Salt Inducible Kinase Inhibitors as a Molecular Approach to Fracture Repair

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Purpose: Over 6.8 million fractures are reported annually in the US, accounting for 20% of all musculoskeletal injuries. Approximately 10% of fractures will experience delayed union or nonunion. Parathyroid hormone (PTH) analogues, anabolic osteoporosis therapeutics, have shown efficacy in stimulating fracture repair in animal models and in patients with chronic nonunion. Previous studies have demonstrated that small molecule salt-inducible kinase (SIK) inhibitors mimic PTH action in vitro and in vivo. Therefore, we hypothesize that YKL-05-099, a small molecule SIK inhibitor, will accelerate fracture callus osteogenesis.

Methods: 126 nine-week-old male C3H/HeJ slow fracture healing mice underwent unilateral intramedullary femoral shaft pinning, followed by a midshaft fracture. The animals received daily 0.1-mL subcutaneous injections of vehicle (phosphate-buffered saline), YKL (15 mg/kg), or PTH 1-34 (100 μg/kg). Animals were euthanized at 10, 14, and 21 days postoperatively. The callus tissues were assessed through RT-qPCR (real-time polymerase chain reaction) to detect fracture repair marker genes. Additionally, the early timepoints were evaluated histologically and through Single-Cell RNA-Seq. Micro-CT images were obtained for image-based callus analysis. Three-point bending testing was conducted on a group of femurs to evaluate tissue biomechanics.

Results: YKL and PTH-treated mice had higher mineralized callus volume fraction (BV/TV) than vehicle-treated mice at all time points (Fig. 1A). This difference was statistically significant when compared to the control group at 14 days (Fig. 1B) but not at 21 days. YKL-treated callus tissue had increased mineral density than the vehicle group at all timepoints, reaching significance at day 21. CT-based structural rigidity analysis demonstrated improved torsional rigidity in mice treated with both PTH and YKL (Fig. 1C). YKL- and PTH-treated animals had more osteoblasts and faster callus bridging than vehicle-treated mice (Fig. 1D). Single-cell RNA-seq analysis of callus tissue revealed the presence of multiple molecularly defined subsets of mesenchymal lineage cells (Fig. 1E). Compared to vehicle, YKL therapy decreased chondrocyte-like precursors and enhanced osteoblast subsets (Fig. 1F).

Conclusion: This study demonstrates that YKL treatment enhances fracture repair in mice. These results support further development of potent and selective small molecule SIK inhibitors to meet this unmet clinical need. As an innovative alternative to the limited options available today, the proposed bone targeting through YKL-05-099 retains efficacy in callus formation and represents a paradigm shift in fracture repair management.

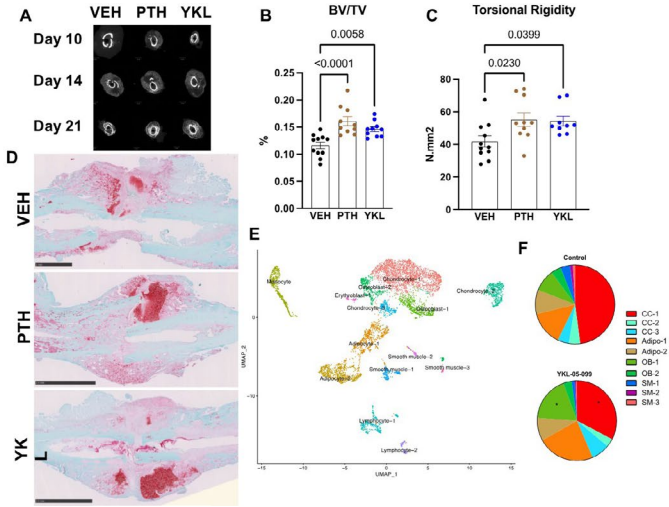

Figure 1. C3H/HeJ (slow-healing) mice were subjected to femur fracture followed by micro-CT over time. Panel **A** shows increased callus mineralization in response to PTH and YKL-05-099 treatment. Panel **B** shows callus mineralization at day 14, and panel **C** shows torsional rigidity (measured by CT-based rigidity analysis). YKL-05-099 treatment increases mineralization and strength versus vehicle. Panel **D** shows safranin O-stained callus histology at day 14. In panel **E**, callus tissue at day 14 was isolated for single cell RNA-seq to identify distinct groups of cells associated with fracture healing. In panel **F**, relative proportion of non-immune cells was determined in distinct animals. YKL-05-099 treatment reduces the relative proportions of cells in chondrocyte