Insulin-like growth factor-1-treated prevents the FasL-induced apoptosis of anulus fibrosus cells activation ERK1/2
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Introduction: The major pathogenesis of neck or low back pain is the intervertebral disk (IVD) degeneration and herniation. In the pathogenic process, the intervertebral disk degeneration causes herniation.

Apoptosis is thought to be a critical component of disc degeneration. The apoptotic pathway of anulus fibrosus cells unlike nucleus pulposus cells, is Fas Type-I cells, which undergo apoptosis through the death-inducing signaling complex and apoptosis of intervertebral disc cells can be attenuated by caspase inhibitors.

IGF-1 is a well characterized growth factor, Some study displays possible points of interaction among the Ras-Raf-MEK-ERK pathway and apoptosis.

In this study, we induced anulus fibrosus apoptosis by antibody FasL and evaluated using various methods. The effect of co-stimulation with FasL and insulin-like growth factor–1 (IGF-1) on the focal adhesion kinase (FAK) expression and FAK and MAP Kinase 1/2 (Erk1/2) phosphorylation were studied. The expression of Aggrecan and collagen II, two major ECM ingredient, was also investigated.

Materials and Methods: Rat cervical anulus fibrosus cell were cultured and treated with antibody FasL, with or without human recombinant IGF-1. Cellular morphology was examined by light microscopy and reverse transcription polymerase chain reaction (RT-PCR). Apoptotic changes were evaluated by transmission electron microscopy, TUNEL staining, and immunostaining of Bax and bcl-2. Real-time PCR was used to investigate the mRNA expression of and FAK, collagen II and aggrecan. Values are normalized to β-actin from three independent experiments. Western blot was performed to assay FAK and Erk1/2 phosphorylation and assess its relation to IGF-1.

Results: Anulus fibrosus cells expressed collagen II and aggrecan in vitro that maintenance cell characteristic(figure1), FasL can induce anulus fibrosus cells apoptosis(figure2). TUNEL staining confirmed increased apoptosis ratio in antibody treated cells. FasL treatment caused FAK, collagen II and aggrecan mRNA expression approximately 2.84, 3.43, 3.91-fold down-regulated when compared to untreated controls. Simultaneous treatment with IGF-1 inhibited the effect of FasL on FAK, collagen II and aggrecan mRNA expression, increased 3.63, 11.32, 2.16-fold after treatment 24 hours. FasL treatment inhibited members of ERK/MAPK pathway, Erk1/2 phosphorylation. But not significant affected FAK phosphorylation. Co-treatment of IGF-1 can activate Erk1/2 (phosphorylation) but not significant activate FAK in short time (figure3).

Discussion: These data suggested that IGF-1 through activation Erk1/2 and up-regulated the FAK expression protects anulus fibrosus cells from FasL-induced apoptosis.


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Figure 1
Hematoxylin and Eosin Staining(HE) and toluidine blue staining confirmed that primary Anulus fibrosus cells possessed characteristic features of fibroblast-like and chondrocyte-like

Cultured anulus fibrosus cells were examined using TEM. Untreated anulus fibrous cells displayed intact round or oval nuclei that were clearly demarcated by a nuclear membrane, and a large amount of rough endoplasmic reticulum and mitochondria in the cytosol. Treatment with anti-Fas antibody resulted in typical apoptotic changes, including chromatin condensation, nuclear deformation and fragmentation, and the formation of apoptotic bodies. Fat vacuoles were found in cytoplasm of the apoptotic cells. Co-treatment with IGF-1 inhibited these apoptotic changes.

Figure 2
Treatments of IGF-1 can activate Erk1/2 phosphorylation but not FAK when co-treatment in 15 minutes and 30 minutes. IGF-1 treatment partially rescued the FasL-induced inhibition of the Erk1/2- MAP kinase pathway.