Regional Differential Genetic Response of Human Articular Cartilage to Impact Injury

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Disclosures:

Introduction: Mechanical factors, such as impact injuries often seen in trauma, have been considered one of the major causes of the resulting cartilage degeneration. Normal physiological movement creates different weight bearing zones within a human knee: the medial condyle bearing the highest and the trochlea bearing the lowest weight. Adaptation to different physiological loading conditions results in different tissue and cellular properties within a knee. We hypothesize that there will be significant differences in gene expression across knee articular cartilage at baseline (prior to injury) and that different regions of the knee respond genetically differently to acute cartilage injury. The objective of this study was to use microarray analysis to examine gene expression differences amongst three anatomical regions of human knee articular cartilage at baseline and following induction of an acute impact articular cartilage injury.

Methods: Healthy cadaveric human en-bloc knee joints were recovered from 7 donors (6 males, 1 female age 23-50) within 24 hours of donor mortality, and were placed at 4C while screened for a standard serology panel for HIV, and Hepatitis B & C (maximum another 48-72 hours). Full thickness cartilage explants (8 mm in diameter) were harvested from 3 anatomical regions: trochlea, lateral condyle, and medial condyle. The osseous end of the plugs were trimmed to a height of 4mm (full height of the osteochondral section) using an oscillating autopsy saw. Within each region, cartilage plugs were divided into the baseline and impact groups. The plugs were cultured individually in a 24-well plate in 2mL of Dulbecco’s Modified Eagle Medium with 10% Fetal Bovine Serum, and 1% antibiotic-antimycotic for 72 hours to habituate the tissue to the new environment (37°C with 5% CO2). After the initial culture, the impact group samples were impacted with 20-25MPa using a drop tower system [1]. Following impaction the cartilage plugs were cultured for an additional 24 hours. Total RNA was extracted from the cartilage plug samples using a modified QIAzol (QIAGEN, Hilden, Germany)-based method. Genome-wide gene expression profiling was done using the Illumina Whole Genome (WG)-DASL® (cDNA-mediated Annealing, Selection, Extension, and Ligation) High Throughput (HT) Assay. The raw expression data (log2 values) were transformed using variance-stabilizing transformation (VST) [2] with the LUMI Bioconductor R package, which takes into account the large number of technical replicates on the Illumina arrays. Normalization was then conducted using the Robust Spline Normalization (RSN) algorithm which combines features of quantile and loess normalization. Quality control was performed using the lumiQ command and differential gene expression was conducted using the limma R code. Gene expression analysis was performed to compare the gene expression profiles (1) between baseline samples (baseline versus baseline) among the three anatomical regions, (2) between impacted samples (impacted versus impacted) among the three anatomical regions, and (3) baseline samples to impact injured samples within each anatomical region (baseline versus impacted). T-tests were run to determine the statistical significance of each probe in a given comparison, resulting in an unadjusted p-value. These analyses were conducted using the Lumi Bioconductor software [3], which normalizes for biological replicates, batch results, and other technical anomalies [4], and accounts for multiple testing to identify genes that met predetermined False Discovery Rate (FDR) thresholds. An FDR-adjusted p-value <0.05 was considered to be statistically significant.
Results:

Baseline regional comparisons (Figure 1): In the comparison of lateral condyle vs. trochlea, 18 genes showed significant differential expression (FDR <0.05). Comparison of the medial condyle vs. trochlea revealed 21 genes with significant differential expression (FDR <0.05). In comparing expression in the medial condyle versus lateral condyle, 10 genes (NR2F2, HOXB2, TBX5, TBX3, FIGN, IRX2, IL4R, C14orf39, EBF3) showed significant differential expression (FDR <0.05).

Impacted regional comparisons (Figure 2): 7 genes were differentially expressed between impacted lateral condyle vs. impacted trochlea cartilage (FDR <0.05). Similar analysis of impacted medial condyle vs. impacted trochlea cartilage showed that 11 genes were differentially expressed (FDR <0.05). Gene expression evaluation of impacted lateral condyle vs. medial condyle cartilage showed 7 genes were significant differentially expressed (FDR <0.05). In injury response within each region: In analyzing baseline versus impacted samples by anatomical regions 130 genes were found to be significantly differentially expressed in the trochlea (supplementary Table 1 FDR≤0.05). No statistically significant changes in gene expression were observed within the condyle regions after injury.

Discussion: Our study shows that baseline differences in regional gene expression exist and effect injury response within those regions. It was found that the lateral and medial condyle regions were more similar compared to the trochlear region, both at baseline and following impact injury. A possible explanation for these observations is that lateral condyle and medial condyle receive similar high weight bearing loads during normal daily life activities, whereas the trochlea receives lower mechanical conditioning. Trochlea is the only region displaying acute genetic response upon injury, which might be rendered by the less differentiated stage of cells in trochlear than in condyles. Several genes that were differentially expressed in the baseline regional comparison overlap with the differences seen in the impact comparisons (LEF1, EMX2, NR2F1, FTCD), suggesting that these anatomical differences are preserved following injury. These finding suggest that mechanical preconditioning may occur which could cause regional genetic changes that might result in different phenotypic expression.

Significance: Our data suggests the trochlear region contains progenitor markers, and shows the largest response to injury. This supports current osteochondral autograft transplantations surgery (OATS) methods of using cartilage from non-weighting bearing regions for transplants. Conversely, our results could explain the incidence of long-term failure in OATS procedures, because of genetic differences observed between the donor and transplant site.

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