

No.11, page 1029-1034, 2010). Its effects on the modulation of osteoblast metabolism markers and tumor necrosis factor alpha (TNF alpha) induced inflammatory response markers were determined by real-time RT-PCR, western blot, enzyme-linked immunosorbent assay, gene reporter luciferase assay and electrophoretic mobility shift assay.

Results: SR1078 is generously provided by Dr. Thomas P. Burris (The Scripps Research Institute, Jupiter Floride, USA). SR1078 increased alkaline phosphatase (ALP), osteocalcin (OC) and collagen type I (COL I) mRNA and activity or protein expression. Moe, SR1078 suppressed TNF alpha-induced production of cyclooxygenase-2 (COX-2), prostaglandins E₂ (PGE₂) and metalloproteinase-9 (MMP-9). Upon examination of signalling pathways, we found that SR1078 was able to block TNF alpha-induced nuclear factor kappa B (NF-kB) activation.

Conclusion: ROR alpha is involved in human osteoblast metabolism by stimulating osteoblast marker expression and inhibiting inflammatory responses. These findings may encourage further exploration of stimulation of ROR alpha as a potential target for the treatment of bone disorders related to inflammation.

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BIOMECHANICAL STRESS AND ESTROGEN IMPACT ON HUMAN OSTEOBLASTS

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Purpose: The etiology and pathogenesis of adolescent idiopathic scoliosis (AIS) remain unknown. There are several proposed etiological hypotheses attributed to a variety of conditions ranging from posture abnormalities to a diet: environmental factors, biochemical factors, mechanical, neurological and hormonal factors muscle and ligament, and recently, the intracellular signaling pathway of melatonin have been proposed. The objectives of this work was to investigate various factors involved in the AIS and to highlight the possible effect of biomechanical stress and estrogen on human osteoblasts derived from AIS patients (undergoing scoliosis surgery) and healthy subjects (surgery for bone trauma).

Methods: Human osteoblasts were derived from tissues obtained at surgery, and cultured in presence or absence of 17-beta estradiol. We used microarray analysis to examine differences in the gene transcription profile between primary human osteoblasts derived from spinal vertebrae of AIS patients and those of healthy individuals (Illumina HT-12 Expression BeadChips™ technology). RNA extracted from AIS patients was compared to the RNA of healthy patients. In addition, osteoblasts were exposed to biomechanical stress (0-2 g/cm²) and investigated for cell proliferation and level of biochemical factors produced by cultured cells, such as NO, COX-2, OPN and ATP

Results: Biomechanical stress differentially influenced cell proliferation: decreased osteoblast proliferation was observed in control cells but not in AIS cells. Following the biomechanical stress, NO, COX-2, OPN and ATP levels were increased in both control cells and cells SIA. Using microarray analysis, we identified that several genes are differentially expressed in AIS osteoblasts. We found that 86 genes were expressed at relatively higher levels in AIS osteoblasts compared to controls, while 59 genes were expressed at lower levels. These genes are involved in various bone regulatory and developmental pathways and interestingly, many of them can be associated with particular biological pathway.

Conclusions: Our study demonstrated that various biochemical factors could be altered by biomechanical stress. Hormonal factor are also involved in the gene expression of certain genes. These factors could be associated to the spinal curve progression and consequently they could impact AIS progression. Our study demonstrated changes in gene transcription between AIS and non-AIS patients and provided a previously unrecognized list of AIS candidate genes. According to their function and their involvement in biological processes, these genes are mainly involved in bone metabolism and embryonic development. Thus, our study suggests various gene interaction and pathways in AIS pathogenesis.

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ELR+ CXC CHEMOKINE SIGNALLING IN CARTILAGE HOMEOSTASIS

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Purpose: The production of ELR+ CXC chemokines is widely studied in arthritis and is postulated to contribute to the inflammatory phenomena that lead to cartilage breakdown and arthritis pathology. Healthy articular chondrocytes however, also express their own chemokine receptors and ligands. The function of CXC chemokine receptors in these cells is puzzling because chondrocytes are encased in a dense extracellular matrix and are not known to migrate in vivo. This study aims to identify the function of this signaling mechanism in articular cartilage.

Methods: Adult human articular chondrocytes (AHAC) were expanded in monolayer culture under standard conditions. Receptor expression was confirmed using semi quantitative RT polymerase chain reaction (RT-PCR), Western blot and immunohistochemistry. CXCR1/2 combined and individual functionality was tested using an in vitro calcium mobilisation assay. CXCR1/2 signaling was blocked at specific receptor level using validated blocking antibodies and siRNA, or at the downstream level using Pertussis toxin, PI3K inhibitors and intracellular calcium chelators. Chondrocyte phenotypic gene expression was assessed using real time RT-PCR. The content of highly sulphated proteoglycans in chondrocyte micromasses was analysed using Alcian blue staining and spectrophotometric quantification normalised for total protein content. CXCL6 and CXCL8 were detected in heparitinase digested, chondroitinase ABC digested and un-digested paraffin sections from healthy and osteoarthritis full thickness human articular cartilage using immunohistochemistry. 8 week old CXCR2-/- mutant mouse knee joint paraffin sections were analysed using Safranin Orange staining, followed by Chambers scoring and ImageJ histomorphometry.

Results: Receptors were expressed in normal human articular cartilage. Blockade of either CXCR1 or CXCR2 individually did not inhibit downstream calcium mobilisation, indicating that CXCR1 and CXCR2 have a higher level of functional redundancy than that observed in neutrophils. Disruption of CXCR1/2 signaling at receptor level or by downstream blockade in chondrocytes resulted in reduced extracellular matrix sulphated glycosaminoglycan content and reduced expression of the chondrocyte differentiation markers COL2A1, Aggrecan, and SOX9. CXCL6 and CXCL8 were found in cartilage extracellular matrix in healthy tissue in distinct localisation patterns, which were disrupted in osteoarthritic tissue and following heparitinase digestion. In vivo analysis of 15 knockout and 15 wild type BALB/C controls revealed that CXCR2-/- mutant mice have significantly thinner epiphyseal growth plates and medial tibial plateaus.

Conclusions: Our findings indicate that CXCR1/2 signaling is required for the maintenance of phenotypic stability in articular chondrocytes. Interactions with heparan sulphate proteoglycans and distribution patterns of ligands within the ECM, together with their disruption during pathology, indicate the presence of a homeostatic mechanism whereby CXCL8 is retained within the articular cartilage matrix via its interaction with heparan sulphate proteoglycans, contributing to chondrocyte phenotypic stability. In vivo analysis suggests that CXCR1/2 signaling may be required during periods of high chondrocyte turnover, such as within the growth plate, whereas in stable conditions, CXCR1 signaling alone is sufficient to compensate for CXCR2 function.

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NF-KB : A POTENTIAL MEDIATOR OF ADAMTS-5 ACTIVATION AND THERAPEUTIC TARGET FOR CARTILAGE BREAKDOWN IN HIGH AGE DIET-INDUCED OSTEOARTHRITIS

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Purpose: The accumulation of Advanced Glycation Endproducts (AGEs) plays an important role in loss of function of many organs, and