Adiponectin Receptor Agonists Redirected the Pathological Trajectory of Tendinopathy from Inflammation to Tendon Regeneration

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INTRODUCTION: Chronic tendinopathy is characterized by persistent inflammation and aberrant differentiation of tendon-derived stem/progenitor cells (TDSCs). Adiponectin, an adipokine with hormone-like properties, demonstrates anti-inflammatory, anti-apoptotic, and tissue-regenerative effects across various cell types. Our unpublished data indicated elevated adiponectin expression in clinical specimens and an animal model of tendinopathy, suggesting its potential role in facilitating tendon repair and attenuating inflammation. Nevertheless, the therapeutic potential of adiponectin in ameliorating the detrimental effects of chronic tendon injury remains unknown. This study aimed to explore the effects of an adiponectin receptor agonist, AdipoRon, on the functions of TDSCs and histopathology of tendons in a degenerative tendon injury model.

METHODS: The study was approved by the animal research ethics committee of the authors' institution (Ref. 23-259-GRF). Achilles TDSCs were isolated from male C57BL/6J mice and treated with AdipoRon or vehicle under inflammation induced by IL-1β (10 ng/mL). Gene expression of inflammatory cytokines, matrix-remodeling markers, tenogenic markers, non-tenogenic markers (osteogenic, adipogenic, chondrogenic), and pro-apoptotic markers was determined by RT-qPCR (n=3-6/group). Multi-lineage differentiation potential was evaluated through osteogenic, adipogenic, chondrogenic, and tenogenic differentiation assays (n=3-4/group). Colony-forming unit and AlamarBlue assays were conducted to assess self-renewal and viability (n=5-6/group). Transwell and wound healing assays were utilized to examine cell migration (n=3-5/group). TUNEL staining (n=4/group), and β-galactosidase activity assay (n=6/group) were performed to investigate cellular apoptosis and senescence, respectively. For in vivo experiments, collagenase was injected into the mid-substances of the Achilles tendon in male C57BL/6J mice to induce injury. AdipoRon was loaded into GelMA hydrogel, and its surface morphology was characterized by SEM. AdipoRon-loaded GelMA or GelMA-only was injected into injured tendons at week 1 post-injury. Tendon samples were harvested at weeks 2 and 4 post-treatment for histological analysis (H&E, polarized light; n=6/group/time point) and immunohistochemistry (IHC) for inflammatory cytokines, matrix-remodeling enzymes, and tenogenic markers (n=6/group/time point). Gait analysis was performed at week 2 using the CatWalk system (n=10/group). Male mice were used to eliminate the effects of estrogen on tendon repair.

RESULTS: AdipoRon treatment in inflamed mouse TDSCs significantly downregulated mRNA expression of pro-inflammatory cytokines and matrix-remodeling enzymes (both $p<0.05$), while markedly upregulated anti-inflammatory cytokines ($p<0.01$) compared to the inflamed control group. Under basal conditions, AdipoRon enhanced the expression of tenogenic markers and suppressed non-tenogenic markers ($p<0.05$ for *Scx* and *Bsp*, $p<0.01$ for other markers). Differentiation assays demonstrated that AdipoRon supplementation promoted tenogenesis under inflammation (Figure 1) while inhibiting osteogenesis, adipogenesis, and chondrogenesis, as evidenced by reduced calcium nodule, lipid droplet and proteoglycan formation (all $p<0.05$). Cell survival was significantly improved, as indicated by increased cell viability, enhanced colony formation (both $p<0.01$) (Figure 1), accelerated wound closure, reduced percentage of TUNEL-positive cells (both $p<0.05$), and decreased β-galactosidase activity ($p<0.01$). A single local administration of AdipoRon-loaded GelMA hydrogel into collagenase-injured tendons significantly enhanced healing at weeks 2 and 4. Treated tendons exhibited reduced hypercellularity, decreased infiltration of small nucleated cells, and improved collagen fiber alignment compared to untreated injured tendons (Figure 2). IHC staining showed reduced expression of pro-inflammatory cytokines (TNF-α, IL-6) (Figure 3), a slight increase in TIMP-1, and elevated expression of tenogenic markers (TNMD, SCX). AdipoRon-loaded GelMA hydrogel also reversed collagenase-induced gait impairments, restoring functional parameters including stand time, swing speed, swing duration, print mean intensity, print area, and stride length ($p<0.01$) towards normal values.

DISCUSSION: Our findings demonstrate that adiponectin receptor activation by AdipoRon effectively redirected the pathological trajectory of tendinopathy from inflammation towards regeneration, with a multifaceted therapeutic response. AdipoRon acted directly on TDSCs to resolve the inflammatory state and steer cell fate away from aberrant differentiation and towards functional tenogenesis. Enhanced cell viability, self-renewal, and migration, coupled with reduced apoptosis and senescence, indicate that AdipoRon bolstered TDSC survival and functionality under inflammatory stress, thereby reinforcing its regenerative potential. Preliminary in vivo results translated these cellular benefits into tangible therapeutic outcomes, wherein a single injection of AdipoRon-loaded GelMA hydrogel facilitated tendon healing with improvement in tendon structure, reduced inflammation, and promoted functional restoration in a degenerative tendinopathy model. In conclusion, adiponectin supplementation, particularly through AdipoRon-loaded GelMA hydrogel, represents a promising novel therapeutic strategy for degenerative tendinopathy. Further studies are needed to elucidate the long-term efficacy and precise molecular mechanisms underlying AdipoRon-loaded GelMA hydrogel in tendon repair.

SIGNIFICANCE/CLINICAL RELEVANCE: AdipoRon facilitated tendon repair by mitigating pathological inflammation, inhibiting aberrant TDSC differentiation and augmenting cellular survival. The significant structural and functional restoration achieved with a single injection of AdipoRon-loaded hydrogel highlights its strong clinical translational potential for treating tendinopathy.

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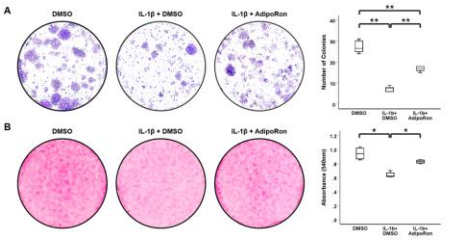

Figure 1. Photographs and boxplots showing (A) the colony-forming ability of TDSCs after 14-day treatment with IL-1β (10 ng/mL) and AdipoRon; and (B) the tenogenic differentiation potential of TDSCs cultured after 10-day treatment in induction medium with IL-1β and AdipoRon. n=4-6/group; * $p<0.05$; ** $p<0.01$

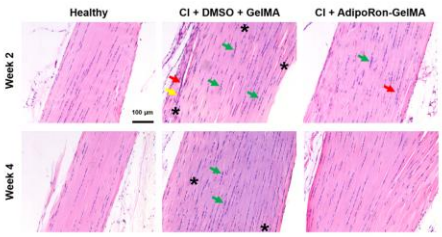

Figure 2. Photomicrographs showing histology of Achilles tendons of mice at weeks 2 and 4 after a single injection of saline, GelMA or AdipoRon-loaded GelMA hydrogel in the collagenase-induced (CI) tendon injury model. Stain: H&E; n=6/group/time point; scale bar: 100 μm; yellow arrow: blood vessels; green arrow: inflammatory cells; red arrow: hypercellular region; *: round cells.

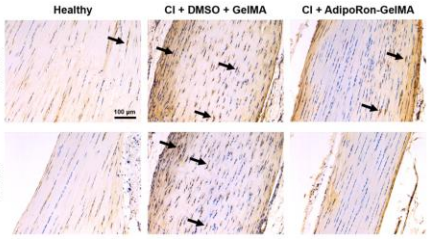

Figure 3. Photomicrographs showing IHC staining of IL-6 in the Achilles tendons of mice at weeks 2 and 4 after a single injection of saline, GelMA, AdipoRon-loaded GelMA hydrogel in the CI tendon injury model. n=6/group/time point; scale bar: 100 μm; black arrow: immunopositive signal.