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Blood-Ocular Barrier Breakdown in Eyes with Ocular Melanoma

A Potential Role for Vascular Endothelial Growth Factor/ Vascular Permeability Factor

Stanley A. Vinores, Michael Küchle, Jeremy Mahlow, Charlie Chiu, W. Richard Green, and Peter A. Campochiaro

From the Wilmer Ophthalmologic Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland

A series of 130 eyes with ocular melanomas, 19 normal eyes, and 18 eyes affected with other disorders leading to blood-ocular barrier (BOB) breakdown were immunohistochemically stained for albumin to localize sites of BOB failure within the retina, ciliary body, and iris. Thirty-nine of the eyes containing melanomas and all of the other eyes were also immunohistochemically stained for vascular endothelial growth factor (VEGF), to investigate its potential role as a mediator for BOB failure. Eyes with melanomas showed widespread leakage through the retinal pigment epithelium, and 58% demonstrated leakage from retinal vessels in the proximity of the tumor. BOB failure remote from the tumor also occurred in retina (50%), optic nerve head (77%), ciliary body (51%), and iris (51%), suggesting that a soluble mediator may be involved. VEGF was demonstrated intraretinally in the proximity of (46%) and remote from (24%) melanomas and in eyes affected by other disease processes, particularly those involving neoplasia or retinal detachments, usually within particular cell populations (ie, retinal vessel walls, ganglion cells, inner or outer nuclear layers, retinal pigment epithelium). VEGF localization in retina, ciliary body, and iris often coincided with sites of extravasated albumin. Preincubation of albumin or VEGF antibodies with normal serum or VEGF peptide, respectively, eliminated or markedly reduced all immunoreactivity. Only 1 of 14 normal postmortem eyes and 0 of 5 normal surgically removed eyes showed VEGF positivity in the retina, 5 of 19 normal eyes had weak positivity in the ciliary body, and VEGF was not demonstrated in the iris of normal eyes. VEGF cannot account for all of the BOB failure associated with ocular melanomas, but appears likely to play a contributing role in many cases. (Am J Pathol 1995, 147:1289–1297)

The blood-retinal barrier (BRB), which is analogous to the blood-brain barrier, consists of an inner component made up of the retinal vascular endothelium and an outer component made up of the retinal pigment epithelium (RPE).[1,2] Leakage through either or both components can lead to macular edema, which is a major cause of visual loss. BRB breakdown leading to cystoid macular edema is known to occur in association with ocular melanomas,[3] even when the tumor is far removed from the posterior pole.[4] A small number of cases were previously examined using immunohistochemical staining for albumin and were found to demonstrate widespread BRB failure, suggesting involvement of a soluble mediator.[5,6] It is not known whether these cases also showed compromise of the blood-aqueous barrier, which is established at the iris and the ciliary body, but the same technique can be used to evaluate the integrity of the blood-aqueous barrier.[7] Vascular endothelial growth factor (VEGF) is secreted by various types of tumor cells,[8–16] and it can cause increased vascular permeability in addition to promoting endothelial cell growth, angiogenesis, and increased

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Address reprint requests to Stanley A. Vinores, Ph.D., 825 Maumenee Bldg., Department of Ophthalmology, Johns Hopkins University School of Medicine, 600 N. Wolfe St., Baltimore, MD 21287-9289.

Table 1. *Pathological Features of Ocular Melanoma Cases*

Pathological feature	Total cases	Cases evaluated for VEGF
Exudative retinal detachment	110/129 (85%)	30/34 (88%)
Cystoid retinal degeneration	94/125 (75%)	29/35 (83%)
Discontinuity of Bruch's membrane	76/130 (58%)	18/35 (51%)
Tumor invasiveness	74/129 (57%)	16/35 (46%)
Lymphocytic infiltrate in tumor	52/130 (40%)	14/35 (40%)
Tumor hemorrhage	41/130 (32%)	8/35 (23%)
Tumor necrosis	28/129 (22%)	7/34 (21%)
Angle closure glaucoma	14/130 (11%)	4/35 (11%)
Rubeosis iridis	5/129 (4%)	1/35 (3%)

Values are expressed as specimens demonstrating a particular feature/total specimens (percentage of specimens positive for each feature).

monocyte activation and chemotaxis.[17–26] In addition to BRB failure, angiogenesis[27,28] and lymphocytic infiltration may occur with ocular melanomas. VEGF is a good candidate for mediating these ocular effects; in this study, we investigated this hypothesis by performing immunohistochemical staining for VEGF in a large number of eyes containing choroidal melanomas in which we also assessed the integrity of the blood-ocular barrier (BOB) by immunohistochemical staining for albumin.

Materials and Methods

Pathological evaluation was performed on 130 eyes with ocular melanomas. Paraffin sections of these eyes, normal eyes removed at autopsy (14), normal eyes surgically removed for orbital tumors (5), and eyes affected by other disease processes known to lead to BRB breakdown (18) were immunohistochemically stained for albumin as previously described.[5,6,29] Thirty-nine of the eyes with melanomas and all of the other eyes were also immunostained for VEGF as follows using an affinity-purified rabbit polyclonal IgG directed against the 20 amino terminal residues of human VEGF (Santa Cruz Biotechnology, Santa Cruz, CA). These antibodies block VEGF activity[30] and specifically react with native and denatured (reduced) VEGF by Western blot.[31] Sections were deparaffinized as above and incubated with 10% normal goat serum (NGS) in 0.05 mol/L Tris-buffered saline (TBS), pH 7.6, for 30 minutes at room temperature. This solution was replaced with a 1:20 dilution of a 100 μg/ml solution of rabbit polyclonal anti-VEGF IgG in 1% NGS in TBS, and the slides were incubated overnight at 4°C in a humidified chamber. After warming to room temperature, the sections were washed with 1% NGS in TBS, incubated 30 minutes with a 1:50 dilution of goat anti-rabbit globulins (Arnel, Brooklyn, NY) in 1% NGS in TBS, washed again, and incubated for 30 minutes with a 1:100 dilution of a rabbit peroxidase-anti-peroxidase complex (Arnel) diluted in 1% NGS in TBS. Tissue sections were thoroughly washed with 0.05 mol/L Tris buffer, pH 7.6, and incubated with a freshly made and filtered solution of 0.38 mg/ml 4-chloro-1-napthol (Sigma Chemical Co., St. Louis, MO) containing 0.3% H_2O_2 and 1.5% ethanol, which was used to dissolve the 4-chloro-1-napthol. The sections were washed with Tris buffer and mounted with Aqua Poly/Mount (Polysciences, Warrington, PA). Immunoreacted sections were evaluated by two separate investigators for extent and localization of staining. Sites of albumin extravasation were compared with areas of VEGF positivity. Although it was impossible to ignore the presence of a melanoma within the ocular sections, the evaluation was otherwise performed without knowledge of the pathological report or other immunohistochemical data. For controls, the anti-VEGF IgG was preincubated for 2 hours at room temperature with a 10-fold excess of VEGF peptide (Santa Cruz Biotechnology). Specimens were determined to be positively stained if they were considered positive by both investigators and there was no staining in the same area on an adjacent control section. Micrographs were taken on a Zeiss Axioskop (Carl Zeiss, Thornwood, NY) using Normarski optics and Kodak T-64 tungsten film (Rochester, NY).

Results

Pathology

A series of 130 eyes with ocular melanomas were evaluated pathologically (Table 1). They were obtained from patients ranging in age from 17 to 91 years. The areas of the tumors on paraffin sections ranged from 4.5 to 240 mm^2. Thirty percent of the tumors were spindle B cell tumors, 9% were epithelioid tumors, and the remainder were composed of

Table 2. *Albumin Extravasation in Ocular Melanoma*

Site of extravasation	Number of specimens evaluated	Total showing BOB breakdown	Mild* albumin extravasation	Moderate** albumin extravasation	Widespread*** albumin extravasation
Outer BRB in proximity of tumor	128	127 (99%)	8 (6%)	10 (8%)	109 (85%)
Inner BRB in proximity of tumor	126	73 (58%)	27 (21%)	28 (22%)	18 (14%)
Outer BRB distal to tumor	126	71 (56%)	10 (8%)	11 (9%)	50 (40%)
Inner BRB distal to tumor	126	61 (48%)	15 (12%)	23 (18%)	23 (18%)
Optic nerve head	80	62 (77%)	19 (24%)	20 (25%)	23 (29%)
Internal to ciliary body epithelium	124	63 (51%)	50 (40%)	11 (9%)	2 (2%)
Iris stroma	125	125 (100%)	25 (20%)	60 (48%)	40 (32%)
Anterior iris	124	63 (51%)	48 (39%)	14 (11%)	1 (1%)
Anterior chamber	125	22 (18%)	16 (13%)	3 (2%)	3 (2%)

*Isolated foci of weak positivity. **Larger areas showing somewhat stronger positive staining. ***The majority of the region in question demonstrates extravascular albumin with moderate to intense staining intensity. Numbers in parentheses represent percentage of total specimens fulfilling the criteria of the particular category.

mixtures of these two cell types with the epithelioid component ranging from 0.15% to 90% of the tumor cell population.

Blood-ocular Barriers

BRB breakdown, demonstrated by the immunolocalization of extravascular albumin, was apparent to varying degrees in all eyes containing melanoma (Table 2). All but one eye showed outer BRB breakdown in the proximity of the tumor, with 85% of eyes showing widespread leakage; 58% also showed inner BRB breakdown in the vicinity of the tumor. In 12 of the eyes demonstrating inner BRB failure, the albumin leakage was confined to the deep capillary bed, and in four eyes it was confined to the superficial capillary bed. Of the eyes with ocular melanomas, 56% showed outer BRB compromise in areas remote from the tumor, with most of the positive cases having widespread leakage (Figure 1a). Forty-eight percent of eyes had inner BRB breakdown in areas remote from the tumor, and in 30 of 61, the leakage was confined to a single capillary bed (deep capillary bed in 24 eyes and superficial capillary bed in 6 eyes). Twenty-seven percent of eyes showed more extensive inner BRB breakdown in the proximity of the tumor (as expected), 20% of eyes showed equivalent amounts of inner BRB compromise in the proximity of the tumor and remote from the tumor, and, surprisingly, in 22% of eyes the inner BRB failure was more conspicuous in areas remote from the tumor; no inner BRB breakdown was evident in 31% of eyes.

In the majority of eyes (Table 2), vascular leakage of albumin was also seen in the optic nerve head (77%) and internal to the ciliary body epithelium (51%). Albumin staining was demonstrated in the iris stroma of all eyes with ocular melanoma, and it extended across the anterior border in 51% of the eyes. Albumin positivity was observed in the anterior chamber of 18%.

In normal eyes, except for occasional minor foci of extravascular albumin positivity, albumin was contained in the choroid and within the vessel lumens in the retina. Albumin positivity was observed in the iris stroma of all eyes, but it was not demonstrated within or adjacent to the ciliary epithelium or the iris pigment epithelium in any case. Preincubation of the anti-albumin antibodies with normal serum eliminated or markedly reduced the immunostaining.

VEGF

Of the 130 eyes with ocular melanoma that were evaluated for BOB integrity, 39 were immunohistochemically stained for VEGF. The pathological features of this group were representative of the entire collection of specimens (Table 1). VEGF was demonstrated in the retina on the same side as the tumor in 46% of eyes containing melanomas, with 27% showing inner retinal positivity and 27% showing positivity in the outer retina or RPE (Table 3). VEGF was also demonstrated in the retina on the opposite side from the tumor, but in a lower percentage of eyes (Table 3). Sometimes immunoreactive VEGF was seen diffusely throughout the retina, but usually it was localized within particular cell types or certain layers of the retina. For example, in different eyes, VEGF was visualized within vessel walls in the inner retina, in photoreceptors (Figures 1c, 2b), in gan-

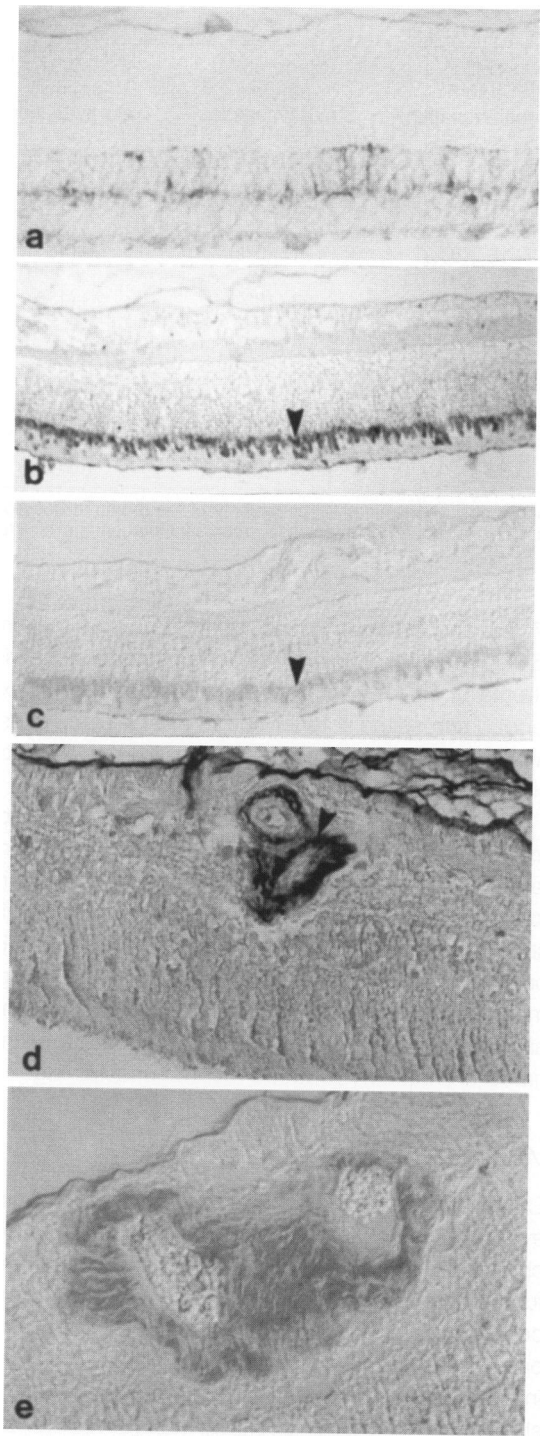

Figure 1. *Immunoperoxidase staining of paraffin sections of human eyes. Sections are not counterstained.* (a) *Extravascular albumin is demonstrated immunohistochemically in the outer retina* (bottom) *on the opposite side of the eye from an ocular melanoma, suggesting outer BRB breakdown.* ×250. (b) *Albumin positivity is localized to the photoreceptor layer* (arrowhead) *on the opposite side of the eye from an ocular melanoma.* ×125. (c) *Another section from the same case* (*compare to* b) *shows that VEGF immunoreactivity is also evident in the photoreceptor layer* (arrowhead) *on the opposite side of the eye from an ocular melanoma.* ×125. (d) *Albumin is seen permeating the walls of a retinal vessel* (arrowhead) *in the eye of a patient with retinoblastoma.* ×125. (e) *VEGF immunoreactivity is also present in retinal vascular walls in the same case* (*compare to* d). ×250.

glion cells (Figure 2f), or within RPE cells (Figure 2i). Its localization coincided with sites of extravasated albumin at the outer BRB in 34% and at the inner BRB in 43% of the cases (compare Figures 1, b and c; 2, a and b, d and f, h and i). The incidence of co-localization of VEGF with extravasated albumin was statistically significant within the retina (P = 0.0069) based on the Fisher's exact χ^2 test.

The ciliary body was positive for VEGF on the tumor side in 53% of eyes with melanoma, and the iris was positive in 21% (Table 3). There was little or no discrepancy in VEGF positivity in ciliary body or iris when comparing both sides of the eye with reference to tumor location. VEGF positivity did not appear to correlate with any of the specific pathological features described in Table 1 or with tumor size, but as in the retina, VEGF immunoreactivity in the ciliary body coincided with sites of BOB breakdown in 45% of the cases (Figure 3, a and b). In all eyes with VEGF positivity in the iris, there was severe or moderate albumin leakage at the same site (Figure 3, e and f); eyes with mild albumin leakage in the iris were consistently VEGF-negative. VEGF staining within the tumors was inconsistent, with only 26% showing positivity within the tumor and 51% in the periphery of the tumor. When staining was observed within the tumor, it was usually seen in areas of necrosis, particularly in tumor vessels and the adjacent stroma. Occasionally, a few tumor cells were positive for VEGF. In the periphery of the tumors, VEGF was frequently detected in the exudate.

Preincubation of the anti-VEGF antibodies with VEGF peptide eliminated or markedly reduced the immunostaining at all locations (Figures 2, c and g, and 3c).

In normal eyes removed at autopsy, only 1 of 14 had weak VEGF positivity in the retina, and 3 of 14 demonstrated weak VEGF staining in the ciliary body (Table 3). In contrast to the eyes with melanomas, which often showed extensive and intense staining, the normal eyes that demonstrated VEGF showed only weak immunoreactivity. Since the eyes affected with melanoma were surgically removed, we felt that surgically removed normal eyes would be more appropriate controls. We therefore, examined eyes that were surgically removed because of orbital tumors and found that the retinas were all negative for VEGF (Figure 2e). The ciliary body in two of five eyes was weakly stained for VEGF with the other cases being negative (Figure 3d). VEGF was not demonstrated in the iris or RPE of any of the control eyes.

Intraocular VEGF immunoreactivity was not uniquely associated with melanomas, but was also seen in the retina, ciliary body, and iris of eyes with

Table 3. *VEGF Immunoreactivity*

Diagnosis	Inner retina	RPE/outer retina	Retina (total)	Ciliary body	Iris
Melanoma (tumor side)	10/37 (27%)	10/37 (27%)	17/37 (46%)	20/38 (53%)	8/38 (21%)
Melanoma (opposite side from tumor)	4/37 (11%)	5/37 (14%) (1 weak)	9/37 (24%) (1 weak)	16/37 (43%) (2 weak)	8/37 (22%) (2 weak)
Normal eyes removed at autopsy	1/14 (7%) (Weak)	1/14 (7%) (Weak)	1/14 (7%) (Weak)	3/14 (21%) (All weak)	0/14 (0%)
Normal eyes removed surgically for orbital tumors	0/5(0%)	0/5 (0%)	0/5 (0%)	2/5 (40%) (Both weak)	0/5 (0%)
Other disorders (listed below)	6/18 (33%)	3/18 (17%)	8/18 (44%)	9/18 (50%)	4/17 (24%)
Retinoblastoma	1 /1 (Vessel walls)	1 /1 (Outer nuclear layer)	1 /1	1 /1	0 /1
Leukemia	2 /2	2 /2 (Photoreceptors)	2 /2	2 /2	1 /2
Keratoplasty/corneal ulcer	1 /1 (Ganglion cells, inner nuclear layer, outer plexiform layer)	0 /1	1 /1	0 /1	0 /1
Keratoplasty (2 weeks post surgery)	0 /1	0 /1	0 /1	1 /1	1 /1 (intense)
Cataract extraction/retinal detachment	1 /1	0 /1	1 /1	0 /1	0 /1
Retinal detachment	1 /1 (Exudate/cystic fluid)	0 /1	1 /1	1 /1	0 /1
Trauma	0 /1	0 /1	0 /1	1 /1 (1 side only)	0 /1
Cystoid macular edema associated with schisis	1 /1	0 /1	1 /1	1 /1 (Weak)	
Uveitis	0 /1	0 /1 (Pars plana is positive)	0 /1	1 /1 (Weak)	1 /1 (Weak)
Retinal phlebitis	0 /1	0 /1	0 /1	1 /1	0 /1
Central retinal artery occlusion	0 /1	0 /1	0 /1	0 /1	0 /1
Central retinal vein occlusion	0 /1	1 /1 (RPE)	0 /1	0 /1	1 /1 (1 side weak, other negative)
Arterial microaneurrysm	0 /1	0 /1	0 /1		
Hypotensive retinopathy	0 /1	0 /1	0 /1	0 /1	0 /1
Parafoveal telangiectasia	0 /1	0 /1	0 /1		

Values are expressed as specimens demonstrating positivity/total specimens (percentage of positive specimens).

Specimens that showed particularly weak labeling are noted. All other positively stained specimens are moderately or intensely labeled. Representative examples of weak staining are shown in Figures 1c and 2f. Examples of intense staining are illustrated in Figures 1d, 2h, and 2i. Prominently stained cell types or locations are also noted. Sections in which a particular structure was absent or destroyed by the tumor were not counted.

other disorders, particularly with those involving neoplasia (Table 3) and, as with ocular melanomas, its localization often coincided with sites of albumin extravasation (Figure 1, d and e).

Discussion

Previous studies using relatively small sample sizes have demonstrated breakdown of the BRB, which was often widespread, and albumin leakage through the optic nerve head associated with ocular melanomas.[5,6] Blood-aqueous barrier breakdown has also previously been quantitated, but not localized, in patients with ocular melanomas.[32] The present study has corroborated these findings in a large number of well characterized cases and has shown that blood-aqueous barrier breakdown frequently occurs in the ciliary body and the iris in many of these cases. BOB failure occurs not only at sites where structural damage inflicted by tumor growth could account for the leakage and subsequent edema, but also at sites far removed from the tumor suggesting that a soluble mediator or mediators

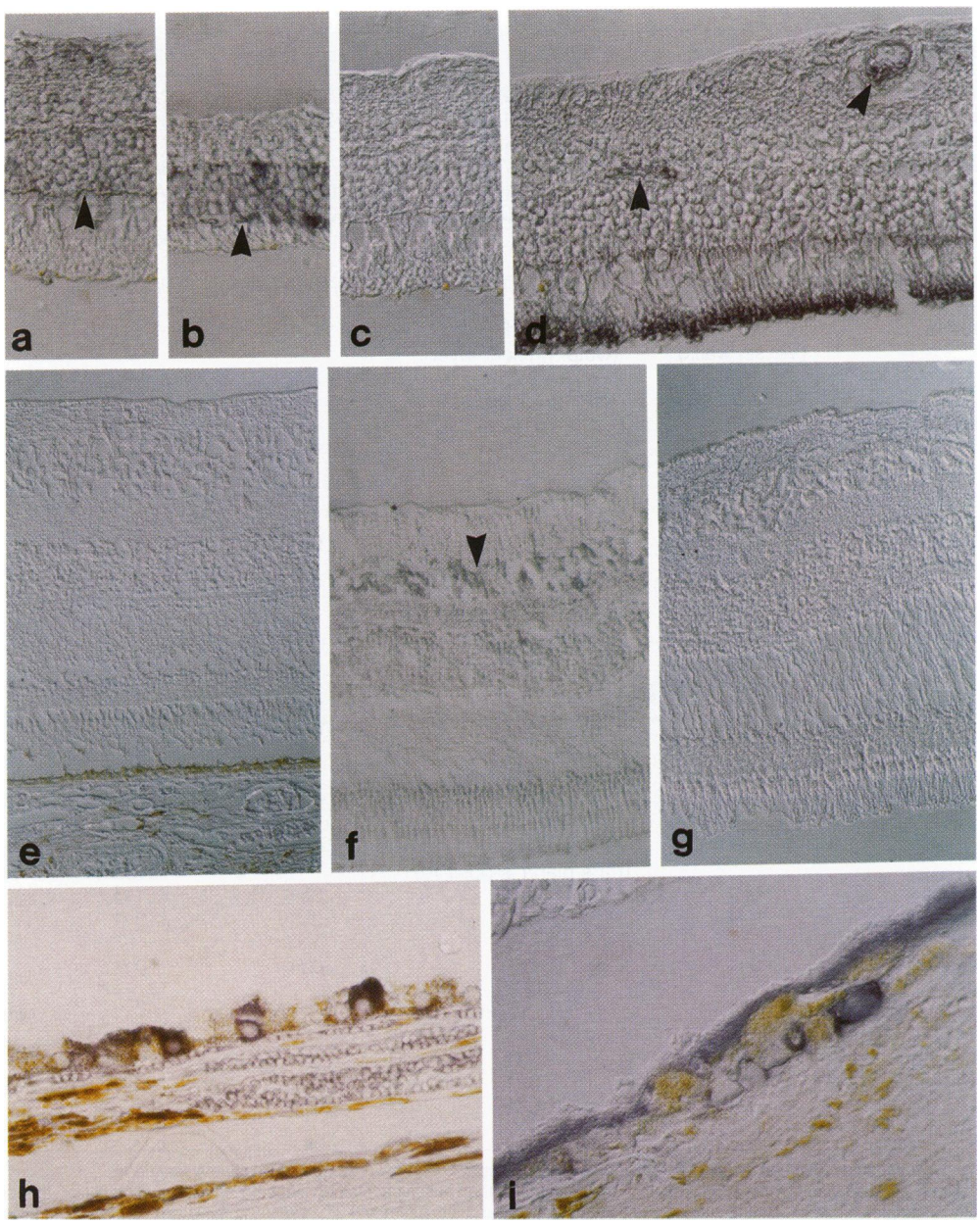

Figure 2. *Immunoperoxidase staining of paraffin sections of a normal human eye* (e) *or human eyes with ocular melanoma to yield a blue-violet reaction product. Sections are not counterstained.* (a) *Staining for albumin to localize BRB breakdown sites shows extravasated albumin predominantly within the outer nuclear layer* (arrowhead). ×250. (b) *Another section from the same case (compare to* a*) shows that immunoreactivity for VEGF is also localized to the outer nuclear layer* (arrowhead). ×250. (c) *Preincubation of the VEGF antiserum with the control peptide eliminates VEGF immunoreactivity in the same case (compare to* b*).* ×250. (d) *Albumin staining is demonstrated within retinal vessels* (arrowheads)*, and weak positivity is seen around these vessels, suggesting vascular leakage. Prominent staining is also evident in the outer retina* (bottom)*, suggesting outer BRB failure.* ×250. (e) *Normal human eye enucleated because of an orbital tumor is negative for VEGF (compare to* b, f, *and* i*).* ×125. (f) *VEGF immunoreactivity is demonstrated in ganglion cells in the same case illustrated in* d*. Ganglion cell layer is designated by arrowhead.* ×125. (g) *Preincubation of the VEGF antiserum with the control peptide eliminates VEGF staining in the same case (compare to* f*).* ×125. (h) *Cytoplasmic positivity for albumin is demonstrated in RPE cells on the opposite side of the eye from the tumor showing permeation of some RPE cells by albumin. The brown color represents melanin.* ×250. (i) *VEGF immunoreactivity in RPE cells directly over the tumor. The brown color represents melanin.* ×250.

may be responsible for the BOB breakdown. The diffuse and frequently widespread BOB failure often seen with ocular melanomas supports this hypothesis.

The present study has also demonstrated a marked increase in staining for VEGF in eyes with melanomas compared with control eyes, that showed little or no staining. This is consistent with studies that have demonstrated increased expression of VEGF in association with several types of tumors,[8,10,12,15,16,19,20,22,25] and its mRNA has been

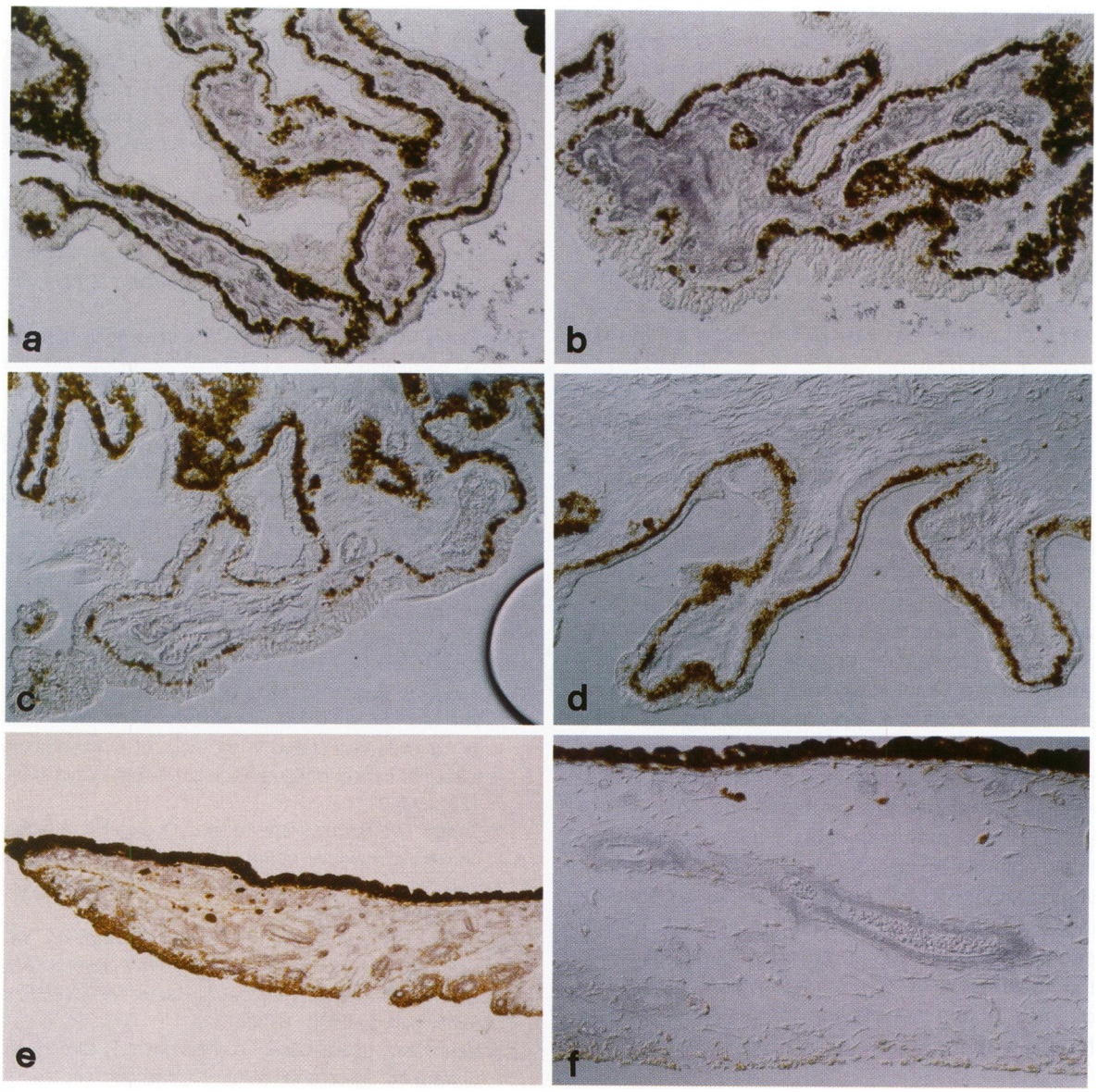

Figure 3. *Immunoperoxidase staining of paraffin sections of a normal human eye* (d) *or human eyes with ocular melanoma to yield a blue-violet reaction product. The brown color represents melanin. Sections are not counterstained.* (a) *Extravascular albumin is demonstrated in the ciliary body.* ×*125.* (b) *VEGF immunoreactivity is visualized in the ciliary body from the same case.* ×*125.* (c) *Preincubation of the VEGF antiserum with control peptide eliminates VEGF immunostaining in the same case.* ×*125.* (d) *The ciliary body of a normal eye enucleated because of an orbital tumor is negative for VEGF.* ×*125.* (e) *Albumin is demonstrated in the vessel walls of the iris showing BOB breakdown.* ×*62.5.* (f) *VEGF is also present in the vessel walls of the iris in the same case.* ×*125.*

demonstrated in intracerebral melanoma cells.[33] VEGF expression can be stimulated by hypoxia,[34–37] and it may promote angiogenesis and vascular hyperpermeability that occurs in association with tumors.[20,21,24] Hypoxic conditions may arise because retinal detachment or occlusion of the choriocapillaris secondary to compression by melanoma cells may restrict access of the overlying retina to the choroidal circulation, resulting in ischemia of the outer retina. The retinal vascular endothelial cell proliferation and BRB breakdown associated with ocular melanomas[28] is consistent with a role for VEGF. The co-localization of VEGF and extravasated albumin in many eyes with ocular melanoma suggests that VEGF augments the leakage of serum proteins at these sites; however, co-localization of VEGF and extravascular albumin is not evident in all cases, raising the possibility that VEGF is but one of several factors that mediate BOB failure. An alternative possibility is the levels of VEGF in the remainder of cases are below the threshold of immunohistochemical detectability.

VEGF was immunolocalized not only within the tumor (a likely source) and surrounding leaky vessels in the retina, ciliary body, and iris (the presumed sites of action), as it was in leaky vessels within tumors,[37–40] but also within ganglion cells, photoreceptors, and RPE cells of eyes with melanomas, often staining more intensely in the latter cell types. This raises the possibility that the tumor may not be the only source of VEGF, but it may release other factors that induce VEGF expression in retinal cells. Transforming growth factor-β,[13] prostaglandins E_1 and E_2,[41] and interleukin-1β[42] are factors that may be produced by tumor cells or in response to tumor cells, and have been shown to induce VEGF production in other cell types. RPE cells[43] and cells in the inner nuclear layer[44] have been demonstrated to synthesize VEGF. VEGF may also stimulate the recruitment and activation of monocytes,[22] which in turn could secrete other mediators of BOB compromise. Immunohistochemistry, however, cannot discriminate between protein synthesized and protein taken up by immunoreactive cells. *In situ* hybridization would resolve this question, but the tissue available for the present study was paraffin-embedded, and severe limitations apply in attempting to identify mRNAs for proteins likely to be present in low concentrations, such as secreted growth factors. Dexamethasone inhibits the synthesis and secretion of VEGF[8,41] and may help reduce macular edema in these and related disorders where VEGF plays a role in mediating BOB compromise. A better understanding of the factors contributing to macular edema could lead to the development of antagonists and the implementation of pharmacological intervention directed at the appropriate targets to prevent or reduce macular edema.

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References

1. Cunha-Vaz JG: The blood-retinal barriers. Doc Ophthalmol 1976, 41:287–327
2. Raviola G: The structural basis of the blood-ocular barriers. Exp Eye Res 1977, 25(Suppl):27–63
3. Kincaid MC, Green WR: Pathology of choroidal melanoma. Retina, vol 1, ed 2. Edited by SJ Ryan. St. Louis, Mosby, 1994, pp 753–761
4. Brownstein S, Orton R, Jackson WB: Cystoid macular edema with equatorial choroidal melanoma. Arch Ophthalmol 1978, 96:2105–2107
5. Vinores SA, Campochiaro PA, Lee A, McGehee R, Gadegbeku C, Green WR: Localization of blood-retinal barrier breakdown in human pathologic specimens by immunohistochemical staining for albumin. Lab Invest 1990, 62:742–750
6. Vinores SA, Amin A, Derevjanik NL, Green WR, Campochiaro PA: Immunohistochemical localization of blood-retinal barrier breakdown sites associated with post-surgical macular oedema. Histochem J 1994, 26: 655–665
7. Küchle M, Vinores SA, Green WR: Immunohistochemical evaluation of the integrity of the blood-aqueous barrier in normal and rubeotic human eyes. Graefe's Arch Clin Exp Ophthalmol 1995, 233:414–420
8. Bruce JN, Criscuolo GR, Merrill MJ, Moquin RR, Blacklock JB, Oldfield EH: Vascular permeability induced by protein product of malignant brain tumors: inhibition by dexamethasone. J Neurosurg 1987, 67:880–884
9. Kondo S, Asano M, Suzuki H: Significance of vascular endothelial growth factor/vascular permeability factor for solid tumor growth, and its inhibition by the antibody. Biochem Biophys Res Commun 1993, 194: 1234–1241
10. Kondo S, Asano M, Matsuo K, Ohmori I, Suzuki H: Vascular endothelial growth factor/vascular permeability factor is detectable in the sera of tumor-bearing mice and cancer patients. Biochim Biophys Acta 1994, 1221:211–214
11. Senger DR, Perruzzi CA, Feder J, Dvorak HF: A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. Cancer Res 1986, 46:5629–5632
12. Senger DR, Van De Water L, Brown LF, Nagy JA, Yeo K-T, Yeo T-K, Berse B, Jackman RW, Dvorak AM, Dvorak HF: Vascular permeability factor (VPF, VEGF) in tumor biology 1993, 12:303–324
13. Pertovaara L, Kaipainen A, Mustonen T, Orpana A, Ferrara N, Saksela O, Alitalo K: Vascular endothelial growth factor is induced in response to transforming growth factor-β in fibroblastic and epithelial cells. J Biol Chem 1994, 6271–6274
14. Goto F, Goto K, Weindel K, Folkman J: Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagen gels. Lab Invest 1993, 69:508–517
15. Brock TA, Dvorak HF, Senger DR: Tumor-secreted vascular permeability factor increases cytosolic Ca^{2+} and von Willebrand factor release in human endothelial cells. Am J Pathol 1991, 138:213–221
16. Weindel K, Moringlane JR, Marmé, Weich HA: Detection and quantification of vascular endothelial growth factor/vascular permeability factor in brain tumor tissue and cyst fluid: the key to angiogenesis? Neurosurgery 1994, 35:439–449
17. Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder

J, Connolly DT: Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 1989, 246:1309–1312
18. Leung DW, Cachianes G, Kuang W-J, Goeddel DV, Ferrara N: Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989, 246:1306–1309
19. Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leimgruber RM, Feder J: Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989, 84:1470–1478
20. Connolly DT, Olander JV, Heuvelman D, Nelson R, Monsell R, Siegel N, Haymore BL, Leimgruber R, Feder J: Human vascular permeability factor. J Biol Chem 1989, 264:20017–20024
21. Connolly DT: Vascular permeability factor: a unique regulator of blood vessel function. J Cell Biochem 1991, 47:219–223
22. Clauss M, Gerlach M, Gerlach H, Brett J, Wang F, Familletti PC, Pan Y-CE, Olander JV, Connolly DT, Stern D: Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. J Exp Med 1990, 172:1535–1545
23. Callow AD, Choi ET, Trachtenberg JD, Stevens SL, Connolly DT, Rodi C, Ryan US: Vascular permeability factor accelerates endothelial regrowth following balloon angioplasty. Growth Factors 1994, 10:223–228
24. McClure N, Healy DL, Rogers PAW, Sullivan J, Beaton L, Haning RV, Connolly DT, Robertson DM: Vascular endothelial growth factor as capillary permeability agent in ovarian hyperstimulation syndrome. Lancet 1994, 344:235–236
25. Berse B, Brown LF, Van De Water L, Dvorak HF, Senger DR: Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. Mol Biol Cell 1992, 3:211–220
26. Senger DR, Connolly DT, Van De Water L, Feder J, Dvorak HF: Purification and NH_2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. Cancer Res 1990, 50:1774–1778
27. Vine AK: Choroidal melanoma with disc and retinal neovascularization. Retina 1983, 3:118–120
28. Michael JC, deVenecia G: Retinal trypsin digest study of cystoid macular edema associated with peripheral choroidal melanoma. Am J Ophthalmol 1995, 119:152–156
29. Vinores SA, Gadegbeku C, Campochiaro PA, Green WR: Immunohistochemical localization of blood-retinal barrier breakdown in human diabetics. Am J Pathol 1989, 134:231–235
30. Sioussat TM, Dvorak HF, Brock TA, Senger DR: Inhibition of vascular permeability factor (vascular endothelial growth factor) with antipeptide antibodies. Arch Biochem Biophys 1993, 301:15–20
31. Santa Cruz Biotechnology, Inc.: Research Product Catalogue. Santa Cruz, CA, 1994, p 77
32. Küchle M, Nguyen NX, Naumann GOH: Quantitative assessment of the blood-aqueous barrier in human eyes with malignant or benign uveal tumors. Am J Ophthalmol 1994, 117:521–528
33. Hatva E, Karpainen A, Mentula P, Jääskeläinen K. Paetau A, Haltia M, Alitalo K: Expression of endothelial cell-specific receptor tyrosine kinases and growth factors in human brain tumors. Am J Pathol 1995, 146: 368–378
34. Goldberg MA, Schneider TJ: Similarities between the oxygen-sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. J Biol Chem 1994, 4355–4359
35. Minchenko A, Bauer T, Salceda S, Caro J: Hypoxic stimulation of vascular endothelial growth factor expression *in vitro* and *in vivo*. Lab Invest 1994, 71:374–379
36. Minchenko A, Salceda S, Bauer T, Caro J: Hypoxia regulatory elements of the human vascular endothelial growth factor gene. Cell Mol Biol Res 1994, 40:35–39
37. Shweiki D, Itin A, Soffer D, Keshet E: Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992, 359:843–845
38. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Dvorak HF, Senger DR: Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. Am J Pathol 1993, 143:1255–1262
39. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Senger DR, Dvorak HF: Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. Cancer Res 1993, 53:4727–4735
40. Dvorak HF, Sioussat TM, Brown LF, Berse B, Nagy JA, Sotrel A, Manseau EJ, Van deWater L, Senger DR: Distribution of vascular permeability factor (vascular endothelial growth factor) in tumors: concentration in tumor blood vessels. J Exp Med 1991, 174:1275–1278
41. Harada S, Nagy JA, Sullivan KA, Thomas KA, Endo N, Rodan GA, Rodan SB: Induction of vascular endothelial growth factor expression by prostaglandin E(2) and E(1) in osteoblasts. J Clin Invest 1994, 93:2490–2496
42. Li J, Perrella MA, Tsai J-C, Yet S-F, Hsieh C-M, Yoshizumi M, Patterson C, Endege WO, Zhou F, Lee M-E: Inducton of vascular endothelial growth factor gene expression by interleukin-1β in rat aortic smooth muscle cells. J Biol Chem 1995, 279:308–312
43. Adamis AP, Shima DT, Yeo K-T, Yeo T-K, Brown LF, Berse B, D'Amore PA, Folkman J: Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. Biochem Biophys Res Commun 1993, 193:631–638
44. Miller JW, Adamis AP, Shima DT, D'Amore PA, Moulton RS, O'Reilly MS, Folkman J, Dvorak HF, Brown LF, Berse B, Yeo T-K, Yeo K-T: Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. Am J Pathol 1994, 145:574–584