Prognostic role of thymidylate synthase expression in gastric cancer: A meta-analysis

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Abstract

Background: thymidine phosphorylase (TP) has been identified as a specific marker enzyme that is expressed in tumor-infiltrating macrophages, being associated with tumor angiogenesis and poor prognosis in patients with intestinal-type gastric cancer. However, the clinical and prognostic significance of TP expression in gastric cancer remains controversial. Publications were identified which assessed the clinical or prognostic significance of TP expression in gastric cancer from January 1995 to May 2013. A meta-analysis was performed to clarify the association between TP expression and clinical outcomes. A total of 13 studies met the inclusion criteria, and comprised 1541 cases. Analysis of these data showed that there was no statistically significant association of TP and tumor differentiation (pooled OR = 0.88, 95% CI: 0.67–1.15, Z = 0.94, P = 0.95, fixed-effect). There was significant association between the expression of TP and clinical parameters such as lymph node metastasis (pooled OR = 2.35, 95% CI: 1.42–3.88, Z = 3.34, P = 0.0009 random-effect) or lymphatic invasion (pooled OR = 1.71, 95% CI: 1.02–2.87, Z = 2.02, P = 0.04 random-effect).Moreover, in identified studies, the expression of TP was correlated with reduced overall survival (relative risk [RR] = 1.73, 95% CI: 1.06–2.82, Z = 2.19, P = 0.03). TP expression is associated with tumor aggressiveness and poor prognosis. If this finding is confirmed by well-designed prospective studies, TP expression may be a useful prognostic indicator for clinical outcome in patients with gastric cancer.

Introduction

Gastric cancer is one of the leading causes of cancer-related deaths worldwide, especially in Far East Asia, in countries such as Japan, China, and Korea [1]. Increasing evidence suggests that solid tumors, including gastric cancer, require neovascularization for growth and metastasis. It is also thought that the degree of tumor angiogenesis is related to clinical outcome, suggesting that angiogenic properties are correlated with tumor aggressiveness [2, 3]. Recent studies have shown that macrophages, as well as malignant cells, are an important source of such angiogenic factors in solid tumors [4]. Kawahara et al reported that TP could be a specific marker enzyme that is expressed in tumor-infiltrating macrophages, being associated with tumor angiogenesis and poor prognosis in patients with intestinal-type gastric cancer [5].

TP, an enzyme originally isolated from platelets and also known as platelet-derived endothelial cell growth factor (PDECGF), catalyses the reversible phosphorolysis of thymidine to deoxyribose 1-phosphate and thymine. It has been proposed that one role for TP is to control the intracellular levels of thymidine, which at high concentrations becomes toxic to cells and causes replication errors in DNA [6]. Direct evidence of a role for TP in angiogenesis comes from experiments in which xenografts from transformed fibroblasts transfected with TP form more highly vascularized tumors than controls [8]. In addition, xenografts of the breast carcinoma line MCF-7, when transfected with TP, form more rapidly growing tumors than controls(8). Increased expression of TP has been reported in a number of tumor types. TP expression was 260-fold higher in invasive cancers than in normal bladder samples and 40-fold higher in invasive cancers than superficial cancers [9, 10].

Expression of TP was significantly associated with microvessel density and poor clinicopathologic parameters in gastric cancer and in other various tumor sites [11]. However, Koriyama et al. showed that the expression of TP varied according to the histological type of gastric cancer [12]. There is insufficient clinical data to confirm a clinical application for TP. In order to address controversial issues, we performed a meta-analysis to determine the association between the expression of TP and clinicopathologic parameters in gastric cancer .

Materials and Methods

Publication search

A comprehensive computer literature search of abstracts about studies in human subjects was performed to identify articles about the prognostic role of TP expression in gastric cancer. The MEDLINE and EMBASE databases, from January 1995 to May 2013, were used with the following key words: "thymidine phosphorylase" or "TP" or "platelet-derived endothelial cell growth factor" or "PDECGF" or "gastric carcinoma" or "gastric cancer", and "overall survival" or "OS". All searched studies were retrieved, and their bibliographies were checked for other relevant studies identified were hand-searched to find additional eligible studies.

Information was carefully extracted from all eligible publications independently by two of the authors according to the inclusion criteria. Disagreements were resolved in consensus. The inclusion criteria were: (1) articles dealing with the expression of TP and either prognostic factors or overall survival (OS) of gastric cancer; (2)articles containing sufficient data to allow the estimation of an odds ratio (OR) or a relative risk (RR) of OS; (3) articles were reported in the English or Chinese (4) articles published as original research. Reviews, comments, duplicated studies, and articles unrelated to our analysis were excluded. Studies with fewer than 50 patients, follow-up less than 2 years, and relevant articles using RT-PCR or ELISA were also excluded.

The following information was extracted from the included papers: author, publication year, patient's country, tumor stage, number of patients, research technique used and definition of TP positivity. Two major groups were created according to the objective. One clarified the association between the expression of TP and clinicopathological parameters, including degree of differentiation, lymph node status and lymphatic invasion. Another group investigated the association between the expression of TP and OS.

Statistical analysis

The meta-analysis was performed as previously described [13]. For ease of analysis, the following data of the expression of TP and clinicopathological factors were combined into single categories: TP-negative and positive; and well and moderate differentiation. ORs with 95% CI were used to evaluate the association between the expression of TP and clinicopathological factors, including tumor differentiation, lymph node status and lymphatic invasion. Survival data were extracted from original papers as described by Parmar et al. [14]. Differences between the expression of TP and OS were quantified using RR with 95% CI. Heterogeneity across studies was evaluated with the Q test and P values. ORs and RRs were calculated by a random-effects model when the P value was less than 0.05. Otherwise, a fixed-effects model was used. The Begg and Egger funnel plot was used to assess publication bias. Statistical analyses were estimated using Review manager software. P values were two-sided, with significance at $P < 0.05$.

Results

Description of studies

A total of 13 publications met the criteria for this analysis [12, 15-26]. The total number of patients was 1541, ranging from 67 to 183 patients per study. Main characteristics of the eligible studies were summarized in Table 1. Thirteen articles dealt with clinicopathological factors. Six studies determined with OS. There was only one kind of methods used to evaluate the expression of TP in gastric cancer specimens: immunohistochemistry (IHC)

Correlation of TP expression with clinicopathological parameters

Nine studies investigated the relationship between the expression of TP and degree of tumor differentiation (Figure 1). There was no statistically significant association of TP and tumor differentiation (pooled OR = 0.88, 95% CI: 0.67–1.15, Z = 0.94, P = 0.95, fixed-effect). There was significant association between the expression of TP and clinical parameters such as lymph node metastasis (pooled OR = 2.35, 95% CI: 1.42–3.88, Z = 3.34, P = 0.0009 random-effect) (Figure 2) or lymphatic invasion (pooled OR = 1.71, 95% CI: 1.02–2.87, Z = 2.02, P = 0.04 random-effect) (Figure 3).

Impact of TP expression on overall survival of gastric cancer patients

The meta-analysis was performed on six studies (868 patients) investigating the association of TP expression and OS. The pooled RR was calculated using the methods described above. As the test for heterogeneity was significant (P < 0.0001), a random-effect model was used to calculate the RR. The presence of TP expression was highly correlated with poor OS (pooled RR = 1.73, 95% CI: 1.06–2.82, Z = 2.19, P = 0.03) (Figure 4). This indicated that TP was an independent prognostic factor in gastric cancer. No significant publication bias was detected.

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Table 1. *Main characteristics of the eligible studies*

Study	Patient's country	Year	Tumor stage (UICC)	Technique	Number of patients	Definition of TP positivity
Takeba-yashi	Japan	1996	I–IV	IHC	158	> 5%
Tanigawa	Japan	1996	I–IV	IHC	154	cytoplasm positive
Maeda	Japan	1996	I–IV	IHC	120	cytoplasm positive
Saito	Japan	1999	II–IV	IHC	126	> 5%
Suda	Japan	1999	IIIB	IHC	67	cytoplasm positive
Kimura	Japan	2001	II	IHC	102	> 5%
Konno	Japan	2002	I–III	IHC	116	> 5%
Yu	China	2004	I–IV	IHC	120	>Score 3
Fan	China	2005	I–IV	IHC	69	> 5%
Li	China	2006	I–III	IHC	90	$\geqq$ Score 4
Koriyama	Japan	2010	I–IV	IHC	156	> 5%
Zhang	China	2011	0–IV	IHC	183	$\geqq$ Score 2
Yang	China	2012	I–IV	IHC	80	>Score 2
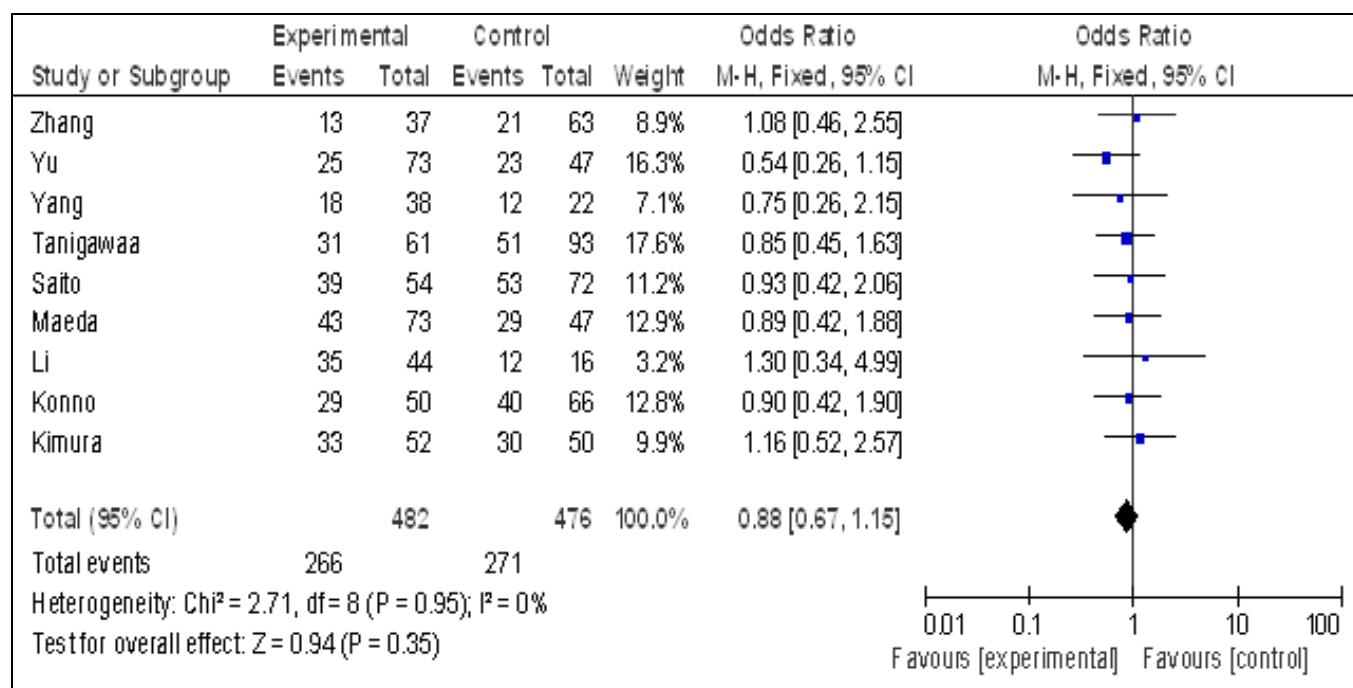
Figure 1. *Forrest plot of ORs for the association of TP expression with tumor differentiation*
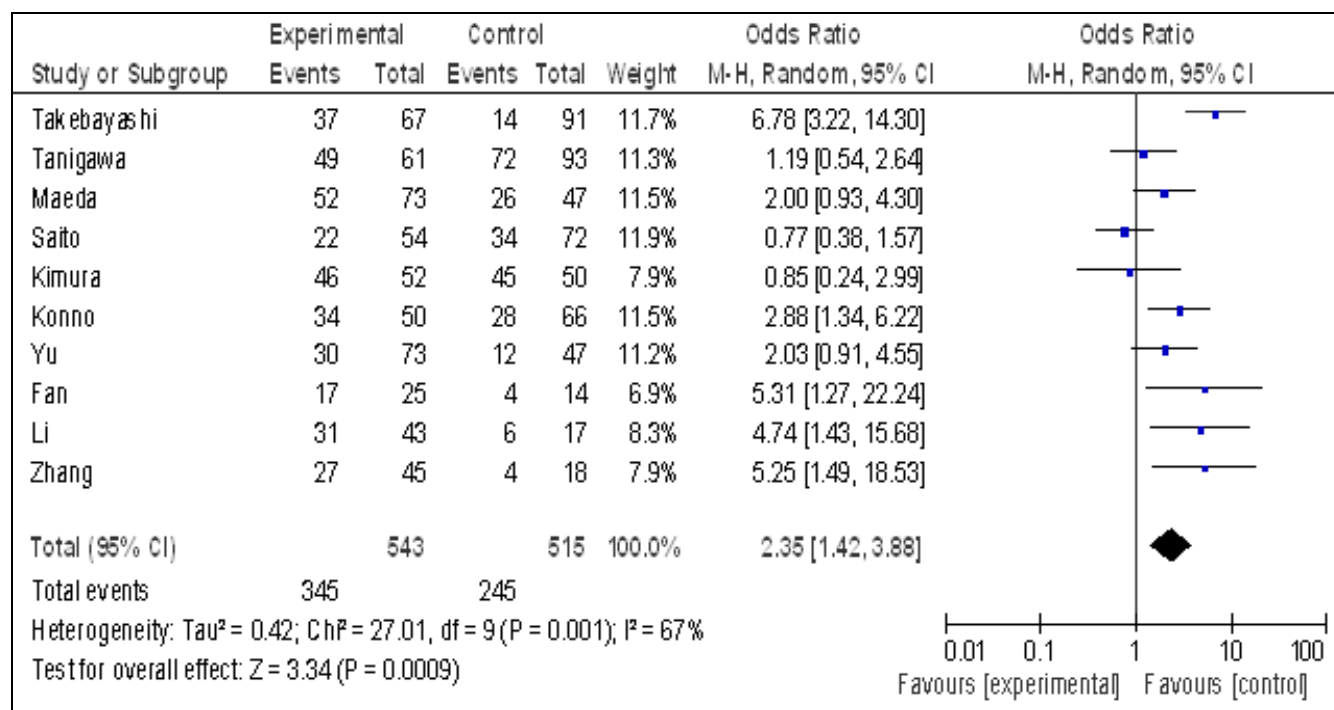
Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Takebayashi	37	67	14	91	11.7%	6.78 [3.22, 14.30]
Tanigawa	49	61	72	93	11.3%	1.19 [0.54, 2.64]
Maeda	52	73	26	47	11.5%	2.00 [0.93, 4.30]
Saito	22	54	34	72	11.9%	0.77 [0.38, 1.57]
Kimura	46	52	45	50	7.9%	0.85 [0.24, 2.99]
Konno	34	50	28	66	11.5%	2.88 [1.34, 6.22]
Yu	30	73	12	47	11.2%	2.03 [0.91, 4.55]
Fan	17	25	4	14	6.9%	5.31 [1.27, 22.24]
Li	31	43	6	17	8.3%	4.74 [1.43, 15.68]
Zhang	27	45	4	18	7.9%	5.25 [1.49, 18.53]
Total (95% CI)		543		515	100.0%	2.35 [1.42, 3.88]
Total events	345		245			

Heterogeneity: Tau² = 0.42; Chi² = 27.01, df = 9 (P = 0.001); I² = 67%
Test for overall effect: Z = 3.34 (P = 0.0009)

Figure 2. Forrest plot of ORs for the association of TP expression with lymph node metastasis.

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Tanigawa	43	67	32	91	14.2%	3.30 [1.71, 6.39]
Takebayashi	40	61	69	93	13.8%	0.66 [0.33, 1.34]
Suda	25	30	33	37	7.8%	0.61 [0.15, 2.49]
Saito	38	54	46	72	13.3%	1.34 [0.63, 2.86]
Maeda	57	73	25	47	12.9%	3.13 [1.41, 6.96]
Koriyama	38	65	43	91	14.4%	1.57 [0.83, 2.99]
Konno	43	50	34	66	11.6%	5.78 [2.27, 14.70]
Kimura	38	52	36	50	12.2%	1.06 [0.44, 2.52]
Total (95% CI)		452		547	100.0%	1.71 [1.02, 2.87]
Total events	322		318			

Heterogeneity: Tau² = 0.38; Chi² = 23.29, df = 7 (P = 0.002); I² = 70%
Test for overall effect: Z = 2.02 (P = 0.04)

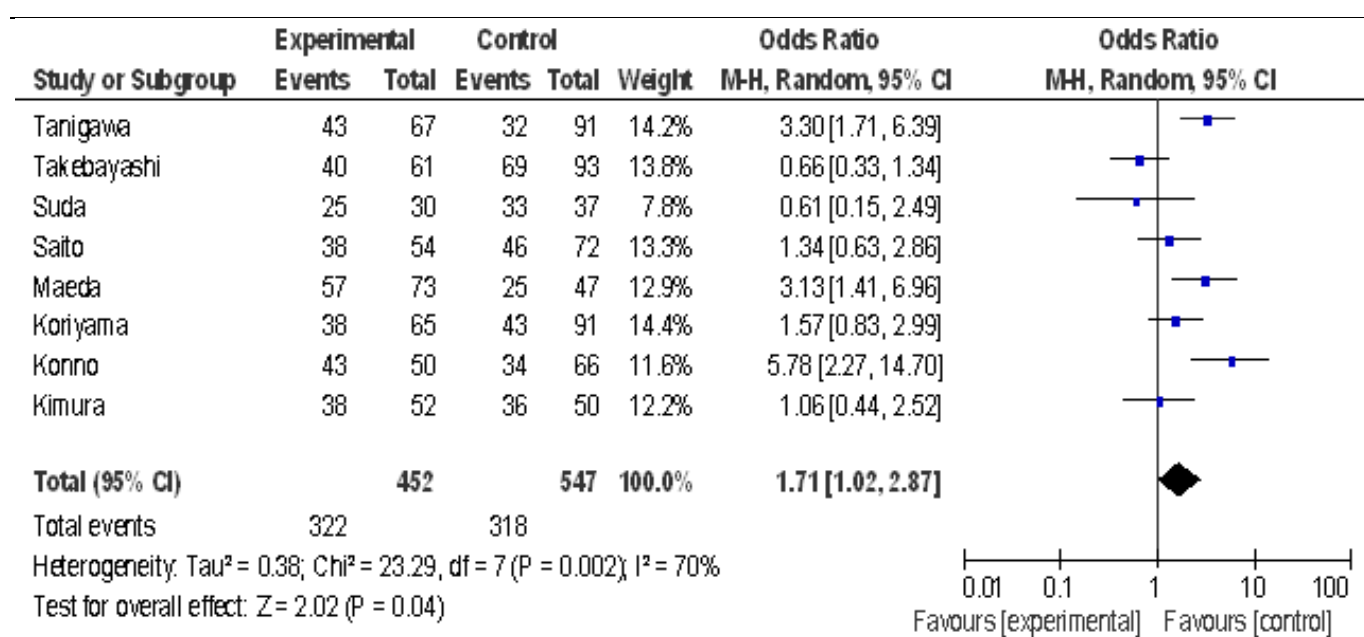**Figure 3.** *Forrest plot of ORs for the association of TP expression with lymphatic invasion.*

Publication bias

Begg's funnel and Egger's test were performed to access the publication bias in this meta-analysis. Funnel plots' shape did not reveal obvious evidence of asymmetry (Figure 5). Egger's test showed no significant publication bias for degree of differentiation (P=0.532), lymph node metastasis (P=0.368) and OS (P=0.152).However, we found that an indication of significant publication bias for Lymphatic invasion (P=0.026) .

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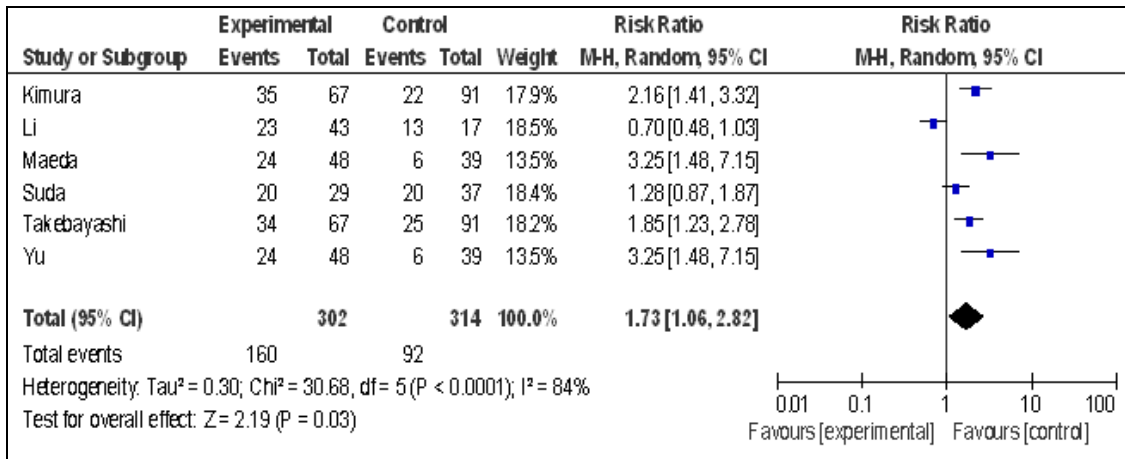

Figure 4. Forrest plot of relative risk (RR) for the association of TP expression with overall survival (OS).
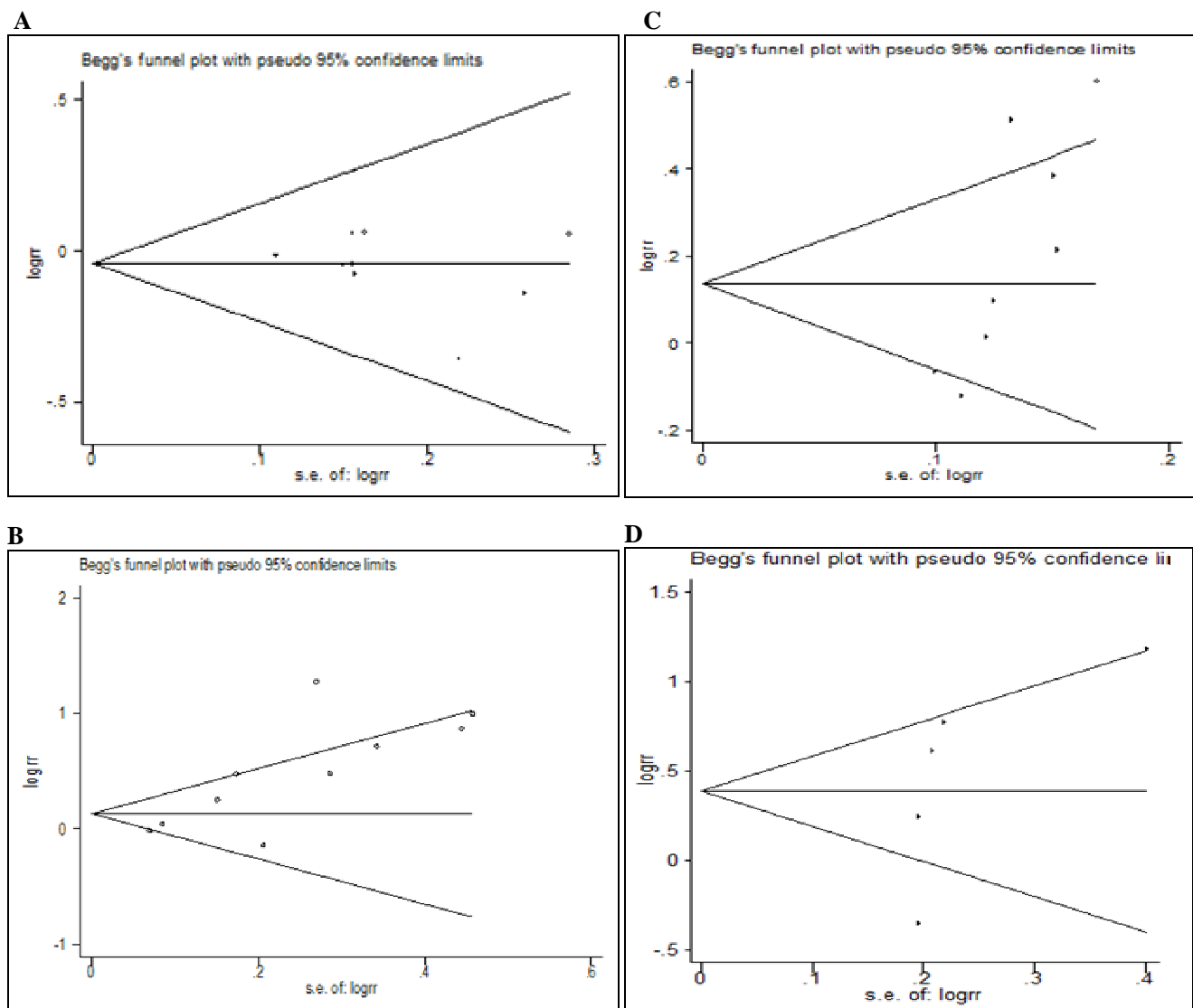

Figure 5. *Begg's funnel plots for assessing the publication bias risk of studies of tumor differentiation (A), lymph node metastasis(B), lymphatic invasion(C),overall survival(D).*

Discussion

To our knowledge, the present meta-analysis is the first article to systematically examine the association between the expression of TP and gastric cancer survival. Our results indicated that the expression of TP, as detected with IHC, was significantly associated with lymph node metastasis , lymphatic invasion and OS, indicating that it might be a marker for poor prognosis of gastric cancer.

TP, an enzyme involved in pyrimidine nucleotide metabolism, has been shown to have angiogenic activity [27]. It can induce endothelial cell migration and tube formation in vitro [28]. Generally, TP expression is correlated with higher microvessel density, higher tumor stage, and more metastasis. An association of TP with tumor grade is evident in bladder, cervical, and renal cell cancer, but not in the other investigated cancers [29]. Furthermore, in most cases, TP appeared to be associated with poor prognosis, although there are conflicting reports for some cancers. For example, seven of the nine studies on colon cancer reported a significant correlation between TP and bad prognosis, while Saito et al. demonstrated that TP is associated with good prognosis [30]. As for gastric cancer, Tanigawa et al. [21] reported that the expression of dThdPase was significantly associated with the intratumoral microvessel counts and the incidence of hematogenous metastasis. However, no association was found between the expression of dThdPase and the overall survival of patients with gastric carcinoma . Takebayashi et al. [11] reported that the gastric cancer patients with dThdPase-positive carcinoma showed a significantly worse prognosis than those with dThdPase-negative carcinoma. This discrepancy might be caused by differences in tissue fixation processing, and staining techniques, experience of the observer in selecting hot spot and the technique of counting the dThdPase-positive cells. Thus, a meta-analysis was performed to try to resolve these apparent discrepancies. It was notable that the association was observed in our meta-analysis of TP expression and lymph node metastasis , lymphatic invasion and OS, suggesting that this protein can be developed for clinical applications.

A dThdPase inhibitor, 6 amino-5-chrolouracil, inhibited angiogenesis induced by dThdPase in a mouse gelatin sponge implant assay [27]. In addition, Takao et al. demonstrated that inhibition of dThdPase suppressed metastasis of dThdPase-transfected KB-3-1 cells in vivo [31]. Taken together with this evidence and our results, the growth of tumor expressing dThdPase may be inhibited by suppressing dThdPase expression.

To be sure, there were some potential limitations in this study. First, in prognostic factors meta-analyses, variability in definitions, outcomes, measurements, and experimental procedures might contribute to between-study heterogeneity [32]. In the current meta-analysis, despite the fact that we tried to optimize standardization, some remaining variability in definitions was unavoidable. Second, as reported above, potential publication bias was a concern. We restricted our review to articles published in English or Chinese language because other languages were not accessible to the readers. This selection could favour the positive studies that are more often published in English while the negative ones tend to be more often reported in native languages [33].

Conclusion

In conclusion, TP expression was associated with increased lymph node metastasis, lymphatic
permeation and unfavorable prognosis in gastric cancer, and TP expression was an independent factor influencing lymph node metastasis , lymphatic invasion and OS. Further studies of TP expression and its potential as a marker for gastric cancer prognosis in clinic are warranted.

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