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Original Article

Correlation between pathological features and protein expressions of TfR1, VEGF and MMP-9 in patients with osteosarcoma

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Abstract: Objective: To analyze the pathological features of patients with osteosarcoma and the correlation of expressions of transferrin receptor 1 (TfR1), vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP)-9 in tumor tissue with the pathological features. Methods: The tumor tissue and paracancerous tissue samples from 31 patients with osteosarcoma were collected before treatment to measure the expressions of TfR1, VEGF and MMP-9. Kaplan-meier curve was used to analyze the relationship of TfR1, VEGF and MMP-9 with the survival time of patients. Log-rank test was used to test the difference, and Spearman test was used to test the correlation. Results: Among the 31 osteosarcoma samples, 23 (74.19%), 24 (77.42%) and 17 (54.84%) samples showed medium-high expressions of VEGF, TfR1 and MMP-9, respectively, which were significantly higher than those in the paracancerous samples ($P<0.05$). Furthemore, the medium-high expression rates of VEGF, TfR1 and MMP-9 were significantly different in patients with different Enneking stages, different histological differentiation degrees, and with or without distant metastasis. Besides, the expression of VEGF was also significantly different in different-size osteosarcoma samples ($P<0.05$). The survival time of patients with low expressions of TfR1, VEGF and MMP-9 was significantly longer than that of patients with medium-high expressions ($P<0.05$). Additionally, in osteosarcoma samples, significant positive correlations were shown between the expressions of TfR1 and VEGF, as well as between TfR1 and MMP-9 ($P<0.05$), but there was no significant correlation between VEGF and MMP-9 ($P>0.05$). Conclusion: TfR1, VEGF and MMP-9 are abnormally expressed in osteosarcoma lesions, suggesting that they all play a role in the occurrence and development of osteosarcoma.

Keywords: Transferrin receptor 1 (TfR1), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), osteosarcoma, indicators

Introduction

Osteosarcoma is a malignant bone tumor that often occurs in children and adolescents [1]. Due to its insidious onset, high incidence, rapid progression, early metastasis and recurrence, the overall survival of patients with osteosarcoma has not been greatly improved, with a 5-year survival rate of less than 20% even after amputation, and the closer the lesion is to the torso, the higher the patient mortality rate. Therefore, early diagnosis and treatment are particularly important. If osteosarcoma can be diagnosed in the early stage, the survival rate and quality of life of patients can be improved to a great extent. In recent years, attention has been paid to the study of pathological indicators of osteosarcoma [2]. Vascular endothelial growth factor (VEGF) is believed to be involved in the process of neovascularization in malignant tumors [3]. Also, as a cell membrane-associated glycoprotein, transferrin receptor 1 (TfR1) plays an important role in iron metabolism, so it is speculated that TfR1 is involved in the occurrence and development of malignant tumors [4]. Matrix metalloproteinase (MMP)-9, a downstream target gene of NF-κB signaling, is a proteolytic enzyme that plays an important

role in inflammation and tumor metastasis. VEGF and TfR1 are regulated by hypoxia-inducible factor (HIF) and miR-210, and VEGF and MMP-9 are important downstream genes of NF-κB signaling. Recently, studies have investigated the pathogenesis of osteosarcoma and the expression of related tumor factors in osteosarcoma, but the correlation of VEGF, TfR1 and MMP expressions in osteosarcoma tissues has not been found [5-7]. Thus, this study collected samples from patients with osteosarcoma to analyze if VEGF, TfR1 and MMP-9 are correlated with the occurrence, development, invasion and metastasis of osteosarcoma.

Materials and methods

General data

Samples were collected from 31 patients with osteosarcoma who underwent radical osteosarcoma surgery in Peking University Cancer Hospital and Institute from April 2019 to April 2021. We obtained lesion tissue sections as osteosarcoma group (OS group) and normal bone tissue 2 cm away from the lesions as control group [8]. This study was approved by the Ethics Committee of Peking University Cancer Hospital and Institute.

Inclusion criteria

(1) Patients' conditions met the World Health Organization's diagnostic criteria for osteosarcoma and confirmed by pathology [9]. (2) Patients were 5-18 years old. (3) Patients were newly diagnosed and did not receive preoperative anti-tumor treatments, such as radiotherapy, chemotherapy, traditional Chinese medicine, targeted therapy and immunotherapy. (4) The clinical data of the patients are complete, and the pathological tissue samples are all paraffin-embedded and in a good condition. (5) Patients were informed about the study and signed informed consent.

Exclusion criteria

(1) Patients had primary malignant tumors in other systems. (2) Patients received antitumor therapy before surgery. (3) Patients died during the perioperative period. (4) Patients had severe organ dysfunction or in critical condition.

Methods

Using an automatic paraffin microtome (LEICA, Germany), the prepared osteosarcoma and paracancerous samples were sliced into serial sections with a thickness of 4 μm and placed in a water tank at a constant temperature (Haier, China). The sections were then placed in a desiccator (Shanghai, MLS-3750) at a constant temperature of 73°C for 2 h and stored for later use. Immunohistochemical analysis was performed using Streptomyces anti-biotin protein-peroxidase (SP) kits (Fuzhou Maixin Biotechnology Development Co., LTD., China) to measure the protein expression of the indicators in osteosarcoma and paracancerous tissues following the operating instructions. The positive sections provided by the kits were used as a positive control, and PBS instead of primary antibody was used as a negative control.

Evaluation criteria [10, 11]

It was seen as positive staining of TfR1 if there were yellow-brown or brown granules in the cell membrane or in the cytoplasm near the cell membrane.

Positive staining of VEGF was identified by yellow-brown or brown granules in the cytoplasm.

Positive staining result of MMP-9 was obtained if the cell membrane or cytoplasm showed yellow-brown or brown granules.

In each sample section, 100 cells were randomly selected from 5 high-magnification fields to observe and calculate the proportion of positive cells. Scoring criteria for positive cells were as follows: 0 point if the positive cells accounted for less than 1% of the counted cells, 1 point for 1-25% positive cells, 2 points for 26-50% positive cells, 3 points for 51-80% positive cells and 4 points for over 80% positive cells.

The scoring criteria for cell staining intensity were as follow: 0 point for no staining marks, 1 point for light yellow cell staining, 2 points for yellow-brown staining, 3 points for significant brown staining.

Immunohistochemical score = score of staining intensity × score of positve cells. An immunohistochemical score ≤3 points refers to low

Table 1. Positive expression of VEFG, TfR1 and MMP-9 in 31 patients with osteosarcoma

Item	n	Positive VEFG	Positive TfR1	Positive MMP-9
Sex				
Male	18	13 (72.22%)	13 (72.22%)	10 (55.56%)
Female	13	10 (76.92%)	11 (84.62%)	7 (53.85%)
Age (years)				
<7	6	5 (83.33%)	4 (66.67%)	3 (50.00%)
7-13	18	12 (66.67%)	14 (77.78%)	10 (55.56%)
>13	7	6 (85.71%)	6 (85.71%)	4 (57.14%)
Distant metastasis				
Yes	15	14 (93.33%)	14 (93.33%)	13 (86.67%)
No	16	9 (56.25%)	10 (62.50%)	4 (25.00%)
Tumor location				
Torso	10	7 (70.00%)	8 (80.00%)	6 (60.00%)
Limbs	21	16 (76.19%)	16 (76.19%)	11 (52.38%)
Tumor diameter (cm)				
<5	16	10 (62.50%)	13 (81.25%)	8 (50.00%)
≥5	15	13 (86.67%)	11 (73.33%)	9 (60.00%)
Histologic grade				
High	9	4 (44.44%)	4 (44.44%)	1 (11.11%)
Intermediate or low	22	19 (86.36%)	20 (90.91%)	16 (72.73%)
Enneking stage				
I	5	2 (40.00%)	1 (20.00%)	1 (20.00%)
II	11	7 (63.64%)	8 (72.73%)	6 (54.55%)
III	15	14 (93.33%)	15 (100.00%)	10 (66.67%)

Note: MMP-9: matrix metalloproteinase-9; VEGF: vascular endothelial growth factor; TfR1: transferrin receptor 1.

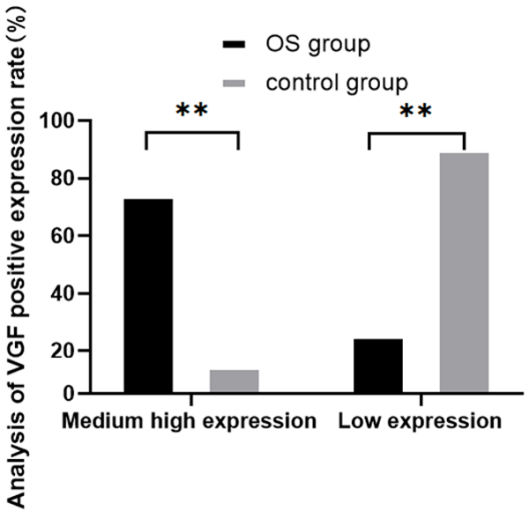

Figure 1. Positive expression of VEGF in osteosarcoma and para-carcinoma tissues. OS: osteosarcoma; VEGF: vascular endothelial growth factor. Compared with OS group, **P<0.01.

expression (- or +). A score of 4-7 points refers to medium expression (++). A score over 7 points refers to high expression (+++).

Statistical processing

The data obtained in the study were processed by statistical software SPSS 23.0. The measurement data that conformed to the normal distribution were expressed as mean ± standard deviation and processed using t test. The counting data were expressed as case number or percentage, and analyzed using Chi-square test. The Kaplan-Meier curve and Log-rank test were used to analyze the relationship of TfR1, VEGF and MMP-9 with the survival time of patients, and the log rank test was used to test the difference. Correlations were analyzed using Spearman's rank-or der correlation. A difference of P< 0.05 was considered statistically significant.

Results

Expression of VEFG, TfR1 and MMP-9 in osteosarcoma

In the 31 patients with osteosarcoma, the positive expression rates of VEGF, TfR1 and MMP-9 were 74.19%, 77.42% and 54.84%, respectively. Details are shown in **Table 1**.

Correlation between VEFG and osteosarcoma

Expression of VEFG in osteosarcoma and paracancerous tissues: Among the 31 osteosarcoma samples, 23 (74.19%) of them showed high expression of VEGF, and 8 (25.81%) showed low expression. Among the 31 samples of paracancerous tissue (control group), 3 (9.68%) of them showed medium-high expression of VEGF, and 28 (90.32%) showed low expression. The high expression rate of VEGF in osteosarcoma tis-

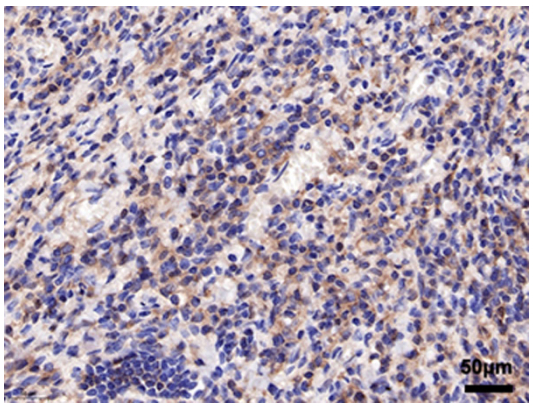

Figure 2. Positive expression of VEGF in osteosarcoma SP×200. VEGF: vascular endothelial growth factor.

sue was significantly higher than that in the paracancerous tissue (χ^2=25.844, P=0.001). See **Figures 1** and **2**.

Relationship between expression of VEGF and pathological features in osteosarcoma tissue: There was no significant difference in the middle-high expression rate of VEGF in the lesion tissue from different tumor locations (P>0.05). However, the medium-high expression rates of VEFG were varied in patients with different osteosarcoma tissue sizes, Enneking stages, histological differentiation degrees, and with or without distant metastasis (P<0.05). See **Table 2**.

Correlation between TfR1 and osteosarcoma

Expression of TfR1 in osteosarcoma and paracancerous tissues: Among the 31 osteosarcoma samples, 24 (77.42%) of them showed medium-high expression of TfR1, and 7 (22.58%) showed low expression. Among the 31 samples of paracancerous tissue (control group), 6 (19.35%) of them showed medium-high expression of TfR1, and 25 (80.65%) showed low expression. The high expression rate of TfR1 in osteosarcoma tissue was significantly higher than that in the paracancerous tissue (χ^2=24.851, P=0.001). See **Figures 3**, **4**.

Relationship between TfR1 expression and pathological features in osteosarcoma tissue: There was no significant difference in the medium-high expression rates of TfR1 in the lesion tissue from different tumor locations or in different sizes (P>0.05). However, differences presented in patients with different Enneking stages, different histological differentiation degrees, and with or without distant metastasis (P<0.05). See **Table 3**.

Correlation between MMP-9 and osteosarcoma

Expression of MMP-9 in osteosarcoma and paracancerous tissues: Among the 31 osteosarcoma samples, 17 (54.84%) of them showed medium-high expression of MMP-9, and 14 (45.16%) showed low expression. Among the 31 samples in the control group, 2 (6.45%) of them showed medium-high expression of MMP-9, and 29 (93.55%) showed low expression. The medium-high expression rate of MMP-9 in osteosarcoma tissue was significantly higher than that in the paracancerous tissue (χ^2=17.733, P=0.001). See **Figures 5**, **6**.

Relationship between MMP-9 expression and pathological features in osteosarcoma tissue: There was no significant difference in the medium-high expression rates of MMP-9 in the lesion tissue from different tumor locations or in different sizes (P>0.05). However, there were significant differences in patients with different Enneking stages, different histological differentiation degrees, and with or without distant metastasis (P<0.05). See **Table 4**.

Relationship of TfR1, VEGF and MMP-9 expressions in osteosarcoma tissue with clinical prognosis

The median survival time of patients with low expression of TfR1, VEGF and MMP-9 was 49 months, 45 months and 51 months, respectively, with 5-year survival rate of 42.86%, 50.00% and 57.14%, respectively. While the median survival time of patients with high expression of TfR1, VEGF and MMP-9 was 27 months, 25 months and 29 months, respectively, with 5-year survival rate of 16.67%, 17.39% and 23.53%, respectively. The results of Kaplan-Meier estimator showed that the survival time of patients with the low expression of TfR1, VEGF and MMP-9 was significantly longer than that of patients with the high expressions (χ^2=10.272, P=0.001; χ^2=8.791, P=0.002; χ^2=10.083, P=0.001, respectively). See **Figure 7**.

Table 2. Relationship between VEGF expression and pathological features in patients with osteosarcoma

Item	Number of cases	Low VEGF expression		Medium-high expression of VEGF		χ^2	P
		n	Rate	n	Rate		
Tumor location						0.136	0.517
Torso	10	3	30.00%	7	70.00%		
Limbs	21	5	23.81%	16	76.19%		
Tumor diameter (cm)						5.011	0.048
<5	16	6	37.50%	10	62.50%		
≥5	15	2	13.33%	13	86.67%		
Distant metastasis						6.560	0.023
Yes	15	1	6.67%	14	93.33%		
No	16	7	43.75%	9	56.25%		
Degree of histological differentiation						5.862	0.027
High	9	5	55.56%	4	44.44%		
Intermediate or low	22	3	13.64%	19	86.36%		
Enneking stage						5.440	0.038
I	5	3	60.00%	2	40.00%		
II, III	26	5	19.23%	21	80.77%		

Note: VEGF: vascular endothelial growth factor. χ^2 is the statistical value of Chi-square test.

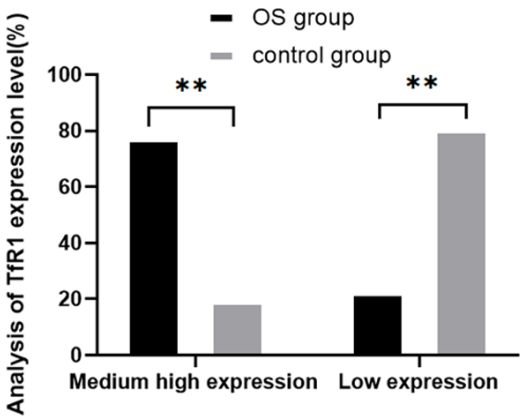

Figure 3. Positive expression of TfR1 in osteosarcoma and para-carcinoma tissues. OS: osteosarcoma; TfR1: transferrin receptor 1. Compared with OS group, **P<0.01.

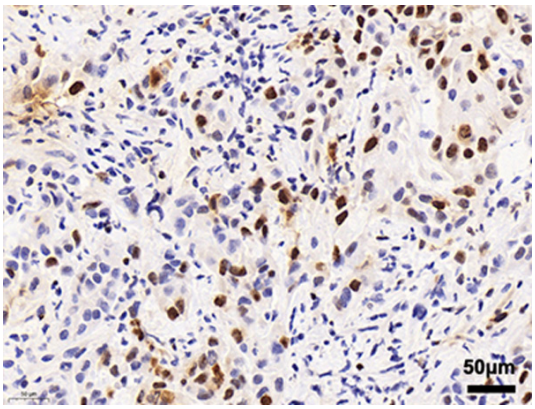

Figure 4. Positive expression of TfR1 in osteosarcoma SP×200. TfR1: transferrin receptor 1.

Correlation among expressions of TfR1, VEGF and MMP-9 in osteosarcoma tissue

Among the 31 osteosarcoma samples, medium-high expression of TfR1 was shown in 24 of them, and low expression in 7 of them; medium-high expression of VEGF was shown in 23 of them, and low expression in 8 of them; medium-high expression of MMP-9 was shown in 17 of them, and low expression in 14 of them. Spearman correlation analysis showed significant positive correlations between the expressions of TfR1 and VEGF, as well as between TfR1 and MMP-9 (P<0.05), but no significant correlation between VEGF and MMP-9 (P>0.05). See **Tables 5**-**7**.

Discussion

At present, the pathogenesis of osteosarcoma is still inconclusive, but scholars have proposed that osteosarcoma, as a malignant tumor, is possibly caused by the combined action of multiple genes and factors, and has a certain cor-

Table 3. Relationship between TfR1 expression and pathological features

Item	Number of cases	Low TfR1 expression		Medium-high expression of TfR1		χ^2	P
		n	Rate	n	Rate		
Tumor location						0.361	0.221
Torso	10	2	20.00%	8	80.00%		
Limbs	21	5	23.81%	16	76.19%		
Tumor diameter (cm)						1.000	0.571
<5	16	3	18.75%	13	81.25%		
≥5	15	4	26.67%	11	73.33%		
Distant metastasis						4.210	0.040
Yes	15	1	6.67%	14	93.33%		
No	16	6	37.50%	10	62.50%		
Degree of histological differentiation						7.888	0.012
High	9	5	55.56%	4	44.44%		
Intermediate or low	22	2	9.09%	20	90.91%		
Enneking stage						11.243	0.005
I	5	4	80.00%	1	20.00%		
II, III	26	3	11.54%	23	88.46%		

Note: TfR1: transferrin receptor 1. χ^2 is the statistical value of Chi-square test.

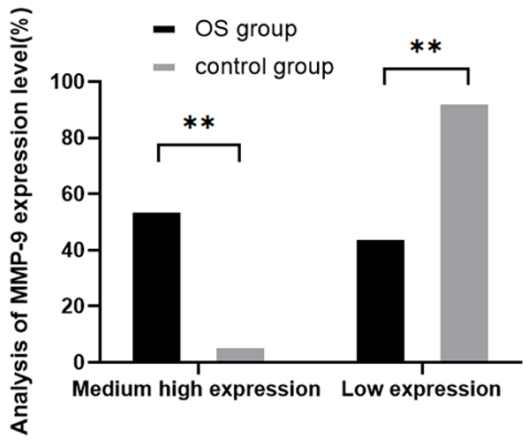

Figure 5. Positive expression of MMP-9 in osteosarcoma and para-carcinoma tissues. OS: osteosarcoma; MMP-9: matrix metalloproteinase-9. Compared with OS group, **P<0.01.

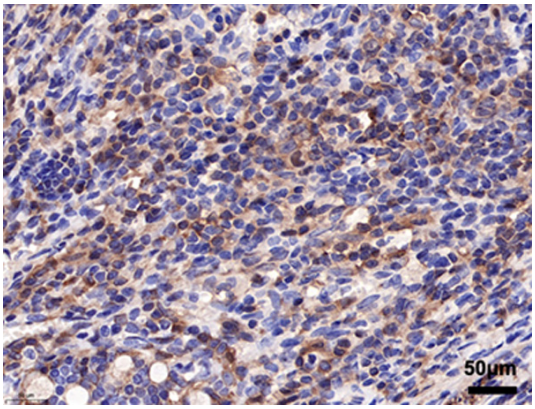

Figure 6. Positive expression of MMP-9 in osteosarcoma SP×200. MMP-9: matrix metalloproteinase-9.

relation with the abnormal changes of proto-oncogenes and tumor suppressor genes [12]. Recent study showed that the formation of blood vessels plays a key role in the rapid growth and distant metastasis of osteosarcoma [13]. Blood vessels, as one of the important components of the interstitium, interact with cancer cells to form a complete micro-ecological system, and provide the nutrients needed for the occurrence and development of tumors [14]. VEGF is a glycosylated secreted polypeptide with high conservation [15]. VEGF can stimulate the differentiation of vascular endothelium and promote angiogenesis after binding with VEGF receptor. In addition, when VEGF acts directly on endothelial cells, it increases the concentration of intracellular calcium ions, thereby improving cell morphology, promoting a large number of cell divisions and building migratory blood vessels. At the same time, VEGF also regulates the activated form of endothelial cell genes, which induces the related factors of endothelial cell gene expression

Table 4. Relationship between MMP-9 expression and pathological features

Item	Number of cases	Low MMP-9 expression		Medium-high expression of MMP-9		χ^2	P
		n	Rate	n	Rate		
Tumor location						0.159	0.690
Torso	10	4	40.00%	6	60.00%		
Limbs	21	10	47.62%	11	52.38%		
Tumor diameter (cm)						0.313	0.576
<5	16	8	50.00%	8	50.00%		
≥5	15	6	40.00%	9	60.00%		
Distant metastasis						11.888	0.001
Yes	15	2	13.33%	13	86.67%		
No	16	12	75.00%	4	25.00%		
Degree of histological differentiation						9.791	0.002
High	9	8	88.89%	1	11.11%		
Intermediate or low	22	6	27.27%	16	72.73%		
Enneking stage						12.922	0.001
I	5	4	80.00%	1	20.00%		
II, III	26	10	38.46%	16	61.54%		

Note: MMP-9: matrix metalloproteinase-9. χ^2 is the statistical value of Chi-square test.

organization and stimulates angiogenesis through the induction of interstitial collagenase and proteolytic enzymes. It has been confirmed that tumor angiogenesis is one of the important prognostic factors of malignant tumors, and VEGF can participate in the growth, invasion and metastasis of tumor cells by inducing the regeneration of blood vessels [15]. In recent years, a large number of studies have pointed out that VEGF is highly expressed in osteosarcoma tissue, and the high expression of VEGF can predict higher Enneking stage and distant metastasis in patients with osteosarcoma. Also, the survival of patients with high VEGF expression is significantly shorter than that of patients with low expression [16, 17]. In addition, study has shown that VEGF receptor 2 is positively expressed in patients with osteosarcoma, and the expression of VEGF receptor 2 is higher in osteosarcoma with lung metastasis, which means that VEGF is not only related to angiogenesis in osteosarcoma, but also osteosarcoma metastasis, suggesting high research significance [18]. In this study, SP method was used to measure VEGF in osteosarcoma and paracancer tissues. The results showed a medium-high expression of VEGF in the osteosarcoma tissue. The positive expression increased when Enneking stage increased, indicating that osteosarcoma was positively correlated with VEGF expression, which is similar to relevant research results [19]. To date, in the clinical TNM staging standard of breast cancer, T2 or T3 stage is determined according to whether the tumor size is greater than 5 cm. Therefore, diameter in 5 cm was used as the demarcation of tumor tissue size in this study. The results showed that the expression of VEGF in tumor with a diameter of over 5 cm was higher than that in tumor with a diameter of ≤5 cm ($P<0.05$). However, a previous study showed that tumor size had no effect on the expression level of VEGF [20]. The difference may be due to different cut-off standard or the study design.

Iron is a trace element necessary for body cells to maintain normal physiological functions. An iron metabolism disorder can easily cause a variety of diseases. Malignant tumor cells increase the demand for iron due to their massive proliferation, during which the cells need to absorb a large amount of iron from the cytoplasm to meet their own proliferation needs [21]. TfR1, as a transferrin receptor on the cell surface, expresses on various types of cells but not on mature red blood cells, especially high in placental red blood cells and in liver [22]. In the process of iron metabolism, TfR1 can recognize the extracellular Tf-$(Fe^{3+})2$ complex, and combine with it to form a TfR1/Tf-$(Fe^{3+})2$ complex, which enters into the cell by endocytosis, and

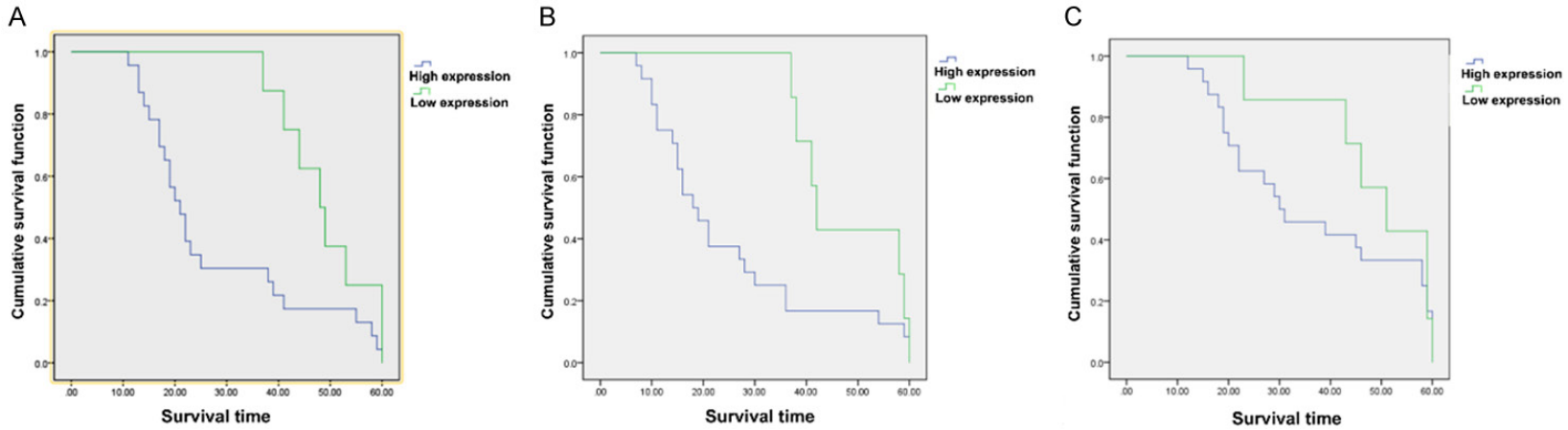

Figure 7. Correlation of TfR1, VEGF and MMP-9 expressions with prognosis of osteosarcoma. TfR1: transferrin receptor 1; VEGF: vascular endothelial growth factor; MMP-9: matrix metalloproteinase-9.

Table 5. Correlation between expressions of TfR1 and VEGF in osteosarcoma tissue (n)

	TfR1		In total	r	P
	Medium-high expression	Low expression			
VEGF				0.615	0.001
Medium-high expression	21	2	23		
Low expression	3	5	8		
In total	24	7	31		

Note: VEGF: vascular endothelial growth factor; TfR1: transferrin receptor 1.

Table 6. Correlation between expressions of TfR1 and MMP-9 in osteosarcoma tissue (n)

	TfR1		In total	r	P
	Medium-high expression	Low expression			
MMP-9				0.385	0.014
Medium-high expression	15	2	17		
Low expression	9	5	14		
In total	24	7	31		

Note: MMP-9: matrix metalloproteinase-9; TfR1: transferrin receptor 1.

Table 7. Correlation between expressions of VEGF and MMP-9 in osteosarcoma tissue (n)

	VEGF		In total	r	P
	Medium-high expression	Low expression			
MMP-9				0.183	0.197
Medium-high expression	12	5	17		
Low expression	11	3	14		
In total	23	8			

Note: MMP-9: matrix metalloproteinase-9; VEGF: vascular endothelial growth factor.

the Fe^{3+} is ingested into cells. Study found that TfR1 is abnormally expressed in a variety of malignant tumors, especially in vigorously dividing tumor cells, where its expression levels are abnormally elevated [23]. The results of this study showed that the expression of TfR1 was significantly increased in osteosarcoma tissue as compred with that in the paracancerous tissue ($P<0.05$), suggesting that TfR1plays an important role in the occurrence and development of osteosarcoma. Further research on the correlation of TfR1with the clinicopathological characteristics found that different tumor sizes and locations had no significant effect on the expression of TfR1 ($P>0.05$), while there were significant differences in the expression of TfR1 in osteosarcoma tissues at different Enneking stages, different histological differentiation degrees, and with or without distant metastasis ($P<0.05$). The expression was significantly elevated with decreasing histological differentiation, increasing Enneking staging and existence of distant metastasis.

The growth and development of tumors is a complex process, which involves in the remodeling of extracellular mechanisms. MMP-9 is one of the important proteins that have been found to be involved in the whole process of tumor growth and development, especially in tumor invasion and metastasis. Knockdown of intratumoral MMP-9 or inhibition of its activity in vitro can limit the invasive and metastasizing ability of tumor cells [24]. Previous study used immunohistochemical method to measure MMP-9 in tissue samples from 153 patients with renal cancer, and showed that the expression of MMP-9 in renal cancer samples was significantly increased, and was positively correlated with tumor size and differentiation degree, suggesting that MMP-9 plays an important role in the progression of renal cancer [25]. Another study also found that patients with high expression of MMP-9 had a poor prognosis, and the disease-free survival time of those patients was significantly shorter than that of patients with low expression [26]. Our results showed that the positive rate of MMP-9 in poorly differentiated tumors was higher than that in moderately differentiated and well-differentiated tumors. In addition, the expression of MMP-9 also increased with the progress of Enneking stage. The positive expression rate of MMP-9 in stage III patients was significantly higher than that in stage I patients, which is similar to the results of related study [27].

Analysis of the correlation of the expressions of TfR1, VEGF and MMP-9 with the prognosis of patients showed that with the increase of those expression levels, the survival time of patients was significantly shortened. The survival time of patients with medium-high expression levels of TfR1, VEGF and MMP-9 was significantly shorter than that of patients with low expression levels ($P<0.05$). It is indicated that the expression levels of TfR1, VEGF and MMP-9 are of important significance for evaluating the prognosis of osteosarcoma. Further analysis of the correlation among TfR1, VEGF and MMP-9 found that the expression of TfR1 and VEGF in osteosarcoma tissue was positively correlated with that of MMP-9, suggesting that the expression levels of TfR1, VEGF and MMP-9 are related to the development, metastasis and invasion of osteosarcoma, and there may be a synergistic relationship between TfR1 and VEGF. The mechanism of action may be related to that TfR1 and VEGF are both important downstream gene of NF-κB and regulated by HIF and miR-210, showing a coordinated effect on the tumor microenvironment and playing an important role in regulating tumor metastasis [28]. In this study, all lesions and paracancerous samples were derived from the same patient, which effectively excluded other disease or individual factors that might have affected the results.

To sum up, the expression levels of TfR1, VEGF and MMP-9 in osteosarcoma tissue are closely related to the occurrence, development, invasion and metastasis of osteosarcoma, which provides a basis for the future use of TfR1, VEGF and MMP-9 as new biomarkers to predict the occurrence, development, degree of disease, distant metastasis and prognosis of osteosarcoma, as well as novel treatment targets for osteosarcoma. However, this study only included a small sample size from one study center and analyzed the expression levels of TfR1, VEGF and MMP-9 and their correlation with osteosarcoma without studying its specific mechanism. So, the mechanism of action of TfR1, VEGF and MMP-9 needs to be explored by further studies with expanded sample size from multiple centers.

Disclosure of conflict of interest

None.

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