

The Role of Focal Adhesion Protein Kindlin-2 in Osteocytes during Distraction Osteogenesis

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INTRODUCTION: Distraction Osteogenesis (DO) effectively stimulates the formation of fresh bone tissue through slow distraction, which has been widely used in orthopedic clinics. However, the molecular mechanisms whereby extracellular mechanical stress signals translate into intracellular biochemical signals during the DO process and promote bone formation remain poorly defined. It is known that osteocytes buried in the bone matrix play an important role in mediating mechanical loading induction of osteogenesis. Kindlin-2, as a key molecule in focal adhesion, plays an important role in regulating bone mass and bone formation. Importantly, we also demonstrated that deleting Kindlin-2 expression in osteocytes impairs the mechanical loading-stimulated bone formation. In this study, we hypothesize that Kindlin-2 may play a vital role in osteocytes during DO procedure.

METHODS: In this study, a custom-made unilateral external fixator was applied on the mouse DO model. Wide-type C57BL/6 mice were used to establish DO model and to determine the dynamic expression of Kindlin-2 during DO procedure. The DO protocol consisted of 5 days of latency, 10 days of distraction at a rate of 0.3 mm daily and several durations of consolidation. Femoral samples were harvested 5, 10, 15, 28, 42 days after the surgery (POD) for micro-computed tomography (μ CT), histology, immunohistochemistry, and in situ hybridization to determine the expression of Kindlin-2 and osteogenic markers. Then, specific knockout of Kindlin-2 in osteocyte transgenic mice, *Dmp1^{Cre/+}; Kindlin-2^{fl/fl}*, were subjected to the same DO procedure as above. Cre-negative *Kindlin-2^{fl/fl}* mice are regarded as controls. In addition to the aforementioned assessments, ELISA examination was conducted to identify specific osteogenic-related cytokines in serum that were affected by Kindlin-2 deficiency. One-way ANOVA test, Pearson correlation analysis and Two-way ANOVA test were performed to compare differences among five groups. The animal experiments were approved by the Animal Research Ethics Committee of The Chinese University of Hong Kong (AEEC No. 22-213-NSF) and the Southern University of Science and Technology (SUSTech-JY202305006).

RESULTS SECTION: We observed that the expression of Kindlin-2 in osteocytes gradually increased in the mid-to-post distraction period but began to decline in the consolidation period through the immunohistochemistry and in situ hybridization examinations. Furthermore, the expression levels of osteogenic markers also positively correlated with Kindlin-2 expression. Interestingly, μ CT analyses of affected femurs revealed that the accumulation of bone volume and bone mineral density (BMD) in the distraction regenerate of *Dmp1^{Cre/+}; Kindlin-2^{fl/fl}* mice were significantly lower than the control group. The concentration of procollagen type 1 N-terminal propeptide (PINP) in peripheral blood was repressed in the Kindlin-2 specific knockout mice.

DISCUSSION: Osteocytes are the most abundant and long-lived cells in bone, which are not only involved in endocrine regulation and calcium/phosphate metabolism but also sense and respond to mechanical stimuli. Osteocytes express various mechano-sensing focal adhesive molecules, while Kindlin-2 is a key molecule in osteocytes that modulates bone regeneration during the DO procedure. Our studies described here show that the deficiency of Kindlin-2 in osteocytes may influence the healing outcome of DO. However, further investigations are warranted to fully understand the molecular mechanism. In conclusion, these findings offer significant insights into the relationship between Kindlin-2 and the mechanical tension that facilitates bone regeneration in distraction osteogenesis. Such insights may pave the way for the creation of innovative approaches that aim to target Kindlin-2, thereby enhancing DO clinical outcomes.

SIGNIFICANCE/CLINICAL RELEVANCE: The knowledge gained through these findings offers valuable insights into the molecular links of Kindlin-2 in mechanical tension-mediated bone regeneration in DO. Furthermore, it may lead to the further development of new strategies for targeting Kindlin-2 to improve outcomes of DO clinical applications with reduced costs to healthcare providers, pain and suffers to patients and their families.

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IMAGES AND TABLES:

