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.

190

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.

191

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.