Comparative Examination of Androgen Receptor Reactivity for Differential Diagnosis of Sebaceous Carcinoma From Squamous Cell and Basal Cell Carcinoma

Fahimeh Asadi-Amoli, MD,1,2 Farid Khoshnevis, MD,1 Hayedeh Haeri, MD,1 Issa Jahan zad, MD,1,3 Reza Pazira, MD,1 and Reza Shahsiah, MD, FASCP1,3

Key Words: Sebaceous carcinoma; Squamous cell carcinoma; Basal cell carcinoma; Androgen receptor; Immunohistochemistry

Abstract

Sebaceous carcinoma (SEB) is the most important malignant neoplasm of the eyelid because of the potential risk of metastasis.1 It arises in the skin of the eyelid, mostly in the meibomian or tarsal glands, followed by the sebaceous glands of the lashes or the glands of Zeis.1,2 SEB is more prevalent in females and in the elderly1,2 and usually manifests as a small, firm nodule resembling a chalazion.1,3,4 Persistent unilateral conjunctivitis or blepharitis may also be noticed along with the loss of eyelashes, which is caused by intraepithelial neoplastic invasion of the hair follicles.1 Invasion of tumor cells into the eyelid, conjunctiva, or nasal cavity may mimic other ophthalmologic disorders and is referred to as the masquerade syndrome.1,5 The various clinicopathologic features that indicate a poor prognosis are lymphovascular and orbital invasion, involvement of both upper and lower eyelids, poor differentiation, multicentric origin, long duration of symptoms, large tumor size, a vastly infiltrative pattern, and pagetoid invasion of the epithelia of skin or conjunctiva.6 Morphologic features of extraocular SEBs are not much different from those arising in the eyelids, plus these tumors demonstrated aggressive behavior in some cases.7

SEB should always be considered in the differential diagnosis of eyelid tumors and must be differentiated from basal cell carcinoma (BCC) and squamous cell carcinoma (SCC),8 particularly BCC with sebaceous differentiation and SCC with hydropic changes.8,9 BCC is the most common malignant neoplasm of the eyelid and rarely metastasizes.1 On the other hand, SCC is not a frequent neoplasm of the eyelid1,11 and occasionally metastasizes.11 Correct and timely diagnosis of SEB can be very helpful, and adequate treatment with primary wide surgical excision significantly improves the prognosis.12

Sebaceous carcinoma (SEB) is the most important malignant tumor of the eyelid. Early diagnosis and proper treatment significantly improve the outcome. SEB should be differentiated histopathologically from basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).

In this study, the expression of androgen receptor (AR) in SEB, SCC, and BCC was evaluated. AR was positive in all 19 SEB cases. Of 18 BCCs, 6 (33%) showed focal nuclear immunoreactivity. The 18 SCCs showed no nuclear immunoreactivity.

AR is a sensitive marker for SEB, especially in less differentiated tumors. Along with other markers and morphologic features, AR can be helpful in the diagnosis of SEB and its differentiation from SCC and BCC.
Cytoplasmic lipids are seen in more differentiated cells in the central part of the normal sebaceous glands and tumors with sebaceous differentiation.13 The presence of intracytoplasmic lipid vacuoles identified by fat stains such as oil red O and Sudan IV may aid in the correct diagnosis of SEB.1,13 Oil red O and Sudan IV staining techniques are best performed on fresh unprocessed tissue, which is not always available because the biopsy sample may be small and not suitable for frozen sections.13 On the other hand, in many situations, SEB is not suspected before the microscopic examination of the tissue is reported. Therefore, in recent years immunohistochemical staining has been extensively used to diagnose SEB and differentiate it from SCC and BCC.13-17

Antibodies such as adipophilin, perilipin, and TIP47 have been developed to recognize proteins associated with lipid droplets. These antibodies can be used for immunohistochemical staining in formalin-fixed, paraffin-embedded specimens.13 Positive staining for epithelial-membrane antigen and BRST-1 is seen in well-differentiated SEBs; positive staining is more prominent in sebaceous-differentiated cells in the center of the nests, morphologically equivalent to normal sebaceous gland central cells.17 Anti–low-molecular-weight keratin antibody CAM 5.2 weakly stains normal sebaceous glands. Nearly 75% of the sebaceous carcinomas show positive staining with this antibody.17 The androgen receptor (AR) is localized in the peripheral cells of normal sebaceous glands, and its reactivity in the central cell components is less intense.18 Likewise, in sebaceous neoplasms as in normal sebaceous glands, AR and cytokeratins (PKK1 and MNF116) are less expressed in more differentiated tumor cells in the center of lobules.19

The aim of this study was to evaluate the status of AR in invasive tumors of the eyelid and its usefulness in differential diagnosis of these tumors.

Materials and Methods

In this descriptive study, the cases with invasive tumor of the eyelid, including SEB, SCC, and BCC, were found in the computer database, and related paraffin blocks were retrieved. Related H&E-stained slides were reevaluated, and cases with unequivocal histopathologic features were included in the study. The histopathologic features of each tumor are described elsewhere.1,8 Invasive tumors with sebaceous differentiation (cells become more lipid laden and foamy as they shift to the center of the nests) or sheets of foamy cells were categorized as SEB. Focal areas of squamous differentiation in a typical case of BCC or SEB were ignored because squamous differentiation can be seen in these tumors.8,20 Otherwise, invasive tumors with squamous differentiation were categorized as SCC. Suitable slides with adequate fixation and the least hemorrhage and necrosis were chosen for immunohistochemical study.

Eventually, 55 formalin-fixed, paraffin-embedded blocks were selected, from which slides with 5 micrometer sections were prepared. Subsequently, the sections were deparaffinized, and antigen retrieval was carried out by heat treatment in 0.294% wt/vol citrate buffer at pH 6.0 in a domestic microwave oven as follows: 900 W for 5 minutes and then 600 W for 10 minutes, avoiding slide dry out. Immunohistochemical staining was performed using the LSAB method (labeled streptavidin-biotin) with the monoclonal antibody (monoclonal mouse anti-human AR clone AR441, DAKO, Glostrup, Denmark) as recommended by the manufacturer. Hyperplastic prostate tissue was used as the positive control sample (nuclear staining in epithelium of prostatic glands).

Subsequently, immunohistochemically stained slides were randomized, and the ratio of positive nuclei to all tumor cells was estimated and recorded for each slide by an expert pathologist (I.J.). Nuclear staining in more than 50% of tumor cells was assessed as diffuse reactive, nuclear staining in 50% or fewer of tumor cells was considered focal reactive, and nuclear staining in fewer than 1% of cells was considered negative.

The data were analyzed by using SPSS software, version 11.5 (SPSS, Chicago, IL). Variables were compared by means of the $\chi^2$ test.

Results

Of the patients represented in the 55 selected cases, 38 were male and the rest were female. Of the 55 patients, 19 had SEB, 18 had SCC, and 18 had BCC. Of 19 SEB cases, 12 (63%) were male, and the rest were female. Of 18 SCC cases, 12 (67%) were male, and the rest were female. Of the 55 patients, 19 had SEB, 18 had SCC, and 18 had BCC. Of 19 SEB cases, 12 (63%) were male, and the rest were female. Of 18 SCC cases, 14 (78%) were male and the rest were female.

All 19 SEBs showed immunohistochemical nuclear AR staining of tumoral cells; furthermore, 13 (68%) of the cases showed a diffuse pattern of AR reactivity Image 1. Some of the BCCs (6/18 [33%]) showed a nuclear reaction to AR Image 2, and the rest were negative. None of the BCCs showed a diffuse pattern of nuclear reaction. All 18 SCCs were AR- and showed no nuclear reaction Image 3.

Focal nuclear AR reactivity was more common in BCC than in SCC ($P < .019$). On the other hand, diffuse nuclear AR reactivity was more common in SEB than in SCC ($P < .001$) and SCC ($P < .001$). There was no relation between the tumor type and sex ($P > .05$) in the present study. The results are summarized in Table 1.

Discussion

Differential diagnosis of SEB from SCC and BCC is based on morphologic findings, but in some cases this differentiation is difficult or even impossible, particularly in small biopsy specimens.
Asadi-Amoli et al. / Sebaceous Carcinoma vs SCC and BCC

In the present study, all of the SEB cases were positive for AR. This finding is consistent with the results reported by Bayer-Garner et al. Although the majority of SEB cases showed diffuse AR reactivity, in contrast with the findings of Bayer-Garner et al., the percentage of AR-reactive tumor cells was less than 90% in most cases. Considering the dimorphic pattern of AR staining in sebaceous cells (Image 1), inclusion of more differentiated tumors could be the major reason for this difference. Meanwhile, variation in formalin fixation and antigen retrieval has a role.

In the present study, focal AR reactivity was found in 33% of BCC cases, whereas 60% to 78% of BCC cases were focally reactive for AR in previous studies. In most of our AR+ BCC cases (5/6), reactive tumor cells constituted more than 5% of tumor cells. In contrast, all BCCs exhibited AR reactivity in fewer than 5% of tumor cells in the study by Bayer-Garner et al. The same AR antibody clone was used for staining in both studies, and, again, the difference could be related to case selection, fixation, and immunohistochemical staining technique. BCC with sebaceous differentiation and mixed basal cell–sebaceous carcinomas are on record. Meanwhile, SEB demonstrates a vast range of differentiation with a dimorphic AR and cytokeratin immunohistochemical reaction pattern of tumor cells. Although a diffuse AR pattern in BCC is less likely for the aforementioned reasons, defining a definitive
Anatomic Pathology / Original Article

A cutoff point of AR reactivity alone for distinguishing SEB from BCC is not easy.

Some salivary gland carcinomas are AR+24; remission of salivary gland carcinomas following antiandrogen therapy has been reported.24,25 As in salivary gland carcinoma, antiandrogen therapy may be of value in SEB.

To date, researchers have found different markers for different cellular components of sebaceous tumors.17,19 Combining AR with a marker for well-differentiated tumor cells (ie, BRST-1 or epithelial membrane antigen) may be of value in the diagnosis of SEB in problematic and difficult cases.

From the 1Department of Pathology, 2Farabi Eye Hospital, and 3Cancer Research Institute, Tehran University of Medical Sciences, Tehran, Iran.

Supported by a grant from Tehran University of Medical Sciences Research Center.

Address reprint requests to Dr Shahsiah: Dept of Pathology, Tehran University of Medical Sciences, Keshavarz Blvd, Tehran, Iran.

**Table 1**

Reactivity of Androgen Receptor in Sebaceous, Squamous Cell, and Basal Cell Carcinoma in Men and Women*

<table>
<thead>
<tr>
<th>Carcinoma Type</th>
<th>Male</th>
<th>Female</th>
<th>Reactive</th>
<th>Nonreactive</th>
<th>Focal</th>
<th>Diffuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sebaceous (n = 19)</td>
<td>12 (63)</td>
<td>7 (37)</td>
<td>6 (32)</td>
<td>0 (0)</td>
<td>13 (68)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell (n = 18)</td>
<td>14 (78)</td>
<td>4 (22)</td>
<td>0 (0)</td>
<td>18 (100)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Basal cell (n = 18)</td>
<td>12 (67)</td>
<td>6 (33)</td>
<td>0 (0)</td>
<td>12 (67)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).

**References**


Acknowledgments: We thank F.A. Ardalan, MD, for excellent comments and F. Mahjoub, MD, and Zahra Omidi for devoted support.
Asadi-Amoli et al / Sebaceous Carcinoma vs SCC and BCC


