RESEARCH ARTICLE

Clinical Predictive Value of Serum Angiogenic Factor in Patients with Osteosarcoma

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Abstract

Objective: To explore serum angiogenic factor expression in patients with osteosarcoma and its relationship with metastasis. Methods: Immunohistochemistry was used to test the expression of CD34 and FVIII-Rag in osteosarcoma tissues of 36 patients (osteosarcoma group) and microvessel density (MVD) was also recorded. In addition, ELISA was used to test the level of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-β1 (TGF-β1) and endostatin (ES) in the osteosarcoma group and in a control group. Results: VEGF and ES level were significantly higher than in the control group before operation (P<0.01), VEGF, bFGF and TGF-β1 correlating with the ES level (P<0.01). Serum VEGF and ES levels of osteosarcoma patients before surgery were closely related to relapse and metastasis; moreover, serum VEGF increased with MVD (P<0.01). Postoperative VEGF and ES levels were lower than the preoperation values (P<0.01); ES level in relapse group was significantly higher than that of the non-relapse group (P<0.01). Conclusion: Preoperative serum VEGF and postoperative ES levels have great predictive value with regard to relapse of osteosarcoma patients.

Keywords: Osteosarcoma - angiogenic facor - microvessel density

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Introduction

Osteosarcoma is a primary malignant carcinoma of bone, which is frequently diangosed in teenagers with high mortality. The histological feature is the formation of osteoid tissue. The disease metastasizes in early stage and minimal pulmonary metastasis occurs in 80% of patients (Lee et al., 2012). Microvessel consist of tiny vessels and capillaries, the distribution of microvessel of carcinoma is among oncocyte. Angiogenic factor has played important role in tumor angiogenesis. Previous research has revealed that vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and transforming growth factor-β1 (TGF-β1) have close relevance to a variety of tumor progression (Kim et al., 2009; Tsubaki et al., 2011). In addition, endostatin (ES) is considered to be related with tumor prognosis and microvessel density. The aim of this research is to explore serum angiogenic factor expression in patients with osteosarcoma, and its relationship with clinical characteristics as well as microvessel density (MVD).

Materials and Methods

Specimen

Pathological tissue and serum of 36 osteosarcoma patients were obtained from tumor specimen bank of Tumor Department from February 2006 to December 2008. There were 20 male and 16 female patients with median age of 21 years (from 13 to 69 years). Tumor location: tumor of 14 patients were in left side of body and 22 in right side; 16 located in femur, 14 in tibia, 4 in fibula and 2 in ribs. Of 36 pathological specimens, 30 were obtained from surgical resection and 6 from biopsy. All of the pathological specimens diagnosed by HE stain had been divided into three levels: 9 patients in levelIwith higher oncocyte differentiation, lower tumor giant cells and osteoid tissue; 14 were in levelIIwith relatively lower differentiation, more tumor giant cells and medium osteoid tissue; There were 14 patients in level III with lower differentiation, the most tumor giant cells and less osteoid tissue. Fifteen patients were treated by amputation and chemotherapy, 11 by amputation and 9 by implantation after tumor resection or integration with the cross ends. One patient was diagnosed by biopsy and had no curative intention. Twenty one patients (relapse group) were detected with relapse and pulmonary metastasis within one year, and the rest 15 had no evidence of relapse and metastasis (non-relapse group). At the same time, we recruited 15 healthy individuals to set a control group, among them 8 are male and 7 female, with median age 21 years (from 12 to 65 years).

ELISA and immunohistochemical staining

The strict manipulation should be followed according to the instructions of R&D Corporation(the ELISA kit was purchased from R&D Corporation) while we adopted ELISA to test the density of VEGF, bFGF, TGF-β1 and

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ES in the serum of osteosarcoma group and control group. The specimen tissue from osteosarcoma group was fixed with 4% paraformaldehyde and embedded by paraffins. The tissue was cut into 4 μm thick serial sections and one tissue was chosen to stain in every five sections. There were 8 sections in one specimen and then the expression of CD34 and FVIII-Rag were tested (the antibody was purchased from sigma), PBS to be negative contrast in stead of the first antibody.

Intratumoral MVD enumeration

The experiment applied CMIAS image analysis system to record 3 MVD numbers by shifting 3 fields at high magnification to observe staining slice of FVIII-Rag and CD34, and then to calculate average value and designated as the value of MDV of each section. For each patient, the MDV value of specimen was set by estimating the average number of 8 slices.

Statistics processing

All the data was analysed by statistics software SPSS10.0, enumeration data by X^2, measurement data by t and relevant analysis by Spearman rank correlation.

Results

General characteristics

General characteristics of patients in osteosarcoma group are listed in Table 1.

Preoperative serum level of VEGF, bFGF, TGF-β1 and ES

Preoperative serum VEGF and ES levels in patients with osteosarcoma were significantly higher than that of control group (P<0.01); No significant difference was detected regarding bFGF, TGF-β1 level with control group (P>0.05) (Table 2). The Spearman analysis demonstrated that the VEGF, bFGF and TGF-β1 level in osteosarcoma group had positive correlation with ES level (P<0.01).

Table 1. General Characteristics of Patients in Osteosarcoma Group

		No=36
Gender	Male	20
	Female	16
Size of tumor (cm)	≥10	22
	<10	14
Recurrence and metastasis	Yes	21
	No	15
Diseased region	Left side	14
	Right side	22
	Femur	16
	Tibia	14
	Fibula	4
	Ribs	2
Specimen source	Surgery resection	30
	Biopsy	6
Treatment	Amputation and chemotherapy	15
	Amputation simplex	11
	Tumor resection, inactivation and reimplantation or integration with the cross ends	9
	Untreated	1
Dahlin's parting	Osteoblastoma type	13
	Chondroblastoma type	9
	Fibroblastoma type	9
	Hybrid type	5
Differentiation degree	Level I	9
	Level II	14
	Level III	13

Table 2. Preoperative Serum VEGF, bFGF, TGF-β1 and ES Levels of Two Groups ($\bar{\chi}$±s)

Group	VEGF(ng/dL)	ES(ng/dL)	bFGF(ng/dL)	TGF-β1(ng/dL)
Osteosarcoma group	1756.3±189.6**	311.5±32.5**	65.4±9.2	120.3±13.5
Control group	389.5±41.6	41.6±5.9	59.9±8.2	112.9±15.6

Compared with control group, **P<0.01

Table 3. The Relationship Between Preoperative Serum VEGF, bFGF, TGF-β1 and ES and Clinical Characteristics ($\bar{\chi}$±s)

	cases	VEGF(ng/dL)	bFGF(ng/dL)	TGF-β1(ng/dL)	ES(ng/dL)
Gender					
Male	20	1769.2±209.6	63.9±9.5	125.9±15.7	287.5±34.3
Female	16	1747.6±185.2	66.8±7.9	119.0±20.5	297.9±39.4
Size (cm)					
≥10	22	1802.6±205.7	62.8±10.5	116.5±21.6	306.7±41.9
<10	14	1695.8±225.4	67.1±9.5	121.5±25.3	294.5±42.5
Recurrence and metastasis					
Yes	21	2789.2±265.4**	67.6±9.9	125.6±19.2	447.9±50.1**
No	15	856.0±101.7	69.2±9.5	132.6±15.5	85.6±9.5
Dahlin's parting					
Osteoblastoma type	13	1805.6±268.4	60.5±7.3	105.6±25.4	297.3±53.6
Chondroblastoma type	9	1779.5±204.6	58.4±5.9	122.6±20.4	285.4±65.3
Fibroblastoma type	9	1815.3±214.5	65.7±9.4	110.4±14.0	301.7±66.0
Hybrid type	5	1684.7±214.8	55.3±10.5	105.2±14.6	277.9±74.0
Differentiation degree					
Level I	9	1710.6±225.6	62.3±9.7	125.8±19.2	288.6±59.5
Level II	14	1779.0±204.0	59.7±6.0	117.0±21.2	295.4±49.5
Level III	13	1756.3±254.6	66.4±8.9	121.6±25.5	281.6±56.0

Compare with non-relapse group, **P<0.01

Table 4. Serum VEGF and ES Level in Patients with Osteosarcoma ($\bar{\chi}$±s)

	Number of patients	VEGF(ng/dL)	ES(ng/dL)
Preoperation	36	1756.3±189.6	311.5±32.5
Postoperation			
Relapse group	21	523.4±65.7**##	66.7±9.5**##
Non-relapse group	15	465.9±59.0**	40.2±5.9**

Compared with preoperation, **P<0.01; Compared with non-relapse group, ##P<0.01

The relationship between serum angiogenic factor expression in preoperative osteosarcoma patients and their clinical characteristics

The VEGF and ES level in serum of peroperation osteosarcoma patients had a close relationship with relapse or metastasis, but there was no correlation with Dahlin's parting and degrees of differentiation (Table 3).

The relationship between MVD enumeration and relevant angiogenetic factors

The enumeration of CD34 and FVIII-Rag immunohistochemical staining demonstrated the ups and downs of MVD. The MVD value of 36 patients in osteosarcoma group was 9.5-88.1, and the median was 40.3. The VEGF level in preoperative serum and MVD were positively correlated (P<0.01).There were no significant relevance between serum bFGF, TGF-β1, ES and MVD preoperatively (P>0.05).

Postoperative serum level of VEGF and ES in osteosarcoma group

Postoperative serum level of VEGF and ES were significantly decreased (P<0.01), the level of VEGF and ES in relapse group was much higher than that of non-relapse group (P<0.01) (Table 4).

Discussion

Osteosarcoma is an angiogenic tumor (Gao et al., 2011; Li et al., 2011; Li et al., 2011; Li et al., 2011; Limmahakhun et al., 2011; Xu et al., 2011; Xu et al., 2011; Yan et al., 2011; Zhang et al., 2011; Zhang et al., 2011). The roles of angiogenic factors such as VEGF, bFGF and TGF-β1 played in the formation of osteosarcoma are not clear. VEGF, as angiogenic factors of vascular endothelial cell, has specific biological function that is to promote the multiplication of endothelial cells and the generation of new vessels in the tumor tissue as well as growth and metastasis of tumor (Nataraj et al., 2012). Normal cells and oncocyte enable to produce VEGF which could act on its receptor of endothelial cells, stimulate the hyperplasia of endothelial cells, accelerate the vascular permeability and finally lead to effusion of plasma proteins (Bălu et al., 2012). Therefore, VEGF is the inducing factor to form the tumor vessels and specifically stimulate the multiplication of vascular endothelial cells. The excessive expression of VEGF has a close relationship with the growth, invasion and metastasis. A variety of experiment in vitro demonstrated that the growth factor, eg., bFGF, TGF-β1 and PDGF are able to enhance the expression and activate the capability of angiogenesis (Kasuya et al., 2011; Yang et al., 2012). The increasing of vascular density presents the accelerated speed of tumor, which could promote the metastasis of cancer. As a result, this could be associated with the occurrence of pulmonary metastasis and poor prognosis of patients. VEGF is able to promote the hyperplasia of endothelial cells and angiogenesis of tumor through the combination with receptor expressed by vascular endothelial cells (Flk-1 and Flt-1) (Hlobilkova et al., 2009).

MVD of osteosarcoma has a close relationship with invasive, relapse and aggravation degree. Thus, it is considered as an index to predict relapse, metastasis and prognosis (Oda et al., 2006). In this research, serum VEGF level of preoperative patients with osteosarcoma has significant positive correlation with relapse, metastasis and MVD, the higher the VEGF in serum, the more reliable in terms of predictive value MVD could be. It is concluded that VEGF level enables to reveal the state of tumor vascularization and predict the early relapse and metastasis as well. The possibility is that osteosarcoma has indirectly induced the expression of VEGF to stimulate angiogenesis. We will take providing polyclonal antibody of VEGF to osteosarcoma cells in vitro culture into consideration to observe its inhibitory action on angiogenesis.

ES, endogenous inhibitory factor with a specific space structure, is able to compete with VEGF and integrates with heparin on the surface of endothelial cell (Sunshine et al., 2012). ES has remarkable inhibition function of restraining tumor angiogenesis and choosing function to proliferation of endothelium. Some research has revealed that ES results in distinct decline of index of tumor cell proliferation. The inhibition is realized through DNA synthetic blockage, depression of dividing and growth of cells as well as angiogenesis. The main mechanism is as follows (Dirkx et al., 2006; Zheng, 2009; Tan et al., 2011): (1) it induces the apoptosis of endothelial cell by means of decline anti-apoptosis gene. (2) it promotes apoptosis of endothelial cell by acidizing connection protein phosphorus and activating of tyrosinase. (3) it restrains endothelial cell integrin to induce apoptosis. (4) ES enables to inhibit G1 phase of endothelial cell. Our research reveal that ES level in osteosarcoma group is higher than control group, and it drops after surgery. In a word, ES is closed related with prognosis.

The balance between stimulating factor and inhibition factor determines the state of angiogenesis of entity tumor (Malamitsi-Puchner et al., 2005), so the equivalence of VEGF and ES probably become the determinant factor. In this research, preoperative serum level of VEGF is positively correlated with ES with statistical significance, and both of them are higher than that of control group; declined levels of both after surgery indicates the preoperation and postoperation test could reflect the activity of cancer angiogenesis. Thus, new method targetting angiogenesis is necessary to be developed to treat osteosarcoma.

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