master Mix and StepOnePlus system (Applied Biosystem, Waltham, MA, USA) with 18S as the internal control. The primers were as follows:
TCF7: forward, aggtcagatgggttggactg; reverse, agggtgcacactgggtttag.

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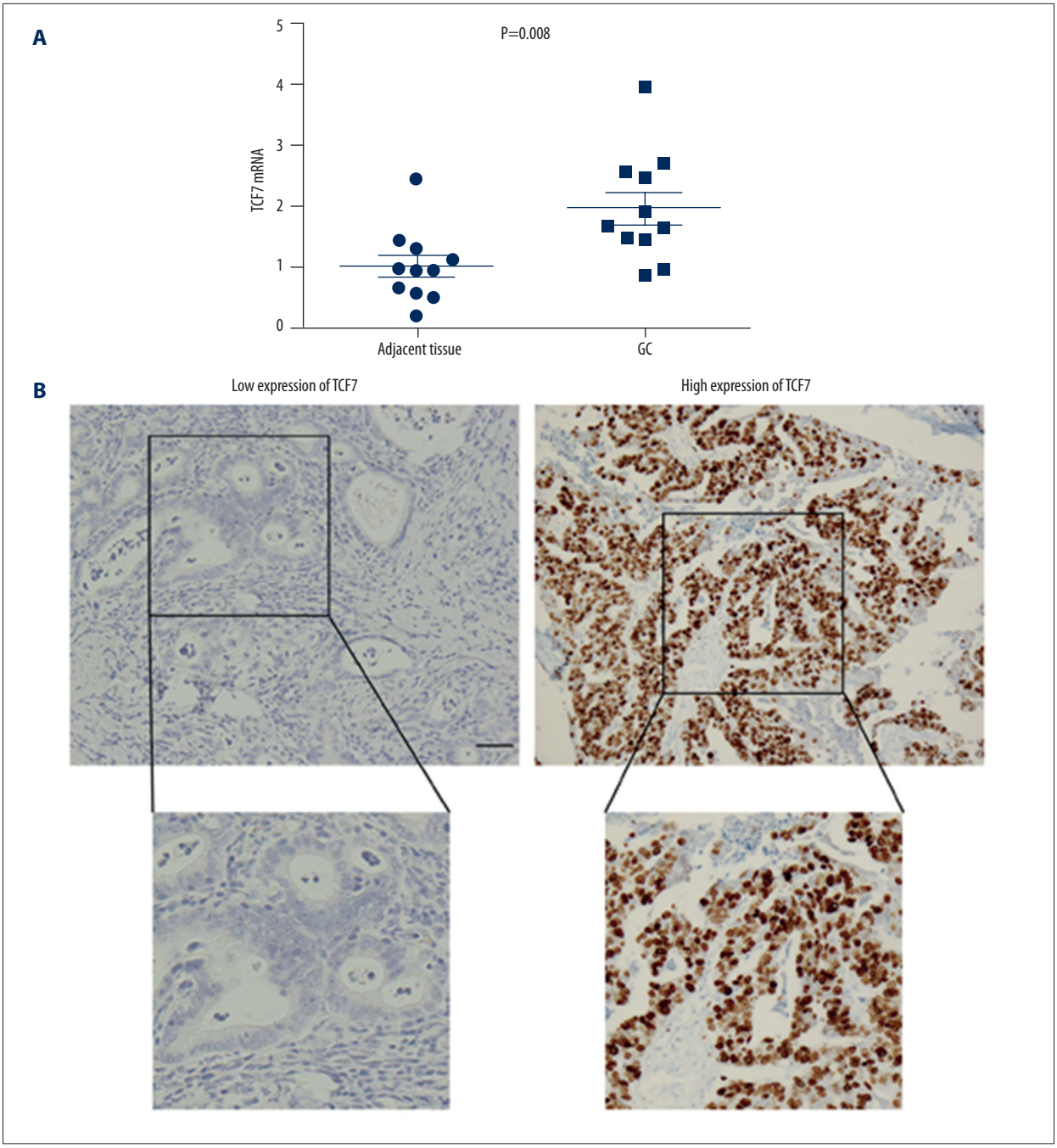

Figure 1. Expression of TCF7 in GC tissues and tumor adjacent tissues. (**A**) TCF7 mRNA levels in adjacent tissues were significantly lower than in GC tissues. The mRNA of TCF7 was detected in 11 pairs of GCs and corresponding adjacent tissues with qRT-PCR. (**B**) The representative images of low expression and high expression of TCF7 in GC. Scale bar: 100 μm.

18S: forward: cagccacccgagattgagca; reverse: tagtagcgacgggcggtgtg.

Statistical analysis

We used SPSS 21 (SPSS, Inc., Chicago, USA) to analyze all data without special illustration. The correlations between TCF7 expression and the clinicopathological factors were analyzed by chi-square test. The overall survival curve was displayed by Kaplan-Meier method and the statistical significances between different groups were compared by the log-rank test. The Cox regression proportional hazards model was applied to identify independent prognostic factors. $P<0.05$ was considered statistically significant.

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Table 1. Basic information of the validation cohort.

Factors	Number	Percentage
Gender		
Male	124	73.81%
Female	44	26.19%
Age		
<60	76	45.24%
≥60	92	54.76%
Tumor diameter (cm)		
≤5	71	42.26%
>5	97	57.74%
Differentiation		
Well + moderate	96	57.14%
Poor	72	42.86%
Tumor invasion		
T1+T2	45	26.79%
T3+T4	123	73.21%
Lymphatic invasion		
No (N0)	62	36.90%
Yes (N1/2/3)	106	63.10%
Distant metastasis		
M0	153	91.07%
M1	15	8.93%
TNM stage		
I–II	64	38.10%
III–IV	104	61.90%
TCF7		
Low	129	76.79%
High	39	23.21%

Table 2. TCF7 was significantly associated with tumor lymphatic invasion and metastasis.

Factors	TCF7		P*
	Low	High	
Sex			
Male	93	31	0.412
Female	36	8	
Age			
<60	57	19	0.714
≥60	72	20	
Tumor diameter (cm)			
≤5	55	16	0.858
>5	74	23	
Differentiation			
Well + moderate	75	21	0.713
Poor	54	18	
Tumor invasion			
T1+T2	33	12	0.540
T3+T4	96	27	
Lymphatic invasion			
No (N0)	54	8	0.022
Yes (N1/2/3)	75	31	
Distant metastasis			
M0	124	29	<0.001
M1	5	10	
TNM stage			
I–II	46	18	0.262
III–IV	83	21	

* Means calculated by chi-square test.

Results

TCF7 expression in GC tissues was higher than in adjacent tissues.

The TCF7 expression in GC tissues was evaluated with qPCR and IHC. The mRNA levels of TCF7 in GC tissues and the corresponding adjacent tissues were first detected with qRT-PCR (Figure 1A). TCF7 in GC tissues had significantly higher mRNA levels than in adjacent tissues (P=0.008), indicating that TCF7 functions as an oncoprotein in GC. Expression of TCF7 in GCs was semi-quantified with IHC and the cohort was divided into groups with low TCF7 expression or high TCF7 expression (Figure 1B). TCF7 was mainly expressed in nuclei of GC, corresponding with its function as a transcription factor. We found that 76.79% (129/168) of patients had low TCF7 expression and 23.21% (39/168) had high TCF7 expression (Table 1).

TCF7 was correlated with distant metastasis

All the clinicopathological factors were included to analyze the correlation with TCF7 expression, including patient sex, age,

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Table 3. TCF7 was significantly associated with low survival rates.

Factors	5-year survival rate	P*
Gender		
Male	27.5	0.954
Female	39.7	
Age		
<60	33.3	0.378
≥60	29.5	
Tumor diameter (cm)		
≤5	29.7	0.801
>5	34.7	
Differentiation		
Well + moderate	39.6	<0.001
Poor	20.8	
Tumor invasion		
T1+T2	60.9	0.020
T3+T4	23.3	
Lymphatic invasion		
No (N0)	33.1	0.018
Yes (N1/2/3)	30.6	
Distant metastasis		
M0	31.7	0.119
M1	31.4	
TNM stage		
I–II	54.7	<0.001
III–IV	15.2	
TCF7		
Low	35.7	0.012
High	12.6	

* Means calculated by log-rank test.

tumor size, differentiation, tumor infiltration, lymphatic invasion, and distant metastasis (Table 2). TCF7 was significantly associated with positive lymphatic invasion (P=0.022) and metastasis (P<0.001), suggesting that patients with high TCF7 were more predisposed to metastasis. This indicated that TCF7 may be involved in the process of tumor invasion and metastasis.

Table 4. TCF7 expression was an independent prognostic factor of GC.

Factors	HR	95%CI	P*
Sex			
Male	1.00	0.85–2.37	0.181
Female	1.42		
Age			
<60	1.00	0.77–1.90	0.412
≥60	1.21		
Tumor diameter (cm)			
≤5	1.00	0.60–1.48	0.809
>5	0.95		
Differentiation			
Well + moderate	1.00	1.30–3.15	0.002
Poor	2.02		
Tumor invasion			
T1+T2	1.00	1.07–3.41	0.028
T3+T4	1.91		
Lymphatic invasion			
No (N0)	1.00	1.00–2.60	0.051
Yes (N1/2/3)	1.61		
Distant metastasis			
M0	1.00	0.58–2.89	0.528
M1	1.30		
TNM stage			
I–II	1.00	1.18–3.07	0.009
III–IV	1.90		

* Means calculated by Cox regression model.

TCF7 was an independent prognostic biomarker in GC

The prognostic value of TCF7 expression in GC was evaluated with univariate analysis and multivariate analysis separately. Kaplan-Meier method was first used to analyze the correlation between clinicopathological factors, including TCF7, and the overall survival rates (Table 3). High expression of TCF7 predicted worse prognosis (P=0.012) (Figure 2A). Additionally, poor differentiation (P<0.001), positive lymphatic invasion (P=0.020), and advanced TNM stage (P<0.001) were all associated with unfavorable prognosis of GC (Figure 2B–2D).

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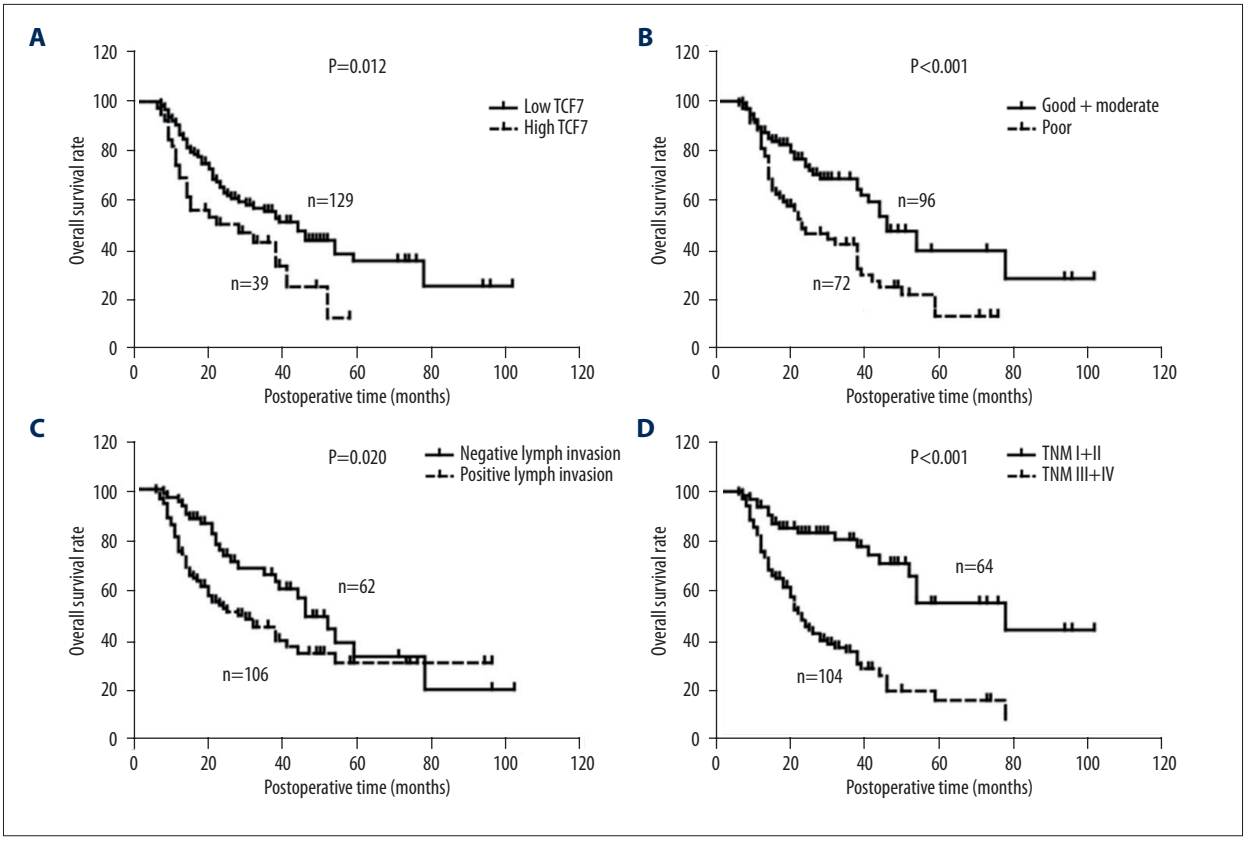

Figure 2. The correlation between TCF7, tumor differentiation, lymph invasion, TNM stage, and survival rates. (**A**) High expression of TCF7 is associated with worse prognosis compared with low expression of TCF7. (**B**) Poor tumor differentiation is correlated with unfavorable prognosis compared with good/moderate differentiation. (**C**) Positive lymph invasion is correlated with worse prognosis compared with negative lymph invasion. (**D**) Advanced TNM stage correlates with poorer prognosis compared with early stage.

With multivariate analysis, we further identified the independent prognostic factors of GC (Table 4). All the clinicopathological factors were enrolled into the Cox regression model, except for TNM stage, because of its interaction with tumor infiltration, lymphatic invasion, and metastasis. In multivariate analysis, TCF7 was confirmed as an independent prognostic factor of GC (HR=1.90, 95%CI =1.18–3.07, P=0.009), suggesting that high expression of TCF7 itself can predict unfavorable prognosis of GC. Poor differentiation (HR=2.02, 95%CI=1.30–3.15, P=0.002) and advanced tumor invasion (HR=1.91, 95%CI=1.07–3.41, P=0.028) were also identified as independent prognostic factors. Positive lymphatic invasion tended to be an independent parameter, but the difference was not statistically significant (P=0.051).

Discussion

The essential functions of Wnt signaling are cell self-renewal and proliferation, so the aberrant Wnt signaling activation usually links to diseases such as cancer and diabetes [13,14]. Constitutive activation of the Wnt signaling pathway is a key cause of many types of cancers, including gastric cancer [9,15]. In the Wnt/β-catenin canonical pathway, Wnt ligands lead to the accumulation of cytoplasmic β-catenin via binding to transmembrane receptors, including Frizzled and lipoprotein receptor-related protein (LRP) 5 and 6. The TCF family plays an important role in Wnt signaling by interacting with the translocated β-catenin and mediating the transcription of target genes [16–18]. There are 19 Wnt ligands in the Wnt family, more than 15 receptors, and numerous intracellular transduction components. The various Wnt signaling components and regulatory networks make the signal transduction of Wnt signaling complex [19].

As an essential mediator in the Wnt signaling pathway, TCF7 can enhance the transcription of Wnt target gene after binding with β-catenin. There is only 1 TCF gene found in Drosophila, while there are 4 TCF genes (TCF7, LEF1, TCF-3, and TCF-4) in mammals. Many TCF variants with distinct properties are produced by the alternative splicing and promoter usage [4,20], resulting in distinct and sometimes redundant functions of

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TCF genes. In the TCF family, the upregulation of TCF7 was observed in many types of cancers, such as prostate cancer [8], adrenocortical tumor [21], pancreatic cancer [22], renal cancer [23], and breast cancer [24]. In our study, we demonstrated that TCF7 was significantly associated with positive metastasis of GC, perhaps because TCF7 promotes the invasion-involved Wnt target gene in GC cells. There are dozens of Wnt target genes in the presence of β-catenin/TCF complex, and the target genes are cell- and context-specific [25]. Several genes activated by Wnt signaling and regulated by TCF7 are involved in tumor cells invasion and metastasis, including c-Myc, MMP-7, and MMP-26 [26]. Our study focused on revealing the clinical significance of TCF7 in GC. Further research is needed to learn how candidate genes are regulated by TCF7 and to define the mechanism by which TCF7 promotes metastasis.

By assessing TCF7 expression in 168 patients with GC and comparing TCF7 expression in 11 pairs of GC tissues and adjacent tissues, we demonstrated that TCF7 was expressed at higher levels in GC tissues than in adjacent tissues. Moreover, TCF7 was significantly associated with positive metastasis of GC patients, as assessed by the chi-square test. With survival analysis, we demonstrated that high TCF7 expression can predict poor prognosis. These results show that TCF7 is an independent prognostic biomarker of GC, suggesting that TCF7 detection of GC stratify high-risk patients and guide precision treatment.

Conclusions

TCF7 expression correlates with metastasis and is an independent prognostic factors of GC. TCF7 detection of GC can help stratify patients with high risk and guide precise treatment.

Conflicts of interest

None.

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