Expression of P53 during Lens Epithelial Cell Apoptosis Induced by Ultraviolet

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Summary: The apoptosis of lens epithelial cells (LECs) induced by ultraviolet and the expression of P53 were investigated. Wistar rats received 100 mW/m^2 ultraviolet irradiation (UVR) (λ=280 nm-315 nm) for 15 min. One, 6, 24 h after irradiation the lens capsules were dissected. The percentages of apoptotic cells were evaluated by the TdT-dUTP terminal nick-end labeling (TUNEL) technique and the expression of P53 was detected by using immunohistochemical assay. The results showed that the percentages of TUNEL-positive nuclei at 24 h after irradiation was significantly higher than in the control group and those 1 h, 6 h after irradiation. The percentages of P53-positive cells at 6 h, 24 h after irradiation were significantly higher than in the control group and those 1 h after irradiation. It was concluded that UVR could induce the apoptosis of lens epithelial cell. The expression of P53 might be responsible for the apoptosis of lens epithelial cells.
Key words: apoptosis; lens epithelial cell; ultraviolet; cataract; P53

Cataract is a disease induced by multi-factors and its pathologic mechanism is still unknown. It has been accepted that ultraviolet irradiation (UVR) is one of the most important pathogenesis. Li *et al* has reported that UVR could induce the apoptosis of lens epithelial cells (LECs) and resulted in lens opacities. In order to study the pathologic mechanism of UVR-induced cataract at molecular and cellular levels, we investigated the apoptosis of LECs and the expression of P53.

1 MATERIALS AND METHODS

1.1 Experimental Animals

Healthy Wistar rats of both sexes aged 5-6 weeks, weighing 52±2 g, were obtained from Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology.

1.2 Reagents and Instruments

The TdT-dUTP terminal nick-end labeling (TUNEL) kits, mouse anti-P53 monoclonal antibody, Strept Aviain-Biotine enzyme Complex kit (Boster Biotechnology Inc. Wuhan, China), Ultraviolet source: FZW—1 Ultraviolet Physicotherapeutic machines (λ=280-315 nm, power 100 mW/cm^2, supplied by Department of Physicotherapy, Tongji Hospital, Huazhong University of Science and Technology).

1.3 Methods

Rats were randomly divided into four groups: control group, three UVR irradiation groups with each group containing 4 rats.

In the UVR irradiation groups, tropicamide sodium was instilled into both eyes every 15 min for 4 times one h before irradiation. Then the animals were anaesthetized by intraperitoneal injection of 1 % sodium pentobarbital (30 mg/kg) and both eyes were fixed below ultraviolet source, irradiated for 15 min.

The rats in the control group and the UVR irradiation groups were killed 1 h, 6 h, 24 h after irradiation. The eyes were harvested. The whole cornea and iris were cut. Lens capsules were dissected carefully and placed on the slides. The slides were marked according to left or right eyes. According to the methods reported by Gavrieli *et al*[2] the slides from right eyes were fixed with 4 % polymethyldehyde for 10 min and washed by PBS twice. After digestion with proteinase K (1 : 100) for 15 min at 37 ℃, each slide was incubated with TdT-Digoxin-dUTP-buffer for 1 h (37 ℃). They were allowed to react with anti-Digoxin-AP 50 μl at 37 ℃ for 30 min. Each slide was stained with 0.5 mg/ml fresh 3, 3-diaminobenzidine tetrachloride (DAB) for 5 min. Brown pellets were observed in TUNEL positive LECs under a microscope.

Each slide from left eyes was fixed with 100 % ethanol and washed with PBS twice. After digestion with proteinase K (1 : 100) for 15 min at 37 ℃, each slide was incubated with anti-P53 antibody overnight at 4 ℃ and washed by PBS twice, co-incubated with second-antibody for 60 min, washed by PBS twice, exposed to ABC for 60 min, stained by DAB, dehydrated and covered with glycerol, and observed under a microscope.

Twenty high power fields under a microscope magnifying 400 times were selected in each slide. The percentage of positive cells was calculated by using ruler-like counter.

All data were expressed as $\bar{x}\pm s$, % and were analyzed by using ANOVA method. A P less than 0.05 was considered statistically significant. The relationship between P53 and TUNEL positive cells was analyzed by using correlation analysis method.

2 RESULTS

2.1 Detection of Apoptosis by TUNEL Method

TUNEL positive LECs were scarcelye detected

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in the slides of the control group. The percentages of TUNEL positive LECs in the UVR irradiation groups were as follows: 1 h after UVR the percentage of TUNEL positive LECs was (0.06±0.04) %, 6 h after UVR (0.04±0.04) %, and 24 h after UVR 14.18 %±0.28 % (table 1). There was significant difference in the percentage of TUNEL positive LECs between the control and experimental groups ($P=0.01$).

2.2 Expression of P53 in LECs

The percentages of P53 positive LECs were 0.06 %±0.07 % in the control group, 0.11 %±0.08 % in the 1 h UVR radiation group, 70.50 %±9.00 % in the 6-h UVR radiation group, and 79.61 %±13.40 % in the 24-h UVR radiation group (table 1), with the difference between control and experimental groups being significant ($P=0.008$), There was no significant difference between the groups at 6 h and 12 h after UVR radiation ($P=0.54$). The percentage of TUNEL positive cells in the UVR radiation groups was positively correlated with the percentage of P53 positive cells ($R=0.67$, $P=0.0074$).

Table 1 Comparison of percentage of TUNEL and P53 positive LECs between the control and UVR radiation groups ($\bar{x}\pm s$, %)

Groups	TUNEL positive LECs (%)	P53 positive LECs
Control	0	0.06±0.07
UVR groups		
At 1 h	0.06±0.048	0.11±0.0
At 6 h	0.04±0.04	70.50±9.00
At 24 h	14.18±0.28	79.61±13.40

3 DISCUSSION

3.1 Relationship between UVR Radiation and Apoptosis of LECs

UV-B (λ=280—315 nm) can directly damage protein, nucleic acid molecule and change their structure and function and initiate apoptosis. Characteristic apoptotic cells can be observed under a microscope and analyzed quantitatively. In this study Wistar rats received UVR. The results evaluated by TUNEL technique indicated that the percentage of apoptotic cells 24 h after UVR radiation was significantly higher than in the control group and the groups at 1 h, 6 h after UVR radiation. UVR can induce the apoptosis of LECs at the early stage. The previous studies showed that Light-oxidative reaction of free radical induced by UVR was one of the main reasons for UV-cataract. Another reason might be that UVR could result in the decrease of the enzyme activity. This study suggested that the apoptosis of LECs induced by UVR radiation might be one of the pathogeneses of UV-cataract.

3.2 Relationship between P53 and Apoptosis of LECs

P53 is the most important gene ever discovered to suppress cancer. It has many kinds of biologic functions[3]. The prior functions are controlling cellular transition from G phase to S phase and inducing apoptosis. P53 doesn't participate all kinds of apoptosis. Previous studies have shown that P53 mainly participated the apoptosis initiated by UVR and DNA damage[4]. The half-life of P53 is very short, about 20-30 min. Normal cells express very low level of p53 protein. But the level is increased after DNA-damage agent. The mechanism is still unknown. P53 can repair the injured cells or induce the apoptosis of injured cells by supervising the damage of DNA. The mechanism might be: P21waf/cip1 is a critical downstream effector in the P53-specific pathway of growth control. At the same time P21 waf/cip1 is a universal inhibitor of cyclin-dependent kinase (CDK) required for G to S transition. The expression of P21 is increased after the increase of P53 inhibiting the activation of CDK. Cells pause in either the G phase of the cell cycle or die by apoptosis. The results in this study indicated that the expression of P53 was low in the normal cells and was not increased immediately after UVR radiation. The decrease of P53 expression was not obvious 1 h after UVR radiation. At 6 h after UVR radiation, the expression of P53 was significantly increased and maintained a high level until 24 h after UVR radiation. The increase of P53 expression was correlated with cell apoptosis.

To sum up, UVR can induce the apoptosis of LECs accompanied with the increase of P53 expression, suggesting the apoptosis of LECs induced by UVR might be regulated by P53 gene.

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