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Insights into osteoarthritis progression revealed by analyses of both knee tibiofemoral compartments

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SUMMARY

Objective: To identify disease relevant genes and pathways associated with knee Osteoarthritis (OA) progression in human subjects using medial and lateral compartment dominant OA knee tissue.
Design: Gene expression of knee cartilage was comprehensively assessed for three regions of interest from human medial dominant OA ($n = 10$) and non-OA ($n = 6$) specimens. Histology and gene expression were compared for the regions with minimal degeneration, moderate degeneration and significant degeneration. Agilent whole-genome microarray was performed and data were analyzed using Agilent GeneSpring GX11.5. Significant differentially regulated genes were further investigated by Ingenuity Pathway Analysis (IPA) to identify functional categories. To confirm their association with disease severity as opposed to site within the knee, 30 differentially expressed genes, identified by microarray, were analyzed by quantitative reverse-transcription polymerase chain reaction on additional medial ($n = 16$) and lateral ($n = 10$) compartment dominant knee OA samples.
Results: A total of 767 genes were differentially expressed $\geq$two-fold ($P \leq 0.05$) in lesion compared to relatively intact regions. Analysis of these data by IPA predicted biological functions related to an imbalance of anabolism and catabolism of cartilage matrix components. Up-regulated expression of IL11, POSTN, TNFAIP6, and down-regulated expression of CHRDL2, MATN4, SPOCK3, VIT, PDE3B were significantly associated with OA progression and validated in both medial and lateral compartment dominant OA samples.
Conclusions: Our study provides a strategy for identifying targets whose modification may have the potential to ameliorate pathological alternations and progression of disease in cartilage and to serve as biomarkers for identifying individuals susceptible to progression.
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Introduction

Although the progression of osteoarthritis (OA) is generally currently unpredictable, altered biomechanical and biochemical properties of the joint organ facilitate progression of disease[1–4]. It is widely accepted that the molecular homeostasis of the joint depends on both the structural integrity of articular cartilage and the appropriate biomechanical stresses[5,6]. A detailed examination of

molecular changes in chondrocytes, the only cell type of articular cartilage during OA disease progression, is of pivotal importance for choosing molecules that could potentially be targeted to achieve a therapeutic benefit.

Joint tissues readily available for research are generally acquired at the time of knee joint replacement and suffer from full-thickness or severe cartilage loss of the medial weight-bearing compartment. This level of OA severity is often associated with an abnormal external knee adduction moment and imbalanced load distribution on the medial compartment of the knee[7]. Therefore, results of molecular analyses of advanced medial compartment knee OA tissue may not only represent direct effects of disease, but also site-related effects driven by altered mechanical loading. In addition, few studies have profiled human tissues for genes associated with OA progression; this is largely due to difficulties obtaining joint tissues with either early- or intermediate-stages of OA severity[8,9]. Another drawback to using patient tissues for gene expression profiling is large inter-patient differences due to variability in genetic background, duration of disease, age and gender.

To overcome these obstacles, we have pursued a two-pronged approach. First we have profiled cartilage gene expression changes relative to a gradient of histological OA severity across the tibial plateau in knees with medial compartment dominant disease[10,11]. This model system was based on evaluation of regions of cartilage and bone across the knee joint from the uninvolved (lateral compartment of the knee) to the involved surfaces (medial compartment of the knee), evaluating for disease severity and site associated gene expression changes. Because initiation of OA usually occurs in a focal subregion where the articular surface is affected most by asymmetric mechanical loading[12], other regions of the articular cartilage in the joint remain macroscopically and structurally normal or much less damaged. With disease progression, regions adjoining the damaged cartilage are affected until the entire joint surface is involved in advanced OA[13]. In our previous study we demonstrated that specific regions across the tibial plateau yielded a gradient of disease severities; intra-individual comparisons of these regions provided a means of overcoming the inter-individual background variation in gene expression studies[10]. This model system also provided a means to overcome the difficulty of obtaining early stage disease tissue by providing relatively normal tissue from the lateral compartment for comparison with the more diseased regions medially.

For the second aspect of this approach, we now profile cartilage gene expression changes relative to a gradient of histological OA severity across the tibial plateau in knees with lateral compartment dominant disease (LOA). Whereas medial compartment dominant knee OA (MOA) is often associated with an abnormal external knee adduction moment and imbalanced load distribution on the medial compartment of the knee, load plays less of a role in development of OA in lateral compartment disease[14,15]. This may be due to the fact that even in valgus knees, the medial compartment remains relatively overloaded until the valgus deformity exceeds 15°[16]. This overcomes a third drawback described above by enabling us to examine gene expression changes relative to worsening OA severity in the lateral compartment that has been shown to be relatively disassociated from loading stress.

For the current study, we hypothesized that this model system, encompassing a full range of histological severity across the tibial plateau, could enable us to identify gene expression changes directly associated with knee OA progression. We hypothesized that the pattern of gene expression for genes directly associated with disease severity, as opposed to joint site or mechanical load, could be the "mirror image", i.e., in medial compartment dominant disease the greatest gene expression changes associated with OA progression should occur in the most degenerative medial cartilage whereas the

inverse should be true for lateral compartment disease. Because load may play less of a role in development of lateral compartment dominant disease than medial compartment OA[7,15], the identification of the severity associated genes expressed in common between medial and lateral compartment dominant disease may more readily identify genes related to biological factors directly associated with OA progression that are independent of load.

Therefore, to identify gene expression patterns directly associated with effects of disease and to differentiate them from patterns of expression that are driven by external mechanical loading and/or site-specific alternations, we evaluated whether OA-related genes identified in medial compartment dominant knee OA joints could be validated in lateral compartment dominant knee OA joints. In the present study, we performed whole genome transcriptome analysis of three regions of interest of articular cartilage in OA and non-OA knee joints and validated gene expression of the 30 most differentially regulated genes in additional independent medial ($n = 16$) or lateral ($n = 10$) compartment dominant knee OA joint cartilage specimens. Ingenuity Pathway Analysis (IPA) was used to discern the gene expression patterns most reflecting the different stages of OA progression. This study identified molecular targets that are involved in the homeostasis of cartilage integrity and provides potential pathways for therapeutic druggable targets or biomarkers in OA.

Materials and methods

Human knee joint tissues

A total of 42 human tibial plateaus were obtained during total knee joint replacement surgery from patients with medial or lateral compartment dominant knee OA ($N = 26$ medial OA, 10 for microarray analysis and 16 for validation of gene expression, mean age 68.9 ± 7.4 years, 70% female; $N = 10$ lateral OA, mean age 73.9 ± 11.45 years, 50% female) and non-OA joints acquired at the time of tumor surgery and above the knee amputation ($N = 6$ non-OA, mean age 39 ± 11.4 years, 50% female). The anatomic orientation was indicated on the freshly isolated specimens by marker pen to ensure consistency of sampling at prespecified regions of interest. All specimens were stored immediately in liquid nitrogen. The study was approved by the institutional review board of all the participating hospitals and Academia Sinica, Taiwan, Written informed consent was obtained from all of the participants.

Regions of interest, cartilage harvest and RNA isolation

The processes of cartilage harvest, sectioning, grinding, and RNA extraction were performed as previously described in Ref. 10. Briefly, regions of interest were sectioned and powdered under liquid nitrogen; 100 mg of articular cartilage powder was used for RNA isolation with 5 ml of Trizol (Invitrogen, CA). The RNA concentration and quality (RNA integrity number, RIN and 28S/18S ratio) were determined by a Nano-Drop (NanoDrop Technologies, DE) and the RNA 6000 Nano Assay on an Agilent 2100 Bioanalyzer (Agilent Technologies, CA), respectively. An adjacent section was preserved for histological evaluation using the OARSI grading system[17] by a scorer blinded to the results of the molecular analyses.

Microarray analysis

400 ng of total RNA per sample was used for one round of cRNA synthesis and amplification. Cyanine 3-labeled cRNAs were purified and hybridized to Agilent whole human genome 44k microarray chips (Agilent Technologies, Santa Clara, CA, USA). All procedures were carried out according to the manufacturer's instructions. The