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Original contribution

Enhanced immunohistochemical detection of neural infiltration in primary melanoma: is there a clinical value?☆

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Summary Neural infiltration in primary melanoma is a histopathologic feature that has been associated with desmoplastic histopathologic subtype and local recurrence in the literature. We tested the hypothesis that improved detection and characterization of neural infiltration into peritumoral or intratumoral location and perineural or intraneural involvement could have a prognostic relevance. We studied 128 primary melanoma cases prospectively accrued and followed at New York University using immunohistochemical detection with antihuman neurofilament protein and routine histology with hematoxylin and eosin. *Neural infiltration*, defined as the presence of tumor cells involving or immediately surrounding nerve foci, was identified and characterized using both detection methods. Neural infiltration rate of detection was enhanced by immunohistochemistry for neurofilament in matched-pair design (47% by immunohistochemistry versus 25% by routine histology). Immunohistochemical detection of neural infiltration was significantly associated with ulceration (P = .021), desmoplastic and acral lentiginous histologic subtype (P = .008), and head/neck/hands/feet tumor location (P = .037). Routinely detected neural infiltration was significantly associated with local recurrence (P = .010). Immunohistochemistry detected more intratumoral neural infiltration cases compared with routine histology (30% versus 3%, respectively). Peritumoral and intratumoral nerve location had no impact on clinical outcomes. Using a multivariate model controlling for stage, neither routinely detected neural infiltration nor enhanced immunohistochemical characterization of neural infiltration was significantly associated with disease-free or overall survival. Our data demonstrate that routinely detected neural infiltration is associated with local recurrence in all histologic subtypes but that improved detection and characterization of neural infiltration with immunohistochemistry in primary melanoma does not add to prognostic relevance.

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1. Introduction

Neural infiltration (NI) is a pathologic feature, detected by routine histology, which has an impact on local recurrence [1,2]. NI can be characterized as tumor cells directly invading neural structures (intraneural) or surrounding the nerve (perineural) [3,4]. Efforts to associate NI with outcome have been contradictory. Although most studies do not show any association between NI and sentinel lymph node metastasis [5], distant spatial recurrence [4,6,7], and overall survival [2,8], some studies have shown NI to be associated with distant metastasis [2] and decreased survival [8]. Neurotropic melanoma (NM) is most commonly considered under the umbrella of spindle cell melanoma [1] or desmoplastic melanoma (DM) as desmoplastic NM [4,9]. DM, a variant of spindle cell melanoma [4], is an uncommon [10] and often locally aggressive tumor [2,5,9] largely attributed to its propensity for NI [6,7,10–12]. Nevertheless, NI is not limited to these tumor histologies and appears in other melanoma subtypes with comparative infrequency [8,13].

The implications of NI in primary melanoma have been confounded with the grouping of NM and DM in most studies [1,2,4,5,9,14], which may account for the conflicting findings of NI with prognosis. In literature, NI has been reported in 30% to 40% of DM [2,10]. However, this phenomenon is scarcely noted in other more common epithelioid histologic subtypes such as superficial spreading and nodular melanomas [11]. Our own database identified NI in only 64 (3%) of 1948 primary melanomas on routine histology. The identification of NI in other histologies could serve as a predictor of local recurrence and can help identify the patients most likely to benefit from wider excision margins [2,7,10,12] or adjuvant radiation therapy [6,7,15–17].

The modest prevalence of NI across melanoma subtypes could be partially attributed to difficulties in the detection of NI during routine histology, particularly with regard to intratumoral location. The malignant cells within the tumor can either mechanically or chemically distort the entangled nerve branches making the diagnosis of intratumoral NI on routine histology potentially challenging. In addition, intratumoral hypercellularity is another confounding factor in detecting NI compared with the less encumbered peritumoral region. Immunohistochemistry (IHC) has already been used successfully to enhance the intratumoral and overall detection of lymphatic vessel invasion (LVI) in malignant melanomas [18]. Immunolabeling using P75 nerve growth factor receptor (P75 NGFR) has been used to identify NI in melanoma, particularly in DM and spindle cell melanoma [11]. P75 NGFR has also been identified as a potential stain to facilitate the diagnosis and differentiation of DM and NM from other neoplasms and melanoma histologic subtypes [19,20]. Concerns regarding diffuse staining of P75 NGFR in all neural-crest derived lesions [19], non-NMs, benign melanocytic tumors after mild fixation [3], and regular staining of spindle cell melanoma [20,21] directed our use of neurofilament (NF) antibody to unambiguously and specifically stain neurons of the central and peripheral nervous system [22,23]. To our knowledge, NF has not been used in cutaneous melanoma to detect NI but has demonstrated a compelling capacity to locate neural filaments, including intratumoral axons, in schwannomas [24,25]. Our aim was to exploit NF to not only detect the presence of NF but also to distinguish peritumoral/intratumoral as well as intraneural/perineural involvement.

2. Materials and methods

2.1. Study population

A total of 128 primary melanoma tissues were retrieved from patients enrolled in the Interdisciplinary Melanoma Cooperative Group (institutional review board no. 10362), a prospectively collected clinicopathological biospecimen database at New York University Langone Medical Center (August 2002–December 2011) [26]. Informed consent for the use of clinical data and tissue was obtained from each patient at the time of enrollment, and all demographic, clinicopathological, and follow-up data were recorded prospectively. All 128 patients in the study underwent additional surgery for wide margins (at least 1 cm) after their initial biopsy and diagnosis. Survival data were recorded prospectively for all patients. Median follow-up time calculated on the basis of survivors (n = 76) was 3.6 years, with all cases having at least 1.4 years of follow-up (range, 1.4-13.7 years).

Patients with available primary melanoma tissues were identified, and NI-positive patients in routine histology were matched to NI-negative patients for stage at initial diagnosis and histologic subtype. Clinicopathological features collected from these cases included primary tumor thickness, ulceration, mitotic rate, sentinel lymph node status, the American Joint Committee on Cancer stage at pathological diagnosis, histologic subtype, primary tumor location, and follow-up data.

2.2. Immunohistochemistry

IHC was performed using antihuman NF protein clone 2 F11 (Dako, Glostrup, Denmark) on formalin-fixed, paraffin-embedded primary melanoma tissues to detect NI. In brief, after deparaffinization and rehydration, heat-induced epitope retrieval was performed in 0.1 M Tris-EDTA buffer (pH 9.0) in a microwave oven at 1200 W at 90% power for 10 minutes. Sections were cooled in tap water for 5 minutes, washed with phosphate-buffered saline, and incubated with blocking serum (VECTASTAIN ABC Kit-Mouse IgG; Vector Laboratories, Burlingame, CA) for 30 minutes. The primary antibody was diluted in buffer (1:200), and the sections were incubated at