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Rapid Communication

Immunohistochemical identification of osteoclasts and multinucleated macrophages

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ABSTRACT

Osteoclasts are bone-resorbing multinuclear cells derived from hematopoietic stem cells which are specialised to carry out lacunar bone resorption. The immunophenotype of giant cell-containing bone lesions in a wide range of osteoclast-like giant cells was similarly assessed. Both multinucleated macrophages and osteoclasts were found to express CD68. Multinucleated macrophages, but not osteoclasts, expressed GrB and Ki67. CD13+/CD14+/CD68+/GrB−/Ki67−/CD56− all giant-cell lesions noted in giant cells of bone. Giant cells have an osteoclast phenotype in most giant cell-rich lesions of bone, which do not express the macrophage-associated antigens GrB and Ki67. Our results indicate that they are formed from osteoclast precursors of mononuclear phagocyte.

1. Introduction

Mononuclear osteoclast precursors circulate in the monocyte fraction and, in the presence of receptor activator for nuclear factor Kappa B ligand (RANKL) and macrophage-colony stimulating factor, differentiate and fuse to form multinucleated cells which have the specific ultrastructural, cytochemical and functional features of osteoclasts [1]. Cells of the mononuclear phagocyte system (MPS) are widely distributed throughout the body and include blood monocytes, tissue macrophages and multinucleated macrophages [2]. The MPS includes several tissue-specific cells which are specialised to perform a particular function. Among these are osteoclasts, multinucleated cells which are specialized to carry out lacunar bone resorption [3].

In keeping with the origin of mononuclear osteoclast precursors from the pluripotent haematopoietic stem cell and membership of the MPS, osteoclasts express leucocyte common antigen (CD14) and several macrophage associated antigens, including the pan-macrophage marker, CD13 [4]. Immunophenotypic markers are commonly used to distinguish osteoclasts from multinucleated macrophages. Both CD14 and CD13 are also expressed by multinucleated macrophages and can be detected in formalin-fixed tissues. In studies using frozen material, it was found that CD68, the vitronectin receptor, is strongly expressed by osteoclasts and that a lack of expression of several macrophage-associated antigens, including CD56, GrB and Ki67, distinguishes osteoclasts from multinucleated macrophages [5]. Using these antibodies, we sought in this study to examine the antigenic phenotype of the human osteoclast and its relationship to that of multinucleated macrophages. Accordingly, we have also sought in this investigation to analyse the immunophenotype of giant cells in these lesions. Although, it has been established that giant cells in giant-cell tumour of bone are osteoclasts, the nature of giant cells in other giant cell-containing lesions of bone has not been determined [6].

2. Materials and methods

All specimens were used in agreement with permission issued by the department of pathology, Fujian Provincial Hospital. Normal and pathological bone and soft-tissue lesions containing osteoclasts or multinucleated macrophages were derived from the archives of the department of orthopedics. In addition, a tissue microarray containing a variety of giant cell-rich bone and soft-tissue lesions was produced by the relevant departments of Fujian Provincial Hospital, with approval from the local ethics council. The number and nature of the samples analysed in this study is shown in Table 1.

All samples were fixed in formalin for at least 24 h; samples containing bone or calcified material were fully decalcified in

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Table 1
The examined samples of giant-cell-containing lesions in this study.

Macrophage polykaryon containing lesions
Tuberculosis (3), sarcoidosis (7), foreign-body granulomas (12), rheumatoid nodule (8), gouty tophus (6), revision arthroplasty (18)
Giant cell-rich lesions of bone
Giant cell tumour of bone (11), benign chondroblastoma (6), chrondromyxoid fibroma (6), non-ossifying fibroma (4), fibrous dysplasia (4), brown tumour (2)
Aneurysmal bone cyst (5), Langerhans cell histiocytosis (5), giant-cell reparative granuloma of small bones (3), giant-cell granuloma of the jaw (4), telangiectatic osteosarcoma (4), giant cell-rich osteosarcoma (6)
The number of specimens analysed in each case is shown in parentheses
Osteoclast-containing lesions
Normal growth plate metaphysic (6), fracture callus (5), hyperparathyroid bone disease (6), osteoarthritis (10) rheumatoid arthritis (3)

strong acid (5% nitric acid) for up to 48 h. Tissue samples were embedded in paraffin wax and routinely processed for H&E staining. Immunohistochemical staining was performed on serial 5 micrometer sections using an indirect immunoperoxidase technique. Tissue sections were dewaxed and rehydrated by successive immersion in xylene, graded ethanol and water. Antigen retrieval was performed by microwave treatment (700 W, 2 × 4 min) in target retrieval solution. Endogenous peroxidise was blocked by 0.2% (v/v) hydrogen peroxide in 80% ethanol and protein block serum prior to 30 min incubation with the monoclonal antibodies. Antigen expression was detected by incubation with labelled polymer and diaminobenzidine. The sections were then counterstained with haematoxylin, dehydrated, cleared and mounted. Incubated with 3% hydrogen peroxide for 5 min to block endogenous peroxidase activity, immersed in 0.01 mol/L citrate buffer (pH 6.0), heated at 120 °C for 15 min to induce antigen retrieval. After blocking with 1% bovine serum albumin (BSA) for 30 min, the sections were then incubated overnight at 4 °C with mouse monoclonal antibody against CD13, CD14, CD68, CD56, Ki67 and GrB (Thermo Fisher Scientific, IgG2b isotype). A subsequent reaction was carried out using second antibodies at 37 °C for 30 min. Then, the sections were washed three times with phosphate buffer solution (PBS) and subsequently the color was displayed with DAB for 5 min. Nuclei were lightly counterstained with hematoxylin. Negative controls consisted of the substitution of antibody diluent alone for primary antibody and immunostaining with an irrelevant antibody, AE1/3, directed against cytokeratin intermediate filaments.

There was an evaluation scale system for the immunohistological staining. According to the proportion of positive tumor cells, immunohistochemistry results divided into: negative, positive cells <5%; weakly positive, positive cells ⩽25%; moderately positive, positive cells 25–50%; strongly positive, positive cells >50%, a percentage may be directly counted.

3. Results

3.1. Immunophenotype of giant cells in giant-cell-rich lesions of bone

These giant cells also expressed CD13 and CD14 but were negative for Ki67, GrB and CD56 (Fig. 1). It was noted that giant cells in a wide range of giant-cell-rich bone tumours and tumour-like lesions of bone all showed strong membrane expression of CD68. CD68+ giant cells in these lesions were found lying not only directly next to a bone surface but also around cartilage in chondroblastoma and chondromyxoid fibroma, and in fibrous tissue, in lesions such as non-ossifying fibroma (Figs. 1 and 2).

3.2. Multinucleated macrophages antigen expression

These giant cells showed membrane expression of GrB and CD14, and cytoplasmic expression of CD13 and Ki67, although Ki67 was also strongly expressed on the surface of multinucleated macrophages. CD56 was absent on multinucleated macrophages in most inflammatory granulomas. Mononuclear cells that expressed GrB, CD56, Ki67 and CD13 were noted in the vicinity of granulomas containing multinucleated macrophages. Multinucleated macrophages also found in a wide range of (extraosseous) pathological lesions were analysed. These included these lesions showed strong expression of CD68 (Fig. 3).

3.3. Osteoclast antigen expression

These multinucleated cells also expressed CD13 and CD14 but did not express GrB, Ki67 or CD56 (Fig. 4). CD14 and CD68 were mainly expressed on the osteoclast surface, whereas CD13 strongly stained the cytoplasm of osteoclasts. Numerous mononuclear cells expressing Ki67, GrB, CD13 and CD14 were seen in the vicinity of remodelling bone. Osteoclasts in specimens of normal growth plate, fracture callus, hyperparathyroid bone disease and remodelling cortical and cancellous bone of osteoarthritic and rheumatoid femoral heads, strongly expressed CD68.

4. Discussion

Our findings in this study indicate that both osteoclasts and multinucleated macrophages express CD13, CD14 and CD68, and that in formalin-fixed tissue, osteoclasts are distinguished by lack of expression of the macrophage-associated antigens GrB and

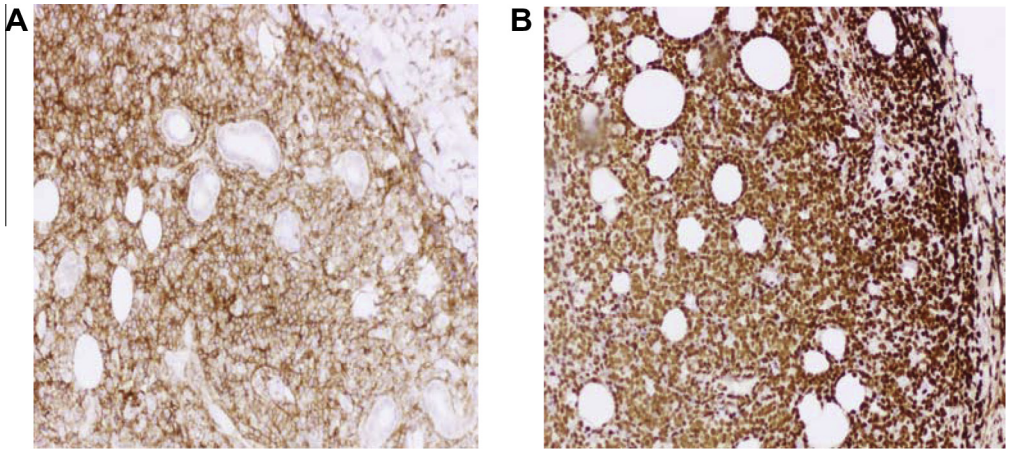

Fig. 1. The imaging immunostaining of giant cells by non-ossifying fibroma for (A) CD13, CD and CD68 but not (B) Ki67, GrB and CD56.