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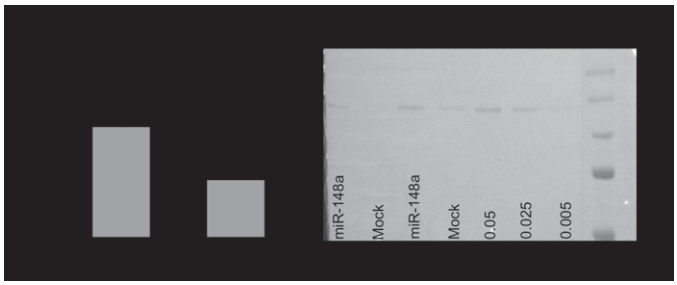
OVEREXPRESSION OF hsa-miR-148A PROMOTES TYPE II COLLAGEN SYNTHESIS BY OSTEOARTHRITIC CHONDROCYTES

L.A. Vonk[1], A.H. Kragten[1], D.B. Saris[1], W.J. Dhert[1,2], L.B. Creemers[1]. *[1]UMC Utrecht, Utrecht, Netherlands; [2]Univ. of Utrecht, Utrecht, Netherlands*

Purpose: MicroRNAs (miRNAs) regulate gene expression through base-specific interactions. The aim of this study was to further identify miRNAs differentially expressed in OA and to investigate the effect of one of these, miR-148, on the chondrogenic potential of chondrocytes from OA cartilge.

Methods: Low-density Taqman arrays were used to identify miRNAs differentially expressed in OA and normal cartilage. OA chondrocytes were isolated from articular cartilage obtained from patients undergoing knee arthroplasty. At passage 2, OA chondrocytes from 6 donors were transfected with a miRNA precursor for hsa-miR-148a or a miRNA precursor negative control. Chondrocytes were reverse-transfected during seeding at high density (1.26×10^6 cells per cm^2) on collagen-coated culture inserts in a 96-wells transwell system. After 2 days, real-time PCR was performed to examine gene expression levels of aggrecan (ACAN), type I collagen (COL1A1), type II collagen (COL2A1) and matrix metallopeptidase 13 (MMP13). After 1 week, glycosaminoglycan (GAG), collagen and DNA content were determined using a DMMB, hydroxyproline and Picogreen assay, respectively. Type II collagen was analyzed at the protein level by Western blot.

Results: 66 miRNAs were differentially expressed in OA cartilage compared to healthy cartilage, including miR-148a, which was downregulated 11 times in OA cartilage compared to normal cartilage. Overexpression of miR-148a had no effect on ACAN and COL1A1 gene expression levels and on the amount of GAG produced by the cells. MiR-148a overexpressed cells showed increased gene expression levels of COL2A1, while MMP13 was decreased. The increase at the mRNA level was reflected by an increase in the total amount of collagen, in particular type II collagen as shown by biochemical analyses.



Conclusions: Several miRNAs are differentially expressed in OA and modulating their expression may lead to a potential therapy for OA. Overexpression of miR-148a increased type II collagen production by OA chondrocytes.

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CHANGES IN GENE EXPRESSION WITH AGE AND OSTEOARTHRITIS IN THE MOUSE KNEE JOINT

R. Loeser[1], A. Olex[1], M.A. McNulty[2], C.S. Carlson[3], M. Callahan[1], C. Ferguson[1], J.S. Fetrow[1]. *[1]Wake Forest Univ., Winston Salem, NC, USA; [2]Universtiy of Minnesota, St. Paul, MN, USA; [3]Univ. of Minnesota, St. Paul, MN, USA*

Purpose: The mechanisms by which aging changes in the joint predispose older adults to develop OA are incompletely understood. The purpose of this study was to analyze differences in gene expression between young and older adult mice to discover genes and biological processes that contribute to age-related differences in the development of OA in a joint injury model. The goal was to study changes in the joint as an organ rather than study an individual tissue.

Methods: Young (12 week-old) and older (12 month-old) adult male C57/BL6 mice underwent surgery to destabilize the medial meniscus (DMM) or sham surgery as a control. 8 weeks after surgery, knee joints from 6 mice in each age and surgical group were evaluated histologically to measure OA severity and joints from 9 mice per group were dissected to isolate RNA from the medial side of the joint (cartilage, meniscus, subchondral bone and joint capsule with synovium) for gene expression microarrays (triplicate arrays for each age and surgical group) and real-time PCR. Computational analysis was performed to determine changes occurring with age alone (sham knee comparisons) and with the development of OA (signal log ratio comparisons of DMM/sham in young vs old).

Results: Both young and older mice exhibited typical features of OA in the DMM knees including cartilage surface fibrillation and clefting, osteophyte formation, and subchondral bone thickening which were consistently more severe in the medial tibial plateau and were more severe in the 12-month-old mice than in the 12-week-old mice. The average articular cartilage structure score was about 2-fold greater in the DMM knees of the older mice than in the younger mice. The sham knees in the young mice were normal while in the older mice early mild OA changes were noted. 861 genes showed age-related differences in expression in the sham knees (430 up and 431 down in old) and 493 genes showed age-related differential expression in the DMM/sham comparison while only 55 genes were expressed similarly in young and older DMM/sham. DAVID analysis revealed that the set of genes up-regulated with age in sham knees included immune response and defense response (e.g. wound healing) genes. These included several chemokine genes such as CXCL13, CCL8(MCP-2), CCL5(RANTES), CXCR2, and CCR7. The genes down-regulated with age were related to metabolic processes and the extracellular matrix including aggrecan, Col2a1, Col9a1, Matn3, and Prkg2. However, opposite changes were noted in DMM knees such that immune response genes were upregulated in young more than old and matrix genes, including aggrecan, periostin and Col3 but not Col2 increased more in the old than young. Genes such as IL-33, CCR7, and DKK3 increased more in young. Only eight genes went down in DMM knees in both age groups including complement factor D (adipsin) and MUP1 while 47 genes went up in DMM knees of both age groups including CCL21, IGF-1, and MMP-3.

Conclusions: When considering the joint as an organ, significant age-related differences in OA severity and in gene expression were noted in the sham control knees and DMM knees of mice that can be used to provide new insights into the effects of age on the development of OA. Differences in expression of immune response genes, not expected in OA, may be of particular interest. These genes went up with aging and with induction of OA in young mice suggesting OA triggers an aging-like response. The results also indicate that age is an important consideration when using the DMM model in mice to study the role of a particular gene in OA.

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NFAT3 SPECIFICALLY REGULATES MIR-140 TRANSCRIPTION IN HUMAN OSTEOARTHRITIC CHONDROCYTES

G. Tardif, D. Hum, H. Fahmi, J-P. Pelletier, J. Martel-Pelletier. *Osteoarthritis Res. Unit, Univ. of Montreal Hosp. Res. Ctr. (CRCHUM), Montreal, QC, Canada*

Purpose: In osteoarthritis (OA), despite major progress regarding the identification and roles of catabolic mediators, further knowledge about factors regulating their expression is needed. In this line of thought, one recently identified class of molecules, the microRNA (miRNA), has been found to add another level of regulation to gene expression by down-regulating its target genes. The miRNA-140, present in an intron of the WWP2 gene, is known to decrease the expression of some genes that play detrimental roles in OA. However, the expression level of miR-140 is significantly decreased in human OA chondrocytes. Therefore, understanding the transcriptional regulation of miR-140 is of importance as it can provide a new basis for the rationalization of a therapeutic strategy for this disease. We thus investigated the transcriptional regulation of miR-140 in human OA chondrocytes.

Methods: Human OA chondrocyte gene and miRNA expression were determined by quantitative PCR, gene silencing following cell transient transfection with specific siRNAs, and miR-140 promoter activity monitored by luciferase activity.

Results: In contrast to the significantly reduced miR-140 expression level in OA compared to normal chondrocytes, the expression of the WWP2 was similar, suggesting that miR-140 has an additional level of regulation. The DNA sequence upstream of miR-140 was found to have promoter activity and predicted binding sites for NMP-4, MAZ and NFAT. Gene silencing of MAZ, NMP4, NFAT1 and NFAT2 did not affect miR-140 or WWP2 expression levels. However, silencing NFAT3 reduced the miR-140 expression level but not that of WWP2, whereas silencing NFAT5 reduced both miR-140 and WWP2 levels. Additional experiments