THE MICROCIRCULATION OF CHOROIDAL AND CILIARY BODY MELANOMAS

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SUMMARY

The microcirculation of ciliary body and choroidal melanomas is remodelled into patterns. The presence of microvascular networks, composed of back-to-back loops that encircle microdomains of tumour, and parallel vessels with cross-linking, are associated with death from metastatic melanoma. The formation of these complex vascular patterns may result from reciprocal interactions between the tumour cell and the extracellular matrix, and pattern formation may reflect an invasive tumour cell phenotype. Ciliary body and choroidal melanomas are among the few forms of cancer treated before a pathologist assigns a grade to indicate whether tumour is likely to follow a benign or aggressive course. There is evidence to suggest that prognostically significant microcirculatory patterns may be detectable by non-invasive imaging techniques that may provide a substitute for biopsy to guide the clinical management of patients with these sight- and life-threatening tumours.

CILIARY BODY AND CHOROIDAL MELANOMAS: CONSIDERING BOTH CYTOLOGY AND ARCHITECTURE

Since Callender's description of cell types in uveal melanoma,[1] ophthalmic pathologists have been preoccupied with the relationship between the appearance of neoplastic melanocytes and the outcome of patients with melanomas of the iris, ciliary body and choroid.[2–11] Studies describing cytological pleomorphism quantitatively by measuring the standard deviation of nucleolar area[10,12–14] concentrated focus on the tumour cell even further.

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Tumour cells interact dynamically with the extracellular matrix and these reactions may be reciprocal, with tumour cells influencing the formation of the matrix and the matrix influencing tumour cell behaviour.[15,16] These progressive and reciprocal interactions are responsible for the generation of tumour stroma.[17] To study the cytology of a neoplasm and ignore the tumour stroma is analogous to considering the wall of a building as a collection of bricks without acknowledging the contribution of the mortar.

In this discussion, attention is focused on the microcirculatory component of the stroma of ciliary body and choroidal melanomas.

THE FORMATION OF PATTERNS IN CILIARY BODY AND CHOROIDAL MELANOMAS: ANGIOGENESIS AND VASCULAR REMODELLING

Angiogenesis plays a particularly important role in the biology of uveal melanomas: choroidal and ciliary body melanomas can only disseminate haematogenously because there are no lymphatics within the eye. However, angiogenesis involves two processes: the *production* of new blood vessels and *remodelling* of the new vascular bed.[18]

Considerable attention has been focused on the prognostic significance of the production of new vessels in the metastatic process[19,20] as pathologists have attempted to determine the relationship between the quantity of tumour vascularisation and outcome. In 1991, Weidner *et al.*[21] reported a significant relationship between the quantity of microvessels in histological sections of breast cancer and subsequent metastasis. For each tumour, the zone of maximum vascular density was identified at low magnification, the number of discrete vessels was counted in a predefined area, and a threshold count that separated patients at low risk from those at high

risk was established. High vascular density assessed from histological material has since been associated with a poor prognosis in some forms of cutaneous melanoma,[22–24] non-small-cell carcinoma of the lung,[25] prostatic[26,27] and bladder cancer,[28–30] nasopharyngeal cancer,[31] and gliomas.[32] The subject of vascular counts and prognosis was reviewed recently.[33]

Vessel counting from histological sections to establish prognosis does not hold for all forms of cancer.[34] Carnochan *et al.*[24] discovered that the density of tumour vessels did not correlate well with the outcome for certain cutaneous melanomas (a more recent study of cutaneous melanoma showed no relationship between vascular counts and outcome[35]), and suggested that 'assessment of other features of the tumour vasculature may provide a useful complement to simple morphometry'. Recently, Page and Jensen,[36] commenting on the failure of two teams of investigators to confirm the prognostic significance of the vessel-counting in breast cancer,[37,38] stated that 'intratumoral angiogenesis may be related more to continual remodelling and migration of vessels than to continual production of new vessels'.

MICROCIRCULATORY PATTERNS OF CHOROIDAL AND CILIARY BODY MELANOMAS

Tissue remodelling generates zones of regional specification or patterns.[39] The remodelling of the microcirculation of ciliary body and choroidal melanomas into patterns was demonstrated in histological sections.[40,41] In addition to normal vessels incorporated into the stroma of these tumours and focal avascular zones, these tumours contain straight vessels, parallel straight vessels, parallel vessels that cross-link, vascular arcs (incomplete loops), arcs with branching, closed vascular loops that encircle small clusters of tumour cells, and microvascular networks composed of back-to-back loops.[40] The detection of these patterns in histological sections is highly reproducible between observers.[41,42] These histological patterns are illustrated in Fig. 1.

MICROCIRCULATORY ARCHITECTURE AND METASTASIS FROM CHOROIDAL AND CILIARY BODY MELANOMAS

In a preliminary matched-pair case–control study of 40 patients with choroidal or ciliary body melanoma, the presence of vascular loops was associated with death from metastatic melanoma,[40] a finding con-

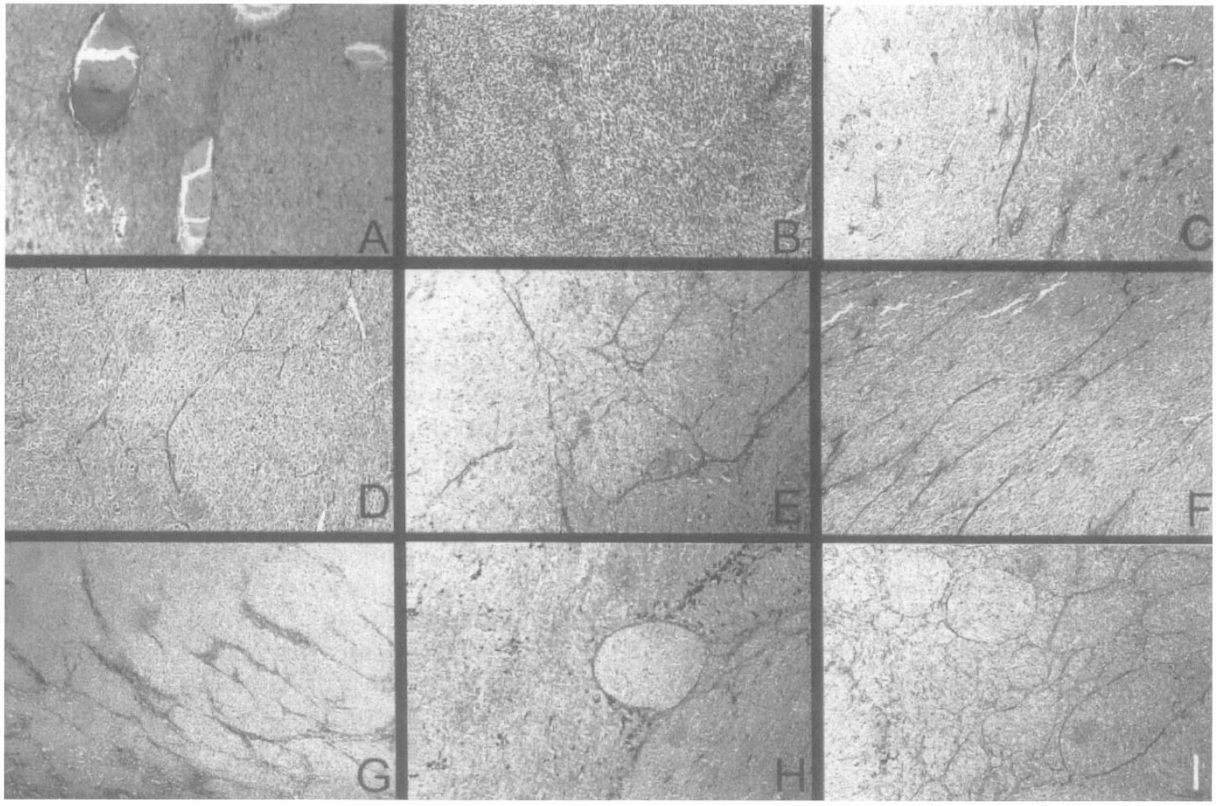


Fig. 1. *Microcirculatory patterns. (A) Tumour surrounds normal vessels. (B) Avascular zone. (C) Straight vessels. (D) Arcs. (E) Incomplete loops or arcs with branching. (F) Parallel vessels with one focus of cross-linking in the lower left corner. (G) Parallel vessels with cross-linking. (H) A vascular loop surrounds a focus of relatively amelanotic tumour just to the right of centre. (I) Networks composed of back-to-back vascular loops. Periodic acid–Schiff (PAS) stain without haematoxylin counterstaining, photographed through a green filter; original magnification ×25 for all figures.*

firmed recently by Sakamoto *et al.*[42] In a larger series of 234 patients, Kaplan–Meier survival curves generated from deaths due to metastatic melanoma indicated that at 10-year follow-up the survival of patients whose tumour lacked the vascular patterns of parallel vessels with cross-linking, loops and networks was significantly better (91.7%, 91.1% and 88.3%, respectively) than for patients whose tumours contained these patterns (56.9%, 55.4% and 50.7%; $p = 0.0001$ for all comparisons, $n = 234$). A Cox proportional hazards model was generated that permitted inclusion of the conventional prognostic factors (including the largest tumour dimension in contact with the sclera, cell type, tumour infiltrating lymphocytes, mitotic figures, gender, and location of the tumour within the eye) and the presence or absence of each of the nine microcirculatory patterns. The most important variable was the network pattern (chi-square = 40.84; $p = 0.0001$). Other significant factors in the model include (in descending order of importance) largest tumour dimension, mitoses, parallel with cross-linking vascular patterns, age, the presence of tumour-infiltrating lymphocytes, and male gender.[41] It was not surprising that loops did not appear in the final Cox model, because networks, the most significant variable in this model, are composed of back-to-back loops.[40]

MICROCIRCULATORY PATTERNS AS A MARKER OF TUMOUR PROGRESSION IN CHOROIDAL AND CILIARY BODY MELANOMAS

There are abundant descriptions of the precursors of cutaneous melanoma,[43] but ophthalmic pathologists traditionally recognise only naevi (including the melanocytoma naevus variant) and melanomas.[43] The existence of uveal melanocytic hyperplasia was advanced as a hypothesis,[44] but has never been demonstrated clinically or histologically in humans. There is no mention in the ophthalmic pathology literature of 'atypical melanocytic hyperplasia' in the choroidal, ciliary body or iris.[45]

To determine whether microcirculatory patterns could assist in refining the classification of uveal melanocytic neoplasms, the microcirculation architecture of choroidal and ciliary body naevi was examined. Tissue blocks from 23 naevi of the ciliary body or choroid were identified from the laboratories of the University of Iowa and the University of Erlangen-Nürnberg. A naevus was defined by the criteria of Naumann *et al.*[46,47] and excluded lesions that contained any spindle B or epithelioid cells. Rummelt *et al.*[48] discovered that choroidal and ciliary body naevi contain only four vascular patterns: normal vessels, avascular zones, straight vessels, and parallel vessels that do not cross-link. None of the naevi contained parallel vessels with cross-linking, arcs, arcs with branching, vascular loops or networks.

A study was designed to test the hypothesis that melanomas could be classified into prognostic groups on the basis of microcirculation architecture patterns.[48] Of the 234 melanomas in this dataset, 49 contained a naevus-like microcirculation and 185 contained vascular patterns not found in naevi. Melanomas *with* a naevus-like microcirculation tend to develop in the choroid posterior to the equator ($p = 0.0007$) and tend to be smaller ($p = 0.0001$) than melanomas *lacking* a naevus-like microcirculation. The mortality from cancer for patients whose tumours *lack* naevus-like microcirculation is 32.4% compared with tumours *with* a naevus-like microcirculation (14.3%, $p = 0.012$). Kaplan–Meier curves plotting the survival of patients whose melanomas had a naevus-like microcirculation versus those whose tumours contained non-naevus patterns revealed that at 15 years' follow-up the survival of patients with naevus-like melanomas is 84.8% versus 60.4% for patients whose tumours lacked a naevus-like microcirculation ($p = 0.0007$). In addition, the median interval between enucleation and death from metastatic melanoma for patients whose tumour had a naevus-like microcirculation is significantly longer (median 6.4 years; range 4.5–16.2 years) than for patients whose tumours featured microcirculatory patterns other than those seen in naevi (median 3.6 years; range 0.5–18 years; $p = 0.018$).

These findings suggested that there are at least three types of melanocytic lesions that develop in the choroid and ciliary body: naevi (which lack the capacity for metastasis and are entirely benign), melanomas with vascular networks (which are strongly associated with metastasis), and melanomas with a naevus-like microcirculation architecture (which have only a limited capacity for metastasis and which tend to have a prolonged survival to death from metastatic melanoma if metastases do develop). The microcirculation architecture, therefore, can be used to define steps in the pathway of tumour progression in choroidal and ciliary body melanoma.[48]

THE COMPOSITION OF MICROCIRCULATORY PATTERNS

Microvascular networks are not unique to uveal melanomas and have been described in a variety of tissue types and tumours.[49–56] The appearance of a microvascular network in tissue sections should not be interpreted, therefore, as an implausible event. Indeed, the presence of a PAS-positive 'chicken-wire' microcirculation is a histological characteristic of myxoid liposarcomas.[57]

The microcirculatory patterns of uveal melanoma were first demonstrated by a modification of the PAS

stain (without haematoxylin counterstaining). The magenta colour of the patterns could be augmented by inserting a green filter into the light path of the microscope to turn the appearance of these patterns black and thus highlight them for observation and photomicroscopy.[40] Not every structure that is PAS-positive in these tumours is part of the microcirculatory system, and pathologists who used this method were cautioned to avoid interpreting all PAS-positive structures as microcirculatory.[40,41]

It is relatively difficult to bleach entire tumour cross-sections that have been stained to demonstrate endothelial-specific markers with immunohistochemical techniques without losing fragments of tissue (especially at the periphery) or inducing tissue folds. The loss of tumour tissue or the presence of artefacts introduced during staining may interfere significantly with the detection of patterns. If one attempts to bleach sections before applying immunohistochemistry, there is a risk of losing antigenic binding sites. With the modified PAS stain, the microcirculation is demonstrated even after removal of melanin from highly pigmented lesions by routine permanganate bleaching.[40]

The claim that the modified PAS stain is an accurate approximation of the microcirculation[40] is based upon four lines of evidence: (*a*) networks have been traced in serial histological sections to vortex veins and the choriocapillaris (Fig. 2); (*b*) correlations have been established between PAS-positive patterns and microcirculatory patterns demonstrated by *Ulex europaeus* agglutinin I[40] by laser scanning confocal microscopy; (*c*) three-dimensional reconstructions of these patterns by computer-assisted laser scanning confocal microscopy confirm convincingly the vascular nature of these patterns[58] (Fig. 3); and (*d*) transmission electron microscopy has con-

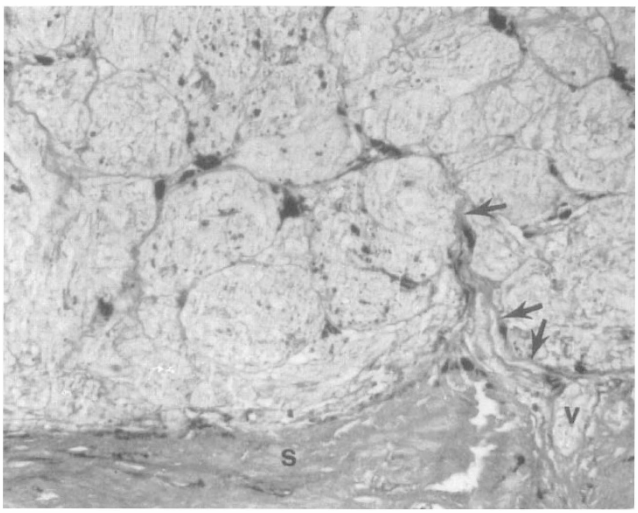
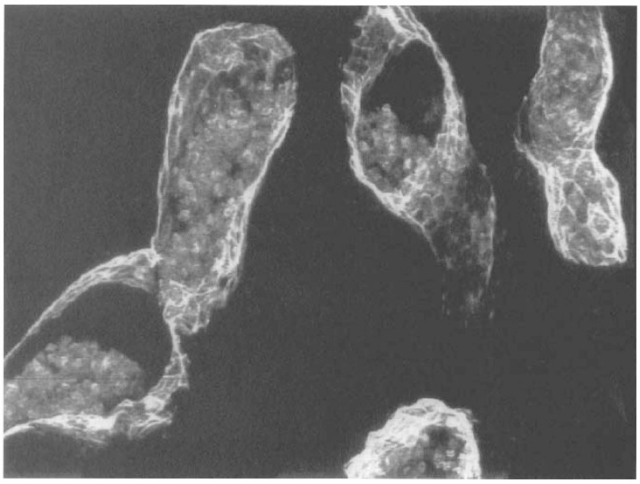
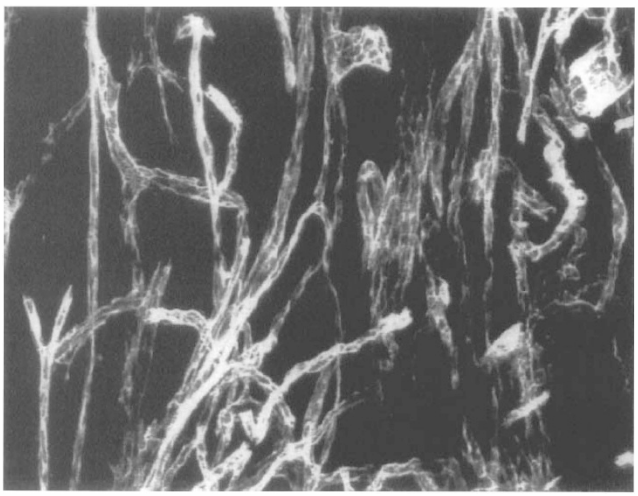


Fig. 2. *Microvascular networks are traced (arrows) to the vortex vein (v) in the lower right corner (s, sclera). PAS without haematoxylin counterstaining; original magnification ×50.*



(a)

(b)



(c)

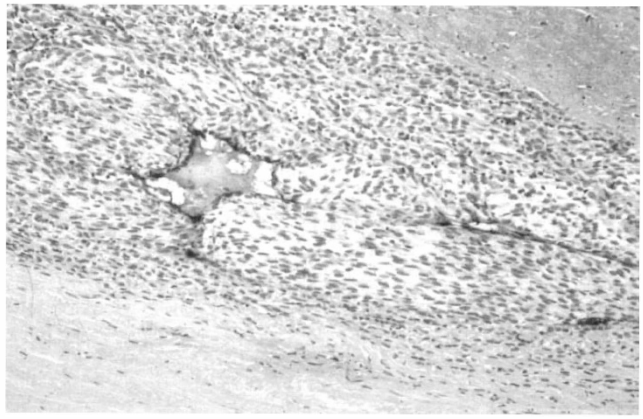
Fig. 3. *Three-dimensional reconstructions by laser-scanning confocal microscopy. (a) Red blood cells are identified in the cut edges of gaping normal vessels. (b) Parallel vessels with a focus of cross-linking (centre). (c) Note the back-to-back loops just left of centre and at the bottom.* Ulex europaeus *agglutinin I–FITC; original magnification ×20 (a) and ×10 (b, c).*

firmed the vascular nature of these structures, demonstrating thin-walled vessels often containing red blood cells in a rouleaux formation.[40,48]

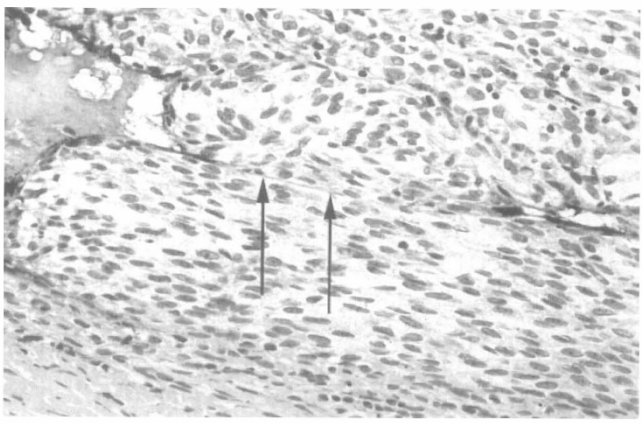
Additional ultrastructural studies of the microcirculation revealed interesting differences between normal choroidal microvessels, the microcirculation in naevi, and melanomas *with* and *without* a naevus-like microcirculation.[48] For example, there was a significant difference ($p = 0.0008$) in the thickness of the microvascular basement membranes in the normal choroid (median thickness 0.47 μm), naevi (median thickness 0.49 μm) and melanomas with a naevus-like microcirculation (median thickness 1.15 μm). In melanomas without a naevus-like microcirculation, there was a tendency for the microvascular basement membranes to be thicker than in vessels in the other three groups. Qualitatively, the basement membrane of vessels in normal eyes, naevi, and melanomas with naevus-like vascular patterns had no evidence of fragmentation, excessive collagen or a multilaminar construction. By comparison the microcirculation of melanomas without a naevus-like microcirculation featured multilaminar basement membrane, increased amounts of vascular-associated collagen, and basement membrane fragmentation. Interendothelial junctions were preserved in normal eyes, naevi, and melanomas with naevus-like microcirculatory patterns, but were absent in vessels in melanomas lacking naevus-like vascular patterns.

These ultrastructural findings are important for two reasons: (*a*) vascular basement membranes in ciliary body and choroidal melanomas may be thickened and fragmented without previous exposure to radiation (these changes were attributed previously to a radiation effect[59–63]); and (*b*) alterations in the basement membrane and loss of endothelial cell junctions suggest a physiological breakdown in the blood–tumour barrier.[64] Recently, it was shown that vessels in choroidal naevi did not leak indocyanine green while the vessels of choroidal melanomas were leaky.[65] Disruption in the blood–tumour barrier of melanomas might facilitate some forms of perfusion-based therapies.[66]

Despite the considerable evidence that the prognostic patterns illustrated in Fig. 1 by PAS staining without haematoxylin counterstaining are microvascular, a team of investigators recently concluded that the microcirculatory nature of these prognostic patterns was in question because paraffin sections of melanomas stained to demonstrate factor VIII failed to demonstrate networks.[67] Factor VIII is known to be a relatively insensitive marker for immature endothelium of the type seen in an angiogenic response in tumours (antigen expression in microcirculatory beds may be related to the relative maturity of the endothelium;[68,69] Fig. 4).



(a)



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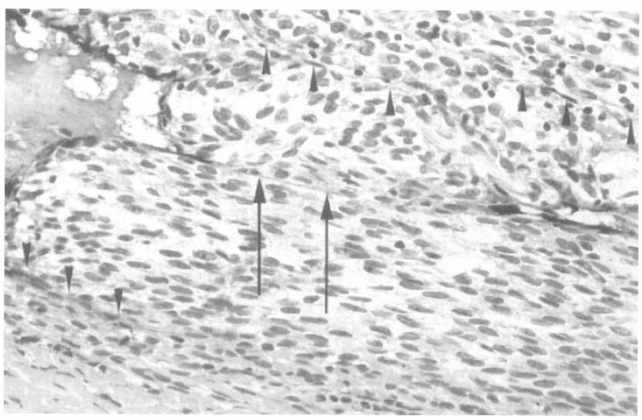

(c)

Fig. 4. *Demonstration of microcirculation using von Willebrand factor (factor VIII). (a) Factor VIII appears to demonstrate two separate vessels at this magnification. (b) At higher magnification a thin-walled vessel continues from the larger vessel (long arrows) on the left but is not stained with factor VIII. (c) Same photomicrograph as (b), but arrowheads now call attention to other small vessels that are incompletely stained by factor VIII. Factor VIII–diaminobenzidine–haematoxylin; original magnification ×25 (a) and ×100 (b, c).*

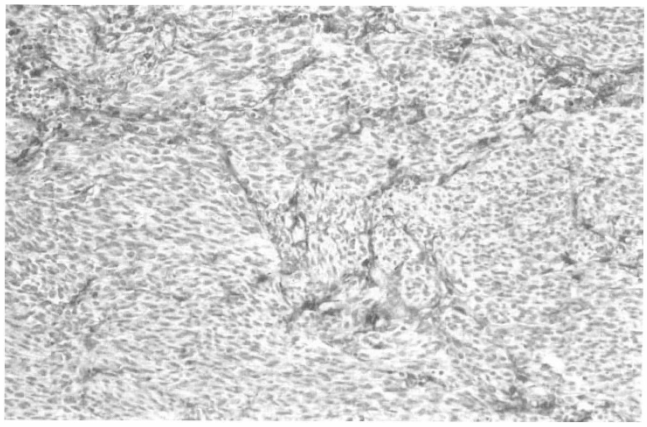

Fig. 5. *Microvascular networks are identified with antibody to CD31. CD31–diaminobenzidine–haematoxylin; original magnification ×25.*

With more sensitive markers for endothelium, it is indeed possible to demonstrate microvascular loops, networks, and parallel vessels with cross-linking in tissue sections of choroidal and ciliary body melanomas (Fig. 5).

CELL TYPE, PROGNOSIS AND MICROCIRCULATORY PATTERNS

Cell type, long considered to be of paramount prognostic importance,[1–11] does not appear in Cox proportional hazards models once microvascular patterns are permitted to enter the models.[41] The relative insignificance of cell type as regards prognosis was confirmed subsequently by Mooy *et al.*[70] Even cytomorphometric measurements such as the mean of the ten largest nucleoli (MTLN)[14,71] do not enter Cox models when vascular patterns are permitted to enter the model,[72] and recently Coleman *et al.*[73] confirmed the finding that MTLN may not be a significant prognostic variable in ciliary body and choroidal melanomas.

Because cell type does not appear in a Cox proportional hazards models does not mean that that cell type is not important in the phenomena of invasion and metastasis and in complex microvascular remodelling that results in the formation of networks: the presence of epithelioid cells is associated with the presence of networks, and the absence of epithelioid cells is associated with avascular zones.[40]

To understand the relationships between cytology, microvascular pattern formation, and invasion and metastasis, it is important to consider the mechanisms by which microvascular patterns form in ciliary body and choroidal melanomas.

THE MECHANISMS OF MICROVASCULAR PATTERN FORMATION IN CHOROIDAL AND CILIARY BODY MELANOMAS

Recently, type VI collagen and hyaluronan were detected around small blood vessels in the microcirculation of choroidal and ciliary body melanomas and surrounding nests of cells separate from the microcirculatory bed.[74] Type VI collagen and hyaluronan both stain positive with PAS stain; type I collagen is not PAS-positive.[75,76] These observations may help to explain why it is possible to demonstrate microcirculatory patterns in routine sections with the PAS stain.[40]

Type VI collagen plays an important role in tissue remodelling,[77] including the generation of tissue patterns and microvascular networks.[78] Type VI collagen is distributed normally in the subendothelial compartment,[79–81] but unlike type I collagen which is normally present in the stoma of the choroid, type VI collagen is normally present in low levels around the choriocapillaris and not present in the choroidal stroma.[82]

The mere presence of type VI collagen in the remodelled microcirculation of ciliary body and choroidal melanomas does not explain the association between the formation of microvascular networks and metastasis. In cultures of eight ciliary body or choroidal melanomas, thin and plump spindled cells (analogous to spindle A and B cells) and epithelioid cells were identified.[74] Invasion assays into type I collagen gels were performed with all eight cell lines. Epithelioid cells and plump spindle cells invaded into type I collagen gels but the thin spindle cells did not invade and remained on the gel surface. Thus, cells that roughly match the Callender spindle B and epithelioid types were shown to be invasive *in vitro*, in contrast to spindle A cells which were not invasive in this assay.

Two lines of evidence suggested that the melanoma cells themselves are capable of synthesising type VI collagen: the direct demonstration of staining for type VI collagen in cultured melanoma cells and in tissue section, and the presence of type VI collagen mRNA as determined by reverse transcriptase–PCR.[74] There was no evidence of type VI collagen production by thin spindle cells.

Tumour cells that had an invasive phenotype *in vitro* (spindle B and epithelioid cells) were the cell types capable of producing type VI collagen. Type VI collagen is thought to contribute to pattern formation and remodelling through the formation of scaffolds into which vessels grow.[83] Therefore, the ability of tumour cells that have invasive characteristics to produce an element of the extracellular matrix associated with vascular remodelling (type VI collagen) may help to explain the association between the appearance of microvascular networks in tissue sections and the development of metastases.

Another line of investigation may shed light on the events that trigger microvascular pattern formation. In many studies, tumours involving the ciliary body have a worse prognosis than those located entirely in

the choroid.[6,7,84] However, in the multivariate Cox model including microcirculatory patterns, tumour location within the eye did not appear as an independent variable.[41] It was discovered subsequently that microvascular networks tend to develop in the ciliary body relative to the choroid.[85] This observation is important because deletion of chromosome 3 may be characteristic of ciliary body melanomas[86] and has been associated with their aggressive behaviour.[86,87] If the aggressive behaviour of melanomas with ciliary body involvement is related to deletion of chromosome 3, if ciliary body tumours develop vascular networks preferentially,[85] and the presence of vascular networks has the strongest association with mortality due to melanoma of all other variables studied,[40,41] then deletion of chromosome 3 may be related to the development of vascular networks.

ADDRESSING A PROBLEM IN THE MANAGEMENT OF PATIENTS WITH CHOROIDAL OR CILIARY BODY MELANOMAS

Understanding the development of tumour stroma in ciliary body and choroidal melanomas is more than a theoretical exercise. Ciliary body and choroidal melanomas are among the few forms of cancer treated before a pathologist assigns a grade to indicate whether tumour is likely to follow a benign or aggressive course. Although pathologists may contribute significant prognostic information after the eye has been removed, the only prognostic information available to ophthalmologists at the time of clinical diagnosis is based on the tumour size[84] and the detection of extraocular extension by conventional ultrasonography or magnetic resonance imaging. Unfortunately, when choosing between enucleation and alternative vision-sparing treatments for their patients, ophthalmologists cannot use information from a histological examination of an incisional biopsy, a procedure that would be likely to disturb vision.

Ophthalmologists have tried to detect the cytological composition of the tumour clinically as a means of obtaining prognostic information that could guide management. Fine-needle aspiration biopsy separates uveal melanomas accurately from conditions that simulate these tumours clinically (e.g. metastases),[88] but its use as a prognostic tool is limited in at least four ways: (*a*) There is a risk of sampling error when determining cell type: the needle track from a fine-needle aspiration biopsy was traced histologically on an enucleation specimen and was localised to a zone of spindle A and B melanoma cells, missing a pocket of adjacent epithelioid cells entirely.[89] (*b*) There are no associations between cytomorphometric measurements on fine-needle aspiration biopsy samples and those from tissue sections of enucleated eyes.[89,90] (*c*) Cell type may not provide the most important prognostic information currently available from the examination of tumour tissue: in Cox regression models that permit the entry of microvascular patterns as well as conventional prognostic features such as cell type, the tumour characteristic most strongly associated with metastasis is the network microcirculatory pattern; cell type did not even appear as an independent variable.[48,72,85] (*d*) Fine-needle aspiration biopsy is invasive; it would be preferable to develop a non-invasive test to determine the patient's prognosis.

In designing a non-invasive substitute for biopsy, it would be reasonable to exploit not only the cytology of the tumour but any prognostic information that might be embedded in the tumour stroma, such as microvascular patterns. There have been two major approaches to detecting prognostically significant microcirculatory patterns clinically: indocyanine green angiography augmented by confocal ophthalmoscopy,[91] and ultrasound power spectrum analysis.[92]

Indocyanine green angiography augmented by confocal ophthalmoscopy[91] utilises a dye that is visible to a certain extent through melanin and the presence of a confocal system permits tomographic 'cuts' through the tumour, so microcirculatory patterns should be visible theoretically if they are within the resolution of the apparatus. Recently, Schneider *et al.*[93] employed such a system to image the microcirculation of posterior choroidal melanomas and demonstrated parallel vessels with cross-linking at the periphery of a tumour. There may be three limitations to this promising new imaging technique: (*a*) only tumours directly in the posterior pole can be imaged, (*b*) in thick tumours it is not known whether the tumour adjacent to the sclera can be imaged, and (*c*) it is not known whether the small, thin-walled vessels of prognostically significant microcirculatory patterns are beyond the resolution of the imaging equipment.

Ultrasound power spectrum analysis can be applied to patients with ciliary body or choroidal melanomas and is independent of the degree of pigmentation or thickness of the tumour. This specialised technique of ultrasound spectrum analysis of digitally recorded echoes returned from a tissue region provides statistical measures that are related to small spatial fluctuations in acoustic impedance.[92] These measures can be used to derive physically relevant characteristics such as the mean size of tissue areas that backscatter ultrasound, and the acoustic concentration.[92] In 1990, Coleman *et al.*[94] suggested that these scatterer patterns might be 'associated with tumour micro-regions such as intervascular nests of cells, rather than directly with the

individual size of cells'. This clinical observation suggested that the stroma of choroidal and ciliary body melanomas might be organised into regions or patterns. The following year, Coleman *et al.*[95] showed that these 'cell distribution patterns' detected by ultrasound power spectrum analysis were related to outcome in patients with choroidal and ciliary body melanomas.

It was particularly interesting that the dimensions of tumour outlined histologically by closed vascular loops measured from digitised laser scanning confocal microscopy images (width 14–116 μm, median 43 μm; length 30–157 μm, median 70 μm[40]) corresponded well with acoustic scatterer patterns of prognostic importance reported by Coleman *et al.* (40–110 μm in diameter).[96] The correspondence between acoustic scatterer sizes and the size of tumour micro-regions outlined histologically by the closed vascular loops in networks suggested that ultrasound spectrum analysis might be capable of detecting this prognostically significant feature of ciliary body and choroidal melanomas clinically, thus forming the basis of a non-invasive substitute for biopsy.

Five patients were examined by ultrasound spectrum analysis before enucleation for ciliary body or choroidal melanoma.[97] Three-dimensional ultrasound images that depict the size and relative concentration of scattering elements within ranges of 50–120 μm were compared with histological sections stained with PAS without haematoxylin[40] to permit the identification of microvascular patterns. Vascular networks were identified histologically in three tumours. The predominant ultrasound features seen in the regions of the histologically identified networks were clusters of scatterers in the range 50–80 μm. In the two cases without networks, lower range scatterers dominated the tumour volume. This pilot study suggested that ultrasound spectrum analysis parameters used to subclassify uveal melanoma may have a biophysical basis related to patterns of tumour microcirculation. Additional studies are being conducted in a prospective fashion to correlate this imaging technique with histological pattern classification.

If ophthalmologists could determine the biological grade of a ciliary body or choroidal melanoma clinically by a non-invasive study, how would this information be used? A patient at high risk for metastasis in the absence of detectable elevations of liver enzymes or abnormal liver scans may benefit from adjuvant chemotherapy to reduce any small foci of extraocular tumour burden.[98–100] What would be the value of knowing that a patient with a ciliary body or choroidal melanoma was at low risk for metastasis? The natural history of a histological low-grade choroidal or ciliary body melanoma is unknown because its course has been interrupted by enucleation. Would a patient classified clinically as having a lesion at low risk for metastasis be observed and monitored by repeated non-invasive 'biopsies' or would the lesion be treated? There are no answers to these questions now, but the availability of a non-invasive substitute for biopsy would make it possible in future to conduct future prospective clinical studies to investigate these problems. It is not even possible to address these issues without an accurate method of grading these tumours clinically.

Would treatment of the primary tumour be different if it were classified clinically at low risk instead of higher risk? The development of a clinical prognostic test based on properties of the microcirculation may contribute to improving existing vision-sparing treatments: radiation therapy may target the tumour vasculature,[61,62,101–104] and the response to hyperthermia may be related to tumour vascularity.[105–111] A better understanding of the microcirculation of these tumours may contribute significantly to the development of new therapeutic strategies. For example, it is known that vascular networks establish a microenvironment of oxygen and nutrients within a tumour and that focal avascular zones produce local tissue hypoxia.[52] Therefore, it may be possible to design new vision-sparing treatments for the primary tumour with emerging strategies such as hypoxia-activated chemotherapeutic agents and other novel anti-angiogenesis therapies.[112]

CONCLUSIONS

The investigations summarised above represent an attempt to balance the ledger in describing the pathology of choroidal and ciliary body melanomas. The pathology of the tumour stroma may contribute significant prognostic information that may be translated into the improvement of clinical classification and management of patients with choroidal and ciliary body melanomas – tumours that threaten not only to shorten the lifespan of the patient but to compromise vision.

The study of tumour cell-matrix and stromal interactions in choroidal and ciliary body melanomas has implications that extend far beyond the boundaries of the practice of clinical ophthalmology.[45] Uveal melanomas represent a model system for the pure haematogenous dissemination of tumours, because there are no lymphatics within the eye. Moreover, the noted tendency for these tumours to spread preferentially to the liver makes choroidal and ciliary body melanomas paradigms for the study of human organ-specific metastasis. Research in ophthalmic oncology may therefore contribute sig-

nificantly to an overall understanding of the mechanisms of invasion and metastasis.

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Key words: Melanoma, Metastasis, Angiogenesis, Prognosis, Type VI collagen, Ultrasound, Indocyanine green angiography, Extracellular matrix.

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