Sprouty4: A Key Regulator of Skeletal Stem Cells in Bone Homeostasis and Healing

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INTRODUCTION:
Skeletal stem cells perform bone maintenance and repair, giving rise to the terminally differentiated cell types that comprise the skeleton including osteolineage cells and adipocytes. The fibroblast growth factor mitogen-activated kinase (FGF-MAPK-ERK) pathway plays crucial roles in stem cell proliferation, quiescence, and differentiation across many organs, including regulating osteogenic and adipogenic differentiation. Despite the critical and widespread roles of the FGF/ERK pathway, there is currently limited information on the pathway's spatiotemporal signaling dynamics in bone and how it is regulated. In this study, we aimed to precisely characterize FGF-MAPK-ERK activity in bone at single cell resolution, how this is impacted during aging, and if perturbation of this pathway affects skeletal stem cell function.

METHODS:
We used a fluorescent reporter mouse line carrying an *H2B-Venus* fusion knocked into the mouse Sprouty4 (*Spry4)* locus, a direct transcriptional target of the FGF-MAPK-ERK signaling which acts as a readout of signaling activity, combined with high resolution laser scanning confocal microscopy to define the spatial pattern of FGF-MAPK-ERK signaling in young and aged mice. The Spry4 allele is a hypomorph, enabling us to also examine the impact of perturbing the FGF-MAPK-ERK pathway on bone homeostasis and fracture healing. Our methods included DEXA and microCT analyses of femurs, flow cytometry to evaluate the effect of *Spry4* loss on skeletal stem cell populations and proliferation, as well as *in vitro* osteogenic and adipogenic differentiation assays. Statistical analysis was performed using both ANOVA and t-test to assess the significance of differences among multiple groups and between paired groups, respectively

RESULTS:
Our findings revealed the significance of *Spry4* in bone physiology. Single-cell RNA sequencing revealed high *Spry4* expression in *Lepr*+ cells, a population of skeletal stem cells. Moreover, we observed an increase in trabecular bone within the femoral bone marrow cavity in the *Spry4* hypomorphic mouse model. Notably, these mice exhibited an increased bone volume/total volume ratio following femur fracture, indicating improved healing. Our results suggest that *Spry4* exerts an influence on stem cells by modulating their proliferation, as assessed by flow cytometry and colony-forming units formation, and their differentiation, as indicated by alizarin red and oil red staining.

DISCUSSION:
The results suggest a significant role for Spry4 as a regulator of FGF-MAPK-ERK signaling in bone physiology, particularly in skeletal stem cells. This study paves the way for a deeper understanding of the mechanisms underlying the action of Spry4 and FGF signaling on skeletal stem cells in bone. This investigation also raises the possibility of FGF/Spry4 as a potential target for improving bone healing following fractures.

SIGNIFICANCE/CLINICAL RELEVANCE:
The study's findings hold clinical relevance as they shed light on the critical role of Spry4 in bone health and healing. With the increasing incidence of bone fractures in aging populations, understanding the mechanisms involved and potential therapeutic targets like FGF/Spry4 becomes increasingly important for improving fracture outcomes.

Fig. Micro-CT analysis of trabecular bones from 2-month-old mice, WT (A) vs Spry4$^{H2B\text{-}Venus/H2B\text{-}Venus}$(B)