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Epithelial dysplasia in pterygium postoperative granuloma

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ABSTRACT

Pterygium postoperative granuloma (PPG) is one of the common complications of pterygium surgery. In order to provide the structural features of PPG, and to further explore its pathogenetic mechanism, we analyzed clinical and pathological characteristics of 12 PPG cases. New blood vessels were observed under a slit lamp in PPG and peripheral conjunctival tissues. In vivo confocal imaging showed that there was extensive neovascularization in the stroma, accompanied by infiltration of dendritic cells and inflammatory cells. Dense fibrous structures were observed in some PPG tissues. H&E staining results confirmed neovascularization and inflammatory cells in PPG tissues. In addition, H&E staining exhibited epithelioid tissue covering some PPG tissues. The immunofluorescence results demonstrated that the PPG epithelium was negative for K19, K10 and Muc5AC. Compared with the normal conjunctiva and pterygium, the expression of collagen IV in PPG basement membrane decreased, the expression of pan-cytokeratin (PCK), claudin 4 and E-cadherin in PPG epithelium was significantly lower, while the expression of vimentin, α-SMA and Snail was significantly increased. Therefore, our results suggest that the expression of epithelial keratin markers and goblet cell specific mucin marker is downregulated in the PPG tissues, and it likely is associated with the occurrence of EMT in granulomatous tissues.

1. Introduction

Granuloma formation after conjunctival surgery is pink tissue hyperplasia with large individual differences, potentially caused by chronic inflammation of the conjunctiva. Ophthalmic surgeries in treating pterygium, strabismus, eye trauma and eye reshaping are the major cause of conjunctival granulomas (Agraval et al., 2017; Espinoza and Lueder, 2005; Kokubo et al., 2016; Romano et al., 2016). Conjunctival granulomas can lead to obvious discomfort or even vision impairment in patients, and usually require further surgical interventions for resection. The incidence of PPG in patients with pterygium is much higher. The granuloma may propagate at the excision loci of the nasal pterygium and the conjunctival flaps. Up until now, there have been few studies on the formation and histological characteristics of PPG.

During or after the pterygium surgery, the intrusion of foreign bodies such as synthetic fiber (Farooq et al., 2011), surgical sutures and talc (Lyon and Taylor, 2007) into conjunctiva may lead to nodular conjunctival granuloma, bacterial and fungal infections at the surgical sites. Microbial infection may also cause purulent lesions in the conjunctival tissues, which results in pyogenic granuloma (Knox et al., 2003). Wu D et al. proposed that there is a correlation between the clinical features and the histological characteristics of granuloma (Wu et al., 2017). However, previous studies only focused on its observed clinical features and histomorphology. In the current study, we used in vivo confocal imaging and immunofluorescent staining to study the structural characteristics and biomarkers of the PPG tissue.

2. Material and methods

2.1. Patients

The study was performed in accordance with the ethical standards included in the Declaration of Helsinki. The study protocol was reviewed and approved by an independent ethics committee of the institution with written informed consent. Demographic and clinical data were obtained, including date of birth, gender, operation and medication information. Patients with ocular infections, trauma, contact lens

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Table 1
Profile of PPG patients.

NO	Age	Sex	Pterygium size in cornea (mm)	Surgical methods of pterygium treatment	Source of Conjunctival Graft	Pterygial Postoperative medical therapy	Interval from pterygium surgery to appearance of PPG (days)	Interval from appearance of PPG to excision (days)	Base diameter of PPG (mm)	Postoperative medical therapy (PPG)
1	43	M	1.0	PE and CT	Superior	Tob, AT	3	14	2.5	Pra, AT
2	43	M	4.0	PE and CT	Superior	Tob	5	16	2	Vid, AT
3	43	F	1.5	PE and CT	Superior	Tob	4	10	3	Tob, Pra
4	50	F	1.0	PE and CT	Superior	Tob	5	7	0.5	Tob, Vid
5	62	M	2.0	PE and CT	Superior	Tob, AT	7	15	4	Vid, AT
6	60	F	1.0	PE and CT	Superior	Tob	2	21	2.5	Tob, AT
7	44	F	2.0	PE and CT	Temporal	Tob	4	19	2	Pra, AT
8	45	F	2.5	PE and CT	Superior	Tob	7	26	3.5	Vid, AT
9	31	M	0.5	PE and CT	Superior	Tob, AT	6	25	3	Tob, Pra
10	36	F	3.0	PE and CT	Superior	Tob	5	5	1.5	Tob, AT
11	27	M	2.0	PE and CT	Superior	Tob	2	15	4	Vid, AT
12	36	F	3.5	PE and CT	Superior	Tob	7	10	3	Tob, Pra

Notes M, Male; F, Female; PE, Pterygium excision; CT, Conjunctiva Transplantation; Tob, Tobradex eyedrops; AT, artificial tears; Vid, Vidisic eyedrops; Pra, Pranoprofen eyedrops.

wear, diabetes, autoimmune disorders or allergy were excluded from the study. All examinations were carried out on the operated eye, using the same room at each visit and maintaining a constant environment.

2.2. Ophthalmologic examination

Every patient recruited in the study underwent a normative ophthalmologic examination involving slit lamp microscope and confocal laser-scanning microscopic evaluation. HRT III Confocal laser-scanning microscope was purchased from Heidelberg Engineering Inc (Baden-Wurttemberg, Germany), pre-installed with a built-in Heidelberg Eye Explorer version 1.5.10.0 software.

Before each examination, 0.5% proparacaine hydrochloride eye drops (Alcon. Inc, Puurs, Belgium) were dripped into the conjunctival fornix. A fixation light was used for the contralateral eye to keep good control over the eye to be examined. A disposable plastic cap was used to maintain a stable distance from the cornea to the microscope lens, and Carbomer gel as a coupling medium. The positioning and constant contact of the eye relative to the plastic cap was monitored by an accessory digital camera, set perpendicular to the eye being examined. Then the granulomas were imaged and recorded using the model of volume.

2.3. Materials and reagents

Antibodies used in this study: Mouse anti-cytokeratin 19 antibody (K19, M0888), cytokeratin 10 antibody (K10, M7002) and cytokeratin antibody (PCK, M3515) were obtained from Dako Cytomation (Copenhagen, CPH, DEN). Rabbit anti-collagen IV antibody (ab6586, Abcam), MUC5AC antibody (ab3649, Abcam) and alpha smooth muscle actin (α-SMA, ab5694, Abcam) were from Abcam (Los Angeles, CA, USA). Rabbit anti-vimentin antibody (HPA001762) was from Sigma-Aldrich Corp (St. Louis, MA, USA). Rabbit anti-E-cadherin (sc7870) antibody and mouse anti-claudin 4 (sc376643) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit anti-Snail (A5243) antibody was purchased from Abclonal Technology (Wuhan, China). Fluorescein Alexa-Fluor 488 and 594 conjugated secondary antibodies (goat anti-mouse or rabbit immunoglobulin [IgG]) were from Thermo Fisher Scientific (Waltham, MA, USA). Immunofluorescence mounting medium VECTASHIELD with DAPI (4,6-diamino-2-phenyl indole) was from Vector Laboratories (Vectorlabs, CA, USA).

2.4. Tissue sectioning

The PPG tissues were embedded in optimal cutting temperature compound (Sakura, CA, USA) after adjustment of their vertical planes parallel to the cutting plane, and 6 μm thick frozen sections were prepared using a cryostat microtome (Leica, NuBloch, Germany). Continuous sections were cut for each specimen.

2.5. Hematoxylin & eosin staining

Frozen sections stored in −80 °C were dried at room temperature for about 5–10 min and fixed in ice cold acetone for 5 min, then rinsed in running water for 3 min. Slides were hematoxylin stained for 5 min and washed several times in running tap water. Then the slides were stained in eosin for 30 s, followed by immersion in 75% alcohol + 2 ml hydrochloric acid for 1 s and rinsed in running tap water for 5 min. The slides were dehydrated by placing them consecutively in 80%, 95%, 100% alcohol for 2 min each time and finally immersed in xylene for 2 min. The air-dried slides were mounted using neutral resin and overlaid with a coverslip.

2.6. Immunofluorescent staining

The frozen sections were thawed and fixed in cold acetone for 10 min at −20 °C. The tissue was rehydrated with phosphate butter solution (PBS) for 5 min and blocked with 2% BSA to prevent non-specific binding. Then the specimens were incubated with the primary antibody or an isotype control (Sigma Chemical Co.) overnight at 4 °C. The sections were incubated with secondary antibody for 1 h at room temperature followed by 3 washes in PBS for 10 min. Again, the sections were washed 3 times and covered with VECTASHIELD (H-1200). Images were captured and stored using a fluorescence microscope (Leica, Wetzlar, Germany) equipped with corresponding software.

3. Results

3.1. Clinical evaluation

A total of 12 PPG patients from the Xiamen Eye Center of Xiamen University were recruited for this study. Their specifics are shown in Table 1. Patient average age was 43.3 ± 10.4 years, and the distance of the head of the pterygium extending onto the cornea was 0.5–4 mm. All the pterygium patients were subjected to surgical resection combined with conjunctival autograft transplantation. Granuloma occurred within one week after surgery (4.75 ± 1.81 days). The time interval between the granuloma occurrence and its resection was quite different (15.3 ± 6.7 days), and the granulomatous base diameter was 2.6 ± 1.0 mm before resection.