Aging-associated Epigenomic Reprogramming Promotes Inflammatory and Catabolic Responses in Tenocytes

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INTRODUCTION: Tendon pathologies, including acute rupture and degenerative conditions such as tendinopathy, disproportionately affect the aging population and represent a major clinical burden **[1]**. Although tendon damage is readily healed in younger individuals, instances of microdamage in aged patients will quickly develop into larger lesions due to their limited healing capacity **[2]**. These conditions are characterized by elevated inflammatory signaling, which disrupts cellular composition and function within the tendon **[3]**. However, the impact of aging on the inflammatory response of tendon-resident cells (i.e., tenocytes) and its contribution to the impaired healing process remain poorly defined. Our previous research demonstrated that tumor necrosis factor-α (TNFα)-induced inflammation triggers global chromatin reorganization in tenocytes, with mature cells displaying extensive DNA rearrangements and reduced expression of hallmark tenocyte genes **[4]**. Building on this, we further hypothesize that maturation alters the intrinsic inflammatory response of tenocytes, thereby promoting pro-inflammatory and pro-degenerative phenotypic changes. This study aims to compare the responses of juvenile versus mature tenocytes to TNFα stimulation and to identify age-associated drivers of degeneration in tendons.

METHODS: Tenocytes were isolated from mouse tail tendon fascicles of Young (4 weeks old) and Mature (45-50 weeks old) male mice following established protocols and treated with 20ng/mL TNFα in basal media for 6 hours at passage 1 (IACUC approved, sex was not considered in this case due to cell-based experimentation) **[5]**. Using three biological replicates per condition, RNA from treatment and control groups was isolated, and accessible chromatin loci were separately extracted via tagmentation. RNA and accessible chromatin were used for library preparation and sequenced via RNA-sequencing (RNA-seq) and Assay for Transposase Accessible Chromatin with sequencing (ATAC-seq), respectively. Significant differentially expressed genes (DEGs) and differentially accessible regions (DARs) were indicated by $|\log_2(\text{fold change})| > 1$ and p-adjust < 0.05. Comparison of TNFα-treated groups relative to controls was performed for each age group separately, and subsequent DEGs/DARs were compared between Young and Mature groups to determine age-associated differences. Age-exclusive DEGs/DARs were determined as genes with significant expression changes within only one age group following TNFα treatment, and these were used for downstream ontology analysis, including gene set enrichment analysis (GSEA), gene ontology analysis (GO), and Ingenuity Pathway Analysis (IPA). Transcription factor motif analysis was run using HOMER software to determine differences in transcription factor (TF) binding site accessibility within each age group, indicated as age-exclusive significant results. All sequencing analysis and statistics were run in R using DESeq2, Bioconductor, and HOMER packages or GraphPad (one-way ANOVA with Tukey's post hoc testing).

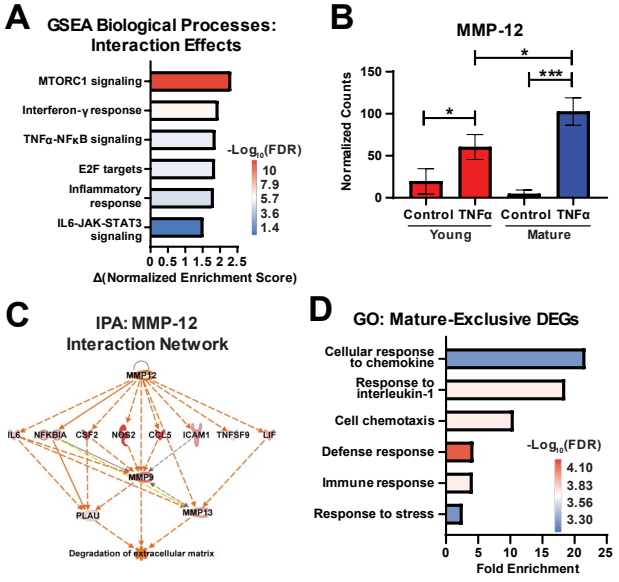

Figure 1: (A) Gene set enrichment analysis (GSEA) of RNA-seq data comparing Mature (Control vs TNFα) to Young (Control vs TNFα) tenocytes. Color indicates significance, and positive values indicate larger magnitude changes in Mature cells. **(B)** Normalized read counts of MMP-12 in Young and Mature cells with TNFα treatment. **(C)** Ingenuity Pathway Analysis (IPA) of RNA-seq DEGs showing the activation of matrix degradation pathways by MMP-12. **(D)** Gene ontology (GO) analysis of DEGs from integrated RNA-seq and ATAC-seq data (Control vs TNFα), exclusive to Mature tenocytes. Color denotes significance. (n = 3 biological rep; *: p<0.05, ***: p<0.001).

RESULTS: RNA-seq and ATAC-seq results revealed extensive age-related differences in tenocytes. TNFα stimulation induced inflammatory gene expression in both age groups; however, Mature cells exhibited a greater magnitude of change across multiple inflammatory pathways identified by GSEA interaction analysis, including immune cell invasion, cytokine production, and damage response **(Fig. 1A)**. Matrix degradation was markedly elevated in Mature tenocytes, with strong upregulation of matrix metalloproteases (e.g., MMP-12) and coordinated activation of multiple catabolic processes **(Fig. 1B, C)**. Integrated RNA-seq and ATAC-seq analyses further identified Mature-specific pathways governing immune activation and invasion **(Fig. 1D)**. Chromatin accessibility analysis via ATAC-seq revealed distinct TF regulation between groups: Young tenocytes significantly downregulated multiple pro-inflammatory RFX-family TFs, whereas Mature cells significantly increased accessibility of pro-inflammatory IRF-family TFs **(Fig. 2A)**. De novo motif enrichment analysis revealed significant downregulation of mechanoregulatory TFs, including TEAD in Mature cells, suggesting a potential loss of mechanical homeostasis with age **(Fig. 2B, C)**. Associated ontology analysis of ATAC-seq data confirmed that inflammatory signal propagation occurred acutely in Mature cells.

DISCUSSION: In this study, we demonstrated that Mature tenocytes exhibited exaggerated pro-inflammatory gene expression compared to Young tenocytes, suggesting a feed-forward loop of inflammation and matrix degradation that may lead to impaired healing in aged tendon tissue. Elevated catabolic activity compromises tissue structure and function, consistent with clinical associations between matrix breakdown and tendon pain **[1]**. Chromatin accessibility analysis further identified TF families driving these age-dependent responses, with Mature cells being preferentially open at pro-inflammatory genetic loci. Conversely, the loss of accessibility for mechanoregulatory TFs such as TEAD suggests diminished capacity to maintain mechanical homeostasis under inflammatory stress, thereby limiting the regenerative potential of aged tendons.

SIGNIFICANCE: Biological aging significantly delays tendon repair, allowing microdamage to accumulate and progress into large-scale mechanical failure and clinically significant lesions. Our findings identify age-dependent, cell-intrinsic pathways that drive matrix degradation and propagate inflammatory signaling in Mature tenocytes upon injury. These mechanisms highlight novel therapeutic targets to restore tendon homeostasis and enhance healing in the aging population.

References: [1] Kwan+, *Int J Mol Sci*, 2023; **[2]** Ireland+, *Mat Bio*, 2001; **[3]** Ellis+, *J Im Regen Med*, 2022; **[4]** Blanch+, *ORS 2025 AM*; **[5]** Zhang+, *APL Bioeng*, 2025.

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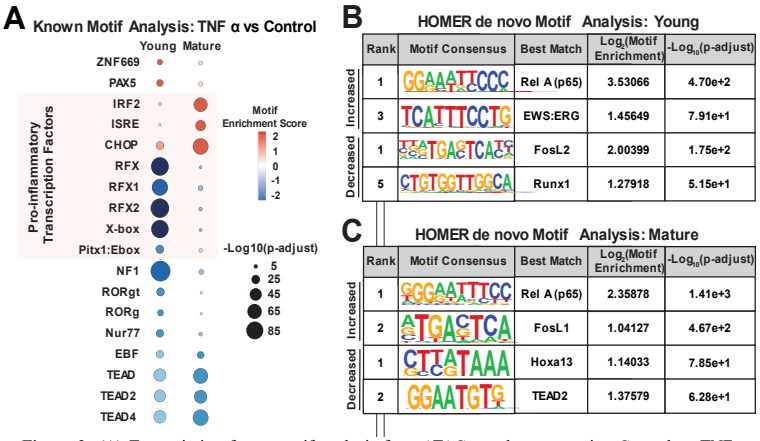

Figure 2: (A) Transcription factor motif analysis from ATAC-seq data comparing Control vs TNFα-treated Young and Mature tenocytes, with pro-inflammatory factors highlighted in pink. Color indicates enrichment score, and circle size reflects significance. **(B, C)** De novo motif analysis using ATAC-seq data from TNFα-treated Young **(B)** and Mature **(C)** tenocytes. (n=2 biological replicates per condition).