INTRODUCTION: “Discogenic pain” is often associated with intervertebral disc (IVD) degeneration, but its precise pathogenesis is still unknown. Intervertebral disc degeneration is biochemically characterized by disorganization of the matrix structure, especially loss of proteoglycans, leading to dehydration of IVD tissues. Degenerated discs become progressively innervated and vascularized and it is those pathological changes that are suggested to contribute to “discogenic pain” [1]. Extracellular matrices regulate nerve growth by guiding axons to the appropriate tissue during development as well as regeneration. For example, aggrecan that is present in non-neuronal tissue such as cartilage or IVD has been shown to inhibit nerve ingrowth in vitro [2, 3].

NG2 proteoglycan (NG2) is a well-characterized transmembrane chondroitin sulfate proteoglycan originally found to be expressed on oligodendrocyte progenitor cells [4] in the central nervous system. NG2 has been recognized as a major component of nerve growth-inhibiting molecules expressed at the site of spinal cord injury. Importantly, NG2 is also a component of non-neuronal tissues such as cartilage and bone during development [5]. We hypothesized that NG2 plays an important role in the regulatory mechanism of innervation in IVD tissue during the degeneration process by inhibiting the growth activity of nerves. The purpose of this initial study was to examine the expression of NG2 in human IVD cells cultured in vitro and the spatial distribution of NG2 in IVD tissues at an early stage of degeneration.

MATERIALS AND METHODS: Cell Preparation: Human IVDs were obtained from cadaveric human spines (4 donors, ages 59-73, AVG 64.8; MRI: Thompson grade, AVG 2-3) from the regional organ bank within 24 hours of death. Nucleus pulposus (NP) and annulus fibrosus (AF) cells were separately isolated by sequential enzymatic digestion and cultured in DMEM/F12 + 10% FBS.

Western Blot: AF and NP cells cultured in monolayer for 7 days were extracted with 1% NP-40 in 10 mM Tris-containing protease inhibitors for 30 min at 4°C. The proteins were separated with SDS-PAGE (4.0% acrylamide) followed by Western blotting using rabbit anti-rat NG2 polyclonal antibody (Chemicon). The crossreactivity to human NG2 has been previously published [5].

NG2 mRNA detection by RT-PCR: On day 7 of culture, total RNA from AF and NP cells was isolated and RT-PCR performed using the primers (sense-5’-TTGCTCTAGTGCTAATGCTT3’ antisense-5’-TGGAGCTGTCGTAGTTGA3’) designed based on the human NG2/MCSFP genes [6]. The primer for GAPDH was used as the internal control. The PCR product of disc cells was separated by 2% agarose gel. The band (219 bp) was purified with QiAquick gel extraction kit (QiaGen) and subjected to sequence analyses.

Tissue Preparation: Anterior and posterior parts of human IVDs were separately removed. Specimens for frozen sections (8 µm) were cut by a cryostat. Serial sections adjacent to the section used for immunohistochemistry were stained with Safranin-O and H-E.

Immunohistochemistry: AF and NP cells were cultured in monolayer for 7 days on a chamber slide. The cells were then fixed in 4.0% paraformaldehyde, permeabilized in 0.1% triton-X and washed with PBS. The cells were stained with rabbit anti-NG2 antibody [Chemicon] overnight at 4°C and Alexa 488-conjugated anti-rabbit IgG (Molecular Probes), for 2 hours. Perineurial fibroblasts from rat sciatic nerve were used for a positive control. The human IVD tissue was also stained using the same protocol. Samples were imaged by confocal microscopy.

RESULTS: NG2 expression on cultured cells: In both AF and NP cells cultured in monolayer, immunoreactivity to the anti-NG2 antibody was clearly identified. The staining was primarily located on the cell membrane as shown in the control, rat perineurial fibroblasts (Fig. 1).

NG2 protein analysis by Western blot: In protein extracts from both the AF and NP cells, a single band with a molecular mass of about 250 kDa (core protein of NG2 [5]) was identified by western blot analysis (Fig. 2).

NG2 mRNA detection by RT-PCR: The results of RT-PCR showed NG2 mRNA expression in both AF and NP cells (Fig. 3). The homology of the DNA sequence of RT-PCR product both from the human AF and NP cells was verified as an expected partial strand of NG2 cDNA (4331th to 4549th mer, or 219 bp).

ACKNOWLEDGMENT: This study was supported by NIH research grants, P50-AR43923 and P01-AR48152.