

Review

Ischemic tissue injury and progenitor cell tropism: significant contributors to the pathogenesis of pterygium

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Summary. Pterygium is a common ocular surface disease characterized by triangular wing-like growth consisting of subconjunctival hypertrophic connective tissue. Pterygium is easily complicated by adhesion to the eyelid and diplopia related to motility restriction of the eyeball. Beyond the cosmetic problems, this condition has a catastrophic effect on quality of life. Post-surgical recurrence rates of pterygium excision have been reported to be very high. Therefore, identifying the distinct pathogenic pathways of the disease may lead to new therapeutic strategies with lower risk of treatment failure. Based on the relatively low vascularity and known-predominance of disease occurrence in the nasal conjunctiva of normal eyes, we proposed that hypoxic ischemic injury can elicit the development of pterygium. Here, we review hypoxia-inducible factor (HIF)-1 α -induced activation of the stromal cell-derived factor-1 (SDF-1)/chemokine receptor type 4 (CXCR4) signaling pathway as a possible mechanism. Supporting this concept of pathogenic mechanism, we also highlight bone marrow-derived progenitor cell tropism as a main contributor to pterygium pathogenesis.

Key words: Pterygium, Hypoxia, HIF-1 α , SDF-1, CXCR4, Progenitor cell

Introduction

Pterygium is a common ocular surface disease characterized by triangular wing-like growth of subconjunctival hypertrophic connective tissue and overlying conjunctival epithelium. The severity of disease varies from mild hyperemia to severe, gross fibrovascular scarring associated with adhesion to the eyelid (symblepharon), which induces diplopia and/or decreased vision (Fig. 1). The major environmental factor for pterygium generation is exposure to ultraviolet (UV) light. Therefore, pterygium has a worldwide distribution more common in peri-equatorial latitudes 37° north and south of the equator, forming a so-called 'pterygium belt' (Krachmer et al., 2011). In addition, numerous studies have implicated other possible pathogenic mechanisms such as oxidative stress (Shimoda et al., 1994; Tsai et al., 2005; Perra et al., 2006), immune reaction (Ioachim-Velogianni et al., 1995; Beden et al., 2003), inflammation (Di Girolamo et al., 2002; Chiang et al., 2007; Tong et al., 2008), growth factors (Kria et al., 1998; Maini et al., 2002; Jin et al., 2003; Solomon et al., 2003; Nolan et al., 2004; Wong et al., 2006), extracellular matrix modulation (Wang et al., 2000; Di Girolamo et al., 2003, 2005; Naib-Majani et al., 2004), and genetic modifications (Kim et al., 1998; Thum et al., 2008; Liu et al., 2010; Riau et al., 2011; Chien et al., 2013; Engelsen et al., 2013). However, pterygium is still an enigmatic disorder whose pathophysiological mechanism is debated to be degenerative (Vass and Tapaszto, 1964; Ansari et al., 1970; Austin et al., 1983) or proliferative (Clear et al., 1979; Greenblatt et al., 1994). Although the

pathogenesis of pterygium seems multi-factorial, the mechanism of the initial phase of conjunctival change to be pterygium is uncertain at present. Investigating the pathologic mechanism of pterygium development, especially during the early phase, may help in the development of medical treatment methods that prevent the future burdens for surgical intervention.

Pterygium is more commonly found nasally than temporally because UV light reflects against the nasal dorsum and focuses on the nasal conjunctival surface. Interestingly, the vascularity of the nasal bulbar conjunctiva is lower than that of other areas of the conjunctiva (superior, inferior and temporal conjunctiva) in normal human eyes (Ha and Kim, 2006) (Fig. 2). Such a relatively low density of vasculature in the nasal area may make normal conjunctiva more vulnerable to hypoxic damage by external stimuli, resulting in structural vessel deformation. As adaptive responses for regeneration, angiogenesis and cell proliferation inevitably occur after hypoxic injury. Among others, hypoxia-inducible factor-1 (HIF-1) is expressed as a representative cellular hypoxic response and targets hypoxia-related genes, including vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-beta1 to promote fibrovascular modulation that is linked to scarring (Phillips et al., 1995; Scheid et al., 2000; Lario et al., 2003). Tissue repair after injury also involves stem cell components (Kollet et al., 2003). HIF-1 from endothelial cells results in *in vivo* stromal cell-derived factor-1 (SDF-1) expression in ischemic tissues and further mediates recruitment of circulating bone

marrow-derived CXCR4 (the main receptor of SDF-1)-expressing progenitor cells to the sites of injury (Ceradini et al., 2004).

In this review, we describe a pathologic mechanism of pterygium development involving ischemic tissue damage, focusing on the early phase. Furthermore, we highlight progenitor cell tropism to the site of pterygia that originates from normal tissue injury and related hypoxic damage.

Ischemic factors in early pathogenesis of pterygium development

After many studies revealed biological evidence of cellular mitogenicity, pterygium has been considered as a form of tumorigenic mimicry. Recently, for example, angiogenin, a representative angiogenic protein originally isolated in colon adenocarcinoma (Fett et al., 1985), was reported to be expressed highly at both mRNA and protein levels in stromal fibroblasts of severe pterygia (Kim et al., 2013a). In carcinoma, there is a scar-like fibrotic focus in the center of a tumor mass, which is known to be associated with high-grade metastasis and poor survival in breast, lung, pancreas, and colon cancer (Hasebe et al., 1998, 2002; Nishimura et al., 1998; Maeshima et al., 2002; Couvelard et al., 2005; Kornegoor et al., 2012). Because the fibrotic focus is centrally located, it is often in a hypoxic state that aggravates tumor progression via HIF-1alpha expression (Van den Eynden et al., 2005). Similarly, pterygium may arise from pinguecula, a hyperkeratotic conjunctival

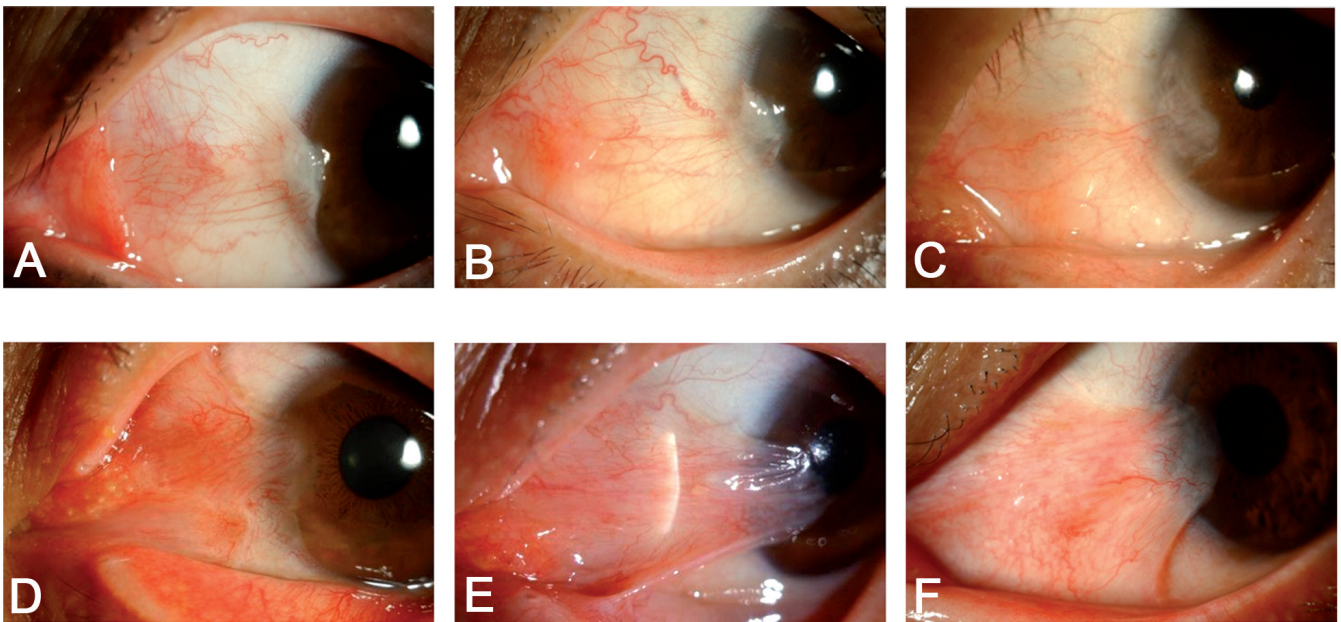


Fig. 1. Photographs of pterygia with diverse morphological appearances. In primary simple pterygia (A-C), there is noted mild hyperemia with scarce fibrous stromal tissue. On the contrary, in severe and recurrent cases (D-F), prominent conjunctival vascularization with vessel engorgement is found. Adhesion between the eyelid and eyeball is noted (D, E), and there is relatively thick and fleshy subconjunctival fibrous scarring (F).

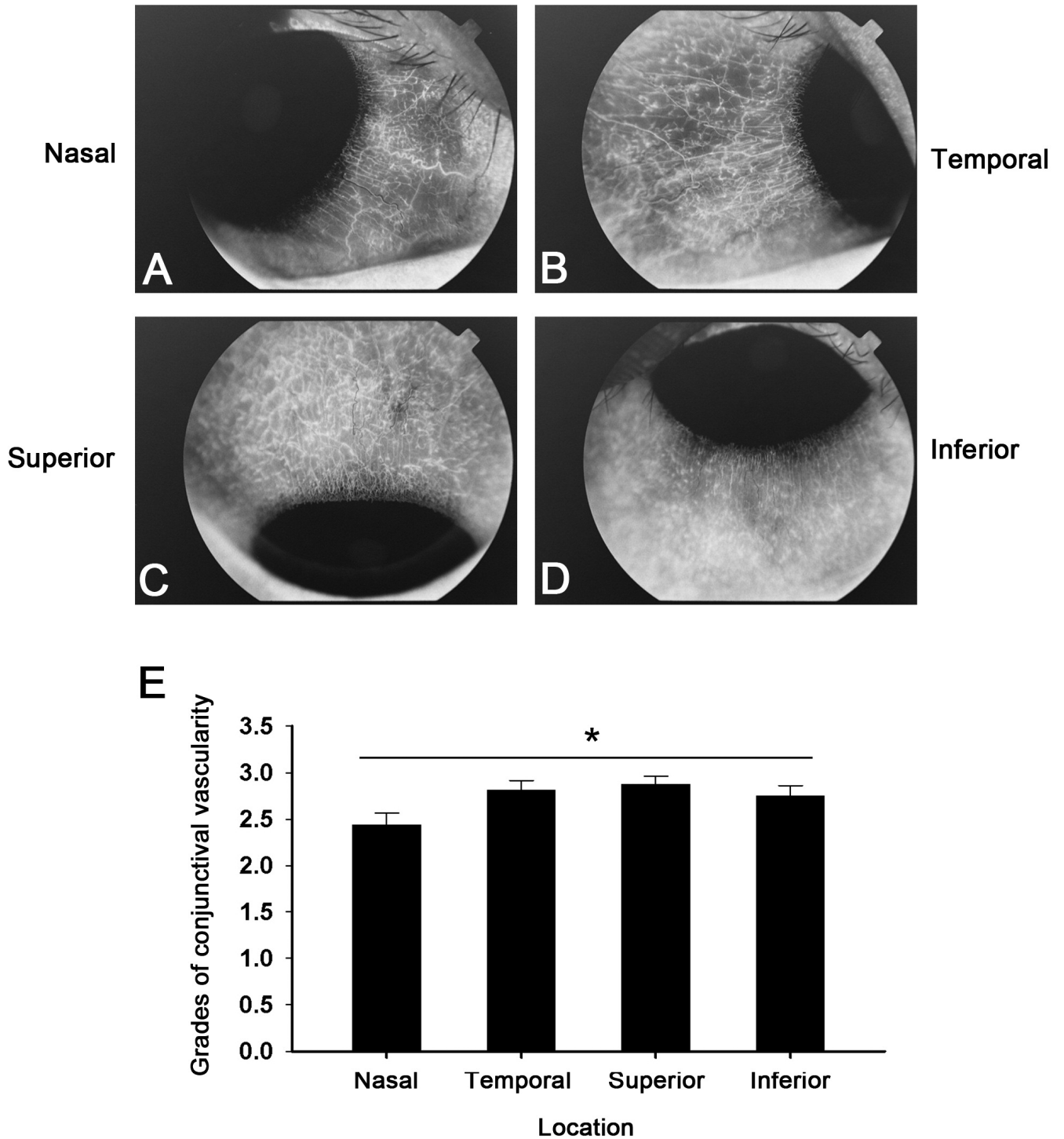


Fig. 2. The grades of conjunctival vascularity in four cardinal directions in normal eyes. **A-D.** Representative photographs of anterior segment fluorescein angiography in a normal human right eye. Compared to temporal (**B**), superior (**C**), and inferior (**D**) areas, the vascularity of the nasal conjunctiva (**A**) is relatively low. **E.** Grades of vascularity are significantly different according to location (* $p < 0.05$), with the lowest grade noted at the nasal area. (Original photos are reprinted from Ha and Kim (2006) with permission from the Journal of Korean Ophthalmological Society).

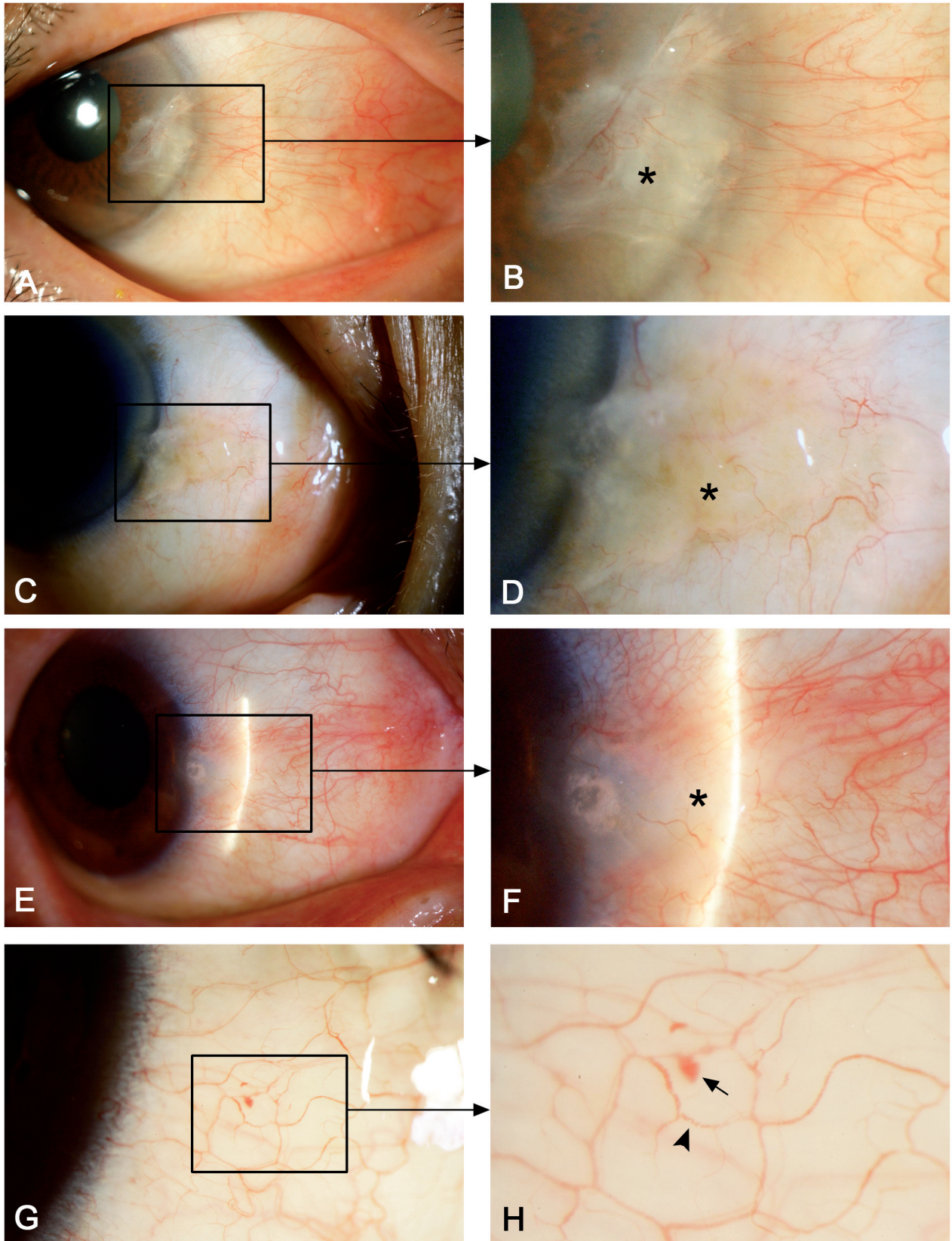
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Fig. 3. Characteristic findings of early pterygia. **A, C, E.** In early pterygia, there is a focus of elastotic degeneration (rectangle) with low vascularity. In high magnification photos (**B, D, F**), tortuosity of vessels and vascular attenuations are noted adjacent to the avascular pinguecula-like lesion (asterisks). As additional features of early-phase pterygium genesis (**G**), a vascular macroaneurysm (arrow, **H**) and vascular narrowing (arrow head, **H**) are identified.

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mass-like lesion. Pinguecula is also termed elastotic degeneration and features central avascularity grossly resembling the fibrotic core of a tumor. It is postulated that hypoxic damage, vascular obliteration, and microaneurysm in the border of pinguecula during the development of early pterygium are signs of local hypoxia (Fig. 3). Previously, delayed perfusion, vessel attenuation, and superficial punctate keratopathy as a sign of ocular surface inflammation were suggested as indicators of early-stage pterygium (Lee et al., 2007).

Seifert and colleagues first reported ingrown capillaries from the stroma into the pterygium epithelium and interpreted them as a reaction to hypoxia (Seifert and Sekundo, 1998). In our previous study, we

verified increased HIF-1 α expression in pterygium tissues compared to normal conjunctiva (Lee et al., 2007). More specifically, a hypoxia-induced proliferative axis, HIF-1 α -induced SDF-1/CXCR4 signaling, has been implicated in very early-stage pterygia (Ha and Kim, 2006) (Fig. 4). Together with immunohistochemical data, the increased concentration of SDF-1 in tears suggests that early pterygial tissue is under the influence of hypoxia (Ha and Kim, 2006). Supporting this phenomenon, a recent study also showed tissue expression of HIF-1 α in conjunction with that of heat shock protein (Pagoulatos et al., 2014).

It is well known that neovascularization is a reactive product against oxygen deficiency and is also a typical

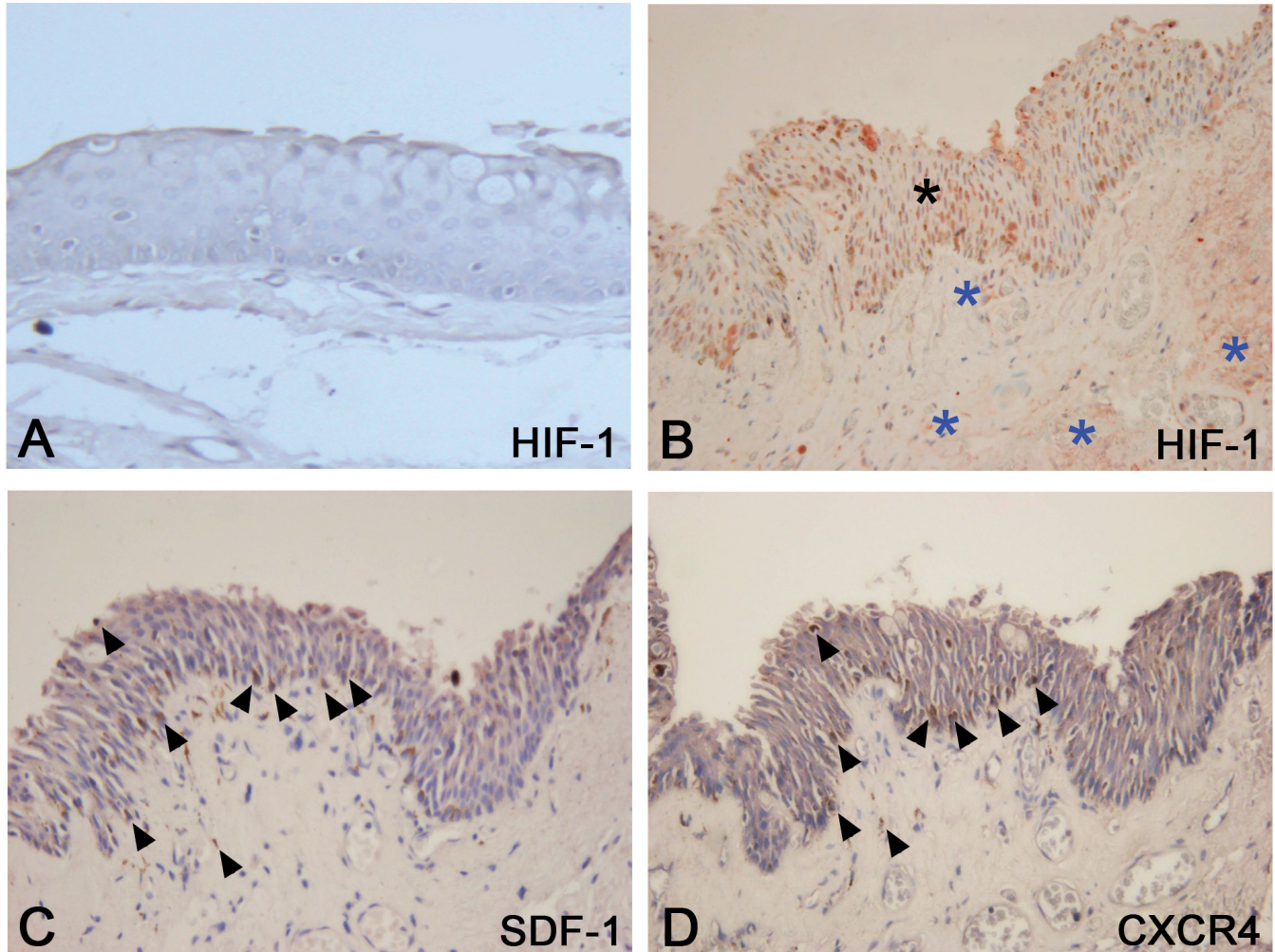


Fig. 4. Immunohistochemical localization of hypoxia-related factors in normal conjunctiva (A) and early pterygium (B-D). Hypoxia-inducible factor-1 α (HIF-1 α) was prominently stained at a whole layer of pterygium epithelium (black asterisk, B) and also in the stromal layer (blue asterisks, B). Similarly, stromal cell-derived factor-1 (SDF-1) was expressed in both epithelial basal cells and stromal vascular endothelium (arrow heads, C), where it co-localized with chemokine receptor type 4 (CXCR4) expression (arrow heads, D). In normal conjunctival tissues, HIF-1 α protein was not expressed (A). (B-D: Original photomicrographs are reprinted from Ha and Kim (2006) with permission from the Journal of Korean Ophthalmological Society). A, $\times 200$; B-D, $\times 100$

clinical feature of pterygium. This feature is triggered by several angiogenic factors, including HIF-1, a strong upstream factor that plays a crucial role in cellular and systemic oxygen homeostasis and induces the transcription of more than 60 proteins, including VEGF and SDF-1, under hypoxic conditions (Aebersold et al., 2001; Semenza, 2003).

Expression of SDF-1 for fibrosis in severe pterygia

HIF-1 α -induced SDF-1 and CXCR4 expression in 'early' pterygium tissues had been previously investigated as a possible mechanism for progenitor cell tropism (Ha and Kim, 2006). However, the SDF-1/CXCR4 axis in 'severe and complicated' pterygia is also an interesting topic. Considered as a product of profibrotic and aggravated wound healing after ocular surface damage by UV light, irritation, and/or inflammation, pterygium is pathogenically considered to be ocular proliferative fibrosis. To elucidate such a mechanism, we investigated the involvement of SDF-1 and CXCR4 signaling in pterygia. SDF-1, the ligand of CXCR4 was initially identified to support the bone marrow niche (Tashiro et al., 1993; Lataillade et al., 2000; Burger and Kipps, 2006) and is also known to enhance wound healing through recruitment of CXCR4-expressing cells into wound areas (Xu et al., 2013). If

exaggerated, SDF-1/CXCR4 signaling may lead to development of hypertrophic scarring (Xu et al., 2007). In our previous study, SDF-1 expression was upregulated in fibroblasts of severe pterygia and also contributed to the accumulation of α -SMA-expressing myofibroblasts in pterygium tissues (Kim et al., 2013b). Further, SDF-1- and CXCR4-expressing cells were found at the same locations in the epithelium and stroma (Fig. 5), with CXCR4-positive cells predominantly located at the perivascular areas of the stroma. These results indicate the existence of CXCR4-expressing progenitor cells homing into pterygium tissues through the circulation system in response to SDF-1. Notably, activity of pterygium fibroblasts was reversed by blockade of the SDF-1/CXCR4 axis using SDF-1-siRNA or a CXCR4 receptor antagonist (AMD3100).

Recruitment of progenitor cells in pterygium pathogenesis

Some pathologic responses in the eye are related to stem cells triggered by various local signals. For example, substance P, which is secreted by corneal sensory neurons in alkali-burned rabbit eyes, is a systemically acting wound messenger that appears to recruit CD29+ stromal-like cells from the periphery to

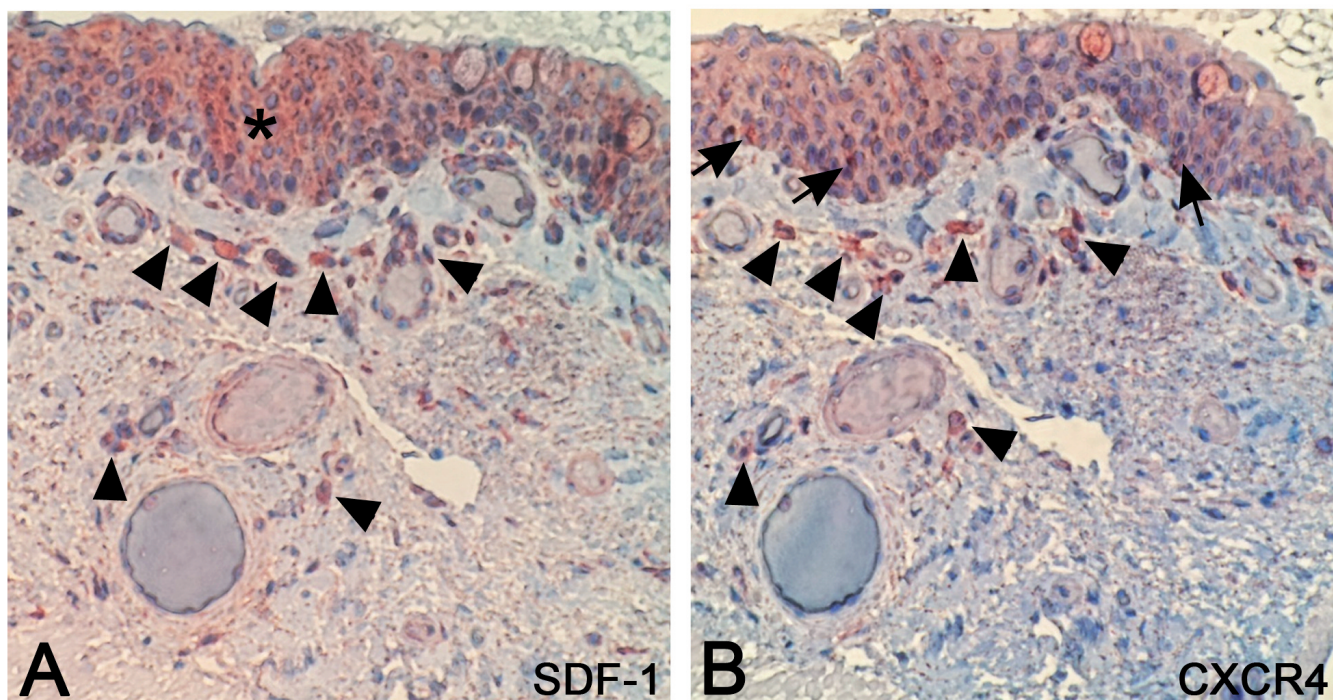


Fig. 5. Immunohistochemical expressions of stromal cell-derived factor-1 (SDF-1, **A**) and chemokine receptor type 4 (CXCR4, **B**) in a representative tissue with severe pterygium. SDF-1- and/or CXCR4-expressing cells were noted in both epithelium (asterisk, **A**; arrows, **B**) and stromal layers (arrow heads), especially in the locationally similar areas. Moreover, in the stromal layers, there was prominent expression of SDF-1 and CXCR4 at the perivascular areas (arrow heads). $\times 200$

the site of injury, resulting in accelerated wound healing (Hong et al., 2009).

Bone marrow-derived mesenchymal and hematopoietic stem cells have been previously suggested to contribute to pterygial fibrovascular stroma formation through differentiation into vascular endothelial cells and tissue fibroblasts, possibly explaining the origin of CXCR4-positive fibroblasts in pterygium. Predominant expression of CXCR4-positive cells at the perivascular areas supports the hypothesis of possible recruitment of circulating cells into the pterygium tissue via vascular structures (Kim, et al., 2013b). In a previous study, we found AC133 and STRO-1 in epithelial and stromal cells, and c-kit expression was identified mainly in the basal epithelium of primary pterygia. However, there is no immunoreactivity of c-kit, AC133, or STRO-1 in normal conjunctiva (Ye et al., 2004). We have also discussed the evidence for bone marrow-derived progenitor mobilization during early-stage pterygium (Lee et al., 2007). In this study, circulating CD34-positive and c-kit-positive mononuclear cells were increased in patients with pterygium and were positively correlated with systemic (in plasma) and local (in tear) cytokines, including substance P, VEGF, and stem cell factor (Lee et al., 2007). Such a phenomenon has been attributed to ocular hypoxia. These studies propose that progenitor cell tropism from bone marrow is strongly involved in pterygium development, and pterygium may not be just a local dysregulation but a possible systemic disorder.

Anti-hypoxia combined with anti-fibrosis for a therapeutic strategy in pterygium

A principal surgical goal for surgeons treating pterygium is to reduce the post-surgical recurrence rate of extensive fibrosis on the ocular surface. Numerous surgical methods have been employed as part of an anti-fibrotic strategy, including adjunctive amniotic membrane transplantation, conjunctival autograft, conjunctival limbal autograft, mitomycin C application, and beta-irradiation (MacKenzie et al., 1991; Starck et al., 1991; Guler et al., 1994; Mastropasqua et al., 1996; Starc et al., 1996; Tan et al., 1997; Solomon et al., 2001). Recently, Liu and colleagues performed a procedure to seal the gap under the nasal conjunctival caruncle, which is a main culprit for fibrovascular emanation (Liu et al., 2012). In a similar vein, we devised an interventional treatment method that involves the insertion of microporous expanded polytetrafluoroethylene (e-PTFE) under the conjunctival caruncle in order to attenuate fibrosis (Kim et al., 2013c). In that study, multiple pores with size of approximately $1.8 \times 10^4 \mu\text{m}^2$ per pore were made in the e-PTFE sheets to promote passage of air and thus minimize the formation of a hypoxic environment beneath the inserted e-PTFE. In a mean follow-up period of 17.2 months after surgery using e-PTFE with multiple pores, the average recurrence rate was 3.3%, compared to 25% in the control group. Additionally, subjective

eyeball redness was more improved in patients with multimicroporous e-PTFE insertion compared to those without.

The cellular proliferative capacity is reportedly augmented 600 times under 2% oxygen compared to 20% oxygen condition (Falanga et al., 1991), and the expression of TGF-beta1, a well-known key fibrogenic factor, is increased at low oxygen concentrations in cultured human dermal fibroblasts (Falanga and Kirsner, 1993). Such proliferative features can potentially be reversed through anti-hypoxic therapy both *in vivo* and *in vitro*. Fortunately, Assaad and colleagues showed that hyperbaric oxygen can manage recurrent pterygia and produce a favorable surgical outcome (Assaad et al., 2011).

Conclusions

Most pterygia arise at nasal conjunctiva, and this might be related to the relatively low vascular density of the nasal bulbar conjunctiva and, thus, increased vulnerability to hypoxic conditions. This finding speculates the involvement of area-matched intrinsic susceptibility of pterygium, especially elicited by hypoxia, in the pathogenesis of pterygium. The expressions and co-localizations of HIF-1alpha, SDF-1, and CXCR4 in tissues of early primary pterygia indicate that the involvement of circulating stem cells is mediated by hypoxic gradients through SDF-1 induction by HIF-1alpha. Moreover, the SDF-1/CXCR4 axis has recently been highlighted as a cicatricial fibrotic condition in severe cases of pterygia.

Taken together, pterygium is thought to proceed during its early phase along intrinsic pathways related to HIF-1-induced hypoxic damage, usually at the epithelial layer. After chronic hypoxic insult, CXCR4-positive circulating cells and bone marrow-derived progenitors may contribute to copious vascularization and fibrosis via transformation to endothelial cell and/or myofibroblasts at the pterygium stroma. This study posits a pathogenic basis of pterygium, and the authors suggest that hypoxia related-progenitor cell homing is one of the principal suspects for such a mechanism.

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