Pterygia Pathogenesis

Corneal Invasion by Matrix Metalloproteinase Expressing Altered Limbal Epithelial Basal Cells

Nicholas Dushku, MD; Molykutty K. John, PhD; Gregory S. Schultz, PhD; Ted W. Reid, PhD

Objective: To assess the potential role of matrix metalloproteinases (MMPs) in the pathogenesis of pterygia by comparing the immunolocalization patterns of MMPs in altered limbal basal stem cells, activated fibroblasts, and areas of elastotic degeneration adjacent to the pterygia.

Methods: Nine primary and 1 recurrent pterygia along with normal superior limbal-conjunctival tissue and cornea were immunostained with mouse monoclonal antibodies specific for MMP-1, MMP-2, MMP-3, MMP-9, membrane type 1 (MT1)–MMP (MMP-14), and membrane type 2–MMP (MMP-15).

Results: Normal conjunctival, limbal, and corneal cells lacked significant immunostaining except for cell surface MT1-MMP. In contrast, altered limbal basal epithelial cells of the 9 primary and 1 recurrent pterygia immunostained for all 6 MMPs. Activated and altered fibroblasts associated with the pterygia immunostained primarily for MMP-1. In contrast, stromal areas of elastotic degeneration (pingueculae) showed variable immunostaining of MMPs.

Conclusions: Altered limbal basal epithelial cells (pterygium cells) immunostained for multiple types of MMPs in contrast to normal conjunctival, limbal, and corneal cells. The pterygium cells invading over Bowman’s layer produce elevated MMP-1, MMP-2, and MMP-9 expression, which probably are the main MMPs responsible for the dissolution of Bowman’s layer. Pterygium cells may also cause activation of fibroblasts at the head of the pterygium, leading to the initial cleavage of fibrillar collagen in Bowman’s layer by the production of MMP-1. Altered fibroblasts in areas of elastotic degeneration (pingueculae) trailing behind the pterygium constitute a second type of tumor, which is noninvasive.

Clinical Relevance: These data of altered MMP expression support the concept that altered basal limbal epithelial cells play a key role in the formation and migration of a pterygium.


In our search for the pathogenesis of pterygia, several important clinical and pathologic characteristics of primary and recurrent pterygia have emerged:

1. Epidemiological studies have firmly established that UV-B radiation correlates as the etiologic agent for pterygia and limbal tumors. Since UV-B is known to be mutagenic for the TP53 tumor suppressor gene, we previously searched for abnormal TP53 expression in pterygia, limbal tumors, and pingueculae from which these 2 growths seem to originate. We found nuclear p53 expression without apoptosis in the limbal epithelia of pterygia, limbal tumors, and pingueculae. This suggested to us that mutation in TP53 or mutations in the p53 pathway for apoptosis may occur as an early event in the tumorlike development in these cells, which is consistent with their causation by UV radiation. As a consequence of mutational damage to the p53-dependent programmed cell death mechanism, mutations in other genes could progressively be acquired by the altered limbal basal epithelial cells. This is consistent with the concept of a multistep development of tumorlike pterygium cells that arise from altered limbal epithelial cells overlying a pinguecula. We also discovered that primary and recurrent pterygia were charac-
PTERYGIA AND NORMAL TISSUE

In compliance with the World Medical Association Declaration of Helsinki, 9 primary and 1 recurrent pterygium were surgically removed in the ambulatory surgery center at the Kaiser Permanente Medical Center, Rancho Cordova, Calif, and processed as described previously.\(^9\,19,23\) Briefly, to identify the invading limbus epithelium with altered limbal basal cells and the zone of dissolution of Bowman’s layer, incisions were made in the cornea 1 to 2 mm central to the leading edge of the pterygium and deep enough to include Bowman’s layer. The incisions were extended into the adjacent conjunctiva for 5 to 6 mm posterior to the surgical limbus and 1 to 2 mm beyond the superior and inferior conjunctival folds. For proper orientation, specimens were sutured onto sterile cardboard and immediately fixed in 10% neutral buffered formalin for 6 to 10 hours, then embedded in paraffin. Serial cross sections of pterygium specimens were made along the longitudinal axis to include the leading edge of cornea-invading altered limbal basal cells over Bowman’s layer, the migrating limbus, and adjacent conjunctiva. Every tenth section was stained with hematoxylin-eosin to locate these 3 areas. For immunostaining, sections were selected that contained cornea-type cells between the dissolved edge of Bowman’s layer and conjunctiva (as indicated by the presence of goblet cells). A specimen of fresh normal human superior limbal-conjunctival tissue served as a normal tissue control. In addition, sections of normal cornea (obtained along with the pterygia) also served as internal controls. Three cadaver eyes placed in 10% neutral buffered formalin 4 to 5 hours after death were used for comparison with the fresh surgical specimens (Table 1, only 5-hour specimen results are shown). Human placenta, which is known to produce MMPs, was used as a positive tissue control.\(^38\) Additional negative controls used ptterygia tissues incubated without the primary antibodies to MMPs.

IMMUNOHISTOCHEMISTRY

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue sections using the avidin-biotin-peroxidase complex method as described previously.\(^9\) Briefly, sections 5 µm thick were cut and deparaffinized in xylene and descending ethanol series. Endogenous peroxidase activity was destroyed by a 20-minute treatment at room temperature with 3% hydrogen peroxide in distilled water. Sections were then incubated for 1 hour at room temperature in a humidified chamber with primary mouse monoclonal antibodies directed against the MMPs. The following mouse monoclonal antibodies were used: MMP-1, MMP-2, MMP-3, and MMP-9, which were all diluted 50-fold (Oncogene Research Products, Boston, Mass), and membrane type 1 (MT1)–MMP and membrane type 2 (MT2)–MMP, which were diluted 100-fold (Chemicon International Inc, Temecula, Calif). Sections were washed and then incubated with a biotinylated secondary antibody directed against the mouse monoclonal antibodies for 1 hour at room temperature in a humidified chamber using the Dako LSAB Kit (Dako Corporation, Carpinteria, Calif). Sections were washed and then incubated with 0.05% 3,3’-diamino-benzidine tetrahydrochloride in 50-mmol/L Tris at pH 7.6 and 0.01% hydrogen peroxide. Sections were counterstained with hematoxylin and photographed with a Zeiss Ultraphot photoscope. To evaluate the specificity of the antibodies, sections were incubated with nonimmune mouse serum substituted for the primary antibodies. Immunostaining for MMP-1, MMP-2, MMP-3, and MMP-9 was considered positive when cytoplasmic and stromal staining was observed. Immunostaining for MT1-MMP and MT2-MMP was considered positive when membrane staining was observed.

These data have led us to propose an integrated model for the formation and pathophysiology of pterygia and pinguecula.\(^9\,19,24\) A key component of this hypothesis is that the true pterygium cells are tumoralike altered limbal epithelial basal cells that have altered TP53 tumor suppressor gene expression. With accumulation of sufficient mutations, the pterygium cells invade onto normal corneal basement membrane and draw conjunctival epithelial cells along with them. If this hypothesis is correct, we would predict that the expression of proteases that degrade basement mem

---

Table 1

<table>
<thead>
<tr>
<th>Protein</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>Positive</td>
</tr>
<tr>
<td>MMP-1</td>
<td>Positive</td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>Positive</td>
</tr>
<tr>
<td>MT2-MMP</td>
<td>Positive</td>
</tr>
</tbody>
</table>

---

\(^{19}\) Recently, it was reported that fibroblasts from pterygia when grown in culture exhibited elevated MMP expression.\(^{25}\) In general, invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-

---

\(^{20}\) Invasive tumor cells are known to overexpress MMPs of various types depending on the tumor.\(^{30}\) These proteases released by tumor cells facilitate invasion by degrading components of their basement membranes and adjacent stromal matrix. Previously, we proposed a model for pterygium migration and dissolution of Bowman’s layer involving proteases.\(^{19,24}\) Recently, elevated expression of MMPs was demonstrated in pterygia.\(^{35}\) Specific localization of MMPs in the altered limbal epithelial basal cells of pterygia had not been reported previously. In this ar-
ticle, we investigated MMP expression in the altered limbal epithelial cells. The data are consistent with these cells contributing to the pathogenesis of pterygia by secreting MMPs, which promote the invasion by the pterygia and the dissolution of Bowman’s layer.37

**RESULTS**

As shown in Figure 1 and Table 1, the specimen of normal conjunctival and limbal tissue (patient CF) did not display immunostaining for any of the MMPs in the epithelial basal cells. However, significant cell surface immunostaining was present with MT1-MMP, and slight staining for MMP-1 and MMP-9 was seen in the stroma (Figure 1A, D). In contrast to the fresh surgical specimens, the 2 cadaver specimens (Table 1) immunostained with most MMPs in the epithelial basal cells and stroma. For example, in the 2 specimens of normal cadaver conjunctiva, limbus, and cornea (patients C34 and C35), immunostaining by the 2 membrane-type MMPs (MT1-MMP and MT2-MMP) was primarily restricted to the membranes of the basal epithelial cells in the cornea, limbus, and conjunctiva and was not present in the stromal compartments of the tissues. Immunostaining of MMP-9 also was restricted to the membranes of basal epithelial cells in the cornea, limbus, and conjunctiva and was not present in the stromal compartments of the tissues. Immunostaining for MMP-2 was present in the epithelium and stroma of the cornea but was not detected in the epithelium of the conjunctiva. The stroma of the conjunctiva was variably positive for MMP-2. Staining for MMP-3 was highly restricted to the epithelium and stroma of the cornea and was not detected in the conjunctiva or limbal tissues. Staining for MMP-1 was present in the epithelium of the cornea and variably present in the corneal stroma and limbal epithelium.

All 10 pterygia specimens (9 primary and 1 recurrent) immunostained with most of the 6 MMPs studied (Table 2). Immunostaining by the MMPs was consistently high in the invading limbus epithelium in the altered limbal basal cells and in the adjacent corneal and conjunctival epithelia, which were infiltrated by invading altered limbal basal cells (Table 2 and Figures 2, 3, and 4). The other MMPs were present in the epithelium of about 8 of 10 pterygia specimens. In the recurrent pterygium, we found a single layer of cuboidal cells, which immunostained with all 6 MMPs, spreading on top of the surface of terminally differentiated squamous cells (data not shown). In addition, MMP expression occurred in some corneal stromal sections at cut, broken, or crushed areas (Figures 2 and 4). Figure 2G is interesting (a higher magnification of Figure 2A) in that it shows staining of MMP-1 in both the basal epithelial cells and the epithelial side of Bowman’s layer.

Matrix metalloproteinase-1 was found to be the most frequently expressed MMP by fibroblasts in pterygia (Table 2 and Figure 4). Matrix metalloproteinase-1 was often present in fibroblasts at the dissolved edge of Bowman’s layer (7/10) and in lobules of pterygeae (6/10) and less frequently present in fibroblasts under the migrating limbus (3/10). Fibroblasts found at the dissolved edges of Bowman’s layer (7/10) and in areas of fibroblast islands frequently immunostained with MMP-1.

### Table 1. MMP Expression in Fresh Normal Conjunctiva and Limbus (Patient CF); Cadaver Conjunctiva, Limbus, and Cornea (Patients C34 and C35); and the Area of Elastotic Degeneration (Pinguecula in Patient C35)*

<table>
<thead>
<tr>
<th>MMP</th>
<th>Patient</th>
<th>Conjunctiva</th>
<th>Limbus</th>
<th>Cornea†</th>
<th>Elastotic Degeneration‡</th>
<th>Fibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Epithelium</td>
<td>Stroma</td>
<td>Epithelium</td>
<td>Stroma</td>
<td>Epithelium</td>
</tr>
<tr>
<td>MMP-1</td>
<td>CF</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>−</td>
<td>+(+)</td>
<td>−</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C35</td>
<td>−</td>
<td>+(+)</td>
<td>−</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td>MMP-3</td>
<td>CF</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>−</td>
<td>+(+)</td>
<td>−</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C35</td>
<td>−</td>
<td>+(+)</td>
<td>−</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td>MMP-2</td>
<td>CF</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>−</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C35</td>
<td>−</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td>MMP-9</td>
<td>CF</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>−</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C35</td>
<td>−</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>CF</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>−</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C35</td>
<td>−</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td>MT2-MMP</td>
<td>CF</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C34</td>
<td>−</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>C35</td>
<td>−</td>
<td>+(+)</td>
<td>+(+)</td>
<td>+(+)</td>
<td>−</td>
</tr>
</tbody>
</table>

* MMP indicates matrix metalloproteinase; MT1, membrane type 1; MT2, membrane type 2; −, negative staining; +(+), some positive staining areas within specimen; B, basal cell staining only; +, mild staining; ++, moderate staining; and S, squamous epithelial cell staining only.
† Specimen CF had no cornea to stain.
‡ Only specimen C35 had areas of elastotic degeneration.
In addition, MMP-1 was expressed by 9 of 10 altered limbal basal cells in cornea over Bowman’s layer (Figures 2-4) and frequently stained Bowman’s layer beneath these basal cells (Table 2).

Areas of elastotic degeneration (pingueculae) usually immunostained for all 6 MMPs (Table 3 and Figure 3), although the fibroblasts in these areas mainly immunostained for MMP-1 and MMP-3.

Table 2. Staining of Pterygia With Monoclonal Antibodies to MMPs*

<table>
<thead>
<tr>
<th>MMP</th>
<th>Epithelium</th>
<th>Fibroblasts</th>
<th>Extracellular Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conjunctiva</td>
<td>Migrating Limbus</td>
<td>Cornea</td>
</tr>
<tr>
<td>MMP-1</td>
<td>10/10</td>
<td>10/10</td>
<td>9/10</td>
</tr>
<tr>
<td>MMP-3</td>
<td>8/10</td>
<td>8/10</td>
<td>7/10</td>
</tr>
<tr>
<td>MMP-2</td>
<td>9/10</td>
<td>9/10</td>
<td>10/10</td>
</tr>
<tr>
<td>MMP-9</td>
<td>8/10</td>
<td>8/10</td>
<td>8/10</td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>8/10</td>
<td>8/10</td>
<td>8/10</td>
</tr>
<tr>
<td>MT2-MMP</td>
<td>8/10</td>
<td>8/10</td>
<td>8/10</td>
</tr>
</tbody>
</table>

* MMP indicates matrix metalloproteinase; MT1, membrane type 1; and MT2, membrane type 2.
† One of the 10 specimens in this study had fibroblast islands.

Figure 1. Matrix metalloproteinase (MMP) immunostaining in normal limbus epithelium. No MMP staining of basal or suprabasal cells. Membrane type 1 (MT1)–MMP staining of some squamous cells. Mild staining of desquamating surface cells (A, C, D, F) and stroma (A, D). Staining of all cut stromal edges: (A) MMP-1, (B) MMP-3, (C) MMP-2, (D) MMP-9, (E) MT1-MMP, and (F) membrane type 2–MMP. Palisades of Vogt are visible in A through D, indicating that the specimens came from the limbal region. Limbal basal cells migrate from left to right (original magnification ×325).

Figure 4. In addition, MMP-1 was expressed by 9 of 10 altered limbal basal cells in cornea over Bowman’s layer (Figures 2-4) and frequently stained Bowman’s layer beneath these basal cells (Table 2).
COLLECTING AND ORIENTING THE SPECIMEN

The identification in pterygia of the migrating limbus with its altered limbal basal cells and their associated activated fibroblasts depends on the correct surgical collection of the specimen. In addition, proper orientation of the specimen is required to demonstrate, with serial cross sections, the key anatomy at the junction where the altered limbal basal cells start to invade onto corneal basement membrane over dissolving Bowman's layer.
NORMAL TISSUE

As in most normal, resting tissues, conjunctival-limbal-corneal epithelial tissues express such small amounts of MMPs that they are nearly undetectable by techniques such as immunohistochemistry40-43 (Figure 1). However, MT1-MMP immunostaining was detectable in the surface epithelial cells of the fresh normal control specimens (Figure 1E). Also, MT1-MMP immunostaining has been found in other normal human tissue.44 In addition, MMP immunostaining occurred in some sections at cut, broken, or crushed areas (Figures 1, 2, and 4), which may be due to an artifactual translocation of the MMPs after surgical wounding.45,46 From these data, we conclude that careful collection of specimens is needed to avoid artifactual MMP staining due to trauma. In addition, fresh surgical specimens are needed, because cadaver eyes, which were 4 to 5 hours old, tended to express abnormal levels of MMPs (Table 1).

PTERYGIA

We discovered previously that pterygia consisted of limbal epithelial tumor cells that expressed p53 and vimentin and displayed a peculiar development and migration pattern.8,9 We also previously demonstrated that the pte-
rygium cells had characteristics of limbal basal epithelial cells. In the present study, we found that the altered limbal basal epithelial cells of pterygia expressed 6 MMPs of various types similar to other invasive tumors, and we speculate that these MMPs are likely to promote corneal invasion of this tumor and contribute to the dissolution of Bowman's layer (Figures 2-4). In addition to migration of a segment of altered limbal epithelium and local infiltration of pterygium cells within adjacent epithelial tissues, we found in our specimen of recurrent pterygium a pattern of surface spread of MMP-expressing cuboidal cells over terminally differentiated squamous cells, which is simi-
lar to those in one of our previous studies.19 The spreading of surface MMP-expressing altered cells has a potential for wider spread than infiltration in the basal layers and could possibly explain some of the recurrences with autografts or wide excisions and the need for supplemental topical chemotherapeutic eyedrops such as mitomycin.19

**MIGRATING LIMBUS EPITHELIUM**

The invasion of the cornea by an entire segment of limbal epithelium with altered basal cells can be explained by MMP-2 and MMP-9 expression by these cells. Elevated expressions of both MMP-2 and MMP-9 are known to dissolve basement membrane components, such as hemidesmosomes, leading to migration and invasion of tumor cells.20,21 Consistent with the expression of MMP-2 and MMP-9 by these cells, the invasion of the cornea by an entire segment of limbal epithelium can be explained by MMP-2 and MMP-9 expression by these cells.

**DISSOLUTION OF BOWMAN’S LAYER**

We previously described 4 different groups of fibroblasts in pterygia6,19,24: (1) a group of collagen-synthesizing fibroblasts under the migrating limbus near the dissolved edge of Bowman’s layer; (2) a group of collagenase-synthesizing fibroblasts surrounding the dissolved edges of Bowman’s layer (Figures 2-4); (3) groups of collagenase-synthesizing fibroblasts located in islands (Ilots de Fuchs)12 (Figure 4) anterior to the leading edges of the pterygium and between corneal basement membrane and Bowman’s layer; and (4) groups of elastic material—synthesizing fibroblasts in basophilic areas where abnormal elastic-type material was present.

None of these fibroblast groups expressed p53 in pterygia, whereas all altered basal cells did synthesize p53,19,23 which suggests that the pterygium cells (ie, altered limbal basal epithelial cells) are the main tumor cells. We found that the p53 overexpression colocalized with the MMP expression (data not shown). Most of the fibroblasts in groups 2, 3, and 4 and a few fibroblasts in group 1 expressed mainly MMP-1 and some MMP-3 but almost none of the other MMPs (Table 2). These findings suggest that in areas of Bowman’s layer dissolution, fibroblasts are making MMPs and most likely play an important role in helping to dissolve Bowman’s layer. These fibroblasts are aided in the dissolution of Bowman’s layer by the MMP-1— and MMP-3—expressing basal cells (Figure 4 and Figure 5) as indicated by MMP-1 and MMP-3 immunostaining of Bowman’s layer in some of the sections. Because the altered limbal basal epithelial cells (the pterygium cells) express transforming growth factor β (TGF-β)24,47 the adjacent MMP-expressing fibroblasts are most likely TGF-β–basic fibroblast growth factor (bFGF) activated cells22 and are not mutationally altered ones19,23,24 (Figure 5).

**ALTERED FIBROBLASTS IN ELASTOTIC AREA: A SECOND TUMOR**

Because fibroblasts in elastotic areas are known to make abnormal elastic material, they have been considered to be altered cells.20,31 We found these fibroblasts making MMP-1 and MMP-3 but none of the other MMPs (Table 2). However, since all areas of elastotic degeneration outside the fibroblasts immunostained for all 6 MMPs (Table 3), we assume that the MMPs came from altered fibroblasts. The altered fibroblast lobules constitute a second stationary tumor (pingueculae) within the main invading pterygium tumor similar to what is present in other ocular and skin tumors with associated areas in the stroma of elastotic degeneration.74 In all of these UV–induced growths, the main tumor cell type is the epithelial cell and not the fibroblast. The fact that tumors consisting of both altered epithelial cells and altered fibroblasts can exist at the same time has been demonstrated in animal experiments where ocular tissue was treated with long-term, low-dose UV radiation.53,54

Recurrent pterygia that return within a few months after surgery do not usually have sufficient UV exposure to develop areas of elastotic degeneration. For this reason, they were assumed to be different from primary pterygia and to produce an exuberant fibroplasia as a result of an abnormal healing reaction.25 In pterygia recurring after several years, we have found elastotic degeneration in all specimens, including the one reported herein.

**THEORY OF PATHOGENESIS OF PTERYGIA**

Based on the data presented in this study and our previous reports, we propose a theory for the pathogenesis of pterygia. Albedo UV light36 (Figure 5) causes mutations in both the UV-sensitive TP53 tumor suppressor genes in the parental limbal basal cells and the elastin gene of the fibroblasts in the limbal epithelium.19 Because of a damaged p53-dependent programmed cell death mechanism,26 mutations in other genes are progressively acquired. This allows the multistep7 development of pterygia and limbal tumor cells from p53-expressing limbal epithelial cells. These cells overlie a pinguecula of altered fibroblasts that make abnormal elastic material and express various MMPs.

Mutations in the TP53 gene or TP53 family in the parental limbal basal cells also result in the overproduction by the pterygium cells of TGF-β via the p53-Rb-TGF-β pathway.24,47 Thus, pterygia are TGF-β–secreting tumors. Excess TGF-β secretion by the pterygium cells can explain many of the tissue changes.

---

**Table 3. Staining of Acellular Areas of Pingueculae, Found Within Pterygia, With Monoclonal Antibodies to MMPs* **

<table>
<thead>
<tr>
<th>MMP</th>
<th>Stromal Lobules With Wavy Elastotic Material</th>
<th>Granular Acellular Stromal Lobules</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>6/7</td>
<td>8/10</td>
</tr>
<tr>
<td>MMP-3</td>
<td>4/6</td>
<td>8/10</td>
</tr>
<tr>
<td>MMP-2</td>
<td>5/6</td>
<td>10/10</td>
</tr>
<tr>
<td>MMP-9</td>
<td>3/6</td>
<td>10/10</td>
</tr>
<tr>
<td>MT1-MMP</td>
<td>5/6</td>
<td>10/10</td>
</tr>
<tr>
<td>MT2-MMP</td>
<td>3/6</td>
<td>7/10</td>
</tr>
</tbody>
</table>

* MMP indicates matrix metalloproteinase; MT1, membrane type 1; and MT2, membrane type 2.
and MMP expressions seen in pterygia. First, pterygium cells (altered limbal basal epithelial cells) produce elevated MMP-2, MMP-9, MT1-MMP, and MT2-MMP, causing dissolution of hemidesmosome attachments. Initially, the pterygium cells migrate centrifugally in all directions onto the adjacent and joined corneal, limbal, and conjunctival basement membranes. Because of the TGF-β production of these cells, they have a reduced number of cell layers and no tumor mass is seen, resulting in an invisible tumor. Later, after an entire group of altered limbal basal cells develop and all hemidesmosomes are dissolved under these cells, they migrate as a suppressed growth onto the cornea followed by conjunctival epithelium, expressing all 6 MMPs and contributing to the dissolution of Bowman’s layer. In addition, TGF-β synthesized by the pterygium cells causes increased monocytes and capillaries within the epithelial and stromal layers (Figure 5). Second, a group of normal fibroblasts gather under the invading limbus epithelium next to the dissolved edges of Bowman’s layer and are activated by a TGF-β–bFGF pathway to produce excess MMP-1 and MMP-3 as they help to dissolve Bowman’s layer. Some of these cytokine-activated fibroblasts migrate anterior to the leading edges of pterygia between Bowman’s layer and the basement membrane of the corneal basal cells to form little islands of fibroblasts that make MMP-1 and locally help to dissolve Bowman’s layer (Figure 4).

The above steps in the formation of a pterygium are seen diagrammatically in Figure 6. Figure 6 shows the migration of the altered limbal basal epithelial cells (MMP expressing) within the body of the migrating limbus and their infiltration into the corneal and conjunctival epithelium. Figure 6 also shows the dissolution of Bowman’s layer under the body of the migrating limbus and the migration of the adjacent conjunctival epithelial cells and stromal structures, such as pingueculae, within the pterygium.

**Conclusions**

The main body of the tumor that is located in pterygia is found in the leading edges and is a migrating transparent microscopic piece of altered limbal epithelium. The migrating limbal epithelium is the occult tumor. If sufficient fibroblasts accumulate under the migrating limbus at the leading edges, the area can be seen clinically with the slitlamp biomicroscope as a gray, glassy cap. Microscopically, the migrating limbal epithelial tumor is always located between the dissolved edges of Bowman’s layer and conjunctival epithelium (as indicated by the presence of goblet cells). From this migrating limbus, altered limbal epithelial basal cells invade centrifugally in all directions into adjacent conjunctival, circumferential limbal, and corneal epithelia. As the migrating piece of limbal epithelium moves onto corneal basement membrane over Bowman’s layer, the adjacent conjunctival epithelium infiltrated with the altered limbal basal cells follows, which creates the gross clinical appearance of the pterygium.

Pterygia are tumors of altered limbal basal cells that secrete TGF-β and produce various types of MMPs similar...
lar to other invasive tumors. The tumor cell proteases degrade components of their basement membranes, which facilitates invasion. The pterygium cells invading over Bowman's layer produce elevated MMP-1, MMP-2, and MMP-9 expressions, which contribute to the complete dissolution of Bowman's layer, which consists primarily of collagen fibril types I and III. Local fibroblasts are activated by the TGF-β and bFGF cytokine pathways to help complete the dissolution of Bowman's layer by MMP-1. However, MMP-1 makes only a single cut in intact fibrillar collagen (eg, fibrillar collagen types I, II, III, VII, VIII, and X), and then the gelatinases MMP-2 and MMP-9 make successive cuts in the altered type I collagen that eventually produces complete destruction of collagen strands. As these 2 groups of cells invade into cornea, they drag along the adjacent conjunctiva and stromal structures, such as pingueculae, which consist of focal areas of noninvasive stationary fibroblast tumors synthesizing abnormal elastic material and MMPs.

Accepted for publication December 28, 2000.

This study was supported in part by grant 61-9783 from the Kaiser Foundation Research Institute, Oakland, Calif (Dr Dushku), and grant EY05587 from the National Institutes of Health, Bethesda, Md (Dr Schultz).

Drs Dushku and John contributed equally to this article.

We thank Samuel Woo, Illustration Services, University of California, Davis, for photography assistance.

Reprints and corresponding author: Nicholas Dushku, MD, Department of Ophthalmology, Kaiser Permanente Medical Center, 1650 Response Rd, Sacramento, CA 95815 (e-mail: Nicholas.Dushku@kp.org).

REFERENCES

18. Ziegler A, Leffell DJ, Kunala S, et al. Mutation hotspots due to sunlight in the
35. Liu YP, Schultz GS, Ren XO, Tan DTH. MMP-2 and MMP-9 levels in pterygia and
27. Solomon A, Li D-Q, Lee S-B, Teng SCG. Increased ex-
33. Newell KJ, Witty JP, Rogers WH, Matrisian LM. Expression and localization of
23. Dushku N, Hatcher SLS, Albert DM, Reid TW. p53 Expression and relation to
117:1593-1599.
9. Dushku N, Reid TW. Immunohistochemical evidence that human pterygia origi-
8. Dushku N, Tyler N, Reid TW. Immunohistochemical evidence that pterygia arise
52. Spencer WH. Ophthalmic Pathology: An Atlas and Textbook . 3rd ed. Philadel-
43. Kawashima Y, Saika S, Yamanaka O, Okada Y, Ohkawa K, Ohnishi Y. Immuno-
44. Knauper V, Murphy G. Membrane-type matrix metalloproteinases and cell surface-
37. Dushku N, John MK, Schultz GS, Reid TW. Pterygia pathogenesis: corneal in-
36. Di Girolamo N, McCluskey P, Lloyd A, Coroneo MT, Wakefield D. Matrix metal-
34. Keaney KF, Fogle JA, Grayson M. Dysgeneses, dystrophies and degenerations of
22. Dushku N, Albert DM, Reid TW. The use of PCR to test for human papilloma vi-
39:541-573.
117:1593-1599.
24. Reid TW, Dushku N. Pterygia and limbal epithelial cells: relationship and mo-
29. Coussens LM, Werb Z. Matrix metalloproteinases and the development of can-
33. Newell KJ, Wittry JP, Rogers WH, Matruxian LM. Expression and localization of matrix-degrading metalloproteinases during colorectal tumorigenesis. Mol Car-
34. Bolon K, Brambilla E, Vandenbunder B, Robert C, Lantuessel S, Brambilla C. Changes in expression of matrix proteases and of the transcription factor c-
35. Liu YP, Schultz GS, Reed XO, Tan DTH, MMP-2 and MMP-9 levels in pterygia and
50. Austin P, Jakobiec FA, Iwamoto T. Elastodysplasia and elastodystrophy as the patho-
38. Vegh GL, Seluck TZ, Fulop V, Genest DR, Mok SC, Berkowitz RS. Matrix metal-
26. Lee S-B, Li D-Q, Gunja-Smith Z, Liu YQ, Tan DTH, Tseng SCG. Increased ex-
25. Liotta LA, Stetler-Stevenson W, Steeg PS. Metastasis suppressor genes. In: De-
59. Liotta LA, Stetler-Stevenson W, Steeg PS. Metastasis suppressor genes. In: De-
38. Vegh GL, Seluck TZ, Fulop V, Genest DR, Mok SC, Berkowitz RS. Matrix metal-
22. Dushku N, Albert DM, Reid TW. The use of PCR to test for human papilloma vi-
70:239-249.
37. Dushku N, John MK, Schultz GS, Reid TW. Pterygia pathogenesis: corneal in-
24. Reid TW, Dushku N. Pterygia and limbal epithelial cells: relationship and mo-
379:19-23.
8. Dushku N, Tyler N, Reid TW. Immunohistochemical evidence that pterygia arise
33. Newell KJ, Witty JP, Rogers WH, Matrisian LM. Expression and localization of
8. Dushku N, Tyler N, Reid TW. Immunohistochemical evidence that pterygia arise
24. Reid TW, Dushku N. Pterygia and limbal epithelial cells: relationship and mo-
29. Coussens LM, Werb Z. Matrix metalloproteinases and the development of can-
32. Shima I, Sasaguri V, Kusakawa J, et al. Production of matrix metalloproteinase-
33. Newell KJ, Wittry JP, Rogers WH, Matruxian LM. Expression and localization of matrix-degrading metalloproteinases during colorectal tumorigenesis. Mol Car-
34. Bolon K, Brambilla E, Vandenbunder B, Robert C, Lantuessel S, Brambilla C. Changes in expression of matrix proteases and of the transcription factor c-
35. Liu YP, Schultz GS, Reed XO, Tan DTH, MMP-2 and MMP-9 levels in pterygia and


ARCHIVES Web Quiz Winner

Congratulations to the winner of our January quiz, Hal Kushner, MD, Daytona Beach, Fla. The correct answer to our January challenge was subperiosteal hematoma. For a complete discussion of this case, see the Case Reports and Small Case Series section in the February ARCHIVES (Sabet SJ, Tarbet KJ, Lenke BN, Smith ME, Albert DM. Subperiosteal hematoma of the orbit with osteoneogenesis. Arch Ophthalmol. 2001;119:301-303).

Be sure to visit the Archives of Ophthalmology World Wide Web site (http://www.archophthalmol.com) and try your hand at our Clinical Challenge Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month’s print edition of the ARCHIVES. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also receive a free copy of the book One Hundred Years of JAMA Landmark Articles.

Figure 1. Coronal computed tomographic scan demonstrating hematoma in a subperiosteal location in the right superior orbit.