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Research Paper

THE ORIGIN OF NEOPLASTIC GIANT CELLS IN FELINE GIANT CELL TUMOR OF BONE

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The maintenance of skeletal integrity is normally the result of the balance of processes of bone modeling and remodeling mediated by the action of different endogenous and exogenous factors that play a direct action mostly to osteoblasts and osteoclasts. Tumors involving skeleton colonize bone structures directly or secondly through the metastatic process of spreading within the bone (Wiswanathan *et al.,* 2009). Tumor cells can strongly accelerate these processes causing an alteration of remodeling and disrupts the normal physiology of the bone that resulting in different degree of osteolytic and osteoblastic bone lesions. Malignant cells within the bone disrupt the normal bone remodeling process throughout activation of different destructive factors. One of this represented by modification of RANK/RANKL/OPG pathway (Barger *et al*., 2007). Is now well understood how the molecular triad represented by receptor activator of NF-kB ligand (RANKL), its receptor RANK and the endogenous soluble RANKL inhibitor, osteoprotegerin (OPG) play a direct roles in the formation, function and modulation of osteoclast activity (Boyle et al., 2003), but are not fully understood the nature and the mechanism involved in the formation of multinucleated giant cells in various giant cell-containing lesions enclosed Giant Cell Tumor of bone (GCT_B). Our work was focused to evaluate these expressions in different spontaneous cases of feline GCT_{BS}.

Keywords: Giant Cell Tumor of bone, Bone, Cat, Multinucleated giant cells

INTRODUCTION

Giant cell tumor of bone (GCT_B) is a rare primary skeletal tumor accounting for about 5% of all primary bone tumors in human species and is a very rare tumor in dogs and cats (Klenke *et al.,* 2010). Giant cell tumor of bone and other type of bone tumors and bone lesions, like chondroblastomas and aneurysmal bone cysts determine osteolytic lesions containing osteoclast-like giant cells (Wülling *et al.,* 2003).It is consider an intramedullary bone tumor composed of three cell types: mononuclear histiocytic cells, multinuclea-

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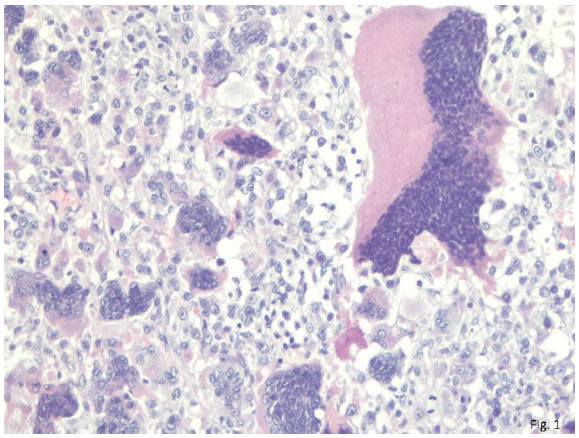
ted osteoclast-like giant cells and mononucleated neoplastic spindle stromal cells (Figure 1) that represent the main proliferating cell population (Klenke *et al.,* 2010). In humans GCT_B has a remarkable preference for the end of long bones where it appears as lytic lesion without matrix calcification. In long bones the tumor usually involves both metaphysis and epiphysis (Wisvanathan *et al*., 2009). In animals GCT_B is a very rare primary neoplasm of domestic animals found frequently in the epiphysis of long bones and generally appear like a monostotic well vascularized lytic lesion (Campanacci *et al*., 1987). The GCT_B usually behaves as a benign tumor, but with significant tendency to recur locally and rarely may produce metastases.Mitotic activity was detected in previous studies rarely and only in mononuclear components (Werner *et al*., 2006). The primary bone cells involved in bone resorption and in various skeletal disorders, like osteoporosis, rheumatoid arthritis and tumor associatedosteolysis are represented by osteoclasts. Osteoclasts derive from monocyte-macropahge lineage precursor cells under stimulation of many factors. Different molecules were identified and extensively characterized that are capable of regulating, proliferation, differentiation, fusion, activation and apoptosis of osteoclasts. Osteoclasts are primary bone-resorbing cells, which play important roles in skeletal homeostasis. Osteoclasts differentiation is strictly regulated by 2 types of cytokines: macrophagecolony-stimulating factor (M-CSF) and receptor activator of nuclearfactor-κBligand (RANKL) (Morgan *et al*., 2005; Yasuda *et al*., 1998). RANKL is a member of a tumor necrosis factor (TNF) family, and is expressed essentially on the surface of osteoblasts and itis in response to various stimulus from hormones and cytokines and itbinds to itsspecific RANK receptor expressed by osteoclast precursors. Osteo-protegerin (OPG) is a specific decoy receptor of RANKL and with a competitive mechanism could inhibits RANK-RANKL interaction (Boyle *et al*., 2003). This RANK/RANKL/OPG axis is involved not only in normal bone remodeling activities but also in different processes of pathologic bone destruction (Vega *et al*., 2007). Litlle is still know about the eactrole of osteoclasts and many skeletal disorders in human and animals (Salerno *et al*., 2008). Osteoclast differentiation and activation is critically dependent on the tumor necrosis factor (TNF) receptor/TNF-like proteins, osteoprotegerin (OPG) and ligand for receptor activator of nuclear factor kB (RANKL). RANKL expression has been observed in GCT-derived stromal cells and these support osteoclast formation. These and other observations have led to widely accepted proposition that the expression RANKL by the neoplastic stromal cell causes the recruitment of osteoclasts (Liu *et al*, 2003). GCT_B cells derived from the tumor stroma have been demonstrated to be rich source of RANKL mRNA while tumor giant cells also produce excessive

Figure 1: Cat, Femur, Giant Cell Tumor of Bone, Typical Pattern of the Tumor with Mononuclear Neoplastic Cells and Multinucleate Giant Cells, Magnification 20x, H&E



levels of RANK mRNA as compared to the production of these factors by normal osteoclasts (Conti *et al*., 2011). Thus GCT_B can be viewed as a neoplasm that produces an autonomous and unregulated overexpression of RANKL and RANK, this unregulated increase in osteoclastic activity results in extensive local bone destruction. The primary biochemical mechanism responsible of bone resorption is the solubilization of the bone minerals and the digestion of the organic bone matrix by acid proteases that promotes the acidification of osteoclast-bone interface: RANKL increases the expression of Metalloproteinase MMP9, involved also in cell matrix dissolution and Carbonate Dehydratase 2 (CAII) (David *et al*., 2001) a zinc metalloenzyme that is expressed at high levels in osteoclasts during bone resorption, in the cell cultured with ILa and this results in a marked increases number of TRAP-positive multinucleated cells. In a recent study (Leonardi *et al*., 2012) we have found a close correlation between CAII overexpression and presence of RANKL bound to RANK. In addition there is evidence that CAII is abundant in giant cells with a distribution in cytoplasm or on inner surface of the border depending on tumor clinical course and this will be a next step of our researches.

MATERIALS AND METHODS

Ten cases of feline primary GCT_B previously diagnosed at the Department of Biopathological Sciences and Hygiene of Animal and Alimentary Productions were examined to determine its immunohistochemical expression of RANKL, RANK and OPG. Specimens were obtained from initial biopsies for primary tumors. All tissue samples were fixed in buffered-formalin and paraffin-embedded. Diagnosis was defined on hematoxylin-eosin stained sections following conventional criteria. Immunohistochemistry (IHC) was performed using the avidin biotin complex (ABC) method. The following primary antibodies were used: RANKL (N-19): sc-7628 [Santa Cruz Biotechnology, inc.] purified goat polyclonal antibody raised against a peptide mapping at the N-terminus of RANKL of mouse origin. NFkB p65 (C-20): sc-372 [Santa Cruz Biotechnology, inc.] purified rabbit polyclonal from C-terminus of NF-kB p65 of human origin and OPG (N-20) [Santa Cruz Biotechnology, inc.] purified goat polyclonal antibody raised against a peptide mapping near N-terminus of OPG of human origin were used to in GCT_B paraffin embedded sections. Paraffin was removed with xylene and then dehydrated in sequential diluted ethanol and then rinsed in distilled water. To inhibit endogenous peroxidase activity the tissue sections were with 3% hydrogen peroxide in tris phosphate-buffered saline (PBS). Non specific reactivity was blocked with the use of normal goat serum for 30 minutes.

RESULTS

Hematoxylin-eosin stained sections of all 10 GCT_B cases had tumor tissue composed of multinucleated giant cells set in a homogenous stroma of mononuclear cells often appearing to arise from spindle-cell stroma. The tumor stromal cells varied in shape from polygonal to slightly spindle-shaped mononucleated cells. Stromal tumors cells staining had pale cytoplasm, indistinct cell borders, their solitary nucleus had a distinct nuclear membrane and an usually prominent nucleolus. The multinucleated giant cells contained several, up to 100, nuclei morphologically identical to those of stromal cells. Mitotic activity was seen only in mononuclear cell components. Scattered areas of degenerative and necrotic tumor cells, hemorrhage and reparative fibrous tissue, foam cells and mild

chronic inflammation were observed in some tumors of this report. Reactive bone formation was seen in rare case from cats where the spiculesof metaplastic bone were generally limited to linear condensations of spindle cells. Mononuclear cells expressed strong positive immunohistochemical membrane staining for RANKL (Figure 2) and OPG. RANKL staining was seen in all 10 examined cases. Staining patterns were found to be identical between positive controls and pathologic specimens. RANKL positive cells showed a diffuse membrane staining and distribution of positive cells. Stromal cells of

Figure 2: Cat, Femur, Giant Cell Tumor of Bone, Mononuclear Cells Expressed Strong Positive Immunohistochemical Membrane Staining For RANKL, High Magnification, H&E Counterstain

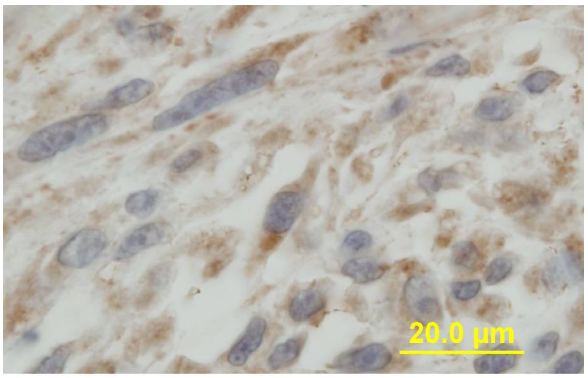
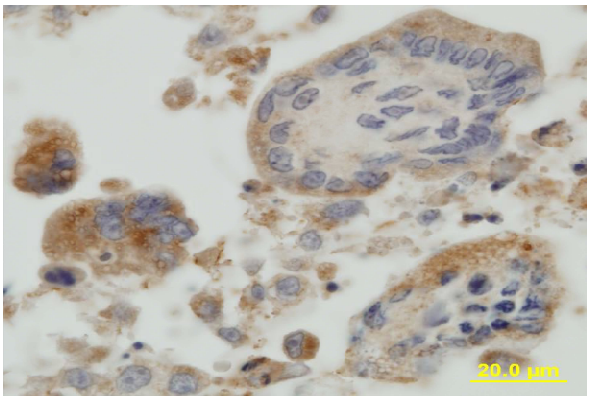


Figure 3: Cat, Femur, Giant Cell Tumor of Bone, Rankl Positive Cells Showed a Diffuse Membrane Staining and Distribution of Positive Cells, High Magnification, H&E Counterstain



GCT_Boverexpress RANKL this results in an increased RANKL/OPG ratio that in turn results in an excessive development of large multinucleated osteoclasts-like giant cells (Figure 3).

DISCUSSION

GCT_B is characterized morphologically by the presence of numerous multinucleate giant cells, some of which mimics osteoclasts, arise from well vascularyzed bone marrow stroma cells of the medullary cavity of bone organs formerly it was consider by some authors that tumor cells of GCT_B arise from macrophage precursors of the bone marrow-derived mononuclear/ macrophage cells, but the pathogenicity still remains elusive. In GCT_B the mononuclear component that fuses to form so-called tumor giant cells arise from spindle-shaped stromal cells that are believed to represent the true neoplastic component of this tumor. The multinucleated giant cells are a constant and prominent part of these tumors, “The giant cells are probably of less significance than mononuclear cells. The basic proliferating cell has round-to-oval or even spindle-shaped nucleus in the field that is diagnostic of true GCT_B”. Our investigation used immunohistochemical staining techniques focused on the biological functions of RANKL, RANK and OPG participants in normal bone metabolism and remodeling activities, to demonstrate that tumor cells in GCT_B arise from bone marrow stroma and not from fusion of mononuclear cells of hemopoietic bone marrow origin. Our findings indicate that the accumulation of multinucleated tumor giant cells occurs by a RANKL-dependent mechanism and that resorption by these cells may be controlled by inhibitors of osteoclastic activity. RANKL was expressed immunohistochemically on the surfaces of pre-osteoblastic stromal cells. The

observation that RANKL is highly expressed in multinucleated tumor giant cells of GCT_B holds that these components are passively recruited by the production of RANKL by the stromal neoplastic components.

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