ABSTRACT

Purpose. To investigate the expression of P53, AE 1/3, vimentin, CD1a in human pterygium by immunohistochemical analysis. Methods. Pterygium samples were obtained from 31 eyes of 31 patients during pterygium excision. Specimens were processed in avidin-biotin-complex immunostaining system with monoclonal anti-P53, AE 1/3, vimentin, CD1a antibody, and counterstained with hematoxylin. Results. The epithelial layers of all the samples examined were positive with AE1/3, vimentin and CD1a. For overexpression of the P53 protein, positive staining was detected in 58% (18/31) of pterygial samples. Conclusions. The pterygial development might, at least in part, be a result of disruption of the normal process of apoptosis occurring in the conjunctiva.

Key Words: Pterygium - Apoptosis – Cytokeratin - Vimentin - CD1a- Immunocytochemistry.

Originally believed to be a degenerative disease, recent thinking on the pterygium has tended to consider this disease as a growth disorder. Maintenance of cellular homeostasis is essentially regulated by two processes, i.e., cellular proliferation and cellular apoptosis. One earlier study has demonstrated similar cellular proliferation patterns between pterygia and conjunctival tissues. If then, pterygium is not a disorder of excess cellular proliferation, it is possible that it results from a failure of appropriate cellular apoptosis. To explore this possibility, we have examined pterygia specimens for the pattern of expression of genes known to be involved in the regulation of apoptosis. In addition, we also studied the cytokeratin, vimentin, and CD1a expression in the epithelia of pterygia.
PATIENTS AND METHODS

A total of 30 patients with unilateral primary were enrolled. Pterygia samples and normal conjunctival specimens were harvested from patients undergoing pterygium excision. Samples were fresh frozen in liquid nitrogen at the time of surgery, in stored at -80°C until required. All specimens were embedded in OTC freezing medium and sectioned into 5μm sections on a cryostat at -20°C and transferred onto poly-L-lysine treated slides.

Immunohistochemistry

Immunoreactive P53, AE1/3, vimentin, and CD1a were detected by the labeled streptavidine-biotin method. Sections were allowed to come to room temperature for 10 minutes at -20°C. Following extensive rinsing in PBS, endogenous peroxidases were quenched in 3% H2O2 for 5 minutes. After washing in PBS, slides were then incubated with blocking reagent for 20 minutes.

Incubation with primary antibody followed rinsing three times with PBS. Incubation was undertaken at 4°C overnight with either a 1:100 dilution of anti-P53 monoclonal antibody; anti-AE 1/3 antibody; anti-vemintin antibody; and anti-CD1a antibody. Following three rinses with PBS, slides then incubated with linking antibody (Dako) for 10 minutes, followed by 10 minutes with streptavidine- horseradish peroxidase diluted as recommended by manufacturer and then incubated for 8 minutes with DAB. After each incubation, samples were rinsed three times with PBS. Samples were then counterstained with hematoxylin for 1 minute and nuclei blued in water. Slides were then dehydrated and mounted.

RESULTS

We have examined the pattern of expression the P53, AE1/3, vimentin, and CD1a in 31 samples, respectively. As shown in Figure 1, the epithelial layers of all the samples examined were positive with AE1/3, vimentin and CD1a. For overexpression of the P53 protein, positive staining was detected in 58% (18/31) of pterygial samples.
Figure 1. Immunohistochemical analysis of pterygium. Specimens of pterygium were stained with antibody directed against AE 1/3 (upper left), P53 (upper right), vimentin (lower left), and CD1a (lower right). Original magnification X200.

DISCUSSION

In the pterygia specimens examined here, we noted P53 positive cells in the basal layer of the epithelium of the pterygia specimens, again suggestive of protein stabilization and inactivation.

Consistent with Dushku and Reid's theory that pterygium arises from the migration of altered limbal epithelial stem cells, we noted vimentin-bearing epithelioid cells over the entire surface of the pterygia in patients in this study. Our results also showed that Langerhans cells are diffusely distributed in pterygial tissue, suggesting that either that Langerhans cells are being retained in abnormal (pterygial) epithelium for a longer period of time than normal or that increased numbers of Langerhans cells are being actively attracted to pterygium by some unknown factor(s) released by the pterygium. Its role merits further research in the future.