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Osteosarcoma with features mimicking malignant fibrous histiocytoma

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Summary. Three osteosarcomas (OS) with features resembling malignant fibrous histiocytoma (MFH) were selected and investigated to identify any clinico-pathological similarities. In all cases there was no significant difference from conventional OS on the radiography and laboratory data. The appearance of MFH-like features within the whole tumour tissue varied from 7% to 55%. It was composed of spindle-shaped cells arranged in short irregular fascicles and a storiform pattern admixed with osteoclast-like giant cells, but devoid of neoplastic osteoid. Such spindle-shaped cells had a poorly developed rough endoplasmic reticulum and expressed a strong alkaline phosphatase activity as well as vimentin. A series of allografts to athymic mice using the MFH-like tissues also showed histologically a proliferation of plump spindle-shaped cells with a storiform pattern lacking osteoclast-like giant cells, and intensely positive for alkaline phosphatase. These findings indicate that the MFH-like features are identified as modulated OS. The constituting cells are most likely to be poorly developed with possible phenotypic alteration in the maturation stage of osteoblastic cell lineage, but different from conventional MFH of bone as regards their distinct histochemical pattern.

Key words: Osteosarcoma – Malignant fibrous histiocytoma – Transplantation

Introduction

Osteosarcoma (OS) can exhibit a wide spectrum of histopathological appearances and on the basis of this a classification has been made into osteoblastic, chondroblastic and fibroblastic subtypes according to the major histological component (Dahlin and Unni 1977). Recently, additional histological subtypes and patterns of OS were variously proposed, all of which were hardly distinguishable from other benign and malignant bone tumours (Mirra 1980; Yunis and Barnes 1986).

On rare occasions OS shows an excessive proliferation of spindle-shaped cells with a pronounced pleomorphism in a storiform pattern without definite osteoid tissue. This pattern is similar to malignant fibrous histiocytoma (MFH) of bone, but the neoplastic osteoid and bone are identified only in other areas of the same tumour. Mirra (1980) designated this MFH-like OS to distinguish it from conventional MFH of bone. The percentage of patients with such MFH-like OS in an entire series of OS varied from 5% to 30% in a number of investigations (Mirra 1980; Huvos 1986; Yunis and Barnes 1986). Ballance et al. (1988) emphasized the MFH subtype of OS as a distinct entity from MFH of bone, and high-lighted the radiographic similarities to conventional, highly aggressive OS. The inclusion of an MFH-like subtype in the classification of OS still remains debated.

In this paper, we report three cases of OS resembling MFH of bone and describe the clinico-pathological features of MFH-like OS as compared with MFH of bone. We discuss whether the histogenetic concept should be listed in the classification of OS.

Materials and methods

Cases examined in this report were selected from 16 consecutive cases of OS (aged 9–75 years, mean 19.3 years) registered in the First Department of Pathology, Tottori University School of Medicine, Japan from 1975 to 1989. Every case was diagnosed by biopsy and histology of surgically resected material. From these, 3 cases of OS with MFH-like features were identified. All clinical and radiographic records are listed in Table 1.

For light microscopy, the surgically resected tumours were fixed with 10% buffered formalin, decalcified in a formic acid-formate buffer and embedded in paraffin. Further, large slab sections of maximally demonstrated areas were prepared for mapping of various histological patterns, including the MFH-like feature.

Tissue blocks obtained from surgical specimens were fixed with 4% paraformaldehyde for 5 h at 4° C, replaced and kept in gum-

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Table 1. Clinical findings of osteosarcoma (OS) with malignant fibrous histiocytoma (MFH)-like features

Case	Age/sex	Clinical sign	Location	Radiographic findings	Treatment	Prognosis
1	14/F	Right knee pain Serum ALPase 76 units/l (37/107)	R-tibia proximal	Mixed sclerotic-lytic Soft tissue mass with calcification	Amputation above knee, neoadjuvant chemotherapy[a]	Alive, postop. 24 months dead lung metastases
2	11/F	Left knee pain Serum ALPase 250 units/l (37/107)	L-femur distal	Mixed sclerotic-lytic Codman's triangle	Wide resection, limb salvation procedure (Kotz's prosthesis) neoadjuvant chemotherapy[a]	Alive, postop. 38 months disease-free
3	75/F	Left knee pain Serum ALPase 117 units/l (22–116)	L-femur distal	Lytic with focal sclerotic lesion	Amputation above the knee, postop. chemotherapy[a]	Alive, postop. 44 months disease-free

ALPase, Alkaline phosphatase
[a] Rosen T-12 protocol

sucrose solution at 4° C. The tissues then were quickly frozen using dry-ice with addition of hexane, sectioned to 4–6 μm thickness with a cryostat (Bright) at −20° C and stained for the following enzymes: alkaline phosphatase (ALPase, by Bengt et al.), acid phosphatase (ANAE, by Leder), adenosine triphosphatase (ATPase, by Müller-Hermelink), 5′-nucleotidase (5′-N, by Muller-Hermelink) and beta-glucuronidase (beta-Gl, by Hayashi et al.).

Immunohistochemical staining was carried out on paraffin sections of 6 μm thickness employing the avidin-biotin peroxidase complex technique after being reacted with antibodies against collagen I (Col I), aminopeptidase M(APM), and dipeptidyl peptidase IV (DDP IV). These antibodies were all primarily prepared in our laboratory (Aki 1989). Their specificities were determined and characterized by recognizing fibroblasts selectively. Anti-factor XIIIa (Hoechst-Behring, Marburg, FRG) for fibroblast and osteoblast marker, anti-alpha-1-antitrypsin (AAT), anti-alpha-1-antichymotrypsin (AACT), anti-lysozyme (Lys, Dakopatts, Copenhagen, Denmark) for monocyte-macrophage marker, and anti-vimentin (Dakopatts) for mesenchymal marker.

Antibodies against Col I were used at a dilution of 1:100. The optimal dilution of other primary antibodies used in this study was as follows: 1:50 for the anti-factor XIIIa; 1:2 for the anti-APM and DDP IV; original kit of anti-AAT, AACT and Lys; and 1:10 for anti-vimentin. The sections were incubated with primary antibodies for 30 min at room temperature, followed by biotinylated anti-mouse/anti-rabbit immunoglobulin for 20 min and then avidin-biotin peroxidase complex (Dakopatts) for 20 min. Finally, the sections were immersed with 0.3% (v/v) hydrogen peroxide and 0.6% (w/v) 3.37-diaminobenzidine solution and counterstained with methyl green. Osteoblastic tumour cells in conventional OS served as controls for 3 cases with MFH-like features to be compared as regards the intensity and extent of staining reactions.

The fresh samples were fixed with 3% glutaraldehyde solution (buffered pH 7.4) for 1 h at 4° C and were post-fixed with 2% phosphate-buffered osmium tetroxide for 1 h. After dehydration with an alcohol series, they were embedded in epoxy resin. Ultrathin sections were stained with lead citrate and uranyl acetate and were observed with a H-800 electron microscope (Hitachi, Japan).

For transplantation study, inbred nu/nu mice of Balb/c genetic background were used at the age of 8 weeks. The tissue fragments with MFH-like features in case 1 were transplanted subcutaneously onto the flank. The transplanted mice were bred and kept under special pathogen-free conditions. A new generation was obtained by transplantation of the xenografted tumour tissue for 5 months. The tissue obtained from the nude mice was examined in the same way as the original tumour.

Results

The clinical data of each case are summarized in Table 1. The patients were all female, ranging in age from 11 to 74-years. All patients complained of pain and swelling with a symptom duration of from 2 weeks to 6 months. The serum ALPase level in each case was from 76 to 250 units/l; case 2 showed a significant elevation and the other two cases (cases 1 and 3) were within normal limits. Radiographic findings (Fig. 1A–C) showed mixed osteolytic and, in part, osteosclerotic lesions of the metaphysis of femur (cases 2 and 3) and tibia (case 1). Destruction of the cortical bone was obvious in all cases.

Grossly, the tumours were solid, white-greyish and gritty to bone-hard with variable amounts of haemorrhage and necrosis (Fig. 2A–C). The marrow cavity of the metaphysis was almost completely obliterated by the above tissue affecting the epiphyseal plate (cases 1 and 2) or diaphysis (case 3). The tumour extended directly through the adjacent cortical bone into the surrounding soft tissues in all cases. Cartilaginous tumour tissue with ground-glass appearance was detected in the soft tissue component and delineated clearly from the hard tissue component (case 2).

Histologically, the MFH-like feature consisted of fibroblast-like spindle-shaped cells and round cells intermingled with varying numbers of osteoclast-like giant cells, and fasciculated growth pattern of spindle-shaped cells in a storiform pattern (Fig. 3). Osteoclast-like giant cells were scattered more or less among the tumour cells (Fig. 4). Occasionally, the tumour cells modulated their morphological features from spindle-shaped to round cells with abundant cytoplasm and became incomplete in a storiform arrangement (all cases), thus impairing the impression of giant cell tumour or giant cell-rich MFH with marked cellular pleomorphism (Fig. 5). How-

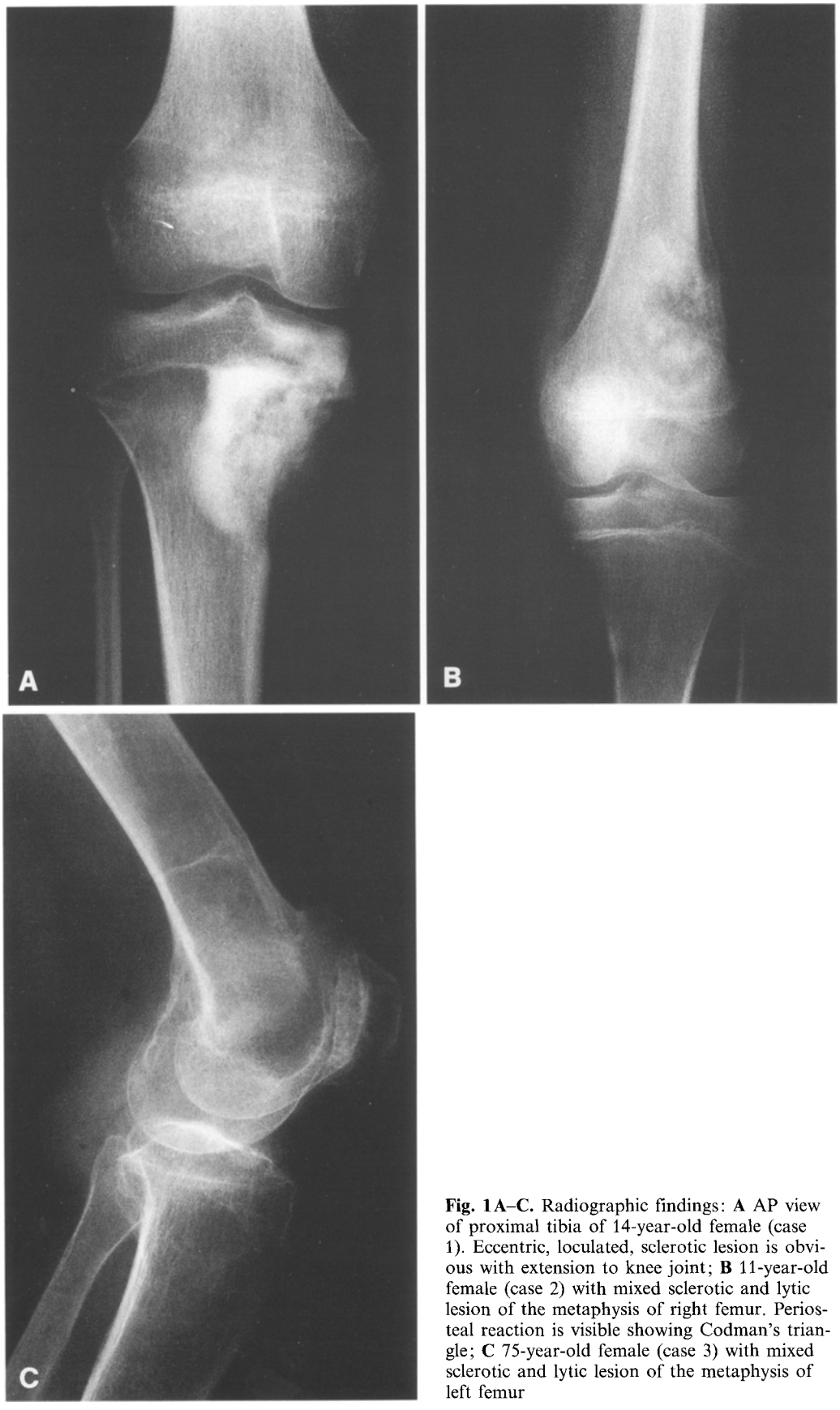


Fig. 1A–C. Radiographic findings: **A** AP view of proximal tibia of 14-year-old female (case 1). Eccentric, loculated, sclerotic lesion is obvious with extension to knee joint; **B** 11-year-old female (case 2) with mixed sclerotic and lytic lesion of the metaphysis of right femur. Periosteal reaction is visible showing Codman's triangle; **C** 75-year-old female (case 3) with mixed sclerotic and lytic lesion of the metaphysis of left femur

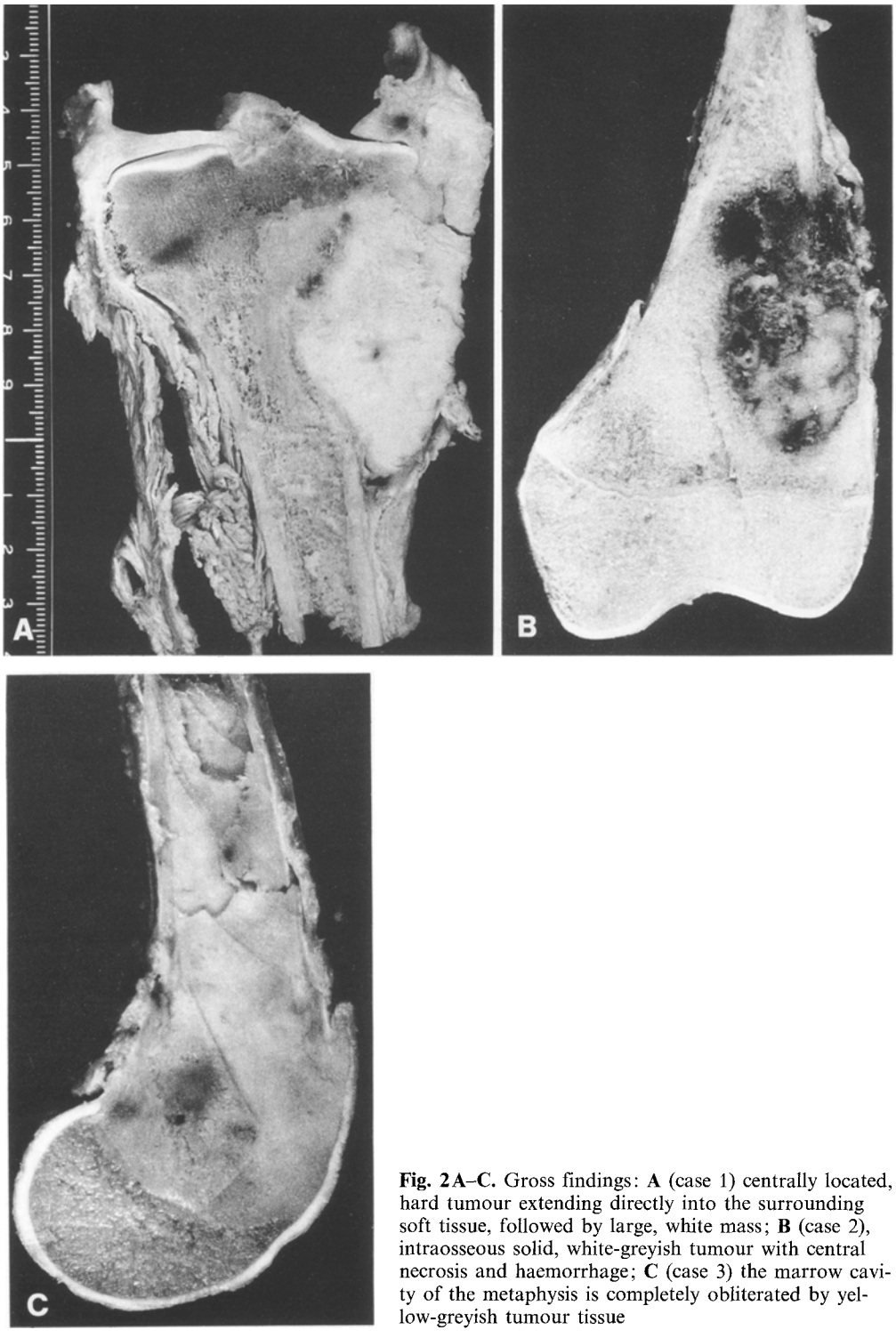
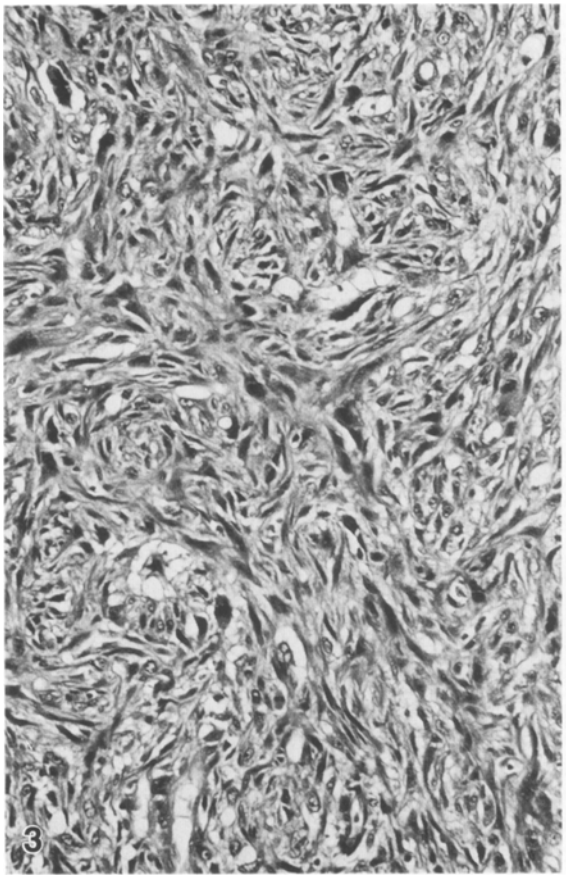
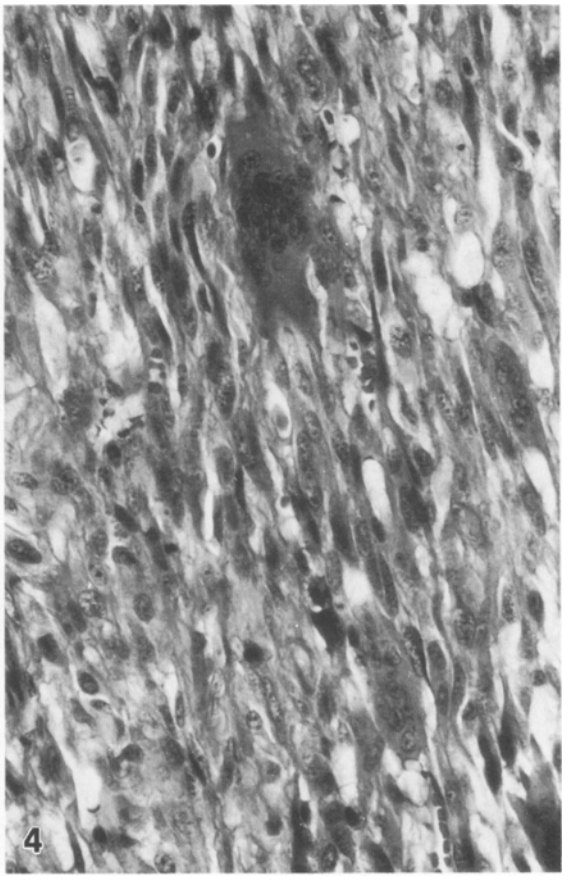


Fig. 2A–C. Gross findings: **A** (case 1) centrally located, hard tumour extending directly into the surrounding soft tissue, followed by large, white mass; **B** (case 2), intraosseous solid, white-greyish tumour with central necrosis and haemorrhage; **C** (case 3) the marrow cavity of the metaphysis is completely obliterated by yellow-greyish tumour tissue

ever, phagocytic figures were not found. Atypical mitotic figures were frequently encountered. These were ill-defined from the osteoblastic area and lacked neoplastic osteoid and bone, but the lace-like osteoid tissue was frequently recognized among the tumour cells adjacent to the osteoblastic component (Fig. 6). An osteoblastic, bone-rich area was found largely in the tumour component located in the marrow cavity (all cases). In case 1, malignant neoplastic chondroid tissue was observed in the soft tissue component and well-delineated from areas of malignant cell proliferation containing bone and osteoid tissue and MFH-like feature (Fig. 7).

According to the histological mapping of the whole tumour tissue, the incidence of MFH-like feature in the three cases was 55%, 40% and 7% (Fig. 8A–C), respectively. Those areas were hardly distinguishable from the osteoblastic area in two cases (cases 1 and 2) and were relatively delineated in case 3.





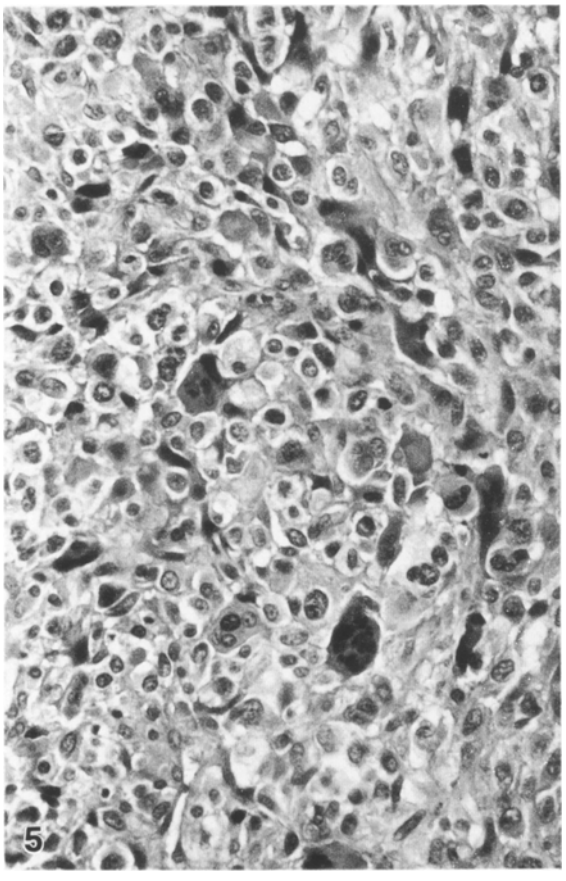


Fig. 3. Malignant fibrous histiocytoma (MFH)-like feature in case 2. Bundles of spindle-shaped cells with pleomorphism are arranged in a typical storiform pattern. H&E, ×100

Fig. 4. Osteoclast-like giant cells are scattered among spindle-shaped tumour cells. H&E, ×200

Fig. 5. In this area, osteoclast-like giant cells are prominent as in giant cell tumour (case 1). H&E, ×100

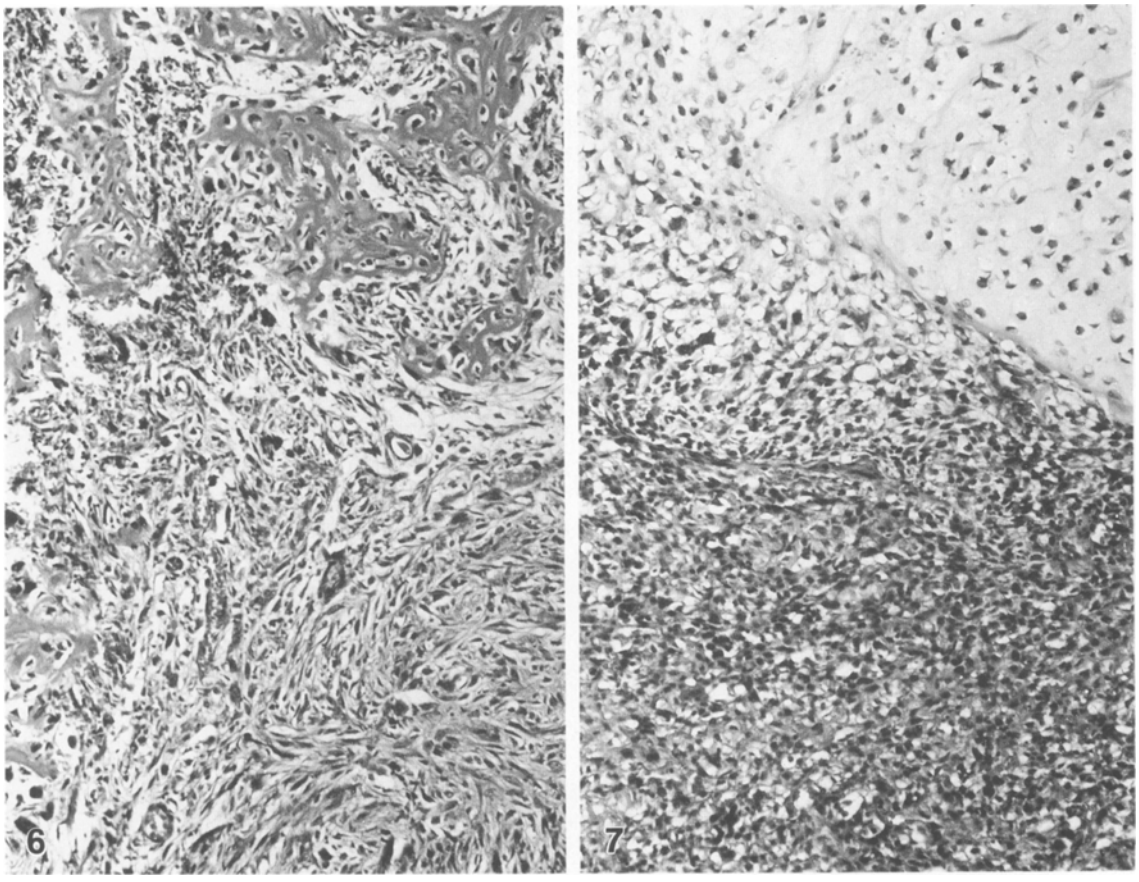


Fig. 6. MFH-like area is generally ill-defined from central osteoblastic area. H&E, ×100

Fig. 7. MFH-like area is well-delineated from the neoplastic chondroid tissue (case 1). H&E

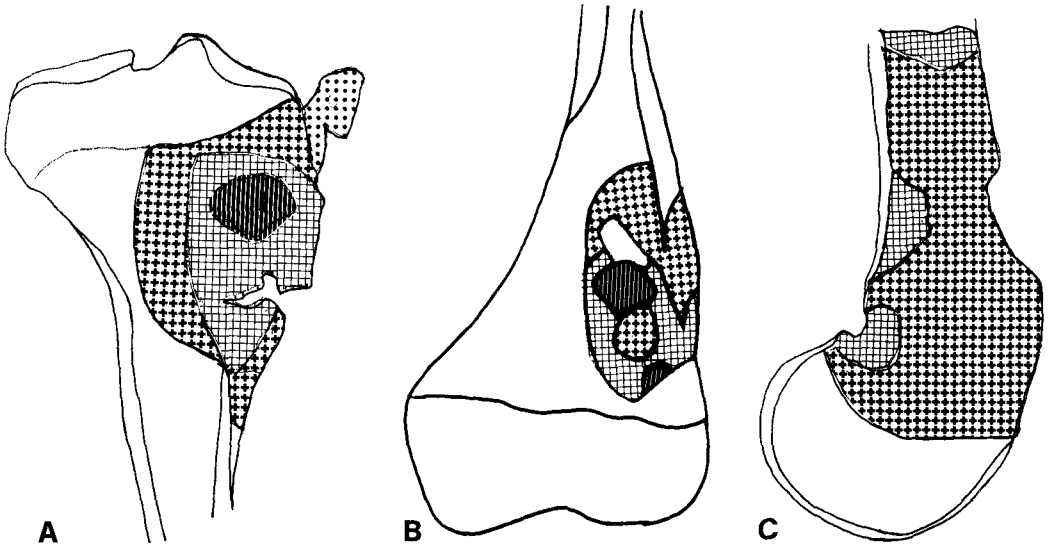
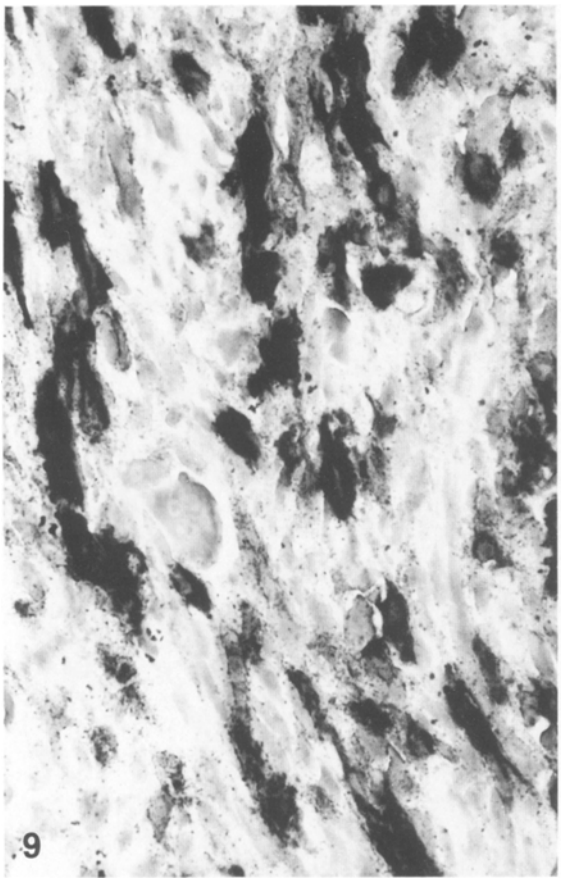
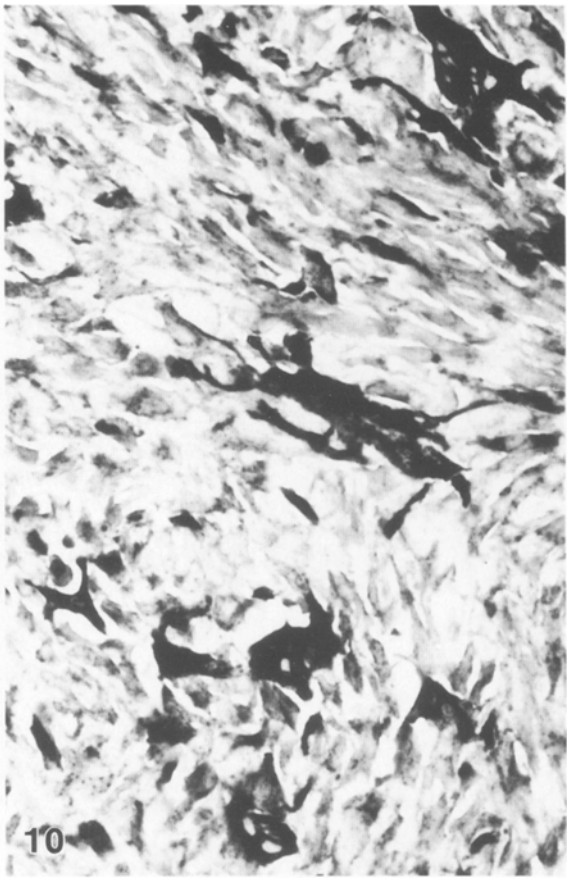


Fig. 8. Schematic diagram of the tumours in each case; the percentage incidence of MFH-like area is shown and determined at a rate of 55% (**A**: case 1), 40% (**B**: case 2) and 7% (**C**: case 3)%, respectively

The results of immunohistochemical staining are shown in Table 2 with additional findings of MFH of soft tissue origin. Spindle-shaped tumour cells in a storiform pattern reacted significantly with anti-AACT antibody for monocyte-macrophage marker and anti-vimentin antibody for mesenchymal marker. In contrast, all types of tumour cells showed negative reaction for the anti-Lys antibody. Both anti-Col I and anti-factor XIIIa antibodies reacted with a small number of cells which were negative for the anti-APM and anti-DDP IV antibodies.

Table 3 shows the cumulative enzyme histochemical





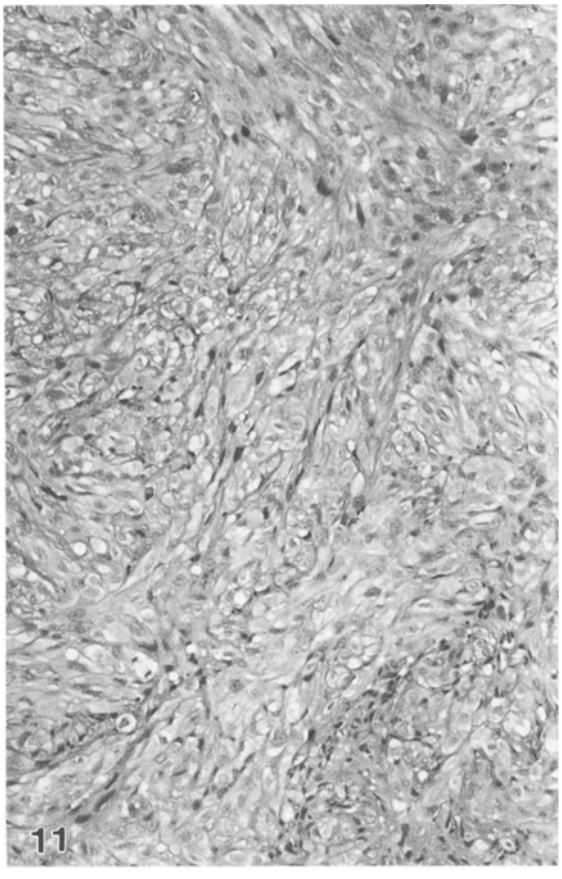


Fig. 9. ALPase reaction of MFH-like area (cases 1 and 2). Most of the tumour cells are significantly positive except for osteoclast-like giant cells. ALPase, ×200

Fig. 10. ATPase reaction of the same areas as those shown in Fig. 8. Positive reaction is shown in osteoclast-like giant cells and a small number of histiocytic cells but not in most of the tumour cells. ATPase, ×200

Fig. 11. Transplanted tumour tissue showing a fasciculating growth pattern of fibroblast-like spindle-shaped cells often with a storiform pattern (case 1, 5th generation). H&E, ×100

Table 2. Comparative immunohistochemical findings between OS with MFH-like feature and MFH of bone

	Antibodies against							
	Col I	XIIIa	APM	DDPIV	AACT	AAT	Lys	Vim
OS with MFH-like lesion								
Case 1	+	−	−	−	+	−	−	+++
Case 2	+++	++	+	+	+	+	−	++
Case 3	+	++	−	−	+	+	−	++
MFH of bone								
Case 1	−	++	−	−	++	++	+	++
Case 2	−	−	−	−	++	++	−	+
Case 3	++	++	+	+	++	++	+	++
Case 4	−	−	−	+	+	−	−	+
Case 5	+	−	−	−	+	+	+	+

Intensity of reaction: +++, strong; ++, moderate; +, slight and focal; −, negative. In strong and moderate reactions, the reactivity extended diffusely on the overall specimens, whereas in slight reaction it was present in some focal areas.
OS, Osteosarcoma; MFH, malignant fibrous histiocytoma of bone; Col I, collagen I; XIIIa, factor XIIIa; APM, aminopeptidase M; DDP IV, dipeptidyl peptidase IV; AACT, alpha-1-antichymotripsin; AAT, alpha-1-antitripsin; Lys, lysozyme; Vim, vimentin

Table 3. Comparative enzyme histochemical findings between OS with MFH-like feature and MFH of bone

Tumour cell components	Enzymes					
	ALPase	ACPase	ANAE	ATPase	5′-N	Beta-Gl
OS with MFH-like lesion ($n=3$)						
Osteoblastic cells	+++	+	−	++	+++	+
Fibroblast-like spindle cells	++	−	+	−	+	−
MFH of bone ($n=4$)						
Fibroblast-like spindle cells	−	+	+	+	+	+
Histiocyte-like round cells	−	++	+++	++	+	+

Intensity of reaction: +++, strong; ++, moderate; +, slight and focal; −, negative. In the strong and moderate reactions, the activity extended diffusely on the overall specimens, whereas in slight reaction, it was present in some focal areas

ALPase, Alkaline phosphatase; ACPase; ANAE; ATPase, adenosine triphosphatase; 5′-N, 5′-nucleotidase; beta-Gl, beta-glucoronidase

results. The spindle-shaped cells and bizarre giant cells were strongly positive for ALPase reaction (Fig. 9). Other enzyme activities were as follows: strongly positive for 5′-N and weakly positive for ATPase on the cell surface and weakly positive for ACPase close to the nucleus. A small number of histiocytic cells which were intermingled and positive for ATPase (Fig. 10) and ANAE seemed to be in the process of reaction.

Ultrastructurally most of the tumour cells in MFH-like foci displayed a spindle-shaped configuration with well-developed rough endoplasmic reticulum dilated to a varying degree in the elongated cytoplasm as described in a previous report (Minamizaki et al. 1990). Amorphous materials with bundles of collagen fibres were rarely seen in the extracellular area. Osteoclast-like giant cells were found more or less with abundant intracytoplasmic vesicles. They had irregularly indented nuclei with perinuclear chromatin clumping.

The latency of transplants varied from 155 to 280 days. The MFH-like area in osteosarcoma (case 1) was successfully grafted and transplanted for five generations. Histological findings of the tissue obtained from the nude mice were almost similar to those of the original tumour, showing a proliferation of interlacing fascicles of fibroblast-like spindle-shaped cells with a storiform pattern in a large proportion of the tissues (Fig. 11). The lace-like osteoid tissue was recognized largely in the central portion of the tumour mass. There was a complete lack of osteoclast-like giant cells. The constituent cells of the transplanted tumour were strongly positive for ALPase reaction.

Discussion

MFH-like OS was first proposed by Mirra (1980) as a different entity from MFH of bone, which was well-described and widely accepted (Huvos et al. 1985). This concept was also supported by Ballance et al. (1988), who emphasized that it was difficult to distinguish it

from conventional and giant cell tumour-like MFHs without radiographic correlation, and that this type of OS showed a predominantly fibro-histiocytic soft tissue component. Our 3 cases out of 16 OS showed histologically similar features to conventional MFH in varying proportions of 55%, 40% and 7%. These were largely in the soft tissue components of the whole tumour tissue and in addition to the central component of conventional OS, and were not clinically different from conventional OS.

In the previous literature, the incidence of MFH subtype, including MFH-like foci, varied from 8% to 30% among all OS (Mirra 1980; Yunis et al. 1986). Huvos et al. (1986) recorded the occurrence at 5–10% of all OS of both bone and soft tissue origin in patients older than 60 years. We encountered 3 cases of OS with MFH-like feature (18.8%) in 16 OS which were all examined by making slab sections of the whole tumour. Therefore, such an area may be noticed in OS much more frequently if sampling is extensive. OS is subsequently reported as a mixed tumour of one or more histological components that may cause difficulties in determining the subtype. Two of 3 OS with MFH-like foci mentioned here represented MFH-like feature in nearly half or more of the whole tumour and thus could be defined as a putative category of MFH-like OS. Histologically, such an area seems to be fibro-histiocytic with a marked pleomorphism and devoid of malignant tumour osteoid and bone. The histological distinction from fibroblastic OS in which the tumour osteoid and woven bone are observed in intimate relation with fibrosarcomatous proliferation without histiocytic reaction is obvious (Mirra 1980). However, Unni (1988) stated that MFH-like OS is within the category of fibroblastic OS. The essential difference between MFH-like and fibroblastic OS may be attributed to a histiocytic reaction, probably due to chemotactic factor produced and secreted by tumour cells.

It was reported that the ALPase enzyme, sensitive to heat treatment, is a better tool to identify and diagnose OS with various histological subtypes (Jeffree and Pice 1965; Yoshida et al. 1982; Yumoto et al. 1988). This is actively demonstrated in normal osteoblasts and their neoplastic counterparts responsible for bone and osteoid formation and was proposed as an intrinsic property of osteoblastic cell lineage (Yoshida et al. 1982). Our cases showed ALPase activity but at varying degrees in spindle-shaped cells exhibiting a characteristic storiform pattern and constituting MFH-like feature. Thus, they were diagnosed correctly as a variant of OS with biopsy specimens. The well-developed rough endoplasmic reticulum seen on electron microscopy implies that the spindle-shaped cells manifest the character of osteoblastic cell lineage. The osteoblastic property was further appreciated from the evidence of positive Col I, factor XIIIa, APM and DDP IV for fibroblast and osteoblast markers. This hypothesis should be attributed to our transplantation study to nude mice. The xenografted tissue also showed an ALPase-positive proliferation of spindle-shaped cells arranged in a storiform pattern and lacking osteoclast-like giant cells and histiocytic cells which seemed likely to be reactive but not neoplastic. It may also be proposed that the spindle-shaped cell is in an early stage of maturation from the evidence of negative staining reaction for osteocalcin (data not shown).

The presence of MFH-like foci has been increasingly reported by accumulating numbers of malignant neoplasms of bone and soft tissue origin such as chondrosarcoma (Jaworski 1984; Wick et al. 1987; Breiwiss and Kaneko 1988), leiomyosarcoma (Hashimoto et al. 1986) and liposarcoma (Kim et al. 1989), and is now understood as a phenomenon of de-differentiation which undergoes anaplastic transformation by successive modulation with a progressive loss of the original histological characters and represents an MFH-like appearance during the final common pathway (Brooks 1986; Roholl et al. 1988; Kosmell 1990). Wick et al. (1987) suggested this phenomenon to be the evolution of a second distinct neoplastic cell clone with more primitive attributes by the observation of dedifferentiated chondrosarcoma having some markers shared by chondrocytes. In the three cases presented here, de-differentiation is not suitable because of a gradual transition from osteoblastic to MFH-like areas. Possible appearance of genuine MFH-like OS, however, is expected as reported in other mesenchymal tumours. Such a particular OS should be limited to the case in which specific ALPase activity has been lost. Further careful study will be necessary to establish the entity of MFH subtype of OS.

In conclusion, MFH-like feature in OS can be defined as the proliferation of osteoblastic cell lineage in the poorly developmental stage, imitating the histological feature of MFH of bone, but differs from conventional Os and MFH. Thus, careful observation is required to distinguish it from conventional MFH of bone and OS in the soft tissue component.

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