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EXPRESSION OF ADIPOCYTOKINES IN OSTEOARTHRITIS OSTEOPHYTES

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Purpose: One risk factor in osteoarthritis (OA) is obesity but little is known about the interaction between bone formation and adipose tissue-derived factors. Adipocytes produce many cytokine-like proteins, e.g. adiponectin, visfatin or resistin. These adipokines can also be produced by other cell types (fibroblasts, osteoblasts, osteoclasts). While an association with the pathogenesis of rheumatoid arthritis has already been shown, their role in osteophyte formation in OA has not been studied yet. The mechanisms of osteophyte formation are mostly unknown but there is growing evidence that inflammation and local stress play a role in this process. In this study, the expression of adipokines in osteophytes and their co-localization with cells of bone remodeling was analyzed.
Methods: Osteophyte tissue and cartilage was obtained from OA patients during joint replacement surgery. Using serial sections of osteophyte tissue and hematoxylin/eosin and Masson trichrome staining, the osteophytes were scored and divided into grade one to five (grade 1: no ossification, consinsting of connective tissue (CT) and cartilage ; grade 2: CT and cartilage about 80%, max. 20% of ossified and mineralized areas; grade 3: the non-ossified osteophyte part consists of about 50% CT and 50% cartilage, more than 20% of ossification and mineralization of the osteophyte; grade 4: ossified, mineralized osteophyte with cartilage, less than 10% CT, no ossified remodeling zones; grade 5: ossified, mineralized osteophyte with cartilage, CT less than 10%, ossified remodeling zones). To identify the localization of adipokines, immunohistochemistry was performed against alkaline phosphatase, collagen type I and II, adiponectin, visfatin, and resistin. TRAP stainings were performed for osteoclast identification. Immunoassay analyses for visfatin were done with ground cartilage and lysates of isolated chondrocytes.
Results: In all osteophyte grades of OA patients (1-5), adiponectin, visfatin and resistin expression was detectable at different levels. In non-ossified osteophytes, adiponectin was localized around blood vessels and in 25% to 50% of CT fibroblasts. Resistin and visfatin were localized in single fibroblasts within the connective tissue.
Reduced amounts of adiponectin were detectable in ossified and mineralized osteophytes in comparison to non-ossified osteophytes. In about 65% of the samples, a co-localization with osteoblasts was visible. Resistin and visfatin showed a co-localization with osteoblasts and osteoclasts at sites of bone remodeling in about 70% of the samples. Visfatin could be detected in chondrocytes in ossified and non-ossified osteophytes, and its expression could be confirmed by ELISA using lysates of cartilage and isolated chondrocytes.
Conclusions: The expression of visfatin in chondrocytes in the osteophyte cartilage and adiponectin in osteophyte connective tissue suggests them to be involved in early stages of osteophyte formation. The co-localization of resistin and visfatin with osteoblasts and osteoclasts in ossified and mineralized osteophytes suggests an involvement in bone formation and remodeling of OA osteophytes in later stages. Therefore, adiponectin, resistin, and visfatin are involved in osteophyte formation at different stages and affect different cell types of cartilage and bone formation during these processes.

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SUBCHONDRAL BONE CHANGE AT OSTEOARTHRITIS ONSET - A COMPARATIVE STUDY ON GUINEA PIGS WITH AND WITHOUT OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is one of the most prevalent joint diseases. To date, the pathogenesis and treatment of OA still remain to be elucidated. Recent research reported that subchondral bone plays an important role in the pathogenesis of knee OA[1]. The objective of this study was to longitudinally observe and characterize the subchondral bone at the very early stage of OA before cartilage degeneration using Dunkin-Hartley (DH) guinea pig spontaneous OA model with Micro-CT and histology, with Bristol Strain 2 (S2) guinea pig as control.
Methods: Eighteen Dunkin-Hartley and eighteen Bristol Strain 2 guinea pigs were divided into 3 groups (6 for each), and they were sacrificed at 1, 2 and 3 months for histological evaluation of cartilage with OARSI score[2] (H&E and Safranin O staining). The knee joints of 3-month group (n=6) were scanned with Micro-CT at age of 1, 2 and 3 months to characterize the subchondral bone of knee joints.
Results: Histological study revealed that the difference of OARSI score between two groups were not significant (Table 1). No significant articular cartilage degeneration was noted at the age of 3 month in both DH and S2 guinea pigs. Only focal hypercellularity in deep zone of articular cartilage were observed in DH guinea pigs of 3 months old.
Micro-CT analysis revealed that both trabecular and cortical bone are thicker in DH group guinea pigs than S2 strain. In addition, trabecular morphology turns to be more plate-like and the organization became less anisotropic in subchondral cancellous bone of DH guinea pig as compared with S2 strain (Figure 1).

	1 month	2 month	3 month
DH	1.5 (0.55)	3.5 (0.55)	4 (1.8)
S2	1.3 (0.52)	3.2 (0.75)	4.5 (1.05)

Table 1 Result of OARSI score evaluation on tibial end knee cartilage of Dunkin-Hartley and Bristol Strain 2 guinea pig.

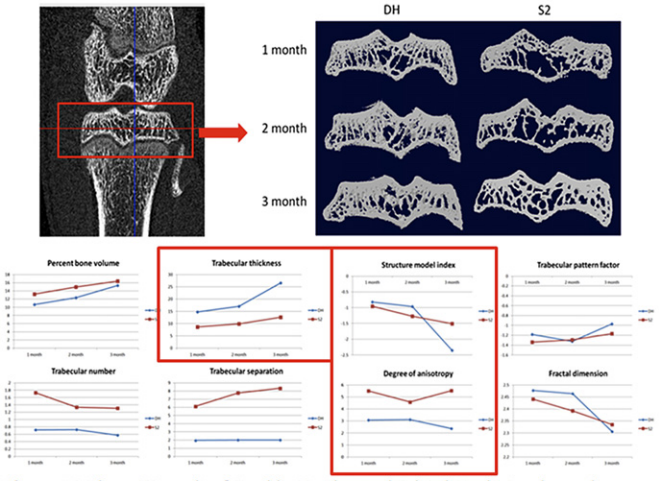

Figure 2 Mricro-CT result of Dunkin-Hartley and Bristol Strain 2 guinea pig.

Conclusions: Subchondral bone change was defined in DH guinea pig spontaneous OA model in terms of over bone formation and trabecular structure turnover at very early stage of OA initiation right before cartilage damage. Further study may be conducted to explore the underlying mechanism in the process of bone remodeling, which may provide new insight to OA pathogenesis and potential treatment solutions.

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SR1078, A SYNTHETIC LIGAND OF NUCLEAR RECEPTOR ROR ALPHA, MODULATES OSTEOBLAST METABOLISM WITH ANTI-INFLAMMATORY EFFECTS

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Objective: To elucidate the effect of one synthetic ligand (SR1078) of nuclear receptor retinoid-related orphan receptor alpha (ROR alpha) in human MG-63 osteoblast-like cells.
Methods: SR1078 represents the first synthetic ligand that is able to function as an ROR agonist (Wang YJ et al., ACS Chemical Biology, Vol.5

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 E_2 (PGE_2) 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