·Basic Research·

Comparison of FGFR1 expression on lens epithelial cells between adults and fetuses

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Abstract

• AIM: To study the differences of fibroblast growth factor receptor 1 (FGFR1) gene on human lens epithelial cells (HLECs) of adults and fetuses.

• METHODS: Indirect *in situ* RT-PCR was adopted for detection of FGFR1 gene. The cDNA of the mRNA in the paraffin sections of fetus and adult HLEC was synthesized by reverse transcription reaction. After PCR amplification, *in situ* hybridization test was performed with synthesized oligonucleotide probe and relative quantification was carried out using image analysis.

• RESULTS: HLECs of adults and fetuses expressed FGFR1 gene, the expression level was higher in fetuses than in adults. The difference between them had significance (P<0.05).

CONCLUSION: FGFR1 exist in HLEC and the expression is age-related, which could be one of causes of the high occurrence of post operational after-cataract in children.

KEYWORDS: human lens epithelial cells; fibroblast growth factor receptor 1; indirect *in situ*/RT-PCR; after-cataract
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INTRODUCTION

The occurrence of after-cataract varies in different age groups, higher in the young, 77.9% in children[1]. BFGF is present in ocular tissues and can promote the proliferation and differentiation of lens epithelial cells. HLECs showed a dose-dependent response to bFGF, proliferation at lower and differentiation/trans-differentiation at higher concentrations[2]. BFGF affected the LECs function by their receptors. At least 5 FGFRs were detected. They were FGFR1, FGFR2, FGFR3, FGFR4 and FGFR5 (FGFRL1)[3]. The FGFRs were found on HLECs of fetuses and cataract patients [4]. In order to clarify the FGFR1 expression is aged-related, we used indirect *in situ* RT-PCR to determine the expression of FGFR1 gene on HLECs of adults and fetuses.

MATERIALS AND METHODS

Materials

Specimen Fresh eyeballs were from 6 fetuses and 5 adults. The eyeballs were fixed in 10% buffered formalin for 12 hours, dehydrated and embedded in paraffin. The specimen was sliced and stored at -20℃.

Reagents Primers and oligonucleutide probe (Sangon Ltd. Scarborough, Canada), RT-PCR kit (roche molecular systems,California, USA), *In situ* hybridization kit and NBI/BCIP kit (Promega, Wisconsin, USA).

Synthesis of primer Upstream primer sequence is 5'-GAA ATGGAGATGAAGATGATCGG; downstream primer sequence is 5'-CCCGAAAGACCACACATCACTC TG.

Synthesis of oligonucleutide probe 5'-AGAGCTGCTCC TCTGGGTTGTGGCTGGGGTTGTAGC.

Methods

Test on the expression of FGFR1 gene *In situ* RT-PCR: 6 sections from fetuses and 5 sections from adults were dewaxed, put in DEPC water, rinsed with PBS, dipped in 0.2 N HCl for 10 minutes, incubated in proteinase K at 37℃ for 15 minutes, rinsed with PBS 5 minutes×3, treated with DNA enzyme (free from RNA enzyme), rinsed with PBS 5 minutes ×2 and dehydrated with ethanol. Reverse transcription: transcription reaction solution includes AMV reverse transcriptase 1U/μL, 1×reverse transcriptase buffer solution, Rnasin lU/μL, downstream primer 1μmoL and dNTPs 250μmoL. The sections were incubated at 42℃ for 60 minutes and then the reverse transcriptase inactivated at 95℃. The sections were dehydrated with ethanol. Amplification reaction solution included: 1×PCR buffer; two primers, 1μmoL each; dNTPs 200μmoL; and Taq DNA polymerase 8U/100μL. Add 50μL amplification reaction solution to each sample. It was put in an amplifier after sealed with plastic film and denaturized at 94℃ for 5 minutes. PCR amplification reaction cycle was carried out as follows: 94℃ for 2 minutes, 55℃ for 2 minutes, 72℃ for

Table 1 Quantitative results of FGFR1 gene by indirect *in situ* PCR Mean±SD

Group	*n*	Cell area(um^2)	Average absorbance	Integral absorbance
Control	52	40.292±15.527	0.040±0.009	58.504±24.737
Adult	34	44.427±21.958	0.052±0.024[a]	123.361±82.383[a]
Fetus	50	41.833±18.126	0.079±0.018[a,c]	197.488±62.241[a,c]

[a]P<0.05 *vs* control group; [c]P<0.05 *vs* adult group

3 minutes, total 30 cycles. After that and extension for 5 minutes at 72℃, the sections were rinsed with PBS, fixed in anhydrous ethanol for 10 minutes and dried in air. Set control test: a: no primer was added; b: no reverse transcriptase was added; c: no Taq polymerase was added; d: no specific probe was added. *In situ* hybridization: The pre-hybridization was carried out at 42℃ for 2 hours. To each section, add 10μL hybridization solution (the probe was denatured at 100℃ for 5 seconds before that and the concentration must not be higher than 2ng/μL), covered the section with a plastic film. After the reaction lasted for 18 hours at 42℃, the plastic film was washed out with 2×SSC. Rinse the sections with 2×SSC (contain 0.2% SDS) at 42℃ for 15 seconds, with l×SSC (contain 0.1%SDS) at 42℃ for 15 seconds, with 0.2×SSC (contain 0.1%SDS) at 42℃ for 15 seconds, with buffer solution I for 5 seconds, with buffer solution II for 30 seconds (confined) and with buffer solution I for 1 second. Add antibody buffer solution I to dilute antibody (l:5 000) and let it stay still for 1 hour. Rinse it with buffer solution I twice, 15 seconds each time, and with buffer solution III for 2 seconds. Color development: NBI/BCIP in 1mL buffer solution III for 30 seconds, terminated, dehydrated and confined. Positive cells were blue or dark blue and negative cells were colorless or light red.

Quantitative examination of the expression of FGFR1 gene Randomly pick up 5-20 cells from each section on which indirect *in situ* RT-PCR had been carried out to perform statistical analysis. Total 34 cells were selected for the adults and 50 cells for the fetuses. Microscope and camera were adopted in image input. Microscope magnification rate: object glass 40 times, projection amplification 2 times, circuit amplification 20 times, total amplification 1600 times. Under this input condition, the reading for each pixel on measuring scale was 0.1695μm. Verify the micro measuring scale and the shadow. Put the slice on objective table and focus to most legible (experiment instrument: Cambridge Quantimet 970 image analyzer). The cell area, mean absorbance and integral absorbance were measured.

RESULTS

Results of Indirect *in situ* RT –PCR There is the expression of FGFR1 gene in HLECs of fetuses and adults (Figures 1 and 2) .

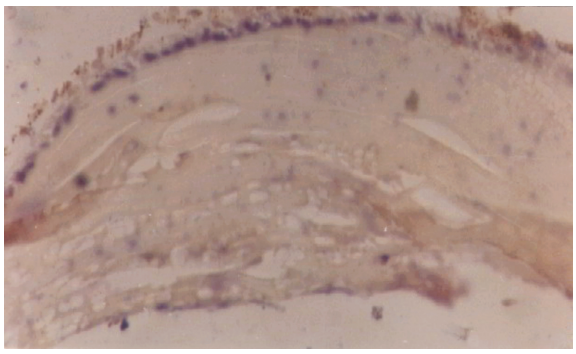

Figure 1 Five months of fetal Strong expression of FGFR1 gene in HLEC (Rt-PCR×400)

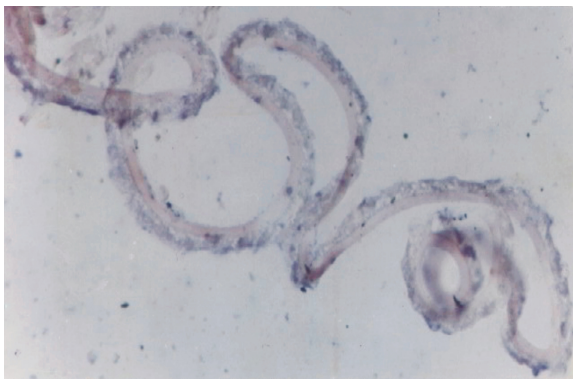

Figure 2 Twenty–eight years of adult Expression of FGFR1 gene in HLEC (Rt-PCR×400)

Results of Image Analysis It showed that there was no significance in cell area between the fetus group and the adult group. Average absorbance and integral absorbance were much higher in the fetus group than that in the adult group (Table 1).

DISCUSSION

There are various receptors on cell surface and can combine with different cytokines or hormones to produce different biological effects. FGFR is an important receptor in the body. It consists of three immunoglobulin structures: extracellular ligand, spiral transmembrane region and intracellular region with tyrosine kinase activity. The expression of four FGF receptor genes can produce 48 kinds of receptor isomers[5]. FGFR1 is the first FGF receptor found in the lens epithelial cells and the expression decreases with the increase of age. However, the expression increases with the stimulation of FGF. It plays an important role in cell development and fibrosis[6].

The number of cell divisions in the body is controlled by

genes. The more the divisions were, the slower the growth of the cells was [7]. The development of lens is the fastest in fetal period, slow down gradually from the fetus to child until it matures. The lens epithelial cells of adult have more cell divisions than that of children, and cell proliferation ability of adult would be more weaken than that of children. BFGF can promote stem cell differentiation [8] and promote the development of lens by binding to the receptor on the lens epithelial cells. The development of Lens requires the interaction between growth factors and extracellular matrix and bFGF increase FAK expression[9].

After cataract surgery, the damage of tissues induces cells to release bFGF and increases FGF receptor activity on lens epithelial cells [6]. Compared with the cells of older people, the cells of the younger are more liable to proliferate and migrate and to have after-cataract. We have found bFGF gene of HLECs expressed differently in fetuses and patients[10]. To compare HLEC receptor expression in fetuses and adults, RT-PCR of mRNA in the sections was carried out. The amplified DNA was detected using probe and image processing was used to compare the cell area, the average absorbance and integral absorbance. The study indicates that the expression of FGFR1 gene in the lens epithelial cells is higher in fetuses than in adults. And it may be one of the reasons why children have a higher incidence of after-cataract.

The effect of bFGF and FGFR in the formation of after-cataract is very important. The proliferation effects of HLEC are taken by the combination of bFGF and FGFR via the MAPK and Akt pathways, Wnt, Notch as well as extracellular matrix cues and possibly the Sal-Warts-Hippo pathway [11]. Recent data indicate that HLEC proliferation is regulated by nuclear factor kappa B. Activation of NF-kappaB could induce posterior capsule opacification [12]. That provides important message for inhibiting the proliferation of HLEC, seeking new drugs that can block the combination of bFGF with its receptor and clinic treatment.

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