RESEARCH ARTICLE

Prognostic Value of Vascular Endothelial Growth Factor Expression in Resected Gastric Cancer

Lei Liu[&]*, Xue-Lei Ma[&], Zhi-Lan Xiao[&], Mei Li[&], Si-Hang Cheng, Yu-Quan Wei

Abstract

Background and Aims: Vascular endothelial growth factor (VEGF) is a potential prognostic biomarker for patients with resected gastric cancer. However, its role remains controversial. The objective of this study was to conduct a systematic review and meta-analysis of published literature. Methods: Relevant literature was identified using Medline and survival data from published studies were collected following a methodological assessment. Quality assessment of eligible studies and meta-analysis of hazard ratio (HR) were performed to review the correlation of VEGF overexpression with survival and recurrence in patients with gastric cancer. Results: Our meta-analysis included 44 published studies with 4,794 resected patients. VEGF subtype for the prediction of overall survival (OS) included tissue VEGF (HR=2.13, 95% CI 1.71–2.65), circulating VEGF (HR=4.22, 95% CI 2.47–7.18), tissue VEGF-C (HR=2.21, 95% CI 1.58–3.09), tissue VEGF-D (HR=1.73, 95% CI 1.25–2.40). Subgroup analysis showed that HRs of tissue VEGF for OS were, 1.78 (95% CI 0.90-3.51) and 2.31 (95% CI 1.82-2.93) in non-Asians and Asians, respectively. The meta-analysis was also conducted for disease free survival (DFS) and disease specific survival (DSS). Conclusion: Positive expression of tissue VEGF, circulating VEGF, VEGF-C and VEGF-D were all associated with poor prognosis in resected gastric cancer. However, VEGF demonstrated no significant prognostic value for non-Asian populations. Circulating VEGF may be better than tissue VEGF in predicting prognosis.

Keywords: Gastric cancer - prognosis - growth factors and signal transduction - VEGF

Asian Pacific J Cancer Prev, **13**, 3089-3097

Introduction

In 2008, nearly 1 billion new gastric cancer cases were estimated. As a result of healthy diet, H. pylori infection reduction and introduction of screening using photofluorography, the incidence rate of gastric cancer has decreased substantially in most parts of the world, but it still remains common in some areas of the world, especially in Eastern Asia. Altogether, gastric cancer still accounts for more than 10% of cancer deaths worldwide, being the second most frequent cause of cancer death following lung cancer (Jemal et al., 2011).

Angiogenic and lymphangiogenic factors play essential roles in the initiation and progression of tumor with vascular endothelial growth factor (VEGF) family ranking first. VEGF family favored the growth of tumors through neovascularization, which brings survival necessities to malignant cells (Kerbel, 2008). Bevacizumab, a monoclonal VEGF antibody has brought prominent survival prolonged in several cancer types when administered with chemotherapy (Jain et al., 2006). However, the reported prognostic value of VEGF was inconsistent among different studies. VEGF-C and VEGF-D are the two most important factors binding to VEGFR-3. A large number of experimental and clinical studies have identified the role of VEGFR-3 signaling in promoting lymph node metastasis via tumor-induced lymphangiognesis (Wissmann and Detmar, 2006). Recently, VEGF-C and VEGF-D have also been studied for their prognostic values for gastric cancer (Takahashi et al., 2002; Gou et al., 2011), yet resulting in controversial conclusions.

The correlation between VEGF expression and prognosis of cancer patients had been demonstrated in hepatocelluar carcinoma (Schoenleber et al., 2009), pancreatic cancer (Smith et al., 2011) and some other cancer types (Kyzas et al., 2005; Zhan et al., 2009) by meta-analysis.

Recently, we found Chen reported the prognostic significance of VEGF for gastric cancer. Their study gave the similar result in Asian group of tissue-VEGF (Chen et al., 2011). Meanwhile, we conducted a systematic review and meta-analysis investigating the prognostic role of VEGF, VEGF-C or VEGF-D in resected gastric cancer patients and we found many interesting meta-analysis results in different race, subtype, and cut-off value.

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Materials and Methods

Search strategy

PubMed was searched on June 30, 2011. The following strategies were used to retrieve articles and abstracts in English, [gastric* OR stomach] AND ([cancer OR tumor OR carcinoma]) AND ([VEGF] OR [vascular endothelial growth factor]).

Study inclusion/exclusion criteria

Studies were considered eligible if they met the following inclusion criteria, (i)studied patients with resected gastric cancer, (ii)measured the expression of VEGF in tumor tissue or blood, and (iii) investigated the association between VEGF expression levels and survival outcome (OS, DFS or DSS). Studies were excluded based on the following criteria, (i) analyzed in various tumors but with no specific results of gastric cancer, (ii)lacked key information for analysis with methods developed by Parmar et al. (1998), Williamson et al. (2002), and Tierney et al. (2007), (iii) were studies with preoperative treatment, such as neo-adjuvant chemotherapy, radiation therapy or other treatments, (iv) or were non-English articles.

Data extraction

Articles were reviewed independently by two investigators (Ma Xuelei and Liu Lei) for data extraction. Disagreements were resolved by consensus. Data were extracted from eligible studies by two investigators (Ma Xuelei and Xiao Zhilan), independently. The primary data were HR and 95% confidence interval (CI) of survival outcomes, including overall survival (OS), disease free survival (DFS) and disease specific survival (DSS). Additional data obtained from the studies included first author, publication year, patients source, study size, VEGF staining positive cases, tumor stage, histological classification, methods to determine VEGF, the VEGF positive or high expression and the conclusion. The statistical data from the studies were obtained, such as HR, 95% CI, p value or the Kaplan–Meier survival curves.

Quality assessment

Studies were scored by two reviewers (Ma Xuelei and Liu Xiaoxiao) independently. Identical scoring was achieved for each single item after discussion. We conducted a quality assessment consisting of 20 items recently developed by Smith et al. (2011) for studies and the scoring criteria were made according to REMARK criteria (McShane et al., 2006). Quality scores were expressed as percentages ranging from 0% to 100%.For each characteristics mentioned, 5% of scores were given to a study.

Statistical Methods

For the quantitative aggregation of the survival results, logHazard Ratio (HR) and standard error (SE) were statistically combined, but these statistical variables were not given explicitly in most studies. Therefore, we calculated the necessary statistics on the basis of available numerical data with methods developed by Parmar et al. (1998), Williamson et al. (2002), and Tierney et al. (2007). TheselogHR and SE were calculated with these methods when any group of the following numerical data were available, (i) the HR and 95% CI, (ii) the p-value for the logrank or Mantel-Haenszel test, (iii)or the Kaplan–Meier survival curves. We performed meta-analysis in each subgroup, categorized by patients' source, VEGF positive staining definition, tumor stage or histological classification. Calculation was accomplished by the software designed by Matthew Sydes and Jayne Tierney with these methods (Medical Research Council Clinical Trials Unit, London, UK) (Tierney et al., 2007).

In this meta-analysis, Forrest plots were used to estimate the effect of VEGF over-expression on survival. Heterogeneity was defined as $p<0.10$ or $I^2>50\%$ (Higgins et al., 2003). When homogeneity was fine ($p\geq0.10$, $I^2\leq50\%$), a fixed effect model was used for secondary analysis. If not, a random effect model was used. An observed HR>1 indicated worse outcome for the positive group relative to the negative group and would be considered statistically significant if the 95% CI did not overlap 1. All above calculations were performed using RevMan5.1 (Cochrane collaboration, Oxford, UK) Publication bias was evaluated using the Begg's funnel plot and Egger's test by STATA 11.0 (STATA Corporation, College Station, TX).

Correlation between quality data and constituents of positive cases or study size were studied using Spearman rank correlation coefficient and whether quality data was associated with patients' source or conclusion were studied using Mann–Whitney test. Both tests were considered statistically significant if $p<0.05$ (two-sided). Calculations were performed on SPSS13.0 (SPSS, Inc., Chicago, IL).

Results

The initial search yielded 243 studies and reviewers identified 93 potential studies for full-text review, 44 eligible studies were included, if one study referred different subtype the study was listed twice (Figure 1). All eligible studies reported the prognostic value of VEGF status for survival in patients with gastric cancer. The total number of patients included was 4794, ranging from 40 to 374 patients per study (median, 109).

VEGF

29 studies were eligible for meta-analysis of prognostic value of VEGF for resected gastric cancer. The specimens

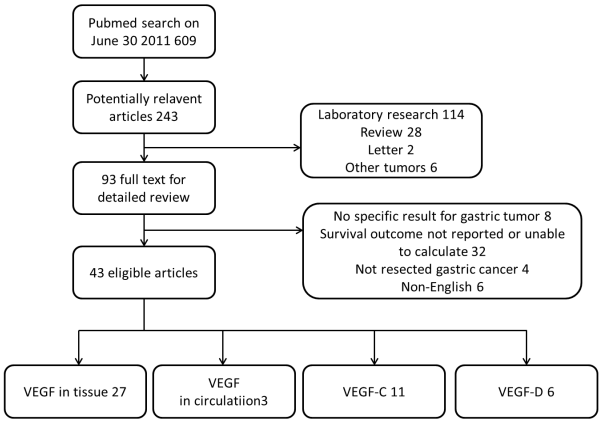

Figure 1. Eligible Studies

Table 1. Main Characteristics of the Included Studies

Article & publication year	Country	Patients number	Positive%[a]	Age (y)	Male%	I|II%[a]	Histology well%[a]	Q	Method to determine biomarker	Survival analysis	HR estimation	Cut-off value	Conclusion
VEGF													
Maeda, K 1997	Janpan	95	35.8	57.8	75.8	52.6	40	13	IHC	OS	estimation	> 5%	positive
Takahashi, R 2003	Janpan	53	26.4	59.5	60.3	NAa	NA	12	IHC	OS	estimation	>10%	positive
Wang, X 2010	China	128	45.3	NA	64.8	30.5	40.6	13	IHC	DFS	HR	>30%	positive
Zhou, Y 2010	China	200	81.5	NA	76.5	26	47.5	14	IHC	OS	estimation	> 0%	positive
Bazas, M 2008	Ukraine	150	42.7	NA	59.3	30	NA	11	IHC	OS	estimation	>20%	positive
Vidal, O 2008	Spain	148	76.4	69	66.9	64.2	52.7	18	IHC	DFS, DSS	HR	> 0%	positive
Zhang, L 2006	China	105	63.8	57.6	66.7	NA	60	10	IHC	OS	estimation	>10%	positive
Kakeji, Y 2002	Japan	188	54.3	NA	66.7	76	70.7	14	IHC	OS	estimation	> 5%	positive
Kolev, Y 2007	Japan	169	50.3	59.6	72.2	70.4	NA	12	IHC	OS,DFS	estimation	>25%	positive
Aoyagi, K 2005	Japan	40	40	59.7	60	NA	17.5	9	IHC	OS	estimation	>50%	positive
Fondevila, C 2004	Spain	156	74.4	67	65	64.1	50.6	20	IHC	OS,DFS	HR	> 0%	positive
Saito, H 1999	Japan	108	42.6	59.7	49.1	NA	67.6	13	IHC	OS	estimation	>10%	positive
Ding, S 2007	China	51	66.7	66	80.4	NA	NA	11	IHC	OS	estimation	> 5%	positive
Maeda, K 1999	Japan	195	30.8	56	NA	24.6	56.4	14	IHC	DFS	HR	> 0%	positive
Shi, H 2003	China	232	52.6	55.6	68.7	NA	55.6	13	IHC	OS	estimation	> 5%	positive
Yang, Q 2010	China	118	54.2	57.8	73.1	NA	59.3	11	IHC	OS	estimation	>10%	positive
Joo, E 2002	Korea	145	31	59.2	68.3	50.3	47.6	15	IHC	DSS	estimation	> 0%	positive
Lieto E 2007	Italy	69	60.9	NA	62.3	62.3	65.2	17	IHC	DSS	HR	>10%	positive
Tanigawa N 1997	Japan	163	48.5	NA	NA	23.3	NA	11	IHC	OS	estimation	> 0%	negative
Urano, N 2006	Japan	146	69.9	NA	NA	76	56.2	12	IHC	OS	estimation	>10%	negative
Wang, J 2011	China	88	36.4	NA	87.5	30.7	38.6		IHC	OS	estimation	>10%	positive
Lee, J 2009	Korea	374	90.1	NA	65.9	73.3	NA	12	IHC	OS, DFS	HR	>10%	negative
Ozdemir, F 2006	Turkey	51	56.9	NA	64.7	NA	NA	10	IHC	OS	estimation	>10%	positive
Ikeguchi, M 1999	Japan	93	29	NA	49.5	100	NA	16	IHC	OS	estimation	>10%	negative
Kimura, H 2001	Japan	102	52	61	64.7	100	38.2	15	IHC	OS	estimation	> 5%	negative
Skarlos, V 2007	Greece	44	84.1	65	60	30	NA	14	IHC	OS, DFS	estimation	>10%	negative
s-VEGF													
Vidal, O.2009	Spain	97	45.4	70	46.4	70.1	54.6	NA	ELISA	OS	HR	320pg/ml	positive
Anastasios J 2002	Greece	58	41.4	68	65.5	31	51.7	NA	ELISA	OS	HR	533pg/ml	positive
Yoshikawa, T 2000	Japan	54	16.7	58.6	66.7	44.4	31.5	NA	ELISA	OS	estimation	100pg/ml	positive
VEGF-C													
Gou, H 2011	China	56	55.4	56.2	62.5	60.7	39.3	13	IHC	OS	curve	>10%	negative
Lee, S 2009	Korea	371	75	60	65.9	46.9	NA	11	IHC	OS	HR	>10%	negative
Han, F 2010	China	204	55	55.8	72.5	50.5	41.2	14	IHC	OS	estimation	>20%	positive
Tsutsumi, S 2005	Japan	102	26.5	64	76.5	90.2	NA	13	IHC	OS	estimation	>20%	positive
Yonemura, Y 1999	Japan	117	25.5	NA	NA	NA	46.2	8	IHC	OS	estimation	>20%	positive
Wang, T 2007	China	80	62.5	57.1	67.5	40	100	11	IHC	OS	estimation	>30%	positive
DA, M 2007	China	68	54.4	55	70.6	45.6	NA	14	IHC	OS	HR	>50%	positive
DING, S 2007	China	51	62.7	66	80.4	NA	NA	11	IHC	OS	estimation	>5%	positive
Takahashi, A 2002	Japan	65	53.8	56.8	67.7	60	NA	10	NA	OS	HR	>50 cell	positive
VEGF-D													
Choi, J 2008	China	104	62.5	59	68.3	52	NA	14	IHC	DFS	estimation	> 0%	positive
Deguchi, K 2010	Japan	72	72.2	NA	69.4	NA	50	13	IHC	OS	estimation	>10%	negative
Deng, J 2009	China	75	65.3	59.5	84	NA	NA	14	IHC	OS, DFS	estimation	NA	negative
Juttner, S 2006	Germany	88	67	62.7	64.8	NA	NA	14	IHC	DFS	HR	> 5%	positive
Shida, A 2005	Japan	143	38.5	52	68.5	NA	35	14	IHC	OS	HR	> 0%	positive
Wang,J 2011	China	88	36.4	NA	87.5	30.7	38.6		IHC	OS	estimation	>10%	positive

Legends of Table I: Positive%, constituents of patients with positive staining; I/II %, constituents of patients with I/II stage gastric cancer; Histology well %: constituents of well-differentiated specimen in histology; Q, quality points; NA, not available

of 26 (Maeda et al., 1997; Tanigawa et al., 1997; Maeda et al., 1998; Ikeguchi et al., 1999; Maeda et al., 1999; Saito et al., 1999; Kimura et al., 2001; Joo et al., 2002; Kakeji et al., 2002; Shi et al., 2003; Takahashi et al., 2003; Fondevila et al., 2004; Aoyagi et al., 2005; Ozdemir et al., 2006; Urano et al., 2006; Zhang et al., 2006; Ding et al., 2007; Kolev et al., 2007; Skarlos et al., 2007; Kolev et al., 2007; Bazas et al., 2008; Lieto et al., 2008; Lee et al., 2009; Yang et al., 2010; Wang et al., 2010; Zhou et al., 2010; Wang et al., 2011) studies were tissue and 3 (Yoshikawa et al., 2000; Karayiannakis et al., 2002; Vidal et al., 2009) were blood from peripheral vein.

Tissue VEGF

The number of patients included was 3411 (male, 66.9%). The study sizes were from 40 to 374 patients (median, 131). The other major characteristics of 26 eligible publications were reported in Table 1. One study was not included because the investigator used enzyme-linked immunosorbent assay (ELISA) method to detect VEGF expression (Kido et al., 2001). Another one study was not included because the patient was similar to the author's another study (Maeda et al., 1997). The average quality score of the 26 eligible studies of VEGF expression was 67.12% (range from 45% to 100%, standard deviation 13.50%). We found no significant difference in quality scores between studies with positive and negative conclusion (Mann Whitney test, p=0.902). Similarly, there was no significant difference in quality scores between studies carried out by Asian and non-Asian investigators (Mann Whitney test, p=0.927). We found no significant correlation between quality scores and study sizes (Spearman's test, r=0.170, p=0.406). Similarly, there was no significant correlation between quality scores and VEGF-positive percentage (Spearman's test, r=0.035, p=0.865).

Studies on Forrest plots and meta-analysis were divided into three groups (OS, DFS and DSS) with 21, 9 and 3 studies, respectively. The combined HR of OS was 2.13 (95% CI 1.71–2.65). There was significant heterogeneity in the result (p=0.002, I2=53%) (Figure2 A).Table 2 illustrated HR, its 95% CI and heterogeneity test results for of all meta-analyses we conducted in our study, including whole-group analysis of tissue

Table 2. HR, 95% CI and Heterogeneity Test Results for All Meta-analyses Conducted in this Study

	Studies number	OS HR	OS HR (95% CI)	OS Heterogeneity test (p, I^2)	Studies number	DFS HR	DFS HR (95% CI)	DFS Heterogeneity test (p, I^2)
Tissue VEGF †	22	1.96	1.70-2.25	0.002*, 54%	7	2.04	1.59-2.60	0.38,6%
‡		2.23	1.78-2.78					
Asian †	18	2.05	1.74-2.42	0.02*, 44%	4	2.03	1.53-2.71	0.82, 0%
‡		2.31	1.82-2.93					
Non-Asian †	4	1.75	1.35-2.26	0.003*, 78%	3	2.05	1.28-3.28	0.06, 64%
‡		1.78	0.90-3.51 (NS)			1.65	0.70-3.92(NS)	
Positive definition≥10%	11	1.73	1.43-2.10	0.21, 24%	3	1.52	0.98-2.38	0.17, 43%
Positive definition<10%	7	2.89	2.15-3.90	0.51, 0%	4	2.31	1.72-3.10	0.91, 0%
Positive definition =10%	8	1.92	1.47-2.52	0.10, 42%				
VEGF (WD[a]<50%)	5	2.65	1.88-3.72	0.69, 0%	1	2.34	1.27-4.33	
VEGF (WD≥50%)	7	2.34	1.80-3.04	0.40, 4%	3	2.3	1.65-3.22	0.76, 0%
VEGF (I/II %≥50%)	10	2.2	1.73-2.79	0.90, 0%	5	1.88	1.35-2.62	0.21, 32%
VEGF (I/II %<50%)								
Circulating VEGF	3	4.22	2.47-7.18	0.84, 0%				
VEGF-C †	10	2.03	1.67-2.46	0.02*, 55%	1	1.78	1.02-3.11	
‡		2.2	1.60-3.04					
VEGF-D	4	1.71	1.17-2.50	0.79, 0%	4	2.3	1.66-3.18	0.79, 0%

Legends of Table II: NS, not significant statistically; WD, well differentiated in histology; †, using fixed effect model; ‡, using random effect model; *, statistically significant

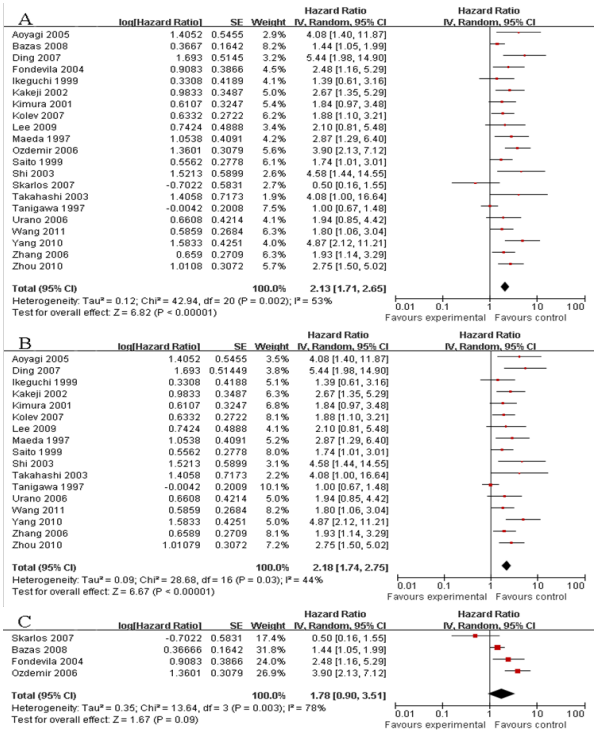

Figure 2. Forest Plots of Meta-analysis for OS Prediction Value of VEGF. Meta-analysis of the association between (A) VEGF expression in tissue and OS; (B) VEGF expression in tissue and OS in Asian population; (C) VEGF expression in tissue and OS in non-Asian population; Each study is shown by the name of the first author and the HR with 95% CIs. The combined HR and 95% CIs are according to random effect model calculations

VEGF, circulating VEGF, VEGF-C and VEGF-D, and subgroup analysis of tissue VEGF grouped by patients source, VEGF positive staining definition, tumor stage or histological classification (Figure 2, Table2). When we grouped these studies by the patients source, the combined HR of Asian and non-Asian group were 2.18 (95% CI 1.74-2.75) and 1.78 (95% CI 0.90-3.51) in random effect model. If we grouped these studies by VEGF positive staining definition, the combined HR of studies with the definition ≥10% was 1.82 (95% CI 1.53-2.17) ,while the combined HR of studies with definition < 10% was 2.89 (95% CI 2.21-3.79) in fixed effect model. Similarly, the combined HR of studies with well-differentiated majority and minority subgroups were 2.39 (95% CI 1.86-3.06) and 2.28 (95% CI 1.69-3.07). All these results suggested that VEGF expression was related with survival outcome of resected gastric cancer. As DSS and DFS statistics of individual meta-analysis were similar to the results of OS, the DSS and DFS related data were shown in Table 2.

Regarding the whole VEGF group, the combined HR of DFS was 2.04 (95% CI 1.59–2.60) in fixed effect model, and no significant heterogeneity was found (p=0.38, I^2=6%). The HR of DSS was 2.59 (95% CI 1.33–5.06) in random effect model with significant heterogeneity (p=0.05, I^2=66%). All these outcomes suggested a statistical significance in correlation of tissue VEGF expression and survival outcome of gastric cancer.

When setting up the funnel plots for OS analysis (Figure 3 A) and DFS analysis (Figure 3 B), we revealed a publication bias in studies regarding OS and tissue VEGF (Egger's, p=0.01, Begg's, p=0.043), regarding DFS and tissue VEGF (Egger's, p=0.044, Begg's, p=0.293) and regarding DSS and tissue VEGF (Egger's, p=0.343, Begg's, p=0.117).

Circulating VEGF

Three studies (Yoshikawa et al., 2000; Karayiannakis et al., 2002; Vidal et al., 2009) with 209 patients were pooled into analysis. The median sample size ranges from 54 to 97.Major characteristics of the 3 eligible publications are reported in Table 1.

All 3 studies used ELISA method to detect VEGF expression. We did not give the quality score because specimens of these studies were assayed by ELISA. A combined HR 4.22 (95% CI 2.47-7.18) was obtained in fixed effect model with a fine homogeneity (p=0.84, I^2=0%). It suggested that serum VEGF expression have a significant association with survival outcome of gastric

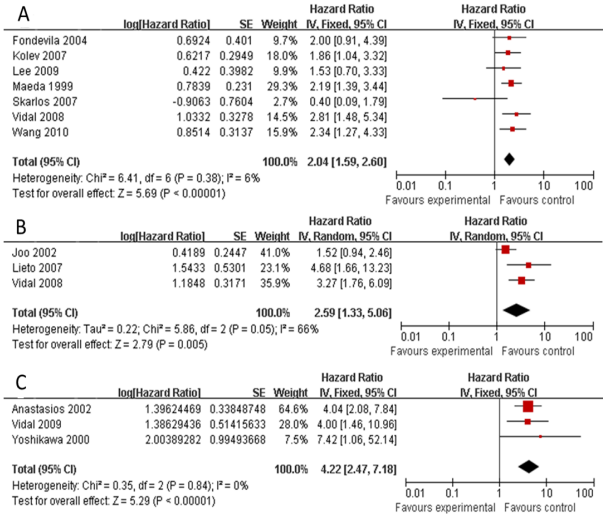

Figure 3. Forest Plots of Meta-analysis for DFS and DSS Prediction Value of Tissue VEGF and OS Prediction Value of Circulating VEGF. Meta-analysis of the association between (A) VEGF expression in tissue and DFS; (B) VEGF expression in tissue and DSS; (C) VEGF expression in circulation and OS; Each study is shown by the name of the first author and the HR with 95% CIs. The combined HR and 95% CIs of (B) are according to random effect model calculations. The combined HR and 95% CIs of (A) (C) are according to fixed effect model calculations

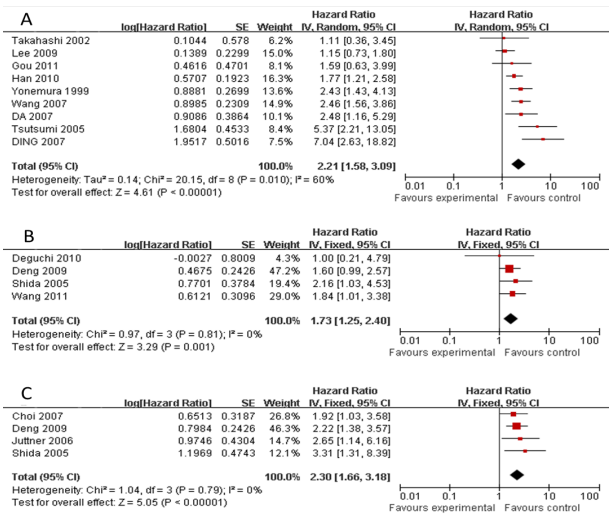

Figure 4. Forest Plots of Meta-analysis for OS or DFS Prediction Value of VEGF-C or VEGF-D. Meta-analysis of the association between (A) VEGF-C expression in tissue and OS; (B) VEGF-D expression in tissue and OS; (C) VEGF-D expression in tissue and DFS. Each study is shown by the name of the first author and the HR with 95% CIs. The combined HR and 95% CIs of (A) are according to random effect model calculations. The combined HR and 95% CIs of (B) (C) are according to fixed effect model calculations

cancer.

As no more than 10 studies were included regarding OS and circulating VEGF, funnel plots were not show. No significant publication bias was observed (Egger's, p=0.307, Begg's, p=0.117).

Tissue VEGF-C

11 studies were eligible for meta-analysis of prognostic value of VEGF-C for resected gastric cancer (Yonemura et al., 1999; Takahashi et al., 2002; Tsutsumi et al., 2005; Shida et al., 2006; Ding et al., 2007; Wang et al., 2007; Da et al., 2008; Lee et al., 2008; Deguchi et al., 2010; Han et al., 2010; Gou et al., 2011). Except Deguchi's report (Deguchi et al., 2010), all studies were dealing with OS. Thus, we only did meta-analysis for OS. In total, the tissue VEGF-C pooled 10 studies with 1164 patient. The median sample size for all studies was 74.0 patients (range=50-371). Major characteristics of the 10 eligible studies are reported in Table 1. One study was not considered because the investigator used RT-PCR to detect mRNA expression of VEGF-C (Shida et al., 2006). The average quality score of the 9 eligible studies of VEGF-C expression was 57.22 (range 40 to 70, standard deviation 9.05). The difference was not significant in quality scores between studies with positive and negative conclusion (Mann Whitney test, p=0.881). There was no significant difference in quality scores between studies carried out by Asian and non-Asian investigators either (Mann Whitney test, p=0.129). Similarly, we found no significant correlation between quality scores and study sizes (Spearman's test, r=0.034, p=0.930) and between quality scores and VEGF-C-positive percentage (Spearman's test, r=0.111, p=0.776).

A combined HR of tissue VEGF-C was 2.21 (95% CI 1.58-3.09) in random effect model with a heterogeneity test result (p=0.01, I^2=60%). The patients source, tumor stage, histological classification, VEGF positive staining definition and other characteristics were used to deal the heterogeneity. Unfortunately none could weaken the heterogeneity (data not shown).

Funnel plots were shown on Figure 3 C. Publication bias were not statistically significant (Egger's, p=0.273, Begg's, p=0.532).

Tissue VEGF-D

In 7 eligible studies, the specimens of 6 studies were tissue (Shida et al., 2005; Juttner et al., 2006; Choi et al., 2008; Deguchi et al., 2010; Deng et al., 2009; Wang et al., 2011) and Tsirlis et al. (2008) was blood from peripheral vein. At last, we pooled 6 studies (4 for OS, 4 for DFS), 567 patients. The median sample size for all studies was 94.5 patients (range72-143). Major characteristics of the 6 eligible publications are reported in Table 1.

One study was not considered because the investigator used RT-PCR to detect mRNA expression of VEGF-D. The average quality score of the 6 eligible studies of VEGF-D expression was 69.0 (range 65 to 10, standard deviation 2.236). The difference was not significant in quality scores between studies with positive and negative conclusion (Mann Whitney test, p=0.221). Either, there was no significant correlation between quality scores and study sizes (Spearman's test, r=0.707, p=0.182). We did not study other difference or correlation described in VEGF and VEGF-C quality assessment because there were no enough studies for VEGF-D.

A combined HR of tissue VEGF-D was DFS (2.30, 95% CI 1.66-3.18), OS (1.73, 95% CI 1.25-2.40). Both results had a fine homogeneity with a heterogeneity test result (p=0.79, I^2=0% for DFS, and p=0.81, I2=0% for OS). The studies suggested that tissue VEGF-D expression

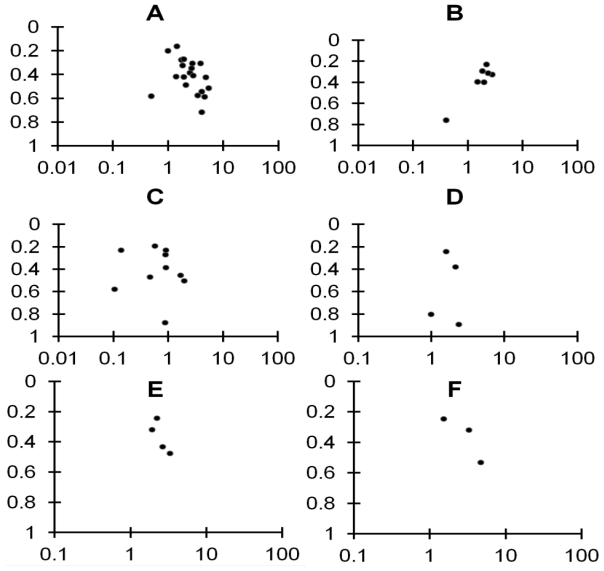

Figure 5. Funnel Plots Depicting Publication Bias. Inverted funnel plots showing the relations between HR and 1/SE of association between (A) VEGF in tissue and OS; (B) VEGF in tissue and DFS; (C) VEGF in circulation and OS; (D) VEGF-C in tissue and OS; (E) VEGF-D in tissue and OS; (F) VEGF-D in tissue and DFS

might have a significant association with survival outcome of gastric cancer.

As no more than 10 studies were included regarding OS, DFS and VEGF-D, funnel plots were not show. No significant publication bias was observed OS (Egger's, p=0.780, Begg's, p=0.602), DFS (Egger's, p=0.280, Begg's, p=0.174).

Discussion

In order to guide clinical decision-making in therapy and prognosis prediction, efforts have been invested in identifying prognostic biomarkers for patients with gastric cancer. Original studies were published aiming at finding prognostic value of VEGF biomarkers for gastric cancer. Our meta-analysis included 27, 3, 10 and 6 published studies, including 3411, 209, 1114 and 567 patients with gastric cancer to yield summary statistics on the association between the prognosis of gastric cancer and expression of tissue VEGF, circulating VEGF, VEGF-C and VEGF-D respectively.

Recently, Chen reported the prognostic significance of VEGF for gastric cancer by meta-analysis yet with only Asian population and only 13 studies concerning prognostic significance of VEGF on OS (Chen et al., 2011). In addition, Chen's study used OR value as the measuring statistics. In fact, according to Tierney et al. (2007), HR is better than OR in meta-analysis as it takes account of not only dichotomous outcomes but also time-to-event outcomes. Our study combined HR for individual meta-analysis categorized by patients' source, VEGF positive staining definition, tumor stage and histological classification. These individual meta-analyses brought better insights into confounding factors identification. Importantly, we gave the meta-analyses of non-Asian group, not Asian group only. Further, we included more recent related studies of survival outcome. Quality assessment was performed to examine the characteristics' influence on study conclusion. Most importantly, we added circulating VEGF, VEGF-C and VEGF-D in meta-analysis.

We found that studies with positive and negative conclusions were not statistically different in quality scores of VEGF, VEGF-C and VEGF-D, respectively. This suggested the reasons for opposite conclusions among these studies may not be caused by quality of studies. Similarly, no difference was discovered in quality scores between studies carried out by Asian and non-Asian investigators, respectively in VEGF and VEGF-C studies. Neither biomarker-positive percent nor study sizes were statistically correlated to quality scores in VEGF and VEGF-C studies, respectively. These suggested the quality of studies was probably not due to location of investigators. Biomarker-positive percent and study sizes of studies may not bring difference to quality of studies, either.

Vascular endothelial growth factor (VEGF) is a major inducer of angiogenesis and vessel permeability (Berse et al., 1992; Ferrara et al., 1992). VEGF binds to VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) that is mainly expressed on vascular endothelial cells. The VEGF-A, usually simply referred to VEGF is believed to play a major role in the tumor growth and metastasis (Saito et al., 1999). In the meta-analysis, OS (HR 2.14), DFS (HR 2.04) and DSS (HR 2.59) analysis results were similar to each other, which all suggested that tissue VEGF expression was significantly related to poor prognosis of resected gastric cancer.

Interestingly, when categorizing studies by geographical location in tissue VEGF individual meta-analysis for OS, non-Asian subgroup did not support the whole-group analysis as HR (1.37, 95% CI 0.71-2.65), meanwhile the meta-analysis for DFS gave a similar analysis, HR (1.65, 95% CI 0.70-3.92). These results suggested that tissue VEGF was not significantly correlate with gastric tumor prognosis in non-Asian subgroup. Considering that only 4 studies were conducted within non-Asian population, we suggested more non-Asian investigators contributed to the further discovery.

Besides, we found three possible confounding factors, VEGF positive staining definition and histological constituent, all with significant statistical test results (Table 2). HR (1.82) value in the subgroup of studies with VEGF positive staining definition ≥10 was larger than the HR(2.89) value in < 10 subgroup. We assumed that, though with low VEGF expression in the tissue, prognosis of patients was obviously poor, whereas, along with VEGF expression elevation, prognosis worsening was indistinct. This might be proved by large sample quantitative analysis of correlation between VEGF expression and survival outcome of gastric cancer. At the same time, we suggested that a specific positive staining definition would be defined for prognostic biomarker discovery in order to obtain comparable results.

Soluble forms of VEGF are detectable in biologic fluids from cancer patients with the elevated levels (Yamamoto et al., 1996; Kraft et al., 1999). Circulating VEGF has been studied in many different cancers recently (Fujisaki

et al., 1998; Salven et al., 1998; Feldman et al., 2000; Poon et al., 2001). The 3 studies of circulating VEGF had revealed a high HR4.22 (95% CI 2.47-7.18) with small heterogeneity (p=0.84, I^2=0%). Whereas, the number of reports which were eligible for HR calculation is small and the cut-off value varied in different literature from 100 to 533 pg/mL in eligible reports and even as high as 1626 pg/mL in an excluded report (excluded because not eligible for calculation of HR) (Seo et al., 2010). From our perspective, the difference might come from calculation (considering platelets contents or not). Therefore, the result should be confirmed by an adequately designed prospective study. Obtaining tissue specimens requires an invasive biopsy or a surgery, while circulating biomarkers could be easily obtained from a minimal invasive. In our opinion, circulating VEGF may become a better parameter than tissue VEGF in predicting prognosis and a potential biomarker for predicting recurrence.

To conclude, VEGF, especially circulating VEGF may be a parameter in predicting prognosis in resected gastric cancer and inhibiting VEGF-mediated angiogenesis might be an effective treatment for gastric cancer. The first anti-VEGF drug (bevacizumab) was approved by the US Food and Drug Administration in 2004 (Ferrara et al., 2004). Thus, anti-VEGF drug may also play an important role in gastric cancer therapy. Indeed, bevacizumab has brought notable benefits to progression free survival in a Phase III Study for first-line therapy of gastric cancer when combined with chemotherapy (Jain et al., 2006).

The VEGF-C level in tissue and serum had proven increased in many malignant tumors, including gastric cancer (Ichikura et al., 2001; Han et al., 2010; Gou et al., 2011). VEGF-C binds to VEGFR-2 and VEGFR-3, which are predominantly expressed on vascular endothelial cells and lymphatic endothelial cells (Wissmann et al., 2006). The tissue VEGF-C expression or the serum level of these factors can predict lymph node metastasis and the prognosis of solid tumors. However the prognostic value of VEGF-C varied in different cancers (Shida et al., 2006; Zhan et al., 2009). In our report, we found the combined HR of tissue VEGF-C was 2.21(95% CI 1.58-3.09) in random effect model, the heterogeneity test result was (p=0.01, I^2=60%). This suggested that VEGF-C was related to poor prognosis of resected gastric cancer. We did not find visible character classification to deal the heterogeneity. Though the result was statistically significant, an included study from Lee et al. (2009) had an opposite result with the largest study size (371) among all the included studies in the meta-analysis. As a result, the research of VEGF-C needs an adequately designed prospective study to confirm.

A combined HR of tissue VEGF-D was OS (1.73, 95% CI 1.25-2.40), DFS (2.30, 95% CI 1.66-3.18). No significant heterogeneity was found in the groups. It suggested that tissue VEGF-D expression had a significant association with poor prognosis in resected gastric cancer. Thus, our result showed that VEGF-D may become a potential biomarker to predict gastric cancer prognosis.

Bias was probably introduced in to this meta-analysis, as the statistics of some studies were obtained from calculation based on the Kaplan-Meier survival curve instead of the given data. Fortunately, the survival curve estimate of the logHR appears to perform reasonably well except in a few cases (Parmar et al., 1998; Tierney et al., 2007). We conducted analyses for publication bias using Egger's and Begg's method. No statistically significant publication bias was found in analyses of outcome except in tissue VEGF group. In the meta-analysis for OS, we find the tissue VEGF group had a significant publication bia (Egger's, p=0.01, Begg's, p=0.043). We tried to reduce bias by conducting individual meta-analysis. As described above, VEGF positive staining definition, histological classification and tumor stage may be the heterogeneity source. However, our meta-analysis could not completely exclude biases. Besides, there were some the limitation in the meta-analysis, such as no adequate data for combination analysis after categorizing studies into subgroups and no identical definition of VEGF positive staining.

In conclusion, the meta-analysis suggested that the positive expression of tissue VEGF, circulating VEGF, VEGF-C and VEGF-D were all associated with poor prognosis of resected gastric cancer all over the world. In Asian population, VEGF was a predictor of poor prognosis for resected gastric cancer but in non-Asian population, VEGF was not. In addition, circulating VEGF may be better than tissue VEGF in predicting prognosis. These results should be confirmed by adequately multi-center designed prospective studies in future.

Acknowledgements

The author(s) declare that they have no competing interests.

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