ONCOLOGY

Vascular Endothelium Growth Factor and Angiogenin in the Serum of Patients with Osteosarcoma and Ewing's Tumor

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The concentrations of vascular endothelium growth factor and angiogenin were measured by enzyme immunoassay in the sera of healthy subjects and patients with osteosarcoma and Ewing's tumor with consideration for the main clinical and morphological characteristics of the diseases. The studied angiogenic factors were characterized by high individual variability in both healthy subjects and age- and sex-matched patients with primary bone sarcomas. The level of endothelial growth factor was higher in control women than in men. The highest concentration of endothelial growth factor was detected in male patients with Ewing's tumor. Possible involvement of vascular endothelium growth factor and angiogenin in the pathogenesis of osteosarcoma and Ewing's tumor is discussed.

Key Words: *angiogenesis; vascular endothelium growth factor; angiogenin; osteosarcoma; Ewing's tumor*

Osteosarcoma and Ewing's tumor are extremely aggressive malignant tumors of the skeleton characterized by early hematogenic metastasizing [3-5]. The mechanisms determining invasion of tumor cells into adjacent tissues, microcirculatory system, and lymphogenic or hematogenic dissemination with subsequent extravasation and formation of secondary tumor foci are still not clear. In addition, the capacity to invasion can vary within a heterogeneous population of primary tumor cells. However it was proven that in some human solid tumors the density of microvascular network per tumor tissue surface unit directly correlated with the incidence of metastases. It is suggested that proliferation and migration of endothelial cells, which, like tumor cells, produce proteases and locally invade the basal membrane and perivascular extracellular matrix, thus forming a new capillary network in the tumor, is a prerequisite for angiogenesis. Angiogenic reaction of the endothelium can be induced by cytokines, *e. g.*, growth factors produced by tumor cells: fibroblast growth factor, platelet growth factors α and β, and epidermal growth factor [1]. A special place among angiogenic cytokines is occupied by vascular endothelium growth factor (VEGF), which binds to endothelial cells and stimulates their proliferation. This factor is produced by some human tumors and promotes tumor neovascularization and, probably, early generalization of the tumor process [8,12]. The central role of VEGF in the regulation of normal and pathological angiogenesis has been recently demonstrated [10-12]. Angiogenic cytokine is an active mitogen for micro- and macrovascular cells of blood and lymph vessels and acts synergistically with fibroblast growth factor-2 [1]. It is now known that angiogenin (AG) is a potential regulator of angiogenesis promoting the growth of primary tumor and metastases [6-9,13,14].

We compared serum concentrations of VEGF and AG in healthy subjects and patients with osteosarcoma and Ewing's tumor.

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MATERIALS AND METHODS

Sixteen patients (13 men and 3 women) aged 15-32 years (19.6±1.4 years) were examined. Clinical and x-ray diagnosis was confirmed by histological examination in all patients. Osteosarcoma was diagnosed in 11 patients (8 men and 3 women) and Ewing's tumor in 5. The disease was first diagnosed in all patients; no specific therapy was carried out before the present study. The sera from 10 healthy subjects (5 men and 5 women) aged 17-36 years served as the control.

Serum VEGF and AG concentrations were measured by enzyme immunoassay using R&D kits.

RESULTS

In patients with malignant bone tumors, the mean serum level of VEGF was higher than in the control group; the mean AG level little differed from that in healthy humans (Table 1). No correlation between blood concentrations of VEGF and AG in patients with osteosarcoma and Ewing's tumor was detected.

Osteosarcoma is one of the main forms of primary malignant skeletal tumors responsible for 30-60% of all primary bone tumors [3]. In the majority (73%) of our patients the tumor was diagnosed at the age of 15-18 years, which is in line with other observations. Serum concentrations of VEGF and AG in patients varied within a wide range (Table 1).

In 10 of 11 patients with osteosarcoma the tumor involved long tubular bones of limbs. The most typical tumor location was metaepiphysis, mainly of the knee joint (9 of 11). Osteosarcoma rarely occurs in flat bones. We observed only 1 patient aged 15 years with tumor in the fifth rib. The concentration of VEGF in this patient was the lowest in our group (78.5 pg/ml); AG concentration was 208.2 ng/ml.

Osteosarcoma is a very malignant tumor characterized by rapid hematogenic metastasizing, most often to the lungs. Two patients with tumors in the distal portion of the femur were hospitalized during generalized stage of the process. Serum concentration of VEGF was relatively low in comparison with the control (92.1 and 106.7 pg/ml); AG concentrations were 338.1 and 235.6 pg/ml, respectively.

The following histological variants of osteosarcoma were distinguished: osteoblastic (n=8), anaplastic (n=2), and mixed (n=1).

The concentrations of VEGF and AG in the blood of patients with osteoblastic osteosarcoma little differed from the mean value in the group. Anaplastic variant was diagnosed in two men aged 15 and 24 years with tumor in the femur; serum VEGF content was 163.4 and 674.3 pg/ml, AG 235.1 and 221.9 ng/ml, respectively. Mixed variant of osteosarcoma was detected in a female patient aged 16 years with typical location of the pathological process in the distal part of the femur; blood VEGF content was 287.6 pg/ml, AG 172.2 ng/ml.

Histological classification of primary bone tumors and tumor-like diseases contains a new article distinguished because of discovery of the neuroectodermal origin of neoplasms: Ewing Tumor (Sarcoma) Family [3]. This group includes Ewing's tumor and primitive neuroectodermal tumor (Askin's tumor). Ewing's tumor is a relatively rare disease; Russian publications report mainly hospital statistical data of specialized institutions engaged in purposeful selection of patients. Angiogenic factors were measured in the sera of 5 male patients with Ewing's tumor.

Ewing's tumor is a disease of children and youths, though it sometimes occurs in older age. The majority of patients aged between 10 and 20 years. In the present study 3 of 5 patients aged 16-18, one 22, and one 28 years. In the two older patients serum concentrations of VEGF were high (464.6 and 814.9 pg/ml), AG concentrations were 462.9 and 146.3 ng/ml.

The location of Ewing's tumor foci in the skeleton is variegated; however the most frequent sites are long tubular bones and pelvic bones. In our patients, the most frequent location of the tumor was long tubular bones (3 of 5 patients). In one case the femur

TABLE 1. Serum Concentrations of VEGF and AG in Healthy Subjects and Patients with Osteosarcoma and Ewing's Tumor

Parameter		Healthy donors		Patients		
				osteosarcoma		Ewing's tumor
		M (n=5)	F (n=5)	M (n=8)	F (n=3)	M (n=5)
VEGF, pg/ml	$M\pm m$	71.5±30.4	226.4±18.2	237.9±80.0	201.9±51.3	446.2±101.7
	range	20.6-149.3	190.3-248.8	78.5-512.3	110.2-287.6	298.8-814.9
AG, ng/ml	$M\pm m$	193.4±59.4	240.9±31.0	269.5±25.4	206.5±17.1	267.3±63.8
	range	112.7-364.4	209.4-303.1	208.2-407.8	172.2-225.1	160.4-462.9
Age, years		18.2±0.6	29.3±4.4	17.1±1.0	26.0±5.0	20.0±2.2

was involved, in the other tibia major; of the flat bones, the ileac and ischial bones were involved. In patients with primary tumors in flat bones, the concentrations of both VEGF and AG in the blood were similar to those in patients with Ewing's tumor in tubular bones (427.8 and 464.6 pg/ml and 374.9 and 462.9 ng/ml, respectively).

Ewing's tumor is characterized by early and fulminant metastases. In our group brain metastases were found in 1 patient aged 22 years with Ewing's tumor in the ischial bone. Serum concentrations of VEGF and AG in this patient were high (464.6 and 462.9 ng/ml, respectively).

Hence, in healthy subjects blood levels of VEGF and AG varied within a wide range but in general were higher in women than in men. In patients with osteosarcoma, the levels of the studied angiogenic factors did not depend on sex. The highest VEGF levels were detected in male patients with Ewing's tumor, which may be related to the tumor histogenesis. No correlation between the concentrations of VEGF and AG in healthy subjects and patients with osteosarcoma and Ewing's tumor were detected.

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