Endostatin, Placental Growth Factor, and Fibroblast Growth Factors-1 and -2 in the Sera of Patients with Primary Osteosarcomas

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Serum levels of endostatin, placental growth factor (PlGF), and fibroblast growth factors-1 and -2 (FGF-1 and FGF-2) were measured in 58 patients with primary osteosarcomas before therapy and in 21 healthy subjects. The incidence of serum FGF-1 in bone tumors was 2.5 times higher than in healthy individuals (p=0.004); significant levels of FGF-2, PlGF, and endostatin were detected in all examined subjects. The mean serum level of endostatin in healthy individuals was significantly lower than in the total group of patients with bone tumors (p=0.005). The level of FGF-1 in osteosarcomas was significantly higher than in chondrosarcomas (p<0.05). No appreciable differences in FGF-2 levels were detected in patients with tumors of different histological structure. The mean serum content of PlGF was virtually the same in healthy individuals and patients with bone tumors. A significant relationship between serum PlGF level and maximum tumor size (p=0.008) was detected in osteosarcoma. No relationships between the levels of FGF-1, FGF-2, PlGF, and endostatin were detected in healthy subjects and patients with primary tumors of the bones. Differences in 3-year overall survival values of patients with bone sarcomas with different initial serum levels of FGF-1 and endostatin were detected.

Key Words: *FGF-1; FGF-2; PlGF; endostatin; bone tumors*

Focused interest of oncologists to the problem of primary osteosarcoma growth and metastases is explained by the fact that these tumors represent one of the most intricate problems of clinical oncology. Molecular biological studies of bone tissue metabolism in health and bone tumors are carried out in many laboratories of the world in order to detect new potential markers of tumor growth. Among these markers are fibroblast growth factors (FGF) [13]. It is known that differentiation of osteoblasts (main bone cells) is regulated (among other factors) by FGF [15]. It is proven that FGF-1 and FGF-2 participate not only in osteoblast differentiation, but also in bone tissue mineralization. Some authors think that unbalanced expression of these growth factors can be a cause of tumor growth [9]. Experimental studies showed that U2OS human osteosarcoma cells express FGF-2 [14], while SaOS-2 human osteosarcoma cells express the FGF-1- and FGF-2-binding receptors [5]. It was also shown that FGF-2 suppresses the growth of Ewing sarcoma cells by inducing their apoptosis [6,10].

It is known that bone sarcomas are malignant tumors liable to rapid hematogenic metastasizing. We have to admit that the mechanisms determining tumor cell capacity to invasion in the adjacent tissues and dissemination with subsequent formation of secondary foci of tumor growth are studied insufficiently. On the other hand, it was shown that stimulants and inhibitors

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of neoangiogenesis in the tumor play an important role in these processes [1,2]. It was shown, for example, that vascular endothelial growth factor (VEGF), the key activator of angiogenesis, realizes its effect synergically with FGF-2 [3]. Placental growth factor (PlGF) is one of VEGF family members. It was experimentally shown that bone morphogenetic protein-2 (BMP-2), one of the main osteogenesis factors, stimulates PlGF secretion in pluripotent mesenchymal cells (osteoblast precursors) and in MG63 osteosarcoma cells. It seems that PlGF does not modify osteoblast differentiation, but realizes the paracrine regulation of angiogenesis and hemopoiesis in bone formation [11].

Endostatin is one of well-known inhibitors of angiogenesis [12]. It was experimentally shown that injection of endostatin to animals with LM 8 murine osteosarcoma after removal of the primary tumor led to a reduction of angiogenic activity, this resulting in shrinkage of metastases in the lungs [8]. Presumably, endostatin can be used in combined therapy for osteosarcoma in humans [4,7,8].

Analysis of experimental findings suggested our clinical study of serum levels of endostatin, PlGF, FGF-1, and FGF-2 in patients with primary malignant osteosarcomas and in healthy subjects of the same age and sex in order to detect the possible relationships of these parameters with clinical and morphological characteristics of the disease.

MATERIALS AND METHODS

The study was carried out in patients with bone tumors (n=58) aged 14-59 and healthy individuals of similar age (n=21); control). The disease was diagnosed for the first time in all the patients and was confirmed by the results of histological study of the tumors. Primary osteosarcomas were diagnosed in 22 (37%) patients, primary chondrosarcoma in 14 (24%), Ewing's sarcoma in 10 (17%), malignant fibrous histiocytoma (MFH) of the bone in 6 (11%), and giant-cell tumor of the bone in 6 (11%).

The tumors were located in tubular bones in 41% and in flat bones in 17 patients.

The maximum size of the tumor could be evaluated in 46 patients, due to which the patients were divided into 2 groups. Group 1 consisted of patients with tumors with the maximum size of no more than 10 cm (n=22) and group 2 included patients with tumors with the maximum size more than 10 cm (n=24).

Endostatin, PlGF, FGF-1, and FGF-2 were measured using commercial R&D kits with an Elx 800 automated reader (Biotek Instruments Inc.).

RESULTS

Significant endostatin levels were detected in all serum specimens from patients and healthy subjects. The mean serum endostatin level in healthy individuals was 109.2±2.4 ng/ml, which was lower than in the total group of patients with bone tumors (131.8±4.8 ng/ml; p=0.005; Table 1). The mean level of endostatin in osteosarcoma was 131.6±8.2 ng/ml, in Ewing's sarcoma 121.7±5.3 ng/ml, in chondrosarcoma 131.1±12.2 ng/ml, in giant-cell tumor 131.2±7.7 ng/ml. In MFH the mean content of endostatin was somewhat higher than in other morphological variants of tumors: 151.2±18.6 ng/ml; however, no significant differences in endostatin levels in tumors of different histological

TABLE 1. Serum Endostatin, PlGF, FGF-1 and FGF-2 in Patients with Primary Tumors of the Bones and Healthy Individuals (M±m)

Subjects examined	Number	FGF-1		FGF-2		Endostatin		PlGF	
		detection rate, %	mean level, pg/ml	detection rate, %	mean level, pg/ml	detection rate, %	mean level, pg/ml	detection rate, %	mean level, pg/ml
Healthy subjects	21	29	43.9±15.6	100	13.4±2.2	100	109.2±2.4	100	20.1±1.4
Bone tumors	58	72*	75.6±13.9	100	16.4±1.7	100	131.8±4.8*	100	21.5±1.0
Osteosarcoma	22	77	96.3±32.2	100	16.2±2.4	100	131.6±8.3	100	22.5±1.8
Ewing's sarcoma	10	80	71.2±12.4	100	18.8±3.6	100	121.7±5.3	100	22.9±2.5
Chondrosarcoma	14	64	38.2±6.9+	100	15.4±4.2	100	131.1±12.2	100	18.3±1.8
MFH	6	33	52.4 and 74.9	100	21.7±8.8	100	151.2±18.6	100	24.6±4.8
Giant-cell tumor	6	100	66.6±15.5	100	9.8±1.3	100	131.2±7.7	100	19.7±1.5

Note. p<0.05 compared to: *healthy subjects, +osteosarcoma.

structure were detected.

Serum endostatin content was the same in involvement of the flat and tubular bones (131.9±7.1 and 131.7±6.2 ng/ml, respectively). The minimum endostatin level was detected in tumor of the tibial bone (7.7 ng/ml), the maximum in involvement of the calcaneum (232.5 ng/ml).

No relationship between serum endostatin level and sex or age was detected in healthy subjects and patients with bone tumors. No relationship between serum endostatin level and maximum tumor size was detected in the total group of patients or in subgroups with different histological structure of the tumor.

Hence, serum endostatin levels in patients with malignant tumors of the bones were significantly higher than in healthy subjects. No significant differences in endostatin levels in patients with tumors of different histological structure were detected.

Significant levels of PlGF were detected in all serum samples from healthy individuals and patients with bone tumors. The mean serum level of PlGF in healthy subjects was 20.1±1.4 pg/ml, in patients with bone tumors 21.5±1.0 pg/ml — virtually the same as in osteosarcoma (22.5±1.8 pg/ml) and Ewing's sarcoma (22.9±2.5 pg/ml). In chondrosarcoma and giant-cell tumor the mean serum levels of PlGF were 18.3±1.8 and 19.7±1.5 pg/ml, respectively (virtually the same as in the control group), while in MFH serum PlGF level was high: 24.6±4.8 pg/ml.

The initial serum levels of PlGF were virtually the same in involvement of the flat and tubular bones (20.8±1.8 and 21.8±1.3 pg/ml, respectively). The minimum PlGF content was detected in clavicular tumor (11.2 pg/ml) and ileac tumor (11.5 pg/ml), the maximum level in tibial tumor (47.1 pg/ml).

No relationship between serum PlGF levels, sex and age were detected in healthy subjects and patients with bone tumors. No relationship between serum PlGF level and maximum tumor size was detected in the total group of patients. However, a detailed statistical analysis showed a direct correlation between serum PlGF level and maximum tumor size (r=0.43; p=0.45).

The level of PlGF in bone tumors with the maximum size more than 10 cm was significantly higher than in smaller tumors (24.1±2.0 *vs.* 19.0±1.1 pg/ml, respectively; p=0.037). Detailed analysis revealed significant differences in serum PlGF levels with consideration for tumor size and histology only for osteosarcoma. In tumors larger than 10 cm in size, the mean serum level of PlGF was 28.1±3.0 pg/ml, in smaller tumors 17.6±1.7 pg/ml (p=0.008).

The incidence of FGF-1 in the control group was 2.5 times lower than in the total group of patients with bone tumors (29 and 72%, respectively; p=0.004). The concentration of FGF-1 in healthy individuals was 43.9±15.6 pg/ml, differing not much from that in patients with bone tumors (75.6±13.9 pg/ml).

Serum FGF-1 in patients with bone tumors was detected in 17 (77%) patients with osteosarcoma, 9 (64%) with chondrosarcoma, 8 (80%) with Ewing's tumor, 6 (100%) with giant-cell tumor, and 2 (33%) with MFH. The mean FGF-1 level in osteosarcoma was significantly higher than in chondrosarcoma (96.3±32.2 pg/ml *vs.* 38.2±6.9 pg/ml; p<0.05) and did not differ much from that in Ewing's sarcoma (71.2±12.4 pg/ml) and giant-cell tumor (66.6±15.5 pg/ml). Serum FGF-1 level was not zero in only two patients with MFH (52.4 and 74.9 pg/ml).

The levels of FGF-1 in patients with involvement of the tubular and flat bones differed negligibly (81.6±17.9 and 56.7±10.9 pg/ml, respectively).

High levels of FGF-1 were detected in the sera of patients aged under 30 years (116.7±41 pg/ml) in comparison with those in patients aged over 50 years (42.7±7.6 pg/ml).

Serum FGF-1 level in the total group of patients did not depend on the maximum size of bone tumor. In Ewing's sarcoma, the level of FGF-1 increased with increasing tumor size (r=0.70, p=0.081).

The incidence of FGF-2 in the serum of healthy individuals and patients was 100%. The mean serum level of FGF-2 in healthy subjects was 13.4±2.2 pg/ml, which virtually did not differ from the level in patients with osteosarcoma (16.2±2.4 pg/ml), chondrosarcoma (15.4±4.2 pg/ml), and Ewing's sarcoma (18.8±3.6 pg/ml). In giant-cell tumor serum level of FGF-2 tended to decrease (9.8±1.3 pg/ml), in MFH to increase (21.7±8.8 pg/ml) in comparison with the control. No relationship between the initial serum levels of FGF-2 and age or sex was detected in healthy subjects and patients with bone tumors. The levels of FGF-2 were virtually the same in involvement of the flat and tubular bones (18.2±3.8 and 15.6±1.9 pg/ml, respectively). The maximum level of FGF-2 was detected in the serum of a patient with tumor of the heel bone (66.6 pg/ml), the minimum in scapular tumor (4.7 pg/ml). No relationship between bone tumor maximum size and serum level of FGF-2 was detected.

No correlation was detected between the initial serum concentrations of FGF-1, FGF-2, PlGF, and endostatin in patients with bone tumors and in healthy individuals.

Forty-two patients were observed during 3 years: 19 with osteosarcoma, 13 with chondrosarcoma, and 10 with Ewing's tumor. Overall 3-year survival for the total group was 59.7±12.2%. In osteosarcoma this value was 78.6±11%, in Ewing's sarcoma 52.5±20.4%, in chondrosarcoma 72.0±17.8%. No significant differences in the survival with consideration for tumor histology were detected (p=0.55). The threshold levels

of FGF-1 and endostatin in the serum, at which 3-year survival of patients was different, were detected by statistical methods. At serum endostatin level of <130 ng/ml the survival was 81.3±12.4%, at the level of ≥130 ng/ml it was 62.2±12.4%, at FGF-1 level of <35 pg/ml 100%, and at FGF-1 ≤35pg/ml 61.5±14.1%. No differences in the survival of patients with osteosarcomas with different serum levels of FGF-2 and PlGF were detected.

Hence, we detected differences in 3-year overall survival of patients with osteosarcomas, correlating with serum concentrations of FGF-1 and endostatin before specific therapy.

It is noteworthy that the incidence of FGF-1 in the sera of patients with bone tumors was significantly higher than in healthy individuals of the same age, while significant levels of FGF-2, PlGF, and endostatin were detected in all examined patients and donors. The mean levels of FGF-1, FGF-2, and PlGF did not differ significantly from the values in the control group, while the mean level of endostatin in patients with bone tumors was significantly higher than in healthy individuals. These data suggest that the expression of FGF-1 and endostatin can be related to bone tumor growth and deserves further investigation on more extensive clinical material. Presumably, this will help to evaluate the disease prognosis and choose the treatment strategy in patients with osteosarcomas.

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