Molecular genetic alterations and viral presence in ophthalmic pterygium (Review)

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Abstract. Pterygium is a lesion of the corneoscleral limbus which tends to grow in size, often recurs after surgical excision and is associated with exposure to solar light. Additionally, a family history is frequently reported. Loss of heterozygosity (LOH), increased P53 expression and the presence of oncogenic viruses, such as human papilloma virus (HPV) and herpes simplex virus (HSV), have been detected in pterygia, supporting the possible neoplastic nature of the lesion. Co-infection by HSV and HPV as well as LOH at some loci have also been correlated with clinical features, such as post-operative recurrence and history of conjunctivitis. A possible model of pterygium formation is proposed, in which genetic predisposition, environmental factors and viral infection(s) participate in a multi-step process. Future research may lead to new ways of pterygium treatment such as anti-viral or gene therapy.

Contents

1. Introduction

Ophthalmic pterygium is a triangular lesion of the corneoscleral limbus, usually developing bilaterally and located nasally to cornea (1,2) (Fig. 1). Despite its benign nature, it can threaten vision in many ways and a surgical excision is commonly indicated, although postoperative recurrence is quite common (3). Pathological features include hyaloid degeneration, accumulation of eosinophilic granular material and intense fibroblastic proliferation with a rich vascular supply. Distorted fibrillar structures stained with elastic tissue pigments, such as Weigert or Verlhof, are commonly observed (4). These structures were initially considered to be derived from elastic fibres, hence the term 'elastotic degeneration', but the fact that incubation with elastase does not disrupt them led to the suggestion that they may represent a form of degenerate collagen (5) (Fig. 2). 'Elastotic degeneration' is commonly observed in cutaneous conditions related to sunlight exposure. Indeed, a strong correlation between pterygium development and exposure to sunlight, as well as to other environmental factors, such as dust and wind, has been universally accepted (1,2).

However, the exact pathogenesis of pterygium still remains unknown, despite numerous theories proposed. Apart from the mechanism of the emergence and development of the lesion, other important questions concern its preferentially nasal location and triangular shape. Recent reports suggest that genetic damage, induced directly by phototoxicity or by viruses, which may in turn be activated by light or other environmental factors, could be an important insult leading to the formation of pterygium or affecting its clinical profile (6-9).

2. Proposed pathogenetic mechanisms for pterygium

Solar light is known to cause various toxic effects to the human eye (8-10). The relation of pterygium development with ultraviolet (UV) radiation was established early in the study of the lesion which was considered an 'ophthalmoheliosis', i.e., an eye condition related to sun exposure (8). The initial concept was that the solar light may lead to the formation of degenerate protein material which could then act as a 'pterygiogenetic' or 'angiogenetic' factor (11). The UV-A and UV-B radiation (with a wavelength of 290-400 nm), especially in the form of scattered light (albedo) have been considered the most dangerous (11,12). There is a strong epidemiological correlation between pterygium and other conditions well known to be related with solar light, such as basal cell carcinoma (BCC) (13), porphyrias (14) and xeroderma pigmentosum (XP) (15) and it has been reported that pterygium may appear as long as 10 years before the emergence of these conditions in a patient (11). The higher incidence of pterygium in males has

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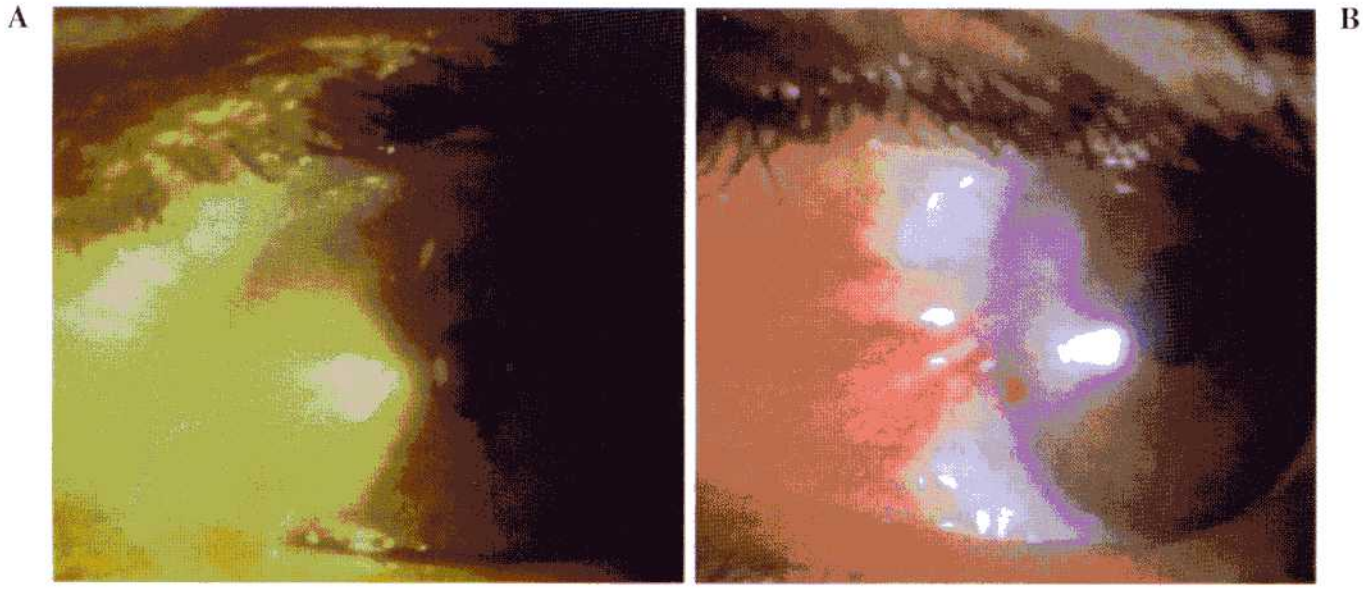

Figure 1. Ophthalmic pterygium and postoperative recurrence. A, Preoperatively; B, Two months postoperatively (recurrence).

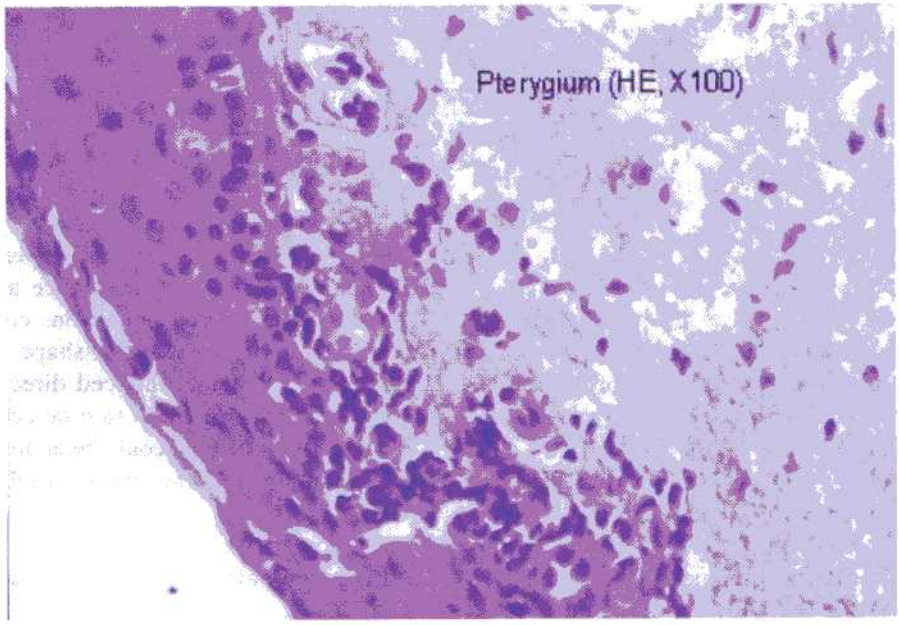

Figure 2. Pathology of ophthalmic pterygium (H&E, x100).

been attributed to the higher levels of exposure to the sun, due to professional reasons (16). Light damage could be related to the formation of free radicals (11). At the cellular level, light damage could be related to modification of the immunological status of the corneoscleral limbus, expressed by a decrease of Langerhans cells in the area (11). Deposition of IgE and IgG in the stroma as well as infiltration with lymphocytes and plasmatocytes have been described in pterygia and it has been proposed that a type I mechanism of immune reaction may be involved (17). According to this model, the binding of IgE leads to the release of platelet activating factor (PAF) and the platelet derived growth factor (PDGF). PDGF has been reported to regulate the action of epithelial growth factor (EGF) (11,17).

The role of the tear film function in the pathogenesis of pterygium is controversial. Inadequate tear film protection of conjunctiva and cornea has been reported as a risk factor (18) but, on the other hand, pterygium is quite common in areas with a humid climate, where conjunctival drying is unlikely (19). There are reports of decreased scores in tests assessing tear secretion or stability (such as Schirmer or Rose Bengal test), but it is difficult to determine whether these alterations are a cause or result of pterygium (18). Lactoferrin, an iron-binding protein present in tears, may play a role in inhibiting

the action of free radicals created by solar light, thus protecting from pterygium advancement (2). The deposition of iron at the corneal aspect of pterygia that have been static for a long time (Stocker's line) may be related with the action of lactoferrin (2).

Apart from light, microtrauma caused by dust particles and wind has been recognised as a factor predisposing to pterygium development and it has been proposed that it may be more intense in the nasal area of the conjunctival cul-de-sac due to the drainage by tears, thus explaining the nasal location of the lesion (11).

Heredity is also considered an important risk factor and a family history of pterygium is commonly described (16). There are reports that the condition could be inherited in an autosomal dominant pattern, it is not clear however if inheritence refers to the condition itself or to increased susceptibility to the effects of solar light (11,16).

The infrared part of solar light spectrum (heat) may also be related with the development of pterygium. It has been shown that 1-h exposure of the rabbit eye to a temperature of 39°C may lead to scleral hyperplasia and fibroblast activation (20,21).

Other mechanisms have been described to explain the development of pterygium including the action of lactic acid contained in sweat (22), which may move along the lateral side of nose and selectively irritate the nasal conjunctiva, a silent thrombosis of conjunctival venous system (11), a contraction of the horizontal rectus muscles leading to venous stasis in conjunctival vessels and conjunctival plication (11) and a reflection of solar light from the lateral side of the nose to the nasal corneoscleral limbus (11). The latter mechanism could perhaps explain the nasal location of pterygium. Another interesting mechanism could be the transcameral focusing of solar light to the inner side of the limbus, where basal cells are not protected by superficial layers (23). There are reports that in pterygia the axes of basal cells become oblique and the cells display morphological abnormalities such as dense nuclei, which are also observed experimentally in rat epithelial cells irradiated with UV light (24). Hyperplasia of cells in epidermis and hair follicles in response to UV light radiation has also been reported (25,26). A model has been developed to explain the triangular shape of pterygium, based on alterations in cell kinetics, and differential responses of corneal and conjunctival epithelium (27).

3. Evidence supporting the neoplastic nature of pterygium

Certain clinical features, such as the tendency to grow in size and the frequent postoperative recurrence have led to the suggestion that pterygium could be a benign neoplastic condition (9). This assumption has been supported by experimental findings. In particular, fibroblasts from pterygia have been shown to behave as neoplastic cells *in vitro*, displaying independence from exogenous growth factors and considerably higher cell concentration compared to normal fibroblasts (28). Moreover, random histological examination of excised pterygia has revealed neoplastic features (29). Loss of heterozygosity (LOH) for microsatellite markers has been detected, implying the involvement of tumor suppressor genes (TSGs) in the pathogenesis of this condition (8,9).

Based on immunohistochemistry with p53 monoclonal antibodies against p53 and vimentin, it has been found that pterygia may arise from a vimentin-expressing, altered limbal epithelial basal cell, the so-called 'pterygium cell' (30). It was reported that nuclear p53 is increased in the limbal epithelium of pterygia, as well as other limbal lesions such as pingueculae, possibly indicating the existence of p53 mutations (31). Such genetic alterations could have been caused by UV radiation, early in the development of these conditions. According to a model for multi-step development of pterygia and limbal tumors, mutations in P53 may also impair apoptotic procedures, thus leading to progressive accumulation of mutations in other genes (p53, *bcl*-2, and *bax*). In that way, development of pterygia could be the result of a disrupted expression of genes associated with the induction or repression of apoptosis in the conjunctiva (32). However, there are controversies concerning the role of p53 expression in the pathogenesis of pterygium (33).

4. Pterygium, loss of heterozygosity (LOH) and microsatellite instability (MI)

Genetic alterations have been studied in pterygia during the recent years. Using microsatellite markers, a high incidence of LOH in pterygia has been reported, especially at areas 9p, 9q and 17q (8,9). LOH is a key pointer to the existence of TSGs, according to 'Knudson's two hit hypothesis' (Fig. 3), and by screening paired blood and tumor samples with markers spaced across the genome, candidate locations for TSGs can be discovered (34). Chromosomal areas 9p, 9q and 17q frequently display LOH in a variety of other neoplastic lesions at various organs, such as breast, lung, oesophagus, kidney urinary bladder, skin and blood, which is consistent with the fact that candidate TSGs located there, such as P16 (9p21) and P53 (17p13.1), play a universal role in the development of neoplasia (8,9).

LOH has not been detected in phenotypically normal conjunctival tissue excised from sites protected from solar radiation by the upper eyelid (12 o'clock at the corneoscleral limbus) implying that genetic alterations may be related with the level of exposure to solar light (9). This conclusion is further supported by the reported correlation between the incidence of LOH at region 9q31-33 and the altitude of residence of patients, which is known to be correlated with the exposure to ultraviolet solar radiation (9,35). Additionally, the fact that LOH incidence at region 9q was found to be correlated with postoperative recurrence of the examined pterygia, perhaps implies that LOH at this area may be useful as a prognostic marker for recurrence (9).

The comparative evaluation of microsatellite markers in paired tumor and peripheral blood samples can reveal the existence of multiple alleles in the former, a finding known as microsatellite instability (MI) (36,37). It has been proposed that MI is an indication of a 'mutator' pathway in oncogenesis, in which a defect in accurate replication or post-replicative correction of mistakes can destabilize oncogenes or TSGs (such as *ras*, P53 or Rb) and can subsequently lead to cancer development (36,38). It is interesting that MI incidence in pterygia was found to be considerably lower compared to LOH incidence (8,9). It therefore seems possible that the

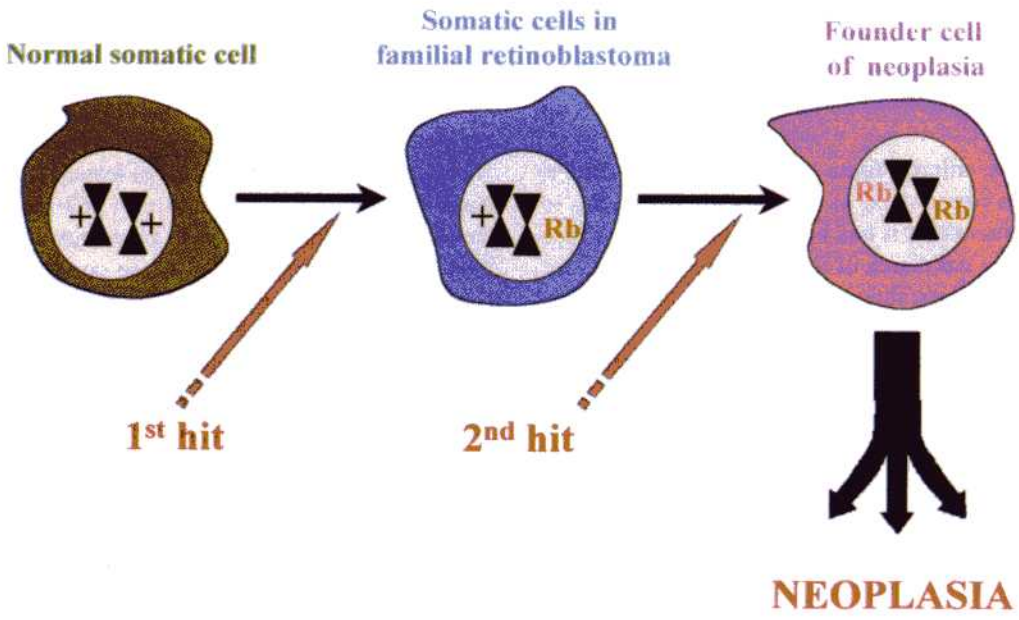

Figure 3. Knudson's 'two hit' hypothesis.

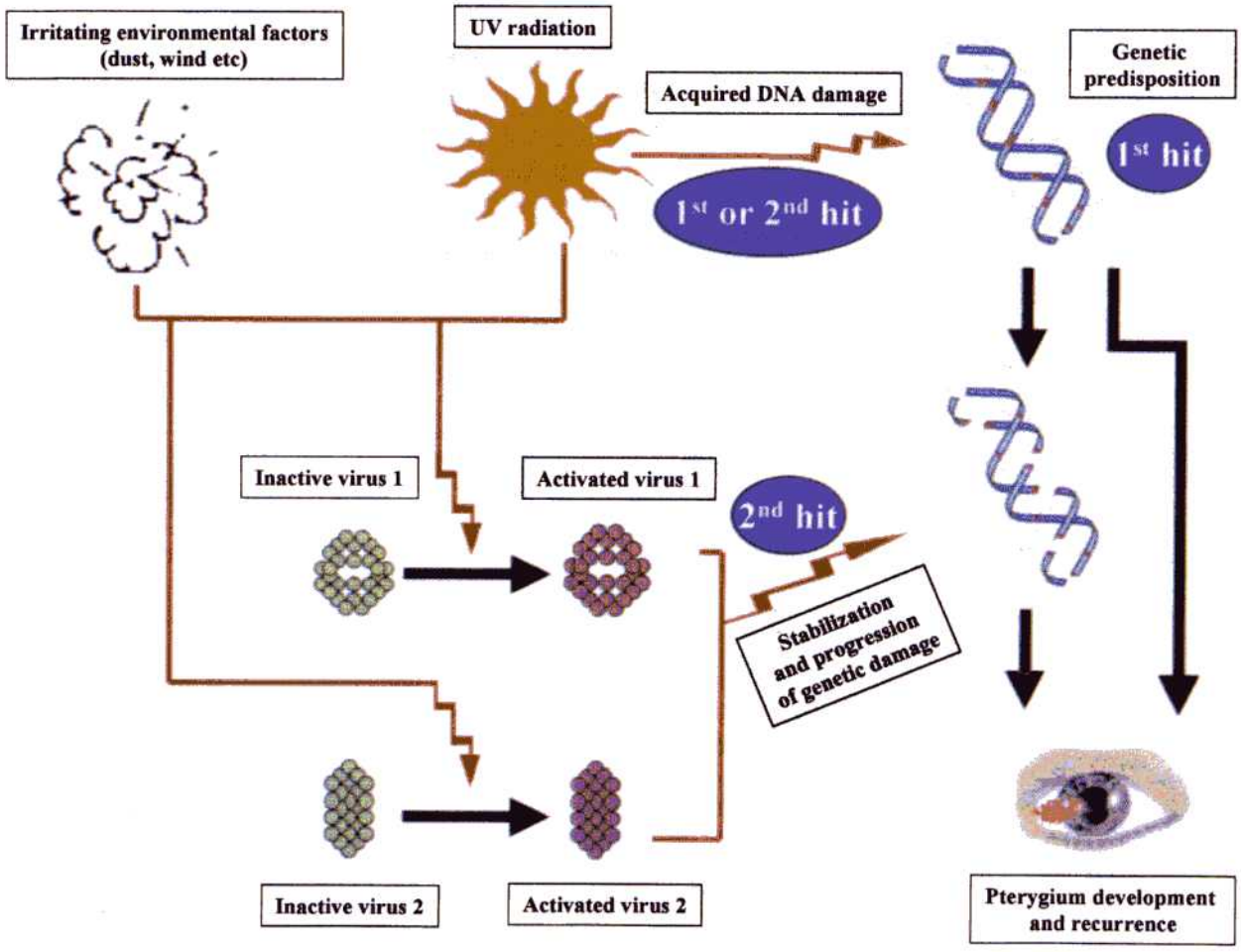

Figure 4. The high incidence of LOH implies a possible 'suppressive' mechanism in the molecular pathogenesis of pterygium. A 'two hit' model is proposed. The '1st hit' could be either inherited (familial occurrence of pterygium) or incurred by UV radiation. The '2nd hit' could be caused either by solar light or by viral infection. Viruses may be activated by UV radiation or environmental microtrauma. It is possible that viral co-operation, as in the case of HSV and HPV, affects the clinical profile of the lesion.

'suppressor' pathway (indicated by LOH) is quite active in pterygium pathogenesis, while the 'mutator' pathway (indicated by MI) does not play a crucial role.

5. Pterygium and viruses

The detection of viral DNA in pterygium provided additional evidence for their possible neoplastic nature. HPV-18 and HPV-16 are associated with papillomas as well as malignant tumors of the eyelids, lacrimal outflow tract and conjunctiva (39,40). However, there are controversial findings concerning HPV involvement in pterygium. Some studies have found HPV in pterygia and respective macroscopically normal conjunctival samples (7,41), while others have not detected the virus or have concluded that HPV DNA is not required as a co-factor in the development of pterygia and limbal tumors (41).

HSV-1 infection is the commonest infective cause of blindness in the USA (42). HSV can cause acute keratitis, blepharitis and conjunctivitis in humans (43). Both DNA hybridization techniques and viral cultures have shown that after the acute phase, HSV infection can become latent (44,45). Initially, latent presence of HSV was detected in neurons. According to the 'skin trigger theory' (46), HSV is continuously released by ganglion cells and is peripherally activated *in situ* after irritating stimuli. However, there are strong indications that the virus can also remain latent in non-neuronal cells (44). Based on experimental evidence, it has been proposed that HSV may be present in chronical eyelid, corneal and conjunctival lesions (43). HSV has also been detected in a number of pterygia with PCR assays, but not in respective conjunctival specimens, suggesting that this virus could have a more specific role in the pathogenesis of pterygium (6). However, it is not certain that HSV association with pterygia is causal. It is possible that HSV detection results from a 'skin-trigger'-type mechanism due to the irritating effect of pterygium.

In some pterygia, a co-infection by HSV and HPV was detected (47). Interestingly, the simultaneous detection of HPV and HSV was correlated with clinical characteristics, such as postoperative recurrence and a history of conjunctivitis. The correlation of the presence of either HPV or HSV alone with the same clinical parameters was much less significant, suggesting that viral co-operation could be active in at least a subset of pterygia (47). There are reports that HSV and HPV can co-exist and possibly co-operate during multistage oncogenesis (48,49).

6. Genetic damage induced by HSV and HPV

There are strong indications that HSV can be involved in the process of multistage carcinogenesis (50,51). It has been proved that the virus can cause both point mutations and more widespread genetic alterations in rodent cells (51). It can also augment the action of chemical carinogens through potentiation of the expression of proto-oncogenes or through inactivation of P53 (52). There are reports for increased nuclear p53 in the limbal epithelium of pterygia indicating the probable existence of p53 mutations in these cells as an early event in their development (31). It is interesting that cellular phenotypical changes induced by HSV can also be accomplished by DNA fragments of the virus that are not translated (51). Unlike with other oncogenic viruses, cells infected by HSV do not constantly express viral genes but are perhaps affected by separate viral attacks, the so-called 'hit-and-run' hypothesis (50). Protein E7 of HPV strains 16 and 18 can bind to the pocket domain of Rb protein, inhibiting its interaction with transcription factor E2F and thus reducing its ability to suppress transcription (34).

7. Interaction between HSV, HPV and environment

UV-B irradiation suppresses cell-mediated immunity and may lead to increased susceptibility to infectious diseases, especially those caused by viruses (53). There are reports that HSV can be reactivated, often in a mutant form, by UV radiation in mammalian cells (54). Various environmental stimuli have been reported to trigger HSV reactivation, including Excimer Laser refractive corneal ablation (55) or chlorinated water in swimming pools (56).

HPV-18 has been isolated from verrucuous carcinoma located in areas with signs of repeated microtrauma (57). E6 protein from a range of cutaneous HPV types has been shown to inhibit apoptosis in response to UV damage (58) in both p53 null and wild-type cells and does not require p53 degradation. p53-degradation by HPV-16 E6 preferentially affects the removal of cyclobutane pyrimidine dimers from non-transcribed strand and sensitizes mammary epithelial cells to UV irradiation (59).

These reports suggest that environmental factors, such as UV radiation or mechanical microtrauma, can enhance the effects of HSV and HPV infection either by reducing host immune response or by intervening in pathogenetic mechanisms at a molecular level.

8. A potential model for the development of pterygium

Based on the aforementioned data, one possible concept for pterygium development is presented in Fig. 4. Taking into account that the 'suppressor' pathway seems to play a major role, a 'two hit' model is proposed. According to this model, the '1st hit' is either inherited (which accounts for the familial predisposition towards the development of the lesion) or caused by environmental noxious stimuli, such as UV light. The '2nd hit' could also be caused by exposure to solar light (adding further damage to a susceptible genetic material) or incurred by the combined activity of viruses, such as HSV and HPV, which could in turn be activated by environmental factors. This model could perhaps explain the detection of HPV and HSV in only a subset of pterygia, although HSV detection may be underestimated, due to its 'hit-and-run' action. Furthermore, this concept could take the form of a multistage process, in which inheritence, solar light and viral infection successively participate, adding to the genetic damage. In support of this possibility comes the finding that, at some loci such as 9q and 17q, LOH incidence in pterygia was found significantly correlated with the time interval pterygium was reported to exist, which was in turn correlated with the rate of postoperative recurrence (9). It would thus seem possible that genetic damage added over the years contributes to the stabilisation of the lesion and renders it more aggressive.

Once a genetic basis for the emergence of a rapidly developing cell population has been established, the nasal location and triangular shape of pterygium could perhaps be attributed to mechanistic factors, such as irritation through tear flow and light focusing or differential population kinetics of the corneal and conjunctival epithelium.

9. Possible future trends in the study of pterygium

Determining whether viral presence in pterygia has a causal role or significantly affects the clinical profile could prove an important step in the study of this lesion. If a causal relation is established, it would seem reasonable to assess the effectiveness of anti-viral therapy in the treatment of pterygium, perhaps as in the form of adjunctive therapy after surgical excision in order to reduce recurrence.

The detection of genetic alterations in excised pterygia (such as LOH at region 9q31-33) could enable predicting postoperative recurrence (9). In pterygia with such alterations, more sophisticated surgical excision could be employed. If detection of recurrence with this method proves accurate, use of potentially toxic adjunctive therapy, such as Mitomycin-C, could be restricted in high risk cases, thus reducing complications.

Apart from TSGs, the role of proto-oncogenes should perhaps be studied in pterygia, taking into account that UV irradiation of mammalian cells in culture has been found to evoke the transcriptional activation of proto-oncogenes, among them members of the *fos/jun* family (60), which are known to play an important role in cell proliferation and differentiation. A differential induction of JunB, JunD, and Egr-1 expression in ocular tissues following ultraviolet irradiation of the rat eye has also been reported (60). Gene therapy, by introducing normal genetic material in affected pterygial cells, could be a subsequent step in the treatment of pterygia.

10. Conclusions

Although first described in ancient times, ophthalmic pterygium keeps the secrets of its pathogenesis and morphology to the present day, despite numerous proposed theories. However, modern concepts of molecular oncogenesis may offer a new perspective in the study of this condition. It is possible that pterygium may result from an interaction between the environment, genetic material and certain viruses, such as HSV and HPV. Detection of genetic alterations in pterygia, which could enable more selective use of toxic therapy, evaluation of the possible role of viruses and anti-viral medication or even gene therapy are some directions for future research in the treatment of this puzzling eye lesion.

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