Increased osteoclast activity is associated with aggressiveness of osteosarcoma

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Received May 16, 2008; Accepted August 1, 2008

DOI: 10.3892/ijo_00000113

Abstract. Osteosarcoma (OS) is a highly malignant primary skeletal tumor with a striking tendency to rapidly destroy the surrounding bone and metastasize, since metastases are frequently present at clinical onset. The basis for the aggressiveness of this tumor is largely unknown. However, recent studies in *in vivo* models indicate that the anti-osteolytic drugs, bisphosphonates, can inhibit the tumor local expansion and the formation of metastases. We further investigated the association between the presence of active osteoclasts and the aggressiveness of OS. We evaluated the presence of osteoclasts and the mRNA of different osteoclast-related genes in tumor biopsies from 16 OS patients and in three OS cell lines and the serum levels of bone resorption markers in the same series and in 28 other patients. Tumor-associated osteoclasts were found in 63 and 75% of cases by histological and mRNA analysis. Among different serum markers, only MMP-9 was significantly higher in OS cases ($p=0.0001$), whereas TRACP 5b was significantly higher in metastatic patients compared to nonmetastatic patients ($p=0.0509$). Serum TRACP 5b was significantly correlated to serum NTX ($p<0.0001$) and cathepsin K mRNA in tumor tissues ($p=0.0153$). In 8 patients we also analyzed TRACP 5b serum level at follow-up and we verified a significant decrease of TRACP 5b after primary tumor removal ($p=0.0117$). In conclusion, tumor-infiltrating osteoclasts are frequently found in OS and increased serum TRACP 5b levels and the presence of active osteoclast at primary sites were positively associated with tumor aggressiveness.

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Key words: osteosarcoma, osteoclast, metastases, tumor aggressiveness, bone turnover markers

Introduction

Osteosarcoma (OS) is the most common primary malignant bone tumor, showing a high incidence in childhood and adolescence (1). OS is a highly aggressive neoplasm composed of proliferating osteogenic cells with remarkable anaplastic features. This translates into a rapid progressive course with an aggressive local behavior leading to rapid invasion and extensive destruction of bone and a striking tendency to develop life-threatening lung and skeletal metastases through the bloodstream (2).

In the past three decades, multiagent chemotherapy has significantly improved the survival of OS patients, resulting in a long-term disease-free survival rate of >60% in patients with localized disease (3). In those patients, the prognosis is strictly dependent on the ability of tumor cells to respond to chemotherapy (4), although other inherent factors may play a significant role (5,6). Moreover, in some 30% of OS, metastases are already present at clinical onset. The basis for this higher aggressiveness is largely unknown, since metastatic OS does not present peculiar phenotypic features in terms of osteogenic differentiation or growth rate (7).

Little is known on the mechanisms by which OS cells destroy the hard matrix of the skeleton. In particular, it is not clear whether other mechanisms play a role in addition to direct tumor-induced bone degradation via metalloproteinase activity (8-10) and if an increased ability to locally invade is associated with a worse prognosis. Since osteoclasts are the primary cells involved in bone matrix solubilization and osteogenic cells are able to activate osteoclasts under physiological and pathological conditions (11), it is reasonable to hypothesize that both mechanisms may combine to enhance local OS invasion and the release of growth factors stored in the bone matrix, in turn promoting cell proliferation and hematogenous spread. As a matter of fact, OS may show multinucleated osteoclast-like cells on histological sections (12,13) and OS cells are able to produce soluble factors that promote differentiation of blood monocytes to osteoclast-like cells *in vitro* (14).

The aim of this study was to investigate the association between tumor local invasiveness through tumor-induced bone resorption activity and tumor systemic aggressiveness. For this purpose, in the same series, we evaluated the expression of pro-osteoclastic differentiation factors and the presence of osteoclasts in OS tissues, and the serum levels of markers of bone resorption. OS behavior appeared to be adversely influenced by osteoclast-mediated bone resorption at the tumor site.

Materials and methods

Study subjects and sample collection. Between May 2002 and July 2005, 44 patients with newly diagnosed OS without evidence of previous benign or malignant bone disease (32 males and 12 females, median age 13, range 5-26) and 17 age-matched individuals (8 males and 9 females, median age 13, range 8-19) were enrolled in this case-control study. A signed informed consent was obtained from patients, all of whom agreed to the use of biological materials for research studies, as approved by the Institutional Ethics Committee. All 44 tumors were diagnosed as high-grade OS (2) and depending on clinical data and imaging study at diagnosis, they were distributed into different groups as follows: 32 OS localized at a single skeletal site, 6 OS metastatic to the lungs and 5 OS with multiple skeletal localizations. The clinical and radiographic characteristics of this series is summarized in Table I (Total group).

Tissue samples for molecular analysis were obtained in 16 of the 44 patients from biopsy specimens before chemotherapy and were immediately partly processed for histopathological evaluation and partly snap-frozen in liquid nitrogen and stored at -80˚C. The clinical and pathological characteristics of this subgroup is reported in Table I (Subgroup).

Serum samples for the analysis of markers associated with bone resorption were taken at the time of diagnosis from untreated patients. Serum was separated from 20 ml venous peripheral blood by centrifugation at 3,000 x g for 20 min at room temperature and stored at -80˚C until analysis. In only 8 patients of the 'Total group', serum samples were also collected at 12 months after the initial diagnosis, after surgical removal of primary tumor and the completion of chemotherapy. All patients were treated according to an established regimen of multiagent chemotherapy combined with surgical removal of the primary lesion (15).

Cell lines. The human OS cell lines Saos-2, MG-63 and U-2 OS, obtained from the American Type Culture Collection (ATCC, Manassas, VA), were maintained in Iscove's Modified Dulbecco's Medium (IMDM, Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (FBS, Mascia Brunelli, Milan, Italy), penicillin (100 U/ml) and streptomycin (100 mg/ml) (Invitrogen).

Histological and molecular analysis of tumor tissues. To evaluate the occurrence of osteoclasts in OS specimens, we verified how many biopsy specimens presented with multinucleated giant cells actively resorbing either host or neoplastic bone. These cells could be easily distinguished from malignant giant cells based on conventional morphological criteria (Fig. 1). Frozen tissues were pulverized in the presence of liquid nitrogen (Mikro-Dismembrator; B. Braun Biotech International, Melsungen, Germany) and mRNA was extracted from powdered tissues by solubilizing using TRIzol RNA isolation reagent (Invitrogen). mRNA from semi-confluence Saos-2, MG-63 and U-2 OS cells were isolated by using the RNeasy mini kit (Qiagen GmbH, Hilden, Germany). Total RNA from cell lines and from tissues was reverse transcribed into cDNA using the Advantage RT-for-PCR kit (Clontech Laboratories, Palo Alto, CA). The RT-PCR for the specified human genes was determined using forward and reverse primers, as follows. The RT-PCR consisted in one denaturation at 94˚C for 5 min and then 30 cycles of amplification (denaturation at 94˚C for 30 sec, annealing at the specific temperature for 30 sec and extension at 72˚C for 45 sec) and then a final extension at 72˚C for 7 min. Forward and reverse primers and the specific temperatures were, respectively: for cathepsin K, 5'-TTCCCGCAGTAATGACACC-3', 5'-TTTCCCCAGTTTTCTCCCC-3' and 63˚C (Accession No. BC016058); for matrix metalloproteinase-9 (MMP-9), 5'-AGCACGGAGACGGGTAT-3', 5'-TTGTCGCTGTCAAAGTTC-3' and 57˚C (Accession No. NM_004994); for macrophage colony stimulating factor (M-CSF), 5'-AGAAGACAGACCATCCAT-3', 5'-TCCACCTGTAGAACAAGA-3' and 52˚C (Acc No. M64592); for Parathyroid Hormone-Realted Protein (PTHrP), 5'-GCGACGATTCTTCCTTCACC-3', 5'-AGAGTCTAACCAGGCAGAGC-3' and 58˚C (Accesion No. BC005961); for Interleukin-6 (IL-6), 5'-GATGCAATAACCACCCCTGACCC-3', 5'-CAATCTGAGGTGCCCATGCTA-3'

Table I. Clinical and pathological features of osteosarcoma patients included in this study.[a]

Variable	Total group (n=44)	Subgroup (n=16)
	No. of patients (%)	
Sex		
Male	32 (73)	11 (69)
Female	12 (27)	5 (31)
Skeletal anatomical site		
Femur	21 (48)	7 (43)
Tibia	9 (20)	4 (25)
Humerus	7 (16)	2 (13)
Fibula	2 (5)	1 (6)
Multiple	5 (11)	2 (13)
Histological subtype		
Osteoblastic	31 (70)	11 (69)
Chondroblastic	6 (14)	2 (13)
Fibroblastic	4 (9)	2 (13)
Telangiectatic	3 (7)	1 (6)

[a]The subgroup includes cases in which tissue samples were also analyzed.

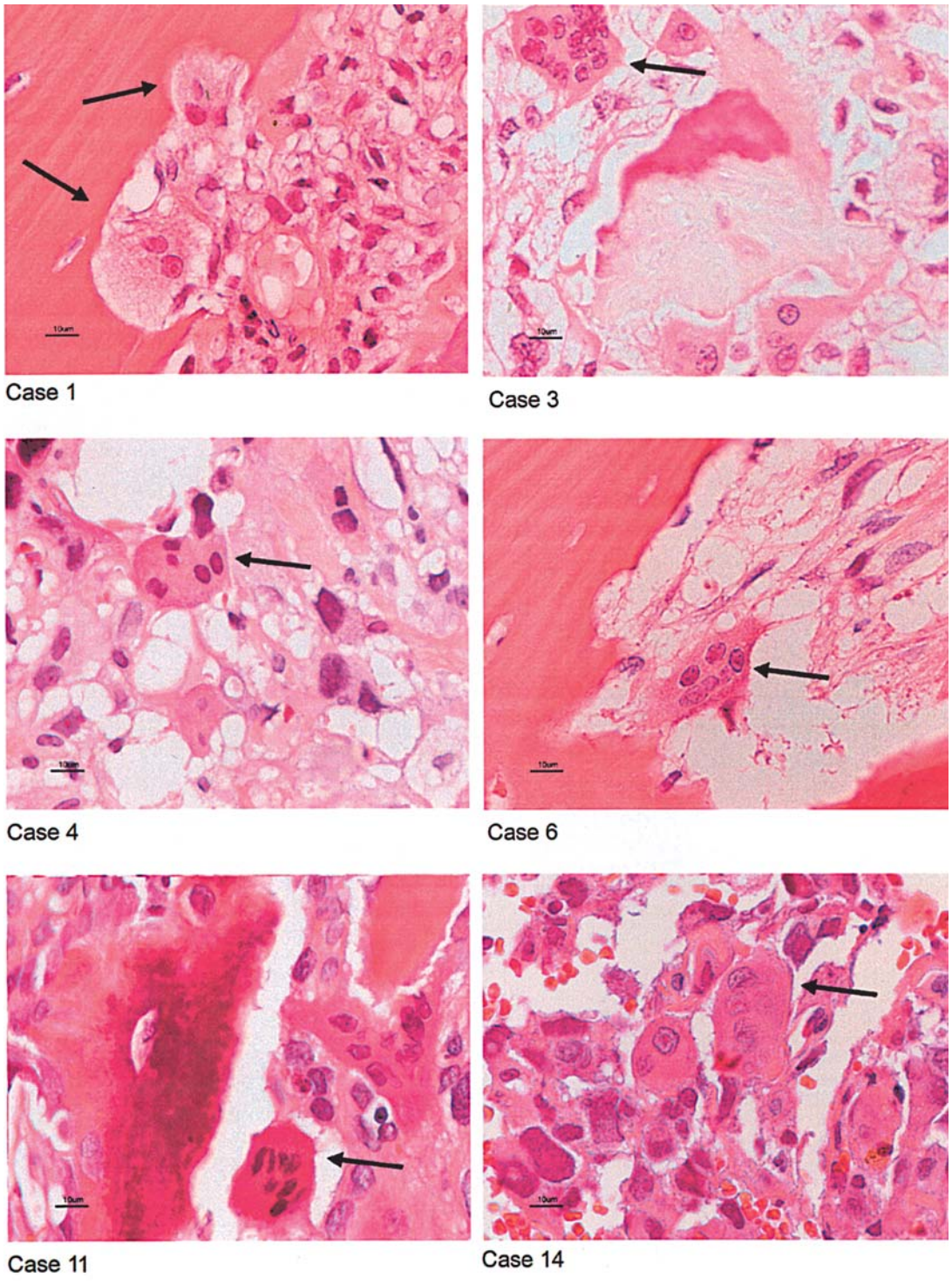

Figure 1. Tumor-infiltrating osteoclasts in tissue samples from six representative osteosarcomas that are positive for the presence of osteoclasts. H&E, black arrows indicate multinucleated osteoclasts (Bar, 10 μm, original magnification, x60).

and 56˚C (Accession No. NM_000600); for the Receptor, Parathyroid Hormone, Type 1 (PTHR1), 5'-CTGGACACTG GCACTGGACTTC-3', 5'-GGCCTGAGCAGGAGCCGTT GAG-3' and 61˚C (Accession No. NM_000316); for receptor activator of nuclear factor-κB ligand (RANKL), 5'-CGTCG CCCTGTTCTTCTA-3', 5'-GAGTTGTGTCTTGAAAATC TGC-3' and 54˚C (Accession No. NM_003701). Parallel reactions were performed for every assay using primers designed to amplify human ß-actin, 5'-ATCTGGCACCACA CCTTCTACAATGAGCTGCG-3' and 5'-CGTCATACTCC TGCTTGCTGATCCACATCTGC-3', forward and reverse primers, respectively (Accession No. NM_001101). Specific cDNA for ß-actin levels was assayed by denaturation at 94˚C for 10 min and then by 30 cycles of amplification of denaturation at 94˚C for 30 sec, annealing at 65˚C for 45 sec and extension at 72˚C for 30 sec and final extension at 72˚C for 10 min. The products were separated by electrophoresis using 2% agarose gel stained with ethidium bromide (0.5 μg/ml). Specific RT-PCR assay was repeated three times for each gene.

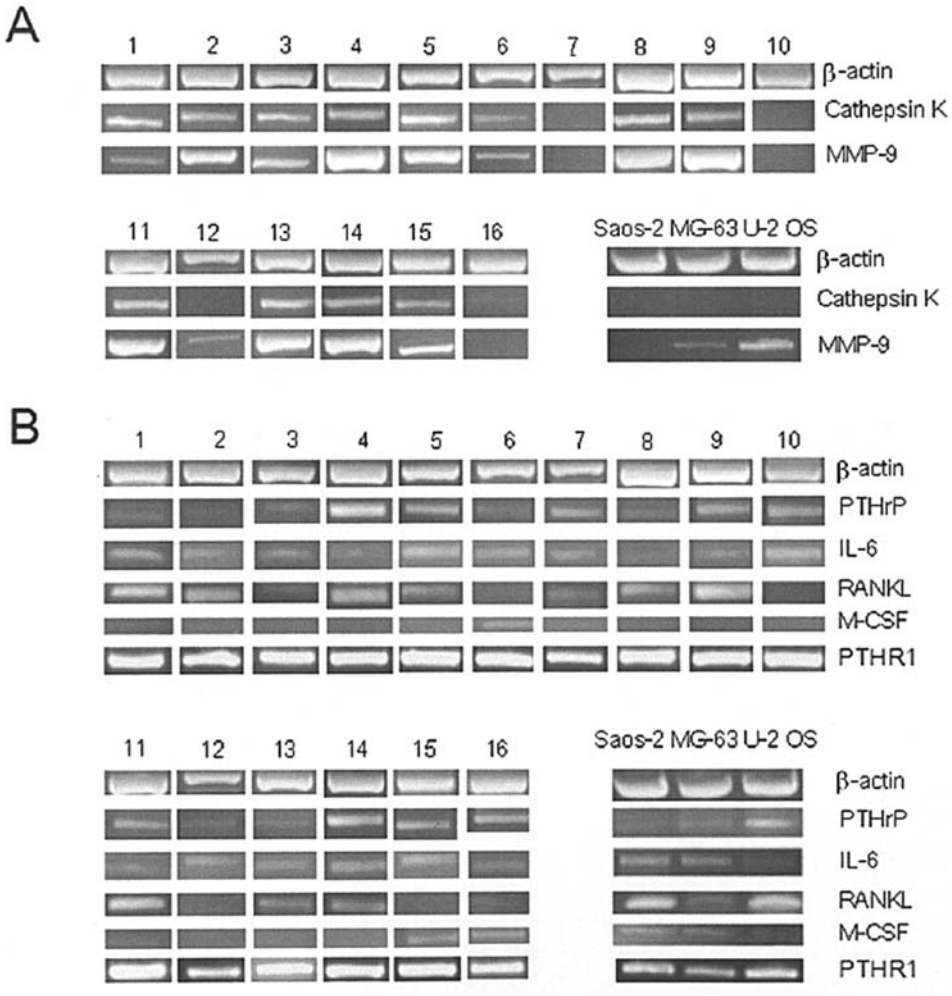

Figure 2. Pro-osteolytic factors and osteoclast markers in tissue samples and cell lines of osteosarcoma. Gel electrophoresis results for cathepsin K and MMP-9 (A). Gel electrophoresis results for PTHrP, IL-6, M-CSF and RANKL pro-osteolytic factors and PTHR1 receptor (B). Representative images.

Determination of serum MMP-9, TRACP 5b and NTX. Serum concentrations of MMP-9 were measured using Quantikine human MMP-9 kit (R&D Systems Inc., Minneapolis, MN) with a specific anti-human antibody which recognizes both active and pro-MMP-9. Serum tartrate-resistant acid phosphatase isoform 5b (TRACP 5b), was determined using a commercial immunoassay (BoneTRAP®; SBA-Sciences, Oulu, Finland). The cross-linked N-terminal telopeptides of Type I collagen (NTX) were measured by ELISA (Osteomark®, Ostex International, Seattle, WA). The results were expressed as nanomoles of bone collagen equivalent (BCE).

Statistical analysis. All statistical analyses were performed using StatView 5.01 software (SAS Institute Inc., Cary, NC). Non-parametric analysis of variance (Kruskal-Wallis test) was applied to detect the effects of multiple clinical variables on the quantitative results and the Mann-Whitney U test was applied as a post-hoc test of multiple analyses, or when only two independent variables were compared. A paired analysis of data (Wilcoxon Rank test) was applied to evaluate the difference of values of TRACP 5b before and after tumor removal. The Spearman Rank correlation test was used to evaluate the association between different serum markers. A two-way contingency table (Fisher exact test) was also used to analyse the association between two qualitative variables. In all statistical calculations, differences were considered significant at p-value ≤ 0.05.

Results

Tumor-associated osteoclasts in OS. Osteoclasts were found in 10/16 tissue samples (63%), both at sites of resorption of host bone and, within the tumor mass, at sites of resorption of malignant osteoid (Fig. 1, representative positive cases for the presence of osteoclasts). The presence of active osteoclasts within OS samples was further confirmed by cathepsin K mRNA analysis by RT-PCR (Fig. 2A). In fact, cathepsin K was present in 9 out of 10 cases with osteoclasts and, overall, in 12 out of 16 cases (75%). Cathepsin K was not produced by OS cells, as suggested by the lack of mRNA transcripts in extracts of OS cell lines. OS, on the contrary, generally expressed MMP-9, as shown by mRNA analysis in OS cell lines and tumor samples (13/16, 81%) (Fig. 2A).

The occurrence of osteoclasts in OS biopsies was positively associated with the presence of lung or bone metastases at diagnosis. In fact, 5/10 cases (50%, cases 1, 2, 4, 9, 14) with tumor-associated osteoclasts presented with metastases at diagnosis, whereas 6 out of 6 cases (100%)

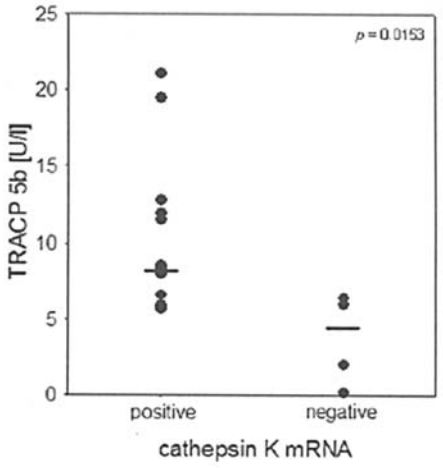

Figure 3. The association between serum levels of TRACP 5b and tissue expression of cathepsin K. The presence of cathepsin K mRNA in OS tissues is indicated as 'positive', while the absence as 'negative'. Median values of the two groups are also shown (black horizontal bars). High TRACP 5b serum levels were significantly associated with a positive expression of cathepsin K mRNA in tumor tissues (Mann-Whitney U test).

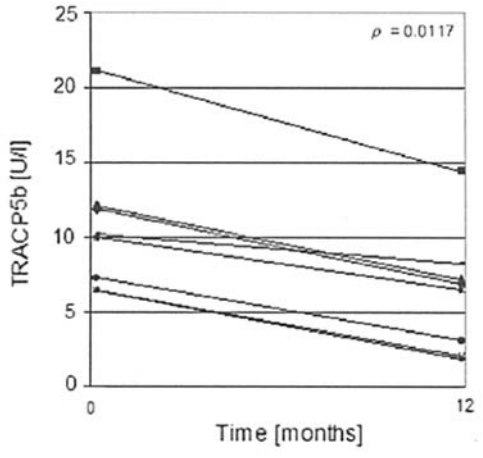

Figure 4. TRACP 5b serum levels: effect of treatment. After surgical removal of the primary tumor and chemotherapy, TRACP 5b serum levels significantly decreased at follow-up (Wilcoxon Rank test).

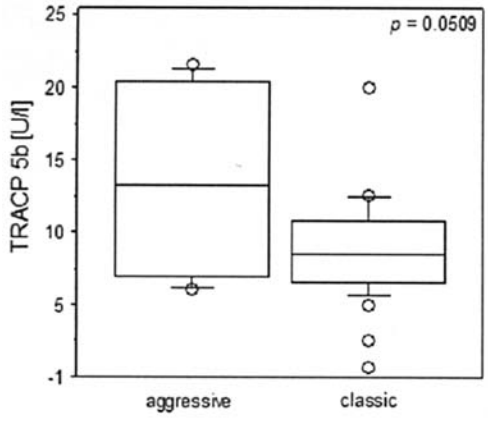

Figure 5. TRACP 5b serum level in 'aggressive' tumors. Serum levels of TRACP 5b in patients who had bone or lung metastases ('aggressive') compared with patients with a single osseous localization ('classic') at diagnosis (Mann-Whitney U test).

without osteoclasts did not have clinical evidence of metastasis at diagnosis (p=0.0253).

Expression of pro-osteolytic factors in OS cells. First we sought to determine if OS cells express molecules that can favour osteoclast differentiation and activity. Among OS samples, 15/16 were positive for parathyroid hormone-related peptide (PTHrP) (94%), 16/16 were positive for IL-6 (100%), 11/16 were positive for RANKL (69%), and 3/16 were positive for M-CSF (19%) (Fig. 2B). Moreover, OS cells exhibited an autocrine PTHrP/PTHR1 loop, as shown by the presence of the PTHR1 in 16/16 samples (Fig. 2B). mRNA for these pro-osteoclastic factors were all expressed at different levels by the three OS cell lines Saos-2, MG-63 and U-2 OS.

Serum markers of bone resorption in OS patients. Among the indicators of bone resorption, only TRACP 5b, but not other markers, were significantly related to subject's age (Spearman correlation test, r=-0.556, p<0.0001), whereas no correlation was found between TRACP 5b, NTX, or MMP-9 serum level and other clinical or pathological features.

In agreement with data obtained by RT-PCR on tissue samples, the levels of MMP-9, a metalloproteinase that is secreted by OS cells, were significantly higher in the serum of OS patients as compared to controls (Table II). On the contrary, the serum values of TRACP 5b, a marker of osteoclast activity and of NTX, an index of bone collagen degradation, were similar in OS patients as compared to controls. TRACP 5b was positively related to NTX in both control and OS groups (p=0.0028, r=0,739; p=0.0014, r=0.073, respectively), supporting that, in OS patients, bone degradation is due to osteoclast-mediated resorption. On the contrary, bone degradation, as shown by NTX levels, did not correlate with the levels of MMP-9, that is secreted both by OS cells and osteoclasts. Moreover TRACP 5b serum levels were positively associated with the presence of cathepsin K mRNA in OS specimens (p=0.0153, Fig. 3). Interestingly, in 8 out of 8 cases analysed after tumor removal and chemotherapy, TRACP 5b serum levels were significantly lower than at diagnosis (p=0.0117, Fig. 4).

Osteoclast activity and aggressiveness of OS. OS presenting with metastases and those with multiple localizations were distinguished from other cases and considered as 'aggressive' (9 males and 2 females) as compared to 'classic' OS (23 males and 10 females). In 'aggressive' OS, serum TRACP 5b levels were significantly higher than in 'classic' OS (Table II, Fig. 5), while neither NTX nor MMP-9 showed significant differences (Table II). As shown in Fig. 6, the percentile distribution of the years showed a perfect overlap among groups, while the differences in TRACP 5b levels remained significant (p=0.0509).

Discussion

OS are aggressive, high-grade osteogenic malignancies that demonstrate characteristic patterns of local growth and metastatic spread. Most of these tumors have broken through the overlying cortical bone and have produced a mass of variable size in the adjacent soft tissues by the time of presentation. Microscopic extensions of tumor can penetrate

Table II. Serum concentrations of bone resorption markers in patients with osteosarcoma (OS) in comparison to healthy individuals matched for age and gender.

	Control		OS	'Classic' OS		'Aggressive' OS
TRACP 5b (U/l)						
Median	9.8		8.2	8.0		12.8
Range	3.7-12.1		0.2-21.1	0.2-19.5		5.5-21.1
n	17		44	33		11
p		NS			0.0509[a]	
MMP-9 (ng/ml)						
Median	406.3		759.3	758.5		579.0
Range	155.0-805.0		178.5-1601.7	284.7-1562.8		178.5-1601.7
n	17		1601.7	32		11
p		0.0001[a]	43		NS	
NTX (nmol BCE)						
Median	84.4		84.9	84.8		85.5
Range	74.4-86.3		74.6-88.7	74.6-88.6		75.4-88.7
n	17		34	26		8
p		NS			NS	

[a]P<0.05; NS, not significant.

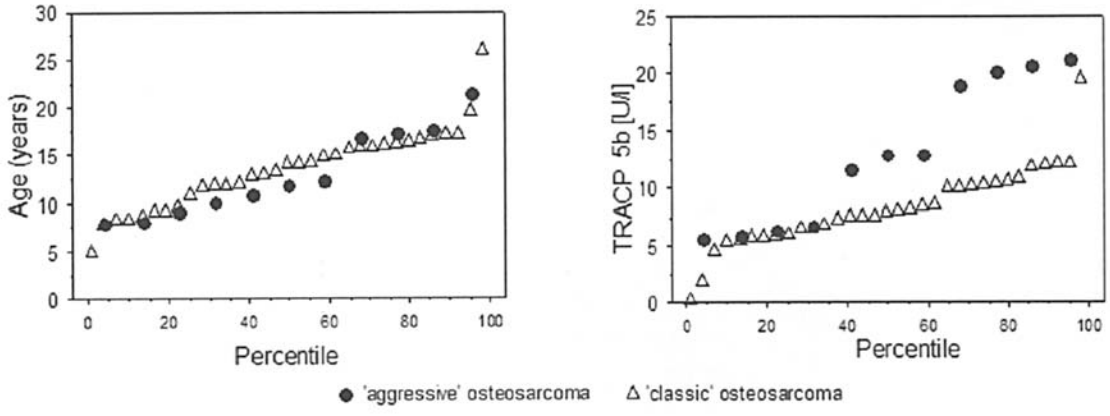

Figure 6. The percentile distribution of age and TRACP 5b serum level in 'aggressive' vs. 'classic' osteosarcomas.

the pseudocapsule outside bone and satellite lesions may occur in the medullary canal at some distance from the primary tumor mass. OS also demonstrate a propensity for developing metastases early in the course of disease. Based on historical data, 80% of patients will have at least microscopic spread of their tumor by the time of diagnosis (3). The dissemination of metastatic OS occurs almost exclusively through the vascular system, and the first evidence of this hematogenous spread is virtually always in the lungs. With the advent of chemotherapy as adjuvant to surgical excision, the prognosis of OS is now largely dependent on tumor responsiveness to anti-cancer drugs (4), although it is clear that other inherent characteristics of the tumor influence for prognosis. Interestingly, local recurrence of OS after resection and multiagent chemotherapy is significantly associated with the rapid development of metastases and a poor outcome (7), suggesting that OS may locally develop characteristics of higher malignancy that may systemically reflect on prognosis. In particular, the ability of OS cells to invade the surrounding bone and reach the vascular bed is a well-recognized characteristic of this tumor. OS shows a high propensity to migrate and invade through the activation of c-MET oncogene, encoding for the scatter factor receptor Met (16). An increased collagen degradation ability by metalloproteinases has also been demonstrated for OS cells (9,10), and this feature appears to be related to a poor prognosis (8).

Little is known, however, on the mechanisms that, in OS, may lead to recruitment and activation of osteoclasts and how this reflects on the clinical course of the disease. In this study, we show that osteoclasts are consistently present in OS, both at the periphery of the tumor at sites of resorption of host bone and, within the tumor tissue, at sites of resorption of neoplastic

osteoid. The presence of active osteoclasts in OS, in addition to the predominant osteogenic stromal component that characterizes this tumor, was confirmed by the RT-PCR finding of cathepsin K mRNA in those cases that, on histology, also appeared to be rich in osteoclasts. Cathepsin K, a cystein protease that degrades Type I collagen, is exclusively expressed at the ruffled border of actively resorbing osteoclasts (17). Cathepsin K is not expressed by OS cells, as confirmed here by the analysis of OS cell lines. Both OS cell lines and tissue samples, on the contrary, constantly expressed MMP-9. This is a metalloproteinase that degrades extracellular bone matrix as a result of secretion by osteoclasts (18) and a number of cancer cell types (19,20), including OS (10,21,22).

To elucidate the role of OS cells in osteoclast formation, we considered a series of osteoclast differentiating factors, including PTHrP, IL-6, and RANKL and found their mRNA to be constantly present both in tissue samples and cell lines, suggesting the presence of multiple mechanisms that OS may activate to enhance osteoclastogenesis. Under this respect, PTHrP seems of particular importance. In other types of cancer, such as breast and lung carcinoma, this growth factor stimulates osteoblasts to secrete RANKL, and this, in turn, is able to induce osteoclast differentiation (23,24). Interestingly, we constantly detected PTHrP and its receptor PTHR1 in all cases of OS, and this is in keeping with recently published data on the significant role of this autocrine pathway in OS progression (25). However, it is not particularly surprising that mRNA for pro-osteolytic factors, like RANKL, M-CSF or IL-6, are expressed in osteosarcomatous tissue. In fact, OS cells are derived from the osteoblast lineage that during normal bone remodelling retains the ability to induce osteoclast differentiation through the secretion of these pro-osteolytic factors.

In order to evaluate the impact of active osteoclasts on bone resorption in OS in a continuous series of patients, including those cases analyzed for the presence of osteoclasts, we analyzed the serum levels of markers that have been associated with bone resorption in bone cancer (26-29). In particular, NTX is cleaved and released from bone collagen by cathepsin K (30,31), MMP-9, secreted by osteoclasts and cancer cells, contributes to the degradation of bone collagen (32), and TRACP 5b is an indicator of the number of active osteoclasts (33-37). Among these markers, only MMP-9 serum levels were significantly higher in OS patients as compared to controls, reflecting their relevant production by cancer cells.

In summary, we were able to show that active osteoclasts are frequently present in OS, that bone destruction and remodeling around and within the primary site of OS might involve osteoclast activity, and that the activity in OS patients is not sufficient to determine a significant difference of TRACP 5b serum levels. However, the induction of osteoclast activity by tumor cells might be particularly increased in those patients with aggressive OS. The possibility that osteoclastogenesis induced by OS may influence not only local tumor invasiveness but also the clinical outcome through an aggressive behavior has been recently suggested by microarray mRNA analysis (38). Consistent with these findings, anti-osteolytic agents, such as zoledronic acid and osteoprotegerin have been found to be effective in *in vivo* models of OS and proposed as adjuvants to conventional treatment (39-41). In our series, OS patients with lung or bone metastases at clinical presentation (therefore defined 'aggressive') showed significantly higher levels of TRACP 5b, a marker that is strictly representative of active osteoclasts, compared to patients with a single osseous localization (defined as 'classic'). Consistent with results obtained by serum analysis, also in tumor biopsies the occurrence of osteoclasts was positively associated with the presence of metastases at diagnosis. To demonstrate that TRACP 5b serum levels are representative of osteoclasts that infiltrate the tumor and may degrade bone collagen we verified that serum TRACP 5b was significantly correlated to NTX, and the presence of cathepsin K in OS tissue was significantly associated with high serum levels of TRACP 5b. On the contrary, TRACP 5b and MMP-9 did not correlate, possibly because MMP-9 is also secreted by OS cells. Moreover, in OS patients, osteoclast activity was strictly related to their presence of the tumor. In fact, in a subset of 8 patients we found decreased serum levels of TRACP 5b after tumor removal and chemotherapy in comparison to the levels analyzed at diagnosis. NTX serum levels did not show a significant difference between 'aggressive' and 'classic' OS, probably because it can be a less sensitive marker of bone resorption then TRACP 5b, as previously demonstrated (42).

Although it is difficult to understand how the presence of osteoclasts at the primary site of OS may influence the behavior of cancer cells, these findings open new insights into OS biology. Similarly to skeletal metastases from carcinomas (26), resorption of host bone induced by OS cells may determine an increased amount of mitogenic, angiogenic, and motogenic growth factors that are normally embedded within the bone matrix, therefore making them available to influence the aggressive behavior of OS cells. An alternative hypothesis might interpret the presence of osteoclasts within the tumor as a manifestation of inflammatory events that could adversely affect the complex interactions between cancer and the immune system (43,44). An imbalance in skeletal homeostasis induced by cancer cells might contribute to the reduced bone mineral density that is almost invariably detected in survivors of OS (45). Although osteoporosis and osteopenia have been generally considered a side effect of chemotherapy, particularly after methotrexate administration (46), the increased osteoclast activity that is found in OS patients at diagnosis may predispose to the reduced bone mass that develops during treatment.

In conclusion, our findings may ultimately contribute to understanding the behavior of OS and its influence on skeletal metabolism, possibly leading to a better chance of cure for this life-threatening cancer. At the present level of knowledge, however, we should limit our conclusion to the demonstration that the presence of active osteoclasts at the primary site of OS and the detection of high levels of TRACP 5b in the serum of OS patients might be considered adverse indicators of prognosis for this tumor, although further investigation is needed. In addition, in those cases with higher TRACP 5b serum level, the increased systemic resorption of bone induced by OS may contribute to osteopenia that is further enhanced by antineoplastic drugs and may ultimately lead to an increased risk of fracture during and after chemotherapy regimens. The use of anti-osteolytic

therapeutic agents may therefore be beneficial and should be included in selected cases of OS.

Acknowledgements

This study was supported by the Italian Association for Cancer Research (AIRC) and the Italian Ministry of Health.

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