Osteoclast inhibition reduces fibrosis expansion and alters cell phenotype in murine model of fibrosis dysplasia (FD)

Yongxing Zhang[1,2], Renee Ormsby[1], Kelly Tsang[1], Felix Killick[1], Julia F. Charles[1,2]

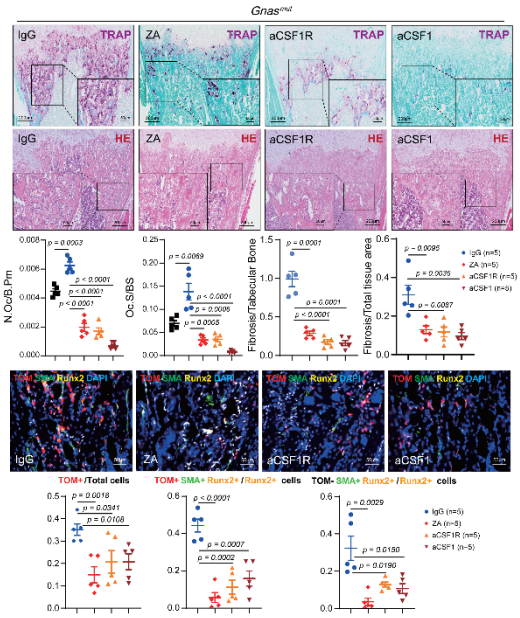
[1] Department of Orthopaedic Surgery, Brigham and Women's Hospital and Harvard Medical School [2] Department of Medicine, Brigham and Women's Hospital and Harvard Medical School

DISCLOSURE: Yongxing Zhang (N), Renee Ormsby (N), Kelly Tsang (N), Felix Killick (N), Julia F. Charles (N)

INTRODUCTION: Fibrous dysplasia (FD) is a rare disorder caused by somatic activating mutations in the GNAS gene, encoding the alpha subunit of the Gs protein. This results in focal expansile bone lesions which cause pain and have an increased risk of fracture. FD lesions are also enriched in osteoclasts (OCs). One hypothesis for FD pathogenesis is that pathological osteoclast-osteoblast coupling drives expansion of WT osteoblastic precursors and inhibits the maturation and mineralization of these cells. Using the *Gnas*$^{R201Hfl/+;}$ *Rosa26*$^{Tdtomato;}$ *Sox9*^{CreER} mouse model of FD, we have previously shown that the mosaic fibrous bone lesions caused by post-natal induction of mutant *Gnas* expression are characterized by accumulation of wild-type SMA+ fibrotic cells and can be prevented using neutralizing antibody to RANKL (aRANKL), which eliminates OCs. To ask whether the effectiveness of aRANKL treatment is mediated by elimination of OCs or by inhibition of non-OC functions of RANKL which have recently been described, we tested whether zoledronic acid and blocking antibodies to the receptor of another key cytokine for OC formation, CSF1R and CSF1, is also able to reduce expansion of WT SMA+ cells and fibrotic bone lesions.

METHODS: Animal experiments were approved by the BWH IACUC. *Gnas*$^{R201Hfl/+;}$ *Rosa26*$^{Tdtomato;}$ *Sox9*^{CreER} mice were treated with tamoxifen (40mg/kg) at P5, resulting in fibrotic lesions. *Gnas*$^{R201Hfl/+;}$ *Rosa26*$^{Tdtomato;}$ *Sox9*^{CreER} pups were then treated with either zoledronic acid (ZA, 0.2mg/kg), aCSF1R/aCSF1 or IgG2a control (25mg/kg) at P5 and P13 (n=5) or at P6-20 every two days (n=5). Mice were euthanized at P21 and long bones were excised for micro-CT analysis and histology. Femurs from both groups were stained using H&E to detect the stromal and fibrotic cells in the bone marrow and tartrate-resistant acid phosphatase activity (TRAP) staining to detect TRAP+ multinucleated osteoclasts lining the trabecular bone. Fibrosis was quantified by manually tracing around the cells in the 500µm region below the growth plate (QuPath, version 0.5.1). Mutant *Gnas* expressing cells are distinguished by the Tomato lineage tracer. Immunocytochemistry was performed to detect αSMA+ and Tomato + cells; cells were counted. Statistical analysis was by the Mann Whitney non-parametric t test where $p<0.05$ is considered significant.

RESULTS: 1) Gnas$^{fl/+}$ mice developed fibrotic lesions; H&E staining showed a significant reduction in stromal cells contributing to fibrosis within the ZA, aCSF1R/aCSF1 treated mice. 2) Anti-osteoclast therapy treated mice showed a significant reduction in TRAP+ osteoclasts. 3) Anti-osteoclast therapy treated mice showed a reduction in both mutant and wildtype SMA+ cells by immunofluorescence as predicted in this model.



DISCUSSION: Anti-osteoclast therapy showed promising results, reducing fibrosis as well as the number of mutant and wild-type SMA expressing stromal cells. aCSF1R/aCSF1 treatment could be an alternative approach for treatment of bone lesions in fibrous dysplasia. Further investigation is required to determine the role of osteoclast-osteoblast coupling in this model.

SIGNIFICANCE/CLINICAL RELEVANCE: This study suggests that the treatment effect of RANKL inhibition is primarily mediated by osteoclast inhibition rather than non-OC functions of RANKL and underscores the important role of OCs in the formation of bone lesions in fibrous dysplasia.