Clinicopathologic and Immunohistochemical Studies of Conjunctival Large Cell Acanthoma, Epidermoid Dysplasia, and Squamous Papilloma

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• PURPOSE: To evaluate clinicopathologically and immunohistochemically a spectrum of conjunctival squamous proliferations.
• DESIGN: Retrospective clinicopathologic study.
• METHODS: One large cell acanthoma, 7 epidermoid dysplasias, and 4 squamous papillomas were evaluated with microscopy and biomarkers Ki-67, p53, epithelial membrane antigen (EMA), Ber-EP4, AE1, AE3, and 8 individual cytokeratins. Normal associated conjunctiva served as a baseline for interpretation.
• RESULTS: The large cell acanthoma recurred 4 times but retained its benign histopathologic features. The cells were 2-3 times larger than the keratinocytes of the normal conjunctiva and did not display atypia. Immunohistochemistry revealed a low Ki-67 proliferation index (PI) in the large cell acanthoma compared with high indices in dysplasias and papillomas. p53 was negative in the nuclei of normal epithelium while positive in all neoplasms, most intensely in the dysplasias. Immunostaining showed similar staining patterns for cytokeratins in large cell acanthoma and normal conjunctiva, except for full-thickness CK14 positivity and CK7 negativity in the lesion. Dysplasias generally lost normal CK7 expression and frequently abnormally expressed CK17. The papillomas displayed a normal cytokeratin pattern but exhibited a higher than normal PI and weak p53 positivity.
• CONCLUSIONS: Conjunctival large cell acanthoma is a morphologically distinctive clonal entity with clinical and immunohistochemical phenotypic characteristics denoting a dysplasia of minimal severity. Because of recurrences without invasion, it requires treatment. Dysplasias exhibited more deviant biomarker abnormalities including frequent aberrant full-thickness CK17 positivity and CK7 negativity. The absence of major cytokeratin derangements in the squamous papillomas may be of ancillary diagnostic value for lesions displaying borderline cytologic features. (Am J Ophthalmol 2013;156:830–846.

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CONJUNCTIVAL INTRAEPITHELIAL NEOPLASIA (CIN) or dysplasia (the term preferred in this article that also encompasses actinic lesions and carcinoma in situ) can lead to invasive carcinoma. CIN is recognized to have 2 major variants: a more common lesion composed of small, ovoid, eosinophilic, vaguely spindled, or atypical basosquamous cells, which may be admixed with larger epidermoid cells[1–5] and may also occasionally be pigmented in patients with dark complexions[6,7]; and a less common and more aggressive mucoepidermoid variant, in which goblet cells or more subtle expressions of mucoid differentiation are present among the epidermoid elements.[8] We generally prefer the term epidermoid (having eosinophilic cytoplasm) because squamous conveys a flattened rather than an oval shape although the two terms are commonly used interchangably. We have retained the term squamous for the papillomas. In this report, a rare example of conjunctival large cell acanthoma[9–12] is contrasted histopathologically and immunohistochemically with more common forms of epidermoid (squamous) dysplasia and papillomas. Our overall objective is to evaluate the role of immunohistochemistry in refining diagnostic principles and in assessing the severity of atypicality for premalignant squamous conjunctival proliferations.

METHODS

THIS RETROSPECTIVE CLINICOPATHOLOGIC STUDY WAS performed under the auspices of the Massachusetts Eye and Ear Infirmary Institutional Review Board (Protocol 12-132H) and conducted in compliance with the rules and regulations of the Health Insurance Portability and Accountability Act and in adherence to the Declaration of Helsinki and all federal and state laws.

After the diagnosis of a conjunctival large cell acanthoma was made, the diagnostic files of the David G. Cogan Laboratory of Ophthalmic Pathology at the Massachusetts Eye and Ear Infirmary were reviewed from July 1, 2008 to June 30, 2012 to identify lesions diagnosed as conjunctival squamous or epidermoid dysplasia, carcinoma in situ, conjunctival intraepithelial neoplasia, and papilloma. Among the cases retrieved was a pigmented dysplasia, which was nonetheless selected for inclusion. Blocks were evaluated for adequacy of remaining tissue to allow

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a battery of immunohistochemical tests. In addition to the large cell acanthoma, 7 routine dysplasias and 4 papillomas were deemed appropriate for this study. Normal conjunctiva present in 11 out of the 12 tissue specimens that were selected served as a baseline for comparison and analysis of the data assembled from the lesions.

Only the conjunctival aspects of the lesions were systematically evaluated immunohistochemically, inasmuch as these portions constituted the majority of the specimens and only a few had small associated strips of what appeared to be corneal epithelium. In most dysplastic lesions, the corneal component had been scraped off, supplemented with an alcohol scrub of the denuded Bowman membrane; the scrapings were not submitted for microscopic examination. Clinical and hospital records as well as clinical photographs were renewed to glean demographic data and other pertinent information on the clinical history and examination findings. In addition to hematoxylin-eosin staining and periodic acid–Schiff (PAS) staining with and without diastase, 4- to 5-μm paraffin-embedded histopathologic sections were prepared that employed the immunoperoxidase method using the chromogen diaminobenzidine with hematoxylin counterstaining. Table 1 lists the immunohistochemical probes that were available for this investigation in the Diagnostic Immunopathology Division of the Department of Pathology at the Massachusetts General Hospital.

Positivity in the immunoperoxidase preparations was determined for the cytokeratins as an all-or-none phenomenon when any intensity of immunohistochemical staining was observed in 50% or more of cells constituting the entire thickness of the epithelium. An assessment of graded intensities of positivity was not attempted except for p53 immunostaining. Specific cell counts for nuclear positivity were performed for p53 and Ki-67 only. Two counts in different areas of each lesion were averaged and reported as a percent of positive cells among those counted per high-power field (so-called proliferation index for Ki-67). In cases of moderate dysplasia, Ki-67 counts were confined to the bottom half of involved epithelium, whereas the p53 counts were made throughout the full thickness of the epithelium in both moderate and severe dysplasias. Cytokeratin (CK) staining patterns of normal and lesional conjunctival epithelium were also evaluated based on selective basilar, suprabasilar, and superficial expression of positivity. The results were collected for each diagnostic category of lesion and the categories were compared with each other and with the staining results manifested by the attached normal segments of conjunctiva in the surgical specimens.

RESULTS

• **CLINICAL FINDINGS:** The clinical features of all 12 lesions in this series are summarized in Table 2.

Large cell acanthoma. Because of its rarity and disputed nature, the clinical history of the patient with the large cell acanthoma is presented in more detail. A 51-year-old woman of Lithuanian and Irish descent developed over a period of 1 year gradual blurring of vision of the left eye associated with a foreign body sensation and occasional eye redness. Her past medical history was remarkable for pre–Waldenstrom macroglobulinemia and hypertension. She had undergone excision of atypical skin lesions in the past, but had never received a diagnosis of skin cancer. She grew up in New England and had extensive sun exposure. Best-corrected visual acuity was 20/20 OU. In 1998, slit-lamp examination showed an irregular, elevated, opalescent lesion located at the nasal limbus OS (unfortunately, a clinical photograph is not available). There was positive punctate corneal and interpalpebral conjunctival epithelial staining with fluorescein and rose bengal. The Schirmer test was abnormal (OD 3 mm, OS 1 mm). The patient was initially diagnosed with keratitis sicca, OS>OD. Debridement of the limbal lesion, which prevented pathologic evaluation of the lesional margins, was done and the patient was given punctal plugs and artificial tears. The lesion recurred after 6 months and was noted to involve more of the corneal surface, prompting another excision (1998). One year later, it recurred for the second time, and was re-excised and submitted for histopathologic evaluation (1999), which we were able to reevaluate and which showed abnormal cells at the surgical margins. During the interim, most of the ocular surface remained stable. The lesion recurred, however, for a third time after 6 years, leading to a third excisional biopsy (2005), which we were unable to review. Seven years after the last excision, the lesion recurred once again (2012). The latest lesion was located at the nasal limbus from 7-11 o'clock, appeared fimbriated, and extended 3 mm onto the cornea. Ocular surface surgery was recommended. Alcohol-assisted superficial keratectomy was performed on the affected corneal epithelium with surgical removal of adjacent involved conjunctiva. The surgical specimen displayed an uninvolved conjunctival margin but the corneal margin was positive. The ocular surface was then reconstructed with an amniotic membrane graft. The patient was treated postoperatively with topical steroids and antibiotics, with rapid resolution of the epithelial defect. There has been no recurrence after 8 months of follow-up.

Squamous dysplasias. The clinical characteristics and epibulbar locations of the remaining 11 cases in this series are briefly described in Table 2, which includes the 7 squamous dysplasias. There were 6 men and 1 woman with an average age of 69 years. All lesions arose at the limbus. The right eye was affected in 5 cases and the left in 2. The patients were symptomatic for an average of 24 months. All lesions were gelatinous or opalescent