Identifying squamous cell carcinoma and cytokeratin-derived amyloid with pan-cytokeratin AE1/AE3 during Mohs micrographic surgery

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INTRODUCTION

Mohs micrographic surgery (MMS) with hematoxylin-eosin (H&E) stained frozen sections remains the gold standard for treatment of cutaneous squamous cell carcinomas (cSCC) of the head, neck, and special sites. Use of immunohistochemistry (IHC) during MMS can be particularly beneficial in treating challenging tumors with high-risk features, such as perineural invasion, lymphovascular invasion, poor differentiation, or tumors with a background of dense inflammation.[1] Although the use of melanoma antigen recognized by T cells 1 during MMS is well established in treatment of melanoma in situ and superficially invasive melanoma, there is a paucity of literature describing utilization rates and outcomes of IHC during MMS for treatment of cSCC.[2] As IHC use during MMS becomes more widely accepted, limitations and potential pitfalls need to be recognized and described. The authors present a case of cSCC treated with MMS and pancytokeratin antibody cocktail AE1/AE3 (PCK) that showed positive staining for cytokeratin-derived amyloid on frozen sections that mimicked tumor. This case highlights the value of IHC for treatment of cSCC during MMS, and the importance of having a robust understanding of IHC staining patterns for accurate determination of margin status.

CASE REPORT

A 30-year-old white woman presented for evaluation of a rapidly growing lesion on the left nasal ala

Abbreviations used:	
cSCC:	cutaneous squamous cell carcinomas
H&E:	hematoxylin-eosin
IHC:	immunohistochemistry
MMS:	Mohs micrographic surgery
SCC:	squamous cell carcinomas
PCK:	pancytokeratin antibody cocktail AE1/AE3

for 3 weeks. The lesion bled intermittently and was painful. Examination found a crateriform, ulcerated, brightly erythematous 0.8-cm papule with an edematous base. A shave biopsy was performed, and histopathology showed an invasive well-differentiated squamous cell carcinoma (SCC) with keratoacanthoma-like features. Given the patient's age and family history of Lynch syndrome in her mother, evaluation of mismatch repair gene mutation was recommended, but the patient declined. The patient was subsequently referred for MMS.

MMS was performed with H&E frozen sections revealing residual well-to-moderately differentiated SCC on stage 1 (Fig 1). Stage 2 H&E frozen sections showed extensive residual inflammation obscuring the base, no residual tumor, and numerous distinct eosinophilic structures in the dermis that we suspected was not tumor but were difficult to interpret (Fig 2). PCK IHC was thus performed on the frozen blocks for stages 1 and 2 to aid in identifying the eosinophilic structures and in determining final margin status. PCK for stage 1 frozen sections found positive staining of residual SCC, as expected,
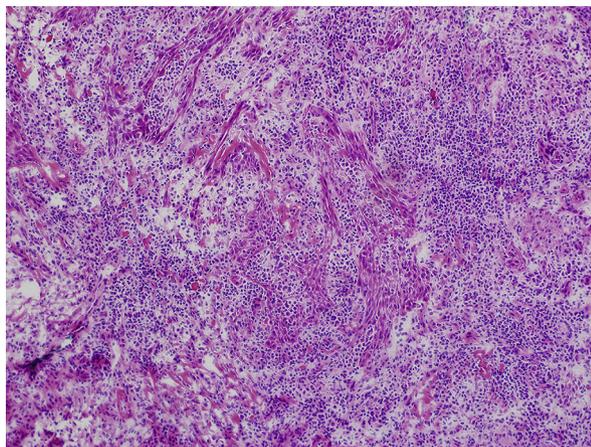
Fig 1. Stage 1 MMS H&E frozen sections show residual well-to-moderately differentiated SCC in a background of dense inflammation. (Original magnification: ×10.)
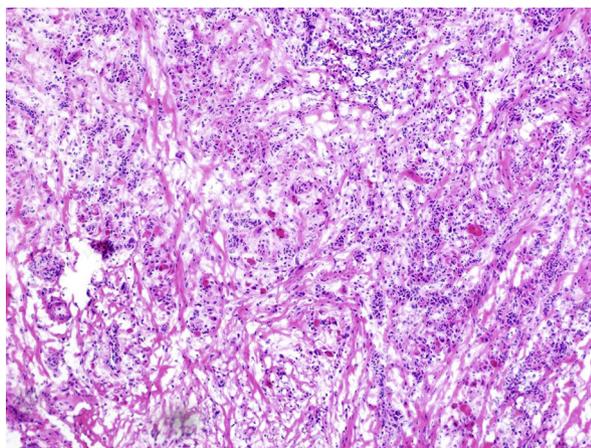
Fig 2. Stage 2 MMS H&E frozen sections show dense inflammatory cells intermixed with light pink–to–dark pink amorphous structures. (Original magnification: ×10.)

Fig 3. A, Stage 1 MMS frozen sections stained with PCK show positive staining for both residual SCC and amyloid. (Original magnification: ×2.) **B**, Stage 1 MMS frozen sections stained with PCK show positive staining for both residual squamous cell carcinoma and amyloid. (Original magnification: ×10.)

but also positive staining for numerous amorphous globular acellular structures (Fig 3, *A* and *B*). On stage 2, PCK showed positive staining for only acellular structures that were also seen in stage 1, which we suspected to be cytokeratin-derived amyloid (Fig 4, *A* and *B*). The PCK staining pattern in stage 2 mimicked tumor on first glance but was distinct from tumor and from the positive normal control pattern of suprabasal keratinocytes and hair follicles. With the addition of PCK, the tumor was considered clear, and the final defect was repaired by a single-stage transposition flap. The frozen tissue blocks were subsequently thawed for formalin-fixed paraffin-embedded permanent sections, and the dermatopathology department was consulted to confirm amyloid. Although Congo red stains were negative, permanent sections with KRT 34BE12 confirmed the presence of keratin-derived amyloid

near tumor in stage 1, and only keratin-derived amyloid in stage 2 (Fig 5).

DISCUSSION

MMS with standard H&E provides 98% to 99% cure rates for primary cSCC with zero (T1) to one (T2a) high-risk features based on Brigham and Women's Hospital staging.[3] Local recurrence rates for high-risk cSCC treated with MMS range between 4% (AJCC8 T3) and 6% (Brigham and Women's Hospital staging T2b/3).[4] However, PCK utilization rate during MMS is unknown, and data on patient outcomes for cSCC treated with MMS plus PCK is lacking. As IHC use during MMS becomes more widely accepted and available for various neoplasms, a robust knowledge of normal staining patterns becomes essential.

The primary benefit of utilizing IHC during MMS for treatment of cSCC is enhanced ability to

A

B
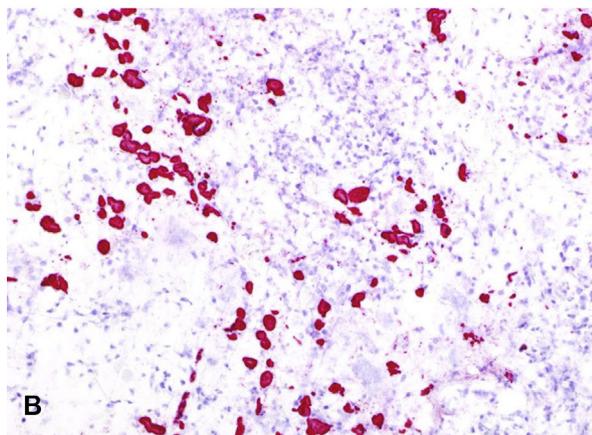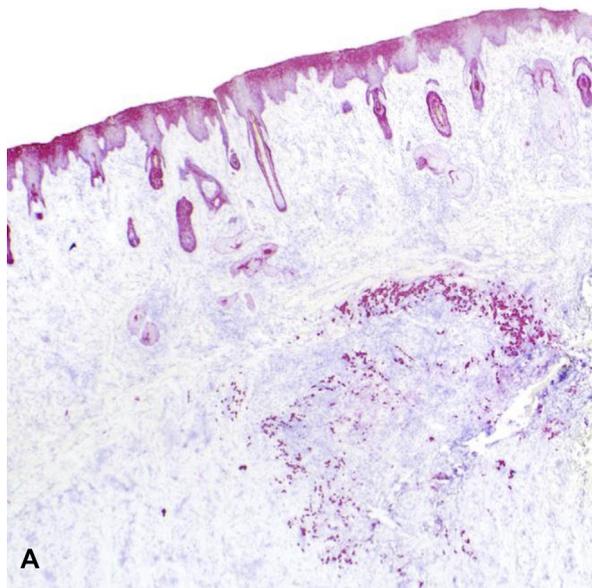
Fig 4. **A**, Stage 2 MMS frozen sections stained with PCK show normal suprabasal staining pattern of keratinocytes and hair follicles, along with positive staining of pink amorphous structures clustered in the dermis. Note the background inflammation that does not stain. **B**, Stage 2 MMS frozen sections stained with PCK show distinct appearance of the individual pink structures, not compatible with background inflammation that does not stain, and also not compatible with tumor. (Original magnifications: **A**, ×2; **B**, ×20.)

Fig 5. Permanent sections with Keratin immunostain that were first thawed from stage 1 and 2 MMS frozen blocks, with KRT highlighting normal cytoplasmic keratin staining of epidermal cells, and confirming cytokeratin-derived amyloid in the dermis. Congo red was negative and not pictured. (Original magnification ×4.)

distinguish epidermal-derived keratinocyte tumors from mesodermal cells devoid of keratin proteins.[2] This can be particularly helpful when tumor is more challenging to visualize on frozen sections, including poorly differentiated tumor cells; tumor cells among dense inflammation or fibrotic tissue; or tumor cells around vessels, within fascial planes, or tracking along nerves.[2,5]

The epitope most commonly targeted by IHC for SCC is cytokeratin, which is an intermediate-filament protein found in epithelial cells and epithelial-derived structures.[1,2] Multiple types of cytokeratin exist and several IHC stains are useful during MMS, including PCK, anticytokeratin 14, MNF116, and p63.[1] PCK has shown the most consistent keratinocyte staining both in terms of intensity of staining and sensitivity, with a mean of 99.1% and 99.9%, respectively.[6]

However, like any diagnostic tool, IHC use in frozen sections carries certain limitations and potential pitfalls. These include considerable added time and cost, although these barriers have become less significant with improved staining techniques and protocols.[7] Additionally, accurate interpretation of IHC requires knowledge of the staining patterns of normal skin, an understanding of validation protocols, appropriate use of internal positive and negative controls, and continuous correlation with H&E sections. IHC is not 100% sensitive or specific for malignant cells, and the potential for false-positive and false-negative results must always be considered.

Amyloidosis is not a distinct single diagnosis per se, but rather a collection of disorders that share a common feature of abnormal extracellular deposition of amyloid.[8,9] Deposition of amyloid in cutaneous tumors is a known phenomenon and has been recognized to occur in several tumor types.[8-10] In this case, the deposit would be classified as a form of localized secondary amyloidosis.[8] The origin of tumor-associated amyloidosis has not been definitively delineated, but some findings suggest the amyloid is derived from degenerated epidermal keratinocytes.[10] The clinical implications of amyloid deposition in this setting are unknown.

This case highlights several important points. First, it demonstrates the utility of IHC during MMS in identifying tumor, especially when confirming high-risk features or ruling out residual tumor, as this can affect accurate staging, counseling on prognostication, and management. Second, it highlights the importance of the surgeon having contextual interpretation of IHC on frozen sections as an adjunct to H&E. Knowledge of the typical keratinocyte staining pattern of AE1/AE3 and other banal structures is critical for accurate frozen section histologic interpretation to avoid false-positive (overcalling) and inappropriate additional stages. This, in turn, may affect the number of stages taken, the final defect size or depth, reconstruction options, and potentially the morbidity and anxiety experienced by the patient. Although IHC during MMS serves as a valuable tool to minimize morbidity while offering a higher degree of certainty in margin control, best practices have not yet been established for use of IHC during MMS and are warranted.

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