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Original contribution

Intratumoral and peritumoral lymphovascular invasion detected by D2-40 immunohistochemistry correlates with metastasis in primary cutaneous Merkel cell carcinoma☆

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Summary Primary cutaneous Merkel cell carcinoma (MCC) is an aggressive neuroendocrine malignancy in which lymphovascular invasion (LVI) correlates with more aggressive phenotype. The prognostic significance of LVI detected by D2-40 immunohistochemistry (IHC) in MCC remains controversial. We aimed to determine how LVI detected by D2-40 IHC compares with LVI detected by hematoxylin and eosin (H&E) staining in predicting MCC metastasis. Clinical and histopathologic features of MCCs diagnosed and treated in 2002 to 2015 were assembled and included 58 MCC tumors from 58 patients. H&E-stained tissue sections and D2-40 IHC studies were reviewed. When LVI was present, the location (peritumoral or intratumoral) and the size of the largest invaded vessel were recorded. LVI findings by H&E staining and D2-40 IHC were compared with each other and with histologic features and clinical outcomes. H&E staining showed LVI in 37 of 58 cases; D2-40 IHC confirmed LVI in 30 of these cases but failed to confirm LVI in 7. D2-40 IHC also detected 14 cases of LVI not identified on H&E staining. Histologically, D2-40–detected LVI was associated with infiltrative growth pattern and nonbrisk lymphoid infiltrate ($P = .005$ and $P = .055$, respectively). There was a statistically significant difference between the frequency of detection of peritumoral LVI by H&E in comparison to D2-40 IHC ($P = .0009$). MCCs in which D2-40 IHC–detected both intratumoral and peritumoral LVI were typically larger than MCCs without (mean,

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24.5 mm versus 17.3 mm; $P = .03$) and more frequently metastasized (87% versus 51%; $P = .03$). D2-40 IHC detection of both intratumoral and peritumoral LVI is associated with metastasis.

1. Introduction

Merkel cell carcinoma (MCC) is an aggressive cutaneous neuroendocrine carcinoma that frequently metastasizes and causes death. Over the past 2 decades, the incidence of MCC has risen steadily, and MCC is currently the second leading cause of skin cancer–related death [1,2]. Risk factors for MCC include fair skin, chronic sun exposure, chronic immune suppression, and advanced age [2-7]. Identification of the Merkel cell polyomavirus (MCPyV) was a critical advance in our understanding of MCC biology. MCPyV DNA integrates into the host genome of approximately 70% to 80% of MCCs and is considered an important pathogenic driver of the disease [8]. Prognostication in MCC follows the guidelines of the TNM staging system set forth by the American Joint Committee on Cancer, which was first published in 2010 [9]. Recently, the guidelines have been updated to include separate clinical and pathological stage groups in addition to regrouping MCC of unknown primary site, providing more accurate prognostication [10]. However, the relative significance of each individual prognostic indicator, including clinical factors (sex, age, anatomic site of disease) [3,4,7] and histopathologic parameters (tumor depth, tumor size, proliferative rate, perineural invasion, and lymphovascular invasion [LVI]) remains controversial [3,4,7,11,12]. There is therefore a critical need to delineate the biomarkers that most robustly inform prognosis in MCC to permit more confident prediction of patient outcome, more effective tailoring of individual patient treatment strategies, and more effective design of clinical trials.

D2-40 (antipodoplanin) is a monoclonal antibody that rarely reacts with vascular endothelium and therefore is highly specific for lymphatic endothelium. Thus, D2-40 is a highly selective marker to delineate LVI by tumor cells [13]. We and others have shown that in cutaneous melanoma, D2-40 immunohistochemistry (IHC) enhances the detection of both intratumoral LVI and peritumoral LVI compared with routine hematoxylin and eosin (H&E) stains alone [14]. Furthermore, detection of LVI with D2-40 IHC correlated with positive sentinel lymph nodes and worse overall survival and disease-free survival in cutaneous melanoma [15]. LVI detection with D2-40 IHC has also been examined in other cancers, including endometrial [16], breast [17], esophageal [18], and colorectal cancers [19], and all such studies have shown correlation of LVI with either sentinel lymph node metastasis or overall survival.

The prognostic relevance of LVI by MCC tumor cells detected using conventional H&E staining is well established [4,20,21]. However, whether adding D2-40 IHC to H&E staining enhances the determination of LVI in MCC remains controversial because comparatively few studies have assessed this [22]. Therefore, in the current study, we assessed 58 MCCs for LVI using both conventional H&E-stained sections and D2-40 IHC to determine whether D2-40 IHC–detected LVI outperforms H&E staining–detected LVI and the value of sublocalizing the location of LVI detected by D2-40 in predicting metastasis and patient survival.

2. Materials and methods

2.1. Selection of cases

With approval from the institutional review board at The University of Texas MD Anderson Cancer Center, we reviewed the institution's pathology files to identify primary MCCs diagnosed and treated in 2002 to 2015. Clinical

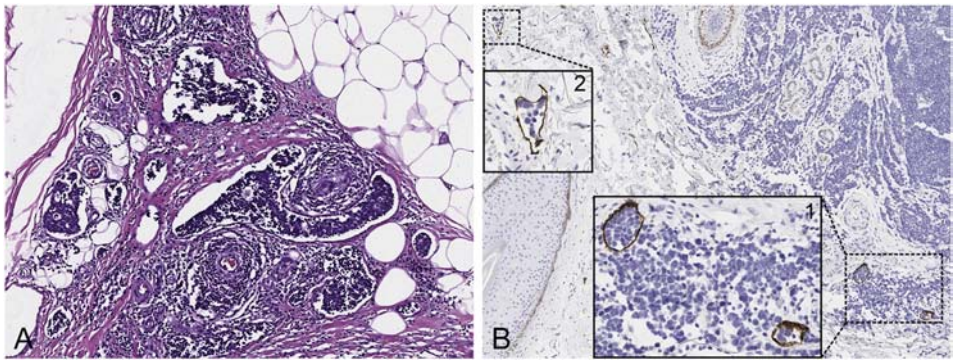

Fig. 1 LVI in MCC. A, H&E staining shows LVI (H&E, original magnification ×200). B, D2-40 IHC shows intratumoral LVI (1) and peritumoral LVI (2) (D2-40 IHC, ×100 in main panel and ×400 in insets).