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RESEARCH ARTICLE

Prognostic significance of VEGF expression in osteosarcoma: a meta-analysis

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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 overexpression with the clinical outcome in patients with osteosarcoma but yielded conflicting results. Electronic databases updated to April 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 osteosarcoma. Survival data were aggregated and quantitatively analyzed. We performed a meta-analysis of eight studies that evaluated the correlation between VEGF overexpression and survival in patients with osteosarcoma. Combined hazard ratios suggested that VEGF overexpression had an unfavorable impact on overall survival (hazard ratio (HR) = 1.75, 95 % confidence interval (CI): 1.21–2.28) in patients with osteosarcoma for overall populations, 2.37 (1.35–3.39) in Asian studies but not in non-Asian studies (HR = 1.51, 95 % CI: 0.89–2.14). No significant heterogeneity was observed among all studies. VEGF overexpression indicates a poor prognosis for patients with osteosarcoma. However, the prognostic value of VEGF on survival in osteosarcoma patients still needs further large-scale prospective trials to be clarified.

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Introduction

Osteosarcoma is the most common malignant bone tumor in adolescents and young adults [1]. The oncological treatment of osteosarcoma consisting of chemotherapy and a surgical excision of the tumor leads to a 5-year overall survival (OS) rate at 60–70 % of patients with the disease localized at presentation. The OS rate decreases to 30 % when metastases are detected at the time of diagnosis [2]. Despite recent advances in surgical, radiotherapy, and chemotherapy treatment protocols, the long-term survival of patients with osteosarcoma still lacks significant improvement for the past decades. The factors that additionally influence the prognosis are the axial localization of the primary tumor, the tumor diameter of more than 8 cm, and the unfavorable histological response to pre-operative chemotherapy [3, 4]. 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. Researchers have focused on the potential role of new biological factors involved in the carcinogenic process as prognostic markers in patients with osteosarcoma.

Angiogenesis, the formation of new blood vessels from existing vasculature, is an important process in many malignancies including osteosarcoma. 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 [5, 6]. 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 osteosarcoma patients.

Many retrospective studies have evaluated whether overexpression of VEGF may be a prognostic factor for survival in patients with osteosarcoma. 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 osteosarcoma.

Materials and methods

Search strategy and study selection

The electronic databases PubMed, Embase, and CNKI (China National Knowledge Infrastructure) were searched for studies to include in the present meta-analysis. An upper date limit of April 1, 2013 was applied; we used no lower date limit. Searches included the terms "osteosarcoma," "osteogenic sarcoma," "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 osteosarcoma tissue with immunohistochemistry (IHC) or reverse transcription-polymerase chain reaction (RT-PCR), (2) provide information on survival, including OS, (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, which was included in the analysis. Two reviewers (X.Y. and T.W.) independently determined study eligibility. Disagreements were resolved by consensus.

Data extraction and quality assessment

The final articles included were assessed independently by two reviewers (X.Y. and T.W.). Data retrieved from the reports included author, publication year, patient source, histo-subtype, 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 not contact the author of the primary study to request the information. We did not use pre-specified 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 [7]. The data extraction and quality assessment were reported in previous meta-analysis [8–12].

Statistical methods

For the quantitative aggregation of the survival results, we measured the impact of VEGF overexpression on survival by HR between the two survival distributions. HRs and 95 % 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. [13].

Heterogeneity of the individual HRs was calculated with χ^2 tests according to Peto's method [14]. 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 [15] 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. [16] and Begg and Mazumdar [17]. 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

A total of 13 studies [18–30] published between 1999 and 2012 were included in the analysis. However, the five studies [14, 18, 20, 22, 23] did not provide the data on overall survival of patients in VEGF overexpression. Eight studies reported the prognostic value of VEGF status for overall survival. The major characteristics of the 13 eligible publications are reported in Table 1. The total number of patients included meta-analysis was 323, ranging from 15 to 91 patients per study (median 32). The studies were conducted in three countries (China, Japan, and Korea) and published between 1999 and 2012. Among the eight studies, six studies (176 patients, 54.5 %) were performed in Asian populations,

Table 1 Main characteristics and results of the eligible studies

First author and year	Patient source	Histo-subtype (Osteo %)	Stage	Number of patients	Method and isoforms	Definition of positivity	Positive (%)	HR estimation	Survival results
Lee 1999	Japan	0.4	NA	30	RT-PCR VEGF165	NA	0.8	Survival curves 9.80 (0.67–26.32)	NS
Handa 2000	Japan	0.43	I-IIB	30	NA	NA	0.93	No data	NS
Kaya 2000	Japan	0.52	I-III	27	IHC	>30 %	0.63	Survival curves 5.88 (0.82–17.32)	NS
Jung 2005	Korea	0.8	NA	25	IHC	CS	0.2	Survival curves 6.08 (1.34–10.82)	Poor
Charity 2006	England	NA	IIB	56	IHC	25 %	0.23	Survival curves 1.43 (0.92–2.22)	poor
Ek 2006	Australia	0.56	NA	25	IHC	CS	0.96	No data	NS
Oda Y 2006	Japan	0.67	NA	30	IHC	CS	0.63	Survival curves 1.85 (0.96–3.64)	Poor
Abdeen 2009	America	0.75	I-III	48	IHC	NA	0.27	No data	NS
Kaya 2009	Japan	NA	NA	15	IHC	50 %	0.53	Survival curves 6.13 (0.98–20.64)	Poor
Boulytcheva 2010	Russia	0.63	NA	40	IHC	10 %	0.15	No data	NS
Zhou 2011	China	NA	II-III	65	IHC	10 %	0.72	No data	Poor
Lugowska 2011	Poland	0.68	NA	91	IHC	50 %	0.39	HR and 95 % CI 2.51 (1.12–5.66)	Poor
Chen 2012	China	0.73	II-III	49	IHC	50 %	0.65	Survival curves 2.34 (1.02–4.52)	Poor

Osteo osteosarcoma, *CS* complex score combining intensity and percentage, *IHC* immunohistochemistry, *RT-PCR* reverse transcription polymerase chain reaction, *NS* not significant, *NA* not applicable, *HR* hazard ratio

and the remaining two studies (147 patients) followed non-Asian patients. All patients in the eligible studies were determined by pathological stage.

All of the studies reported the prognostic value of VEGF status for survival in patients with osteosarcoma. Of the eight studies, four directly reported HRs (multivariate analysis), while the other four studies provided survival curves. Among them, the proportion of patients exhibiting VEGF overexpression in individual studies ranged from 20 % to 96 %. Six of the eight studies identified VEGF overexpression as an indicator of poor prognosis, and the other two 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 Fig. 1. Overall, the combined HR for all eight eligible studies evaluating VEGF overexpression on OS was 1.75 (95 % CI: 1.21–2.28), suggesting that VEGF overexpression was an indicator of poor prognosis for osteosarcoma patients. No significant heterogeneity was observed among the studies ($Q = 4.39$, $I^2 = 15.3$ %, $P = 0.310$). When grouped according to geographic settings of individual studies, the combined HRs of Asian studies and non-Asian studies were 2.37 (95 % CI: 1.35–3.39) and 1.51 (95 % CI: 0.89–2.14), respectively, indicating that VEGF is an indicator of poor prognosis of OS in Asian patients but not in non-Asian patients. No significant heterogeneity was observed among the studies on VEGF overexpression on Asian studies ($Q = 3.48$, $I^2 = 0.0$ %, $P = 0.360$) and non-Asian studies ($Q = 4.53$, $I^2 = 0.0$ %, $P = 0.370$)

Publication bias

Begg's funnel plot and Egger's test were performed to assess the publication bias in the literature. All eight eligible studies investigating VEGF overexpression on OS yielded a Begg's test score of $P = 0.019$ and an Egger's test score of $P = 0.001$; meanwhile, according to the funnel plot (Fig. 2), the publication bias was found. These results suggested that there were publication biases in these subgroup analyses.

Table 2 Meta-analysis: HR value of OS in osteosarcoma subgroups according to patient source

	Number of studies	Patients	Random effects HR (95 % CI)	χ^2 heterogeneity test (P value)
Overall for OS	8	323	1.75 (1.21–2.28)	0.310
Asian	6	176	2.37 (1.35–3.39)	0.360
Non-Asian	2	147	1.51 (0.89–2.14)	0.370

HR hazard ratio, *OS* overall survival

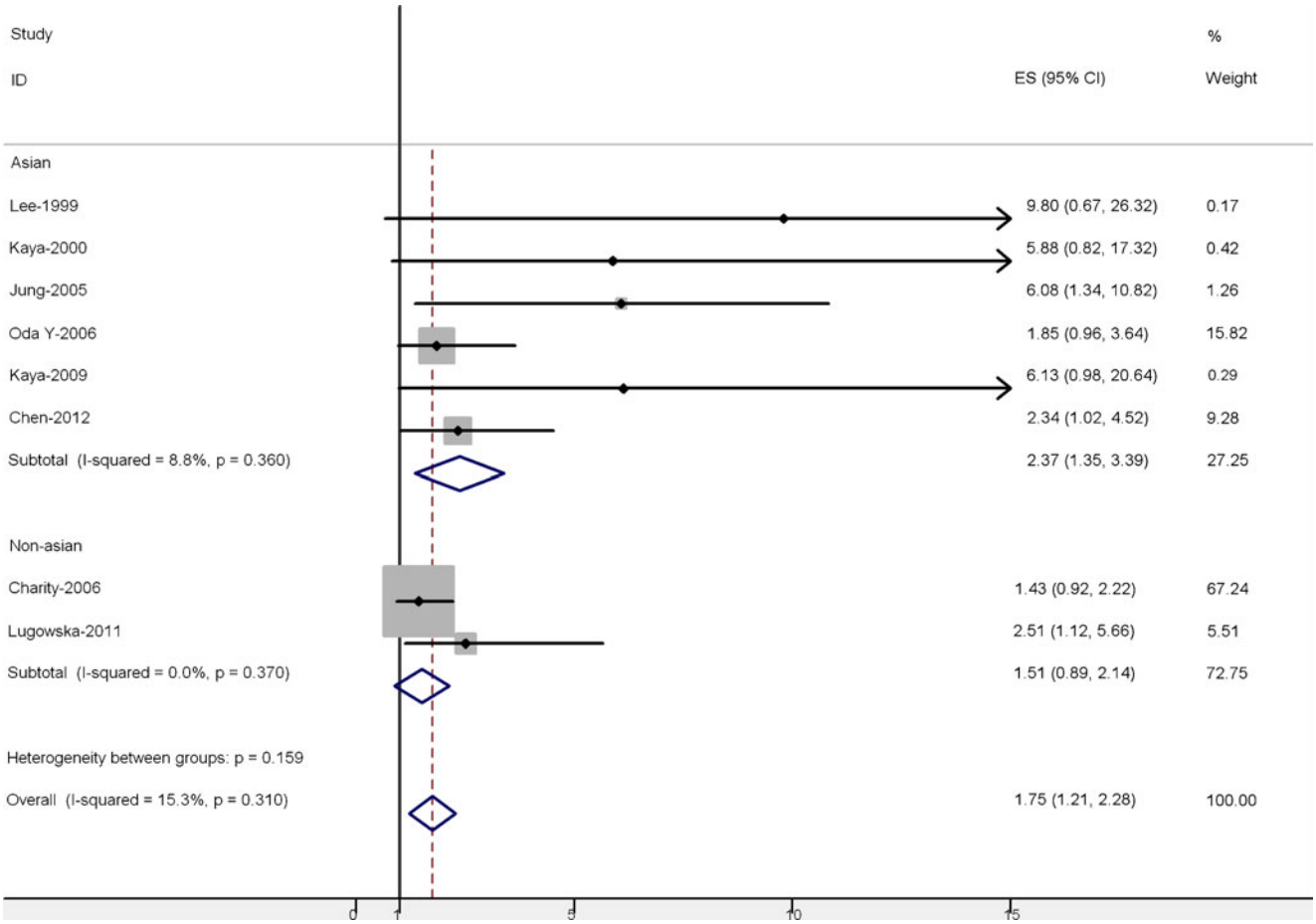

Fig. 1 Meta-analysis (forest plot) of the eight evaluable studies assessing VEGF in osteosarcoma stratified by patient source for overall survival

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 pre-existing vasculature, respectively. VEGF-A exists as a homodimer or can heterodimerize with either VEGF-B or non-VEGF factors such as placenta growth factor [31–33]. VEGF-A and

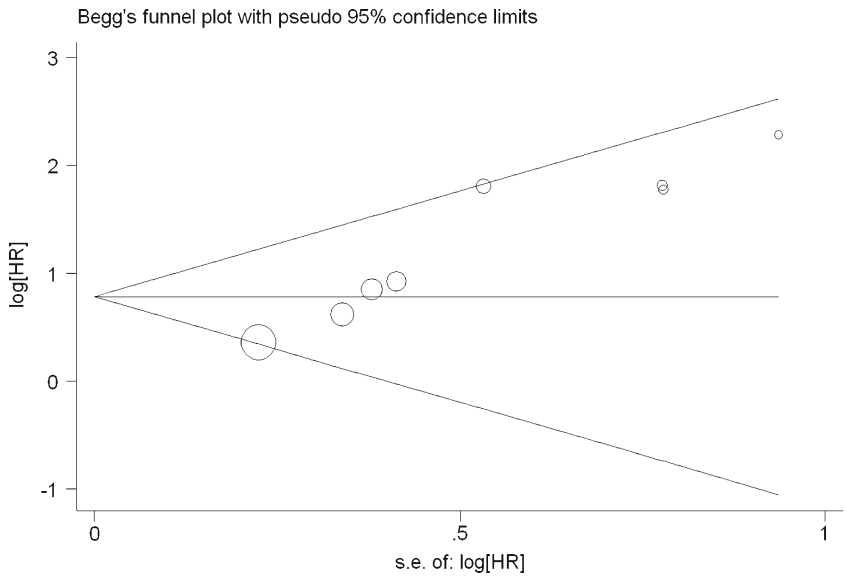

Fig. 2 Funnel plot of the eight evaluable studies assessing VEGF in osteosarcoma for overall survival

VEGF-B promote vascular angiogenesis primarily through activation of vascular endothelial cell associated VEGFR-1 (Flt1) and VEGFR-2 (Flk1/KDR).

The present meta-analysis has combined eight publications including 323 patients to yield statistics, indicating a statistically significant role of VEGF on overall survival in osteosarcoma patients. In subgroup analysis according to the geographic settings of individual studies, statistically significant detrimental effect of VEGF was found in Asian patients but not in non-Asian patients. In our meta-analysis, patient cohorts were mainly from Eastern Asian countries (176 patients, 54.5 %), only two study patient sources were non-Asian. In our meta-analysis, the combined hazard ratios mainly represented the Eastern Asian; however, the results of western countries remained unclear.

There were several meta-analyses studying the prognostic value of VEGF in other cancer types, such as head and neck squamous cancer [34], lung cancer [35], colon cancer [36], gastric cancer [37], and hepatocellular carcinoma [38]. 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 (TKIs), and antisense strategies [39]. Bevacizumab (a humanized monoclonal antibody) is an effective anti-angiogenic agent that is the first to be approved by the American Food and Drug Administration as the first-line treatment for metastatic colorectal cancer [40], and it blocks secreted VEGF and prolongs overall survival of patients with advanced NSCLC in combination with standard chemotherapy in a randomized phase 3 trial [41] and patients who respond well to Bevacizumab with recurrent malignant gliomas [42].

The heterogeneity issue was complicated in the systematic review and meta-analysis. We found no significant heterogeneity among all studies and subgroup analysis included. 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.

Publication bias [43] 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 support publication bias; the obtained summary statistics likely approximate the actual average. 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 expression and survival of patients with osteosarcoma. As determined in our meta-analysis, we concluded that VEGF expression was associated with poor overall survival in osteosarcoma, 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 osteosarcoma.

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Conflicts of interest None

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