Immunohistochemical Expression of Platelet Growth Factor and Vascular Endothelial Growth Factor in Patients With Melanoma With and Without Redness (Brenner Sign)

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Objective: To assess whether an erythematous eruption in the vicinity of or distant from a melanoma lesion might be related to the vascular endothelial growth factor, the platelet-derived endothelial cell growth factor, or both.

Methods: Biopsy specimens from 13 patients with primary melanoma, 6 of whom had erythematous eruptions and 7 who did not, were studied by immunohistochemistry for the expression of vascular endothelial growth factor and platelet-derived endothelial cell growth factor.

Results: Vascular endothelial growth factor was positive in 3 of 6 patients (50%) with melanoma and redness (Brenner sign) and in 4 of 7 patients (57%) with melanoma without redness. Platelet-derived endothelial cell growth factor was positive in all 6 patients (100%) with melanoma and redness and in 4 of 7 patients (57%) with melanoma without redness.

Conclusion: Platelet-derived endothelial cell growth factor may have a part in the pathogenesis of the redness observed in patients with melanoma, called Brenner sign, by affecting vasculature function.

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The rise in incidence of malignant melanoma is among the greatest for all kinds of cancer, with an annual rise of 3% to 8% in newly diagnosed melanomas worldwide.¹ The increase is especially great in regions of the world where intense sun, especially in the Middle East, and a trend toward more revealing clothing expose people to untoward amounts of UV radiation. Despite efforts to achieve early detection and recognition of the unfavorable prognostic factors and the development of novel treatment modes, progressive disease has a dismal prognosis. Tumor progression and development cannot be attributed to a single process. The escape of the tumor from the regulatory mechanisms includes excessive production of cytokines and autocrine growth factors, substituting for cytokines and exogenous growth factors,² self-tolerance to melanoma antigens, and down-regulation of class II major histocompatibility complex molecules.³ Neoplasms including melanoma are known to induce angiogenesis, which is believed to have a role in the development and spread of the melanoma.⁴ ⁵ Previous publications⁶ ⁷ described an observation of patients with malignant melanoma who had a particular reddish appearance that could not be attributed to any medical cause including photosensitivity. In one study 8 of 14 patients (57%) with melanoma exhibited an erythematous rash adjacent to and in the general vicinity of the melanoma that was confluent with the surrounding tissue and had no distinguishable borders.⁸ These authors labeled the rash Brenner sign (Figure 1).

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In an effort to identify visible signs that can alert the clinician to the possible presence of melanoma, we investigated the expression of 2 cytokines secreted by melanoma cells—vascular endothelial growth factor (VEGF) and platelet-derived endothelial cell growth factor (PDGF)—and their role in the pathogenesis of the rash.

Methods

Patients

All biopsy specimens were obtained from the tissue files of 13 patients in the Pathology Department, Tel Aviv-Sourasky Medical Center, Tel Aviv, Israel. The study was approved by the...
hospital ethics committee. The formalin-fixed, paraffin-embedded melanocytic lesions represented 13 primary melanomas. One group of patients with melanoma who had the erythematous rash called Brenner sign included 6 patients (4 men and 2 women), with an age range of 31 to 86 years, and a Breslow level range of 0.1 to 10 mm. The group of patients with melanoma without the Brenner sign consisted of 7 patients (3 men and 4 women), with an age range of 43 to 74 years, and a Breslow level range of 0.9 to 23 mm. There was no difference between the 2 groups in the distribution of subtypes of melanoma or in the outcome.

Immunoreactivity to VEGF and PDGF was evaluated by light microscopy following immunochemical staining. Ten high-power fields were viewed for each sample and graded as follows: negative, no cells stained; low, 1% to 10% cells stained; moderate, 15% to 30% cells stained; and high, 40% or more cells stained.

**IMMUNOHISTOCHEMISTRY**

Formalin-fixed, paraffin-embedded tissues were stained by an automated immunostainer (Nexes-Immunohistochemistry; Ventana Medical Systems, Tuscon, Arizona), after which they were deparaffinized and subjected to a 3-step indirect process based on the labeled (strept) avidin-biotin (LABORATORY-SA) peroxidase complex method, using the I-VIEW detection kit (Ventana Medical Systems). Sections were pretreated with buffer (pH 6.0, Target Retrieval; Zymed, San Francisco, California) for 12 minutes at 97°C by temperature-controlled microwave treatment using a processor (model H28900; Energy Beam Sciences, Inc, Ayawa, Maine). The Ventana Medical System dispensed the primary antibodies; consecutive sections were incubated for 32 minutes with polyclonal antibodies: 1:90 dilution of VEGF (CT) clone (Z-CVF3: Zymed), and 1:50 dilution of PDGF-α CLONE: C-20 (Santa Cruz, California). 3,3′-Diaminobenzidine tetrahydrochloride, which produces a dark brown precipitate chromogen, was used to localize the immunoreactions. Slides were counterstained with hematoxylin, dehydrated, and coverslipped with a permanent mounting medium for microscopic examination.

**RESULTS**

Immunoreactivity for VEGF was positive (moderate and high staining grades) (Figure 2) in tissue specimens of 3 of 6 patients (50%) with melanoma and redness and in 4 of 7 patients (57%) with melanoma without redness. No VEGF was detected in any of the melanomas with a Breslow depth of 1 mm or less.

In contrast, immunoreactivity for PDGF was positive (moderate and high staining grades) (Figure 3) in tissue specimens of 6 of 6 patients (100%) with melanoma and redness and in 4 of 7 patients (57%) with melanoma without redness. There was no difference between the 2 groups in Breslow depth.

**COMMENT**

An erythematous rash accompanying malignant melanoma lesions, confined mainly to the face, was first described by Brenner and Wolff and Tamir and Brenner. An erythematous rash accompanying malignant melanoma lesions, confined mainly to the face, was first described by Brenner and Wolff and Tamir and Brenner. Another study confirmed this observation, reporting the...
rash in 57% of patients with melanoma classified as stages I through III, and in none of the keratinocyte-derived tumor group or healthy individuals. Subject to further investigation and confirmation of the significance of this phenomenon, the rash could serve as an additional tool for the diagnosis of melanoma (asymmetry, border, color variegated, and diameter > 6 mm) as well as a measure of progression or regression of the disease. Explanation of this phenomenon probably lies in the pathogenesis of malignant melanoma. Melanoma cells can express cytokines and growth factors, having autocrine, paracrine, or both effects that permit autonomous growth of cells, including tumor cells as well as fibroblasts, monocytes, lymphocytes and granulocytes, keratinocytes, and endothelial cells.

Angiogenesis is a crucial component of growth, invasive progression, and metastatic spread of solid tumors, including melanoma. The interaction and reciprocal signaling between melanoma cells and endothelial cells are thought to guide the progression and growth of tumor cells. Melanoma cells produce a plethora of angiogenic cytokines, including VEGF, PDGF, and others. These cytokines are in constantly changing equilibrium with antiangiogenic agents.

Platelet-derived growth factors are a family of dimeric disulfide-bonded growth factors that activate tyrosine kinase receptors that are expressed on mesenchymal origin cells such as pericytes, fibroblasts, glial cells, and smooth muscle cells. Platelet-derived growth factors participate in the process of solid tumor development in several ways, including recruitment of tumor stroma fibroblasts, stimulation of tumor angiogenesis, autocrine stimulation of tumor cell growth, and paracrine stimulation of vascularized stroma growth.

Pericytes are formed in the connective tissue and remain there. These smooth muscle–like cells, smaller than fibroblasts, are located mainly along the blood capillaries and stabilize the capillary wall. Platelet-derived growth factors regulate embryonic pericyte recruitment. Neovessels in melanoma show perivascular PDGF-receptor staining of pericytes and possibly perivascular fibroblasts. Melanoma-derived PDGF is associated with enhanced tumor growth rate and increased coverage of tumor vessels with pericytes (not at late tumor growth phase), but with no increase in vessel density. This may reflect a pericyte-mediated protection from regression of immature vessels, because pericyte deficiency in mice causes endothelial hyperplasia, vessel dilation, vessel leakage, and rupture. The effect of PDGF on tumor vasculature is functional; it increases functionality of tumor vessels, decreases the fraction of nonperfused vessels, and promotes endothelial cell maturation through pericyte-derived signals. Our findings of positive PDGF immunoreactivity in tissue specimens of all 6 patients (100%) with melanoma and redness and in 4 of 7 patients (57%) without redness are in accord with the known activity of PDGF in melanoma. The erythema observed in cases of melanoma may reflect the higher percentage of mature endothelial cells and functional and perfused vessels.

Vascular endothelial growth factor is a glycosylated dimeric polypeptide that comprises a multifunctional cytokine expressed by most tumors. It acts on endothelial cells through tyrosine kinase receptors by being a selective endothelial cell mitogen and by causing an increase in microvessel permeability. Melanocytic tumors but not benign melanocytic proliferations display strong VEGF expression. Vascular endothelial growth factor is expressed in thicker melanoma, appears later in melanoma development, and correlates with the presence of metastases. It is also associated with an increase in vascular density. In our study the immunoreactivity for VEGF did not differ statistically between patients with melanoma with (50%) and without (57%) redness, indicating that the Brenner sign cannot be attributed to VEGF. Because VEGF is expressed in thick melanoma, no melanoma with a Breslow depth 1 mm or less was found to express VEGF.

Platelet-derived growth factors may have a role in the pathogenesis of the redness by affecting the function of the vasculature rather than its abundance. The number of patients is too small to draw definitive conclusions about these differences, but there seems to be a pattern that warrants further study. We continue to investigate other cytokines, angiogenic growth factors, and their promoters such as mitogen-activated protein kinase, and vascular leak promoters such as angiopoietin 2. We are also examining the red area for increased vascular density. While our search for the pathogenesis of Brenner sign continues, there is evidence that it could possibly serve as a red flag to primary care physicians, as another prognostic factor, or as a marker of the efficacy of treatment.

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REFERENCES


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