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Giant Cell Tumor of Bone: A Unique Paradigm of Stromal–Hematopoietic Cellular Interactions

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Abstract Giant cell tumor of bone is a progressive, potentially malignant process which destroys skeletal tissue by virtue of its osteoclast complement. As a biological entity it provides a unique natural model of bone resorption by osteoclasts whose recruitment and development is controlled by a neoplastic population of fibroblast-like cells. Understanding of the etiopathogenesis of this tumor could provide new insights into the mechanisms underlying osteoblast–osteoclast interactions in normal and diseased bone. Recent studies have shown that the stromal cell component in giant cell tumors is the only proliferating subpopulation of cells, and the giant cells themselves are nonproliferative and reactive. These stromal cells express several genes associated with the osteoblastic phenotype, synthesize, to a limited degree, certain matrix proteins associated with bone, and express several factors which are presumably involved in the recruitment of osteoclasts. In culture, giant cell tumor–associated stromal cells promote the fusion of monocytes and the proliferation of osteoblasts either by the secretion of factors or cell–cell contact. Hence, giant cell tumor of bone is a self-contained biosystem in which cells of both the stromal and hematopoietic lineages interact in a fashion similar to that observed in normal skeletal remodeling. The neoplastic nature of the stromal component, however, drives the hematopoietic precursors to undergo fusion, produces aggressive bone resorption, and results in extensive skeletal destruction. Examination of the various components of this system could lead to new directions for investigations aimed at a better understanding of osteoblast–osteoclast interactions.

Key words: giant cells, osteoblasts, osteoclasts, hematopoiesis, stromal cells

Significant interest in skeletal disorders such as osteoporosis, Paget's Disease, hyperparathyroidism, and hypercalcemia of malignancy has led to an abundance of investigations into the regulation of bone formation and resorption. In general, these investigations adhere to the concept that bone resorption is produced by multinucleated osteoclasts whose development and activity are under the influence of osteoblasts and possibly osteoblast precursors. As osteoclasts are derived from hematopoietic stem cells, while osteoblasts develop from a mesenchymal stem cell pool, the potential association between the stromal and hematopoietic cellular systems is an area of significant interest. In normal skeletal metabolism these associations presumably take place within the bone marrow compartment. However, one of the more interesting natural models in which to study a unique variation of hematopoietic and stromal cell interactions occurs in the primary bone neoplasm, giant cell tumor of bone (GCT). This is a tumor of fibrohistiocytic origin in which the neoplastic mononuclear stromal cell appears to be derived from the undifferentiated mesenchymal stem cell. While some of the cells in this tumor express typical monocyte-associated markers such as MOS-1 and MOP-9 (LEU M3), most studies suggest that the monocytes, lymphocytes, and leukocytes present are reactive to the fibrohistiocytic tumor cell population [Ling et al., 1988; Aqel et al., 1988].

Goldring et al. [1987] have shown that cells cultured from giant cell tumors of bone can be characterized based on their proliferative capacities, presence of granulocyte-monocytic antigenic markers, receptors for skeletal hormones, and secretion of soluble cell products. Three major cell types have been identified: (1) A population of mononuclear cells with a fibroblastic morphology, resembling connective tissue stromal cells, and which proliferate in culture. These

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cells are thought to be the neoplastic element of the tumor. (2) A second population of mononuclear cells which lack receptors for skeletal hormones, do not proliferate in culture, and are believed to be of the monocyte-macrophage lineage. (3) A third population of cells consisting of large multinucleated giant cells which possess phenotypic features of osteoclasts, such as the expression of receptors for calcitonin [Goldring et al., 1987]. Although there have been arguments to suggest that the giant cell population in this tumor is not precisely analogous to in osso osteoclasts, most investigators have assumed that the mechanisms which lead to the fusion of the mononuclear cells, and the development of the giant cells in this tumor, are under similar regulatory control mechanisms to those which occur in normal bone remodeling [Goldring et al., 1987].

Recently, Oreffo et al. [1993] have identified a cell line derived from mononuclear cells from a giant cell tumor of bone. This cell line, C433, was shown to have the following characteristics: (1) it represents undifferentiated cells, not recognized by any known antigenic markers for leukocytes; (2) it contains tartrate-resistant acid phosphatase; (3) it responds to the osteotropic factors 1,25 dihydroxyvitamin D_3, insulin-like growth factors I and II, but not the parathyroid hormone; (4) it forms sarcomas when transplanted into nude mice; and (5) it produces an activity that stimulates isolated avian and rat osteoclasts to resorb bone [Oreffo et al., 1993]. Since it is apparent from these and other investigators that the mononuclear cells in giant cell tumors of bone are composed of two types—one set that does not persist in culture and is positive for Ia and monocyte-macrophage antigens [Ling et al., 1988] and another that persists in culture and resembles connective tissue stromal cells, produces collagen, and has receptors for parathyroid hormone [Goldring et al., 1987]—a closer look at how these cell types interact in this tumor, how molecular aberrations may lead to over expression of cytokines or growth factors emanating from the neoplastic stromal cells, and how the mononuclear and multinucleated cells respond and behave within this milieu may provide further insight into the basic mechanisms of bone formation and remodeling as they apply to other skeletal conditions.

The unique behavior of GCTs depends on the neoplastic nature of the stromal cell population. To characterize the phenotype of this fibroblastic stromal cell, a series of experiments have been conducted on cellular outgrowths of human GCTs [Oreffo et al., 1993; Feng, 1990; Robinson et al., in press]. Confluent cultures of spindle-shaped cells have been developed from explanted tissue specimens of freshly biopsied or extirpated lesions. While in culture, the number of round-shaped cells have been shown to decrease rapidly in sequential passages over a 2 week period, and the giant cell population is lost after the first passage. By 2 weeks, the cultures consist of purely fibrohistiocytic cells [Oreffo et al., 1993; Feng, 1990; Robinson et al., in press].

Giant cell tumor–associated stromal cells express low levels of alkaline phosphatase activity approximately equivalent to the levels measured in human bone marrow stromal fibroblasts [Robinson et al., in press]. These alkaline phosphatase activities are much lower than those observed for primary cultures of cancellous bone-derived osteoblasts or ROS 17/2.8 cells. 1,25 dihydroxyvitamin D increases the expression of this enzyme approximately twofold and tends to slow the rate of cellular proliferation. Combined treatment with basic fibroblast growth factor and dexamethasone, which has been reported to enhance osteoblastic phenotypic expression in bone marrow stromal cells, tends to increase the level of alkaline phosphatase expression approximately twofold without blunting the cellular proliferation rate. Treatment with retinoic acid in concentrations greater than 10^{-8}M completely inhibits proliferation while *increasing* alkaline phosphatase expression approximately sixfold [Robinson et al., in press]. Northern hybridization against total RNA isolated from giant cell tumor–associated stromal cells shows low levels of alkaline phosphatase expression, no expression for the matrix proteins osteopontin or bone sialoprotein, and high levels of procollagen αI (1). There is also significant expression of mRNA for the protooncogenes c-jun and c-fos but not for c-src or c-kit [Robinson et al., in press]. These observations are consistent with the hypothesis that these tumor-associated mesenchymal stromal cells express a phenotype which is significantly similar to osteoblast-like cells.

Conditioned media from giant cell tumor–associated stromal cells increases the rate of proliferation of several osteoblast-like cells of both rodent and human origin including MC3T3-E1, ROS 17.28, RCT-3, primary human osteoblast-like cells, and primary human stromal bone

marrow fibroblasts. However, these media fail to have a proliferative effect on nonosteoblastic cells including NIH-3T3, ROS 25/1, as well as primary outgrowths of human skin fibroblasts [Robinson et al., in press]. Hence these fibroblast-like stromal cells have certain autocrine effector capabilities.

The ability of giant cell tumor–associated stromal cells to secrete factors which influence cells of the hematopoietic lineage have been investigated in U-937 pre-monocytes and in factor-specific clonal cell lines [Oreffo et al., 1993; Robinson et al., in press; Kito, 1991]. The findings show that GCT-conditioned media induces a 55% acceleration in the proliferation rate of these monocyte-like cells and the development of multinucleated cells after 6 hr of exposure [Robinson et al., in press]. Tartrate-resistant acid phosphate activity in the U-937 cells increases 10-fold as a result of the GCT stromal cell–conditioned media treatment. These findings are also observed when U-937 are cocultured with giant cell tumor–associated stromal cells. None of these results are observed in U-937 cells when conditioned media from human fibroblasts, primary human osteoblasts, MC3T3-E1 osteoblast-like cells, or NIH 3T3 cell–conditioned media are used. Moreover, cocultures of MC3T3-E1 cells and U-937 cells also fail to show these effects. Thus it appears that giant cell tumor–associated stromal cells are capable of secreting into the medium substances which induce differentiation or fusion of monocytes [Oreffo et al., 1993; Robinson et al., in press; Kito, 1991]. Since previous studies have shown that MC3T3-E1 cells [Horowitz et al., 1989] as well as primary human osteoblasts [Skjødt and Russell, 1992] are capable, upon induction, of secreting specific cytokines possibly involved in osteoclastic differentiation, the fact that conditioned medium from GCTs produces similar effects suggests that the same or similar cytokines may be secreted, but in a constitutive fashion. The lack of a requirement for induction by endotoxin or lectin suggests that the stromal cell population in GCTs may overexpress these factors, or possess a unique mechanism for autocrine stimulation not observed in normal osteoblasts.

Based upon the above findings and those of previous reports, an etiology for the pathogenesis of giant cell tumors of bone can be suggested. The scenario to be proposed is that of a neoplastic population of early mesenchymal stem cells which are capable of rapid division and the expression of markers of the osteoblastic lineage. These cells then acquire the capacity to express and secrete factors which have the ability to recruit osteoclast progenitors or increase the activity of fully differentiated osteoclasts. The neoplastic cells may also exert these effects through direct cell–cell contact with mononuclear cells of the hematopoietic lineage. The ability to influence these hematopoietic precursors is upregulated in comparison to that which is seen during normal homeostatic bone metabolism.

Perhaps the most interesting aspect of giant cell tumors of bone, and what makes the stromal–hematopoietic cellular interactions within them particularly intriguing, is how the neoplastic nature of the stromal cell directs the biological interactions which take place. The expression of the protooncogenes c-jun and c-fos can support a concept that the regulation of promoter binding is one potential intracellular mechanism which could be altered. For example, a viral infection or spontaneous transformation could affect the processing of environmental signals at the nuclear level to affect cellular behavior. Observations of giant cell tumors containing viral-like filaments in the nuclei have been reported [Fornasier et al., 1985]. In the case of GCT-associated stromal cells, the outcomes of such an alteration could be to upregulate paracrine processes which influence the behavior of hematopoietic cells. Another possibility is that there are abnormalities in the fos and jun elements (AP-1 site) in which altered binding of vitamin D to the vitamin D–responsive enhancer element (VDRE/AP-1) would repress the ability of vitamin D–mediated differentiation-related genes to function and could also account for the loss of control of certain osteoclastic activities [Lian et al., 1991]. Thus, the response observed to 1,25 dihydroxyvitamin D in the above experiments might indicate either a heterogeneous population in which some cells are more highly differentiated than others, or the presence of other vitamin D–responsive DNA segments which are not inhibited by the fos-jun heterodimer.

From a clinical perspective, giant cell tumor of bone has always been considered one of the most problematic primary neoplasms of the skeleton. Important questions concerning the histogenesis, clinicopathological diagnosis, and treatment remain subjects of considerable controversy. One of the problems related to the understanding of this tumor is that its biology is

poorly appreciated. It fits neither into the definition of a malignant nor a benign tumor, although most clinicians who treat it have great respect for the damage it can do as a result of its bone resorbing capacity and local aggressiveness. Although there are instances of giant cell tumors which metastasize, most produce their damage at the primary site of occurrence.

Although giant cell tumors of bone, while not common, are sufficiently prevalent to justify directed efforts towards scientific exploration and treatment, it may have more important broad implications as a model system for understanding the interactions between the hematopoietic and musculoskeletal systems with respect to metabolic bone disorders. Since it is currently thought that the mononuclear stromal cell population is responsible for the biological behavior of the cell types within this tumor, the system is analogous to the interactions which may take place during normal bone remodeling. Because the local aggressiveness of this tumor and its resultant bone destruction is much more exaggerated than that which occurs during even the most active types of osteoporosis, giant cell tumor of bone may be a biologically amplified system of normal homeostatic mechanisms gone awry. Thus, by developing an understanding of the biological intricacies of this tumor, otherwise subtle mechanisms of normal cellular function in bone may possibly be revealed.

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