Characterization of Meniscal Pathology with Molecular and Proteomic Analyses
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Introduction:
Over one million operative procedures are performed each year in the United States to address meniscal-associated problems. Most procedures require partial resection, resulting in decreased joint function, which inevitably leads to osteoarthritis (OA), pain, and disability. Although the meniscus has been studied for years, a comprehensive genomic and proteomic analysis of meniscal pathology is lacking, and would greatly improve our understanding of the etiopathogenesis and progression of this disease. Further, diagnostic, prognostic, and treatment monitoring biomarkers may be identified using these testing strategies. Therefore, our objective was to use microarray and LC-MS/MS to assess the transcriptome and proteome of normal and pathologic meniscal tissue.

Methods:
Meniscal Tissue Collection: All procedures were performed with IRB approval (IRB#1042248). Meniscal tissue was collected from the knees of five patient groups (n=3/group) – age range in parentheses: (1) Aged-normal (AN) (64-78): no previous injury, operative procedures, or gross meniscal/articular damage; (2) Young meniscal debridement (YMD) (28-36): minimal articular cartilage damage; (3) Older meniscal debridement (OMD) (53-57): chronic degenerative tears, moderate articular cartilage damage; (4) Mild radiographic OA, received a total knee arthroplasty (TKA) (MOA) (44-61); (5) Severe radiographic OA, received TKA (SOA) (50-69). All tissue was collected from white/white and white/red zones of posterior medial menisci and placed in RNAlater.
Tissue Extraction: Total RNA was extracted from the tissue using the TRIzol solution after the upper aqueous phase was removed for RNA.

Results:
Microarray Analysis (Figures 1-3): The microarray identified 70 genes that had at least a 1.5x fold change in expression between the AN and clinical groups. Representative genes listed are associated with extracellular and intracellular matrix (ECM, ICM) synthesis, vascularity, tissue signaling, and apoptosis.

Proteomics Analysis (Figures 4-6): Proteomics analysis identified 173 unique proteins in the meniscal tissue. Proteins were marked for further evaluation based on fold difference, spectral count, potential in vivo function, and correlation with microarray data.

Discussion:
To our knowledge, this is the first study to characterize the proteome and transcriptome of normal and pathologic meniscal tissue using microarray and LC-MS/MS on the same tissue sample. The data from this study have begun to indicate an array of pathological processes that occur during the etiopathogenesis and progression of meniscal disease. As expected, pathologic tissue showed increased expression and synthesis of ECM/ICM and vascularity related constituents at the gene and protein levels; both indicative of attempts at tissue repair. Further, the increase in thyroid hormone signaling indicates another potential component of this reparative attempt. However, gene expression data also indicated increased apoptosis in the pathologic tissues. Increased release of reactive oxygen species from apoptotic cells coupled with the decrease in tissue EC-SOD noted suggests that an important mechanism of disease involves increased oxidative stress in pathologic meniscal tissues.

Determining and characterizing the pathways that are involved in the disruptive nature of meniscal pathology will allow us to begin to define algorithms for comprehensive and accurate diagnostic, therapeutic, and prognostic strategies for meniscal disorders in clinical patients. The data from this study indicate the need to continue using these techniques on a larger subset of samples to identify significant differences between pathologic and normal meniscal tissues. Continuing studies in our laboratory are further characterizing and validating the changes observed in this study to improve our understanding of meniscal pathology.