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Transcriptional analysis of micro-dissected articular cartilage in post-traumatic murine osteoarthritis

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SUMMARY

Objective: Identify gene changes in articular cartilage of the medial tibial plateau (MTP) at 2, 4 and 8 weeks after destabilisation of the medial meniscus (DMM) in mice. Compare our data with previously published datasets to ascertain dysregulated pathways and genes in osteoarthritis (OA).
Design: RNA was extracted from the ipsilateral and contralateral MTP cartilage, amplified, labelled and hybridized on Illumina WGv2 microarrays. Results were confirmed by real-time polymerase chain reaction (PCR) for selected genes.
Results: Transcriptional analysis and network reconstruction revealed changes in extracellular matrix and cytoskeletal genes induced by DMM. TGFβ signalling pathway and complement and coagulation cascade genes were regulated at 2 weeks. Fibronectin (*Fn1*) is a hub in a reconstructed network at 2 weeks. Regulated genes decrease over time. By 8 weeks fibromodulin (*Fmod*) and tenascin N (*Tnn*) are the only dysregulated genes present in the DMM operated knees. Comparison with human and rodent published gene sets identified genes overlapping between our array and eight other studies.
Conclusions: Cartilage contributes a minute percentage to the RNA extracted from the whole joint (<0.2%), yet is sensitive to changes in gene expression post-DMM. The post-DMM transcriptional reprogramming wanes over time dissipating by 8 weeks. Common pathways between published gene sets include focal adhesion, regulation of actin cytoskeleton and TGFβ. Common genes include Jagged 1 (*Jag1*), Tetraspanin 2 (*Tspan2*), neuroblastoma, suppression of tumourigenicity 1 (*Nbl1*) and N-myc downstream regulated gene 2 (*Ndrg2*). The concomitant genes and pathways we identify may warrant further investigation as biomarkers or modulators of OA.

Introduction

Osteoarthritis (OA) is a polygenic, multifactorial disease of synovial joints, characterised by articular cartilage degradation and changes in other joint tissues[1–3].

The molecular events occurring during disease initiation and early progression are poorly understood. Initial large-scale gene expression studies used osteoarthritic human cartilage from end-stage disease[4–7]. These studies demonstrated changes in gene expression in late stage OA but were unable to examine early molecular events as it is very difficult to procure human cartilage from different stages of disease and pair it with non-diseased age-matched tissue. Animal models provide genetically controlled and aged-matched tissue samples to allow the study of OA pathogenesis[8]. Broad molecular approaches are able to identify single

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pathogenic molecules and unravel networks of interacting genes/proteins that are dysregulated in disease[9].

The destabilisation of the medial meniscus (DMM) surgical model of OA is a widely used and validated model of post-traumatic OA[10]. We have used it to examine gene expression profiles shortly after joint destabilisation. We found early gene expression changes are highly mechanosensitive and largely attenuated by joint immobilisation[11]. When analysing the whole joint tissue, immobilised joints do not have increased expression of pathogenic proteases such as *Adamts5*, *Adamts4*, *Adamts1* and *Mmp3*, show attenuated expression of inflammatory response genes like *Ccl2*, *Il1b* and *Il6* and are protected from disease. Similarly, joint immobilisation led to decreased expression of those genes in the cartilage at 6 h post-DMM. These results indicate the rapid response of cartilage to DMM. We now extend these results showing gene changes in this highly mechanosensitive tissue occurring beyond 6 h, over an 8-week course. We were interested in identifying "phases" of disease progression that might provide novel targets and new biomarkers for investigation in the human condition.

Methods

Animals and surgical model of OA

All procedures complied with the Animals (Scientific Procedures) Act 1986 and with guidelines of the local ethics committee. C57BL/6J male mice were purchased from Charles Rivers (Margate, UK). Mice were housed 4–5 per standard individually ventilated cages and maintained under 12-h light/12-h dark conditions at an ambient temperature of 21°C. Animals were fed a certified mouse diet (RM3; Special Dietary Systems) and water *ad libitum*. In total 187 animals were used in this study (Supplementary Fig. 1).

DMM surgery was performed on 10-week-old mice during daylight hours following the protocol described previously in Ref. 11. The right knee (ipsilateral) was operated and the left (contralateral) remained unoperated, acting as a control. For microarray, three groups of eight mice were operated on per time point (Fig. 1). For histological analysis, 10 mice were operated on per time point. For PCR, an extra three groups of five mice were operated on for each time point. An additional 15 mice were used to quantify the amount of RNA extractable from each joint tissue. Eleven of these mice were whole joint extractions and the other four were micro-dissected. No pooling was performed for meniscus or subchondral bone samples. Where pooling was performed, the experimental unit was regarded as one (Supplementary Fig. 1).

Histological analysis

Knee joints from operated and contralateral limbs were dissected and processed for histological analysis at 2, 4, 8 and 12 weeks. Two blinded independent observers scored the cartilage morphology, as described previously in Ref. 11.

RNA extraction and whole genome expression analysis (WGA)

Micro-dissection of joint tissues was performed as previously described in Ref. 11 at 2, 4 and 8 weeks following DMM surgery. Detailed protocol is provided in the supplementary file. RNA extraction, amplification, cDNA labelling, hybridisations on an Illumina Mouse WG-6 v2.0 Expression BeadChip (Illumina), microarray processing, data analysis and construction of regulatory pathways was performed as described in the Supplementary File. Data are deposited in NCBI's Gene Expression Omnibus[12] with GEO Series accession number GSE53857 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53857).

Comparison of previously published microarray datasets

We compared our 2-week dataset with other arrays owing to the size of our dataset and availability of other mouse arrays at this time point. We selected previously published datasets fulfilling the following criteria:

a. They were from pure knee cartilage RNA[5–7,13–16], or enriched for cartilage[17];
b. They were of mouse[14,17], rat[13,15] or human origin[5–7,16];
c. The rodent sets were from models of surgical induced knee instability; and
d. Where possible we preferred the 2-week time point for comparison[14,17].

We used the gene-lists corresponding to the significantly dysregulated probes of each study. Additionally, we compared the overlap between our array and a gene-set specific for inflammatory-related genes[18]. Details are found in the Supplementary File. The Venn diagrams were constructed using Venny[19]. Hypergeometric distribution probability mass function (p.m.f.) calculations of overlaps observed between two sets were done in R.

Real-time (RT)-PCR

RT-PCR was performed using RNA from biological replicates at each time-point as described before in Ref. 11. The list of assays used is available upon request.

Results

Determining the relative abundance of articular cartilage RNA in the whole joint

We established a method to isolate RNA from distinct tissues within the mouse knee joint[11]. The tibial epiphysis contributes almost half of the whole joint RNA (44% ± 20%), with lesser contributions from meniscus (2.4% ± 1.5%) and cartilage (0.174% ± 0.082%) [Fig. 1(A)]. Although the articular cartilage contributes a small percentage of total joint RNA, it is clearly implicated in early OA. Gene expression changes localised to this tissue may be lost when taken with the whole joint tissue. The RNA isolation technique resulted in 9 ± 4.3 ng RNA/joint from the pooled medial tibial plateau (MTP) with a RIN of 8.4 ± 0.4 [Fig. 1(B)].

We extended our original observations by performing a microarray analysis of genes regulated specifically in the MTP cartilage beyond 2 weeks.

Histological cartilage degradation in operated joints relative to controls

Operated joints showed progressive increase in pathological changes over the 12-week time course [Fig. 1(C) and (D)]. Within the operated joints, histological scores were consistently higher in the medial compartment compared to the lateral compartment [Fig. 1(D)]. No significant pathological changes were detected in the contralateral knees and there was no difference between the medial and lateral compartment of the contralateral knees at any time point.