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A Meta-Analysis of HIF-1α and Esophageal Squamous Cell Carcinoma (ESCC) Risk

Guogui Sun · Wanning Hu · Yifang Lu · Yadi Wang

Abstract To investigate the correlation of hypoxia-inducible factor-1α (HIF-1α) expression with clinical prognosis and efficacy of radiochemotherapy in esophageal squamous cell carcinoma (ESCC). Studies assessing the clinical or prognostic significance of HIF-1α expression in ESCC published prior to December 2011 were selected by searching PubMed, EMBASE, Cochrane Library, and (China National Knowledge Infrastructure) CNKI. A meta-analysis was performed to clarify the impact of HIF-1α expression on clinicopathological parameters or survival in ESCC. A total of 16 studies met the inclusion criteria, which included 1261 patients with ESCC. Accordingly, the level of HIF-1α expression in esophageal tissues of patients with ESCC was significantly higher than that in normal patients (odds ratio, OR=33.111, 95 % confidence interval, CI= 11.912–92.040). The expression of HIF-1α correlated with the depth of invasion (OR=1.701, 95 % CI=1.076–4.705), clinical TNM stage (OR=2.160, 95%CI=1.516–3.077), as well as lymph node metastasis (OR=2.393, 95 % CI= 1.319–4.344), regardless of differentiation grading (OR= 1.185, 95 % CI=0.859–1.635). Furthermore, there was a significant association of increased HIF-1α expression with poorer radiochemotherapy outcomes, 2-year overall survival (OR=0.219, 95 % CI=0.104–0.461) and survival (OR= 0.320, 95 % CI=0.115–0.887, $P<0.05$) in patients with ESCC. In addition, HIF-1α expression correlated with VEGF expression in the ESCCs (OR=4.635, 95%CI= 2.591–8.292). Increased expression of HIF-1α plays an important role in the malignant biology of ESCC resulting in significantly poorer radiochemotherapy outcomes and 2-year overall survival. HIF-1α expression may be a prognostic factor, as well as a potential target for therapy in patients with ESCC.

Keywords ESCC · HIF-1α · Meta-analysis · Survival

Introduction

Cancer, the leading cause of death all over the world, has become a worldwide public health problem [1]. Esophageal cancer (EC) is one of the most common human cancers, with a 5-year survival rate of ≤10 % in China [1, 2]. There are two main histological types of EC, esophageal squamous cell carcinoma (ESCC) and adenocarcinoma (EAC). ESCC is the predominant histological subtype of EC worldwide [1].

Although the etiology for the carcinogenesis remains to be elucidated, carcinogen exposure, nutritional deficiency and biological virus infection are thought to lead to ESCC [3]. However, only a portion of the population exposed to these risk factors develop ESCC, indicating a variation in individual susceptibility to esophageal cancer [3]. Recent studies have revealed that variations in genes governing carcinoma metabolism, DNA repair, cell proliferation and apoptosis may play a role in individual susceptibility to the occurrence and progression of ESCC [2, 4–6]. Therefore, identification of genes that increase susceptibility may lead to a better understanding of the mechanism underlying ESCC.

Intra-tumoral hypoxia is a hallmark of solid cancer [7]. A hypoxic microenvironment initiates multiple

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cellular responses, such as proliferation and angiogenesis [7, 8]. Hypoxia-inducible factor-1 (HIF-1) is a key transcription factor that regulates the cellular responses to hypoxia. Recent studies demonstrated that HIF-1 plays an important role in the progression of cancer through the activation of a gene profile that is involved in the crucial aspects of cancer biology [8, 9]. HIF-1 is a heterodimeric transcription factor consisting of α subunit and β subunit. β subunit is constitutively expressed, and α subunit, which determines HIF-1 activity, is regulated by oxygen tension [7–10]. Recent studies have shown that HIF-1α is over-expressed in various human cancers with tumors of advanced grade, implying that HIF-1α may be an independent prognostic factor of cancer [7–9]. Although the majority of studies displayed a significant association of HIF-1α with lymph node involvement in ESCC, some conflicting results were present, thus, the clinical significance of HIF-1α remains unsettled. A meta-analysis was carried out to clarify this issue.

Materials and Methods

Publication Search PubMed, the Cochrane database and CNKI were searched using the following terms: (i) esophagus or oesophagus and cancer or neoplasm or carcinoma; (ii) hypoxia-inducible factor-1α or HIF-1α. The references cited in the articles or reviews were also screened. The search included literature published from the initiation of database to December 2011.

Inclusion Criteria This meta-analysis investigated the significance of HIF-1α in ESCC. Studies in the meta-analysis met the following criteria: (i) examined the relationship between HIF-1α and clinic-pathological parameters of ESCC, (ii) utilized inmmunohistochemistyr to determine the expression of HIF-1α inparaffin-embedded surgical specimens (IHC), (iii) sufficient data to estimate an odds ratio (OR) with 95 % confidence interval (CI) using multivariates analysis, (iv) therapeutic response evaluated by RECIST 7.0, (v) ESCC was confirmed histopathologically according to UICC in 1997, (vi) assessed HIF-1α expression in the para-cancerous tissues or normal tissues, (vii) patients had no previous chemotherapy or radiotherapy, (viii) appropriate statistical methodology was used.

Exclusion Criteria Studies that met the following criteria were excluded: (i) not case–control studies, (ii) case reports, letters, reviews and editorial articles, (iii) studies based on raw data with no usable data, (iv) duplicated data, (v) non-human subjects, (vi) Immunohistochemistry (IHC) was not used to evaluate the HIF-1α expression.

Data Extraction Two investigators independently extracted data from eligible studies, and disagreements were resolved by discussion amongst the team of pathologists. For each study, the first author, journal, publication year, number of eligible patients, TNM stage, laboratory method, antibody used for IHC, HIF-1α status and survival results were recorded. Quality of data extraction was confirmed by two authors.

Statistical Analysis The meta-analysis in this study was performed using the statistical software STATA 11.0. With regard to enumerative data, odds ratio (OR) and 95 % confidence interval (95 % CI) were applied to analyze the therapeutic efficacy. The heterogeneity of the data from eligible studies was evaluated by χ^2-test. The meta-analysis was performed using a fixed-effect model to determine the heterogeneous insignificance for the data. Statistical significance was defined as a *P*-value less than 0.05. The random-effect model was the optimal choice to perform the meta-analysis of the eligible studies, as significant heterogeneity existed among studies, both statistical and clinical. Due to poor-quality studies the sensitivity test was indicated, while depictive analysis was applied for the clinical data that had significant heterogeneity. The funnel chart was created using the Begg's test, and the logistic model was constructed for the symmetrical test.

Results

Clinico-Pathological Characteristics A total of 16 of 138 studies addressing HIF-1α expression in ESCC published from 1990 to 2011 met the criteria for this review [11–24]. These 16 studies included 223 controls and 1261 patients with ESCC, including 655 cases of well/moderate-differentiation, 268 of poor-differentiation, 246 of stage I/II, and 404 of stage III/IV. Clinicopathological characteristics of eligible studies are summarized in Table 1. IHC was the only method used for the evaluation of HIF-1α expression in esophageal cancer specimens. The most commonly used antibody was a Santa Cruz antibody against HIF-1α, SC-13515. A wide range of dilutions were used from 1:200 to 1:1000. The definition of positive HIF-1α staining varied among the studies, and the cut-off values in the studies ranged from 5 % to 30 %.

Association of HIF-1α with Clinico-Pathological Characteristics Seven studies examined the difference in HIF-1α expression between ESCC and normal tissues. The random-effect model was used for the meta-analysis. A significant difference was detected between

Table 1 Main characteristics of eligible studies

First author	Year	Stage	N pts	Method	Antibody in country, dilution	Definition of HIF-1α-positive	Clinicopathological factors
Liu JL	2007	I–IV	70	IHC	USA, 100	>3	Differentiation, T, LN, TNM
Wu XA	2007	I–IV	45	IHC	China, 80	>10 %	CRT, OS
Yu ZT	2008	I–IV	42	IHC	USA, 100	>10 %	T, LN,
Li DJ	2008	I–III	62	IHC	China, 80	>1 %	Differentiation, T, LN, TNM
Zhang HZ	2007	I–IV	50	IHC	USA,100	>1 %	Differentiation, T, LN, TNM, VEGF, CRT, OS
Fu ZC	2010	II–III	95	IHC	China, 50	>1 %	Differentiation, TNM, OS
Chen YS	2006	I–IV	50	IHC	China, 80	>1 %	Differentiation, LN, TNM
Kurokawa T	2003	I–IV	130	IHC	USA, 400	>3	Differentiation, T, LN, TNM, OS
Sohda M	2004	I–IV	65	IHC	USA, 50	>10 %	Differentiation, T, LN, TNM, OS
Matsuyama T	2005	I–IV	215	IHC	USA, 100	>3	Differentiation, T, LN, TNM, VEGF, OS
Tzao C	2008	I–IV	85	IHC	USA, 200	>3	Differentiation, T, LN, TNM, VEGF
Ogawa K	2011	–	25	IHC	Japan, 200	>10 %	LN, CRT
Kimura S	2004	I–IV	82	IHC	USA, 1000	>10 %	Differentiation, T, LN, TNM, VEGF
Guo LJ	2007	–	55	IHC	China, 100	>1	Differentiation, CRT, OS
Wu XA	2006	I–III	46	IHC	China, 100	>10 %	Differentiation, T, LN, VEGF
Hu JL	2010	II–IV	144	IHC	China, 50	>10 %	Differentiation, T, LN, TNM,

N pts number of patients; *IHC* immunohistochemistry; *score 1–7* different scores with combination of percentage of positives cells and intensity; *T* depth of invasion; *LN* lymph node status; *TNM* TNM stage; *VEGF* vascular endothelial growth factor; *CRT* chemotherapy or radiotherapy; *OS* overall survival

ESCC and normal tissues (OR=33.111, 95%CI: 11.912–92.040, P=0.000) (Fig. 1 and Table 2).

Eleven studies examined the correlation between HIF-1α expression and histologic differentiation. Since no significant heterogeneity existed (P=0.215), a fixed-effect model was used for the meta-analysis. As shown in Fig. 2 and Table 2, the combined OR was 1.185 (95 % CI: 0.859–1.635, P=0.300) for poorly versus well/moderately differentiated tumors. The association between HIF-1α and invasive depth was investigated in 9 studies. The outcomes were significantly heterogeneous (P=0.000), and a random-effect model was used for the meta-analysis. The combined OR was 2.250 (95 % CI=1.076–4.705, P=0.001, Fig. 3 and Table 2) for T$_{3-4}$ versus T$_{1-2}$. The association between HIF-1αand metastatic lymph nodes was explored in 13

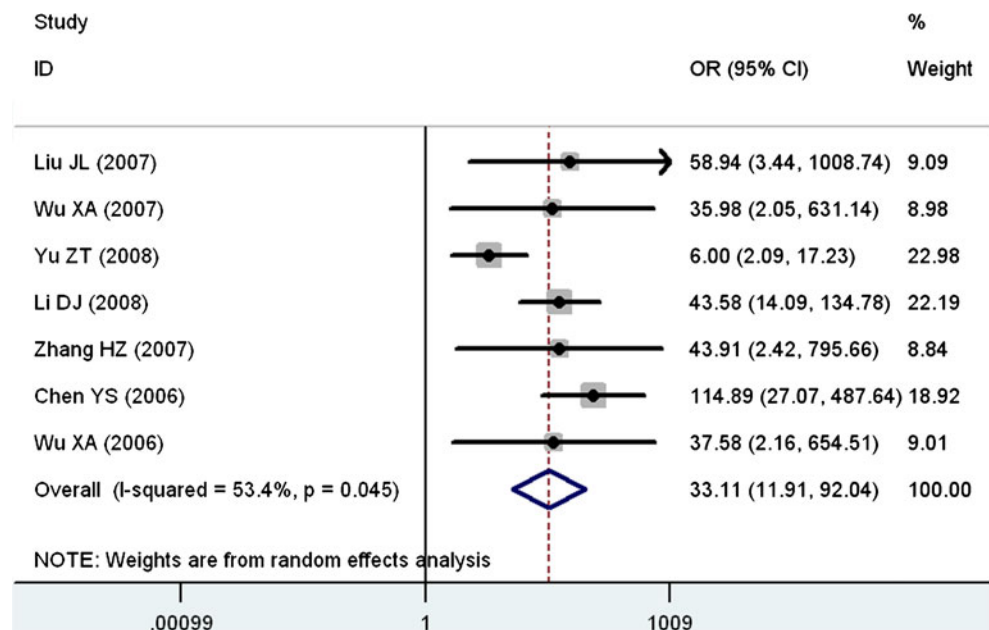**Fig. 1** Forest plot of OR to assess the association of HIF-1α expression with ESCC

Table 2 Meta-analysis outcomes of the eligible studies

Group	N pts	OR/RR	95 % CI	χ^{2a}	P^a	Z^b	P^b	P^c
HIF-1α expression	365	33.111	11.912–92.040	12.880	0.045[d]	6.710	0.000	0.091
Differentiation	923	1.185	0.859–1.635	13.150	0.215	1.040	0.300	0.550
Depth of invasion	797	1.701	1.247–2.320	34.810	0.000 [d]	3.350	0.001	0.171
Lymph node status	1095	2.393	1,319–4,344	47.510	0.000 [d]	2.870	0.004	0.234
TNM stage	650	2.160	1.516–3.077	9.860	0.197	4.260	0.000	0.617
VEGF	263	4.635	2.591–8.292	2.560	0.465	5,710	0.000	0.289
CRT	163	0.219	0.104–0.461	0.130	0.716	3.990	0.000	0.224
2-year survival	151	0.320 [e]	0.115–0.887	4.360	0.113	2.190	0.028	0.104

N pts number of patients; *OR* odds ratio; *RR* relative risk; *CI* confidence intervals; *VEGF* vascular endothelial growth factor; *CRT* chemotherapy or radiotherapy

a = Test for heterogeneity, b = Test for overall effect, c = When *P*<0.05, statistically significant publication bias exists according to Egger's test, d = A random-effects model was used when the *P* value for heterogeneity was<0.10; otherwise, a fixed-effects model was used, e = RR

studies. The heterogeneity in the subgroups was significant (*P*= 0.000), so a random-effect model was used for the meta-analysis. The combined OR for N[(+)] versus N[(−)] was 2.393 (95 % CI=1.319–4.344, *P*=0.000, Fig. 4 and Table 2). Begg's funnel model was used for the bias analysis, and no bias was detected in the correlation between HIF-1α and lymph node metastasis. Egger's test consistently supported the fact that no analytical bias appeared in the meta-analysis (Fig. 5, Table 2). The relationship between HIF-1α and tumor stage was evaluated in 8 studies. No significant heterogeneity was detected (*P*= 0.197), and a fixed-effect model was applied in the meta-analysis. The combined OR for stage III–IV group versus stage I–II group was

2.160 (95 % CI=1.516–3.077, *P*=0.000, Fig. 6 and Table 2).

HIF-1α as a Prognostic Factor for ESCC Four studies in which the correlation between HIF-1α expression and the curative effects of chemo-radiotherapy on ESCC were investigated and did not display significant heterogeneity (*P*=0.716). Hence, a fixed-effect model was used for the meta-analysis. HIF-1α was associated with a reduced therapeutic benefit of chemoradiotherapy in patients with ESCC (OR=0.219, 95 % CI: 0.104–0.461, *P*=0.000, Table 2). The function of HIF-1α as a prognostic factor for ESCC was explored in 3 studies. No significantly statistical heterogeneity was detected
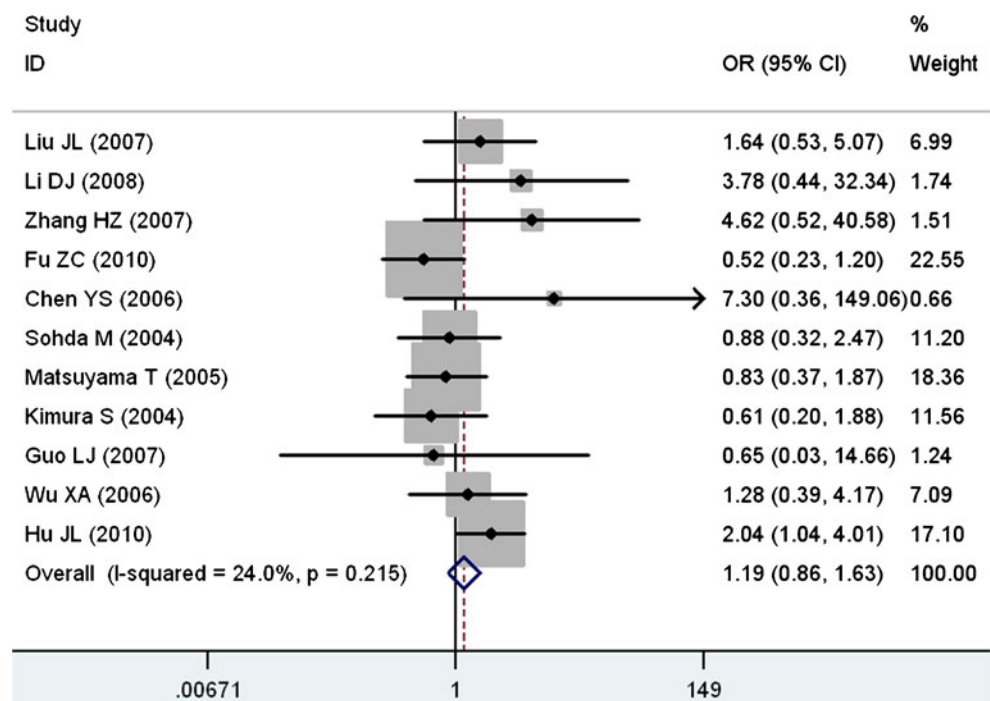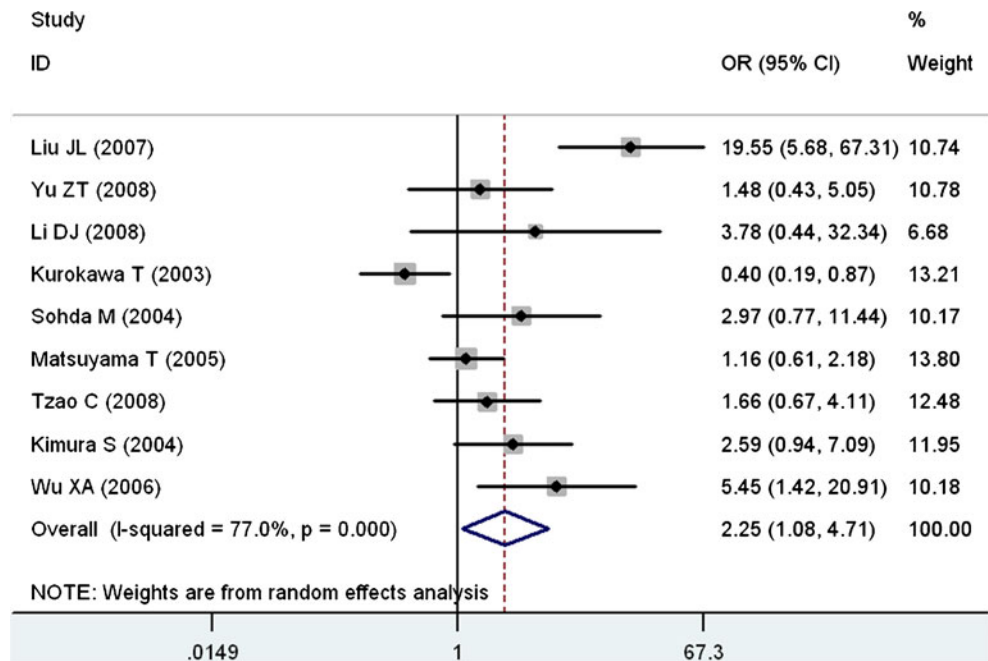
Fig. 2 Forest plot of OR to assess the association of HIF-1α expression with ESCC differentiation

Fig. 3 Forest plot for OR to assess the association of HIF-1α expression with the depth of ESCC invasion

(P=0.237), and a fixed-effect model was applied to calculate OR, 1.78 (95 % CI: 0.104–0.461, P=0.000). These results indicated that HIF-1αcorrelated with poor survival in patients with ESCC (Table 2).

Correlation of HIF-1α Expression with VEGF Expression Of patients with ESCC, 175 exhibited positive expression of VEGF, while HIF-1α was detected in 276 cases. 95 cases of ESCC presented HIF-1α$^{(-)}$VEGF$^{(-)}$. The meta-analysis indicated that a substantial correlation between HIF-1α and VEGF existed, in which OR was 4.635 (95 % CI: 2.591–8.292, P=0.000, Fig. 7, Table 2).

Discussion

Tumor hypoxia negatively regulates tumor cell growth and leads to a malignant phenotype by up-regulating the expression of genes encoding angiogenic, metabolic and metastatic factors [25, 26]. Of clinical importance, insufficient oxygen affects the efficiency of chemotherapy and radiotherapy [9, 27]. HIF-1 is a master transcriptional activator of oxygen-regulated genes, which is constitutively up-regulated in several tumor types [10].

Meta-analysis is a quantitative approach which statistically integrates the findings of individual researchers.

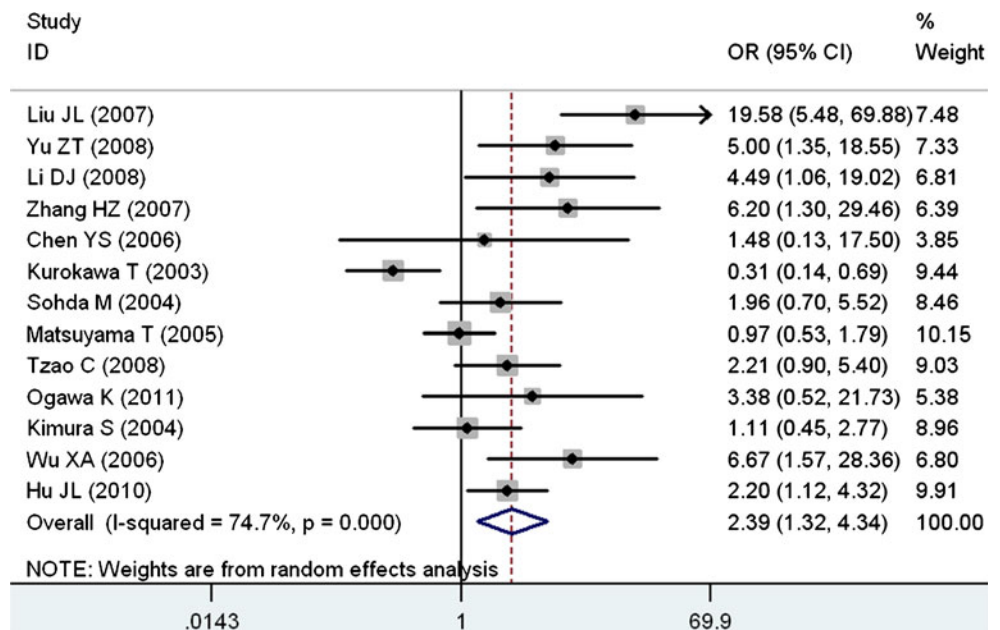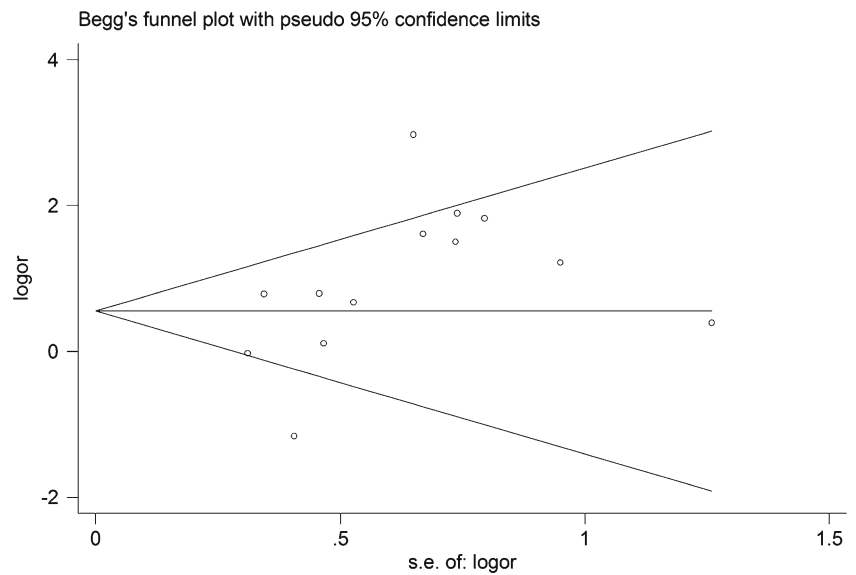**Fig. 4** Forest plot of OR to assess the association of HIF-1α expression with lymph node metastasis of ESCC

Fig. 5 Begg's funnel plot to assess the association of HIF-1α expression with lymph node metastasis

Recently, it has been used for the evaluation of cancerous prognosticators [2, 28–30]. In this meta-analysis, 16 studies addressing the expression of HIF-1α in relation to ESCC clinico-pathological parameters and survival were evaluated. Expression of HIF-1α was determined by IHC. HIF-1α was found to be associated with tumor invasion, lymph node metastasis, and advanced pathological stage for ESCC, regardless of tumor differentiation. This was consistent with previous studies in vitro, which demonstrated that HIF-1α expression may enhance tumor cell metastasis by inhibiting cell adhesion and the apoptotic cascade [13, 31]. Although the statistical significance of the correlation between HIF-1α expression and pathological stage may have varied with lymph node metastasis or depth of invasion, the increased

expression of HIF-1α in ESCCs with positive lymph node metastasis or T3/T4 invasion still supports the potential role of an HIF-1α inhibitor in the adjuvant therapy of ESCC.

The prognostic value of HIF-1α for survival in patients with ESCC was only evaluated in a few studies included in this meta-analysis. Three eligible studies were evaluated to generate the combined hazard ratio value of 1.78 (95 % CI: 0.104–0.461, $P = 0.000$), which suggested that HIF-1α is a prognostic factor for patients with ESCC. However, the results of these studies were conflicting; hence, a re-evaluation of these data will be necessary in order to reach a reasonable conclusion.

In the majority of the studies that were included in this analysis, the univariate analysis was applied with the

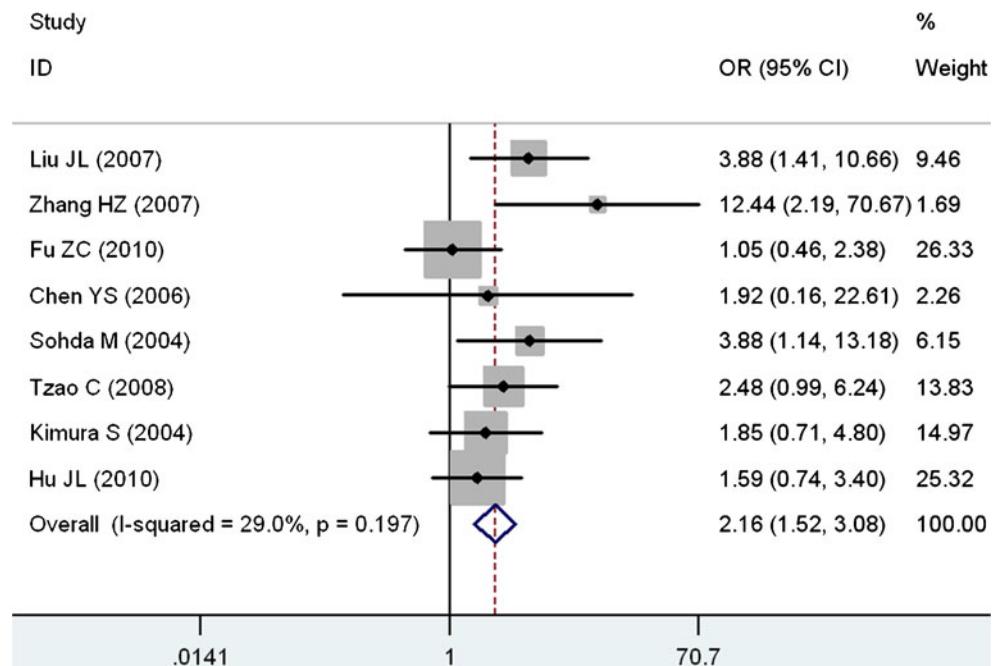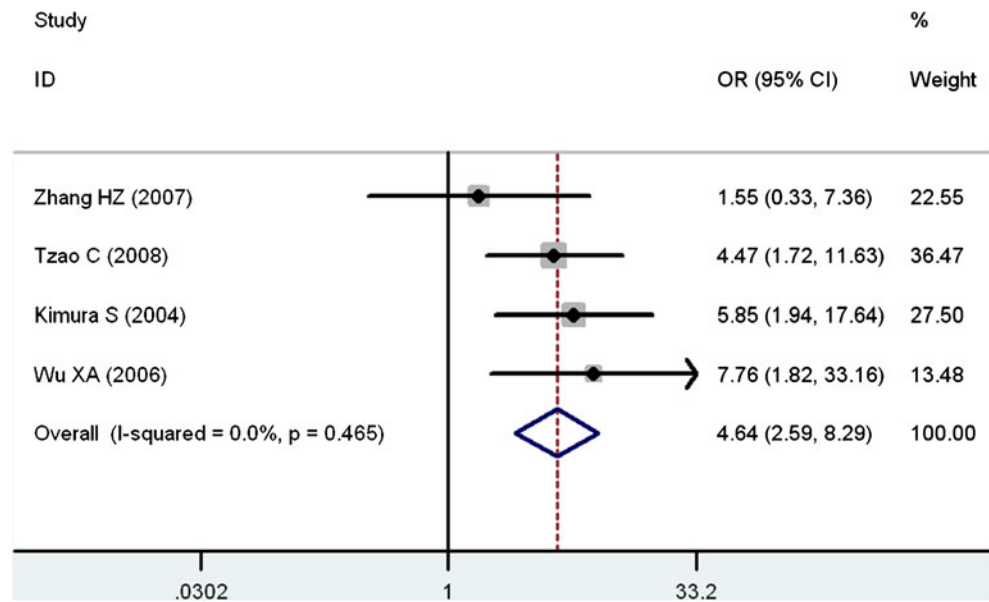Fig. 6 Forest plot of OR to assess the association of HIF-1α expression with TNM stage

Fig. 7 Forest plot of OR to assess the association of HIF-1α expression with VEGF expression

relatively poor convincingness. Pooling the results of these studies may have introduced a bias to the analysis. These findings are important for the clinical application and further prospective studies of HIF-1α in patients with ESCC. Interestingly, the results of this meta-analysis demonstrated that increased HIF-1α expression was correlated with a decreased response to chemoradiation in patients with ESCC. Li et al. found that HIF-1α has the potential to predict the treatment outcome of prostate cancer [32]. Studies in cultured cells or animal models demonstrated that cells overexpressing HIF-1α tend to be resistant to apoptosis [17, 33]. Hence, ESCC with a high level of HIF-1α expression may be less sensitive to radiochemotherapy.

Hypoxic microenvironments are frequently found in solid tumors as a result of morphologically and functionally inappropriate vascularization, irregular bloodflow, anemia and the high oxygen consumption of rapidly proliferating malignant cells [31]. Hypoxic tumor cells undergo oxygen-regulated transcriptional activation of a specific set of genes [34, 35]. HIF-1α is a transcription factor that regulates the transcription of genes associated with cell proliferation and angiogenesis [36–38]. Our results showed a correlation between HIF-1α and VEGF in ESCC tissues, which is in agreement with HIF-1α's function as a transcriptional factor for these angiogenic factors.

The lack of reliability of IHC as a technique to assess the expression of HIF-1α may have introduced some heterogeneity issues into the meta-analysis. IHC is the method most commonly used technique to assess the expression of HIF-1α due to the fact that it is readily available and inexpensive, as well as able to detect variations in a relatively low proportion of tumor cells and formation-fixed tissues for retrospective studies. Furthermore, additional variations

exist among these studies in regard to IHC, including the wide range of primary antibodies used with varying dilutions, protocols, and cut-off points, which were selected arbitrarily by investigators.

There are limitations to this meta-analysis. Among the studies included in the meta-analysis there was also variability in the length of follow-up and the inconsistency in the inclusion of clinico-pathological parameters. Only studies published in English or Chinese were included, which may have led to a bias in publication inclusion. Due to the fact that most of the studies analyzing survival analysis were not performed by multivariate analysis, the meta-analysis could only be performed by univariate analysis.

Conclusions

In conclusion, this meta-analysis demonstrates that HIF-1α is associated with tumor invasion, lymph node metastasis, pathological stage, chemo/radiotherapy sensitivity, and survival of patients with ESCC. These results warrant the prospective validation of HIF-1α expression as a prognostic marker for patients with ESCC.

Competing interests The authors declare that they have no competing interests.

Authors' contributions GS and WH conceived the study and drafted the manuscript. GS and WH collected and analyzed the data, GS and YW also secured funding. YL and YW contributed to the

quality control of study inclusion and discussion. All authors read and approved the final manuscript.

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