

RESEARCH ARTICLE

Prognostic Impact of Elevation of Vascular Endothelial Growth Factor Family Expression in Patients with Non-small Cell lung Cancer: an Updated Meta-analysis

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Abstract

Background: The vascular endothelial growth factor family has been implicated in tumorigenesis and metastasis. The prognostic value of each vascular endothelial growth factor family member, particular VEGF/VEGFR co-expression, in patients with non-small lung cancer remains controversial. **Materials and Methods:** Relevant literature was identified by searching PubMed, EMBASE and Web of Science. Studies evaluating expression of VEGFs and/or VEGFRs by immunohistochemistry or ELISA in lung cancer tissue were eligible for inclusion. Hazard ratios (HRs) and 95% confidence intervals (CIs) from individual study were pooled by using a fixed- or random-effect model, heterogeneity and publication bias analyses were also performed. **Results:** 74 studies covering 7,631 patients were included in the meta-analysis. Regarding pro-angiogenesis factors, the expression of VEGFA (HR=1.633, 95% CI: 1.490-1.791) and VEGFR1 (HR=1.924, 95% CI: 1.220-3.034) was associated separately with poor survival. Especially, VEGFA over-expression was an independent prognostic factor in adenocarcinoma (ADC) (HR=1.775, 95% CI: 1.384-2.275) and SCC (HR=2.919, 95% CI: 2.060-4.137). Co-expression of VEGFA/VEGFR2 (HR=2.011, 95% CI: 1.405-2.876) was also significantly associated with worse survival. For lymphangiogenesis factors, the expression of VEGFC (HR=1.611, 95% CI: 1.407-1.844) predicted a poor prognosis. Co-expression of VEGFC/VEGFR3 (HR=2.436, 95% CI: 1.468-4.043) emerged as a preferable prognostic marker. **Conclusions:** The expression of VEGFA (particularly in SCC and early stage NSCLC), VEGFC, VEGFR1 indicates separately an unfavorable prognosis in patients with NSCLC. Co-expression VEGFA/VEGFR2 is comparable with VEGFC/VEGFR3, both featuring sufficient discrimination value as preferable as prognostic biologic markers.

Keywords: Vascular endothelial growth factor - vascular endothelial growth factor receptor - prognosis - NSCLC

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Introduction

Lung cancer is the leading cause of cancer-related mortality around the world (Alberg and Samet, 2003). Less than 15% of the patients will be cured and enjoy long-term survival. The poor prognosis has shown little improvement in recent decades (Molina et al., 2008). In light of disappointing therapeutic effect it is likely that the use of clinical or molecular markers will become important in predicting response to treatment and outcome. The main prognostic factors in NSCLC are disease stage, amount of weight lost, microvessel density, ERCC1, RRM1, BRCA1, p53, bcl-2, KRAS, Ki-67, (18)F-FDG, AKT, mTOR and EGFR mutation (Paesmans et al., 1995; Kaira and Yamamoto, 2010). However, these biological prognostic factors didn't well predict clinical outcome or their discriminate value is insufficient to predict the optimal therapeutic course for an individual. Therefore,

it is important to identify ideal predictive/prognostic biologic markers for patients undergoing treatment.

The hypothesis "Tumor growth is angiogenesis dependent" was first proposed by Folkman in 1971 (Folkman, 1971), which was confirmed by subsequent observations that tumors are strictly limited in size in the absence of neovascularization (Gimbrone et al., 1972). Angiogenesis, the formation of blood vessels from pre-existing vessels at a later stage (Ferrara and Kerbel, 2005), is critical for the development and subsequent growth of tumors and is a prerequisite for metastasis. The VEGF family comprises four ligands (VEGFA, VEGFB, VEGFC and VEGFD), which exhibit specific binding profiles with three transmembrane VEGF receptors (VEGFR1, 2 and 3) and promote intracellular tyrosine kinase cascades when activated (Ferrara, 2002; Hicklin and Ellis, 2005). VEGFA and its receptor (VEGFR1, VEGFR2) play a major role in physiological as well as pathological angiogenesis

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including tumor angiogenesis. While VEGFC/D and their receptor VEGFR3 can regulate angiogenesis at early embryogenesis but mostly function as critical regulators of lymphangiogenesis during lifetime (Alitalo and Carmeliet, 2002; He et al., 2005).

The effect of VEGFs and/or VEGFRs expression on survival in patients with NSCLC has been studied for over decades. However, conflicting results regarding the ability of VEGF to predict survival have been reported one by one from different laboratories. In this study, we sought to conduct a systematic review with meta-analysis to primarily estimate the prognostic impact of each VEGF family member for survival in NSCLC patients. The secondary goal of our study was to explore whether the VEGF/VEGFR co-expression with sufficient discriminate value as preferable predictive/prognostic biologic marker for an individual undergoing treatment.

Materials and Methods

Literature search

An electronic literature search was performed to identify potentially relevant published article in Pub Med, EMBASE, Web of Science. We used lung cancer/lung tumor/lung neoplasm/lung carcinoma and vascular endothelial growth factor/VEGF and prognosis/prognostic as searching terms. No lower date limit was used and last search was updated on May 9, 2014. All potentially eligible studies were retrieved. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki, which was approved by the ethics committee of Provincial Hospital Affiliated to Shandong University.

Inclusion and study criteria

To be eligible studies for inclusion in this meta-analysis had to meet the following inclusion criteria: (i) measure VEGF and/or VEGFRs expression in the primary lung cancer tissue with immunohistochemistry (IHC) or enzyme linked immune sorbent assay (ELISA); (ii) the study has sufficient data on survival for extracting; (iii) the same patient series was included in more than one publication, only the more recent or most complete study was included in the analysis.

In addition, the expression of different VEGFs and/or VEGFRs obtained from the same patient population by the same authors in different years was also included; There was no pre-specified sample size or follow-up period used to determine study inclusion. Criteria used to determine duplicate populations included study period, treatment information, and any additional inclusion criteria. Language was restricted for review title and abstract in English, but was not restricted for data collection.

Data extraction

Data were extracted using a predefined form, recording: first author, year of publication, number of patients, histology, disease stage, detective method, cut-offs and positive ratios of positive expression, outcome of univariate or multivariate analysis (HRs, 95% CIs) and the original author's results (Table 1). If the above data of any categories was not reported in the primary study, items

were treated as "NR" (not report). We did not contact the author of the primary study to request the information. The required information was extracted independently from primary studies by two reviewers (Chunlong Zheng and Chen Qiu) according to a standard data record worksheet designed in advance.

Statistical

All calculations were performed with the hazard ratios (HRs) and the associated 95% confidence intervals (CIs) of OS, RFS, DSS, or DFS. The most accurate and easiest method was to collect the reported HRs and CIs from primary articles directly. When these statistical variables were not given explicitly in an article, which were either extracted from the Kaplan-Meier survival curves indirectly or calculated, if available number (the total numbers of events and the total numbers of patients in each group) were given, assuming that the rate of censored patients was constant during the study follow-up. The method and spreadsheet used for these calculations were provided by Tierney et al (Tierney et al., 2007). HRs defined as the risk of death or progression for high expression vs low expression. In studies that reported HRs for low level vs high level, the reciprocal of the HR calculated and *p* value were taken to calculate the associated 95% CI for meta-analysis.

Heterogeneity of the individual HRs was performed with Q statistic test and I² statistic test. All of the studies included were categorized by VEGF and VEGFRs isoform, histology, disease stage, patient race. Individual meta-analysis was conducted in each subgroup. If HRs were found to have fine homogeneity (*p* (Q)>0.05, I²<56%) (Walter, 1997; Hardy and Thompson, 1998; Dwyer et al., 2001; Higgins and Thompson, 2002), a fixed effect model was used for secondary analysis; if not, a random-effect model was used. In this meta-analysis, Inverse Variance fixed effects and I-V heterogeneity random effects analysis were used to estimate the effect of VEGF family high expression on survival. By convention, an observed HR>1 implies worse survival with positive expression, and the impact on survival was considered to be statistically significant if the 95% CI didn't overlap with 1. Similarly, if HR<1 means good survival, and statistically significant if the 95% CI didn't overlap with 1. Horizontal lines represent 95% CIs. Each square 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. (1997) and Begg et al. (1994). Moreover, contour-enhanced funnel plot (Peters et al., 2008) was performed constructed to assess publication and/or selection bias. If studies appear to be missing in areas of low statistical significance, then it is possible that the asymmetry is due to publication bias. If studies seem to be missing in areas of high statistical significance, then publication bias is a less likely cause of the funnel asymmetry. Intercept significance was determined by the test suggested by Begg and Egger (*p*<0.05 was considered

Table 1. Characteristics and Results of the Eligible Studies

First Author	Year	Patients Source	No. of Pts	Histology	Stage	Method	Cut-off (%)	Positive (%)	HR Estimation	HR (95% CI)	Results
Kim	2013	south korea	75	NSCLC	I-IIB	IHC	20	VEGFA 28%	Report	0.86 (0.111-6..658)	+ve
Yurdakul	2012	Turkey	91	NSCLC	I-IV	IHC	CS	VEGFA 37.4%	Surv.curves	1.25 (0.80-1.96)	-ve
Wang	2012	China	210	NSCLC	III	IHC	CS	VEGFA 49.0%	Report	1.461 (1.015-2.104)	+ve
Starnes	2012	USA	102	NSCLC	I	IHC	50	KDR 49%	Report	1.258 (0.510-3.102)	-ve
Anagnostou	2011	Greece	97 59 47	NSCLC NSCLC ADC	I-IV I-III	IHC	CS	VEGFA 66.0% VEGFC 80.6% VEGFD 92.2% VEGFR3 94.2%	Report	A 1.4 (0.25-7.9) C 4 (1.11-14.27) D 0.61 (0.26-1.42) R3 0.85 (0.08-8.46) A 0.11 (0.002-5.2) C 10.6 (4.3-26.5) D 0.75 (0.23-2.4) R3 0.01 (0-0.34) A 8.6 (1.4-53.6)	-ve +ve -ve -ve -ve +ve -ve In-verse +ve
Ucvet	2011	Turkey	117	NSCLC	IA-IIIIB	IHC	25	VEGFA 70.1%	Surv.curves	0.92 (0.50-1.69)	-ve
Donnem	2011	Norway	335	NSCLC	I-IIIa	IHC	CS	A+R2 29%	Surv.curves	A+R2 1.85 (1.16-2.96)	+ve
Dai	2011	China	98	NSCLC	I-IIIa	IHC	CS	VEGFC 59.2%	Report	1.862 (1.464-2.386)	+ve
Chen	2011	Taiwan	49	NSCLC	I	IHC	10	VEGFC 49%	Report	3.98 (1.23-12.89)	+ve
Yamashita	2010	Japan	117	NSCLC	I	IHC	A 5 C 10	VEGFA 73.5% VEGFC 48.7%	Report	1.529 (0.711-3.289) 1.359 (0.708-2.604)	-ve -ve
Rades	2010	Germany	61	NSCLC	II-III	IHC	10	VEGFA 65.6%	Surv.curves	0.61 (0.26-1.46)	-ve
Lin	2010	China	185	NSCLC	I	IHC	30	VEGFA 49.7%	Report	1.83 (1.25-2.69)	+ve
Chen	2010	China	92	NSCLC	IIIa	IHC	30	VEGFC 65%	Report	2.523 (1.057-6.024)	+ve
Bircan	2010	Turkey	46	NSCLC	I-IV	IHC	NR	VEGFA 93.5%	Report	2.452 (0.457-13.166)	-ve
Sun	2009	China	82	NSCLC	I-IIIa	IHC	10	VEGFC 74.4%	DC	1.441 (0.511-4.066)	-ve
Carrillo	2009	Spain	48	NSCLC	I-IV	IHC	CS	VEGFD 22.9% VEGFR1 41.7%	Report	0.22 (0.08-0.67) 3.56 (1.56-8.1)	In-verse +ve
Bonnesen	2009	Denmark	102	NSCLC	I-IIIIB	IHC	CS	VEGFA 96.1% VEGFR2 93.1%	Surv.curves	0.85 (0.48-1.50) 0.73 (0.45-1.16)	-ve -ve

Table 1 (cont). Characteristics and Results of the Eligible Studies

Ko	2008	Korea	118	NSCLC	I-III	IHC	CS	VEGFC 60.2% VEGFD 52.5%	Surv.curves	0.65 (0.31-1.35) 0.95 (0.49-1.81)	-ve -ve
Kadota	2008	Japan	147	NSCLC	I-III	IHC	0.3	VEGFA 46.9% VEGFC 44.2%	Report	2.006 (1.144-3.519) 1.801 (1.024-3.167)	+ve +ve
Zhou	2007	China	118	NSCLC	I	IHC	25	VEGFA 36.5 %	DE	2.960 (1.551-5.650)	+ve
Yoo	2007	Korea	219	NSCLC	I-III	IHC	5	VEGFA 92.7%	Report	1.063 (0.365-3.096)	-ve
Yilmaz	2007	Turkey	50	NSCLC	I-IIIA	IHC	50	VEGFA 26%	Report	4.651 (1.538-14.069)	+ve
Saintigny	2007	France	92	NSCLC	I-III	IHC	CS	VEGFC 74% VEGFR3 42% C+R3 43.5%	Report	1.237 (0.644-2.376) 1.759 (0.876-2.512) 1.819 (0.925-3.578)	-ve -ve -ve
Ohta	2007	Japan	122	NSCLC	I	IHC	A 50 C 10	VEGFA 80.3% A+C 23%	Report	1.503 (1.048-2.117) 1.365 (0.964-1.966)	+ve +ve*
Donnem	2007	Norway	335	NSCLC	I-IIIA	IHC	CS	VEGFA:42% VEGFC:31% VE-FD:68% VEGFR1: 25.7% VEGFR2: 58% VEGFR3: 57%	Surv.curves	A 1.83 (1.33-2.54) C 1.43 (0.89-2.34) D 1.08 (0.58-2.01) R1: 1.66 (1.2-2.3) R2: 1.50 (1.07-2.09) R3: 1.686 (1.153-2.446)	+ve -ve -ve +ve +ve +ve
Maekawa	2007	Japan	55	ADC	I-III	ELI- SA	C 93.75 D 62.50 R3	NR	Report DC Report	2.306 (0.530-10.038) 0.294 (0.096-0.896) 0.371 (0.049-2.793)	-ve In-
Zhang	2006	China	42	NSCLC	I-IV	IHC	10	VEGFC 54.8%	Surv.curves	3.07 (1.22-7.69)	+ve
Seto	2006	Japan	60	NSCLC	I	IHC	10	VEGFA 30% ft-1 32% KDR 48% A+KDR23%	Surv.curves Surv.curves Surv.curves DE	3.29 (1.23-8.82) 1.96 (0.88-4.37) 2.24 (1.00-5.02) 2.26 (1.297-3.938)	+ve -ve +ve +ve
Enatsu	2006	Japan	78	ADC	I-III	IHC	10	VEGFA:68% VEGFC 42%	Report	0.35 (0.093-1.332) 0.471 (0.141-1.574)	-ve -ve
Tomita	2005	Japan	60	NSCLC	I-III	IHC	20	VEGFA 58%	Report	0.950 (0.729-2.484)	-ve
Renyi	2005	Hun- gary	103	NSCLC	I-IIIA	IHC	30	VEGFC 54.4%	DC	1.756 (0.627-2.724)	-ve

Table 1 (cont). Characteristics and Results of the Eligible Studies

Li	2005	China	126	NSCLC	I-IV	IHC	CS	VEGFA 77.8%	Surv.curves	1.81 (1.01-3.27)	+ve
Kim	2005	USA	74	NSCLC	I-II	IHC	CS	VEGFA 71.6%	DE	2.147 (0.939-4.907)	+ve*
Huang	2005	Japan	97 76	NSCLC	I II-III	IHC	CS	VEGFA 48.5% VEGFC 40.2% VEGFA 44.6% VEGFC 34.0%	Report	2.37 (1.07-5.24) 2.10 (1.01-4.37) 1.27 (0.69-2.35) 0.94 (0.54-1.64)	+ve +ve -ve -ve
Nishi	2005	Japan	100	ADC	I-III	IHC	NR	VEGFA 22%	Surv.curves	2.71 (1.41-5.21)	+ve
Kojima	2005	Japan	129	ADC	I	IHC	CS	VEGFA 45.7% VEGFC 43.4% VEGFR3 56.6% C+R3: 31.8%	Report	2.011 (0.942-4.293) 1.646 (0.760-3.565) 1.494 (0.602-3.705) 2.513 (1.315-4.801)	-ve -ve -ve +ve
Zhang L	2004	China	43	NSCLC	I-III	IHC	10	VEGFA 65.1%	Surv.curves	2.90 (1.04-8.07)	+ve
Yi	2004	China	84	NSCLC	I-IV	IHC	10	VEGFC 55.9% R3 59.5%	Report	1.005 (0.593-1.703) 3.370 (1.813-6.265)	-ve +ve
Tanaka	2004	Japan	162	NSCLC	I	IHC	CS	VEGFA 38.9%	Report	1.644 (0.815-3.314)	-ve
Ogawa	2004	Japan	125	NSCLC	I-IIIa	IHC	CS	VEGFC 60.7%	Report	1.724 (1.087-2.734)	+ve
Nakashima	2004	Japan	153 85 52	NSCLC ADC SCC	I-IIIb	IHC	30	VEGFA 51.0% VEGFC 41.8% VEGFA 54.1% VEG- FC45.9% VEGFA 51.0% VEGFC 40.4%	DE	2.012 (1.181-3.427) 1.460 (0.898-2.373) 3.816 (1.602-9.092) 2.024 (0.970-4.224) 0.913 (0.319-2.611) 3.946 (1.316-11.835)	+ve -ve +ve -ve -ve +ve
Mineo	2004	Italy	51	NSCLC	Ib-IIa	IHC	25	VEGFA 82.4%	Report	3.617 (1.054-14.610)	+ve
Li	2004	China	96	NSCLC	I-IIIb	IHC	CS	VEGFA 64.6%	Surv.curves	1.82 (1.01-3.29)	+ve
Iwasaki	2004	Japan	71	NSCLC	I-III	ELI- SA	18ng/	VEGFA 39%	Report	2.060 (1.024-4.778)	+ve
Saad	2004	USA	50	ADC	I	IHC	20	VEGFA 66%	Surv.curves	3.37 (1.39-8.16)	+ve
Liang	2003	China	55	NSCLC	I-IV	IHC	30	VEGFA 74.6%	Surv.curves	1.63 (1.18-2.25)	+ve
Li	2003	China	76	NSCLC	I-IV	IHC	10	VEGFC 72.4%	Surv.curves	2.55 (1.22-5.34)	+ve

Table 1 (cont). Characteristics and Results of the Eligible Studies

Arinaga	2003	Japan	180	NSCLC	I-III	IHC	30	VEGFC 76.1% VEGFR3: 22% C+R3: 22%	DC Report Surv.curves	1.554 (0.989-2.442) 1.270 (0.993-1.620) 2.32 (1.03-5.24)	-ve +ve* +ve
Ito	2002	Japan	80	AC	I-IV	IHC	10	VEGFA 40%	Surv.curves	1.30 (0.20-8.32)	-ve
Tanaka	2002	Japan	236	NSCLC	I-III	IHC	CS	VEGFA 34%	Report	1.380 (0.788-2.417)	-ve
Kojima	2002	Japan	94 38	ADC SCC	I	IHC	CS	VEGFA 60.6% VEGFA 60.5%	Report	3.374 (0.911-12.488) 1.541 (0.518-4.588)	-ve -ve
Inoshima	2002	Japan	132	NSCLC	I-IV	IHC	CS	VEGFA 49.2%	Report	2.327(1.260- 4.262)	+ve
Minami	2002	Japan	47	ADC	I	IHC	30	VEGFA 29.8%	Report	0.834(0.386- 1.371)	-ve
Shou	2001	Japan	119	NSCLC	I-III	IHC	NR	VEGFA 63.9%	Surv.curves	1.77 (0.92- 3.41)	-ve
Osaki	2001	Japan	33	SCC	NR	IHC	NR	VEGFA 36.4%	Surv.curves	4.56 (1.15-18.13)	+ve
Niklinska	2001	Poland	89	NSCLC	I-IIIa	IHC	70	VEGFA 20%	Report	2.56 (1.01-6.49)	+ve
Masuya	2001	Japan	104 34 59	NSCLC SCC ADC	I-IIIB	IHC	30	VEGFA 51.9%	DE	1.944 (1.028-3.678) 9.730 (2.298-41.194) 0.819 (0.322-2.083)	+ve +ve -ve
Liao	2001	China	127	NSCLC	I-III	IHC	25	VEGFA 40.9%	Report	1.176 (0.687-2.013)	-ve
Kang	2001	Korea	61	NSCLC	I-IIIB	IHC	25	VEGFA 88.5%	Report	1.083 (0.324-3.626)	-ve
Kajita	2001	Japan	62	NSCLC	I-IV	IHC	5	VEGFC 38.7%	Report	2.24 (0.860-5.830)	-ve
Han	2001	USA	85	NSCLC	I	IHC	20	VEGFA 71%	Report	1.82 (1.19-4.33)	+ve
Yano	2000	Japan	108	NSCLC	I-IV	IHC	25	VEGFA 45%	DE	0.487 (0.229-1.037)	-ve
Sheng	2000	Japan	98 57 31	NSCLC ADC SCC	I-IV	IHC	5	VEGFA 51% VEGFA 49.1% VEGFA 61.3%	Surv.curves	3.27 (1.41-7.67) 5.12 (1.02-25.76) 4.86 (1.02-22.86)	+ve +ve +ve
Ohta	2000	Japan	122	NSCLC	I	IHC	10	VEGFC 45.1%	Report	1.34 (0.58-3.10)	-ve
Odaka	2000	Japan	67	NSCLC	I	IHC	20	VEGFA 38.8%	DE	10.744 (2.386-48.660)	+ve
O'Byrne	2000	UK	223	NSCLC	I-III	IHC	70	VEGFA 46.6%	DE	1.63 (1.080-2.460)	+ve
Konishi	2000	Japan	181	NSCLC	I- IIIB	IHC	CS	VEGFA 25.4%	Report	1.771 (1.080-2.905)	+ve
Oshika	1998	Japan	84 25	NSCLC SCC	NR	IHC	NR	VEGFA 40% VEGFA 32.0%	Surv.curves	3.95 (1.29-12.12) 3.17 (1.33-7.53)	+ve +ve

Table 1 (cont). Characteristics and Results of the Eligible Studies

Imoto	1998	Japan	91 34	NSCLC SCC	I-III	IHC	5	VEGFA 53% VEGFA 38.2%	Report Surv.curves	2.590 (1.349-4.973) 3.67 (1.91-7.07)	+ve +ve
Giatromanola	1998	Greece	114	NSCLC	I-III	IHC	NR	VEGFA 68%	Surv.curves	1.56 (1.02-2.38)	+ve
Shibusu	1998	Japan	44	ADC	I	IHC	CS	VEGFA 61.3%	Surv.curves	3.65 (1.01-13.25)	+ve
Volm	1997	Germany	109	SCC	I-IIIa	IHC	CS	VEGFA 59% flt-1 68%	Surv.curves	2.76 (1.18- 6.40) 1.14(0.54-2.41)	+ve -ve
Fontanin	1997	Italy	104	NSCLC	I-III	IHC	40	VEGFA 44%	Surv.curves	2.61 (1.01- 6.73)	+ve
Takanami	1997	Japan	118	ADC	I-IV	IHC	NR	VEGFA 58%	Report	1.54 (0.91- 2.67)	+ve*

*VEGF,vascular endothelial growth factor. NSCLC,non-small cell lung cancer; adenocarcinoma; AC, adenocarcinoma; SCC, squamous cell carcinoma. IHC, immunohistochemistry; ELISA, enzyme linked immunosorbent assay. NR, not reported. CS, complex score combining intensity and percentage. OS, overall survival; RFS,recurrence-free survival; DFS,disease free survival; DSS, disease-specific survival. DE: data extrapolated; DC, data of +/- convert +/- .Results, author's results; Positive, there was an inverse relationship between VEGFs and survival(poor prognosis); Inverse, there was a direct relationship between VEGFs and survival(good prognosis); -ve, there is no relationship. *,marginal value

represent significant statistically publication bias). All calculations were performed using STATA version 12.0 (Stata Corp., College Station, TX, USA).

Results

Literature search

The results of the literature research were presented in Figure 1. The initial search strategy identified 2470 potential relevant studies. 2302 of which were excluded after the initial review of their titles and abstracts: 633 articles duplicated; 767 had no direct link with the main subject (i.e. other tumors, indicators or not relevant articles); 392 of them were reviews and editorials; 423 articles of them were drug treatment, and 87 were case reports or case series. Then 168 full manuscripts were retrieved for detailed evaluation. 94 studies were excluded because of the lack of sufficient data (n=36), serum level (n=43), molecular level (n=13) and duplicated (n=2). Finally, 74 studies (Fontanini et al., 1997; Takanami et al., 1997; Volm et al., 1997; Giatromanolaki et al., 1998; Imoto et al., 1998; Oshika et al., 1998; Shibusu et al., 1998; Konishi et al., 2000; O'Byrne et al., 2000; Odaka et al., 2000; Ohta et al., 2000; Sheng et al., 2000; Yano et al., 2000; Han et al., 2001; Kajita et al., 2001; Kang et al., 2001; Liao et al., 2001; Masuya et al., 2001; Niklinska et al., 2001; Osaki et al., 2001; Shou et al., 2001; Inoshima et al., 2002; Ito et al., 2002; Kojima et al., 2002; Minami et al., 2002; Tanaka et al., 2002; Arinaga et al., 2003; Li et al., 2003; Liang et al., 2003; Iwasaki et al., 2004; Li et al., 2004; Mineo et al., 2004; Nakashima et al., 2004; Ogawa et al., 2004; Saad et al., 2004; Tanaka et al., 2004; Yi and Pan, 2004; Zhang et al., 2004; Huang et al., 2005; Kim et al., 2005; Kojima et al., 2005; Li et al., 2005; Nishi et al., 2005; Renyi-Vamos et al., 2005; Tomita et al., 2005; Enatsu et al., 2006; Seto et al., 2006; Zhang et al., 2006; Donnem et al., 2007; Maekawa et al., 2007; Ohta et al., 2007; Saintigny et al., 2007; Yilmaz et al., 2007; Yoo et

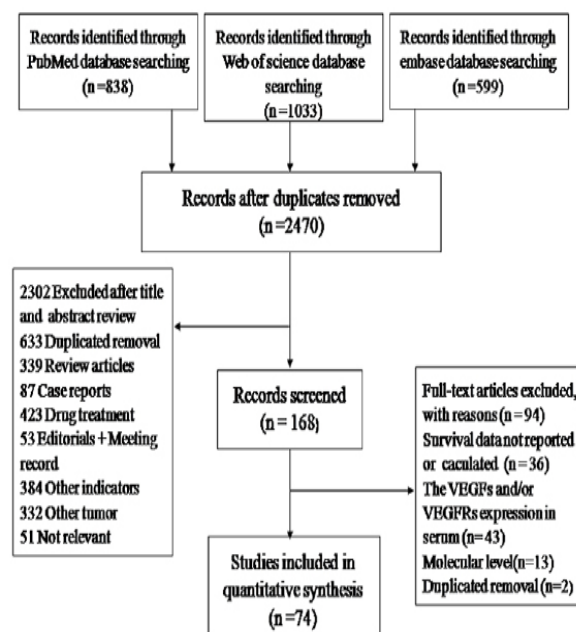


Figure 1. Flow Chart of Review Relevant Article for Meta-analysis

al., 2007; Zhou et al., 2007; Kadota et al., 2008; Ko et al., 2008; Bonnesen et al., 2009; Carrillo de Santa Pau et al., 2009; Sun et al., 2009; Bircan et al., 2010; Chen et al., 2010; Lin et al., 2010; Rades et al., 2010; Yamashita et al., 2010; Anagnostou et al., 2011; Chen et al., 2011; Dai et al., 2011; Donnem et al., 2011; Ucvet et al., 2011; Starnes et al., 2012; Wang et al., 2012; Yurdakul et al., 2012; Kim et al., 2013b) published between 1997 and 2014 were included according to the inclusion and exclusion criteria.

Baseline characteristics

The main characteristics of the studies included in the meta-analysis were shown in Table 1. A total 74 studies comprising 7631 patients were included in this meta-analysis. All studies reported the prognostic value

of VEGFs and/or VEGFRs status for survival in NSCLC patients, which total number of patients included was 7631, ranging from 33 to 335 patients per study (median, 98). These publications fall into several different patient cohorts: 61 studies dealt with all types of NSCLC, 12

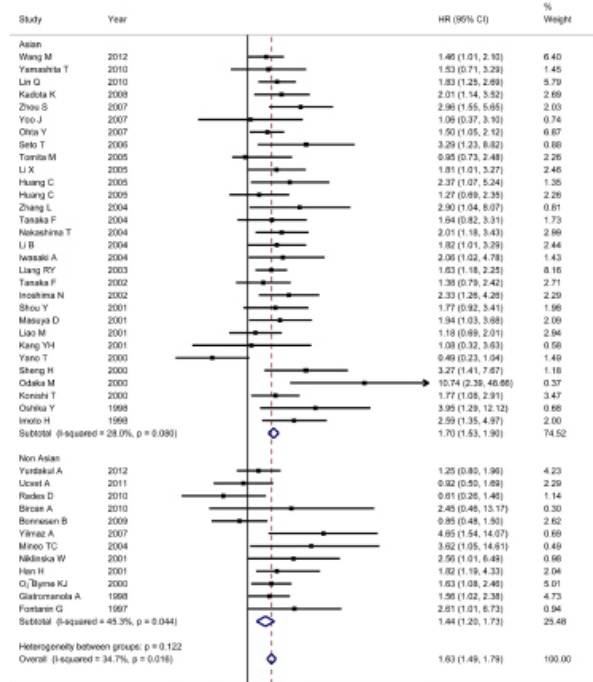


Figure 2. Hazard Ratio for Overall Survival of NSCLC Patients Stratified by Ethnicity with VEGFA Expression

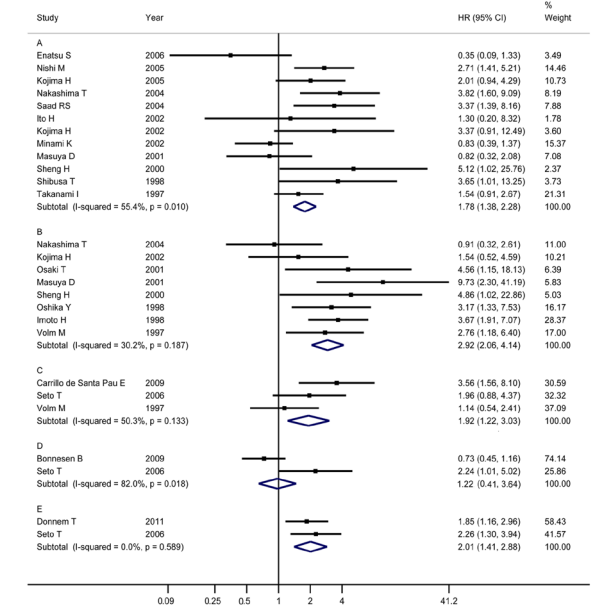


Figure 3. Hazard Ratio for Overall Survival of Different Patients with VEGFs or VEGFRs Expression. A) Hazard ratio of VEGFA expression in ADC; B) Hazard ratio of VEGFA expression in SCC; C) Hazard ratio of VEGFR1 expression in NSCLC; D) Hazard ratio of VEGFR2 expression in NSCLC; E) Hazard ratio of VEGFA/VEGFR2 co-expression in NSCLC

studies dealt with ADC, 8 studies dealt with SCC. In all patients with NSCLC, surgery was performed as the

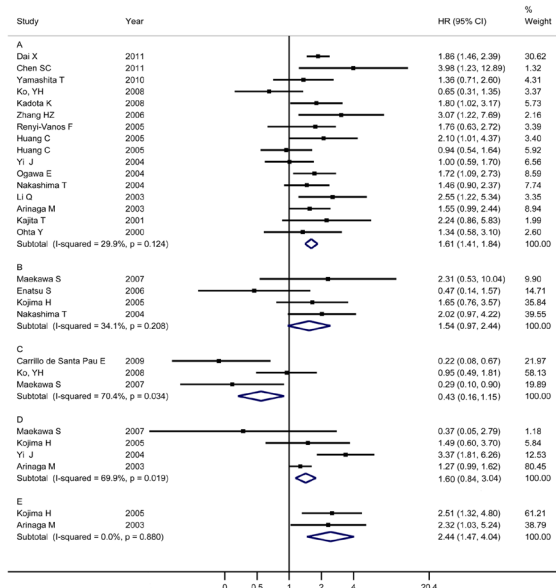


Figure 4. Hazard Ratio for Overall Survival of NSCLC Patients with VEGFC Expression. A) Hazard ratio of VEGFC expression in NSCLC; B) Hazard ratio of VEGFC expression in ADC; C) Hazard ratio of VEGFRD expression in NSCLC; D) Hazard ratio of VEGFR3 expression in NSCLC; E) Hazard ratio of VEGFC/VEGFR3 co-expression in NSCLC

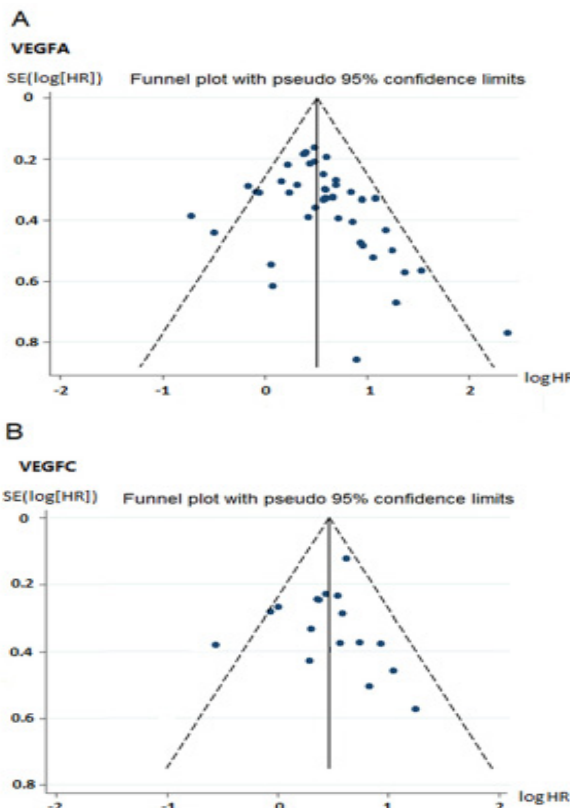


Figure 5. Funnel Plots Showing Publication Bias. A) Publication bias for VEGFA expression in NSCLC, p=0.006 (Begg's), p=0.045 (Egger's); B) Publication bias for VEGFC expression in NSCLC, p=0.344 (Begg's), p=0.996 (Egger's)

foremost treatment measure. Seventy-two studies used IHC and two studies used ELISA to determine VEGFs and/or VEGFRs expression. Among the 41 studies evaluating VEGFA expression in NSCLC, 29 studies (3525 patients, 75.68%) were performed in Asian populations, the remaining 12 studies (1133 patients, 24.32%) followed European or American patients. The proportion of patients exhibiting VEGFA expression in individual studies ranged from 20 to 96.1% by IHC. The rest of VEGFC, VEGFD, VEGFR1, VEGFR2 and VEGFR3 were detected in 20, 5, 4, 4 and 7 studies, respectively (Table 2).

Angiogenesis (VEGFA, VEGFR1, VEGFR2) and prognosis

The combined HR of VEGFA expression in NSCLC (n=41) was recorded as 1.633 (95%CI: 1.490-1.791, Q=62.76, $p=0.016$, $I^2=34.7\%$, Figure 2), indicating that positive immunostaining for VEGFA was significantly associated with adverse survival in the pooled patient group overall. When grouped according to the territorial scope of each studies, 1.704 (95%CI: 1.532-1.896) in Asian and 1.443 (95%CI: 1.202-1.732) in non-Asian (Table 2); When grouped according to the stage of NSCLC, the pooled HR was 1.887 (95%CI: 1.554-2.292), 1.552 (95%CI: 1.373-1.754), 1.683 (95%CI: 1.165-2.431) for stage I, I-III, I-IV, respectively (Table 2). The combined HR of stage I (1.887) was larger than stage I-III (1.552) and stage I-IV (1.683), suggesting that VEGFA expression could be an important prognostic factor for early stage NSCLC.

Studies evaluating VEGFA levels were separate aggregated to ADC and SCC for subgroup analyses, A statistically significantly correlation with survival was observed, particularly in SCC. The pooled HR was 1.775 (95%CI: 1.384-2.275, Q=24.65, $p=0.010$, $I^2=55.4\%$, Figure 3A) in ADC (n=12), whereas 2.919 (95%CI: 2.060-4.137, Q=10.03, $p=0.187$, $I^2=30.2\%$, Figure 3B) in SCC (n=8).

With regard to the effects of the VEGFR1 expression on survival in NSCLC (n=4), the pooled HR was 1.745 (95%CI: 1.339-2.274, Q=4.30, $p=0.231$, $I^2=30.2\%$, Table 2), and taken the one DSS included out (n=3), the combined HR was 1.924 (95%CI: 1.220-3.034, Q=4.03, $p=0.133$, $I^2=50.3\%$, Figure 3C), suggesting that VEGFR1 high expression is significantly associated with low survival rates.

Interestingly, highly significant statistically influence of VEGFA/VEGFR2 co-expression on survival in NSCLC patients was detected, with the combined HR was 2.011 (95%CI: 1.405-2.876, Q=0.29, $p=0.589$, $I^2=0.0\%$, Figure 3E). However, statistically significant effect of VEGFR2 expression on survival wasn't observed, the pooled HR was 1.270 (95% CI: 0.793-2.034, Q=8.13, $p=0.043$, $I^2=63.10\%$, Table 2) for overall, and 1.218 (95%CI: 0.408-3.640, Q=5.57, $p=0.018$, $I^2=82.0\%$, Figure 3D) for OS.

Lymphangiogenesis (VEGFC, VEGFD, VEGFR3) and prognosis

The pooled results from the 20 studies evaluating VEGFC expression in NSCLC was 1.609 (95%CI: 1.420-1.823, Q=25.29, $p=0.191$, $I^2=20.9\%$, Table 2), and 1.611 (95%CI: 1.407-1.844, Q=21.41, $p=0.124$, $I^2=29.9\%$,

Figure 4A) when five non-OS excluded, indicating that VEGFC expression was an indicator of poor prognosis for NSCLC patients. When the four studies investigating VEGFC expression limited to the patients with ADC, which statistically significant effect on survival wasn't observed, with the pooled HR 1.536 (95%CI: 0.967-2.440, Q=4.55, $p=0.208$, $I^2=34.1\%$, Figure 4B).

The combined HR of seven eligible studies evaluating VEGFR3 expression in NSCLC was 1.513 (95%CI: 1.267-1.808, Q=11.10, $p=0.085$, $I^2=46\%$, Table 2), and when three non-OS excluded, the pooled HR was 1.596 (95%CI: 0.837-3.045, Q=9.97, $p=0.019$, $I^2=69.9\%$, Figure 4D), which statistically significant effect on survival wasn't detected. In addition, the data collected was not sufficient to determine the prognostic value of VEGFC in patients with ADC and SCC, separately.

To our surprise is the VEGFC/VEGFR3 co-expression was highly significant prognostic value in NSCLC, with the pooled HR was 2.436 (95%CI: 1.468-4.043, Q=0.020, $p=0.880$, $I^2=0.0\%$, Figure 4E).

With regard to the effects of the VEGFD expression on survival in NSCLC (n=5), the pooled result was a marginal value (HR=0.596, 95%CI: 0.336-1.058, Q=9.68, $p=0.046$, $I^2=58.7\%$, Table 2). When the two DSS excluded (n=3), the combined HR was 0.427 (95%CI: 0.159-1.150, Q=6.75, $p=0.034$, $I^2=70.4\%$, Figure 4C). These results show that VEGFD expression was more likely associated with good outcome. Unfortunately, the more sufficient data were required to determine the prognostic value of VEGFD expression.

Heterogeneity analysis

This systematic review with meta-analysis was inspected by heterogeneity test. Highly significant heterogeneity was found among 8 studies of stage I-IV NSCLC with VEGFC expression, 4 studies of NSCLC with VEGFR2 expression, 5 studies of NSCLC with VEGFD expression, 7 studies of NSCLC with VEGFR3 expression (Table 2).

Publication bias

The publication bias in the literature was quantitative evaluated by Begg's and Egger's test. The absence of publication bias was found in 15 studies investigating VEGFC expression in patients with NSCLC, with a Begg's test score of $p=0.344$ and an Egger's test score of $p=0.996$ (Figure 5B). Similar results were found in the four studies with VEGFR1 expression ($p=0.308$ and 0.671), four studies including patients with VEGFR2 expression ($p=0.734$ and 0.929) and eight studies investigating VEGFR3 expression in patients with NSCLC ($p=0.764$ and 0.872).

However, the funnel plot revealed an apparent asymmetry in 41 eligible studies investigating NSCLC patients with VEGFA expression ($p=0.006$ and 0.045) (Figure 5A) and five studies investigating VEGFD expression ($p=0.086$ and 0.004), suggesting the presence of a potential publication bias. The bias of publication could be explained by a language bias, inflated estimates by a flawed methodological design in smaller studies, what really counts is lack of publication of trials with opposite

Table 2. Overall and Stratified Analysis on the Association of VEGF Family with NSCLC Prognosis, Heterogeneity and Publication Bias

	No. of study	No. of Pts	HR (95% CI)		Heterogeneity Test		Publishi bias		Survival index	
			fixed Effects (p)		Q, p value	I ²	Begg's Test	Egger's test		
			Random Effects (p)							
Proangiogenesis Factors										
VEGFA										
NSCLC	45	5239	1.650(1.511-1.801); 0.000	1.650(1.511-1.801); 0.000	64.01	0.033	29.70%	0.028	0.124	Overall OS
Asian	41	4658	1.633(1.490-1.791); 0.000	1.633(1.490-1.791); 0.000	40.26	0.016	34.70%	0.006	0.045	
Non-Asian	29	3525	1.704(1.532-1.896); 0.000	1.704(1.532-1.896); 0.000	20.11	0.080	28.00%			
I	12	1133	1.443(1.202-1.732); 0.000	1.443(1.202-1.732); 0.000	10.60	0.044	45.30%			
I-III	9	1013	1.887(1.554-2.292); 0.000	1.887(1.554-2.292); 0.000	31.53	0.139	24.50%			
I-IV	25	2905	1.552(1.373-1.754); 0.000	1.552(1.373-1.754); 0.000	17.81	0.013	23.90%			
	8	740	1.824(1.360-2.447); 0.000	1.824(1.360-2.447); 0.000	0.76	0.859	60.70%			
ADC	4	581	1.775(1.384-2.275); 0.000	1.775(1.384-2.275); 0.000	24.65	0.010	0.00%			
SCC	12	941	2.919(2.060-4.137); 0.000	2.919(2.060-4.137); 0.000	10.03	0.187	55.40%	1	0.637	Non-OS OS OS
VEGFR1	8	356	1.745(1.339-2.274); 0.000	1.745(1.339-2.274); 0.000	4.30	0.231	30.20%	0.308	0.671	One DSS included OS
NSCLC	4	552	1.924(1.220-3.034); 0.005	1.924(1.220-3.034); 0.005	4.03	0.133	50.30%			
VEGFR2	3	217	1.270(0.793-2.034); 0.319	1.270(0.793-2.034); 0.319	8.13	0.043	63.10%	0.734	0.929	Overall OS
NSCLC	2	217	1.218(0.408-3.640); 0.724	1.218(0.408-3.640); 0.724	5.57	0.018	82.00%			
VEGFA+VEGF2	2	395	2.011(1.405-2.876); 0.000	2.011(1.405-2.876); 0.000	0.29	0.589	0.00%			One DSS included
Lymphangiogenesis Factor										
VEGFC										
NSCLC	20	2347	1.609(1.420-1.823); 0.000	1.609(1.420-1.823); 0.000	25.29	0.191	20.90%	0.097	0.642	Overall OS
I	15	1649	1.611(1.407-1.844); 0.000	1.611(1.407-1.844); 0.000	21.41	0.124	29.90%	0.344	0.996	
I-III	4	385	1.739(1.170-2.584); 0.006	1.739(1.170-2.584); 0.006	3.09	0.379	2.80%			
I-IV	8	1000	1.581(1.352-1.850); 0.000	1.581(1.352-1.850); 0.000	11.22	0.129	37.60%			
ADC	4	264	1.597(1.154-2.209); 0.000	1.597(1.154-2.209); 0.000	6.87	0.076	56.30%			
VEGFD	5	698	1.536(0.967-2.440); 0.208	1.536(0.967-2.440); 0.208	3.87	0.423	0.00%			
NSCLC	4	347	1.903(1.069-3.389); 0.029	1.903(1.069-3.389); 0.029	4.55	0.208	34.10%			Non-OS OS
VEGFR3	5	653	0.596(0.336-1.058); 0.077	0.596(0.336-1.058); 0.077	9.68	0.046	58.70%	0.086	0.004	Two DSS included OS
NSCLC	3	221	0.427(0.159-1.150); 0.092	0.427(0.159-1.150); 0.092	6.75	0.034	70.40%	1	0.142	
VEGFC+VEGF3	7	972	1.513(1.267-1.808); 0.000	1.513(1.267-1.808); 0.000	11.10	0.085	46.00%	0.764	0.872	Overall OS
NSCLC	4	448	2.193(1.462-3.290); 0.000	2.193(1.462-3.290); 0.000	9.97	0.019	69.90%	1	0.851	
NSCLC	3	401	2.436(1.468-4.043); 0.001	2.436(1.468-4.043); 0.001	0.48	0.786	0.00%	1	0.976	One DSS included OS
	2	272			0.02	0.882	0.00%			

*NSCLC, non-small cell lung cancer; ADC, adenocarcinoma; SCC, squamous carcinoma. VEGFA,C,D; VEGFR1,2,3; vascular endothelial growth factor receptor 1,2,3.OS: overall survival, DSS: disease specific survival, Non-OS: non-overall survival, including RFS, DSS, or DFS. Boldface, effect model selected of statistical analysis according to the corresponding evidence

results (Table 2).

Discussion

Our meta-analysis showed that high VEGFs and/or VEGFRs expression did indeed predict poor survival in patients with NSCLC. For angiogenesis, our result clearly indicated that VEGFA expression has a significant correlation with poor survival in patients with NSCLC. When the analyses were restricted to the stages of NSCLC, VEGFA expression could be an important prognostic factor for early stage NSCLC (HR=1.887 for stage I). When the analyses were restricted to the histologies of NSCLC, a significant prognostic significance (HR=2.919) was found in lung SCC patients. Data analysis revealed that VEGFR1 expression associated with low survival rate, but the VEGFR2 expression wasn't detected statistically significant effect on survival in NSCLC patients. For lymphangiogenesis, the expression of VEGFC predicted a poor prognosis in NSCLC patients. However, the VEGFC overexpression was only associated with poor survival. Unfortunately, neither the VEGFC expression nor the VEGFR3 expression was sufficient to determine the prognostic value in lung SCC patients.

The emergence of the targeted therapies for NSCLC has generated a need for accurate histologic subtyping of NSCLC (Kim et al., 2013a) because of the different clinicopathological and molecular characteristics of ADC and SCC (Miller et al., 2004; Inamura et al., 2010). Patients with SCC were not recommended to receive bevacizumab (Avastin) because of a 30% mortality rate due to fatal hemorrhage (Johnson et al., 2004; Cohen et al., 2007; Yan et al., 2011; Stead et al., 2012). There were very few previous literatures reported the prognostic impact in lung SCC patient with VEGFA expression. Our pooled analysis showed that VEGFA overexpression was associated with worse survival in patients with SCC (HR=2.919; 95%CI: 2.060-4.137). Therefore, the severe bleeding may be a significant response. Several ongoing clinical randomized controlled trials do include squamous participants (Schiller et al., 2009; Spratlin et al., 2010; Sternberg et al., 2010; Doebele et al., 2012) in recent years. The role of angiogenesis inhibition in the adjuvant setting is currently being tested by the ECOG (Eastern Cooperative Oncology Group) 1505 trial, in which NSCLC patients (including 31% SCC) with completely resected tumors are randomly assigned to chemotherapy alone or in combination with bevacizumab. The safest setting to pursue further evaluation of bevacizumab in patients with SCC seems to be after surgical resection. Our conclusion supports the new viewpoint that anti-VEGF therapies may be a reliable targeted therapy for postoperative SCC patients.

There were very few previous literatures reported the prognostic impact of VEGF/VEGFR co-expression in patients with NSCLC. Our meta-analysis explored the prognostic impact of VEGF/VEGFR co-expression on survival in patients with NSCLC. The combined HR of VEGFA/VEGFR2 co-expression in NSCLC was recorded as 2.011 (95%CI: 1.405-2.876, Q=0.29, p=0.589, I²=0.0%), indicating that positive immunostaining for

VEGFA/VEGFR2 co-expression was significantly associated with adverse survival. The VEGFC/VEGFR3 co-expression also had highly significant prognostic value in NSCLC, with the pooled HR was 2.436 (95%CI: 1.468-4.043, Q=0.020, p=0.880, I²=0.0%). Empirically, HRs>2 are considered strongly predictive (Hayes et al., 2001). In a word, both VEGFA/VEGFR2 co-expression and VEGFC/VEGFR3 co-expression were sufficient discriminate value as preferable prognostic biologic marker for an individual undergoing treatment.

Our data were consistent with the three previous meta-analysis (Delmotte et al., 2002; Zhan et al., 2009; Jiang et al., 2014), which separately included 15, 51, 16 studies. Besides, the previous analyses were insufficient to determine the prognostic value of VEGFD, VEGFRs and VEGF/VEGFR co-expression in NSCLC. We have improved these deficiencies by incorporating more related studies in recent years. What is more, we have found the several biologic markers (i.e. VEGFA expression in SCC, VEGFA/VEGFR2 co-expression in NSCLC, VEGFC/VEGFR3 co-expression in NSCLC, etc.) with preferable discriminate value for an individual prognosis.

In addition, heterogeneity and potential publication bias were assessed in accordance with published guidelines. There were several potential sources of heterogeneity: the different of baseline characteristics of patients included (age, tumor size, and stage), the adjuvant treatment they might have received; the duration of follow-up, the differences in the cutoff value (5%, 10%, 20%, 25%, 50%, complex scores) of IHC method used, the distribution of immunostaining used for scoring was not explicitly stated in the text (i.e. cytoplasmic, membranous, nuclear, stromal, etc.), the primary antibody used wasn't identical, the different criteria used for immunohistochemical classification. But the Der Simonian and Laird method we used (random effect model) took them into account (DerSimonian and Laird, 1986).

The extract method of HRs and 95% CI was one potential source of bias. The exact value of VEGF and/or VEGFRs expression status requires to be determined by appropriate multivariate analysis. Data for multivariate survival analysis reported were included in the present systematic review with meta-analysis; if these data were not available, we extrapolated them from the survival curves as univariate analysis incorporated, necessarily making assumptions about the censoring process. The choice of study and detected method was another potential source of bias. Firstly, the prognostic value should be confirmed by adequately designed prospective study. Unfortunately, few prospectively designed prognostic studies concerning biomarkers have been reported. Our collected many retrospective studies to reveal their clinical significance. Secondly, the variability of IHC method was an inconvenient source of selection bias. In future studies, the assessment of these prognostic factors should be better standard, especially for patients whom adjuvant therapy is recommended (Vermeulen et al., 2002).

The major concern for all forms of meta-analysis is publication bias (Dubben and Beck-Bornholdt, 2005). The present analysis found significant publication bias among 47 studies of NSCLC patients with VEGFA expression,

which funnel plot revealed an apparent asymmetry. The bias of publication could be explained by a language bias, inflated estimates by a flawed methodological design in smaller studies. Nevertheless, the most important point is a lack of publication of trials with opposite results. We attempted to minimize publication bias by making our literature search as complete as possible, using three databases (PubMed, EMBASE and Web of Science). The various published studies, which conclusions were discrepancy, could have encouraged researchers to publish their data whether positive or negative, such publication bias will be limited.

In summary, the expression of VEGFA (particularly in SCC and early stage NSCLC), VEGFC and VEGFR1 indicates an unfavorable impact on survival of patients with NSCLC, respectively. However, the expression of VEGFD seems to have no significant impact on survival of NSCLC patients, and the VEGFR2 expression also wasn't associated with low survival rate in our meta-analysis. Furthermore, the co-expression of VEGFA/VEGFR2, VEGFC/VEGFR3 reveals sufficient discriminate value for an individual as preferable prognostic biologic markers. These results should be confirmed by adequately further study. The findings of this study will encourage more people to identify the VEGF/VEGFR co-expression as ideal prognostic indicators in clinical practice in the future.

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