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A gene expression study of normal and damaged cartilage in anteromedial gonarthrosis, a phenotype of osteoarthritis

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SUMMARY

Objective: To identify osteoarthritis (OA) relevant genes and pathways in damaged and undamaged cartilage isolated from the knees of patients with anteromedial gonarthrosis (AMG) – a specific form of knee OA.

Design: Cartilage was obtained from nine patients undergoing unicompartmental knee replacement (UKR) for AMG. AMG provides a spatial representation of OA progression; showing a reproducible and histologically validated pattern of cartilage destruction such that damaged and undamaged cartilage from within the same knee can be consistently isolated and examined. Gene expression was analysed by microarray and validated using real-time PCR.

Results: Damaged and undamaged cartilage showed distinct gene expression profiles. 754 genes showed significant up- or down-regulation (non-False discovery rate (FDR) $P < 0.05$) with enrichment for genes involved in cell signalling, Extracellular Matrix (ECM) and inflammatory response. A number of genes previously unreported in OA showed strongly altered expression including *RARRES3, ADAMTSL2* and *DUSP10.* Confirmation of genes previously identified as modulated in OA was also obtained e.g., *SFRP3, MMP3* and *IGF1.*

Conclusions: This is the first study to examine a common and consistent phenotype of OA to allow direct comparison of damaged and undamaged cartilage from within the same joint compartment. We have identified specific gene expression profiles in damaged and undamaged cartilage and have determined relevant genes and pathways in OA progression. Importantly this work also highlights the necessity for phenotypic and microanatomical characterization of cartilage in future studies of OA pathogenesis and therapeutic development.

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Introduction

Knee osteoarthritis (OA) is common with more than 37% of those aged over 60 showing radiographic disease[1]. Cartilage damage in OA is likely to result from the aggregate effect of multiple genetic, environmental, mechanical and cell biological factors driving changes in gene expression[2]. These gene expression changes alter chondrocyte activity and phenotype, further driving OA progression. Studies of gene expression in OA have highlighted a number of differentially regulated genes and pathways (e.g., Matrix Metalloproteinases (MMPs) and tissue remodelling) providing valuable clues to the gross changes that differentiate OA from normal cartilage[3–9].

Gene expression analysis of human OA is limited by inter-patient variability due to confounding factors including genetics and drug therapy; cartilage is generally removed from an end-stage joint and compared to normal cartilage from an individual without OA. The site of cartilage excised is not always consistent across patients or controls; and end-stage disease is heterogeneous in both its phenotype and the mechanisms underlying its onset and progression, thus key pathways and gene expression changes may be masked or misrepresented[3,5–7,9]. In order to overcome the heterogeneity of human OA a number of animal models have been utilized[10]. These rely on a known mechanism to induce disease (e.g., destabilization of the medial meniscus) thus providing a relatively consistent OA phenotype in terms of cause and time of onset. These models provide important clues to the genes expression changes at different stages of OA pathogenesis[4,11]. However

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comparisons to human disease may be limited due to the lack of equivalent disease phenotype in humans (i.e., acute injury and fast progressing).

Studying gene expression in damaged and undamaged cartilage from within the same joint compartment would be advantageous. The most common phenotype of knee OA, Anteromedial Gonarthrosis (AMG) provides a spatially reproducible pattern of disease. AMG affects up to 60% of patients presenting with knee OA and is characterized by: full thickness cartilage loss in the anterior third of the medial tibial plateau; partial thickness loss of the middle third; and a macroscopically and histologically normal region in the posterior third[12]. Thus AMG provides a spatial representation of cartilage degradation in OA, allowing comparison of undamaged and damaged cartilage from microanatomically defined regions [Fig. 1].

This study aims to characterize gene expression in AMG, a defined and common phenotype of OA[13]. Studying AMG has the advantage of reproducible identification and isolation of damaged and undamaged cartilage from within the same joint. Additionally AMG provides a consistent human phenotype of disease. Through study of these defined regions of damaged and undamaged cartilage we aim to identify genes and pathways that can be therapeutically targeted. We also aim to assess whether regional variation in gene expression occurs across cartilage from the same joint. This study will provide much needed insight into both AMG pathogenesis and OA progression.

Materials and methods

Tissue collection

Patients were identified if attending our specialist centre for unicompartmental knee replacement (UKR) for AMG. Ethical approval was obtained to approach these patients and obtain consent for use of their resected tissue in research (Ethics Reference C01.071). Evidence of these patients having tricompartmental OA made them unsuitable for both UKR and this study.

Ten medial tibial plateaus were collected (median age 64 years, six right knees and four left knees, six females and four males). Cartilage from damaged and undamaged regions was removed and snap frozen for microarray analysis. A uniform and anatomically aligned section was taken through all regions, wax embedded and the blocks stored for confirmation of cartilage phenotype using Safranin-O. A further six patients were recruited and cartilage collected for qPCR confirmation studies.

RNA extraction and preparation

Damaged and undamaged cartilage was identified and isolated using our previously defined spatial model of AMG [Fig. 1]. Cartilage was ground in liquid nitrogen and RNA extracted using the RNeasy lipid kit (Qiagen, Crawley, UK) according to the manufacturers instructions. All samples underwent on-column DNAse treatment. RNA yield and quality was determined using an Agilent Bioanalyzer and Nanodrop Spectrophotometer. The median RNA integrity number (RIN) value was 7.3 (range 6.5–8.8), one patient was excluded due to a low RIN value. Paired (damaged and undamaged) samples were used for microarray at equal concentrations. Prior to labelling, the samples were concentrated to 8.3 μl in a rotary evaporator.

Microarray

Samples were prepared as per Agilent's Two-Colour Microarray-Based Gene Expression Analysis (Quick Amp Labelling v5.7)

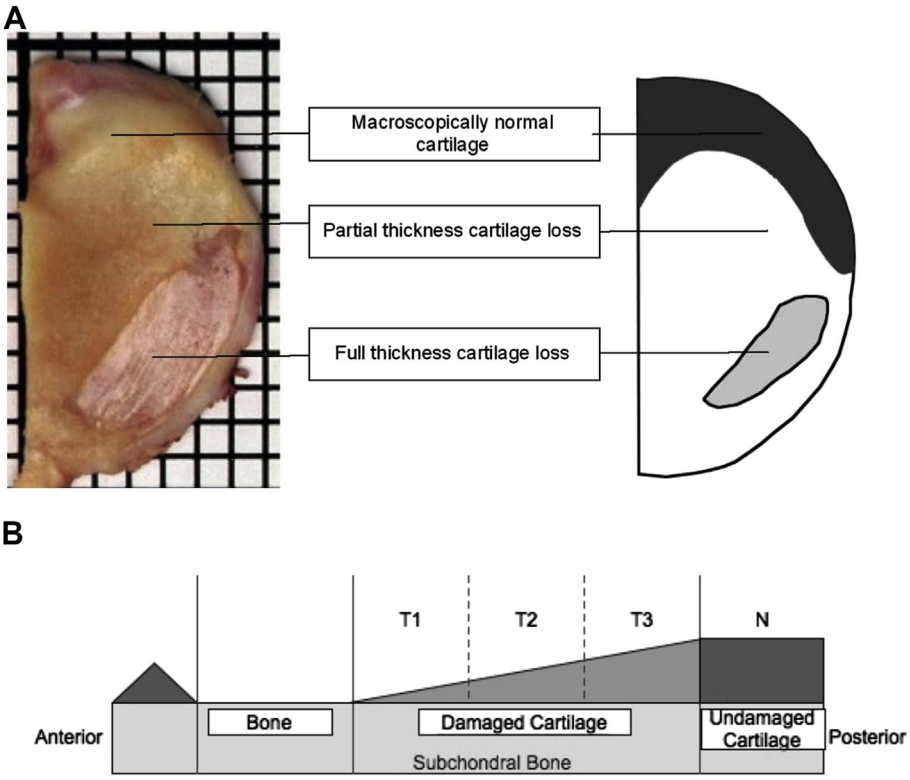

Fig. 1. AMG. A. A medial tibial plateau resection specimen from a patient having Unicompartmental knee arthroplasty (UKA) for AMG. The schematic on the right helps better demonstrate the areas of full thickness cartilage loss, partial thickness cartilage loss and macroscopically normal cartilage. B. Schematic representation of AMG. The damaged area can be divided into equal thirds (T1, T2, T3). The undamaged cartilage is region N. T1-T3 and N were collected for microarray analysis.

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