

# Osteopontin-mediated Cyclo-oxygenase 2 Expression in Osteoclasts

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## INTRODUCTION:

Bone homeostasis is dependent on a precise balance of osteoclast-mediated bone resorption and osteoblast mediated bone replacement. An imbalance in osteoclast-mediated bone resorption can result in osteoporosis and lead to a greater incidence of bone fractures. Most osteoporosis therapies are designed to limit osteoclast formation and activity. Prior research also indicates that depletion of osteoclasts can impair fracture healing (Lin & O'Connor 2017). Further, cyclooxygenase-2 (COX-2) is normally expressed in bone and fracture callus osteoclasts (Lin & O'Connor, 2014) and COX-2 is an essential factor in proper and timely fracture healing. Therefore, the role of COX-2 in osteoclast activity and the potential role of COX-2 expressed from in osteoclasts in fracture healing are areas of significant research interest. This study investigated pathways which can induce COX-2 expression in osteoclasts.

## METHODS:

The murine macrophage cell line, RAW 264.7, or bone marrow monocytes (BMM) harvested from hind limbs of 8 to 16-week old mice were subjected to directed osteoclast differentiation in vitro using RANKL (and M-CSF for BMM primary cultures). Osteoclast cultures were also treated with osteopontin (OPN), vehicle (PBS) as a negative control, and LPS as a positive control. The cultures were then subjected to a variety of experimental procedures to measure COX-2 expression (promotor activity and protein levels) as well as osteoclast differentiation and resorption activity. For osteoclast differentiation, the cultures were stained for TRAP and the number and size of osteoclasts determined. COX-2 promoter activity was measured using a luciferase reporter. Protein expression was assessed by immunofluorescence and western blot. For immunofluorescence, osteoclast cultures were stained with anti-COX-2 antibodies, phalloidin and DAPI. COX-2 expression was measured in individual osteoclasts via fluorescent microscopy. For resorption activity, the osteoclast cultures were grown on calcium phosphate coated plates; cells were removed at the endpoint, and the number and size of resorption pits analyzed. For statistical analyses t-tests and/or one-way ANOVA were employed. This study was approved by the Rutgers University IACUC.

## RESULTS SECTION:

Little or no COX-2 expression was evident in osteoclasts cultured on TCP treated with (or without) vehicle. In a preliminary screening, several bone-related proteins (fibronectin, osteonectin, osteopontin, periostin and vitronectin) were tested for their ability to induce COX-2 expression in osteoclasts. All but vitronectin induced COX-2 expression. OPN was chosen for further investigation of osteoclast COX-2 expression. At 100, 200 and 300 nM concentrations, OPN induced significant increases in COX-2 expression in osteoclasts in vitro as assayed by immunofluorescence ( $p \leq 0.001$ ; **Figure 1**). By western blot, COX2 expression increased significantly after treatment with 200 or 300 nM OPN ( $p \leq 0.001$  &  $p = 0.007$ , respectively; **Figure 1**). Moreover, OPN increased *Ptgs2* (i.e., COX-2 gene) promoter activity 3.6-fold via luciferase assay ( $p \leq 0.001$ ). Integrin  $\beta 3$  is a known OPN receptor. COX-2 induction by OPN was significantly decreased ( $p \leq 0.001$ ) in osteoclasts from BMM cells of mice with a targeted deletion of integrin  $\beta 3$  (Lyz2-Cre /floxed integrin  $\beta 3$ ). OPN treatment also increased osteoclastogenesis in normal BMM cells as indicated by increased numbers of osteoclasts in culture visualized through TRAP staining ( $p = 0.005$ ; **Figure 2**).

## DISCUSSION:

Most studies have focused on the role of osteoclasts bone matrix degradation. In this study, we demonstrated that OPN can induce COX-2 expression in osteoclasts and increase osteoclastogenesis in vitro, suggesting an autocrine or paracrine role for COX-2 in promoting osteoclastogenesis. This is an important distinction that may help identify new therapeutic approaches to treat bone injury and disease. Further investigation into OPN mediated COX-2 regulation of osteoclast activity is underway to better understand the role of osteoclast COX-2 expression in osteoclast formation, function, and bone fracture healing.

## SIGNIFICANCE/CLINICAL RELEVANCE:

Our data suggests that osteoclast expression of COX-2 is an important factor in osteoclastogenesis and can be regulated through OPN signaling, at least in vitro. The study results suggest that regulating osteoclastic COX-2 expression as a therapeutic approach may be useful to augment fracture healing or to diminish bone loss in patients with osteoporosis.

## IMAGES AND TABLES:

