VARIABLE EXPRESSION OF NEURAL ADHESION MOLECULE (CD56) IN INTERVERTEBRAL DISCS OF CHONDRODYSTROPHOID CANINES: ASSOCIATION WITH CLUSTER FORMING NP CELLS

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INTRODUCTION:
Despite the significant impairment associated with degenerative disc disease, a clear understanding of its pathogenesis is still lacking. The nucleus pulposus (NP) of the intervertebral discs (IVDs) contain a mixed population of cell types at various stages of differentiation. The NP is formed either by or with the help of cells from the embryonic notochord, which appear to be replaced during development by a population of chondrocyte-like cells. Studies that explore this cellular heterogeneity are important in determining treatment options and cell types for disc repair. Recently, neural adhesion molecule (CD56) gene, a marker for neuron has been reported as one candidate for NP cell marker [1]. CD56 has also been reported as a marker for superior selectivity of mesenchymal stem cells [2]. In order to understand the relevance of CD56 in the IVDs, the present study explores the variability of CD56 expression in the IVDs of chondrodystrophic canines.

METHODS:
Lumbar and caudal IVDs were collected from six mature beagles (age about 1.5 year). Histological analysis was performed using Hematoxylin-Eosin(H-E), and Safranin-O stain. Immunohistochemistry was performed for expression of filamentous actin (F-actin) by fluorescently tagged phalloidin, for gap junction by connexin-43 and CD56. In order to assess the difference in matrix production ability between lumbar and caudal NP cells, NP cells were isolated by enzymatic digestion and expanded in monolayer culture in standard Dulbecco’s modified Eagle’s medium (DMEM)/F-12 supplemented with 10% foetal bovine serum, penicillin, and streptomycin. At passage 1, IVD cells were seeded into alginate beads at a cell density of 10^5 cells per milliliter. Protoplastic (PG) and DNA content were assessed using DMMB assay and DAPI after 14 days. Influence on cell cycle was assessed in CD56 (+) NP cells by flow-cytometric BrdU/7AAD assay. Statistical differences were analyzed by ANOVA using the Fisher’s PLSD as a post hoc test.

RESULTS:
Histological sections indicated clusters of large cells in caudal NP, containing from 3 to over 30 cells. Some of these caudal NP cells contained vacuole-like inclusions and these clusters stained intensely with Safranin-O. In contrast, lumbar NP contained relatively few cells, usually alone or in small clusters (~3-8 cells in two-dimensional section). These cells generally resembled chondrocytes, with smaller diameters (~15 μm) and no inclusions (Fig.1 a-d). F-actin was largely absent in lumbar IVD but was more prevalent in NP cell clusters of caudal IVD (Fig.1 e-f). Connexin-43 was scattered over the part of caudal NP cell clusters, with a concentration in the vicinity of cell-cell junction (Fig.1 g). CD56 (+) cell was scarce in chondrocyte-like cells but was more prevalent in caudal NP cell clusters (Fig. 2). In vitro cell culture study showed significant increase of PG production in caudal NP cells containing CD 56 (+) cells (lumbar: 0.668 ± 0.212, caudal: 1.30 ± 0.769) (Fig. 3). Percentage of CD 56(+) NP cells was significantly higher in caudal IVD compared to other phases in cell cycle analysis (Fig. 4).

DISCUSSION:
Chondrodystrophic canines are among the few species that possess chondrocyte-like cells in the lumbar IVDs. Hunter et al. has reported that NP cell clusters interconnected with F-actin and expressing connexin-43 may be a feature of notochordal cell population [3]. We identified these NP cell clusters in caudal IVDs of chondrodystrophic canines but significantly less in lumbar IVDs. CD56 a marker identified as highly expressed in caudal IVDs in chondrodystrophoid canines in microarray and immunohistochemical analysis of canine nucleus pulposus and anulus fibrosus. Spine 2009;34:1448-56.

REFERENCES: