Effects of Aging on the Molecular Profile of Cultured Tendon Cells

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Introduction

- Rotator cuff tears affect millions each year, with a higher incidence in the elderly. Surgical repair can improve function and reduce pain, but repair failure is common [1].
- Biologic augmentation has been investigated [2-4].
- Autologous biceps cells delivered to the repair site during supraspinatus repair improve healing in juvenile and aged rats, but do not affect healing in adult rats [5].
- Molecular mechanisms behind differential effects are not well understood.
- Objective:** to determine differences in the **RNA signature of primary tendon-derived cells** from the long head of the biceps of juvenile, adult, and aged animals.
- Hypotheses:** 1) **juvenile cells exhibit a more stem-cell like molecular profile** than aged or adult tendon-derived cells, 2) **aged cells have increased expression of genes associated with tendon homeostasis** and differentiation compared to juvenile cells.

Methods

27 Fisher (F344) rats used (IACUC approved) across 3 age groups: juvenile, adult, and aged

Cell culture: Animals were euthanized and intra-articular biceps tendons were collected. Biceps tendon cells harvested via tissue morselization and cell migration.

Cell Staining: P1 cells were fixed, permeabilized, stained with Phalloidin and DAPI, and imaged (Leica TCS SP8 Multiphoton Confocal).

RNA Isolation: P1 cells were lysed and homogenized in TRIzol, RNA isolated and purified.

Rat Transcriptome Array and Bioinformatics Analysis: cDNA was synthesized and run (Clariom™ Rat Transcriptome Array). Bioinformatics processing: Transcriptome Analysis Console (TAC) Software and DAVID (n=5/group).

qRT-PCR: qPCR run in quadruplicate using TaqMan assays (n=8/group). Data analyzed with $\Delta\Delta C_t$ method; expression levels were compared between age groups with one-way ANOVAs and post-hoc Tukey tests.

Results

- Majority of gene expression clustered into six distinct patterns when comparing between ages.
- Majority of differential gene expression between juvenile and aged cells (not shown).

- No qualitative differences were observed in cell morphology between age groups.

- qPCR confirmed that genes associated with stemness (*Fgf10*, *Postn*, *Gpnmb*, *Osr1*) are downregulated with age.
- Genes related to inflammation are differentially expressed with age (decreased *Il6* and *Il6st*, increased *Cd200* and *Cd28*).

Conclusions

- Distinct molecular profiles exist for juvenile, adult, and aged biceps tendon-derived cells.
- Juvenile cells: increased expression of genes associated with mesenchymal stem cells** compared to aged cells.
- Aged cells:** stem cell markers decreased, suggesting a **greater population of terminally differentiated tendon cells**; few consistent increases in tendon marker expression, suggesting **population heterogeneity**; demonstration of **anti-inflammatory signature**.

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[1] Galatz LM+ J Bone Joint Surg Am 2004. [2] Hernigou P+ Int Orthop 2014. [3] Chen JM+ Tissue Eng 2007. [4] Longo UG+ Br Med Bull 2010. [5] Huegel J+ J Orthop Res. 2017. [6] Lin TW+ J Biomech 2005. [7] Lin TW+ J Biomech 2006.