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Review Article

Prognostic and clinicopathological value of Gli1 expression in esophageal squamous cell carcinoma: a meta-analysis

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Abstract: Lots of clinical and basic researches focused on Glioma associated oncogene 1 (Gli1) which plays an important role on the development and prognosis of esophageal squamous cell carcinoma (ESCC), but did not reach consensus. This study aims to provide valuable reference for prognosis estimation and therapeutic approach in ESCC patients. Eleven studies with 749 cases of esophageal carcinoma were included to analyze the relationship between Gli1 expression and clinicopathological features, 3/5-year survival, and overall survival (OS) using pooled risk ratios (RRs) and hazard ratio (HR) with 95% confidence intervals (CIs). This meta-analysis suggested that up-regulated Gli1 was associated with advanced clinical stage (RR = 3.71, 95% CI: [2.34, 5.86]), higher T stage (RR = 3.43, 95% CI: [1.41, 8.36]), and lymph node metastasis (RR = 1.98, 95% CI: [1.22, 3.21]). This study also indicated that positive Gli1 expression was associated with poorer survival in ESCC, with 3-year survival (RR = 2.37, 95% CI: [1.57, 3.60]), 5-year survival (RR = 3.40, 95% CI: [2.21, 5.25]) and overall survival (OS) (HR = 2.42, 95% CI: [1.76, 3.34]). On the whole, the over-expression of Gli1 could predict poor survival in ESCC patients and detection of Gli1 may provide a new thought for monitoring the prognosis of ESCC.

Keywords: Gli1 over-expression, esophageal squamous cell carcinoma, prognosis

Introduction

Cancer has been a costly public health burden all around the world. Esophageal carcinoma is the sixth most common cause of cancer death in the world [1, 2]. Approximately half of the newly diagnosed esophageal carcinoma happened in China [3]. Esophageal squamous cell carcinoma (ESCC) is the major pathological type in Asian countries, which accounts for approximate 90% of esophageal carcinoma [4]. Most of esophageal carcinoma patients have advanced stages at initial diagnosis. Nevertheless, the overall 5-year survival varies from 15% to 25% despite of the improvement of diagnostic techniques and treatment approaches [2]. Although the same therapy was given to esophageal carcinoma patients in the same stage, the clinical outcomes vary from each other, which may be attributed to diversity of biological behavior of tumors [5]. Studies have shown that some genetic alterations were related to the progression of esophageal carcinoma, while seldom of them have been clearly demonstrated to be correlated with clinicopathological features of esophageal carcinoma [6]. Consequently, there is a need to find better prognostic indicator in order to improve treatment for esophageal carcinoma.

Aberrant activation of Sonic Hedgehog (Shh) signaling pathway is verified to be associated with tumorigenesis and tumor progression in malignant cancers, such as skin tumor [7, 8],

pancreatic carcinoma [9, 10], esophageal carcinoma [11], lymphoma [12], brain carcinoma [13], colonic carcinoma [14], gastric carcinoma [15], prostate carcinoma [16]. Smoothened (Smo) protein is a transmembrane protein acting as on-off switch in Shh signaling pathway. Gli1 is a downstream transcriptional factor of Smo protein which can transduct extracellular Shh signal into intracellular Gli1 signal and elicit Gli1-dependent transcription of intranuclear target genes to activate Shh signal pathway [17]. Thus abnormal up-regulated expression of Gli1 caused by aberrant activation of Shh signaling pathway could be related to development of malignant cancers.

Gli1 has been reported to be an unfavorable prognostic factor in stomach, pancreas, breast, ovary, liver and bladder cancers [18-25]. Overexpression of Gli1 is common in Barrett's and adenocarcinoma of esophagus [26-28]. Gli1 could contribute to invasion and metastasis of ESCC through promoting epithelial-to-mesenchymal transition (EMT) [29, 30], which is an early event in the metastatic progression of a number of types of epithelial cancers [31, 32]. Moreover, Gli1 can also bind to the promoter and enhance expression of cyclin-dependent kinase 2 (CDK2), which is a cell cycle regulator, promoting cell proliferation in esophageal tumorigenesis [26]. Furthermore, Gli1 expression has been reported to be associated with lymph node metastasis, tumor progression and resistance to chemo-radiotherapy in esophageal carcinoma [18, 27, 28]. Yoshikawa suggested that Gli1 nuclear expression is a strong independent predictor of early relapse and poor prognosis in ESCC after chemo-radiotherapy [11]. However, potential predictive role of Gli1 in prognosis of esophageal carcinoma remains unclear. Our study aims at integrating evidence to elucidate relationship between Gli1 expression and clinicopathological features of esophageal carcinoma, so as to provide evidence for prognosis estimation and therapeutic approach.

Methods

Literature search strategy

MEDLINE, EMBASE, PubMed, the Cochrane Library, and the China National Knowledge Infrastructure were searched for the studies by using the key words at the last time on November 11, 2016. The search strategy included the following keywords variably combined with "esophagus cancer (or esophagus carcinoma)", "Gli1 (or Glioma associated oncogene 1)" and "prognosis (or prognostic)". We also searched the studies referring to the reference of the eligible studies or relevant reviews. Finally, we removed duplicates and got the initial articles.

Study selection criteria

We considered studies as eligible if they met all of the following inclusion criteria: (1) esophagus cancer patients were diagnosed by pathological examination; (2) expressions of Gli1 were measured; (3) studies could provide adequate information of the survival analysis or clinical features of patients related to the Gli1 expression. Review articles, case reports, laboratory articles, letters, or the papers lack of necessary information for what we needed were excluded.

Data extraction

Articles were reviewed independently by two investigators for article inclusion and exclusion according to the criteria we mentioned above. Disagreements were resolved by consultation with the third investigator. We extracted the HR and 95% confidence interval (CI), *p* value, Kaplan-Meier survival curves of survival outcomes, first author, publication year, study design, study size, origin of population, patients age and sexuality, clinical stage, T stage, methods to detect Gli1, definition of Gil1 positive and expression rate in each studies.

Quality assessment

Two investigators evaluated the quality of the eligible studies independently by the Newcastle-Ottawa Scale (NOS). The NOS score (full score = 9) more than 5 was defined as high quality study. Controversial studies were discussed together by the whole team.

Statistical methods

The log (HR) and standard error of the log (HR) were used for aggregation of the survival results. In addition to directly extraction, the

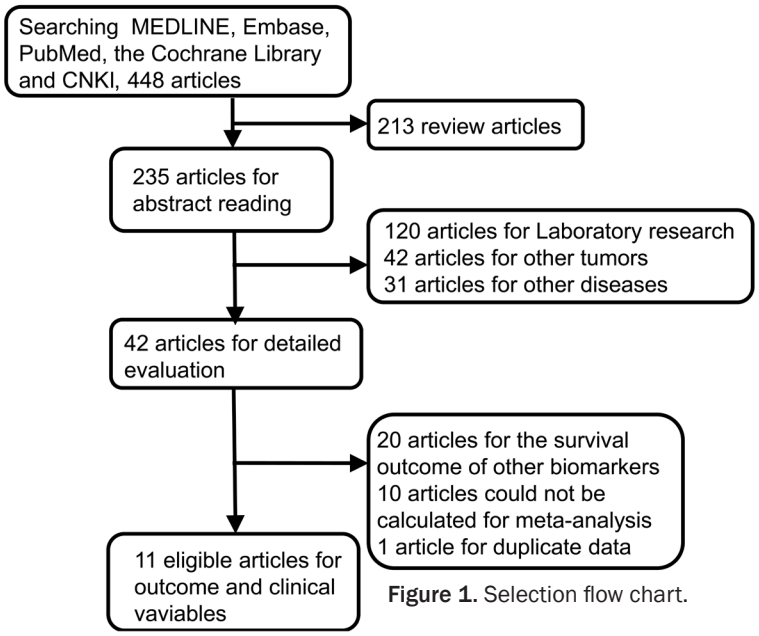

Figure 1. Selection flow chart.

necessary statistics were also calculated based on the available data with methods proposed by Parmar [33], Williamson [34], and Tierney [35]. Multivariate Cox hazard regression analysis data were preferred in our analysis, if not available, we extracted Univariate Cox hazard regression analysis or Kaplan-Meier survival curves by applying Engauge Digitizer version 4.1 with log-rank *p* value of survival outcomes instead. Then further meta-analysis of OS was performed. Calculation was accomplished by the software designed by Matthew Sydes and Jayne Tierney with their methods (Medical Research Council Clinical Trials Unit, London, UK)[35]. Risk ratio (RR) was used to evaluate the association between positive Gli1 expression and clinical features, such as gender (male vs. female), histological grade (poor/undifferentiated vs. well/moderate), T stage (T3/T4 vs. T1/T2), lymph node metastasis (Yes vs. No), clinical stage (III/IV vs. I/II), etc.

Effect of Gli1 expression on survival outcome and the correlation between Gli1 expression and the clinical features were estimated by Forrest plots. Obvious heterogeneity was defined as $p < 0.05$ for the χ^2 test or $I^2 > 50\%$. When there was no statistically significant heterogeneity, a fixed effect model was used for analysis; otherwise, a random effect model was used [36]. Begg's funnel plots and Egger's tests were used to evaluate publication bias, and $p > 0.05$ was considered no potential publication bias [37]. All above calculations were performed using Stata version 12.0 (Stata Corporation, College Station, TX, USA).

Results

Eligible studies

Total 448 published articles including 213 reviews were yielded by searching in the databases of MEDLINE, EMBASE, PubMed, the Cochrane Library, and the China National Knowledge Infrastructure. We screened abstracts of the rest 235 articles and excluded 120 articles for laboratory research, 42 articles for other cancers and 31 articles for other diseases. Then remaining 42 articles were selected for detailed evaluation. Among them, 20 were removed for analyzing survival focused on unrelated biomarkers, 10 were removed for data not available for meta-analysis and 1 was deleted for possible duplicated data. Finally, 11 eligible articles [11, 18, 28, 38-45] were analyzed in our study. Flow chart of study identification is presented in **Figure 1**.

Study characteristics

We collected data from eleven studies including 749 cases of esophageal carcinoma (744 ESCC and 5 esophageal adenocarcinomas) from China, Japan and South Korea. They were all ranged from 2006 to 2016. Gli1 expression was evaluated by immunological histological chemistry (IHC) method except for Li's study [40] by reverse transcription-polymerase chain reaction (RT-PCR) method. The sample size ranged from 12 to 127. Total six studies [11, 18, 28, 38, 42, 45] analyzed the relationship between Gli1 expression and OS, two [11, 45] of which also investigated correlation between Gli1 expression and disease free survival (DFS) and one [28] of which also investigated Gli1 expression and progression free survival (PFS). Further detailed features were presented in **Table 1**, quality assessments were listed in **Table 2** and main outcomes were summarized in **Table 3**.

Table 1. Characteristics of all identified studies

First author	Year	Country	Pathological type	Number (M/F)	Mean age	Method	Antibody source	Definition of Gil1 positive	Expression rate (%)	Survival analysis
Yang ZT	2016	Korea	ESCC	127 (120/7)	NR	IHC	Abcam	Weak staining ≥50% or moderate staining ≥20%	28.35	OS, DFS
Zhu WG	2011	China	ESCC	100 (85/15)	55	IHC	Santa Cruz Biotech	≥10%	72	OS, DPFS, LPFS
Mori Y	2006	Japan	ESCC	104 (92/12)	63	IHC	C-18 Santa Cruz	>25%	50	OS
Wei LY	2011	China	ESCC (30)/Ade (5)	35 (29/6)	60	IHC	Eugene	NR	71.4	OS
Yoshikawa R	2008	Japan	ESCC	69 (58/11)	60.7	IHC	Sana Cruz Biotech	≥5%	10.14	OS, DFS
Cui HW	2015	China	ESCC	12 (9/3)	53.61	IHC	NR	NR	83.33	NR
Li JP	2013	China	ESCC	68 (44/24)	54	RT-PCR	NR	Score >3*	70.60%	NR
Ju L	2013	China	ESCC	50 (50/0)	62	IHC	Santa Cruz Biotech	NR	68	NR
Sun B	2011	China	ESCC	60 (36/24)	60.6	IHC	Bioss antibodies	≥5%	88.33	NR
Wei LY	2016	China	ESCC	88 (75/13)	56.52	IHC	Eugene	NR	71.59	OS
Xiao F	2016	China	ESCC	36 (20/16)	NR	IHC	Santa Cruz Biotech	≥5%	55.6	NR

NR, not reference; IHC, immunohistochemistry; RT-PCR, reverse transcription-polymerase chain reaction; ESCC, esophageal squamous cell carcinomas; Ade, adenocarcinoma; DFS, disease free survival; OS, overall survival; LPFS, loco-regional progression free survival; DPFS, distant progression free survival; NOS, Newcastle-Ottawa Scale. *IHC staining score: 1, 0~10%; 2, 10% ~ 50%; 3, >50%. The dyeing strength score: 0 = undetectable; 1 = yellow (blue); 2 = moderate yellow (blue); 3 = brown (purple blue). The final score = IHC staining score x the dyeing strength core, and total score of 0 ~ 3 divided into negative, more than 3 is positive.

Table 2. Quality assessment of all identified studies

First author	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design	Assessment of outcome	Enough follow-up for outcomes to occur	Adequacy of follow up of cohorts	NOS score
Yang ZT	1	1	1	1	1	1	1	1	8
Zhu WG	1	0	1	1	1	1	1	1	7
Mori Y	1	0	1	1	1	1	1	0	6
Wei LY	1	0	1	1	1	1	0	1	6
Yoshikawa R	1	0	1	1	1	1	1	1	7
Cui HW	1	1	1	1	1	1	0	0	6
Li JP	1	1	1	1	1	1	0	0	6
Ju L	1	1	1	1	1	1	0	0	6
Sun B	1	1	1	1	1	1	0	0	6
Wei LY	1	0	1	1	1	1	1	1	7
Xiao F	1	1	1	1	1	1	0	0	6

Table 3. Summary of the outcomes presented in this meta-analysis.

Group	No. of studies	No. of total patients	RR/HR (95% CI) (Gli1 positive VS. Gli1 negative)	P for heterogeneity	I^2	References
Gender (male vs. female)	8	562	1.06 (0.62, 1.80)	0.731	0.0%	[18, 28, 39, 40, 41, 43-45]
Histological grade (poor/undifferentiated vs. well/moderate)	8	542	1.26 (0.58, 2.75)	0.037	53.2%	[18, 28, 38-41, 44, 45]
Clinical stage (III/IV vs. I/II)	7	393	3.71 (2.34, 5.86)	0.937	0.0%	[18, 38-40, 42-44]
T stage (T3/T4 vs. T1/T2)	4	299	2.23 (1.87, 5.60)	0.077	56.2%	[18, 40, 43, 44]
Lymph node metastasis (Yes vs. No)	7	514	1.98 (1.22, 3.21)	0.091	45%	[28, 38, 40, 41, 43-45]
3-year survival	6	523	2.37 (1.57, 3.60)	0.509	0.0%	[11, 18, 28, 40, 41, 43]
5-year survival	5	454	3.40 (2.21, 5.25)	0.244	26.6%	[18, 28, 40, 41, 43]
OS	6	523	2.42 (1.76, 3.34)	0.385	5.0%	[11, 18, 28, 40, 41, 43]

Correlation between Gli1 expression and clinicopathological features

Gender and histological grade: Total eight studies involving 562 patients were used to analyze the correlation between Gli1 expression and gender (male vs. female), histological grade (poor differentiated vs. well/moderate differentiated) respectively, as shown in **Figure 2A** and **2B**. Results showed that no significant relationship was discovered between Gli1 expression and gender (RR = 1.06, 95% CI: [0.62, 1.80]), histological grade (RR = 1.26, 95% CI: [0.58, 2.75]) in ESCC patients. A fixed effect model was applied for gender considering the heterogeneity (I^2 = 0.0%, P = 0.731), while a random effect model was used for histological grade due to the obvious heterogeneity (I^2 = 53.2%, P = 0.037).

Clinical Stage: There were seven studies [18, 38-40, 42-44] including 393 patients offering data of clinical stage (stage III/IV vs. stage I/II) and Gli1 expression. Results disclosed that overexpression of Gli1 was more prevalent in stage III/IV when compared with that in stage I/II (RR = 3.71, 95% CI: [2.34, 5.86]) (**Figure 2C**). A fixed effect model was applied considering the heterogeneity (I^2 = 0.0%, P = 0.937).

T stage: Four studies [18, 42-44] containing data of T stage (T3/T4 vs. T1/T2) and Gli1 expression levels were included for analyzing correlation between Gli1 expression level and T stage. Result showed up-regulation of Gli1 expression is significantly associated with advanced T stage (T3/T4) (RR = 3.43, 95% CI: [1.41, 8.36]) (**Figure 2D**). A random effect model was applied considering the heterogeneity (I^2 = 56.2%, P = 0.077).

Lymph node metastasis: Relation between status of lymph node metastasis (positive vs. negative) and Gli1 expression was analyzed based on data provided by seven studies [28, 40-45]. Result showed positive lymph node metastasis was significantly associated with up-regulated Gli1 expression (RR = 1.98, 95% CI: [1.22, 3.21]) (**Figure 2E**). A fixed effect model was applied considering the heterogeneity (I^2 = 45%, P = 0.091).

Correlation between Gli1 expression and 3-year and 5-year survival

Total six studies [11, 18, 28, 42, 43, 45] provided 3-year survival data and five of them [18, 28, 42, 43, 45] provided 5-year survival data. The results displayed mild heterogeneity (3-year survival: I^2 = 0.0%, P = 0.509; 5-year survival: I^2 = 26.6%, P = 0.244), so a fixed effect model was applied to calculate the pooled RRs and their corresponding 95% CIs. Elevated Gli1 expression level was associated with poor 3-year survival (RR = 2.37, 95% CI: [1.57, 3.60]) and 5-year survival (RR = 3.40, 95% CI: [2.21, 5.25]) in ESCC patients, as shown in **Figure 3**.

Correlation between Gli1 expression and OS

Data involving OS were extracted from six studies [11, 18, 28, 42, 43, 45] including 523 patients. Given the mild heterogeneity (I^2 = 5.0%, P = 0.385), a fixed effect model was used to calculate the pooled HR and its corresponding 95% CI. The pooled HR for OS was 2.42 (95% CI: [1.76, 3.34]) demonstrating that Gli1 over-expression was associated with poor OS of ESCC patients.

Publication bias

Both Begg's funnel plot and Egger's test were used to assess the publication bias in all studies evaluating gender, histological grade, clinical stage, T stage, lymph node metastasis,

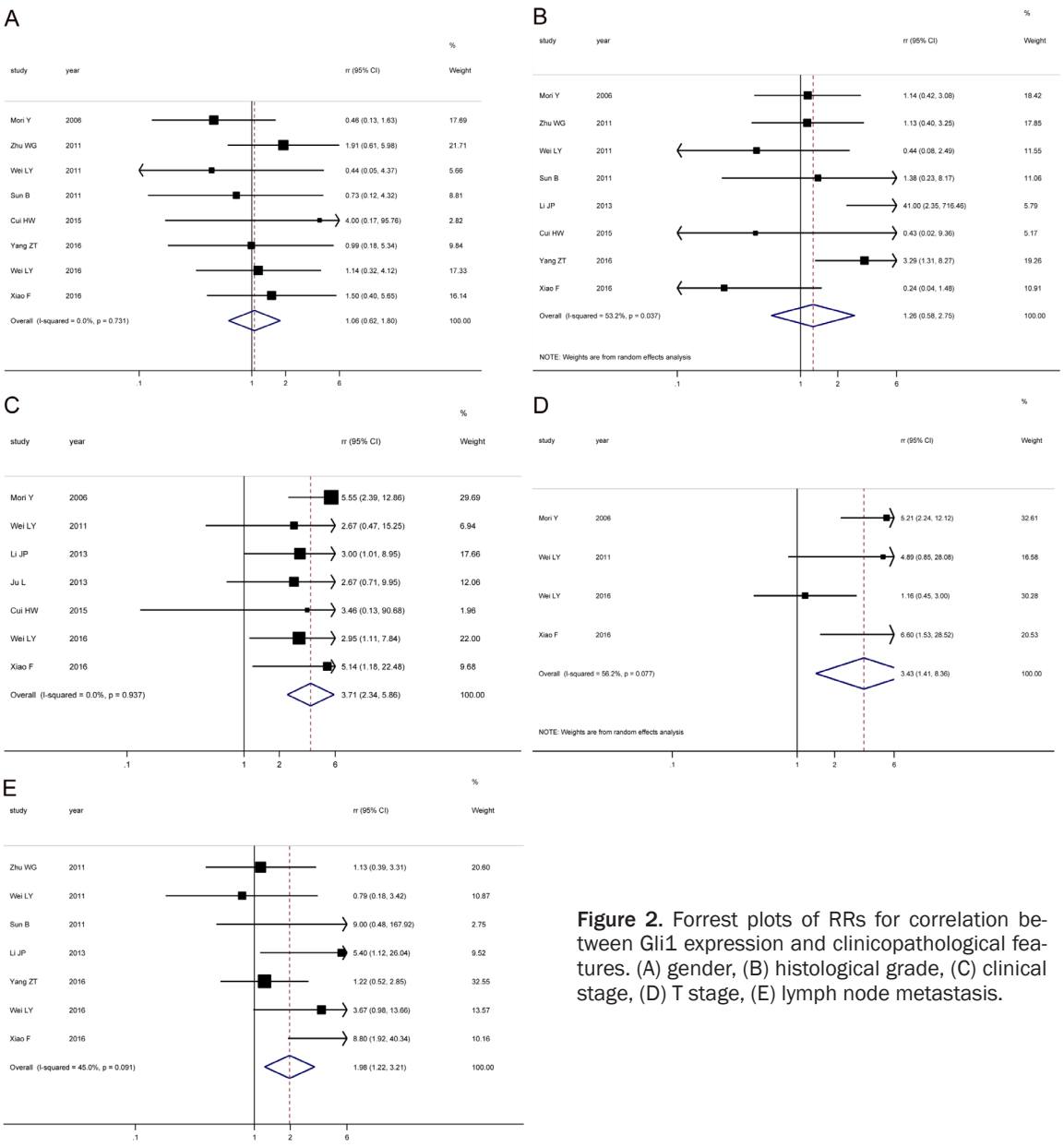

Figure 2. Forrest plots of RRs for correlation between Gli1 expression and clinicopathological features. (A) gender, (B) histological grade, (C) clinical stage, (D) T stage, (E) lymph node metastasis.

3-year and 5-year survival and OS, respectively (**Figure 4**). The Begg's funnel plot did not demonstrate any evidence of statistically significant asymmetry in the meta-analysis of gender (p = 0.621), histological grade (p = 0.621), clinical stage (p = 0.881), T stage (p = 0.497), lymph node metastasis (p = 0.099), 3-year and 5-year survival (p = 0.243) and OS (p = 0.188). Moreover, there was also no evidence of publication bias in Egger's test of gender (p = 0.928), histological grade (p = 0.749), clinical stage (p = 0.502), T stage (p = 0.717), lymph node metastasis (p = 0.120), 3-year and 5-year survival (p = 0.025) and OS (p = 0.286).

Discussion

Gli1, as a key transcription factor of Shh signaling pathway, is proved to be associated with a variety of malignant tumors in recent years. Tremendous researches have disclosed correlations between Shh pathway and some classic signaling pathway of tumorigenesis and tumor progression [8, 10, 46-48], eliciting its potential role in predicting prognosis of malignant tumors and acting as potential target for cancer treatment. Although Gli1 is reported to be an unfavorable prognostic factor in some solid cancers like stomach, pancreas, breast, ovary,

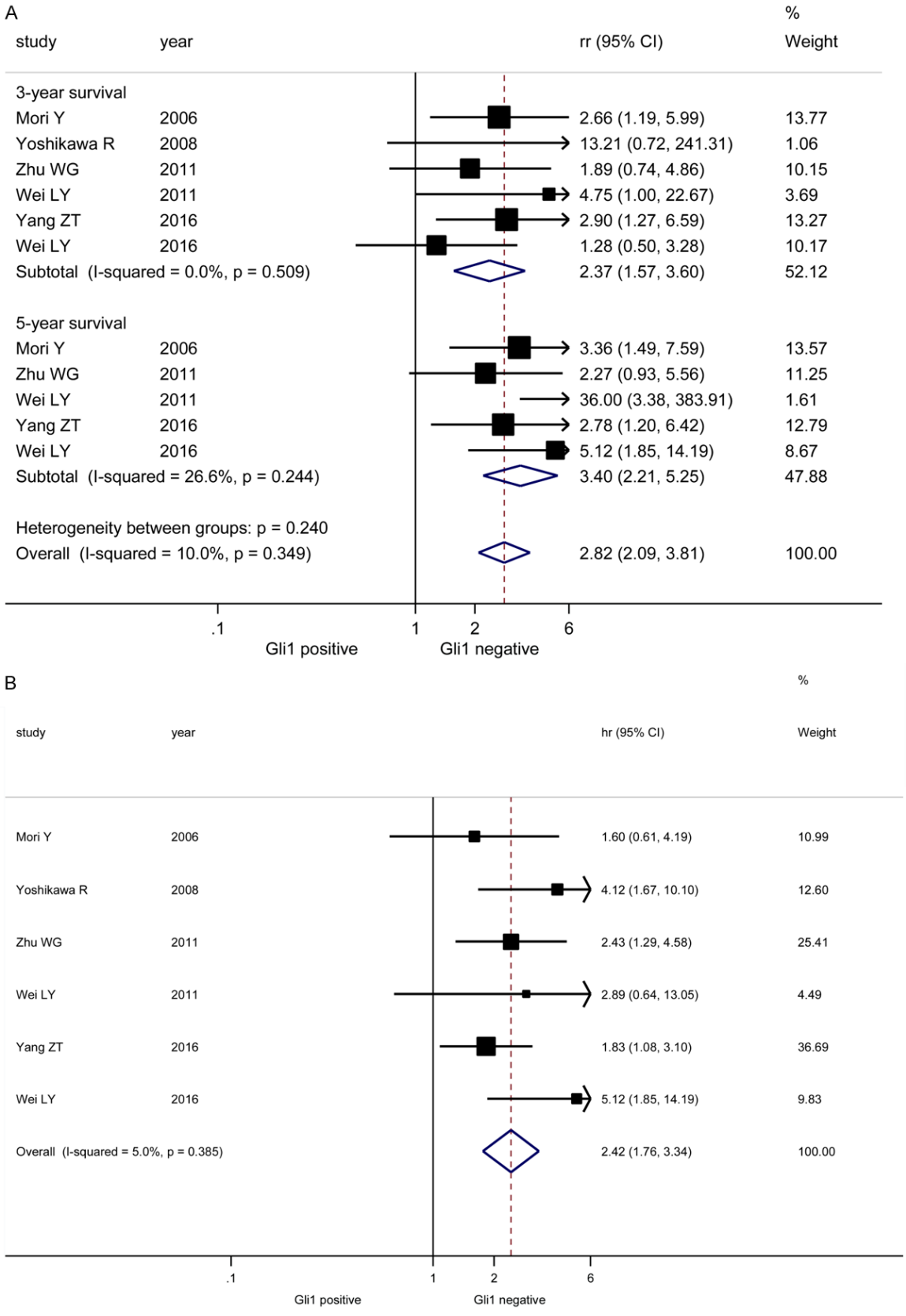

Figure 3. Forrest plots of RR and HR for Gli1 expression about the survival outcomes. (A) 3-year and 5-year survival, (B) OS.

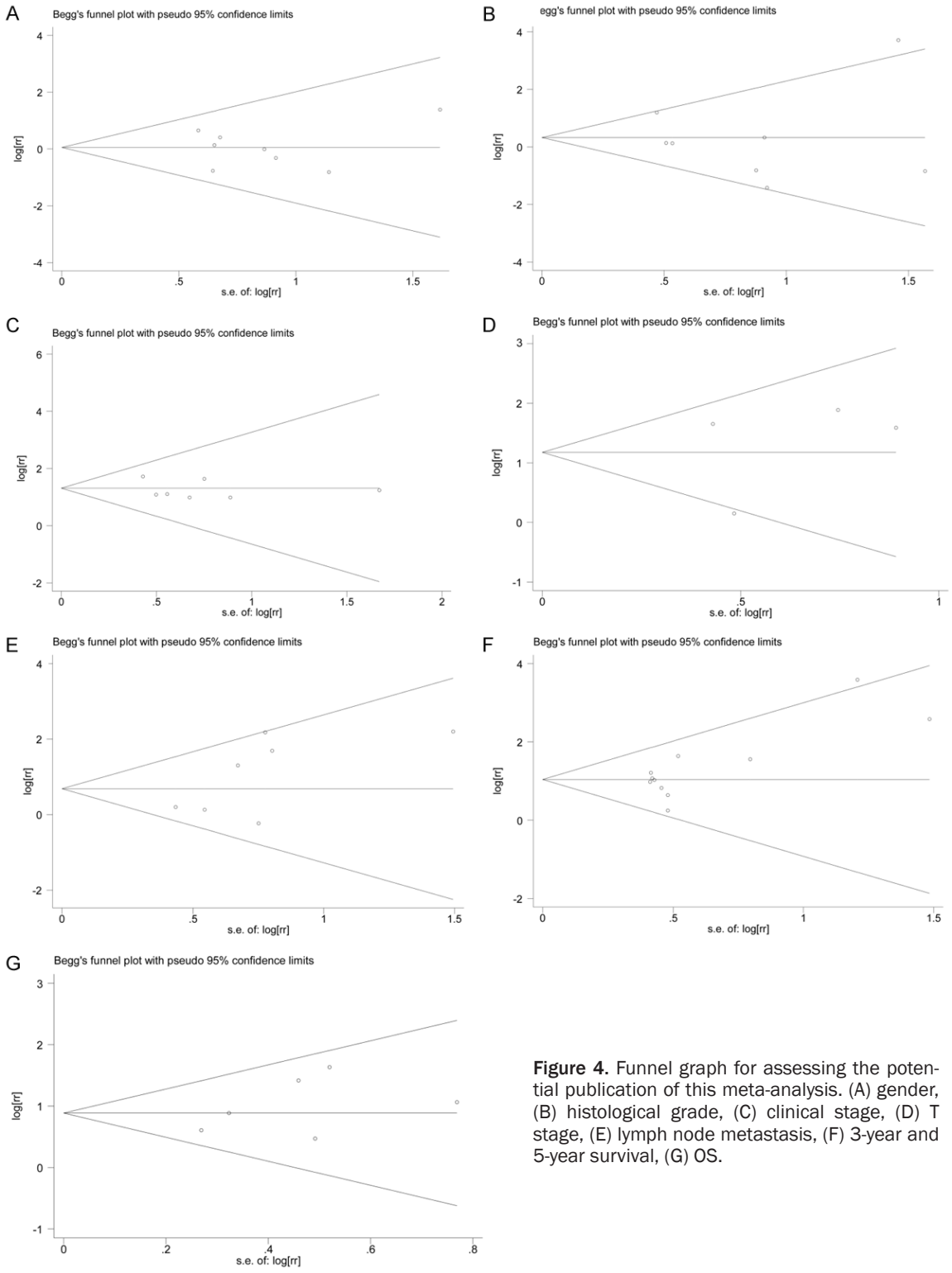

Figure 4. Funnel graph for assessing the potential publication of this meta-analysis. (A) gender, (B) histological grade, (C) clinical stage, (D) T stage, (E) lymph node metastasis, (F) 3-year and 5-year survival, (G) OS.

liver and bladder cancers [18-25], it remains unclear that whether up-regulation of Gli1 expression is associated with unfavorable prognosis in patients suffering from esophageal cancer. So far, there have been two meta-analyses analyzing Gli1 expression and prognosis of malignant cancers published. Cheng's study focused on summarizing prognostic role of Gli1

in all solid tumors with the defect of possible heterogeneities between different cancer types [49]. While Lu's study explained prognostic role of Gli1 over-expression in gastric cancer [19]. Thus our study is the first meta-analysis to expound that Gli1 is a reliably unfavorable prognostic factor in ESCC patients and revealed correlations between Gli1 expression and some clinicopathological features.

In our study, results demonstrated that up-regulated Gli1 expression was related with poor outcome in 3-year survival, 5-year survival and OS in ESCC patients. Gli1 expression was correlated with advanced clinical stage, higher T stage and positive lymph node metastasis of ESCC but unrelated to gender and histological grade. Enhanced expression of Gli1 was more common in patients at advanced stages or with lymph node metastasis, therefore Gli1 may be considered as a potential biomarker to predict prognosis in advanced stages of ESCC. Moreover, Yang's [45] study and Yoshikawa's [11] study reported Gli1 over-expression predicted worse DFS in esophageal cancer patients. Zhu's [28] study found Gli1 was correlated with shorter both locoregional progression free survival (LPFS) and distant progression free survival (DPFS). Despite eligible articles in our study involving DFS or PFS were very limited, Gli1 expression may be related with shorter DFS and PFS in ESCC according to these published data. In summary, our study supported that Gli1 indicated poor prognosis in ESCC patients.

Lymph node metastasis is one of the main metastasis modes of ESCC, and also one of the most common causes of recurrence and death in ESCC patients. Most patients with ESCC would have lymph node metastasis, at locations such as mediastinum, abdomen, trachea, hilum of lung and bronchi [50, 51]. Our research showed improved expression of Gli1 often accompanied with lymph node metastasis in ESCC patients. Therefore, doctors should pay more attention to the inspection of lymphatic drainage area in the examination of patients with the high expression of Gli1. Making early intervention such as preventive radiotherapy of lymphatic drainage area in ESCC patients without lymph node metastasis, whether can bring more benefits to the survival of patients, is very interesting. These may provide a new thought for the clinical diagnosis and treatment decision for ESCC patients.

Although the satisfactory results were showed above, there were also several limitations in our meta-analysis. Firstly, there were lack of randomized controlled trials, and most studies included were retrospective studies. Secondly, the crowd of the researches was concentrated in Asia, so the clinical features and outcomes of this study are more applicable for Asian population. Thirdly, due to the differences of antibodies and definitions of Gil1 positive in the detection of the Gli1 expression, potential bias might occur in our analysis. Finally, there were 5 esophageal adenocarcinoma patients who could not be separated from our analysis. These short comes can be better solved with more relevant researches published.

Our meta-analysis integrated convincing evidence to elucidate relationship between Gli1 expression and prognosis of ESCC. The high expression of Gli1 could predict poor survival in patients with ESCC. Doctors should pay more attention to the patients with high expression of Gli1, who may occur with progression, invasion and metastasis. On the whole, our meta-analysis is the first one to explain Gli1 expression as aggressive biological behavior in ESCC patients, and the correlation between Gli1 expression and lymph node metastasis may have important significance on making treatment decisions. The detection of Gli1 provides more convincing evidences for guiding the diagnosis and estimating prognosis of ESCC patients.

Disclosure of conflict of interest

None.

Abbreviations

Gli1, Glioma associated oncogene 1; ESCC, esophageal squamous cell carcinoma; RRs, risk ratios; HR, hazard ratio; CIs, confidence intervals; OS, overall survival; Shh, Sonic Hedgehog; Smo, Smoothened; EMT, epithelial-to-mesenchymal transition; CDK2, cyclin-dependent kinase 2; IHC, immunological histological chemistry; RT-PCR, reverse transcription-polymerase chain reaction; DFS, disease free survival; PFS, progression free survival; LPFS, locoregional progression free survival; DPFS, distant progression free survival.

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