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Mechanism of PEDF promoting the proliferation of lens epithelial cells in human eyes

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ABSTRACT

Objective: To investigate the regulation effect of pigment epithelium-derived factor (PEDF) on the growth of human lens endothelial cells (LECs) and related mechanisms *in vivo* and *in vitro*.
Methods: In the part of *in vivo* study, 82 eyes of 82 patients with age-related cataract were included to collect the central lens anterior capsule (diameter at 5.0–5.5 mm) with the informed consent of surgery for patients. The selected specimens were divided into the LECs low density group and high density group with 20 specimens for each group based on hematoxylin and eosin staining results. The relative expression level of *PEDF* mRNA in LECs was detected by reverse transcription PCR. In the part of *in vitro* study, LEC line (HLE-B3) was cultured and 50 ng/mL PEDF was added in media for 72 h in PEDF culture group, while normally cultured cells were used as the control group. The percentage of LECs at G_0 and S phases and apoptotic rate of cells were assayed by using flow cytometry with annexin V-FITC/7-AAD double staining method. Intracellular expression of vascular endothelial growth factor (*VEGF*) mRNA was detected by real-time fluorescence quantitative PCR.
Results: The central anterior subcapsular LECs density and relative expression level of *PEDF* mRNA were lower than those of high density group. There were no significant differences between two groups ($P = 0.168$). The apoptotic rate in the PEDF culture group was significantly reduced in comparison with the control group ($P < 0.001$). In addition, the expression level of *VEGF* mRNA was lower in the PEDF culture group compared with the control group ($P < 0.001$).
Conclusions: In human eyes, PEDF may function as cytotropic factor to promote survival of LECs through anti-apoptosis and reducing-expression of VEGF. Decrease of PEDF content in LECs probably modulates the pathophysiological process of lens cells and further cataractogenesis.

1. Introduction

The lens epithelial cell (LEC) is one of the key mechanisms on regulating the growth and aging of lens cell and also associated with cataractogenosis and its development. Its biochemical behavior allows molecular regulation by a variety of intracellular and extracellular signal transduction [1–4]. The pigment epithelium-derived factor (PEDF) is a kind of factor with multiple effects widely distributed in embryo and adult [5,6]. The previous research indicated a significantly antagonism action existed between PEDF and vascular endothelial growth factor (VEGF), while both of them had the effect of nutrition protection detected in histiocyte [7,8]. According to the earlier study of our research, the PEDF levels in human aqueous humor and in LECs presented positive correlation with the aging level of body and the degree of cataract attack [9–12]. However, whether PEDF has regulating effect on the growth of LECs and its relevant molecular mechanism has not been reported so far. The present study aimed to evaluate the regulating effect of PEDF on the growth of LECs and its relevant molecular mechanism by *in vivo* and *in vitro* experimental study.

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2. Materials and methods

2.1. Clinical materials

2.1.1. In vivo experiment

A total of 82 eyes of patients (male: 31 cases of 31 eyes, female: 51 cases of 51 eyes) with cataract treated by operation in our hospital from January 2011 to December 2012 were collected under the following standards: (1) the age ranged from 52 to 92; (2) non-congenital cataract, non-metabolic cataract and non-secondary cataract; (3) without gestational diabetes mellitus, fundus lesions, uveitis and glaucoma; (4) no eye trauma and the history of intra-ocular surgery; (5) no anterior lens capsules turbidity; (6) no other lens turbidity unless age-related lens. According to LOCSⅡ standard, the turbidity degree of lens cortex, lens nucleus and posterior subcapsular can be divided into from C2 to C5 level, N2 to N3 level and with or without turbidity, respectively. We declared that the study was approved by the ethics committee in our hospital. All subjects in this study knew and understood the content and risk of the research and signed the informed consent.

2.1.2. In vitro experiment

The human LE cell line, HLE-B3, provided by the experiment center of our hospital was cultured in complete medium mixed with 10% of fetal bovine serum for subculture. Then the optical microscope was used to observe the cell growth in good condition. After 70%–90% of cell density, the medium was digested by pancreatin and kept under $-80\ ^{\circ}C$ or liquid nitrogen for further use.

2.1.3. Main reagents and apparatus

In this study, PEDF, Trizol Reagent, RT-PCR reagent, the primers of PEDF, VEGFM and β-actin, Phosphatidylserine kit, fluorescence quantitative PCR reagent, ultraviolet spectrophotometer, flow cytometry and real-time PCR were used in this study.

2.2. Methods

2.2.1. Detection of PEDF mRNA expression by RT-PCR method

The central lens anterior capsules containing epithelial cells under the sac with diameter at 5.0–5.5 mm were obtained by emulsified continuous curvilinear capsulorhexis emulsification during cataract extraction. The obtained anterior capsules samples were divided into two parts, one of which was fixed by adding 4% paraformaldehyde for the later detection of cell density, and the other of which was kept by immerging to Trizol Reagent and at $-20\ ^{\circ}C$ for the test of mRNA expression.

To roll out the samples, after hematoxylin eosin staining, the detection of cell densities of them was conducted under three different visual angles. The mean of results was calculated as the cell density of LEC. Then the samples were divided into the low density group ($<4\,000/mm^2$) and the high density group ($>4\,500/mm^2$) with 20 samples for each group. Each 5 samples of each group were combined into 1 sample for test of the mRNA expression, thus both groups contained 4 samples. The RNA of the lens capsule membrane tissue was extracted and synthesized into cDNA by using RT-PCR kit. According to literature, the sequences of PEDF sense primer and reverse primer, internal reference β-actin sense primer and its reverse primer were 5′-TGTGCAGGCTTAGAGGGACT-3′, 5′-GTTC ACGGGGACTTTGAAGA-3′, 5′-GGTGGCTTTTAGGATGG-CAAG-3′ and 5′-ACTGGAACGGTGAAGGTGACAG-3′, respectively. By adopting the semi-quantitative reverse transcription PCR technology to increase mRNA and after the electrophoretic imaging, the amplified fragment was analyzed and calculated for the relative expressions of PEDF and mRNA. This experiment was repeated 5 times.

2.2.2. Annexin V method for detection of LECs growth and apoptosis

HLE-B3 was cultured in DMEM complete medium containing 10% fetal calf serum and 50 ng/mL PEDF in the culture group for 72 h, and in the control group, the HLE-B3 was cultured for 72 h by DMEM complete medium only containing 10% fetal calf serum. The cell cycles and circumstances of apoptosis of both groups were detected by using annexin V-FITC/7-AAD double-staining method.

About 0.5×10^6–1×10^6 of suspension cells were collected in two groups, respectively. One extractive was added in 100 μL buffer solution and 100 μL Annexin V-FITC for the incubation avoiding light. The other extractive was added in 400 μL buffer and 5 μL 17-ADD solution and quantitatively assayed for its cell cycle and apoptosis condition by using flow cytometry. The suspension cell without adding annexin V-FITC and 7-AAD was used in the negative control group. This experiment was repeated 5 times.

2.2.3. Real-time fluorescence quantitative PCR for detection of VEGF and mRNA expressions

The cell suspension solution of the PEDF group and the control group was extracted, respectively, and added in the Trizol Reagent for extracting RNA. The experiment was continuously, strictly conducted under operation instruction of real-time fluorescence quantitative PCR kit. According to literature, the sequences of VEGF sense primers and reverse primers were 5′-TTCAGAGCGGAGAAAGCATT-3′ and 5′-GAG-GAGGCTCCTTCCTGC-3′, respectively. The size of amplified fragment was 166 bp, while β-actin, as internal reference, was 161 bp. By using the real-time fluorescence quantitative PCR, the parameters were set as follows: pre-degeneration at 95 °C for 5 min, denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s and extension at 72 °C for 20 s, reaction circulation for 40 times. The results were analyzed by adopting CT method. This experiment was repeated 5 times.

2.3. Statistical analysis

The data during research were analyzed by using SPSS13.0 and the measurement data were expressed as mean ± SD. The differences between groups were tested by t-test and when $P < 0.05$, it was considered as having statistical differences ($\alpha = 0.05$).

3. Results

3.1. Comparison of age, LECs density and relative transcript level of PEDF mRNA between low density group and high density group

The comparison of age between both groups revealed no significant difference ($P = 0.168$). The LECs density of the high