FABP4 as a Novel Target for the Treatment of Tendinopathy

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INTRODUCTION: Excessive inflammation and erroneous tendon-derived stem cell (TDSC) differentiation contribute to degenerative failed healing of tendinopathy. FABP4 is a pro-inflammatory adipokine and its circulating level is upregulated in various inflammatory and metabolic diseases. Inhibition of FABP4 has been reported to improve these pathological conditions. Our data have shown that overexpression of FABP4 was associated with excessive inflammation in the clinical samples and animal model of tendinopathy. This study aimed to examine the effects of FABP4 administration on the histopathology of tendon and functions of TDSCs. The effects of genetic knockout or pharmacological inhibition of FABP4 on injury progression in a degenerative failed healing model of tendinopathy were also investigated.

METHODS: The animal research ethics committee approved the study. FABP4 or saline was injected into the C57BL/6J mouse Achilles tendons and the samples were collected for histology and immunohistochemistry staining (IHC) of inflammatory cytokines at week 1 and week 8 post-injection. The samples collected at week 8 were also subjected to micro-CT imaging. Achilles TDSCs were isolated from C57BL/6J mice. The effect of IL-1β on the expression of FABP4 in TDSCs was examined by immunofluorescent staining (IF) and qRT-PCR. TDSCs were treated with FABP4 or FABP4 inhibitor (BMS309403). The number of colony-forming unit (CFU) was counted by crystal violet staining after 10 days. The mRNA expression of inflammatory marker (*Tnfa*), ER stress markers (*Chop*, *Grp78*), tenogenic markers (*Tnmd*, *Scx*) and osteogenic marker (*Runx2*) in IL-1β-treated TDSCs was examined by qRT-PCR after treatment for 48 hours. *FABP4* knock-out (KO) and wild-type (WT) mice on C57BL/6J background were injected with collagenase and the Achilles tendons were collected at week 3 post-injection for histological analysis. In another experiment, BMS309403 or vehicle were injected multiple times into the Achilles tendon of WT mice starting from week-1 post-CI. The Achilles tendons were harvested at week 2 after BMS309403 or vehicle treatment. The tendon samples were collected for histology and IHC of inflammatory cytokines. BMS309403 was also loaded into GelMA hydrogel and its surface morphology was examined by SEM. At week 1 after CI tendon injury, the injured tendons were injected either with saline, GelMA-only or BMS309403-loaded GelMA once. At week 2 after treatment, the Achilles tendons were harvested for histology and gait analysis.

RESULTS SECTION: A single injection of FABP4 increased the cellularity and vascularity in tendons at week 1 and week 8, accompanied with cell rounding, loss of cell and collagen fiber alignment, infiltration of small, nucleated cells, and fat accumulation (n=3-5/group) (Figure 1). The expression of IL1-β, IL-6, TNF-α, and IL-10 increased in mouse Achilles tendons at week 1 post-FABP4 injection (n=5/group). Ectopic bone formed in tendon at week 8 after FABP4 injection (n=6/group). Mouse TDSCs expressed very low levels of FABP4 protein (n=6/group) and mRNA (n=4-5/group) under basal condition. However, IL-1β significantly increased the protein and mRNA expression of FABP4 in mouse TDSCs ($p<0.05$) (Figure 2). FABP4 reduced CFU of TDSCs (n=4-5/group) ($p<0.05$). It increased the expression of *Chop* and *Bglap* but reduced the expression of *Col1a1* in inflamed TDSCs (n=4-6/group) (all $p<0.05$). In contrast, treatment with BMS309403 reduced the expression of *Tnfa*, *Chop*, *Grp78* and *Runx2* but promoted the expression of *Tnmd* and *Scx* in IL-1β-treated TDSCs (n=4-6/group) (all $p<0.05$). Collagenase induced tendon degeneration and expression of inflammatory cytokines in mouse Achilles tendons, with concomitant increase in the expression of FABP4 (n=5/group). Injured mouse Achilles tendons with *FABP4* KO showed lower infiltration of small, nucleated cells and higher collagen birefringence compared to the injured WT tendons and hence alleviated CI tendon injury (n=6/group). Pharmacological inhibition of FABP4 with multiple injections of BMS309403 also alleviated CI tendon damages (Figure 3) and reduced the expression of IL-1β, IL-6 and TNF-α after CI injury at week 2 after treatment (both n=4/group). A single local injection of BMS309403-loaded GelMA hydrogel promoted tendon healing (n=6/group). CI tendon injury changed the gait patterns in the injured limb at week 2 after treatment (n=8/group). BMS309403-loaded GelMA hydrogel reversed the changes of gait parameters and there was no significance compared to the healthy Control group (n=8/group).

DISCUSSION: FABP4 injection induced pathological changes, increased expression of inflammatory cytokines and ectopic bone formation in tendon resembling tendinopathy. TDSCs expressed FABP4 mRNA and protein, which increased during tendon inflammation. FABP4 decreased self-renewal capacity of TDSCs. It also reduced the tenogenic differentiation potential and increased the ER stress of TDSCs under inflammation. FABP4 inhibition with BMS309403 suppressed inflammation, oxidative stress and osteogenesis but promoted tenogenesis of TDSCs under inflammation. Both genetic and pharmacological inhibition of FABP4 promoted tendon healing and the effect was likely mediated by the reduced expression of inflammatory cytokines after tendon injury. Preliminary results showed that a single injection of BMS309403-loaded GelMA promoted tendon healing at a lower cumulative dose, with improvement in tendon histology and gait pattern. In conclusion, pharmacological inhibition of FABP4, in particular, BMS309403-GelMA, may be used as a novel treatment for degenerative tendinopathy. Further research is needed to confirm the efficacy and underlying mechanisms of BMS309403-loaded GelMA hydrogel with more comprehensive assessments and with longer follow-up.

SIGNIFICANCE/CLINICAL RELEVANCE: In conclusion, BMS309403 promoted tendon healing via suppressing inflammation, inhibiting erroneous differentiation, and enhancing self-renewal of TDSCs. Pharmacology inhibition of FABP4, in particular, in the form of BMS309403-GelMA, may be used as a novel treatment for degenerative tendinopathy.

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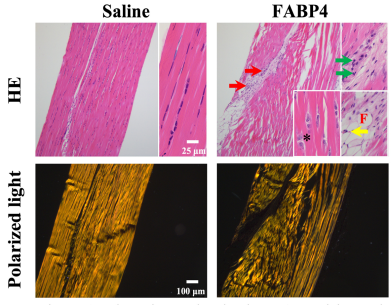

Figure 1. Photomicrographs showing H&E staining and corresponding polarized images of mouse Achilles tendons at 1 week after saline or FABP4 (0.5 μg) injection. n=3-5/group; Scale bar = 100 μm (insert 25 μm), yellow arrow: red blood cells; red arrow: hypercellular area; green arrow: small nucleated cells; black asterisk: cell rounding, F: fatty tissue.

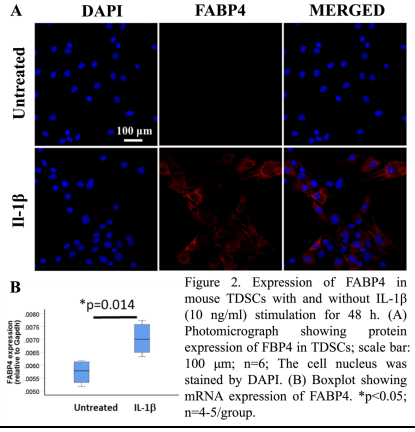

Figure 2. Expression of FABP4 in mouse TDSCs with and without IL-1β (10 ng/ml) stimulation for 48 h. (A) Photomicrograph showing protein expression of FBP4 in TDSCs; scale bar: 100 μm; n=6; The cell nucleus was stained by DAPI. (B) Boxplot showing mRNA expression of FABP4. *p<0.05; n=4-5/group.

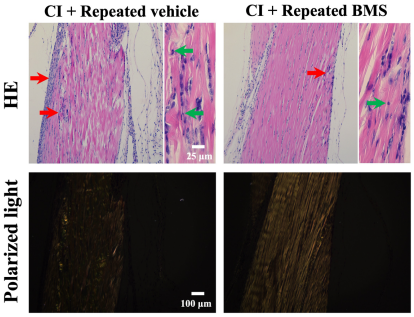

Figure 3. Photomicrographs showing H&E staining and corresponding polarized images of representative samples of WT mouse Achilles tendons at week 2 after repeated injection of vehicle or BMS309403 in the CI tendon injury model. n=4/group; Scale bar = 100 μm (insert 25 μm); red arrow: hypercellular area; green arrow: small nucleated cells.