Periosteal osteosarcoma and parosteal chondrosarcoma evaluated by double immunohistochemical staining

Report of 2 cases

Tokuhiro Chano[1], Keiji Matsumoto[1], Michihito Ishizawa[1], Shigeru Morimoto[1], Sinsuke Hukuda[1] and Hidetoshi Okabe[2]

Differentiation of periosteal osteosarcoma and parosteal (periosteal) chondrosarcoma by conventional histology may be difficult. One case each of clinically and histologically proven periosteal osteosarcoma and parosteal chondrosarcoma were evaluated by a double-immunohistochemical staining method using proliferating cell nuclear antigen (PCNA) and S-100 protein (S-100). Conventional histology showed proliferation of both osteoblastic and chondroblastic cells in the periosteal osteosarcoma, while there was a growth of only chondroblastic tumor cells in the parosteal chondrosarcoma. Immunohistochemical studies indicated that the nuclei of chondroblastic cells recognized by S-100 were PCNA-negative, while osteoblastic stromal cells were PCNA-positive in the periosteal osteosarcoma. In contrast, chondroblastic cells in the parosteal chondrosarcoma were both S-100- and PCNA-positive. Our findings suggest that periosteal osteosarcoma is characterized by the proliferation of osteoblastic stromal cells, whereas parosteal chondrosarcoma is characterized by the proliferation of chondroblastic cells. This method of double immunohistochemical staining, using PCNA and S-100, may be useful in differentiating these chondroblastic tumors.

Departments of [1]Orthopedics and [2]Surgical Pathology, Shiga University of Medical Science, Seta, Otsu, Shiga 520-21, Japan. Tel +81-775 48 2252. Fax -775 48 2254
Submitted 93-09-22. Accepted 93-11-25

Periosteal osteosarcoma and parosteal (periosteal) chondrosarcoma are rare bone tumors, that arise from the external surface of the bone. Their differential diagnosis is controversial. Although Bertoni et al. (1982) stated that these tumors can be distinguished by their clinical, roentgenological, and pathological features, differential diagnosis is sometimes difficult.

We applied the double-immunohistochemical staining method using proliferating cell nuclear antigen (PCNA) and S-100 protein (S-100) for the analysis of proliferating cells in each type of tumor. PCNA is generally considered to be a marker useful for identifying proliferating cells in normal and neoplastic tissues (Celis et al. 1984). S-100 is a marker for tumors of cartilagenous origin (Okajima et al. 1988).

The objectives were: 1) to determine the character of proliferating cells in each tumor, and 2) to apply this immunohistochemical staining for differential diagnosis, using specimens obtained from 2 patients.

Patients and methods

Periosteal osteosarcoma

A 17-year-old man was admitted to our hospital with a tender mass on the left tibia. Radiographs revealed thickness of cortical bone with saucer-like osteolysis at the anterior border of the left tibial diaphysis without involvement of the marrow (Figure 1). A wide excision of the tumor was done on April 18, 1985. The histological diagnosis was periosteal osteosarcoma.

Parosteal chondrosarcoma

A 79-year-old man with a large multirecurrent mass on the left humerus was admitted to our hospital. Magnetic resonance imaging (MRI) showed a multilobular tumor of high intensity (T2-weighted imaging), without involvement of the marrow at the shaft of the left humerus. A disarticulation of the left shoulder was performed on October 19, 1989. The clinicopathological diagnosis was chondrosarcoma of periosteal (parosteal) origin, Grade 2. This case

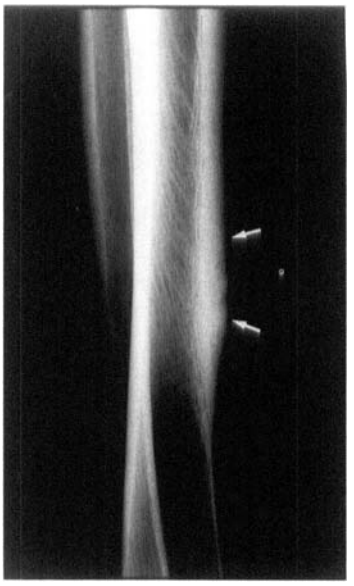


Figure 1. Periosteal osteosarcoma. Radiographs reveal thickness of cortical bone with saucer-like osteolysis (white arrows), without involvement of marrow.

has been described in detail (Matsumoto et al. 1993).

The specimens we studied were derived from these 2 periosteal tumors.

The specimens were fixed in 10% buffered formalin, embedded in paraffin, and cut into 5 µm-thick sections. Consecutive sections were stained with hematoxylin and eosin. They were also stained with alcian blue to determine the location of the chondroid matrix. Other sections were stained immunohistochemically. For the first immunohistochemical staining we used the avidin-biotin-peroxidase complex (ABC) method (Hsu et al. 1981). The primary antiserum, anti-PCNA mouse monoclonal antibody/PC10 (Novocastra Laboratories, Newcastle, England), was applied in a 1:100 dilution for 1 hour at room temperature. The sections were stained with fresh diaminobenzidine, and thus the marked nuclei stained brown. For the second immunohistochemical staining, we applied the monoclonal alkaline phosphatase-antialkaline phosphatase (APAAP) complex (Mason and Sammons 1978). The primary antiserum, anti-cow S-100 rabbit polyclonal antibody (DAKO Co., Copenhagen, Denmark), was applied in a 1:600 dilution overnight at room temperature. The sections were stained with Fast Red TR base, and thus the marked cells stained red. These sections were followed by counterstaining with methyl green, dehydration, clearing with xylene and mounting.

For the analysis of cell kinetics, 2 areas were defined: 1) the area rich in S-100-positive cells, and 2) the area rich in S-100-negative cells. More than 1000 cells in each area were observed to determine the PCNA labeling index (PCNA LI)—that is, the percentage of cells labeled by PCNA. We also determined the S-100 labeling index (S-100 LI)—that is, the percentage of cells labeled by S-100. In addition, the percentage of cells positive for both markers was calculated, defined as the double-labeling index (Double LI).

Results

Periosteal osteosarcoma

Conventional histologic findings showed that the lesion was located on the surface of the cortical bone. The periosteum over the lesion was essentially preserved. Bone marrow involvement was not evident. Areas of fine lace-like osteoid were associated with entrapped malignant cells. Cartilage tissue was seen in some parts as well-defined lobules. The cellular features were those of a low-grade malignant cartilaginous tumor (Figure 2).

Immunohistochemical examination showed S-100-positive cells in both chondroid matrix and fine lace-like osteoid (S-100 LI: 30%), and PCNA-positive cells were rarely seen in the area rich in S-100-positive cells (PCNA LI: 3.4%). In contrast, numerous PCNA-positive cells were observed in the area rich in S-100-negative cells (PCNA LI: 56%). Conventional histology revealed that PCNA-positive cells were equivalent to osteoblastic stromal cells. In the same area, S-100 LI was 0% . Tumor cells positive for both markers could not be observed in each area.

Parosteal chondrosarcoma

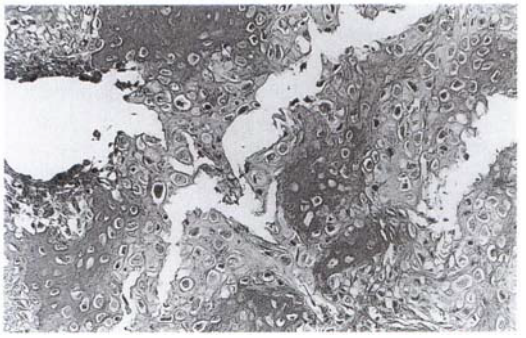
Conventional histologic findings showed a lobular, moderately cellular, cartilaginous tumor with a matrix composed mainly of hyaline cartilage. The chondrocyte nuclei were sometimes bizarre, but mitotic cells were scanty. No bone or osteoid-forming tumor cells were observed (Figure 3).

Immunohistochemically, S-100 was positive in almost all areas. S-100-positive as well as PCNA-positive cells were abundant (PCNA LI: 57% , S-100 LI: 65%). Double LI was about 50%.

Discussion

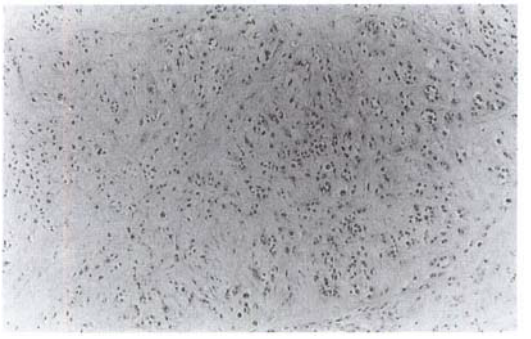
Parosteal (periosteal) chondrosarcoma was first reported by Lichtenstein (1955), while periosteal osteosarcoma was first described by Unni et al.

Figure 2. Periosteal osteosarcoma.

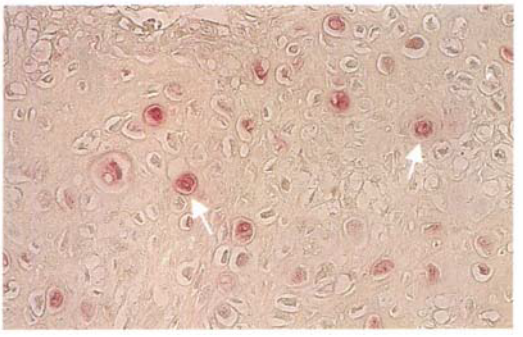


Conventional histology shows that the tumor was composed of fine lace-like osteoid and spindle-shaped, osteoblastic stromal cells. Hematoxylin and eosin, ×40.

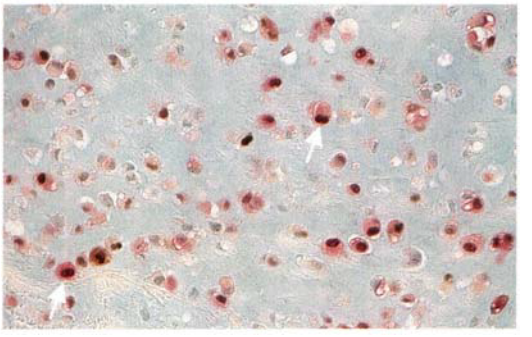
Figure 3. Parosteal chondrosarcoma.



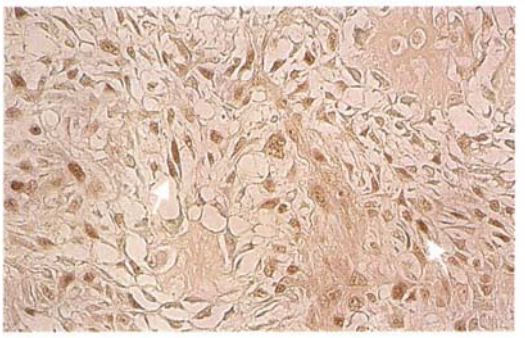
Conventional histology shows a lobular, moderately cellular, cartilaginous tumor. Hematoxylin and eosin, ×25.



Chondroblastic cells are positive for S-100 (red) and rarely positive for both markers. Double-staining using PCNA and S-100, ×80.



Not only PCNA-positive cells (brown) but also S-100-positive cells (red) are abundant. Double-staining using PCNA and S-100, ×80.



Numerous PCNA-positive cells (brown) were observed in osteoblastic stromal cells. Double-staining using PCNA and S-l00, ×80.

(1976). There has been considerable controversy concerning their natures. Schajowicz (1977) suggested that most cases of periosteal osteosarcoma reported by Unni et al. (1976) were really juxtacortical chondrosarcomas. Nojima et al. (1985) reported that the tumors described as juxtacortical chondrosarcomas by Schajowicz (1977) were essentially identical with periosteal osteosarcomas. The clinical and histological features of parosteal (periosteal) chondrosarcoma are still controversial. Bertoni et al. (1982) concluded that both a chondroblast-rich form of periosteal osteosarcoma and a pure periosteal chondrosarcoma exist, and can be distinguished by their clinical, radiographic and pathological features. Mirra et al. (1989) also suggested that some of Schajowicz's (1977) cases of juxtacortical chondrosarcoma contained 2 distinct entities, periosteal osteosarcoma and true parosteal chondrosarcoma based on his series of patients and a literature review in 1989.

PCNA-identified cyclin (Mathews et al. 1984) is the auxiliary protein for DNA polymerase-δ (Bravo et al. 1987, Prelich 1987). The immunolocalization of PCNA can therefore be used as an index of cell proliferation in normal and neoplastic tissues (Celis et al. 1984). Anti-PCNA monoclonal antibody, PC10, established by Waseem and Lane (1990), can recognize the proliferating cells in formalin-fixed paraffin-embedded tissues. S-100 is an acidic, cal-

cium-binding protein and that can serve as a marker for tumors originating in the cartilage, notochord and T-zone histiocyte (Okajima et al. 1988).

Osteosarcoma is a malignant tumor composed of proliferating malignant cells that produce osteoid or bone. Chondrosarcoma is a malignant tumor in which malignant cartilage cells proliferate and produce a cartilage matrix. This study clearly demonstrated the characteristic differences of proliferating cells in each case. In the case of periosteal osteosarcoma, the proliferating cells labeled by PCNA did not produce S-100. The chondroblastic cells that produce S-100 in this case have little proliferative ability. In contrast, in the case of parosteal chondrosarcoma, the proliferating tumor cells produced S-100.

Our observations confirm the suggestion that periosteal osteosarcoma and parosteal (periosteal) chondrosarcoma are two distinct entities (Bertoni et al. 1982, Nojima et al. 1985, Mirra et al. 1989, Matsumoto et al. 1993). Double-immunohistochemical staining may be useful for their differentiation.

References

Bertoni F, Boriani S, Laus M, Campanacci M. Periosteal chondrosarcoma and periosteal osteosarcoma. Two distinct entities. J Bone Joint Surg (Br) 1982; 64 (3): 370–6.

Bravo R, Frank R, Blundell P A, Macdonald Bravo H. Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. Nature 1987; 326 (6112): 515–7.

Celis J E, Bravo R, Larsen P M, Fey S J. Cyclin: a nuclear protein whose level correlates directly with the proliferative state of normal as well as transformed cells. Leuk Res 1984; 8 (2): 143–57.

Hsu S M, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981; 29 (4): 577–80.

Lichtenstein L. Tumors of periosteal origin. Cancer 1955; 8 (5): 1060–9.

Mason D Y, Sammons R. Alkaline phosphatase and peroxidase for double immunoenzymatic labelling of cellular constituents. J Clin Pathol 1978; 31 (5): 454–60.

Mathews M B, Bernstein R M, Franza B R Jr, Garrels J I. Identity of the proliferating cell nuclear antigen and cyclin. Nature 1984; 309 (5966): 374–6.

Matsumoto K, Hukuda S, Ishizawa M, Saruhashi Y, Okabe H, Asano Y. Parosteal (juxtacortical) chondrosarcoma of the humerus associated with regional lymph node metastasis. A case report. Clin Orthop 1993; 290: 168–73.

Mirra J M, Picci P, Gold R H. Bone tumors. Clinical, radiologic, and pathologic correlations. Lea & Febiger, Philadelphia 1989: 1726–7.

Nojima T, Unni K K, McLeod R A, Pritchard D J. Periosteal chondroma and periosteal chondrosarcoma. Am J Surg Pathol 1985; 9 (9): 966–77.

Okajima K, Honda I, Kitagawa T. Immunohistochemical distribution of S-100 protein in tumors and tumor-like lesions of bone and cartilage. Cancer 1988; 61 (4): 792–9.

Prelich G, Tan C K, Kostura M, Mathews M B, So A G, Downey K M, Stillman B. Functional identity of proliferating cell nuclear antigen and a DNA polymerase-delta auxiliary protein. Nature 1987 ; 326 (6112): 517–20.

Schajowicz F. Juxtacortical chondrosarcoma. J Bone Joint Surg (Br) 1977; 59 (4): 473–80.

Unni K K, Dahlin D C, Beabout J W. Periosteal osteogenic sarcoma. Cancer 1976; 37 (5): 2476–85.

Waseem N H, Lane D P. Monoclonal antibody analysis of the proliferating cell nuclear antigen (PCNA). Structural conservation and the detection of a nucleolar form. J Cell Sci 1990; 96 (Pt 1): 121–9.