capable of internalizing lipoproteins, via LOX-1 (lectin-like oxidized low density lipoprotein receptor 1). Once cholesterol is accumulated in cells, its efflux is mediated through reverse cholesterol transport via nuclear factors LXRa and LXRβ (liver X receptors) and the ATP-binding-cassette transporter A1 (ABCA1) gene. ABCA1 serves as a lipid pump that effluxes cholesterol from cells to apolipoprotein A1 (ApoA1). As the accumulation of lipids in chondrocytes may signify a causal relationship to development and/or progression of OA, we investigated the expression of genes regulating reverse cholesterol transport, as ABCA1, ApoA1, LXRa and LXRβ in human chondrocytes. We also investigated the effect of a synthetic LXR agonist on apoptosis, ABCA1 and ApoA1 mRNA expression, lipid accumulation, as well as on COL2A1, AGC, MMP-3, MMP-13 and IL-6 expression levels.

Methods: Articular cartilage samples were obtained from 27 patients with primary OA undergoing knee replacement surgery, while normal cartilage was obtained from 8 individuals undergoing fracture repair surgery, with no history of joint disease. Total cellular RNA was extracted from all samples and ABCA1, ApoA1, LXRα and LXRβ mRNA and protein expression levels were evaluated using real-time PCR and Western blot analysis respectively. The effect of the synthetic LXR agonist TO-901317 was studied after treatment of osteoarthritic chondrocytes and subsequent investigation of ABCA1, ApoA1, MMP-13, MMP-3 as well as COL2A1 and AGC mRNA expression levels. IL-6 was measured with ELISA. Cholesterol efflux was evaluated in osteoarthritic chondrocytes radiolabeled with [1,2(n)-^{3}H] cholesterol after LXR treatment, while intracellular lipid accumulation was studied after Oil-red-O staining. Apoptosis was evaluated using flow cytometry.

Results: We found that ApoA1 and ABCA1 mRNA and protein expression levels were significantly lower in osteoarthritic compared to normal cartilage ($p<0.01$ and $p<0.001$ respectively). In addition, LXRα and LXRβ mRNA expression levels were also found to be significantly lower in osteoarthritic cartilage ($p<0.05$ and $p<0.01$ respectively). Treatment of osteoarthritic chondrocytes with the LXR agonist TO-901317 resulted in: (i) significantly increased ApoA1 and ABCA1 mRNA expression levels ($p<0.01$), (ii) significant increase in cholesterol efflux ($p<0.05$) (iii) elimination of intracellular lipids deposits, which had been observed before agonist treatment (iv) significant reduction of MMP-3 and MMP-13 protein levels ($p<0.001$ and $p<0.01$, respectively), (v) significant increase by 2 and 6.6-fold in COL2A1 and AGC mRNA expression levels ($p<0.05$), (vi) significant decrease in IL-6 levels ($p<0.01$) and (vii) significant reduction of apoptosis ($p<0.005$).

Conclusions: Our findings suggest that impaired expression of genes regulating cholesterol efflux may be a critical player in osteoarthritis, while the ability of the LXR agonist to facilitate cholesterol efflux, results in reduction of catabolic and inflammatory molecules and increase in anabolic genes' expression, suggesting its potential use for therapeutic intervention in osteoarthritis.

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CARTILAGE-SPECIFIC DELETION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA IN MICE IS ASSOCIATED WITH GROWTH PLATE DEFECTS

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Purpose: The majority of long bones develop through a process called endochondral ossification, which involves strict coordination of chondrocyte proliferation, differentiation and apoptosis within the growth plate, thus resulting in the replacement of cartilage by bone. Interplay of the multitude of intracellular and extracellular factors acting from within and on chondrocytes results in very tight regulation of these processes, and disturbances of the fine balance controlling endochondral bone growth results in growth and development-related abnormalities such as dwarfism and skeletal deformities. The exact mechanisms through which chondrocyte function/behaviour is controlled during cartilage growth and development are largely unknown. Peroxisome proliferator-activated receptor gamma (PPARγ), a transcription factor, plays a key role in lipid homeostasis and recent studies suggest that PPARγ is involved in the maintenance of bone homeostasis by contributing to osteoclastogenic and osteoblastogenic pathways. However, the specific in vivo function of PPARγ in chondrogenesis, and cartilage growth and development is largely unknown. Therefore, for the first time, my study will examine the specific in vivo contribution of PPARγ to cartilage growth and development processes using cartilage-specific PPARγ knockout (KO) mice.

Methods: Cartilage-specific PPARγ-deficient mice were generated using the Lox P/Cre system. Using mice at post-natal day zero (P0) and embryonic 16.5 days old (E16.5), the following techniques were used: (1) Histological staining of long bones with Safranin-O/Fast Green stain to determine the role of PPARγ in ossification patterns, chondrocyte cell shape, and organization of growth plates. (2) Staining of whole skeletons of newborn mice with alcian blue/alizarin red to determine skeletal phenotypic changes. (3) Real-Time PCR and western blotting to determine the expression of extracellular matrix (ECM) markers. (4) Immunohistochemistry for Collagen X (to determine formation of osseous center), p57 (a marker of hypertrophic differentiation), and PECAM (cell surface marker for endothelial cells to account for vascularization).

Results: Newborn wild-type (WT) mice showed that PPARγ is expressed in all zones of the growth plate of tibias. Although cartilage-specific PPARγ KO mice were viable and healthy at least three weeks post-birth, they showed reduced length, weight, skeletal growth and length of long bones at birth compared to WT mice. Newborn heterozygous (het) and homozygous (hom) cartilage-specific PPARγ KO mice showed growth plate abnormalities including abnormal growth plate organization and cell shape, reduced cellularity, loss of columnar organization, and shorter hypertrophic zones in a gene-dose dependent manner. Additionally, immunohistochemistry for p57 showed reduced chondrocyte differentiation in het and hom mice compared to WT mice. Immunohistochemistry for PECAM and Collagen X in long bones of E16.5 mice revealed reduced vascularity and delayed formation of osseous center in PPARγ KO mice compared to WT mice. Furthermore, in vitro studies using PPARγ-deficient chondrocytes showed increased expression of ECM degradation products including matrix metalloproteinase (MMP)-13 and ADAMTS-5 (aggrecanase), and decreased expression of ECM building products including aggrecan and type-II collagen.

Conclusions: This is the first report to demonstrate that PPARγ-deficiency in cartilage results in growth plate abnormalities. Overall, we show that PPARγ plays a potential role in cartilage growth and development in vivo and its deficiency will result in serious musculoskeletal deformities in vivo.

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TRANSGLUTAMINASE 2 INDUCED BY RETINOIC ACID DECREASE HUMAN CHONDROCYTE APOPTOSIS INDUCED BY HYDROGEN PEROXIDE

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Purpose: Osteoarthritis is a disease characterized by destruction and failure of the extracellular matrix, which serves as the functional component of the articular cartilage. The production of extracellular matrix is solely dependent on the chondrocytes, which makes up the only cell type in the articular cartilage. Therefore, chondrocyte death and survival are essential for maintaining the articular cartilage. Transglutaminase 2 (TGase 2) is an enzyme catalyzing Ca^{2+}-dependent protein cross-linking. TGase 2 has been shown to be induced and activated during apoptosis. We have previously shown that TGase 2 expression is increased in human chondrocytes undergoing apoptosis. Furthermore, inhibition of TGase 2 by monodansylcadaverine (MDC); a competitive substrate of TGase 2; and TGase 2 siRNA have increased chondrocyte apoptosis. These findings suggested a possible protective role of TGase 2 in chondrocyte apoptosis. Retinoic acid (RA) and its various synthetic analogs affect mammalian cell growth, differentiation, and apoptosis. RA consistently induces TGase 2 expression and activation, and it was recently shown that increased TGase 2 expression protected NIH3T3 cells from apoptosis. The purpose of this study was to demonstrate whether TGase 2 is induced in human chondrocytes with RA and explore the role of TGase 2 in human chondrocyte apoptosis.

Methods: *1. Human chondrocytes culture and apoptosis assay*. Human chondrocyte culture, apoptosis induction and analysis of TGase 2 expression was performed as previously described. Briefly, human chondrocytes were obtained from the articular cartilage of patients undergoing total knee arthroplasty and cultured in monolayer. Chondrocyte apoptosis was induced by treating with H_2O_2 (1mM) for 24 hours. Apoptosis was assessed by two methods, biochemically by Annexin-V FACS analysis, and morphologically by nuclear staining for 4'6-Diamidine-2'-phenylliondole (DAPI).

2. RA treatment. Human chondrocytes were grown in medium containing 10% bovine serum and 1% streptopenicillin at 37°C in a humidified incubator with 5% CO_2. The cells were starved in medium containing 1% serum for 48 hours prior to the treatment with 5 μM RA.

3. TGase 2 assay. The expression and enzyme activity of TGase 2 was examined with western blot and immunocytochemistry. Total cellular proteins were isolated and western blotted as described previously. Enzyme activity was evaluated by determining the incorporated biotinylated pentylamine using horseradish peroxidase-conjugated streptavidin and measuring the absorbance at 492 nm using a microplate spectrophotometer.
Results: *1. RA up-regulated TGase 2 expression and enzyme activity in human chondrocytes.* Human chondrocytes treated with RA resulted in up-regulation of TGase 2 protein as shown by the western blot and immunocytochemistry. RA-induced TGase 2 expression increased time dependently. The enzyme activity of TGase 2 was also increased in RA treated cells compared with normal human chondrocyte cells.
2. TGase 2 induced by RA results in decreased apoptosis in human chondrocytes. Apoptosis in RA-treated chondrocytes was decreased, measured by Annexin-V FACS analysis, when compared with H_2O_2-treated cells chondrocytes. Similar patterns were observed in 3 independent experiments using chondrocytes obtained from different patients.
Conclusions: We have previously reported that endogenous TGase 2 expression was increased in human chondrocytes undergoing apoptosis. Inhibition of TGase 2 by MDC and TGase 2 siRNA was also shown to increase apoptosis and suggest a possible protective role of TGase 2 in chondrocyte apoptosis. The protective role of TGase 2 was further validated in this study as the RA-induced overexpression of TGase 2 decreased apoptosis of human chondrocytes. These results implicate a protective role of TGase 2 against apoptosis in human chondrocytes and the possibility of TGase 2 as a potential modulator of osteoarthritis.

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VISFATIN/NAMPT: A POTENTIAL TARGET FOR NGF-TRIGGERED PAIN IN OSTEOARTHRITIS

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Purpose: Obesity is the main risk factor for knee OA. The two main features of the pathophysiology of obesity-induced OA are based on a local component (mechanical stress) and a systemic component (pro-inflammatory adipokines). Nerve growth factor (NGF) is present within OA synovial fluid and may be involved in pain associated with OA. We previously showed that visfatin/NAMPT, an adipokine, has pro-degradative effects. However, its role in OA pain has not been evaluated yet.
Methods: Primary cultures of newborn mouse articular chondrocytes or cartilage explants were stimulated by increasing amounts of visfatin/NAMPT, IL-1 beta, prostaglandin E_2 (PGE_2) or by cyclic mechanical compression (0.5 Hz, 1 MPa). mRNA NGF levels were assessed by real-time quantitative PCR and NGF released into media was determined by ELISA.
Results: Unstimulated articular chondrocytes expressed low levels of NGF. Mechanical stress induced NGF mRNA expression and release in conditioned media. Visfatin/NAMPT, a pro-inflammatory adipokine produced by chondocytes in response to IL-1 beta, stimulated NGF expression (2 fold) and release (3.7 fold). When stimulated by IL-1 beta, a dose-dependent increase in NGF mRNA expression (5.7 fold increase with 10 ng/ml IL-1) and NGF release (x ng/ml, 19 fold increase with 10 ng/ml) in chondrocyte conditioned media was observed. Neither siRNA visfatin/NAMPT nor APO 866, an inhibitor of NAMPT enzymatic activity, prevented the production of NGF induced by IL-1 beta. Interestingly, PGE_2, which is produced by chondrocytes in response to IL-1 beta and visfatin/NAMPT, did not stimulate NGF production. Consistently, indomethacin, a cyclooxygenase inhibitor, did not counteract IL-1-induced NGF production.
Conclusions: These results suggest that obesity-induced OA pain may involve NGF mediated by the overexpression of visfatin/NAMPT and mechanical stress. These effects seem to be independent of the well-known pro-inflammatory mediators involved in OA pain, IL-1b and PGE2. Thus, along with reduction of weight, visfatin/NAMPT could be an interesting target for pain in OA

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HUMAN OSTEOARTHRITIS CHONDROCYTES EXPRESS AND RESPOND TO THE PARATHYROID HORMONE RECEPTOR 1

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Purpose: Parathyroid hormone (PTH) is an endogenous peptide hormone that elevates calcium levels by releasing calcium salts stored in bone and preventing their renal excretion. Although its role in bone metabolism has been thoroughly studied, little is known about its effects on cartilage. In this study, we sought to test the hypothesis that human chondrocytes do express the PTH 1 receptor (PTH1R) and are responsive to PTH *ex vivo*.
Methods: RNA was purified from freshly isolated, primary chondrocyte samples from OA knee cartilage. A dedicated reverse transcription polymerase chain reaction (RT-PCR) to amplify the coding region of the parathyroid hormone 1 receptor (PTH1R) mRNA was run. Similarly, western blotting was done with cell lysate; specific antibodies for the PTH1R protein were used to detect the presence of the receptor on human chondrocytes. In addition immune-cytochemistry as well as immune-histochemistry was done to identify the receptor in situ. Human chondrocytes were isolated and maintained serum-free for 1h in the presence of 100μM IBMX (PDE inhibitor), and subsequently stimulated with PTH 1nM-100nM + IBMX and the cAMP levels were quantified by ELISA. In addition, articular cartilage explants were cultured in 6 replicates for 17 days, with or without 10nM PTH treatment, and 5 μCi ^{35}sulphate was added for the last 24 hours. Soluble proteoglycans were released by 4M GuHCl and incorporated sulfate was measured. Neo-epitopes of pro-peptides of collagen type II (PIINP) were quantified as a measure of formation in the conditioned medium.
Results: We identified an approx. 2 kb band after RT-PCR, which was sequenced and aligned with the coding region of PTH1R mRNA. Furthermore, we detected a protein reacting against PTH1R antibody consistent with the transcript sequence in chondrocyte samples from osteoarthritis patients. PTH1R immunoreactivity was primarily localized in the cell membrane of chondrocytes, observed by immunochemistry. When stimulated with PTH, the cultured chondrocytes accumulated intracellular cAMP levels significantly ($P < 0.003$) in a dose-depend manner. The maximum concentration of PTH (100 nM) resulted in a 23-fold increase compared with baseline. In the explant cultures of OA articular cartilage, a two-fold increase of PIINP was observed in the supernatant after PTH stimulation when compared to non-stimulated cartilage samples. Furthermore, 10 nM PTH increased incorporation of ^{35}sulphate by 40% ($p=0.002$).
Conclusions: The current data strongly suggest that PTH, in addition to osteoblasts and bone turnover, also has direct anabolic effects on chondrocytes and cartilage. Human articular cartilage chondrocytes express both the mRNA and protein molecules of the parathyroid hormone 1 receptor. We have shown that PTH can not only avert but also facilitate cartilage generation in both in vitro and in vivo situations. Presented data indicate the potency of PTH and intrigues further investigation of PTH as a potential DMOAD.

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REVERSIBILITY OF IMMOBILIZATION-INDUCED ARTICULAR CARTILAGE DEGENERATION AFTER REMOBILIZATION IN RAT KNEE JOINTS

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Purpose: Joint immobilization (Im) causes articular cartilage degeneration. Im-induced cartilage degeneration is generally recognized as disuse atrophy caused by decreased chondrocytes activity. In our rat immobilized knee model with a plate and screws, the changes in the non-contact (NC) area was similar to disuse atrophy, but the changes in the transitional (T) area and contact (C) area were quite different. Reversibility of Im-induced cartilage degeneration is still controversial. The differences may be originated from methods of Im and measurement sites. The purpose of this study was to clarify the reversibility of Im-induced cartilage degeneration after remobilization.
Methods: Unilateral knee joints of adult male rats were rigidly immobilized at 150° of flexion with a plate and screws for 1, 2, and 4 weeks. After the experimental periods, the fixation devices were removed and the rats were allowed to move freely in standard cages for 16 weeks. Only screws were