The Emerging Role of p38 for expression of Sclerostin in Giant Cell Tumor

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Introduction: Giant cell tumor (GCT) of the bone is a relatively uncommon lytic bone tumor comprised of multinucleated, osteoclast-like giant cells. Although GCT is relatively benign, there is potential for aggressive local invasion and destruction. Furthermore, metastases to the lung and other distant sites occur in 2% of cases. First-line treatment includes surgical curettage, however, local recurrence occurs in up to 50% of cases, requiring repeat surgeries and adjuvant therapy. Bone is a dynamic organ within the human body. Recent advancements in bone biology, histology, and genetics have revealed important regulators of osteoclastogenesis and bone resorption. Bone maintenance and turnover is regulated by bone cells, namely osteoclasts, osteoblasts, and osteocytes. The delicate interplay of these bone cells is integral to the pathogenesis of GCT. Sclerostin, primarily physiologically produced in osteocytes, is an emerging important negative regulator of bone mass. It acts by inhibition of the Wnt pathway to induce osteoblastic apoptosis and ultimately decreases bone deposition. Sclerostin expression is regulated by a multitude of factors, including mechanical loading, parathyroid hormone, cytokines such as prostaglandin E2, calcitomin, and others.

We hypothesize that sclerostin is a mediator of bone loss in the characteristic osteolytic bone lesions seen in GCT. We further posit that primary GCT cell lines directly produce sclerostin causing increased local osteolysis and facilitating tumor growth and spread. Furthermore, we hypothesize that sclerostin is a downstream target of the pro-inflammatory cascade as regulated through the p38 pathway and may be inhibited by SB203580, offering a new therapeutic potential for this anti-cancer drug and suggesting an additional mechanism of sclerostin regulation.

Methods: A. Culture of 143B and Primary Giant Tumor Cell lines
The primary giant cell tumor cells were obtained from patients via tumor surgery at the department of orthopaedic surgery at the Columbia University Medical Center.
GCT cell lines were cultured in MEM-α supplemented with 10% FBS, 1% penicillin-streptomycin solution.
B. Western blot
Breast cancer cells were rinsed with PBS and lysed with LIPA buffer. Protein concentrations of the samples were determined by Bio-rad protein assay. Equal amounts of cellular protein extracts were electrophoresed under reducing conditions and then transferred to a polyvinylidene-difluoride Western blotting membrane (Bio-rad). The following primary antibodies were used: P-ERK1/2 (T202/Y204), ERK1/2, P-p38(T180/Y182), p38, P-JNK(T183/T185), JNK and Actin (Cell signaling).
C. Immunocytochemistry (ICC)
To detect the activation of p38 and expression of Sclerostin in Primary GCT cell lines, cells were seeded into 4-well chamber slides (BD Science). After 24 hours, the P-p38 (T180/Y182) and Sclerostin (abcam) was detected using routine immunofluorescence methods.
D. Quantitative PCR
Total RNA was harvested using Trizol reagent (Invitrogen) according to the manufacturer’s protocol. Real-time Quantitative PCR was performed with SYBR Select MasterMix using QuanStudio 6 Flex (Life technologies).

**Results:** ICC indicated that primary GCT cell lines express sclerostin and activated p38. Western blot also indicated that activation of p38 in GCT cell lines (Figure 1). RT-PCR analysis showed significant upregulation of sclerostin in GCT-TJ100511 and GCT-BJ033114 cell lines as compared to 143B osteosarcoma cell line (Figure 1). Treatment of GCT-BJ033114 with SB203580 resulted in downregulation of sclerostin expression (Figure 2).

**Discussion:** We demonstrate strong evidence for a novel mechanism of bone loss in osteolytic GCTs owing to the production of sclerostin. Additionally, contrary to the current belief that sclerostin is predominantly produced by osteocytes, we found that sclerostin can be produced by specific cells after cancerous transformation. We further show that sclerostin expression is inhibited by SB203580, a p38 inhibitor, suggesting sclerostin may be regulated by the pro-inflammatory cascade and may be further categorized as a pro-inflammatory cytokine that mediates osteolysis.

**Significance:** Our data suggest that pharmacologic treatment of osteolytic tumors require both antiresorptive agents as well as anti-sclerostin antibodies in order to prevent pathological fractures.
Figure 2. SB203580 reduces expression level of sclerostin in GCT-BJ033114 cell line.