

Expression of NG2 proteoglycan in the degenerated intervertebral disc in dachshunds

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ABSTRACT. The pathogenesis of intervertebral disc (IVD) degeneration is not fully understood. The biomolecular signaling pathways involved in the IVD degeneration require further investigation. The aim of this study was to investigate the expression of NG2 proteoglycan in the degenerated IVD. IVD samples were obtained from 16 Dachshunds that were confirmed to have IVD herniation and subsequently underwent hemilaminectomy. The samples were subjected to histological and immunohistochemical (IHC) examinations. IHC revealed positive results for the expression of NG2 proteoglycan in all examined samples. The results showed the expression of NG2 proteoglycan by the degenerated IVDs.

KEY WORDS: dachshund, immunohistochemistry, intervertebral disc (IVD) degeneration, NG2 proteoglycan

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The detailed pathogenesis of intervertebral disc (IVD) degeneration and its underlying mechanism in dogs is not fully known [5]. Although the process of IVD degeneration has been described histopathologically [3, 10], the biomolecular signaling pathways involved in the IVD degeneration require further investigation [7, 21, 22].

The mechanism of degeneration may be associated with a variety of changes that involve the cells and extracellular matrix (ECM) of all parts of the IVD [5, 9]. The normal IVD is composed of cells embedded in the ECM [5, 6, 10]. The ECM is mainly formed from collagen and proteoglycan, which assemble into multiple matrix compartments [6, 10]. Chondroitin sulfate proteoglycans (CSPGs), such as neurocan, aggrecan, versican, brevican, phosphacan and NG2 (nerve/glial antigen 2), are expressed throughout the developing and adult central nervous system (CNS) in rat and human [13, 20, 28].

NG2 (or CSPG4), is a transmembrane CSPG that participates in both extracellular and intracellular events in various tissues [1, 8, 16, 19, 26]. Moreover, NG2 binds to type VI collagen, which is the main constituent of the pericellular matrix in the IVD annulus fibrosis (AF) and nucleus pulpo-

sus (NP) and articular cartilage [1, 18]. The changes in the pericellular microenvironment contribute to the pathoetiology of connective tissue diseases, such as osteoarthritis and IVD degeneration [1]. The expression of NG2 proteoglycan has been observed in cells of the human IVD with a high level of advanced degeneration [1]. There is no report on the expression of NG2 in IVD cells in dogs.

The purpose of this study was to investigate the expression of NG2 proteoglycan in the degenerated IVD of dachshund dogs using immunohistochemistry.

Herniated disc samples were obtained from 16 client-owned dachshunds that underwent surgical treatment for IVD herniation. The animals were subjected to routine physical, clinical and neurological examinations, and blood samples were collected for CBC (complete blood count) and serum biochemical analysis. Radiography, myelography and computed tomography (CT) or magnetic resonance imaging (MRI) were performed. All the animals were subjected to decompression surgery, and the extruded disc samples were removed and preserved for histological and immunohistochemical examinations.

All histological samples were immediately fixed in 10% neutral buffered formalin and subsequently embedded in paraffin wax. Serial sections (4 μ m) were cut and fixed onto silanized glass slides for routine hematoxylin and eosin (H&E) staining and were examined using light microscopy. The sections were assessed by two independent observers. Histopathological grading was performed according to the modified Bergknot histological grading system [15].

Immunohistochemistry (IHC) was conducted on formalin-fixed, paraffin-embedded sections. Antigen retrieval

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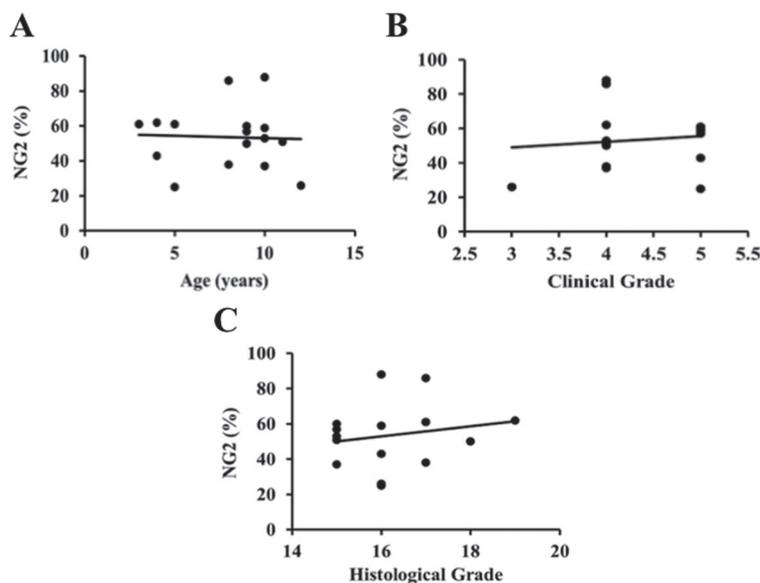


Fig. 1. Correlation between the NG2 proteoglycan expression level and age of dogs (A), clinical grade (B) and histological grade (C). NG2 expression was not correlated with age of dogs, clinical grades and histological grades ($r=-0.04$, $P=0.887$; $r=0.111$, $P=0.68$ and $r=0.178$, $P=0.51$, respectively, confidence interval (CI)=95%). $P>0.05$ was considered to be nonsignificant.

and tissue section processing were carried out as standard procedures. To determine the NG2 proteoglycan expression, a rabbit polyclonal anti-human CSPG4 antibody (diluted 1:100, Sigma-Aldrich, St. Louis, MO, U.S.A.) was used. In negative control sections (*canine* lung tissue), the primary antibody was omitted or replaced with its respective serum. Samples of *canine* melanoma expressing the antigen were used as a positive control [17]. All sections were stained in the same session.

The adoptive scoring system was that described by Itoh *et al.* [11]; cell counts were performed by 2 independent observers. More than 100 cells in each sample were examined to evaluate the proportion of cells that showed a positive reaction for NG2 proteoglycan.

Statistical analysis was performed using Pearson linear regression to determine the association between age, clinical grades and degenerative grades with the level of NG2 proteoglycan expression. The linear correlation coefficient (r) and 95% confidence interval (CI) were recorded. Values of $P<0.05$ were considered significant.

A total of 16 male client-owned dachshunds were included in this study. Their ages ranged from 3 to 12 years (mean 7.9 years). A preoperative neurological examination was performed for all dogs, and it showed that the overall median clinical grade was IV. The clinical neurological grades were III (1/16, 6.25%), IV (8/16, 50%) and V (7/16, 43.75%). There was no correlation between the age of animals ($r=-0.04$, CI=95% and $P=0.887$) (Fig. 1A), clinical grades ($r=0.111$, CI=95% and $P=0.68$) (Fig. 1B) and histological grades ($r=0.178$, CI=95% and $P=0.51$) (Fig. 1C) and the level of NG2 expression by the degenerated IVD samples.

The surgically obtained IVD samples consisted of the AF

and NP. These was marked chondrocyte proliferation in the AF (up to the outer layers of the AF) in all cases, while the proliferation of chondrocytes in the NP comprised small clones (2–7 cells) in 6/16 dogs (38%), medium sized clones (8–15 cells) in 4/16 dogs (25%), huge clones (>15 cells) in 5/16 dogs (31%) and formation of scar tissue in 1/16 dogs (6%). Notochordal cells were present (1–50%) in 5/16 dogs (31%) and completely absent in 11/16 dogs (69%). Representative histopathological examples are shown in Fig. 2.

The expression of NG2 using IHC was observed in all herniated disc tissues. The brown color in immunohistochemical staining was restricted to the cytoplasm and cell membrane of the chondrocyte, and the nucleus was stained blue with hematoxylin. Representative immunohistochemical examples are shown in Fig. 3.

The results of this study demonstrated, for the first time, that canine intervertebral disc cells express NG2 proteoglycan and that it can be detected by immunohistochemical examination.

The occurrence of IVD degeneration and the transformation of the NP from the gelatinous to dried form at the thoracolumbar discs during early life in chondrodystrophic dogs may have caused the lack of correlation between age of animals (3–12 years) and the level of NG2 expression in this study. Furthermore, the expression of NG2 proteoglycan might be restricted to dogs of a certain age, but it might be expressed by cells in fetal, normal and degenerative adult IVDs as reported in human osteoarthritic articular cartilage [10, 18, 24].

The clinical grades due to spinal cord dysfunction depend upon the location, size, severity and rate of development of the lesion that occurs as a result of the compressive force of

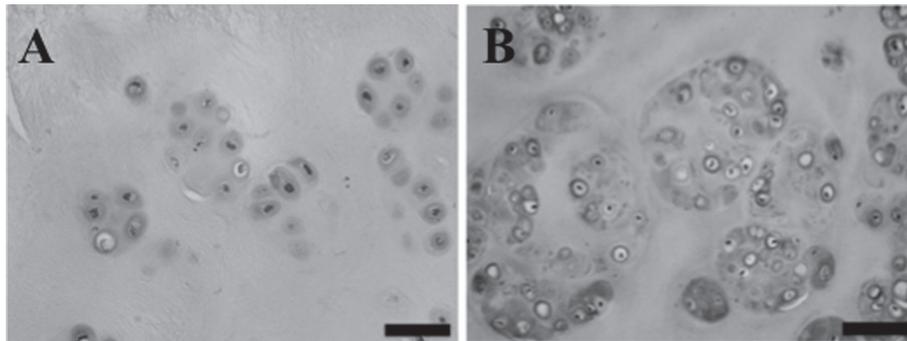


Fig. 2. Representative histological images showing chondrocyte-like cells proliferation in the nucleus pulposus (NP) of degenerative IVDs (H&E staining). (A) Moderate sized clones (8–15 cells) in the case of grade IV IVD degeneration. (B) Huge clones (more than 15 cells) in the case of grade V degeneration. Bar: 200 μm (A), 100 μm (B).

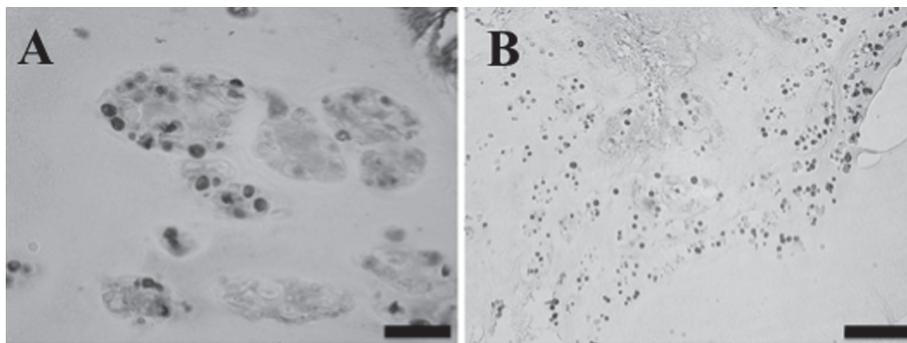


Fig. 3. Immunohistochemical staining of canine degenerative IVD samples with an anti CSPG4 antibody showing positively labeled NG2-expressing chondrocyte-like cells (brown-colored cells). (A) grade IV, (B) grade V degenerated IVD samples. Bar: 200 μm (A), 100 μm (B).

extruded disc material on the spinal cord parenchyma and/or the nerve roots [23]. So, as shown in the results of this study, the clinical grades are not correlated with the degree of degeneration of IVDs and level of NG2 expression. In addition, there is a difference between IVD degeneration and herniation. The former can give rise to a number of diseases, one of them IVD herniation [4], which may occur in normal (non-degenerated) discs due to severe trauma [10].

Although a previous study reported a positive relationship between the degenerative grade of IVDs and the level of NG2 expression [1], the results of the present study did not confirm this relationship. This may be due to the degree of degeneration of almost examined samples which was either grade IV or V, while the other grades were not represented in this study. Moreover, the small number of animals included in this study is considered one of its limitations.

The expression of NG2 was restricted only to the cytoplasm and cell membrane of the chondrocytes. This is in agreement with previous studies that described the structure of NG2 as a large extracellular domain (2224 amino acids), a single small transmembrane domain (25 amino acids) and a short cytoplasmic domain (76 amino acids) [25, 27].

Although the loss of notochordal cells from the NP is as-

sociated with the aging process and disc degeneration, the results of this study showed the presence of notochordal cells in samples from 5/16 dogs, which ranged in age from 3–12 years. The persistence of notochordal cells in chondrodystrophic dogs was reported in previous studies [6, 10, 12]. The normal NP in chondrodystrophic dogs has 2 types of notochordal cells. Large cells represent about 13% of total NP cells, and small cells account for the rest. The large cells are able to synthesize 1.5 times proteoglycan than the small cells [6]. The degenerative process of the IVD is characterized by a decrease in proteoglycan level and increase in collagen fibers as a result of the replacement of the notochordal cells with chondrocyte-like cells. So, the large notochordal cells may have a more important role in maintaining the integrity of IVD than smaller cells [2, 6], some of which may persist in the NP of the IVD of chondrodystrophic breeds.

Increase of the number of chondrocyte-like cells in the NP is in agreement with previous studies, which stated that the NP is replaced by chondrocyte-like cells embedded in a large amount of extracellular matrix at an early age in chondrodystrophic dogs in a process called chondrification or chondroid metaplasia of the NP [5, 6, 10, 14].

In conclusion, the results of this short study indicated that

NG2 proteoglycan is expressed by the degenerated IVD.

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