·Basic Research·

PTEN mRNA expression in proliferative LECs of the rabbit

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Abstract

• AIM: To observe the effects of chromosome 10 (PTEN) and the tumor suppressor gene in proliferative LECs of the rabbit.

• METHODS: Forty-two white rabbits were randomly divided into test group (36 rabbits) and control group (6 rabbits). The transparent lenses of treated rabbits were operated with extra-capsular cortex extraction, and the controls were kept untouched. The rabbits were sacrificed 1 day, 3 days, 1 week, 2 weeks, 1 month and 2 months after surgery. Immunohistochemistry was applied to detect proliferative cellular nuclei antigen (PCNA) as well as hybridization *in situ* and reverse-transcriptase polymerase chain reactions (RT-PCR) were applied to detect phosphatase as well as tensin homology deleted on PTEN mRNA in lens equartor.

• RESULTS: PCNA expression increased significantly to a high level at 1 week, began to reduce 2 weeks later, and recovered to the normal level 1 month or 2 months after the surgery. PTEN mRNA expressed positively in normal rabbit LECs. The relative PTEN mRNA contents reduced greatly 1 day after operation and remained low level at 3 day. It began to increase slightly at 1 week, kept rising at 2 weeks and regained the normal expression after 1 month or 2 months. There was inverse correlation between the PTEN mRNA and PCNA expression.

• CONCLUSION: PTEN mRNA expresses positively in normal rabbit LECs plasma. PTEN participates in LECs proliferation and correlates with lens proliferative conditions.

• KEYWORDS: crystalline; lens; epithelial; animal; experiment; PTEN

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INTRODUCTION

Cataract is one of the most common eye diseases, which may cause visual disorder and blindness. At present, extra capsular cataract extraction (ECCE) combined with the implantation of intraocular lens (IOL) is the most effective method to treat cataract. After the surgery, nearly a prefect capsular sac remains, which separates the aqueous humor from vitreous and contains IOL. The surgery provides an optical passage because there is only transparent IOL and very thin, cell free capsular membrane in the visual axial part. However, the lens epithelial cells (LECs), which remain after the surgery may invade the capsular membrane by migration and proliferation, which can reach the surface of IOL and the posterior capsular, causing posterior capsular opacification (PCO)[1]. Although single sheet of cells is not enough to affect visual acuity (VA), secondary matrix and cell changes may affect visual function. PCO incidence rate has declined greatly by various methods, but it is still an intractable problem. The remaining LECs after the surgery proliferate as other cells according to cell proliferative regularity, which is influenced by many factors. If the molecular mechanism of the LECs proliferation can be clarified and its proliferation can be inhibited in the molecular level, PCO prevention may be greatly improved.

PCO starts by the environmental stimulation signals. Many studies have shown that cellular factors are very important in the PCO formation. These factors combined with the corresponding receptors on the cell membrane, by which the signals are transferred into the nucleus, promoting or inhibiting special target gene expression, and regulating cell growth. The signal transduction form is determined by the property of the cellular factor receptors.

Phophatase and tensin homology deleted on chromosome 10 (PTEN) gene was discovered in 1997 [2]. Phosphatase of PTEN is homogenous with double specific protein tyrosine phophatase (PTP). The phosphatase of PTEN is, so far, the first discovered inhibiting gene with phosphatase, which can dephosphate tyrosine, serine or threonine. Lipid phosphatase of PTEN can inhibit phosphatidylinositol-3 kinase (PI-3K),

protein kinase B (PKB) activity and inhibit cell proliferation, which plays the role of negative feed back to PI-3k / PKB signal system. Meanwhile, PTEN protein is highly homogenous with tensin protein and auxilin. PTEN processes the activity of protein phosphatase, dephospharating focal adhesion kinase (FAK) and Src-homology collagen (Shc), suppressing the cell invasion and migration.

At present, PTEN is mostly studied in tumor. There is no study on the PTEN mRNA on the proliferation yet. Proliferating cell nuclear antigen (PCNA) was used as proliferation control index to test the PTEN expression in different periods after surgery in rabbits. The results provide us with experimental data about the molecular mechanisms of the proliferation of LECs and the way to impede the proliferation by cell signal transduction and control.

MATERIALS AND METHODS

Randomized 42 healthy white rabbits (average weight 1.5kg) into two groups: the test group (36) and control group (6). In control group, 6 rabbits without any treatment were killed and the lenses were extirpated for test. In test group, lens cortex absorption by the same surgeon was performed on 36 anesthetized rabbits. Every 6 rabbits were killed and the lenses were prepared for test 1 day, 3 days, 1 week, 2 weeks, 1 month and 2 months after the operation. In every group among 6 rabbits, 6 lenses from 3 rabbits (42 lenses in all) were fixed by 40g/L formaldehydum polymerisatum and embedded by paraffin wax. Equatorial portion was obtained within 2mm of equator from the 6 lens of the other 3 rabbits in each group, and were frozen in the temperature of -70℃.

The expressions of PCNA in equator LECs in different periods were detected by immunohistochemistry (SABC). As for the immunohistochemistry results, the average absorption optical density (A value) in unit area of PCNA was analyzed by Metamorph software.

The expressions of PTEN mRNA in equator LECs were detected by hybridization *in situ* and the RT-PCR method. The average A value in unit area of the results of hybridization *in situ* was analyzed by Metamorph software. RT-PCR results were analyzed by Kodak ID image software and the relative mRNA quantities were obtained. The linear correlation between PTEN mRNA and PCNA was tested.

Statistical Analysis The t-test and linear correlation test were carried on SPSS10.0 statistical software.

RESULTS

The Results of Hybridization *in situ* The expression of PTEN mRNA in the cytoplasm of normal rabbit epithelial cells was positive. The expressions in the equatorial region on the postoperative 1-3 day (0.20±0.02 and 0.19±0.02) were weaker than preoperative ones (P<0.01, Table 1). The expressions of PTEN gradually increased 1 week after the operation. Until 1-2 months postoperatively, the expressions of PTEN restored to its preoperative level. There was inverse correlation between PTEN mRNA and PCNA (r = -0.979).

Table 1 Average absorption optical density of PTEN mRNA and PCNA

Time	PTEN mRNA			PCNA(A)		
	mean ± SD	*n*	*p*	mean ± SD	*n*	*P*
Control	0.54±0.03	6	—	0.46±0.04	6	—
1d post	0.20±0.02	6	<0.01	0.86±0.02	6	<0.01
3d post	0.19±0.02	6	<0.01	0.83±0.02	6	<0.01
1wk post	0.25±0.04	6	<0.01	0.79±0.02	6	<0.01
2wk post	0.42±0.03	6	<0.01	0.67±0.03	6	<0.01
1mo post	0.53±0.04	6	>0.05	0.49±0.03	6	>0.05
2mo post	0.54±0.04	6	>0.05	0.50±0.02	6	>0.01

Table 2 RT-PCR product of PTEN mRNA and PCNA absorption

Time	PTEN mRNA			PCNA(A)		
	mean ± SD	*n*	*P*	mean ± SD	*n*	*P*
Control	55.70±5.90	6	—	0.46±0.04	6	—
1d post	101.33±5.09	6	<0.01	0.86±0.02	6	<0.01
3d post	98.24±1.44	6	<0.01	0.83±0.02	6	<0.01
1wk post	123.21±5.24	6	<0.01	0.79±0.02	6	<0.01
2wk post	130.09±2.11	6	<0.01	0.67±0.03	6	<0.01
1mo post	155.11±7.23	6	>0.05	0.49±0.03	6	>0.05
2mo post	140.51±6.99	6	<0.01	0.50±0.02	6	<0.01

The Results of RT-PCR The expression of PTEN mRNA in the normal rabbit LECs was positive, and the relative quantity was 155.70 ±5.90, but the expression on the postoperative 1 day was weakly positive, which was 101.33 ±5.09 (P<0.01) in comparison with preoperative ones (Table 2). The expression gradually increased after 1 week. Until 1-2 months, it restored to its preoperative level. There was negative correlation between PTEN mRNA and PCNA (r =-0.94863).

DISCUSSION

The obvious solution to cataract is operation. However, PCO is the most common complication after cataract surgery, which is caused by the remaining LECs proliferation and migration, occupying the posterior capsule and refracting the parallel rays irregularly. Sudhaker reported that PCO incidence was 11.5% in one-year follow-up[3], while Moisseiev's result was 41% after four-year follow-up [4]. And PCO incidences correlate well with age. It is about 37% in the

population over 60 and 70% in the population under 40[5]. PCO occurs shortly after cataract surgery in children. Design and material of IOL also influence the PCO process [6-8]. The PCO incidence reduces with the development of effective treatments, but there is still a long way to go before doctors can handle the problem.

PCO, caused by the remaining LECs proliferation and differentiation, is related complexly with extracellular matrix secretion as well. The mechanisms of cell proliferation play important roles in opacification, which is also the key point to solve the trouble.

PTEN gene locates on human chromosome 10q23.3, and its protein expresses in cellular plasma. In 1997, the PTEN gene was identified as a tumor suppressor gene on the long arm of chromosome 10. Since then, important progress has been made to the understanding of the role of the PTEN protein in the normal development of the molecular pathogenesis. PTEN functions in the development of different biologic features, such as loss of cell-cycle control and uncontrolled cell proliferation, escape from apoptosis. Most of the tumor-suppressive properties of PTEN are dependent on its lipid phosphatase activity, which inhibits the phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway through dephosphorylation of phosphatidylinositol-(3,4,5)-triphosphate [9]. In addition to the treasure of data elucidating the functional roles of PTEN, recent studies suggest a diagnostic significance of PTEN gene alterations as a molecular marker for poor prognosis in anaplastic astrocytomas. Furthermore, the possibility of selective targeting of PTEN mutant tumor cells by specific pharmacologic inhibitors of members of the Pten/PI3K/Akt pathway opens up new perspectives for a targeted molecular therapy of malignant tumors. PTEN also functions as inhibitor of mitogen-activated protein kinase (MAKP) and FAK [10,11].

PTEN is one of the highlights in cancer research in recent years, but its effects in LECs proliferation and its mRNA expression in LECs have not been reported.

In our research, positive expressions of PTEN protein have been detected in rabbit LECs, and there was inverse correlation between it and PCNA. On the basis of previous data, hybridization *in situ* and RT-PCR were used to detect PTEN mRNA. Positive expression of PTEN mRNA can be detected in normal rabbit LECs. The cells began to initiate repairation and proliferation soon after the injury. PCNA expression increased sharply and reached the peak value 1 day after operation, with most of the remaining cells ready for proliferation. PCNA expression reduced gradually on 1 or 2 months after operation. On the other hand, PTEN mRNA expression decreased definitely in the first 3 days, recovery PTEN mRNA level could be seen at the end of the first week, and returned to the normal level after 2 weeks. There was inverse correlation between PTEN mRNA and PCNA expression, i.e., counteraction connects these two proteins in LECs proliferation. The results of hybridization *in situ* were in complete accordance with those of RT-PCR (r_{Hybrid}=-0.979, r_{RT-PCR}=-0.949).

PTEN, as one of the tumor suppressor gene, is of great importance in cell proliferation and apoptosis. The knowledge of PTEN will be deepened with the development of molecular biological technology. It may throw light on PCO prevention by promoting PTEN gene expression and inhibiting LECs proliferation.

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