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Using p63 Immunohistochemistry during Mohs micrographic surgery for cutaneous squamous cell carcinoma

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Short Report

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Abstract

Background

Immunohistochemical (IHC) stains are increasing in use during Mohs micrographic surgery (MMS) when treating high-risk cutaneous squamous cell carcinoma (cSCC). This case series sought to understand the benefits of intraoperative p63 IHC stains during MMS for poorly differentiated cSCC.

Methods

Intraoperatively, a staining protocol for AE1/AE3 and p63 IHC staining was implemented for 15 cSCC cases. One additional case used only p63 IHC staining. Stains were reviewed and graded by the Mohs surgeon and dermatopathologist as either minimal/no staining or strong straining.

Results

Upon review, no p63 cases stained strongly for eccrine duct or follicular structures, compared to 100% of AE1/AE3 cases. Additionally, 89% and 62.5% of cases strongly stained tumor cells with p63 and AE1/AE3, respectively. After a mean follow-up time of 12 months, there have been two local recurrences and two deaths to date. Both deceased patients died of causes unrelated to cSCC.

Conclusion

Intraoperative IHC with p63 during MMS for cSCC has not been previously explored in literature. We demonstrate the successful use of p63 IHC in combination with AE1/AE3 IHC in 15 cases with cSCC. The strong staining of adnexal tissue with AE1/AE3 can be visually distracting. IHC with p63 is promising considering the high rate of positivity in cSCC, more intense staining, and minimal staining of adnexal tissue. While larger studies are needed, intraoperative p63 IHC staining in combination with AE1/AE3 IHC staining during MMS is likely beneficial in identifying poorly differentiated cSCC.

Introduction

Cutaneous squamous cell carcinoma (cSCC) is increasing at a rate of approximately 2–4% per year (1). Mohs micrographic surgery (MMS) is the recommended treatment for high-risk cSCC to ensure thorough and complete peripheral and deep margin assessment. Poorly differentiated cSCC can be difficult to visualize microscopically using traditional hematoxylin and eosin (H&E) staining on both frozen and permanent section histology (1). Immunohistochemical (IHC) staining during MMS is becoming more prevalent with an increase from 4.0% in 2014 to 7.7% in 2021 (2). IHC stains can be used during MMS to improve visualization of high-risk cSCC. A common IHC stain used during MMS for cSCC identification is multicytokeratin AE1/AE3 which stains for cytokeratin intermediate filament proteins found in epithelial

cells (3). The use of AE1/AE3 IHC staining during MMS results in easier visualization of cSCC at surgical margins, potentially resulting in a decrease in recurrence rates and morbidity for cSCC (3).

While AE1/AE3 IHC staining is effective, using p63 IHC to visualize poorly differentiated SCC during MMS has also been reported (4). AE1/AE3 IHC can have lower intensity staining in poorly differentiated cSCC and can stain non-tumor structures more intensely. This suggests a benefit to the use of p63 IHC stain in combination with AE1/AE3 IHC stain for visualizing poorly differentiated cSCC during MMS. Herein, we describe the intraoperative use of p63 IHC stains during MMS for cSCC.

Materials and Methods

We present a case series of16 patients treated for cSCC. On initial biopsy, all cases were paraffin embedded, stained with H&E and diagnosed as poorly differentiated cSCC. Additionally, two of the biopsy specimens were stained with AE1/AE3 and p40 IHC stains, one biopsy specimen was stained with AE1/AE3, p63, and p40 IHC stains, and one biopsy specimen was stained with AE1/AE3, p40, and CD163 IHC stains.

Intraoperatively, we executed a staining protocol for AE1/AE3 and p63 IHC staining for 15 cases. For one case, only the p63 IHC staining was used. Debulking specimen was removed and stained with both AE1/AE3 and p63 IHC in five cases and with only p63 IHC in one case.

Based on previous scoring protocols, slides were reviewed and graded on a scale from 0 to 3+ (0 = no staining, 1 + = 25% staining, 2 + = 50% staining +/- faint staining, 3 + = robust staining of all visible cells) by a Mohs surgeon (FLS) and dermatopathologist (KAM) (5). Slides graded as 0 or 1 + were considered minimal/no staining and slides graded as 2 + or 3 + were considered strong staining. There was no discordance in scoring.

Results

Stage and stain results are summarized in Table 1.

Table 1
Stage results (top) and AE1/AE3 and p63 stain results (bottom).

Stage Results: # of cases with no residual tumor seen with IHC staining				
IHC stain	**Stage I**	**Stage II**	**Stage III**	**Stage IV**
AE1/AE3 & p63	11	1	2	N/A
p63 only	0	1	0	N/A
AE1/AE3 only	0	0	0	1
Stain Results: # of cases with strong or minimal/no staining				
Stain	**Adnexal tissue (e.g., eccrine duct, folliculosebaceous)**		**Tumor**	
	Strong staining (2+, 3+)	**Minimal/no staining (0, 1+)**	**Strong staining (2+, 3+)**	**Minimal/no staining (0, 1+)**
AE1/AE3	15	0	5	3
p63	0	16	8	1

Overall, AE1/AE3 IHC staining resulted in much stronger eccrine duct and folliculosebaceous unit staining while p63 IHC staining resulted in minimal eccrine duct and folliculosebaceous unit staining (Fig. 1a-c). All AE1/AE3 IHC staining showed strong staining for adnexal tissue while no p63 cases stained strongly for adnexal tissue. More tumor cells stained strongly with p63 compared to AE1/AE3 at rates of 89% and 62.5%, respectively. Notably, three slides showed minimal/no staining of tumor with AE1/AE3 IHC but strong staining of tumor with p63 IHC, thereby showing that p63 IHC identified tumor tissue that AE1/AE3 IHC did not. One case with intense AE1/AE3 IHC adnexal staining also showed strongly stained keratin fragments in the absence of tumor, thought to represent a ruptured follicle (Fig. 1d).

After a mean follow-up time of 12 months (range 0.0–34.0 months), there have been two local recurrences and two deaths to date. Both instances of recurrence were cases performed with both AE1/AE3 and p63 IHC staining. Both deceased patients died of causes unrelated to cSCC.

Discussion

While it has been briefly reported, intraoperative IHC with p63 during MMS for cSCC has not been previously explored in literature. We demonstrate the successful use of p63 IHC in combination with AE1/AE3 IHC in 15 cases with cSCC.

The strong staining of adnexal tissue with AE1/AE3 can be visually distracting. Immunohistochemistry with p63 is promising considering the high rate of positivity in cSCC, more intense staining as seen in

eight of nine cases (89%) as well as the minimal staining of adnexal tissue. Considering three cases only stained tumor intensely with p63 IHC and not AE1/AE3 IHC, p63 IHC is useful in ensuring tumor is not missed. Interestingly, one case did stain tumor more intensely for AE1/AE3 which is why we continue to use the stains in combination.

While larger studies are needed, intraoperative p63 IHC staining in combination with AE1/AE3 IHC staining during MMS is beneficial in identifying poorly differentiated cSCC.

Declarations

Full Author Disclosures:

None of the authors have disclosures for this case series.

Competing Interests

None of the authors have competing interests for this case series.

Author Contributions Statement

FLS and TW implemented the staining protocol. FLS and KAM reviewed and graded the slides. EE wrote the manuscript and prepared the table and figures. All authors reviewed the manuscript.

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Figures

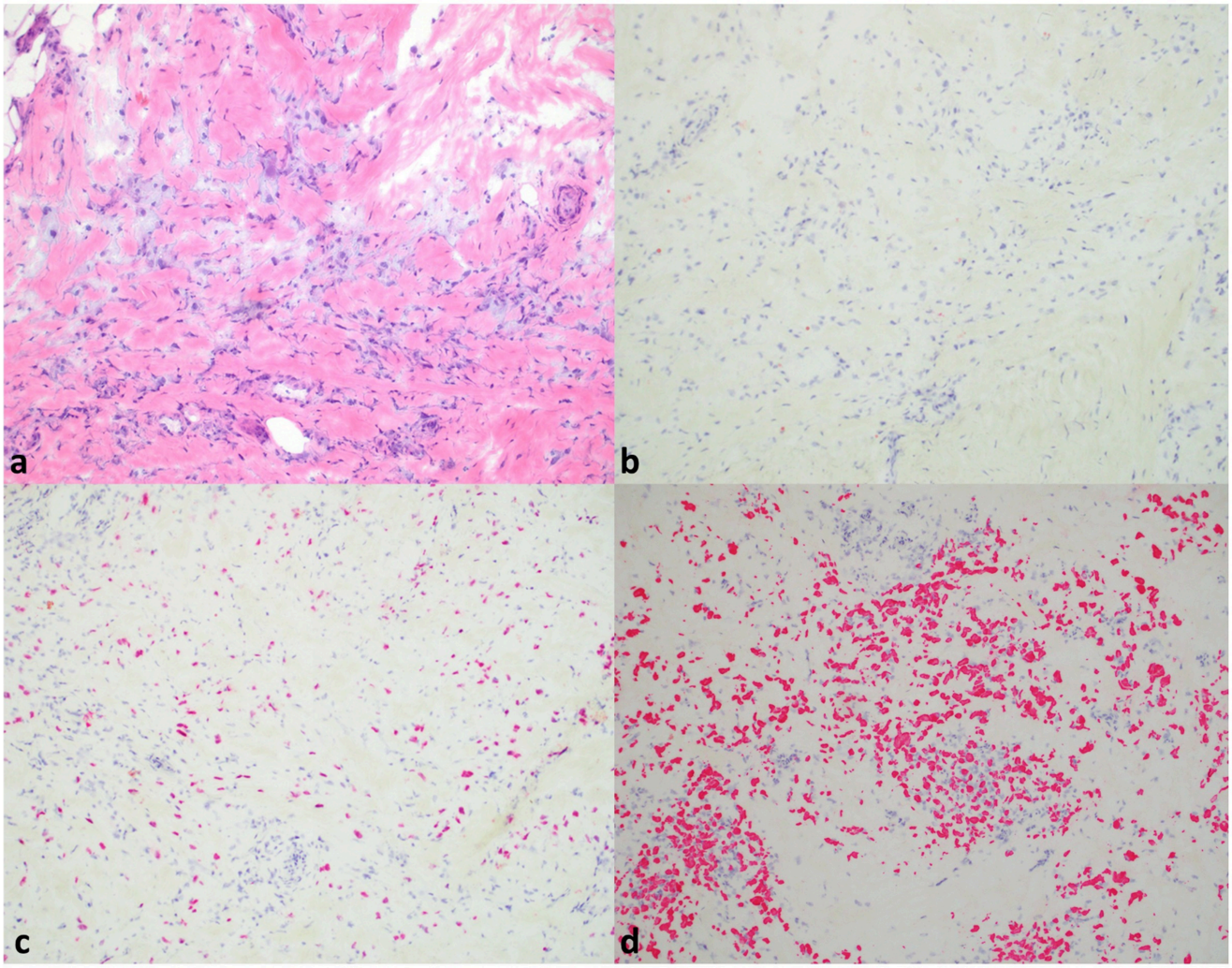


Figure 1

cSCC with (a) H&E staining, (b) AE1/AE3 IHC showing minimal staining of the tumor, and (c) p63 IHC showing strong staining of the tumor. Strong non-tumor staining of amorphous keratin granules with (d) AE1/AE3 IHC.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table128Sept2026.tif