INTRODUCTION: Intervertebral disc diseases such as lumbar canal stenosis and herniation are a significant cause of morbidity and impair activities of daily living. Conventional treatment options include pain modulators and surgical procedures including discectomy or fusion. However, these treatments do not focus on the etiology of disc degeneration, which is poorly understood. One possible approach for regulating intervertebral disc degeneration is the inhibition of apoptosis. Apoptosis may play an important role in the reduction of disc cell numbers during disc aging and degeneration leading to diminished generation, organization, and repair of extracellular matrix.

Caspase 3, the most prominent effector caspase, is localized downstream in the caspase cascade and represents the main effector molecule in apoptosis. It irreversibly executes programmed cell death. To date, in vivo studies targeting reduction of disc cell apoptosis for treating intervertebral disc degeneration have not been conducted. We investigated anti-apoptotic effects of caspase 3 in disc degeneration and tried to uncover potential therapeutic advantages of this novel approach.

METHODS: All animal procedures were performed under the guidance of our animal research committee. We investigated the effects of caspase 3 short interfering RNA (siRNA) on rabbit nucleus pulposus (NP) cells in a serum-starved medium. Effects of direct injection of Alexa 555-labeled caspase 3 siRNA into the intervertebral disc were also determined in vivo using the rabbit annular needle puncture model.

RESULTS: NP cells transfected with caspase 3 siRNA showed a significant decrease in serum-starved apoptotic cells (figure 1). After local injection of caspase 3 siRNA into intervertebral discs, red fluorescence was observed in NP after treatment with Alexa-labeled caspase 3 siRNA. Messenger RNA and caspase 3 protein were downregulated in the caspase 3 siRNA group (figure 2). MRI and histological examination showed that degenerative changes were significantly suppressed in the caspase 3 siRNA group 4 and 8 weeks after injection (figure 3, 4). Quantification of TUNEL staining showed that caspase 3 siRNA group had significantly fewer apoptotic NP cells compared to control group (figure 5).

DISCUSSION: The present study evolved from the premise that downregulating disc cell apoptosis could recover or slow down the processes of disc degeneration. In this study, caspase 3 siRNA-transfected cells showed a significant decrease of caspase 3 gene expression until 4 days after transfection, leading to a reduction of apoptotic cells in a serum starvation model. These findings demonstrated the potential to improve disc cell survival after knockdown of caspase 3 expression. Moreover, local injection of caspase 3 siRNA into the disc effectively suppressed the caspase 3 protein in vivo. Both MRI and histological examination showed inhibition of intervertebral disc cell apoptosis and delay of disc degeneration. Percutaneous introduction of siRNAs into the disc is simple and less invasive and is advantageous in clinical settings.

CONCLUSIONS: Our findings indicate caspase 3 knockdown in intervertebral disc cells is effective in preventing apoptotic cell death, thus regulating intervertebral disc degeneration.

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