Salt inducible kinase (SIK) inhibition induces chondrogenesis during fracture repair in a mouse model

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INTRODUCTION: Over 20 million people in the United States have osteoporosis, which causes 1.5 million fragility fractures each year[1]. Current bone-anabolic treatments, such as intermittent parathyroid hormone (PTH) administration, effectively stimulate bone formation but is limited by the need for daily subcutaneous injections[2]. Antiresorptive drugs focused on the inhibition of osteoclastic bone erosion, although modestly improve bone mineralization, are often discontinued after 3-5 years due to associated side effects[3]. These challenges have driven the search for orally available bone anabolic agents that can similarly enhance bone formation without the limitation of subcutaneous injections or therapy discontinuation. Salt-inducible kinases (SIKs), specifically SIK2 and SIK3, represent attractive targets, as they act downstream of the PTH receptor and their suppression genetically or through an orally administered small molecule inhibitor (i.e. SK-124) increases bone mass in mouse models[4]. In this study, we investigated the potential bone anabolic effects of SK-124 in a mouse model of stabilized fracture healing. We evaluated the effects of SK-124 on mice that we previously showed to develop fibrotic nonunion[5] (due to activation of hedgehog signaling in muscle fibroadipogenic progenitors i.e. *Clec3b*$^{cre.ERT2/+}$*; R26*^{SmoM2}), and their wild-type littermates.

METHODS: All experiments were approved by IACUC.
Mice: *Clec3b*^{CreERT2} knock-in mice were developed with CRISPR-Cas9. *R26*$^{SMO/EYFP/+}$ mice (that conditionally express a Smoothened transgene resulting in constitutive activation of hedgehog signaling) were purchased from Jackson Laboratories. Between 3 and 4 weeks of age, *Clec3b*^{CreERT2}*; R26*$^{SMO/EYFP/+}$ female mice (MUT) and their Cre-lacking littermates (WT) received 3x tamoxifen (75mg/kg) intraperitoneally (WT_VEH n=6; WT_SK-124 n=3; MUT_VEH n=3; MUT_SK-124 n=4). Due to limitations in SK-124 availability and mouse breeding, we prioritized using female mice for this initial phase of the study. We plan to include male mice in future experiments to assess potential sex-specific effects.
Stabilized Fracture Repair: Six weeks after tamoxifen injections, mice underwent stabilized fracture surgeries. Under anesthesia, the right femoral diaphysis was subjected to full osteotomy with a high-speed drill. The fractured bones were stabilized with a sterile intramedullary pin. The correct alignment of fractured bone was confirmed with X-ray. Starting 3 days after surgeries, *Clec3b*$^{CreERT2/+}$*; R26*$^{SMO/EYFP/+}$ and *Clec3b*$^{+/+}$*; R26*$^{SMO/EYFP/+}$ mice were administered with either SK-124 (40 mg/Kg in 15% hydroxypropyl β-cyclodextrin) or vehicle every day for 3 weeks via oral gavage. Following euthanasia, both the fractured and contralateral intact femora were collected for analysis.
μ*CT Analysis:* Scans (μCT 45, Scanco Medical, Switzerland) were performed at 7.4 μm voxel size. Callus mineralization was determined at 7.4μm resolution. The region spanning 1 mm (135 slices) on either side of the fracture line was selected as the region of interest (ROI). In intact femora, proximal diaphysis (~2mm) and distal metaphysis (~1.7 mm) were analyzed for trabecular and cortical parameters.
Histology: Following micro-CT imaging, bone samples were decalcified in EDTA and frozen in OCT for standard histology with hematoxylin & eosin and safraninO stains. Imaging was conducted using Zeiss Axioscan7 Slide Scanner.
Statistical Analysis: All comparisons were made in R, using one way ANOVA with post-hoc Tukey testing.

RESULTS SECTION: Analysis of intact femora showed that mice in the SK-124 treatment groups had increased trabecular bone density (p=0.0004, p=0.0009) and trabecular thickness (p=0.001, p=0.0005) compared to mice that received vehicle regardless of genotype, confirming the osteoanabolic effects of the treatment[4] (Figure 1). However, we did not observe significant changes in diaphyseal cortical thickness of the intact femurs.

Consistent with our previous findings, *MUT* mice developed significantly (p=0.027) smaller calluses (TV) than their wild-type littermates (Figure 1). However, SK-124 treatment did not increase bone volume or bone volume fraction (BV/TV, p=0.9067) in either MUT or WT mice. Histologic analysis revealed that, administration of SK-124 significantly increased the cartilage content (p=0.0061, p=0.0096) in the fracture calluses of both mouse groups (Figure 2) and fibrosis (p=0.016) in MUT mice.

DISCUSSION: Our results confirm that orally administered SK-124 increases bone mass in uninjured mouse long bones. However, SK-124 did not similarly boost mineralization in fracture calluses in either wild-type mice or a genetically induced model of fibrotic nonunion. The significant amount of cartilage we detected in both treatment groups is unusual, as cartilage is a transient tissue in endochondral healing of long bone fractures, typically formed between 7 and 14-days post-fracture and replaced with bone by 21 days. Loss of both SIK2 and SIK3 in mice results in the expansion of the growth plate[4]; thus, SIK-inhibition likely stimulates chondrogenic activity (either through increased proliferation or prolonged hypertrophy) in addition to enhancing bone formation, resulting in persistence of cartilage in the fracture callus. Our future work will focus on determining whether delayed administration of SK-124 (initiated after the cartilaginous callus begins to calcify) could improve fracture repair.

SIGNIFICANCE/CLINICAL RELEVANCE: SIK-inhibition is a novel therapeutic strategy that could potentially improve fracture healing in addition to reducing fracture risk.

REFERENCES: (1) Cummings SR et al., *Lancet*. 2002; (2) Reid I., *Lancet* 2022; (3) Camacho P et al., 2016; (4) Sato T., 2022; (5) Aydin et al., ORS 2025. (6) US Preventive Services, 2018; (7) Morin SN, 2025.

IMAGES AND TABLES:

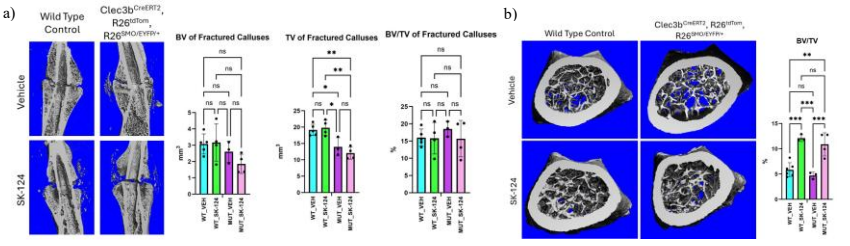

Figure 1: μCT analysis demonstrates (a) no significant difference in the callus mineralization (p=0.7601) and (b) significant increase (p=0.0004, p=0.0009) in trabecular bone density of intact femora (ns: p>0.05, * p<0.05, ** p<0.01, *** p<0.001)

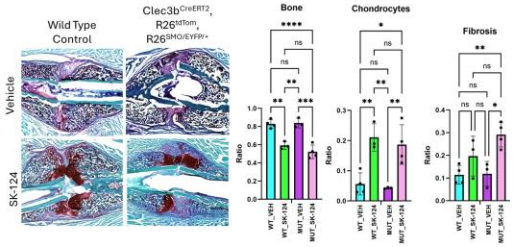

Figure 2: SafraninO-stained images and histological quantification show that SK-124 increases fibrosis (p=0.016) and chondrogenesis (p=0.0061, p=0.0096) in the fracture callus (ns: p>0.05, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).