Senescence in tendon cells causes loss of cell-matrix homeostasis and can be alleviated with senotherapeutics

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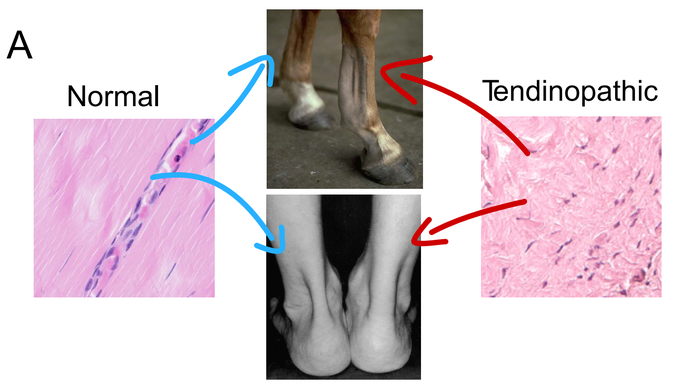
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Background

Tendons are frequently injured, particularly as we get older.

Horses also suffer age-associated tendon injuries, and the equine superficial digital flexor tendon (SDFT) provides an excellent model to study tendon ageing, with homologous energy-storing function and structure to human Achilles.

During ageing, the tendon extracellular matrix undergoes structural and compositional changes which contribute to tendon degeneration. While the drivers of these changes are unknown, cells isolated from aged tendons exhibit markers of senescence.



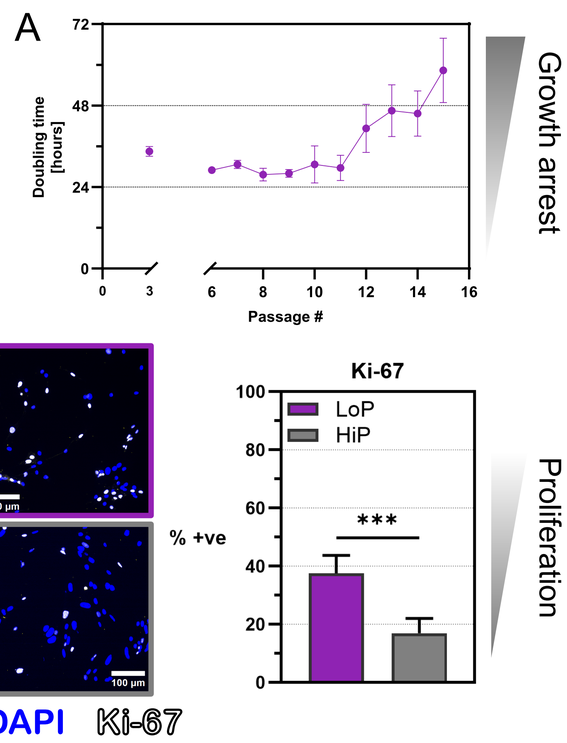
(A) The equine SDFT has similar structure, function, and disease aetiology to human Achilles tendon. (B,C) Staining of tendon fibroblasts from younger/older horses for canonical markers of senescence.

Aim & Methods

Aim: Uncover the role of cellular senescence in age-related tendon degeneration.

Tendon fibroblasts isolated from healthy SDFTs are either frozen (low-passage) or replicatively stressed to the point of senescence (high-passage).

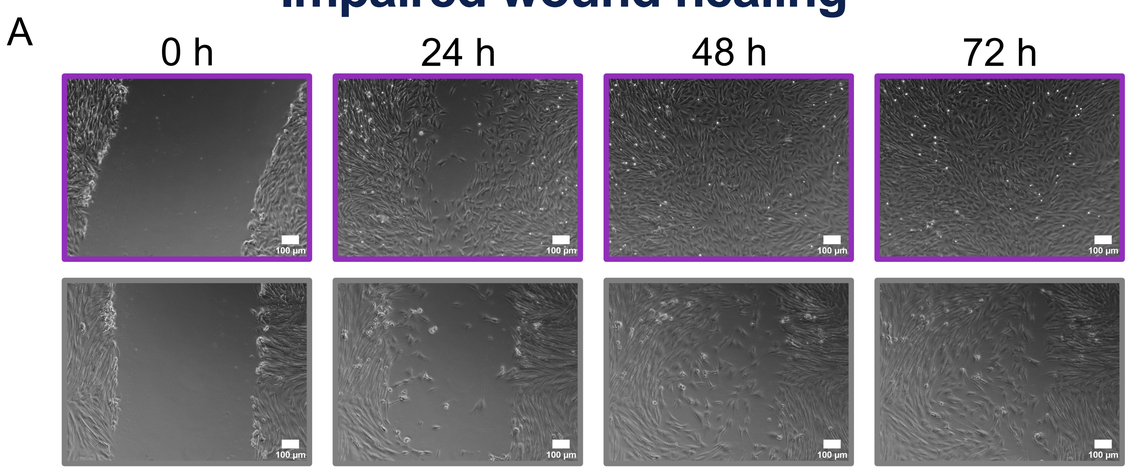
By inducing senescence in healthy cells in vitro, we decipher links between cellular senescence and tendon degeneration through transcriptomics and functional assays; and trial senotherapeutic treatments to reverse the effects of senescence.



(A) Repeated passaging replicatively stresses tendon fibroblasts to senescence. (B,C) High-passage cell populations from younger tendons replicate senescence marker expression of cells taken from older tendons.

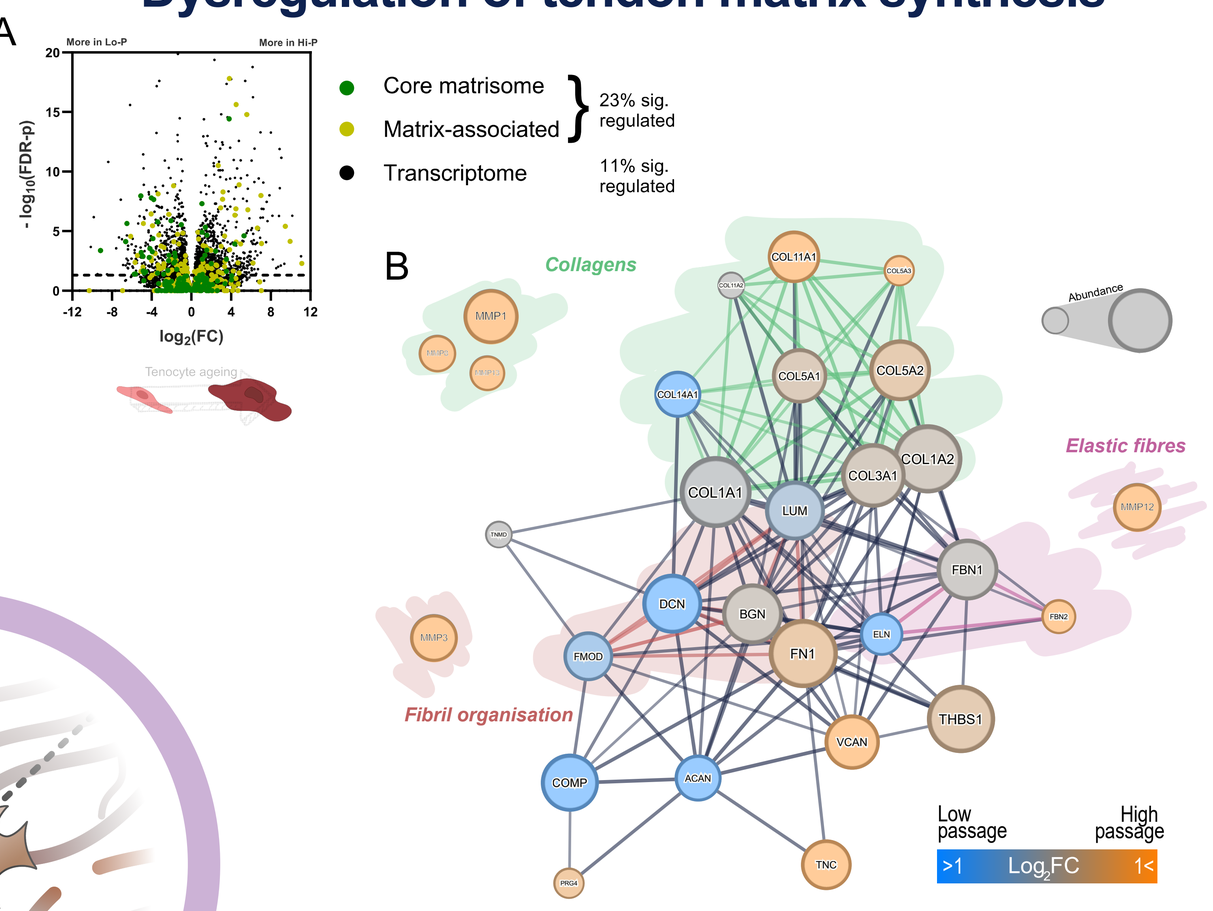
Results

Impaired wound healing



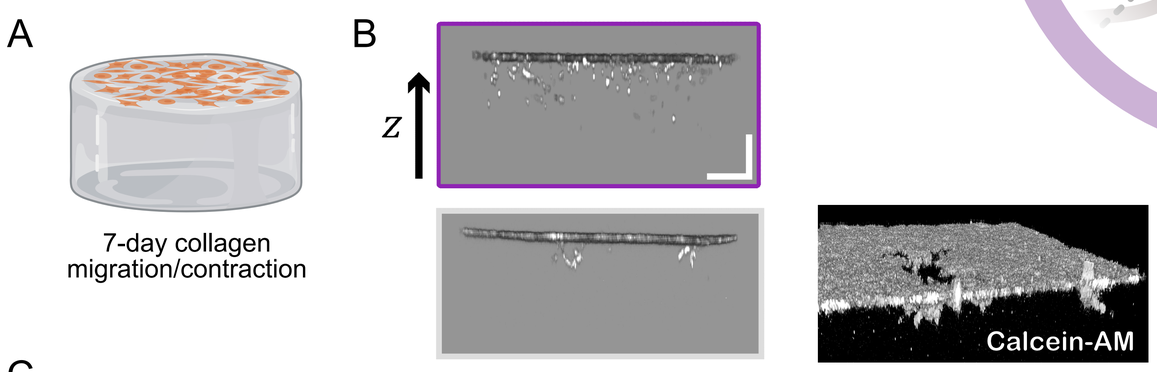
(A,B) Scratch assays with live-cell imaging demonstrate decreased cell motility and impaired capacity for senescent tendon fibroblasts to close an artificial wound site.

Dysregulation of tendon matrix synthesis



(A) Bulk RNA-sequencing shows that the matrisome is disproportionately affected by senescence versus the rest of the transcriptome. (B) Several tendon matrix proteins and MMPs are dysregulated at the transcript level in senescent cells.

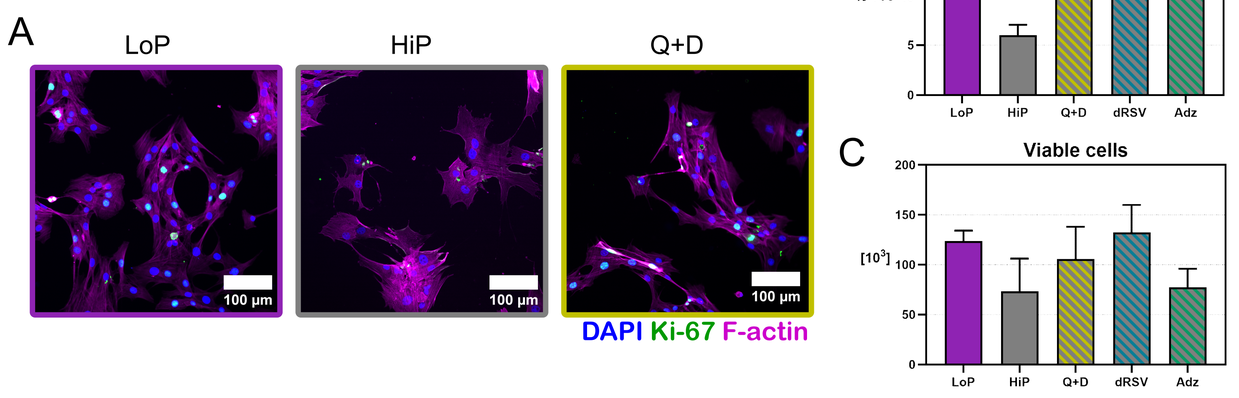
Altered cell-matrix interaction



(A) Tendon fibroblasts are seeded atop a collagen gel, with migration (B) and contraction (C) analysed over 7 days. (D) Altered collagen interaction coincides with increased expression of inflammatory cytokines.

Senotherapeutic regeneration

***S.lytic**: Quercetin/Dasatinib, selectively kills senescent cells*
***S.reversant**: Dihydroresveratrol, restores proliferative capacity*
***S.morphic**: Adezmapimod, suppresses pro-inflammatory secretion*



(A) 7-day treatments of high-passage populations with senotherapeutic compounds have the capacity to improve cell proliferation (B, via S-phase marker Ki-67 staining), and viability (C, via trypan blue staining).

Glossary

***Senescence:** Pro-inflammatory DNA-damaged cells which accumulate in ageing tissues*

***SDFT:** Equine structural & functional homologue to human Achilles tendon*

***High-passage:** Cellular model of ageing via replicative senescence*

Conclusions

- *In vitro senescence provides a tool to link cellular ageing to tendon degeneration.*
- *Senescent tendon fibroblasts have impaired tendon cell function and dysregulate tendon matrix homeostasis.*
- *Senotherapeutics may revert phenotypes of tendon fibroblast senescence.*