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Expression of Met/Hepatocyte Growth Factor Receptor Gene and Malignant Behavior of Musculoskeletal Tumors

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Overexpression of the hepatocyte growth factor receptor (Met/HGF receptor), a transmembrane tyrosine kinase encoded by the* met *proto-oncogene, has been associated with tumor progression in different human carcinomas. More recently, the Met/HGF receptor has also been described in tumor cell lines of mesenchymal origin, suggesting the existence of an autocrine loop that may contribute to the pathogenesis of sarcomas. In this study, we analyzed the expression of Met/HGF receptor by Western blotting and immunohistochemistry in frozen samples of 87 primary tumors of bone and soft tissues. Among benign tumors, overexpression was consistently found only in giant-cell tumor, a locally aggressive lesion that may also, although rarely, spread to the lung. Among malignant lesions, the presence of the Met/HGF receptor was detected in a relevant percentage of primaries and in almost all of the recurrences. The highest levels of Met/HGF receptor were found in osteosarcoma, a highly aggressive tumor that typically permeates the host bone and rapidly expands to the soft tissues. On the contrary, only low levels of Met/HGF receptor were found in chondrosarcoma, a slowly growing tumor that usually expands without massive destruction of the surrounding structures. These data indicate an association of Met/HGF expression with local aggressiveness in human mesenchymal tumors. The finding of Met/HGF receptor overexpression in all of the osteosarcomas suggests a role for the* met *proto-oncogene in the pathogenesis of this tumor. (Am J Pathol 1996, 149:1209–1219)

Structural and/or functional genetic changes in oncogenes and tumor suppressor genes may be involved in the initiation or progression of human cancer through an altered expression of their products. Characterization of these genes may offer valuable clinical information. In sarcomas, inactivation of *Rb* and *p53* tumor suppressor genes has been shown to play a key role in the pathogenesis,[1–3] whereas the relevance of an altered expression of oncogenes has not been fully clarified, with the notable exception of the observed amplification and/or overexpression of c-*myc*, c-*sis*, and c-*fos* in osteosarcoma[4–6] and the generation of bone lesions, including bone tumors, in c-*fos*-overexpressing transgenic mice.[7]

The *met* proto-oncogene was originally identified as the transforming gene of a human osteosarcoma cell line (MNNG-HOS), which had acquired tumorigenicity after treatment with a chemical carcinogen.[8] *met* encodes a 190-kd receptor composed of two disulfide-linked chains, an extracellular 50-kd α-subunit, and a 145-kd β-subunit[9] showing tyrosine kinase activity.[10] Both chains are derived from a 170-kd precursor that is glycosylated and cleaved to give the mature heterodimer.[11] The *met*-encoded transmembrane tyrosine kinase Met has been identified as the receptor for the hepatocyte growth factor/scatter factor (HGF/SF).[12] HGF/SF is a pleiotropic polypeptide with a number of biological activities in

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several cell types, including mitogenesis,[13] morphogenesis,[14] angiogenesis,[15,16] cell scattering, and stimulation of cell motility and invasiveness.[17] *In vivo*, HGF/SF and its receptor are thought to be involved in embryogenesis, tissue reorganization, and tumor progression (for reviews, see Refs. 18–20). In addition, transfection of normal cells with functional Met/HGF receptor has been shown to confer an invasive phenotype,[21] and co-transfection of NIH-3T3 cells with *met* and HGF/SF has resulted in an efficient tumorigenesis.[22] These data suggest that the Met/HGF receptor and its ligand may be involved in the pathogenesis and/or progression of neoplasms. In particular, as the highest concentrations of the Met/HGF receptor are found in epithelial cells,[23,24] and the synthesis/secretion of HGF/SF were first detected in mesenchymal cells,[15,25] HGF/SF and Met/HGF have been suggested as a model for a paracrine signaling system in the context of mesenchymal-epithelial cell interactions.[26] In this model, HGF is synthesized by mesenchymal cells, with the target epithelial cells expressing Met/HGF. This hypothesis has prompted several investigators to analyze the expression of Met/HGF receptor in neoplasms of epithelial origin. Overexpression of this receptor has been found in different types of carcinoma,[23,27–29] and a possible linkage of *met* expression with tumor progression has been suggested in these neoplasms.[30–31] However, we and others[32,33] have recently described the presence of the Met/HGF receptor also in sarcoma cells, demonstrating the existence of an autocrine loop that may contribute to the tumorigenic process of sarcomas. Moreover, the creation of a HGF-mediated autocrine loop in cells of mesenchymal origin has been recently shown to induce invasive properties *in vitro* and metastatic ability *in vivo*.[34]

The aim of this study was to extend our previous *in vitro* observations to tissue samples of benign and malignant musculoskeletal lesions to clarify the actual role of the *met* proto-oncogene expression in the clinical behavior of these lesions.

Materials and Methods

Patients and Specimens

A total of 87 musculoskeletal tumor specimens were available for this study. All of the patients were operated on at the Istituti Ortopedici Rizzoli between 1990 and 1992. They included 2 osteoblastomas, 2 chondroblastomas, 1 non-ossifying fibroma, 13 giant-cell tumors (7 primaries and 6 local recurrences), 4 soft tissue malignant fibrous histiocytomas (2 primaries and 2 local recurrences), 7 soft tissue fibrosarcomas (5 primaries and 2 local recurrences), 49 osteosarcomas (27 primaries, 5 local recurrences, and 17 metastases), and 9 chondrosarcomas (8 primaries and 1 local recurrence). All of the primary lesions were from previously untreated patients. The clinical and pathological features of benign and malignant tumors are reported in Tables 1 and 2, respectively.

Table 1. *Clinical and Pathological Features of Benign Musculoskeletal Tumors*

Case	Age/sex	Site	Diagnosis	Stage*
1	25/F	Femur	OBL	1
2	26/M	Pelvis	OBL	2
3	16/M	Humerus	CBL	3
4	28/F	Scapula	CBL	1
5	20/M	Tibia	FNO	1
6	28/M	Tibia	GCT	1
7	46/F	Tibia	GCT	2
8	20/F	Femur	GCT	2
9	24/F	Humerus	GCT	3
10	59/F	Femur	GCT	3
11	49/M	Femur	GCT	2
12	15/F	Humerus	GCT	3
13	65/M	Femur	GCT, rec	2
14	29/F	Femur	GCT, rec	3
15	15/M	Tibia	GCT, rec	2
16	27/M	Humerus	GCT, rec	2
17	24/M	Radius	GCT, rec	2
18	42/M	Tibia	GCT, rec	3

Stage is according to the criteria of the Musculoskeletal Tumor Society. F, female; M, male; OBL, osteoblastoma; CBL, chondroblastoma; FNO, non-ossifying fibroma; GCT, giant-cell tumor; rec, local recurrence.

In each case, tissue samples taken from representative areas of the tumor were partly immediately frozen in liquid nitrogen and partly processed for histopathological evaluation. Hematoxylin-and-eosin-stained sections were analyzed by conventional criteria[35] to establish the histological type and grade.

Immunohistochemistry

The avidin-biotin-immunoperoxidase method was used to immunostain 5-μm cryostat sections that had been previously fixed in 4% paraformaldehyde for 10 minutes at room temperature. Briefly, sections were treated with methanol containing 1% H_2O_2 for 30 minutes to block endogenous peroxidase activity and incubated overnight with the murine D024 monoclonal antibody, directed against an epitope of the extracellular domain of the *met* gene product,[24] at a 1:800 dilution. After 30 minutes of incubation with a biotinylated secondary monoclonal antibody at room temperature, the final reaction product was visualized by diaminobenzidine plus H_2O_2. Sections

Table 2. *Clinical and Pathological Features of Malignant Musculoskeletal Tumors*

Case	Age/sex	Site	Diagnosis	Grade	Stage*
1	14 /M	Foot	FSA†	4	IIB
2	76 /F	Thigh	FSA	3	IIA
3	38 /M	Shoulder	FSA	2	IA
4	67 /F	Gluteus	FSA	4	IIB
5	75 /F	Arm	FSA	4	IIB
6	57 /F	Leg	FSA, rec	3	IIB
7	32 /M	Thigh	FSA, rec	3	IIA
8	57 /M	Thigh	MFH	4	IIB
9	42 /F	Thigh	MFH	4	IIB
10	55 /M	Thigh	MFH, rec	4	IIB
11	48 /F	Thigh	MFH, rec	4	IIB
12	30 /F	Scapula	CSA	1	IB
13	78 /M	Femur	CSA	2	IA
14	55 /M	Pelvis	CSA	3	IIB
15	68 /F	Metatarsus	CSA	2	IB
16	60 /M	Femur	CSA	3	IIB
17	34 /M	Pelvis	CSA	2	IB
18	63 /F	Humerus	CSA	2	IB
19	63 /M	Pelvis	CSA, rec	3	IIB
20	79 /M	Femur	CSA	3	IIB
21	25 /M	Femur	OSA	4	III
22	22 /F	Tibia	OSA	4	IIB
23	21 /F	Femur	OSA	2	IB
24	61 /M	Pelvis	OSA	4	IIB
25	66 /M	Humerus	OSA	3	III
26	18 /F	Femur	OSA	1	IA
27	44 /M	Scapula	OSA	4	IIB
28	37 /F	Tibia	OSA	4	III
29	61 /M	Humerus	OSA	4	IIB
30	27 /M	Femur	OSA	4	IIB
31	9 /F	Fibula	OSA	4	IIB
32	11 /F	Femur	OSA	4	IIB
33	26 /M	Femur	OSA	4	IIB
34	24 /F	Tibia	OSA	4	IIB
35	12 /F	Tibia	OSA	4	III
36	33 /M	Femur	OSA	4	IIB
37	14 /M	Pelvis	OSA	4	III
38	41 /F	Fibula	OSA	3	IIB
39	20 /F	Humerus	OSA	3	IIB
40	13 /F	Pelvis	OSA	4	IIB
41	33 /M	Pelvis	OSA	4	IIB
42	17 /M	Femur	OSA	4	IIB
43	23 /F	Tibia	OSA	2	IB
44	10 /F	Femur	OSA	4	IIB
45	25 /M	Femur	OSA	2	IB
46	16 /M	Femur	OSA	4	IIB
47	20 /M	Pelvis	OSA	4	III
48	9 /F	Tibia	OSA, rec	3	III
49	20 /F	Femur	OSA, rec	4	III
50	20 /F	Tibia	OSA, rec	4	III
51	12 /F	Femur	OSA, rec	3	III
52	24 /M	Femur	OSA, rec	4	IIB
53	15 /M	Lung	OSA, met	4	III
54	16 /F	Lung	OSA, met	4	III
55	19 /F	Lung	OSA, met	4	III
56	17 /M	Lung	OSA, met	4	III
57	10 /F	Lung	OSA, met	4	III
58	14 /F	Lung	OSA, met	4	III
59	17 /F	Lung	OSA, met	4	III
60	15 /F	Lung	OSA, met	4	III
61	15 /M	Humerus	OSA, met	4	III
62	21 /M	Lung	OSA, met	4	III
63	24 /F	Lung	OSA, met	4	III
64	25 /M	Lung	OSA, met	4	III
65	21 /M	Lung	OSA, met	4	III
66	19 /M	Lung	OSA, met	4	III
67	14 /F	Lung	OSA, met	4	III
68	33 /M	Lung	OSA, met	4	III
69	12 /F	Tibia	OSA, met	4	III

Stage is according to the criteria of the Musculoskeletal Tumor Society. M, male; F, female; FSA, fibrosarcoma; MFH, malignant fibrous histiocytoma; CSA, chondrosarcoma; OSA, osteosarcoma; rec, local recurrence; met, metastasis.

were counterstained with Gill's hematoxylin. Specimens in which the incubation with the primary antibody had been omitted were used as a negative control.

Western Blotting

Western blot analysis was performed as previously described.[33] Briefly, pulverized tissues were solubilized in boiling Laemmli buffer[36] containing the reducing agent β-mercaptoethanol. In these conditions, the 50-kd α-subunit and the 145-kd β-subunit of the Met/HGF receptor are distinct. Equal amounts of proteins (200 μg) were loaded into each lane. Proteins were separated by polyacrylamide gel electrophoresis and transferred to nitrocellulose sheets. Western blot analysis was carried out as described by Towbin et al.[37] Blots were probed with either the DQ-13 monoclonal antibody (EMBL Data-Bank reference X54559), raised against a peptide corresponding to the 19 carboxyl-terminal amino acids (from Ser 1372 to Ser 1390) of the human Met/HGF receptor sequence, or with the DL-21 monoclonal antibody,[24] raised against the extracellular domain of the receptor. For HGF analysis, anti-HGF H04 antiserum, kindly provided by Dr. A. Galvani (Farmitalia Carlo Erba, Milano, Italy), was used. Horseradish-peroxidase-conjugated rabbit anti-mouse immunoglobulins were used as secondary antibody. The reaction was revealed by chemiluminescence (Amersham, Amersham, UK), and the relative expression of the receptor was quantified by laser densitometric scanning of x-ray films.

Southern Blotting

DNA was extracted and Southern blot analysis was performed as previously described.[9] DNA (10 μg) was digested with the *Eco*R1 restriction enzyme for 12 hours at 37°C and loaded onto each lane. Hybridization was carried out at 65°C using cDNA encompassing the entire coding sequence of the *met* gene as a probe. Probes were generated using the Mega Prime method (Amersham) in the presence of [^{32}Pα]CTP. Nylon sheets were washed twice in 1× standard saline citrate (SSC/0.1% sodium dodecyl sulfate) (SDS) at room temperature for 15 minutes and twice at 65°C for 20 minutes. Filters were rehybridized with a ^{32}P-labeled β-globin probe to estimate the amount of DNA loaded. The intensity of labeled bands was estimated by laser densitometric scanning of x-ray films.

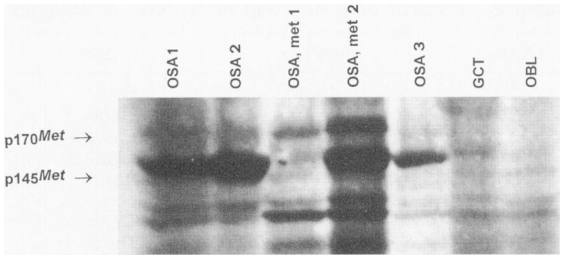

Figure 1. *Different expression of the Met/HGF receptor in human bone tumor samples by Western blot analysis. The assay was carried out with DQ-13 monoclonal antibody, which detects both the p145 β-subunit and the p170 precursor of the receptor when proteins are separated on SDS-polyacrylamide gel electrophoresis in the presence of a reducing agent. OSA, primary osteosarcoma; OSA, met, metastasis of osteosarcoma; GCT, giant-cell tumor; OBL, osteoblastoma.*

Results

The expression of the Met/HGF receptor in bone tumors was investigated by Western blot analysis in 87 patients with musculoskeletal tumors, including 18 benign and 69 malignant lesions. A representative experiment, in which different levels of expression of this protein were detected, is shown in Figure 1. Both the 145-kd β-chain (p145^{Met}) and the 170-kd precursor of the receptor (p170^{Met}) were labeled with the DQ-13 antibody.

Among benign tumors, the Met/HGF receptor was not detected either in osteoblastoma or in non-ossifying fibroma (Table 3). Of the two cases of chondroblastoma, the one featuring an aggressive behavior with a striking tendency to invade the surrounding tissues (Figure 2a) was highly positive for the protein, whereas the other, showing clinical

Table 3. *Expression of Met/HGF Receptor in Benign Musculoskeletal Tumors Analyzed by Western Blotting*

	Number of cases	Number of positive cases	Relative score		
			+	++	+++
Osteoblastoma	2	0	0	0	0
Non-ossifying Fibroma	1	0	0	0	0
Chondroblastoma	2	1 (50%)	0	0	1
Giant cell tumor, primaries	7	4 (57%)	3	1	0
Giant cell tumor, recurrences	6	6 (100%)	4	1	1

+, weakly positive tumor; ++, positive tumor; +++, strongly positive tumor.

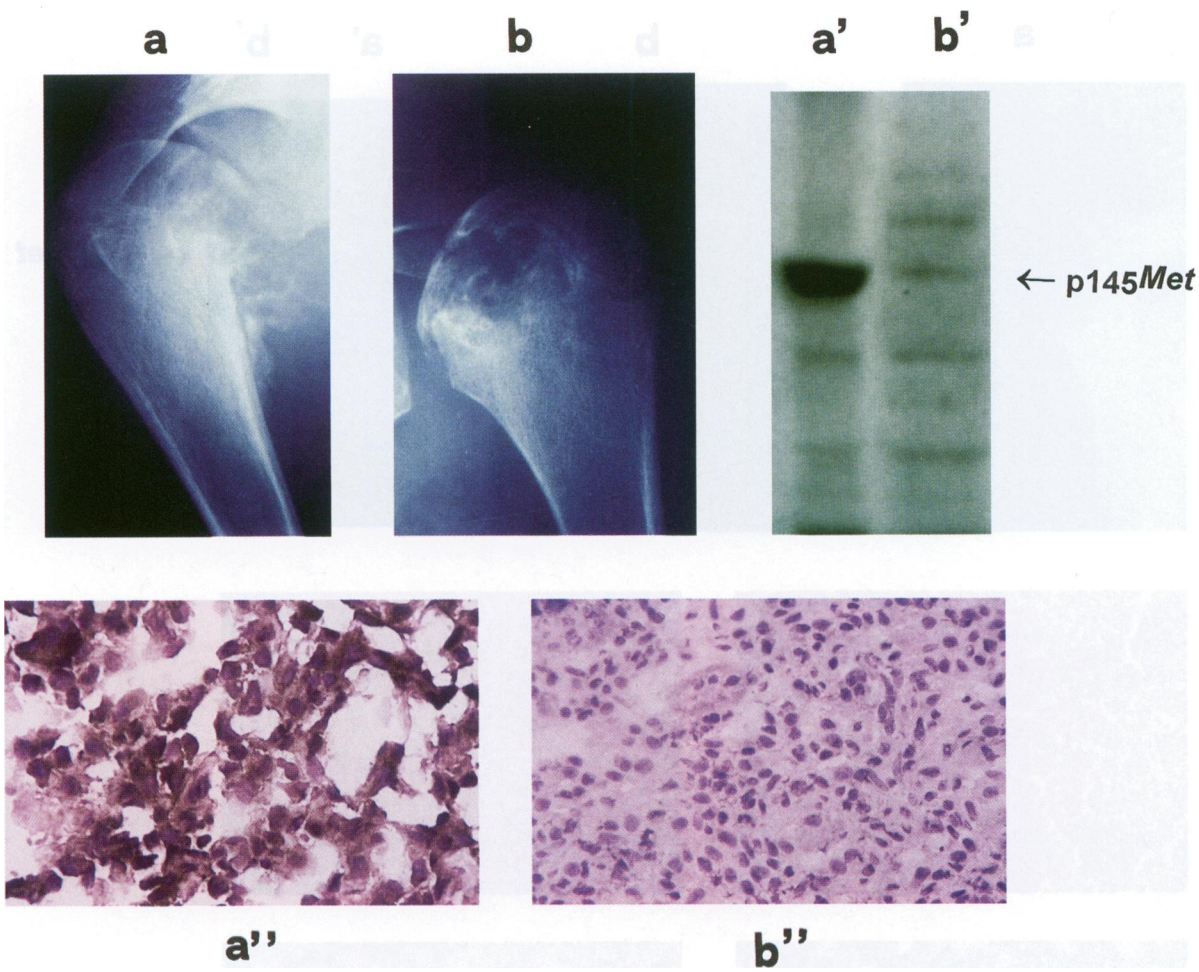

Figure 2. *Two cases of chondroblastoma of the proximal humerus showing different clinical and pathological features.* a: *An aggressive lesion, expanding through the cortical bone to the surrounding soft tissues, corresponds to an increased expression of Met/HGF receptor, as revealed by Western blotting* (a') *and immunohistochemistry* (a''). b: *A small osteolytic lesion, which is entirely confined to the epiphyseal bone, corresponds to a lack of expression of Met/HGF receptor by Western blotting* (b') *or immunohistochemistry* (b'').

and pathological characteristics of a benign inactive tumor (Figure 2b) did not express the Met/HGF receptor. Among giant-cell tumors, detectable levels of $p145^{Met}$ were found in four out of seven primary lesions. Figure 3 shows two representative cases, an active lesion overexpressing the Met/HGF receptor (Figure 3a) and a quiescent lesion that was negative for the receptor (Figure 3b), respectively. All of the cases of local recurrences of giant-cell tumor showed expression of Met/HGF receptor.

The expression of Met/HGF receptor in malignant musculoskeletal tumors is summarized in Table 4. Among soft-tissue sarcomas, detectable levels of $p145^{Met}$ were observed in 43% of primary and in 75% of recurrent lesions. Among bone sarcomas, a striking difference was observed between chondrosarcoma and osteosarcoma. In chondrosarcoma, low levels of Met/HGF receptor were found in two out of eight primary lesions and in the single recurrence analyzed. On the other hand, in osteosarcoma, all of the primaries and the local recurrences as well as most of the metastases showed overexpression of $p145^{Met}$. Moreover, in all of the osteosarcomas, particularly in primaries and recurrences, Met/HGF receptor was expressed at remarkably high levels. Metastatic lesions had expression of Met/HGF receptor equal to or lower than primary tumors. To clarify whether Met/HGF receptor could be down-modulated by HGF, a few cases of metastatic and primary osteosarcoma were tested. Metastases showed a higher expression of HGF than primaries (data not shown).

To establish whether overexpression of the Met/HGF receptor in osteosarcoma was due either to *met* gene rearrangement or amplification, Southern blot analysis was performed using a cDNA encompassing the entire *met* coding sequence as a probe (Figure 4). DNA was extracted from 13 osteosarcoma

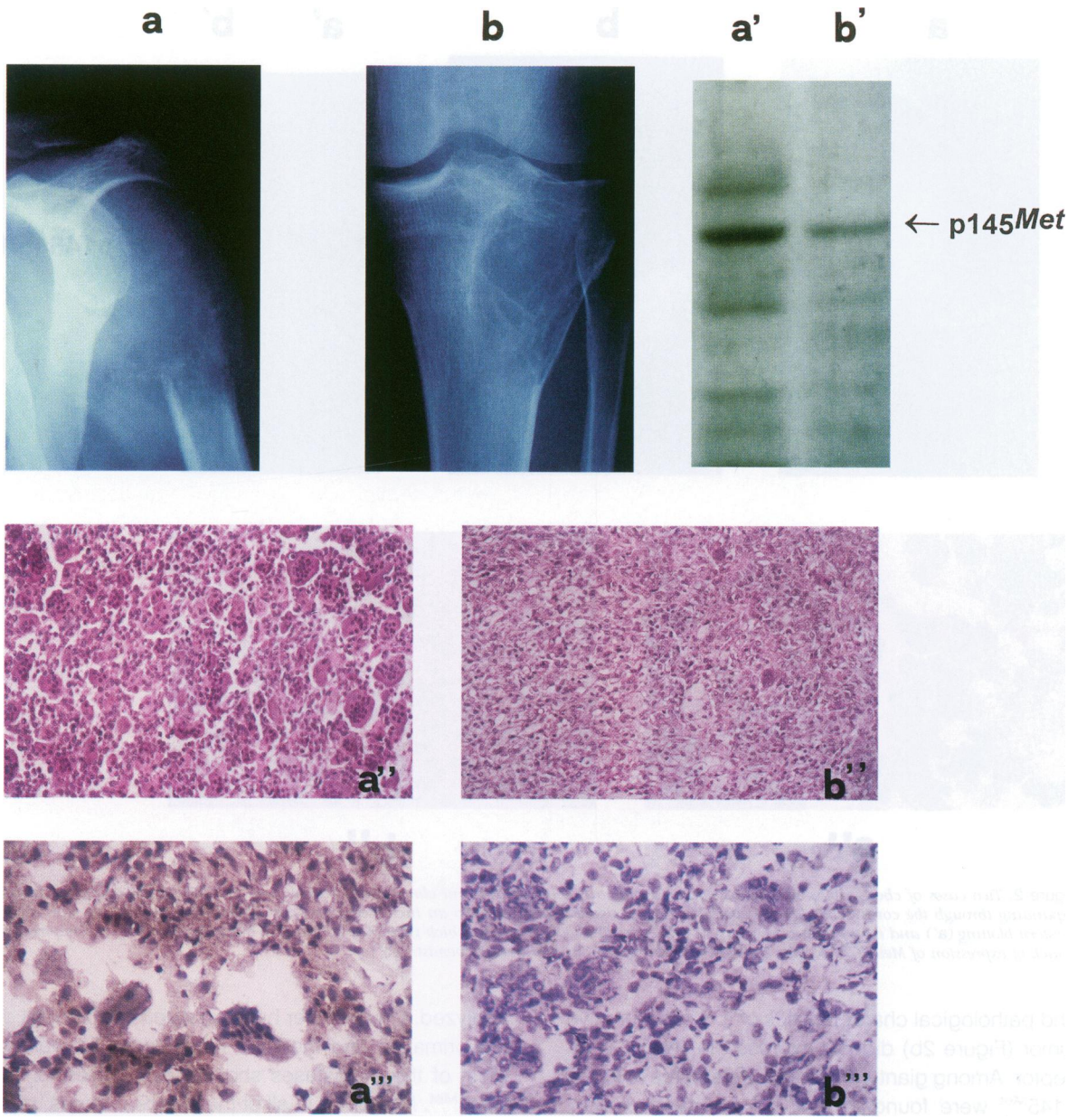

Figure 3. *Two cases of giant cell tumor of bone, showing different clinical and pathological features.* a: *An osteolytic lesion of the proximal humerus, showing a histological picture characterized by a striking number of multinucleated giant cells* (a'), *corresponds to an increased expression of Met/HGF receptor, as revealed by Western blotting* (a'') *and immunohistochemistry* (a'''). b: *An inactive lesion of the proximal tibia, showing only a few scattered giant cells on histology* (a'), *corresponds to very low levels of expression of Met/HGF receptor by Western blotting* (b'') *and no expression by immunohistochemistry* (b''').

samples showing different levels of Met/HGF receptor expression. Identical restriction patterns were detected in all of the cases after *Eco*R1 digestion, indicating that no major rearrangement of the *met* gene was present. In fact, the same fragments were obtained from a reference cell line (GTL-16) in which *met* had been cloned and sequenced, and no rearrangement had been observed.[38] No evidence for amplification was found by measuring the intensity of the DNA bands obtained from the tumors overexpressing the Met/HGF receptor.

In 68 cases, including all of the osteosarcomas, the presence of the receptor was also analyzed by immunohistochemistry on cryostat tissue sections to confirm the data obtained by Western blotting and to analyze the tissue distribution of the Met/HGF receptor. In particular, to ascertain whether overexpression of the *met* gene was a consequence of very high

Table 4. *Expression of Met/HGF Receptor in Malignant Musculoskeletal Tumors Analyzed by Western Blotting*

		Number of cases	Number of positive cases	Relative score		
				+	++	+++
Soft tissue sarcomas	Primaries	7	2 (29%)	1	1	0
	Recurrences	4	3 (75%)	1	2	0
Chondrosarcoma	Primaries	8	2 (25%)	1	1	0
	Recurrences	1	1 (100%)	0	1	0
Osteosarcoma	Primaries	24	24 (100%)	0	3	21
	Recurrences	5	5 (100%)	0	0	5
	Metastases	17	12 (71%)	4	5	3

+, weakly positive tumor; ++, positive tumor; +++, strongly positive tumor.

levels of expression in a few cells or the result of low but diffuse expression of the receptor, immunohistochemical analysis of frozen tissue sections was performed with a monoclonal antibody specific for an extracellular epitope of the receptor. The percentages of positive tumors were substantially equal by Western blotting and by immunohistochemistry (Tables 5 and 6), and the staining was always found to be homogeneously distributed. Among malignant tumors, immunohistochemistry confirmed the striking difference in Met/HGF receptor expression between chondrosarcoma and osteosarcoma observed by Western blotting. The Met/HGF receptor was poorly expressed in chondrosarcoma, a neoplasm characterized by a weak invasive ability (Figure 5, a and a′), whereas all of the cases of osteosarcoma, a tumor that rapidly invades and destroys the surrounding tissues, showed a high expression of Met/HGF receptor (Figure 5, b and b′). As previously shown by Western blotting and by immunohistochemistry in osteosarcoma, overexpression of Met/HGF receptor was present in all of the primaries and local recurrences and in the majority of metastases.

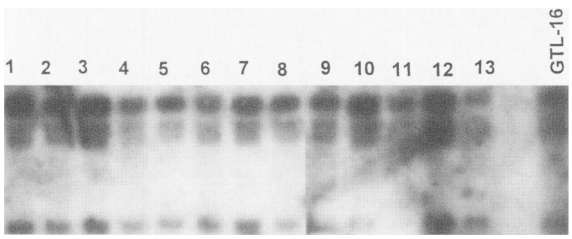

Figure 4. *Southern blot analysis of* met *proto-oncogene in osteosarcoma samples (1 to 13). Identical restriction patterns were detected in all of the cases after* EcoRI *digestion. The same fragments were obtained by DNA digestion of a reference cell line (GTL-16) in which* met *was cloned and sequenced.*[38]

Table 5. *Expression of Met/HGF Receptor in Benign Musculoskeletal Tumors Analyzed on Cryostatic Section Using DO24 Monoclonal Antibody*

	Number of cases	Number of positive cases	Relative score		
			+	++	+++
Osteoblastoma	2	0	0	0	0
Non-ossifying Fibroma	1	0	0	0	0
Chondroblastoma	2	1 (50%)	0	0	1
Giant cell tumor, primaries	6	2 (33%)	2	0	0
Giant cell tumor, recurrences	5	3 (60%)	0	2	1

+, scattered positive cells, weak positivity; ++, diffuse, weak positivity; +++, diffuse, strong positivity.

Table 6. *Expression of Met/HGF Receptor in Malignant Musculoskeletal Tumors Analyzed on Cryostatic Sections Using DO24 Monoclonal Antibody*

		Number of cases	Number of positive cases	Relative score		
				+	++	+++
Soft tissue sarcomas	Primaries	4	1 (25%)	1	0	0
	Recurrences	1	1 (100%)	0	1	0
Chondrosarcoma	Primaries	3	1 (33%)	1	0	0
	Recurrences	1	1 (100%)	1	0	0
Osteosarcoma	Primaries	20	19 (95%)	6	8	5
	Recurrences	4	4 (100%)	0	4	0
	Metastases	17	14 (82%)	7	4	3

+, scattered positive cells, weak positivity; ++, diffuse, weak positivity; +++, diffuse, strong positivity.

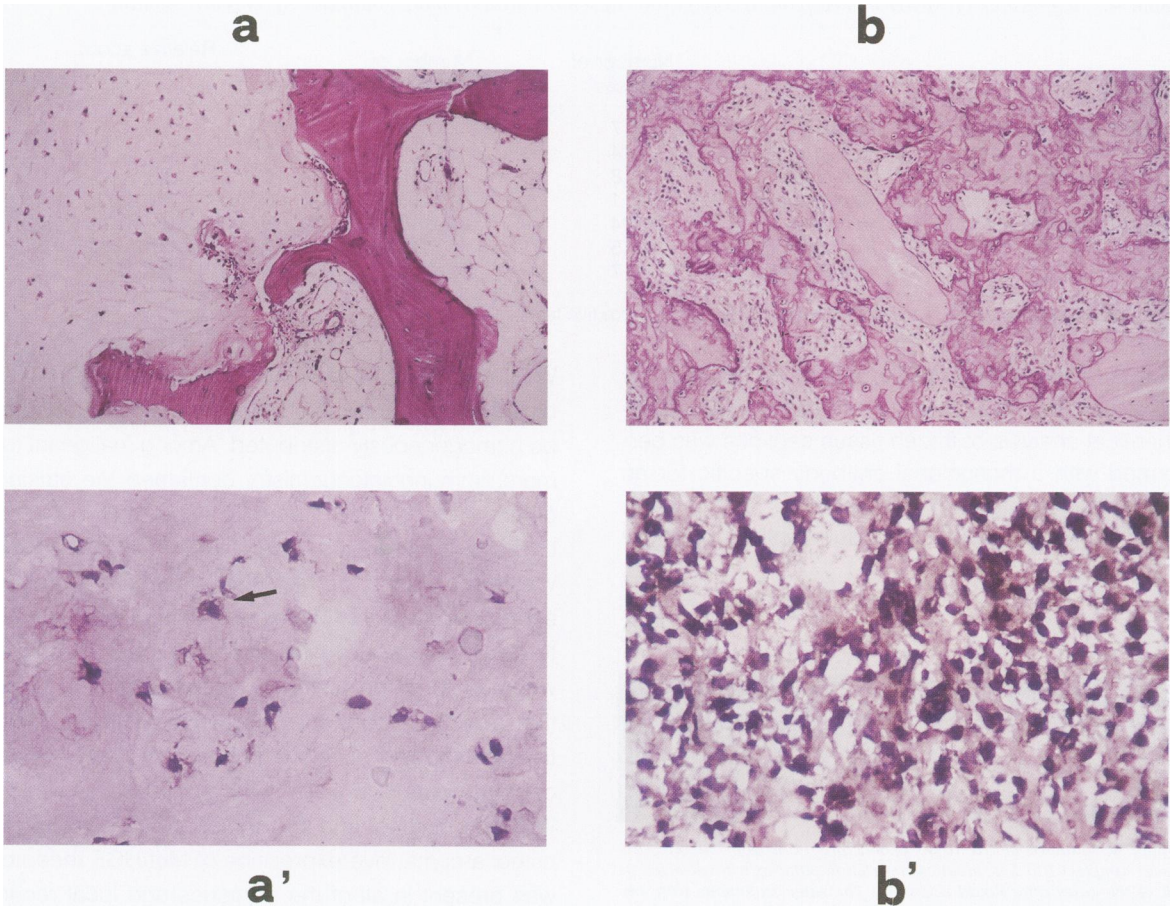

Figure 5. *Different expression of the Met/HGF receptor in bone sarcomas.* a: *A grade 2 chondrosarcoma pushing, but not infiltrating, through the bone, shows an extremely weak positivity for the receptor* (arrow) *by immunohistochemistry* (a'). b: *On the contrary, in a case of grade 4 osteosarcoma, showing a massive substitution of the host bone with neoplastic osteoid, a high level of expression of the Met/HGF receptor is revealed by immunohistochemistry* (b').

Discussion

Tumor invasion and metastasis are complex multistep processes involving a variety of cell features, such as cell adhesion, activation and secretion of proteases, cell motility, cell growth, and neoangiogenesis, all contributing to the malignant potential of neoplastic cells. Some of these mechanisms are associated with signal transduction mediated by the Met/HGF receptor. In fact, HGF/SF is reported to act both as a mitogenic and a motogenic factor *in vitro*[13,17] (for a review, see Refs. 18–20) and is also able to induce blood vessel formation and to contribute to tumor angiogenesis *in vivo*.[15,16] Therefore, inappropriate expression of its receptor, the *met* proto-oncogene product Met/HGF receptor, may lead to tumor invasion and metastasis. Met/HGF receptor has been found to be overexpressed in tumors of epithelial origin[23,27–29] and to be associated with tumor progression in prostatic and colorectal carcinoma.[30,31] In carcinomas, HGF/SF and its receptor may operate in a paracrine mode, HGF/SF being produced by mesenchymal reactive cells and the Met/HGF receptor being expressed by tumor cells. Recently, the existence of an autocrine mechanism mediated by HGF/SF has been suggested for mesenchymal neoplasms. In fact, co-transfected fibroblasts expressing endogenous HGF/SF and the Met/HGF receptor become tumorigenic,[22] and the autocrine activation of *met* in these cells enhances cell motility, collagenase activity, and invasiveness *in vitro* as well as metastatic ability *in vivo*.[34] Moreover, overexpression of the Met/HGF receptor has been described in sarcoma cells,[32,33] suggesting a role of the *met* proto-oncogene in these neoplasms.

In this paper, we analyzed the expression of the Met/HGF receptor in human musculoskeletal neoplasms and found a relationship between the expression of this receptor and local aggressiveness. Met/HGF receptor was overexpressed in 5 of 12 benign tumors (42%) and in 28 of 39 malignant le-

sions (72%; $P = 0.06$). However, in these neoplasms, Met/HGF receptor expression appeared to be consistently associated with the invasive ability of the single tumor rather than with the benign *versus* malignant histological classification criteria. In fact, no expression of Met/HGF receptor was detected in osteoblastoma, in non-ossifying fibroma, or in one of the two cases of chondroblastoma, all showing typical features of inactive tumors. On the contrary, the second case of chondroblastoma, showing an aggressive clinical behavior, had high levels of expression of the Met/HGF receptor. The receptor was also expressed in the majority of the primaries and in all of the local recurrences of giant-cell tumor, a neoplasm that, although histologically benign, is characterized by a considerable degree of local aggressiveness, a rare but consistent ability to give lung metastases, and a striking tendency to sarcomatous progression.[39] The presence of the Met/HGF receptor in benign but locally aggressive neoplasms suggests a role for this protein in determining the invasiveness of mesenchymal tumor cells featuring otherwise benign characteristics. The importance of the Met/HGF receptor for the local aggressiveness of mesenchymal tumors is further confirmed by the analysis of malignant neoplasms. In fact, the level of expression of the Met/HGF receptor was low in chondrosarcoma, a slowly growing tumor that rarely infiltrates the surrounding normal tissues. On the contrary, the highest level of Met/HGF receptor positivity was found in osteosarcoma, the most aggressive primary malignant bone tumor, which typically permeates and disrupts the host bone and rapidly expands to the soft tissues. Immunostaining for the Met/HGF receptor confirmed the data obtained by Western blotting and indicated that the receptor, when expressed, is present in the majority of tumor cells.

In a preliminary report, we had shown the presence of both HGF/SF and its receptor in a small number of mesenchymal tumors[33] and suggested that their interaction might occur either via a paracrine or an autocrine mechanism. The extracellular matrix is reported to be a high-capacity, low-affinity reservoir for pro-HGF/SF,[40,41] and the proteolytic cleavage of the matrix-bound precursor[42,43] may activate this growth factor. Therefore, in primary bone tumors, HGF/SF might be freely available in the microenvironment and stimulate the tumor cells through a paracrine or autocrine interaction. Cells overexpressing Met/HGF receptor at their surface may be influenced in their motile-invasive behavior. It has been previously shown that transfection of a functional Met/HGF receptor to fibroblasts may transfer the motogenic but not the mitogenic program triggered by HGF/SF.[21] Moreover, our previous *in vitro* studies have demonstrated that HGF/SF may stimulate the motility of osteosarcoma cells[33] and their invasive ability through Matrigel-coated filters and collagen matrix (manuscript in preparation). Similar results have been recently reported for leiomyosarcoma cells,[44] further suggesting that HGF-Met/HGF receptor signaling is an important property for invasion in mesenchymal tumors cells. These data are in agreement with the evidence provided here on clinical material.

In osteosarcoma, we were unable to demonstrate an association between Met/HGF receptor expression and tumor progression. Metastatic lesions showed expression of the Met/HGF receptor equal to or even slightly lower than primary tumors. This can be partly explained by the extremely malignant nature of osteosarcoma, a neoplasm that virtually always spreads to the lungs early during its course.[45] In this tumor, the phenotypic characteristics of metastatic and primary lesions are therefore likely to be similar. Moreover, it should also be considered that HGF/SF is abundantly produced by lung cells,[46] and this might determine a down-modulation of the receptor.[32]

The observation that, in osteosarcoma, overexpression of *met* proto-oncogene is present in all of the primary and recurrent lesions and in the majority of metastases may be relevant to understand the pathogenesis of this tumor. Evidence has been provided suggesting that signal transduction pathways involving tyrosine kinase are implicated in bone physiology and pathology. The c-*src* and c-*fms* oncogenes are involved in bone resorption and remodeling,[47–49] and bone cell transformation has been induced by v-*fps*.[50] Moreover, the loss of p53, which is found in 50 to 60% of osteosarcomas,[2] has been recently shown to increase the likelihood of enhancing the expression of Met/HGF.[51]

In conclusion, this study demonstrates the expression of the Met/HGF receptor in human musculoskeletal tumors. The presence of the Met/HGF receptor appears to be associated with the invasive ability and the local aggressiveness rather than with the metastatic potential of these tumors. In osteosarcoma, the consistent occurrence of *met* overexpression suggests the importance of its role for the pathogenesis of this tumor.

References

1. Reissmann PT, Simon MA, Lee WH, Slamon DJ: Studies of the retinoblastoma gene in human sarcomas. Oncogene 1989, 4:839–843

2. Wadayama B, Toguchida J, Yamaguchi T, Sasaki MS, Kotoura Y, Yamamuro T: P53 expression and its relationship to DNA alterations in bone and soft tissue sarcomas. Br J Cancer 1993, 68:1134–1139
3. Mulligan LM, Matlashewski GJ, Scrabel HJ, Cavenee WK: Mechanisms of p53 loss in human sarcomas. Proc Natl Acad Sci USA 1990, 87:5863–5867
4. Fahrer C, Brachmann R, Von Der Helm K: Expression of c-*sis* and other cellular proto-oncogenes in human sarcoma cell lines and biopsies. Int J Cancer 1989, 44: 652–657
5. Ladanyi M, Kum Park C, Lewis R, Jhanwar SC, Healey JH, Huvos AG: Sporadic amplification of the MYC gene in human osteosarcomas. Diagn Mol Pathol 1993, 2:163–167
6. Ikeda S, Sumii H, Akiyama K, Watanabe S, Ito S, Inoue H, Takechi H, Tanabe G, Oda T: Amplification of both c-*myc* and c-*raf-1* oncogenes in a human osteosarcoma. Jpn J Cancer Res 1989, 80:6–9
7. Ruther U, Komitowski D, Schubert FR, Wagner EF: c-*fos* expression induces bone tumors in transgenic mice. Oncogene 1989, 4:861–865
8. Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM, Vande Woude GF: Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature 1984, 311:29–33
9. Giordano S, Ponzetto C, Di Renzo MF, Cooper CS, Comoglio MP: Tyrosine kinase receptor indistinguishable from the c-*met* protein. Nature 1989, 339:155–156
10. Gonzatti-Haces M, Seth A, Park M, Copeland T, Oroszlan S, Vande Woude GF: Characterization of the *TPR-MET* oncogene p65 and the *met* protooncogene p140 protein tyrosine kinases. Proc Natl Acad Sci USA 1988, 85:21–25
11. Giordano S, Di Renzo MF, Narsimhan R, Cooper CS, Rosa C, Comoglio PM: Biosynthesis of the protein encoded by the c-*met* proto-oncogene. Oncogene 1989, 4:1383–1388
12. Bottaro DP, Rubin JS, Faletto DL, Chan AM-L, Kmiecik TE, Vande Woude GF, Aaronson SA: Identification of the hepatocyte growth factor receptor as the c-*met* proto-oncogene product. Science 1991, 251:802–804
13. Kan M, Zhang G, Zarnegar R, Michalopoulos G, Myoken Y, Mckeehan WL, Stevens JL: Hepatocyte growth-factor, hepatopoietin-A stimulates the growth of rat-kidney proximal tubules epithelial cell (rpte), rat nonparenchymal liver cells, human melanoma cells and mouse keratinocytes, and stimulates anchorage independent growth of SV40-transformed rpte. Biochim Biophys Res Commun 1991, 174:331–337
14. Montesano R, Matsumoto K, Nakamura T, Orci L: Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. Cell 1991, 67:901–908
15. Bussolino F, Di Renzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L, Gaudino G, Tamagnone L, Coffer A, Comoglio PM: Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J Cell Biol 1992, 119:629–641
16. Grant DS, Kleinman HK, Goldberg ID, Bhargava MM, Nickoloff BJ, Kinsella JL, Polverini P, Rosen EM: Scatter factor induces blood vessel formation *in vivo*. Proc Natl Acad Sci USA 1993, 90:1937–1941
17. Weidner KM, Behrens J, Vandekerckhove J, Birchmeier W: Scatter factor: molecular characteristics and effect on the invasiveness of epithelial cells. J Cell Biol 1990, 111:2097–2108
18. Rubin JS, Bottaro DP, Aaronson SA: Hepatocyte growth factor/scatter factor and its receptor, the c-*met* proto-oncogene product. Biochem Biophys Acta 1993, 1153:357–371
19. Weidner KM, Hartman G, Naldini L, Comoglio PM, Sachs M, Fonatsch C, Rieder H, Birchmeier W: Molecular characteristics of HGF/SF and its role in cell motility and invasion. Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the c-*Met* receptor. Edited by ID Goldberg and EM Rosen. Birkauser Verlag, Basel, 1993, pp 311–328
20. Comoglio PM: Structure, biosynthesis and biochemical properties of the HGF receptor in normal and malignant cells. Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the c-*Met* receptor. Edited by ID Goldberg and EM Rosen. Birkauser Verlag, Basel, 1993, pp 131–165
21. Giordano S, Zhen Z, Medico E, Gaudino G, Galimi F, Comoglio PM: Transfer of motogenic and invasive response to scatter factor/hepatocyte growth factor by transfection of human *met* protooncogene. Proc Natl Acad Sci USA 1993, 90:649–653
22. Rong S, Bodescot M, Blair D, Dunn J, Wakamura T, Mizuno K, Park M, Chan A, Aaronson S, Vande Woude GF: Tumorigenicity of the *met* proto-oncogene and the gene for hepatocyte growth factor. Mol Cell Biol 1992, 12:5152–5158
23. Di Renzo MF, Narsimhan RP, Oliviero M, Bretti S, Giordano S, Medico E, Gaglia P, Zara P, Comoglio PM: Expression of the Met/HGF receptor in normal and neoplastic human tissue. Oncogene 1991, 6:1997–2003
24. Prat M, Narsimham RP, Crepaldi T, Nicotra MR, Natali PG, Comoglio PM: The receptor encoded by the human c-MET oncogene is expressed in hepatocytes, epithelial cells and solid tumors. Int J Cancer 1991, 49:323–328
25. Stoker M, Gerardi E, Perryman M, Gray J: Scatter factor is a fibroblast-derived modulator of epithelial cell motility. Nature 1987, 327:239–242
26. Sonnenberg E, Meyer D, Weidner KM, Birchmeier C: Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. J Cell Biol 1993, 123:223–235
27. Di Renzo MF, Oliviero M, Ferro S, Prat M, Bongarzone I, Pilotti S, Belfiore A, Costantino A, Vigneri R, Pierotti MA, Comoglio PM: Overexpression of the c-MET/HGF receptor gene in human thyroid carcinomas. Oncogene 1992, 7:2549–2553
28. Ebert M, Yokoyama M, Friess H, Büchler MW, Korc M:

Coexpression of the c-*met* proto-oncogene and hepatocyte growth factor in human pancreatic cancer. Cancer Res 1994, 54:5775–5778
29. Di Renzo MF, Poulsom R, Oliver M, Comoglio PM, Lemoine NR: Expression of the Met/hepatocyte growth factor receptor in human pancreatic cancer. Cancer Res 1995, 55:1129–1138
30. Humphrey PA, Zhu X, Zarnegar R, Swanson PE, Ratliff TL, Vollmer RT, Day ML: Hepatocyte growth factor and its receptor (c-MET) in prostatic carcinoma. Am J Pathol 1995, 147:386–396
31. Di Renzo MF, Olivero M, Giacomini A, Henri Porte, Chastre E, Mirossay L, Nordlinger B, Bretti S, Bottardi S, Giordano S, Plebani M, Gespach C, Comoglio PM: Overexpression and amplification of the Met/HGF receptor gene during the progression of colorectal cancer. Clin Cancer Res 1995, 1:147–154
32. Rong S, Jeffers M, Resau JH, Tsarfaty I, Oskarsson M, Vande Woude GF: Met expression and sarcoma tumorigenicity. Cancer Res 1993, 53:5355–5360
33. Ferracini R, Di Renzo MF, Scotlandi K, Baldini N, Olivero M, Lollini PL, Cremona O, Campanacci M, Comoglio PM: The *Met*/HGF receptor is over-expressed in human osteosarcomas and is activated by either a paracrine or an autocrine circuit. Oncogene 1995, 10: 739–749
34. Rong S, Segal S, Anver M, Resau JH, Vande Woude GF: Invasiveness and metastasis of NIH 3T3 cells induced by Met-hepatocyte growth factor/scatter factor autocrine stimulation. Proc Natl Acad Sci USA 1994, 91:4731–4735
35. Dahlin DC, Unni KK: Bone Tumors: General Aspects and Data on 8542 Cases, ed 4. Springfield, IL, Charles C. Thomas, 1986
36. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227:680–685
37. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheet: procedure and some applications. Proc Natl Acad Sci USA 1979, 76:4350–4353
38. Ponzetto C, Giordano S, Peverali F, Della Valle G, Abate M, Vaula G, Comoglio PM: c-*met* is amplified but not mutated in a cell line with an activated *met* tyrosine kinase. Oncogene 1991, 6:553–559
39. Campanacci M, Baldini N, Boriani S, Sudanese A: Giant-cell tumor of bone. J Bone Joint Surg 1987, 69A: 106–114
40. Masumoto A, Yamamoto N: Sequestration of a hepatocyte growth factor in extracellular matrix in normal adult rat liver. Biochem Biophys Res Commun 1991, 174: 90–95
41. Naldini L, Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C, Narsimhan RP, Hartmann G, Zarnegar R, Michalopoulos GK, Birchmeier W, Comoglio PM: Scatter factor and hepatocyte growth factor are indistinguishable ligands for the *Met* receptor. EMBO J 1991, 10:2867–2871
42. Naldini L, Tamagnone L, Vigna E, Sachs M, Hartmann G, Birchmeier W, Daikuhara Y, Tsubouchi H, Blasi F, Comoglio PM: Extracellular proteolytic cleavage by urokinase is required for activation of hepatocyte growth factor/scatter factor. EMBO J 1992, 11:4825–4833
43. Miyazawa K, Shimomura T, Kitamura A, Kondo J, Morimoto Y, Kitamura N: Molecular cloning and sequence analysis of the c-DNA for a human serine protease responsible for activation of hepatocyte growth factor. J Biol Chem 1993, 268:10024–10028
44. Jeffers M, Rong S, Vande Woude GF: Enhanced tumorigenicity and invasion-metastasis by hepatocyte growth factor/scatter factor-Met signaling in human cells concomitant with induction of the urokinase proteolysis network. Mol Cell Biol 1996, 16:1115–1125
45. Goorin MA, Abelson HT, Frei E: Osteosarcoma: fifteen years later. N Engl J Med 1985, 313:1637–1643
46. Yanagita K, Nagaike M, Ishibashi H, Niho Y, Matsumato K, Nakamura T: Lung may have an endocrine function producing hepatocyte growth factor in response to injury of distal organs. Biochem Biophys Res Commun 1992, 182:802–809
47. Yoshida H, Hayashi S, Kunisada T, Ogawa M, Nishikawa S, Okamura H, Sudo T, Shultz LD, Nishikawa S: The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. Nature 1990, 345:442–445
48. Soriano P, Montgomery C, Geske R, Bradley A: Targeted disruption of the c-*src* proto-oncogene leads to osteopetrosis in mice. Cell 1991, 64:693–698
49. Ferracini R, Leonardi A: New perspectives on osteopetrosis. Ital J Mineral Electrolyte Metab 1993, 3:199–205
50. Cogliano A, Birek C, Tenenbaum HC: Inducibility of neoplastic transformation by Fujinami sarcoma virus in an *in vitro* chick embryo model for osteosarcoma: i) effect of differentiation and ii) investigation for *in vivo* growth potential in athymic mice. Bone 1991, 12:365–375
51. Rong S, Donehower LA, Hansen MF, Strong L, Tainsky M, Jeffers M, Resau JH, Hudson E, Tsarfaty I, Vande Woude GF: Met proto-oncogene product is overexpressed in tumors of p53-deficient mice and tumors of Li-Fraumeni patients. Cancer Res 1995, 55:1963–1970