Cutaneous squamous cell carcinoma with subtle perineural invasion detected with cytokeratin and epithelial membrane antigen immunohistochemistry

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INTRODUCTION

Perineural invasion (PNI), the infiltration of tumor within the perineural space, is an independent risk factor for adverse outcomes in cutaneous squamous cell carcinoma (cSCC) including recurrence, metastasis, and death.[1,2] During microscopically controlled excision, histopathologic features seen on intraoperative tissue sections can present challenges in assessing the presence of PNI. Benign findings such as normal perineurium may mimic PNI in routine sections. Conversely, subtle PNI may be mistaken for benign findings. Immunohistochemistry (IHC) may be more accurate than routine sections in diagnosing PNI, with significant implications for staging, prognosis, and management. We present a case in which IHC proved essential in identifying cSCC with PNI, affecting the course of therapy.

Abbreviations used:

cSCC:	cutaneous squamous cell carcinoma
EMA:	epithelial membrane antigen
IHC:	immunohistochemistry
MMS:	Mohs micrographic surgery
PNI:	perineural invasion
RT:	radiation therapy

CASE

A 68-year-old man with chronic lymphocytic leukemia presented in 2014 with a bleeding, 1.2- × 1.0-cm ulcerated plaque on the left frontal scalp. This lesion was present for 3 months, arising 3 cm inferior to a healed scar from a cSCC that was surgically treated 17 months prior (Fig 1). On physical examination, no neurologic deficits or palpable lymphadenopathy were noted. Biopsy confirmed the diagnosis of cSCC.

The patient had a history of chronic lymphocytic leukemia for which he completed chemotherapy and was in complete remission. He also had multiple prior nonmelanoma skin cancers that were treated with Mohs micrographic surgery (MMS). Seventeen months before this presentation, he was treated with MMS for an infiltrative cSCC of the left frontal scalp with focal PNI. Clear surgical margins were achieved, and the defect was repaired with linear closure. Adjuvant radiation therapy was recommended but declined by the patient.

The current cSCC (in 2014) was treated with MMS, during which infiltrative cSCC was noted to extend deeply into the subcutis and frontalis muscle. On the fourth stage of MMS, a prominent nerve at the deep surgical margin (within fascia) was

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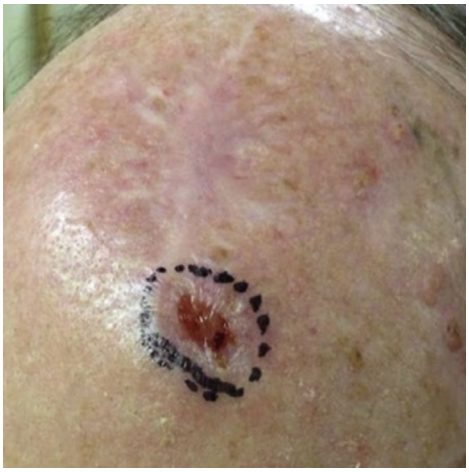

Fig 1. Presentation of cSCC as an ulcerated, bleeding plaque measuring 1.2 × 1.0 cm on the left frontal scalp, 3 cm inferior to a healed surgical scar.

ensheathed by a layer of monomorphic cells resembling perineurium. Other than these cells of uncertain significance, the surgical margins were clear of tumor. MMS was stopped, and the defect resurfaced with a porcine xenograft. Given the uncertain nature of the cells surrounding the nerve with concern for subtle PNI, the excised MMS tissue specimens were sent for permanent sections and IHC analysis.

On formalin-fixed, paraffin-embedded hematoxylin-eosin–stained sections, there were lobules and strands of cytologically atypical keratinocytes alternating with well-differentiated squamous foci and keratin cysts (Fig 2, *A*). Large dermal and subcutaneous nerve roots (up to 0.3 mm diameter) were encapsulated by monomorphic and bland-appearing cells originally interpreted as normal perineurium (Fig 2, *B*). Because cSCC with PNI can mimic normal perineurium morphologically, immunohistochemical labeling was performed.

IHC labeling for epithelial membrane antigen (EMA) and cytokeratin MNF116 was used to assess for the presence of PNI (Fig 3). EMA is an ubiquitously expressed protein in the epidermis, normal perineural cells, and cSCC.[3] In contrast, cytokeratin MNF116 stains keratinocytes, including cSCC[4] but not nerves or perineurium. Thus, only malignant cSCC would be identified by positive staining for both EMA and cytokeratin. In this case, the cells surrounding the nerve were positive for both stains, showing that cSCC was present in the perineural space (Fig 3).

The presence of tumor cells within the perineural space confirmed that cSCC with PNI was present at the deep surgical margin within a large caliber nerve root. Given the increased risk of local recurrence, the patient was treated with adjuvant radiation therapy (RT). He completed 5000 cGy of electron beam RT 5 months after MMS without significant sequelae. Three years later, there was no evidence of recurrence or neurologic deficit.

DISCUSSION

Approximately 2% to 14% of cSCCs exhibit PNI,[1,2] defined histologically by tumor cells within the perineural space.[5] PNI in cSCC correlates with a more aggressive clinical course, leading to higher recurrence and mortality rates.[1,2] In the American Joint Committee on Cancer Eighth Edition guidelines, PNI is one of 4 factors that elevates cSCC to the high-risk T3 stage.[6] Treatment with MMS, which allows for complete microscopic margin control, is optimal for such high-risk cSCC.[7] Nevertheless, there may be false-negative margins with routine sections in cases of subtle PNI. In the case presented here, the patient had a prior cSCC with focal PNI that was surgically excised with negative margins 3 cm distal to the presenting lesion. Thus, there was increased risk of PNI and aggressive biological behavior in this satellite lesion. As this case demonstrates, accurate identification of PNI provides essential prognostic information and supports the use of adjuvant therapy that may improve patient outcomes.[8]

Benign findings, including inflammation, fibrosis, and normal perineurium, can mimic PNI in routine sections.[9] Conversely, as our case highlights, subtle PNI can mimic benign findings and may be missed without special stains. Previous reports suggest that IHC has an increased sensitivity for detecting infiltrative cSCC[10] and may facilitate detection of subtle PNI.[9] Thus, if definitive conclusions for cSCC with PNI cannot be made intraoperatively with routine sections, use of IHC stains can be instrumental in increasing accuracy of PNI detection in high-risk cases. An additional factor to consider in the determination of PNI is the phenomenon of re-excision PNI, a benign finding of mature squamous epithelial cells within the perineurium of superficial nerves within the scar of a previous excision.[9] Immunohistochemically, malignant PNI and re-excision PNI may be indistinguishable, and clinical-pathologic correlation is therefore essential. In this case, the location of the affected nerves outside the prior surgical scar and the presence of adjacent cSCC infiltrating the subcutis and muscle ruled out benign re-excision PNI.

This case shows the beneficial role that IHC for cytokeratin and EMA play in identifying cSCC with

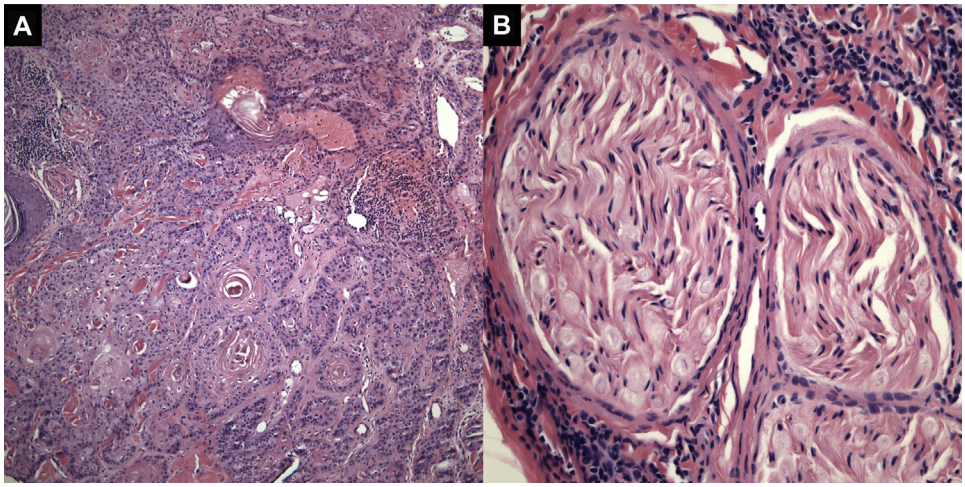

Fig 2. Permanent section of cSCC. **A**, Lobules of atypical keratinocytes are present in association with well-differentiated squamous foci and keratin cysts. **B**, Bland-appearing cells mimicking normal perineurium surround large nerve roots up to 0.3 mm in diameter. (Hematoxylin-eosin stain; original magnifications: **A**, ×100; **B**, ×400.)

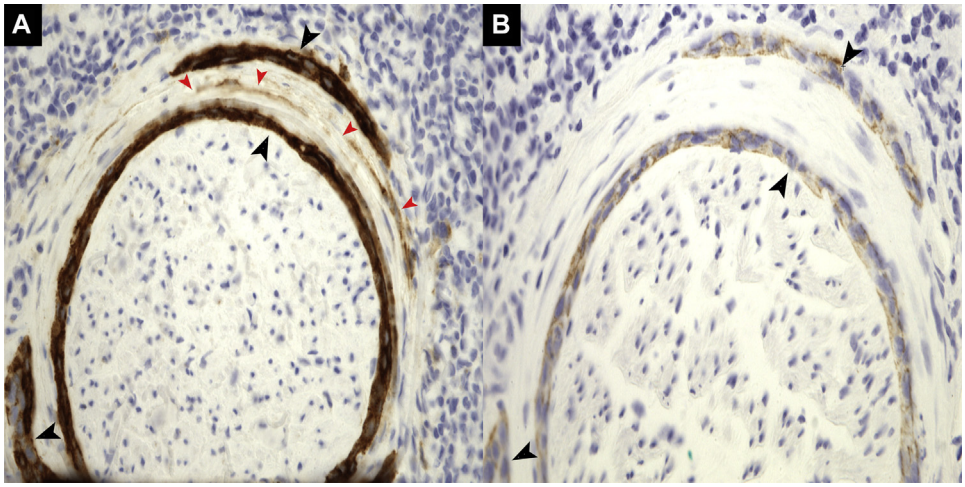

Fig 3. IHC stains confirm diagnosis of perineural cSCC with positive staining of both EMA and MNF116 within a large caliber nerve root. **A**, EMA stain: both cSCC (*black arrows, dark brown cells*) and normal perineurium (*red arrows, light brown cells*) stain positively for EMA. **B**, MNF116 stain: only cSCC (*black arrows*) also stains positively for cytokeratin MNF116.

subtle PNI. Such identification can facilitate the timely use of adjuvant RT and contribute to disease control.

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