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Bone Chondroblastoma: An Immunohistochemical and Ultrastructural Study

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Summary. **To elucidate the histogenesis of chondroblastoma, we examined seven cases of chondroblastoma immunohistochemically and ultrastructurally. Immunohistochemically, the majority of mononuclear tumor cells were positive for S-100 protein, but far fewer were Type II collagen-positive mononuclear tumor cells. This immunohistochemical pattern was similar to that of embryonal cartilage cells. Moreover, the ultrastructural features of mononuclear tumor cells closely resembled those of embryonal cartilage cells. Osteoclast-like multinucleate giant cells were confirmed to be histiocytic in nature and a reactive element. Our findings suggest that chondroblastoma develops from the remnant of embryonal cartilage.**

INTRODUCTION

Chondroblastoma was first described as a distinct entity by Jaffe and Lichitenstein[1] in 1942. Nowadays the tumor is defined as an almost invariably benign neoplasm showing a marked predilection for epiphysis and comprised predominantly of ovoid, round and spindle cells, some of which resemble immature chondrocytes (chondroblasts).[2,3] However, the histogenesis of chondroblastoma is still controversial. Although most investigators consider that it is derived from cartilaginous cells,[4-8] there are some who state that the tumor cell is histiocytic origin.[9,10] Even the dominant theory of cartilaginous cell origin contains contradiction, because there is no chondral element in the normal epiphysis of bone where most chondroblastomas occur. We here describe the immunohistochemical and ultrastructural findings of chondroblastomas in comparison with those of various kinds of cartilaginous tissue, and discuss the histogenesis of chondroblastoma.

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MATERIALS AND METHODS

Seven cases of unequivocal chondroblastoma were obtained from surgical pathology files at Niigata University School of Medicine and surrounding community hospitals. The clinical data of patients with chondroblastoma used in the present study are summarized in Table 1. Surgically curetted specimens were fixed in 10% formalin and processed for embedding in paraffin. Sections were cut at a 3-μm thickness and stained with hematoxylin and eosin, alcian blue, and toluidine blue.

For immunohistochemical examination, deparaffinized sections were incubated in methanol with 0.3% hydrogen peroxide to eliminate endogenous peroxidase activity. After incubation with 20% normal swine serum for 30 min at room temperature, the specimens were stained by the immunoperoxidase method with a biotin-streptavidin-peroxidase system (BSA) (Nichirei, Tokyo, Japan). A rabbit anti-Type II collagen antibody was obtained from Advance (Tokyo, Japan). We performed pretreatment with 1% trypsin for immunostaining of the Type II collagen. A rabbit anti-S-100 protein antibody and a mouse monoclonal antibody to human macrophage, CD68 (KP-1), were obtained from Dako (Glostrup, Denmark). For negative controls, nonimmune rabbit or mouse serum was used in place of the primary antibodies.

For electron microscopic examination, fresh specimens obtained from four cases of chondroblastoma (Cases 4-7) were fixed in phosphate-buffered 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide,

Table 1. Clinical features of patients with chondroblastoma

Case	Age and sex	Symptoms	Location	Radiographic feature	Treatment	Follow-up (yr)
1	23, M	Pain in hip	Femoral neck	Osteolytic	Intralesional curettage	NED (16)
2	10, F	Pain in ankle	Talus	Osteolytic	Intralesional curettage	NED (15)
3	13, F	Pain in shoulder	Humeral neck	Osteolytic	Intralesional curettage	NED (13)
4	12, M	Pain in knee	Proxinal epiphysis of tibia	Osteolytic	Intralesional curettage	NED (7)
5	19, M	Pain in hip	Greater trochanter of femur	Osteolytic	Intralesional curettage	NED (7)
6	16, F	Pain in hip	Greater trochanter of femur	Osteolytic	Intralesional curettage	NED (1)
7	19, M	Pain in knee	Patella	Osteolytic	Intralesional curettage	NED (1)

NED: no evidence of disease

and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate.

For a comparative study, we examined a mesenchymal chondrosarcoma, embryonal cartilage, growth plate and joint cartilage. Embryonal cartilage at gestational ages of 40 and 54 days was obtained at induced abortion. Growth plates and joint cartilage were obtained from the tibia of 12 aged males and the femoral head of 14 aged males, respectively, at surgery for malignant tumors.

RESULTS

Light microscopic findings

All seven tumors showed basically the same histologic features, and were composed of round to polygonal, mononuclear cells, osteoclast-like giant cells (Fig. 1) and chondroids (Fig. 2). Although the cytologic spectrum was large, most mononuclear cells had a well-defined cytoplasmic border, lightly staining or focally clear cytoplasm. The shape of the nucleus was generally ovoid or round, but nuclear clefts, grooves, or invaginations were occasionally found. Mitotic figures were 1-3/10 high power fields. Osteoclast-like giant cells were evenly distributed between clusters of mononuclear cells. The chondroid matrix was a typically amorphous pinkish substance deposited as small, generally less than 1-mm nodules within the stroma of the tumors.

Immunohistochemical findings

The majority of mononuclear tumor cells were strongly positive for S-100 protein (Fig. 3), which was also detected in the embryonal cartilage, growth plates, joint cartilage and well-differentiated cartilaginous cells of mesenchymal chondrosarcoma. However, osteoclast-like giant cells of chondroblastoma and the majority of undifferentiated cells of mesenchymal chondrosarcoma were negative for S-100 protein.

Although Type II collagen was detected in the cytoplasm of neoplastic or nonneoplastic well-differentiated cartilaginous cells and in the adjacent matrix, only a few mononuclear tumor cells of chondroblastoma and embryonal cartilaginous cells were positive for Type II collagen in the cytoplasm (Fig. 4). Osteoclast-like giant cells were negative for Type II collagen. CD68 was detected in osteoclast-like giant cells alone (Fig. 5).

Ultrastructural findings

Ultrastructurally, mononuclear tumor cells of chondroblastoma were classified into two types: Type A cells were characterized by abundant glycogen particles and less intracytoplasmic organelles (Fig. 6); Type B cells were characterized by abundant rough endoplasmic reticulum and multiple Golgi complexes (Fig. 7). There were also intermediate transitional forms between Types A and B. Type A cells closely resembled embryonal cartilage cells (Fig. 8). Neoplastic or nonneoplastic well-differentiated cartilaginous cells were characterized by abundant, markedly dilated rough endoplasmic reticulum. Undifferentiated mesenchymal cells of mesenchymal chondrosarcoma had far less intracytoplasmic organelles than Type A cells chondroblastoma (Fig. 9). Osteoclast-like giant cells were characterized by abundant mitochondria

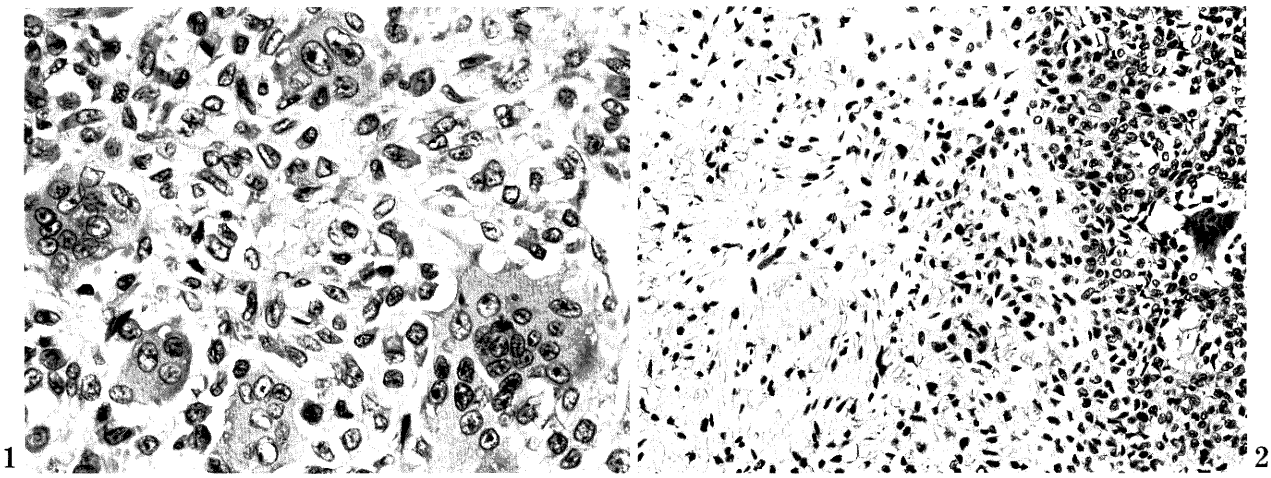


Fig. 1. Chondroblastoma composed of polygonal mononuclear tumor cells, with osteoclast-like giant cells. HE.

Fig. 2. Chondroblastoma showing a chondroid matrix. HE.

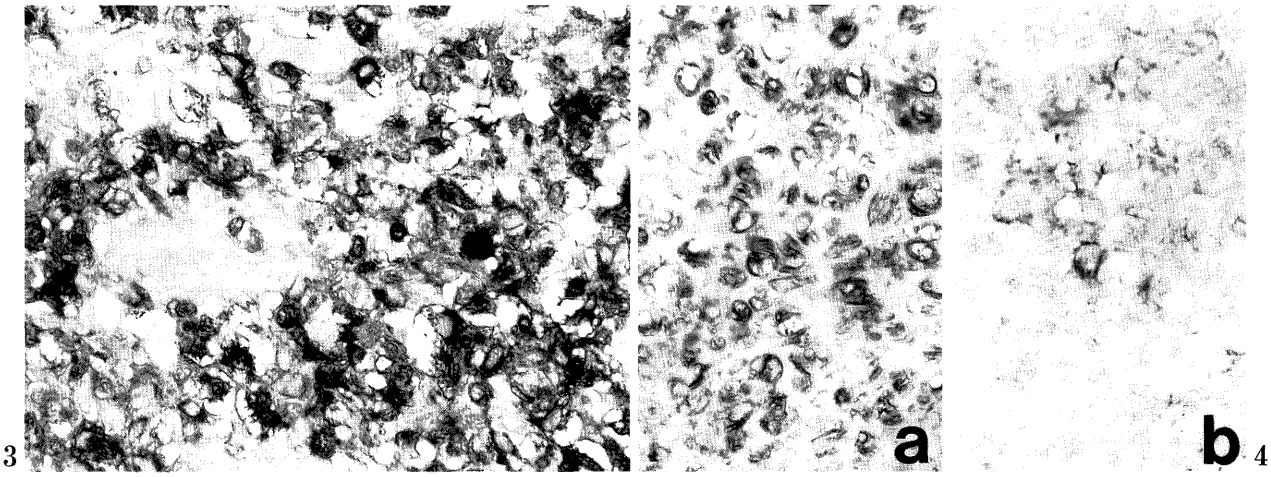


Fig. 3. Immunostaining of S-100 protein. Chondroblastoma showing strongly positive staining for S-100 protein. Methyl green counterstain.

Fig. 4. Immunostaining of Type II collagen. Some chondroblastoma cells (**a**) and a few embryonal cartilage cells (**b**) are positive for Type II collagen. Methyl green counterstain.

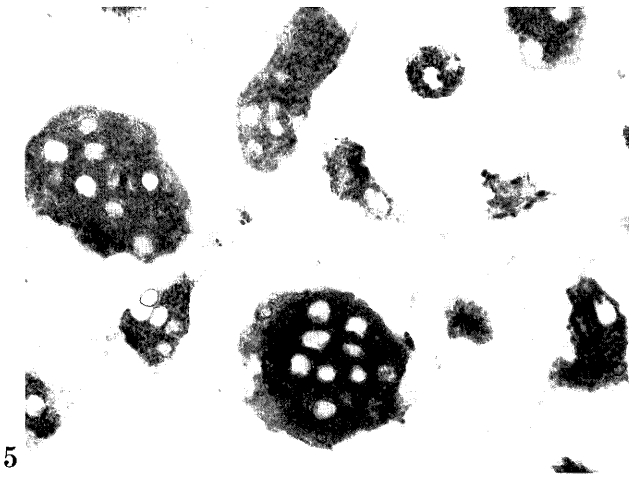
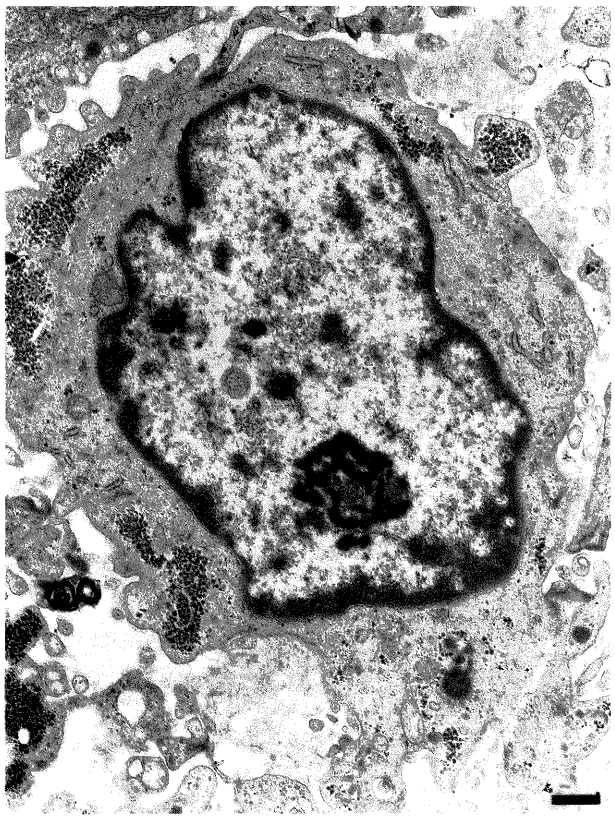


Fig. 5. Immunostaining of CD68. CD68 is detected only in osteoclast-like giant cells. Methyl green counterstain.



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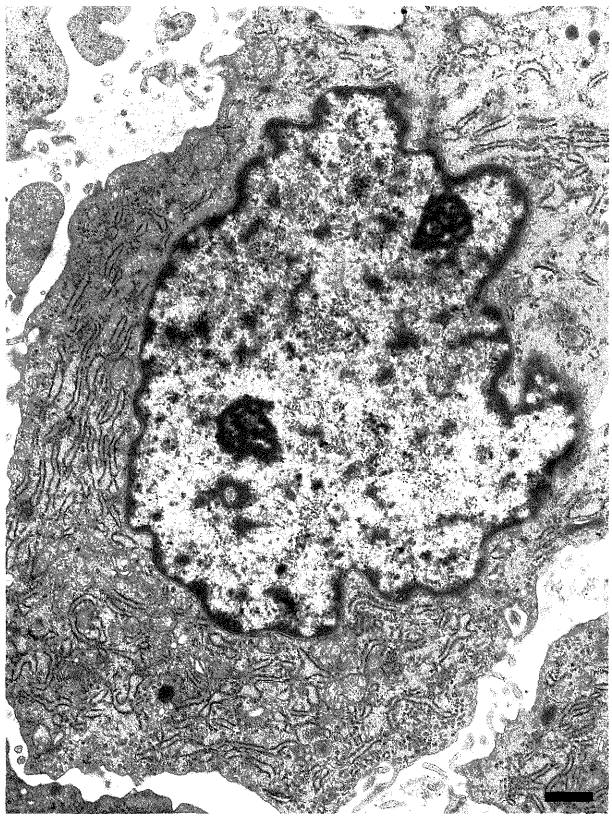

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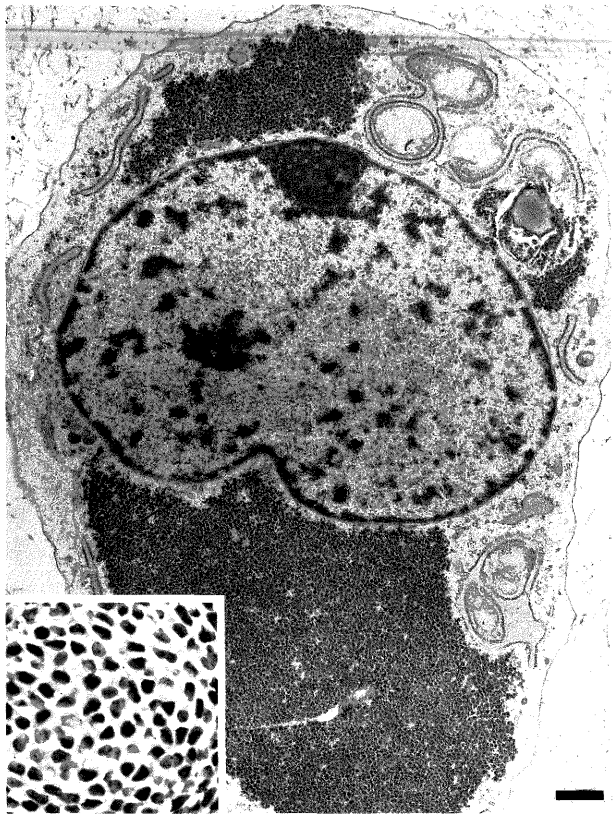

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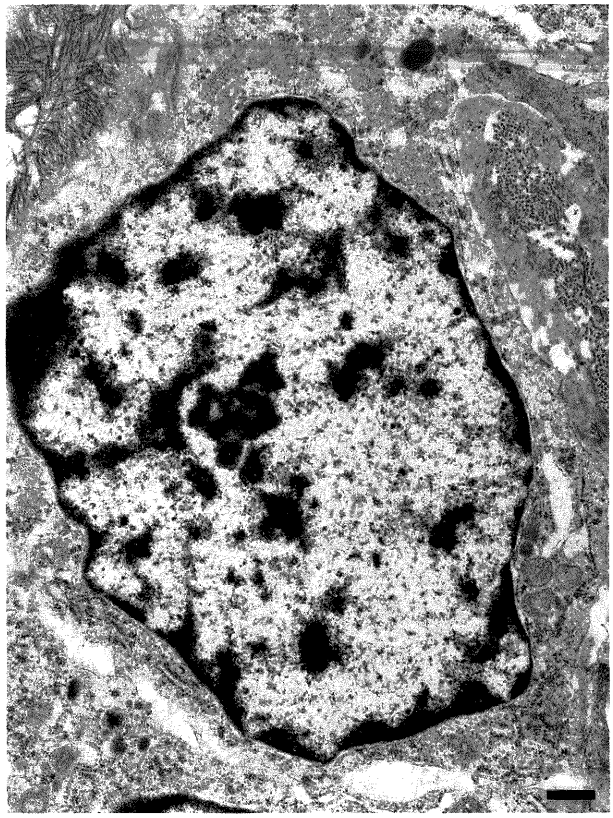

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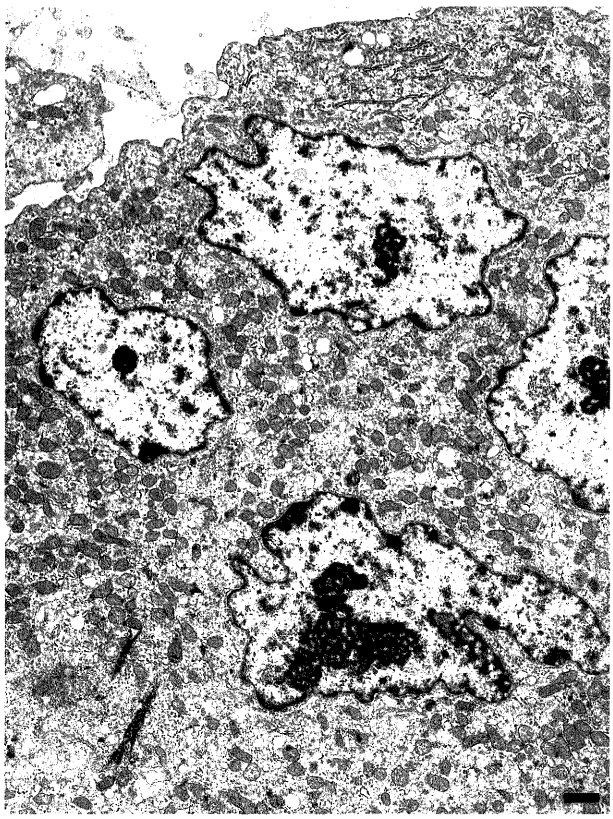

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Fig. 6. Type A chondroblastoma cell is characterized by abundant glycogen particles and fewer intracytoplasmic organelles. Bar=1 μm.

Fig. 7. Type B chondroblastoma cell is characterized by abundant rough endoplasmic reticulum and multiple Golgi complexes. Bar=1 μm.

Fig. 8. Embryonal cartilage cell resembling Type A chondroblastoma cell. Bar=1 μm. **Insert:** Embryonal cartilage. HE.

Fig. 9. Undifferentiated mesenchymal cells of mesenchymal chondrosarcoma showing a marked sparsity of intracytoplasmic organelles. Bar=1 μm.

Fig. 10. Osteoclast-like giant cells of chondroblastoma characterized by abundant mitochondria and considerable endoplasmic reticulum and lysozomes. Bar=1 μm.

and considerable endoplasmic reticulum and lysozomes (Fig. 10).

DISCUSSION

Although some investigators have speculated that chondroblastoma is of histiocytic origin,[9,10] our immunohistochemical and ultrastructural studies confirmed that mononuclear tumor cells of chondroblastoma had a cartilaginous character. Only osteoclast-like giant cells expressed CD68—which is a marker of histiocytes, but they did not show any cartilaginous character. These cells are presumably nonneoplastic reactive elements.

The presence of S-100 protein in the neoplastic cells supports their chondroid differentiation.[11,12] Type II collagen is the main component of cartilage, and is also detected in the cytoplasm of cartilaginous tumor cells and in the adjacent matrix.[13] In the present examination, Type II collagen was detected in a few mononuclear tumor cells of chondroblastoma and a few embryonal cartilage cells in addition to well-differentiated cartilaginous cells and the chondroid matrix as shown by Edel et al.[14] We found a tendency for undifferentiated mesenchymal cells to express neither S-100 protein nor Type II collagen. Poorly differentiated cartilaginous cells, such as chondroblastoma cells and embryonal cartilage cells, are positive for S-100 protein, but rarely positive for Type II collagen. Well-differentiated cartilaginous cells are positive for both S-100 protein and Type II collagen.

Ultrastructural findings of mononuclear tumor cells of chondroblastoma have been reported by a few investigators.[4-7] Although there are some differences among their descriptions, we do not consider these differences essential, and believe that they are due to the differentiation phase foward a cartilaginous direction within the tumor. Type A cells of chondroblastoma have features common to embryonal cartilage cells: namely, abundant glycogen particles and less intracytoplasmic organelles. Type A cells are presumably the most primitive tumor cells and in the proliferating phase, while Type B cells are rather in the functional phase, when they produce some proteinous substances such as Type II collagen.

It is generally accepted that mesenchymal chondrosarcoma is developed from "primitive mesenchyme".[7,15] On the other hand, even the most primitive tumor

cells in chondroblastoma already have a cartilaginous character. Mononuclear tumor cells of chondroblastoma are closely similar to embryonal cartilage cells both immunohistochemically and ultrastructurally. Indeed, there is no chondral element in the normal epiphysis of bone. However, we must remember the following: 1) The epiphysis is initially composed of cartilaginous tissue, and then replaced by bony tissue; 2) The chondroblastoma principally occurs in young people; and 3) This tumor is a benign neoplasm, which does not usually show marked dedifferentiation. Therefore, we believe that chondroblastoma probably develops from the remnant of embryonal cartilage.

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