COMPARATIVE ELECTRON MICROSCOPIC AND IMMUNOHISTOCHEMICAL STUDY OF STROMAL CELLS IN GIANT CELL TUMOR OF BONE

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ABSTRACT

Giant cell tumor of bone is an osseous neoplasm that is histologically benign but clinically shows local aggression and high rate of recurrence. The histogenesis of this lesion remains unclear. The histological appearance does not predict the clinical outcome and there are still many unanswered questions with regard to both its treatment and prognosis. In order to further clarify this lesion, we examined ultrastructurally and immunohistochemically the tumor mononuclear cells in ten patients operated on in our hospital for matrix metalloproteinase-9. Positive reaction was detected in the spindle-like stromal cells of giant cell tumor of bone and these cells had the ultrastructural characteristics of fibroblastic cells. The other mononuclear cells did not express matrix metalloproteinase-9 and showed ultrastuctural characteristics of macrophage-like cells. The positive reaction for matrix metalloproteinase-9 in all patients clearly shows that this protease may play a key role in the pathophysiology of giant cell tumor of bone.

Key words: *giant cell tumor of bone, ultrastructural study, matrix metalloproteinase-9*

INTRODUCTION

Giant cell tumor of bone (GCTB) is a primary intramedullary bone tumor that is histologically benign but clinically shows local aggression with significant osteolysis (3,5,13,14). The tumor is named for its numerous multinucleated giant cells of monocytic-macrophageal origin which are principally responsible for the extensive bone resorption that is characteristic of GCTB (2,8). However, the neoplastic components of GCTB are the spindle-like stromal cells, which promote giant cell formation and largely direct the pathogenesis of the tumor (2,8). It accounts for about 5% of primary bone tumors and predominantly affected the epiphyses of long bones (3,13,14). This tumor appears most often in the third and fourth decade of life with a slight predilection for females (3,13,14). Surgical treatment options include intralesional excision or segmental resection (3,6,9,13,14).

In order to further clarify the GCTB, we examined this lesion in ten patients operated on in our hospital

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ultrastructurally and immunohistochemically for matrix metalloproteinase-9 (MMP-9).

MATERIALS AND METHODS

We examined material from ten patients with GCTB that were operated on in our hospital (6 female and 4 male). The medico-legal office and local Ethic Committee approved this study.

Immunohistochemistry preparation protocol for MMP-9: Tissue samples were fixed in 10% buffered formalin for 24 h, then dehydrated in increasing concentrations of ethanol. Alcohol was removed using cedar oil until samples became translucent. Samples were rinsed in xylene and embedded in paraffin. Two to three serial paraffin sections, 7 μm in thickness, were mounted on slides previously coated with chrome-gelatine. Sections were deparaffinized, dehydrated, and washed in phosphate buffered saline (PBS, pH 7.4). Endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 min at room temperature. The sections were rinsed in PBS and nonspecific binding sites were blocked with 2.5% horse serum in PBS (Vector Laboratories Inc., Burlingame, CA) for 20 min. Primary polyclonal antibody for MMP-9 (Sigma Co., St. Louis, MO) at a dilution 1:500 was added and sections were incubated overnight at 4°C, rinsed in PBS and incubated with biotinylated horse anti-rabbit IgG (Vector Laboratories Inc., Burlingame, CA), diluted 1:400 in 1.5% horse serum for 60 min at room temperature. Sections were rinsed as before and incubated with streptavidin-HRP (Vector Laboratories Inc., Burlingame, CA) for 45 min at room temperature. Antibody binding was visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma Co., St. Louis, MO) as chromogen for 10 min. Sections were counterstained with Mayer's haematoxylin, dehydrated in graded series of ethanol, cleared in xylene, and cover-slipped with Canada balsam. For controls, the primary antibody was replaced with isotype-matched anti-rabbit IgG. For representation of classical pathological diagnosis we used routine staining method haematoxylin-eosin.

Electron microscopy preparation protocol: Tumor tissues were fixed in 3% glutaraldehyde for 2 h. After that the tissue samples were postfixed in 1% OsO_4 in PBS for 2 h. Then the slices were dehydrated and embedded in Durkupan (Fluka, Buchs, Switzerland). All slices were processed with disectional microscope and cut with ultramicrotome (LKB, Stockholm-Bromma, Sweden). Finally, they were mounted, covered, contrasted, and examined with an electron microscope Hitachi 500.

RESULTS

Classical histological appearance of multinucleated giant cells admixed with mononuclear stromal cells was established in all cases.

Our results showed positive immunohistochemical reaction for MMP-9 in the spindle-shape cells of GCTB (Fig. 1). The polygonal cells were negative for MMP-9.

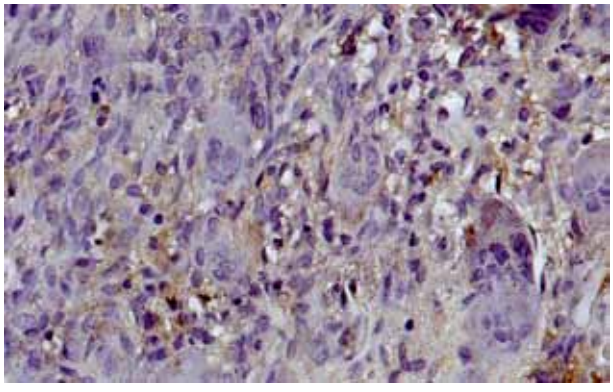

Fig. 1. Immunohistochemical localization of MMP-9 (x400)

Ultrastructurally, the spindle-shaped cells resembled fibroblastic cells and mitotic activity was observed. The formation of deep infoldings of the nuclei was observed. Their nuclei also displayed delicate chromatin structure with clearly visible heterochromatin clumps in the peripheral nuclear area. The cytoplasm contained expanded rough endoplasmic reticulum, a well-developed Golgi apparatus, free ribosomes, polysomes, and irregularly shaped mitochondria (Fig. 2). The ultrastructural features of the second type of mononuclear cells were similar to macrophages (Fig. 3). They had oval nuclei with low chromatin density. The cell membrane had pseudopodial extensions. The cytoplasm of these cells presented relatively abundant rough endoplasmic reticulum, well developed Golgi apparatus, variable number of mitochondria, lysosomes, and free ribosomes.

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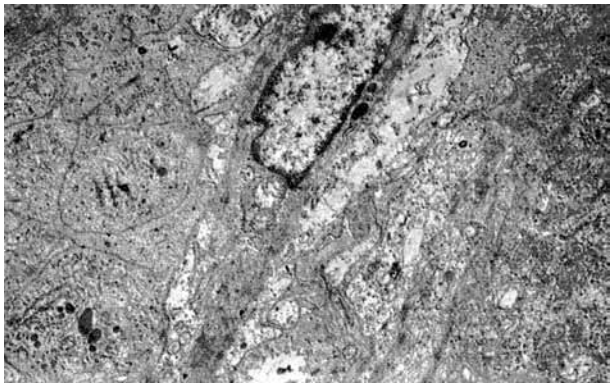

Fig. 2. Electron micrograph of a mononuclear spindle-shaped cell (x 8700)

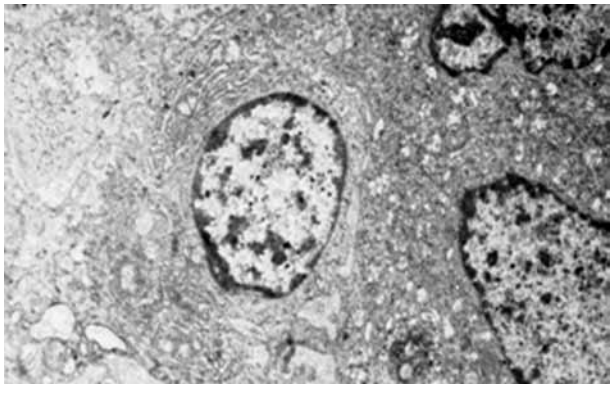

Fig. 3. Electron micrograph of a mononuclear polygonal cell (x 9000)

DISCUSSION

GCTB is described as a locally invasive tumor with a high rate of recurrence and a possibility of mainly pulmonary metastases or transformation into a malignancy (3,6,9,13,14). In the current literature GCTB is described as a predominantly osteoclastogenic stromal cell tumor of mesenchymal origin (8). It is composed of three cell types – the spindle-like stromal cells, mononuclear monocyte cells, and multinucleated giant cells (8,16). The multinucleated giant cells which mimic osteoclasts are principally responsible for the extensive bone resorption that is characteristic of GCT (2). However, the stromal cells are the main neoplastic component of GCTB and have been shown to express and secrete a variety of chemotactic factors to enlist pathologic components (2,8). Mononuclear monocyte cells are considered to be either reactive macrophages or osteoclast precursors (2,8). To further examine this lesion we studied mononuclear cells in GCTB ultrastructurally and immunohistochemically for MMP-9.

Matrix metalloproteinases (MMPs) are proteases which play a central role in the catabolism of extracellular matrix macromolecules (4,7,10). MMPs play an important regulatory role in the accelerated breakdown of the extracellular matrix, tissue morphogenesis, cell differentiation, tumor invasion and metastasis (4,7,10). In this study we have shown that the spindle-like stromal cells of GCTB express MMP-9 in all studied specimens, which clearly shows that this protease has a definite role in the pathogenesis of GCTB. Previous studies presented that bone matrix destruction via type-I collagen degradation (by osteoclasts) leaves behind denatured type-I collagen (gelatin), which the gelatinases MMP-2 and MMP-9 degrade and cause osteolysis (11,15). Ultrastructurally the spindle-like mononuclear cells resemble fibroblastic cells, and some have mitotic activity, which corresponds to the other electron microscopic studies of GCTB (12,17). These cells also have well-developed rough endoplasmic reticulum, which indicates that they have a high degree of protein synthesis. The deep nuclear infoldings of the nuclei are consistent with the view that these cells are truly neoplastic. However, similar findings are also observed during reparative processes of connective tissues (1). The other mononuclear cells do not express MMP-9 and have the ultrastructural characteristics of macrophages, also presented by others (10,12,17). There is no convincing ultrastructural evidence to suggest that these cells are neoplastic in nature (1).

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