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Original contribution

Microvessel density, lymphovascular density, and lymphovascular invasion in primary cutaneous melanoma—correlation with histopathologic prognosticators and *BRAF* status☆

Phyu Phyu Aung MD, PhD[a,1], Dominick Leone MPH, MS[b], John Kyle Feller MA[a], Shi Yang MD[c], Marier Hernandez MD[d], Ron Yaar MD[e], Rajendra Singh MD[f], Thomas Helm MD[g], Meera Mahalingam MD, PhD, FRCPath[a,*]

[a]*Dermatopathology Section, Department of Dermatology, Boston University School of Medicine, Boston, MA 02118*
[b]*Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118*
[c]*Department of Pathology, Boston University School of Medicine, Boston, MA 02118*
[d]*Department of Dermatology, UT Southwestern Medical Center, Dallas, TX 75390*
[e]*Aurora Diagnostics, Greensboro, NC 27408*
[f]*Departments of Dermatology and Pathology, Mt Sinai School of Medicine, New York, NY 10029*
[g]*Department of Dermatology, State University of New York at Buffalo, Buffalo, NY 14203*

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Summary The relationship between microvessel density (MVD), lymphovascular density (LVD), and lymphovascular invasion (LVI) in primary cutaneous melanoma (PCM) remains unclear. Given this, a total of 102 PCMs were assessed for MVD (vascular endothelial growth factor receptor 2 and Endocan), LVD (D2-40), and LVI (immunostaining with D2-40/S-100 and hematoxylin and eosin); tumoral S-100A13, vascular endothelial growth factor receptor 2, and Endocan; and *BRAF* status. LVD was associated with MVD ($P = .01$). MVD was higher in PCMs with depth greater than or equal to 2 mm and ulceration ($P = .04, .05$), whereas LVD was higher in PCMs with depth greater than or equal to 2 mm and mitoses ($P = .03, .02$). After adjusting for MVD and LVD, only ulceration was associated with LVI ($P < .02$). A *BRAF* mutation was seen in 30.4% cases, and when present, both LVD and host response ($P = .0008$ and .04, respectively) were significantly associated with MVD. Immunostaining with S-100A13 was noted in 99% of cases and a significant association noted only with ulceration ($P = .05$). Immunostaining increased LVI positivity (46.5% versus 4.9% by hematoxylin and eosin, $P < .0001$). MVD and LVD are not associated with LVI, appear to be closely related with each other, and are associated with select markers of poor prognosticative value. The association between a host response and LVD and MVD in PCMs with a *BRAF* mutation suggests that they exhibit potential for strategizing immunotherapies.

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* Corresponding author. Dermatopathology Section, Department of Dermatology, Boston University School of Medicine, 609 Albany St, J-401, Boston, MA 02118, USA.
E-mail addresses: aungpatho@gmail.com (P. P. Aung), nleone@bu.edu (D. Leone), j.kyle.feller@gmail.com (J. K. Feller), shiyang@bu.edu (S. Yang), marierhernandez@gmail.com (M. Hernandez), ryaar@alum.bu.edu (R. Yaar), skinpathology@gmail.com (R. Singh), thelm@buffalomedicalgroup.com (T. Helm), mmahalin@bu.edu (M. Mahalingam).
[1] Current address: Departments of Pathology and Dermatology, Dermatopathology Section, U. T. - M. D. Anderson Cancer Center, Houston, TX 77030.

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1. Introduction

In cutaneous malignancies, microvessel density (MVD) has been shown to correlate with tumor grade in canine mast cell tumors [1]. In a study of human cutaneous nonmelanoma skin cancers, although MVD was found to be higher in squamous cell carcinoma than basal cell carcinoma and Bowen disease, a correlation with histopathologic prognosticators was not performed [2]. Although this correlation has indeed been demonstrated in malignant melanoma, results have been inconclusive and conflicting [3-10]. Using a plethora of markers, studies reporting an association between increased vascularity and an unfavorable outcome [3-6] have been refuted by others showing that increased MVD is significantly associated with improved patient survival [7-10].

Utilization of the lymphatic endothelial marker, D2-40, for measurement of lymphovascular density (LVD) in cutaneous malignancy has been reported in squamous cell carcinoma of the head and neck region with conflicting results regarding its prognosticative value [11]. This appears to be true for LVD in melanomas as well. In support of this, in 1 study, metastatic melanomas had significantly more LVD, which was associated with poor disease-free and overall survival, whereas in another, decreased LVD was present in thicker and more proliferative tumors (Ki-67) [6,7].

The incidence of lymphovascular invasion (LVI), based on hematoxylin and eosin (H&E) staining alone in melanoma has been shown to range from 0% to 6%. Select studies have shown that this detection rate increases with the use of select immunohistochemical stains targeting endothelial cells [12-14]. The relevance of detection of LVI in primary cutaneous melanoma (PCM) lies in that it has been shown to be significantly associated with time to regional nodal metastatic disease as well as first metastasis and disease-related death [15-17].

The premise of the current study was to ascertain precisely the relationship between MVD, LVD, and LVI in PCM. MVD was assessed using vascular markers including vascular endothelial growth factor receptor 2 (VEGFR-2) and Endocan; LVD was assessed by D2-40; and LVI was assessed by both H&E and double staining of S-100 and D2-40. We also assessed for tumoral expression of the proangiogenic marker (S-100A13), VEGFR-2, and Endocan. Markers used in the current study were selected for the following reasons: VEGFR-2, an autocrine growth factor receptor for VEGF, shown to be the dominant effector of VEGF function in the metastatic melanoma microenvironment [18,19]; Endocan or endothelial cell specific molecule 1, a soluble proteoglycan secreted by endothelial cells, overexpression of which has been shown in vitro to be a poor prognosticator in melanoma [20]; and S-100A13, a proangiogenic molecule and a calcium-binding protein involved in the release of fibroblast growth factor family [21]. An additional aim was to ascertain the correlation between MVD, LVD, and established histopathologic prognosticators as well as the *BRAF* status and S-100A13 expression in PCM.

2. Materials and methods

2.1. Sample selection

This study was approved by the Boston University School of Medicine Institutional Review Board (docket no. H-31284). Archival tissues with a diagnosis of PCM (n = 102) were retrieved from the pathology files of the Skin Pathology Laboratory, Boston University School of Medicine (Boston, MA), between January 2010 and December 2012. Inclusion criteria were randomly selected cases of invasive PCM with a depth of at least 1 mm (a cut-off selected to facilitate quantification of intratumoral MVD). Histopathologic sections of all cases were reviewed by 2 board-certified dermatopathologists (initial sign out on all by a dermatopathologist; cases were then rereviewed and diagnosis confirmed by the senior author). All patient data were deidentified.

The median age of the patients was 67 years (range, 19-103 years) of which 70% (n = 72) were men. Mitosis was present in 90 of 102 and absent in 12 of 102. Host response was present in 48 of 102 and absent in 54 of 102. Ulceration was present in 28 of 102 and absent in 74 of 102. Regression (including partial or active regression and defined by the presence of fibrosis or a heavy lymphocytic infiltrate with loss or degeneration of tumor cells) was present in 81 of 102 and absent in 21 of 102. LVI detected by H&E stain was noted in 5 of 102 cases.

All were of the American Joint Committee on Cancer (AJCC) clinical grade T2a and above at the time of initial diagnosis including 44-T2a, 9-T2b, 19-T3a, 16-T3b, 7-T4a, and 7-T4b. The median thickness of all tumors assessed was 2.4 mm (range, 1.1-8.3 mm).

2.2. Immunohistochemistry

Immunohistochemistry was performed on 4-μm thickness formalin-fixed, paraffin-embedded sections using commercially available markers D2-40 at 1:200 (Dako, Carpinteria, CA), VEGFR-2 at 1:500 (clone 55B11; Cell Signaling Technology, Danvers, MA), S-100A13 at 1:500 (Sigma-Aldrich, St Louis, MO), S-100 at 1:1000 (Dako), and Endocan MEP14 at 1:500 (Lunginnov, Lille, France). Double immunolabeling was performed using EnVision DuoFLEX Doublestain System (Dako) in combination with Vector Blue AP Substrate Kit (Vector, Burlingame, CA) and a Methyl Green counterstain (Vector). All stained slides were reviewed and scored by the first author (P. A.) and the senior author (M. M.) in a blinded fashion, and any disagreements reviewed together to achieve a consensus score.