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RESEARCH ARTICLE

Prognostic significance of VEGF immunohistochemical expression in oral cancer: a meta-analysis of the literature

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Abstract Vascular endothelial growth factor (VEGF) is considered as a prime mediator of angiogenesis and has been implicated in carcinogenesis and metastasis. Various studies examined the relationship between VEGF protein overexpression with the clinical outcome in patients with oral cancer, but yielded conflicting results. Electronic databases updated to March 2013 were searched to find relevant studies. A meta-analysis was conducted with eligible studies which quantitatively evaluated the relationship between VEGF overexpression and survival of patients with oral cancer. Survival data were aggregated and quantitatively analyzed. We performed a meta-analysis of 17 studies (n=1,207 patients) that evaluated the correlation between VEGF overexpression detected by immunohistochemistry and survival in patients with oral cancer. Combined hazard ratios suggested that VEGF overexpression had an unfavorable impact on overall survival (hazard ratio [HR]= 1.89; 95 % confidence interval [CI], 1.24–2.55) and disease-free survival (HR=2.08; 95 % CI, 1.14–3.02) in patients with oral cancer: 1.77 (1.09–1.44) in oral squamous cell carcinoma (SCC) patients and 4.28 (1.35–7.21) in adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC) of the salivary glands. No significant heterogeneity was observed among all studies. VEGF overexpression indicates a poor prognosis for patients with oral SCC, ACC, and MEC of the salivary glands.

Keywords Vascular endothelial growth factor · Oral cancer · Prognosis · Meta-analysis

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Introduction

Oral cancer is one of the most frequent cancers in the world and a high prevalence occurs in regions where people habitually smoke cigarettes, chew betel quid, and drink alcohol [1, 2]. Squamous cell carcinoma (SCC) is the most common malignant tumor of the oral cavity and head and neck. Adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC) of the salivary glands are not uncommon. Their annual incidence accounts for about 3 % of all oral cavity and head and neck cancer [3]. Despite recent advances in surgical, radiotherapy, and chemotherapy treatment protocols, the long-term survival of patients with oral cancer still lacks significant improvement for the past three decades. The main prognostic factors are clinicopathological characteristics of the disease, including tumor size, stage, and grade. However, the prognostic factors do not fully predict individual clinical outcome. There is the need for better markers to identify patients with poor prognosis at the time of diagnosis. Researches have focused on the potential role of new biological factors involved in the carcinogenic process as prognostic markers in patients with oral cancer.

Angiogenesis, the formation of new blood vessels from existing vasculature, is an important process in many malignancies including oral cancer. It is the result of an intricate balance between pro-angiogenic and anti-angiogenic factors. VEGF (also referred to as VEGF-A, vascular permeability factor) is a critical pro-angiogenic factor in cancer. The role of VEGF in the regulation of angiogenesis is the object of intense investigation for more than a decade. The VEGF family is composed of several subtypes, including VEGF-A, VEGF-B, VEGF-C, and VEGF-D which exist as numerous splice variant isoforms [4, 5]. Many anti-angiogenic compounds are being developed, most of which target VEGF and/or its receptors. It is necessary to establish whether VEGF expression is a prognostic marker in oral cancer.

Many retrospective studies have evaluated whether immunohistochemical (IHC) overexpression of VEGF may be a prognostic factor for survival in patients with oral cancer. However, the results of the studies are inconclusive and no consensus has been reached. It is unknown whether differences in these investigations have been mostly due to their limited sample size or genuine heterogeneity. Thus, we conducted a meta-analysis of all available studies relating VEGF with the clinical outcome in patients with oral cancer.

Materials and methods

Search strategy and study selection

The electronic databases PubMed, Embase, and China National Knowledge Infrastructure were searched for studies to be included in the present meta-analysis. An upper date limit of March 1, 2013 was applied; we used no lower date limit. Searches included the terms "oral or mouth," "cancer or carcinoma or tumor or neoplasm," "VEGF," "vascular endothelial growth factor," and "prognosis." We also reviewed the Cochrane Library for relevant articles. The references reported in the identified studies were also used to complete the search.

Studies eligible for inclusion in this meta-analysis met the following criteria: (1) measure VEGF expression in the primary oral cancer tissue with immunohistochemistry (IHC); (2) provide information on survival (i.e., disease-free survival [DFS] and/or overall survival [OS], studies investigating response rates only were excluded); (3) have a follow-up time exceeding 5 years; and (4) when the same author reported results obtained from the same patient population in more than one publication, only the most recent report or the most complete one was included in the analysis. Two reviewers (S.Z. and X.Y.) independently determined study eligibility. Disagreements were resolved by consensus.

Data extraction and quality assessment

The final articles included were assessed independently by two reviewers (S.Z. and X.Y.). Data retrieved from the reports included author, publication year, patient source, histology, disease stage, test method, definition of positivity (cutoff value), VEGF positive, and survival data (Table 1). If data from any of the above categories were not reported in the primary study, items were treated as "not applicable." We did no contact the author of the primary study to request the information. We did not use prespecified quality-related inclusion or exclusion criteria and did not weigh each study by a quality score because the quality score has not received general agreement for use in a meta-analysis, especially observational studies [6].

Statistical methods

Included studies were divided into two groups for analysis: those with data regarding OS and those regarding DFS. For the quantitative aggregation of the survival results, we measured the impact of VEGF overexpression on survival by hazard ratio (HR) between the two survival distributions. HRs and 95 % confidence intervals (CIs) were used to combine as the effective value. If the HRs and their 95 % CIs were given explicitly in the articles, we used crude ones. When these variables were not given explicitly, they were calculated from the available numerical data using methods reported by Parmar et al. [7].

Heterogeneity of the individual HRs was calculated with χ^2 tests according to Peto's method [8]. Heterogeneity test with inconsistency index (I^2) statistic and Q statistic was performed. If HRs were found to have fine homogeneity, a fixed effect model was used for secondary analysis; if not, a random-effect model was used. DerSimonian–Laird random effects analysis [9] was used to estimate the effect of VEGF overexpression on survival. By convention, an observed HR >1 implies worse survival for the group with VEGF overexpression. The impact of VEGF on survival was considered to be statistically significant if the 95 % CI did not overlap with 1. Horizontal lines represent 95 % CIs. Each box represents the HR point estimate, and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with CI represented by its width. The unbroken vertical line is set at the null value (HR=1.0).

Evidence of publication bias was sought using the methods of Egger et al. [10] and of Begg et al. [11]. Intercept significance was determined by the t test suggested by Egger ($P<0.05$ was considered representative of statistically significant publication bias). All of the calculations were performed by STATA version 11.0 (Stata Corporation, College Station, TX).

Results

Study selection and characteristics

Seventeen studies [12–28] published between 1998 and 2012 were eligible for this meta-analysis. All reported the prognostic value of VEGF status for survival in oral cancer patients. The total number of patients included was 1,207, ranging from 31 to 176 patients per study (median 71). The major characteristics of the 17 eligible publications are reported in Table 1. The studies were conducted in four

Table 1 Main characteristics and results of the eligible studies

First author, year	Patient source	Histology	Stage	N pts	Definition of positivity	Positive (%)	HR estimation	Survival results
Maeda T, 1998	Japan	SCC	I–IV	45	5 %	42.2	OS 3.90 (1.02–14.9)	Poor
Smith BD, 2000	USA	SCC	I–IV	56	NA	41.1	OS 3.21 (1.63–6.32) DFS 2.66 (1.27–5.57)	Poor
Yu F, 2003	China	ACC	I–IV	31	50 %	51.6	DFS 1.48 (0.55–3.94)	NS
Li X, 2003	China	SCC	I–IV	53	25 %	52.8	OS 2.82 (1.22–6.52)	Poor
Kishimoto K, 2003	Japan	SCC	NA	62	20 %	53.2	OS 3.46 (1.11–10.79)	Poor
Lim JJ, 2003	South Korea	ACC	I–IV	40	60 %	75	OS 8.18 (2.42–27.65)	Poor
Lim JJ, 2005	South Korea	SCC	I–IV	84	20 %	67.9	OS 1.80 (0.78–4.18)	NS
Zhang J, 2005	China	ACC	I–IV	80	50 %	67.9	OS 3.82 (1.49–9.82)	Poor
Kim SH, 2006	South Korea	SCC	I–III	38	50 %	52.6	DFS 2.47 (0.58–10.53)	NS
Chuang HC, 2006	China	SCC	I–III	94	NA	44.7	DFS 4.91 (1.71–14.08)	Poor
Chien CY, 2006	China	SCC	I	176	NA	44.3	DFS 3.39 (1.49–7.72)	Poor
Cho JH, 2007	South Korea	SCC	I–II	33	50 %	60.6	OS 1.45 (0.32–6.66) DFS 1.83 (0.51–6.49)	NS
Shi L, 2007	China	MEC	I–IV	75	50 %	54.7	DFS 1.67 (0.63–4.40)	NS
Cheng SJ, 2011	China	SCC	I–IV	100	40 %	NA	OS 3.14 (1.02–11.35)	Poor
Seki S, 2011	Japan	SCC	I–IV	90	NA	33.7	OS 1.24 (0.60–2.58)	NS
Ou Yang KX, 2011	China	MEC	I–IV	70	CS	78.6	OS 4.31 (1.12–9.85)	Poor
Huang C, 2012	China	SCC	I–IV	80	CS	78.8	OS 1.69 (1.20–4.78)	Poor

SCC squamous cell carcinoma, *ACC* adenoid cystic carcinoma, *MEC* mucoepidermoid carcinoma of the salivary glands, *CS* complex score combining intensity and percentage, *NS* not significant, *NA* not applicable, *HR* hazard ratio, *N pts* number of patients, *OS* overall survival, *DFS* disease-free survival

countries (China, Japan, South Korea, and the USA) and published between 1998 and 2012. Among the 17 studies, 16 studies (1,151 patients, 96.3 %) were performed in Asian populations, and the remaining one study (56 patients) followed up non-Asian patients. All patients in the eligible studies were determined by pathological stage including SCC (12 studies), ACC (3 studies), and MEC (2 studies).

All of the studies reported the prognostic value of VEGF status for survival in patients with oral cancer. Of the 17 studies, 9 directly reported HRs (multivariate analysis), while the other 8 studies provided survival curves. Among them, the proportion of patients exhibiting VEGF overexpression in individual studies ranged from 33.7 to 78.8 %. Estimation using survival curves was segregated according to either OS or DFS. An HR on OS and DFS could be extracted for 12 publications and 7 publications of all studies, respectively. Twelve of the 17 studies identified VEGF overexpression as an indicator of poor prognosis, and the other four studies showed no statistically significant impact of VEGF overexpression on survival.

Meta-analysis

The results of the meta-analysis were shown in Table 2 and Figs. 1 and 2. Overall, the combined HR for all 12 eligible studies evaluating VEGF overexpression on OS was 1.89 (95 % CI, 1.24–2.55), suggesting that VEGF overexpression detected by IHC was an indicator of poor prognosis for oral cancer. No significant heterogeneity was observed among the studies (Q=7.39, I^2=0 %, P=0.767). When grouped according to histological types, the combined HRs of oral SCC and ACC/MEC of the salivary glands were 1.77 (95 % CI, 1.09–1.44) and 4.28 (95 % CI, 1.35–7.21), respectively, indicating that VEGF is an indicator of poor prognosis of OS in all histological types of oral cancer. In addition, statistically significant effect of VEGF overexpression on DFS (HR=2.08; 95 % CI, 1.14–3.02) in patients with oral cancer was also observed. No

Table 2 Meta-analysis: HR value of OS and DFS in oral cancer subgroups according to histology

	Nb Patients	Random effects HR (95 % CI)	χ^2 heterogeneity test (P)
Overall for OS	12	1.89 (1.24–2.55)	0.767
SCC	9	1.77 (1.09–1.44)	0.829
ACC and MEC	3	4.28 (1.35–7.21)	0.813
Overall for DFS	3	2.08 (1.14–3.02)	0.871

HR hazard ratio, *Nb* number of studies, *ACC* adenoid cystic carcinoma, *MEC* mucoepidermoid carcinoma of the salivary glands, *OS* overall survival, *DFS* disease-free survival

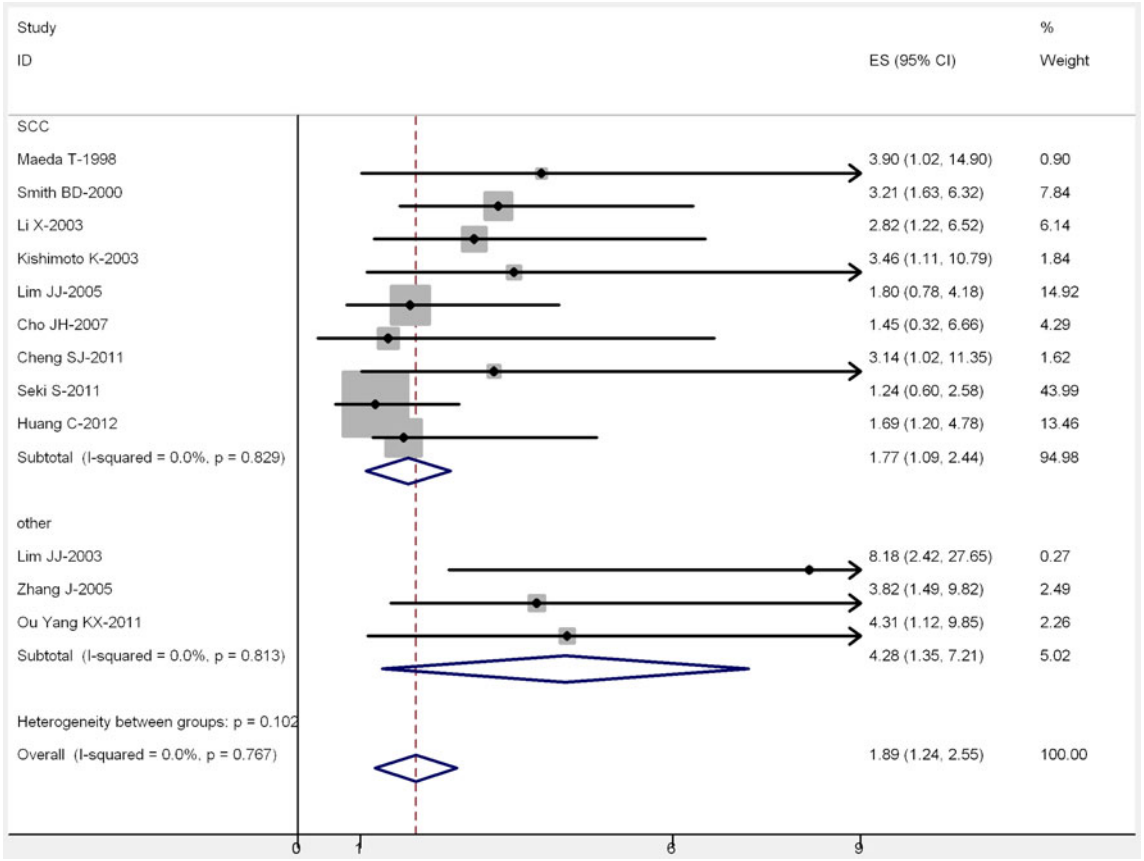

Fig. 1 Meta-analysis (forest plot) of the 12 evaluable studies assessing VEGF in oral cancer stratified by histological types for overall survival

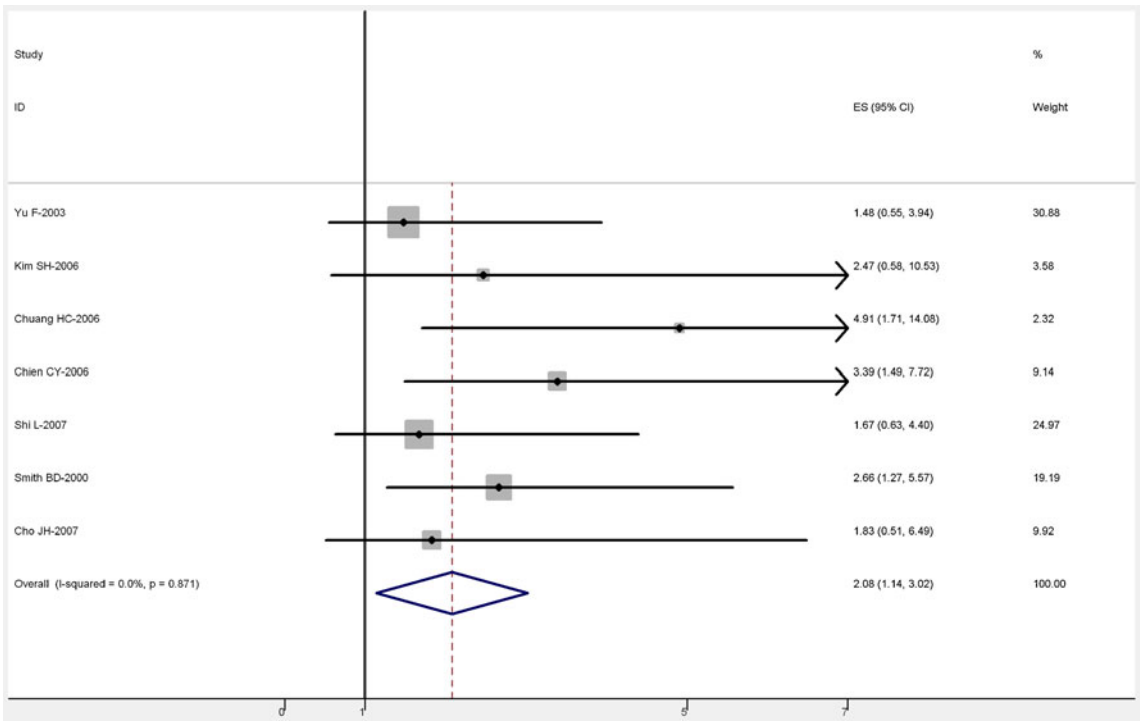

Fig. 2 Meta-analysis (forest plot) of the seven evaluable studies assessing VEGF in oral cancer for disease-free survival

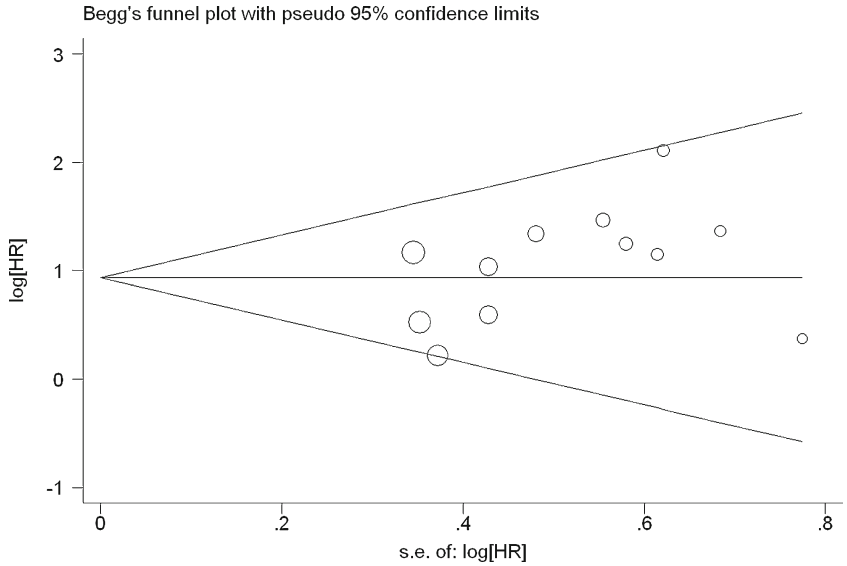

Fig. 3 Funnel plot of the 12 evaluable studies assessing VEGF in oral cancer for overall survival

significant heterogeneity was observed among the studies on VEGF overexpression on DFS (Q=2.48, I^2=0.0 %, P=0.871).

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias in the literature. All 12 eligible studies investigating VEGF overexpression on OS yielded a Begg's test score of P=0.304 and an Egger's test score of P=0.130, meanwhile according to the funnel plot (Figs. 3 and 4), the absence of publication bias was found. Similar results were found for investigating VEGF overexpression on DFS (a Begg's test score of P=0.881 and an Egger's test score of P=0.674). These results suggested that there were no publication biases in these subgroup analyses.

Discussion

Members of the VEGF family promote two very important processes in vivo, angiogenesis and lymphangiogenesis, which involve growth of new blood and lymphatic vessels from preexisting vasculature, respectively. VEGF-A exists as a homodimer or can heterodimerize with either VEGF-B or non-VEGF factors such as placenta growth factor [5, 29, 30]. VEGF-A and VEGF-B promote vascular angiogenesis primarily through activation of vascular endothelial cell-associated VEGFR-1 (Flt1) and VEGFR-2 (Flk1/KDR) [15]. On the other hand, VEGF-C and VEGF-D which are ligands for VEGFR-2 and VEGFR-3 promote angiogenesis and lymphangiogenesis [31, 32].

The present meta-analysis has combined 17 publications including 1,207 patients to yield statistics, indicating a

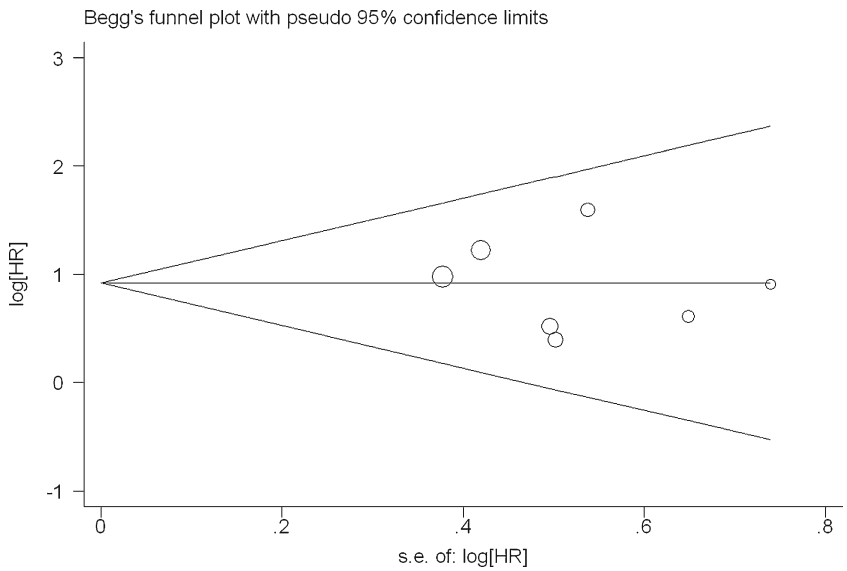

Fig. 4 Funnel plot of the seven evaluable studies assessing VEGF in oral cancer for disease-free survival

statistically significant role of VEGF detected by IHC on overall survival and disease-free survival in oral cancer. In subgroup analysis according to the different histological types of oral cancer on OS, statistically significant detrimental effect of VEGF was found in patients with oral SCC, ACC, and MEC of the salivary glands. In our meta-analysis, patient cohorts were mainly from Eastern Asian countries (1,151 patients, 95.3 %); only one study was from the USA. The combined hazard ratios only represented the Eastern Asian population in our meta-analysis; however, the results of western countries remained unclear.

The previous meta-analysis [33] on the head and neck squamous cancer evaluated the correlation between VEGF (detected by immunohistochemistry) and 2-year overall survival and found that VEGF positivity seems to be associated with worse overall survival (HR=1.88) for the risk of death in 2 years. Our data were partially consistent with the results of a previous meta-analysis. However, that meta-analysis included 12 studies about head and neck squamous cancer, and only eight studies were conducted on oral cancer. In addition, that meta-analysis only represented the 2-year survival analysis. In our present study, we have focused on oral cancer by including more recent related studies and by generally using a more comprehensive search strategy. In our study, we performed the prognostic analysis on 5-year survival and disease-free survival. Study selection and data extraction were performed independently and reproducibly by two reviewers. We also explored heterogeneity and potential publication bias in accordance with published guidelines.

There were several meta-analyses studying the prognostic value of VEGF in other cancer types, such as head and neck squamous cancer [33], lung cancer [34–36], colon cancer [37], gastric cancer [38], and hepatocellular carcinoma [39]. The association of VEGF overexpression with poor outcomes provides a rationale for anti-angiogenic use in the treatment of cancer. VEGF has become a leading therapeutic target for the treatment of cancer. Potentially therapeutic strategies to inhibit VEGF pathway include monoclonal antibodies directed against VEGF, tyrosine kinase inhibitors, and antisense strategies [40]. Bevacizumab (Avastin) is a humanized monoclonal antibody directed against VEGF [41]. It binds to all isoforms of VEGF-A, thus blocking its binding to VEGFR, but it does not bind to other VEGF molecules, such as VEGF-B or VEGF-C.

The heterogeneity issue was complicated in the systematic review and meta-analysis. We found no significant heterogeneity among all studies included and subgroup analysis. Another potential source of bias is related to the method of HR and 95 % CI extrapolation. If these statistics were not reported by the authors, we calculated them from the data available in the article. If this was not possible, we extrapolated them from the survival curves, necessarily making assumptions about the censoring process. Data for multivariate survival analysis reported in the article were included in the present systematic review with meta-analysis; if these data were not available, data calculated from survival curves by univariate analysis were included. These results should be confirmed by an adequately designed prospective study. Furthermore, the exact value of VEGF overexpression status needs to be determined by appropriate multivariate analysis. Unfortunately, few prospectively designed prognostic studies concerning biomarkers have been reported; thus, our collection of many retrospective studies revealed more significance. Furthermore, our meta-analysis relied on publication, not on individual patient data. Studies may have differed in the baseline characteristics of patients included (age, stage, treatment received, and the duration of follow-up).

Publication bias [42] is a major concern for all forms of meta-analysis; positive results tend to be accepted by journals, while negative results are often rejected or not even submitted. The present analysis does not support publication bias; the obtained summary statistics likely approximate the actual average. However, it should be noted that our meta-analysis could not completely exclude biases. For example, the study was restricted to papers published in English and Chinese, which probably introduced bias.

In conclusion, our meta-analysis is the first study to systematically estimate the association between VEGF positivity and oral cancer survival. As determined in our meta-analysis, we concluded that VEGF expression detected by IHC was associated with poor overall survival and disease-free survival in oral cancer, and there is no significant heterogeneity among all studies. To strengthen our findings, well-designed prospective studies with better standardized assessment of prognostic markers should help to explore the relation between VEGF overexpression and survival of oral cancer.

Conflicts of interest None

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