Partial Epigenetic Reprogramming Rescues Aged Tendon Healing

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INTRODUCTION: Aging significantly impairs tendon homeostasis, increasing the risk of tendon injuries. Moreover, aged tendons demonstrate functional and mechanical deficits following tendon injury [1]. However, the mechanisms that drive impaired healing are unknown, resulting in a paucity of treatment strategies to restore tendon structure-function after injury in aged individuals. Therefore, we sought to define changes in the tendon cell environment during healing in young and aged mice and identify divergent transcriptional programs that may underpin age-related healing deficits. In addition, age-related changes in the epigenetic landscape, leading to aberrant cell functions are well described in many tissues. Importantly, recent work has demonstrated that aged cells can be 'rejuvenated' via partial reprogramming to erase age-related epigenetic changes (Fig. 1). This approach relies on cyclic expression of the Yamanaka factors: *Oct4*, *Sox2*, *Klf4* and *cMyc* (OSKM)[2]. Unlike full reprogramming which induces pluripotency and can lead to teratoma formation, partial reprogramming aims to rejuvenate cells without the loss of their identity or intrinsic capabilities. We hypothesize that in vivo partial epigenetic reprogramming will rescue aged cell functions and improve functional outcomes during aged tendon healing (Fig. 1B).

METHODS: All animal studies were IACUC approved. To define the changes in the cellular and molecular environment of aged tendon healing, young (10-16 weeks, Jackson Labs) and aged (21months) NIA C57Bl6 mice underwent surgical transection and repair of the flexor digitorum longus (FDL) tendon. As previous work has not identified changes in the healing cell environment as a function of sex, female mice were used in this study. Healing tendons (n=10-12 per age per timepoint) were harvested at day 7, 10, 14, 21, and 28 post-surgery for 10X Chromium single cell RNA sequencing (scRNAseq), followed by analysis using the Seurat (v 5.3) pipeline in RStudio. Clusters were visualized as UMAP projections, and *FindAllMarkers* was used to identify differentially expressed genes (DEGs) within each cluster. Clusters were annotated using cell-type-specific markers[3]. Gene set enrichment was performed with the enrichR R package using the GO (Gene Ontology) Biological Processes 2025 database.

Partial epigenetic reprogramming was performed in male and female heterozygous OSKM transgenic mice ([R26rRTA; Col1a12lox-4F2A]; Jackson labs) expressing a single copy of the Oct4-Sox2-Klf4-cMyc (OSKM) cassette driven from the 3'UTR of the Col1a1 locus [2]. Cyclic OSKM induction was achieved by 2 days of doxycycline (Dox; 1mg/mL) administration via drinking water, followed by 5 days of Dox withdrawal. Given that the onset of decreased cellular density in the flexor digitorum longus (FDL) occurs starting at 18 months, cyclic induction in OSKM mice started at 18 months of age and continued until 21 months (n=10) (Fig. 1A). Controls (n=10-13) received only drinking water. Flexor tendon repair was performed at 21 months, and healing samples were harvested 14 days post-surgery for quantitative assessment of functional and mechanical properties. OSKM gene expression was validated by RealTime Quantitative PCR in tendons (n=3 per group).

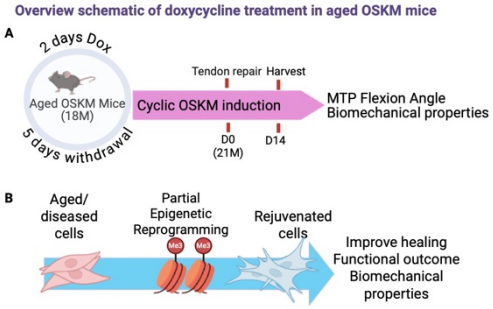

Figure 1A. Schematic representation of the cyclic induction of OSKM. (B) Proposed hypothesis that partial epigenetic reprogramming will rejuvenate aged tendon cells without erasing cellular identity and function leading to improved healing and functional outcomes.

RESULTS: To understand what drives age-related impairments in tendon healing, we performed scRNAseq in young and aged mice. Surprisingly, there were no substantial differences in the proportion of the major cell populations involved in tendon healing between young and aged tendons (Fig. 2A & B). However, aged cells, independent of cell type, exhibited upregulation of genes related to protein unfolding, degradation, and chaperone mediated cellular proteostasis, suggesting altered cellular homeostasis that likely impedes physiological wound healing initiation and resolution (Fig. 2C).

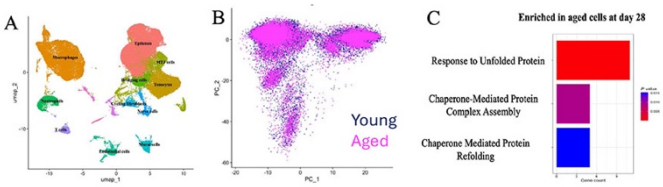

Fig. 2 Single cell RNA sequencing of young and aged tendons. A) UMAP projection of unsupervised cell clusters from integrated data from healing samples across all timepoints. B) PCA component of young and aged cells showing no changes in cell populations between the groups. (C) GO enriched processes based on significantly upregulated DEGs in aged cells at day 28.

In addition to changes in the cellular and molecular environment, there are clear epigenetic changes during tendon aging, including an aberrant increase of 5-hydroxymethylcytosine (5hmC) (Fig. 3A). Supported by these findings, we utilized the OSKM mouse model to further investigate if partial epigenetic reprogramming would rescue aged healing. Following three months (18→21 months of age) (Fig. 1B) of partial epigenetic reprogramming, OSKM mice treated with Dox demonstrated a significant 60.8% increase in Range of Motion (p=0.02, Fig. 3B**)** in parallel with a significant 76% decrease in gliding resistance (p=0.02) as compared to controls at day 14 post-surgery. While no difference in max load was observed between the groups, stiffness showed an upward trend (54%, p=0.07) in Dox-treated repairs as compared to controls. Collectively, these data suggest that alterations in proteostasis and changes in the epigenetic landscape underpin age-related impairments in tendon healing.

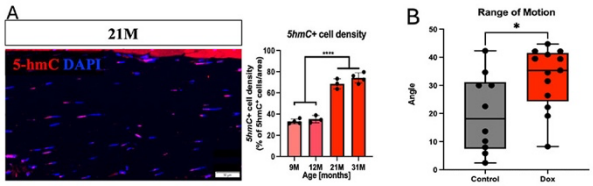

Fig. 3 Aberrant accumulation of epigenetic changes in aged tendons. A) Increased staining of 5-hmC in aged (21M) mice and increased percentage of 5-hmC+cells (red) in 21M and 31M tendons, indicating aberrant accumulation of epigenetic changes during tendon aging. B) Partial epigenetic reprogramming (Dox) rescues tendon function relative to age-matched control as measured by increased range of motion (n=8-10). (*) $p < 0.05$ by Student's T-Test.

DISCUSSION: Although there was no global difference in cell-type abundances between young and aged tendon repairs, scRNA sequencing analysis revealed significant differences in the healing trajectories between young and aged animals. As compared to young mice, aged mice exhibited a markedly different transcriptional profile characterized by the expression and enrichment of protein unfolding and chaperone-mediated processes, suggesting disrupted protein homeostasis, and an insufficient wound healing response to tendon injury relative to young tendons. As such, we focused on targeting age-related changes in the epigenetic landscape via partial reprogramming, which was sufficient to rescue age-related functional deficits in healing without compromising tendon mechanics. Together, these findings indicate that alterations in proteostasis and the epigenetic landscape likely contribute to healing deficiencies observed in aged tendons. Partial reprogramming, thus, has the potential to restore the intrinsic capabilities of cells that are critical to promoting a physiological healing process (Fig.1B). On-going work will define the specific age-related epigenetic modifications that underpin the insufficient response to injury in aged tendons to identify translationally relevant targets to enhance aged tendon healing.

SIGNIFICANCE/CLINICAL RELEVANCE: Improved understanding of the molecular mechanisms that drive impaired tendon healing during aging is crucial for developing effective therapeutic strategies.

REFERENCES: 1. Korcari, A., et al., Connect Tissue Res, 2023. **64**(1): p. 1-13.; 2.Ocampo, A., et al., Cell, 2016. **167**(7): p. 1719-1733 e12.; 3. Nichols, A.E.C., et al., Nat Commun, 2025. **16**(1): p. 5448.