

Prognostic role of telomerase activity in gastric adenocarcinoma: A meta-analysis

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Abstract. Activation of telomerase is involved in carcinogenesis in most types of cancers. However, the prognostic value of telomerase activity (TA) in patients with gastric carcinoma (GC) remains controversial. We conducted a meta-analysis to assess the relationship between TA and the clinical outcome of GC. A meta-analysis of 18 studies (886 patients) was performed to evaluate the association between TA and metastasis-related parameters in GC patients by searching databases, including PubMed, MEDLINE, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical Literature database (CBM) (last search updated in October 2011). We used the odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of the association between TA and metastasis of GC. Our analysis results indicated that high telomerase activity expression tended to be associated with the presence of lymph node metastasis (866 patients) (OR=2.03, 95% CI 1.21-3.39, p=0.007), the depth of invasion (886 patients) (OR=1.87, 95% CI 1.30-2.70, p=0.0007), distant metastasis (407 patients) (OR=2.71, 95% CI 1.59-4.63, p=0.0002), tumor size (466 patients) (OR=2.14, 95% CI 1.31-3.50, p=0.002) and TNM stage (711 patients) (OR=2.39, 95% CI 1.30-4.41, p=0.005). However, high TA expression was not associated with the presence of histologic differentiation (791 patients) (OR=1.51, 95% CI 0.73-3.11, p=0.26). In conclusion, telomerase overexpression not only plays a key role in primary initiation, but also promotes invasion and metastatic progression of GC.

These findings raise the possibility of using TA to screen for the prognosis of gastric cancer.

Introduction

Gastric carcinoma (GC) is estimated to be the second most common cause of cancer-related death in the world, although both the incidence and mortality have declined in the past 50 years (1). The prognosis of patients with GC remains poor due to the high rate of tumor invasion into underlying tissue and lymph node metastasis, which are major prognostic indicators of neoplastic recurrence after treatment (2). Thus, there is an urgent need to identify cancer metastasis earlier and more accurately. Accumulating evidence indicates that progression beyond the initial stages of the malignant transformation in gastric adenocarcinoma is associated with cellular immortality, which occurs in other neoplasms (3).

The key factor responsible for cellular immortality is telomerase, which is a specialized ribonucleoprotein complex that adds telomeric DNA onto the ends of chromosomes. By synthesizing the repetitive telomeric sequence using its RNA template, telomerase prevents cellular senescence in somatic cells (4). Moreover, human telomerase reverse transcriptase (hTERT), as the rate-limiting step in the activation of telomerase, is known to be an accurate measure of telomerase activity (TA). The presence of hTERT is therefore required for aberrant cell proliferation and carcinogenesis in most cancer types (5). Thus, telomerase is considered to be a potential marker of oncogenesis (6).

The prognostic role of high TA and overexpression of hTERT has been reported by many authors (7,8). Although several studies focusing on telomerase have referred to clinicopathological variables, including tumor size, site, histologic grade, depth of tumor invasion, lymph node metastasis, distant metastasis and TNM stage, the relationship between telomerase and tumor progression or metastasis in patients with GC remains controversial. It is uncertain whether reported results depend on the number of patients or ethnic heterogeneity present in each trial. Therefore, it is appropriate to undertake a meta-analysis of existing trials to achieve insight into the metastatic value of TA and hTERT in GC.

In the present study, we enrolled clinicopathological parameters (such as depth of tumor invasion and lymph node

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Abbreviations: hTERT, human telomerase reverse transcriptase; GC, gastric carcinoma; TA, telomerase activity; OR, odds ratio; CI, confidence interval

Key words: telomerase, gastric carcinoma, prognosis, metastasis, meta-analysis

metastasis) from case-control studies to predict the clinical outcome of GC. The results demonstrated that telomerase over-expression may play a key role in metastatic progression of GC.

Materials and methods

Literature search. This meta-analysis followed the proposal set by the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group (9), and was performed by searching PubMed, MEDLINE, EMBASE, Web of Science databases, Cochrane Library and the Chinese Biomedical Literature database (CBM) (last search updated in October 2011). The search strategy included the following terms: (telomerase [MeSH] or Telomerase Catalytic Subunit [TEXT WORD] or Telomerase Reverse Transcriptase [TEXT WORD] or hTERT [TEXT WORD]) and (Stomach Neoplasms [MeSH] or Gastric Cancer [TEXT WORD] or Gastric Neoplasms [TEXT WORD] or Stomach Cancer [TEXT WORD]). Searches also included scanning reference lists in relevant articles and conference proceedings as well as correspondence with authors when additional data were required. Two reviewers (Lü and Deng) independently screened titles and abstracts of each identified reference, and categorized papers based on the full text to evaluate their eligibility for inclusion.

Inclusion criteria. The inclusion criteria for primary studies were as follows: i) the data were from prospective or retrospective case-control studies and included correlations of telomerase or hTERT to GC; ii) each study presented a proven diagnosis of GC in humans; iii) each study measured telomerase activity or hTERT evaluation using immunohistochemistry (IHC), a telomeric repeat amplification protocol assay (TRAP), a telomeric repeat amplification protocol/enzyme-linked immunosorbent assay (TRAP-ELISA), a membrane-array assay, a reverse transcription-polymerase chain reaction (RT-PCR) or real-time fluorescent quantitative PCR (qRT-PCR); iv) the papers had to provide the sample size, ethnicity and other sample information; v) if data were shared between multiple studies, only the most recent or largest population was included (10), and vi) the publication was in English.

Data extraction. The following items were collected from the reports: first author, year of publication, sample size, ethnicity, TA or hTERT assessment method, cutoff value of TA or hTERT positivity, and telomerase or hTERT expression related to clinicopathological parameters, including gender, age, tumor size, histologic grade, depth of invasion, lymph node metastasis, distant metastasis and TNM stage. Depth of tumor invasion was confirmed using histologic examination, and infiltration into serosa indicated a poor prognosis. The presence of lymph node metastasis in early GC was not a good sign. Distant metastasis was a definite prognostic marker of tumor recurrence. We required that each study definitively reported at least two of the following criteria: the depth of invasion, the presence of lymph node metastasis and the presence of distant metastasis. Data extraction was performed independently by two individuals (Lü and Deng), and any disagreement was resolved by consensus or by consultation with additional reviewers (Yang and Zhang).

Table I. Newcastle-Ottawa quality assessment scale.

Selection
1) Is the case definition adequate?
a) Yes, with independent validation*
b) Yes (<i>record linkage or based on self reports</i>)
c) No description
2) Representativeness of the cases
a) Consecutive or obviously representative series of cases*
b) Potential for selection biases or not stated
3) Selection of controls
a) Community controls*
b) Hospital controls
c) No description
4) Definition of controls
a) No history of disease*
b) No description of source
Comparability
1) Comparability of cases and controls on the basis of the design or analysis
a) Study controls for <u>metastasis</u> *
b) Study controls for any additional factor* (<i>age, gender, grade</i>)
Exposure
1) Ascertainment of exposure
a) Secure record (<i>surgical records</i>)*
b) Structured interview blind to case/control status*
c) Interview not blinded to case/control status
d) Written self report or medical record only
e) No description
2) Same method of ascertainment for cases and controls
a) Yes*
b) No
3) Non-response rate
a) Same rate for both groups*
b) Non-respondents described
c) Rate different and no designation

A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability. Underlined and quoted phrases are provided in the scale to allow for adjustment to particular studies. Italicized phrases indicate our interpretation of the question relevant to this study.

Qualitative assessment. Quality assessment was performed with the Newcastle-Ottawa quality assessment scale (NOS) for case-control studies (Table I). A 'star system' has been used to judge data quality based on three broad perspectives: the selection, comparability and outcome of interest for cohort studies. Stars are added up to compare the study quality in a quantitative fashion (11). Based on these criteria, the content validity was evaluated by Lü and Deng, and any disagreement was resolved via discussions between Lü and Deng or with the other authors (Yang and Zhang) for adjudication.

Statistical analysis. Statistical analysis was performed using RevMan 5.0 according to the principles set out in the Cochrane Handbook for Systematic Reviews of Interventions. The methodological quality of each study was assessed with the QUADAS tool recommended by the Cochrane Collaboration, and the kappa statistic (κ) for inter-rater reliability was

calculated. Agreement was assessed using the κ statistic for evaluating methodological quality (12). For dichotomous outcomes, the meta-analysis was performed using crude odds ratios (ORs) with 95% confidence intervals (CIs) to assess the strength of association between telomerase activity or hTERT and metastasis of GC. The data were reported in a binary manner, elucidating the telomerase activity or hTERT value as either 'high' or 'low'. The pooled ORs were conducted to assess the depth of invasion, lymph node metastasis and distant metastasis. For analyzing clinical outcome, well and moderate differentiation were merged, poor and undifferentiated were merged, T1 and T2 were merged, T3 and T4 were merged, stage I and stage II were merged, and stage III and stage IV were merged. Assessment of heterogeneity was assessed by the Chi-square test (χ^2) and inconsistency index test (I^2). Heterogeneity was not considered statistically significant when $p > 0.10$ in the χ^2 -test, and acceptable heterogeneity was defined as $I^2 < 50\%$ in studies. For studies lacking a measure of heterogeneity, a Mantel-Haenszel fixed effect model was used for the primary meta-analysis (13); otherwise, a DerSimonian-Laird random effects model was adopted (14). Assessment of publication bias for each of the pooled study groups was tested using a funnel plot.

Results

Selection and characteristics of the studies. At the beginning, 215 records were examined according to the search strategies. In total, 152 articles were eliminated after scanning the titles or abstracts since they were review articles, case reports, commentaries and letters or since they were irrelevant to this analysis. After further review, an additional 45 articles were excluded: first, 2 studies overlapped with others. Second, 9 studies were experiments on cell cultures or animals. Finally, 34 studies lacked usable data that correlated telomerase or hTERT with lymph node status or TNM stage to create 2x2 tables. Thus, a total of 18 eligible studies related to GC patients were finally identified in our meta-analysis with good agreement between reviewers ($\kappa = 0.73$) (15-32) (Fig. 1).

In the remaining studies, all measurements were performed using the primary tumor, and all of the patients had not received chemotherapy or radiotherapy before enrollment. Although research was conducted at tertiary referral centers, almost all studies were performed in Asia and one in South America (22). Sample size varied from 20 to 95 participants, and the average age across all of the studies was 59.3 years, with a variation ranging from 32 to 89 years. The number of GC patients with T3 and T4 invasion ranged from 11 to 78; the number of GC patients with lymph node metastasis ranged from 7 to 75; the number of GC patients with distant metastasis ranged from 2 to 32. Four studies used IHC, 9 studies used TRAP, 1 study used TRAP-ELISA, 1 study used a membrane-array assay, 2 studies used RT-PCR and 1 study used qRT-PCR. The quality assessment of studies was performed using the NOS ranged from 5 to 7 (with a mean star rating of 5.9), with a higher value indicating better methodology. The scale is listed in Table II. The cutoff value of telomerase or hTERT expression was determined using different methods in each study. The basic feature description of the 18 studies is summarized in Table II, and

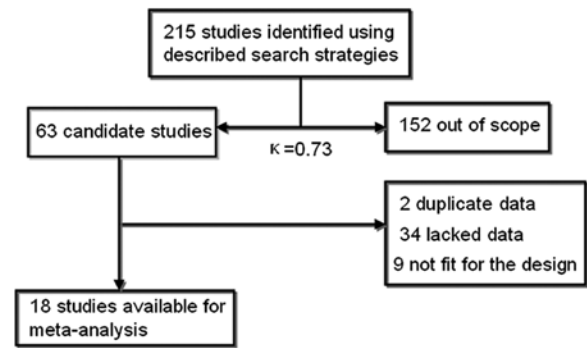


Figure 1. Flow chart of the meta-analysis.

the correlation between telomerase or hTERT expression and clinicopathological factors is listed in Table III.

Quantitative synthesis

Correlation between TA and clinicopathological characteristics. When stratifying variables by lymph node metastasis, there was a slight heterogeneity in the data ($\chi^2 = 26$, $I^2 = 38.5\%$, $p = 0.05$). Patients with lymph node metastasis in GC displayed a significantly higher TA expression in 17 studies (866 patients) (OR=2.03, 95% CI 1.21-3.39, $p = 0.007$; Fig. 2A). When stratifying for the depth of tumor invasion in GC, 18 studies (886 patients) were reported without significant heterogeneity ($\chi^2 = 21.34$, $I^2 = 20.3\%$, $p = 0.21$). We observed that patients with T3 and T4 GC had a significantly higher TA (OR=1.87, 95% CI 1.30-2.70, $p = 0.0007$; Fig. 2B). When stratifying for distant metastasis in GC, 9 studies (407 patients) were combined, and there was no significant heterogeneity in the data ($\chi^2 = 11.95$, $I^2 = 33\%$, $p = 0.15$). Patients with distant metastasis had a significantly higher TA in GC (OR=2.71, 95% CI 1.59-4.63, $p = 0.0002$; Fig. 2C). We also observed a correlation between TA and other clinical characteristics, including tumor size > 5 cm in 9 studies (466 patients; OR=2.14, 95% CI 1.31-3.50, $p = 0.002$; Fig. 2D), poor histologic differentiation in 16 studies (791 patients; OR=1.51, 95% CI 0.73-3.11, $p = 0.26$; Fig. 2E), and a higher (III + IV) clinical stage in 14 studies (711 patients; OR=2.39, 95% CI 1.30-4.41, $p = 0.005$; Fig. 2F). When stratifying the variables by poor histologic differentiation of GC, there was heterogeneity ($I^2 = 60.4\%$), then the DerSimonian-Laird random effects model was used. There was no significant correlation between TA and histologic differentiation ($p = 0.26$).

Publication bias. Funnel plots were used to estimate the publication bias of the meta-analysis. As shown in Fig. 3, the shape of the funnel plot did not reveal obvious asymmetry.

Discussion

GC remains the second leading cause of cancer-related mortality worldwide (33), in part because of its high rate of metastasis and recurrence. It is critical to explore molecular biomarkers to guide clinical decision-making with regard to the treatment of GC. TA is thought to be a critical step in the evolution of most tumor types (34-37), including GC (38,39). Considerable clinical research has been conducted with the aim of assessing the correlation between TA expression and clinicopathological outcome in patients with GC, but the

Table II. Main characteristics of the 18 studies included in the meta-analysis.

Author/(Ref.)	Year of publication	Language	Population	Study from PubMed	No. of patients (M/F)	Median age (years)	TA/hTERT detection method	Cutoff for TA positivity (%)	Result	Study quality points
Yang <i>et al</i> (15)	2001	English	China	Yes	29/13	52.9	TRAP assay	>6-bp ladder	All negative	7/9
Liu <i>et al</i> (27)	2008	Chinese	China	Yes	27/13	54.0	RT-PCR	>0.6	3,4,6 positive	6/9
Okusa <i>et al</i> (28)	1998	English	Japan	Yes	22/14	62.3	TRAP-ELISA	>5%	5 positive	6/9
Hu <i>et al</i> (16)	2009	English	China	Yes	28/18	56.3	TRAP assay	>0.2 units	All positive	5/9
Shin <i>et al</i> (29)	2002	English	Korea	Yes	35/30	55.4	RT-PCR	NR	2,4 positive	6/9
Mori <i>et al</i> (30)	2000	English	Japan	Yes	32/14	61.7	TRAP assay	>6-bp ladder	3 positive	5/9
Wu <i>et al</i> (17)	2006	English	China	Yes	41/23	60.5	Membrane-array assay	ROC curve	All negative	7/9
Wang <i>et al</i> (18)	2004	English	China	Yes	30/11	57.2	IHC	>5%	1,2,4,6 positive	6/9
Yoo <i>et al</i> (19)	2003	English	Korea	Yes	38/13	61.3	IHC	>10%	2,3 positive	6/9
Yasui <i>et al</i> (20)	1998	English	Japan	Yes	10/10	68.9	IHC	Focal or diffuse staining	NR	5/9
Kameshima <i>et al</i> (21)	2000	English	Japan	Yes	19/8	66.9	TRAP assay	>0.6 μ g	All negative	6/9
Gigek <i>et al</i> (22)	2009	English	Brasil	Yes	36/19	NR	IHC	No positive cells were observed	All negative	5/9
Hu <i>et al</i> (23)	2004	English	China	Yes	25/10	55.2	qRT-PCR	>5.39	2 positive	6/9
Ahn <i>et al</i> (24)	1997	English	Korea	Yes	57/38	54.3	TRAP assay	>6-bp ladder	All negative	7/9
Zhan <i>et al</i> (31)	1999	English	China	Yes	50/44	63.0	TRAP assay	>6-bp ladder	All negative	6/9
Gümüş-Akay <i>et al</i> (32)	2007	English	Turkey	Yes	NR	NR	TRAP assay	NR	2,3,4 negative	5/9
Hiyama <i>et al</i> (25)	1995	English	Japan	Yes	37/19	55.0	TRAP assay	>0.6 μ g	1,4,6 positive	7/9
Tahara <i>et al</i> (26)	1995	English	Japan	Yes	13/7	64.0	TRAP assay	>6-bp ladder	4,5 positive	6/9

Results: 1, tumor size; 2, histologic grade; 3, depth of invasion; 4, lymph node metastasis; 5, distant metastasis; 6, TNM stage. Study quality is listed using the results of the Newcastle-Ottawa questionnaire. NR, not reported.

Table III. Main characteristics of 18 studies relating TA expression to clinicopathological factors.

Author/(Ref.)	Year of publication	Language	Country	No. of positive/ (negative)	No. of patients with TA-positivity					
					Size >5 cm (<5 cm)	Histo P/U (W/M)	T T3/T4 (T1/T2)	N positive/ (negative)	M positive/ (negative)	TNM TIII/IV (TI/TII)
Liu <i>et al</i> (27)	2008	English	China	26 (14)	-	-	20 (6)	22 (4)	11 (15)	24 (2)
Yang <i>et al</i> (15)	2001	English	China	40 (2)	26 (14)	30 (10)	26 (14)	20 (20)	-	17 (23)
Wu <i>et al</i> (17)	2006	English	China	52 (12)	23 (29)	49 (3)	40 (12)	37 (15)	14 (38)	33 (19)
Hu <i>et al</i> (23)	2004	English	China	18 (17)	10 (8)	14 (4)	7 (11)	14 (4)	10 (8)	-
Zhan <i>et al</i> (31)	1999	English	China	81 (13)	46 (35)	43 (38)	68 (13)	51 (30)	-	60 (21)
Hu <i>et al</i> (16)	2009	English	China	41 (5)	28 (13)	33 (8)	21 (20)	5 (36)	30 (11)	-
Wang <i>et al</i> (18)	2004	English	China	32 (9)	19 (13)	25 (7)	13 (19)	23 (9)	-	27 (5)
Gümüş-Akay <i>et al</i> (32)	2007	English	China	42 (1)	-	22 (20)	31 (11)	27 (15)	-	-
Ahn <i>et al</i> (24)	1997	English	Korea	85 (10)	-	63 (22)	60 (25)	68 (17)	-	61 (24)
Yoo <i>et al</i> (19)	2003	English	Korea	37 (14)	18 (19)	20 (17)	11 (26)	26 (11)	-	-
Shin <i>et al</i> (29)	2002	English	Korea	30 (35)	-	20 (10)	10 (20)	22 (8)	7 (23)	13 (17)
Mori <i>et al</i> (30)	2000	English	Japan	19 (27)	-	10 (9)	16 (3)	14 (5)	6 (13)	12 (7)
Tahara <i>et al</i> (26)	1995	English	Japan	17 (3)	-	8 (9)	14 (3)	12 (5)	2 (15)	12 (5)
Hiyama <i>et al</i> (25)	1995	English	Japan	56 (10)	28 (28)	51 (5)	42 (14)	34 (22)	-	24 (32)
Okusa <i>et al</i> (28)	1998	English	Japan	22 (14)	-	10 (12)	20 (2)	18 (4)	8 (14)	16 (6)
Yasui <i>et al</i> (20)	1998	English	Japan	17 (3)	-	9 (8)	16 (1)	-	-	11 (6)
Kameshima <i>et al</i> (21)	2000	English	Japan	19 (8)	8 (11)	7 (12)	11 (8)	8 (11)	-	7 (12)
Gigek <i>et al</i> (22)	2009	English	Brasil	44 (11)	-	-	23 (21)	43 (1)	18 (26)	43 (1)

Positive, patient have TA expression; negative, patients have no TA expression. T, depth of invasion. N, lymph node metastasis. M, distant metastasis. Histo, histodifferentiation: P, poor differentiation; U, undifferentiation; W, well differentiation; M, moderate differentiation; '-' corresponds to missing data and was not analyzed in the meta-analysis.

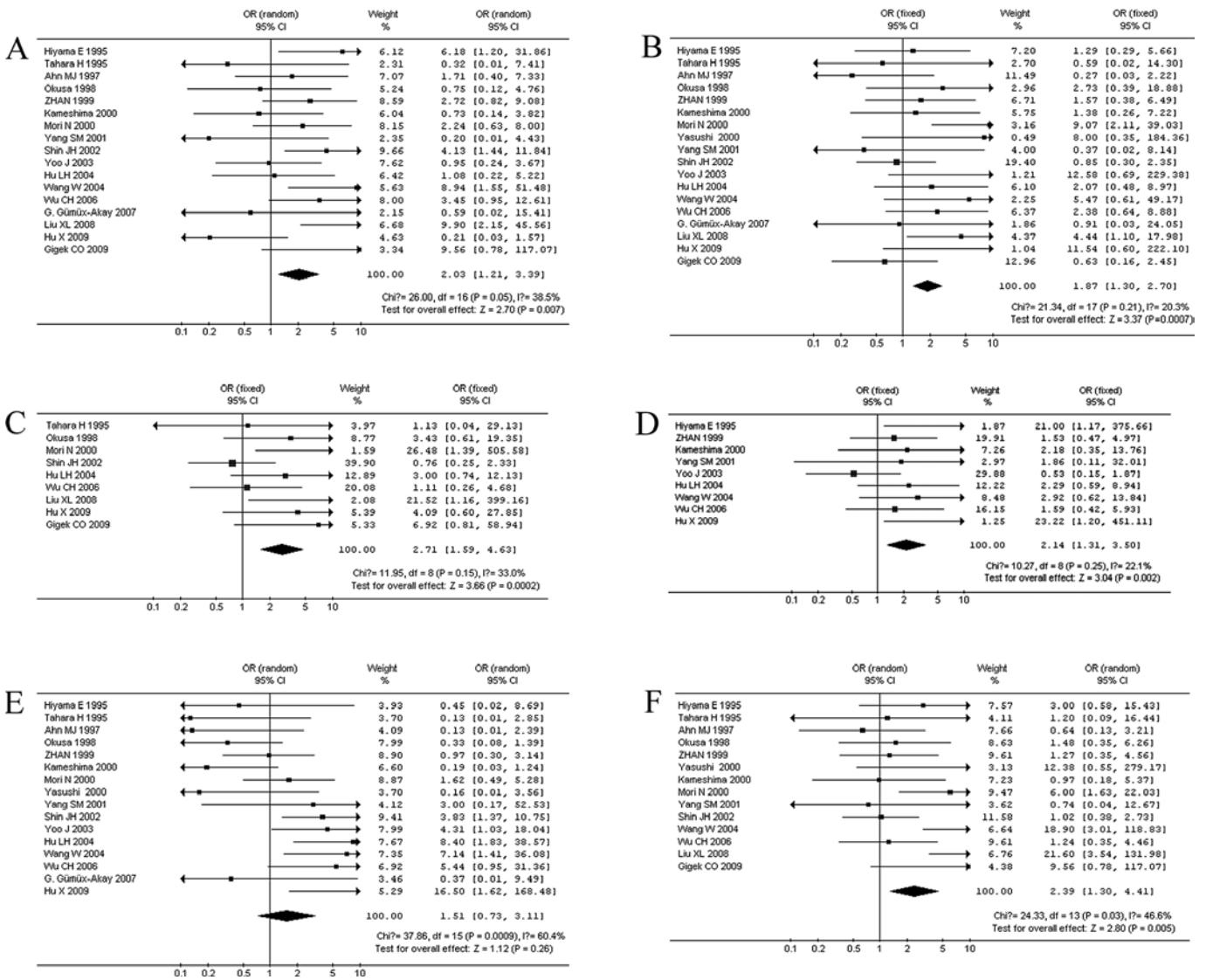


Figure 2. Meta-analysis of the relation between TA expression and clinicopathological parameters. (A) Lymph node metastasis, (B) depth of tumor invasion, (C) distant metastasis, (D) tumor size, (E) histologic differentiation and (F) clinical stage.

results have been controversial. Several studies have shown that the TA in GC tissues is related to depth of tumor invasion and lymph node metastasis (25,29); however, other studies found no association between clinical outcome and TA (21,24).

Recently, many studies have indicated that hTERT is the rate-limiting step in the activation of telomerase, and its expression level is directly proportional to expression levels of TA (40,41). In our analysis, TA was measured by TRAP assay in 9 studies and TRAP-ELISA in 1 study, whereas hTERT was measured by RT-PCR in 2 studies, qRT-PCR in 1 study, a membrane-array assay in 1 study and IHC in 4 studies. TA expression was detected using all of these methods. The cutoff value of TA positivity obtained from different methods was recognized as a standard to assess TA expression. The pooled statistical data showed that the prognostic utility of TA was consistent with clinical characteristics, including depth of tumor invasion ($p=0.0007$), lymph node status ($p=0.007$), distant metastasis ($p=0.0002$) and TNM stage ($p=0.005$). In addition, we identified and evaluated the association of TA expression with tumor size and tumor grade. Our findings also showed that there was a strong association between high TA

expression potential and tumor size ($p=0.002$), but not tumor grade ($p=0.26$). Combining several independent studies, our estimates supported the idea that TA and hTERT overexpression were strongly related to gastric tumor invasion and metastasis. Therefore, the role telomerase plays in inducing tumor progression is not only based on its well-documented effects on tumor proliferation rate (42).

Our findings were consistent with the reports on TA expression in melanoma (43), breast cancer (44), hepatocellular carcinoma (45) and giant-cell tumors of the bone (7), in which TA contributed to the poor survival of patients. We demonstrated at the cellular level that hTERT transfection in U2OS (a hTERT-negative cell line) re-activated its telomerase activity and further promoted its invasive and metastatic potential. The mechanism that enhances these malignant phenotypes may be correlated with the increasing adhesive ability of these tumor cells to the extracellular matrix (46). High TA may activate the glycolytic pathway to promote tumor growth and metastasis (43). TA suppression may render cells more susceptible to anchorage-independent growth inhibition, and unstable tumors require a higher TA level to prevent genomic deterioration and

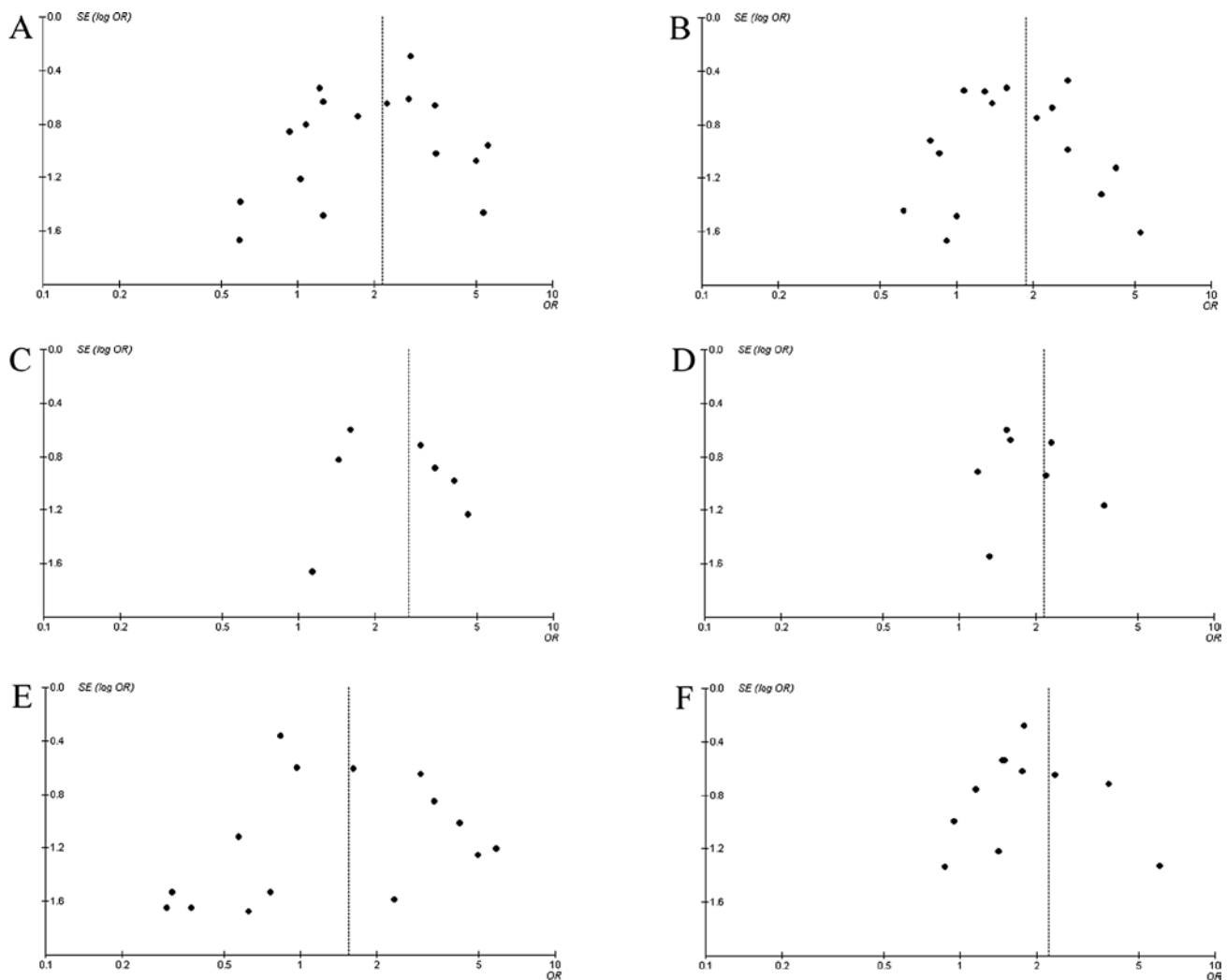


Figure 3. Funnel plot analysis of publication bias for clinicopathological parameters. (A) Lymph node metastasis, (B) depth of tumor invasion, (C) distant metastasis, (D) tumor size, (E) histologic differentiation and (F) clinical stage.

to induce more aggressive cancer cells during carcinogenesis (7). The mechanism described above offered a possible interpretation of the observed strong statistical association between TA overexpression and tumor metastasis.

Caution must be taken to note the limitations of this study. First, most of the patients with GC enrolled in our meta-analysis came from Asia, which may be attributed to the apparent decrease in the incidence and mortality rates for GC in the past 50 years in Western countries compared to Japan and China (47). Second, reports in languages other than English were excluded. The risk of language bias had to be considered, but may not result in any notable bias in the assessment of interventional effectiveness (48). Third, data containing negative results may be less likely published, although we took care to access all available data. Fourth, the eligible data do not assess whether TA may influence the prognosis of patients according to distinct therapeutic schedules.

Based on the results of this analysis, we conclude that telomerase overexpression is not only involved in the carcinogenesis in the initiation of GC, but also promotes the invasion and metastasis of GC. These results improve our understanding of telomerase as a potentially important molecular target in clinical diagnostics and therapeutics of gastric cancer.

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