·Basic Research·

PCNA, Bcl –2 and TERT expression in epiretinal membrane of rat traumatic proliferative vitreore – tinopathy

Li-Ping Xue[1], Feng-Ying Kang[2], Shi-Xing Hu[1,3], Xin-Guo Deng[1], Shao-Chun Lin[1]

[1] Department of Clinical Pharmacology, Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou 510080, Guangdong Province, China

[2] Department of Ophthalmology, Affiliated Hospital of Weifang Medical College, Weifang 261031, Shandong Province, China

[3] Eye Hospital, China Academy of Traditional Chinese Medicine, Beijing 100040, China

Correspondence to: Shi-Xing Hu. Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou 510080, Guangdong Province, China; Eye Hospital, China Academy of Traditional Chinese Medicine, Beijing 100040, China.markhu@263.net

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Abstract

• AIM: To explore the dynamic expression and correlation among telomerase catalytic subunit (TERT), proliferating cell nuclear antigen (PCNA)and antiapoptosis protein Bcl-2 which relate to cell proliferation in epiretinal membrane (ERM)of rat traumatic proliferative vitreoretinopathy (PVR).

• METHODS: S-P technique was applied for immunohistochemical staining of ERM of traumatic PVR with TERT, PCNA and Bcl-2 antibody. HE staining was also carried out. The staining results were analyzed with image analysis system.

• RESULTS: The positive rate and average A of PCNA protein were up regulated at first and then down regulated, with the peak value in 14 days Group, which was significantly different from those in 7 days Group and 28 days Group. The positive rate and average A of TERT and Bcl-2 were also up regulated at first and then down regulated, with the peak value in 14 days Group and 21 days Group, which were significantly different from those in 7 days Group. There was significant correlation among PCNA, Bcl-2 and TERT protein expression ($P \leqslant 0.01$).

• CONCLUSION: TERT and Bcl-2 take part in the regulation of proliferative cells in ERM of traumatic PVR, with high correlation with the dynamic changes of cell proliferation.

• KEYWORDS: traumatic proliferative vitreoretinopathy; proliferating cell nuclear antigen; telomerase catalytic subunit; Bcl-2; animal model; immunohistochemistry

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INTRODUCTION

Proliferative vitreoretinopathy (PVR) is responsible for the failure of the surgical treatment of retinal detachment, vitreous hemorrhage and ocular trauma as well as their major complications after operation. It also causes retinal vascular diseases and results in disease deterioration and severe vision damage. PVR is a cell media excess reaction to wound, and its pathogenic character is cellular proliferation and retinal detachment resulting from formation of membrane with tractive function. Its exact mechanism is still unknown, which brings problems to proper therapy. Therefore, it is necessary to discuss the mechanism of PVR from the perspective of molecular biology and the regulation mechanism of proliferative cells. Proliferating cell nuclear antigen (PCNA) is a kind of cell cycle protein which lies in nucleolus and regulates DNA composing and cell proliferation. Both Bcl-2 and telomerase catalytic subunit (TERT) are antiapoptosis gene. Their expressions relate to cell proliferation, differentiation and tumor formation. So this experiment is designed to observe the dynamic expression and correlation among TERT, PCNA and Bcl-2 in epiretinal membrane (ERM) of rat traumatic PVR, so as to provide a new vision for preventing PVR with drug therapy or gene means and to provide the basis for therapy time choice.

MATERIALS AND METHODS

Materials Forty healthy SD rats (2 months old, 200-300g), without limitation of female or male (provided by Experimental Animal Center of Weifang Medical College), were raised in normal condition and were prepared for the model of posterior penetrating eye injury. Self blood was poured into their vitreous cavities. Seven consecutive paraffin sections at 4μ m thick were prepared, among which one was stained with, and the others with TERT, PCNA and Bcl-2 immunohistochemistry. Mice anti-PCNA mAb

Table 1 PCNA, Bcl-2, TERT positive cell rate and average A

t/d	PCNA		Bcl-2		TERT	
	Positive cell (%)	A	Positive cell (%)	A	Positive cell (%)	A
7d	38.0 ± 7.3	10.3 ± 2.5	31.5 ± 5.8	7.6 ± 1.3	4.5 ± 2.7	6.9 ± 2.1
14d	64.1 ± 13.5	15.3 ± 4.1	48.2 ± 9.7	9.6 ± 3.3	14.2 ± 4.6	9.1 ± 1.3
21d	63.0 ± 7.0	16.4 ± 3.8	46.8 ± 6.7	9.4 ± 5.3	13.0 ± 4.8	8.3 ± 2.8
28d	45.5 ± 18.3	11.1 ± 2.4	42.4 ± 6.0	9.57 ± 2.3	9.2 ± 2.3	7.4 ± 1.6

There was marked correlation among PCNA, Bcl-2 and TERT

(Zymed, USA), Mice anti-Bcl-2 mAb (Sant Cruz, USA), rabbit anti-TERT (Sant Cruz, USA), general S-P kit (Zymed, USA), EDTA, trypsin and DAB kit were all purchased from Beijing Zhongshan Company.

Methods TERT immunohistochemistry was performed according to the kit direction. Antigen was repaired with 1g/L trypsin at 37℃ for 30 minutes, with PBS substitute I antibody and colon cancer tissue as negative and positive controls respectively. PCNA and Bcl-2 immunohistochemistry were also performed according to the kit direction. Antigen was repaired with 1mmol/L EDTA at 92-98℃, heated by microwave oven for 15 minutes, and then naturally cooled down to room temperature, with PBS substitute I antibody as negative control, galactophore cancer tissue as PCNA positive control and amygdalae lymph node tissue as Bcl-2 positive control. The positive cell of PCNA had clear nucleolus, with shape of rotund or elliptoid, brown granule or spots staining, and the density of staining was not uniform. Cells with brown granule or spots in plasma were regarded as both TERT and Bcl-2 positive cells. Total cells and positive cells on proliferative tissue under 5 fields high microscopy were counted. The average A of positive cells was computed under 3 fields of 200 times per section. All the statistical results were shown with mean and standard deviation, and SPSS 10.0 statistical software was used to analyze the data. One-way ANOVA and SNK-q tests were used to analyze the difference among groups and their correlation. $P \leqslant 0.05$ was regarded as the standard of significant difference.

RESULTS

At postoperative 1 day there was dense clot in vitreous cavity, and at 7 day the clot in vitreous cavity did not decompose yet. Dense white-gray cloud and proliferative strip occurred in vitreous cavity at 14 day and 21 day. The white-gray cloud was partialy absorbed and the proliferative strip became thicker at 28 day. Under microscope, fibrous proliferative tissue, degenerated red cells, fibroblast and macrophages were seen at 7 day. The proliferative tissue became denser and the cell density was increased at 14 day and 21 day, the major inflammatory cells belonged to macrophage, retina was drawn by proliferative tissue, and wrinkle and detachment occurred. At 28 day the grayish cloud was almost absorbed and the proliferative tissue was denser and thicker, but the cell density was slightly decreased. Fibre increased and thick fibre beam could be seen.

Dynamic Change of PCNA The positive rate and average A of PCNA protein were up regulated at first and then down regulated after operation. The peak was found in 14 days Group, which was significantly different from those in 7 days Group and 28 days Group (Table 1).

Dynamic Change of Bc1-2 The positive rate and average A of PCNA protein were upregulated at first and then down-regulated after operation. The peak value appeared in 14 days Group and 21 days Group, which was significantly different from those in 7 days Group and 28 days Group (Table 1).

Dynamic Change of TERT The positive rate and average A of PCNA protein were up regulated at first and then down regulated after operation. The peak was in 14 days Group and 21 days Group, with significant difference with those in 7 days Group (Table 1).

DISCUSSION

Tian *et al*[1] thought that the PVR model induced by pouring self blood into vitreous cavity is an almost natural process of PVR. The major character of PVR is cell proliferation. Guan *et al*[2] considered that not only cell proliferation, but also cell apoptosis occurs during the development of PVR, and the apoptosis rate decreases with the development of the disease. PCNA is regulated by cell cycle and closely related to the state of cell proliferation. Bcl-2 is a kind of antiapoptosis gene. The study of Liu *et al*[3] shows that there is high level expression of PCNA and Bcl-2 in cultured human retinal pigment epithelium (RPE) and ERM of PVR. Our experiment reveals that there is PCNA and Bcl-2 expression in ERM of traumatic PVR and it shows up-regulation at first and then down-regulation. The possible reasons are that inflammation medium and cell factor in vitreous cavity decrease in scar stage and the disease

becomes stable. In the second generation of cultured human RPE at 24, 48 and 72 hour, the positive cell rates of PCNA are 28%, 46% and 38%, respectively. The further study [4] shows that inhibition of antisense oligonucleotides encoding PCNA mRNA to cell proliferation can reach 81%. Research of Wang *et al* [5] indicates that the most inhibition of antisense PCNA to cell proliferation is 38.8%, while there is no evidence showing the inhibition of antisense Bcl-2 to cell proliferation. Further study is needed to ensure whether we can restrain cell proliferation and prevent PVR by antisense nucleic acid technique. Mandava *et al* [6] considered that nucleotide enzyme aiming to PCNA can prevent or treat PVR effectively, which shows good stability without poisonous side effect in vitreous cavity. Our study shows that the positive rate of PCNA in traumatic PVR ERM fluctuates with the course of diseases. At 2-3 week after trauma cells are in the most active proliferative state, therefore it is the best treatment time. At 4 week proliferative membrane has been formed and cells decrease in proliferative state, and the effect of anti-proliferation therapy is not good enough.

Telomerase is made up of telomerase associate protein, TERT and RNA component. Rubin's study [7]also shows that TERT is necessary for telomerase activity and its mRNA level is consistent. Telomerase can be activated by several surrounding agents. Our study also shows that abnormal proliferative cells express weak telomerase activity. There is significant correlation between Bcl-2 and TERT, and both of their changes consist to the fluctuation of cell proliferation. The overexpression of Bcl-2 and the activation of telomerase may take part in regulating cell proliferation in ERM of traumatic PVR. DNA damage is the key of apoptosis mediated by several factors. The overexpression of Bcl-2 can activate telomerase that may stabilize DNA, and this may be one of the possible mechanisms to antiapoptosis and may promote cell proliferation indirectly. Study [8] *in vitro* shows that the cultured human RPE expresses TERT at low level (13%). Xiang and Zeng's study [9] shows that applying telomerase inhibitor (camptothecin) and hot shock protein inhibitor (geldanamycin) on cultured human RPE, the asset rate is 72.3% and 71.9% respectively with MTT technique. This study provides new thoughts for medicine or gene method for preventing and threating PVR. At present therapies targeting telomerase to anti-tumor and anti-proliferation have been further developed. Antisense nucleotide, nuclease, ribonucleoside analogue, protein kinase inhibitor and cell differentiation inducer all show great potential prospect as telomerase inhibitor.

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