



## BRIEF REPORT

# Expression of receptor activator of NF- $\kappa$ B ligand and osteoprotegerin in peripheral giant cell granulomas of the jaws

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**BACKGROUND:** Peripheral giant cell granuloma is a tumor of the jaw characterized by the presence of multinucleated giant cells and mononuclear cells within a fibrous stroma. These lesions are considered to be of a reactive nature rather than neoplastic. Although peripheral giant cell granulomas is a well-described clinical entity, little is known on its pathogenesis. The aim of this study was to investigate the receptor activator of NF- $\kappa$ B ligand (RANKL) and osteoprotegerin (OPG) expression and immunolocalization in giant cell granulomas.

**METHODS:** RANKL and OPG protein expression was evaluated in 22 peripheral giant cell granulomas samples, by means of immunohistochemistry. Staining was evaluated semi-quantitatively, according to the extent and intensity of the stain.

**RESULTS:** RANKL was expressed in all cases with a cytoplasmic staining pattern, whereas OPG expression was detected in 21 of the 22 cases examined. Active multinucleated giant cells exhibited intense immunoreactivity for both proteins.

**CONCLUSION:** RANKL and OPG are expressed in peripheral giant cell granulomas of the jaw in a manner supporting the osteoclastic nature of giant cells whereas the possible osteoclastic lineage of stromal monocytes remains ambiguous.

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**Keywords:** giant cell granuloma; jaw; OPG; RANKL

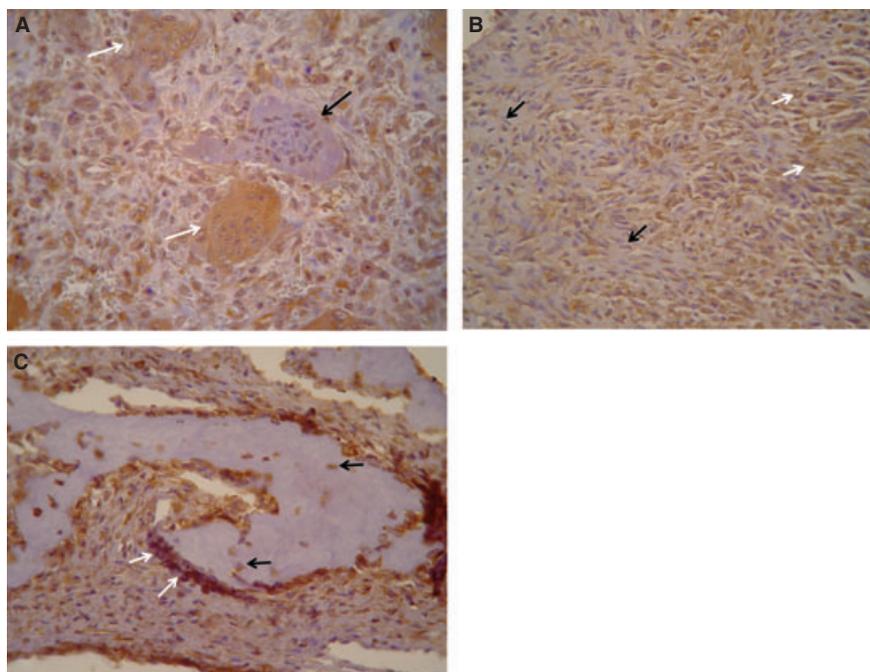
## Introduction

Peripheral giant cell granulomas (PGCGs) of the jaws are probably reactive in nature lesions that arise mainly from the connective tissue of the gingiva or

the periosteum of the alveolar ridge (1). They may present as firm, soft, bright nodules, predominantly bluish red with a smooth shiny or mamillated surface. PGCGs are basically asymptomatic, unless they interfere with occlusion. X-rays are important for determining whether the lesion is of gingival (i.e. peripheral) origin or of intraosseous (central) origin with spread towards the surface (2). The histological study of PGCGs focuses on three elements: epithelium, connective tissue zone and medullary or core region. The surface squamous epithelium may be ulcerated. The subepithelial connective tissue zone consists of connective tissue with abundant small-caliber blood vessels. An acute inflammatory infiltrate is often encountered. The medullary or core region includes the giant cells (2).

Giant cells in giant cell granulomas (GCGs) have been considered as phagocytes reacting to hemorrhage (3), foreign body cells (4) or osteoclasts (5, 6). Two types of giant cells have been described: type A cells correspond to poly-nuclear eosinophilic cells with a diffuse and abundant cytoplasm; their nuclei are prominent and the chromatin is distributed along the inner membrane. Type B cells are characterized by a regular and well-defined, more abundant and dense cytoplasm. The nuclei exhibits poorly defined borders and tend to central accumulation, with strong hyperchromatism (2). The mononuclear stromal cells may participate on the formation of giant cells. Two members of the tumor necrosis factor (TNF) – ligand family, receptor activator of NF-kappa B ligand (RANKL) and osteoprotegerin (OPG) may play an important role in the formation of giant cells (3). In general, RANKL, a transmembrane molecule produced by osteoblasts/stromal cells, binds to RANK, which is present on the surface of osteoclast precursors to stimulate the latter to differentiate forwards osteoclasts (7). OPG, also produced by osteoblasts/stromal cells, competitively binds to RANKL, neutralizing and interrupting stromal cell-derived RANKL signals, resulting in the reduction of osteoclastogenesis (8–10).

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**Figure 1** Immunohistochemical detection of RANKL protein in giant cell granulomas. (A) RANKL-selective immunopositivity in type A giant cells (white arrows) of a peripheral GCG. Lack of immunostaining in a type B giant cell (black arrow) ( $\times 250$ ). (B) RANKL immunopositive reaction of mononuclear cells (white arrows). Lack of reaction of spindle shaped mononuclear cells (black arrows) ( $\times 250$ ). (C) RANKL immunopositivity of osteoblasts (white arrows) and osteocytes (black arrows) ( $\times 250$ ).

The aim of this study was to evaluate protein expression of RANKL and the decoy receptor OPG in a series of PGCGs to examine their possible involvement in PGCGs pathogenesis.

### Materials and methods

Formalin-fixed paraffin-embedded blocks from 22 cases of PGCGs were retrieved from the file of the Department of Oral Pathology, Faculty of Dentistry, University of Athens. Antibodies raised against human RANKL (N-19) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and OPG (N-20) (Santa Cruz Biotechnology) were applied in a standard three step immunohistochemical procedure at dilutions of 1:50 and 1:75, respectively, with an overnight incubation at 4°C. Thyroid cancer specimens, expressing RANKL and OPG proteins, were used as external controls. Specificity of the immunohistochemical staining was demonstrated by complete absence of staining product using the secondary antibody alone and substituting TBS for the primary antibodies. Staining was semi-quantitatively evaluated according to the extent and intensity of the stain by two expert observers (S.T.B and K.I.T), blinded to the antibody applied to each section, using an  $\times 40$  objective.

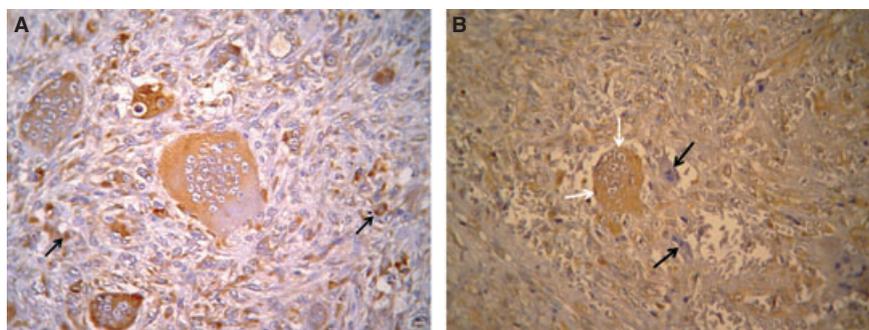
### Results

Hematoxylin and eosin-stained sections of all PGCGs showed a variable number of multinucleated giant cells that were scattered in a fibrous stroma containing round

and spindle shaped mononuclear cells. The immunohistochemical results indicated that RANKL was expressed in all cases with a cytoplasmic staining pattern. The RANKL immunostaining in giant cells was homogenous and selectively observed in type A giant cells, while type B giant cells were immunonegative (Fig. 1A). Mononuclear cells with round vesicular nuclei were RANKL-positive whereas no staining was noticed in spindle-shaped fibroblastic cells (Fig. 1B). When bone trabeculae were present, osteoblasts and osteocytes were positive for RANKL (Fig. 1C). These areas served as internal positive controls. OPG expression was detected in 21 of the 22 cases examined. It was observed in inflammatory cells, either mononuclear or neutrophils as well as in stromal cells (Fig. 2A). Most type A giant cells exhibited non-homogenous cytoplasmic staining (perinuclear, peripheral or patchy) (Fig. 2B), whereas type B giant cells were OPG-immunonegative.

### Discussion

The immunohistochemical expression of OPG and RANKL in stromal cells, as observed in the present investigation, is consistent with their purported osteoblastic lineage (3, 11, 12). The restriction of immunoreactivity in type A giant cells is in accordance to the active role of this type of giant cells. In giant cell tumors of bone, giant cells also appeared to express abundant cytoplasmic OPG protein, whereas the stromal element, but not the giant cells, expressed RANKL protein and mRNA; as far as OPG stromal staining is concerned, it exhibited a cytoplasmic and extracellular matrix pattern



**Figure 2** Immunohistochemical detection of OPG protein in giant cell granulomas. (A) OPG immunostaining of inflammatory/stromal cells, among which many mononuclear cells (black arrows) ( $\times 250$ ). (B) The pattern of OPG immunoreactivity in giant cells. Immunopositive (white arrows) and immunonegative giant cells (black arrows) are observed ( $\times 250$ ).

(13). Osteoclast formation is influenced by interactions between stromal cells which express RANKL and RANK-expressing mononuclear phagocyte osteoclast precursors (11). Stromal mononuclear cells in GCGs of the jaw are thought to consist of a heterogeneous population of macrophage-and-fibroblast-like cells (1). Despite many data supporting the capacity of the latter to differentiate along fibroblast/osteoblast lines (3) and, because of their RANKL expression (3, 11, 12), induce osteoclast differentiation from mononuclear phagocyte precursors, in the examined considerable number of cases, spindle shaped cells were RANKL-negative. RANKL-negative spindle cells are most likely mature fibroblasts, quite abundant to peripheral lesions such as PGCGs. It is the round mononuclear stromal cells that correspond to the proliferative department of the lesions and it was those cells that expressed RANKL and OPG. RANKL was indeed expressed in stromal cells of PGCGs which corresponded morphologically rather to macrophage than fibroblasts.

In conclusion, RANKL and OPG are expressed in a manner indicating their association in regulating osteoclastogenesis in PGCGs of the jaw.

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