Comprehensive Transcriptome Analysis of Aging-related Change in Early Phase of Post-traumatic Osteoarthritis

Tomoaki Fukui, M.D., Ph.D.1, Alesha B. Castillo, Ph.D.2,3, Ashley Russell, B.S.2,3, Jasper HN Yik, Ph.D.1, Dominik R. Haudenschild, Ph.D.1.
1Lawrence J. Ellison Musculoskeletal Research Center, Department of Orthopaedic Surgery, University of California Davis Medical Center, Sacramento, CA, USA, 2Rehabilitation R&D, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA, 3Division of Plastic and Reconstructive Surgery, Department of Surgery, Stanford University School of Medicine, Stanford, CA, USA.


Introduction: Osteoarthritis (OA) is a degenerative disease of the articular joints characterized by cartilage degeneration. It is the most common form of arthritis, and affects a large population. Risk factors for OA include age and a history of joint trauma. Aging-related changes in the joint occur independent of the mechanical wear on the articular cartilage (ref.) At this time, the relationship of age to the response of the joint to traumatic injury remain largely unknown, especially during the early acute phase of the injury. The objective of the current study is to elucidate the aging-related changes in gene expression following knee injury using comprehensive transcriptome survey at an early time point after injury. To accomplish this, microarray analysis was performed on RNA from injured and uninjured knees of young and old mice.

Methods:
Animal model of joint injury
Male C57BL/6 mice were used for experiments at 10-12 weeks old as young mice, and at 54 weeks old as old mice (n=8). The right knees were injured with a single mechanical compression (Ref.1), which causes a transient anterior subluxation of the tibia and the anterior cruciate ligament injuries and leads to post-traumatic OA (PTOA) within 8 weeks. The contralateral uninjured left knees served as controls. The mice were euthanized 7 days after injury, total knees dissected to isolate articular cartilage and bone, and RNA extracted. All animals were maintained and used in accordance with National Institutes of Health guidelines on the care and use of laboratory animals. The Palo Alto Veterans Affairs Medical Center and University of California Institutional Animals Care and Use Committees approved all experimental procedures performed at the respective institutions.

Microarray analysis
The total RNA was hybridized to an Affymetrix GeneChip® Mouse Gene 1.0 ST Array. For analysis, GeneSpring software was used with all gene expression values for unsupervised clustering of the arrays and principal components analysis. ANOVA with conservative false-discovery rates was used to identify those genes that were differentially expressed (p1.5-fold change. This filtered list was imported into Cluster 3.0 and JavaTreeView to cluster and visualize gene expression data, and also imported into Ingenuity Pathway Analysis to identify canonical signaling pathways, cellular processes, and networks.

Results:
Transcriptome analysis
Unsupervised clustering of all data in the 32 arrays showed that the gene expression patterns agreed well with both the injury status and age of the mice, as shown by the principal component analysis (Fig1A). We next compared specific experimental groups to identify genes differentially regulated by age (Old Uninjured vs. Young Uninjured, OU v YU), by injury in young mice (YU vs. Young Injured, YU v YI), and by injury in old mice (OU vs. OI), revealing approximately 7500 genes regulated by age, 3700 genes regulate by injury in young mice, and 6000 genes regulated by injury in old mice (Fig1B). We included all fully annotated genes with >1.5-fold change in further analyses.

**Comparative analysis between old and young uninjured knees**

Hierarchical clustering of genes differentially regulated with age are shown in Fig2A. Of the 309 differentially regulate genes, 206 were up-regulated and 103 were down-regulated in OU against YU. The top 5 genes with the highest absolute fold change in expression (Fig2B) include anabolic genes such as collagen 2a1 that were down-regulated by aging, and genes related to signal transduction that were up-regulated by aging. Top 10 diseases and functions which those regulated genes are related to were shown in Fig2C.

**Injury response in old and young mice**

Comparative analysis between injured knees vs. uninjured knees in old and young mice found 511 genes up-regulated and 28 genes down-regulated by injury in old mice, and 323 genes up-regulated and 30 genes down-regulated in young mice (Fig1B). A total of 319 genes were regulated by injury in both old and young mice, and 218 genes and 34 genes were regulated by injury specifically in old and young mice, respectively (Fig3). The top 5 genes up- or down-regulated genes differentially in only old or young mice are listed in Figure3.

**Discussion:** To identify the basic difference between old and young mice, a comparison of OU and YU was performed and hierarchical clustering indicated definite molecular differences between old and young mice. The tendency was that up-regulated genes in old mice are mostly related to intracellular signal transduction, while up-regulated genes in young mice included many extracellular matrix components such as collagen type II, XI, and IX. This suggests that young mice have greater anabolic capacity, (perhaps still in the growth phase) compared to old mice. The age-dependent response to injury was investigated by comparison of injured knees and uninjured knees in old and young mice and the injury responses in old and young mice. As shown in Figure 3, anabolic genes including collagen II and collagen X, which were down-regulated in YU against OU, were up-regulated in only old mice. It apparently seems that old mice have higher ability to recover against injury, however the expression level of these anabolic genes in microarray was lower in OI than YU or YI. Perhaps the significant up-regulation of anabolic genes in old mice is due to low basal expression and may imply greater susceptibility to injury in old mice compared to young mice. The absolute greater increase in old mice fits with what we believe happens in injured bone in old mice, there are fewer progenitors, but progenitors that are there can still produce a robust response though they cannot overcome the age-related deficit.

**Significance:** In the current study, comprehensive transcriptome analysis was performed with injured and uninjured knees of 8 old mice and 8 young mice. The information obtained from this study will be able to contribute to understanding how and why OA is initiated and progresses following trauma, and to better understand the age-related loss of wound healing capacity upon joint injury. This may help identify therapeutic targets for treating PTOA that are unique to patient age.
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B

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Genes regulated by injury