Studies on intervertebral disc degeneration in the chondrodystrophoid dog breed

Abstract of Doctoral Thesis

Munetaka Iwata

Division of Veterinary Surgery, Department of Veterinary Science,

Faculty of Veterinary Medicine,

Nippon Veterinary and Life Science University

Intervertebral disc (IVD) degeneration greatly affects the quality of life. The nucleus pulposus (NP) of chondrodystrophic dog breeds (CDBs) is similar to the human NP, because the cells disappear with age and are replaced by fibrochondrocyte-like cells. Because IVD develops as early as within the first year of life, we used canines as a model to investigate the in vitro mechanisms underlying IVD degeneration. The mechanism underlying age-related IVD degeneration, however, is poorly understood. Specifically, we evaluated the potential of a three-dimensional (3D) culture of healthy NP as an in vitro model system to investigate the mechanisms of IVD degeneration. Agarose hydrogels were populated with healthy NP cells from beagles after performing magnetic resonance imaging (MRI), and mRNA expression profiles and pericellular extracellular matrix (ECM) protein distribution were determined. After 25 days of 3D culture, there was a tendency for redifferentiation into the native NP phenotype.

Several research groups have suggested that Wnt/β-catenin signal plays an important role in IVD degeneration. However, the role of Wnt/β-catenin signals in IVD cells is not yet well understood. Here, we demonstrate that Wnt/β-catenin signal would enhance Runx2 expression in intervertebral degeneration and lead to IVD degeneration and calcification. NP tissue was obtained from 12-month-old male Beagle dogs after evaluation of the degeneration based on the MR signal intensity. Histological analysis

showed that lack of Safranin-O staining and increased calcified area and MMP13-positive cells with progression of the degeneration. Furthermore, β-catenin- and Runx2-positive cells also increased with the progression of the degeneration. Real-time reverse-transcription polymerase chain reaction (RT-PCR) analysis showed that the MRI signal intensity and mRNA expression levels of β-catenin and Runx2 are correlated in NP tissues. Moreover, to evaluate the role of the Wnt/β-catenin pathway in the regulation of NP cells degeneration, we studied the effects of LiCl on cultured canine NP cells. Using western blotting analysis, we found that the levels of β-catenin were consistently upregulated by LiCl. A supplementation of 20 mM LiCl induced β-catenin accumulation and Runx2 expression. In contrast, FH535, an inhibitor of β-catenin/TCF activity, inhibits the upregulation. These results indicate that Wnt/β-catenin signals have a significant role in degeneration and calcification in IVD through Runx2 expression.