Helpful immunostains for the differential diagnosis of PSC include EMA, Ber-Ep4, AR, and adipophilin.

Sebaceous Carcinoma of the Eyelid
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Background: Periocular sebaceous carcinoma (PSC) is a rare but aggressive neoplasm that tends to clinically and histopathologically mimic other conditions. PSC can be challenging to diagnose using histomorphology alone given its overlap with 2 more common tumors that occur in this area (basal cell carcinoma [BCC] and squamous cell carcinoma [SCC]). Use of immunohistochemistry can help resolve this differential diagnosis.

Methods: A review of the literature was performed, focusing on the epidemiology, morphology, and immunohistochemical features of PSC.

Results: The most useful immunostains in the differential diagnosis of PSC are epithelial membrane antigen, Ber-Ep4, androgen receptor (AR), and adipophilin. To discern PSC from BCC, one should use EMA, Ber-Ep4, AR, and adipophilin, whereas discerning PSC from SCC can be achieved by evaluating AR and adipophilin. In addition, p53 and ERBB2 (formally known as HER2/neu) are other potentially useful immunohistochemical markers for the differential diagnosis of PSC.

Conclusions: Use of new immunohistochemical techniques, as well as the elucidation of molecular alterations, such as the presence of ERBB2 amplification, will advance our understanding of PSC.

Introduction
Cutaneous sebaceous carcinoma is a rare disease that makes up an estimated 0.05% of all cutaneous malignancies.1,2 However, because the eyelid and periocular regions harbor a high density of sebaceous glands either associated with eyelashes (Zeis) or the tarsal plate (meibomian), it is not surprising that, when considering all cutaneous sebaceous carcinomas, up to 40% of these tumors arise in this particular anatomical site.3 In the United States, sebaceous carcinoma represents 5% of all malignant neoplasms arising in the periocular region, followed basal cell carcinoma (BCC), which makes up 90% of eyelid malignancies.4,6

In general, periocular sebaceous carcinoma (PSC) presents in the elderly (average of 70 years of age).1-3 Predisposing factors, such as immunosuppression, prior history of radiotherapy to the head and neck, and Muir–Torre syndrome, occur in a minority of patients.7 PSC can clinically present as a diffuse thickening of the eyelid, often exhibiting inflammatory features. More advanced cases are characterized as a discrete, palpebral mass. The clinical presentation of early-stage PSC has the potential of mimicking non-neoplastic lesions, notably chalazion, keratoconjunctivitis, and other inflammatory, non-neoplastic processes.5,8,9 Thus, those with PSC are at an increased risk for delayed diagnosis.

Historically, a diagnosis of PSC was associated with a poor prognosis and a mortality rate that reached 50%.10,11 However, with increased disease awareness and the introduction of better diagnostic
techniques, rates of survival have improved (mortality rates of 2%–11%). Early identification is crucial and relies on a high level of clinical suspicion, together with an accurate histopathological diagnosis that often depends on small or limited specimens on biopsy.

**Histomorphology**

When examining the invasive component of PSC at low magnification, 4 basic morphological patterns can be recognized: trabecular, lobular, papillary, and BCC-like (Figs 1 and 2). Similar to BCC, a mixture of 2 or more of these patterns can be seen in a single lesion. However, all of the classic features of BCC are not found, such as peripheral palisading, tumor–stromal cleft artifacts, and mucinous/myxoid spindle cell stroma. The trabecular pattern of PSC has a tendency to reveal areas of central comedo-like necrosis. An in situ component of PSC may be encountered, either as a standalone lesion as well as adjacent or overlapping an invasive lesion (see Figs 1C–F). The intraepithelial lesions are often bowenoid in appearance and can exhibit exuberant pagetoid spread (see Figs 1D and 1E). They can also radially spread by undermining the normal conjunctival epithelium (see Fig 1F). Keratinization is not usually a feature of PSC. The presence of changing keratinization should prompt the health care professional to consider squamous cell carcinoma (SCC) with sebaceous differentiation rather than PSC.

In a similar fashion to cutaneous sebaceous carcinoma, cytologically PSC tumors are composed of 3 basic cell types: basaloid, sebaceous/vacuolated, and intermediate forms. These cell types are usually intermixed at varying ratios, which will determine the level of differentiation of a given tumor, among other factors (see Figs 2D, 2E, and 2G). Broadly speaking, the percentage of basaloid cells will be inversely correlated with the level of differentiation, which ranges from well-differentiated tumors to poorly differentiated and anaplastic/de-differentiated tumors.

Another factor to consider when grading a lesion is the architecture of the tumor. Well-differentiated PSC tumors are often nodular, whereas poorly differentiated tumors are more infiltrative. Taking into account all of these features, including grading, architecture, presence or absence of an in situ component/pagetoid spread, as well as presence of spread to distant sites, the following 4-tiered classification has been proposed: PSC in situ, low-grade, high-grade infiltrating PSC with or without pagetoid spread, and PSC with extraocular/extracutaneous spread, including distant metastases.

In addition to its ability to clinically mimic other lesions, PSC can also overlap the histopathology of several benign and malignant entities encountered in the periocular region. Among the benign processes, the health care professional should consider sebaceous adenoma, follicular adnexal tumors (eg, desmoplastic trichoepithe-
When considering malignancies, the 2 main possibilities are BCC — particularly with sebaceous differentiation — and SCC with sebaceous differentiation (rare). The latter also includes a basaloïd variant. A rare but plausible differential diagnostic possibility — particularly for poorly differentiated types of PSC — is Merkel cell carcinoma, which is a malignant neoplasm with a predilection to arise in the head and neck area. When confronted with in situ PSC with abundant pagetoid spread, other pagetoid malignancies such as pagetoid SCC in situ, melanoma in situ, and intraepidermal Merkel cell carcinoma should be considered.

Morphologically, the nuclear features in PSC tumor cells have a distinctive shape — namely, they are round, oval, or indented — and contain fine, indented, or smudgy chromatins with 1 or 2 small nucleoli (see Figs 1E and 2G). These features are not found in Merkel cell carcinoma, which have washed-out, neuroendocrine-type chromatins. By contrast, melanoma cells often display large nuclei with vesicular chromatins and macronucleoli. In a proportion of poorly differentiated types of PSC (approximately 23%–77%), morphological assessment will be hampered by the presence of overlapping features with other entities included in the differential diagnosis. In such situations, immunohistochemical stains may be helpful.

**Immunohistochemistry**

Among the malignant tumors that arise in the periorbital region, the 3 most common are BCC, squamous cell carcinoma (SCC), and PSC. Several immunohistochemical markers are useful in the differential diagnosis, including epithelial antigens, as are hormonal markers and other markers highlighting fat vacuole production. Immunohistochemical stains will also re-

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**Fig 2A–I.** — Panel exhibiting immunohistochemical staining patterns of 3 markers useful for the differential diagnosis of PSC. (A–C) Epithelial membrane antigen often exhibits patchy but intense membranous and cytoplasmic positivity in PSC. An internal control is present and represented by normal sebaceous glands (C, arrow). A: H & E, ×20. B: H & E, ×20. C: H & E, ×200. (A, D, E) Although this case exhibits a basal cell carcinoma–like pattern (D and E: H & E, ×200), (F) Ber-Ep4 is completely negative. F: H & E, ×200. An internal control is present in the germinative portion of an adjacent follicular structure (arrow). (G) The presence of intermediate cells with lipid vacuoles (arrows) is highlighted with adipophilin. G: H & E, ×400. (H, I) A “vacuolar-type” staining pattern is seen. H: H & E, ×200. I: H & E, ×400.

H & E = hematoxylin and eosin, PSC = periorbital sebaceous carcinoma.
The 2 epithelial markers deemed to be most useful in the differential diagnosis of PSC, BCC, and SCC are epithelial membrane antigen (EMA) and Ber-Ep4.7,24

**Epithelial Membrane Antigen:** Also known as mucin 1, cluster designation 227, and cancer antigen 15-3 (particularly when detected in serum), EMA is a 75-kDa transmembrane glycoprotein coded by the 1q21 locus. It is commonly used as an epithelial marker in conjunction with cytokeratins. In normal skin and conjunctiva, EMA is expressed in glandular structures, including sebocytes (see Fig 2C, arrow), as well as in squamous epithelium, but not in follicular structures.25 EMA exhibits a membranous and cytoplasmic signal (see Figs 2B and 2C) and is prominently expressed in the majority of cases of PSC and SCC, while being predominantly negative in BCC.7,24

**Ber-Ep4:** Ber-Ep4 is a monoclonal antibody directed toward the epithelial cell adhesion molecule, a transmembrane glycoprotein coded by the 2p21 locus and normally expressed at the basolateral membrane of cells by the majority of epithelial tissues, except in adult squamous epithelium and by some specific epithelial cell types (eg, hepatocytes).26,27 This marker is expressed in the secretory portion of eccrine glands and follicular germinative cells in normal skin and eyelids (see Fig 2F, arrow).28 This antibody exhibits a predominant membranous pattern of staining; it is most useful in distinguishing BCC from PSC and SCC because it is frequently positive for Ber-Ep4 (70%–100%).24,28 By contrast, PSC and SCC are negative for Ber-Ep4 in 74% to 94% and 100% of cases, respectively (see Fig 2F).24,28

**Cytokeratin:** Intermediate filaments known as cytokeratins are expressed in carcinomas (and rarely in a few mesenchymal tumors). Because cytokeratin is also expressed in other entities included in the differential diagnosis of PSC, expression of CAM 5.2 (a cytokeratin cocktail) is not particularly useful to resolve the differential diagnosis for PSC.29 Cytokeratin 7 is expressed in a higher percentage in PSC cases (88%) compared with BCC cases (28%); this is especially true in SCC cases (9%), so it could be used as an adjunctive tool.24 In addition, the expression of cytokeratin 19 may be helpful in distinguishing sebaceous carcinomas (17% focally positive) from BCC (64% strongly positive and 14% focally positive).30 However, given the ubiquity of cytokeratins in epithelial malignancies, the ability of cytokeratins to discriminate among the common tumors around the eye is limited.

**Thomsen–Friedenreich (T) and its Precursor (Tn) Antigen:** These markers are composed of O-glycosylated, mucin-type complex carbohydrates and are — in addition to the glycoporphin protein system — part of the MNs blood group system. They are expressed in carcinomas from a visceral origin while being hidden from the immune system via the addition of covalently linked carbohydrates and sialic acid in the majority of normal tissues, with the exception of mature sebocytes, the luminal aspect of the sweat glands, and in the intercellular junctions of the spinous layer of the epidermis.31,32 A small study specifically testing the immunohistochemical expression of the Thomsen–Friedenreich (T) antigen found a strong expression of this marker in the majority of sebaceous carcinoma cases (n = 8) tested as well as in the sebaceous adenoma (n = 15) and sebaceoma (n = 9) samples, while being negative in the all but 1 of the tested BCC cases (n = 13); only 1 case of BCC was positive for sebaceous differentiation.13,32 Although no follow-up studies were found in the literature regarding this marker, one could consider incorporating T-antigen immunohistochemistry to resolve this differential diagnosis.

**Lipid-Processing Markers**

Sebaceous carcinomas are related to the production of lipids (mainly triglycerides). Historically, the health care professional was only able to rely on the often cumbersome histochemical demonstration of lipids utilizing Oil red O or Sudan black IV, inconvenient stains that required a frozen tissue sample and were associated with both poor rates of sensitivity (40%) and stain fading over time.33 However, another group of immunohistochemical markers demonstrating lipid production are now available. This group of markers is composed of 3 lipid droplet–associated proteins comprised of the so-called perilipin, adipophilin, and TIP47 group (also known as perilipins 1, 2, and 3, respectively). These molecules are associated with the phospholipid monolayer membrane surrounding the triglyceride core of the intracytoplasmic lipid droplets and are related in the formation, maintenance, modifi-

### Table. — Three Commonly Used Immunohistochemical Markers for the Differential Diagnosis of Periocular Sebaceous Carcinoma

<table>
<thead>
<tr>
<th>Type of Carcinoma</th>
<th>Epithelial Membrane Antigen</th>
<th>Ber-Ep4</th>
<th>Androgen Receptor</th>
<th>Adipophilin</th>
<th>p53</th>
<th>ERBB2</th>
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<td>Periocular sebaceous</td>
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<td>−/-</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>−/-</td>
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<td>+</td>
<td>−</td>
<td>−</td>
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<td>+/-</td>
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cation, and involution of such structures. All of these markers appear specific for lipid droplets in normal tissues as well as in a variety of malignant neoplasms with lipogenic features. Although adipophilin appears to be the most sensitive marker of the group, perilipin is considered to have superior rates of specificity, and both immunostains have proven to be of particular utility in the differential diagnosis of PSC,23,25,33,39,40

**Adipophilin:** Also known as adipocyte differentiation–related protein and perilipin 2, adipophilin is a lipid vesicle–associated protein coded by a gene in 9p22.1. This marker is expressed in normal sebocytes. However, when using this stain, the health care professional must be aware of its different staining patterns, including its diffuse cytoplasmic signal pattern, which is often encountered in well-differentiated areas of PSC tumors; its vacuolar, vesicular-rimming pattern (see Figs 2G and 2I); or the presence of both of these, thus highlighting the intermediate cells of a PSC tumor; and a fine particulate, granular, or dusty pattern (considered nonspecific and could be found in other tumors [eg, SCC, BCC]). The health care professional must remember the poorly differentiated areas of PSC tumors will often be lipid-poor and basaloid cell-rich; thus, they may be predominantly negative or weakly positive for adipophilin in an overall patchy pattern.7,23,25,33,39 Adipophilin can be useful when differentiating PSC that is predominantly immunoreactive, showing either vacuolar or diffuse patterns, or is predominantly negative or weakly positive with a finely granular pattern in the setting of BCC and SCC.

**Perilipin:** Perilipin is a transmembranous protein related to lipid vesicles coded by the 15q26 locus that is expressed in sebocytes. Perilipin appears to be more specific but less sensitive than adipophilin.33,39 The different patterns of staining are similar to those of adipophilin, and the health care professional could use this marker in cases for which adipophilin was unsatisfactory.

**Androgen Receptor**
Androgen receptor (AR) is coded by Xq12 and forms part of a family of nuclear-located, steroid hormone transcription factors/receptors that bind testosterone and dihydrotestosterone. In normal skin and conjunctiva, AR is predominantly expressed in follicular and adnexal structures, especially in the sebaceous glands.25,41,42 Because it is a transcription factor and a hormone receptor from the steroid class, its immunohistochemical signal is predominantly nuclear (see Fig 3). This marker can be of particularly help when differentiating between PSC (predominantly positive, 33%–83%), SCC (predominantly negative, 90%–100%), and BCC (occasionally negative, 50%–86%).27,28

**Underlying Mutations/Genetic Aberrations**

**p53 Protein:** The p53 protein is a transcription factor coded by *TP53* (17p13.1), which induces apoptosis when DNA damage occurs. Approximately 67% of PSC tumors will harbor missense or nonsense *TP53* mutations that are not of the ultraviolet (UV) signature type (unrelated to UV damage).33 Patients with germline *TP53* mutations (Li–Fraumeni syndrome) frequently develop PSC, along with other malignancies that characterize the condition.44 Because the gene mutation rate is quite high in the setting of PSC, many of these tumors (40%–72%) will show an overexpression (accumulation) of p53 protein on immunohistochemistry translated by a strong nuclear signal.7,45 By contrast, this pattern of p53 staining occurs in fewer than 20% of BCC cases and 50% to 60% in SCC cases.7,46,47 Thus, some have advocated for use of p53 immunohistochemistry as a diagnostic aid in the differential diagnosis of PSC.7 Both the presence of *TP53* mutations and
the consequent p53 overexpression are more frequently encountered in PSC than extraocular sebaceous carcinoma and are inversely correlated with the presence of alterations of the mismatched repair (MMR) proteins and Muir–Torre syndrome.45

Mismatched Repair Proteins: Assessing the expression of MMR proteins with immunohistochemistry for mutL homolog 1, mutS homolog 2, mutS homolog 6, and PMS1 homolog 2 (MLH1, MSH2, MSH6 and PMS2, MMR system components) in cutaneous neoplasms — particularly in sebaceous adenoma — is becoming an easy and convenient way to screen for Muir–Torre syndrome, a variant of the Lynch syndrome spectrum.48-50 However, by contrast to the extraocular forms of sebaceous neoplasms, most PSC tumors appear to be unrelated to loss of MMR expression. Therefore, PSC should be considered as a poor “index lesion” to screen for Muir–Torre syndrome.55,51,52 Nevertheless, periorcular sebaceous adenoma appears to follow the same trend as extraocular sebaceous neoplasms in terms of its relationship with Muir–Torre syndrome.53

ERBB2 (formally known as HER2/neu): PSC has been shown to exhibit 2+ and 3+ patterns of ERBB2 (formally known as HER2/neu) immunohistochemical positivity in up to 82% of cases, with 75% of ERBB2 tumors exhibiting 2+ and 3+ patterns of expression, and overexpression of ZEB2, as well as loss of E-cadherin expression. The epithelial to mesenchymal transition is an embryonic process to which poorly differentiated PSC have been shown to disrupt the intercellular and cell stroma adhesion machinery with loss or diminished membranous E-cadherin expression and beta-catenin protein expression in 53% to 83% and 56% to 61% of cases, respectively.55,56 Loss or diminished expression of these markers was correlated with the presence of the epigenetic promoter methylation of CDH1 (16q22.1).55

Similarly, PSC also appears to overexpress ZEB2 in up to 68% of cases.57 ZEB2 is a transcription factor gene closely related to the epithelial to mesenchymal transition phenomenon that also interacts with CDH1—producing gene repression and the subsequent decrease of E-cadherin expression. The epithelial to mesenchymal transition is an embryonic process to which poorly differentiated neoplasms regress to enhance their invasiveness and metastatic potential by increased cell migration. The presence of the immunohistochemical overexpression of ZEB2, as well as loss of E-cadherin expression, and CDH1 promoter methylation in the setting of PSC have been correlated with poor rates of survival.55-57 Loss, diminution, and aberrant E-cadherin expression as well as CDH1 promoter methylation can also be found in both BCC59-62 and SCC,61,63 so these findings preclude use of ZEB2 as a diagnostic marker in this setting.

Conclusions

Several markers are differentially expressed in periorcular sebaceous carcinoma (PSC) compared with lesions in the differential diagnosis. A panel of immunohistochemical stains is recommended because different combinations of immunoprofiles characterize these entities, of which epithelial membrane antigen (EMA), Ber-Ep4, androgen receptor (AR), and adipophilin appear to be the most useful. An optimal stain panel to discriminate PSC from basal cell carcinoma (BCC) is presented by EMA, Ber-Ep4, AR, and adipophilin, while distinguishing PSC from squamous cell carcinoma (SCC) can be achieved by exploring the expressions of AR and adipophilin.

The 3 most typical immunophenotypes are the following: (1) PSC is positive for EMA, AR, and adipophilin but negative for Ber-Ep4, (2) SCC shows a predominant immunoprofile of EMA positivity but is negative for AR, Ber-Ep42, and adipophilin, and (3) BCC is predominantly negative for EMA and adipophilin but positive for Ber-Ep4. Two other potential immunohistochemical markers that may be useful in the differential diagnosis are p53 and HER2/neu, both of which will be predominantly expressed in PSC. Mismatched repair protein immunohistochemical expression aberrations appear to be absent in the majority of cases of PSC, because they appear to harbor TP53 mutations. PSC as an index case to screen of Muir–Torre syndrome is currently not recommended.

The introduction of new immunohistochemical stains for PSC and the expansion of our knowledge regarding the underlying molecular aberrations of PSC, such as the presence of ERBB2 amplifications, represent an exciting new era in understanding of this entity. These advancements could facilitate the early detection of PSC and possibly result in improved patient outcomes.

References

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