Human Pathology (2005) **36**, 945–954

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Phenotypic and molecular studies of giant-cell tumors of bone and soft tissue

Yu Sin Lau[a], Afsaneh Sabokbar[a], C.L.M.H. Gibbons[b], Henk Giele[b], Nicholas Athanasou[a,*]

[a]*Department of Pathology, University of Oxford, Nuffield Orthopaedic Centre, OX3 7LD Oxford, UK*
[b]*Nuffield Department of Surgery, University of Oxford, Nuffield Orthopaedic Centre, OX3 7LD Oxford, UK*

Received 1 March 2005; accepted 6 July 2005

Keywords:
Giant-cell tumor;
Calcitonin;
Osteoprotegerin;
Osteoclast;
Bone resorption

Summary Giant-cell tumor of bone (GCTB) and giant-cell tumor of soft tissue (GCTST) are tumors that contain a prominent osteoclastlike giant-cell component. The precise relationship between these morphologically similar tumors is unclear, and the cellular mechanism whereby giant cells accumulate within these and other locally aggressive tumors is uncertain. In this study, we have examined the cytochemical, functional, and molecular phenotype of the mononuclear and multinucleated components of GCTB and GCTST. Giant cells in GCTB and GCTST exhibited an osteoclast phenotype expressing tartrate-resistant acid phosphatase and vitronectin receptor and being capable of lacunar resorption. The mononuclear stromal cells derived from GCTB and GCTST exhibited an osteoblast phenotype, expressing alkaline phosphatase, and the receptor activator for nuclear factor κB ligand (RANKL), a factor that is essential for osteoclast formation. These cells also expressed osteoprotegerin (OPG), an inhibitor of osteoclastogenesis, and TRAIL, a receptor that binds OPG. Lacunar resorption by giant cells isolated from GCTB and GCTST was inhibited by OPG, zoledronate, and calcitonin. These findings indicate that the mononuclear and giant-cell components of GCTB and GCTST have similar phenotypic features and that the accumulation of osteoclasts in these giant-cell–rich tumors occurs by a RANKL-dependent process. RANKL expression by osteoblastlike mononuclear stromal cells in these tumors stimulates osteoclast formation and resorption; this would account for the osteolysis associated with these giant-cell–rich tumors. Inhibitors of osteoclast formation and activity are likely to be effective in controlling the osteolysis associated with GCTB and possibly other giant-cell–rich lesions.

1. Introduction

Benign and malignant tumors that contain numerous osteoclastlike giant cells are generally categorized on the basis of tissue location and histomorphology. Giant-cell–rich tumors can arise in bone, joint, or soft tissue. The mechanism whereby giant cells accumulate within these lesions and contribute to the osteolysis that occurs when these tumors arise either within bone or adjacent to bone is not certain. Two morphologically similar giant-cell–rich tumors that arise in bone and soft tissue are giant-cell tumor of bone (GCTB) and giant-cell tumor of soft tissues (GCTST), respectively [1].

* Corresponding author. Department of Pathology, Nuffield Department of Orthopaedic Surgery, University of Oxford, Nuffield Orthopaedic Centre, OX3 7LD Oxford, UK.
E-mail address: nick.athanasou@noc.anglox.nhs.uk (N. Athanasou).

0046-8177/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2005.07.005

GCTB typically presents as an osteolytic tumor arising at the epiphyseal end of a long bone in a skeletally mature individual. It contains numerous regularly distributed osteoclastlike giant cells lying in a well-vascularized spindle cell stroma. Several studies have shown that the giant cells in GCTB are mature osteoclasts [2-4]. GCTST is a rare soft tissue tumor that closely resembles GCTB histologically [1,5]. It is now commonly subclassified as either a giant-cell tumor of low malignant potential or a more aggressive giant-cell–rich malignant fibrous histiocytoma (MFH) [1,5-7]. It arises most frequently in the thigh, trunk, or upper extremity as a superficial or deep soft tissue lesion. GCTST arising in deep soft tissue can, as with other giant-cell–rich soft tissue lesions such as giant-cell tumor of tendon sheath, erode into bone and cause considerable osteolysis. The mononuclear and multinucleated cell (MNC) populations in GCTST have not been fully characterized, and the mechanism of giant-cell accumulation in GCTST remains unclear.

Osteoclasts are formed from marrow-derived circulating precursors that express a monocyte/macrophage phenotype [8,9]. These mononuclear phagocyte precursors express the receptor activator for nuclear factor κB (RANK) and, in the presence of macrophage colony-stimulating factor (M-CSF) and the ligand for RANK (RANKL), differentiate into MNCs that express the specific cytochemical and functional phenotype of mature bone-resorbing osteoclasts [10,11]. Giant cells in GCTB have been shown to express RANKL [5,12], but the precise role of the RANKL pathway in osteoclast formation in GCTB and GCTST is uncertain.

At present, surgery is the only treatment to control the osteolysis that occurs when a GCTB or other osteoclast-rich giant-cell lesion arises in bone. Complete surgical excision of GCTB and other giant-cell–rich lesions is not always possible, and local recurrence is a major problem [1,4]. This has led to exploration of the use of pharmacological inhibitors of osteoclastic bone resorption to control tumor osteolysis [13]. In this study, we have analyzed the phenotypic and molecular aspects of mononuclear and MNC populations of GCTB and GCTST. Our aim has been not only to examine the relationship between these two lesions but also to determine the cellular and humoral mechanisms underlying the recruitment of osteoclast precursors into giant-cell–rich lesions of bone and soft tissue. We have also evaluated whether pharmacological inhibitors of osteoclast formation and bone resorption may be of value in the treatment of osteolysis associated with these giant-cell–rich tumors.

2. Materials and methods

Alpha minimum essential medium (MEM) and fetal bovine serum (FBS) were purchased from Gibco Laboratories (Paisley, Scotland, UK); MEM containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin (MEM/FBS) was used for cell culture experiments. Salmon calcitonin was obtained from Sigma Diagnostics (St. Louis, MO); M-CSF, osteoprotegerin (OPG), and RANK:Fc were obtained from R&D Cytokines (Abingdon, UK). Zoledronate was obtained from Novartis (Basel, Switzerland). Collagenase and the diagnostic tartrate-resistant acid phosphatase (TRAP) kit were obtained from Sigma Diagnostics. All reagents used in reverse transcription and DNA amplification were obtained from Invitrogen, Life Technologies (Paisley, UK).

2.1. Isolation and culture of giant cells from GCTB and GCTST

Tissue from 4 cases of GCTST involving the calf (3 cases) and thigh and from 4 cases of GCTB involving the lower end of femur (2 cases), upper tibia, and upper fibula was obtained at the time of surgery at the Nuffield Orthopaedic Centre, Oxford. The tumors were sampled for histological examination; the remaining tissue was used for the studies outlined below.

Osteoclastic giant cells were isolated from the above tumors as previously described [4,14,15]. GCTB and GCTST tumor tissue was washed in sterile phosphate-buffered saline. Fragments of the tumors were then placed in collagenase (diluted 1 mg/mL in αMEM) and incubated at 37°C for up to 45 minutes. The number of giant cells was counted in a hematocytometer, and the volume was adjusted to obtain approximately 30 giant cells. The cell suspension was then added to dentine slices and glass coverslips in a 96-well plate. After 3 hours incubation at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, the dentine slices and glass coverslips were washed in MEM/FBS to remove any nonadherent cells. The slices were then transferred into 24-well plates containing fresh MEM/FBS and incubated in the presence and absence of the resorption inhibitors calcitonin (10^{-8} to 10^{-6} mol/mL), RANK:Fc (50-150 ng/mL), OPG (50-150 ng/mL), and zoledronate (10^{-8} mol/mL). Cultures were maintained for 24 and 48 hours at 37°C. Cultures on glass coverslips were fixed in acetone and stained histochemically and immunohistochemically for osteoclast markers (as detailed below). Cultures on dentine slices were treated with 1 mol/L ammonium hydroxide, washed in distilled water, and ultrasonicated to remove adherent cells; these slices were then stained with 0.5% toluidine blue to reveal areas of lacunar resorption and examined by light microscopy. The number of resorption pits was counted, and the percentage surface area of lacunar resorption on each dentine slice was measured using image analysis software (Adobe Photoshop) as previously described [16].

2.2. Histochemical and immunohistochemical characterization of giant cells derived from GCTB and GCTST

Cultures on glass coverslips of cells isolated from GCTB and GCTST were characterized histochemically for the