Tendon Pathology Alters Chromatin Organization and Mechano-sensitivity in Human Tenocytes

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Disclosures

None

Introduction

Fibrous connective tissue injury or degeneration (e.g. tendinosis) is prevalent, with few treatments that restore function. Pathological changes in tendon alter the tissue chemo-physical environment, impacting endogenous cell behavior[1,2]. For instance, degeneration alters collagen orientation and changes tissue stiffness,[1,2] and lower local oxygen levels present in damaged tissues may promote early tendinopathy[2]. Furthermore, in the early phase of the injury/disease process, pro-inflammatory cytokines in the local milieu promote tendon cell catabolic response. These pathological changes in tissues impact cells across length scales, including at the level of chromatin organization. In a previous study, using super-resolution imaging, we found that tendinosis results in nano-scale chromatin reorganization in human tenocytes, with chromatin increasingly localized to the nuclear periphery, compared to healthy age matched controls[3]. Moreover, when healthy human tenocytes were cultured on a soft microenvironment (~3 kPa), we observed similar chromatin reorganization as seen in degenerative tenocytes[3]. Based on these findings, here we extended these studies to further investigate how cues from pathological chemo-physical environment (e.g. dynamic changes in stiffness, induction of hypoxia, and presence of inflammatory factors) impact chromatin remodeling in tenocytes.

Methods

Human tenocytes were isolated from young [Young (y), 42 years] or degenerative [Tendinosis (t), 35 years] tendons according to established protocols[2]. To investigate how changes in oxygen tension affect nanoscale chromatin organization, young healthy tenocytes were seeded on chambered-coverglass (500 cells/mm^2) for one day, followed by four days of culture under normoxic (21% O_2) or hypoxic (1% O_2) conditions. Next, to investigate how pro-inflammatory cytokines impact chromatin, young tenocytes were cultured on chambered-cover glasses for 1 day followed by additional culture for 24-hours with/without exposure to IL-1β (0.1-1ng/ml) or TNFα (1-10 ng/ml). Finally, to investigate how degeneration impacts mechano-sensitivity of tenocytes, young or tendinosis tenocytes were seeded onto a "stiffening" hydrogel system that provides rapid dynamic changes in substrate stiffness (for example, ~3→30 kPa) in the presence of cells[5]. For this, cells were pre-cultured for 24 hours followed by additional 24 hours of culture after stiffening. For all studies, fixed cells were immunostained for histone-H2B (H2B, Proteintech), and then incubated with secondary antibodies custom labeled with activator-reporter dye pairs (Alexa Fluor 405-Alexa Fluor 647, Invitrogen) for super-resolution stochastic optical reconstruction microscopy (STORM) imaging (Nanoimager, ONI)[3,4]. Obtained STORM images were analyzed by dividing the inner border of the nucleus (border: 15-20% determined from the image intensity profile across the diameter of the nucleus) from the rest of the nucleus and rendered these segments using custom MATLAB code and the Nanoimager software (ONI) respectively. For quantitative analysis, in MATLAB, Voronoi tessellation of the H2B localizations was adapted to segment super-resolution images[3,4].

Results

Consistent with our previous findings[3], super-resolution imaging H2B heat maps showed that, while dense chromatin was distributed through the nucleus in young healthy tenocytes (Young), H2B was more condensed and primary localized to the nuclear periphery in young degenerative nuclei (Tendinosis) (Figure 1a-c). Strikingly, when healthy tenocytes were cultured under the hypoxic conditions, chromatin relocalized to the nuclear periphery and became more condensed (Figure 1a-c). This suggests that altered oxygen tension in tendon after injury or during degeneration may promote aberrant chromatin remodeling. Given that injury and degeneration increase inflammation, we next investigated how pro-inflammatory cytokines impact nanoscale chromatin organization in young healthy tenocytes. These studies showed that exposure to pro-inflammatory

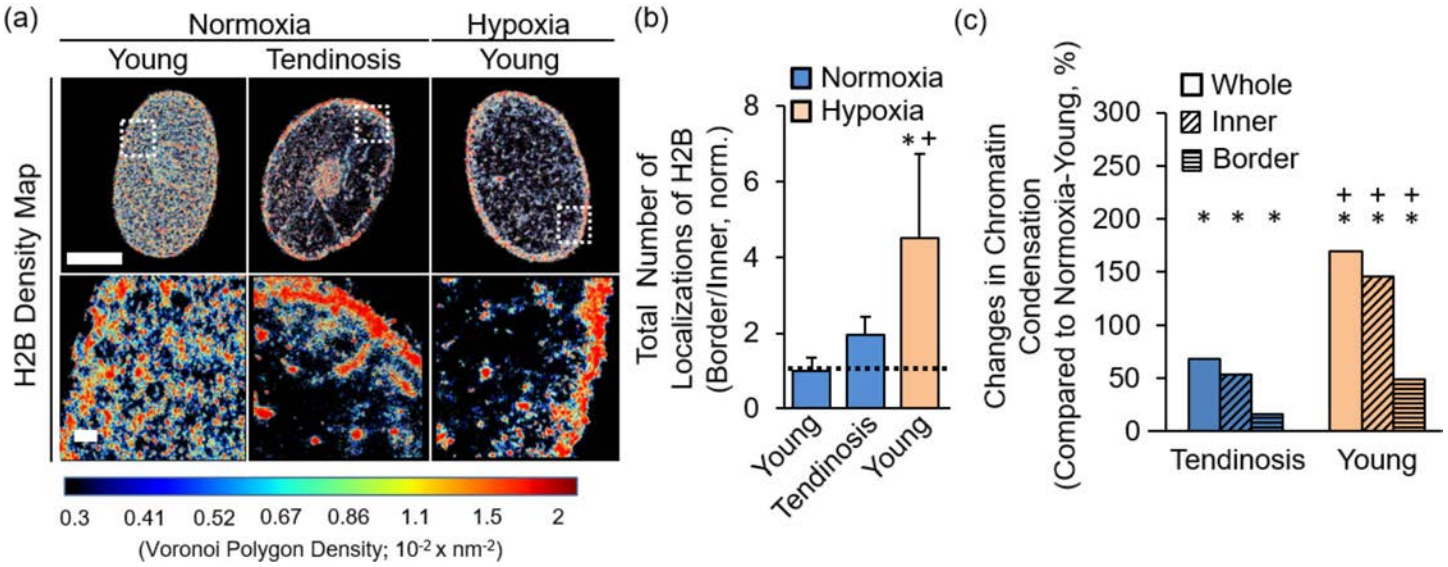

Figure 1: (A) Heat maps showing H2B localization density in young or tendinosis under normoxia or hypoxia culture condition (scale bars: top = 5μm, bottom = 500 nm). Quantification of the ratio of the total number of H2B localizations in the border to the inner **(B)** and changes in chromatin condensation (compared to young tenocyte in the normoxia culture condition). n = 5 cells, *:p < 0.05 vs. Young-normoxia, +:p < 0.05 vs. Tendinosis-normoxia.

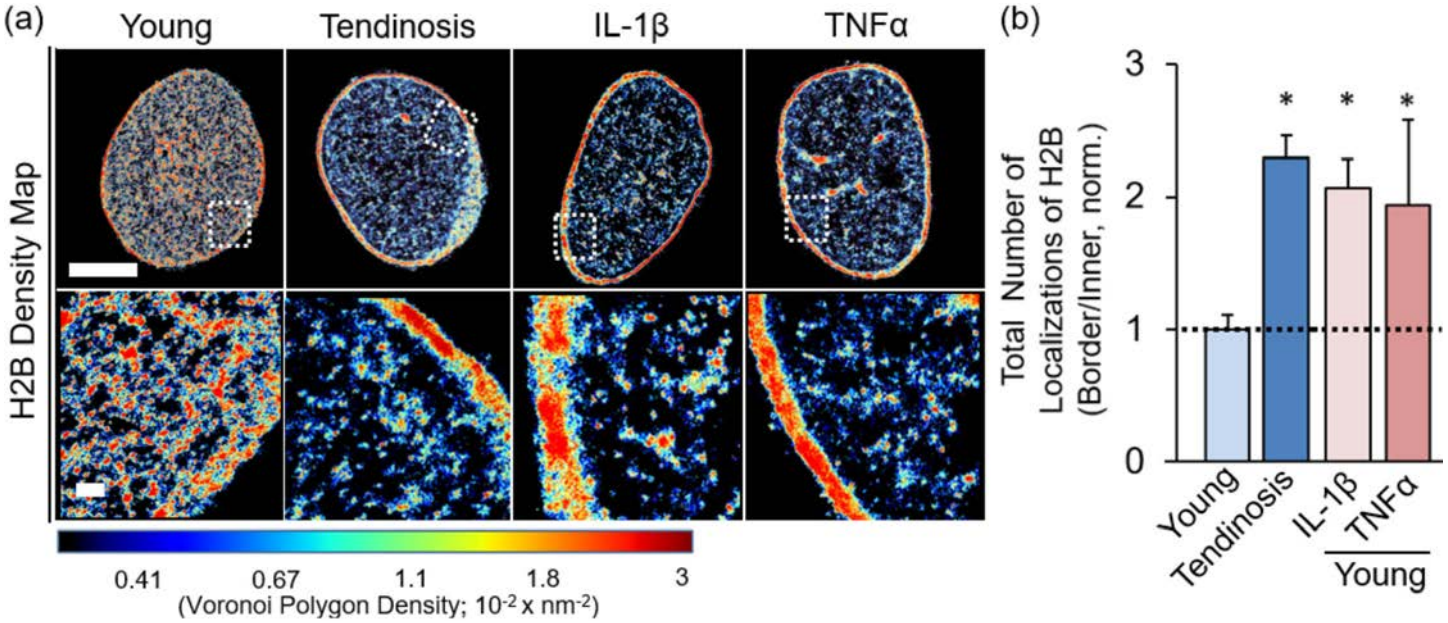

Figure 2: (A) Heat maps showing H2B localization density in young, tendinosis, or young human tenocytes with IL-1β (1 ng/ml) or TNFα (10 ng/ml) treatment. (B) Quantification of the ratio of the total number of H2B localizations in the border to the inner. n = 5 cells, *:p < 0.05 vs. Young human tenocyte (normalized). (scale bars: top = 5μm, bottom = 500 nm).

cytokines (IL-1β or TNFα) for one day resulted in rapid led chromatin reorganization to the nuclear periphery (Figure 2a-b) and increased chromatin condensation (not shown). This suggests that pro-inflammatory factors present in the wound environment impact chromatin organization in tenocytes during repair. Finally, we examined how a change in tissue stiffness impacts chromatin mechano-response in tendons. As was seen in soft substrates[3], H2B in young or tenocytes with tendinosis was primarily localized to the nuclear periphery within 4 hours after stiffening (Figure 3a-b). With 24 hours of stiffening, H2B became more uniformly dispersed and de-compacted in young healthy tenocyte nuclei. Conversely, in tendinopathic cells, no change in the spatial organization of chromatin and less de-compaction was observed (Figure 3a-c). This may suggest that prolonged changes in chromatin organization in diseased tenocytes may be associated with a loss of mechanical sensitivity and chromatin reorganization capacity with tissue degeneration.

Discussion

In this study, we show that tendon degeneration alters nanoscale chromatin organization in tenocytes and impacts their mechanical sensitivity. These data indicate that alterations in the chemo-physical environment that arise with tendon injury or degeneration induces phenotypic and chromatin alterations that are apparent at the nanoscale. Chromatin

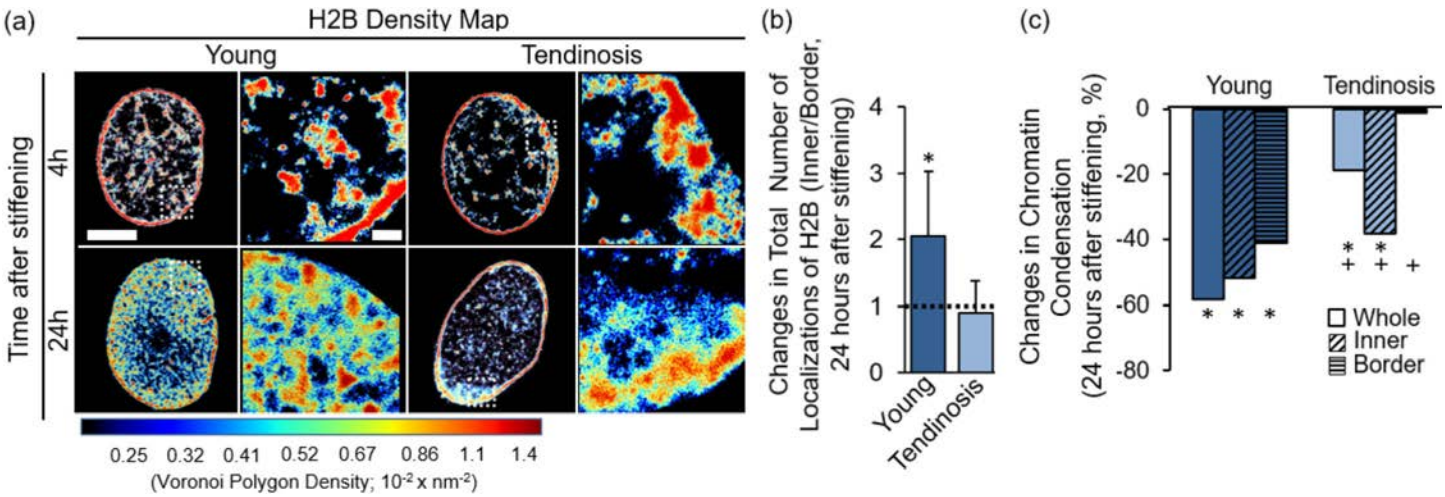

Figure 3: (A) Heat maps showing H2B localization density in young or tendinosis cultured on stiffening hydrogel system (4 hours or 24 hours after stiffening, scale bars: left = 5μm, right = 500 nm). Quantification of the ratio of the total number of H2B localizations in the inner to the border **(B)** or changes in chromatin condensation at 24 hours after stiffening **(C)**. n = 5 cells, *:$p < 0.05$ vs. before the stiffening, +:$p < 0.05$ vs. Young tenocyte.

reorganization to the nuclear periphery has been described as silent chromatin (heterochromatin) suppressing transitional activation[6]. Current work is identifying specific genetic loci that move to the periphery and the transcriptional activity of these loci in diseased tenocytes.

Significance

Our data show that degeneration alters the nanoscale chromatin organization of tenocytes and changes their mechano-sensitivity. This study may inform new directions to identify novel therapeutic targets for the treatment of connective tissue pathologies.

Acknowledgements

This work was supported by the NIH (R01 AR056624, K01 AR077087) and NSF (CMMI-1548571)

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