Inhibition of Piezo1 Improves Tendon Healing

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INTRODUCTION: Tendon injuries represent one of the most common musculoskeletal disorders. Both endogenous tendon-derived stem cells (TDSCs) and biomechanical environment play essential roles in tendon homeostasis and regeneration. The stem cells can convert mechanical signals into biological signals but how they cooperate during tendon healing remains elusive. Piezo1 is a mechanosensitive ion channel that can translate different kinds of mechanical stimuli into biological signals. Recent study reports that Piezo1 enhances tendon stiffness through modifying collagen cross-linking, while knockout of Piezo1 reduces tendon stiffness. However, no study has investigated the role of Piezo1 during tendon healing process yet. In the present study, we attempted to identify the spatial and temporal expression of Piezo1 after tendon injury created at the Achilles of rats, and to demonstrate whether regulating Piezo1 activity improve tendon healing. We tested the hypotheses that Piezo1 expression/ activity may increase after tendon injury, and inhibiting Piezo1 activity by GsMTx4 (a peptide as Piezo1 blocker) may alleviate the mis-differentiation of stem cell during tendon healing.

METHODS: A total of 110 SD rats were used in our experiments. In the first part, 24 SD rats were used to establish a rat Achilles full-thickness tendon defect animal model. We collected samples at week 2, 4, 8, and 12. H&E staining, SOFG staining and μCT were conducted to characterize the phenotype after tendon injury. In the second part, stem cell differentiation assays (tenogenic, chondrogenic, and osteogenic differentiation) were used to test the effects of activation and inhibition Piezo1 on cell fate determination *in vitro*. In the third part, we fabricated GelMA hydrogel and characterized it using SEM, degradation test, and swelling test. 96 SD rats were randomly assigned to defect (blank repair) group, GelMA group, GelMA loaden 50μg GsMTx4 group, and GelMA loaden 100μg GsMTx4 group. H&E staining, IHC, and gait analysis were applied to evaluate the effects of GsMTx4 for tendon healing (n=6/group/timepoint). We also measured the bone volume around injured site at week 12 by μCT to test the effects of GsMTx4 on heterotopic ossification (n=5/group/timepoint). One-way analysis of variance (ANOVA) with Tukey's post-hoc testing and student's t test were used for statistical analysis. Significance was considered at $P<0.05$.

RESULTS SECTION: Piezo1 was highly expressed in intact Achilles tendon compared to other ion channels, and Piezo1 expression was significantly increased after Achilles tendon injury at the mRNA level, while there was no significant difference in the Piezo2 channel. We performed immunohistochemistry (IHC) and immunofluorescence (IF) for Piezo1 in intact and injured Achilles tendons and found that Piezo1 protein was significantly increased after Achilles tendon injury, and the expression level gradually decreases during the tendon healing. In addition, Piezo1 was co-stained with Nestin$^+$ and CD90$^+$ stem cells in the injured tendons and tendon stem cells, and we also confirmed that modulation of Piezo1 activity could change tendon stem cell fate in vitro. In vivo study, inhibition Piezo1 activity by GsMTx4 reduced pain, promoted tendon healing, and alleviated heterotopic ossification.

DISCUSSION: Our study had identified that Piezo1 is highly expressed in the Achilles tendon, and Piezo1 is elevated after Achilles tendon injury. Piezo1 channel have a close relationship with tendon stem cell fate. In addition, inhibition of Piezo1 activity by GsMTx4 results in better tendon healing process. Ongoing studies will define the downstream of Piezo1 in tendon healing process. Furthermore, conditional knockout Piezo1 mice will be used for further experiments.

SIGNIFICANCE/CLINICAL RELEVANCE: Piezo1 inhibition could be a therapeutic treatment for tendon diseases.

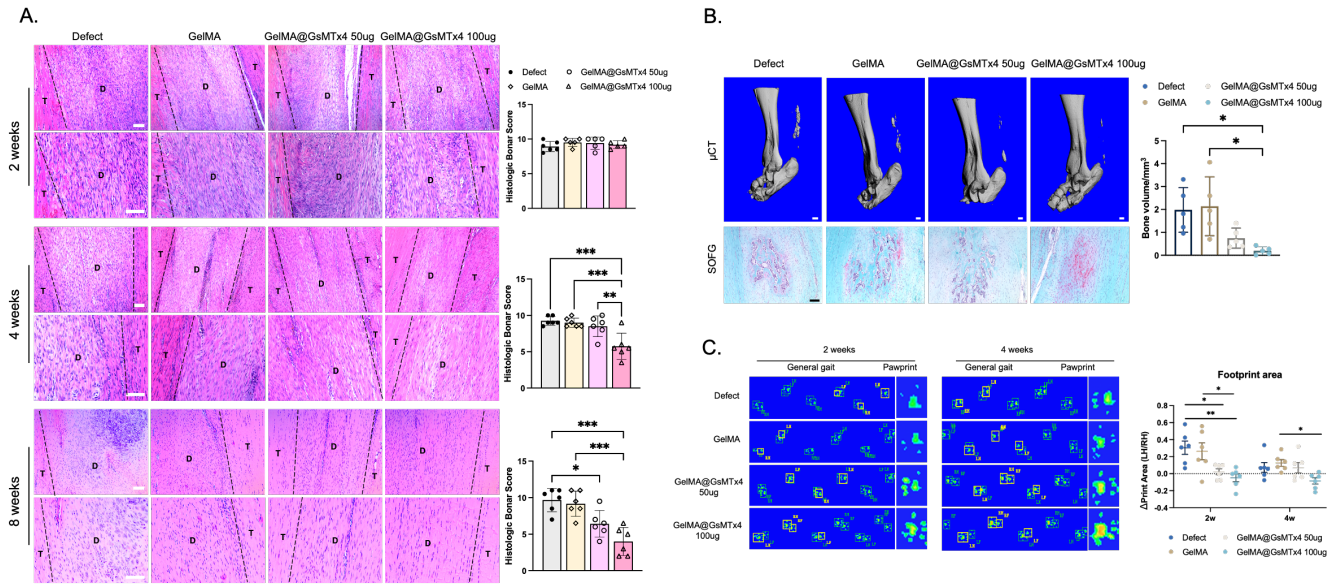

Fig. Piezo1 inhibition by GsMTx4 enhances tendon healing. A. Representative images of H&E staining in different groups at week 2, 4 and 8 after injury. D, defect; T, tendon. N=6. **B.** μCT and SOFG staining at week 12 after injury. N=5. **C.** Catwalk gait analysis in different groups at week 2 and 4 after injury. N=6. One-way ANOVA with Turkey test; Data were shown as mean± SD. *P <0.05, **P <0.01, ***: P<0.001.