Whole Transcriptome sequencing of Bone/Cartilage of Tibia and Meniscus Reveals Altered Lipid Metabolism Through Significant Upregulation of Leptin and Perilipins in Osteoarthritis Pathology

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Introduction: Joint pain is a primary cause of making osteoarthritis (OA) the most disabling disease worldwide. Mechanisms related to pain in OA are poorly understood; however, there is sufficient preclinical and clinical evidence to interpret that a strong peripheral nociceptive drive from the affected joint maintains pain and central sensitization in OA joints[1, 2]. Mechanistic elucidation of structural alterations in joint innervation and nerve damage that occur in the course of OA progression can help the identification of novel targets for pain. Aligning with this strategy REJOIN, NIH HEAL consortium was set to map sensory nerve patterns in human knees adapt to aging and disease. Given that OA is a failure of the 'joint as an organ', scrutinizing tissue-specific transcriptional changes that contribute to joint sensitization by influencing joint innervation patterns is crucial in meeting the objective of REJOIN project.

Methods: Total RNA-seq of bone/cartilage from medial and lateral tibial plateau and medial and lateral meniscus of 15 consented OA donors (males: 5; females: 10) planned for knee arthroplasty was conducted, wherein the samples were thawed in RNAlater™-ICE and total RNA-isolation was performed using mirVana miRNA isolation kit (Invitrogen, Waltham, MA), followed by ribodepletion with the Illumina Ribo-Zero plus rRNA Depletion Kit (Illumina, #20037135), and library construction with the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, #E7760L). Libraries were evaluated on the Tapestation 4200 (Agilent) for average size and Qubit 4.0 (Life Technologies) for concentration. The libraries were then pooled equimolar and sequenced on an Illumina NovaSeq X Plus. All the protocols were approved by IRB of the University of Florida, and each sample was run in quadruplet.

Results: Raw transcriptome reads were aligned to the GRCh38 reference genome using rsem and star packages on HiPerGator, followed by calculation of gene expression for each sample using rsem. Statistical analysis was performed using R software. After normalization, filtering, and annotation of RNA reads, data dispersion was transformed using either a regular log or a variance stabilizing transformation depending on visualization of plotted standard deviations. Using macroscopic grading keys (modified version of Outerbridge[3], and Pauli[4] and Cooper's[5] classification specific to cartilage and meniscus in OA and injury), tissue samples were binarized into degenerated or non-degenerated categories to compare their transcriptome. RNA-seq data on 94 bone/cartilage and meniscus samples (59 bone/cartilage + 35 menisci) revealed 85 genes that were significantly expressed (log Fold Change >2 or <-2) (Figure 1A). Significantly upregulated genes in the degenerated bone/cartilage and meniscus included leptin (q value: 3.22E-22; 6.6-fold), perilipins (PLIN1: q value: 4.96E-25; 5-fold, PLIN4: q value: 8.49E-22; 4.3-fold and PLIN5: q value: 9.23E-22; 4.7-fold) and carbonic anhydrase4 (q value:1.44E-13; 5.89-fold). Bone/cartilage specific upregulated genes included collagens (COL9A1: q value:0.000193; 6.8-fold, COL9A3: q value: 2.96E-10; 9.17-fold), CILP gene (q value: 7.08E-18; 8.7-fold), alarmins (S100A8: q value: 3.30E-08; 9.17-fold, S100P: q value: 0.0156; 7.46-fold) Immunoglobulin heavy and kappa chain variables (IGHV4-30-2: 4.84-fold, IGKV1D-13: 3.5-fold, IGKV1D-12: 3.12-fold) along with angiopoietin-like protein7 (ANGPTL7: 3.2-fold) and vascular endothelial growth factor-A (VEGFA: 2.16-fold) were among significantly downregulated genes.

Discussion: Gene Ontology (GO) analyses involving significantly upregulated genes revealed enrichment in several Reactome Pathways/Wiki Pathways/Elsevier Pathways including adipogenesis, transcriptional regulation of white adipocyte differentiation and showed a strong involvement of leptin and adiponectin (Table 1). Interestingly, recent research studies have reported a strong correlation between increased leptin and knee pain[6,7]. Also, leptin is able to act via the sympathetic nervous system (SNS), and its receptors have been identified on dorsal root ganglia neurons which needs deeper mechanistic evaluation. Given that infra-patellar fat pad and synovium are major sources of leptin production in OA knees, RNA-seq data of these tissues (which is currently under evaluation as 2nd phase of this study) is expected to provide more insights on these findings.

Clinical significance: Although pro-inflammatory role of adipokines, particularly leptin, in OA is well known, their connection with pain is relatively new learning. Upon further validating their mechanism in pain, adipokines may serve as potential targets for pain in OA.

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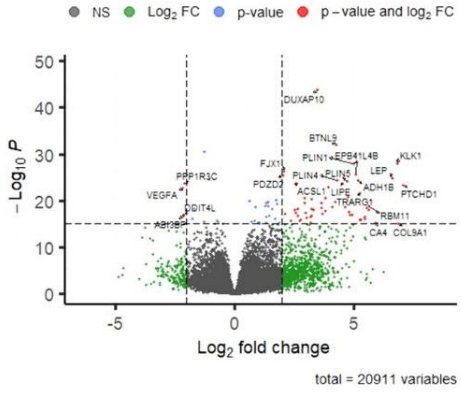

Figure 1. Volcano plot of differentially expressed genes between degenerated and non-degenerated bone/cartilage and meniscus

Upregulated pathways	p value
Collagen Degradation (Reactome Pathways)	0.0003577
ECM Proteoglycans (Reactome Pathways)	0.0005042
Adipogenesis <Reactome Pathways)	0.001052
Leptin And Adiponectin WP3934 (Wiki Pathways)	0.004492
Transcription Factor Regulation In Adipogenesis WP3599 (Wiki Pathways)	0.009858
Leptin -> CD25/IL6/IL10 Production (Elsevier Pathways)	0.004043

Table 1. Key pathways with their *p*-values, generated during GO analysis of the differentially expressed genes in degraded bone/cartilage and meniscus samples.

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