Articular Cartilage and Intervertebral Disc Degeneration in Mice Lacking Early Growth Response Protein-1 (EGR-1)

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Introduction: Developmental and degenerative changes in articular cartilage and intervertebral discs (IVDs) are not well understood. Early growth response protein-1 (Egr-1) is a transcription factor induced by stress or injury, mitogens, and differentiation factors. It has been shown to be regulated by various cytokines, growth factors and of more interest by ischemic/hypoxic stress as well as shear stress and mechanical injury. These latter regulators have been linked to the pathogenesis of osteoarthritis. Furthermore, Egr-1 has been shown to regulate the expression of collagens and enzymes affecting the extracellular matrix [1-4]. The aim of this study was to determine whether Egr-1 deficiency affects articular cartilage and IVDs.

Materials and Methods: Experimental animals - Experimental studies used 6- to 7-month-old adult female wild-type (+/+) C57Bl/6 or Egr-1-deficient (-/-) mice. Egr-1 -/- mice have a C57Bl/6 genetic background. All animals were sacrificed at the same age interval (8- to 9-months). Mice were immediately frozen and stored at -20°C.

Histological preparation and staining - Prior to dissection, posterior-anterior and lateral X-rays of whole mice were done. The mice were then dissected carefully, removing the right knee joint and cervical to lumbar spine. These specimens were immediately placed in neutral-buffered 10% formalin. These fixed samples were then decalcified, dehydrated, embedded in paraffin and cut into 4 μm sections. One section from each specimen was stained with the following: hematoxylin-eosin (H&E), Safranin-O/Fast green, and Weigert’s hematoxylin/alcan blue/picrosirius red. The slides were examined under light microscopy and digital pictures were taken for appropriate comparison.

Bone mineral density (BMD) - Mice were sacrificed and imaged using a PIXImus Bone Densitometer System #56069 (GE Lunar corporation), which measures bone densitometry using dual-energy x-ray absorptiometry. The femur and lumbar vertebrae were selected and analyzed using a Windows 98 Based Software.

Micro computed tomography (CT) - Micro CT data were acquired on a SkyScan T1072 X-ray Microscope-Microtomograph (SkyScan, Aartselaar, Belgium). CTan Software (from SkyScan) was used to analyze the sample.

Results: We observed that the knee joints of Egr-1 knockout mice were significantly different than that of the wild type. Particularly, the smooth contour of cartilage found on the wild type was more irregular and degenerative in the Egr-1 -/- mice (Fig 1). Furthermore, a lower concentration of proteoglycans (predominantly aggrecan) was observed in articular cartilage of Egr-1 -/- mice as evidenced by reduced staining of Safranin-O.

The IVD of Egr-1 -/- mice showed evident changes from the wild type. Particularly, the smooth contour of articular cartilage found on the wild type was more irregular and degenerative in the Egr-1 -/- mice (Fig 1). Furthermore, a lower concentration of proteoglycans (predominantly aggrecan) was observed in articular cartilage of Egr-1 -/- mice as evidenced by reduced staining of Safranin-O.

Furthermore, there was a trend for increased lumbar vertebrae BMD as well a significant increase in BMD in the femur (15% P= 0.09) in the Egr-1 -/- mice. The relative bone volume (BV/TV) was significantly smaller in Egr-1 -/- mice as was trabecular number (Tb.N) and trabecular separation (Tb.Sp) while there was increased bone surface to bone volume (BS/BV).

Discussion: Our findings reveal that mice lacking Egr-1 have approximately 15% greater trabecular bone mineral density and significantly greater cortical bone mineral content than normal mice. Safranin-0 staining of articular cartilage reveals loss of proteoglycan staining of the knockout mouse. Loss of height in IVD and cellular components of the NP as well as increased cell numbers in cartilage endplates between L4-L5 show early manifestations that bear some resemblance to those of human degenerative disc diseases However, further studies are needed to determine the mechanism by which Egr-1 leads to articular cartilage and IVD degeneration.

References:

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