Aging Alters Epigenetic and Mechanobiological Status in Murine Tenocytes

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Introduction

Age-related fibrous connective tissue degeneration (e.g., tendinosis) is a significant and costly clinical problem. While the increased prevalence of tendinopathy in older populations is well known, the molecular mechanisms by which this degeneration occurs is less well understood[1]. Degeneration alters the bio-physical environments of fibrous tissues, including changes in stiffness and changes in mechanical loading conditions.[2] These changes alter the biophysical inputs to resident cells (called tenocytes), impacting their phenotype and contributing to pathology. Chromatin organization, which is regulated by epigenetic changes, plays an important role in gene expression and cell differentiation.[3] Moreover, altered bio-physical environments with tissue degeneration regulate epigenetic status in cells. For instance, in tenocytes, epigenetic changes in genome architecture that contribute to pathology may be explained by a stiffening environment that occurs with age.[4] However, this is not yet fully understood in the context of tendon aging and degeneration. Here, we investigate the underlying age-dependent epigenetic and chromatin structural changes in tenocytes and their baseline mechanobiological status.

Methods

Tenocytes were harvested from young (< 5 wks) and old (> 40 wks) mouse tail tendons (four donors per group) and cultured in basal growth media until passage 2. Cells were seeded and fixed (after two days) on 8-well chambered cover-glasses and stained for transcriptional repression markers (H3K27me3: the tri-methylation of lysine 27 on histone H3), activation markers (H3K4me3: the tri-methylation of lysine 4th of histone H3), and histone-H2B (for super-resolution STORM imaging). H2B staining and Voronoi cluster analysis-based chromatin density were determined using our established protocols[5, 6]. Baseline cellular contractility was determined by traction force microscopy (TFM) carried out on 10 kPa poly-acrylamide gels with cells seeded for 1 day[7]. A wound closure assay (WCA) was performed on cells seeded in a 6-well dish with imaging via brightfield at 4-hour increments. Wound closure was quantified by percent closure of a predefined area at time of scratch using Image J. Dynamic tensile loading (DL) was applied to cells seeded on aligned poly ε-caprolactone (PCL) nanofibrous scaffolds using a custom bioreactor[3] at 5% strain, 1Hz, for 5 hours—cell seeded scaffolds were immediately placed in Trizol for mRNA extraction. RT-PCR was performed to assess GAPDH, Type-I collagen (Col1), Scleraxis (Scx), Matrix metalloproteinase 3 (Mmp3), and Tumor necrosis factor-α (Tnfα). Fold change is calculated by the delta-delta-C_T method, relative to Young-Control. Statistical analyses were performed using a student's t-test or two-way ANOVA with Tukey's post hoc testing.

Results

The baseline mean intensity of H3K27me3 (epigenetic repressor) was higher in the aged group. Conversely, basal levels of H3K4me3 (epigenetic activator) decreased with donor age (Figure 1A). Consistently, STORM data showed an increase in Voronoi density (and thus increased nanoscale chromatin density and condensation) (Figure 1B). TFM showed an increase in cellular contractility in old cells, as measured by total force and average traction stress (Figure 2A). WCA results showed an increase in migratory capacity of old tenocytes as compared to young (Figure 2B, C). The application of DL significantly increased gene expression of Col1 in both young and old tenocytes (Figure 3.) and the load-induced Col1 gene expression level was higher in young donors than old donors (Figure .3). Interestingly, only the expression of Tnfα in the young group was significant in response to the mechanical perturbation (Figure 3). No significant changes in Scx1 and Mmp3 expression with DL were observed in either donor (Figure 3).

Discussion

The immunofluorescence of histone modifications broadly indicates a general decrease in transcriptional activity in aged tenocytes. This is consistent with quantitative STORM imaging of H2B – showing an increase in condensed chromatin and reduced levels of gene expression. We used TFM and WCA

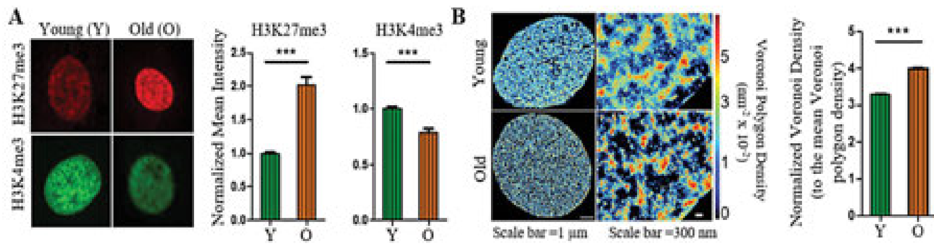

Figure 1. Region-dependent PCM presence is apparent at P14. Collagen VI staining (pink) reveals no differences in intensity between the tensile (Tens) and compressive (Comp) regions of the FDL tendon at P7 **(A)**. By P14 **(B)**, the compressive region contains higher intensity staining compared to the tensile region, which persists at P21 **(C)**. Signal intensity quantification shows that these changes are significant **(D)**. Solid lines denote $p < 0.05$.

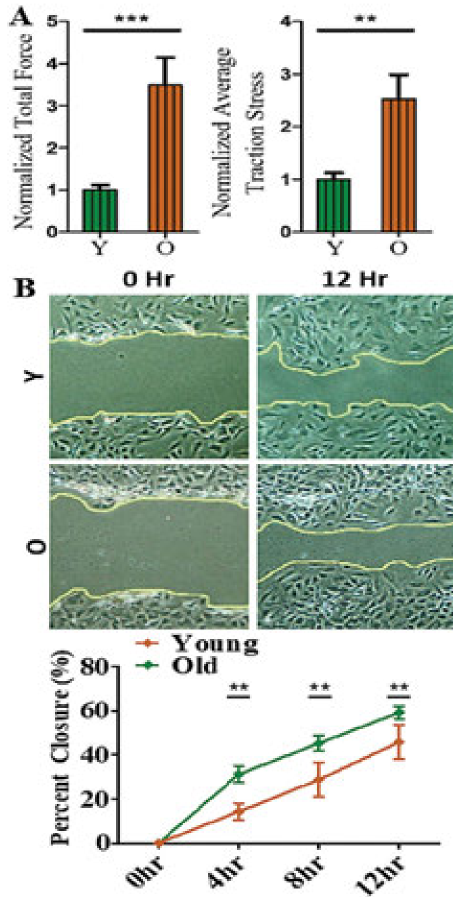

Figure 2. (A) Total traction force and normalized average traction stress (n=37 both groups, $p < 0.05$). **(B)** WCA representative images and results quantified as percent wound closure over time ($n = 3$ both groups, **: $p < 0.01$).

to measure the mechanobiological status of the tenocytes, and the results suggest an altered baseline contractility and migratory capacity in cells with aging. An increase in traction stress in old cells correlates well with the increase in migratory behavior observed in the WCA. Interestingly, Col1 was upregulated in both young and old tenocytes in response to 5% strain DL, but no effects were observed in Scx and Mmp3. More interestingly, Tnfα (a pro-inflammatory cytokine) was significantly upregulated in only the young group in response to DL, but no significance was observed in the old group. The effects of young and old tenocytes to DL are interesting, and ongoing studies are further focused on determining the effects of applying varying strain levels of the DL on young and old tenocytes. Overall, these data indicate age-dependent mechano-sensitivity in tenocytes and ongoing studies are focused on exploring the impact of degeneration on the tenocyte epigenome and in generating a detailed landscape of age-dependent gene expression by profiling accessible chromatin through ATAC-Seq and by understanding the impact of histone modifications through ChIP-Seq.

These findings contribute to a broader understanding of changes in epigenetic and mechanobiological status in tenocytes within aging tendon and its role in tissue degeneration for therapeutic applications. Future work will focus on novel epigenetic methods to rejuvenate tenocytes to promote healthy tissue regeneration and physiological function.

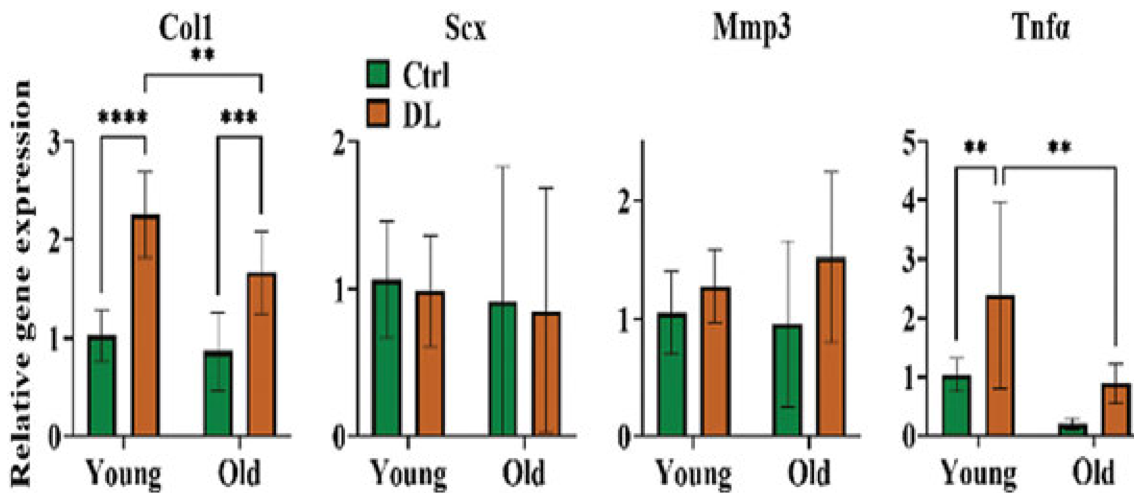

Figure 3. Gene expression levels of Col1, Scx, Mmp3, and Tnfα ($n = 10$ from two donors per group, *: $p < 0.05$). Y: Young, O: Old, Ctrl: Control, DL: Dynamic Loading.

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