



Comprehensive analysis of the oncogenic roles of vascular endothelial growth factors and their receptors in stomach adenocarcinoma

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ARTICLE INFO

Keywords:

Vascular endothelial growth factors
Vascular endothelial growth factor receptors
Stomach adenocarcinoma
Prognosis
Bioinformatics

ABSTRACT

Background: Vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) play complicated oncogenic roles in multiple tumors by initiating and promoting tumor angiogenesis and lymphangiogenesis. The main goal of our study was to comprehensively investigate the oncogenic roles of VEGFs and VEGFRs in stomach adenocarcinoma (STAD).

Methods: The present study applied multiple bioinformatic tools to comprehensively explore the expression levels, prognostic values, genetic alterations and immune infiltrations of VEGFs and VEGFRs in STAD patients.

Results: We found that *VEGFA*, *VEGFC*, placenta growth factor, *FLT1*, *KDR*, *FLT4*, and Neuropilin 1 were overexpressed in STAD, while the expression of *VEGFB* and *VEGFD* were decreased. Survival analysis revealed that higher transcription levels of VEGF/VEGFRs were obviously correlated with worse clinical outcome in STAD patients. Additionally, high alteration frequencies of VEGFs and VEGFRs (27%) were observed in STAD patients, and alterations of VEGFs and VEGFRs improved their prognosis. The expression of VEGFs and VEGFRs was remarkably associated with immune cell infiltration and immune checkpoint expression in STAD patients.

Conclusion: Our study systematically explored the transcriptome profiles and distinct prognostic values of VEGFs and their receptors in STAD and contributed to a better understanding of the oncogenic roles of VEGF/VEGFR members in STAD.

1. Introduction

Solid tumor tissue is comprised of neoplastic cells and surrounding stromal cells, and this heterogeneous composition contributes to maintaining the complexity of tumor microenvironment (TME) [1]. Owing to the rapid development rate, tumor cells demand a constant supply of nutrients to maintain a higher metabolic rate. These pathological processes are mainly dependent on the induction of angiogenesis [2]. During physiological processes, angiogenesis is crucial for embryonic processes and wound healing. However, abnormally accelerated angiogenesis processes in TME provide supplemental oxygen and nutrients to tumor cells, which leads to the development of tumor [3]. Besides, the pericyte coverage of tumor vasculature is often lacking or loose, which enhanced the vascular

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<https://doi.org/10.1016/j.heliyon.2023.e17687>

Received 18 February 2023; Received in revised form 19 June 2023; Accepted 26 June 2023

Available online 6 July 2023

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permeability and thereby promoting the metastasis of cancer cells [4]. Therefore, angiogenesis has been recognized as a primary driving force to participate in the pathological process of many severe human diseases, especially malignant tumors, and inhibition of the tumor angiogenesis pathway is a promising strategy for clinical anti-tumor therapy [5].

The process of angiogenesis is mainly modulated by multiple growth factors and their correlated receptor tyrosine kinases (RTKs). Among them, vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) are especially important [6]. Accumulating studies have indicated that VEGFs and VEGFRs are principal drivers in the initiation and progression of tumor angiogenesis [7,8]. There are five VEGFs in the mammalian genome, including *VEGFA* (also known as *VEGF*), *VEGFB*, *VEGFC*, *VEGFD* (also known as *FIGF*) and placenta growth factor (*PGF*), which are mainly responsible for the processes of angiogenesis and lymphangiogenesis in physiological or pathological condition [9]. In addition, previously published researches have proved that, with the activation of VEGF/VEGFR pathways, the proliferation, differentiation and migration of tumor cells are significantly promoted [10,11]. In terms of VEGFR, there are two subtypes: tyrosine kinase receptor and non-tyrosine kinase receptor. The former includes *VEGFR1* (also known as *FLT1*), *VEGFR2* (also known as *KDR*), and *VEGFR3* (also known as *FLT4*), and the latter, Neuropilin 1 (*NRP1*) and *NRP2* [12]. *VEGFR1*, a common receptor for *VEGFA*, *VEGFB* and *PGF* ligands, is mainly expressed on the surface of vascular endothelial cells, macrophages, dendritic cells and tumor cells [13]. The activation of *VEGFR1* mediated by VEGFs is related to cellular proliferation, migration, apoptosis, and angiogenesis [14]. *VEGFR2*, a receptor for *VEGFA*, *VEGFC* and *VEGFD*, is predominantly expressed on vascular endothelium cells, with the activation of *VEGFR2* significantly enhanced mitogenesis, angiogenesis, vascular invasion and vascular permeability [15]. *VEGFR3* is a tyrosine kinase receptor of *VEGFC* and *VEGFD* that is mainly expressed on lymphatic endothelial cells and serves as a vital role in regulating lymphangiogenesis [16]. *NRP1* and *NRP2* are co-receptors that selectively bind certain VEGFs and regulate the functions of *VEGFR2* and *VEGFR3*, thereby enhancing the migration of endothelial cells in angiogenesis [17].

Stomach adenocarcinoma (STAD) accounts for the fifth most frequently diagnosed cancer, accounting for more than 700,000 cancer-related deaths around the world [18]. In recent years, several anti-angiogenic agents targeting the VEGF/VEGFR signaling and conferring survival benefits in patients with STAD have been approved for clinical treatment of STAD [19]. Nevertheless, only a subset of STAD patients benefited from the clinically approved anti-angiogenic drugs in use currently, and many who initially respond develop resistance over time. Thus, we performed the present study to explore the oncogenic roles of distinct VEGF/VEGFR members in STAD.

2. Materials and methods

2.1. Oncomine

Oncomine (<https://www.oncomine.org/>) database is a web-based project providing genome-wide expression analysis across diverse cancer types [20]. In the present study, Oncomine project was applied for analyzing the transcription levels of distinct VEGF/VEGFR members in distinct cancers. *P*-value <0.05, fold change (FC) > 1.5, and gene rank in the top 10% were considered to be statistically significant.

2.2. The Cancer Genome Atlas data

The Cancer Genome Atlas (TCGA, <https://www.cancer.gov/tcga>) is a public resource containing many types of data from over 20,000 tumor and normal samples. In the present study, we first obtained the sequencing profiles of RNA in Fragments Per Kilobase Million format and clinicopathological parameters of STAD patients from TCGA database. We then analyzed the expression patterns of distinct VEGF/VEGFR members and their correlations with patients' clinical characteristics in STAD by R software (v4.1.1, <https://www.r-project.org/>). *P*-value <0.05 was considered to be statistically significant.

2.3. Kaplan-Meier Plotter

The Kaplan Meier plotter (<http://kmplot.com/analysis/>) is a web-based platform constructed for evaluating the prognostic values of more than 50,000 genes across 21 types of malignant tumor, including STAD [21,22]. In this study, we applied this platform to explore the influences of distinct VEGF/VEGFR members on clinical outcome of STAD patients, including overall survival (OS), first-progression survival (FP), and post-progression survival (PPS). The hazard ratio (HR) with 95% confidence intervals (CIs) and log-rank *p*-values were automatically calculated to the best cutoff value, and log-rank *p*-value <0.05 was recognized statically significant.

2.4. cBioPortal

The c-Bio Cancer Genomics Portal (cBioPortal, v3.7.11, <https://www.cbioportal.org/>) is an integrated online-based platform that contains multidimensional cancer genomic datasets from TCGA project [23]. We used the Stomach Adenocarcinoma (Pfizer and UHK, Nat Genet 2014) [24] and Stomach Adenocarcinoma (TCGA, PanCancer Atlas) [25] datasets in cBioPortal to explore the genetic alterations of distinct VEGF/VEGFR genes and their prognostic values in STAD patients. A log-rank test *p*-value <0.05 was recognized statically significant.

2.5. TIMER

Tumor IMMune Estimation Resource (TIMER, <https://cistrome.shinyapps.io/timer/>) is a platform for systematical exploration of tumor immunological features, which precalculated the infiltrations of several tumor infiltrated immune cells (TIICs) (Neutrophils, B cells, Macrophages, CD4⁺ T cells, CD8⁺ T cells, and Dendritic cells) for more than 10,000 tumors from 32 types of malignant tumor [26]. In our study, TIMER database was used for assessing the correlations between the infiltration levels of TIICs and gene expression levels of distinct VEGF/VEGFR members in STAD. Besides, we also evaluated the correlations of immune checkpoint expression level with expression levels of distinct VEGF/VEGFRs members in STAD by TIMER. The relationships were evaluated by Spearman's test, and p-value <0.05 was recognized statically significant.

3. Results

3.1. Transcriptional levels of VEGF/VEGFR members in pan-cancer

We utilized OncoPrint project to evaluate the differential transcriptional levels of distinct VEGF/VEGFR members between STAD and corresponding normal samples. As a result, VEGFB, VEGFD, and FLT4 showed significantly downregulated expression levels in STAD compared to control specimens (Fig. 1). Besides, the transcriptional levels of VEGFC, PGF, KDR, NR1, and NR2 were obviously elevated in STAD compared to control specimens. However, no differential expression information of VEGFA and FLT1 in STAD was observed in the OncoPrint dataset.

3.2. mRNA expression levels of VEGF/VEGFR members and their correlations with clinicopathological characters in patients with STAD

The sequencing data of 407 specimens (including 375 cancerous and 32 normal specimens) and corresponding clinicopathological parameters were retrieved from TCGA. Differential expression analysis found that the mRNA expression levels of VEGFA, VEGFC, PGF, FLT1, KDR, FLT4, and NR1 were obviously elevated in STAD compared to control specimens, while the mRNA expression levels of VEGFB and VEGFD were significantly downregulated, and no expression difference was found for NR2 (Fig. 2). We further evaluated the correlation of distinct VEGF/VEGFR members' expression with the pathological stage of STAD patients. As displayed in Fig. 3, the transcriptome levels of VEGFB, VEGFC, VEGFD and PGF were upregulated in stage II tumors. The mRNA expression of FLT1, NR1, and NR2 showed an increasing trend with increasing tumor stage. Contrariwise, VEGFA, KDR, and FLT4 groups did not show significant

Analysis Type by Cancer	VEGFA	VEGFB	VEGFC	VEGFD	PGF	FLT1	KDR	FLT4	NR1	NR2
	Cancer vs. Normal									
Bladder Cancer	4 2			4	2	1 1	2 1		1	6
Brain and CNS Cancer	12	1	1	3	7	4 4	1 1	1	5	7 2
Breast Cancer	9 1	7	2 13	20	1	1 4	3	4	2 8	5
Cervical Cancer	3					1				
Colorectal Cancer	17		1	9	5 1	2	1 1	1 3	6	
Esophageal Cancer	2	2			2 2	3 2		2 2	1 1	1 1
Gastric Cancer		1	1	3	8	1 4	2	2	4 1	4 3
Head and Neck Cancer	2 2	1	5 1	1	2 6	1 4	1	1	1	8
Kidney Cancer	7 3	4	2 1		3 3	6 7	1 9	2 3	3 4	3 1
Leukemia	3 8	2		1	2	3	4	2 1	1 1	
Liver Cancer			2			1 2	1			
Lung Cancer	5 2		1 3	11	7	3 8	10	3	4	1 2
Lymphoma	14 1	1 1	7 1		5 2	6 1	5	4	6	5 1
Melanoma	2 1		1		1 2	1	1		1	1
Myeloma	4				1	1		2	2	1
Other Cancer	7	1	4 2	1	5 3	4 6	5 2	2	4 1	5
Ovarian Cancer	8	1	2	1	3		4		3	1
Pancreatic Cancer	2 1		3	1 2	1 1	2		2	1	2 2
Prostate Cancer	1 4	1		1	1	1 2	1		1	6
Sarcoma	1	1 4	2	2	1 2	1 4	2		6 2	4 1
Significant Unique Analyses	101 26	10 18	29 29	3 57	54 25	37 50	18 35	11 21	37 32	53 28
Total Unique Analyses	452	435	449	357	450	452	447	425	417	424

Fig. 1. The transcription levels of VEGF/VEGFR members in different types of cancers (OncoPrint). Red represents the target gene is upregulated, and blue indicates that the target gene is downregulated.

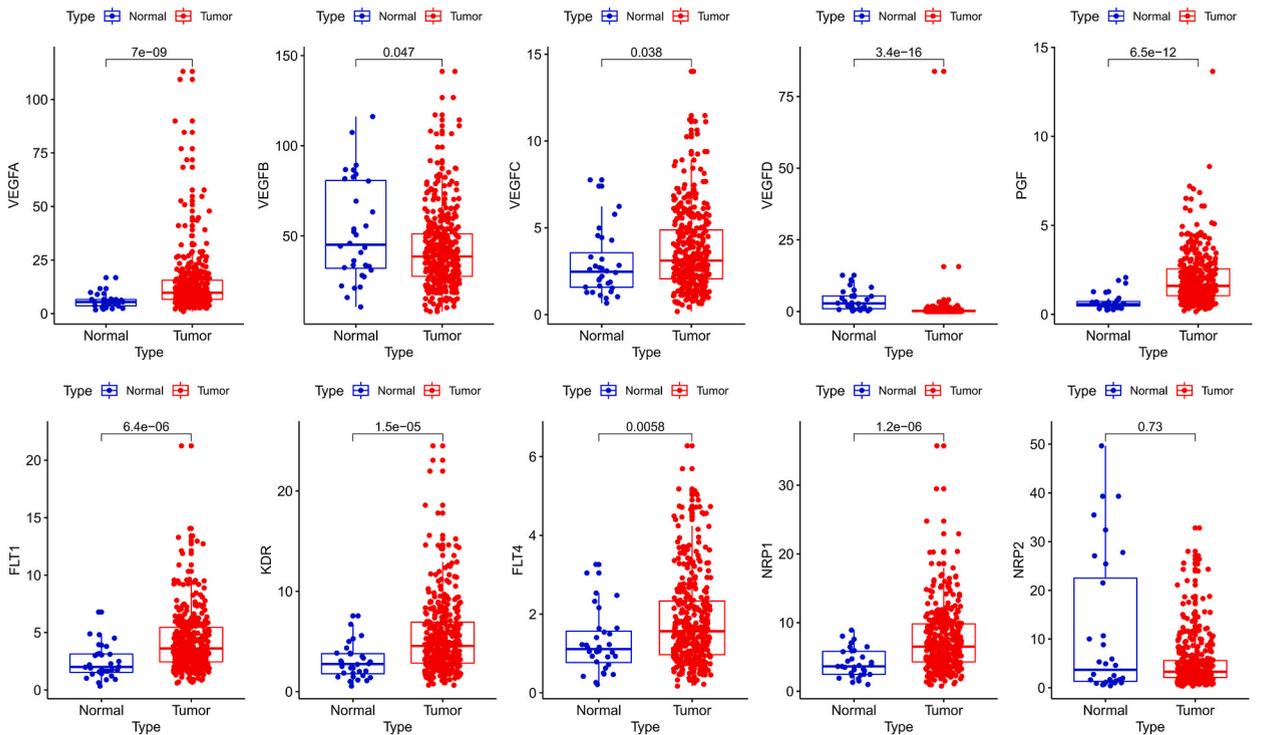


Fig. 2. The mRNA expression levels of VEGF/VEGFR members in STAD (TCGA). The red dots indicate the STAD samples, and the blue dots indicate the normal samples.

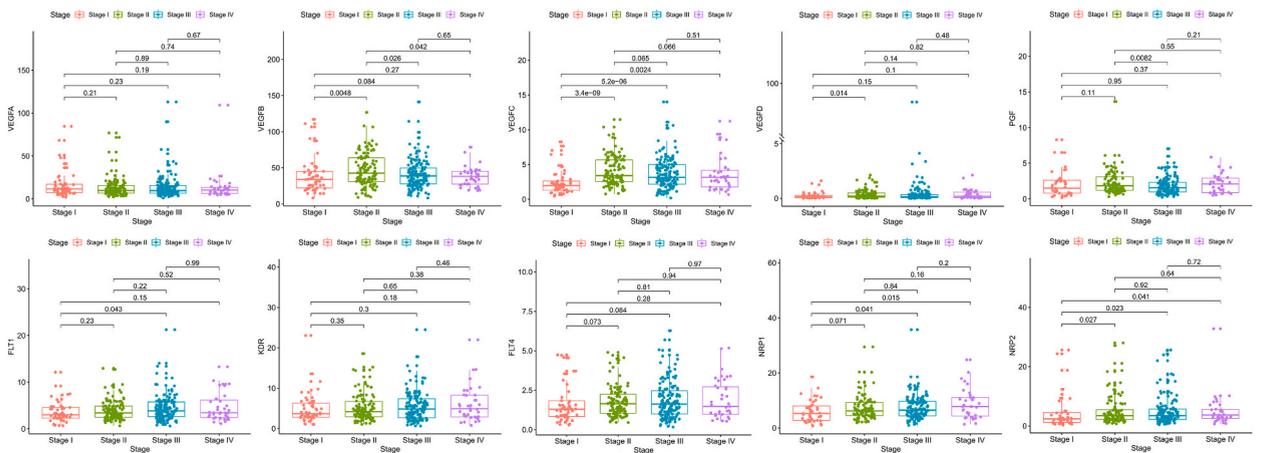


Fig. 3. The correlations between VEGF/VEGFRs expression and tumor stage in STAD patients (TCGA).

differences.

3.3. Correlations of the increased expression levels of distinct VEGF/VEGFRs with poor clinical outcome of STAD patients

We applied the Kaplan Meier plotter for determining the influences of distinct VEGF/VEGFR members on clinical outcomes of STAD patients and found that each member of the VEGF/VEGFR family genes were obviously correlated with the clinical outcomes of STAD patients. As shown in Fig. 4, patients with STAD possess higher VEGF/VEGFRs expression showed worse OS, FP, and PPS than those possess low expression ($p < 0.05$).

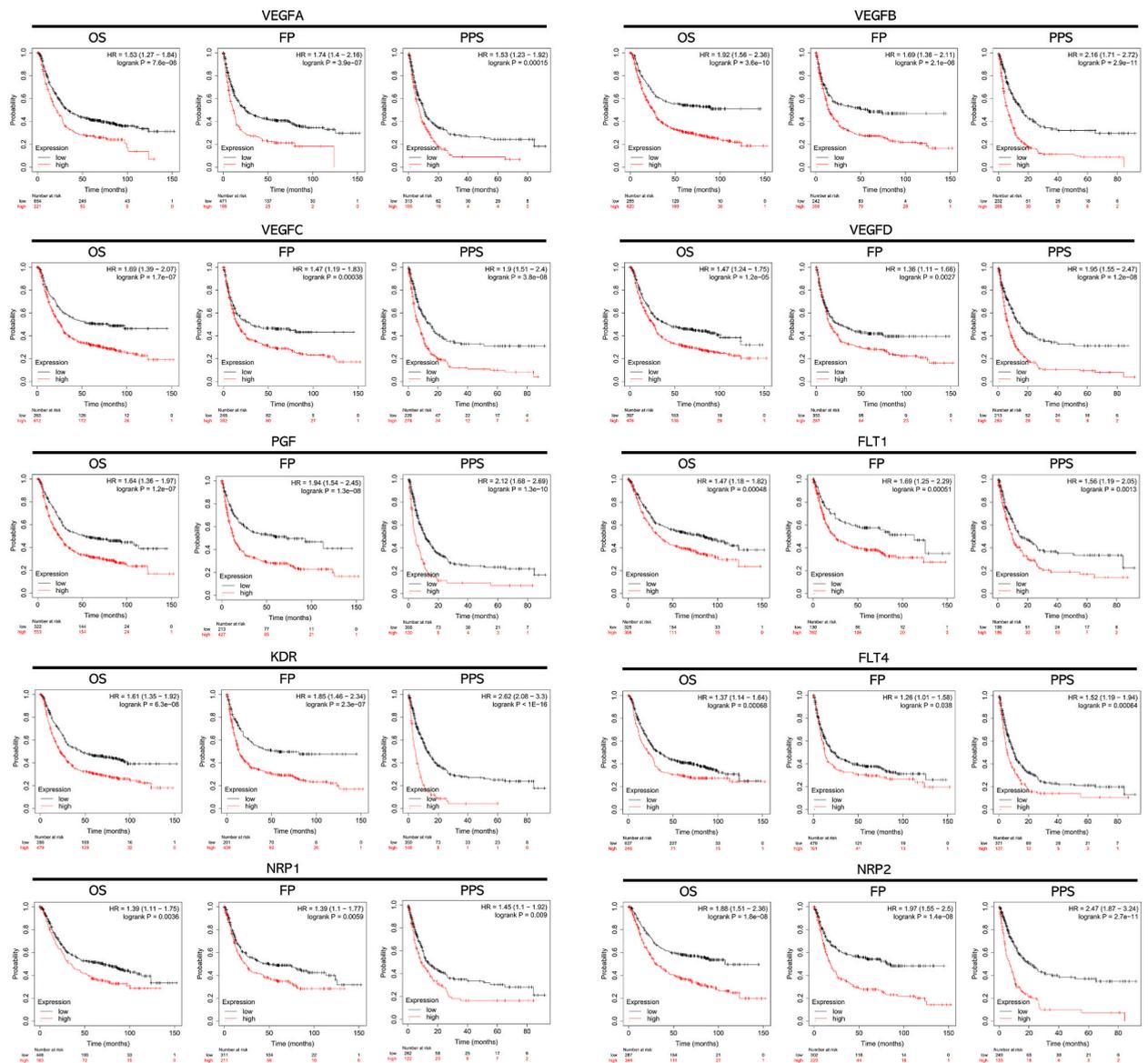


Fig. 4. The prognostic values of distinct VEGF/VEGFR members in patients with STAD (Kaplan-Meier Plotter).

3.4. Genetic alterations of VEGF/VEGFR members in STAD patients

The genetic variations of VEGF/VEGFR members in STAD were explored via cBioPortal project. As a result, the VEGF/VEGFR members were altered in 145 samples of 540 STAD patients from two datasets, and the individual sequence alteration frequencies varied from 0.6 to 6% (Fig. 5A and B). Subsequently, we found that STAD patients in the altered subgroup shared an obviously increased progression-free survival and disease-specific survival compared with those in unaltered subgroup (Fig. 5C and D). However, STAD patients with or without alterations in VEGF/VEGFR members had no relationship with OS and disease-free survival (Fig. 5E and F), and the survival curve of each VEGF/VEGFR member in STAD patients was displayed in Supplementary Fig. 1.

3.5. VEGF/VEGFR members positively correlated with immune cell infiltration in STAD

Considering the VEGF/VEGFR signaling-mediated angiogenesis is connected with immune reaction in TME, we further utilized TIMER for exploring the relationships of distinct VEGF/VEGFR members expression with the abundances of TIICs. As shown in Fig. 6, VEGFA showed a significant negative connection with the Macrophage, B cell, CD4⁺ T cell, CD8⁺ T cell, and Dendritic cell abundances in STAD. VEGFB, VEGFC, FLT1, KDR, FLT4, and NRP2 were all positively associated with Macrophage, CD4⁺ T cell, CD8⁺ T cell, Dendritic cell, and Neutrophil abundances in STAD patients, but there was no significant correlation with B cell. Additionally, all six

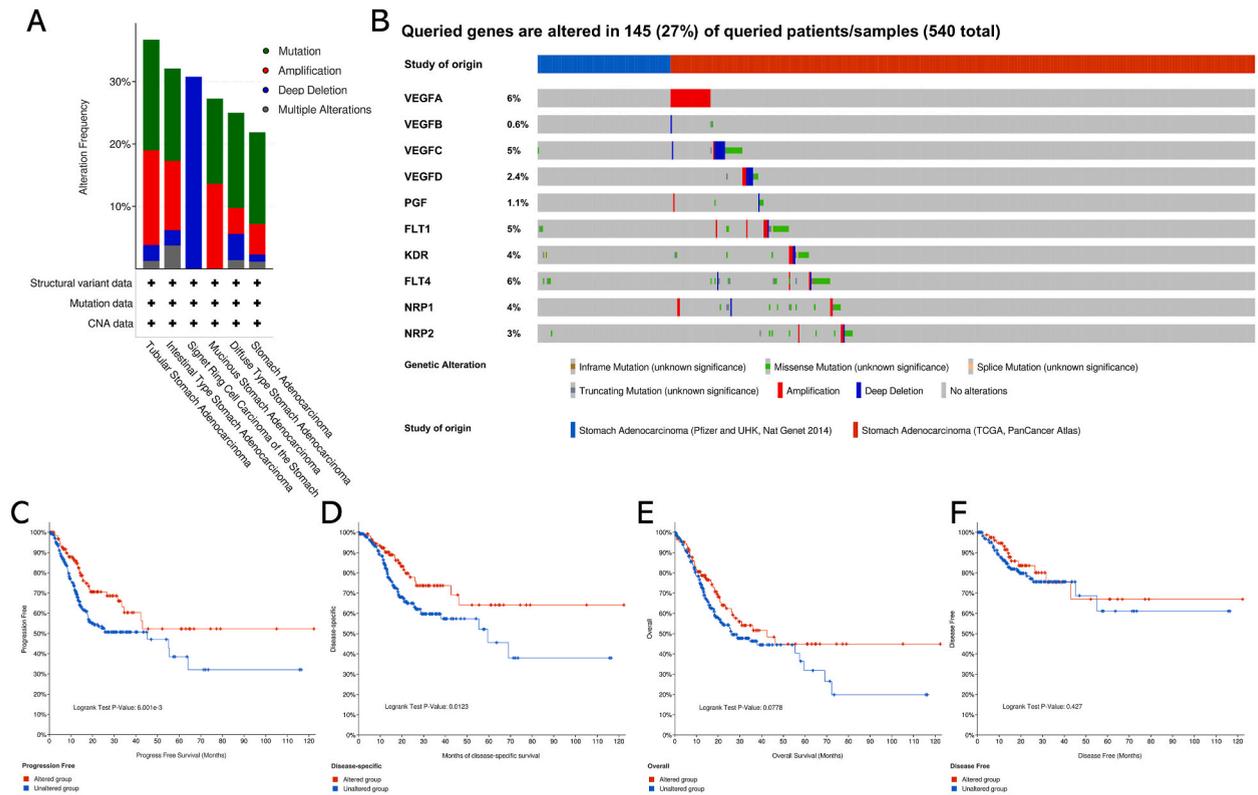


Fig. 5. The genetic alteration analysis of VEGF/VEGFRs in patients with STAD (cBioPortal). (A) Summary of alterations in VEGF/VEGFRs. (B) OncoPrint tab summary of alteration types and proportions of distinct VEGF/VEGFR members in STAD. Kaplan–Meier plots comparing (C) progression free survival (PFS), (D) disease specific survival (DSS), (E) overall survival (OS) and (F) disease-free survival (DFS) in patients with/without VEGF/VEGFRs gene alterations.

host immune cells have an obvious association with *FIGF* and *NRP1* expression in STAD. With regard to *PGF*, CD4⁺ T cell and Macrophage showed a positive correlation in STAD, while B cell showed a negative correlation.

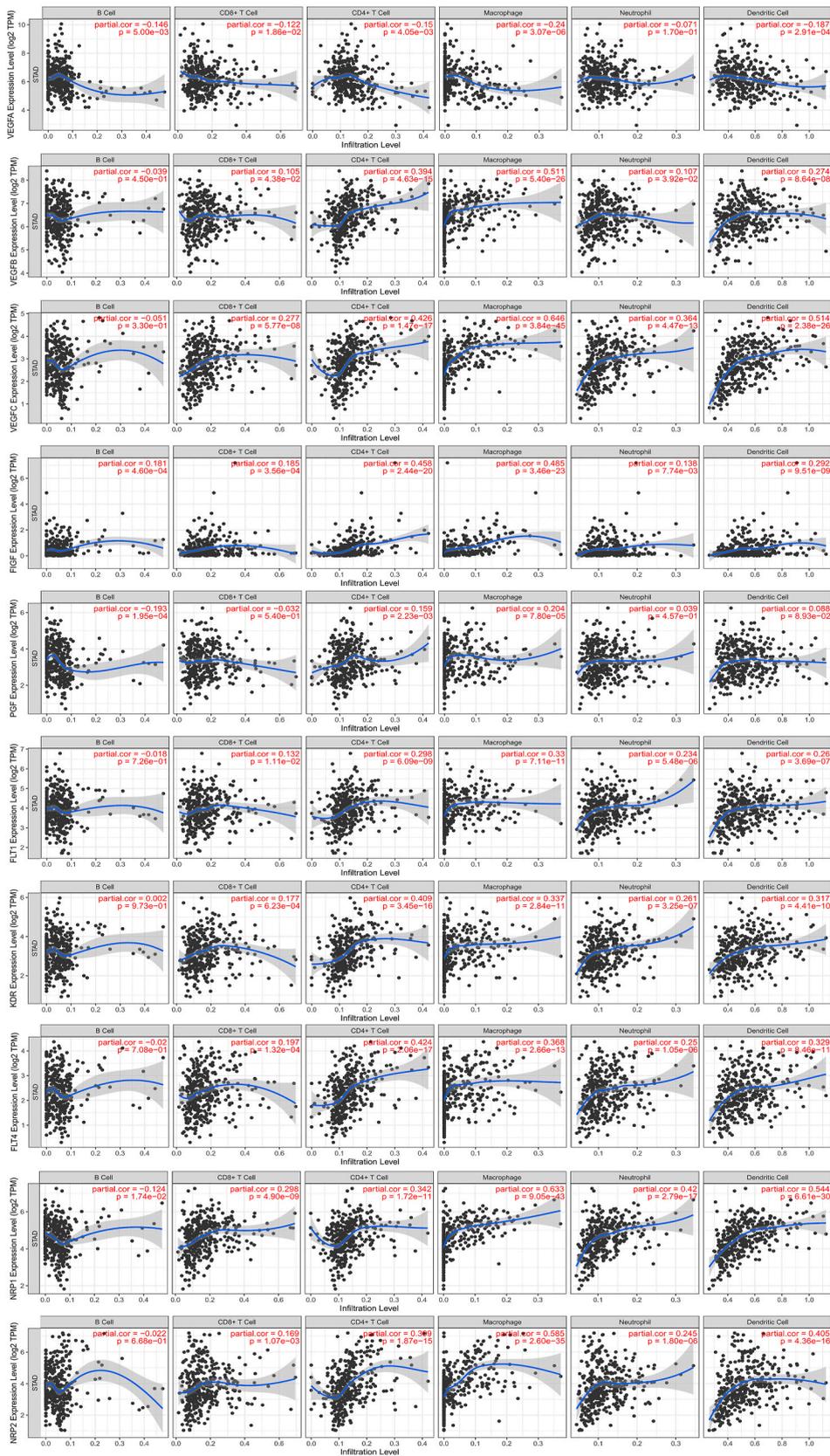
3.6. Correlations of VEGF/VEGFR members with immune checkpoints in STAD

Studies have reported that abnormal angiogenesis induces immunosuppression in TME by undermining the functions of tumor-infiltrating lymphocytes (TILs). Therefore, the correlations of distinct VEGF/VEGFR members with immunosuppression biomarkers, including PDCD1 (PD1), CD274 (PD-L1), CTLA4 and HAVCR2 (TIM-3), were evaluated by TIMER. As displayed in Fig. 7, *VEGFA* expression was in connection with CD274 and CTLA4 in STAD. *VEGFB* and *VEGFD* were positively connected with PDCD1 and HAVCR2. Besides, expression of *PGF* was found to be positively associated with HAVCR2 expression. In terms of the rest members, *VEGFC*, *FLT1*, *KDR*, *FLT4*, *NRP1*, and *NRP2* were all significantly positively correlated with all analyzed immune checkpoints in patients with STAD. These results suggested that VEGF/VEGFR-mediated oncogenic roles in STAD might partially through upregulating immune checkpoint expression to suppress the functions of TIL.

4. Discussion

VEGFs not only induce tumor angiogenesis and increase vascular permeability, but also directly activate downstream signaling pathways of tumor cells by binding to VEGFRs. To date, it is widely accepted that VEGF/VEGFR-mediated angiogenesis is crucial for the progression of STAD, and several VEGF/VEGFR pathway-targeted drugs, such as bevacizumab, ramucirumab and apatinib, have shown a beneficial effect on STAD treatment [27]. However, typical of a heterogeneous disease, only a few patients with STAD show sensitivity to anti-VEGF/VEGFR treatment, and the clinical application of VEGF/VEGFR pathway-targeted drugs today is still limited. Therefore, we launched the present research to comprehensively analyze the oncogenic roles of distinct VEGF/VEGFR members from the viewpoints of expression characteristics, clinical relevance, prognostic properties, somatic alteration, and immune infiltration. As far as we know, this is the first study to analyze the oncogenic roles of different VEGF/VEGFR members in human STAD. We hope that our work will provide an optimal treatment strategy, and therefore improve the clinical outcome of STAD patients.

VEGFA, also called *VEGF*, is the prototype member of the VEGF family. *VEGFA* mainly binds to *VEGFR2* and thereby mediates blood vessel formation [8]. Studies have reported that *VEGFA* was overexpressed in STAD specimens compared with the tumor-adjacent



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Fig. 6. The correlations between distinct VEGF/VEGFR genes and immune cell infiltration (TIMER).

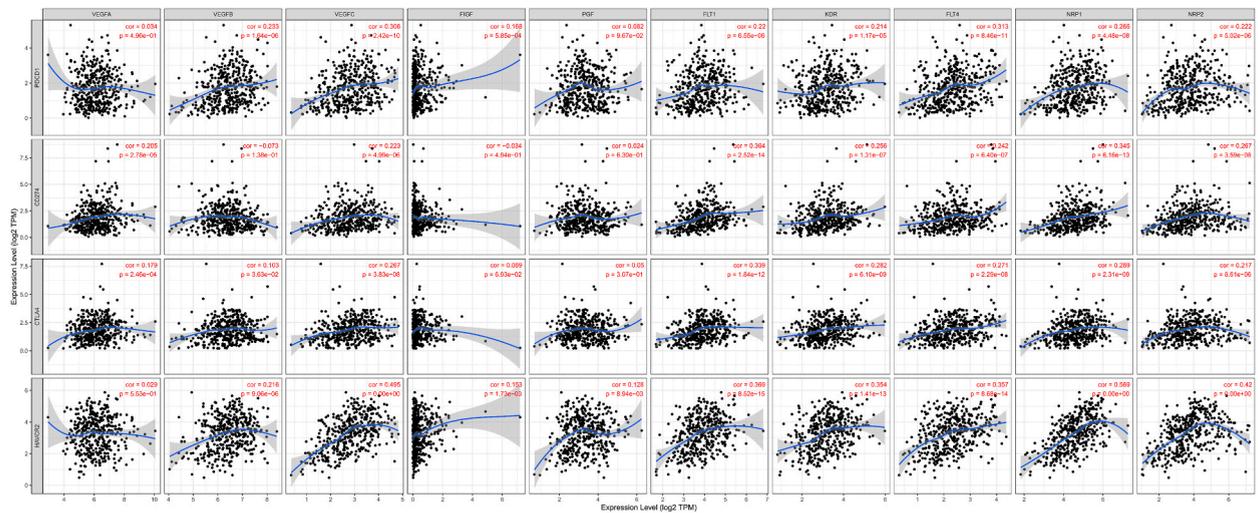


Fig. 7. The correlations between distinct VEGF/VEGFR genes and immune checkpoint expression (TIMER).

normal specimens. The literature also shows that overexpression of *VEGFA* in STAD patients was obviously associated with tumor differentiation and vascular invasion [28,29]. *In vitro* and *in vivo* analyses found that *VEGFA* overexpression contributes to cell proliferation and tumor growth of NCI-N87 gastric cancer cells [30]. Moreover, overexpression of *VEGFA* promoted peritoneal metastasis of gastric cancer cells by activating the ERK/JNK signaling *in vivo* [31]. Our study revealed that *VEGFA* was obviously overexpressed in STAD compared to normal specimens. Survival analysis found that patients with STAD possess higher *VEGFA* expression showed worse OS, FP, and PPS compared to those possess low *VEGFA* expression. Moreover, *VEGFA* overexpression was negatively connected with immune infiltrations of CD4⁺ T cell, B cell, Macrophage, CD8⁺ T cell, and Dendritic cell, and simultaneously positively correlated with CD274 and CTLA4 expression.

VEGFR1 is a ligand of *VEGFR1*, which is mainly expressed on the endotheliocyte, myocardium, and skeletal muscle [32]. To date, the significance of *VEGFB* in pathological angiogenesis is still extremely controversial and deserves more attention. Several studies indicated that *VEGFB* predominantly contributes to the development of the cardiovascular system and its function for angiogenesis is not essential [17]. On the contrary, various studies also demonstrated that *VEGFB* is a vascular remodeling molecular boosting cancer metastasis and plays a prognostic marker for cancer patients [33]. In STAD, a recent research reported that *VEGFB* was highly expressed in cancer specimens, and its overexpression was obviously related to with aggressive histological stage and worse clinical outcome [34]. In our research, we found that *VEGFB* expression in STAD was reduced compared to normal controls, and high *VEGFB* expression was remarkably related to short OS, FP, and PPS in STAD patients. Immune infiltration analysis suggested that *VEGFB* overexpression was obviously positively correlated with Macrophage, CD8⁺ T cell, Dendritic cell, CD4⁺ T cell, and Neutrophil infiltration level. Moreover, *VEGFB* expression is also significantly associated with expression levels of PDCD1, HAVCR2, and CTLA4.

VEGFC has a high affinity for *VEGFR3* and is essential for lymphangiogenesis. Recent studies have demonstrated that *VEGFC* serves as an oncogene in multiple cancer types, including STAD [35]. *VEGFC* overexpression is a predictive biomarker for lymphatic metastasis for STAD patients, and patients possess higher *VEGFC* expression shared worse survival rates than those possess low *VEGFC* expression [36]. Kallistatin has been reported to suppress *VEGFC* expression to restrain the NF- κ B signaling, thus inhibiting the lymphangiogenesis and lymphatic metastasis of STAD *in vivo* [37]. In addition, *VEGFC* overexpression contributes to distant metastasis and cisplatin resistance of STAD [38]. Our study revealed that *VEGFC* expression in STAD was obviously elevated, and its expression was significantly varied across different tumor stages. We also found that *VEGFC* overexpression was remarkably related to worse OS, FP, and PPS in STAD patients. Additionally, our findings suggested that *VEGFC* expression was significantly associated with immune infiltrations of CD8⁺ T cell, CD4⁺ T cell, Macrophage, Dendritic cell, and Neutrophil, and there are positive correlations between *VEGFC* expression and immune checkpoint biomarkers (PDCD1, CD274, CTLA4, and HAVCR2). These results above are consistent with the idea that *VEGFC* acts as an oncogene in STAD.

VEGFD, also known as *FIGF*, presents similar properties to *VEGFC* and promotes the development of lymphatic vessels in tumors by binding *VEGFR3*. *VEGFD* expression was found to be obviously higher in gastric cancer cell lines than in normal controls, and overexpression of *VEGFD* was remarkably associated with hepatic metastasis in STAD patients undergoing surgery [39,40]. Besides, a meta-analysis across 30 studies revealed that *VEGFD* serves as a predictive marker for worse clinical outcome in STAD [41]. *In vitro*, curcumin restrains the lymphangiogenesis of gastric cancer cells via inhibiting the *HMGB1/VEGFD* axis [42]. Our results indicated that *VEGFD* expression in STAD was remarkably decreased. However, no significant correlations between *VEGFD* expression and tumor stage was observed in patients with STAD. Patients with STAD in the high *VEGFD* expression subgroup showed worse OS, FP, and PPS

than those in the low *VEGFD* subgroup. Besides, *VEGFD* was found to be remarkably associated with all TIICs' abundance and immune checkpoint biomarker expression. Given that *VEGFC* and *VEGFD* all participate in lymphangiogenesis by binding *VEGFR3*, targeting the *VEGFC/VEGFD-VEGFR3* pathway to inhibit lymphatic metastasis might be an alternative strategy for anti-cancer therapy of STAD, especially for those who have developed resistance to anti-angiogenic therapies.

PGF, also called *PLGF*, is also a member of the VEGF family and was first identified in human placental tissues. The present study proved that *PGF* expression was significantly elevated in STAD, and overexpression of *PGF* was remarkably related to positive lymph node metastases, advanced tumor stage, and decreased survival time in STAD patients [43]. *PGF* knockdown was demonstrated to suppress migration and induce apoptosis in STAD by depressing PI3K/Akt signaling pathway [44]. In addition, *PGF* acts as a predictor for ramucirumab treatment, and patients with STAD in the *PGF*-low group showed higher ramucirumab sensitivity than the *PGF*-high group [45]. Recent study also determined that *PGF* expression was remarkably increased in STAD, and *PGF* overexpression was remarkably connected with worse OS, FP, and PPS in STAD. Besides, high *PGF* expression was significantly correlated with high CD4⁺ T cell, Macrophage, and B cell infiltration levels in STAD. *PGF* expression was also positively connected with PDCD1 and HAVCR2 expression in STAD.

There are three types of VEGFRs: *VEGFR1*, *VEGFR2*, and *VEGFR3*. Studies have indicated that mRNA and protein expression of *VEGFR1* were significantly elevated in STAD compared to adjacent non-cancerous specimen, and high *VEGFR1* acts as a predictor for poor clinical outcome in STAD patients [32,46]. Blockade of *VEGFR1* contributes to enhancing paclitaxel sensitivity in gastric cancer cells [47]. *VEGFR2*, also called *KDR*, is an angiogenesis regulatory gene that has been proved to be closely related to tumorigenesis and metastasis in diverse malignant tumors, including STAD [48]. Furthermore, the survival rates of STAD patients possess higher *VEGFR2* expression are obviously lower than those possess low *VEGFR2* expression [46]. In addition, selectively targeting *VEGFR2* contributes to enhancing the T cell-mediated antitumor immunity in STAD [49]. *VEGFR3*, also called *FLT4*, is mainly responsible for the development of lymphatic vessels. *VEGFR3* overexpression has been found to be significantly associated with depth of invasion and lymphatic metastasis in STAD [50]. Besides, elevated *VEGFR3* expression also has been found to be significantly related to unfavourable OS in STAD patients, with the silencing of *VEGFR3* significantly inhibiting proliferation and inducing apoptosis in gastric cancer cell lines [46,51]. Similarly, our research revealed that the expression levels of these three VEGFRs in STAD are all remarkably higher than those in normal specimens. In addition, all of these VEGFRs are remarkably correlated with poor OS, FP, and PPS in STAD patients. We also found that these three VEGFRs positively correlate with Macrophage, CD8⁺ T cell, Dendritic cell, CD4⁺ T cell, and Neutrophil infiltration levels. Expression of these VEGFRs are remarkably associated with PDCD1, CD274, HAVCR2, and CTLA4, respectively. These results confirmed the oncogenic roles of VEGFRs in human STAD.

NRP1 and *NRP2* are transmembrane non-tyrosine kinase glycoproteins that modulate the activity of RTKs by selectively binding to certain isoforms of VEGFR. *NRP1* is mainly expressed in vascular endothelial tissue, and dysregulation of *NRP1* has been found to be participated in the development of diverse malignant tumors, including STAD [52]. A recent study involving 1225 cases revealed that *NRP1* overexpression in STAD was closely related to aggressive clinical stage, low differentiation, and lymph node metastasis [53]. Upregulation of *NRP1* predicts worse clinical outcomes and promotes tumor metastasis through epithelial-mesenchymal transition in STAD [54]. *NRP2* is specifically expressed in the venous and lymphatic endothelial cells, and *NRP2* overexpression increase the proliferation and migration of gastric cancer cells by enhancing VEGF activity [55]. In addition, knockdown of *NRP2* remarkably suppressed migration and invasion of gastric cancer cells *in vitro* and sensitized gastric cancer cells to 5-FU toxicity *in vitro* via the β -catenin signaling [56]. Likewise, our findings revealed that *NRP1* mRNA expression level in STAD is significantly higher than that in non-cancerous controls. However, the difference of *NRP2* expression between cancer and control samples with no significance. Interestingly, we found that the expression patterns of *NRP1/NRP2* in differential cancer stages was similar to the expression patterns of *VEGFR1/VEGFR2/VEGFR3*. Survival analysis revealed that high expressions of *NRP1* and *NRP2* are obviously related to unfavourable OS, FP, and PPS in STAD. Immune infiltration analysis demonstrated that *NRP1* and *NRP2* are positively correlated with all host immune cells except for B cell. In addition, we also found that *NRP1* and *NRP2* overexpression correlate with high PDCD1, CD274, HAVCR2 and CTLA4 expression in STAD. Collectively, these results imply that NRP dysregulation in STAD leads to the development of tumors, and the carcinogenic effect of VEGFRs was partially enhanced by NRPs. Co-targeting NRPs might improve the efficiency of anti-VEGF therapy in STAD.

In recent decades, immunotherapy has gained significant progress as an anti-cancer treatment for several malignant tumors. Accumulating studies focused on the association between gene expression and tumor immunity have been published. For example, *NFE2L2* has been identified to serve as a crucial role in the progression of brain lower grade glioma by regulating the infiltration of TIL [57]. *BRAP* has been reported to be overexpressed in liver cancer specimens compared with non-cancerous control, and overexpression of *BRAP* is remarkably associated with immune infiltrating cells and immune checkpoint biomarkers in liver cancer [58]. As for VEGF/VEGFRs, studies have reported that *VEGFA* contributes to inhibiting the immunity of T and NK cells via promoting the proliferation of Treg cells, thereby enabling the cancer cells to escape the immunological surveillance in STAD [59]. Besides, studies also found that *VEGFC* expression was obviously negatively associated with the density of dendritic cells in STAD. *VEGFC* contributes to immune escape of STAD by regulating the function of dendritic cells [60]. *VEGFR2* was highly expressed by effector Treg cells, with the silencing of *VEGFR2* significantly enhanced CD8⁺ T cell infiltration abundances by suppressing *VEGFR2*-mediated effector Treg cell proliferation [61]. *NRP2* was reported to serve as a molecular mediator that links efferocytosis and immunosuppression by regulating phagocytosis in macrophage, knockout of *NRP2* in macrophage suppressed the immunosuppressive effects in the myeloid compartment [62]. Likewise, our findings indicated that the expression of VEGF/VEGFRs in STAD was remarkably associated with abundances of TIICs, including B cell, Macrophage, CD4⁺ T cell, Dendritic cell, CD8⁺ T cell, and Neutrophil. We also found that the expression of VEGF/VEGFRs was remarkably correlated the immune checkpoint biomarkers, including PDCD1, CD274, CTLA4, and HAVCR2. These results above indicate that VEGF/VEGFRs might mediate the initiation and progression of STAD partially through regulating the

abundances of TIICs and immune checkpoint biomarkers expression; therefore, targeting the VEGF/VEGFR signaling might enhance the efficacy of immunotherapy in STAD.

Inevitably, several limitations exist in our study. Firstly, the present study is a retrospective study based on public database, multicenter prospective studies are required to further validate our findings. Besides, clinical and fundamental investigations are needed for exploring the biological mechanisms underlying VEGF/VEGFR-induced tumorigenesis in STAD.

5. Conclusion

In our study, comprehensive bioinformatic analyses were performed to investigate the expression levels, prognostic values, genetic alterations, and immune infiltrations of distinct VEGF/VEGFR members in patients with STAD. We found that the expression of *VEGFA*, *VEGFB*, *VEGFC*, *VEGFD*, *PGF*, *VEGFR1*, *VEGFR2*, *VEGFR3* and *NRP1* were significantly dysregulated in STAD. Increased expression of VEGF/VEGFRs is a potential predictive biomarker of poor prognosis in STAD patients. Besides, we found that the expression of VEGF/VEGFRs was remarkably associated with the infiltration of diverse TIICs in STAD. Our findings elucidate the complexity of the VEGF/VEGFR-mediated tumorigenesis and provide reliable evidence for future comprehensive analysis on the biological function and molecular mechanisms of the VEGF/VEGFR pathway in human STAD, which may provide clues to identify novel prognostic biomarkers among VEGF/VEGFRs in STAD.

Authors' contributions

Jianxin Li: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ting Han: Conceived and designed the experiments; Analyzed and interpreted the data. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in The Cancer Genome Atlas (TCGA) repository (https://portal.gdc.cancer.gov/repository?facetTab=files&filters=%7B%22op%22%3A%22and%22%2C%22content%22%3A%5B%7B%22op%22%3A%22in%22%2C%22content%22%3A%7B%22field%22%3A%22cases.primary_site%22%2C%22value%22%3A%5B%22stomach%22%5D%7D%7D%2C%7B%22op%22%3A%22in%22%2C%22content%22%3A%7B%22field%22%3A%22cases.project.program.name%22%2C%22value%22%3A%5B%22TCGA%22%5D%7D%7D%2C%7B%22op%22%3A%22in%22%2C%22content%22%3A%7B%22field%22%3A%22cases.project.project_id%22%2C%22value%22%3A%5B%22TCGA-STAD%22%5D%7D%7D%2C%7B%22op%22%3A%22in%22%2C%22content%22%3A%7B%22field%22%3A%22files.data_category%22%2C%22value%22%3A%5B%22transcriptome%20profiling%22%5D%7D%7D%2C%7B%22op%22%3A%22in%22%2C%22content%22%3A%7B%22field%22%3A%22files.data_type%22%2C%22value%22%3A%5B%22Gene%20Expression%20Quantification%22%5D%7D%7D%2C%7B%22op%22%3A%22in%22%2C%22content%22%3A%7B%22field%22%3A%22files.experimental_strategy%22%2C%22value%22%3A%5B%22RNA-Seq%22%5D%7D%7D%5D%7D).

Ethics statement

Not applicable (No human clinical data were used in the study).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e17687>.

Abbreviations

VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
STAD	Stomach adenocarcinoma
TME	Tumor microenvironment
RTK	receptor tyrosine kinase

PGF	Placenta growth factor
NRP1	Neuropilin 1
NRP2	Neuropilin 2
TCGA	The Cancer Genome Atlas
OS	Overall survival
FP	First-progression survival
PPS	Post-progression survival
HR	Hazard ratio
CI	Confidence interval
cBioPortal	c-Bio Cancer Genomics Portal
TIMER	Tumor IMMune Estimation Resource
TIL	Tumor-infiltrating lymphocyte
TIIC	tumor infiltrated immune cell

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