Histological And Molecular Analysis Of Cell Signaling Pathways In The Growth And Differentiation Of The Mouse Lumbar Intervertebral Disc

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Introduction: Intervertebral disc (IVD) degeneration is the most common cause of back pain. The IVD has been described to have mainly two types of components; the outer concentric rings of fibrous cartilage which form the annulus fibrosus (AF) and inner gelatinous population of cells known as the nucleus pulposus (NP). There isn't much information regarding the growth and development of the IVD and the molecular changes that leads to disc degeneration. We wish to understand the cellular and molecular mechanisms of disc growth and development in mouse.

Materials and Methods: 8 μm thick coronal and transverse cryosections, from lumbar vertebrae (LV) of 1-48 weeks old male mice were collected. Histology was analyzed by H&E and alkaline phosphatase staining. Physical growth of the discs was measured using a 1mm graticule. The number and thickness of the layers of the annulus fibrosus (AF), was measured using DIC optics. Cell proliferation and death was determined by phospho Histone H3 (PH3) and TUNEL staining, respectively. Immunolocalization of components of the TGFβ, BMP, FGF and Shh pathways was carried out using confocal microscopy. β-gal staining of the lumbar disc from Topgal mice was used to determine the Wnt signaling.

Results: Growth of the IVD was parallel to that of the vertebral column, being most rapid between birth and 3 weeks, and plateauing off by 9 weeks of age. Proliferating cells were found until 3 weeks of age in both the AF and NP (Fig-A) suggesting that the addition of new cells leading to the disc growth occurs only during this short period. DIC imaging also revealed that the number of layers of AF increased from 1-2 weeks of age, after which each layer became very thick due to the extracellular matrix secreted by them. The number of AF layers decreased in the IVDs of older mice. During the first week, the AF became divided into a mineralized AF component over the vertebral bodies, which stained positively for alkaline phosphatase, and non-mineralized or fibrous AF component between the vertebrae (Fig-B). Cells in the mineralized component of AF also became hypertrophied with age. Apoptosis was only found in the NP cells. TGFβ as well as BMP signaling pathways were active in the NP as well as the AF cells for a very long time into adulthood. Shh was secreted by the NP and fibrous AF cells but it acted on the mineralized AF cells, as the receptors for Shh, which are patched (ptc), were present only on the mineralized AF cells. Furthermore, it was observed that the FGF signaling was present only during the early postnatal life when the growth was maximum, 4 weeks onwards FGF signaling went off and the BMP and Shh signaling increased much more. PTHrP is known to be regulated by the hedgehog (Indian hedgehog) signaling in the growth plates of the long bones, so we also analyzed the expression of PTHrP ligand in the IVD with age. The expression of PTHrP was observed only in the mineralized AF cells and the expression increased with age when these cells become very hypertrophied. This pattern correlated to that of the patched expression on the mineralized AF. β-gal staining of the Topgal mouse revealed that the Wnt signaling was present only during the early period of growth in the fibrous AF cells.

Discussion: The IVDs grow coordinately with the vertebrae. Both components of the disc grow, and then involutes with age. The AF becomes divided into a mineralized and non-mineralized component during growth. We have assayed for activation of intercellular signaling pathways in different components of the IVD with age. There is differential activity of the BMP, FGF, Wnt, Shh, and TGFβ pathways in the disc, consistent with roles in the differentiation of different components of the disc. Analysis of active cell signaling pathways suggests that BMP signaling in the NP and fibrous AF cells stimulates Shh signaling while FGF signaling inhibits this pathway. Shh signaling increased only after the FGF signaling was switched off. Shh then acts via patched, the receptors for Shh on the hypertrophic AF which leads to increased expression of PTHrP pathway in the mineralized AF. It is interesting to note that while PTHrP is involved in the proliferation of growth plate chondrocytes, it seems to have a role in hypertrophy and matrix secretion in the mineralized AF of IVD. These signaling pathways seem to be actively involved in disc maintenance.

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