Notch Signaling in Osteoclasts is Essential for Resorption, but Dispensable for Osteoblast Coupling

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Introduction

Bone loss due to excessive osteoclast resorptive activity is a major source of patient morbidity and mortality. In addition to their role as bone resorbing cells, osteoclasts promote the differentiation of bone-forming osteoblasts. This coupling role is apparent in both genetic and pharmacologic suppression of the osteoclast numbers, both of which result in a concomitant decrease in the number of osteoblasts. Conversely, approaches that inhibit osteoclast activity while preserving the osteoclast number preserve osteoblast function. Notch signaling plays a crucial role in osteoclast maturation and function. Our lab has previously demonstrated that stimulating the Notch pathway in committed osteoclasts precursors results in large multinuclear cells with increased resorptive activity whereas chemical inhibition of the same caused smaller osteoclasts with impaired resorption. Herein, we investigated osteoclast resorptive and coupling functions in the context of genetically inhibited Notch signaling.

Methods

Notch activation requires cleavage of its intracellular domain NICD to translocate into the nucleus and interact with co-activators such as Mastermind-like1 (MAML) for transcriptional activation. Wild type (WT) [LysM-Cre/− dnMAML−/−] and dnMAML [LysM-Cre/−dnMAMLMye+/−] mice were utilized in this study. dnMAML mice express a dominant negative form of MAML that inhibits the transcriptional complex. Furthermore, this dnMAML expression is restricted to myeloid lineage cells, which include osteoclasts and their precursors. Cells from tibial and femoral bone marrow of both wild type and dnMAML+ mice were isolated and cultured for osteoclast differentiation with Monocyte/Macrophage Colony-Stimulating factor (MCSF) and mouse Receptor Activator of Nuclear Factor kβ Ligand (RANKL). The osteoclasts were later used for tartrate-resistant acid phosphatase (TRAP) staining, gene expression analysis using quantitative PCR (q-PCR) and functional assays such as bovine cortical bone resorption and osteoblast stimulation. Osteoblast precursors from wild type mice were cultured in presence of condition media obtained from both wild type and dnMAML osteoclasts separately. Both alkaline phosphatase activity and mineralization content were determined in the osteoblasts. All mice were maintained as per the guidelines issued by Institutional animal care and use Committee (IACUC) of the University of Pennsylvania.

Results

Using the dnMAML+ osteoclasts we observed that inhibition of Notch signaling results in osteoclasts precursors that fail to mature and function without significant alterations in early osteoclastic gene expression. Osteoclasts from the dnMAML group showed defect in maturation process and were smaller in size and fewer in number compared to the wild type (Figure 1A). The functionality of dnMAML+ cells upon stimulation with Notch signaling by Jagged-1 showed down-regulation of Hes1 (marker for Notch activation) compared with the wild type which is consistent with suppressed Notch

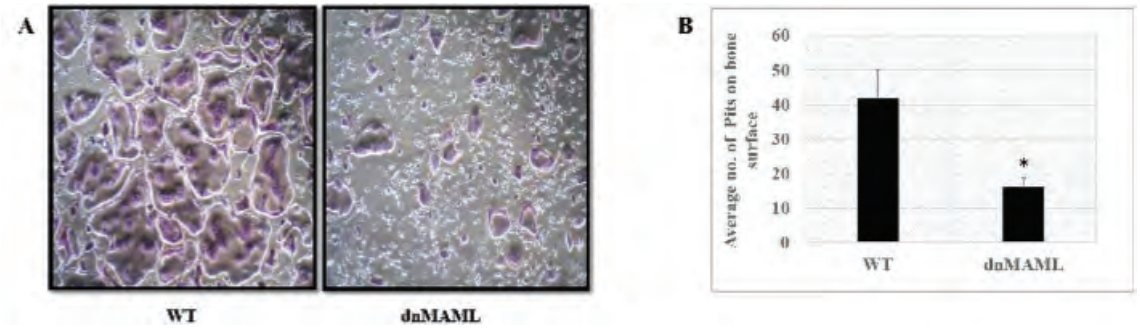

Figure 1. Reduced osteoclast maturation and resorptive function by dnMAML expressing osteoclasts. **(A)** Osteoclasts from the WT are giant and fully mature while the osteoclasts from Notch defective mice (dnMAML) fail to fuse effectively and remain immature using TRAP stain (purple). **(B)** The osteoclast precursors from both WT and dnMAML mice were differentiated on bone slices, stained using toluidine blue and quantified. dnMAML osteoclasts shows significant lesser number of resorption pits (*p<0.05).

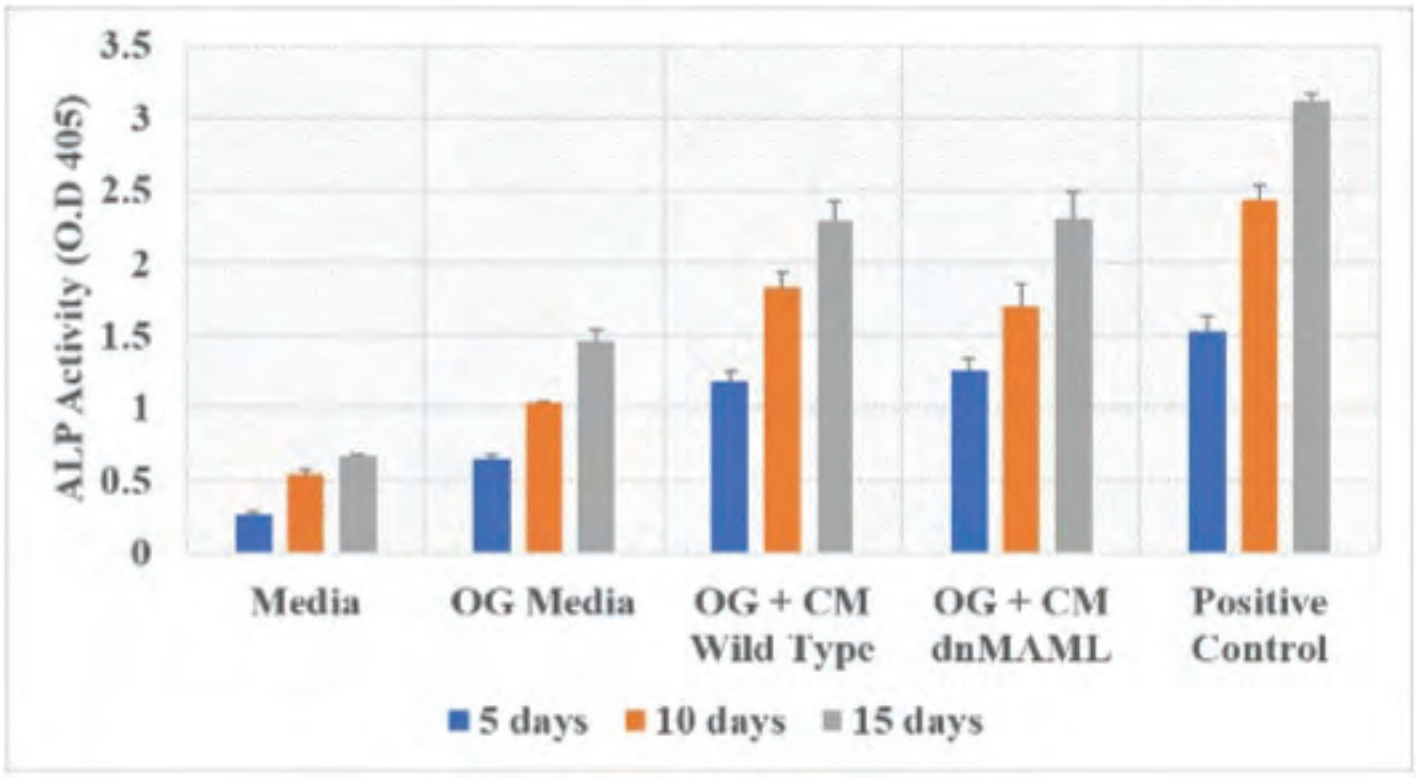

Figure 2. dnMAML osteoclasts retain their osteoblast stimulating ability. Cultured osteoblast precursors were cultured under osteogenic conditions in the presence of unconditioned medium or medium conditioned by WT or dnMAML osteoclasts. WT and dnMAML osteoclast conditioned medium similarly enhanced osteogenesis. OG media is osteogenic media while positive control is OG media + bone morphogenetic protein.

signaling. Next, functional characterization of genetically modified osteoclasts (dnMAML+) was performed using bone slices. dnMAML expressing osteoclasts showed significantly lower resorption and formed fewer resorption pits compared to the wild type (Figure 1B). dnMAML+ showed lesser TRAP positivity using the bone slices and were found to be smaller and immature. TRAP activity in the condition media collected at regular intervals from osteoclasts seeded over bone slices showed marked reduction in the enzymatic activity due to lesser bone resorption by dnMAML+ osteoclasts. Lastly, no significant difference was observed in alkaline phosphatase activity (Figure 2) and mineralization ability of wild type osteoblasts stimulated with either WT or dnMAML osteoclast conditioned medium.

Discussion

In our present work, we have demonstrated that osteoclasts derived from dnMAML mice showed no significant differences in early osteoclastic gene expression compared to wildtype. However, osteoclasts with defective notch signaling had decreased TRAP production and decreased bone resorption. Lastly, dnMAML+ osteoclasts preserved osteoblast function and activity. These observations suggest that inhibition of Notch signaling impairs osteoclast maturation rather than early commitment and gene expression. Importantly, this suggests that the regulation of Notch signaling could allow for the persistence of immature, weakly resorbing osteoclasts that retain their osteoblast-stimulating activity.

Significance

The current work provides a mechanistic insight into osteoclast function and important pre-clinical data informing potential use of Notch signaling inhibition in osteoclasts to improve bone mass.