

ORIGINAL ARTICLE

Immunohistochemical staining for adipophilin, perilipin and TIP47

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Background: The presence of lipid in the cell cytoplasm is useful for supporting the diagnosis of sebaceous gland carcinoma (SGC). Currently this requires histochemical stains that are carried out on frozen sections of unprocessed tissue. Recently, several anti-adipocytic antibodies that recognise proteins associated with lipid vesicles have been described. These antibodies can be applied to paraffin-wax sections.

Aim: To assess the ability of anti-adipocytic antibodies to identify intracytoplasmic lipid in SGC.

Methods: Immunohistochemistry with a monoclonal antibody to adipophilin and polyclonal antibodies to perilipin and TIP47/PP17 was carried out on archival, formalin-fixed, paraffin-wax-embedded sections of 26 samples of SGC. The immunostaining was compared with 22 other eyelid tumours (11 basal cell carcinomas (BCC), 10 squamous cell carcinomas (SCC) and 1 Merkel cell tumour).

Results: Immunohistochemical staining was positive in 23, 10 and 2 cases of 26 SGC with adipophilin, perilipin and TIP47, respectively. The positive staining identified cytoplasmic lipid vesicles. Anti-adipophilin was positive in five other eyelid tumours (4 BCC and 1 SCC) staining small cytoplasmic granules that can be easily distinguished from the staining in SGC.

Conclusions: Immunohistochemical staining for adipophilin and perilipin is a useful ancillary technique for the demonstration of lipid in SGC that may be applied to paraffin-wax sections.

Sebaceous gland carcinoma (SGC) is a rare tumour of the skin. However, it is the second most common malignancy of the eyelid, accounting for 1–5% of all eyelid neoplasms.^{1–3} SGC usually originates in the Meibomian gland, but may also arise from the gland of Zeiss or sebaceous glands of the eyelid skin.^{3–4} Ocular SGC commonly occurs in elderly patients and shows a preponderance in female sex.^{3–7} The prognosis is poor compared with most other malignant eyelid tumours, with local recurrence in up to 18% of patients and metastases in 8–40%.^{3–10} The prognosis improves considerably with early diagnosis and surgery.^{11–13} However, the clinical appearance of SGC is variable and this tumour is notorious for masquerading as other benign or malignant lesions that may result in delayed diagnosis. Specifically, SGC may be indistinguishable from squamous cell carcinoma (SCC) or basal cell carcinoma (BCC), or may mimic a range of benign conditions including chalazion and blepharconjunctivitis³ (fig 1A,B).

SGC can also present a challenge to the histopathologist. This is a rare tumour that may show a nodular or diffuse growth pattern. Previous studies have reported an incorrect initial histological diagnosis in 40–75% of patients.^{1–8 12 14} Misdiagnosis is more common when sections are interpreted by pathologists inexperienced in tumours occurring in the ocular area rather than an ophthalmic pathologist.^{11 12} In addition, failure to identify pagetoid spread of tumour cells in the epithelium may also result in incorrect reporting of excision margins.¹⁵

The presence of intracytoplasmic lipid in SGC by fat stains such as Oil Red O and Sudan IV may aid the diagnosis of SGC, distinguishing it from other poorly differentiated carcinomas. These stains require fresh or unprocessed tissue as lipid is dissolved during standard processing. However, even short periods of formalin fixation may result in less intense staining with conventional lipid stains.¹⁶ The ability to undertake conventional lipid stains depends on there being sufficient tissue for frozen sections in addition to

paraffin-wax sections, which is not always possible with small incisional biopsy specimens.

Recently, antibodies that recognise proteins associated with lipid droplets have been described, including adipophilin, perilipin and TIP47/PP17. Adipophilin is present in milk fat globule membranes and on the surface of lipid droplets in various normal cell types, including the zona fasciculata of the adrenal, Sertoli cells and the glandular breast tissue in lactation.¹⁷ Adipophilin is also present in hepatocytes in alcoholic steatosis.¹⁷ The perilipins are a family of phosphoproteins found on the surface of intracellular lipid droplets and in the adrenal gland, Leydig cells and both brown and white fat.¹⁸ TIP47/PP17 is a cargo protein associated with the trafficking of the mannose-6-phosphate receptor between endosomes and the Golgi apparatus.¹⁹ TIP47/PP17 has been seen in human milk fat globule membranes, placenta, and has been investigated as an oncodevelopmental marker in cervical carcinoma.²⁰ Our study aims to assess the ability of antibodies to these lipid-associated proteins to identify lipid in SGC in formalin-fixed paraffin-wax-embedded sections by immunocytochemistry.

MATERIALS AND METHODS

Tissue samples

Archival, formalin-fixed, paraffin-wax-embedded tissue samples were retrieved from the ophthalmic pathology files of the Western Infirmary, Glasgow, UK. All samples were anonymised and full ethical approval in accordance with local policy was obtained for the use of these tissue samples. The samples included 26 SGC, 11 BCC, 10 SCC and 1 Merkel cell tumour.

The pathology was reviewed and samples of SGC were categorised according to their degree of differentiation (well,

Abbreviations: BCC, basal cell carcinoma; CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; SCC, squamous cell carcinoma; SGC, sebaceous gland carcinoma; TBS, tris-buffered saline

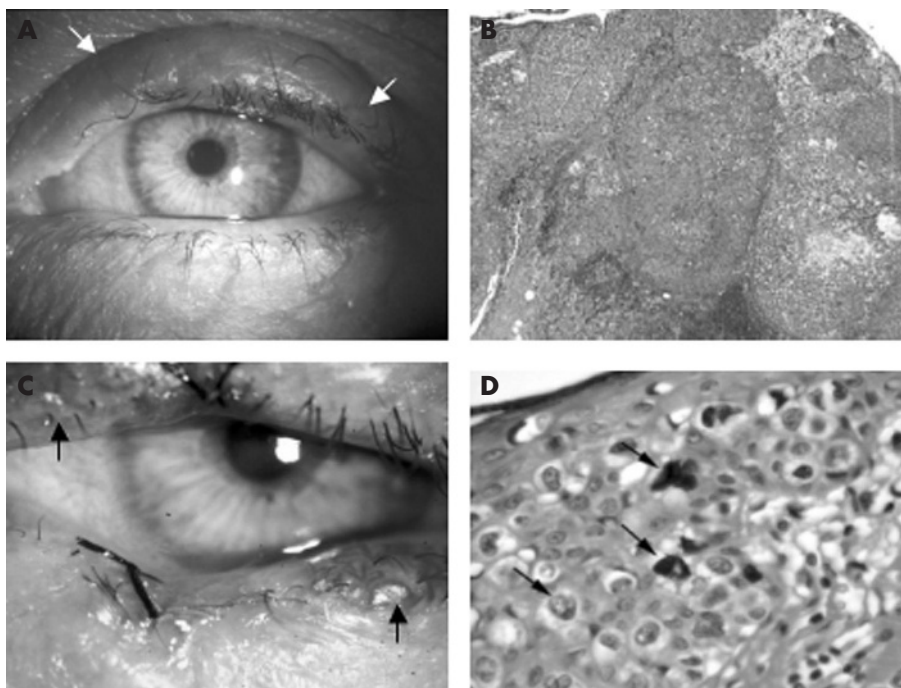


Figure 1 Clinical and histopathological appearances of nodular and diffuse sebaceous gland carcinoma (SGC). (A) Left eye showing swelling of the upper eyelid (arrows) clinically thought to be a chalazion. Excision biopsy of the lesion showed a nodular SGC. (B) Moderately differentiated SGC with a predominantly nodular growth pattern (haematoxylin and eosin (H&E), $\times 100$). (C) Left eye showing patchy inflammation at upper and lower lid margins (arrows). This is associated with patchy loss of eyelashes. A suture at the centre of the lower lid indicating the previous biopsy site. Biopsy showed diffuse pagetoid spread in the epithelium. (D) Diffuse SGC. Atypical vacuolated cells are evident in the epidermis (H&E, $\times 400$).

moderately or poorly differentiated) and predominant growth pattern (nodular or diffuse). Wherever available, Oil Red O sections were also reviewed.

Immunohistochemistry

Table 1 shows the information on the three antibodies used in this study.

Immunohistochemical staining was carried out on 3 μ m sections that were immunostained using the DakoCytomation Chemate Envision system (DAKO, Ely, Cambridge, UK). Briefly, sections were washed with Tris-buffered saline containing Tween buffer (tris-buffered saline (TBS)/TB), then blocked with normal goat serum (1:20) for 20 min. Sections were then incubated with primary antibody for 30 min, followed by a further wash in TBS or TB. After this, the sections were incubated with Dako Envision for 30 min. After a final wash in TBS or TB, colour was developed with diaminobenzidine for 10 min and sections were counterstained with haematoxylin. For antigen retrieval, slides were microwaved under pressure in 1 mmol/l ethylenediamine tetra-acetic acid, pH 8.0 for 5 min. Appropriate control material (according to manufacturer’s instructions)

was used for each run. For negative controls, the primary antibody was omitted.

Interpretation

The slides were evaluated by light microscopy and the presence and location of positive staining for each antibody was recorded. Staining that was weak or difficult to interpret was regarded as equivocal.

Statistical analysis

The sensitivity and specificity were calculated for each antibody and for Oil red-O staining, where available, as previously described.²¹ Sensitivity was calculated as positive SGC/negative SGC+positive SGC. Specificity was calculated as negative non-SGC/positive non-SGC+negative non-SGC. Equivocal results were not included in the calculations.

RESULTS

Table 2 summarises the results.

Histological review of SGC

The diagnosis of SGC was confirmed in all 26 samples. Of these, 11 samples were poorly differentiated, 13 moderately differentiated and 2 well differentiated. The growth pattern was nodular in 13 samples and diffuse in 13 samples with Pagetoid involvement of the epithelium (fig 1C,D).

Oil Red O staining was available in 22 samples and was positive in 8 (4 poorly, 2 moderately and 2 well differentiated). Oil red O staining was not carried out on the other eyelid tumours.

Adipophilin

Moderate to strong positive immunostaining for adipophilin was identified in 23 of the 26 samples of SGC and seemed to stain the membrane of intracytoplasmic vesicles considered to represent lipid droplets (fig 2A,B). The remaining three

Table 1 Anti-adipocytic antibodies

Antibody	Antigen	Dilution	Source
Mouse Monoclonal	Adipophilin	1:5	Progen, Heidelberg, Germany Biotechnik GmbH, Heidelberg, Germany
Guinea-pig polyclonal	Perilipin	1:100	Progen
Guinea-pig polyclonal	TIP47/PP17	1:50	Progen

Table 2 Staining of eyelid tumours for Oil Red O and anti-adipocytic antibodies

Tumour type	Oil Red-O	McAb adipophilin	PcAb perilipin	PcAb TIP47/PP17
SGC				
WD, N	2/2	2/2	0/2	1/2
MD, N	1/5	4/6	5/6	1/6
MD, D	1/6	7/7	2/7	0/7
PD, N	1/4	5/5	2/5	0/5
PD, D	3/5	5/6	1/6	0/6
All	8/22	23/26	10/26	2/26
SCC	N/A	1/10	0/10	0/10
BCC	N/A	4/11	0/11	0/11
MCC	N/A	0/1	0/1	0/1

BCC, basal cell carcinoma; D, diffuse growth pattern; McAb, monoclonal antibody; MCC, Merkel cell tumour; MD, moderately differentiated; N, nodular growth pattern; PcAb, polyclonal antibody; PD, poorly differentiated; SCC, squamous cell carcinoma; SGC, sebaceous gland carcinoma; WD, well differentiated.

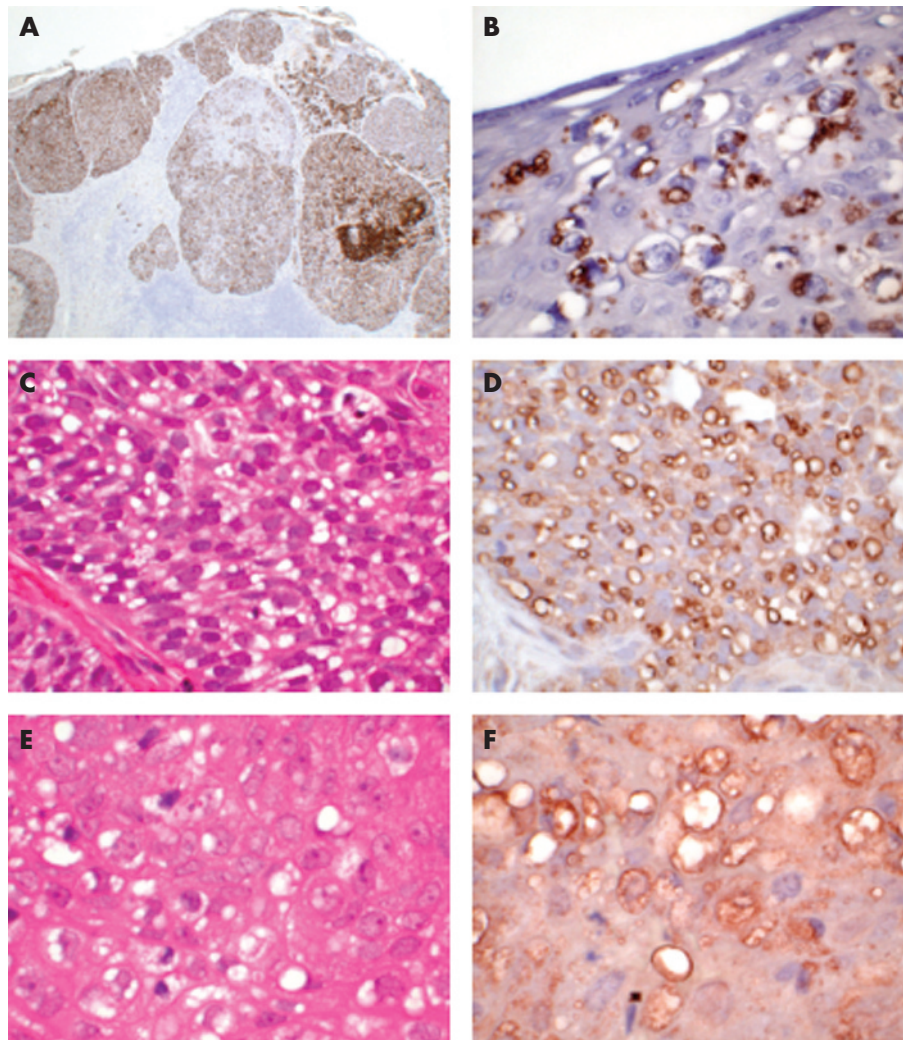


Figure 2 Immunohistochemical staining for adipophilin, perilipin and TIP47/PP17 in sebaceous gland carcinoma (SGC). (A) Immunohistochemical staining for adipophilin of SGC shown in fig 1B. There is widespread strong positivity, with numerous cytoplasmic vacuoles (anti-adipophilin, $\times 100$). (B) Immunohistochemical staining for adipophilin of SGC shown in fig 1D. There is strong positivity staining of vacuoles highlighting the Pagetoid infiltration of the epidermis (anti-adipophilin, $\times 400$). (C) Moderately differentiated nodular SGC showing numerous intracytoplasmic vacuoles (haematoxylin and eosin (H&E), $\times 400$). (D) Immunohistochemical staining for perilipin outlines numerous small lipid vacuoles within the tumour cells (anti-perilipin, $\times 400$). (E) Moderately differentiated nodular SGC showing numerous intracytoplasmic vacuoles (H&E, $\times 400$). (F) Immunohistochemical staining for TIP47/PP17 outlines numerous small lipid vacuoles in the tumour cells (anti-TIP47/PP17, $\times 400$).

samples were negative. These consisted of two moderately differentiated nodular SGC and poorly differentiated diffuse SGC. Staining for Oil Red O was positive in one of these three samples.

Positive immunostaining for adipophilin was seen in four BCCs and one SCC. The pattern of staining, however, differed from that seen in SGC, taking the form of focal (often perinuclear) intracytoplasmic granules. The remaining 17 samples (7 BCC, 9 SCC and 1 Merkel cell tumour) were negative.

Therefore, immunostaining for adipophilin has a sensitivity of 88.5% and a specificity of 77%.

Perilipin

Moderate to strong positive immunostaining for perilipin was identified in 10 of the 26 samples of SGC (7 moderately differentiated—5 nodular and 2 diffuse; and 3 poorly differentiated—2 nodular and 1 diffuse), again staining the membrane of intracytoplasmic vesicles (fig 2C,D). Four samples showed weak staining that was interpreted as equivocal. The remaining 12 samples were negative.

The other eyelid tumours were all negative for anti-perilipin immunostaining. Thus, the specificity of perilipin was 100%, although the sensitivity was only 45.5%.

TIP47/PP17

Moderate to strong positive immunostaining was evident in two samples of nodular SGC (1 well differentiated and 1 moderately differentiated). Staining was in the form of intracytoplasmic vesicles as with adipophilin and perilipin (fig 2E,F). Equivocal staining was observed in two further samples of nodular SGC. Staining was negative in the other 22 samples of SGC. All non-SGC were negative with this antibody.

Therefore, anti-TIP47/PP17 had a sensitivity of 8.3%, but a specificity of 100% (95% confidence interval 83.9% to 100%).

Anti-adipocytic antibodies as an immunohistochemical panel

Anti-perilipin was positive in two samples that were negative for anti-adipophilin.

Thus, in combination, anti-adipophilin and anti-perilipin have a sensitivity of 96%. The two samples that were anti-TIP47/PP17 positive were also anti-adipophilin positive. Nine samples of SGC showed positive immunostaining with more than one antibody (7 samples with anti-adipophilin and anti-perilipin; 2 samples with anti-adipophilin and anti-TIP47). One sample stained with all three antibodies. Staining with more than one antibody occurred in both moderately and poorly differentiated tumours and in nodular and diffuse tumours.

DISCUSSION

The morphological diagnosis of SGC is supported by the presence of intracytoplasmic lipid in the tumour cells by fat stains such as Sudan IV or Oil Red O. These histochemical stains require unprocessed tissues because the graded alcohols used in processing dissolve tissue lipid. However, there exist inherent limitations to the use of unprocessed tissue for this purpose. There may be no clinical suspicion of SGC at the time of biopsy such that unprocessed tissue is not available when the diagnosis is suspected on histological examination, or the biopsy may be a small incisional biopsy. Even if residual formalin-fixed tissue is available, it is well recognised that even though formalin fixation does not dissolve tissue lipids short periods of formalin, fixation can reduce the intensity of Oil Red O staining considerably. In this study, we have shown that anti-adipocytic antibodies can identify lipid vesicles in the cytoplasm of the cells of SGC. The three antibodies used in this study identify proteins

associated with fat vesicles; the antigens, unlike the lipid itself, are preserved during tissue processing. Immunohistochemistry for two of these fat-associated proteins was superior to Oil Red O staining of frozen sections and the sensitivity was improved by using both anti-adipophilin and anti-perilipin immunostaining. Oil red O staining was positive in only 36% (8/22) of SGC, whereas staining for adipophilin and perilipin was positive in 88% (23/26) and 38% (10/26), respectively. Staining for either adipophilin or perilipin was positive in 96% (25/26) of cases. The third antibody, TIP47/PP17, was less sensitive than Oil Red O, with positive staining in only 8% (2/26) of SGC. In addition to confirming the diagnosis of SGC, anti-adipophilin highlighted the intraepithelial component of SGC with Pagetoid spread and may have a useful role for confirming excision margins. No association of positive staining with degree of differentiation or pattern of spread was found. Similar to Oil Red O, these antibodies showed positive staining in other lipid-containing cells including normal sebaceous units such that the entire histopathological picture is required to make a diagnosis of SGC.

Histologically, SGC must be distinguished from other more common eyelid tumours including poorly differentiated SCC and BCC.²² BCC with sebaceous differentiation can present particular diagnostic difficulties. It is worth noting, however, that these tumours are extremely rare and that this diagnosis must be made with extreme caution in the eyelid, where SGC is more likely.²² Merkel cell tumour, an uncommon and aggressive tumour, should also be considered in the differential diagnosis.²³ In this study, there was focal positive immunostaining for adipophilin in four BCC and one SCC. The staining, however, was weak and focal, with small intracytoplasmic granules often in a perinuclear location. This staining was easily distinguishable from the widespread and moderate to strong positive staining seen in SGC. As the pattern of staining is different, this probably represents a cross reaction with some other antigen rather than the presence of a small amount of cytoplasmic lipid. It is surprising, however, that this occurred with anti-adipophilin, the only monoclonal antibody in the study. No staining of the other eyelid tumours was observed with antibodies to perilipin or TIP47/PP17.

Several previous studies have used immunohistochemistry to differentiate SGC from BCC and SCC. SGC is usually positive with antibodies to epithelial membrane antigen (EMA)¹⁶ and androgen receptor,²⁴ but negative for carcinoembryonic antigen (CEA).¹⁶ EMA may, however, be focal or negative in poorly differentiated tumours.²⁴ Furthermore, some skin adnexal tumours may share this immunoprofile.²⁵ SCC is usually positive with antibodies to CEA and negative for EMA. BCC is negative for EMA and CEA, but there may be focal positivity for androgen receptor in up to 60% of samples. Expression of CK7 is also helpful as this is positive in most cases of ocular SGC, but is usually negative in SCC and BCC.²⁶⁻²⁷

In conclusion, antibodies to adipophilin and perilipin that identify proteins associated with lipid vesicles are a useful ancillary technique for the identification of lipid in SGC of the eyelid. This immunohistochemical technique is applicable to paraffin wax sections. It is more sensitive and avoids the need for unprocessed tissue and frozen sections required for traditional histochemical stains such as Oil Red O. These antibodies may have an important role either alone or as an addition to an immunohistochemical panel for the diagnosis of SGC.

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Take-home messages

- Identification of intracytoplasmic lipid is helpful in the diagnosis of sebaceous gland carcinoma.
- Oil Red O and Sudan IV require fresh or unprocessed tissue and results may be less than optimal after short periods of formalin fixation.
- Immunohistochemical staining for lipid-associated proteins adipophilin and perilipin carried out on paraffin wax-embedded sections is a more sensitive way of identifying intracytoplasmic lipid in sebaceous gland carcinoma than conventional histochemical stains.

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