LABORATORY STUDY

Dimorphic immunohistochemical staining in ocular sebaceous neoplasms: a useful diagnostic aid

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Abstract

Purpose **We studied whether patterns of immunostaining in formalin-fixed, paraffin-embedded tissue could help to distinguish between sebaceous neoplasms of the eyelid and other eyelid neoplasms.**
Methods **We applied antibodies to human milk fat globule-1 (HMFG1), cytokeratins (PKK1 and MNF116), epithelial membrane antigen (EMA) and carcino-embryonic antigen (CEA) to normal eyelid tissue and to a range of sebaceous lesions of the eyelid; these included sebaceous hyperplasia, sebaceous adenoma and sebaceous epithelioma, in addition to well to poorly differentiated sebaceous carcinoma.**
Results **The central and peripheral cellular components of normal sebaceous glands and neoplastic sebaceous lesions showed a distinctive dimorphic staining pattern with the antibody panel used. The central foamy 'sebaceous' cells expressed HMFG1 and EMA, but not PKK1 or MNF116, whereas the smaller, peripheral basal and ductal cells expressed PKK1 or MNF116 but not HMFG1 or EMA. CEA expression in sebaceous cells was unhelpful diagnostically.**
Conclusion **Normal sebaceous glands and all sebaceous neoplasms show a dimorphic cell population that can be identified using a small panel of antibodies on formalin-fixed, paraffin-embedded tissue. This distinctive staining pattern can be assessed retrospectively, even in small biopsies, and largely removes the need for fat stains on frozen sections to differentiate sebaceous lesions from other ocular neoplasms. The results also support the suggestion that ocular sebaceous neoplasms arise from a common stem cell, rather than from either sebaceous or basal/ductal cells.**

Key words Diagnosis, Eye, Eyelid, Immunohistochemistry, Sebaceous carcinoma, Sebaceous neoplasm

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Cutaneous sebaceous glands have a lobular structure within which two major cell types can be distinguished. Basal cells are continuous with the lining cells of the gland's major ducts and act as stem cells by differentiating into the central, lipid-filled (and hence vacuolated) 'sebaceous' cells. Holocrine secretion of sebum results from rupture of mature sebaceous cells into the ducts of the gland. The sebaceous glands of the ocular adnexa include the tarsal Meibomian glands, the glands of Zeis which open onto the lid margin in association with the eyelashes, the sebaceous glands of the eyelid skin and those of the caruncle.

A variety of lesions arise from proliferations of sebaceous gland cells, the most serious of which is carcinoma. Ocular sebaceous carcinoma is a relatively rare neoplasm in the West, although the incidence is said to be much higher in China.[1] Clinically, diagnosis of sebaceous carcinoma can pose a problem due to its mimicry of other localised or diffuse eyelid conditions. These may be inflammatory or neoplastic, including chalazion, basal cell or squamous carcinoma (nodular growth pattern) and chronic blepharitis or conjunctivitis (diffuse growth pattern).[1] Treatment can be difficult because intra-epithelial (pagetoid) spread may be extensive and recurrence is likely if this is incompletely excised.

Histologically, sebaceous carcinoma may be well, moderately or poorly differentiated with paucity or even absence of the distinctive, lipid-filled sebaceous cells.[2–4] Often the only suggestion of sebaceous differentiation is cytoplasmic vacuolation of the neoplastic cells, an appearance which can overlap with the (probably artefactual) foamy cytoplasm sometimes seen in squamous and basal cell carcinomas and in melanoma. Of sebaceous carcinomas occurring in the ocular adnexa, 40–80% are associated with intra-epithelial spread,[4] but isolated intra-epithelial sebaceous carcinoma can also occur in the absence of a nodular tumour in the conjunctiva and skin of the eyelid.[5]

In addition to carcinoma, there are several other sebaceous proliferations that can occur around the eye. Although these are unlikely to cause diagnostic confusion, they provide a spectrum of differentiation which is useful for studying the evolution of sebaceous lesions:

Table 1. *Patient details*

Age (years)	Sex	Site (Size, mm)	Diagnosis
37	M	Right caruncle (4 × 3 × 2)	Sebaceous hyperplasia
62	M	Right caruncle (10 × 5 × 4)	Sebaceous adenoma
72	M	Right inner canthus (4 × 3 × 2)	Sebaceous epithelioma
48	M	Left upper lid (6 × 5 × 2)	Sebaceous epithelioma
27	M	Left lower lid (4 max)	Sebaceous carcinoma (wd)
57	F	Right upper lid (8 × 6 × 4)	Sebaceous carcinoma (pd)
88	F	Right upper lid (15 × 15 × 5)	Sebaceous carcinoma (pd)
57	F	Right upper lid (NA)	Sebaceous carcinoma (md)
80	M	Right orbit (12 max)	Sebaceous carcinoma (pd)
73	F	Right lower eyelid (NA)	Sebaceous carcinoma (md)
83	F	Right lower lid (NA)	Sebaceous carcinoma (md)
68	F	NA (NA)	Sebaceous carcinoma (md)
79	F	Right upper lid (2 × 2 × 1)	Sebaceous carcinoma (wd)
47	F	Left lower lid (NA)	Sebaceous carcinoma (md)
50	M	Right upper lid (extensive)	Sebaceous carcinoma (pd)
85	F	Right upper and lower lids (extensive)	Sebaceous carcinoma (wd)
60	M	Left upper lid (NA)	Sebaceous carcinoma (md + ies)
55	M	Right lower lid (5 max.)	Sebaceous carcinoma (pd + ies)
63	F	Upper eyelid (NA)	Sebaceous carcinoma (md + ies)

wd, well-differentiated; md, moderately differentiated; pd, poorly differentiated; ies, intra-epithelial spread; NA, not available.

Sebaceous hyperplasia is usually found on the forehead and cheeks of older adults, but can also occur on the caruncle.[6] Gland lobules are increased in size and number, but show normal maturation and an orderly arrangement around the central duct.

Sebaceous adenomas present as single or multiple skin nodules on the head, trunk, neck and legs of older individuals. Up to 50% are associated with internal malignancy, even when solitary (Torre-Muir syndrome). If incompletely excised, sebaceous adenomas can regrow rapidly, simulating malignancy.[7] Histologically, they are well circumscribed, with multiple lobules of varying size and shape. Maturation is slightly disordered, with increased basal cells, reduced sebaceous cells and the added presence of intermediate cells.[8]

Sebaceous epitheliomas are less well defined, but are thought to represent benign sebaceous lesions with a significant basal cell component. In practice, if over half the cells are of basal type then the lesion is designated a sebaceous epithelioma rather than an adenoma.[9] Histologically, they show loss of the normal lobular arrangement with a variable proportion of sebaceous cells among the basal cells. However, sebaceous epitheliomas lack the poor circumscription, infiltrative margins and cytological atypia of sebaceous carcinomas and tend to maintain peripheral palisading.

Traditionally, definitive diagnosis of sebaceous differentiation has required demonstration of intracytoplasmic lipid by fat stains such as Sudan III or Oil red-O. Not only does this require frozen sections, but inferior results can occur on tissue that has been stored for even a short time in formalin. When the possibility of sebaceous differentiation is raised following histological examination of the routinely stained section, the tissue may already have been in formalin too long for fat stains to be useful or, frequently, no unprocessed tissue is available for fat stains, since eyelid biopsies are often small, making frozen section inappropriate or impossible.

In this study we have used immunohistochemistry to investigate sebaceous differentiation in a spectrum of eyelid neoplasms that showed evidence of sebaceous differentiation either on conventional light microscopy or using fat stains on frozen tissue. Our findings, of consistent staining patterns throughout the spectrum of lesions, show that an appropriate panel of antibodies can largely replace frozen section in ocular sebaceous lesions, particularly where the results of standard staining are equivocal.

Methods

We identified cases of ocular sebaceous neoplasms arising within a 6 year period. The majority of these cases had been diagnosed using a combination of standard histological techniques supplemented by a fat stain on frozen tissue sections where appropriate – for example, more poorly differentiated carcinomas. The range of lesions studied and selected clinical features are shown in Table 1. Normal Meibomian and other sebaceous glands, eyelid skin, adnexal structures and conjunctiva adjacent to the tumours were used as internal controls.

Table 2. *Immunohistochemical reagents*

Reagent	Source	Supplier	Dilution
Anti-human CEA (polyclonal)	Rabbit	Dako	1:600
Anti-human cytokeratin clone MNF116	Mouse	Dako	1:100
PKK1	Mouse	Labsystems	1:100
Anti-human EMA	Mouse	Dako	1:200
HMFG1	Mouse	Novocastra	1:100

All antibodies were monoclonal unless otherwise stated.
CEA, carcino-embryonic antigen; EMA, epithelial membrane antigen; HMFG1, human milk fat globule-1.

All the specimens had been fixed in 10% formalin and embedded in paraffin using standard procedures. Four micrometre sections were cut for staining with haematoxylin and eosin (H&E) and for immunohistochemistry. The antibodies used are shown in Table 2. Initially the cytokeratin antibody PKK1 was used, but following the death of this clone, the cytokeratin antibody MNF116 was substituted and this gave very similar results.

Sections were washed in Tris-buffered saline (TBS) at pH 7.6 and incubated overnight at 4 °C with primary monoclonal antisera. Polyclonal antibodies were incubated for 30 min at room temperature. Slides were then washed in TBS, incubated with biotinylated anti-rabbit or anti-mouse antibody for 30 min at room temperature and rewashed in TBS. Antibody localisation was demonstrated using the avidin-biotin complex method (Vecta stain). The slides were incubated with avidin-biotin complex for 30 min at room temperature and then washed again in TBS before developing the final 3,3'-diaminobenzidine reaction product and counterstaining with haematoxylin.

Results

In normal Meibomian, Zeis and other sebaceous glands (Fig. 1) the basal and duct cells were positive for MNF116 and/or PKK1, but were negative for HMFG1 and EMA. In contrast, the foamy sebaceous cells were positive for HMFG1 and EMA, but were negative for MNF116 and/or PKK1. Normal conjunctival epithelium was positive for EMA, CEA and HMFG1.

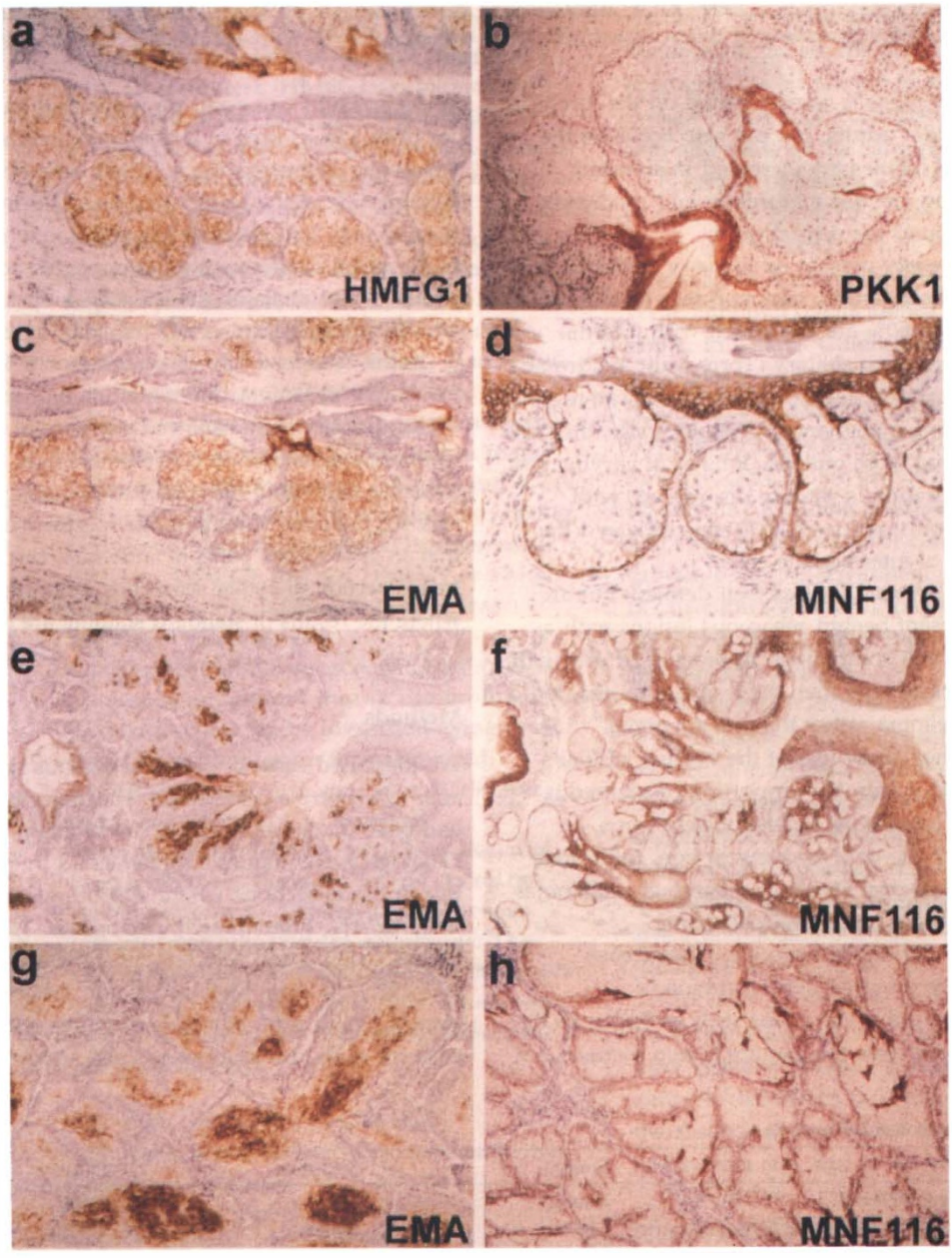

Fig. 1. *Normal Meibomian glands demonstrating brown immunostaining for HMFG1 and EMA in foamy sebaceous cells (a, c) and for PKK1 and MNF116 in basal/ductal cells (b, d). This dimorphic pattern of staining in sebaceous and basal/ductal cells is maintained in sebaceous hyperplasia (e, f) and sebaceous adenoma (g, h).*

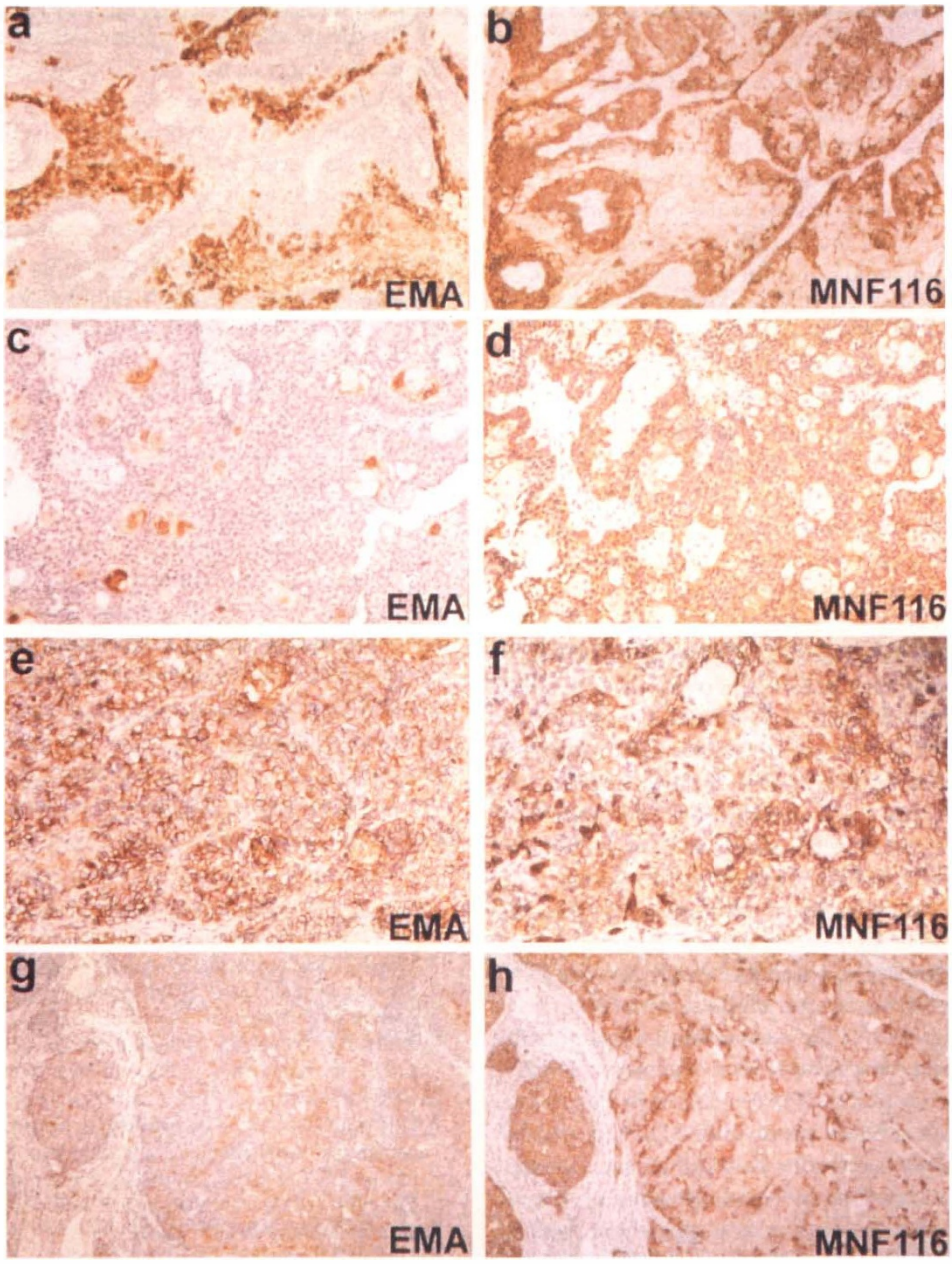

Fig. 2. *The dimorphic staining pattern of sebaceous cells positive for EMA and basal/ductal cells positive for MNF116 is conserved in sebaceous epithelioma (a, b), and in well-differentiated (c, d), moderately differentiated (e, f) and poorly differentiated (g, h) sebaceous carcinoma. Note the separate tumour nodule (g, h) in which the majority of the cells are of basal/ductal type.*

In benign sebaceous lesions and well-differentiated invasive carcinoma, sebaceous and basal cells could be distinguished using H&E, facilitating positive identification of these cell types with the immunohistochemical stains. In each of these lesions the dimorphic phenotype was maintained, with basal cells and sebaceous cells showing the same immunophenotype as their normal counterparts (Figs. 1, 2). In the more poorly differentiated carcinomas it was difficult or impossible to distinguish between the two cell types using H&E, but a dimorphic population of cells was readily detected using immunohistochemistry (Fig. 2). In all the lesions studied, basal cells were positive for MNF116 and/or PKK1 and were negative for HMFG1 and EMA. Also in all the lesions, foamy sebaceous cells expressed HMFG1 and EMA but not MNF116 or PKK1. In sebaceous carcinomas immunohistochemical staining revealed admixtures of 'basal' and 'sebaceous' tumour cells in patterns that demonstrated the loss of architectural polarity of these cell types in malignant lesions. The range of patterns included sebaceous cells surrounded by basal cells, separate nodules of basal and sebaceous cells and mixtures of the two cell types in small groups. In the majority of cases individual cells had the phenotype of either basal or sebaceous cells, but occasionally intermediate cell types were present, being positive for HMFG1, EMA and MNF116/PKK1.

CEA expression was not seen in the basal cells in any of the lesions, but it was focally and weakly expressed in sebaceous cells in all but the single case of sebaceous hyperplasia.

Discussion

Several previous studies have examined aspects of the immunophenotype of ocular sebaceous carcinomas.[10–12] However, there has been a need for a well-defined panel of antibodies to aid in the diagnosis of poorly differentiated sebaceous lesions of the ocular adnexa. Previous authors have found difficulty consistently distinguishing sebaceous carcinomas from other skin neoplasms using their particular panel of antibodies.[12]

We have identified a straightforward and readily available panel of antibodies that consistently demonstrates a dimorphic staining pattern in basal and sebaceous cells in the full range of sebaceous lesions. This pattern of differentiation is maintained even in poorly differentiated cases when the two cell populations are not clearly discernible using routine H&E staining. The reciprocal staining pattern means that it is possible to demonstrate distinct positive and negative areas within a given lesion which, when interpreted together, help to confirm the presence of sebaceous differentiation by forming a mirror-image staining pattern.

The most important cells in the diagnosis of sebaceous carcinoma are the 'sebaceous' cells, classically recognised by the presence of numerous, fine, lipid-containing vacuoles. These cells consistently express both EMA and HMFG1, but are negative for MNF116 (PKK1). The absence of MNF116 (PKK1) staining in the central sebaceous cells is very useful since the majority of lesions in the differential diagnosis of sebaceous lesions, such as eccrine or apocrine tumours, metastases and extra-mammary Paget's disease, are MNF116 positive. Differential diagnoses of the intra-epithelial component of sebaceous carcinoma include extra-mammary Paget's disease and adenocarcinoma (where the cells contain mucin), but these cells are positive for MNF116, which excludes sebaceous carcinoma. In addition, the absence of MNF116 staining in sebaceous cells is especially useful in a lesion invading the conjunctival epithelium, which normally expresses MNF116, EMA and HMFG1.

Squamous carcinomas are usually positive for cytokeratin (usually high molecular weight) and EMA, and also often for CEA. Occasional tumours may show positivity for HMFG1. Basal cell carcinomas are usually negative for EMA and CEA, but positive for cytokeratins (especially low molecular weight). Merkel cell tumours have a distinctive peri-nuclear dot positivity for low-molecular-weight cytokeratin, but are also positive for neurofilament and NSE. None of these potential differential diagnoses show the reciprocal staining pattern described above.

We found small focal areas of CEA expression in the central foamy cells in all but the case of sebaceous hyperplasia and one of the intra-epithelial carcinomas. This was unexpected because sebaceous cells have been reported to be negative for CEA.[11] The positivity may represent areas of imminent cystic degeneration, or alternatively it may represent ectopic production of tumour markers or gain of antigenic determinants, which are well recognised phenomena in neoplasia.

The dimorphic cell population clearly illustrated in this study suggests that sebaceous tumours do not arise from basal or sebaceous cells alone, but presumably originate from undifferentiated stem cells which continue to partially differentiate into basal and sebaceous cells even in poorly differentiated tumours. With decreasing tumour differentiation there is a more chaotic admixture of cell types, which our immunohistochemical panel can distinguish. Only sebaceous tumours and normal sebaceous glands show the dimorphic pattern we have described. Other adnexal tumours do not show this pattern. Occasional eccrine neoplasms (in particular, hidradenomas and spiradenomas) show HMFG1 and EMA staining, usually as spidery patterns highlighting intercellular canaliculi. Sebaceous tumours thus cannot be confused with other adnexal tumours on the basis of this staining pattern.

The small panel of antibodies described here provides a consistent means of diagnosing sebaceous differentiation in routinely processed neoplasms of the eyelid, without the requirement for tissue to be specially retained for frozen sections and fat stains. Having become familiar with the use of this antibody panel, we have recently been able to dispense with frozen sections and fat stains in the diagnosis of ocular sebaceous lesions.

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