Fine Tuning of Senescent Microenvironment by Sympathetic Outflow Orchestrates Tendon Repair

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INTRODUCTION: Tendon injuries are prevalent musculoskeletal disorders causing chronic pain, functional loss, and high re-tear rates after repair. Senescent cells are increasingly recognized as active regulators of tissue regeneration by modulating inflammatory resolution and orchestrating extracellular matrix remodeling. However, their role in tendon repair and the mechanisms underlying their regulation remain unclear. The objective of this study was to determine whether nervous system activity regulates the senescent microenvironment during tendon healing, and to elucidate the cellular pathways involved.

METHODS: A flexor digitorum longus tendon injury model was established in 8-week-old male p16-tdTomato senescence reporter mice (n = 30) to track senescent cell dynamics. Cellular composition was determined by *in situ* co-immunofluorescence and flow cytometry. Transcriptional profiles of tendon tissues were analyzed by Single-cell RNA sequencing. Tissue clearing and 3D imaging were performed to assess spatial relationships between regenerating nerve fibers and senescent cells. Sympathetic denervation was achieved in 8-week-old male TH-iDTR mice (n = 6), and β_2-adrenergic receptor (Adrb2) deletion in $p16^+$ senescent cells was induced in 8-week-old male p16-Cre^{ERT2}::$Adrb2^{flox/flox}$ mice (n = 6). Data was analyzed using GraphPad Prism 9. Comparisons between two groups used unpaired two-tailed Student's t-test, and multiple group comparisons used one-way ANOVA with Tukey's post hoc test. Data are mean ± SD; $p < 0.05$ was considered significant. All animal procedures were approved by the IACUC of Shanghai Jiaotong University with protocol number A2024434-002.

RESULTS: Senescent cells progressively accumulated from day 0 to day 14 post-injury (n = 6/ total 5 time points), peaking at day 7. The majority of peritendinous senescent cells were $F4/80^+$ macrophages (76.4% ± 4.7% of $p16^+$ cells, n = 6, $p < 0.001$). Single-cell RNA sequencing revealed an early SASP-rich profile (IL-6, TNF-α, CCL2) shifting to a reparative phenotype enriched in Arg1, Col I, and Col III in senescent macrophages during late healing. Tissue clearing and 3D imaging demonstrated spatial co-localization of regenerating nerve fibers with senescent cells, predominantly sympathetic nerves (co-localization coefficient r = 0.72 ± 0.04, n = 3, $p < 0.01$). Sympathetic denervation in TH-iDTR mice (n = 6) abolished Adrb2 activation in $p16^+$ macrophages and blocked the phenotypic shift. p16-CreERT2:$Adrb2^{flox/flox}$ mice (n = 6) phenocopied these effects, showing persistent SASP expression, reduced collagen organization score (1.8 ± 0.4 vs. 3.9 ± 0.3, $p < 0.001$), and lower ultimate tensile strength (6.4 ± 0.5 MPa vs. 10.7 ± 0.6 MPa, $p < 0.001$). β_2-adrenergic signaling upregulated LACC1 expression (3.1 ± 0.4-fold vs. control, n = 5, $p < 0.001$), thereby sustaining purine flux (inosine +42.5% ± 5.1%, $p < 0.01$). Conversely, loss of SNS input or Adrb2 signaling reduced LACC1, suppressed purine flux (–37.4% ± 4.8%, $p < 0.01$), and maintained a chronic inflammatory phenotype of senescent macrophage which led to delayed collagen alignment and reduced tendon strength.

DISCUSSION: Sympathetic outflow promotes tendon healing by driving β_2–LACC1-mediated reprogramming of senescent macrophages from a pro-inflammatory to a reparative phenotype, linking neural regulation to immune-metabolic control in soft tissue repair. A limitation of this study is that all experiments were performed in adult male mice, and sex- or age-related differences in SNS–senescence interactions remain to be investigated.

SIGNIFICANCE / CLINICAL RELEVANCE: This work identifies a novel neuro–immune–metabolic mechanism that could be targeted to enhance tendon and rotator cuff repair by harnessing the beneficial plasticity of senescent cells.

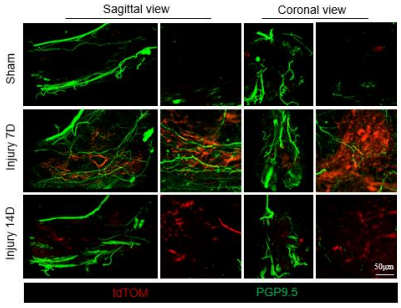

Figure 1 3D light-sheet imaging of injured tendon of p16-tdTomato mice suggests co-localization of senescent cells (tdTOM in RED) and regenerating nerve fibers (PGP9.5 in GREEN).