Eyelid Sebaceous Carcinoma: Clinicopathologic and Multiparametric Immunohistochemical Analysis That Includes Adipophilin

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• PURPOSE: To evaluate the fine cytopathologic features and immunohistochemistry of eyelid sebaceous carcinoma.
• DESIGN: Retrospective clinicopathologic study.
• METHODS: Clinical records and microscopic glass slides of 12 patients diagnosed with sebaceous carcinoma were reviewed. Paraffin-embedded tissue recuts were immunoreacted for epithelial membrane antigen (EMA), Ber-EP4, p53, Ki-67, and adipophilin for cytoplasmic lipid. Invasive growth and intraepithelial spread were analyzed separately. Cytoplasmic and nuclear characteristics were correlated with the results of the immunohistochemical profiling.
• RESULTS: Five patients experienced recurrences, but no metastases or tumor-related deaths were discovered. The nuclei in 11 invasive tumor cells were typically round with finely divided, granular, or smudgy chromatin without prominent margination at the nuclear membrane; they exhibited small punctate nucleoli. Positivity for EMA (both diffuse and focal), p53 (72% of nuclei), and Ki-67 (45% proliferation index) was demonstrated. Adipophilin positivity in vesicular and granular forms was detected in paraffin sections in all invasive tumors, most prominently in moderately differentiated and well-differentiated lesions. Among 9 cases exhibiting intraepithelial extensions, 6 showed mostly granular positivity and 3 vesicular positivity. p53 identified residual atypical intraepithelial cells when conjunctival epithelial sloughing occurred.
• CONCLUSIONS: Immunohistochemistry can make significant contributions to the diagnosis of sebaceous carcinoma. p53 and vesicular granular adipophilin positivity were highly reliable in supplementing the routine microscopic diagnosis of infiltrative tumors and both can be used in paraffin sections, thereby obviating cumbersome oil red O staining of frozen sections. The cells found in intraepithelial spread were strongly EMA and p53 positive, with more granular than vesicular adipophilin positivity. (Am J Ophthalmol 2014;157:186–208. © 2014 by Elsevier Inc. All rights reserved.)

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IN THE LARGEST PUBLISHED PATHOLOGIC SERIES FROM Europe of 5504 eyelid lesions excised between 1989 and 2007, sebaceous carcinoma accounted for only 29 examples (0.5% of total series) of all benign and malignant epithelial conditions (excluded from the series were 3340 chalazia).[1] Among 6000 eyelid cases in the pathologic files of a major American eye institute, 40 cases (0.7%) were sebaceous carcinomas.[2] Sebaceous carcinoma is nonetheless the second most common eyelid malignancy (5% of malignancies in this site), after basal cell and ahead of epidermoid (squamous) cell carcinomas, in the United States.[3–5] Because of the generous number of sebaceous glands in the eyelids (intratarsal meibomian glands, Zeis glands attached to the cilia, and also those present in the caruncle and the brow), the periocular region is the most frequent single site for this malignancy throughout the entire integument (40% of all sebaceous carcinomas).[6] Sebaceous carcinoma has also been discovered to occur as a primary lacrimal gland neoplasm.[7,8] Delays in the correct diagnosis of sebaceous carcinoma can lead to recurrences, metastases, and ultimately death. Progressive education about this disease, leading to correct diagnosis and prompt, appropriate surgery, has reduced the death rate from 50% of cases in early studies[9,10] to around 2%-10% in more recent ones.[3,11] Tumors removed before 1970 in one series had a death rate of 24%, which fell to zero thereafter,[2] probably because of heightened awareness as the result of a seminal publication in 1968.[9] The morbidity of multiple surgeries attendant upon recurrences owing to incomplete eradication, however, still persists.

This investigation is aimed at devising an approach to further improve expeditious diagnostic accuracy and to determine the full extent of local tumor spread in the conjunctival epithelium. We have studied 12 patients with eyelid sebaceous carcinomas by concentrating on the fine details of cytomorphology and the use of 4 immunohistochemical markers: epithelial membrane antigen (EMA), p53, Ki-67, and adipophilin. Early[12] and subsequent[13–15] attempts to employ cytokeratin analysis have not caught on, in part possibly owing to unfamiliarity with these markers and to the ability of keratinocytes to modulate their cytokeratin expression in various diseases. Some

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authorities have concluded that other markers are more reliable than cytokeratins.[14] Additional papers on eyelid tumors[16–20] have contradicted each other with respect to the differential value and staining profiles of other immunohistochemical markers for sebaceous carcinoma vs mimicking epithelial cancers. For example, epithelial membrane antigen has been claimed to be negative in squamous cell carcinoma,[20] while sebaceous carcinoma has been asserted to be Ber-EP4 positive[19]; these erroneous "findings" have also been carried over into major textbooks.[21,22] In fact, it is now accepted that Ber-EP4 is negative in sebaceous carcinoma and EMA is negative in basal cell carcinoma, but positive in squamous cell carcinoma.[23] That "primary" conjunctival epithelial sebaceous carcinoma has been alleged to be p53 negative while intraepithelial spread associated with infiltrating disease is p53 positive is an intriguing finding that has never been reassessed.[16] It may have been the result of confusion with primary clear cell epidermoid carcinoma of the palpebral conjunctiva.[24,25]

The results of adipophilin staining, which is capable of identifying intracellular lipid in formalin-fixed, paraffin-embedded sections, has not yet been reported in the ophthalmic literature in connection with eyelid sebaceous carcinoma. If proven to be helpful, its use would obviate oil red O staining of fresh tissue, which is frequently not performed because of failure to suspect a sebaceous tumor at the time of surgery. Furthermore, identification of oil red O positivity in conjunctival biopsy specimens for the presence of intraepithelial spread is notoriously fraught with pitfalls. The differential diagnostic specificity of the 2 morphologic forms of cytoplasmic adipophilin positivity (vesicular vs granular) is to an extent unsettled.[23–26] We have attempted to resolve some of the foregoing immunohistochemical discrepancies to enhance the early and accurate diagnosis of eyelid sebaceous carcinoma and thereby facilitate optimal surgical therapy. We recognize, however, that reconciling discrepancies in contemporary immunohistochemical studies in regard to eyelid sebaceous carcinoma will continue to unfold as new data are brought forward.

METHODS

THIS CLINICOPATHOLOGIC RETROSPECTIVE STUDY WAS conducted under the auspices of the Massachusetts Eye and Ear Infirmary's Institutional Review Board (Protocol #13-056H), in compliance with the rules and regulations of the Health Insurance Portability and Accountability Act and all applicable federal and state laws, and in adherence to the tenets of the Declaration of Helsinki. After searching through the records of the David G. Cogan Laboratory of Ophthalmic Pathology at the Massachusetts Eye and Ear Infirmary from 2007-2013, 12 cases were considered acceptable to be selected for this study. This selection was based on histopathologic features displayed in formalin-fixed, paraffin-embedded, and hematoxylin-eosin-stained sections on glass slides. An essential criterion for inclusion was the availability of a paraffin block for further immunohistochemical studies derived from at least 1 surgery in the course of the disease that often included recurrences. The 12 patients had undergone a total of 30 surgical procedures. The grand total of separate specimens obtained and reviewed from all surgeries was 119 (including conjunctival map biopsies), of which 44 contained histopathologic evidence of either intraepithelial or infiltrative growth, or both. Patients' medical records and clinical photographs were also reexamined in search of possible correlations.

Immunohistochemical staining was performed on 25 out of a total of 44 histopathologically tumor-positive specimens with available paraffin blocks on file. These were considered the most representative examples of the salient and often duplicative features; this restriction therefore reduced redundancy and cost. The results of multiple positivities of the same feature were aggregated for a single summary notation in Table 2 for each case. The studies were conducted in the Diagnostic Immunopathology Laboratory of the Massachusetts General Hospital. Immunostaining was done using the Leica Bond III (Leica Microsystems, Bannockburn, Illinois, USA) with appropriate controls to validate antibody quality. The following probes were used: epithelial membrane antigen (mouse monoclonal; Leica Biosystems, Newcastle, UK; prediluted); p53 (mouse monoclonal; Leica Biosystems; prediluted) for nuclear protein accumulation attributable to mutated tumor suppressor gene; adipophilin (clone AP125; Fitzgerald Industries Intl, Acton, Massachusetts, USA; 1:75) for cytoplasmic lipid; Ki-67 (mouse monoclonal; Dako, Carpinteria, CA; 1:200) for nuclear DNA replication; and Ber-EP4 (mouse monoclonal; Dako; 1:50) for basal cell carcinoma cytoplasm, and pancytokeratin (AE1/AE3: Leica Biosystems; 1:1000 and Cam 5.2: BD Biosciences, Franklin Lakes, New Jersey, USA; 1:100). Endogenous peroxidase activity was blocked by H_2O_2 before antibody incubation. The chromogen diaminobenzidine was used and the tissues were counterstained with hematoxylin. Nuclear staining was recorded as percent positivity for p53 and Ki-67 (proliferation index), whereas cytoplasmic or cell membrane staining was noted to be positive or negative for EMA and Ber-EP4, respectively. Positive cytoplasmic staining for adipophilin was scored as 3+ (>50% of cells), 2+ (26%-50%), 1+ (6%-25%), and negative (≤5%).

RESULTS

• **CLINICAL FINDINGS:** The clinical features of the cases involved in this study are summarized in Table 1. Among