

# Ophthalmic Pterygium

## A Stem Cell Disorder with Premalignant Features

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**Pterygia are common ocular surface lesions thought to originate from limbal stem cells altered by chronic UV exposure. Traditionally regarded as a degenerative condition, pterygia also display tumor-like features, such as a propensity to invade normal tissue and high recurrence rates following resection, and may coexist with secondary premalignant lesions. This study was initiated to determine the rate of concurrent ocular surface diseases in patients with pterygia recruited from the practice of a single surgeon operating in a Sydney metropolitan hospital. One hundred pterygium specimens were histopathologically reviewed and selected cases were immunohistochemically assessed to confirm diagnosis. Along with previously documented typical features including epithelial proliferation, goblet cell hyperplasia, angiogenesis, inflammation, elastosis, stromal plaques, and Bowman's membrane dissolution, we identified five cases of ocular surface squamous neoplasia, six cases of primary acquired melanosis, two compound nevi (one suspect invasive melanoma), and one dermoid-like lesion. In 18 specimens, clusters of basal epithelial cells that coexpressed cytokeratin-15/-19 and p63- $\alpha$  were identified at the head of the pterygium, coinciding with clinical observation of Fuchs' flecks. Our data show that significant preneoplastic lesions may be associated with pterygium and that all excised pterygia should undergo histological examination. The presence of p63- $\alpha$ -positive epithelial cell clusters supports the hypothesis that pterygia develop from limbal epithelial progenitors. (*Am J Pathol* 2011, 178:817–827; DOI: 10.1016/j.ajpath.2010.10.037)**

Pterygium is a wing-shaped ocular surface lesion traditionally described as an encroachment of bulbar conjunctiva onto the cornea.<sup>1</sup> Historically, pterygia were considered degenerative lesions, exemplified by degradation of Bowman's layer and elastosis. Currently, however, pterygia are described as a proliferative disorder resembling an aberrant wound healing response.<sup>2</sup> Histopathologically, pterygia are characterized by a hyperplastic, centripetally directed growth of altered limbal epithelial cells accompanied by Bowman's layer dissolution, epithelial-mesenchymal transition, and an activated fibroblastic stroma with inflammation, neovascularization, and matrix remodeling, mediated through the concerted actions of cytokines, growth factors, and matrix metalloproteinases.<sup>2–9</sup> Despite advances in understanding of its pathogenesis, pterygium remains an ophthalmic enigma. Intriguingly, pterygia have a predilection for the nasal limbus and affect only humans, possibly reflecting the unique ocular morphology of humans, compared with nonhuman primates and other animals.<sup>10</sup> Although there is no consensus regarding the pathogenesis of pterygia, epidemiological evidence,<sup>11–14</sup> its association with sun-related disorders such as pinguecula and cataracts,<sup>15</sup> climatic droplet keratopathy,<sup>16</sup> and squamous cell and basal cell carcinomas,<sup>17,18</sup> together with our *in vitro* studies,<sup>5–8</sup> support the concept that UV radiation plays a major role in development of pterygium.<sup>19</sup> Furthermore, the limbal predilection may be explained by the phenomenon of peripheral light focusing, in which incidental light passes through the anterior chamber and is focused at the distal (nasal) limbus where limbal stem cells (LSCs) reside.<sup>20</sup>

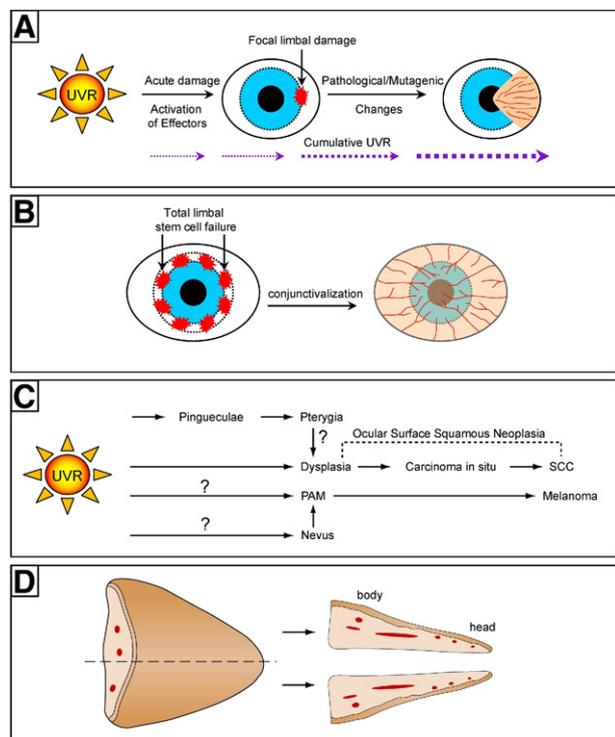
A healthy corneal surface is maintained by self-renewing, lineage-specific stem cells (SCs) that reside in the limbus, a narrow annular transition zone that circumscribes the cornea. This regenerative capacity is regulated by exquisite programs that govern stem cell quiescence, proliferation,

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**Figure 1.** The role of cumulative UV radiation exposure in pterygium development. **A:** Model for the pathogenesis of pterygium: focal limbal damage from UV radiation triggers migration of altered LSCs toward the central cornea. **B:** In total LSC deficiency, damage to the limbal niche or depletion of stem cell reserves results in conjunctivalization of the cornea from all directions. **C:** Model of how ocular surface squamous neoplasia and melanoma might arise from pterygia. **Question marks** with pathways indicate absence of direct supporting clinical or experimental evidence. **D:** Bisection and orientation of pterygium specimens as assessed in the current study.

migration, and differentiation. Failure to maintain a normal microenvironment as a result of extrinsic (eg, UV radiation) or intrinsic (eg, cytokines) signals can result in the development of ocular disorders.<sup>2-6,19,21,22</sup> The importance of an intact limbus and its stem cells was recognized four decades ago by Davanger and Evensen,<sup>23</sup> who proposed that pterygia represent a specific zone of LSC deficiency. Our hypothesis for pterygium development takes into account peripheral light focusing<sup>2,9,19,20</sup> at the nasal limbus, which activates and/or mutates LSCs, resulting in clonal expan-

sion, local cell proliferation, and invasion into the cornea (Figure 1A). Alternatively, focal UV radiation may destroy the LSC repository, which acts as a barrier that segregates cornea from conjunctiva, thereby opening the flood gates for conjunctival ingress and pterygium formation. Furthermore, an intrinsic weakness in the LSC reserves is implied by less prominent limbal palisades in the nasal and temporal limbus,<sup>24,25</sup> suggesting that these regions might be more susceptible to damage and less likely to undergo effective repair. An analogous mechanism may occur in patients with total LSC deficiency,<sup>26</sup> in which the absence of LSCs allows conjunctival invasion of the cornea to occur from 360 degrees (Figure 1B). In support of this posit, consecutive rounds of limbal excision affected wound healing, encouraged neovascularization, and promoted conjunctival ingress in rabbit corneas.<sup>27</sup>

Ophthalmologists have traditionally regarded pterygia as benign lesions, because they grow slowly. Unless a pterygium is sufficiently large as to obscure the visual axis or causes astigmatism, decisions to treat are often based on a patient's cosmetic concerns. An argument against this view, however, is the local invasiveness and high rate of recurrence when pterygia are inappropriately managed.<sup>28</sup> Current management strategies for pterygia involve surgical excision, followed by wound closure with grafts or by application of adjunctive therapy to the bare scleral bed.<sup>29,30</sup> Once excised, pterygia are commonly discarded without histological evaluation. This practice is not recommended, in the face of reported identification of unsuspected and potentially malignant secondary disorders in association with pterygia<sup>31-33</sup> (Table 1). These studies suggest that pterygia might have the propensity to evolve into precursors of squamous cell carcinoma and malignant melanoma of the ocular surface (Figure 1C).

In this study, we examined the histopathology of pterygia from patients treated by a single surgeon operating in a metropolitan hospital in Sydney, Australia. Histological features of pterygia and concurrent ocular diseases were recorded. All unusual cases were reviewed by an experienced anatomical pathologist and were further investigated by immunohistochemical methods. Additionally, we describe novel cell clusters in some pterygia that expressed putative LSC markers.

**Table 1.** Premalignant Ocular Disease Reported in Association with Pterygium and Pinguecula

References	Study population	Tissues	Sample size	Associated ocular disease	%
Sevel and Sealy <sup>31</sup>	Cape Town, South Africa	pterygia	n = 100	squamous cell carcinoma	12
Clear et al <sup>17</sup>	Malawi	pinguecula and pterygia	n = 167	carcinoma <i>in situ</i>	17
				hyperplasia or mild dysplasia	75.4
				moderate dysplasia	11.4
				carcinoma <i>in situ</i>	12.6
Erie et al <sup>34</sup>	Mayo Clinic, Minnesota	pterygia	n = 92	carcinoma <i>in situ</i>	9.8
Perra et al <sup>33</sup>	Ecuador	pterygia	n = 80	PAM without atypia	6.3
				PAM with atypia	2.5
				nevi	2.5
Hirst et al <sup>32</sup>	Queensland, Australia	pterygia	n = 533	OSSN	9.8

PAM, primary acquired melanosis; OSSN, ocular surface squamous neoplasia.

**Table 2.** Clinicodemographic Data for 100 Patients with Pterygia

Variable	Value
Age (years)	
Mean $\pm$ standard deviation	50 $\pm$ 15
Range	21–83
Sex (no. of cases)	
Male	62*
Female	35*
Eye (no. of cases)	
Right	45
Left	55
Location of lesion (no. of cases)	
Nasal	96
Temporal	4†
Type of lesion (no. of cases)	
Primary	59
Recurrent	41

\*Two females and one male had bilateral disease and pterygium surgery on separate occasions.

†All temporal pterygia were recurrent.

These cell clusters may provide the first histological evidence supporting the view that pterygium is a disease of stem cell origin.

## Materials and Methods

### Patients

Patients undergoing routine pterygium excision surgery by a single surgeon (M.T.C.) were recruited from Prince of Wales Hospital, Randwick, Sydney, Australia, and from the surgeon's private practice. Clinicodemographic features recorded included patient age and sex and location (nasal or temporal) and type of lesion (primary or recurrent) (Table 2). The study population was of mixed ethnic background, but the majority of patients were of European continental origin. Patients underwent routine ophthalmic examination and documentation of the pterygium, including anterior segment photography (iPIX camera; Designs For Vision, Ronkonkoma, NY) and *in vivo* confocal microscopy (HRT 3 Rostock cornea module; Heidelberg Engineering, Heidelberg, Germany). There were no clinical signs of dysplasia in any

of the patients, although one patient demonstrated obvious pigmentation in the head of his pterygium. All patients underwent pterygium excision with reconstruction of the resulting wound using an autologous free limbal-conjunctival graft.<sup>35</sup> Informed consent was obtained from each patient before tissue and data collection. This study was approved by the institutional Human Research Ethics Committee and adheres to the tenets of the Declaration of Helsinki.

### Histopathological Evaluation

Pterygia ( $n = 100$ ) were FFPE and oriented such that sections were cut longitudinally through the head and the body of the pterygium (Figure 1D). Sections (4  $\mu$ m) were stained with H&E, then evaluated by two experienced senior ocular scientists (J.C. and N.D.). Unusual, suspect, and atypical cases were reviewed by a pathologist (R.C.) to provide a histopathological diagnosis.

### Immunohistochemistry

Pterygia with atypical features were investigated further by immunohistochemistry. Tissues were stained with putative markers for melanocytes, LSCs, or cytokeratins (Table 3). Briefly, 4- $\mu$ m paraffin sections were dewaxed in xylene and rehydrated through a graded series of ethanol baths. Sections were subjected to antigen retrieval by heating in a microwave oven in 0.1 mol/L sodium citrate buffer (pH 6.0), followed by incubation in 3% H<sub>2</sub>O<sub>2</sub> in methanol to block endogenous peroxidase activity. After blocking in 20% normal goat serum in Tris-buffered saline (pH 7.6) for 30 minutes, sections were incubated overnight in primary antibody (Table 3) at 4°C. Tissues were next incubated in biotinylated goat anti-rabbit or goat anti-mouse IgG (1:200 dilution; Dako, Glostrup, Denmark) for 30 minutes, followed by streptavidin-conjugated horseradish peroxidase (1:100 dilution; Dako) for 1 hour at room temperature. Sections were thoroughly washed with Tris-buffered saline between each step. Immunoreactivity was visualized using 3-amino-9-ethylcarbazole (AEC; Sigma-Aldrich, St. Louis, MO), and nuclei were counterstained with Mayer's hematoxylin

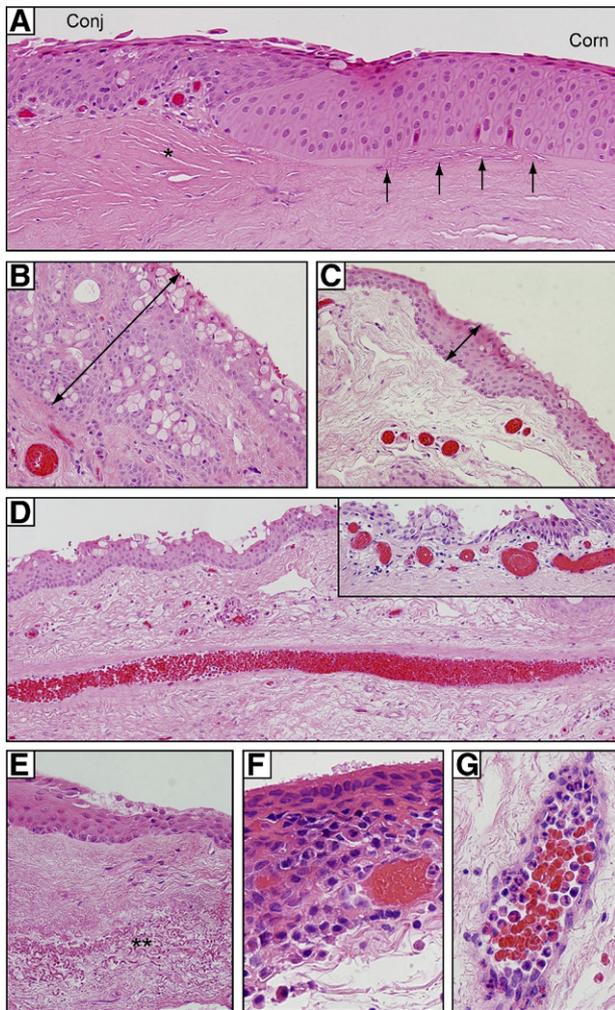
**Table 3.** Primary Antibodies Used for Immunohistochemistry

Targeted epitope	Antibody type	Host*	Clone	Manufacturer†	Catalog no.	Dilution
Human melanosome	IgG <sub>1</sub> , $\kappa$	M	HMB-45	DAKO	M0634	1:50
Melan A	IgG <sub>1</sub> , $\kappa$	M	A103	DAKO	M7196	1:100
S100B	IgG	R	—	DAKO	Z0311	1:900
P63 (pan)	IgG <sub>2a</sub>	M	4A4	DAKO	M7247	1:50
P63-alpha	IgG	R	—	CST	4892	1:20
Keratin-15	IgG <sub>2a</sub>	M	MS-1068	TFS	LHK15	1:150
Keratin-19	IgG <sub>1</sub>	M	4A36	USB	C9097-24B	1:150
Ki-67	IgG	R	—	TFS	RB-1510R7	1:200
Connexin 43	IgG <sub>1</sub> , $\kappa$	M	CX-1B1	Zymed	13-8300	1:100
Isotype	IgG <sub>1</sub>	M	—	DAKO	X0931	1:100
Isotype	IgG	R	—	DAKO	X0903	1:900

\*M, mouse; R, rabbit.

†DAKO, DakoCytomation; CST, Cell Signaling Technology; TFS, Thermo Fisher Scientific; USB, United States Biological; Zymed, Zymed Laboratories.

(Dako). Sections were mounted in aqueous mounting medium (Crystal Mount; Biomedica Corporation, Foster City, CA), then coverslipped in DPX mounting medium (VWR International, Poole, UK). For double-labeling, tissues were incubated in a mixture of primary antibodies (Table 3), followed by incubations in goat anti-mouse<sup>Alexa488</sup> and goat anti-rabbit<sup>Alexa594</sup> (Invitrogen, Carlsbad, CA) and counterstained with DAPI (0.3 μmol/L final). Sections were coverslipped in Vectashield anti-fade mounting medium (Vector Laboratories, Burlingame, CA), then imaged. Negative control reactions included tissues that were incubated with an isotype antibody instead of an epitope-specific primary antibody. Photomicrographs were taken with a DP70 digital camera system mounted on an Olympus BX51 microscope



**Figure 2.** Typical histological features of pterygia. **A:** In the advancing head of a pterygia, conjunctival-like epithelium (Conj) merges abruptly into corneal epithelium (Corn). The underlying Bowman's layer (arrows) is fragmented and precedes a fibrovascular stroma (asterisk). **B, C:** Goblet cell hyperplasia is apparent in pterygia (B), compared with donor-matched conjunctiva (C). Note the thickness of the epithelial layer (double-headed arrows in B and C). **D:** Prominent central feeder vessel; inset shows dilated subepithelial vessels. **E:** Elastotic changes (double asterisk) in pterygium stroma. **F:** Inflammatory infiltrates in the epithelium. **G:** Stromal vessels loaded with polymorphonuclear leukocytes. All sections were stained with H&E. Original magnification: ×200 (A and D); ×400 (B, C, D inset, and E); ×1000 oil emersion (F and G).

**Table 4.** Histological Findings in 100 Cases of Pterygia

Histological findings	Pterygia (no.)		
	Primary (n = 59)	Recurrent (n = 41)	Total
PAM without atypia	4	1	5
PAM with atypia with a subconjunctival nevus	1	0	1
Epidermolysis bullosa nevus	0	1	1
OSSN	3	2	5
Dermoid-like lesion	0	1	1
Plaques	4	2	6
Basal stem cell-like clusters	8	10	18

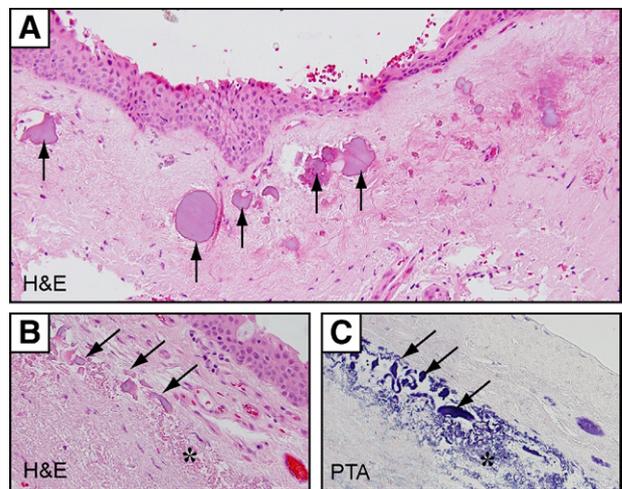
PAM, primary acquired melanosis; OSSN, ocular surface squamous neoplasia.

(Olympus; Sydney, Australia) and processed with Photoshop version 9 (Adobe Systems, San Jose, CA).

**Results**

*Typical and Common Histopathological Findings in Pterygia*

Common histological features observed included a proliferative and locally invasive front of pterygium epithelium that abruptly transitioned into corneal epithelium at the advancing edge (Figure 2A). At the junction between the pterygium epithelium and normal cornea, the stroma was often characterized by feeder blood vessels (Figure 2A, asterisk) that preceded the fibroblastic stroma. The advancing pterygium edge was demarcated by a fragmented Bowman's layer (Figure 2A, arrows). Goblet cell hyperplasia was prominent in pterygium epithelium (Figure 2B), compared with autologous normal conjunctiva (Figure 2C). Feeder vessels extending the length of the lesion were regularly noted (Figure 2D), as well as sub-



**Figure 3.** Stromal plaques in pterygia stained with H&E (A and B) or phosphotungstic acid (PTA) (C). Irregular-shaped stromal plaques (arrows) with an amorphous appearance were frequently associated with elastotic changes (asterisk). Plaques appear lilac in H&E-stained sections and deep blue in PTA-stained sections. Elastin fibers also stained blue with PTA. Original magnification: ×200 (A); ×600 (B and C).

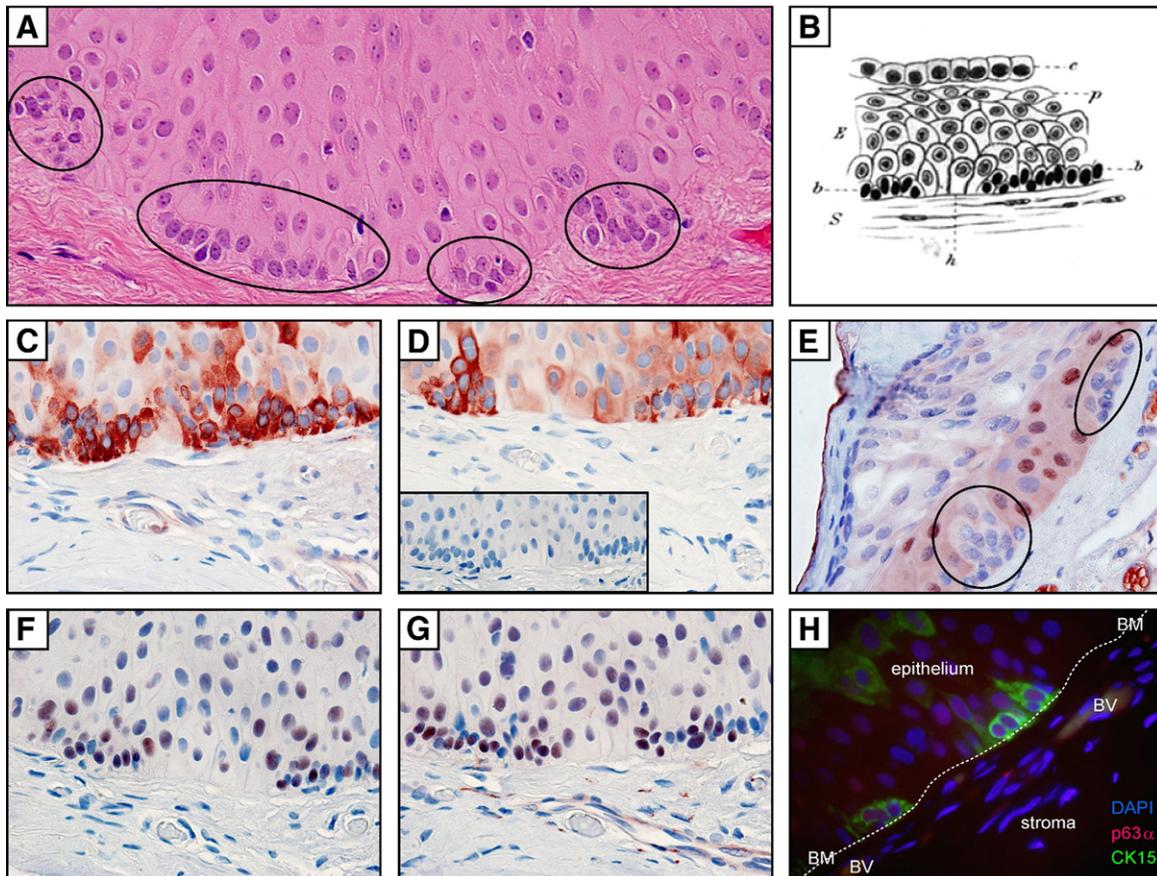
epithelial neovascularization (Figure 2D, inset). Stromal elastosis (Figure 2E, double asterisk) and both intra- and subepithelial (Figure 2F) and intravascular inflammation were present in 60% of cases.

### Uncommon and Novel Histopathological Findings in Pterygia

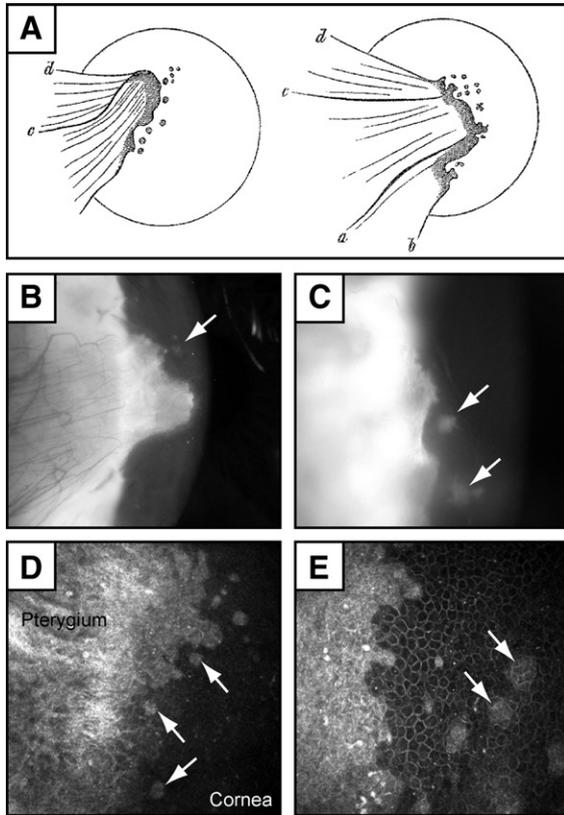
Uncommon histological features included basophilic stromal plaques within the pterygium body in 6% of cases (Table 4) that localized to elastotic zones (Figure 3, arrows). These plaques varied in size and shape, and were generally lilac in color after H&E staining (Figure 3, A and B, arrows) or dark blue when stained with phosphotungstic acid (Figure 3C, asterisk). However, we could not identify their composition further with other histological stains, including tetrachrome, Safranin O, von Kossa stain, or alizarin crimson (data not shown).

Small clusters of basal cells were observed in 18% of our pterygium specimens. Cells within these aggregates were smaller, had increased nuclear-to-cytoplasm ratio, consisted of 8 to 15 cells anchored to the basement membrane, and were invariably associated with corneal-

like epithelium near the head of the pterygium (Figure 4A, ovals). Morphologically, these cells appeared primitive and less differentiated than their suprabasal counterparts, suggesting that they may be stem cell like. Such observations prompted us to partially phenotype these cells by immunostaining, using well-accepted markers of LSCs. Indeed, cells within these microclusters demonstrated immunoreactivity to CK-15, CK-19, p63 (pan), and p63 $\alpha$  (Figure 4, C, D, F, and G, respectively) and were double-immunoreactive to CK-15/p63 $\alpha$  (Figure 4H) and CK-19/p63 $\alpha$  (not shown), but lacked immunoreactivity to Ki-67 (proliferation marker) (Figure 4E) or Cx43 (gap junction protein) (data not shown). Our findings suggest that, although these cells are not proliferating (lacked Ki-67 expression), they retain proliferative potential (strong p63 $\alpha$  expression) and may become activated when appropriate signaling mechanisms are initiated during pterygium development. These cell clusters were documented by Ernst Fuchs<sup>36</sup> more than a century ago in his seminal article “*Ueber das Pterygium*” at both a microscopic (Figure 4B) and macroscopic level (Figure 5A) as small spots or flecks at the head of pterygia. Commonly known as Fuchs’ flecks (or Fuchs’ patches, Fuchs’ islets), these pterygium cell clusters



**Figure 4.** Stem cell microclusters in pterygium tissue. **A:** H&E-stained section of a pterygium illustrating multiple mini-aggregates of basal epithelial cells (ovals). **B:** Pterygium epithelium as illustrated by Ernst Fuchs<sup>36</sup> in 1892. **C–G:** Pterygium immunolabeled with CK-15, CK-19, Ki-67, p63 (pan), and p63 $\alpha$  (C–G, respectively), using an indirect immunoperoxidase method with 3-amino-9-ethylcarbazole chromogen; red denotes positive labeling. Staining is absent in sections incubated with control IgG (D, inset). **H:** Indirect immunofluorescent double-labeling of pterygium epithelial cell clusters with CK-15 (green), p63 $\alpha$  (red), and counterstained with DAPI (blue); note basement membrane (BM, indicated by dotted line) and blood vessels (BV). Original magnification,  $\times 1000$  oil emersion (all photomicrographs). Image B is reproduced with permission from Springer (original publication: Fuchs E. Ueber der Pterygium. Graefes Archiv Ophthalmol 1892, 38:1–89).



**Figure 5.** Clinical appearance of Fuchs' flecks in pterygia. **A:** Original illustrations by Ernst Fuchs<sup>36</sup> show small spots or *flecken* in the cap region at the head of pterygia. **B–E:** Pterygia with Fuchs' flecks (**arrows**) under slit lamp (**B** and **C**) and *in vivo* confocal microscopy (**D** and **E**). Slit lamp photographs were taken with an iPIX camera (Designs For Vision, Ronkonkoma, NY) and confocal micrographs with an HRT 3 Rostock cornea module (Heidelberg Engineering, Heidelberg, Germany). Confocal images were taken with a 63× objective. The field of view is 400µm × 400µm for (**D**) and 300µm × 300µm for (**E**). Image **A** is reproduced with permission from Springer (original publication: Fuchs E. Ueber der Pterygium. Graefes Archiv Ophthalmol 1892, 38:1–89).

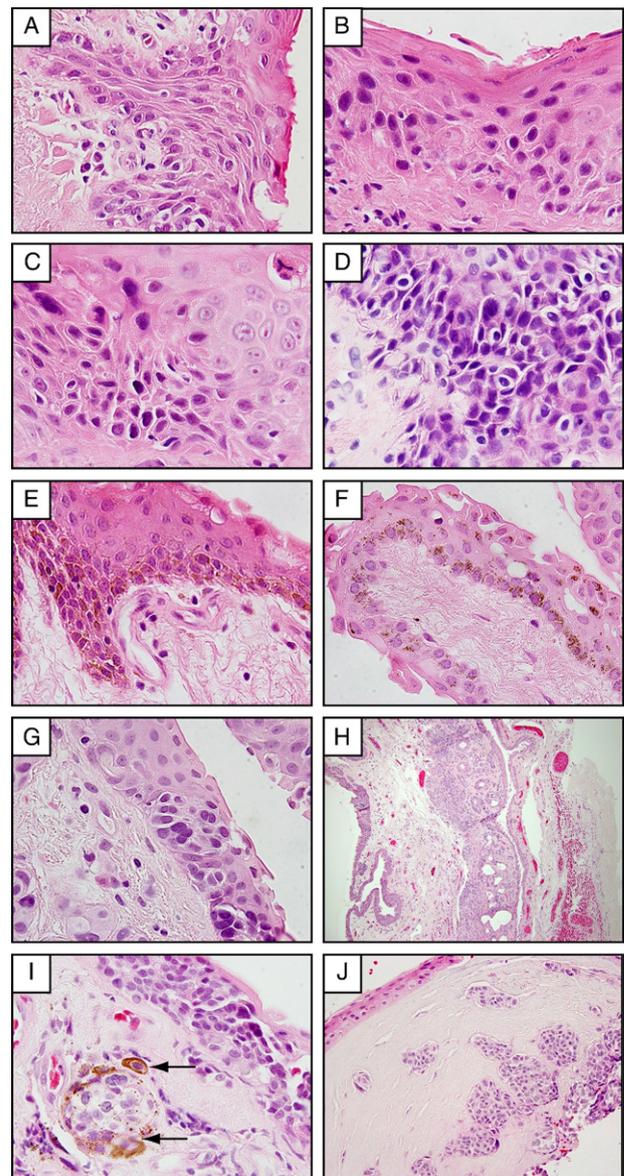
could be visualized in our patients by slit-lamp examination (Figure 5, B and C) and by confocal microscopy (Figure 5, D and E).

### Atypical Histopathological Findings in Pterygia

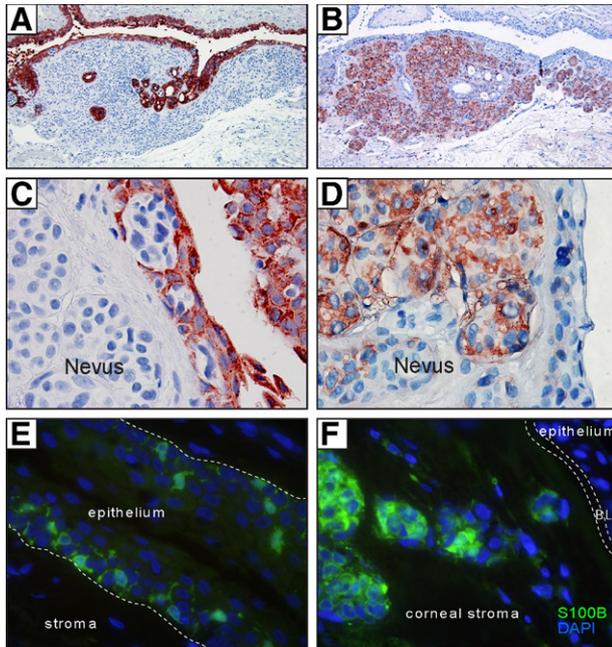
Atypical epithelial and melanocytic lesions were identified in 12% of pterygia (Figure 6 and Table 4). Rete ridge-like down-growths were occasionally noted in the hyperplastic epithelium (Figure 6A). Foci of epithelial dysplasia ranging from mild to moderate to severe (Figure 6, B, C, and D, respectively) were observed in pterygium epithelium, where cells displayed increased nuclear-to-cytoplasmic ratio and loss of polarity affecting the basal (Figure 6B), the suprabasal (Figure 6C), or the entire epithelium (Figure 6D). Nonetheless, the metastatic potential of these lesions is low, given that they do not breach the basement membrane (Figure 6, A–D).

Melanocytic lesions were identified in 7% of our samples, which included racial pigmentation (7%) (Figure 6E), primary acquired melanosis (PAM) without (5%) and with atypia (1%) (Figure 6, F and G, respectively), and two cases of conjunctival nevi (Figure 6, H–J). One nevus

extended from the pterygium body into the corneal stroma, with well-circumscribed nonpigmented melanocytic nests found at the surgical margin (Figure 6, I and H). Nonpigmented nevi may be difficult to detect clinically, especially if masked by an inflamed pterygium. CK-19 staining was therefore used to demarcate pterygium epithelium, distinct from the underlying symmetrical nevus (Figure 7A). Under higher magnification, CK-19-negative melanocytes were identified as uniform, rounded, occasionally displaying hyperchromatic nuclei, and generally confined to cohesive epithelioid nevocytic



**Figure 6.** Atypical histological features in pterygia included pterygium epithelium demonstrating papillary folding (**A**) and dysplastic changes ranging from mild (**B**) to moderate (**C**) to severe (**D**). Melanocytic lesions included racial pigmentation (note layers of pigmented basal melanocytes) (**E**), PAM without atypia (**F**), PAM with atypia (**G**), subconjunctival nonpigmented compound nevus (**H**), and a nonpigmented nevus (**I**) from a patient with epidermolysis bullosa that invaded the corneal stroma (**J**). **Arrows** in **I** point to melanophages that have acquired pigmented granules and surround a melanocytic nest. Sections were assessed after H&E staining. Original magnification: ×1000 oil emersion (**A–G** and **I**); ×100 (**H**); ×400 (**J**).



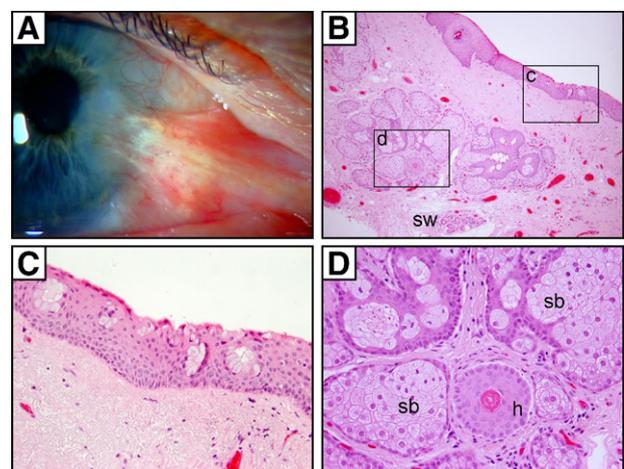
**Figure 7.** Cytokeratin and S100B staining of nevi in pterygia. Indirect immunoperoxidase (A–D) and immunofluorescence (E and F) techniques demonstrate CK-19 immunoreactivity in pterygium epithelium (A and C) or S100B immunoreactivity in nevus melanocytes (B, D, and F) and in dendritiform cells scattered within the epithelium (E). In panels A–D, positive labeling is denoted by red color (from 3-amino-9-ethylcarbazole chromogen) and nuclei counterstaining in blue (hematoxylin). In panels E and F, S100B expression is denoted by green immunofluorescence and DAPI counterstaining in blue. In E, the hatched line indicates the pterygium basement membrane; in F, it indicates Bowman’s layer (BL). Original magnification:  $\times 100$  (A and B);  $\times 1000$  oil emersion (C–F).

nevi without pagetoid spread (Figure 7C). When samples were labeled with melanocytic marker S100B, stromal-epithelial junctional involvement became more obvious (Figure 7, B and D). Similar staining patterns were observed with other melanocytic markers, such as melan A and HMB-45 (data not shown). S100B-positive cells were also detected within the pterygium epithelium; however, in contrast to the epithelioid melanocytes within the nevus, these intraepithelial cells were dendritiform in morphology (Figure 7E). The second nevus displayed cell invasion of the epithelium (pagetoid spread; not shown) with prominent and lateral nonpigmented melanocytic spread. This specimen, although diagnosed as a benign lesion, was viewed as a suspect invasive melanoma, because of the spread of S100B-positive melanocytic nests from the main disease foci within the conjunctiva into the corneal stroma (Figure 7F).

Last, we identified cutaneous features within a recurrent pterygium from a 67-year-old man (Figure 8). This unusual wing-shaped lesion had the macroscopic appearance of a typical pterygium, except that hairs were visibly growing out of the body (Figure 8A). On microscopy, we observed a nonkeratinizing squamous epithelium with goblet cells adjacent to well-differentiated cutaneous elements such as hair follicles and sebaceous and sweat glands (Figure 8, B–D). These features are reminiscent of limbal dermoids,<sup>37</sup> but this patient had no history of such lesions before he developed a pterygium.

## Discussion

By standardizing the orientation of our specimens (Figure 1D), we identified several previously documented common, uncommon, and novel histopathological features in pterygia. Common findings included a prominent migratory front of actively proliferating and locally invasive epithelium with evidence of Bowman’s layer dissolution (Figure 2), which we have previously shown to be mediated by the activity of UV-induced matrix metalloproteinase.<sup>3,6</sup> Other features included a reactive fibrovascular stroma with evidence of epithelial-mesenchymal transition,<sup>38</sup> elastosis, and intravascular, subepithelial, and intraepithelial leukocyte infiltration (Figure 2), likely mediated through UV-induced cytokines and growth factors.<sup>2,9</sup> As in previous studies, leukocyte infiltrates in our pterygium specimens were neutrophils, T cells, plasma cells, macrophages, and mast cells.<sup>39,40</sup> The stroma comprising an altered extracellular matrix has also been studied and shown to consist of excess deposits of collagens, heparin sulfates, versican, laminin, and fibronectin.<sup>41,42</sup> In addition, we also observed foci of amorphous basophilic material in some pterygia, which were frequently associated with elastotic changes within the stroma. Plaques and associated elastotic changes appeared blue after treatment with phosphotungstic acid, suggesting that they may have a similar chemical composition (Figure 3C). Although these deposits have been documented by others,<sup>43,44</sup> their composition and precisely how they form are still unknown. Stromal plaques have also been described in climatic droplet keratopathy,<sup>45</sup> and it has been hypothesized that these plaques may be derived from UV-denatured plasma constituents.<sup>46</sup> Proteomic analysis of stromal plaques found in climatic droplet keratopathy revealed that they were rich in annexin A2 and glyceraldehyde 3-dehydrogenase.<sup>47</sup> We can only speculate that the stromal plaques in pterygia may be similar in composition to those reported in climatic droplet keratopathy,



**Figure 8.** Cutaneous elements in a recurrent pterygium. Clinical image of a pterygium with hairs growing from the body (A) and its corresponding H&E-stained paraffin sections (B–D). The regions encompassed by the rectangles (c and d) in panel B are magnified in panels C and D, respectively. Goblet cell clusters within the conjunctival portion of the pterygium (C) overlie cutaneous elements (D) such as sebaceous glands (sb), hair follicles (h), and sweat glands (sw). Original magnification:  $\times 100$  (B);  $\times 400$  (C and D).

given that both diseases are strongly associated with UV exposure.<sup>16</sup> One reason for their presence in only a proportion of specimens might be related to the stage of pterygium development and amount of cumulative UV exposure.

Controversy also surrounds the origins of the osmophilic elastoid bands within the pterygium substantia propria. Although it was originally described as actinic elastosis (elastotic degeneration of collagen) through the actions of UV radiation, because similar changes have been described in skin exposed to solar radiation,<sup>48</sup> Austin et al<sup>44</sup> now claim that elastodysplasia plays a role through excess production of elastin derived from UV-activated or injured fibroblasts. Evidence to support this theory was presented by Wang et al,<sup>49</sup> who detected mutations in the 3'-untranslated region of tropoelastin in pingueculae and UV-irradiated conjunctival fibroblasts.

The concept that pterygia arise from LSCs has been a topic of discussion for decades. In a seminal article in 1971, Davanger and Evensen<sup>23</sup> concluded that "a failure in the limbal structure may be the cause of pterygia." Some 20 years later, Tseng et al<sup>26</sup> speculated that pterygia represent a focal zone of LSC dysfunction (Figure 1, A and B). Our model of chronic focal UV radiation to the limbal margin<sup>50</sup> provides an explanation for how LSCs and their niche might be damaged, and Dushku et al<sup>51</sup> postulated that pterygia arise from altered limbal basal epithelial cells that took on a migratory phenotype.

More than a century ago, Fuchs<sup>36</sup> identified small clusters of conjunctival-like basal cells dispersed among the corneal-like epithelium in pterygia (Figure 4B). Such islands of pterygium epithelial cells were also reported in our previous work.<sup>9</sup> In the present study, we further characterized these small, morphologically primitive cells as resembling LSCs through their coexpression of CK-15, CK-19, and p63 $\alpha$  (Figure 4H) and localized them to the advancing head of the pterygium. In location and size, they are similar to Fuchs' flecks,<sup>36</sup> which can be visualized by slit lamp and by *in vivo* confocal imaging (Figure 5). Our observations support the notion that pterygium is a disease of LSCs, and the presence of these cell clusters may account for the clinical behavior of pterygium. We hypothesize that these primitive cell clusters may be activated to proliferate, forming an invasive pterygium (Figure 1A).

It is unclear why pterygium epithelial cell clusters are present in only ~20% of our specimens, but we suspect that i) some may have escaped detection, because their small size makes them difficult to identify without serial sectioning, or that ii) their presence may be associated with actively growing pterygia, but it is our normal practice to resect lesions when they are quiescent. Further studies are also required to establish if pterygium epithelial cell clusters are functionally, phenotypically, or genetically altered as a result of UV radiation.

Finally, within a clinically apparent pterygium, we found cutaneous elements (hair follicles, sebaceous glands, and sweat glands) adjacent to goblet cells in pterygium epithelium (Figure 7). This unusual finding supports the notion that stem cells within the limbus might

retain the capacity to differentiate into skin-like tissue. The precise mechanism for this reprogramming is unclear, but we speculate that UV radiation may be the trigger, because we have recently noted the development of cutaneous elements in corneas of UV-irradiated mice (unpublished observation). Alternatively, these cutaneous elements might have originated from the caruncle, which is known to contain hairs and sebaceous and sweat glands, as well as lacrimal glands and goblet cells.<sup>52</sup>

Ocular surface squamous neoplasia (OSSN) is a spectrum of diseases that ranges from epithelial dysplasia to carcinoma *in situ* to squamous cell carcinoma.<sup>53,54</sup> It is commonly found in elderly men with a history of chronic UV exposure.<sup>55</sup> Increased incidence of OSSN has also been reported in HIV-positive individuals, with OSSN showing a female predominance and sometimes presenting at a younger age.<sup>56,57</sup> As with pterygia, UV exposure is thought to contribute to the pathogenesis of OSSN.<sup>55,58</sup> We observed mild to severe epithelial dysplasia in 5% of our pterygia, but no carcinoma *in situ* or squamous cell carcinoma. This is in contrast to studies from Malawi and South Africa,<sup>17,31</sup> where >29% of pterygia had concurrent OSSN (Table 1), leading to suggestions that tumors may arise from preexisting lesions (such as pterygia). Recently, Hirst et al<sup>32</sup> noted the full spectrum of OSSN in ~10% of their pterygia from Brisbane, Australia (approximately double the rate of our Sydney cohort). One possible explanation for this disparity within an Australian population is greater UV exposure in Brisbane, compared with Sydney, because Brisbane is closer to the equator (latitude 27°28' S versus 33°51' S). Increased UV exposure may also explain higher OSSN rates in pterygia in African populations, but racial factors (continental origin) and concurrent HIV infections might also play a role. The relationship between pterygia and neoplasms is not completely understood, but UV-induced mutations in tumor suppressor genes may play a role.<sup>19</sup> The tumor suppressor protein p53 is responsible for regulating cell-cycle arrest<sup>59</sup> and is hypothesized to be mutated in pterygia, based on its overexpression in pterygium tissues.<sup>60-62</sup> In OSSN, p53 is similarly overexpressed,<sup>63</sup> and signature UV-induced mutations (CC to TT transitions) have been detected in these lesions.<sup>64</sup> Whether p53 mutations are present within our newly identified pterygium cell clusters remains to be investigated. In one study, no *TP53* mutations were observed in pterygium specimens after laser-capture microdissection of the entire epithelium.<sup>65</sup>

Primary acquired melanosis of the conjunctiva is a clinical classification of melanocytic lesions that falls between nevus and invasive melanoma. PAM occurs predominantly in middle-aged persons of European continental origin, but can be present in patients as young as 15 years of age.<sup>66</sup> PAM is further grouped into lesions with and without cytological atypia, and it may account for 11% of all conjunctival tumors.<sup>67,68</sup> Histological grading of PAM is important in its management, because PAM with atypia progresses to melanoma,<sup>66</sup> and lesions with epithelioid features have been associated

with invasion and metastasis,<sup>68</sup> but PAM without atypia do not progress to melanoma.<sup>66</sup> In the present series of 100 pterygia, we observed seven cases of racial pigmentation, six cases of PAM (with and without atypia), and two cases of nevi (one patient had a history of cutaneous melanoma; the other had epidermolysis bullosa). These frequencies are comparable to those in a study by Perra et al,<sup>33</sup> who reported seven cases of PAM and two cases of nevi in a series of 80 pterygia. These numbers, although small, are of concern for the ophthalmologist, because PAM with atypia is considered a premalignant condition and 13% to 50% of PAM cases may progress to melanoma,<sup>66,69</sup> a potentially fatal disease if undetected. Shields et al<sup>67</sup> reported that 13% of patients with PAM had bilateral disease. In PAM with atypia, the mean interval for melanoma development is 39 months.<sup>66</sup> Therefore, close monitoring by clinicians with longer follow-up periods are required in patients with pterygia in which PAM with atypia has been identified.

Amelanotic melanoma and PAM of the conjunctiva are rare, but cases have been reported.<sup>70,71</sup> The nevi in our case series were not pigmented. These lesions are clinically and histologically difficult to diagnose. Immunohistochemical markers such as S100B, HMB-45, and melan A may help identify and differentiate between benign and malignant melanocytic lesions. In particular, HMB-45-positive staining is reported to be associated with atypia and is recommended for differentiating between PAM with and without atypia.<sup>72,73</sup> The Wilms tumor gene<sup>74</sup> and Ki-67 staining<sup>73</sup> may be used to distinguish between benign and malignant nevi. In our series, all melanocytes labeled intensely with S100B, whereas the other melanocytic markers, HMB-45 and melan A, had a similar distribution but with less intense staining. The nevus associated with epidermolysis bullosa was faintly immunoreactive to HMB-45, but did not stain for Ki-67 (data not shown), suggesting that it might be a benign nevus. Nonetheless, long-term follow-up is required given its location at the surgical margin within the corneal stroma.

In summary, we have provided evidence that pterygium is a disease of stem cells in which clusters of pterygium epithelial cells expressing putative limbal stem cell markers correspond to Fuchs' flecks at the head of the pterygium. Our study also showed that preneoplastic diseases (such as PAM with atypia and OSSN) may coexist with pterygia, and we speculate that cumulative genetic damage from chronic UV exposure may be a shared etiology between these conditions. These preneoplastic conditions could remain undiagnosed if the excised pterygium is discarded, and we recommend that all pterygia be subjected to thorough histological evaluation. Incomplete excision of PAM with atypia or OSSN is of concern, given the potential for transformation and recurrence.<sup>66,75</sup> In such cases, use of topical chemotherapeutic agents,<sup>76</sup> such as interferon  $\alpha$ -2b alone<sup>77</sup> or in combination with retinoic acid,<sup>78</sup> is proving to be highly efficacious and may be useful in the treatment of residual disease. Annual follow-up for the remainder of the patient's life is advisable.<sup>66,75</sup>

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