Effects of Aging on the Molecular Profile of Cultured Tendon Cells

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Introduction

Rotator cuff tears affect millions of individuals each year, with a higher incidence in the elderly. Although surgical repair can improve function and reduce pain, rotator cuff repair failure is common[1]. To improve surgical outcomes, biologic augmentation via delivery of cells or growth factors has been investigated[2-4]. Recently, autologous biceps cells delivered via nanofibrous scaffold to the repair site during supraspinatus repair were shown to improve healing in juvenile and aged rats, but did not affect healing in adult rats[5]. However, the molecular mechanisms behind these differential effects are not well understood. Therefore, the objective of this study was to determine the differences in the RNA signature of primary tendon-derived cells cultured from the long head of the biceps of juvenile, adult, and aged animals. Our hypotheses were: 1) tendon-derived cells from juvenile animals would exhibit a molecular profile more characteristic of stem cells than tendon-derived cells from adult or aged animals, and 2) tendon-derived cells from aged rats would have increased expression of genes associated with tendon homeostasis and differentiation compared to cells derived from juvenile or adult rats.

Methods

27 Fisher (F344) rats were used (IACUC approved) across three age groups: juvenile (4 weeks), adult (8 months), and aged (16 months) (n = 9/age group). Animals were sacrificed and the intra-articular biceps tendons were collected.

Cell Culture

Biceps tendon cells were harvested from the tissue via morselization and cell migration. Cells were expanded in culture using basal media and split at confluence. Subcultured (P1) cells were allowed to reach 75-85% confluence (average 12 days in culture) at which time they were lysed and homogenized in TRIzol.

RNA Isolation

RNA was isolated using the TRIspin method and processed via RNA Clean & Concentrator 5 columns (Zymo Research).

Rat Transcriptome Array and Bioinformatics Analysis

cDNA made with 250ng of RNA using the Affymetrix WT PLUS Kit and was run on a Clariom™ D Rat Transcriptome Array 1.0 (Applied Biosystems, n = 5/age group). Bioinformatics processing was performed using Transcriptome Analysis Console Software and DAVID analysis (cut-offs set at $|FC| > 2$ and $p < 0.05$ for all pair-wise age comparisons). *qRT-PCR*: Reverse transcription was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). To validate microarray results, qPCR was run in quadruplicate using TaqMan Assays on a QuantStudio 12K Flex Real-Time PCR system (ThermoFisher, n = 8/age group). Data was analyzed using the $\Delta\Delta Ct$ method, and expression levels were compared between age groups with one-way ANOVAs and post-hoc Tukey tests. *Cell Staining*: At 50% confluence, P1 cells were fixed, permeabilized, and stained with Alexa Fluor 488 Phalloidin and DAPI. Slides were imaged with a Leica TCS SP8 Multiphoton Confocal.

Results

Principle component analysis demonstrated that cell expression profiles grouped into distinct regions by age (Figure 1). The majority of gene expression clustered into six distinct patterns when comparing between ages (data not shown). The majority of differential gene expression exists between juvenile and aged cells (640 genes significantly decreased, 531 increased), while the

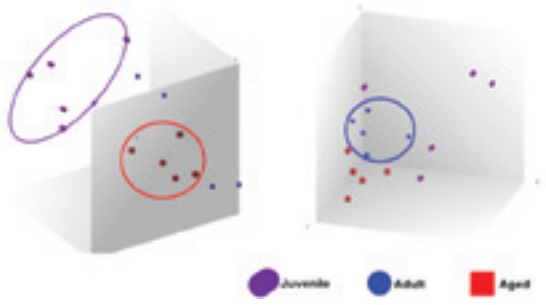

Figure 1. Two Principle Component Analysis plots from Rat Transcriptome Analysis. Juvenile cells shown as purple cylinders, adult cells are shown as blue spheres, and aged cells are represented as red cubes. Age groups are circled to demonstrate distinct clusters.

fewest differences exist between adult and aged cells (54 genes significantly decreased, 101 genes increased). Comparison of juvenile cells to adult cells identified 216 significantly increased genes and 203 significantly decreased genes. No qualitative differences were observed in cell morphology between age groups (data not shown). Gene ontology identified differences in genes related to: 1) cell adhesion, wound healing, and chondrocyte differentiation between juvenile and adult cells, 2) cell division and cell adhesion between juvenile and aged cells, and 3) wound healing and vasculogenesis between adult and aged cells. qPCR confirmed that genes associated with stemness are downregulated with age, including *Postn*, *Fgf10*, *Osr1*, and *Gpmnb* (Figure 2). Additionally, genes related to inflammation are differentially expressed with age, including increased *Cd28* and *Cd200* expression and decreased *Il6* and *Il6st* expression (Figure 3).

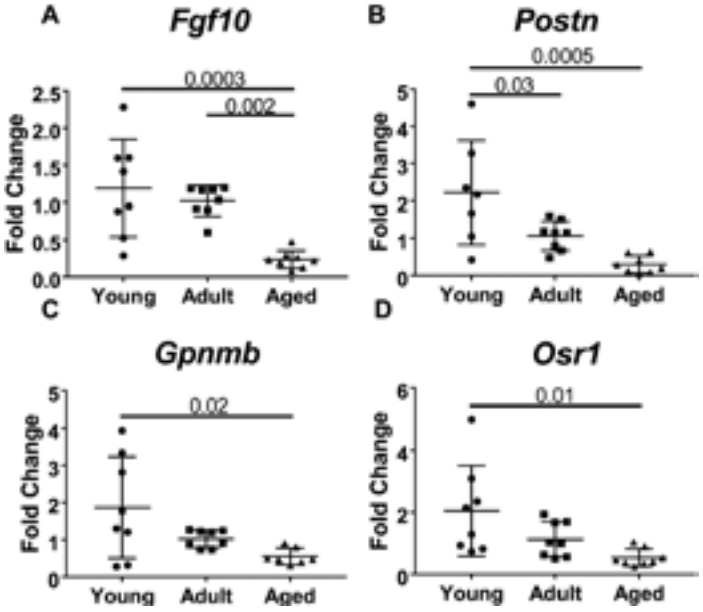

Figure 2. Decreased expression of stem related mRNAs measured via qPCR. **(A)** *Fgf10* **(B)** *Postn* **(C)** *Gpnmb* and **(D)** *Osr1* expression decreased with aging. Significance is denoted with solid lines (n = 8/group).

Discussion

Results demonstrate distinct molecular profiles for juvenile, adult, and aged biceps tendon-derived cells. Juvenile cells showed increased expression of genes associated with mesenchymal stem cells, such as *Postn and Fgf10*, supporting our first hypothesis. Furthermore, although stem cell associated markers are present in both juvenile and adult cells, they are significantly decreased in aged cells, suggesting that a greater population of aged tendon cells may have terminally differentiated. However, contrary to our second hypothesis, there were no consistent increases in the expression of tendon markers in aged cells, suggesting that there may be significant population heterogeneity. Interestingly, aged cells demonstrate a decreased pro-inflammatory signature, including decreased expression of pro-inflammatory cytokine *Il6* and its signaling receptor *Il6st,* as well as an increased anti-inflammatory milieu, including increases in both *Cd28* and *Cd200* expression

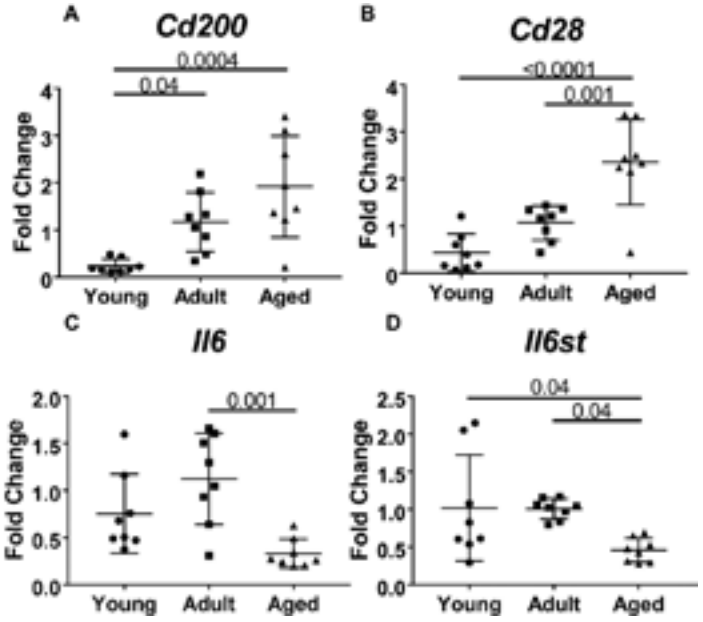

Figure 3. qPCR confirms changes in inflammatory response with age. **(A)** *Cd200* and **(B)** *Cd28* increased with age, while **(C)** *Il6* decreased in aged cells only and **(D)** *Il6st* decreased with aging. Significance is denoted with solid lines (n = 8/group).

compared to juvenile cells. Previous work demonstrated that Il6-null mice (simulating an aged phenotype) have increased native tendon mechanical properties[6], but show a similar healing response as WT mice[7], suggesting a role for this cytokine in how delivered cells integrate into and contribute to new tendon formation. This study specifically explored RNA level changes in biceps tendons in culture, and we have not yet shown that these findings relate to changes at the protein level. However, these age-specific expression signatures can begin to uncover the mechanisms behind functional differences previously shown between age groups after cell delivery[5]. Future research will investigate protein level changes as well as how these changes relate to functional differences in tendon healing with age. It will be important to discern how both population heterogeneity and inflammation affect the contribution of scaffold-delivered biceps cells for rotator cuff repair.

We previously demonstrated age-specific differences in supraspinatus healing after autologous biceps cell delivery[5]; the current study demonstrates that these cell populations display distinct molecular differences.These differences should be considered when addressing musculoskeletal regenerative medicine, particularly in the context of augmented tendon repair. Furthermore, modulating the molecular profile of adult or aged cells may further improve tendon repair.

References

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