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Original Article

Analysis of basic fibroblast growth factor expression in the lens of cataract patients of different ages and its ability to promote epithelial cell proliferation

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Abstract: Objective: To investigate the differences in basic fibroblast growth factor (bFGF) expression levels in lens epithelial cells (LECs) of elderly, middle-aged and young cataract patients after surgery, and to analyze the correlation between age and epithelial proliferation ability of exogenous bFGF. Methods: In this prospective study, 90 cataract patients diagnosed and treated from August 2017 to November 2018 were selected and divided into a youth group (n=14), middle-aged group (n=31) and elderly group (n=45) according to their age. The expression of bFGF in the lens of patients was measured by immunohistochemistry. After primary culture of patients' LECs, 15 ng/mL of exogenous bFGF was added. The expression level of PNCA in each group was detected by immunohistochemistry, and the correlation between age and proliferation of LECs induced by exogenous bFGF was analyzed by Pearson correlation test. Results: The positive rate of bFGF in the lens of cataract patients after surgery in elderly group was higher than that of the other two groups ($P<0.05$). The PCNA positive area rate of LECs in the three groups with adding bFGF was higher than that without bFGF ($P<0.05$). The younger the patients were, the stronger the role of bFGF protein in promoting LEC proliferation. Conclusion: The positive rate of bFGF in the lens of cataract patients after surgery in the elderly group was higher than that of the middle-aged and youth groups. Exogenous bFGF can promote the proliferation of LECs in all patients, and the younger the age of the patients, the stronger the proliferation promoting effect of exogenous bFGF protein.

Keywords: Basic fibroblast growth factor, cataract, lens epithelial cells, immunohistochemistry

Introduction

Cataracts mostly occur in middle-aged and elderly people. Cataracts are caused by a disorder of lens metabolism caused by heredity, age and chronic diseases, resulting in lens turbidity [1]. The incidence of cataracts is highest in people over 40 years old, and with the continuous development of the disease, the incidence of blurred vision will increase, affecting the vision of patients [2, 3]. Senile cataracts are related to the proliferation of lens epithelial cells (LECs) in nuclear cataracts, and the reproductive capacity of LECs is different under different environmental conditions [4]. Basic fibroblast growth factor (bFGF) can promote the proliferation of LECs, but this conclusion needs to be further verified [5, 6]. Therefore, a prospective study was conducted on cataract patients to investigate the differences in bFGF level of LECs and the proliferation promoting effect of bFGF in different age cataract patients after surgery, so as to provide reference for the clinical treatment of cataracts.

Materials and methods

Clinical data

In this prospective study, 90 cataract patients diagnosed and treated from August 2017 to November 2018 were selected and divided into a youth group (n=14, 15-45 years old), middle-aged group (n=31, 45-59 years old) and elderly group (n=45, ≥60 years old) according to their age.

Inclusion criteria: (1) All enrolled patients met the diagnostic criteria for cataracts and were

confirmed by surgical examination [7, 8]; (2) All the enrolled patients were prepared for phacoemulsification cataract surgery, and all the patients were able to tolerate it. This study was approved by the Ethics Committee of Yichang Aier Eye Hospital, and all patients signed informed consent.

Exclusion criteria: (1) Patients complicated with autoimmune system diseases, solid malignant tumors or other diseases; (2) Patients complicated with cognitive dysfunction, mental abnormality or severe liver and kidney abnormalities; (3) Patients complicated with other eye diseases, such as myopia and retinopathy; (4) Patients with secondary cataracts with other causes.

Methods

Specimen collection: Patients in the youth group, middle-aged group and elderly group were routinely treated with surgery. After intraoperative capsulorhexis, the anterior capsular of the lens with a diameter of 5 mm or 6 mm was removed and divided into two parts for follow-up study. One part was fixed with 1-2 drops of cold methanol for 10 min, and then washed three times continuously with PBS solution for immunohistochemistry detection [9, 10], and the other part was taken out and immediately soaked in a complete medium containing 10% double antibody for primary cell culture.

Immunohistochemistry: The lens specimens fixed with methanol were embedded, sectioned, dewaxed and activated with 3% methanol and hydrogen peroxide, and peroxidase respectively for 10 min. After PBS washing, goat serum was used to block the specimen for 20 min, then bFGF primary antibody (Hangzhou Sijiqing Biological Products Co., Ltd.) or PCNA primary antibody (Hangzhou Sijiqing Biological Products Co., Ltd.) was added and incubated overnight at 4°C. On the second day, the sections were washed with PBS, the secondary antibody was added dropwise and incubated at room temperature for 2 h. After using diaminobenzidine (DAB) for color development (Fuzhou Maixin Biological Technology Co., Ltd.), positive cells were found under an inverted microscope (Olympus, Japan). The cytoplasm of the positive cells was brownish yellow, and the color was obviously darker than the background. The positive area rate and positive rate of bFGF or PNCA were quantitatively analyzed by JDIAS medical multifunctional image analysis software [11-13].

Detection of cell proliferation: The lens tissue was digested with trypsin for 5 min, the digestion was terminated after adding the complete medium, and the digested cells were collected for cell culture. This procedure was repeated 5-8 times to collect enough epithelial cells. The cell suspension ($1*10^5$) was dropped onto a sterile slide in a 24-well plate. After 30 min, the complete medium was added into the petri dish, and bFGF (15 ng/mL, Semefei Company) was added to stimulate the cells and the cells were cultured for 24 h at 37°C and 5% CO_2. After fixing the cells with 4% paraformaldehyde, the expression rate of PCNA positive cells was measured by immunohistochemistry.

Correlation analysis: Pearson correlation analysis was used to analyze the correlation between age and proliferation ability of LECs stimulated by bFGF in cataract patients after surgery (The greater the absolute value of correlation coefficient is, the stronger the correlation is: the closer the correlation coefficient is to 1 or -1, the stronger the correlation degree is, the closer the correlation coefficient is to 0, the weaker the correlation degree is).

Statistical analysis

SPSS 18.0 software was used to process the data in this study. The enumeration data were expressed as n (%), with χ^2 test. The measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± sd), with one-way ANOVA combined with Bonferroni post-hoc test. Pearson correlation analysis was used to investigate the relationship between the proliferation ability of LECs and the positive rate of bFGF in cataract patients. There was a significant difference at $P<0.05$.

Results

Comparison of general data

There was no significant difference among the three groups in general data such as gender, average age, average course of disease, body mass index, and combined underlying diseases ($P>0.05$). See **Table 1**.

Table 1. Comparison of general data

Group	Youth group	Middle-aged group	Elderly group	Z/F	P
Gender (n)				1.108	>0.05
Male/female	8/6	16/15	25/20		
Average age (year)	32.59±4.31	54.24±5.77	78.46±7.81	2.551	>0.05
Average course of disease (year)	4.51±0.69	4.54±0.71	4.55±0.78	1.837	>0.05
BMI (kg/m^2)	23.25±3.23	22.41±3.21	22.09±2.87	1.364	>0.05
Underlying diseases				0.916	>0.05
Hypertension	0	4	6		
Type 2 diabetes	1	3	5		
Coronary heart disease	0	2	7		

Note: BMI: body mass index.

Table 2. Comparison of positive rate of bFGF (n, %)

Group	Number	Positive rate of bFGF
Youth group	14	3 (21.43)
Middle-aged group	31	14 (45.16)[a]
Elderly group	45	23 (51.11)[a,b]

Note: Compared with youth group, [a]P<0.05; compared with middle-aged group, [b]P<0.05. bFGF: basic fibroblast growth factor.

Comparison of positive rate of bFGF

The positive rate of bFGF in the lenses of cataract patients after surgery in the elderly group was higher than that in the other two groups (P<0.05). The positive rate of bFGF in the middle-aged group was higher than that in the youth group (P<0.05). See **Table 2**; **Figure 1**.

Effect of bFGF on the proliferation of LECs

There was no statistical significance in the PCNA positive area rate of without adding bFGF LECs in youth group, middle-aged group and elderly group (P>0.05). The PCNA positive rate of LECs in the three groups with adding bFGF was higher than that without bFGF (P<0.05). See **Table 3**.

Relationship between exogenous bFGF-induced LECs proliferation and bGF positive rate of epithelial cells

The results of SPSS Pearson correlation analysis showed that after adding bFGF (15 ng/mL), the degree of LEC proliferation was negatively correlated with the patient's age (r=0.693, P=0.000). See **Figure 2**.

Discussion

The etiology of senile cataracts is relatively complex, and due to the older age of the patients, they are often accompanied with a variety of underlying diseases, coupled with a variety of cataract types, the proliferation and growth of LECs are affected by many factors [14, 15]. The lens is relatively complex in the human body, and the tissue has no blood supply, and is mainly regulated by cytokines [16]. LECs are widely distributed, mainly between the central part and the equatorial part of the eye. The LECs at the equator have a wide range of biological activities, slow cell proliferation and growth cycle, which can promote the generation of new lens cells and differentiate into different lens fiber layer structures, and help to maintain the transparency of the optical region [17, 18]. In this study, bFGF expression was the highest in the elderly group, while the positive expression of bFGF was the lowest in the youth group, indicating that there was a significant difference in the expression of bFGF in patients of different ages, and the highest positive rate of bFGF was found in the elderly patients, which played an important role in the occurrence and development of the disease.

bFGF is a powerful and cell-proliferation promoting factor. By binding with its specific receptor, it can phosphorylate the receptor protein tyrosine kinase, activate the cascade signal transduction system of related proteins through guanosine triphosphate binding protein, and cause obvious changes in various cell cycle regulatory factors [19, 20]. At the same time, bFGF can promote cell proliferation and growth

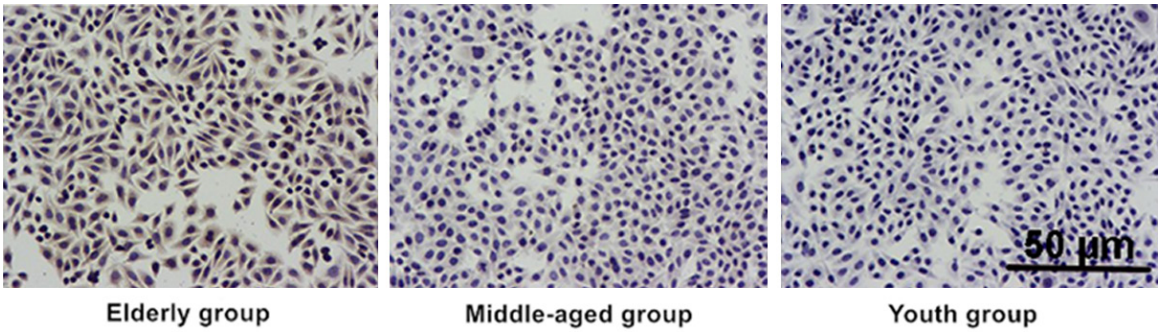

Figure 1. Representative images of lens immunohistochemistry of bFGF in the elderly, middle-aged and youth groups (200×), bFGF: basic fibroblast growth factor.

Table 3. Effect of bFGF on the proliferation of LECs ($\bar{x} \pm sd$)

Group	PCNA positive area rate of LECs		t	P
	With bFGF	Without bFGF		
Youth group (n=14)	15.43±3.25	4.28±0.71	6.493	0.000
Middle-aged group (n=31)	10.71±3.18	4.31±0.78	5.358	0.000
Elderly group (n=45)	6.45±2.32	4.70±0.77	7.121	0.000
F	6.414	1.210		
P	0.000	0.648		

Note: LECs: lens epithelial cells; bFGF: basic fibroblast growth factor.

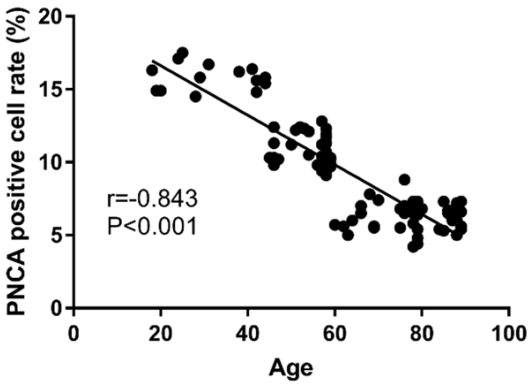

Figure 2. Correlation analysis of LEC proliferation induced by exogenous bFGF and age in cataract patients. LEC: lens epithelial cell; bFGF: basic fibroblast growth factor.

and improve the self-repair ability of tissues [21]. Bharathidevi et al. showed that bFGF was relatively widely distributed in the human eye and could be distributed in the lens and surrounding tissues. Meanwhile, sustained high expression of bFGF would cause damage to LECs and surrounding tissues [22]. In this study, there was no statistical significance in the PCNA positive area rate of LECs when exogenous bFGF proteins were not added in youth group, middle-aged group and elderly group (P>0.05). After the addition of exogenous bFGF, the PCNA positive area rate of LECs in the three groups was higher than that without bFGF (P<0.05), indicating that locally high concentration of bFGF can cause the proliferation of LECs, and it has a promoting effect in the old, middle and young age groups.

In order to further analyze the relationship between the proliferation of LECs induced by bFGF and the age of patients, a Pearson correlation analysis was conducted. The results showed that after the addition of bFGF (15 ng/mL), the degree of LEC proliferation was negatively correlated with the age of the patients (r=0.693, P=0.000). This may be due to the fact that the proliferation of LECs by bFGF is weakened with the increase of patients' age. With the increase of age, the bFGF level will increase, leading to the decrease of the body's phosphorylation susceptibility, thus weakening the proliferation of LECs. Therefore, the determination of patients' bFGF level should be strengthened before and after surgery for diagnosed cataract patients, and the prognosis of patients should be evaluated, so as to provide a reference for the formulation and adjustment of a postoperative treatment plan. Meanwhile, cataract patients can be assisted clinically by blocking bFGF and its receptor-receptor binding effect to look for positive preventive measures.

The inadequacy of this study lies in the small number of samples and lack of verification on mRNA and protein levels, thus the results may have some distortion. At the same time, there is a lack of in-depth discussion on the mechanism. Subsequently, the sample size should be increased for further in-depth research and added mechanistic investigation.

In conclusion, bFGF plays an important role in the proliferation of LECs in cataract patients after surgery, and the younger the patient, the stronger the proliferation promoting effect of exogenous bFGF protein.

Disclosure of conflict of interest

None.

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