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Review Article

Clinicopathological and prognostic significance of TFF3 immunohistochemical expression in gastric cancer: a meta-analysis

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Abstract: The molecular biomarker trefoil factor 3 (TFF3) is reported that plays important role in the pathogenesis of gastric cancer (GC). However, it is still controversial whether TFF3 expression can be regarded as a prognostic factor for GC patients. Here we performed a meta-analysis to evaluate the value of TFF3 for its survival prognostic indicator and predictive correlation with clinicopathological features in GC. Eligible studies were identified from PubMed, Embase and Web of science. The odd sratio (OR) and hazard ration (HR) with their 95% confidence intervals (CI) were calculated using Review Manager version 5.3. Finally 8 studies involving 1170 patients with GC were included in this meta-analysis. Our results showed that TFF3 overexpression was significantly associated with poorer overall survival (HR=1.86, 95% CI=1.26-2.74, P=0.002) and disease free survival (HR=2.29, 95% CI=1.48-3.56, P=0.0002) in GC. Moreover, TFF3 overexpression was also significantly associated with histological type (OR=1.69, 95% CI=1.31-2.17, P<0.0001), lymph node metastasis (OR=2.05, 95% CI=1.61-2.62, P<0.00001), the depth of invasion (OR=1.37, 95% CI=0.99-1.88, P=0.05) and tumor TNM stage (OR=2.00, 95% CI=1.36-2.97, P=0.0005). However, none of other demographic parameters such as age and gender were associated with TFF3 expression. In conclusions, our results indicating that TFF3 overexpression could be regarded as a novel prognostic factor for gastric cancer patients, which may help to better guide clinical decision-making.

Keywords: TFF3, gastric cancer, prognosis, meta-analysis

Introduction

Gastric cancer (GC) is one of the most common malignancies and ranks the second cause of cancer deaths worldwide [1]. Although the diagnostic capabilities and therapeutic methods have improved, but the prognosis for GC patients still remains poor, especially in those in the advanced stage [2]. Current biomarkers (such as CEA and CA-199) lack specificity and sensitivity for earlier diagnosis and prognostic significance of GC, which limited efficiency of current treatment for advanced GC and lack of molecular markers for targeted therapy. Therefore, detecting new molecular mechanisms involved in gastric carcinogenesis is critical for the improvement of diagnosis, therapy, and prognosis prediction of GC.

Recently, the mechanism of Trefoil factor 3 (TFF3) inducing tumorigenesis and progression has become a hotspot [3]. TFF3 is a member of the TFF gene family which mainly expressed in the gastrointestinal tract and other epithelial tissues, it encodes a series of small mucin-associated polypeptides [4], and play an important role in maintaining mucosal integrity [5]. In the gastrointestinal tract, TFF3 is a major component of goblet cells and mainly expressed in the cytoplasm [6, 7]. Normal gastric mucosa is essentially negative for TFF3, however, TFF3 strongly positive was observed in goblet cells of intestinal metaplasia [8]. Several studies have been suggested that TFF3 was involved in carcinogenesis and/or progression of human malignancies [7, 9-14]. Many retrospective articles have been evaluated whether TFF3 overexpression may be a prognostic factor for survival in patients with GC. However, the results of these articles are inconclusive and no consensus has been reached. It is necessary to investigation whether TFF3 expression can be regarded as a prognostic biomarker in GC. Accordingly, we performed a meta-analysis to evaluate the association between various clinicopathological characteristics and TFF3 expression in patients with GC.

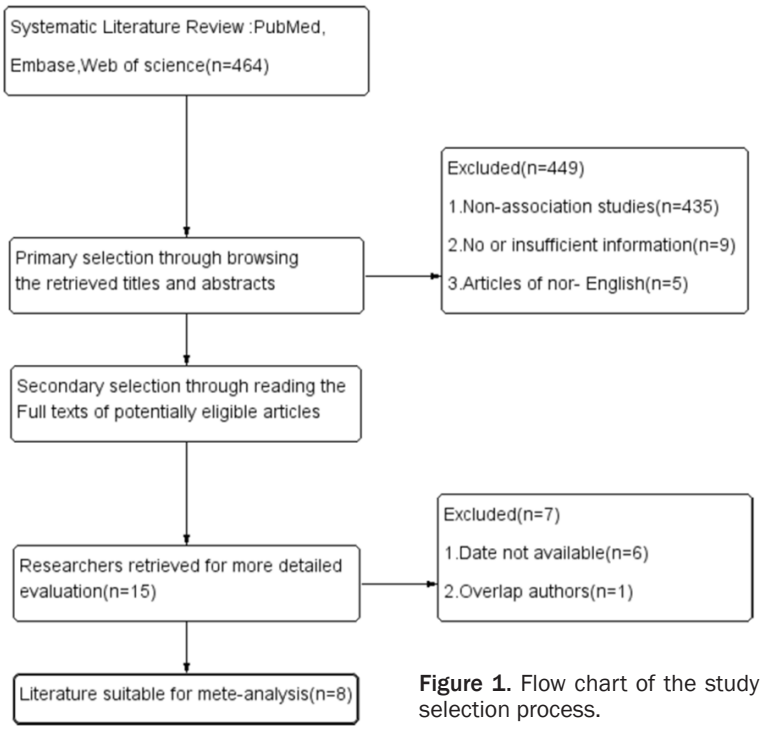


Figure 1. Flow chart of the study selection process.

Material and methods

Search strategy and study selection

Eligible articles published were searched in the PubMed, Embase and Web of science for the last time on November, 2016. The search strategies using the following terms "gastric cancer, stomach cancer, gastric carcinoma, gastric tumor or gastric neoplasm", "TFF3 or trefoil factor 3", and "prognosis, survival or outcome". The references cited by the primary studies were also reviewed to make up missing search of the strategies. The most recently or larger sample size studies was selected when duplicated data were published.

Inclusion and exclusion criteria

Eligible studies must meet the following criteria: (1) the expression of TFF3 in the primary gastric cancer tissue was evaluated by immunohistochemistry (IHC); (2) studies investigated the association between TFF3 expression and prognosis of patients (overall survival [OS] and/or disease free survival [DFS]); (3) studies described the correlation between TFF3 and clinicopathological features in GC; (4) the median follow-up period was no less than 24 months; (5) only published studies written in English. The exclusion criteria were: (1) studies were reviews, letters or conference papers; (2) studies were not performed in humans; (3) articles were failed to report sufficient data for determining desired meta-analysis outcomes.

Data extraction

All candidate articles were carefully assessed by two independent reviewers (Gang Liu and Tao Wan) for possible inclusion, any disagreements were resolved by discussion between the two reviewers or consultation with a third reviewer (Zheng-jie Huang). The following information was captured from all included studies: first author, country, publication year, number of patients, patient ages and genders, TNM stage, positive expression rate of TFF3, detection method, cut-off value, follow-up time, hazard ratios (HRs) for overall survival (OS) or disease-free survival (DFS) and their 95% confidence intervals (CIs), clinicopathological features. HRs and 95% CIs were directly extracted from articles or estimated from Kaplan-Meier survival curves by the open digitizing program (Engauge Digitizer) and Tierney's methods [15]. The quality of included studies was assessed by using Newcastle-Ottawa Quality Assessment Scale (NOS) [16]. The NOS was composed of eight questions with a full score of 9, and studies with scores ≥6 were considered as high quality.

Statistical analysis

The meta-analysis was performed using Review Manager version 5.3 (CochraneCollaboration, Oxford, UK). Heterogeneity across eligible studies was evaluated by Cochran's Q test and I^2 test (A p-value <0.10 for the Q-test or I^2>50% represented statistically significant heterogeneity). The fixed-effects model or random-effects model was used depending on the above heterogeneity analysis. HRs and 95% CIs were applied to estimate the impact of TFF3 on OS or DFS, while ORs and 95% CIs were used to assess the association between TFF3 expression and clinicopathological characteristics in GC. Subgroup analyses were stratified by sur-

Table 1. Characteristics of the included studies

Author	Country	Years	N	TFF3 positive (%)	Method (cut-off value)	TNM	Fellow-up (months)	Outcome	HR (95% CI)	Date extract
Yamachika	Japan	2002	209	114 (54.5)	IHC (>10%)	I-IV	72	OS	1.49 (0.89-2.49)	Curve
Dhar	American	2005	111	49 (44.1)	IHC (Score >4)	I-IV	120	DFS	3.05 (1.32-7.04)	Direct
Im	Korea	2013	292	129 (44.18)	IHC (Score >3)	I-III	84	OS	1.17 (0.66-2.07)	Direct
Ding	China	2013	142	63 (44.37)	IHC (Score >3)	NA	60	OS	3.41 (3.39-8.14)	Direct
Meng	China	2013	90	46 (51.1)	IHC (>5%)	I-IV	108	OS	1.13 (0.73-1.74)	Curve
Xu	China	2013	126	59 (46.83)	IHC (Score >3)	I-III	50	DFS	2.06 (1.23-3.45)	Direct
								OS	2.09 (1.14-3.82)	
Li	China	2014	108	57 (52.78)	IHC (Score ≥3)	NA	50	OS	2.02 (1.20-3.39)	Direct
Gu	China	2015	92	42 (45.65)	IHC (>5%)	I-III	84	OS	2.33 (1.20-4.50)	Direct

HR, hazard ratio; CI, confidence interval; N, number of patients; IHC, immunohistochemistry; NA, not available; OS, overall survival; DFS, disease-free survival.

Table 2. The quality assessment of included studies based on the NOS

Author	Representativeness of the exposed cohort	Selection unexposed cohort	Ascertainment of exposure	Outcome not present at the start of the study	Control for Important factors	Assessment of outcomes	Adequacy of follow-up	Length of follow-up	Quality scores
Yamachika	*	*	*	*	*		*	*	7
Dhar	*	*	*	*	**	*	*	*	9
Im	*	*	*	*	*	*	*	*	8
Ding	*	*	*	*	*	*	*	*	8
Meng	*	*	*	*	*		*	*	7
Xu	*	*	*	*	*	*	*	*	8
Li	*	*	*	*	*	*	*	*	8
Gu	*	*	*	*	**	*	*	*	9

NOS, Newcastle-Ottawa Quality Assessment Scale.

vival analysis method, age, gender, histological type, lymph node metastasis, the depth of invasion and TNM stage. In order to verify the robustness of conclusions, sensitivity analyses were conducted by sequential exclusion of each included study. In addition, publication bias was also estimated using funnel plots. All of the generated *p* values <0.05 was defined as statistically significant.

Results

We initially identified 464 relevant studies through the search strategy. After the implementation of the inclusion criteria mentioned above, finally a total of 8 studies [6, 7, 13, 14, 17-20] involving 1170 patients were included in current meta-analysis. The studies selection process was shown in **Figure 1**. The 8 eligible studies were published between 2002 and 2015, of these, 5 studies were from China, 1 from Japan, 1 from Korea, and 1 from American. The sample sizes ranged from 90 to 292. All 8 studies utilized the IHC method for TFF3 expression detection. Among them, 7 studies explored the prognostic role of TFF3 in OS, and 2 studies investigated the prognostic impact of TFF3 in DFS. HRs and 95% CIs were extracted directly from the 6 studies [7, 13, 14, 18-20] and estimated by Kaplan-Meier survival curves in 2 studies [6, 17]. The main characteristics of included studies have been presented in **Table 1**.

As displayed in **Table 2**, the quality assessment of all eligible studies was performed by the modified Newcastle-Ottawa Scale. The median quality scores of these studies was 8, indicating that the methodological quality was relatively high. Of note, for the correlation between TFF3 high expression and survival outcome in GC, no study attempted to control other confounding prognostic factors, such as variation of treatment.

Impact of TFF3 overexpression on survival outcome of GC

The pooled HRs indicated that TFF3 overexpression was significantly correlated with poor

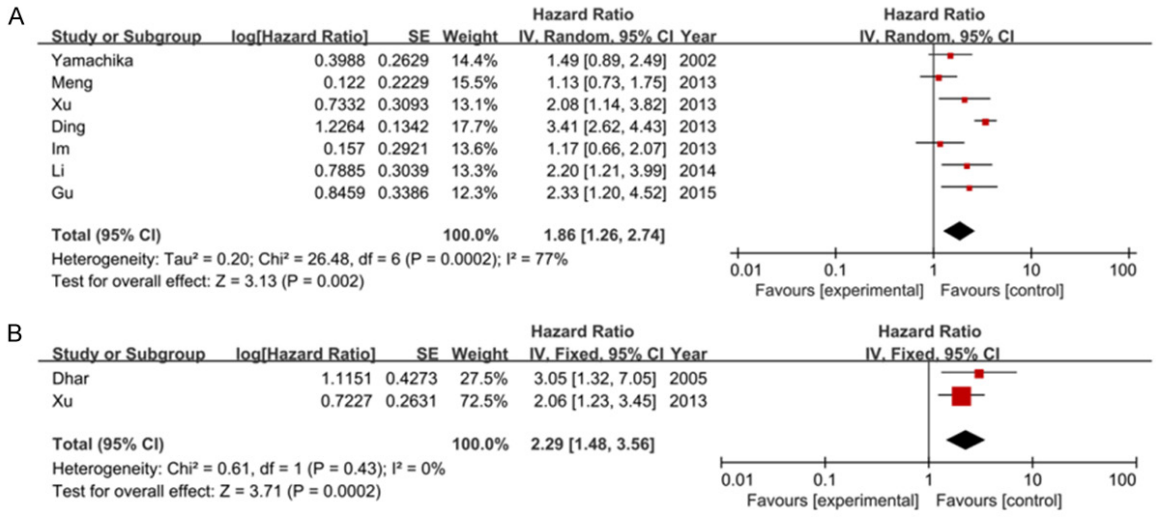
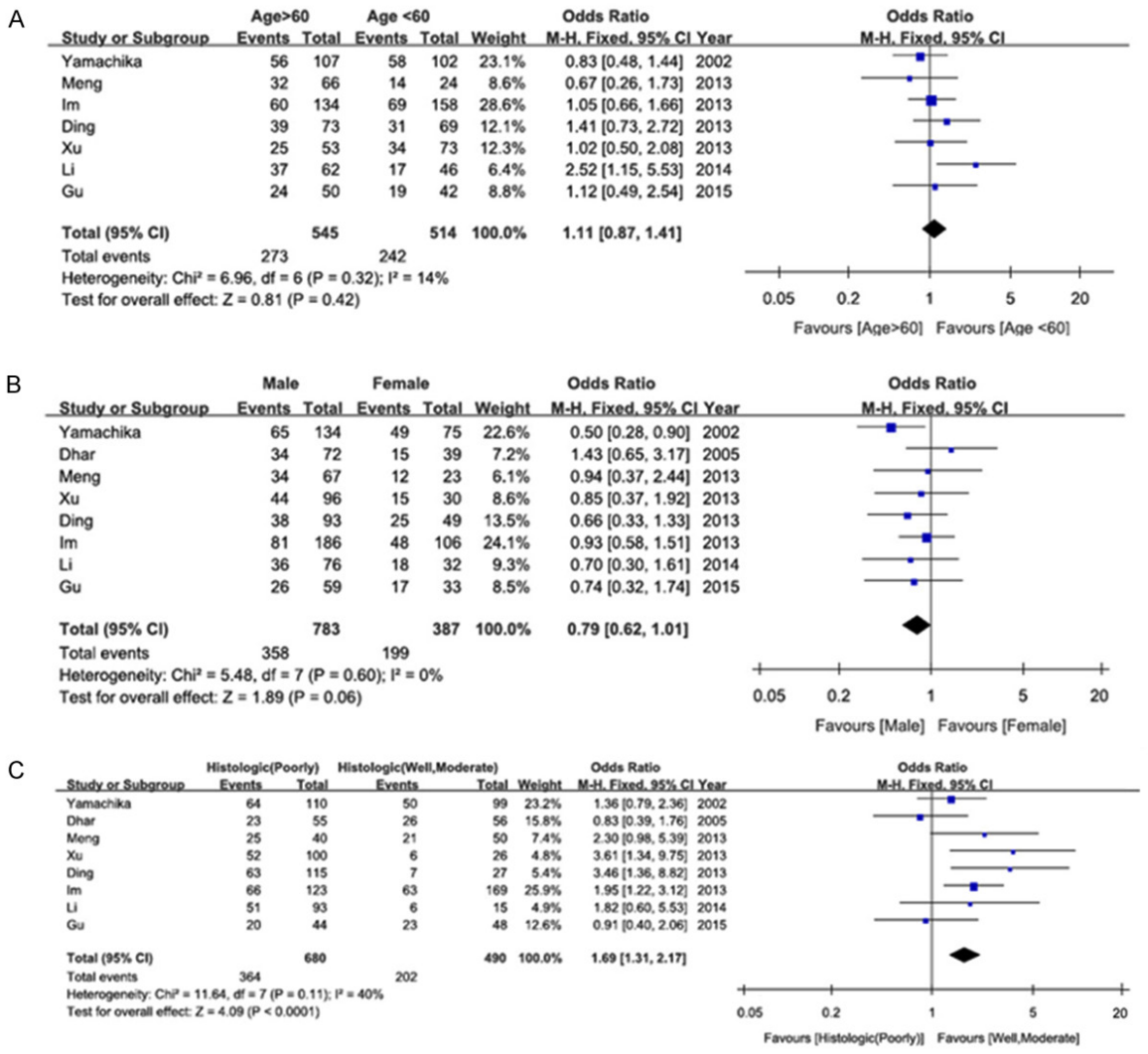


Figure 2. Forest plots for the association between TFF3 overexpression and OS (A)/DFS (B) in GC.



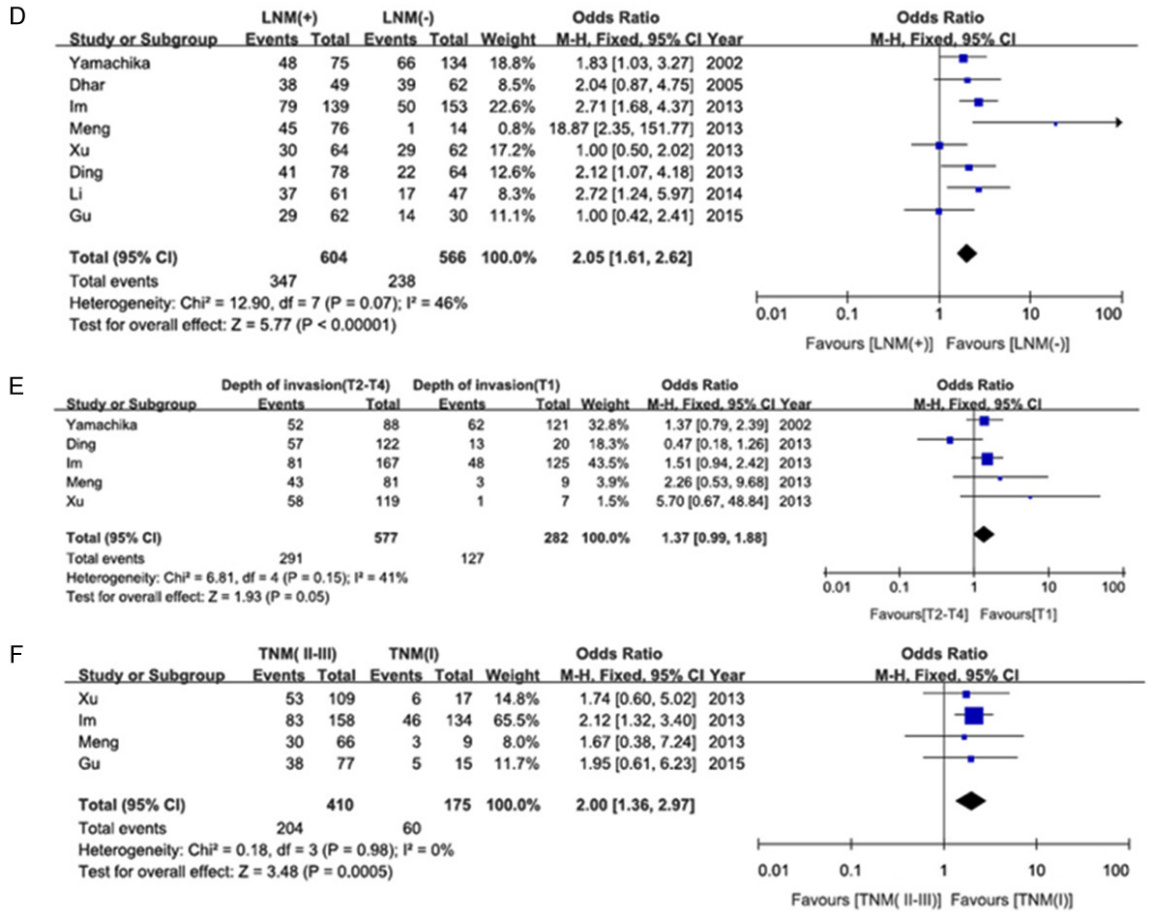


Figure 3. Forest plots for the correlation of GLUT-1 and Clinicopathological factors in GC:age (>60 vs. <60) (A); gender (male vs. female) (B); histological type (poor vs. well+moderate) (C); lymph node metastasis (Positive vs. Negative) (D); depth of invasion (T2-T4 vs. T1) (E); tumor TNM stage (II-III vs. I) (F).

OS inGC patients (HR=1.86, 95% CI=1.26-2.74, P=0.002). Owing to the test for heterogeneity in the 7 studies was significant, therefore, arandom-effects model was used (I^2=77%; P=0.0002) (**Figure 2A**). Furthermore, we also evaluated the correlation between TFF3 expression and DFS from 2 included studies. The result from the DFS analyses was consistent with that from the OS analysis (HR=2.29, 95% CI=1.48-3.56, P=0.0002), and no significant heterogeneity among the 2 studies was found (I^2=0, P=0.43) (**Figure 2B**).

The correlation between TFF3 expression and clinicopathological features in GC

As shown in **Figure 3**, the correlation between TFF3 overexpression and age, gender, histological type, lymph node metastasis, the depth of invasion and tumor TNM stage also was explored in our meta-analysis. According to the results of evidence synthesis, we found that TFF3 overexpression was significantly correlation with histological type (OR=1.69, 95% CI= 1.31-2.17, P<0.0001), lymph node metastasis (OR=2.05, 95% CI=1.61-2.62, P<0.00001), the depth of invasion (OR=1.37, 95% CI=0.99-1.88, P=0.05) and tumor TNM stage (OR=2.00, 95% CI=1.36-2.97, P=0.0005). However, none of other clinicopathological parameters such as age (OR=1.11, 95% CI=0.87-1.41, P=0.42) and gender (OR=0.79, 95% CI=0.62-1.01, P=0.06) were associated with TFF3 expression. The significant heterogeneity was not observed in all subgroup analyses of TFF3 expression with histological differentiation. Thus, a fixed-effects model was employed for the subgroup analysis.

Sensitivity analysis and publication bias

Each single study was deleted each time to reveal the influence of individual study on the observed overall effect size, and the results of

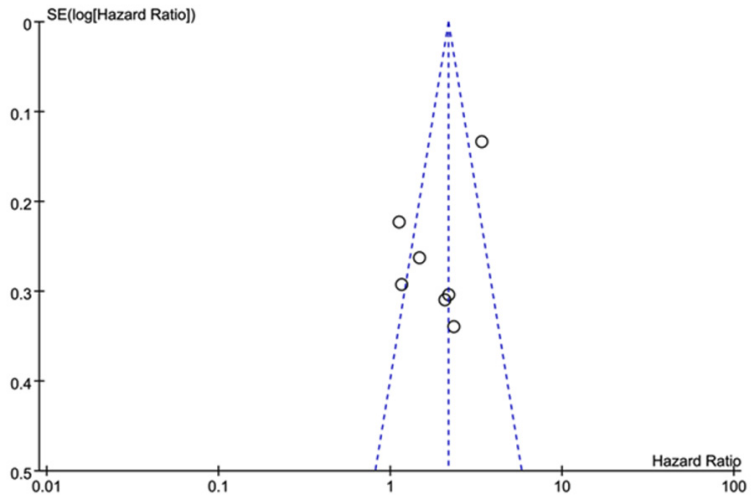


Figure 4. Publication bias using funnel plots for OS.

sensitivity analysis indicated that the pooled HRs and ORs were not significantly influenced by any single study. Moreover, no significant publication bias for OS was demonstrated by visual inspection of the funnel plot. The above test results demonstrated that the findings of the current meta-analysis are credible (**Figure 4**).

Discussion

Considering of the high morbidity and mortality of gastric cancer, researchers have been dedicated to identify available new prognostic markers to achieve better clinical decision-making regarding therapy and outcomes in past decades. In order to understand the prognostic significance of a potential biomarker, it is greatly necessary to acquire a relatively large sample size and conduct comprehensive evaluation by gather and synthesize as much data as possible on the topic [21].

As a novel biomarker, many studies indicated that TFF3 overexpression was associated with poor survival outcome of GC patients [6, 7, 13, 14, 17-20]. However, there was no consensus be reached on the conclusion. As far as we know, our meta-analysis clarifies the controversial issue for the first time. The results form evidence synthesis indicated that TFF3 overexpression could be regarded as an available prognostic factor for OS and DFS in GC, in addition, it also revealed that TFF3 expression was significantly associated with high risk of histological type, lymph node metastasis, the depth of invasion and tumor TNM stage.

TFF3 is a soluble peptide and member of the trefoil peptide family, which is conserved among species and has trefoil domain and C-terminal dimerization domain [22]. Some studies reported that TFF3play a key role in the reconstitution of the mucosal barrier to protect the epithelial layer against environmental injury [23, 24].

Furthermore, TFF3 could preserve the integrity of the gastric mucosal epithelium by activating the PI3K/Akt signaling axis [25], as well aspromote tumorigenesis via activating the Leptin/ObRb/signal transducers and activatorsof transcription 3 (STAT3) pathway. Recently, other studies also reported that the mRNA expressions of vascular endothelial growthfactor (VEGF) and hypoxiainducible transcriptionfactor-1α (HIF-1a) are up-regulated through TFF3 overexpression, which implicated a theory that TFF3 might be applied as a potential targeted therapy for GC [10, 11, 26]. All the same, more studies are required to analyze the specific molecular mechanism of TFF3 overexpression promoting the initiation and development of gastric cancer.

Although our findings are promising, the meta-analysis has several limitations. First, the sample size of most included studies was relatively small. Second, the relatively high variability for detection the expression of TFF3 protein by different studies could partly be attributed to the inconsistent cut-off points, staining procedure and antibodies of IHC. Third, most of the included studies were retrospective studies rather than randomized prospective studies. Therefore, an well-designed prospective study with stricter quality criteria will contribute to further improve the reliability of pooled conclusions.

In conclusion, our study demonstrated that TFF3 high expression was significantly associated with poor OS and DFS in GC. Moreover, TFF3 expression was also significantly correlated with poor histological type, presence of lymph node metastasis and advanced TNM stage. This information may be valuable for screening anti-TFF3 therapy in future clinical trials.

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Disclosure of conflict of interest

None.

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