COL10 expression in the limb cartilage. Under the OA induction in the wild-type joints, C/EBPβ was induced at the frontline of cartilage destruction, whereas in the C/EBPβ+/− joints, the destruction as well as chondrocyte hypertrophy and COL10 expression were significantly suppressed. In the ex vivo culture of C/EBPβ−/− costal chondrocytes, COL10 expression was significantly decreased compared to the wild-type culture. The mRNA level and promoter activity of COL10 were enhanced by the C/EBPβ transfection, and the core responsive region of the COL10 promoter was identified between -81 and -76 bp relative to the transcriptional start site. Since C/EBPβ and Runx2 are known to function as mutual transcriptional co-factors, we further generated C/EBPβ and Runx2 compound deficient (C/EBPβ+/−; Runx2+/−) mice. The C/EBPβ+/−; Runx2+/− mice exhibited severer dwarfism than the C/EBPβ+/−/+ mice. Although chondrocyte hypertrophy and COL10 expression were comparable between the two genotypes, cartilage degradation and MMP13 expression were markedly suppressed by the Runx2 insufficiency. In the culture of SW1353 cells, co-transfection of C/EBPβ and Runx2 enhanced MMP13 expression, but not proliferation or COL10 expression, as compared to a single transfection of C/EBPβ or Runx2. The promoter activity of MMP13 was synergistically enhanced by the co-transfection, and the core responsive region was identified between -111 and -89 bp, which contains a C/EBP-binding motif, but not a Runx2-binding motif.

Conclusions: C/EBPβ is a crucial transcription factor for chondrocyte hypertrophy and cartilage degradation. Runx2 contributes to the latter step as the co-factor, but not to the former step, indicating distinct transcriptional control of these sequential steps during enchondral ossification by C/EBPβ and Runx2.

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MI R-140 IS EXPRESSED IN DIFFERENTIATED HUMAN ARTICULAR CHONDROCYTES AND MODULATES IL-1 RESPONSES

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Purpose: MicroRNAs (miRNAs) are a class of noncoding small RNAs that act as negative regulators of gene expression. The miRNAs exhibit tissue-specific expression patterns and changes in their expression may contribute to pathogenesis. The objectives of this study were to identify miRNAs expressed in articular chondrocytes, determine changes in osteoarthritic cartilage and address the function of miR-140.

Methods: To identify miRNAs specifically expressed in chondrocytes, we performed gene expression profiling using miRNA microarrays and quantitative PCR with human articular chondrocytes compared to human mesenchymal stem cells (MSC). The expression pattern of miR-140 was monitored during chondrogenic differentiation of hMSC in pellet cultures and in human articular cartilage from normal and osteoarthritic knee joints. We tested effects of IL-1β on miR-140 expression. Double-strand (ds) miR-140 was transfected into chondrocytes to analyze changes in the expression of genes associated with osteoarthritis.

Results: Microarray analysis showed that miR-140 has the largest difference in expression between chondrocytes and MSC. During chondrogenesis cultures of MSC miR-140 expression increased in parallel with Sox9 and Col2a1. Normal human articular cartilage expressed miR-140 and this was significantly reduced in OA tissue. In vitro treatment of chondrocytes with IL-1β suppressed miR-140 expression. In contrast to miR-140, miR-146 has a broader tissue distribution, it is increased in response to IL-1, it is upregulated in OA. Transfection of chondrocytes with ds-miR-140 downregulated IL-1β-induced ADAMTS-5 expression and rescued the IL-1β-dependent repression of Aggrecan gene expression. Moreover, we performed searches in three databases (“TargetScan”, “PicTar”, “miRBase”) and this yielded 223-975 potential miR-140 targets. Only 9 potential targets were identified in all three databases.

Conclusions: This study shows that miR-140 has a chondrocyte differentiation-related expression pattern. The reduction in miR-140 expression in OA cartilage and in response to IL-1β may contribute to the abnormal gene expression pattern characteristic of OA.

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THE ADIPOKINE, RESISTIN, INDUCES A HIGH LEVEL OF EXPRESSION OF PRO-INFLAMMATORY CYTOKINES AND CHEMOKINES IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: To provide a picture of the effect of resistin on human articular chondrocyte gene expression of cytokines and chemokines.

Methods: Chondrocytes were obtained from conserved area of cartilage from donors undergoing total knee joint replacement surgery. Chondrocytes were isolated and plated at a density of 2.5 × 10^5 cells/cm^2 in Dulbecco’s modified eagle’s medium (DMEM)/F12 media plus 10% fetal bovine serum (FBS), 50 mg/ml ascorbate and antibiotics (50 U/ml penicillin and 50 mg/ml streptomycin) for 24 h. Serum was removed and cells were allowed to recover for 24 h before adding resistin from BioVision (Mountain view, CA) for 24 h. Changes in gene expression were analyzed by quantitative real-time polymerase chain reaction.

Results: Resistin treated human articular chondrocytes showed significant increases in the expression of a large group of cytokines and chemokines, including IL-1a, IL-1b, IL-6, IL-8, CCL3, CL4, CCL8, CXCL1, CXCL3, CXCL6. As expected, the mRNA for matrix metalloproteinase (MMP)-1, MMP-3 and MMP2 also increased, but not as much as the above genes. Genes were placed in three
categories: genes with relative fold-change of mRNA less than 10 fold (A), genes with relative fold-change of mRNA between 10 and 100 fold (B), genes with relative fold-change of mRNA more than 100 fold (C).

Conclusions: These results indicated that resistin, the adipocyte-derived cytokine, could be an important link between obesity and osteoarthritis (OA) joint disease. Resistin is a proinflammatory and destructive mediator of joint inflammation in human joint, and might be considered as a potential therapeutic target in joint degenerative diseases such as OA.

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PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORα AGONISTS DECREASE INFLAMMATORY AND DESTRUCTIVE RESPONSES OF OSTEARTHRITIC SYNOVIIUM, CARTILAGE AND HOFFA’S FAT PAD

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Purpose: Although osteoarthritis (OA) was originally described as a noninflammatory arthropathy, inflammatory responses in synovium and cartilage may contribute to disease progression. Inflammation is caused by direct biomechanical perturbation or reaction to cartilage matrix degradation. Recent evidence has emerged for a role of the Hoffa’s fat pad in the development and progression of OA by the secretion of pro-inflammatory cytokines. The Hoffa’s fat pad is located in the knee, intraarticular and extrasynovial, under the patella. Peroxisome Proliferator-Activated Receptor (PPARα) is a member of class I nuclear receptor superfamily of ligand-dependent transcription factors. Anti-inflammatory effects of PPARα agonists have been described in adipose tissue, liver, blood vessels, kidney, macrophages and mesangial cells. We hypothesized that addition of synthetic PPARα agonist Wy-14643 exerts anti-inflammatory effects in OA joint tissues.

Methods: Explants of synovium (n=9), cartilage (n=9) and Hoffa’s fat pad (n=10) were obtained from OA patients that underwent total knee arthroplasty. Explants were cultured in DMEM-high glucose with ITS, with(out) 100 μM PPARα agonist. 10 ng/ml IL-1β was added as a pro-inflammatory stimulus. Gene expression analysis was performed on cartilage explants (MMP1, MMP3, MMP13, aggrecan, collagen type II) and on synovium explants (MMP1, MMP3, MMP13, IL-1β). The culture supernatant of cartilage and synovium samples was analysed for NO production. Supernatant of cartilage was analysed for GAG release. The culture supernatant of Hoffa’s fat pad was analysed with ELISA for leptin, adiponectin, resistin, MCP-1, TNFα, IL-1β, IL-6, IL-2 and IL-10. A decrease or increase was defined as more than 25 % of the control value. Results were analysed with non-parametric tests.

Results: Addition of PPARα agonist decreased mRNA expression of cartilage with 76 % for MMP1 (p=0.12), 68 % for MMP3 (p=0.02) and 87 % for MMP13 (p=0.01). Addition of IL-1β decreased collagen type II gene expression a 15-fold. Addition of the PPARα agonist decreased this expression even more with 49 % (p=0.02). Aggrecan mRNA expression was not influenced by the PPARα agonist. NO production of cartilage showed a trend towards a decrease by the addition of PPARα agonist. The release of GAG in cartilage culture supernatant was lower after addition of 100 μM PPARα agonist (p=0.06). A trend towards decrease could be observed for MMP1, MMP3 and MMP13 after adding PPARα agonist. IL-1β mRNA expression and NO production by synovium was not influenced by the PPARα agonist. In culture supernatant of Hoffa’s fat pad, MCP-1 (p=0.01), IL-4 (p=0.03) and IL-10 (p=0.03) were decreased after adding PPARα agonist. 7 donors showed a decrease and 2 an increase for TNFα production (p=0.18). Leptin, resistin and IL-1β showed a trend towards more donors with a decrease. Adiponectin and IL-6 were not influenced.

Conclusions: Addition of 100 μM PPARα agonist Wy-14643 decreased IL-1β induced MMP gene expression, NO production and GAG release in cartilage and had an effect on collagen type II, but not on aggrecan production. A non significant decrease was seen for MMP expression in synovium. The production of pro- and anti-inflammatory cytokines by Hoffa’s fat pad was decreased by adding 100 μM PPARα agonist. In general, the use of PPARα agonist Wy-14643 showed promising results in inhibiting inflammatory processes in OA joint tissues. The use of these synthetic ligands for treatment of OA should be further explored.

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OSTEOARTHRITIS SUSCEPTIBILITY GENES ARE ASSOCIATED WITH VARIATIONS IN HIP MORPHOLOGY

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Purpose: It has been suggested that many cases of primary hip OA are actually caused by mild morphological variations in the shape of the hip. Interestingly, genes that have been found to affect susceptibility for OA such as FRZB, GDF5 and DIO2, are involved in endochondral ossification and regulate the process of skeletal formation.

In this study, we have quantified the shape of the hip in sibling pairs of the GARPs study, who have symptomatic OA at multiple joint locations. We estimated familial aggregation of hip shape and investigated whether shape is affected by the genetic variation at the OA susceptibility genes.

Methods: A Statistical Shape Model was created of the shape of the proximal femur and pelvis of radiographs of the hips of sibling pairs of the GARPs study. The method results in a set of independent modes that together quantitatively describe the total shape, while each mode separately describes a specific characteristic of the shape. This preliminary data concerns 74 sibling pairs (148 subjects), of which 29 subjects had radiographic signs of hip OA (KL>1). Familial aggregation (heritability) of shape was estimated from the variance within and between siblings. Associations between shape and SNPs of the FRZB, GDF5 and DIO2 genes were tested using mixed model regression with height, gender, age, BMI, and OA status (KL>1 for 0, 1 or 2 hips) as co-variables. In these analyses we modeled familial and left and right hip dependencies through random effect variables.

Results: 5 out of 30 modes showed significant familial aggregation describing over 60% of the total variation in shape. OA status associated significantly with femurs with a stocky appearance (mode 6, p=0.03), a short superior neck (mode 11, p=0.002) and a small offset between superior neck and head (mode 17, p=0.035). It is unclear whether these associations are the result of the OA disease process or point to a factor underlying the OA process. Modes 5 (p=0.012) and 16 (p=0.004) showed a dose response association with respectively DIO2 OA susceptibility SNPs rs225014 and rs12885300. Both modes are descriptors of the OA disease process or point to a factor underlying the OA process. Modes 1 (p=0.03) and 5 (p=0.03) showed association with respectively DIO2 OA susceptibility SNPs rs225014 and rs12885300. Both modes are descriptors of the OA disease process or point to a factor underlying the OA process. Modes 5 (p=0.012) and 16 (p=0.004) showed a dose response association with respectively DIO2 OA susceptibility SNPs rs225014 and rs12885300. Both modes are descriptors of the OA disease process or point to a factor underlying the OA process.