Age-Dependent Inflammatory Responsiveness and Chromatin Dynamics in Mouse Tenocytes

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INTRODUCTION: Tendon injuries frequently occur due to their critical load-bearing role, often leading to debilitation and limited treatment success, causing discomfort and recurring injuries **[1]**. The inflammatory stage of tendon healing promptly initiates after acute injury, marked by clot formation within the damaged tissue **[2]**. While the conventional injury response involves transient phases of inflammatory signaling in tendons, degenerated tissues associated with aging struggle to resolve these cues, resulting in prolonged matrix degradation and shifts in cell phenotypes **[2,3]**. However, the precise mechanisms underlying how inflammatory conditions impact tendon resident cells (tenocytes) and how aging influences these responses remain enigmatic. Thus, this study aims to uncover disparities in cellular reactions to inflammatory cues (e.g., tumor necrosis factor-α, TNFα) between young and mature tendon cells. Specifically, employing super-resolution microscopy (e.g., STORM), we investigate how inflammatory conditions regulate age-related histone reorganization within tendon cells. This investigation probes the underlying epigenetic mechanisms of aging, shedding light on potential epigenetic-targeted therapies to address tendon injuries.

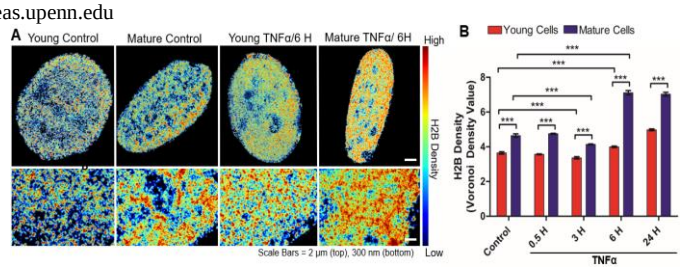

Figure 1: (A) Heat map of nuclear H2B localization density in young and mature mouse tenocytes under normal (Control) or inflammatory (TNFα, 6-hour) culture conditions. (B) Quantification of condensed chromatin values via STORM imaging analysis at multiple timepoints with inflammatory media-treated and untreated cells. n = 7 cells/group, ***: $p < 0.001$.

METHODS: Mouse tenocytes were isolated from young (<5 weeks, male) and mature (>45 weeks, male) tail tendons following established protocols **[4]**. To identify age-dependent responsiveness to inflammatory signaling, young and mature tenocytes were cultured in basal media (control) or TNFα-containing basal media (20ng/mL TNFα) for durations of 0.5, 3, 6, or 24-hours (H) prior to fixation for STORM and immunofluorescence (IF) imaging. To determine chromatin condensation status through histone H2B localization, fixed cells were immunostained for histone-H2B (Invitrogen) and a custom secondary antibody featuring activator-reporter dye pairs (Alexa Fluor 405-Alexa Fluor 647) for established STORM imaging **[5]**. Super-resolution determination of TNFα receptor density in young and mature cells was performed with tenocytes immunostained for TNFα-Receptor 1 (TNFαR1, Enzo) or -Receptor 2 (TNFαR2, Abcam) before secondary antibody incubation. STORM image reconstruction and analysis were done using the Nanoimager software (ONI), and quantification of Voronoi tessellation results for each super-resolution image was implemented in MATLAB to derive the density values **[5]**. Further, fixed cells from all treatment groups (as outlined above) underwent immunostaining for markers of transcriptional repression (or chromatin condensation, H3K27me3: the tri-methylation of lysine 27 on histone H3) or activation (or chromatin decondensation, H3K9ac: the acetylation of lysine K9 on histone H3 and H3K4me3: the tri-methylation of lysine 4 on histone H3). Mean florescence intensities of these markers within nuclei were quantified using Image-J. Statistical analyses employed a student's t-test or one-way ANOVA with Tukey's post hoc testing.

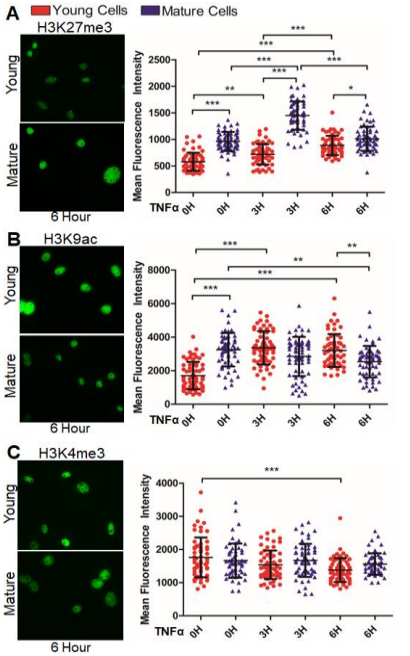

Figure 2: (A-C) Representative IF images (left, 6-hour TNFα treatment) and quantification of mean fluorescence intensity (right, control (0H), 3-hours (3H), or 6-hours (6H)) with TNFα-containing media. Groups were stained for H3K27me3 (A), H3K9ac (B), or H3K4me3 (C). n = 60 cells/group, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

RESULTS: Super-resolution images depicting H2B localization revealed an elevated presence of nanoscale H2B density (indicating increased chromatin condensation) within mature cells compared to their young counterparts in control and all inflammatory treatment conditions **(Fig. 1A, B)**. The duration of inflammatory treatment progressively increased the global chromatin condensation in both age groups. Notably, mature cells exhibited a substantial increase in heterochromatin after 6-hours (6H) and 24-hours (24H), significantly distinguishing them from young cells **(Fig. 1B)**. Given that chromatin architecture is influenced by alterations in histone modification status, the STORM imaging results led us to question how inflammatory signals affect post-translational modifications on histone complexes in young and mature cells. The gene repression-associated histone modification marker (H3K27me3) revealed an overall increase in chromatin condensation over treatment times, with notably more pronounced changes in mature cells than in young cells **(Fig. 2A)**. Conversely, histone modification markers for gene activation (H3K9ac) exhibited contrasting trends in chromatin condensation in response to the TNFα treatment **(Fig. 2B)**, whereas H3K4me3 exhibited only moderate adjustments in both cell types **(Fig. 2C)**. To unravel contributors to age-related responsiveness to inflammatory treatment, TNFα receptor densities were quantified between the age groups. While no discernible differences in TNFαR1 distributions were noted between young and mature cells (not shown), mature tenocytes exhibited a significant increase in TNFαR2 surface density compared to young cells **(Fig. 3A, B)**.

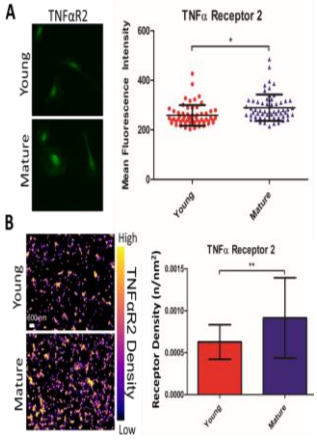

Figure 3: (A) Representative IF images and mean fluorescence intensity quantification for young and mature cells stained for TNFαR2, n = 60 cells/group. (B) Heat map images of TNFαR2 density (left, scale: 600 nm)) and quantification (right) for young and mature cells stained for TNFαR2, n=30 cell regions/group. *: $p < 0.05$, **: $p < 0.01$.

DISCUSSION: This study uncovers a distinct tenocyte response to inflammatory cues (TNFα) that correlates with age. Aging introduces alterations in the nanoscale chromatin organization and the rates of specific histone modifications within these cells. However, the application of inflammatory treatment across various time points leads to changes in these factors characterized by either opposing rates of change or entirely contrasting trends. TNFα prompts chromatin compaction, which are gene regions typically considered transcriptionally inactive. Both young and mature tenocytes exhibit an elevation of H3K27me3 with progressive TNFα treatment, although this increase is more pronounced in mature cells. Interestingly, while young cells increase in H3K9Ac under inflammation, mature cells experience a modest decrease. The interplay between changes in H3K27me3 and H3K9ac observed in young and mature cells might underlie the variations seen in chromatin condensation with H2B-STORM. These findings also suggest that the inflammatory microenvironment in aged tendons could potentially drive abnormal tenocyte behavior. Notably, TNFαR2 is elevated in mature tenocytes, possibly indicating receptor density disparities as a contributing factor to the heightened inflammatory response observed in aged populations. Ongoing studies are delving into how these nanoscale chromatin organizational changes influence transcription and protein expression in the context of tendon aging and disease.

SIGNIFICANCE: The study assesses the response of tenocytes to inflammation across different age groups, shedding light on the mechanisms underlying inflammation-related changes in cell phenotype to guide future treatment approaches for tendon-related disorders, including tendinopathy.

REFERENCES: [1] Mafulli+, Am J Sport Med, 2000; [2] Ellis+, J Immunol Regen Med, 2022; [3] Ireland+, Mat Bio, 2001; [4] Lee+, BMC Musculoskelet Dis, 2018; [5] Heo+, Nat BioE, 2023. **ACKNOWLEDGEMENTS:** This work was supported by the NIH (P50 AR080581, K01 AR077087) and NSF (CMMI-1548571).