Expression and clinical significance of cathepsin K in intervertebral disk degeneration in humans

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Abstract

BACKGROUND: Several studies have confirmed that activation of intervertebral disc enzymes is closely related to matrix degradation. Matrix metalloproteinase and tissue inhibitor of metalloproteinase have been shown to exert important roles in the process of extracellular matrix degeneration in intervertebral disk. Besides these two enzyme systems, whether other proteases that exhibit degrading effects on extracellular matrix are involved in the intervertebral disk degeneration remains poorly understood.

OBJECTIVE: To detect the cathepsin K expression in normal and degenerated human intervertebral disc cells and investigate the correlation between cathepsin K and intervertebral disc degeneration.

METHODS: Cathepsin K expression was detected in intervertebral disc tissue from 30 patients with lumbar intervertebral disc protrusion using immunohistochemistry SP method and ELISA. At the same time, the intervertebral disc tissue from 15 healthy adult cadavers and/or spine fracture patients was taken as control. Cathepsin K protein expression in normal and degenerated human intervertebral disc tissues were compared.

RESULTS AND CONCLUSION: Cathepsin K expression was observed in normal and degenerated intervertebral disc tissues. The expression level was significantly higher in degenerated tissue than normal tissue ($P < 0.05$). These findings demonstrate that Cathepsin K possibly participates in the intervertebral disc degeneration.

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INTRODUCTION

Cathepsin K, a lysosomal cysteine protease, is mapped to chromosome1q21.2, resides in various histiocytes, and participates in the catabolism of connective tissue. The correlation of cathepsin K and intervertebral disc degeneration can be determined by comparing cathepsin K expression between normal and degenerated intervertebral disc tissues. But pertinent literature has not been found at home and abroad. The present study detected cathepsin K expression in human intervertebral disc cells using immunohistochemistry SP method and ELISA method and further investigated the correlation between cathepsin K and intervertebral disc degeneration to add theoretical evidence for studying the mechanism underlying intervertebral disc degeneration and postponing and treating this disease.

MATERIALS AND METHODS

Design

A protein level-based, independent, controlled, block design experiment.

Time and setting

This study was performed at the laboratories of the First Affiliated Hospital, Medical School, Shihezi University between May and September 2007.

Materials

The intervertebral discs used were from patients who received surgery at the Department of Orthopedics, Orthopedics Hospital of Nanyang between October 2006 and March 2007 and fresh cadavers. The degenerated intervertebral disc group comprised 30 samples from lumbar intervertebral disc protrusion patients who underwent discectomy, consisting of 18 males, 12 females, averaging 52 years of age (25–76 years). The normal control group comprised 15 samples from spine fracture patients or healthy adult cadavers, consisting of 10 males, 5 females, averaging 23 years of age (17–40 years). Written informed consent regarding sample use was obtained from each patient or relatives. The samples harvested from discectomy or spine surgery were processed by MRI T2-weighted signal. The samples from cadavers were pathologically confirmed. Rat anti-human cathepsin K monoclonal antibody (ready-to-use) and SP immunohistochemistry kit were purchased from Wuhan Boster, China. Cathepsin K ELISA kit was provided by ENDOGEN Inc, USA.

Methods

Immunohistochemical staining and results evaluation

Paraffin sections (5 μm) were dewaxed with dimethylbenzene and rehydrated with 3% H_2O_2 at room temperature for 15 minutes. Endogenous peroxidase was blocked with 3% H_2O_2, and antigen retrieval was performed, followed by three phosphate buffered saline washes, each 3 minutes. Immediately after antibodies were added, sections were incubated at 4 ℃ overnight. Immunohistochemistry was performed in strict accordance with kit instruction. A negative and positive control was separately used for each batch of sections. Phosphate buffered saline was used for negative control. Breast carcinoma sections confirmed were used as positive controls.

Results evaluation

The cathepsin K was located in the cytoplasm, as brown positive staining results. The percentage of

cells with positive cathepsin K expression was calculated, and cathepsin K staining intensity was observed. The positive staining intensity was determined based on the scores of positive cell percentage multiplied by staining intensity. Five 400-fold visual fields were randomly selected. A score of 0 represents < 15% of cells with positive cathepsin K expression; a score of 1 indicates 16%–50% of positive cells; a score of 2 suggests 51%–70% of positive cells, and a score of 3 indicates > 70% of positive cells. The staining intensity was graded by a 3-point scale: 1: light yellow; 2: brown; 3: dark brown. The results of positive cell percentage multiplied by staining intensity were graded as follows: 0–1 points: negative; 2–3 points: weakly positive; 4–5 points: moderately positive; 6–7 points: strongly positive.

ELISA determination

The samples from the degenerated intervertebral disc and normal control groups were triturated after addition of 8.6 g/L cold physiological saline (Mass sample: Mass physiological saline = 9: 1) and prepared 10% tissue homogenate. Then, a 15 minute 3 000 r/min centrifugation procedure was performed. The supernatant was detected in strict accordance with kit instruction. Absorbance values were obtained through the use of an ELISA reader. A curve chart was made based on absorbance value of standard sample and corresponding concentrations. From this curve chart, corresponding capthesin K concentrations were found based on the absorbance value of capthesin K.

Main outcome measures

Cathepsin K expression in normal and degenerated intervertebral disc tissue.

Design, enforcement, and evaluation

All authors co-contributed to experimental design, enforcement and data evaluation.

Immunohistochemistry data were processed using nonparametric rank sum test. ELISA data were expressed as Mean ± SD. *t* test was employed for comparison between two samples. All statistical analyses were performed using SPSS 13.0 software. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Detection of cathepsin K expression by immunohistochemistry

Immunohistochemistry results showed that cathepsin K was located in the cytoplasm. In the degenerated intervertebral disc group, most of samples exhibited cathepsin K-positive cells, with a positive rate > 60% (Figure 1). In the normal control group, few cathepsin K-positive cells were observed, with a positive rate < 20% (Figure 2). Nonparametric rank sum test data showed significant difference between these two groups in terms of cathepsin K-positive expression ($P < 0.05$).

Detection of capthesin K expression by ELISA

ELISA data were compared using *t* test. Results showed that capthesin K expression was significantly higher in the degenerated intervertebral disc group than in the normal control group ($P < 0.05$).

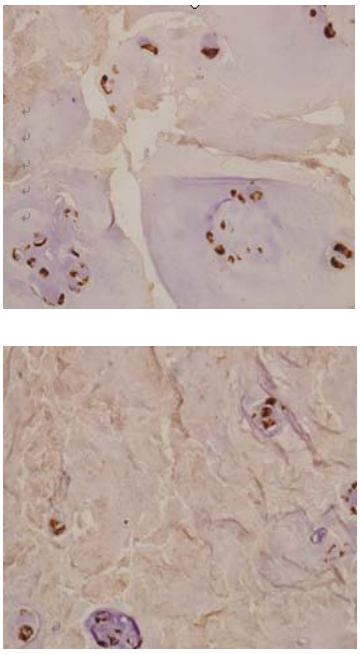

Figure 1 Cathepsin K expression in degenerated intervertebral disc tissue, as detected by immunohistochemistry (× 400)

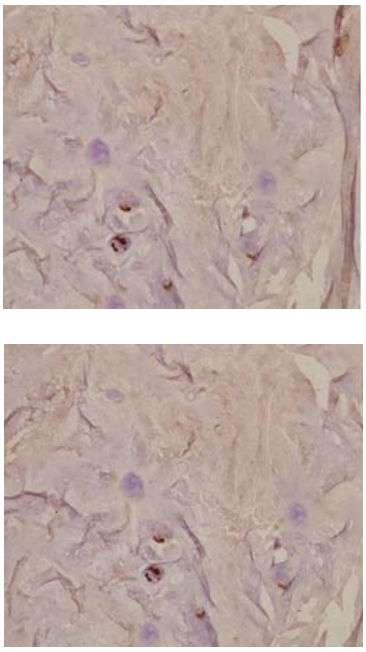

Figure 2 Cathepsin K expression in normal intervertebral disc tissue, as detected by immunohistochemistry (× 400)

DISCUSSION

Intervertebral disc degeneration has been considered the pathological basis of intervertebral disc protrusion and other diseases. People have further studied intervertebral disc

degeneration from molecular biology, biochemistry, and immunology. But precise mechanisms are disputed. Considerable work needs to be performed. Matrix metalloproteinase and tissue inhibitor of metalloproteinase exert important roles in the process of extracellular matrix degeneration in intervertebral disk. Besides these two enzyme systems, other proteases that exhibit degrading effects on extracellular matrix are likely to be involved in the intervertebral disk degeneration.

Cathepsin K, a lysosomal cysteine protease, is mapped to chromosome1q21.2, and resides in various histiocytes. Cathepsin K has been presently found not only in osteoclasts, but also in osteoblasts[1]. In addition, it is also distributed in inflammatory granuloma tissue, pulmonary tissue, follicular epithelial cells, endometrium, and fat tissue. It is secreted by cells in the inactive form and transformed into active form by proteolytic cleavage at the N-terminal side[2] and exhibits the role of proteinase.

Capthesin K is acidic, while matrix metalloproteinase is neutral or basic[3]. An *in vivo* study demonstrates that the pH value of degenerated intervertebral disc ranges 5.7–6.3[4]. The intervertebral disc is the largest tissue that has no blood supply. The absorption of nutritive substance and discharge of metabolic products are primarily dependent on the osmosis of cartilage end plate. With aging, the cartilage end plate gradually calcifies and peripheral blood vessels are progressively reduced, which prevents the entry of nucleus pulposus nutritive substance and the discharge of metabolic waste. At this time, anaerobic metabolism strengthens, lactic acid accumulates, and pH value in the extracellular environment is decreased. Such an acidic environment not only damages cell metabolism, influences cell biosynthesis, but also creates suitable conditions for exerting the role of extracellular proteinase and degrading extracellular matrix. These findings indicate that capthesin K plays a more important role in the process of degrading extracellular matrix than matrix metalloproteinase.

The present results revealed that capthesin K expression was significantly higher in degraded intervertebral disc tissue than in normal intervertebral disc tissue. Konttene *et al*[5] confirmed that in the capthesin family, capthesin G expression was also higher in the degraded intervertebral disc tissue than in normal intervertebral disc tissue, and proposed that capthesin G exhibit intervertebral disc degrading effects. Capthesin family has been shown to participate in the catabolism of connective tissue. All these provide direct or indirect theoretical evidence for the fact that capthesin K is involved in intervertebral disc degeneration. Although upregulated capthesin K expression in degraded intervertebral disc was demonstrated in this study, the regulatory factor, the activation and inhibition mechanisms of capthesin K, as well as correlation between capthesin K and other members in capthesin family need to be further clarified, which would add more theoretical evidence for studying the mechanism underlying intervertebral disc degeneration and postponing and treating this disease.

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组织蛋白酶 K 在人椎间盘中的表达及意义

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摘要

背景：研究证实，椎间盘内酶类的激活与基质成分的降解密切相关。已知基质金属蛋白酶和金属蛋白酶组织抑制剂两个酶系统在椎间盘细胞外基质改变过程中发挥着重要作用，除两者外，其他对细胞外基质有降解作用的蛋白酶是否也参与了椎间盘的退变过程？

目的：检测正常和退变人椎间盘细胞中组织蛋白酶 K 的表达，探讨组织蛋白酶 K 与椎间盘退变的相关性。

方法：应用免疫组化 S-P 法和 ELISA 法对 30 例腰椎间盘突出症患者椎间盘组织中组织蛋白酶 K 进行检测，并取 15 例健康成人(尸体)和脊柱骨折手术患者正常椎间盘组织作对照，观察组织蛋白酶 K 在正常和退变人椎间盘细胞中的蛋白表达。

结果与结论：正常椎间盘和退变椎间盘中均有组织蛋白酶 K 表达，退变椎间盘中组织蛋白酶 K 表达水平明显高于正常组，差异有显著性意义($P < 0.05$)。提示组织蛋白酶 K 可能参与了椎间盘退变的病理改变。

关键词：组织蛋白酶 K；椎间盘；退变；基质；骨组织工程

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