The presence of extracellular matrix degrading metalloproteinases in the fetal intervertebral disc

J. Rutges1, P. Nikkels2, C. Oner1, K. Ortink1, A. Verbout1, R. Castelein1, W. Dhert1, L. Creemers1
1Orthopaedics, University Medical Center Utrecht, Utrecht, Netherlands; 2Pathology, University Medical Center Utrecht, Utrecht, Netherlands

j.rutges@umcutrecht.nl

Introduction: Matrix metalloproteinases (MMPs) are strongly associated with degenerative diseases such as osteoarthritis and intervertebral disc (IVD) degeneration. However, MMPs also play a role in non-pathological circumstances, for instance in angiogenesis, bone development and remodeling. Moreover, high levels of MMP-1 activity have been found in the synovial fluid of fetal equine knees. MMP-3 is found in chondrocytes of fetal joints, MMP-2 and MMP-14 knock-out mice develop dwarfism, osteopenia and arthritis. As MMPs are involved in cell migration and can regulate connective tissue architecture, we postulate these could be associated with fetal development of the intervertebral disc.

Materials and Methods: Sixteen fetal human lumbar spine segments were obtained at autopsy within 24 hrs of death of the patient (average age 24.7 wks after gestation, range 15.5-40.3 wks). A control group was composed of five non-degenerated (Thompson grade I) L4-L5 IVDs (average age 12.7 yrs, range 3.3 – 21.8 yrs). IVD samples were processed to mid sagittal paraffin sections and tissue extracts containing both annulus fibrosis (AF) and nucleus pulposus (NP) tissue. Notochord (NC) cells were identified by immunohistochemistry for epithelial membrane antigen (EMA) and pan keratin (AE1/AE3). IHC for MMP-1, MMP-2, MMP-3 and MMP-14 was graded by two independent observers.

The amount of pro- and active MMP-2 was determined by gelatin zymography. Five μg protein from tissue extracts was electrophoresed through a 8% acrylamide/0.1% gelatin gel. After overnight incubation at 37 C, gels were stained with Coomassie Blue and analysed by densitometry.

Results: Histology showed clear differences between fetal and undegenerated IVDs. Fetal IVDs show more vascularisation (Fig 1A, 1B, arrows) and more intense staining of proteoglycans, blue (Fig 1B). Abundant staining for MMP-1 was seen in the nuclear and cartilage endplate (CE) was less intense. In the non-degenerated IVDs MMP-1 was moderately positive in the NC cells, NP and CE, no staining was seen in the CE. In the fetal IVDs the intensity of the MMP-14 staining in the NC and NP cells was strong, the staining of the AF and CE was moderate. In the nondegenerative IVDs NC cells were positive for MMP-14, the AF, NP and CE were negative. MMP-3 was clearly seen in the NC, AF and NP of fetal IVDs and a moderate staining was seen in the NP cells. In the nondegenerative IVDs clear MMP-3 staining was seen in the NC cells and moderate staining in NP cells, CE and AF. A moderately positive staining was seen for MMP-2 in the NC and NP cells of the fetal IVDs and no staining was found in the AF. MMP-2 staining in the non-degenerative IVDs was variable throughout the nondegenerated IVD, with a moderate to weak staining in the entire IVD. Gelatin zymography for pro- and active MMP-2 showed a significant negative correlation between age and active MMP-2, p < 0.0000, correlation coefficient (CC) 0.80, no correlation was found between levels of pro-MMP-2 and age (Figure 3). With respect to fetal development only (first 3 age groups), a negative correlation was found for activity and age, p = 0.013 CC = 0.61.

Discussion: This study clearly shows the presence of MMP-1, 2, 3 and 14 in the fetal human IVD. The intensity of immunohistochemical staining of these MMPs seems stronger in fetal IVDs compared to nondegenerated IVDs. The most abundant expression was seen in the NC and NP cells. Zymographic analysis of MMP-2 showed that during fetal development MMP-2 activation rather than production decreases, while in nondegenerative IVDs its production is decreased. Throughout IVD development MMPs might be involved in cell migration, regulation of extracellular matrix turnover and activation of other MMPs. The MMPs found in the fetal IVD could very well be involved in these processes, in particular MMP-1 and MMP-14, which are both also capable of activating several other MMPs, including MMP-2.