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

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Introduction

- Age-related fibrous connective tissue degeneration (e.g. tendinosis) is a significant clinical problem. Development, aging, and degeneration alter the mechanical environments of fibrous tissues, changing the biophysical inputs to resident cells and impacting their phenotype **[1]**.
- For instance, with degeneration of fibrous tissues, organized fibrous tissue structure begins to fail and micro-domains of non-fibrous material emerge in flexor tendons **(Fig. 1)**, characterized by cells that locally change in phenotype and begin producing aberrant proteoglycan-rich ECM (arrows) **[1, 2]**.

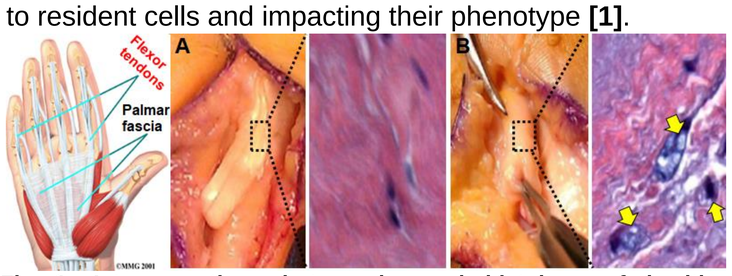

Fig 1. Intra-operative photographs and histology of healthy 35-year-old (A) or 42-year-old female patient undergoing tendinosis (B) flexor tendons (Red: collagen, Blue: proteoglycan-rich ECM.

- With tendon degeneration, tissue stiffness **[2]**, and in vivo areas of tendon vascularity **[3]** are reduced impacting cell phenotype.
- While it has been well understood that chromatin organization plays an important role in gene expression and differentiation to impact cell phenotype **[4]**, these studies not been applied to the context of tissue aging and degeneration regulate these processes is not known.
- ***Here, using a super-resolution nanoscopy [i.e. stochastic optical reconstruction microscopy (STORM)], we assessed how tissue degeneration altered chromatin organization at the nano-scale in tenocytes isolated from human donors that were young, or diagnosed with tendinosis (a common tendon pathology, associated with repetitive use and mechanical overload [2]).***

Materials and Methods

- ***Cell Preparation:*** Human tenocytes were isolated from young [Young (y), 35 years, female] or degenerative [Tendinosis (t), 42 years, female] flexor tendons with established protocols **[2]**.
- ***Assessment of Histone-H2B Localization at the Nano-scale:*** For STORM imaging (Nanoimager, ONI), cells were plated on chambered-cover glasses (500 cells/mm^2) followed by 2 days of culture in basal growth media. 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) **[4]**. STORM images were analyzed and rendered using Nanoimager software (ONI).

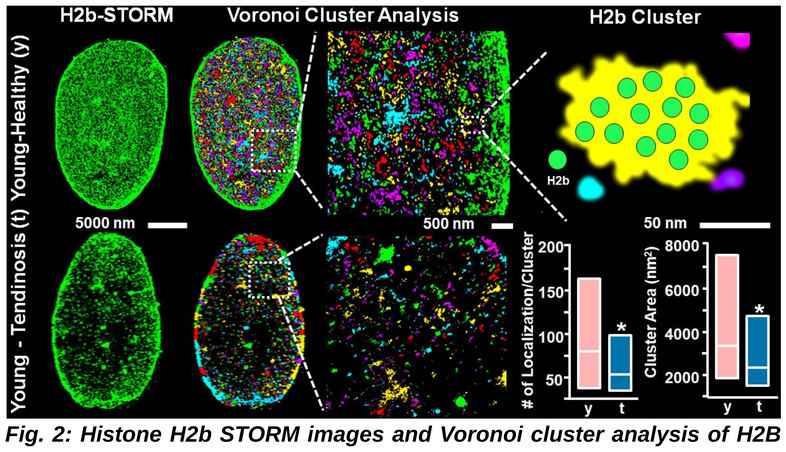

*Fig. 2: Histone H2b STORM images and Voronoi cluster analysis of H2B localizations in young (y), or tendinosis (t) human flexor tendon cell nuclei. Quantifications show the number of H2b localizations per cluster and the cluster area [n ≥ 10,275 clusters from 5 cells, *p<0.01 vs. Young Healthy (y)]; the box and line correspond to the interdecile range (IDR, 10th~90th percentile) and median respectively.*

- For quantitative analysis, Voronoi tessellation of the H2b localizations and H2b density maps were implemented in MATLAB to segment super-resolution images **[4]**.
- ***Substrate Stiffness & Hypoxic Culture:*** To investigate the effect of substrate stiffness, young (y) healthy tendon cells were cultured on cover glass (Glass), 5 kPa (Soft), or 55 kPa (stiff) methacrylated hyaluronic acid hydrogels (MeHA) **[Fig. 3 (A), 5]**, followed by 2 days of culture, and were then imaged by STORM. Additionally, to investigate the effect of hypoxic culture conditions, young (y) healthy tendon cells were seeded in glass substrates, and cultured under hypoxic (1% O_2) or normoxic (21% O_2) conditions for 4 days.

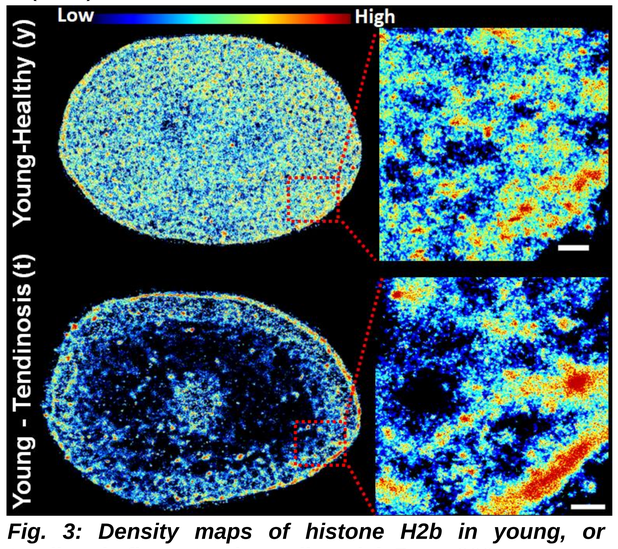

Fig. 3: Density maps of histone H2b in young, or tendinosis flexor tendon cell nuclei. Bar = 300 nm.

Results

- STORM images of H2B and Voronoi tessellation analysis showed that, on glass, H2B localizations clustered to form distinct and spatially separated nanodmains in young healthy tenocyte nuclei [Young (y)]. Conversely, degenerated [Tendinosis (t)], tenocyte nuclei contained smaller domains **(Fig. 2)**.
- Interestingly, H2B nanodomains were localized at nuclear periphery in the degenerative [Tendinosis (t)] tenocyte nuclei **(Fig. 3)**.
- When young healthy tenocytes (y) were cultured on soft substrates, nanodomain size decreased to levels comparable to that seen in degenerative (tendinosis) or aged tenocytes and, strikingly, these H2B nanodomains relocated to the nuclear periphery on the 5 kPa substrates (soft) **(Fig. 4A-D)** .

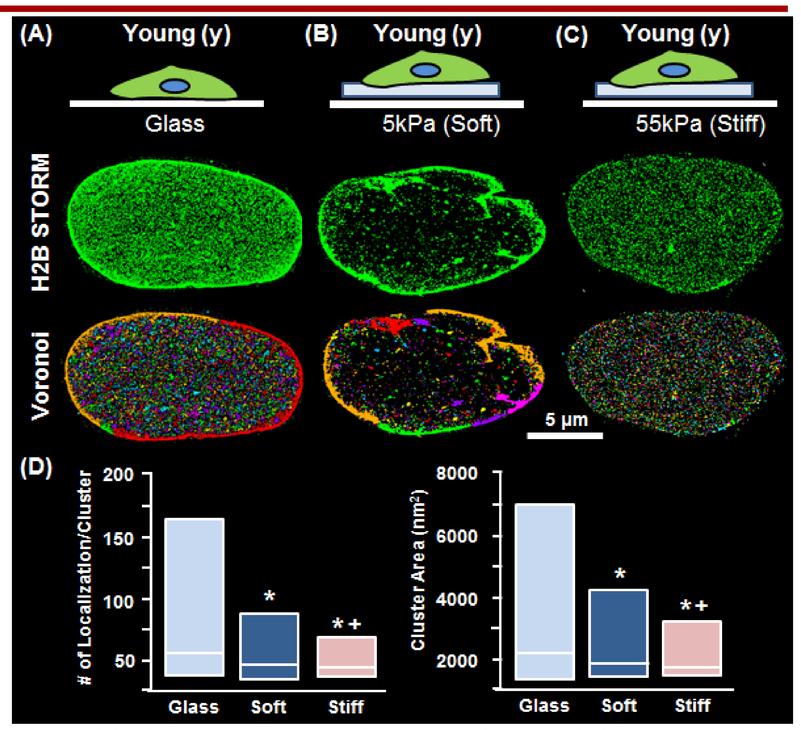

*Fig. 4: (A) Histone H2B STORM images and Voronoi cluster analysis of localizations in young (y) tendon nuclei seeded on glass (A), 5kPa (Soft, B), or 55kPa (Stiff, C) substrates. (D) Quantification of the number of H2B localizations per cluster and the cluster area [n ≥ 12,334 clusters from 5 cells, *p<0.01vs. Glass, +p<0.05 vs. Soft]; the box and line correspond to the interdecile range (IDR, 10th~90th percentile) and median respectively.*

- When young healthy tenocytes (y) were cultured under the hypoxic conditions, nanodomain size decreased to levels comparable to that seen in degenerative (tendinosis), and strikingly, these H2B nanodomains relocated to the nuclear periphery **(Fig. 5)**.

Discussion

- In this study, we show that degeneration alter chromatin organization and mechano-responsivity in tenocytes.
- Interestingly, tendon degeneration decreased the number and area of localizations, while disease increased the concentration of these nanodomains to the periphery.
- This region of the genome is understood to be generally heterochromatic (condensed) and inactive. Interestingly, when healthy young cells were plated onto soft substrates, this bias in nanodomain localization to the periphery increased, suggesting perhaps that tendon micro-damage and softening drives aberrant cell behavior.
- When young healthy tenocytes (y) were cultured on soft substrates (5kPa) under normoxic conditions (21% O_2) or stiff substrates (glass) under hypoxic conditions (1% O_2), nanodomain size decreased to levels comparable to that seen in degenerative (tendinosis) and, these H2B nanodomains relocated to the nuclear periphery on the conditions.
- These data supports that 'degenerative' biophysical environments in tendons impact chromatin localization in tenocytes.

Ongoing studies: We now are focused on elucidating how this nano-scale organization impacts expression and phenotype in tendon aging and disease.

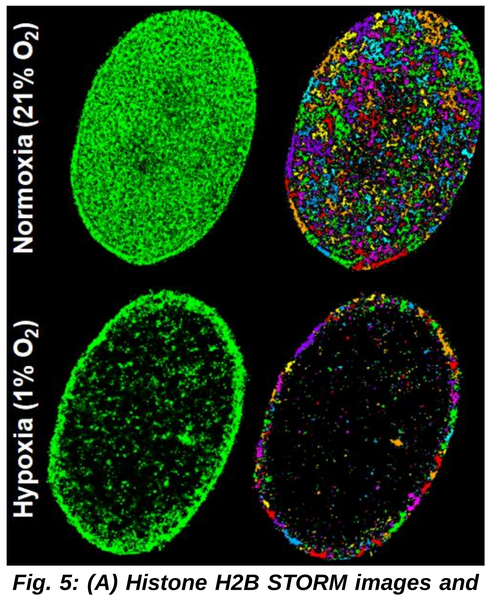

Fig. 5: (A) Histone H2B STORM images and Voronoi cluster analysis of localizations in young (y) tendon nuclei seeded on glasses and cultured under normoxia or hypoxia culture conditions.

References

[1] Han+, Nat Mater 2016; [2] McBeath+, Aging Cell 2019; [3] Tempfer+, Front Physiol 2015; [4] Heo+, eLife 2016; [4] Ricci+, Cell 2015; [5] Cosgrove+, Nat Mater 2016; [6] Heo+, ORS 2019.

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