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Molecular differentiation between osteophytic and articular cartilage – clues for a transient and permanent chondrocyte phenotype

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SUMMARY

Objective: To identify the molecular differences between the transient and permanent chondrocyte phenotype in osteophytic and articular cartilage.
Methods: Total RNA was isolated from the cartilaginous layer of osteophytes and from intact articular cartilage from knee joints of 15 adult human donors and subjected to cDNA microarray analysis. The differential expression of relevant genes between these two cartilaginous tissues was additionally validated by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) and by immunohistochemistry.
Results: Among 47,000 screened transcripts, 600 transcripts were differentially expressed between osteophytic and articular chondrocytes. Osteophytic chondrocytes were characterized by increased expression of genes involved in the endochondral ossification process [bone gamma-carboxyglutamate protein/osteocalcin (BGLAP), bone morphogenetic protein-8B (BMP8B), collagen type I, alpha 2 (COL1A2), sclerostin (SOST), growth arrest and DNA damage-induced gene 45ß (GADD45ß), runt-related transcription factor 2 (RUNX2)], and genes encoding tissue remodeling enzymes [matrix metallopeptidase (MMP)9, 13, hyaluronan synthase 1 (HAS1)]. Articular chondrocytes expressed increased transcript levels of antagonists and inhibitors of the BMP- and Wnt-signaling pathways [Gremlin-1 (GREM1), frizzled-related protein (FRZB), WNT1 inducible signaling pathway protein-3 (WISP3)], as well as factors that inhibit terminal chondrocyte differentiation and endochondral bone formation [parathyroid hormone-like hormone (PTHLH), sex-determining region Y-box 9 (SOX9), stanniocalcin-2 (STC2), S100 calcium binding protein A1 (S100A1), S100 calcium binding protein B (S100B)].
Immunohistochemistry of tissue sections for GREM1 and BGLAP, the two most prominent differentially expressed genes, confirmed selective detection of GREM1 in articular chondrocytes and that of BGLAP in osteophytic chondrocytes and bone.
Conclusions: Osteophytic and articular chondrocytes significantly differ in their gene expression pattern. In articular cartilage, a prominent expression of antagonists inhibiting the BMP- and Wnt-pathway may serve to lock and stabilize the permanent chondrocyte phenotype and thus prevent their terminal differentiation. In contrast, osteophytic chondrocytes express genes with roles in the endochondral ossification process, which may account for their transient phenotype.

Introduction

The generation of phenotypically stable hyaline repair cartilage is still a challenge for regenerative medicine. The aim of therapeutic strategies is not confined to the induction of chondrogenesis but also includes the perpetuation of a stable, permanent cellular phenotype that is typical for articular chondrocytes. Unfortunately, cartilage repair approaches that focus on the recruitment of bone marrow stem cells are not only confronted with insufficient chondrogenesis but also with the problem of chondrocyte hypertrophy and inadvertent endochondral ossification[1–4]. Thus, the cellular differentiation programme within cartilage repair tissue recapitulates cellular processes that are similar to the fetal growth plate, fracture callus or forming osteophytes[5,6]. All these tissues have in common that their cartilaginous tissue is only an intermediate state toward bone formation and their cells only adopt

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a transient chondrocytic phenotype. In contrast, chondrocytes of healthy articular cartilage are characterized by a postmitotic status and retain their unique phenotype for a period of many decades. To date, the mechanisms that stabilize the articular chondrocyte phenotype have not been fully identified. We hypothesize upon a distinction between transient and permanent chondrocytes, and the aim of this study was to investigate the differences in their gene expression profile.

Since phenotypically instable transient cartilage repair tissue is available only to a limited degree and characterized by a heterogeneous composition, we focussed on the cartilaginous cap of mature osteophytes which can be considered a prototype for cartilage repair tissue that arises in a more predictive and uniform pattern. Osteophytic chondrocytes originate from mesenchymal stem or precursor cells, undergo chondrogenic differentiation leading to the formation of cartilaginous tissue, that is, however, prone to be replaced by endochondral ossification in the mature osteophyte[5,7–11]. Therefore, osteophytic chondrocytes represent a well-suited substrate to investigate the transcriptional differences between transient chondrocytes and permanent chondrocytes from articular cartilage.

Materials and methods

Tissue samples and preparation

Human articular and osteophytic cartilage probes were obtained as matched pairs from the same respective knee joints of 15 individual patients [with a mean ± standard deviation (SD) age of 68.2 ± 6.7 years] undergoing total knee arthroplasty for osteoarthritis (OA) at the University Hospital Erlangen. The diagnosis of primary OA was based on clinical and radiographic evaluations according to standard criteria and patients with any secondary OA or rheumatoid arthritis were excluded.

Articular cartilage was isolated from the dorsal part of the femoral condyles with a macroscopically intact joint surface characterized by an Outerbridge score of 0 or 1. Osteophytic cartilage was isolated from the same respective joints from the cartilaginous cap of the osteophyte outgrowths located at the edges of the femoral condyles. Osteophytes were distinguished from the marginal transition area of the joint surface by the existence of a concave ridge toward the joint surface. In order to selectively isolate the cartilaginous cap of the osteophyte and to exclude any abrasion of bone trabeculae and calcified tissue of the deepest cartilaginous zone, only minimal forces were applied with a scalpel by cutting tangentially to the surface to yield thin consecutive slices of less than 1 mm thickness. Informed consent was obtained from each patient prior to surgery, and the institutional ethics committee approved the study protocol.

Microarray

Total RNA was isolated from articular or osteophytic cartilage tissue as described previously in detail[11]. The quality of isolated RNA was assessed using the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA), and the concentration was determined with a Spectrophotometer (ND 1000; NanoDrop Technologies, Rockland, DE). RNA preparations from a total of 15 different donors were used. Three paired sets of RNA preparations of articular and osteophytic cartilage each of five donors in equal quantities were used for analysis. The appropriate pooling of RNA samples had been shown to be statistically valid for microarray experiments.[51] Gene expression profiling was performed with the Affymetrix Human Genome (HG) U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. The Genechip U133 Plus 2.0 is a comprehensive whole human genome expression array with over 47,000 transcripts. Genes highly expressed by articular and osteophytic chondrocytes were identified by comparing gene expression levels between the two groups.

Quantitative reverse transcriptase polymerase chain reaction (RT-PCR)

To confirm the validity of the microarray, the individual RNA preparations of 15 donors were additionally analyzed by real-time RT-PCR using an ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA) and Verso One-Step QRT-PCR Rox Kit (Abgene, Hamburg, Germany) by quantifying the expression levels of functionally relevant genes including ACAN (aggrecan), BGLAP (bone gamma-carboxyglutamate protein/osteocalcin), COL1A2 (collagen type I, alpha 2), COL10A1 (collagen type X, alpha 1), FRZB (frizzled-related protein), GADD45ß (growth arrest and DNA damage-induced gene 45ß), GREM1 (Gremlin-1), MMP13 (matrix metallopeptidase 13), PTHLH (parathyroid hormone-like hormone), RUNX2 (runt-related transcription factor 2), SOX9 (sex-determining region Y-box 9), and ß2M (ß 2-microglobulin). The relative quantification of gene expression was performed using the standard curve method. For each sample, the relative amount of target mRNA was determined and normalized to that of ß2m mRNA. Primer and probe sets are shown in Table I.

Immunohistological analysis

Osteochondral specimens from the boundary region of articular cartilage and osteophyte were fixed in 4% paraformaldehyde for

Table I
Primer/probe sets of target genes used for quantitative RT-PCR (TaqMan Gen Expression Assays, Applied Biosystems)

Genes	Gene expression assay ID	Reference sequence
ACAN	Hs00153936_m1	NM_013227
SOX9	Hs00165814_m1	NM_000346
RUNX2	Hs00231692_m1	NM_004348
COL1A2	Hs00164099_m1	NM_000089
COL10A1	Hs00166657_m1	NM_000493
PTHLH	Hs00174969_m1	NM_002820
MMP13	Hs00233992_m1	NM_002427
FRZB	Hs00173503_m1	NM_001463

	Forward	Reverse	Probe
GREM1	GTGACGGAGCGCAAATACC	CCTTCCTCGTGGATGGTCT	AGACTGGTGCAAAACCCAGCCG
BGLAP	CGGTGCAGAGTCCAGCAAA	AGCGCCTGGGTCTCTTCACT	TGCAGCCTTTGTGTCCAAGCAGGAG
GADD45ß	ATTGACGAGGAGGAGGAGGA	CGTTGTCACAGCAGAAGGAC	ATCGCCCTGCAAATCCACTTCACG
ß2M	TGACTTTGTCACAGCCCAAGATA	AATCCAAATGCGGCATCTTC	TGATGCTGCTTACATGTCTCGATCCCA