the synovium expressed complement activating proteins, and found a strong upregulation of COMP, lumican, osteomodulin, biglycan, decorin and fibromodulin. In addition, biglycan expression was strongly enhanced (2.5-fold) in human CHECK-samples from patients with joint damage.
Conclusions: All in all, these data suggest an active role for the synovium in OA pathology, and identifies pathways that are likely to be involved. One of the strongest associations was of the complement-pathway with cartilage damage. In addition, TGFβ-, BMP- and wnt-signaling in the synovium, may contribute to further joint damage. The enhanced expression of cartilage damaging MMP-1, MMP-3 and MMP-13 again suggests an active role of the synovium in OA pathology. Future studies will focus on association of gene expression patterns with progression of damage of CHECK-patients.

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IDENTIFICATION AND CHARACTERISATION OF MICRORNAS INVOLVED IN CHONDROCYTE DIFFERENTIATION AND OSTEOARTHRITIS

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Purpose: The majority of bones develop through the process of endochondral ossification where a cartilaginous template is calcified and remodelled into bone. During this process chondrocytes undergo proliferation and differentiation. Many of the signalling pathways and transcription factors which control this developmental programme have been established. MicroRNAs are 20-24 nucleotide non-coding RNA molecules that post-transcriptionally regulate gene expression. There is strong evidence that they can influence both chondrogenesis and the initiation and progression of osteoarthritis (OA), however the exact mechanisms are still undetermined. In this study we aimed to profile expression of miRNAs in a cell model of chondrocyte differentiation and use this to prioritise miRNAs for functional analyses in OA. Using deep sequencing we also aimed to identify novel miRNAs expressed in human articular cartilage.
Methods: The ATDC5 murine embryonic carcinoma cell line was induced to differentiate through chondrogenesis *in vitro*. An Exiqon miRNA microarray was used to profile the expression of all known murine miRNAs across this cell model. Expression of regulated miRNAs was verified in the mouse and chick embryo by *in situ* hybridisation. The. 3' UTRs of potential target genes were subcloned downstream of a luciferase gene for experimental. Novel miRNAs expressed in human articular cartilage were identified using deep sequencing with Illumina's GAIIX system and validated in primary chondrocyte culture in vitro.
Results: The expression of a number of microRNAs was regulated across chondrogenesis. This includes 39 microRNAs co-expressed with microRNA-140, known to be involved in cartilage homeostasis and osteoarthritis. Of these, microRNA-455 resides within an intron of *COL27A1* which encodes a cartilage collagen. Comparing human osteoarthritic cartilage with femoral neck fracture controls, both microRNA-140-5p and microRNA-455-3p show increased expression in osteoarthritic cartilage. *In situ* hybridisation shows microRNA-455-3p expression in the developing limbs of chicks and mice and in human osteoarthritic cartilage. The expression of microRNA-455-3p is regulated by TGFβ ligands and the microRNA regulates TGFβ signalling. *ACVR2B*, *SMAD2* and *CHRDL1* are direct targets of miR-455-3p and may mediate its functional impact on TGFβ signalling. Deep sequencing of the small RNA pool from chondrocytes extracted from primary human osteoarthritic cartilage identified 16 potential novel miRNAs. These have been validated in cultured chondrocytes using qRT-PCR, Northern blot and Dicer knockdown.
Conclusions: MicroRNA-455 is expressed during chondrogenesis and in adult articular cartilage where it can regulate TGFβ signalling, suppressing the Smad2/3 pathway. Diminished signalling through this pathway in ageing and osteoarthritic chondrocytes is known to contribute to cartilage destruction. We propose that the increase in microRNA-455 in osteoarthritis exacerbates this process and contributes to disease pathology. Novel miRNAs expressed in cartilage may regulate cartilage homeostasis and contribute to disease.

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THE ANALYSIS OF THE GENOME-WIDE DNA METHYLATION PROFILE OF HUMAN ARTICULAR CHONDROCYTES REVEALS DIFFERENT FORMS OF OA.

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Purpose: To identify and analyze the genome-wide DNA methylation profiles of human articular chondrocytes from a population-based case-control study of OA.
Methods: DNA methylation profiling was performed using the Infinium HumanMethylation27 beadchip (Illumina Inc.), which allows interrogation of 27,578 highly informative CpG loci. Previously, cartilage isolated DNA from 23 OA patients and 19 healthy controls was bisulfite-modified, using the EZ DNA methylation kit (Zymo Research) and hybridized according to the manufacturer's instructions. DNA methylation β-values were normalized using GenomeStudio v3.0 (Illumina Inc.). Appropriate bioinformatics analyses were carried out using both R bioconductor software packages and Babelomics suite v 4.2 (babelomics.bioinfo.cipf.es).
Results: A first approach based on an unsupervised clustering method for the most variable CpG loci (n=508) showed three distinct groups of samples, called cluster 1 (5 OA patiens), cluster 2 (6 OA patients) and cluster 3 (12 OA patients and 17 healthy controls). Specifically, cluster 2 formed a particularly tight cluster with a characteristic DNA methylation profile (Figure 1). The analyses of the biological relevance of the differentially methylated genes in cluster 2 compared with non-cluster 2 by means of a gene set enrichment approach, showed that the biological processes significantly altered were those related to the superoxide metabolic process, morphogenesis/angiogenesis and regulation of cell proliferation, all of them hypermethylated in cluster 2; on the contrary, those mechanisms related to both IL-8 biosynthetic process and apoptosis appeared significantly hypomethylated in cluster 2.
Conclusions: The genome-wide methylation analysis shows a clearly distinct epigenetic profile for OA. The DNA methylation profile could be one of the reasons of the existence of different forms of OA and could also be related to both the prevalence and progression of this disease.