Neurotrophin receptors and perineural invasion in desmoplastic melanoma

Noah Frydenlund, BS,[a] Dominick A. Leone, MPH,[b] Brendon Mitchell, MA,[e] Shi Yang, MD,[c] April Deng, MD, PhD,[f] Mai P. Hoang, MD,[g] and Meera Mahalingam, MD, PhD, FRCPath[d]
Boston and Worcester, Massachusetts; and Gainesville, Florida

Background: Perineural invasion (PNI) in desmoplastic melanoma is associated with increased local recurrence and reduced disease-free survival. The biological mechanisms underlying PNI remain unclear although several lines of evidence implicate neurotrophins and their receptors.

Objectives: We investigated the expression of p75NGFR and TrkA, and the presence of functional RET polymorphism (RETp) as they relate to PNI in desmoplastic melanoma.

Methods: In all, 43 cases of desmoplastic melanoma were immunohistochemically evaluated for TrkA and p75NGFR expression and RETp was detected by direct DNA sequencing.

Results: PNI was present in 67% of cases. On univariate analysis, p75NGFR was associated with PNI (expression detected in 79% of PNI-positive cases compared with 36% of PNI-negative cases, $P = .005$), increased Breslow depth ($P = .007$), and greater Clark level ($P = .01$). RETp was noted in 28% of cases but was not significantly associated with PNI ($P = .27$) or other histopathologic variables. TrkA expression was absent in all cases. PNI was associated with increased Breslow depth and Clark level ($P = .01$ and $P = .009$, respectively). Controlling for the association between p75NGFR and depth, p75NGFR remained associated with an increased propensity for PNI (odds ratio 4.68, $P = .04$).

Limitations: The sample size was limited.

Conclusion: In desmoplastic melanoma, p75NGFR expression is significantly associated with PNI and a more locally aggressive phenotype. (J Am Acad Dermatol 2015;72:851-8.)

Key words: desmoplastic melanoma; immunohistochemistry; neurotrophin; p75NGFR; perineural invasion; RET; TrkA.

Perineural invasion (PNI) refers to the invasion of the perineural sheath by tumor cells, followed by their subsequent proliferation and axial spread within the perineural space.[1] In melanoma, PNI is most frequently seen in desmoplastic melanoma, although epithelioid melanomas may, albeit rarely, display PNI.[1-3] The clinical relevance of PNI in cutaneous malignancies has been best established in squamous cell carcinoma in which it is considered a high-risk tumor characteristic,[4] and in melanoma PNI has been linked to increased depth of invasion and greater risk of local recurrence.[1,5,6]

Abbreviations used:

IHC:	immunohistochemistry
NGF:	nerve growth factor
OR:	odds ratio
PNI:	perineural invasion
RETp:	functional RET polymorphism

From the Division of Graduate Medical Sciences,[a] School of Public Health,[b] Department of Pathology,[c] and Dermatopathology, Section, Department of Dermatology,[d] Boston University School of Medicine; University of Florida College of Medicine[e]; Department of Pathology, University of Massachusetts Medical School, Worcester[f]; and Department of Pathology, Massachusetts General Hospital, Boston.[g]

Funding sources: None.
Conflicts of interest: None declared.
Accepted for publication January 16, 2015.

Reprints not available from the authors.
Correspondence to: Meera Mahalingam, MD, PhD, FRCPath, Dermatopathology Section, Department of Dermatology, Boston University School of Medicine, 609 Albany St, J-401, Boston, MA 02118. E-mail: mmahalin@bu.edu.

Published online March 6, 2015.
0190-9622/$36.00

http://dx.doi.org/10.1016/j.jaad.2015.01.026

The biological basis for PNI remains to be elucidated. Neurotrophins and their receptors have emerged as popular candidates for mediating PNI.[5,7-9] Particular attention has been paid to nerve growth factor (NGF) and both its high and low affinity receptors (TrkA and p75NGFR, respectively). Expression of TrkA has been linked to PNI in select cutaneous and noncutaneous malignancies.[8,10-13] There is at least 1 report of TrkA detected by immunohistochemistry (IHC) in primary and metastatic melanoma,[14] and another demonstrating TrkA expression in vitro in melanoma cell lines.[15] The use of staining for p75NGFR for distinguishing spindled melanomas from other cutaneous spindled lesions has been previously demonstrated.[16-21] In 2010, Chan and Tahan[5] used IHC to show that p75NGFR expression was present in 8 of 10 PNI-positive melanomas compared with 1 of 9 of PNI-negative cases, although the study was not confined to desmoplastic melanoma and the small sample size precluded extensive analysis. Lastly, the proto-oncogene RET (which encodes the receptor tyrosine kinase for glial cell line–derived neurotrophic factor) has been suggested to play a role in mediating PNI in pancreatic cancers.[9,22] Notably, a functional RET polymorphism in exon 11 (RETp) has been shown to be more frequently expressed in desmoplastic melanoma, suggesting that it contributes to the enhanced propensity of desmoplastic melanoma for PNI.[23]

Given the above, aims in the current study were to evaluate the relationship between TrkA, p75NGFR, RETp, and PNI in a cohort of desmoplastic melanomas. In addition, we investigated their relationship to established histopathologic prognosticators (Breslow depth, Clark level, pure vs mixed subtype, host response, ulceration, and regression).

CAPSULE SUMMARY

- Perineural invasion is frequently observed in desmoplastic melanoma and may be a negative prognostic finding.
- p75NGFR expression in desmoplastic melanoma is associated with an increased incidence of perineural invasion beyond its previous affiliation with spindle cell morphology.
- p75NGFR positivity in desmoplastic melanoma indicates a locally aggressive tumor, and should prompt a high degree of suspicion toward perineural invasion.

METHODS

Sample selection

This study was approved by the institutional review board of Boston University School of Medicine (docket numbers H-32816, H-32952). Archival annotated tissue samples with a diagnosis of desmoplastic melanoma (n = 43) were retrieved from the pathology files of the Skin Pathology Laboratory, Boston University School of Medicine. Inclusion criteria were randomly selected cases of primary melanoma with adequate tumoral tissue to allow for analyses. Histologic sections of all cases were reviewed by 2 board-certified dermatopathologists (initial sign-out on all by a dermatopathologist; cases were then re-reviewed, and the diagnoses confirmed by the senior author). All patient data were de-identified.

Immunohistochemical analyses of TrkA expression

Formalin-fixed, paraffin-embedded tissue of primary melanomas (n = 43) were baked at 75°C for 30 minutes. Sections were deparaffinized with xylene and rehydrated in a series of decreasing concentrations of ethanol solution. Heat-induced antigen retrieval was carried out in retrieval solution (Dako, Carpinteria, CA) with a pH of 6.1 in a 98°C bath for 20 minutes. All immunostained slides were reviewed and scored by the first author (N. F.) and the senior author (M. M.) in a blinded fashion with respect to each other's scores and any disagreements were reviewed together to achieve a consensus score. The slides were treated with dual endogenous enzyme block (Dako) then incubated with anti-TrkA (14G6) rabbit monoclonal antibody (Cell Signaling Technology, Danvers, MA) at a dilution of 1:200 for 50 minutes at room temperature, followed by treatment with polymer horseradish peroxidase (Dako) for 20 minutes. Color development and contrast were achieved using DAB and hematoxylin, respectively.

Positive TrkA expression was defined as either membranous or mixed (membranous and cytoplasmic) staining of tumor cells. Those cells displaying only cytoplasmic staining were considered negative. Membranous staining of basal epidermal cells, eccrine epithelial cells, mature sebocytes, and the outer root sheath of hair follicles served as positive internal controls where they could be visualized.

p75NGFR and RET polymorphism

Sections from the same formalin-fixed paraffin-embedded tissue blocks of desmoplastic melanoma cases used in the current study were analyzed previously for p75NGFR expression and RET G619S polymorphism.[24] Briefly, p75NGFR expression was determined via IHC analysis using an anti-p75NGFR