**Piezol loss of function reduces Scx+ cell migration and accelerates recovery of tendon stiffness after injury**

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DISCLOSURES: Nicole A. Chittim (N), Tino Stauber (N), Maja Bollhalder (N), Maya Ben-Yehuda Greenwald (N), Greta Moschini (N), Jess G. Snedeker (1-Karl Storz Endoscopy GmbH, ZuriMED Technologies AG; 3C-Octapharma AG, Novartis AG; 4-ZuriMED Technologies AG; 9-ZuriMED Technologies AG (Board of Directors))

INTRODUCTION: Tendon is strong, stiff, yet flexible connective tissue which connects muscle to bone, enabling optimal skeletal movement. Tendon injuries heal slowly and often never recover full mechanical properties. Our lab recently discovered PIEZO1 to be a crucial shear stress sensor in tendons, positively correlating with mechanical properties during tendon homeostasis1. While we have established a role of PIEZO1 in tendon adaptation to exercise, we speculated that it may play a separate role in healing. For instance, studies in skin wound repair have shown that PIEZO1 is essential to cell migration2. On this basis, we hypothesized that tendon healing would be dysregulated by knocking out Piezo1 in Scx+ cells, leading to altered healing.

METHODS: Animal experiments were approved by the Zurich Veterinary Office (license: ZH018/2018). We used Piezo1−/− mice with a Scleralis (Sxc)-Cre driver to selectively knockout Piezo1 from Sxc-containing tissues. We surgically induced a full-thickness, central third defect in the patellar tendon of the left leg of SxcCre;Piezo1−/− (“knockout”) mice and floxed littermate controls (WT). The right leg underwent sham surgery for an intra-subject control. After 2 or 4 weeks of healing, patellar tendons were isolated and mechanically tested to failure. Tensile testing occurred in 1% RT PBS and followed the protocol: preload to 0.1 N, precondition 25x to 1% strain, 30 s rest, stretch to failure at 1.0% L/s. Data was analyzed with a custom-written MATLAB Stress-Strain code (R2019a). We isolated patellar tendon cells from Sxc-GFP mice using collagenase and, at passage 3-5, transfected the cells with siRNA to knock down Piezo1 and used a non-selective siRNA as a scramble control. We performed a scratch assay by seeding the cells on a collagen-coated 12-well plate, serum starvation with 1% for 24 hours, scratching the surface with a p200 pipette tip, and imaging with brightfield and confocal microscopes at 0, 4, 8, 12, 24, and 46 hours post-scratch. Images were analyzed with FIJI v.2.14. All statistical analysis was performed in GraphPad Prism v.9.5.1.

RESULTS: After 2 weeks of healing, the stiffness of the injured patellar tendon of the SxcCre;Piezo1−/− mice had recovered to 89% of the sham stiffness on average (n=7 mice); those of the WT mice recovered to 72% of the sham stiffness on average (n=7). These data demonstrated a large Cohen’s d effect size (0.98), indicating a practical significance between the two groups. The elastic modulus (E-mod) recovered to 67% and 40% of sham E-mod for SxcCre;Piezo1−/− and WT, respectively (p=0.02). After 4 weeks, the stiffness of the injured patellar tendon on average recovered to 89% and 82% of the SxcCre;Piezo1−/− sham and WT sham stiffnesses, respectively (n=3 WT & 4 SxcCre;Piezo1−/−). This indicates a moderate (0.5) Cohen’s d effect size for practical significance. The E-mod of SxcCre;Piezo1−/− recovered to 76% and WT to 47% of sham E-mod (p=0.14). Results from the in vitro scratch assay (n=12 replicates from 4 mice) align with the mechanical data: after 4 and 12 hours, the knockdown cells exhibit significantly more migration into the defect (i.e., the width of the scratch has closed more) than the control cells (p=0.03 and 0.05, respectively). After 46 hours, the control cell migration has caught up to that of the knockdown cells. When examining the Scx+ cells specifically at timepoints of faster migration, we found significantly fewer Scx+ cells in the scratch of the knockdown cells after 12 h than in that of the control cells when normalized to the amount of Scx+ cells in the scratch at 0 h (p<0.001).

DISCUSSION: Although the SxcCre;Piezo1−/− mice exhibited a functionally relevant increase in mechanical recovery of stiffness and strength after 2 weeks over WT, our 4-week data suggest that this does not result from fibrotic healing. Cross-sectional area (CSA) of the injured patellar tendons was 160% of sham for WT and 144% of sham for SxcCre;Piezo1−/− after 2 weeks of healing. This suggests that assembly of new collagen matrix may be more ordered in the SxcCre;Piezo1−/− tissue. After 4 weeks of healing, the CSA’s reduced to 111% of sham for WT and 119% of sham for SxcCre;Piezo1−/−. These data indicate that there is both healing and remodeling taking place in the tendons of both groups over time. Speculating that reduced migration of Scx+ cells could potentially explain the improved healing in the SxcCre;Piezo1−/−, we performed a confocal imaging of the Scx+ cell migration in a scratch assay. Indeed, there were fewer Scx+ cells migrating into the scratch of the Piezo1 knockdown tenocytes than into that of the control tenocytes. This is consistent with previous work3 describing accelerated tendon healing in a model with depletion of Scx+ cells, giving credence to our current results. While the sample size for the 4-week in vivo data is currently small, our data suggest that a lack of the PIEZO1 channel in Scx+ cells may slow the migration of Scx+ cells to a tendon injury, which in turn could result in the observed acceleration in mechanical recovery.

SIGNIFICANCE: Our experiments suggest a novel role for PIEZO1 in tendon healing response that could potentially be exploited for therapeutic purposes.


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