ONCOLOGY

Matrix Metalloproteinases 2, 7, 9 and Tissue Inhibitor of Matrix Metalloproteinase-1 in the Sera of Patients with Bone Tumors

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Comparative enzyme immunoassay of matrix metalloproteinases (MMP-2, -7, -9) and their tissue inhibitor-1 (TIMP-1) in the sera of 26 healthy individuals and 54 patients with primary osteal tumors before therapy revealed elevated TIMP-1 levels in the patients with classical central and periosteal osteosarcomas in comparison with the control. In patients, the level of MMP-9 significantly decreased compared to that in healthy individuals, while the levels of MMP-2 and MMP-7 remained unchanged. No differences in serum levels of MMP and TIMP-1 associated with gender, age, primary osteal tumor location and size were detected. Overall 3-year survival of patients with classical central osteosarcoma with serum level of MMP-9 below its median was higher than that of patients with MMP-9 level equal to above the median (90.9±8.7 and 50.8±23%, respectively).

Key Words: *bone tumors; MMP-2, -7, -9; TIMP-1; serum*

Osteosarcomas are one of the most intricate sections of clinical oncology. These tumors are characterized by rapid growth, early hematogenic metastasizing, and unfavorable prognosis [6]. The mechanisms determining sarcoma cell capacity to invasion in the adjacent tissue and dissemination with subsequent formation of secondary foci of tumor growth are not yet amply studied. Presumably, the matrix metalloproteinase (MMP) family is involved in these processes. MMP participate in destruction of the extracellular matrix during malignant tumor growth [1,10]. Activities of MMP are regulated by their tissue inhibitors (TIMP) [2]. More than 20 MMP [12] and 4 TIMP are now identified. The balance between MMP and TIMP expression is not constant in normal processes associated with tissue growth and development and in various pathologies, including tumor diseases [2,5,14]. The MMP–TIMP imbalance was detected in some diseases [2,7,10], but the role of MMP and TIMP in the mechanisms of bone tumor growth and progress remains not quite clear. Experiments showed that osteosarcoma cells produce MMP-2, MMP-9, TIMP-1 [3,4,11,13,15], which are presumably involved in invasive growth of tumors [7-9].

Analysis of experimental data prompted this research. We compared serum levels of MMP-2, MMP-7, MMP-9, and TIMP-1 in normal individuals (control group) and patients with primary osteosarcomas in order to detect possible relationships between these characteristics and the main clinical morphological characteristics of the disease and its prognosis.

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MATERIALS AND METHODS

Fifty-four patients with primary osteosarcomas (29 men and 25 women) aged 14-59 years were examined. The disease was diagnosed for the first time in all patients and was confirmed by histological findings. Classical central osteosarcoma was detected in 21 (39%) patients, periosteal osteosarcoma in 4 (8%), Ewing's sarcoma in 11 (20%), primary chondrosarcoma in 6 (11%), malignant fibrous bone histiocytoma (MFH) in 3 (6%), and giant-cell bone tumor (GCT) in 9 (16%) patients.

The tumors were located in tubular bones in 48 patients and in flat bones in 6. It should be noted that in all patients with osteosarcomas the tumors were detected in tubular bones in.

The control group consisted of 26 healthy subjects (14 men and 12 women) of similar age.

All serum values were measured by enzyme immunoassay. The sera were obtained by the standard method before therapy. TIMP-1 was assayed using Biosource reagents, MMP-2, -7, and -9 were measured using R&D reagents according to manufacturer's instructions. The measurements were carried out on an EL_X800 automated universal microplate reader (Bio-Tek Instruments, Inc.).

The values in different clinical morphological groups were compared using Mann–Whitney's test. Relationships between different parameters were evaluated using Spearman's rank correlation test (R). Survival was evaluated using Kaplan–Meyer's method. The differences/correlations were considered significant at $p<0.05$ in all cases.

RESULTS

MMP-2, -7, -9, and TIMP-1 were detected in the sera of virtually all healthy individuals and patients with bone tumors (Table 1). Serum levels of TIMP-1 were significantly higher in patients with classical central and periosteal sarcomas in comparison with controls ($p=0.038$ and $p=0.007$, respectively).

On the other hand, serum levels of MMP-9 in patients with bone tumors were significantly lower than in healthy individuals ($p<0.05$). In addition, serum levels of MMP-9 differed significantly ($p<0.05$) in patients with chondrosarcoma and periosteal sarcoma.

No appreciable differences were detected between the serum levels of MMP-2 and MMP-7 in healthy individuals and patients with bone tumors. Significant differences were detected only for serum MMP-7 levels in patients with GCT and Ewing's sarcoma, in whom the level of this marker was lower ($p<0.05$).

No differences in serum levels of the studied MMP and TIMP-1 associated with patient's gender, age, and primary tumor location and size were detected.

A significant positive correlation between serum content of TIMP-1 and MMP-9 was found in patients with classical central osteosarcoma, periosteal osteosarcoma, and Ewing's sarcoma ($r=0.37$; $p=0.024$). No relationships between serum levels of MMP-2, MMP-7, and MMP-9 in patients with bone tumors were detected.

The overall 3-year survival and relapse-free survival with consideration for the initial serum content of TIMP-1, MMP-2, -7, and -9 could be carried out only in patients with classical central osteosarcoma

TABLE 1. Serum MMP-2, -7, -9, and TIMP-1 in Patients with Primary Osteosarcomas and Healthy Individuals

Group	N	TIMP-1, ng/ml		MMP-2, ng/ml		MMP-7, ng/ml		MMP-9, ng/ml	
		range of values	median	range of values	median	range of values	median	range of values	median
Healthy individuals	26	352-571	436	144-318	196	1.07-5.10	2.35	92-915	501
Bone tumors	54	295-785	490	92-262	160	1.20-8.17	2.95	169-1057	377*
classical central osteosarcoma	21	369-701	484*	92-243	158	1.75-5.56	2.94	210-1057	372
periosteal osteosarcoma	4	571-637	609*	130-193	149	2.16-8.18	2.88	495-593	552
Ewing's tumor	11	357-662	486	116-203	161	1.20-4.49	2.62	221-558	378
chondrosarcoma	6	294-569	455	117-262	168	1.79-6.15	3.32	169-428	311°
MFH	3	367-785	584	142-178	149	2.24-3.98	2.97	283-491	435
GCT	9	339-702	457	117-241	159+	2.21-5.97	3.41	259-612	377

Note. $p<0.05$ in comparison with *healthy individuals, +patients with Ewing's sarcoma, °patients with periosteal osteosarcoma.

(21 cases). Five patients with osteosarcoma died from the disease progress over this period, 11 patients developed metastases in the lungs. The patients were divided into 2 groups for each of the studied parameters: group 1 included patients with marker level below its median ("low" level), group 2 those with its level equal to or above the median ("high" level). No appreciable differences in the survival of patients with different serum levels of TIMP-1, MMP-2, -7 before therapy were recorded. Analysis of the overall 3-year survival of patients with classical central osteosarcoma in relation to their serum levels of MMP-9 showed significantly better overall survival of patients with "low" level of MMP-9 in comparison with those with "high" levels (90.9±8.7 and 50.8±23%, respectively; p=0.039).

Hence, serum level of TIMP-1 in patients with classical central osteosarcoma and particularly with periosteal osteosarcoma was significantly higher than in healthy individuals. The level of TIMP-1 was inessential for relapse-free and overall survival of patients. On the other hand, the initial serum level of MMP-9 was significantly lower in patients with bone sarcomas, and the overall 3-year survival of patients with classical central osteosarcoma with "high" serum level of MMP-9 was significantly worse than of patients with "low" level of MMP-9. These data suggest that the expression of TIMP-1 and MMP-9 can be associated with the growth and metastasizing of classical central osteosarcoma, which is the object of further research.

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