Tendon resident macrophages are dependent on fibroblast-derived CSF1 and are essential for normal collagen fibrillogenesis

Catherine A. Bautista[1], Courtney A. Nuss[1], Louis J. Soslowsky[1], Nathaniel A. Dyment[1]
[1]University of Pennsylvania, Philadelphia, PA
dyment@pennmedicine.upenn.edu

Disclosures: Catherine A. Bautista (N), Courtney A. Nuss (N), Louis J. Soslowsky (N), Nathaniel A. Dyment (N)

INTRODUCTION: Resident macrophages are essential for the development of many tissues throughout the body. Most resident macrophage populations require CSF1 receptor (CSF1R) signaling for their differentiation and survival. Recent studies in our laboratory have demonstrated that Csf1rGFP^{+} resident macrophages reside adjacent to tendon fibroblasts throughout tendon development, growth, and homeostasis [1]. The proportion of resident macrophages relative to the total tendon cell population increases over the course of postnatal growth, suggesting the importance of these cells during this time. Additionally, our gene expression analyses suggest there is crosstalk between resident macrophages and neighboring fibroblasts. Therefore, in this study we investigated the impact of tendon fibroblast-derived CSF1 on the resident macrophage population as well as the effects of resident macrophage depletion on tendon development.

METHODS: All procedures were IACUC approved. Mouse models. Scleraxis-lineage CSF1 knockout mice were generated by breeding Csf1$^{flox/flox}$ mice [2] with Scx^{Cre} mice [3] to obtain Scx^{Cre};Csf1$^{flox/flox}$ knockouts ("cKO"), as well as Cre-negative Csf1$^{flox/flox}$ littermate controls ("WT"). Scx^{Cre};Csf1$^{flox/flox}$ mice were crossed with Csf1rGFP mice for cryohistology. Experimental design. Achilles tendons ("AT") and patellar tendons ("PT") from 12-week-old (12w) mice were harvested for cryohistology (n = 3-4) and transmission electron microscopy ("TEM"; n = 6) whereas P14 ATs were used for qPCR (n = 3). Cryohistology. Nuclei and mean GFP intensity within each nuclear mask were quantified using Fiji (3-4 sections/sample) from sagittal sections. TEM. Transverse sections were stained with UranyLess and 1% phosphotungstic acid and imaged. Collagen fibril diameters (12 images/sample) were measured using custom MATLAB scripts. qPCR. AT midsubstance was dissected from sagittal sections for qPCR using a Fluidigm IFC for 96 genes of interest (n = 4). Statistics. WT and cKO groups were compared by two-sample t-test for ΔC_T, and Mann Whitney U test for cell density, % GFP, and collagen fibril diameter metrics.

RESULTS: Loss of tendon fibroblast CSF1 expression resulted in Csf1rGFP^{+} resident macrophage depletion. Using the Csf1rGFP reporter, we found a depletion of Csf1rGFP^{+} resident macrophages following tendon fibroblast-derived CSF1 ablation (**Fig. 1A**), with significant decreases in Csf1rGFP^{+} cell density (**Fig. 1B**) and proportion (**Fig. 1C**) in cKO ATs ($p<0.05$). Similar trends were found in PTs. Resident macrophage depletion resulted in larger diameter collagen fibrils. We hypothesized that resident macrophages may affect collagen fibrillogenesis either directly via ECM remodeling or indirectly by signaling to tendon fibroblasts. In fact, we found that cKO tendons had a wider range of collagen fibril diameters and a higher frequency of larger diameter fibrils (**Fig. 2**). Average fibril diameter was significantly higher in cKO PTs (92.7±4.9 nm vs. 80.9±5.7 nm; p=0.004) but not significantly different in cKO ATs (88.9±6.2 nm vs. 86.0±8.7 nm; p=0.7). Gene expression changes in P14 cKO ATs. To investigate changes in gene expression in cKO tendons during early postnatal growth, we performed qPCR on P14 ATs. As expected, we found significant downregulation of genes that are prominent in macrophages, including *Csf1r*, *Il1b*, *C1qc*, *Cx3cr1*, *C1qa*, *Pf4*, and *Adgre1* in cKO tendons (**Fig. 3**). We also found significant downregulation of ECM-related genes (*Lair1*, *Ctss*, *Mrc1*, *Ctsc*) that are enriched in resident macrophages compared to tendon fibroblasts. Additionally, we found a decrease in several cytokines (*Il1b*, *Tnf*, *Ccl2*) involved in inflammatory processes. Interestingly, the cytokine *Il2*, which is associated with activated T cells, was significantly upregulated in cKO tendons.

DISCUSSION: This study demonstrated that tendon resident macrophages require fibroblast-derived CSF1 for their survival (**Fig. 1**). This macrophage depletion model was specific to the tendon body, as macrophages still existed in the peritendinous tissues. Resident macrophage depletion resulted in a wider distribution of collagen fibril diameters driven by the formation of larger diameter fibrils compared to wild-type controls (**Fig. 2**). Surprisingly, despite lacking normal numbers of resident macrophages and possessing an altered collagen fibril diameter distribution, there were no detectable changes in mechanical properties in 12w cKO tendons (data not shown). These findings suggest that resident macrophages do not have a critical role in tendon formation and maturation at the ages examined in this study. It is possible, however, that the effects of resident macrophage depletion and aberrant collagen fibrillogenesis require more time to become evident, which is a focus of future studies.

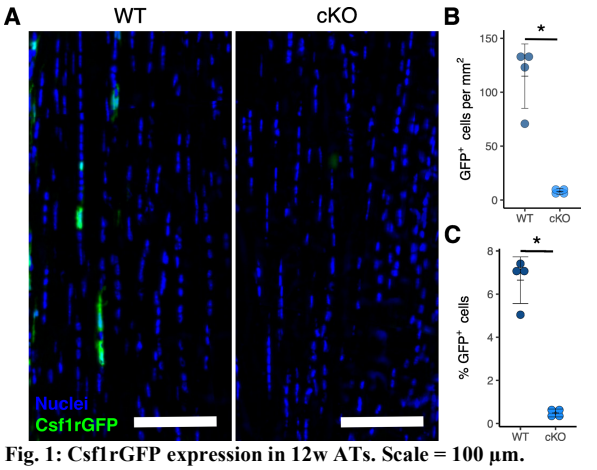

Fig. 1: Csf1rGFP expression in 12w ATs. Scale = 100 µm.

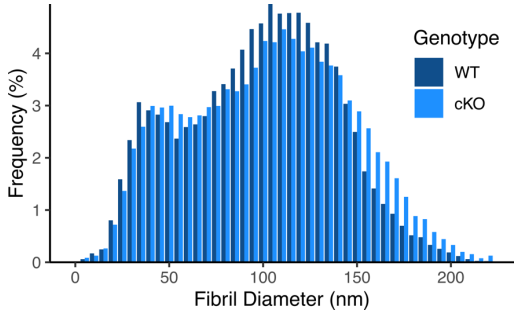

Fig. 2: Collagen fibril diameter distribution of 12w ATs.

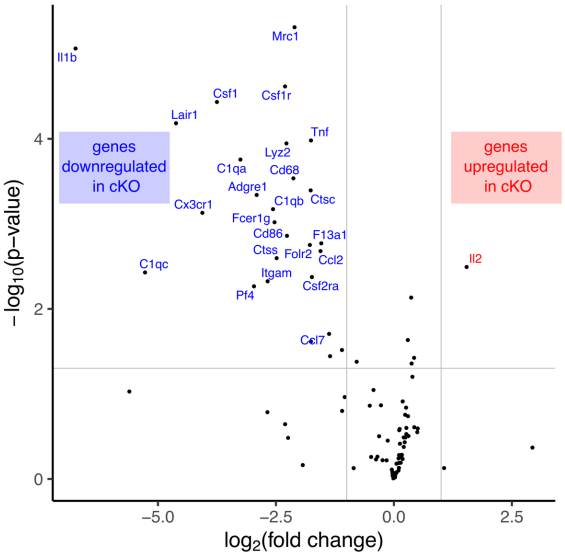

Fig. 3: Volcano plot comparing gene expression in P14 ATs.

SIGNIFICANCE: This study gives new insight into potential roles of resident macrophages during tendon development and growth and their interaction with fibroblasts and the ECM, which may inform future studies in the contexts of disease pathogenesis and tendon repair.

REFERENCES: 1) Bautista et al., 2023. 2) Harris et al., 2012. 3) Blitz et al., 2013. 4) Sasmono et al., 2003.

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