Fibroblast-Specific Deletion Of Tak1 Reduces Adhesion Formation During Flexor Tendon Healing Via Inhibition Of Myofibroblast Activation

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Introduction: The clinical outcomes of flexor tendon injury repairs are often complicated by extensive scar formation that compromises tendon gliding, reduces finger range of motion, and impairs hand functions. Fibrotic scars contain myofibroblasts that produce excessive extracellular matrix proteins such as COL1A1 and COL3A1. TGF-β, a potent activator of fibroblasts has been implicated in tendon adhesion formation.[1] Though animal studies targeting components of the TGF-β signaling pathway have shown effective reduction in adhesion formations, little progress has been made in translating these findings from bench to bedside due to our limited knowledge of the precise role of TGF-β signaling during tendon healing. [2] TGF-β regulates target genes through multiple mechanisms. In addition to canonical SMAD signaling, TGF-β signals via activation of MAPKs. In particular, TGF-β-Activated Kinase 1 (TAK1), a MAP Kinase Kinase Kinase that is a component of the TGF receptor complex, activates p38 MAP Kinase and JNK.[3] In addition, β-catenin can be activated by TGF-β via MAP Kinase signaling.[4] β-catenin has also been implicated in the activation of myofibroblasts and in the development of fibrotic diseases. Thus, several lines of evidence suggest that TAK1 may be an important effector of TGF-β-mediated scar formation and fibrotic disease.[5] In this study, we examine the hypothesis that deletion of Tak1 can inhibit the conversion of fibroblasts to myofibroblasts. Here, both in vitro and in vivo approaches are employed to investigate the role of Tak1 in regulation of tendon injury repair.

Specifically, we utilized a murine model of primary flexor digitorum longus (FDL) tendon repair in which Tak1 is conditionally deleted in fibroblasts using the Fsp1-Cre transgene in combination with the Tak1 floxed allele. In vitro, we investigated the role of Tak1 in regulation of fibroblast proliferation, migration, contraction, and collagen production.

Methods: Animals: To determine the expression pattern of Fibroblast specific protein 1 (Fsp1), ROSA-mT/mG mice were bred with Fsp1-Cre mice; Tak1fx/fx mice were bred with Fsp1-Cre mice to generate mice with gene deletion specifically in fibroblasts. Surgeries: 8- to 10-week-old mice were used for primary flexor tendon repair surgeries. FDL tendon of the left hindpaw was transected and repaired via a reversed Kessler method by 8-0 sutures. Specimens were harvested on days 0, 3, 7, 10, 14, 21 and 28 post-surgery.

Histology, immunohistochemistry (IHC) and fluorescence microscopy: Specimens were fixed in 4% PFA and decalcified in 14% EDTA. Paraffin-embedded sections were stained with ABH/orange G. IHC was performed with anti-active β-catenin antibodies. Cryosections were viewed by fluorescence microscopy.

Biomechanical studies: Metatarsal joint flexion was measured by digital imaging at an incremental load of 0-19g static weights. Tensile strength was measured with an Instron 8841 Testing System.

qPCR: Total RNA was extracted from injured tendon or cells and reverse transcribed to cDNA. Gene expression was quantified by qPCR using murine specific primers to Acta2, Axin2, Col1a2, Col3a1, and Tak1.

Statistical analysis: Results are shown as mean ± S.E.M. Statistical analysis was performed with a two-way ANOVA. P<0.05 were considered statistically significant.

Results: In Vivo Experiments: Consistent with earlier reports, Fsp1+ cells were observed in spleen, forestomach, and skin of Fsp1cre; mT/mG mice. Upon injury, Fsp1+ cells populated the reparative tissues at the injury site. In contrast, native tendon tissue did not have any Fsp1+ cells. Deletion of Tak1 in fibroblast populations reduced the gene expression of Tak1 in Fsp1cre; Tak1fx/fx mice to ~50% of the level observed in WT mice (Tak1fx/fx) from 7 to 28 days post-repair. Histology showed reduced scar formation in Fsp1cre;Tak1fx/fx mice during tendon healing. At day 7, less granulation tissue had accumulated at the injury site in Fsp1cre;Tak1fx/fx mice compared to WT mice. Between days 14 and 28, Fsp1cre;Tak1fx/fx mice had reduced reparative tissue. Consistent with histology, expression of the matrix genes Col1a2 and Col3a1 were significantly lower in Fsp1cre;Tak1fx/fx mice throughout the repair process (Fig 1C). Tendon repairs in Fsp1cre;Tak1fx/fx mice also had reduced expression of Acta2, a marker for myofibroblasts, suggesting that TAK1 is involved in fibroblast activation during tendon repair (Fig 1D). Biomechanical studies showed improved range of motion in tendon repairs in Fsp1cre;Tak1fx/fx mice (Fig 1A, B).
Axin2, an established target of the Wnt/β-catenin signaling pathway, was significantly reduced in Fsp1cre;Tak1fx/fx mice from day 10 post-repair onward. Consistent with reduced Axin2, sections from the Fsp1cre;Tak1fx/fx mice showed decreased staining of active β-catenin at the repair site at day 14 compared to sections from WT mice. Altogether the findings show that deletion of Tak1 in Fsp1+ cells reduces scar formation during tendon healing.

In Vitro Experiments: Inhibition of TAK1 reduces the transformation of fibroblasts to myofibroblasts upon TGF-β1 treatment in vitro. Tak1 deletion in TGF-β1-treated MEFs resulted in decreased expression of Acta2 (Fig 2 C). Several other key functions of activated myofibroblasts, such as proliferation, migration, contraction, and collagen production were also inhibited by the loss of Tak1 (Fig 2). We also found that addition of TGF-β1 to MEF cultures enhanced the activation and nuclear accumulation of β-catenin. In contrast, Tak1 inhibition in MEF cultures resulted in decreased myofibroblast gene expression and reduced activation of Wnt/β-catenin signaling.

Discussion: The present studies demonstrate that Tak1 is a critical mediator in tendon adhesion formation and fibroblast to myofibroblast conversion. Loss of Tak1 in fibroblasts results in decreased scar formation, and increased range of motion. The decreased scar formation is associated with reduced expression of genes involved in the fibrotic response, including Col1a2, Col3a1, and Acta2. Acta2, a marker of the myofibroblast, is associated with scarring and tissue contraction. In vitro experiments confirmed that inhibition of Tak1 reduces Acta2 expression and results in reduced fibroblast proliferation, migration, and contraction. Furthermore, both the in vivo and in vitro experiments suggest that β-catenin signaling is involved in the formation of tendon adhesions and is downstream of TGF-β/TAK1 signaling.

Significance: Our studies suggest that inhibition of Tak1 could serve as a novel approach for therapeutic intervention for the treatment of adhesion formation. Tak1 links TGF-β and β-catenin signaling during the development of fibrotic responses after tendon injury.

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