

## ORIGINAL ARTICLE

# Immunohistochemical detection of the receptor activator of nuclear factor Kappa B ligand and c-fos in giant cell granuloma

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## ABSTRACT

**Background:** Giant cell granuloma (GCG) is an intraosseous giant cell fibroblastic lesion that predominantly affects the jaw bones in children and adults. Despite its frequent local progression and destructive effect, it is traditionally considered reparative or reactive in nature. The receptor activator of nuclear factor Kappa B ligand (RANKL), a member of the tumor necrosis factor family and the transcription factor c-fos play a major role in osteoclast proliferation and differentiation. In this study, we examined the expression of RANKL and c-fos in lesional tissues from seven patients with GCG. **Materials and Methods:** Automated immunohistochemical staining was performed on formalin-fixed paraffin-embedded sections from 7 cases, using antibodies against RANKL, c-fos and p53. **Results:** All tissues showed nuclear staining for c-fos and cytoplasmic staining for RANKL. The staining was strong, diffuse and observed in both mononuclear lesional cells and giant cells. No staining was observed with p53. **Conclusion:** Expression of RANKL and c-fos in this lesion, similar to what has been reported in giant cell tumors of bone, suggests a similar pathogenesis and hence a potential response to anti-RANKL inhibitors. A larger study is needed to confirm these findings and define the relationship of this lesion to other giant cell-rich bone lesions.

**Key words:** C-fos, giant cell granuloma, receptor activator of nuclear factor Kappa B ligand

## INTRODUCTION

Central giant cell granuloma (GCG) is a bony lesion of the jaw that affects patients mostly in the first or second decade of life. It commonly arises in the mandible or maxilla, but has been reported in other craniofacial bones and extragnathic sites. In the jaw, it appears as an intraosseous lesion that may expand the bone and displace the teeth. Plain X-rays reveal a well-demarcated radiolucent lesion with a trabeculated or multiloculated “soap bubble” appearance.<sup>[1]</sup> The histologic appearance is similar to giant cell tumor of the bone and aneurysmal bone cyst and consists of proliferating spindle cells admixed with multinucleated osteoclast giant cells in a fibrous background. GCG is postulated

to arise as a reparative consequence to trauma, dental implants and reaction to hemorrhage.<sup>[1-3]</sup>

Although considered as reactive or non-neoplastic, GCG can become locally aggressive and exhibits a high recurrence rate of 11–35%.<sup>[4]</sup> The reactive nature of this lesion has recently been questioned because of their potential aggressive nature and bone destruction. Giant cells are derived from osteoclasts which undergo a series of differentiation steps or activation under the influence of signaling pathways that result in the activation of several transcription factors such as receptor

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activator of nuclear factor Kappa B ligand (RANKL), which induces the activation of c-fos and related transcription factors. RANKL is important in bone remodeling. During bone development, RANKL is expressed by osteoblasts which together with osteoclasts, coordinate and stimulate bone synthesis.<sup>[5]</sup> C-fos is a member of the transcriptional activating protein complex 1 and is essential for bone remodeling and osteoclast activation.<sup>[6]</sup> RANKL, c-fos and related factors induce the fusion of osteoclasts to become mature giant cells.<sup>[7]</sup> In this article, we have studied the expression of RANKL and c-fos in GCG using immunohistochemical methods in an attempt to understand the pathogenesis of this lesion.

## MATERIALS AND METHODS

After institutional approval, the archives of the Pathology Department were searched for all cases of GCGs in all age groups and genders in a period of 3 years (2007–2009). Information about patient's age, sex, tumor location and size was obtained from the pathology reports. Unstained sections for immunohistochemistry were handled blindly without identifiable information.

### Immunohistochemistry

Immunohistochemical stains with RANKL, c-fos and p53 antibodies were performed on formalin-fixed paraffin-embedded sections from tumor specimens of patients. According to the manufacturer, anti-RANKL antibody (Abcam, San Francisco, USA) was a polyclonal antibody raised against a recombinant protein. C-fos (Abcam, San Francisco, USA) was a polyclonal antibody raised against the c-terminal of the protein. p53 (Carpinteria, CA, USA) detects both mutated and wild-type p53 proteins. Immunohistochemical stains were performed with Leica automated instrument (Leica Biosystems, Richmond, Illinois, USA). Deparaffinized slides were sequentially incubated with the primary antibody, a secondary antibody and a polymer. This procedure was performed for all tumor cases and positive controls. Staining of sections containing normal brain and non-neoplastic bone with osteoclasts served as positive controls for c-fos and RANKL, respectively. P53 staining was previously validated with known positive controls. Negative controls were similarly treated except that the primary antibody was omitted.

## RESULTS

### Patients

Seven patients with a diagnosis of GCG were retrieved; their ages ranged from 15 to 50 years. All patients were females and tumors were clinically labeled as "aggressive." Six cases had lesions in the mandible and only one case in the maxilla [Table 1]. Six patients presented with bone and soft tissue swelling and only one case was discovered as incidental. All

lesions showed an evidence of bone destruction by radiography. All lesions showed similar histologic appearance with mixed proliferation of spindle/mononuclear cells and multinucleated giant cells in a fibrous background containing blood vessels and few inflammatory cells [Figure 1]. Areas of hemorrhage and hemosiderin deposits were present, but no necrosis, marked cytologic atypia or increased mitotic rate were identified.

### Immunohistochemistry

RANKL exhibited strong (i.e., more or equal to positive control) diffuse staining in 6/7 cases and one case had weak staining (i.e., less than of positive control). The staining was cytoplasmic and identified in both giant cells and mononuclear/spindle cells. Staining for c-fos revealed positive staining in all cases, similar to positive control. The staining was nuclear and seen in both giant cells and mononuclear/spindle cells [Figure 1]. P53 immunostain was negative in all cases. All positive controls reacted appropriately with normal or non-lesional bone, demonstrating adequately visible immunoreactivity for RANKL and c-fos.

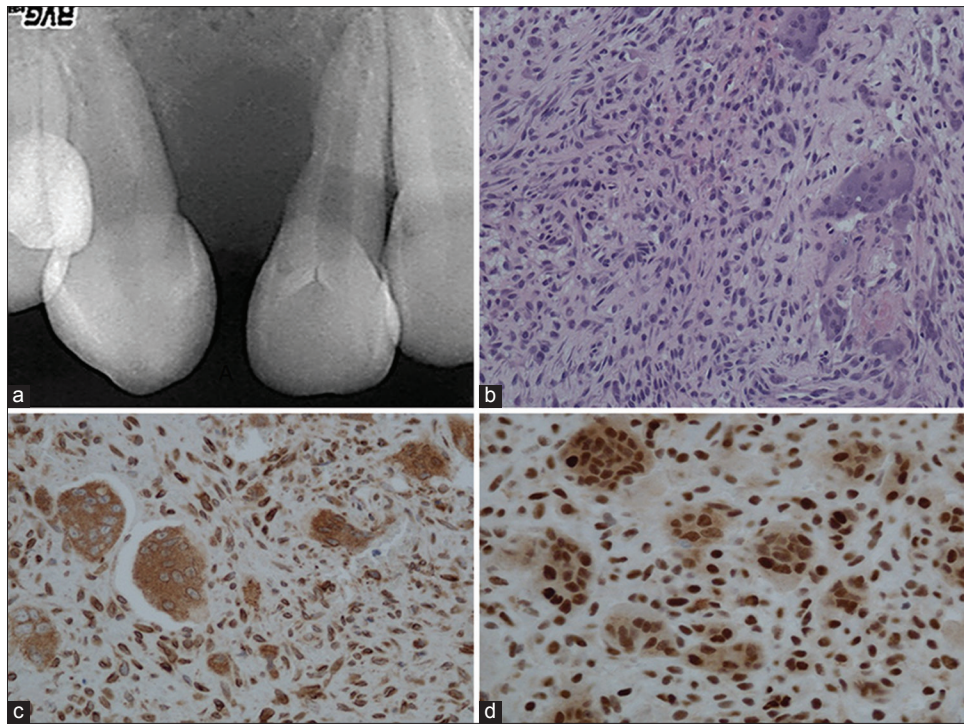
## DISCUSSION

Although GCGs are traditionally sub-classified into peripheral and central, their pathogenesis and histologic appearances are essentially similar. Central giant cell tumors have the potential of causing significant bone destruction and subsequent recurrence. The aggressiveness of these lesions depends on the number of giant cells. Giant cell-rich lesions are more aggressive than lesions with fewer giant cells.<sup>[2]</sup> Giant cells differentiate from osteoclasts that are derived from the monocytes/macrophages lineage and hence are positive for CD68.<sup>[8]</sup> They secrete tartrate-resistant acid phosphatase and tissue metalloproteinases that cause bone destruction.<sup>[8,9]</sup>

Differentiation of mononuclear cells into osteoclasts and subsequent fusion of osteoclasts to form giant cells are dependent on the activity of RANKL signaling pathway. RANKL is a membrane-bound tumor necrosis factor-related molecule expressed by osteoblast/stromal cells.<sup>[10]</sup> Differentiation of osteoclasts is initiated by cytokines,

**Table 1: Clinical features of giant cell reparative granuloma cases**

Number	Age	Sex	Location of lesions	Maximum tumor dimension (cm)
1	37	Female	Right mandible; teeth roots #28-29	2
2	50	Female	Teeth #26-27	1.3
3	16	Female	Left mandible	2
4	31	Female	Tooth #32	2
5	15	Female	Right maxilla; right palate	3.0
6	17	Female	Right mandible	3.0
7	27	Female	Left maxilla	2.5



**Figure 1:** (a) Plain X-ray revealing a radiolucent well-defined intraosseous lesion widening the space between the teeth. (b) The typical histology of giant cell granuloma shows proliferation of spindle cells and mononuclear cells with a focal presence of multinucleated giant cells (H&E stain,  $\times 100$ ). (c) Immunohistochemical stain for receptor activator of nuclear factor Kappa B ligand displaying cytoplasmic positivity in the mononuclear cells and multinucleated giant cells (IHC stain,  $\times 200$ ). (d) Immunohistochemical stain for c-fos displaying nuclear positivity in the mononuclear cells and multinucleated giant cells (IHC stain,  $\times 200$ )

i.e. interleukin-1 and macrophage colony-stimulating factor that stimulate the activation of RANKL. Binding of RANKL to its receptor on the surface of precursor cells triggers the formation of a complex that leads to the activation of NF- $\kappa$ B and mitogen-activated protein kinases. This leads to the activation of c-fos and the induction of nuclear factor of activated T-cells, cytoplasmic 1, which is a master transcription factor of osteoclast differentiation and induces the expression of osteoclast-specific genes.<sup>[11,12]</sup> This pathway is a nonspecific pathway that has been elucidated in other giant cell-rich lesions including giant cell tumor of bone.<sup>[10-13]</sup>

Immunohistochemical expression of RANKL in this study and a previous study confirming the presence of RANKL mRNA in GCG indicate that it may play a role in the pathogenesis of this lesion as well.<sup>[9]</sup> This fact has an important management implication with the use of RANKL-targeted therapy.<sup>[14]</sup> Denosumab, a clinically available RANKL-targeted therapy, was found to be beneficial in the treatment of giant cell tumors and aneurysmal bone cysts; both are giant cell-rich lesions with bone destructive effects caused by the activation of RANKL signaling pathway.<sup>[15,16]</sup> The use of RANKL-targeted therapy may prove to be useful in treating recurrent or large GCG that cannot be satisfactorily treated with curettage.<sup>[1]</sup>

The p53 gene is the most frequently mutated gene in human cancer and immunohistochemical expression of p53, in general, corresponds to p53 gene mutation. The p53 protein expression

is usually seen in high-grade malignancies. Although the expression of the oncogene “c-fos” in GCG is consistent with its aggressive nature, the lack of p53 expression suggests that it is still a low-grade lesion.<sup>[17]</sup>

## CONCLUSION

Although limited by the small number of cases, expression or activity of RANKL and c-fos is identified in GCG in this pilot study. Activation of this pathway in GCG suggests a relationship with other giant cell-containing tumors of the bone. Additional studies with larger number of cases are needed to verify these findings. Such studies are of profound importance because of the potentially destructive nature of these lesions and possible benefit from targeted therapy against RANKL.

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## Conflicts of interest

There are no conflicts of interest.

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