·Basic Research·

Survivin and p53 expression in primary and recurrent pterygium in Chinese patients

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Abstract

• AIM: To assess the expression of anti-apoptotic protein survivin and tumor suppressor p53 protein in primary and recurrent pterygium and to investigate the relationship between them.

• METHODS: Survivin was assessed immunohistochemically using rabbit polyclonal antibody and p53 using mouse monoclonal antibody in a study sample of 20 cases of primary pterygium, 10 cases of recurrent pterygium and 10 cases of normal conjunctiva.

• RESULTS: In our study, 35% of primary (7 of 20) and 40% of recurrent (4 of 10) pterygium specimens were positive for survivin staining; 45% of primary (9 of 20) and 50% of recurrent (5 of 10) pterygium specimens were positive for p53 expression; and all normal conjunctiva showed no staining of either survivin or p53. The p53 and survivin immunoreactivity in primary and recurrent pterygium groups was greater than those in normal conjunctiva group ($P<0.05$). There were no differences in p53 and survivin immunoreactivity between groups of primary and recurrent pterygium ($P>0.05$). The expression of survivin clearly segregated with p53-positive pterygium as compared with p53-negative cases [8 of 14 cases (57.1%) *vs* ersus 3 of 16 cases (15.2%)] . The Fisher's exact test analysis confirmed a highly statistically significant correlation between survivin and p53 expression ($P<0.05$).

• CONCLUSION: The survivin and p53 are overexpressed with correlation between them in primary and recurrent pterygium.

• KEYWORDS: pterygium; survivin; p53

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INTRODUCTION

Pterygium is a common, benign, fibrovascular ocular disease. It is characterized by the encroachment of a fleshy triangle of conjunctival tissue into the cornea. A population-based survey conducted in rural Beijing demonstrated a pterygium prevalence of 3.76% in subjects aged 55-85 years [1]. The pathogenesis of pterygium is not fully understood. Recent data have provided evidence implicating that a genetic component [2], viral infections, immunological mechanisms, cytokines, growth factors, extracellular matrix remodelling, anti-apoptotic mechanisms and several angiogenic factors may play roles in the pathogenesis of this disease [3,4]. Pterygium has long been considered as a chronic degenerative condition. More recently, tumor-like characteristics such as mild dysplasia, local invasiveness, and high recurrence rate have been found in pterygium. Findings of p53 in the epithelium of pterygium specimens further evidence that pterygium is a UV-related neoplastic growth disorder rather than a degenerative process.

The p53 protein is a tumor suppressor protein that is encoded by the TP53 gene, which located to the short arm of chromosome 17 [5]. The p53 is essential for regulating cell division and preventing tumor formation. In normal cells, p53 protein is a short-lived and is maintained at low, often undetectable levels; but mutations in p53 gene lead to increased stability of its protein in the cell, which can be detected by antibodies to several epitopes of p53. Many researchers have found abnormal level of p53 protein in the epithelium of both primary and recurrent pterygium. The reported prevalence of p53 positive staining in pterygium by immunohistochemistry ranges from 7.9% to 100% [6]. Survivin is a member of the inhibitor of apotosis (IAP)

family and a strong inhibitor of apoptosis protein, which is over-expressed in most tumors [7,8]. Several reports revealed that the expression of wild-type p53 was associated with strong repression of the survivin promoter in various cell types[9].

Previous studies by Maxia C *et al* [10,11]. have shown that the expression of survivin is increased in primary ptergium as compared with the normal conjunctiva tissue and have a significant link with the expression of p53, cyclooxygenase-2 (COX-2) and 8-hydroxydeox- yguanosine (8-OHdG). However, the role of survivin in recurrent pterygium and the differences of the survivin expression between the primary and recurrent pterygium are not clear. The objective of this study is further to investigate the expression of survivin and p53 proteins in active primary and recurrent pterygium in Chinese patients and a possible link between these two proteins.

MATERIALS AND METHODS

Patients Active primary pterygium were harvested from 20 patients (11 males and 9 females), whose age ranged from 40 to 76 (mean 57.15±9.816) years. Recurrent pterygium were harvested from 10 patients (5 males and 5 females), whose age ranged from 43 to 67 (mean 53.0±8.781) years. All patients underwent excision by bare sclera technique at the Second Xiangya Hospital of Central South University (Changsha, China). All the lesions were located on the nasal side and only the head of primary pterygium was used as pterygium sample. Normal conjunctiva samples as controls were collected from medial bulbar conjunctiva of 10 patients (6 males and 4 females) without pterygium and pinguecula while undergoing retinal detachment surgery, whose age ranged from 40 to 62 (mean 48.5±7.619) years. Relevant clinical features of the patients weare summarized in Table 1. Patients received 3g/L Norfloxacin eye drops (Wujing, Wuhan) three times a day for (2-3 days) preoperatively and 0.4g/100mg Benoxil topical anesthetic (Saten, Japan) before surgery. No durgs drugs or chemical agents were used during surgical procedure. The study protocol was approved by the local research and ethic committee, and informed consent was obtained from all subjects in this study according to the Declaration of Helsinki.

Methods Tissue segments were fixed by 40g/L paraformaldehyde overnight and embedded in paraffin. Sections of 4μm were cut and treated for the immunohistochemical demonstration of p53 and survivin using the Strept Avidin Peroxidase conjugated method as described previously [11,12]. Briefly, all slides were deparaffinized and rehydrated with a gradient of ethanol concentrations and phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked by immersion for 10 minutes in 30mL/L H_2O_2 at room temperature. Antigen retrieval was performed by microwave heating in 10mmol/L citrate buffer solution (pH 6.0) for 15 minutes. Sections were treated for 10 minutes with 100mL/L normal goat serum in PBS. The ready-to-use rabbit anti-human survivin polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA) and mouse anti-human p53 monoclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA) diluted at 1:50 were used as primary antibodies and incubated for 60 minutes at room temperature (RT). Biotinylated goat anti-rabbit and anti-mouse IgG were used as secondary antibodies and incubated 15 minutes at RT. The samples were further incubated in Horseradish Peroxidase Streptavidin for 15 minutes at RT. The sites of antibody-antigen reaction were visualized with a brown chromogen produced by incubation with 3,3'-diaminoben-zidine tetrachloride (DAB) (Santa Cruz Biotechnology, USA) and hydrogen peroxide mixture within 5 minutes. The slides were counterstained with hematoxylin and mounted in Neutral balsam (Zhongshan Golden- bridge Biotechnology Co., Beijing, China). Similarly, sections of human gastric

Table 1 Demography of the pterygium patients

Sample	Variables	Number of patients
Primary pterygium(20)		
Age(yr)	>55	11
	≤55	9
Gender	Male	11
	Female	9
Survivin expression		
	Positive	7
	Negative	13
p53 expression		
	Positive	9
	Negative	11
Recurrent pterygium (10)		
Age(yr)	>55	4
	≤55	6
Gender	Male	5
	Female	5
Survivin expression		
	Positive	4
	Negative	6
p53 expression		
	Positive	5
	Negative	5
Normal Conjunctiva (10)		
Age(yr)	>55	2
	≤55	8
Gender	Male	6
	Female	4

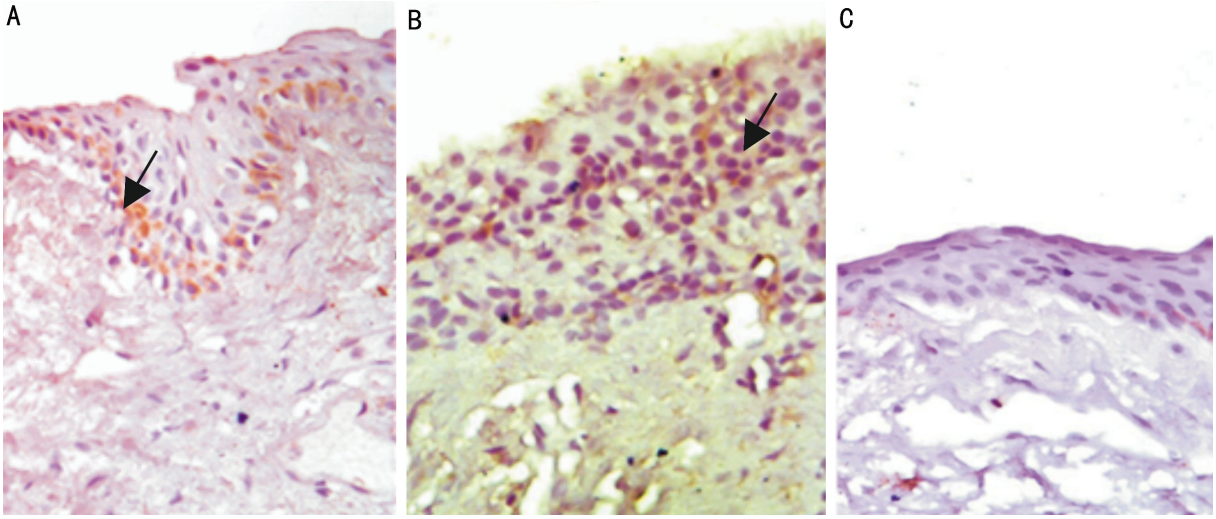

Figure 1 A: Immunohistochemical staining for survivin in primary pterygium; B: Rrecurrence pterygium; C: Nnormal conjunctiva. Staining was located in the basal and middle layers in the cytoplasm of the epithelial cells. The arrow indicates the strong immunoreactivity and the original magnification was 400×

carcinoma were used as positive control tissue for survivin, while sections of human mammary carcinoma were used as positive control for p53. Negative controls were performed by replacing the primary antibody with PBS. Micrographs were captured by a digital camera Zeiss coolpix 4200 (Germany) on a microscope OLYMPUS PM-10AK (Japan) and processed by pPhotoshop software. Results were evaluated independently by three observers in five high power (×400) microscopic fields and scored for the percentage of epithelial immunoreactive cells. The measurements were averaged. Those with more than 10% of cells stained were considered positive.

Statistical Analysis A possible correlation between the expression of survivin and p53, and the differences of expression between pterygium and conjunctiva were assessed with Fisher's exact test by the SPSS statistical software package, version 17.0 (SPSS Inc., Chicago, IL). P<0.05 was considered statistically significant.

RESULTS

Survivin expression In our study, 35% primary (7 of 20) (Figure 1A) and 40% recurrent (4 of 10) (Figure 1B) pterygium specimens were positive for survivin staining. There was no difference in survive immunoreactivity between primary and recurrent ptergium groups (P>0.05). Staining was limited to the cytoplasm in the epithelial cells;; no immunostaining was observed in the subepithelial fibrovascular layers. In normal conjunctiva group, all specimens were negative for staining (Figure 1C). The survivin immunoreactivity in groups of primary (P=0.038) and recurrent (P=0.043) pterygium was greater than that in normal conjunctiva group. The positively control section from human gastric carcinoma demonstrated immunoreactivity for survivin in the cytoplasm of tumor cells. The negative control sections have no reactivity developed.

Table 2 Relationship between survivin and p53 expression

p53	Survivin		Total
	Positive	Negative	
Positive	8	6	14
Negative	3	13	16
Total	11	19	30

p53 expression 45% primary (9 of 20) (Figure 2A) and 50% recurrent (5 of 10) (Figure 2B) pterygium specimens were positive for p53 expression. There was no difference in p53 immunoreactivity between primary and recurrent groups (P>0.05). Staining was limited to the nuclei in the epithelial cells;; no immunostaining was observed in the subepithelial fibrovascular layers. In normal conjunctiva group, all specimens were negative for staining (Figure 2C). The p53 immunoreactivity in groups of primary (P=0.012) and recurrent (P=0.016) pterygium was greater than that in normal conjunctiva group. The positively control section from human mammary carcinoma demonstrated immunoreactivity for p53 in the nuclei of tumor cells. The negative control sections have no reactivity developed.

Relationship between survivn and p53 The expression of survivin clearly segregated with p53-positive pterygium as compared with p53-negative cases [8 of 14 cases (57.1%) *vs* 3 of 16 cases (15.2%), shown in Table 2. The Fisher's exact test analysis confirmed a highly statistically significant correlation between survivin and p53 expression (P=0.035).

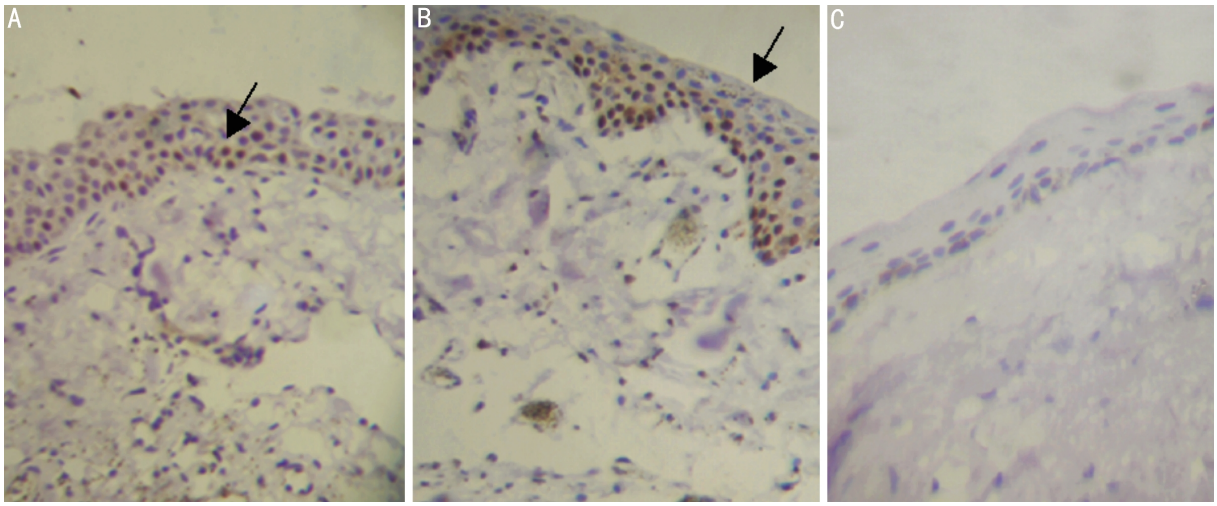

Figure 2 A:Immunohistochemical staining for p53 in primary pterygium; B: Rrecurrence pterygium; C: Nnormal conjunctiva. Staining was located in the basal and middle layers in the nuclei of the epithelial cells. The arrow indicates the strong immunoreactivity and the original magnification was 400×

DISCUSSION

In our study, 45% primary (8 of 20) and 50% recurrent (5 of 10) pterygium specimens were positive for p53 expression, whereas the expression of these protein in normal human conjunctiva was negative. These data are in agreement with previous studies reporting increased p53 expression in primary and recurrent pterygium [6,11,13,14,]. p53 is a known to be a tumor suppressor protein that plays a role in regulation of cellular proliferation and apoptosis. The reported prevalence of p53 positive staining by immunohistochemistry ranges from 7.9-% to 100% [6] . The p53 protein antibody cutoff level and race affected the results of the p53 staining. The present used p53 protein antibody included pAb 240, DO7, bp53.12, DO1 and CM-1; while DO7 only was used in our study. The prevalence of p53 protein expression in previous immunohistochemical reports with the use of DOO7 ranged from 21.6% to -74.5%[6,11,14-17]. The epidemiology of pterygium shows a relationship with UV exposure, postulated to be *via* the formation of radical oxygen species (ROS). Tasi *et al* [18] found a DNA damage biomarker, 8-OHdG overexpression in primary pterygium. Moreover, studies by Ismaeel *et al* [19] demonstrated the presence of 8-OHdG in recurrent pterygium. In addition, Perra *et al* [17] observered the concomitant presence of altered p53 in 8-OHdG immunoreacitve cells, providing further evidence that oxidative stress resulting in apparent p53 genetic instability plays an important role in the development of pterygium.

The inhibitor of apoptosis protein family functions as inhibitors of apoptotic pathways and have greater suppress apoptosis effecttion than other family of apoptotic inhibitors including the bcl-2. Survivin is a member of the inhibitor of apotosis (IAP) family and can blocks apoptosis induced by a variety of apoptotic triggers. Survivin has been reported suppressing apoptosis via inhibit caspase-3 and -7 [20]. In our study, 35% primary and 40% recurrent pterygium specimens were positive for survivin staining, whereas the expressions of these protein in normal human conjunctiva wereas negative. Our results are in agreement with previous data in primary pterygium by Perra *et al* [10,11]. To our knownledge, this is the first study to demonstrate a significative significant survivin overexpression in recurrent pterygium. Several reports revealed that the expression of wild-type p53 was associated with strong repression of the survivin promoter in various cell types[9]. In our study, the expression of survivin clearly segregated with p53-positive pterygium as compared with p53-negative cases, confirming a highly statistically significant correlation between survivin and p53 expression. Overexpression of survivin is probably related to p53 genetic instability induced by ultraviolet light [17]. Furthermore,, there was no difference in p53 and survivin immunoreactivity between primary and recurrent pterygium groups in our study. The observation of p53 immunoreactivity and high proliferative activity in the epithelium overlying the pterygium, reported by Chowers *et al* [21] , was not associated with recurrence of pterygium. These findings suggest that anti-apoptotic mechanism may be not responsible for pterygium recurrence.

In conclusion,, our study suggests that the regulation of survivin by p53 plays a role in an anti-apoptoticmechanism of pterygium. Recently, several novel experimental therapeutic strategies to reduce tumor growth have been

developed to target survivin [8,9]. Thus, targeting survivin could be a potential mechanism-based therapeutic strategyies to inhibition the formation of pterygium.

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