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Original Article

The relationship between HELQ expression and intervertebral disc degeneration

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Abstract: Background: Degeneration of the intervertebral disc, mainly of nucleus pulposus, is strongly implicated as a cause of low back pain. However, the mechanism of disc degeneration is not clear. Objective: This study is to investigate the expression level of HELQ protein in nucleus pulposus tissues and the relationship between HELQ expression and intervertebral disc degeneration. Methods: 84 samples of lumbar intervertebral disc degenerated of different degrees were obtained from patients underwent lumbar spinal surgery for low back pain and neurological symptoms. Degeneration degrees of all samples were classified by radiological and histopathologic methods. Immunohistochemistry (IHC) staining was performed to evaluate the expression level of HELQ protein in nucleus pulposus tissues. And the relationship between HELQ expression and radiological degeneration grades, histopathological degeneration grades of nucleus pulposus were analyzed. Results: HELQ protein was detected in human nucleus pulposus. The HELQ expression level was significantly negative correlation with radiological degeneration grades (r=-0.333, *P*=0.002) and histopathological degeneration grades (r=-0.519, *P*=0.000) of the nucleus pulposu. No correlation were detected between HELQ expression and patients' age (*P*=0.374), gender (*P*=0.063) and lumbar spine segments (*P*=0.676). Conclusions: The expression level of HELQ protein was significantly lower in nucleus pulposus with higher degeneration degree than those with lower degeneration degree. The results indicated that down-regulated expression of HELQ may have a close relationship with intervertebral discs degeneration.

Keywords: Intervertebral disc degeneration, nucleus pulposus, HELQ

Introduction

Low back pain (LBP) is one of the most common symptoms of spinal disorders, with an annual prevalence of 30% [1]. It significantly affects the quality of patients' life [2]. One reason of LBP is intervertebral disc degeneration (IDD) [3-5]. Evidently, association between IDD and LBP has been well-established [6]. The disc degeneration usually begins from the inner of nucleus pulposus (NP), and the senescence of nucleus pulposus cell is the cause of intervertebral disc degeneration [7]. However, cell senescence is closely related to DNA damage [8-10]. Previous studies have pointed out that DNA damage may play an important role in IDD [11-13].

Interstrand cross-links (ICLs) is a kind of DNA damage, which are particularly toxic because of disrupting genetic information on both strands and potently inhibiting DNA replication and transcription [14]. Helicase-like enzymatic activities are predicted to be useful for several steps in ICL repair. HELQ is a DNA helicase that translocates in the 3'-5' direction and can displace oligonucleotides of 70 nt or more from DNA [15]. It is indicated that HELQ is involved in cellular resistance to interstrand cross-links (ICLs) in human cells and plays a key role in ICL repair [16]. However, whether HELQ is involved in intervertebral disc degeneration and the mechanism of it are still unclear.

In this study, an analysis of HELQ protein expression in NP of degenerative disc by immunohistochemical staining was performed, and the relationship between HELQ expression and histopathological parameters of disc degeneration was evaluated. The purpose of this study is to

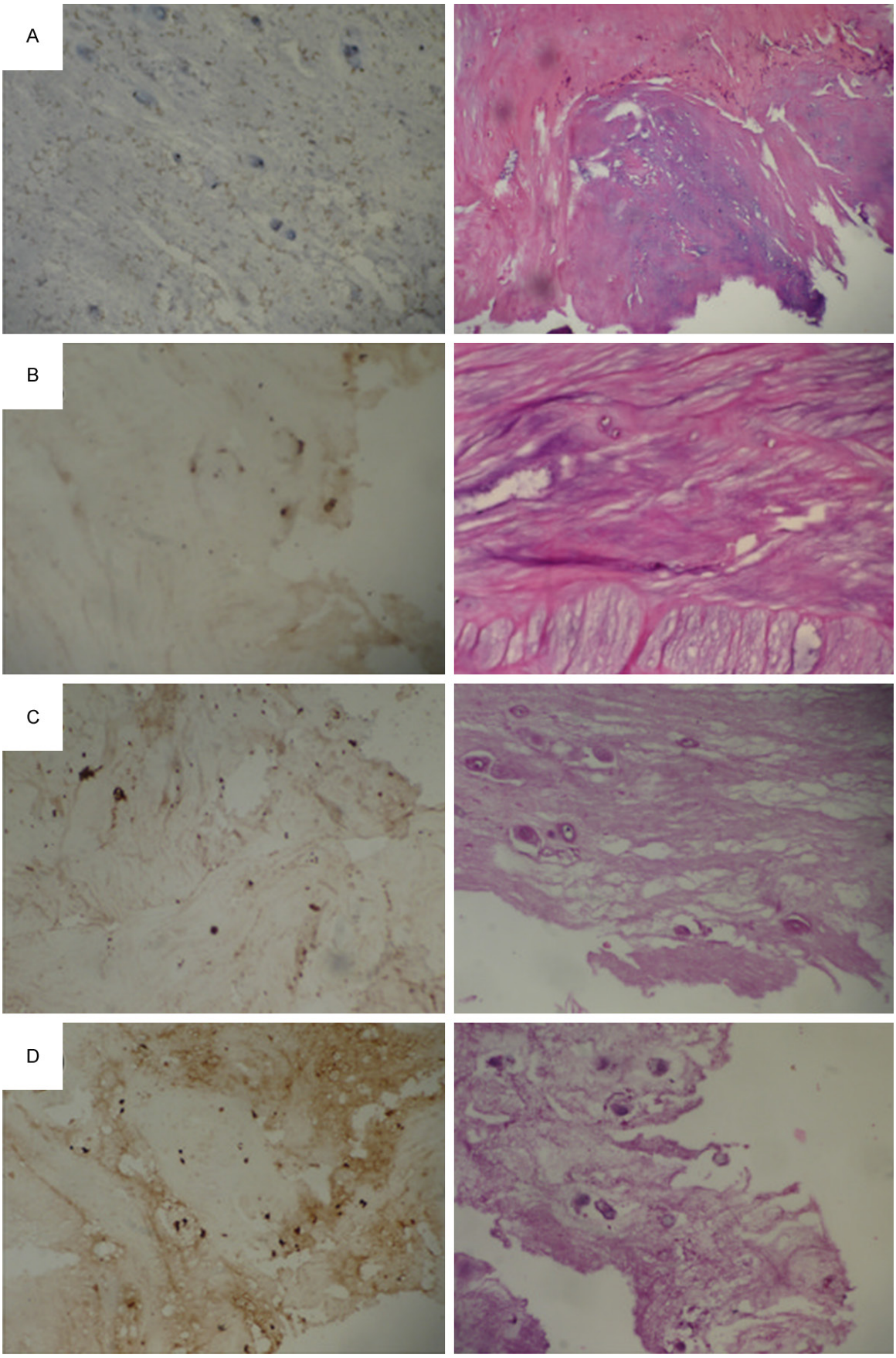
A
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Figure 1. Immunohistochemical and H&E staining findings for HELQ in NP. HELQ was expressed in the cartilage and fibroblast cells. Patients with intervertebral disc degeneration (radiological degeneration grade V and histopathological degeneration grade 4) had the negative HELQ expression in the (A) cartilage and fibroblast cells (×40). Patients with intervertebral disc degeneration (radiological degeneration grade IV and histopathological degenerative grade 3) had weakly positive HELQ expressed in the (B) cartilage and fibroblast cells (×40). While patients with Intervertebral disc degeneration like (C) (radiological degeneration grade III and histopathological degenerative grade 2) and (D) (histopathological degeneration grade 1), the HELQ expressed more and more evident. Photomicrographs (640×480 pixels) were obtained from non-coincident fields for each case at a magnification of 400×, using Panasonic DMC-FX33 digital camera adjusted to the same parameters.

clarify the role of HELQ expression in intervertebral disc degeneration. Our findings indicated that HELQ expression was significantly lower in NP with higher degeneration degree than those with lower degeneration degree and there was a negative correlation between HELQ expression and radiological and histopathological classification of NP. These findings suggested that HELQ may be a therapeutic target for IDD.

Materials and methods

Specimens

Nucleus Pulposus from patients with lumbar disc degeneration were harvested. All the patients underwent lumbar spinal surgery at the first affiliated hospital of Nanchang university from October 2013 to March 2014. Each patient signed informed consent for their participation. This study followed the Tenets of the 1975 Declaration of Helsinki and it was approved by the Ethics Committee of the hospital. All the patients received lumbar discectomy because of disc degeneration. If the IDD combined with lumbar spine tuberculosis, infections, tumor, severe systemic disease, severe rheumatic diseases, immune diseases and a history of inflammatory joint disease, the patients were excluded from this study. 84 NP specimens from 64 patients with disc herniation and 20 patients with lumbar spondylolisthesis were involved in this study. All the discs were graded III to V according to radiological classification based on magnetic resonance imaging (MRI) [17] (**Figure 2**).

Immunohistochemical staining

Paraffin sections (4 μm) were stained with hematoxylin and eosin (HE) for histological evaluation. Immunohistochemical staining was performed using a modified avidin-biotin peroxidase complex method. 4 μm sections from formalin-fixed, paraffin-embedded tissues were mounted on poly-L-lysine-coated slides and then deparaffinized. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 30 min at room temperature. After antigen retrieval through immersed in boiling 0.01 M sodium citrate buffer (pH 6.0) for 10 min, the sections were incubated overnight at 4°C with anti-DNA helicase HELQ 308 antibody (ab173400 Abcam Cambridge, USA) at the dilution of 1:200. After washing in PBS for 5 min, dropping the Maxvision, and incubating the sections for 1 hour at room temperature. Finally, the specimens were reacted with EnVision™ System HRP (DAB) (Dako). Hematoxylin was used as the counter stain.

Evaluation of immunohistochemical staining

Both the extent and intensity of immunopositivity were considered when judging HELQ protein expression. The extent of positivity was scored as follows: 1, <10%; 2, ≥10-50%; 3, ≥50-75% and 4, ≥75% of the cells in the NP. The intensity was scored as follows: none, 0; weak, 1; moderate, 2; and intense, 3. The final score was obtained by multiplying the extent of positivity to the intensity scores, producing a range from 0 to 12. The results were divided into 4 grades: Negative expression: <4 (grade 0); Positive expression: ≥4-<6 (grade 1), ≥6-<9 (grade 2), 9≥-<12 (grade 3).

Histopathological classification

Histopathological classification was analyzed according to Iou's histopathological classification method [18]. The degenerative nucleus pulposus were classified into four grades. The histopathological manifestation of those degenerative nucleus pulposus tissues included the changes of Collagen fiber, Keratin sulfate, Chondroitin sulfate and content of moisture. According to this histopathological classification method, the morphological change of the nucleus pulposus, the content of chondroitin sulfate and keratin sulfate were observed respectively by H&E staining and alcian blue

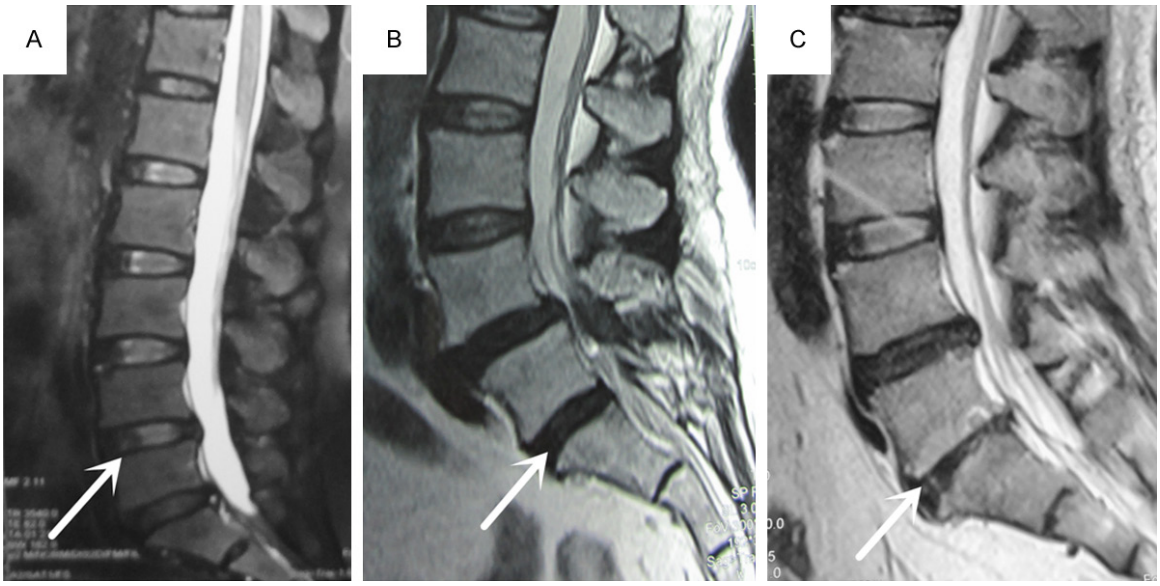

Figure 2. MR image of intervertebral discs classified according to the Pfirrmann grading system. (A) Grade III. (B) Grade IV, and (C) Grade V.

Table 1. Correlation between MRI grades and histopathological grades

MRI grades	Histopathological grades				r	*P*
	1	2	3	4		
III	5	6	8	0	0.547	0.000*
IV	1	11	36	7		
V	0	0	3	7		

*Significant difference between the MRI grades and Histopathological grades (P=0.000).

CEC staining. These 84 sections of NP were interpreted independently by two pathologists. Once disagreement arose, the images were viewed in conference before a consensus was reached.

Statistical analysis

The results were expressed as mean ± SD. All the statistical analyses were performed using the IBM SPSS statistics 19.0 software (Armonk, NY, USA). Analysis of variance (ANOVA) was used for evaluating age and Pearson Chi-Square was used for assessing gender and segments. Spearman's correlation coefficient was used to analyze the correlation of HELQ expression and radiological degeneration classification, histopathological degeneration classification. $P<0.05$ was considered statistically significant.

Results

The expression of HELQ protein in NP

Eighty-four lumbar intervertebral discs were obtained from patients (40 males and 44 females), with a mean age of 52.6 ± 10.2 years old (rang 28-77 years). Immunohistochemical staining of NP specimens showed that the protein of HELQ was expressed in the NP. And HELQ protein was mainly expressed in the cartilage and fibroblast cells. However, the extent and intensity of immunopositivity were not the same in different degeneration grades of NP. The expression level of HELQ protein was significantly lower in NP with higher degeneration degree than those with lower degeneration degree.

Degenerative classification of NP tissues

There are four grades according to the histopathological degeneration classification. 6 cases were classified in grade 1, 17 in grade 2, 47 in grade 3 and 14 in grade 4 (**Figure 1**). For the radiological classification, 19 discs were classified in grade III, 55 discs in grade IV and 10 discs in grade V according to Pfirrmann grading system (**Figure 2**). Statistically significant positive correlation was found between the histopathological grades and the radiological grades for the specimens (r=0.547,

Table 2. Relationship between HELQ protein expression and patients' demographic characteristics

	0	1	2	3	*P*-value
N	5	16	38	25	
Age	47.6 ± 9.2	55.1 ± 8.2	53.3 ± 9.7	50.8 ± 11.9	0.374†
Gender (male/female)	3/2	7/9	13/25	17/8	0.063‡
Segments: L3/4 (N)	0	2	3	2	
L4/5 (N)	5	9	24	14	0.676‡
L5/S1 (N)	0	5	11	9	

†ANOVA, ‡Pearson Chi-Square. No significant difference between the HELQ protein expression and patients' age, gender and segments (*P*>0.05).

P=0.000). The histopathological degeneration classification and radiological degeneration classification of NPs were showed in **Table 1**.

Relationship between HELQ protein expression and clinical parameters

The demographic characteristics of the patients were demonstrated in **Table 2**. Based on the Spearman's correlation analysis, no significant correlation was found between the HELQ protein expression and patients' age, gender and lumbar spine segments (*P*>0.05).

Correlation of HELQ protein expression with radiological degeneration classification

Figure 2 showed the different radiological degeneration grades of NP. Based on the analysis, HELQ expression was significantly higher in NP with lower degeneration degree. There was statistical significant negative correlation between HELQ expression and radiological degeneration grades (r=-0.333, P=0.002).

Correlation of HELQ protein expression with histopathological degeneration classification

The expression of HELQ proteins was decreased in NP with higher degeneration degree according to histopathological degeneration classification. Significant negative correlation was detected between HELQ expression and histopathological degeneration grades (r=-0.519, P=0.000).

Discussion

Currently, different methods have been used to describe the degeneration of intervertebral disc [19], such as X-ray, nucleography, computed tomography (CT) and MRI. And the radiological classification of the disc based on MRI [17] was widely used in clinical practice [20, 21]. Thus, we included the degenerative intervertebral discs into this study based on the MRI of the lumbar spine. In addition, a histopathological degeneration classification based on the amount of Matrix components and the form of Collagen fiber was used in the study. The results showed that radiological classification of NPs was significantly correlated with the histopathological classification of them (P<0.05). Therefore, we believed the two classifications can sufficiently reflect the degenerative degree of the intervertebral disc.

Genetics is an important risk factor for disc degeneration. Many studies have identified positive familial aggregation, which indicates some degree of genetic influence [22]. And there are many genes were reported to be associated with intervertebral disc degeneration [23-25]. The HELQ gene in Mammalian is a 3'-5' DNA helicase with strand displacement activity. HELQ, originally designated HEL308, which is on chromosome 4q21 and encodes a polypeptide of 1101 amino acids. It is a DNA helicase that translocates in the 3'-5' direction and can displace oligonucleo tides of 70 nt or more from DNA [15]. Interstrand cross-links (ICLs) present a unique problem to the repairing apparatus of the cell because this type of lesion involves both strands of DNA. Failure to remove these lesions from DNA eventually leads to cell death [26]. HELQ is involved in cellular resistance to ICLs in human cells [14]. Carrie A. Adelman et al. reported a critical role for HELQ in germ cell maintenance and tumor suppression in mammals, which attributed to the role of replication-coupled DNA repairing [16]. Based on the present study, The HELQ proteins were detected in human nucleus pulposus. With the increasing of the degree of degeneration, the expression of HELQ protein

was significantly decreased. The results suggested that there may be a closely relationship between HELQ and IDD, which may be associated with the intrinsic characteristics of HELQ.

The results of this study demonstrated for the first time that there may be a correlation between HELQ protein expression and disc degeneration grade. Based on the results, it was showed that the expression of HELQ protein decreased when the degree of disc degeneration increased. That maybe due to the senescence of NP cells, which is related to DNA damage. And HELQ plays an important role in DNA damage. In addition, the results indicated that no correlation was found between the expression of HELQ and the age, lumbar segments and gender of the patients. It was different from the results of previous studiy. In previous study, it was found that alteration of HELQ may be relevant to aging [14].

Also, there are several limitations in this study. Firstly, the HELQ expression was examined only by immunohistochemical staining. More methods were needed to test the expression level of HELQ. Secondly, the sample size is not large enough, and further study with a large number of patients will be necessary.

In conclusion, this study revealed that HELQ protein was expressed in the human nucleus pulposus. And there was a negative correlation between HELQ expression and radiological and histopathological degeneration classification of NP. It is proposed that HELQ may serve as a new therapeutic target for intervertebral disc degeneration. However, the mechanism of down-regulated expression of HELQ in degenerative lumbar disc remains unclear. More studies are needed to identify the molecular mechanism of IDD related to HELQ expression.

Disclosure of conflict of interest

None.

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