Osteopathy in Gorham-Stout Disease Animal Model

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INTRODUCTION: Gorham-Stout Disease (GSD) is a rare condition associated with abnormal lymphatic invasion into bone leading to massive bone loss. To date, the mechanism of bone loss identified in GSD remains to be elucidated. Most recently, a lymphatic-specific KRAS somatic activating mutation was identified in GSD patients. A mouse model recapitulating this mutation in Prox1 expressing lymphatic cells showed detrimental lymphatic valve formation. To better understand the abnormal bone loss, present in GSD patients, we generated a tamoxifen-induced lymphatic endothelial cell KRAS somatic activating mutation in mice and characterized their skeletal phenotype.

METHOD: Prox1-CreER^{T2} expressing mice were bred with KRAS$^{LSL\text{-}G12D}$ mice to create KRAS inducible somatic activating mutation in lymphatic endothelial cells (iLEC^{KRAS}) mouse line. KRAS mutation was induced on P0 and P7 in iLEC^{KRAS} mice using tamoxifen treatment. After 4-6 weeks of age, mice were assessed for height and weight followed by collection of bone and soft tissue. Before termination, mice were assessed using Lunar PIXIMUS DEXA scan. Tissues were assessed histologically for lymphatic vessels while bone was examined through micro-CT analysis. Isolated bone marrow cells were purified for culture of osteoclasts. Osteoclast differentiation and function were assessed through TRAP activity and resorption pit assays. RT-qPCR was assessed from frozen bone tissue of both wild-type (WT) and iLEC^{KRAS} mice for bone related markers. Data were analyzed using GraphPad Prism 9 Software and presented as the mean ± SEM.

RESULTS SECTION: iLEC^{KRAS} mutant animals exhibited decreased body weight compared to WT littermates. In addition, we observed extensive pleural effusion in the thoracic cavity in comparison to WT littermates. Soft tissue immunohistochemical analysis of LYVE-1 (lymphatic endothelial cell marker) revealed lymphatic vessel invasion into the kidneys. Subsequently, bone mineral density, content, and area were all found to be decreased in iLEC^{KRAS} mutants compared to WT mice. These results were also observed in iLEC^{KRAS} mutant spine, femur, and tibia compared to WT controls. In addition, a decrease in total fat and lean muscle mass was also observed. Further, assessment of bone mRNA expression showed a decrease in Runx2 and Collagen Type I in iLEC^{KRAS} mutant compared to WT mice. However, the expression of osteoclast-related markers (TRAP, DC-Stamp, and Cathepsin-K) was increased in iLEC^{KRAS} mutants compared to WT animals. When assessing bone marrow-derived macrophages *in vitro*, we found a decrease in cell proliferation associated with decreased total osteoclast count in iLEC^{KRAS} mutant compared to WT mice. Interestingly, TRAP activity per osteoclast was increased in iLEC^{KRAS} mutant compared to WT mice.

DISCUSSION: Our findings show a lymphatic specific KRAS mutation lead to a decrease in bone mass *in vivo*. This mutation induces cell autonomous changes in osteoclast differentiation *ex vivo*. For this reason, we predict KRAS mutant lymphatic endothelial cells actively secrete bone regulating factors capable of modulating bone homeostasis. In conclusion, our study is the first to report abnormal bone phenotype found in iLEC^{KRAS} mutant mice. Future studies are directed to identify lymphatic secreted factors responsible for changes in osteoclast differentiation and function, and bone resorption observed in the GSD mouse model.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides a platform for the investigation of lymphatic KRAS mutation and its association with the osteopathic phenotype observed in GSD patients.

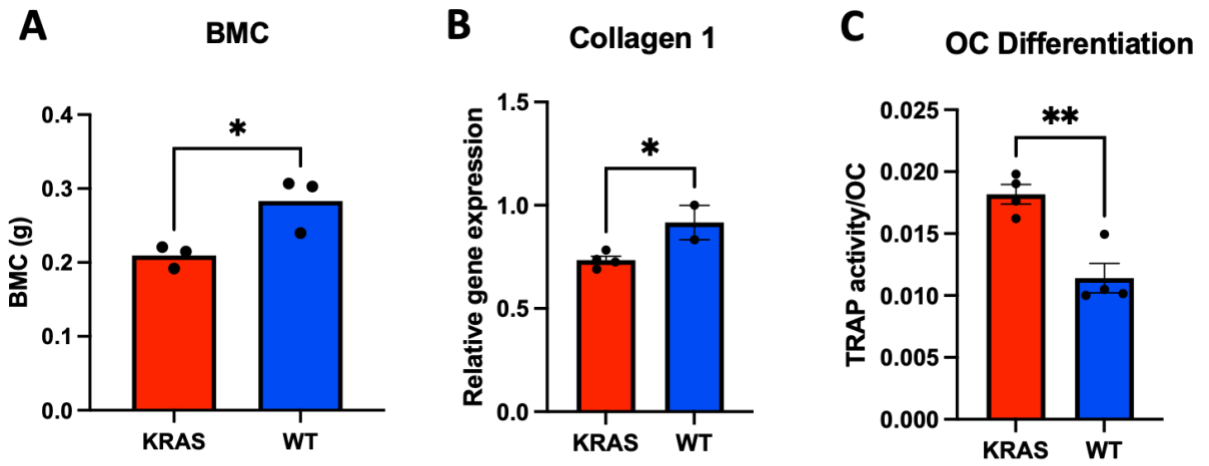

Figure 1. Osteopathy in iLEC^{KRAS} GSD animal model. Lymphatic specific KRAS activating mutation led to a decrease in bone mineral content (BMC) in iLEC^{KRAS} when compared to wild-type (WT) mice (**A**). Bone mRNA expression of collagen Type I (Collagen 1) was lower in iLEC^{KRAS} When compared to WT littermates (**B**). TRAP activity per osteoclast (OC) was higher in KRAS animals when compared to WT (**C**). N=3. *P<0.05, **P<0.01 compared to WT mice. Data presented as mean ± SEM.