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Original Article

Prognostic role of Smad4 expression in gastric cancer: a meta-analysis

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Abstract: Smad4 is located on chromosome 18q21 and acts as a central mediator in TGF-β superfamily signaling to inhibit relevant tumor development and progression. However, the prognostic role of Smad4 in gastric cancer (GC) was still controversial. Our aim of this meta-analysis was to evaluate the potential association between Smad4 expression and clinicopathological differences in GC patients. A total of 6 studies with 1021 GC patients up to April 2016 were included in this study. Our data demonstrated that Smad4 expression did related to the gender (pooled OR=0.812, 95% CI=0.381-1.732, P=0.591, random effect), Lauren histology (pooled OR=1.015, 95% CI=0.221-4.659, P=0.985, random effect) and tumor differentiation (pooled OR=0.709, 95% CI=0.403-1.248, P=0.233, random effect) of GC patients. But reduced Smad4 was significantly associated with the lymph node metastases (pooled OR=0.580, 95% CI=0.339-0.990, P=0.046, random effect) and TNM stage (pooled OR=0.645, 95% CI=0.426-0.977, P=0.039, fixed effect), which finally led to a shorter overall survival (OS) rate (RR=0.388, 95% CI=0.168-0.895, P=0.026) in GC patients, compared to the preserved cases. These results strongly suggested that Smad4 might serve as a novel biomarker for prognostic indicator, and could be a potential therapy for diagnosis and treatment in GC.

Keywords: Smad4, gastric cancer, clinicopathological feature, prognosis, meta-analysis

Introduction

Gastric cancer (GC) is a multi-factorial and malignant disease, which led to 24, 590 GC cases newly diagnosed and 10, 720 GC deaths in 2015 [1]. Due to lack of specific symptoms at early stage, the GC patients are often diagnosed at late stage with high rates of lymph node metastases [2]. Its occurrence is associated with environmental factors, such as Helicobacter pylori infection, Epstein-Barr viruses, smoking and unhealthy diet. Additionally, genetic factors are also found to participate in GC development and progression [3-5]. Despite continuously improving strategies including surgical intervention, radiotherapy, chemotherapy and biologic therapy, the prognosis of GC patients is still poor. Some serum tumor markers are applied for GC screening such as carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9) and carbohydrate antigen 72-4 (CA72-4), but there are still some defects of low sensitivity and specificity [6]. Therefore, the elucidation of pathogenic mechanisms and finding effective biomarkers with high specificity and sensitivity of GC are extremely urgent [7].

Smad4, originally named DPC4 (deleted in pancreatic carcinoma 4), which was firstly reported as a pancreatic cancer suppressor gene by Hahn SA et al. in 1996 [8]. The term "Smad" was combined with two orthologous proteins, sma from Caenorhabditis elegans and Mad from D. melanogaster [9]. Smad4 is located on chromosome 18q21 and acts as a central mediator in TGF-β superfamily signaling to inhibit relevant tumor development and progression [10, 11]. In pancreatic and colorectal cancer, Smad4 gene was deleted or mutated, and associated with malignant progression [12, 13]. Afterwards, Smad4 were

gradually unveiled in other types of cancers, such as hepatocellular carcinoma, esophageal cancer, and seminoma germ cell tumor [14-16]. In 2014, Smad4 was identified as a mediator of TGF-β superfamily signaling by Kang et al., which was critical for the TGF-β-driven upregulation of N-cadherin and the resultant invasive phenotype of human pancreatic ductal epithelial cells during EMT [17]. The loss of Smad4 expression in colorectal cancer promoted the progression and metastasis through activation of PI3K/Akt pathway, and led to 5-FU resistance [18].

In 1997, Powell et al. firstly reported that Smad4 genetic alterations played a significant role in gastric tumorigenesis [19]. Xu et al. demonstrated that Smad4 functioned as a tumor suppressor in the gastrointestinal tract, and provided a valuable model for screening factors that promoted or prevented gastric tumorigenesis [20]. Some other studies also confirmed its importance as a tumor suppressor in GC. However, the function of Smad4 in GC was still controversial. For example, the study of Sakellariou et al. implied that Smad4 played different roles in the progression of GC, depending on the degree of tumor differentiation and aggressiveness. Smad4 might behave as a tumor promoter in low grade GC [21]. In 2005, Kim also found that Smad4 might play different roles in human gastric carcinogenesis, especially between intestinal type and diffuse type of gastric adenocarcinoma, and the protein expression of Smad4 was higher than that in overall gastric adenoma [22]. So no certainty outcomes were determined up to now. To determine the potential function of Smad4 and address controversial issues in GC, our present meta-analysis was performed. The relationships between Smad4 and some clinicopathological variables such as the gender, Lauren type, tumor differentiation, lymph node metastasis and TNM stage were examined. In addition, the prognostic significance of Smad4 for the OS of GC patients was also discussed.

Materials and methods

Literature search

We searched the literatures from the electronic databases including PubMed, Embase, Medline and Cochrane library. The searching deadline was April 2016. The keywords were searched by using the following terms and their combinations: “Smad4” or “DPC4” and “gastric cancer” or “gastric carcinoma”. Studies were included in our meta-analysis if: (1) studies were written in English; (2) articles were published as original research; (3) there were quantitative information that reported the potential relationships between Smad4 expression and clinicopathological factors (or OS); (4) articles must be the full-text manuscripts. The excluded criteria were: (1) studies were researched by RT-PCR or other non-immunohistochemistry methods; (2) studies were not clinical articles; (3) we could not acquire the relevant original data.

Data extraction

The eligible included articles were assessed by two investigators (Yuliang Jiang and Zhihua Xu). General information extracted from the eligible articles included: authors, the publication years, the methods of measuring the Smad4 expression in GC tissues, tumor stages, the total number of GC patients in each study, the number of Smad4 positive or negative in GC patients, the correlations between Smad4 and its potential related clinicopathological parameters (or the 5-year OS rate).

Statistical analysis

We used OR and 95% CI to evaluate the associations between Smad4 expression and clinicopathological parameters in GC patients including the gender, Lauren type, tumor differentiation, lymph node metastasis and TNM stage. To facilitate our analyses, we combined the following data into the single categories: Tumor stage was divided into two groups: high T stage (3-4 stage) and low T stage (1-2 stage). Similarly, Lauren type (intestinal and non-intestinal) and sub-groups of tumor differentiation (well as differentiated, moderate and poor as undifferentiated) were simplified. RR and 95% CI were used to measure the impact of Smad4 expression on the OS of GC patients. We extracted the survival data from the qualified articles as described by Parmar or calculated from the available data [23]. The heterogeneity was evaluated by the Q-test using *P*-value. When the P>0.05, the OR and RR were calculated by a fixed effects model. Other-wise, a random-effects model was applied. Public-

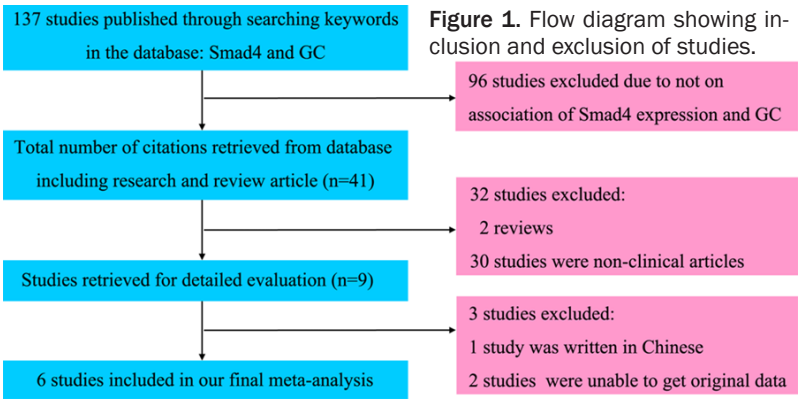

Figure 1. Flow diagram showing inclusion and exclusion of studies.

Table 1. Characteristics of included studies in this meta-analysis

Study	Year	Methods	Tumor stage	Total	Smad4 positive (n)	Smad4 negative (n)
Che	2001	IHC	I-IV	249	62	187
Okano	2004	IHC	-	166	62	104
Kim	2004	IHC	I-IV	304	266	38
Kim	2005	IHC	-	88	60	28
Sakellariou	2008	IHC	I-IV	63	18	45
Zizi-Sermpetzoglou	2014	IHC	I-IV	151	131	20

ation bias was assessed using Funnel plots and Egger' linear regression test. All *P*-values were two-tailed and P<0.05 was meaningful. All of the statistical calculations were performed by STATA version 11.0 (Stata Corporation, College Station, TX, USA).

Results

Study characteristics

As shown in **Figure 1**, using a series of literature searching strategies, 137 articles were initially identified up to April 2016. Among them, 96 articles lacked the correlations with Smad4 expression and GC. 32 other studies were excluded as 2 were review articles and 30 studies were non-clinical articles. For further detailed evaluation, 1 study was written in Chinese and other 2 articles were unable to get sufficient original data. Finally, 6 studies met our meta-analysis [21, 22, 24-27]. A total of 1021 patients ranged from 63 to 304 per study with 599 Smad4 positive and 422 Smad4 negative GC patients. Immunochemistry (IHC) was the only method to assess Smad4 expression in GC specimens. The main characteristics of these 6 included studies were described in **Table 1**.

Correlation of Smad4 expression with clinicopathological parameters

As illustrated in **Figure 2A**, 4 eligible studies indicated that no associations between Smad4 expression and the gender of GC patients were observed (pooled OR=0.812, 95% CI= 0.381-1.732, P=0.591, random effect), with no significant heterogeneity. Similarly, Smad4 did not relate to the Lauren histology (pooled OR=1.015, 95% CI=0.221-4.659, P=0.985, random effect) and tumor differentiation (pooled OR=0.709, 95% CI=0.403-1.248, P=0.233, random effect) (**Figure 2B**, **2C**). However, Smad4 expression was significantly associated with the lymph node metastasis (pooled OR=0.580, 95% CI=0.339-0.990, P=0.046, random effect) and TNM stage (pooled OR=0.645, 95% CI=0.426-0.977, P=0.039, fixed effect) (**Figure 3A**, **3B**).

Correlation of Smad4 expression with overall survival

We extracted the related date from the Kaplan-Meier curves or calculated from the available data if RRs were not described directly. 4 studies were included in this section, and the heterogeneity was not significant (P>0.05). Our data demonstrated that low expression of Smad4 was statistically related to the 5-year OS of GC patients (RR=0.388, 95% CI=0.168-0.895, P=0.026) (**Figure 3C**). This meant that Smad4 might act as a tumor suppressor in GC, and reduced protein level of Smad4 in GC tissues might led to a shorter overall survival rate in GC patients.

Publication bias and sensitivity analysis

The likelihood of publication bias was assessed by applying Begg's funnel plot and homologous *P*-values. The shape of the funnel plots was symmetrical and *P*-values were all less than 0.05, which suggested that no evidence

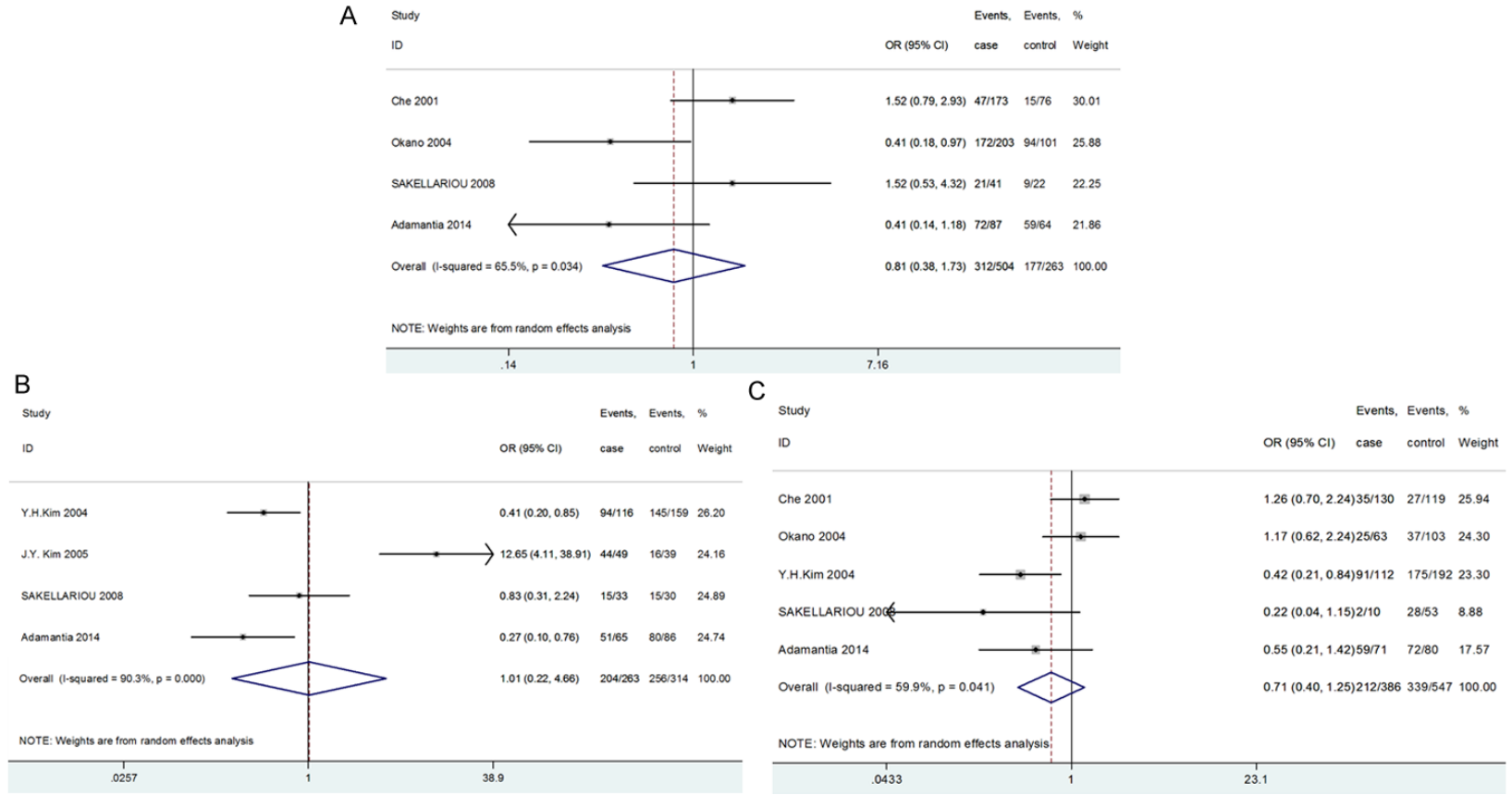

Figure 2. Forrest plot of ORs for the association of Smad4 expression with the A: Gender; B: Lauren type; C: Tumor differentiation in GC patients.

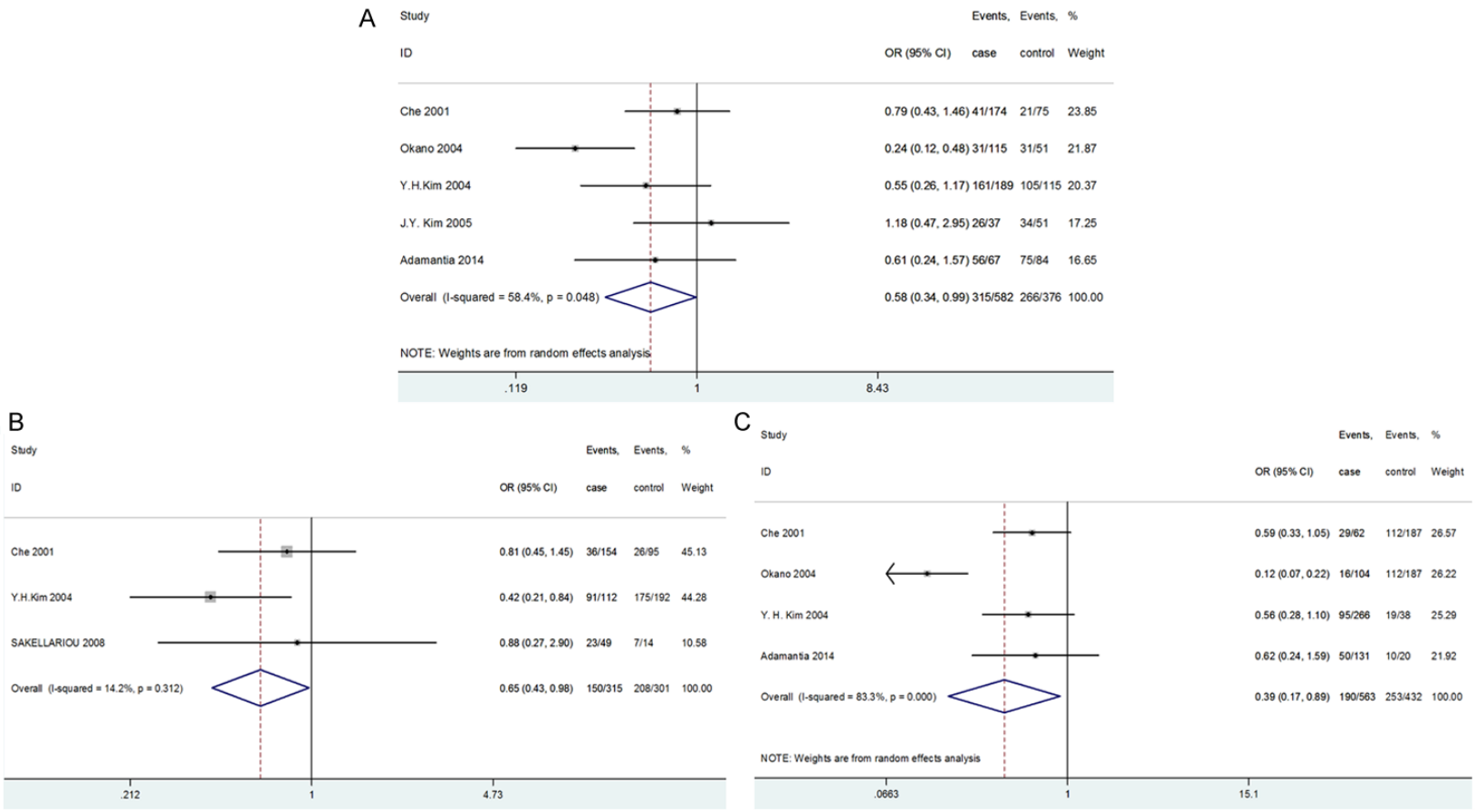

Figure 3. Forrest plot of ORs for the association of Smad4 expression with the A: Lymph node metastasis; B: TNM stage; C: OS in GC patients.

of publication bias existed among these studies. Our results implied that the relationships between abnormal Smad4 expression and all clinicopathological parameters had no published bias (**Figure 4**). To validate the stability of the pooled results, sensitivity analyses were performed. The pooled OR and RR were not significantly changed, which further verified the stabilities of our meta-analyses (**Figure 5**).

Discussion

Smad4, as one of the Smads family of TGF-β transducer signaling, has been shown to depress cancer proliferation and promote apoptosis [28]. When TGF-β combined with its receptor triggers phosphorylation of Smad2/Smad3, Smad4 is formed and then stimulates relevant target genes expression. TGF-β serves as a potent tumor suppressor due to blocking the cell cycle and cellular proliferation through Smad4 [29]. Activation of the TGF-β/Smad4 depend signaling results in embryonic development, fibrosis, tumor development, immune function and wound healing. Recently, down-regulation of Smad4 was shown to play an important role in the induction of EMT in head and neck squamous cell carcinoma [30].

Early in 1997, significant 18q allelic loss (56% of 34 informative cases) was noted in GC using microsatellite markers near the Smad4 locus by Powell [19]. Afterwards, Takaku et al. inactivated mouse homologue Smad4 and demonstrated its role in the malignant progression of benign adenomas to invasive adenocarcinomas [31]. With the thorough studies of Smad4, its role and potential mechanism in GC were gradually illustrated and known. Up to now, a total of 137 articles which referred to Smad4 in GC were identified from the electronic databases. With further detailed evaluation, 6 studies were finally included in our meta-analysis. We assessed the associations between Smad4 expression and clinicopathological factors. It is clear that the intestinal type GC has a better 5-year overall survival than diffuse type and mixed type, and undifferentiated histological type plays a vicious role in GC progression [32, 33]. But our results indicated that no correlations of Smad4 expression with the gender, Lauren histology and tumor differentiation were found. This connection was partly in agreement with previous report which showed that loss of Smad4 did not related with these 3 clinicopathological features in GC [21]. Similar results were also found in colorectal cancer by Isaksson-Mettävainio et al. in 2006 [34]. However, some other previous reports also revealed that Smad4 expression was significantly associated with the histological type or tumor differentiation. For example, Kim et al. pointed that the expression of Smad4 was significantly lower in diffuse-type gastric adenocarcinoma than intestinal-type gastric adenocarcinomas, which was also associated with tumor differentiation. They speculated that Smad4 played different roles in GC and silencing Smad4 might be an important event in the tumorigenesis of GC of the intestinal type and in high grade tumors [22, 26].

In GC, the number of metastatic lymph nodes can act as an outstanding prognostic indicator, and stages are determined with the combination of the depth of invasion and the numbers of metastatic lymph nodes. Therefore, lymph node metastasis was thought to be a key etiology of recurrence and distant metastasis after resection of primary gastric tumors [35, 36]. Our data showed that low Smad4 level was statistically associated with lymph node metastases and TNM stage of GC patients. In 2009, Leng et al. demonstrated that Smad4 expression obviously related with the lymphatic metastasis, and provided a mechanism by which a balance between Smad4 and Smad7 in human GC was critical for metastasis, and apoptosis of tumor cells [37]. Similar conclusion was also supported by Okano, who implied that the reduction of Smad4 was one of the determinants of lymph node metastases in GC, which led to a poor patient outcome [24]. In this study, we also concluded that Smad4 expression acted as an independent prognostic factor in GC, and reduced Smad4 expression had a poorer clinical outcome than those with preserved expression.

In interpreting the results from our meta-analysis, there were some limitations should be presented. Firstly, IHC was the only applied method in our selected studies, and the cutoff value was defined differently. Secondly, the number of studies and case samples were relatively small, which might lead to higher heterogeneity. We believe that there will be more clinical researches in the future that can effectively focus on Smad4 in GC. If in that case,

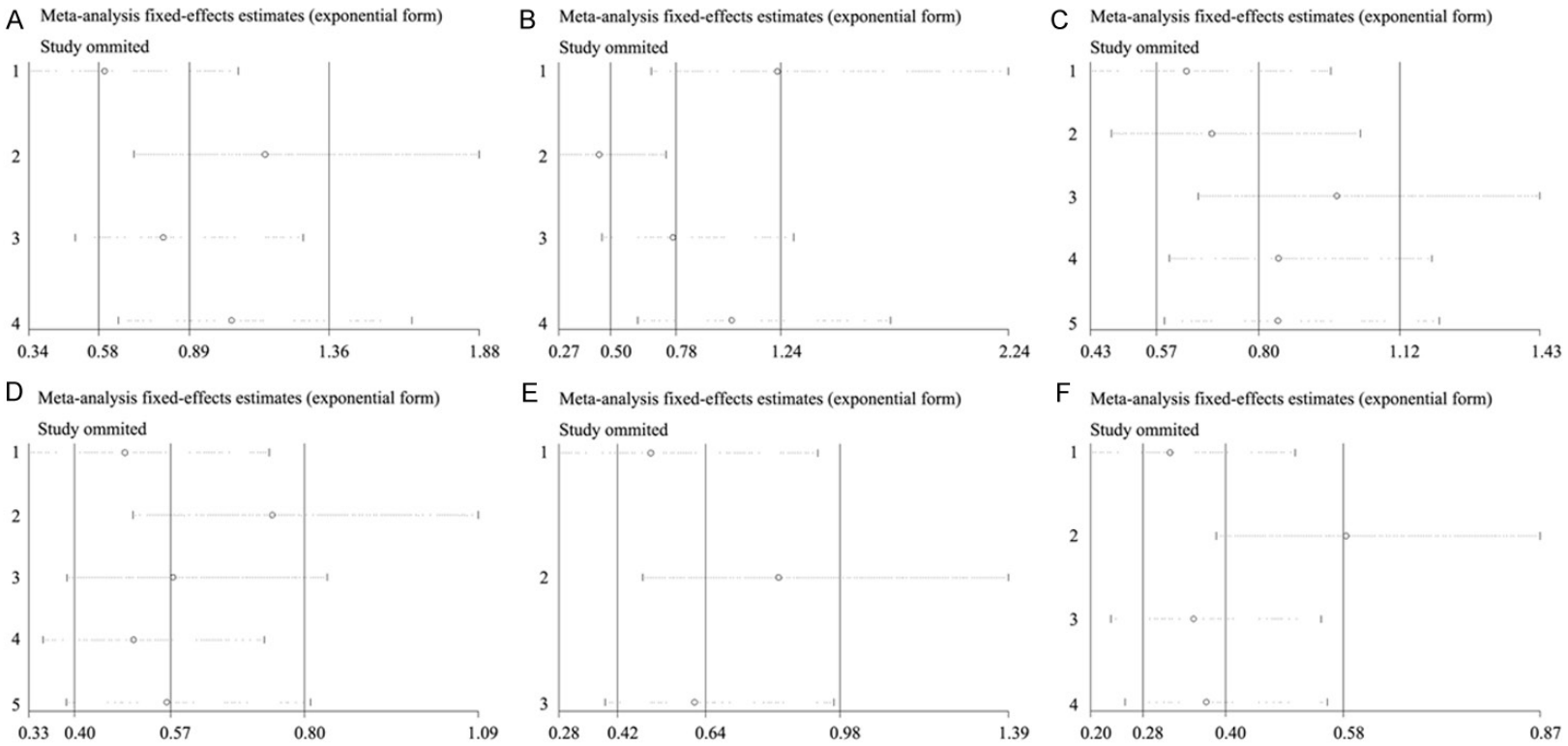

Figure 4. Funnel plots for publication bias. All the graphical funnel plots appeared to be symmetrical. A: Gender; B: Lauren type; C: Tumor differentiation; D: Lymph node metastasis; E: TNM stage; F: OS in GC patients.

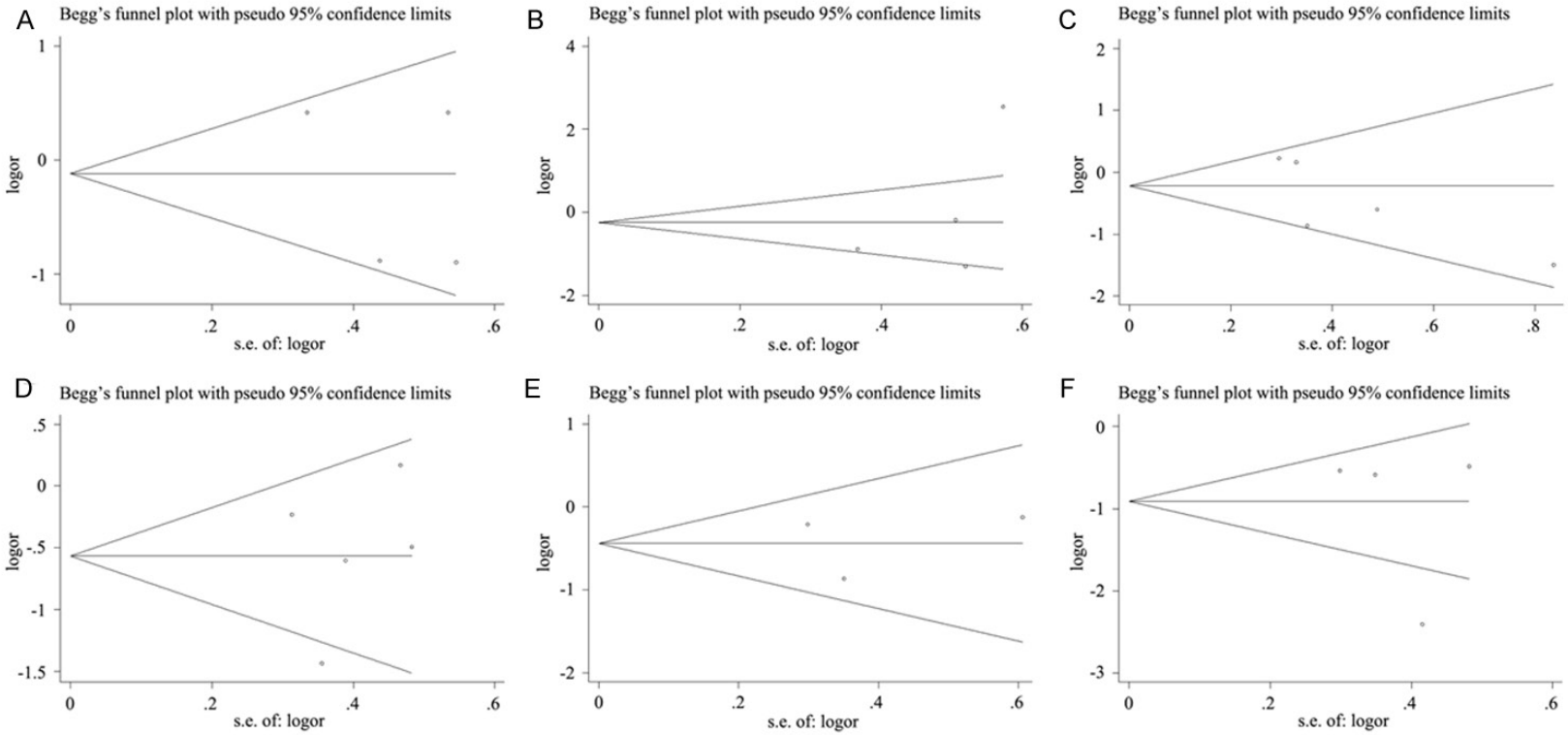

Figure 5. Sensitivity analyses in our meta-analysis. A: Gender; B: Lauren type; C: Tumor differentiation; D: Lymph node metastasis; E: TNM stage; F: OS in GC patients.

we will improve subgroup analysis precisely. Thirdly, some other clinicopathological parameters such as the tumor size, location, venous invasion and hepatic metastasis were not analyzed in this study because of inadequate data. If more studies related to those factors are available, we will renew the according results. Finally, the data of OS was achieved from survival curves rather than original data of variance directly. The results which we calculated for OS from available data or Kaplan-Meier curves might be less reliable.

Though larger well-designed studies with more ethnic groups and larger population studies are required, to the best of our knowledge, this meta-analysis is the first one to evaluate the prognostic role of Smad4 expression in GC. Our data implied that Smad4 did not relate to the gender, Lauren histology and tumor differentiation of GC patients, but reduced Smad4 expression was significantly associated with the lymph node metastasis and TNM stage, which led to a poorer clinical outcome in GC patients. These results strongly suggested that Smad4 might serve as a novel biomarker for prognostic indicator, and could be a potential therapy for diagnosis and treatment in GC.

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Disclosure of conflict of interest

None.

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