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VEGF overexpression is associated with optic nerve involvement and differentiation of retinoblastoma

A PRISMA-compliant meta-analysis

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Abstract

Background: Vascular endothelial growth factor (VEGF) plays an important role in the pathogenesis of cancer. Although numerous studies have investigated the association between VEGF expression and pathogenesis of retinoblastoma, the results remained inconsistent. To illuminate the association, we performed a meta-analysis study.

Methods: According to the PRISMA guideline, eligible studies were searched in the Medicine, Embase, Web of Science, Chinese National Knowledge Infrastructure, and Wanfang databases. Stata 14.0 software was used to calculate the relevant statistical parameters.

Results: Seventeen studies with 296 controls and 470 patients with retinoblastoma were included from 17 eligible literatures. Overall, significant association between VEGF overexpression and susceptibility of retinoblastoma was observed in Chinese population (odds ratio [OR] = 21.67, 95% confidence interval [CI] = 13.96–33.62). Subgroup analysis based on control sample type showed that VEGF overexpression was significantly associated with the risk of retinoblastoma (Normal retina tissue, OR = 23.97, 95% CI = 9.67–59.42; retinoblastoma adjacent tissue, OR = 20.85, 95% CI = 12.64–34.37). Significant associations of VEGF overexpression with optic nerve involvement and differentiation of retinoblastoma were found (Optic nerve involvement, OR = 6.90, 95% CI = 4.01–11.88; Differentiation, OR = 0.18, 95% CI = 0.12–0.28). In addition, only 1 study was included to analyze the role of VEGF protein expression in the prognosis of retinoblastoma, and the result showed that VEGF expression was significantly associated with the prognosis of retinoblastoma, which should be verified in the future studies.

Conclusions: Our findings demonstrated that VEGF overexpression was significantly associated with the risk of retinoblastoma. Besides, the results suggested that VEGF overexpression might have a crucial effect on the optic nerve involvement and differentiation of retinoblastoma.

Abbreviations: CIs = confidence intervals, CNKI = Chinese National Knowledge Infrastructure, IHC = immunohistochemistry, KIF14 = kinesin family member 14, NOS = Newcastle–Ottawa scale, ORs = odds ratios, OS = overall survival, RBL2 = member retinoblastoma-like 2, VEGF = vascular endothelial growth factor.

Keywords: clinical characteristics, expression, prognosis, retinoblastoma, vascular endothelial growth factor

1. Introduction

Retinoblastoma is the most common intraocular cancer of childhood with approximately 8000 new cases diagnosed in the word every year.[1] Retinoblastoma is classified into 2 clinical forms: bilateral tumor which accounts for approximately 75% of the patients with retinoblastoma and unilateral tumor which accounts for 25% of the cases.[2] The symptom of leukocoria is found in more than half of the children with retinoblastoma, while strabismus is another common sign and is related with macular involvement.[3] In addition, advanced intraocular tumors may be painful due to secondary glaucoma.[3] Other symptoms are also found in the diagnosis of retinoblastoma such as: retrolental fibrodysplasia, persistent hyperplastic primary vitreous, congenital cataracts, and Coats disease.[4] Recently, numerous studies have been carried out to explore the molecular mechanisms of retinal tumorigenesis. According to the results, the germline mutations of RB1 gene might be the main cause of bilateral retinoblastoma, and the mutations of RB1 gene were inherited from parents in 25% of the cases. Approximately 75% children with retinoblastoma possessed the mutations of RB1 gene as new germline mutation and did not have positive family history.[5] It has been reported that the germline mutations of RB1 gene leaded to the most bilateral tumors. In the whole-genome sequencing of parents' blood cells, RB1 somatic mutations were detected in the majority of retinoblastoma cases, and unilateral tumor accounted for 75% cases.[5] However, most of retinoblastoma in parents were early stage, and the progress of

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the retinoblastoma was unclear.[5] Although the germline mutations of RB1 gene increased the risk of retinoblastoma, other genetic variations and epigenetic changing might affect the progress of retinoblastoma. In fact, single RB1 gene mutation did not directly result in the occurrence of retinoblastoma; other gene mutations such as Rbl1, Rbl2, and Cdkn1b were required in the establishment of mouse retinoblastoma model.[6] Therefore, the inactivation of RB1 gene and other genetic variations might result in the presence of retinoblastoma together.[7] Genomic hybridization studies have found several genetic variations in some genes such as: kinesin family member 14, p53 regulator MDM4, transcription factors E2F3 and DEK, cadherin 11, and member retinoblastoma-like 2.[8–10] These genes were related to the growth, proliferation, and apoptosis of cancer cells. Previous study reported that RB1 gene suppressed E2F transcription factor, which was one of the causes of retinoblastoma formation.[11,12] Furthermore, no RB1 mutations were found in some cases of unilateral tumor, and the upregulation of oncogene MYCN expression was detected.[9] Epigenetic processes such as microRNA regulation, DNA methylation, histone modification, and ATP-dependent chromatin reorganization were implicated in the susceptibility of retinoblastoma.[12–14] Let-7, a tumor suppressor miRNA, repressed the expression of Ras family, HMGA2, and c-Myc, and its reduced expression was reported in retinoblastoma.[15] The differential expression of other microRNAs such as miR-34a and miR-106a was also found in retinoblastoma.[16] Furthermore, the abnormal methylation of gene promoters such as p16INK4A, MGMT, GSTP1, RASSF1A, APC, DAPK, RARβ, CDH11, and CDH13 was detected in retinoblastoma.[17] In the meantime, the overexpression of VEGF protein was found in retinoblastoma tissues compared with normal retina tissues.[18] These epigenetic changes affected the expression of the genes and disrupted the regulation of signaling pathways. These genes might become the new targets in the treatment of retinoblastoma, and the studies regarding these genes were helpful for illuminating the molecular mechanism of retinoblastoma. Thus, confirming the expression levels of these genes in retinoblastoma tissue was very crucial.

The VEGF is an endothelial cell-specific peptide mitogen and angiogenic factor, and hypoxia and various cytokines promote the VEGF expression.[19] Previous study have demonstrated that VEGF was overexpressed in many tumors, which stimulated angiogenesis both in vitro and in vivo.[20] VEGF was mainly produced by parenchymal cells and promoted the proliferation of endothelial cells, which induced migration, differentiation, and proliferation of cancer cells.[21] VEGF also stimulated the production of nitric oxide and induced vasodilatation through modulating vascular smooth muscle cells contraction.[22] As we all known, growth of cells was strictly dependent on blood support, and abnormal angiogenesis provided more material and oxygen for tumor cells. VEGF often became a molecular target in the treatment of cancers because of its function of promoting angiogenesis. For example, aflibercept was approved as an inhibitor of VEGF protein in the treatment of metastatic colon cancer. Hence, VEGF might be a potential therapeutic target for the treatment of retinoblastoma. However, the association between VEGF expression and pathogenesis of retinoblastoma should be confirmed firstly. In published studies, the overexpression of VEGF has been detected in retinoblastoma tissues, and it induced vascular leakage in neovascular capillary beds.[23] However, the associations of VEGF overexpression and clinical features of retinoblastoma were not inconsistent, positive and negative results were both presented in the published reports. Therefore, we performed a meta-analysis to investigate the effects of VEGF expression on the pathogenesis of retinoblastoma.

2. Materials and methods

2.1. Literature search

Medicine, Web of Science, Embase, Chinese National Knowledge Infrastructure, and Wanfang databases were searched to acquire eligible literatures until August 9, 2018. The search terms were used to retrieve relevant literatures: ("Vascular endothelial growth factor" or "VEGF") and "retinoblastoma" and ("expression" or "gene expression"). The articles in English and Chinese language were searched for the meta-analysis. In addition, the reference lists of included articles and reviews were scanned to obtain eligible literatures.

2.2. Selection criteria

The study was considered eligible if it met the following inclusion criteria: case–control studies or studies that assessed the association of VEGF expression with risk and clinical features of retinoblastoma; the patients were clearly diagnosed with retinoblastoma; the detection method of VEGF expression was immunohistochemistry (IHC); included studies should contain enough data for the meta-analysis; studies were written in English or Chinese language. The exclusion criteria were as follows: meta-analysis, case report, letter, and review; studies that were conducted in cells or animals.

2.3. Data extraction and methodological quality assessment

First author's name, publication year, country, detection method of protein expression levels, the frequency of VEGF protein positive and negative in control and case group, disease stage, histology, ethnicity, differentiation, cut-off value, optic nerve involvement, and choroid involvement were extracted from included studies. The information was independently extracted by 2 investigators. Methodological quality of included studies was evaluated with Newcastle–Ottawa scale (NOS, http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) table. The methodological quality assessment of included studies was classified into 3 parts: selection of case and control, comparability, and exposure of case and control. Potential disagreements were discussed by 2 investigators.

2.4. Statistical analysis

The Chi-squared test was conducted to discuss the correlation between VEGF expression and risk of retinoblastoma in retinal tissue. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to evaluate the strength of the association between VEGF expression and risk of retinoblastoma.[24] the Q-test and Higgins I^2 statistic were applied to analyze the heterogeneity among included studies, while $P < .05$ and $I^2 > 50\%$ were considered as significant heterogeneity.[25,26] If significant heterogeneity was found, random effects model based on DerSimonian and Laird method was applied; otherwise fixed effects model based on Mantel–Haenszel method was used.[27,28] Begg test and Egger linear regression test were used to evaluate potential publication bias. In addition, sensitivity analysis was conducted to observe the stability of the overall results.[29] All P-values were 2-sided, and $P < .05$ was considered as statistically

significant. All statistical data were calculated using Stata 14.0 software (Stata Corp LP, College Station, TX).

3. Results

3.1. Study selection and methodological quality assessment

A total of 456 articles were identified from the electronic databases. According to the PRISMA guideline, 110 articles were remained after the removal of 346 repeated articles. Then, irrelevant reports were eliminated via reading title and abstract of articles.[30] In addition, after reading full-text of articles, 16 papers were not related to the association of VEGF expression with risk of retinoblastoma, and 4 articles did not have enough data for meta-analysis. Therefore, 20 articles were removed, and 17 articles with 296 controls and 470 patients with retinoblastoma were finally included.[31–47] In the remaining 17 articles, 14 articles were related with the risk of retinoblastoma, and 3 reports were about the association between VEGF expression and clinical features of retinoblastoma. According to the quality criteria, 7 articles acquired 8 scores, while 4 articles obtained 6 scores. The flow chart of literatures searching was presented in Figure 1. The detailed information of included studies could be found in Tables 1 and 2.

3.2. Meta-analysis

No heterogeneity among included studies was detected in the analysis of the association between VEGF overexpression and risk of retinoblastoma ($I^2 = 0.0\%$, $P = .93$). VEGF overexpression was significantly associated with the susceptibility of retinoblastoma in Chinese population (OR = 21.67, 95% CI = 13.96–33.62) (Fig. 2). Subgroup analysis based on control sample type, significant association was detected in both 2 control sample type (Retinoblastoma tissue vs normal retina tissue, OR = 23.97, 95% CI = 9.67–59.42; Retinoblastoma tissue vs retinoblastoma adjacent tissue, OR = 20.85, 95%CI = 12.64–34.37). To investigate the role of VEGF overexpression in clinical characteristics of

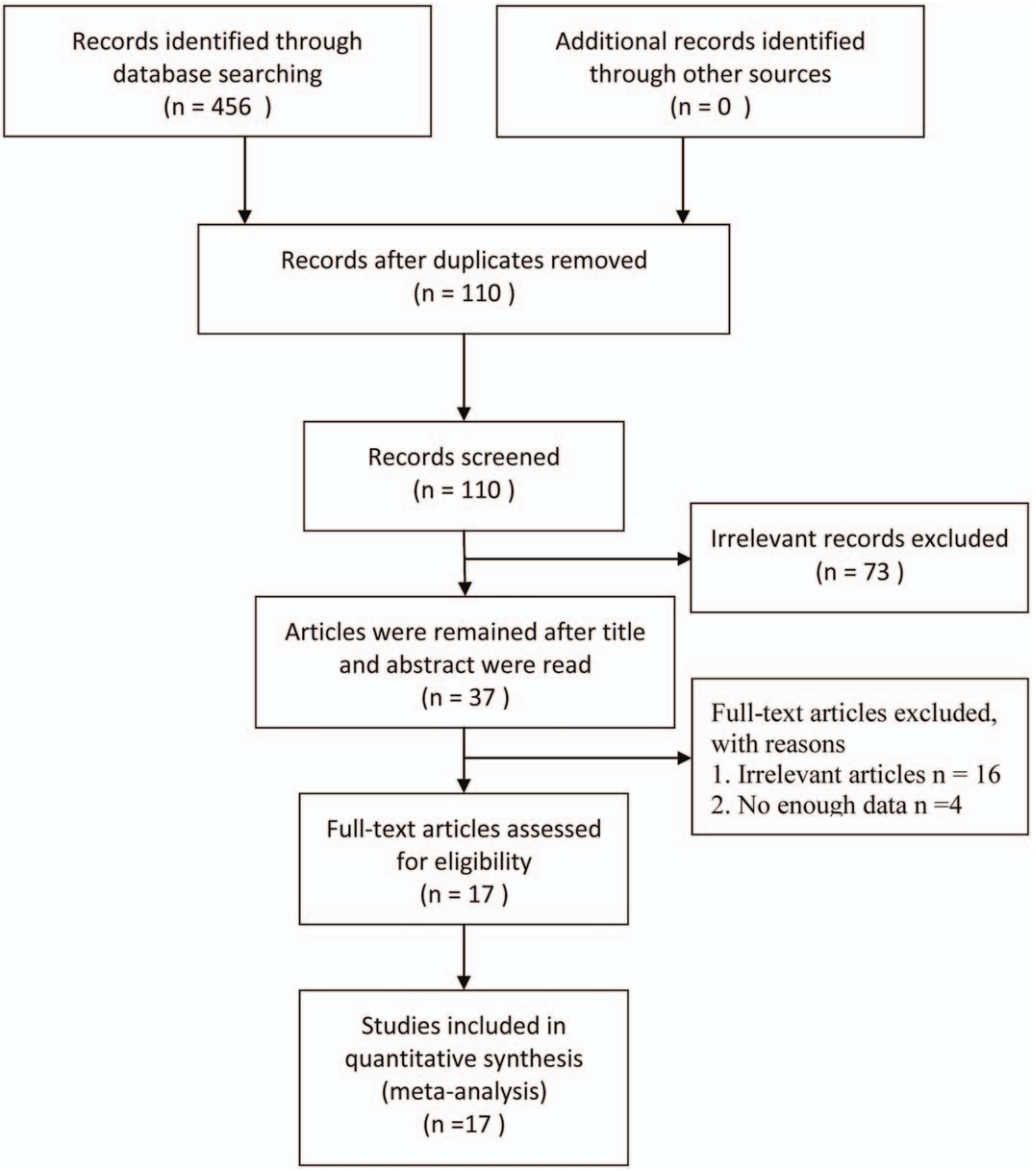

Figure 1. Flow chart of literature selection.

Table 1

Characteristics of included studies for the association between risk of retinoblastoma and VEGF protein expression.

						Control			Case				
Author	Publication year	Country	Ethnicity	Method	Histology	Sample type	VEGF −	VEGF +	Sample type	VEGF −	VEGF +	Cut-off (%)	NOS
Liang[31]	2017	China	Asians	IHC	RB	NT	27	3	RBT	28	72	NR	6
Tong[32]	2017	China	Asians	IHC	RB	AT	15	1	RBT	6	10	50	8
Liu[33]	2014	China	Asians	IHC	RB	AT	24	6	RBT	9	21	30	8
Meng[34]	2011	China	Asians	IHC	RB	AT	43	5	RBT	12	36	50	8
Wang[35]	2010	China	Asians	IHC	RB	NT	9	1	RBT	12	22	10	6
Zhou[36]	2010	China	Asians	IHC	RB	AT	36	4	RBT	11	29	25	8
Fang[37]	2010	China	Asians	IHC	RB	AT	29	4	RBT	9	24	25	8
Niu[38]	2009	China	Asians	IHC	RB	NT	9	1	RBT	22	38	10	6
Yu[39]	2009	China	Asians	IHC	RB	AT	41	6	RBT	10	37	25	8
Jian9[40]	2004	China	Asians	IHC	RB	AT	21	1	RBT	8	14	25	8
Fu[41]	1999	China	Asians	IHC	RB	NT	9	1	RBT	3	37	25	6

AT = adjacent tissue, IHC = immunohistochemistry, NOS = Newcastle–Ottawa scale, NR = no report, NT = normal tissue, RB = retinoblastoma, RBT = retinoblastoma tissue, VEGF = vascular endothelial growth factor.

retinoblastoma, the associations of VEGF expression with optic nerve involvement, choroid involvement, and differentiation were assessed with ORs and 95% CIs. The results indicated that VEGF overexpression could significantly promote the nerve invasion of retinoblastoma cells (OR = 6.9, 95% CI = 4.01–11.88) (Fig. 4A). Corresponding to the result, VEGF overexpression prevented the further differentiation of retinoblastoma (OR = 0.18, 95% CI = 0.12–0.28) (Fig. 5A and Fig. 6A). No significant heterogeneity was observed among the studies on VEGF overexpression for clinical characteristics of retinoblastoma (Optic nerve involvement, $I^2 = 19.50\%$, $P = .24$; Differentiation, $I^2 = 0.00\%$, $P = .72$) (Table 3).

Table 2

Characteristics of included studies in the meta-analysis for the association between clinical features of retinoblastoma and VEGF protein expression.

						No		Yes		
Author	Publication year	Country	Ethnicity	Method	Histology	VEGF −	VEGF +	VEGF −	VEGF +	Cut-off (%)
Tong[32]	2017	China	Asians	IHC	RB	6	2	0	8	50
Youssef[42]	2014	Egypt	Mixed	IHC	RB	11	19	2	24	10
Liu[33]	2014	China	Asians	IHC	RB	9	8	0	13	30
Rodjan[43]	2012	Netherland	Caucasians	IHC	RB	3	3	6	3	NR
Li[44]	2012	China	Asians	IHC	RB	10	18	4	20	25
Radhakrishnan[45]	2011	India	Asians	IHC	RB Stage III	11	3	5	3	10
Meng[34]	2011	China	Asians	IHC	RB	12	15	0	21	50
Yuan[46]	2010	China	Asians	IHC	RB	6	8	2	15	35
Zhou[36]	2010	China	Asians	IHC	RB	11	16	0	13	25
Fang[37]	2010	China	Asians	IHC	RB	9	13	0	11	25
Yu[39]	2009	China	Asians	IHC	RB	10	22	0	15	25
Ge[47]	2007	China	Asians	IHC	RB	10	11	0	11	30
Jiang[40]	2004	China	Asians	IHC	RB	8	6	0	8	25
Fu[41]	1999	China	Asians	IHC	RB	3	24	1	13	25
Choroid involvement				No	Yes					
Youssef[42]	2014	Egypt	Mixed	IHC	RB	5	16	8	27	10
Rodjan[43]	2012	Netherland	Caucasians	IHC	RB	8	6	1	0	NR
Radhakrishnan[45]	2011	India	Asians	IHC	RB Stage III	5	1	11	5	10%
Differentiation					Poor differentiation	Well differentiation				
Liang[31]	2017	China	Asians	IHC	RB	2	43	23	32	NR
Youssef[42]	2014	Egypt	Mixed	IHC	RB	6	14	7	29	10
Liu[33]	2014	China	Asians	IHC	RB	2	16	7	5	30
Li[44]	2012	China	Asians	IHC	RB	4	24	10	14	25
Meng[34]	2011	China	Asians	IHC	RB	3	26	9	10	50
Wang[35]	2010	China	Asians	IHC	RB	4	17	8	5	10
Yuan[46]	2010	China	Asians	IHC	RB	2	16	6	7	35
Zhou[36]	2010	China	Asians	IHC	RB	4	21	7	8	25
Fang[37]	2010	China	Asians	IHC	RB	2	19	7	5	25
Yu[39]	2009	China	Asians	IHC	RB	3	27	7	10	25
Ge[47]	2007	China	Asians	IHC	RB	3	17	7	5	30
Jiang[40]	2004	China	Asians	IHC	RB	2	12	6	2	25
Fu[41]	1999	China	Asians	IHC	RB	1	26	2	11	25

IHC = immunohistochemistry, NR = no report, RB = retinoblastoma, VEGF = vascular endothelial growth factor.

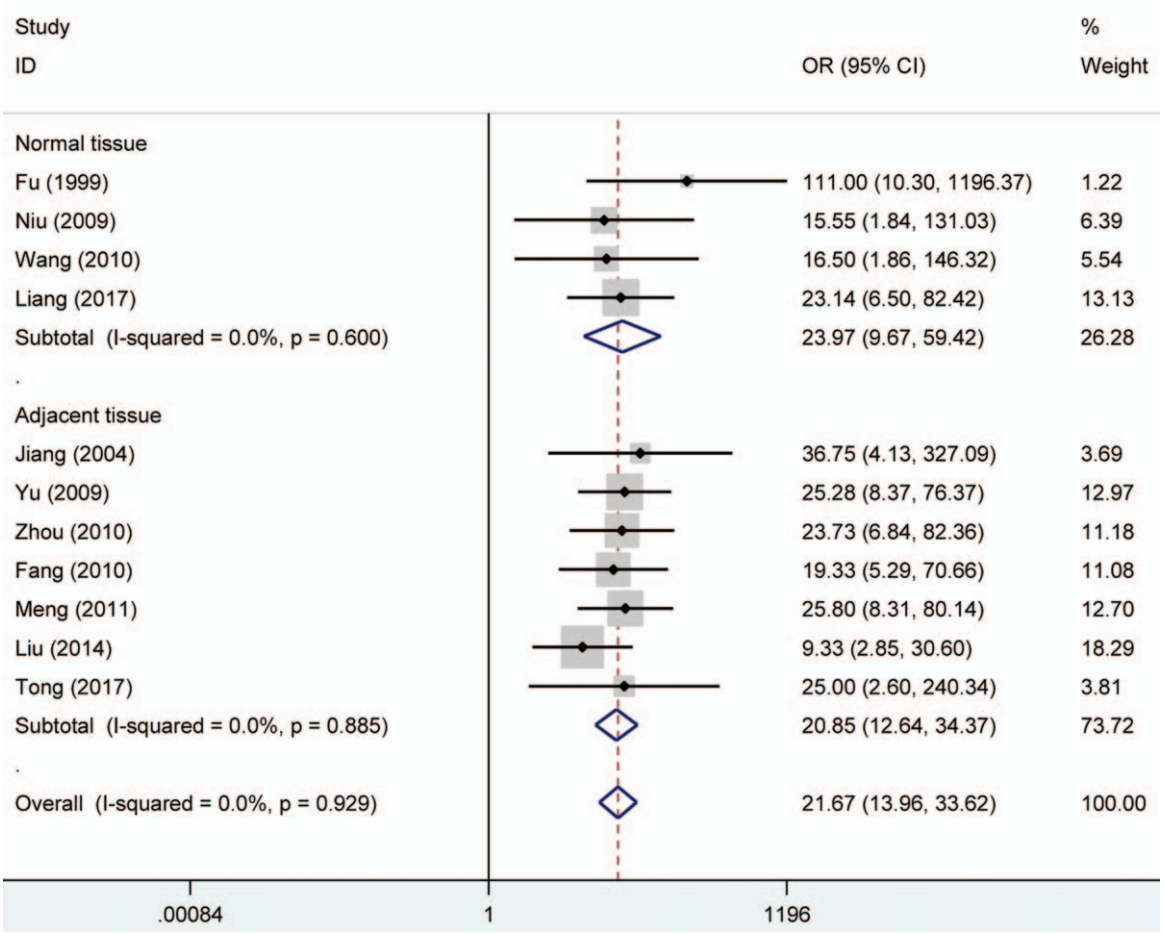

Figure 2. A forest plot of comparison: vascular endothelial growth factor expression in control tissues and retinoblastoma tissues. CI = confidence interval, OR = odds ratio.

In addition, only 1 study was conducted to investigate the potential value of VEGF expression in the prognosis of retinoblastoma.[45] The study of Radhakrishnan et al found the overall survival (OS) for VEGF-positive retinoblastoma patients (33.3%) was lower than that of VEGF-negative retinoblastoma patients (54.69%), while progression-free survival of VEGF-positive retinoblastoma patients was 33.33% and that of VEGF-negative retinoblastoma patients

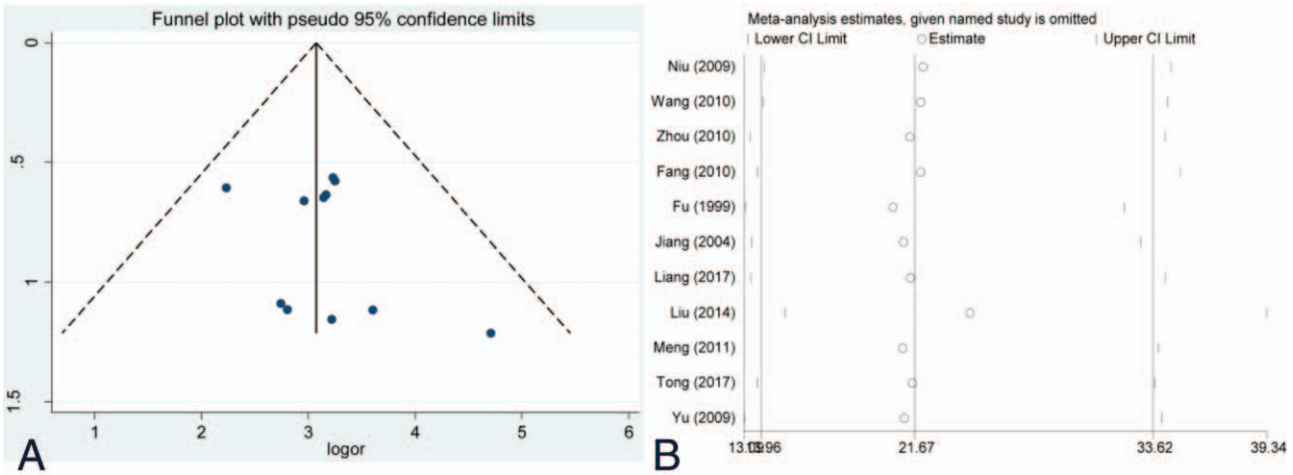

Figure 3. Begg test and sensitivity analysis of association between vascular endothelial growth factor protein expression and susceptibility of retinoblastoma. (A) Funnel plot. (B) Plot of sensitivity analysis. CI = confidence interval, OR = odds ratio.

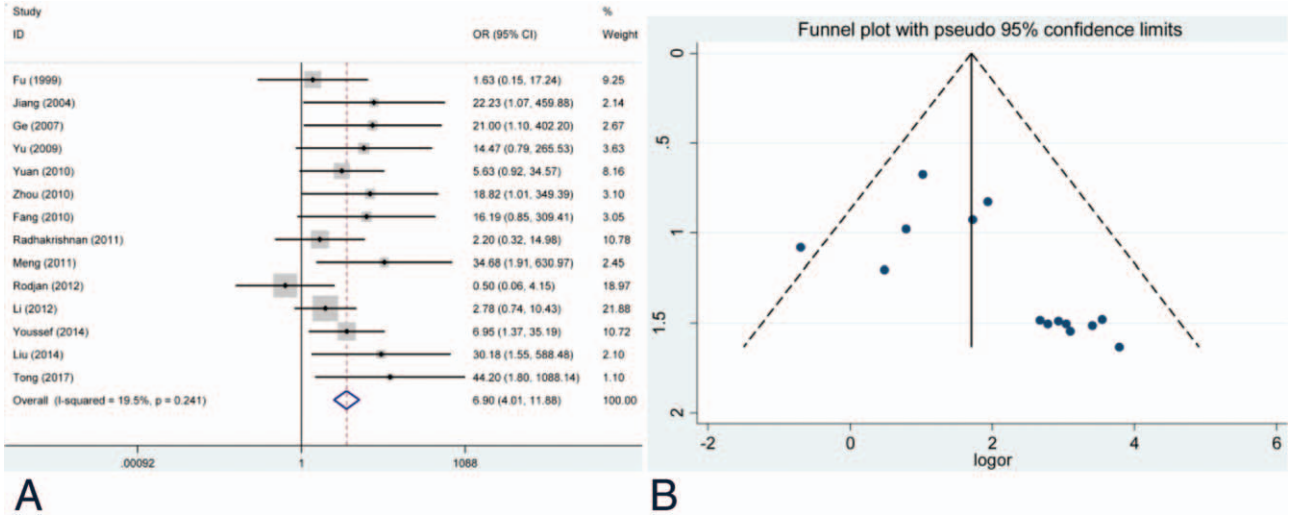

Figure 4. Forest plot and funnel plot of association between vascular endothelial growth factor protein expression and optic nerve involvement of retinoblastoma. (A) Forest plot and (B) funnel plot. CI = confidence interval, OR = odds ratio.

was 54.69%. Event-free survival rate was 0% and 56.25% for of VEGF-positive and VEGF-negative retinoblastoma patients, respectively.

3.3. Publication bias

Sensitivity analysis revealed that the overall ORs was stable by omitting a single study each time. $P < .05$ was found in the Egger test; therefore, subgroup analysis based on sample type was conducted and publication bias was reduced partly (Fig. 3). For observing the influence of publication bias in the overall OR, trim and fill analysis was conducted and the significant association was still found. Hence, publication bias had a little impact on the overall OR for the analysis of retinoblastoma risk.

Moreover, significant publication bias was detected on the basis of Begg test ($P < .05$) for the clinical characteristics of retinoblastoma (Figs. 4B, 5B, 6B). Interestingly, Egger test did not observe significant publication bias ($P > .05$). However, according to the results of sensitivity analysis, the overall OR was still robust. Given that weak heterogeneity was found across the included studies, trim and fill analysis was not conducted.

4. Discussion

Currently, some efficacious treatments such as plaque radiotherapy, chemotherapy, thermotherapy, photocoagulation, cryotherapy, external beam radiotherapy, and enucleation have been used to treat the retinoblastoma.[48] Because of the use of these multimodal therapies, the cure rates reached 95% in developed countries.[48] Enucleation was commonly used in case of late-stage unilateral retinoblastoma. But if retinoblastoma was bilateral, chemotherapy and aggressive focal therapy would be applied to save partial vision.[49,50] However, no standard chemotherapy regimen for retinoblastoma was designed up to

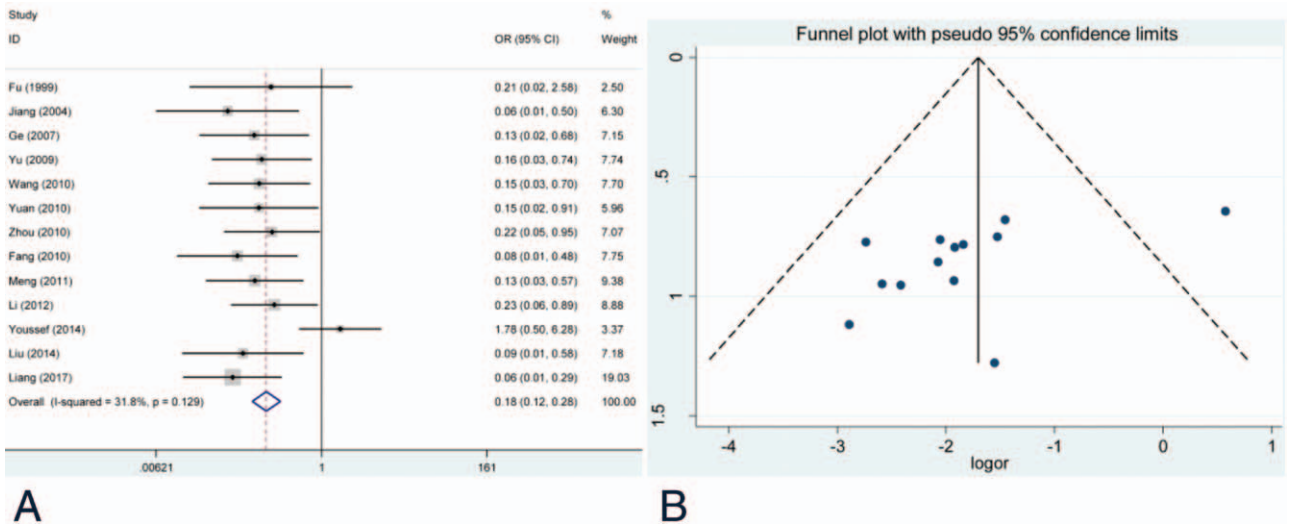

Figure 5. Forest plot and funnel plot of association between vascular endothelial growth factor protein expression and differentiation of retinoblastoma. (A) Forest plot and (B) funnel plot. CI = confidence interval, OR = odds ratio.

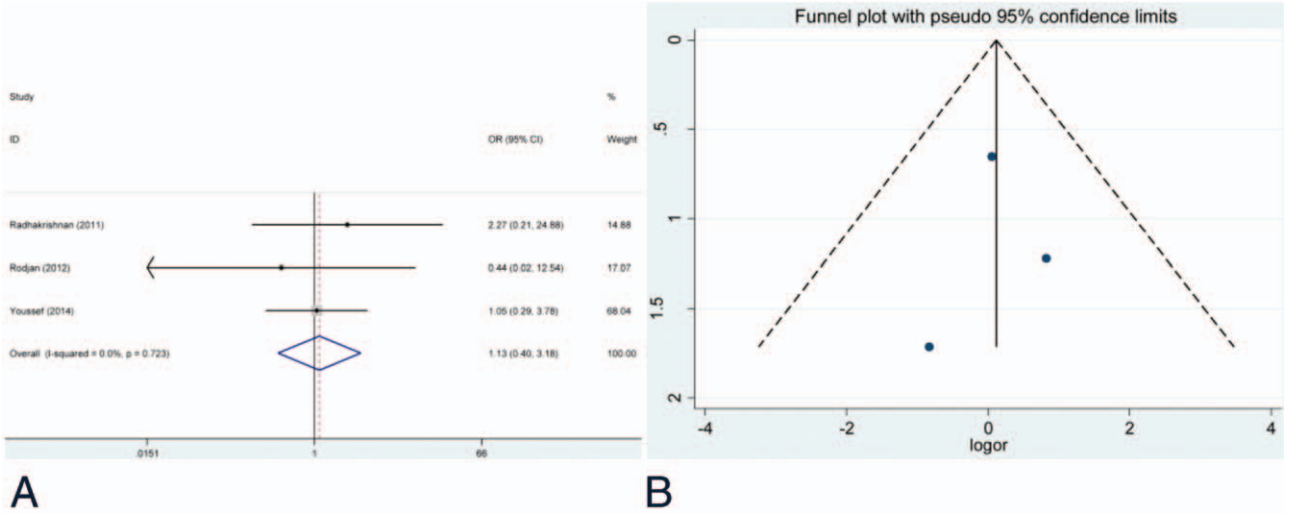

Figure 6. Forest plot and funnel plot of association between vascular endothelial growth factor protein expression and choroid involvement of retinoblastoma. (A) Forest plot and (B) funnel plot. CI = confidence interval, OR = odds ratio.

now. In clinical treatment, the combination of vincristine, carboplatin, and etoposide were the most commonly used drugs to treat the retinoblastoma. These drugs might increase the risk of tumorigenesis; hence, the combination should be used in the centers of retinoblastoma tissues. In addition, chemotherapy regimens should be designed based on the laterality and stage of the retinoblastoma and experience of doctors.[49–52] In addition to the conventional treatments, drugs that targeted protein molecules were often used. Topotecan, a topoisomerase I inhibitor, has been used to treat the gynecological cancers and small-cell lung carcinoma.[53] The in vitro study revealed that topotecan reduced the cell viability of retinoblastoma and activated the apoptotic signaling pathways of cancer cells.[54] Furthermore, some antiangiogenic agents were developed to restrain angiogenesis, and further inhibited the growth of cancer cells. VEGF was overexpressed in many cancers and promoted tumor angiogenesis, so many drugs or antibodies that targeted VEGF protein were designed to treat cancers. Bevacizumab, the anti-VEGF monoclonal antibody, has been used in the treatment of non-small cell lung cancer and colon cancer.[55] The antibody was also used in the treatment of retinoblastoma in the xenografted mice. The results indicated that bevacizumab significantly reduced microvessel density and suppressed the growth of retinoblastoma cell.[56] Although VEGF played an important role in tumorigenesis, several clinical studies should be carried out to develop more drugs which targeted VEGF protein, especially in retinoblastoma. But before this, the levels of VEGF expression in retinoblastoma tissue with different stages should be confirmed.

Overall, VEGF overexpression was associated with the increased risk of retinoblastoma in Chinese population. In the analysis of risk of retinoblastoma, all included studies obtained positive results. However, studies that were carried out in other countries and ethnicities were not found. Included studies of the risk of retinoblastoma were all performed in Chinese population.[31–41] Hence, further studies in Caucasians or other populations should be performed to illuminate the association. Published studies have suggested that VEGF was implicated in the risk and prognosis of cancers such as lung cancer, head and neck carcinoma, and osteosarcoma.[56–58] Moreover, significant correlation between degree of tumor vascularity and VEGF expression was detected in published studies.[59] Kvantna et al have found that VEGF was overexpressed in retinoblastoma tissues, but not in tumor vessels.[60] Previous study has found the low expression of VEGF in human retina and choroid tissues.[61] According to the type of control sample, stratified analysis was carried out and significant association was observed in normal retina tissue and retinoblastoma adjacent tissue. However, the

Table 3

Summary of ORs of the associations between vascular endothelial growth factor protein expression and clinical characteristics of retinoblastoma.

Group	Number of RB patients	ORs	95%CIs	Heterogeneity		Publication bias	
				I^2 (%)	*P*-value	Begg (*P*-value)	Egger (*P*-value)
Risk (overall)	470	21.67	13.96–33.62	0.00	.93	.70	.001
Risk (Control sample type)							
Normal tissue	234	23.97	9.67–59.42	0.00	.60	.50	.25
Adjacent tissue	236	20.85	12.64–34.37	0.00	.89	.88	.01
Clinical analysis							
Optic nerve involvement	485	6.90	4.01–11.88	19.50	.24	.003	.39
Differentiation	565	0.18	0.12–0.28	31.80	.13	.05	.36
Choroid involvement	93	1.13	0.40–3.18	0.00	.72	.60	.86

CI = confidence interval, ORs = odds ratios, RB = retinoblastoma.

stage of included retinoblastoma patients was unclear, so subgroup analysis based on case sample could not be conducted. In addition, the cut-off values of eligible studies were inconsistent, and no universally accepted criteria of IHC was strictly applied which might affect the accuracy of overall results.

Appropriate therapeutic regimens and drugs should be developed on the basis of the stage of cancer patients. According to the results of the meta-analysis, the expression of VEGF was higher in poor differentiated retinoblastoma than that in well differentiated retinoblastoma. Therefore, medicines which targeted VEGF might be used in the well differentiation of retinoblastoma with caution. In addition, VEGF overexpression was significantly associated with the optic nerve involvement of retinoblastoma. Six included studies have acquired opposite results about optic nerve involvement of retinoblastoma,[39,41,43–46] while seven studies obtained the same results.[32–34,36,37,40,42,47] In addition, Arean et al found that VEGF overexpression was not significantly associated with the optic nerve involvement of retinoblastoma, while no significant association between VEGF overexpression and choroid involvement was found.[18] Weak heterogeneity and publication bias were found in the analysis of clinical features. Studies which were carried out in Caucasians or Africans were still insufficient. Furthermore, VEGF expression was not significantly associated with choroid involvement of retinoblastoma, which was consistent with the results of included studies. In developing countries, retinoblastoma was often diagnosed in advanced stage, and the therapeutic regimen might be carefully developed to save the vision and relieve the pain of retinoblastoma patients.[62] So the role of antiangiogenic agents in retinoblastoma patients might be further investigated according to different levels of VEGF expression in cancer patients with different stages. Furthermore, the results of Radhakrishnan's study indicated that VEGF overexpression was a promising prognosis factor for retinoblastoma.[45] The same result was also found in other cancers.[56–58] However, only 22 evaluable retinoblastoma patients were included in this study, in which the sample size was too small, and expansion of sample size was necessary.

Some limitations should be considered in the meta-analysis. First, publication bias might affect the accuracy of pooled results, in which positive results tended to be accepted by journals. Second, there was not a standard cut-off value in defining levels of VEGF expression in tissues. Third, the studies that carried out in Caucasians and Africans were too few to obtain appropriate pooled results. Fourth, the information of retinoblastoma stage and subtype was not enough. Hence, larger scale studies with enough clinical information should be carried out in different populations.

Despite these limitations above, our meta-analysis demonstrated that VEGF overexpression was significantly associated with increased risk of retinoblastoma. In addition, VEGF overexpression was related to the optic nerve involvement and differentiation of retinoblastoma.

Author contributions

Data curation: Qizheng Wu.
Formal analysis: Qizheng Wu, Xiao Sun.
Funding acquisition: Guangying Zheng.
Investigation: Qizheng Wu.
Methodology: Qizheng Wu, Xiao Sun.
Project administration: Guangying Zheng.
Software: Qizheng Wu.
Supervision: Xiao Sun, Guangying Zheng.
Validation: Xiao Sun.
Writing – original draft: Qizheng Wu, Xiao Sun.
Writing – review & editing: Guangying Zheng.

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