Androgen Receptor Identification in the Diagnosis of Eyelid Sebaceous Carcinomas

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• PURPOSE: To assess the role of androgen receptor detection in diagnosing eyelid sebaceous carcinomas and to compare it with that of adipophilin.
• DESIGN: Retrospective, clinicopathologic study.
• METHODS: Ten sebaceous carcinomas (8 invasive, 2 intraepithelial only) were stained immunohistochemically for androgen receptors and were compared with adipophilin staining. Receptor staining also was performed on benign sebaceous tumors (a sebaceoma and an adenoma) and as controls on eyelid basal cell carcinomas, eyelid squamous cell carcinomas, conjunctival squamous dysplasias, and conjunctival melanomas.
• RESULTS: All 8 patients with an invasive component of sebaceous carcinoma underwent a biopsy in which the tumor cells showed diffusely positive results for androgen receptors (>20% of cells and usually >40%) and positive results for adipophilin. Eight cases displaying an intraepithelial (or pagetoid) component of spread also showed diffusely positive results for androgen receptors and adipophilin in at least 1 of multiple biopsy samples from each patient. However, in 8 of 21 separate conjunctival biopsy specimens with intraepithelial cytologic atypia, adipophilin results were negative. A sebaceoma and a sebaceous adenoma also showed positive results for both of these biomarkers. Among the controls, squamous carcinomas and melanomas showed negative results for androgen receptors and adipophilin. Basal cell carcinomas displayed focal receptor positivity in fewer than 5% of cells and showed negative results for adipophilin.
• CONCLUSIONS: Androgen receptors and adipophilin can separate sebaceous tumors immunohistochemically from squamous carcinomas and melanomas, which showed negative results for both, and from basal cell carcinomas, which showed positive receptor results in a distant minority of cells. Regarding intraepithelial (or pagetoid) spread, androgen receptor detection was more sensitive and reliable than adipophilin in highlighting this component of the disease. (Am J Ophthalmol 2014;157:687–696. © 2014 by Elsevier Inc. All rights reserved.)

APART FROM THEIR ROLE IN REGULATING SEXUAL and reproductive functioning, androgen receptors also are present in many tissues and affect important aspects of the body's nonsexual economy. Dermatopathologists have correlated male pattern baldness with androgen receptors,[1] which have been identified in the pilosebaceous-apocrine unit. Work with androgen receptors has been limited in ophthalmology to elucidating their impact on the regulation of Meibomian gland functioning that assists in maintaining the integrity of the ocular surface.[2,3] Among tumors, androgen receptor physiology in tumors has been studied most thoroughly in prostatic neoplasms.[4,5] Only a few nonophthalmic skin tumors have been evaluated immunohistochemically for androgen receptors, such as basal cell carcinomas, trichoblastic lesions, chondroid syringomas, and benign and malignant sebaceous tumors.[6–10]

Although sebaceous malignancies of the eyelids logically by extrapolation may be expected to have similar immunohistochemical properties to those found elsewhere in the skin, this presumption has not yet been clearly proved scientifically. Indeed, the Meibomian glands are unique in the skin for not being associated with either a strong or vellus hair (only the Zeis glands are attached to the eyelashes). Furthermore, intraepithelial (or pagetoid) spread is seen rarely in nonophthalmic skin examples,[11] which may alter tumor cell biomarker expression and obscure the correct diagnosis. In the current article, we explore the diagnostic value of androgen receptors with respect to benign and malignant ocular adnexal sebaceous tumors[9,12,13] and compare the immunostaining results with those obtained with adipophilin.[12,13] We also investigated whether sebaceous tumors can be distinguished with androgen receptors from other tumors, such as squamous and basal carcinomas and melanomas.[9]

METHODS

THIS RETROSPECTIVE STUDY WAS CONDUCTED UNDER THE auspices of the Massachusetts Eye and Ear Infirmary Institutional Review Board (protocol no. 13-056H) and in compliance with the rules and regulations of the Health Insurance Portability and Accountability Act, in

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adherence to the tenets of the Declaration of Helsinki, and according to all relevant federal and state laws.

Eight of the 10 selected patients had infiltrating eyelid sebaceous cell carcinomas and 8 had a component of intraepithelial spread. Seven cases have been reported previously for adipophilin expression,[12] but not for androgen receptors, and therefore have been included in this study. These cases were acceptable because there was adequate tissue remaining in the paraffin blocks for further immunostaining. Three new patients not previously reported also are included in this series. The glass slides from all of the earlier biopsies were reviewed. For invasive disease, the paraffin block of the most representative section stained with hematoxylin and eosin, mucicarmine and periodic acid Schiff was chosen for additional immunostaining for androgen receptors and adipophilin. A total of 21 separate tissue specimens from all 8 patients manifesting intraepithelial spread were immunostained for androgen receptors and adipophilin to establish their variable rates of expression and their relative values as biomarkers for diagnosis (if discernible).

As controls, an eyelid sebaceoma that has been published,[13] but without androgen receptor testing, and an unreported caruncular sebaceous adenoma with papillary features also were evaluated for comparison. Additionally, 5 eyelid-infiltrating epidermoid (squamous)-cell carcinomas, 5 basal-cell carcinomas, and 5 conjunctival melanomas were investigated for androgen receptors. Seven conjunctival juxtalimbal epidermoid (squamous) dysplasias, including 5 that have been reported previously[14] without investigating androgen receptors, also were used as controls.

Immunohistochemical evaluations were performed on archived formalin-fixed, paraffin-embedded tissue blocks after 5-μm tissue sections were obtained. Infiltrating lesions were judged to have overall positive results for sebaceous carcinoma if androgen receptors and adipophilin (either in its vesicular or granular forms) each were discovered in 5% or more of cells or stated otherwise, the lesions were regarded as having negative results if 95% or more of cells were nonimmunoreactive. All tumors reached the level of positivity. Immunohistochemical stains were processed in the standard manner with appropriate nonophthalmic tissue controls on a Leica Bond III autostainer (Leica Biosystems, Melbourne, Australia) at the Diagnostic Immunopathology Laboratory of the Massachusetts General Hospital (Boston, Massachusetts, USA). The tissue controls validated the quality and specificity of antibody staining. The antibody probes for detecting the following biomarker antigens were used: androgen receptor (mouse monoclonal, clone AR441, 1:50; Dako, Carpinteria, California, USA) detectable in the nucleus; adipophilin (mouse monoclonal, clone AP125, 1:75; Fitzgerald Industries International, Acton, Massachusetts, USA) for cytoplasmic lipid; and microphthalmia-associated transcription factor for the nuclei of melanoma cells (mouse monoclonal, clone D5, ≥ 1:120; Cavendish Scott Ltd, Hertforshire, United Kingdom). For androgen receptors, heat-induced epitope retrieval was carried out before immunohistochemical staining using Bond epitope retrieval solution 2 (ph, 9.0; Leica Biosystems, Newcastle-upon-Tyne, United Kingdom) for 20 minutes. All immunohistochemical stains were developed using the chromogen diaminobenzidine, and tissues were counterstained with hematoxylin. The tissue slides were visualized using routine light microscopy.

RESULTS

• **SEBACEOUS CARCINOMAS:** Of the 10 patients in this study with sebaceous carcinoma of the eyelids, 8 were women and 2 were men. Their ages ranged from 44 to 98 years, with a mean of 76 years. The lower eyelids were involved in 6 cases and the upper eyelid in 4 cases. Three patients (30%) experienced recurrences, but there have been no tumor-associated deaths. Six patients (60%) underwent biopsies or excisions that showed evidence of infiltrating sebaceous carcinoma (Figure 1, Top left), 2 patients (20%) had infiltrating disease alone, and 2 patients had only intraepithelial disease. Two benign sebaceous lesions also were studied for androgen receptor expression: a caruncular sebaceous adenoma in a 68-year-old woman (Figure 1, Top right) and a sebaceoma in a 53-year-old man (this case was published previously without androgen receptor analysis[13]).

For a background of normal eyelid adnexal glandular immunostaining, 10 normal uninvolved regions of full-thickness eyelid specimens, removed for the treatment of basal cell and epidermoid carcinomas, sebaceous tumors, floppy eyelids, or ectropion or entropion, were evaluated. The epidermis and palpebral conjunctival epithelium showed negative results for androgen receptors and adipophilin. Androgen receptor nuclear positivity was noted among many basal and occasional suprabasal cells in the Meibomian and Zeis glands (Figure 1, Bottom right), but not in the nonkeratinizing squamous duct cells (Figure 1, Bottom left). The smaller lobules (alveoli) exhibited more positive nuclei centrally than the larger ones. The most intense staining was found in the inner secretory cells of the apocrine glands of Moll (Figure 1, Bottom left inset), which was consistently observed in 7 separate specimens. Five accessory lacrimal glands of Wolfring (tarsus) were present in surgical excisions and showed negative results for androgen receptors.

A summary of the main histopathologic diagnostic categories and their immunostaining characteristics is provided in the Table. The 6 cases showing both infiltrating and intraepithelial sebaceous carcinoma displayed vacuolated cells in each component, with oval or round nuclei manifesting a fine stippling of the chromatin and a small punctate nucleolus, as has been reported previously and illustrated in detail.[11] The invasive component in these