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Expression and significance of VEGF and p53 in degenerate intervertebral disc tissue

Xiao-Yu Lu[1], Xiao-Hong Ding[1], Li-Jun Zhong[1], Hong Xia[2], Xiao-Dong Chen[3], Hai Huang[1*]

[1]*Rehabilitation Medicine, Hospital of Special Surgery, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, Guangdong Province, China 510010*
[2]*1st Department of Spine, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, Guangdong Province, China 510010*
[3]*Pathology Department, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, Guangdong Province, China 510010*

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ABSTRACT

Objective: To investigate the mechanism of expression and significance of vascular endothelial growth factor (VEGF) and p53 in degenerate intervertebral disc tissue. **Methods:** Pathological sections collected from 156 patients with lumbar disc herniation after surgery were tested by immunohistochemistry method, for evaluation of the expression of VEGF and p53 in degenerate intervertebral disc tissue. **Results:** 98 cases (62.8%) with vascular infiltration phenomenon are found, and positive rates of VEGF and p53 in degenerate intervertebral disc tissue are 73.42% (116/156) and 58.97% (92/156); co-expression rate is 53.2%(83/156); the expression rates of VEFG and p53 are significantly higher in the tissue with blood vessel infiltration than in the tissue without infiltration; there is a close relationship of VEGF with p53. **Conclusions:** VEGF and p53 gene synergetic express in degenerate intervertebral disc tissue, working together in neovascularization and infiltration, and accelerating intervertebral disc tissue degeneration.

1. Introduction

Degeneratie lumbar disc herniation is the basic cause of lumbar disc herniation. The study shows that there is a relationship of lumbar disc herniation with neovascularization. Many vascular growth factors take part in modulation and formation of neovascularization, now there is still not much reports about the relationship of infiltration with the expression of VEGF and p53. Regarding this, the present study try to investigate their relationship.

2. Materials and methods

2.1. Patients general data

Pathological sections were collected from 156 patients (male 100, female 56) with lumbar disc herniation after surgery, admitted in our hospital during January, 2009 to July, Their average age was 45.5 year old (25-66). 15 cases of L3/4 extrusion, 54 cases of L4/5 extrusion, 71 cases of L5S1 extrusion. 4 cases (male 2, female 2) of normal Intervertebral disc tissue (L1-5) were set as control, average 32.2 year old (25-40). The tissue sections were fixed by 10% neutral formalin, embbedded by paraffin, athe 4 μm slices consecutively were observed under light microscope after HE dying.

2.2. Immunohistochemistry study

Immunostain SP Kit was from Maixin Technology Co. monoclonal antibodies ofvascular endothelial growth factor (VEGF) and p53 were bought from Dako, USA. Wax block was deal with as routine, dewaxing and hydration organization the tissue sections, then 3% H_2O_2 was added for blocking endogenous peroxidase for 20 min, diluted primary antibodies (1:100) was added at 4 ℃ after antigen repairing by microwave, secondary antibodies with biotin labeling was added for incubation at room temperature for 30 min.

*Corresponding author: Hai Huang, Rehabilitation medicine, Hospital of Special Surgery, Guangzhou general hospital of guangzhou military command, Guangzhou, Guangdong Province, China 510010.
Tel: +86-20-36653995
E-mail: huanghaigz@126.com

Then LSA was added for incubation at room temperature for 30 min; dyed by DBA and hematine sealed by neutral gum, each group of dyeing all has the positive control and PBS negative contrast.

2.3. Statistics processing

Data was analysed using SPSS 16.0 software. group differences were analyse using *chi*-square method. $P<0.05$ was regarded as statistically significant.

3. Results

3.1. Pathological histology study

Degenerat intervertebral disc tissue was presented as loose and edema of hyalineye yellow ligament; hyperplasia of nucleus pulposus cartilage cell and fiber ring; degeneration necrosis in part of the cartilage cell. 98 cases (62.8%) with vascular infiltration phenomenon were found, most of new blood vessels were in intervertebral disc edge with narrow cavity, formed by single or a number of endothelial cells. Microscopically, the blood vessels and granulation tissue grew towards interertebral disc. It was visible that lymphocytes and macrophages scattered around. Degenerate intervertebral disc tissue fibroblast proliferation was relatively active, for the performance of a significant increase in the number, arrangement mixed and disorderly with more matrix, and the cell atypia was small. There was only regular form of cartilage cells in normal intervertebral disc, located in cartilage lacunar, the surrounding matrix was rare and did not see the angiogenesis.

3.2. Immunohistochemistry results

156 cases with degenerate intervertebral disc were found that VEGF expression positive rate was 73.42% (116/156); the positive expression of p53 was 58.97% (92/156). VEGF positive cells located in the cytoplasm, the cells were mainly capillary endothelial cell, cartilage type cells and mononuclear macrophage cells in nucleus pulposus. p53 positive cells located in the cell nucleus, mainly was the nucleus pulposus. In 4 normal cases of cartilage disc tissue, there was not VEGF and p53 positive expression.

3.3. VEGF and p53 expression in intervertebral disc degenerate organization with and without vascular infiltration

In group A, the degenerate intervertebral disc tissue, with vascular infiltration, VEGF expression positive rate was 89.8%, the positive expression of P53 was 48.3%; in group B, for intervertebral disc degenerate without vascular infiltration, VEGF and P53 expression positive rate were 78.6% and 25.9%. Comparing to degenerate intervertebral disc tissue with vascular invasion, VEGF and p53 expression were obviously higher than that wihout vascular infiltration, the statistically significant difference were $P<0.01$ and $P<0.01$ ($\chi^2=32.94, 41.84$), repectively.

3.4. VEGF and p53 expression relationship in degenerate intervertebral disc tissue

In 116 VEGF positive cases with degenerate intervertebral disc, there were 83 cases of p53 positive, 33 cases of p53 negative; VEGF negative in 40 cases of degenerate intervertebral disc, 9 cases of p53 positive, 31 cases of p53 negative, the common expression rate was 53.2% (83/156), VEGF expression and p53 expression is closely related.

4. Discussion

Degenerate lumbar intervertebral is the basic cause for lumbar intervertebral disc, the main pathological changes of lumbar degeneration are the decrease in the number of cells and intervertebral disc matrix degradation[1]. In the fetus and infant period, the intervertebral disc fiber outer ring can have some blood supply, but disappear in adult period, thus intervertebral disc becomes the largest organizationwithout blood supply. Vascular structures are visible in degenerate intervertebral disc, especially in lumbar intervertebral disc. Research on intervertebral disc degeneration model discovered that, two weeks after stabbing the pig intervertebral disc, the vascular invasion granulation tissue began to form, the following biochemical test showed the characteristics of degeneration, thus it was concluded that vascular infiltration and intervertebral disc degeneration are closely related[2]. Some scholars think capillary is the reason for emergence of the intervertebral disc degeneration. Because the intervertebral disc degeneration is the essence of the degradation of matrix, the physiological state of the intervertebral disc is a no blood supply organization[3], and vascular invasion perhaps can satisfy required activation conditions of collagen enzyme and substrate degradation enzyme, resulting in the rapid degradation of matrix, then followed by rapid degeneration and biological performance loss of intervertebral, until fracture hernia out[4]. We observed the 156 cases of intervertebral disc prominent organization, found 98 cases of vascular invasion, accounting for 62.8%; angiogenesis was not found in the normal intervertebral disc tissue. Also the vascular infiltration and intervertebral disc degeneration are closely related, the emergence of the capillary may be the cause of the intervertebral disc degeneration in lumbar intervertebral disc degeneration, clinical treatments including four goals, 1)

relieving spinal cord, nerve root tension and conglutination; 2) achieving clinical anatomy reset; 3) eliminating the surrounding tissues of aseptic inflammatory edema; 4) restoring spinal stability. The clinical surgical indications must be strictly controlled, so the conservative treatment is still the first choice. For early rehabilitation therapy focused onb strength enhancement of trunk muscle and improvement of the joint activity.

The mechanism still is not very clear about the source and the formation of new blood vessels in intervertebral disc tissue, there are reports that the capillary is newly formed, VEGF and platelet-derived growth factor may have synergy in the formation of new blood capillary process, participated in the formation of new blood vessels process in intervertebral disc. They observed the 50 lumbar disc protrusion specimens, VEGF immune positive rate was 88%, the cell source mainly were vascular endothelial cells, free type protrusion type positive rate is obviously higher than that of the other outstanding type. In the 156 cases with intervertebral disc degeneration of this experiment, VEGF expression positive rate is 73.42% (116/156), cell source mainly are capillary endothelial cells, cartilage type cells within the nucleus pulposus and mononuclear macrophages. And 4 cases of normal intervertebral disc tissue have no VEGF positive expression, expression positive rate of VEGF in intervertebral disc with vascular infiltration is obviously higher than that without infiltration ($P<0.01$). It is showed that VEGF plays an important role in new blood vessels formation and infiltration, accelerates the intervertebral disc tissue degeneration. Study showed that VEGF can induce intervertebral disc tissue angiogenesis, plays an important role in intervertebral disc spontaneous absorption process. VEGF is a highly specific vascular endothelial cell mitosis element, has double function which can increase the microvascular permeability, promote the plasma fibrin extravasation for the formation of blood vessels, provide a fiber network for cella migration; and combine with two special endothelial cell receptor FLT and FLK/KDR union, directly stimulate migration and proliferation of endothelial cells, so as to promote the formation of blood vessels[5,6].

P53 gene is concerned as a cancer suppressor gene, working in kinds of cells process, including inducing apoptosis, DNA repairing, cell cycle, genome stability[7]. Recent research shows that p53 gene can regulate the expression of angiogenesis regulation factor, like VEGF and platelet reagin-1 (TSP-1), mutant p53 protein can through the expression of TSP-1, or enhance the activity of protein kinase C, increase quantity of VEGF expression, to promote the formation of new blood vessels[8]. This study found that 156 cases of intervertebral disc degeneration, the positive expression rate of p53 was 58.97% (92/156); and 4 cases of normal intervertebral disc tissue did not present positive p53 expression; the expression of p53 in intervertebral disc degeneration with vascular infiltration is obviously higher than that of no vascular infiltration ($P<0.01$). p53 may be involved in neovascularisation and infiltration processes in degeneration of intervertebral disc tissue. Co-expression rate of VEGF and p53 are 53.2% (83/156); VEGF and p53 protein expression is closely related ($P<0.01$), VEGF and p53 coordinated expression, in intervertebral disc degeneration,, involved in the protruded intervertebral disc, the formation of new blood vessels and infiltration process.

In short, vascular invasion has close ties to intervertebral disc. The capillary may be the cause of the intervertebral disc degeneration, VEGF and p53 gene in intervertebral disc degeneration coordinated their expression, which may participate neovascularisation and infiltration in the degeneration of intervertebral disc, and then accelerate its degeneration.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1]Shang XP, Sun XC, Wang YX, Ju BB. Association of BCL-2 polymorphism with the presence and severity of lumbar disc degeneration in the Chinese Han population. *Clin Lab* 2012; **58**(3-4): 261-266.

[2]Wakelee H, Kernstine K, Vokes E, Schiller J, Baas P, Saijo N, et al. Cooperative group researchefforts in lung cancer 2008: focus on advanced-stage nonsm all cell lung cancer. *Clin Lung Cancer* 2008; **9**(6): 346-351.

[2]Cheung KM, Samartzis D, Karppinen J, Schiller J, Baas P, Saijo N, et al. Are "patterns" of lumbar disc degeneration associated with low back pain?: new insights based on skipped level disc pathology. *Spine* 2012: **37**(7): 430-438.

[4]Omair A, Lie BA, Reikeras O, Brox JI. An association study of interleukin 18 receptor genes (IL18R1 and IL18RAP) in lumbar disc degeneration. *Open Orthop J* 2012; **6**: 164-171.

[5] Vourtsis SA, Spyriounis PK, Agrogiannis G, Papalois A, Ionac M. Does VEGF have an effect on the survival of a long random skin flap by its application at the recipient area? *Chirurgia (Bucur)* 2012; **107**(4): 494-500.

[6]Seifert K, Büttner A, Rigol S, Eilert N, Wandel E, Giannis A. Potent small molecule Hedgehog agonists induce VEGF expression *in vitro*. *Bioorg Med Chem* 2012: DOI: S0968-0896(12)00648-7.

[7]Ryan BM, O D 'onovan N, Duffy MJ. Survivin: a new target for anti-ancer therapy. *Cancer Treat Rev* 2009; **35**(7): 553-562.

[8]Fan J, Wang L, Jiang GN, He WX, Ding JA. The role of survivin on overall survival of non-small cell lung cancer, a meta analysis of published literatures. *Lung Cancer* 2008; **61**(1): 91-96.