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Expression and significance of VEGF and p53 in rat degenerated intervertebral disc tissues

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ABSTRACT

Objective: To discuss the expression and significance of vascular endothelial growth factor (VEGF) and p53 expression in rat degenerated intervertebral disc (IVD) tissues. **Methods:** A total of 78 even-aged Sprague-Dawley (SD) rats (observation group) were chosen to establish a rat IVD degeneration model with anterior limbs and tail-removing method. Three months after modeling, rats were executed and degenerated IVD tissues were obtained for pathological biopsy so as to observe VEGF and p53 expression thereof. Meanwhile, IVD tissues from 8 healthy rats were gained for comparison. **Results:** Capillary infiltration was found in 62.8% of cases in observation group. Positive expression of VEGF and p53 was seen in 74.4% and 59.0% of cases respectively with a co-expression rate of 53.8%. VEGF and p53 expression in degenerated IVD tissues with capillary infiltration was higher than that in non-infiltration group ($P < 0.05$). **Conclusion:** A coordinate expression of VEGF and p53 in rat degenerated IVD tissues is demonstrated, indicating that they are both involved in neovascularization and infiltration, which accelerates IVD degeneration.

1. Introduction

Lumber intervertebral disc degeneration, the fundamental cause of lumbar intervertebral disc protrusion (LIDP), has been proved by studies to be associated with neovascularization[1]. Though reports have demonstrated that multiple vascular endothelial growth factors (VEGF) are involved in the process of neovascularization[2], a few researches concerning VEGF and p53 expression in degenerated IVD tissues and its relationship to capillary infiltration have been found. In this study, a total of 78 even-aged SD rats were enrolled for animal molding and degenerated IVD tissues were obtained for pathological biopsy after 3 months. Preliminary discussion was made and presented as follows.

2. Material and methods

2.1. Experimental animals

A total of 78 3-month-old male Sprague-Dawley (SD) rats (provided by Experimental Animal Center of Suzhou University) were enrolled and fed in sanitary single cages (observation group). No IVD diseases were revealed by MRI examination. Meanwhile, another 8 3-month-old healthy rats from the same batch were chosen as control group.

2.2. Reagent

S-P immunohistochemical kit purchased from Maixin Bio-Tech Co., Ltd and monoclonal antibodies VEGF and p53 produced by America Dako Company were utilized. According to immunohistochemical S-P method, measures were taken as follows: paraffin blocks were processed routinely. After dewaxing and hydration of histologic sections, and adding 3% hydrogen peroxide to block endogenous peroxidase, sections were incubated for 20 min. After microwave antigen repair, diluted primary antibodies were added (working concentrations for VEGF and p53

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were both 1:100) and sections were kept in fridge of 4 °C overnight. Then they were incubated at room temperature for 30 min after adding biotin labeling second antibodies, and another 30 min room temperature incubation was performed after horseradish–peroxidase–labeled pronase avidin solution were added. Sections were observed under light microscope after colored by DAB, counterstained by hematoxylin and sealed with neutral balsam. Both positive control and PBS negative control were set for each group.

2.3. Methods

Rat IVD degeneration model was established by removing anterior limbs and tails under aseptic condition after rats were anesthetized[3]. Hemostasis was performed strictly and rats were returned to cages after regaining consciousness. After wounds healing, we increase feeding height gradually to force rats stand up on their posterior limbs so as to develop the habit of up–right walking. Three months after modeling, IVD degeneration was observed and then rats were executed. Degenerated IVD tissues obtained were flushed with distilled water for three times after removing IVD surrounding muscles and adipose tissue. Specimen was dehydrated in 10% neutral formalin and then embedded in paraffin. Serial sections of 4 μ m thick were observed under light microscope after routine HE staining.

2.4. Statistical processing

Data were processed using SPSS 12.0 software, χ^2 test was performed for comparison of rates, and statistical difference was set at $P < 0.05$.

3. Results

3.1. Histopathological observation

Degenerated IVD tissues, observed under microscope, were characterized by loose, edema and hyaline degeneration of ligamentum flavum, proliferation of nucleus pulposus cartilage cells and annulus fibrosus, and necrosis in part of cartilage cells (Figure 1). Vascularization was found in 49 cases in observation group, accounting for 62.8%. New vessels, composed of single vascular endothelial cell or multiple endothelial cells, had narrow lumens and mostly locate in IVD edges. Microscopic examination showed outside–in infiltrative growth of vessels and granulation tissues, together with scattered surrounding lymphocytes and macrophage. Fibroblast proliferation, relatively active in degenerated IVD tissues, was manifested with remarkable increase in cell numbers, random sequencing, increased surrounding matrix and small cellular atypia. In comparison, normal IVD tissues only revealed cartilage cells with regular shape. These cells locate in cartilage lacunae, with little surrounding matrix. Angiogenesis was not observed (Figure 2).

2.2. Immunohistochemical results

In observation group, positive expression of VEGF and p53 was found in 74.4% and 59.0% of cases respectively, while no positive expression of VEGF or p53 was observed in control group. VEGF positive cells locate in cytoplasm, mainly from capillary endothelial cells, nucleus pulposus chondrocyte–like cells and mononuclear macrophage. In comparison, P53 positive cells locate in karyon, mainly being nucleus pulposus chondrocyte–like cells.



Figure 1. HE staining of degenerative intervertebral disc tissues (×200).



Figure 2. HE staining of degenerative intervertebral disc tissues with capillary infiltration (×100).

Table 1

Comparison of VEGF and p53 expression in two groups[n (%)].

Group	n	VEGF		p53	
		+	-	+	-
Observation group	78	58(74.4)	20(25.6)	46(59.0)	32(41.0)
Control group	8	0(0.0)	8(100.0)	0(0.0)	8(100.0)

3.3. VEGF and p53 expression in degenerated intervertebral disc tissues in infiltration and non–infiltration groups

Positive expression of VEGF and p53 in infiltration group was 89.8% and 49.0% respectively, which was remarkably higher than that in non–infiltration group (78.4% and 24.1% respectively). Difference between groups were statistically significant ($P < 0.01$) (Table 2).

3.4. Relation of VEGF expression to p53 expression in degenerative intervertebral disc tissues

Among 58 degenerative IVD tissues with positive VEGF

expression, positive expression of p53 was found in 42 cases and negative expression in 16 cases. Co-expression rate was 53.8% (42/78), indicating that VEGF expression was closely associated with p53 expression ($P < 0.01$).

Table 2

VEGF and p53 expression in degenerative intervertebral disc tissues with and without blood capillary infiltration[n (%)].

Group	n	VEGF		P53	
		+	-	+	-
Infiltration group	49	44(89.8)*	5(10.2)	24(49.0)*	20(51.0)
Non-infiltration group	29	22(75.9)	7(24.1)	7(24.1)	22(75.9)

* $P < 0.01$.

4. Discussion

Pathologic changes of lumbar intervertebral disc degeneration, the fundamental cause of lumbar intervertebral disc protrusion, are mainly characterized by depletion in cell numbers and IVD matrix degradation[3]. The outer layer of annulus fibrosus has partial blood supply during fetus period and infancy, and it degenerates from adulthood[4]. It was reported that in a pig IVD degeneration model, 2 weeks after injury, capillary infiltration and formation of granulation tissue in injured sites, followed by degeneration could be seen, demonstrating a close association between capillary infiltration and IVD degeneration[5]. In this group of data, capillary infiltration was found in 62.8% of cases in observation group, while neovascularization did not appear in control group. This also denotes that capillary infiltration is closely associated with IVD degeneration which might be result from capillary growth.

Several researches point out that capillaries in herniated IVD tissues are newly formed, and VEGF and platelet-derived growth factor (PDGF) may jointly participate in the process of new capillary formation, with a synergistic effect[6]. In this experiment, positive VEGF expression was found in 74.4% of cases in observation group, mainly from capillary endothelial cells, intranuclear chondrocyte-like cells and mononuclear macrophage. In comparison, no positive expression of VEGF was observed in normal rat IVD tissues. Positive expression rate of VEGF in rat degenerative IVD tissue with capillary infiltration was higher than that in non-infiltration group. The difference was statistically significant ($P < 0.01$). This reveals that VEGF is involved in neovascularization and capillary infiltration, which accelerates IVD degeneration. P53, a cancer suppressor gene, is associated with multiple cellular processes including inducing apoptosis, DNA repair, genome stabilization and regulation of genetic transcription[7,8]. In this study, p53 positive rate in observation group was 59.0%, while no positive expression of p53 was observed in control

group. P53 expression in degenerated IVD tissues with capillary infiltration was significantly higher than that in non-infiltration group ($P < 0.01$), indicating that p53 is likely involved in neovascularization and capillary infiltration in degenerated IVD tissues. Coexpression rate of VEGF and p53 in degenerative IVD tissues was 53.8%, which suggests that VEGF expression is closely associated with p53 expression ($P < 0.01$). A coordinate expression of VEGF and p53 was observed, which indicates that they are both involved in the process of neovascularization and capillary infiltration.

This research demonstrates a coordinate expression of VEGF and p53 in degenerated IVD tissues, indicating that they are both involved in the process of neovascularization and capillary infiltration, which accelerates degeneration of rats' IVD tissues.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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