59

CARTILAGE-SPECIFIC DELETION OF PPAR-GAMMA IN MICE RESULTS IN EARLY ENDOCHONDRAL OSSIFICATION DEFECTS AND ACCELERATED AGING-DEPENDENT DEVELOPMENT OF OSTEOARTHRITIS

R. Monemdjou[1], F. Vasheghani[1], G. Perez[1], M. Blati[1], N. Taniguchi[2], M. Lotz[2], R. St-Arnaud[3], H. Fahmi[1], J-P. Pelletier[1], J. Martel-Pelletier[1], F. Beier[1], M. Kapoor[1]. *[1]Univ. of Montreal, Montreal, QC, Canada; [2]The Scripps Res. Inst., La Jolla, CA, USA; [3]Shriners Hosp. for Children, Montreal, QC, Canada*

Purpose: Purpose: Long bones develop through a strict coordinated process of endochondral ossification within the growth plate resulting in the replacement of cartilage by bone and defect in this coordinated process may result in skeletal abnormalities such as dwarfism, kyposis and also age-related defects such as osteoarthritis (OA) and osteoporosis. PPARγ, a transcription factor, plays a key role in lipid homeostasis but its in vivo role in cartilage/bone development is unknown. Therefore, we determined the specific *in vivo* role of PPARγ in endochondral bone ossification, cartilage/bone development and in OA using cartilage-specific PPARγ knockout (KO) mice.
Methods: Cartilage-specific PPARγ KO mice were generated using LoxP/Cre system. Skeletal and histological staining of long bones was performed to determine skeletal changes, growth plate organization, bone density, calcium deposition and joint phenotypic changes during aging. Immunohistochemistry for Collagen X, BrdU, p57, Sox9, PECAM and MMP-13 was performed. RT-PCR on isolated chondrocytes was performed to determine the expression of ECM markers.
Results: PPARγ KO mice at birth showed reduced length, weight, skeletal growth and length of long bones compared to wild-type (WT) mice. Histomorphometric analysis of embryonic and adult mutant mice demonstrate reduced long bone growth, calcium deposition, bone density, and vascularity as well as delayed primary and secondary ossification. Mutant growth plates were disorganized with abnormal chondrocyte shape, proliferation, and differentiation, reduced cellularity, loss of columnar organization, and shorter hypertrophic zones. Isolated chondrocytes and cartilage explants from mutant mice further show decreased expression of VEGF-A, extracellular matrix (ECM) production products, aggrecan and collagen II, and increased expression of the catabolic enzyme, MMP-13. Furthermore, aged PPARγ KO mice exhibit an accelerated OA-like phenotype associated with enhanced cartilage degradation, synovial inflammation, and increased expression of MMP-13, accompanied by increased staining for MMP-generated aggrecan and collagen II neoepitopes.
Conclusions: For the first time, we demonstrate that loss of PPARγ in the cartilage results in endochondral ossification defects and subsequently accelerated OA in mice. PPARγ is essential for normal development of the cartilage.

60

SIRT1-DEFICIENT MICE EXHIBIT AN ALTERED CARTILAGE PHENOTYPE AND UNDERGO INCREASED CARTILAGE BREAKDOWN AND APOPTOSIS

O.H. Gabay[1], C. Sanchez[2], M. Dvir-Ginzberg[3], V. Gagarina[1], E. Lee[1], K.J. Zaal[1], M.W. McBurney[4], D.J. Hall[1]. *[1]NIH, Bethesda, MD, USA; [2]Bone and Cartilage Res. Unit, Inst. of Pathology B23, Univ. of Liege, Liege, Belgium; [3]Lab. of Cartilage Biology, Inst. of Dental Sci., Faculty of Dental Med., Hebrew Univ. – Hadassah Ein Kerem, Jerusalem, Israel; [4]Cancer Therapeutics, Ottawa Hosp. Res. Inst., 501 Smyth Road, Box 926, Ottawa, ON, Canada*

Purpose: Cartilage degenerative diseases such as osteoarthritis, are age-related. The histone deacetylase Sirt1 is thought to be an anti-aging protein. We previously demonstrated that Sirt 1 regulates apoptosis and cartilage-specific gene expression in human chondrocytes. Sirt 1 is also a potent inhibitor of the matrix metalloproteinases. To determine if Sirt 1 plays a protective role on cartilage homeostasis in vivo, we investigated Sirt1 KO mice to characterize their cartilage and try to understand the mechanisms underlying a phenotype, such as cartilage breakdown or apoptosis.
Methods: Articular cartilage was harvested from hind paws and knees of 1-week to 6-month-old mice carrying wildtype, null, or point mutations affecting the Sirt 1 gene. The cartilage was processed for histology and immunohistochemistry, or used to establish cultures of chondrocytes.
Results: We found that articular cartilage tissue sections from SirT1 KO mice at any age exhibited low levels of type 2 collagen, aggrecan and glycosaminoglycans. In contrast, protein levels of MMP-8, MMP-9 and MMP-13 were elevated in the SirT1 KO mice, leading to an increase of cartilage breakdown. The apoptotic process was shown to be elevated in these mice. Moreover, PTP1b, protein tyrosine phosphatase b, a chondrocyte proapoptotic protein elevated in OA, was elevated in the SirT 1 KO mice.
Conclusions: The findings from this animal model demonstrate that SirT1 KO mice present an altered cartilage phenotype. The apoptotic process and the cartilage breakdown were elevated in these mice.

61

ELIMINATION OF MITOCHONDRIA RELATED BIM/BID PROTEINS REDUCES CHONDROCYTE APOPTOSIS AFTER MECHANICAL INJURY

E. Eisner[1], C.G. Davis[2], J.J. Shelton[1], R.S. Hotchkiss[2], C.C. Chen[1], J. Borrelli[1]. *[1]UT SouthWestern Med. Ctr., Dallas, TX, USA; [2]Washington Univ., St. Louis, MO, USA*

Introduction: Chondrocyte apoptosis following blunt mechanical injury is a well-known risk factor for the onset of posttraumatic osteoarthritis. Recent studies suggest that chondrocyte apoptosis can be prevented after traumatic injury, but the molecular mechanism by which this inhibition is possible is unclear. Apoptotic death of mammalian cells is known to proceed by at least two complex pathways: Fas ligand (utilizing caspases 3, 6, 7, 8) pathway and mitochondrial or Bcl-2 mediated pathways (Bcl-2, Bcl-xL, Bax/Bak, Bim, and Bid). Bim is the primary mediator for Bcl-2 related cytokine withdrawal and deregulation of calcium flux where Bid is the key regulator of mitochondrial pathway for Fas related apoptosis through caspase-8 activation. In order to study the role of Bim and Bid proteins in the chondrocytes apoptosis following tissue injury, we developed a novel model of cartilage injury for Bim/Bid double knockout mice. We hypothesized that the deletion of Bim/Bid in mice would prevent chondrocyte apoptosis following mechanical injury.
Methods: Fourteen Bim/Bid double knockout (C57BL/6 Bim−/−Bid−/−) and fourteen matching wild-type (C57BL/6, Jackson Lab) were included in this study. The animals were maintained in the animal facility for one week before surgery using established protocols approved by the IACUC. Each mouse was 8-weeks-old at the time of surgery. The xiphoid was exposed through a 1 cm incision centered over the distal end of the mouse sternum. A modified Kelly clamp was used to apply a crushing force of approximately 22 MPa to the exposed xiphoid (Fig. 1). The clamp was applied three times (two minutes each time) with a one-minute pause between clamping. The animals were sacrificed at 48 hours and the xiphoid harvested. Histology, TUNEL and Caspase 3: Cell matrix damage was determined using H&E and safranin-O/fast green, while apoptosis was determined using TUNEL, and activated caspase-3 staining. Xiphoid cartilage was fixed and in paraffin and sectioned into 5 μm sections. The total numbers of nuclei were determined by counter-staining with propridium iodine (PI). Images of the fluorescent specimens were captured with a CCD camera. Percentage of TUNEL+ (versus PI+) cells was analyzed using Image J. Adjacent cartilage specimens were stained with H&E, Safranin-O/fast-green and activated caspase 3 to confirm the TUNEL findings. Statistical Analysis: Non-paired Student's t-test was performed to determine the statistical significance ($p < 0.05$).
Results: There was a significant increase of TUNEL+ (apoptotic) chondrocytes in wild-type xiphoid as compared to the Bim−/−/Bid−/− specimens (Figs. 2A,B). Additionally, wild-type specimens had considerably more caspase-3 positive cells than the Bim−/−/Bid−/− knockout specimens (Figs. 2C,D). TUNEL was used to quantify the amount of apoptosis occurring in the injured specimens. At 48 hours, a statistically significant 54% decrease ($p = 0.027$) was seen in the Bim/Bid double knockout mice as compared to the wild-type C57BL/6 mice (Fig. 3). No notable differences were found between sham-operated wild-type and sham-operated Bim−/−Bid−/− groups.

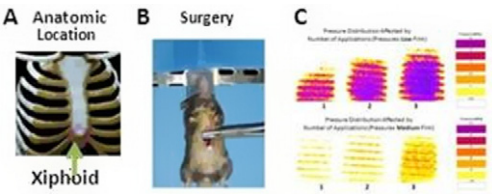

Fig. 1.