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Original Article

Clinicopathological implications of VEGF/VEGFR2 expression and microvessel density in soft tissue sarcoma

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Abstract: Background: Previous studies have shown that angiogenesis, which is regulated by vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) and could assessed by microvessel density (MVD), is important for tumor growth and progression. Nonetheless, the relation between angiogenesis and prognosis of soft tissue sarcoma (STS) remains controversial. Methods: VEGF, VEGFR2 and CD34 protein expression were analyzed by immunohistochemistry in 94 paraffin-embedded tumor tissues of soft tissue sarcoma. MVD was measured by counting vessels stained with CD34 antibody. The associations among VEGF/VEGFR2 protein expression, MVD and prognosis were analyzed by statistical analyses. Results: High levels of VEGF protein expression was found to be significantly correlated with high levels of VEGFR2 ($P<0.001$) and MVD in the high VEGFR2 expression group, but not high VEGF expression group, was observed to be significantly greater than that in the low VEGFR2 expression group ($P=0.007$). In the univariate analyses, VEGF ($P=0.003$) and VEGFR2 ($P=0.002$) were significant negative prognostic indicators of overall survival. While in multivariate analysis, high expression of VEGF was an independent significant negative prognostic factor for overall survival among patients with soft tissue sarcoma. Conclusion: VEGF is a significant independent negative prognostic factor for patients with soft tissue sarcoma and might be a potential adjuvant therapeutic target.

Keywords: Vascular endothelial growth factor, vascular endothelial growth factor receptor 2, microvessel density, overall survival, soft tissue sarcoma

Introduction

Soft tissue sarcomas (STSs) are tumors originating from the mesenchymal lineage. Despite the STS group of tumors share a similar ancestry, they cover more than 50 different histological entities. As the low incidence which only amounts to 0.5% of the annual cancer incidence makes it difficult to focus more on the individual histological entities, STSs usually were treated as a single group [1]. The STSs belong to the high aggressive cancer types with a lethality of 40%-50% [1]. The mainstay of sarcomas treatment is complete surgical resection following with chemoradiotherapy or not, and the cure rate is about 50%. Although chemotherapeutic agents result in short-lived responses but usually could not improve survival. Novel and targeted agents are needed to improve the outcome of these patients. It has been demonstrated that angiogenesis, generally assessed by the micro vessel density (MVD), is involved in the formation of new blood vessels and plays an important role in the growth and metastasis of several solid tumors [2-5]. Angiogenesis inhibitors provide a new and exciting therapeutic option for patients with STS [6-8]. However, the association between MVD and the clinical outcome of STSs is currently controversial and inconclusive [6, 9, 10]. The angiogenesis related pathway in STS also needs to be further confirmed to improve the treatment strategy [6].

The vascular endothelial growth factor (VEGF) is a potent angiogenic factor, which could stimulate endothelial cell proliferation, survival and vascular maturation [11]. VEGF and its recep-

Table 1. Prognostic relevance of clinicopathological variables/VEGF/VEGFR2/MVD for overall survival in 94 patients with softtissue sarcomas

Variables	Patients (n)	Patients (%)	Mean survival (months)	95% *CI*	*P* values
Gender					0.973
Female	51	54.3	36	28-44	
Male	43	45.7	37	27-46	
Age (years)					0.133
≤60	60	63.8	39	32-47	
>60	34	36.2	30	20-40	
Tumor localization					0.268
Extremities	30	31.9	46	36-55	
Trunk	40	42.6	32	23-41	
Head/Neck	9	9.6	22	9-35	
Visceral	4	4.3	40	21-59	
Retroperitonenm	11	11.7	36	16-56	
Histological entity					<0.001
Fibrosarcoma	49	52.1	49	40-59	
Synovial sarcoma	21	22.3	30	20-40	
Liposarcoma	18	19.1	20	10-29	
Undifferentiated Pleomorphic sarcoma	6	6.4	13	7-19	
Tumor size					0.348
<5 cm	36	38.3	40	30-49	
5-10 cm	38	40.4	36	27-46	
>10 cm	20	21.3	27	17-38	
Malignancy grade					0.005
G1-G2	48	51.1	42	35-50	
G3-G4	46	48.9	28	19-36	
TNM stage					0.004
I+II	52	55.3	43	35-50	
III+IV	42	44.7	26	18-35	
Tumor depth					0.012
Superficial	25	26.6	50	37-63	
Deep	69	73.4	31	25-38	
Metastasis at diagnosis					0.005
No	78	83.0	40	33-48	
Yes	16	17.0	20	9-31	
VEGF					0.003
Low	58	61.7	42	35-50	
High	36	38.3	25	17-33	
VEGFR2					0.002
Low	54	57.4	44	36-52	
High	40	42.6	25	17-32	
MVD					0.375
Low	47	50.0	39	30-48	
High	47	50.0	34	25-42	

Note: The cutoff value for low and high: VEGF low <6, VEGF high ≥6, VEGFR2 low <6, VEGFR2 high ≥6, MVD low <42, MVD high ≥42; TNM: tumor node metastasis; VEGF: vascular endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor 2; MVD: microvessel density (univariate analyses, log rank test).

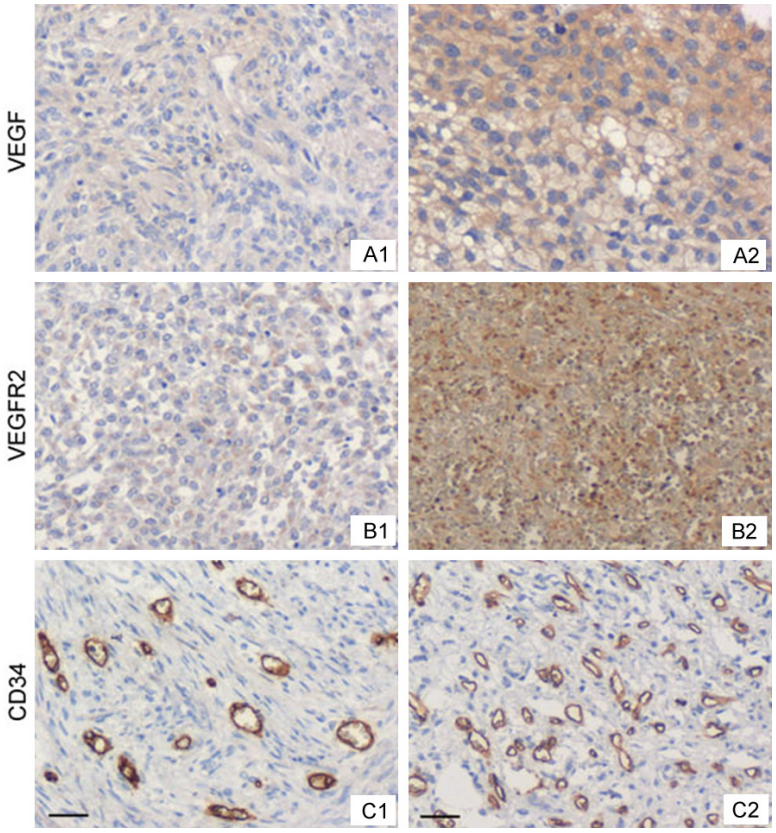

Figure 1. Immunohistochemical expression patterns of VEGF, VEGFR2 and CD34 for MVD in soft tissue sarcoma tissues. A1. Low VEGF expression (<6). A2. High VEGF expression (≥6). B1. Low VEGFR2 expression (<6). B2. High VEGFR2 expression(≥6). C1. Low MVD (<42/field). C2. High MVD (≥42/field). Magnification, ×20. VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; CD34, cluster ofdifferentiation 34; MVD, microvessel density. Scale bar: 50 μm.

tors (VEGFRs) are well-known targets in antiangiogenic treatment. VEGF isomer activates VEGFR1 and is involved mostly in embryonic angiogenesis. VEGF signaling through VEGFR2, which is the most important receptor in tumor angiogenesis, is the major angiogenic pathway promoting vascular proliferation, survival, and metastasis. Various studies have demonstrated that the expression of tumor VEGF and high levels of serum VEGF have been correlated with outcome in many solid tumors, including STS [12-15]. The levels of VEGF in tumor tissue or blood samples from STS patients were associated with tumor grade, metastasis, response to treatment, overall survival (OS) and the risk of recurrence [6, 13, 15-18]. In addition, Itakura E et al found that high expression of VEGFR-2 was associated with longer overall survival [19].

To evaluate the influence of angiogenic activity on the prognosis of patients with soft tissue sarcoma, the expression of VEGF, VEGFR2 and cluster of differentiation (CD) 34 as the marker of MVD were investigated in 94 cases of STS in this study. We investigated the relationship existing among these factors and assessed whether expression of VEGF, VEGFR2 and MVD is correlated with clinicopathological features and disease outcomes.

Materials and methods

Patients and tissue samples

A total of 94 patients with soft tissue sarcoma were enrolled in this retrospective study. All patients were diagnosed and treated at the Fourth Hospital of Hebei Medical University (Shijiazhuang, China) between January 2005 and September 2015. The patients enrolled inthis study all were histopathological diagnosis of soft tissue sarcoma, newly diagnosed and untreated, no history of other tumor. All the patients selected in this study included 43 males and 51 females and the age from 10 to 83 years (mean age, 50.7 years). This study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University. All the patients were followed up by Regular reexamination or phone interview until death or September 2016. The median follow-up was 20 (range 3-87) months. Overall survival was defined as the time from curative surgery to death. All tissue samples were formalin-fixed and paraffin-embedded. Clinical information about the samples is described in detail in **Table 1** and Supplementary Data.

Immunohistochemistry

For immunohistochemistry, paraffin-embedded tissue sections were deparaffinized and rehy-

drated. Sections were placed in 0.01 mol/L of citrate buffer and exposed to microwave heating of 20 min for antigen retrieval. Endogenous peroxidase activity is blocked with 0.3% hydrogen peroxide for 10 minutes. Sections were incubated at 4°C overnight with primaryantibody after blocked in 5% goat serum for 1 hour. Then, slides were incubated with a biotinylated secondary antibody for 1 hours and then with avidin-peroxidase complex for 0.5 hour. The slides were visualized with 3,3'-diaminobenzene (DAB) and counterstained with hematoxylin. Samples incubated with the primary antibody diluents instead of primaryantibodies were used as negative controls. The antibodies used in the study were as follows: VEGF (rabbit monoclonal working solution, Zhongshan Golden Bridge Biotechnology, China, ZA-0580), CD34 (rabbit monoclonal working solution, Zhongshan Golden Bridge Biotechnology, China, ZA-0550,) and VEGFR2 (1:100, rabbit polyclonal, Abcam, Cambridge, ab2349).

Evaluation of immunohistochemistry

For VEGF or VEGFR2 assessment, we randomly selected five independent fields from each slide; the average density positive cells of these five fields were calculated as the protein expression level. Sections were evaluated and scored separately by two pathologists blinded to the clinical parameters. In brief, the intensity ofimmunostainingwas scored as 0 (no immunostaining), 1 (weak), 2 (moderate), and 3 (strong). The extent of staining was scored as 0 (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%). Values for the intensity and extent were multiplied to construct the final score ranging from 0 to 12. High expression of VEGF or VEGFR2 in tumor cells was defined as score ≥6 (representative picture is shown in **Figure 1**).

To analyze the microvessel density, the number of CD34-positive vessels was counted. CD34 is expressed in the endothelial cells of microvessels. Initially, we identified the area with high concentration of vessel at low magnification (×40; ×100). Then MVD was counted at a greater magnification ×200 (×20 objective lens and ×10 ocular lens). Not less than three fields were counted, and the average was calculated. All vessels (both mature and immature) positive for CD34 were calculated. In addition, the specimens were divided into the following two groups: High ≥42 (median value) vessels per highpower field; and low <42 (median value) vessels per highpower field(representative picture is shown in **Figure 1**).

Statistical analysis

All Statistical analysis was performed using SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA). The significance of the association between molecular marker expression and various clinicopathological parameters was evaluated using Chi-squared tests. Mean differences in microvessel counts were compared with the use of the paired t-test. The Kaplan-Meier method with log-rank tests was used to calculate OS. Multivariate analyses using the Cox proportional hazards model were carried out to evaluate independent prognostic factors. The significance level used for all statistical tests was $P<0.05$.

Results

Clinicopathological variables

Table 1 summarizes the clinicopathological variables in the patients included. Among all the patients 51 were female and 43 were male, the median age was 50 (rang 10-83) years. The soft tissue sarcomas comprised 94 tumors including fibrosarcoma (n=49), synovial sarcoma (n=21), liposarcoma (n=18) and undifferentiated pleomorphic sarcoma (n=6). The tumor origins were distributed as follows: 42.6% trunk, 31.9% extremities, 9.6% head/neck, 4.3% visceraland 11.7% retroperitoneal. As shown in **Table 1**, histological entity ($P<0.001$), malignancy grade ($P=0.005$), TNM stage ($P=0.004$), tumor depth ($P=0.012$) and metastasis at diagnosis ($P=0.005$) were all significant prognostic indicators for OS.

Positive correlation between VEGF/VEGFR2 expression or MVD and clinicopathological features

The associations between clinicopathological features and VEGF/VEGFR2 expression levelsor MVD are given in **Table 2**. There was no significant association identified between VEGF/VEGFR2 expression levels or MVD for gender, tumor location, histological entity, tumor size, tumor depth or metastasis at diagnosis. High

Table 2. Correlation between clinicopathological features and VEGF/VEGFR2 expression levels or MVD

Variables	Patients (n)	VEGF expression			VEGFR2 expression			MVD		
		Low (n (%))	High (n (%))	*P* values	Low (n (%))	High (n (%))	*P* values	Low (n (%))	High (n (%))	*P* values
Gender				0.532			0.587			0.535
Female	51	30 (58.8)	21 (41.2)		28 (54.9)	23 (45.1)		27 (52.9)	24 (47.1)	
Male	43	28 (65.1)	15 (34.9)		26 (60.5)	17 (39.5)		20 (46.5)	23 (53.5)	
Age (years)				0.008			0.272			0.668
≤60	60	43 (71.7)	17 (28.3)		37 (61.7)	23 (38.3)		31 (51.7)	29 (48.3)	
>60	34	15 (44.1)	19 (55.9)		17 (50.0)	17 (50.0)		16 (47.1)	18 (52.9)	
Tumor localization				0.204			0.943			0.280
Extremities	30	19 (63.3)	11 (36.7)		16 (53.3)	14 (46.7)		12 (40.0)	18 (60.0)	
Trunk	40	22 (55.0)	18 (45.0)		25 (62.5)	15 (37.5)		20 (50.0)	20 (50.0)	
Head/Neck	9	7 (77.8)	2 (22.2)		5 (55.6)	4 (44.4)		7 (77.8)	2 (22.2)	
Visceral	4	1 (25.0)	3 (75.0)		2 (50.0)	2 (50.0)		3 (75.0)	1 (25.0)	
Retroperitonenm	11	9 (81.8)	2 (18.2)		6 (54.5)	5 (45.5)		5 (45.5)	6 (54.5)	
Histological entity				0.069			0.149			0.894
Fibrosarcoma	49	31 (63.3)	18 (36.7)		31 (63.3)	18 (36.7)		26 (53.1)	23 (46.9)	
Synovial sarcoma	21	16 (76.2)	5 (23.8)		13 (61.9)	8 (38.1)		9 (42.9)	12 (57.1)	
Liposarcoma	18	11 (61.1)	7 (38.9)		9 (50.0)	9 (50.0)		9 (50.0)	9 (50.0)	
Undifferentiated Pleomorphic sarcoma	6	1 (16.7)	5 (83.3)		1 (16.7)	5 (83.3)		3 (50.0)	3 (50.0)	
Tumor size				0.721			0.673			0.378
<5 cm	36	22 (61.1)	14 (38.9)		19 (52.8)	17 (47.2)		21 (58.3)	15 (41.7)	
5-10 cm	38	25 (65.8)	13 (34.2)		22 (57.9)	16 (42.1)		16 (42.1)	22 (57.9)	
>10 cm	20	11 (55.0)	9 (45.0)		13 (65.0)	7 (35.0)		10 (50.0)	10 (50.0)	
Malignancy grade				<0.001			<0.001			0.004
G1-G2	48	38 (79.2)	10 (20.8)		36 (75.0)	12 (25.0)		31 (64.6)	17 (35.4)	
G3-G4	46	20 (43.5)	26 (56.5)		18 (39.1)	28 (60.9)		16 (34.8)	30 (65.2)	
TNM stage				0.003			0.010			0.213
I+II	52	39 (75.0)	13 (25.0)		36 (69.2)	16 (30.8)		29 (55.8)	23 (44.2)	
III+IV	42	19 (45.2)	23 (54.8)		18 (42.9)	24 (57.1)		18 (42.9)	24 (57.1)	
Tumor depth				0.450			0.763			0.243
Superficial	25	17 (68.0)	8 (32.0)		15 (60.0)	10 (40.0)		15 (60.0)	10 (40.0)	
Deep	69	41 (59.4)	28 (40.6)		39 (56.5)	30 (43.5)		32 (46.4)	37 (53.6)	
Metastasis at diagnosis				0.943			0.076			0.583
No	78	48 (61.5)	30 (38.5)		48 (61.5)	30 (38.5)		40 (51.3)	38 (48.7)	
Yes	16	10 (62.5)	6 (37.5)		6 (37.5)	10 (62.5)		7 (43.8)	9 (56.2)	

Note: The cutoff value for low and high: VEGF low <6, VEGF high ≥6, VEGFR2 low <6, VEGFR2 high ≥6, MVD low <42, MVD high ≥42; TNM: tumor node metastasis; VEGF: vascular endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor 2; MVD: microvessel density (VEGF and VEGFR2: Chi-squared tests; MVD: the paired t-test).

Table 3. Multivariate cox model analysis of overall survival

Variables	Beta value	Hazard ratio	95% *CI*	*P* values
Histological entity	0.799	2.223	1.463-3.380	<0.001
Metastasis at diagnosis	0.534	1.706	0.817-3.560	0.155
Tumor depth	0.408	1.503	0.758-2.979	0.243
Malignancy grade	-0.713	0.490	0.184-1.309	0.155
TNM stage	0.352	1.422	0.603-3.355	0.422
VEGFR2 expression	0.103	1.109	0.527-2.334	0.785
VEGF expression	0.982	2.670	01.186-6.013	0.018

Note: TNM: tumor node metastasis; VEGF: vascular endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor 2. Other adjusted factors: Gender P=0.973, Age P=0.133, Tumor localization P=0.268, Tumor size P=0.348, MVD P=0.375 (**Table 1**).

Table 4. Correlation between VEGF and VEGFR2 protein expression

VEGF expression	VEGFR2 expression		*P* value
	Low	High	
Low	48	10	<0.001
High	6	30	

Note: VEGF: vascular endothelial growth factor; VEGFR2: vascular endothelial growth factor receptor 2 (Chi-square test: r value 0.000).

levels of VEGF protein expression were significantly correlated with the age of the patients (P=0.008), malignancy grade (P<0.001) and tumor node metastasis (TNM) stage (P=0.003). VEGFR2 high expression were associated with malignancy grade (P<0.001) and TNM stage (P=0.010). While higher MVD was only correlated with the malignancy grade (P=0.004). Representative immunohistochemical expression patterns of VEGF, VEGFR2 and CD34 are shown in **Figure 1**.

High expression of VEGFR2 was associated with high MVD

The results of correlations among VEGF, VEGFR2 and MVD are shown in **Table 4**, high levels of VEGF protein expression significantly correlated with high levels of VEGFR2 (P<0.001). The mean MVD of the high VEGFR2 expression group was significantly greater than that of the low VEGFR2 expression group (63 vs 41, P=0.007, **Figure 2B**), while the mean MVD between the high and low VEGF protein expression groups was demonstrated no significant difference (53 vs 49, P=0.572, **Figure 2A**). These data imply that VEGF signaling through VEGFR2 may play a role in leading endothelial cells proliferation and microvessel formation.

High expression of VEGF/VEGFR2 correlated with poor STS prognosis

The expression of VEGF and VEGFR2 in TST tissues were significantly correlated with OS via Kaplan-Meier analysis. The log-rank test further demonstrated the significant differences between groups with VEGF or VEGFR2 protein high and low expression, indicating that a high level of VEGF or VEGFR2 was tightly correlated with a shorter survival (**Figure 3A**, **Figure 3B**, **Table 1**, P=0.003, P=0.002). However, no significant difference was identified in the overall survival rate between the high and low MVD groups (**Table 1**, **Figure 3C**, P=0.375). Then multivariate analysis was also performedusing the Cox proportional hazards model including Histological entity, Metastasis at diagnosis, Tumor depth, Malignancy grade, TNM stage, VEGF expression and VEGFR2 expression. As the result presented in **Table 3**, the high level of VEGF expression tightly correlates with poor STS prognosis and is a significant independent prognostic indicator of OS.

Discussion

Based on the current study, angiogenesis is important for tumor growth and metastasis. Angiogenic factors including VEGF and VEGFRs are general associated with increased MVD and poor clinical outcomes. In the present study, we found that high VEGF and VEGFR2 protein expression but not microvessel count in the tumor tissue were significantly correlated with a short OS of patients with soft tissue sarcoma. In addition, a positive correlation between VEGF and VEGFR2 expression was identified, and MVD of the high VEGFR2 expression group was significantly higher than that of the low VEGFR2 expression group.

Previous studies have described that the expression of tumor VEGF expression was correlated with clinical outcome in various types of malignant tumor [20-22], including soft tissue sarcoma [23, 24]. However, the results of various reports indicate the relationship between

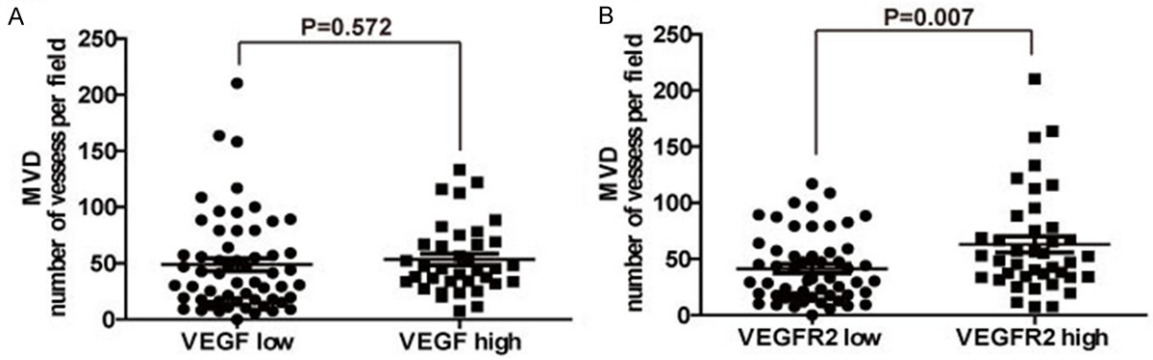

Figure 2. MVD in the low and high VEGF (A) or VEGFR2 (B) expression groups. The data are presented as mean ± SEM. VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; MVD, microvessel density; SEM, standard error of measurement.

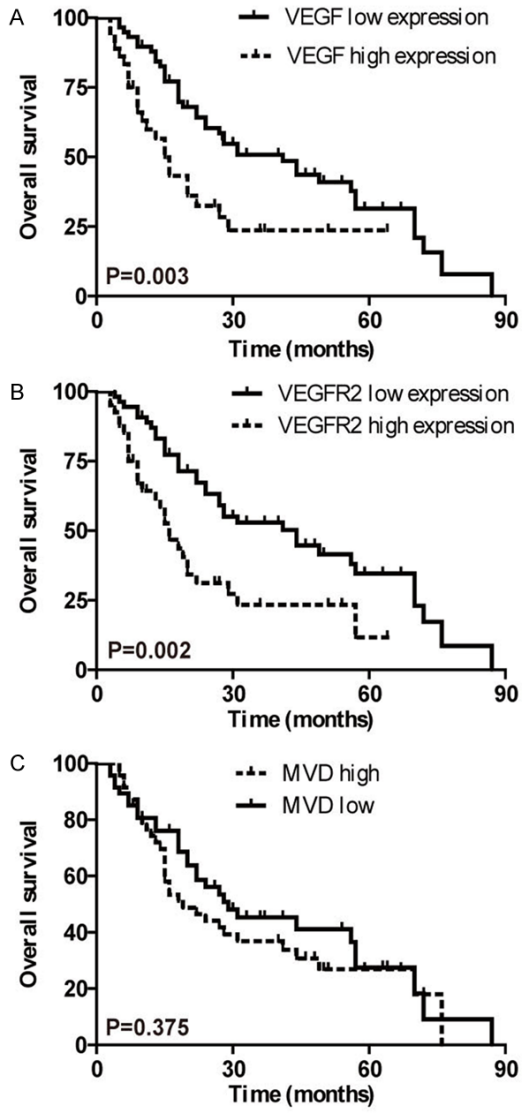

Figure 3. Kaplan-Meier survival analysis of overall survivalin all patients according to VEGF (A), VEGFR2 (B) expression or MVD (C). Thelog-rank test was used to calculate *P* values.

VEGF expression in STS and tumor angiogenesis or clinical outcomes is inconclusive and conflicting [6, 9, 25]. For example, a study indicated VEGF expression was correlated with grade but not independently correlated with overall survival [13]. While Iyoda and colleagues reported that VEGF was an independent prognostic factor for disease free survival in patients with STS of the thorax [14]. In the current study, the OS rate of patients with STS in the high VEGF or VEGFR2 expression group was significantly lower than that of the low expression group. Moreover, Cox proportional hazard multivariate analysis revealed that only the high expression of VEGF was a significant independent prognostic indicator of OS in patients with STS. Our data was consistent with the findings of K Yudoh et al, who demonstrated that VEGF expression correlated with OS in patients with STS [6]. Similar results have also been reported in gastric cancer [26].

However, our study found that the count of microvessels stained with CD34 was not correlated with the prognosis of STS. While in many kinds of cancers, neovascularity assessed by MVD in the tumor tissue has a prognosis value [5]. Furthermore, the data showed that MVD was not correlated with the expression of VEGF in STS. MVD in the high VEGFR2 expression group was significantly higher than that of the low VEGFR2 expression group. While high levels of VEGF protein expression significantly correlated with high levels of VEGFR2. Compared with VEGFR1 and VEGFR3, VEGFR2 is the most important receptor in tumor angiogenesis, VEGF signaling through VEGFR2 is considered as the major angiogenic pathway, promoting vascular proliferation, survival, and metastasis.

The reason why the level of VEGF expression did not correlate with the density of microvessels in STSs still remains unclear. Further studies are imminently needed to clarify this discrepancy in soft tissue sarcomas.

The heterogeneity of the soft tissue sarcoma population is the major disadvantage of our study which is usually appeared in sarcoma studies. In this study, only Fibrosarcoma, Synovial sarcoma, Liposarcoma and Undifferentiated Pleomorphic sarcoma are included. The numbers are not large enough as representative data to do meaningful discussion according to histological subgroups. The results need to be confirmed by larger data.

In conclusion, the results of the current study indicate that VEGF expression levels could be a prognostic factor for patients with soft tissue sarcoma. In addition, it could be deduced that VEGF and VEGFR2 play critical roles in angiogenesis progression of soft tissue sarcoma, but whether these angiogenesis factors could serve as potential therapeutic targets remains unclear. The detailed mechanism of VEGF and VEGFRs on regulating angiogenesis of STS has to be further clarified. Furthermore, the relevant translational study in this area is also urgently needed.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Siegel R, Ward E, Hao Y, Xu J and Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009; 59: 225-249.

[2] Folkman J, Watson K, Ingber D and Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 1989; 339: 58-61.

[3] Xiang T, Xia X and Yan W. Expression of matrix metalloproteinases-2/-9 is associated with microvessel density in pancreatic cancer. Am J Ther 2017; 24: e431-e434.

[4] Qu W, Fu JD, Yang F, Yang GL, Zhang YL, Wang XY, Gu HX, Zhang HY and Wang L. Clinical implications of PTEN and VEGF expression status, as well as microvessel density in esophageal squamous cell carcinoma. Oncol Lett 2015; 10: 1409-1415.

[5] Chang Y, Niu W, Lian PL, Wang XQ, Meng ZX, Liu Y and Zhao R. Endocan-expressing microvessel density as a prognostic factor for survival in human gastric cancer. World J Gastroenterol 2016; 22: 5422-5429.

[6] Yudoh K, Kanamori M, Ohmori K, Yasuda T, Aoki M and Kimura T. Concentration of vascular endothelial growth factor in the tumour tissue as a prognostic factor of soft tissue sarcomas. Br J Cancer 2001; 84: 1610-1615.

[7] Sleijfer S, van der Graaf WT and Blay JY. Angiogenesis inhibition in non-GIST soft tissue sarcomas. Oncologist 2008; 13: 1193-1200.

[8] Martin-Liberal J, Judson I and Benson C. Anti-angiogenic approach in soft-tissue sarcomas. Expert Rev Anticancer Ther 2013; 13: 975-982.

[9] West CC, Brown NJ, Mangham DC, Grimer RJ and Reed MW. Microvessel density does not predict outcome in high grade soft tissue sarcoma. Eur J Surg Oncol 2005; 31: 1198-1205.

[10] Saenz NC, Heslin MJ, Adsay V, Lewis JJ, Leung DH, LaQuaglia MP and Brennan MF. Neovascularity and clinical outcome in high-grade extremity soft tissue sarcomas. Ann Surg Oncol 1998; 5: 48-53.

[11] Giatromanolaki A, Sivridis E and Koukourakis MI. Angiogenesis in colorectal cancer: prognostic and therapeutic implications. Am J Clin Oncol 2006; 29: 408-417.

[12] Potti A, Ganti AK, Tendulkar K, Sholes K, Chitajallu S, Koch M and Kargas S. Determination of vascular endothelial growth factor (VEGF) overexpression in soft tissue sarcomas and the role of overexpression in leiomyosarcoma. J Cancer Res Clin Oncol 2004; 130: 52-56.

[13] Chao C, Al-Saleem T, Brooks JJ, Rogatko A, Kraybill WG and Eisenberg B. Vascular endothelial growth factor and soft tissue sarcomas: tumor expression correlates with grade. Ann Surg Oncol 2001; 8: 260-267.

[14] Iyoda A, Hiroshima K, Baba M, Fujisawa T, Yusa T and Ohwada H. Expression of vascular endothelial growth factor in thoracic sarcomas. Ann Thorac Surg 2001; 71: 1635-1639.

[15] Graeven U, Andre N, Achilles E, Zornig C and Schmiegel W. Serum levels of vascular endothelial growth factor and basic fibroblast growth factor in patients with soft-tissue sarcoma. J Cancer Res Clin Oncol 1999; 125: 577-581.

[16] Hayes AJ, Mostyn-Jones A, Koban MU, A'Hern R, Burton P, Thomas JM. Serum vascular endothelial growth factor as a tumour marker in soft tissue sarcoma. Br J Surg 2004; 91: 242-247.

[17] Yoon SS, Segal NH, Olshen AB, Brennan MF and Singer S. Circulating angiogenic factor levels correlate with extent of disease and risk of recurrence in patients with soft tissue sarcoma. Ann Oncol 2004; 15: 1261-1266.

[18] DuBois S and Demetri G. Markers of angiogenesis and clinical features in patients with sarcoma. Cancer 2007; 109: 813-819.

[19] Itakura E, Yamamoto H, Oda Y and Tsuneyoshi M. Detection and characterization of vascular endothelial growth factors and their receptors in a series of angiosarcomas. J Surg Oncol 2008; 97: 74-81.

[20] Sluiter NR, de Cuba EM, Kwakman R, Meijerink WJ, Delis-van Diemen PM, Coupe VM, Belien JA, Meijer GA, de Hingh IH and te Velde EA. Versican and vascular endothelial growth factor expression levels in peritoneal metastases from colorectal cancer are associated with survival after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. Clin Exp Metastasis 2016; 33: 297-307.

[21] Xia H, Shen J, Chen S, Huang H, Xu Y and Ma H. Overexpression of VEGF-C correlates with a poor prognosis in esophageal cancer patients. Cancer Biomark 2016; 17: 165-170.

[22] Lin C, Zhang Z, Xu Y, Wang R, Chen S, Gao J, Wang D, Huang Q, Tu X and Wang L. High tumor vascular endothelial growth factor expression is associated with poorer clinical outcomes in resected T3 gastric adenocarcinoma. Am J Clin Pathol 2016; 146: 278-288.

[23] Lebellec L, Bertucci F, Tresch-Bruneel E, Bompas E, Toiron Y, Camoin L, Mir O, Laurence V, Clisant S, Decoupigny E, Blay JY, Goncalves A and Penel N. Circulating vascular endothelial growth factor (VEGF) as predictive factor of progression-free survival in patients with advanced chordoma receiving sorafenib: an analysis from a phase II trial of the french sarcoma group (GSF/GETO). Oncotarget 2016; 7: 73984-73994.

[24] McAuliffe JC, Lazar AJ, Yang D, Steinert DM, Qiao W, Thall PF, Raymond AK, Benjamin RS and Trent JC. Association of intratumoral vascular endothelial growth factor expression and clinical outcome for patients with gastrointestinal stromal tumors treated with imatinib mesylate. Clin Cancer Res 2007; 13: 6727-6734.

[25] Kilvaer TK, Valkov A, Sorbye S, Smeland E, Bremnes RM, Busund LT and Donnem T. Profiling of VEGFs and VEGFRs as prognostic factors in soft tissue sarcoma: VEGFR-3 is an independent predictor of poor prognosis. PLoS One 2010; 5: e15368.

[26] Kimura Y, Morohashi S, Yoshizawa T, Suzuki T, Morohashi H, Sakamoto Y, Koyama M, Murata A, Kijima H and Hakamada K. Clinicopathological significance of vascular endothelial growth factor, thymidine phosphorylase and microvessel density in colorectal cancer. Mol Med Rep 2016; 13: 1551-1557.