· Original article ·

Differences of bFGF gene expression in lens epithelial cells between fetuses and cataract patients

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Received:2009-07-02 Accepted:2009-12-15

Abstract

• AIM: To study the differences of basic fibroblast growth factor (bFGF) gene expression in lens epithelial cells (LECs) between fetuses and cataract patients.

• METHODS: *In situ* hybridization was used to detect bFGF mRNA in the LECs that were cultured and in tissue sections from fetuses and in the LECs from the anterior capsule of cataract patients. Image analysis was used for the relative quantitative analysis of bFGF mRNA.

• RESULTS: bFGF gene existed in the LECs that were cultured and in tissue sections from fetuses and in the LECs from the anterior capsule of cataract patients. The integral absorbance for the fetal cultured cells, the fetal tissue sections and the capsule membrane cells of cataract patients were 627.1 ± 268.7, 131.5 ± 42.8 and 79.2 ± 26.3 respectively. The integral absorbance of fetal cultured LECs was significantly higher than that of fetal section LECs ($P<0.01$). The integral absorbance of cataract LECs was significantly lower than that of fetal LECs ($P<0.01$).

• CONCLUSION: The *in vitro* culture of LECs can improve bFGF gene expression. The bFGF gene expression in fetal LECs is significantly higher than that in cataract LECs.

• KEYWORDS: lens epithelial cells; environmental scanning electron microscope; *in situ* hybridization; basic fibroblast growth factor; image analysis
DOI:10.3969/j. issn. 1672-5123. 2010. 01. 003

Liu YF, Liu HW, Zhou Y. Differences of bFGF gene expression in lens epithelial cells between fetuses and cataract patients. *Int J Ophthalmol (Guoji Yanke Zazhi)* 2010;10(1):9-11

INTRODUCTION

Posterior capsule opacification is mainly caused by the proliferation of residual lens epithelial cells(LECs) after operation which is caused by post operational inflammation and repair of injury, in which cytokines play an important role. The occurrence of after-cataract changes in different age groups, younger population has a higher occurrence, and children have an occurrence of 77.9% [1]. The bFGF protein can be detected in human LECs and exists in the LECs of cataract patients as well[2]. In this study, we compared the difference in bFGF gene expression in LECs between fetuses and cataract patients and explored the effect of cell culture on bFGF gene expression.

MATERIALS AND METHODS

Materials Reagents digoxin 1410bp bFGF cDNA probe (Academy Of Military Medical Sciences, Beijing, China), *In situ* hybridization kit and NBI/BCIP kit (Promega, Wisconsin, USA).

Methods

Preparation of specimen fetal lens capsule slides From 6 spontaneous abortuses, the left eyeballs were taken. The sclera was cut open. The anterior capsules were taken and fixed in 40g/L paraformaldehyde solution for 5 minutes and then rinsed with PBS for later use.

Cultured fetal LECs From the above 6 spontaneous abortuses, the right eyeballs were taken. The anterior capsules were cut to pieces for cell culture. Dropped the cell suspension of second generation of fetal LECs on the coverslip and cultured cells for a few days. The cells were fixed in 40g/L paraformaldehyde solution for 5 minutes and rinsed with PBS. Capsules of cataract patients: the anterior capsules from 6 cataract patients and capsules were fixed in 40g/L paraformaldehyde solution for 5 minutes and then rinsed with PBS for later use.

Examination by environmental scanning electron microscope The cultured LECs and capsules were fixed by 20g/L glutaraldehyde for 24 hours and washed by PBS for 3 times. They were examined and photographed by FEI Quanta 200 environmental scanning electron microscope.

Test on the expression of FGF gene by *in situ* hybridization Performed the experiment according to the methods described in reference[3]. Negative control was set up. The positive cells were blue or indigo and the negative cells were colorless or slight pale red.

Image analysis Cambridge Quantimet 970 image analyzer was used for image analysis and integral absorbance of cells calculated.

Statistical Analysis Data were shown in mean ± SD and SPSS 11 was used for F test between groups.

RESULTS

The bFGF Gene Expression The LECs of culture and tissue sections from fetuses expressed bFGF gene. The LECs from the anterior capsule of cataract patients expressed bFGF gene, too.

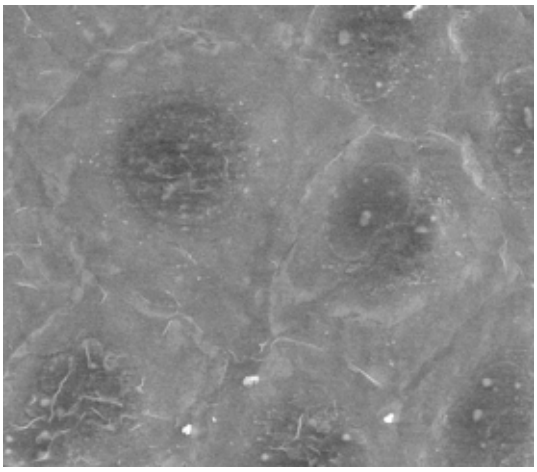

Figure 1 Cultured lens epithelial cells (×1500)

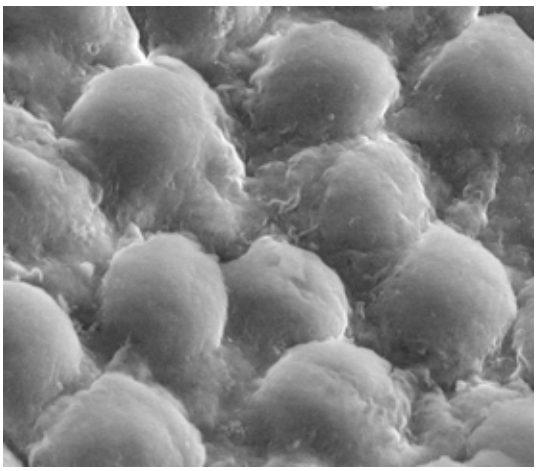

Figure 2 Lens epithelial cells in capsule(×5000)

Cells Structure of ESEM The cultured cells were flat and big, the diameter was 20-22μm (Figure 1). The cells in capsule were prominent and small, the diameter was 5-6μm (Figure 2).

LEC Integral Absorbance The integral absorbance of the LECs according to image analysis on 30 cultured fetal cells, 30 tissue section cells and 30 cataract capsule cells were 627.1±268.7, 131.5±42.8 and 79.2±26.3 respectively. The integral absorbance of the cultured cells was higher than that of tissue section cells and there was great significance in the difference between the two groups ($P < 0.01$). The integral absorbance of the LECs of cataract patients was lower than that of the LECs of fetuses and there was great significance between the two groups ($P < 0.01$).

We also determined average absorbance which is the absorbance per unit area of cells. They were 0.031, 0.070 and 0.022 for fetal cultured cells, fetal tissue section cells and cataract capsule cells respectively. The average absorbance of fetal tissue section cells was higher than that of the cultured cells. However, since the cultured cells had a larger volume, the integral absorbance of the cultured cells was still higher than that of the tissue section cells. Both the average absorbance and the integral absorbance of the LECs of cataract patients were lower than that of the fetal cells.

DISCUSSION

There are many differences in morphology and many proteins between cultured lens epithelial cells and the lens epithelial cells of capsule [4]. The lens is an avascular tissue, separated from the aqueous and vitreous humors by its own extracellular matrix, the lens capsule. Cell culture can injure and stimulate the tissues itself, thus can activate the synthesis of bFGF. The bFGF stimulates the proliferation of lens epithelial cells through acting on the FGF receptor in the cell membrane [5]. The bFGF within the cell may be transported to the cell outside. However, bFGF genes can not code signal peptide sequence and how it arrives outside the cell is unclear. One of the possible causes is that the cells are dissolved. The lens capsule is also a source of bFGF, Matrix metalloproteinase 2 (MMP-2) may induce bFGF released from capsular [6]. The bFGF can combine with extracellular matrix components such as heparin sulfate fibronectin and heparin sulfate proteoglycan. The biosynthetic genovariation of heparin sulfate can affect the binding of FGF with its receptor to reduce the effect bFGF [7]. Some substances, such as IL1, may promote the formation of bFGF [8].

The application of anti-sense technology can reduce bFGF mRNA expression and inhibit the promotion effect of bFGF on LECs proliferation [9]. It is reported that the promotion effect of bFGF on LECs proliferation actualizes through MAPK/ERK signaling system [10]. In addition, bFGF can promote FAK expression and conduct the signals of extracellular matrix into lens epithelial cells to enhance cell proliferation and migration [11]. But, LECs proliferation was not only promoted by bFGF, but also by other factors [12].

In situ hybridization studies on fetal tissue section, cell culture and cataract capsule have shown that there is bFGF gene expression in human lens epithelial cells, which proves that bFGF exists in these cells and plays physiological actions when it is released to outside of cells after injury and stimulation. Posterior capsule opacification is possibly caused by injury-repair reaction after operation which induces the injured or stimulated lens epithelial cells to release bFGF. The released bFGF plus the bFGF in humor aqueous and other factors in the eye reach a certain concentration level promote cell proliferation. The capacity of the synthesis of bFGF decreases with the increase of age. The occurrence of after cataract is higher in children than in adults.

Acknowledgements: We are grateful to Dr. Xie Jiayi from Lab of Biological Imaging and Nanomedicine, National Center for Nanoscience and Technology, Beijing, China for ESEM support.

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碱性成纤维细胞生长因子基因在胎儿与白内障患者晶状体上皮细胞内表达的比较

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摘要

目的:研究碱性成纤维细胞生长因子基因在胎儿和白内障患者晶状体上皮细胞内表达的区别。

方法:采用原位杂交方法,用 cDNA 探针检测胎儿培养及组织切片中的晶状体上皮细胞和白内障患者前囊中的晶状体上皮细胞的 bFGF 的 mRNA,并用图像分析进行相对定量,比较胎儿培养细胞、组织切片细胞及患者囊膜细胞的积分光吸收度值。

结果:胎儿的培养及组织切片中晶状体上皮细胞和白内障患者前囊中的晶状体上皮细胞都存在 bFGF 基因表达。胎儿体外培养晶状体上皮细胞、胎儿组织切片晶状体上皮细胞和白内障患者晶状体上皮细胞积分光吸收度值分别为 627.1±268.7,131.5±42.8 和 79.2±26.3。胎儿体外培养晶状体上皮细胞积分光吸收度值显著高于胎儿组织切片晶状体上皮细胞($P<0.01$);白内障患者晶状体上皮细胞积分光吸收度值显著低于胎儿晶状体上皮细胞($P<0.01$)。

结论:晶状体上皮细胞体外培养可增加 bFGF 基因表达;胎儿晶状体上皮细胞 bFGF 基因表达显著高于白内障患者晶状体上皮细胞。

关键词:晶状体上皮细胞;环境扫描电镜;原位杂交;碱性成纤维细胞生长因子;图像分析