

# Notch Signaling is Required for Osteoclast Differentiation and Function

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## Introduction

Osteoclasts are multinucleated giant cells responsible for the resorption of bone.<sup>1</sup> As such, they are a critical component along with bone-forming osteoblasts and bone-regulating osteocytes in the process of bone remodeling. For this reason, osteoclasts are an attractive target in instances where increased bone mass is desired. In particular, anti-resorptive agents, which are chemicals and biologicals that inhibit osteoclast differentiation and/or activity are the most commonly proscribed treatments for the prevention of fractures in patients with osteoporosis, a disease marked by decreased bone mineral density. Side-effects and limited improvements in bone quality under current anti-resorptive therapies highlight the need for better, more nuanced control over osteoclast function. One promising potentially targetable pathway is mediated by the mammalian homologues of Notch.

Regulated signaling from Notch is recognized as an important regulator of tissue patterning during development and stem cell niche maintenance. Notch signaling results from interaction with a membrane-bound Notch family receptor with a membrane-bound ligand such as Jagged1 or Delta-like1. After binding, the intracellular domain of Notch (NICD) is released from the membrane by a gamma-secretase complex (targeted inhibition by DAPT).<sup>1</sup> NICD then translocates to the nucleus where it engages in transcription in a complex containing CSL and Mastermind-like (targeted inhibition by SAHM1) (Fig.1). Notch signaling also impacts the differentiation of osteoblasts.<sup>1,2,3</sup> As such, there is growing interest in manipulation of Notch signaling to improve outcomes in fracture healing. Notch signaling, however, may also influence the differentiation and function of osteoclasts, and understanding of the manner in which osteoclasts, which are

essential both in physiological bone turnover and remodeling of a healing fracture callus, may be affected by therapies manipulating Notch signaling is essential for proper exploitation of this pathway. The role of Notch signaling in osteoclasts and its utility in bone loss disorders such as osteoporosis, however, are controversial as there is evidence for both stimulatory and inhibitory effects.<sup>1,2,3,4,5,6</sup> Studies of specific activation of Notch family members with antibodies have provided strong evidence for a positive role for Notch signaling in osteoclastogenesis. Because of this, we hypothesized that Notch signaling is necessary for osteoclastogenesis and stimulation of Notch signaling will result in increased osteoclast formation and function.

## Methods

Notch signaling was stimulated by plating bone marrow macrophages on goat anti-human IgG (control), Jagged1-Fc (JAG), or Delta-like1 (DLL) immobilized on the culture surface at 10µg/mL. Notch signaling was inhibited by adding DMSO (control), DAPT, or SAHM1 to the culture medium at a final concentration of 10µM. Osteoclastogenesis was performed by culturing bone marrow macrophages in

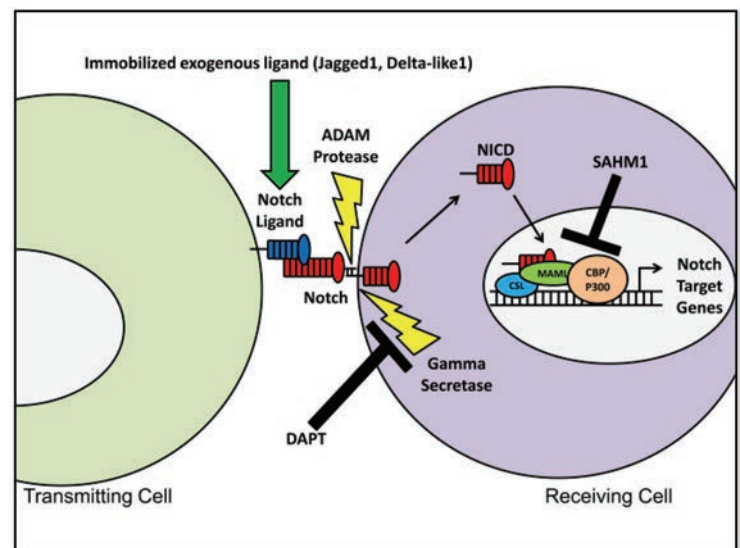


Figure 1. Summary of Notch signaling.

medium containing 35ng/mL M-CSF and 100ng/mL RANKL. After differentiation, cells were stained for tartrate-resistant acid phosphatase (TRAP) activity and quantified for osteoclast size, osteoclast number, osteoclast precursor number, nuclei per cell, and TRAP-stained area. Osteoclastic resorption was measured by differentiating osteoclasts on hydroxyapatite-coated culture surfaces; cells were cultured for 4 or 6 days, and resorbed areas were quantified.

## Results

Under M-CSF/RANKL-stimulated osteoclastogenesis, JAG, but not DLL, promoted a significant increase in osteoclast size and TRAP-stained well area compared to IgG. Conversely, both DAPT (inhibition of NICD cleavage activation) and SAHM1 (inhibition of NICD mediated transcriptional activation) significantly reduced osteoclast size and stained area compared to DMSO control. As measured by quantitative RT-PCR, JAG stimulation resulted in a significant increase in TRAP, cathepsin K, and MMP9 expression, while DAPT significantly decreased in cathepsin K expression. Both JAG and DLL significantly increased resorbed area compared to IgG control at day 4. At this time, DMSO control, DAPT, and SAHM1 groups were similar. At 6 days, however, IgG resorption matched JAG resorption, though DLL stimulation still showed significantly higher resorption than IgG control. DAPT and SAHM1 groups had significantly less resorption than DMSO control. In partial explanation for the enhanced osteoclast size and resorption seen in JAG and DLL stimulated osteoclasts, JAG and DLL both significantly increased expression of pro-fusion genes CD200 and DC-STAMP. DAPT significantly reduced expression of both CD200 and DC-STAMP, and SAHM1 significantly reduced expression of CD200.

## Discussion

These data indicate that Notch signaling is necessary for normal osteoclast maturation and function, and Notch stimulation by JAG and DLL can increase osteoclast formation and function potentially by enhancing the fusion of osteoclast precursors.

## Significance

Dysregulated osteoclastic resorption resulting in bone loss and insufficiency fractures represents a costly challenge both in terms of financial and quality-of-life considerations. While current anti-osteoclast therapies are effective in preventing further bone loss, they are often insufficient in promoting new bone growth. Controlling the Notch pathway may provide the advantage of being able to simultaneously regulate bone growth (osteoblast and chondroblast lineages) and resorption for positive therapeutic effect.

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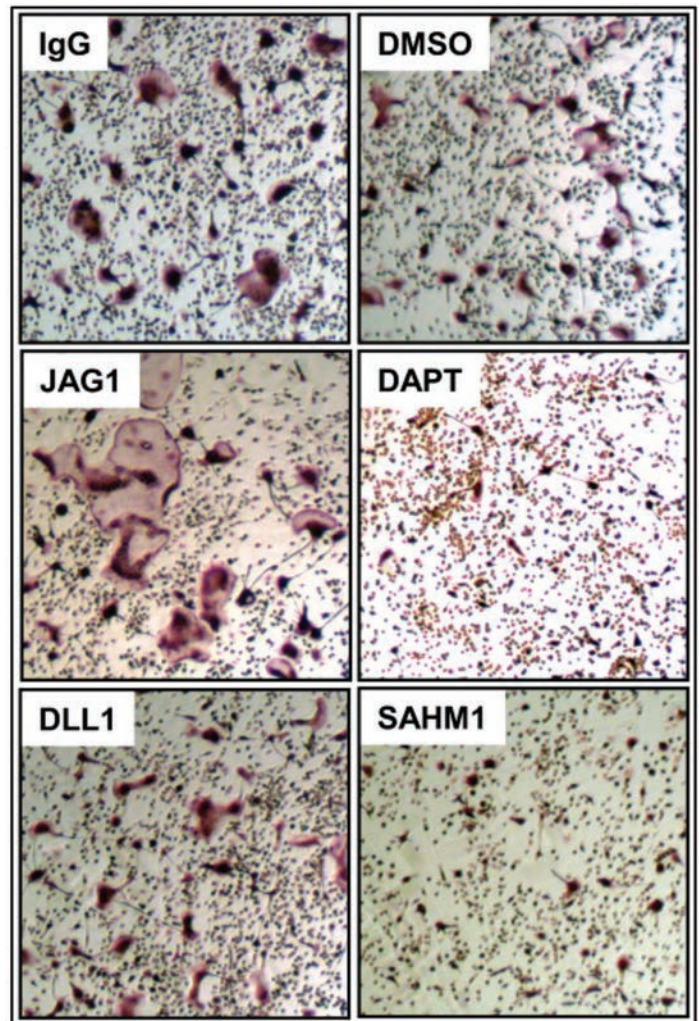


Figure 2. Osteoclastogenesis under Notch stimulation and inhibition.

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