VASCULOGENIC MIMICRY AND TUMOUR-CELL PLASTICITY: LESSONS FROM MELANOMA

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The gene-expression profile of aggressive cutaneous and uveal melanoma cells resembles that of an undifferentiated, embryonic-like cell. The plasticity of certain types of cancer cell could explain their ability to mimic the activities of endothelial cells and to participate in processes such as neovascularization and the formation of a fluid-conducting, matrix-rich meshwork. This ability has been termed 'vasculogenic mimicry'. How does vasculogenic mimicry contribute to tumour progression, and can it be targeted by therapeutic agents?

ANGIOGENESIS

PERIODIC ACID SCHIFF STAIN (PAS stain). A histochemical assay used to identify extracellular matrix, on the basis of the presence of glycogen and related mucopolysaccharides.

The clinical management of cutaneous and uveal melanoma, and of many other types of cancer, would benefit greatly from the identification of valid predictors of disease progression and metastatic potential. Recent studies aimed at characterizing the molecular 'signature' of melanoma tumour cells have resulted in a classification scheme for malignant cutaneous melanoma[1] and uveal melanoma[2]. Microarray analyses comparing gene-expression patterns between highly aggressive and poorly aggressive human cutaneous and uveal melanoma cell lines have shown that aggressive tumour cells express genes that are associated with many cellular phenotypes. These include genes that are usually expressed by precursors of endothelial, epithelial, pericyte, fibroblast, haematopoietic, kidney, neuronal, muscle and several other cell types[1–3]. These findings indicate that aggressive melanoma cells might revert to an undifferentiated, embryonic-like phenotype. However, the biological importance of these unexpected findings remains unresolved. Indeed, these observations have prompted further investigation into the potential relevance of a 'plastic' tumour-cell phenotype, and they challenge our current thinking of how to identify and target tumour cells that can masquerade as other cell types.

Many of the biological properties that are relevant to embryogenesis are also important for tumour growth. For example, during embryonic development, the formation of primary vascular networks occurs by the process of vasculogenesis — the differentiation of mesodermal progenitor cells (angioblasts and haemangioblasts) to endothelial cells and their organization into a primitive network (for reviews, see REFS 4–6; FIG. 1A). The subsequent remodelling of the vasculogenic network into a more refined microvasculature occurs through angiogenesis — the sprouting of new capillaries from pre-existing networks. Similarly, it is widely accepted that during cancer progression, tumours require a blood supply for growth and also use the blood supply for metastatic dissemination (for reviews, see REFS 7–14, and the article by Isaiah J. Fidler on page 451 of this issue).

On the basis of the molecular profile of aggressive melanoma cells, together with new *in vitro* observations (using three-dimensional matrices) and correlative histopathological findings, our laboratory and others introduced the concept of 'vasculogenic mimicry' in 1999 (REF. 15). At that time, vasculogenic mimicry described the ability of aggressive melanoma cells to express endothelium-associated genes and to form extracellular matrix (ECM)-rich vasculogenic-like networks in three-dimensional (3D) culture, as shown by PERIODIC ACID SCHIFF STAINING (PAS staining). The formation of these networks seemed to recapitulate the embryonic development of vasculogenic networks

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Summary

- The molecular 'signature' of aggressive melanoma cells is illustrative of an undifferentiated cell with a gene-expression profile that is similar to that of embryonic-like cells.
- Vasculogenic mimicry describes the ability of aggressive melanoma cells to express endothelium-associated genes and form extracellular matrix (ECM)-rich vasculogenic-like networks in three-dimensional culture. These networks recapitulate embryonic vasculogenesis, and they have been detected in human aggressive tumours.
- Vasculogenic mimicry in melanoma involves several signalling molecules that are also involved in embryonic vasculogenesis, including vascular endothelial (VE)-cadherin, erythropoietin-producing hepatocellular carcinoma-A2 (EPHA2), phosphatidylinositol 3-kinase, focal adhesion kinase, matrix metalloproteinases and laminin 5 γ2-chain.
- The biological implications of vasculogenic mimicry *in vivo* are unclear, but recent studies indicate that the formation of vasculogenic-like networks that are rich in laminin could serve as an intratumoral fluid-conducting meshwork.
- Vasculogenic mimicry has been observed in non-melanoma tumour types, including carcinomas of the breast, prostate, ovary and lung, synoviosarcoma, rhabdomyosarcoma and phaeochromocytoma, and in cytotrophoblasts forming the placenta.
- Endostatin, an angiogenesis inhibitor, abrogates endothelial-cell-driven angiogenesis, but not vasculogenic mimicry, in melanomas.
- Identification of the pathways that regulate this undifferentiated, highly plastic phenotype could lead to the development of new therapeutic strategies for cancer.

(FIG. 1A), and they were associated with the distinctly patterned, ECM-rich networks that are observed in aggressive tumours of patients with cancer[2,15–23]. Additional studies by several investigators have reported intriguing observations regarding vasculogenic mimicry in various tumour types. But how do these networks form and what is their contribution to tumorigenesis?

Initially, researchers observed patterned loops and arcs that tightly encircled spheroidal clusters of cancer cells in xenograft models and in biopsies of human aggressive melanoma. These loops and arcs formed networks that were found to be lined with tumour cells and also to be rich in laminin. The networks probably contain other components of the ECM that have not yet been identified. Studies of tumour-tissue sections showed that the spheroidal tumour clusters contained either small, channel-like spaces between them, or seemed to be partially or totally juxtaposed by ECM. Some of these channel-like spaces were originally known as 'vascular channels', because they were found to contain erythrocytes and plasma. They were thought to provide a mechanism of perfusion and a dissemination route within the tumour that functioned either independently of or simultaneously with angiogenesis (or other sources of vascularization such as vessel co-option).

Individuals with melanomas that have undergone vasculogenic mimicry have a poor prognosis[17–23], but little is known about the biological relevance of this phenomenon. Additional studies have reported vasculogenic mimicry in several other tumour types, including breast, lung, prostatic and ovarian carcinoma, and these studies have attempted to determine the molecular mechanisms underlying this unique process. These include the demonstration of a viable blood flow between tumour-cell-lined vascular spaces and endothelium-lined and/or mature vasculature[24].

The importance of these findings relates to the aetiology of vasculogenic mimicry — in particular, the ongoing investigations aimed at identifying the factors that regulate this process. Vasculogenic mimicry seems to involve dysregulation of the tumour-specific phenotype and the concomitant transdifferentiation of aggressive tumour cells into other cell types, such as

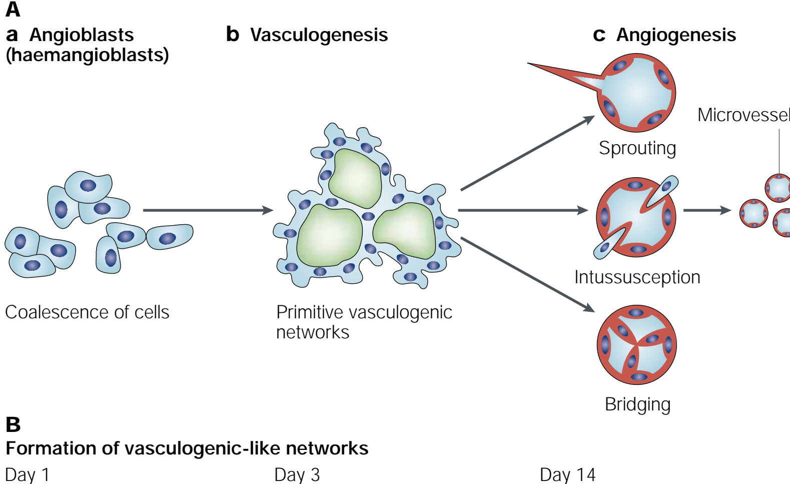

Figure 1 | **Vasculogenesis, angiogenesis and tumour-cell vasculogenic mimicry.** **A** | Schematic overview of vasculogenesis and angiogenesis, showing how endothelial-cell precursors (angioblasts and haemangioblasts) coalesce and differentiate into endothelial cells (**a**), and form primitive vasculogenic networks (vasculogenesis) (**b**). Remodelling of these networks occurs through angiogenesis (**c**), which involves sprouting, intussusception and/or bridging, resulting in the formation of microvessels. Adapted, with permission, from REF. 5 © (2002) Macmillan Magazines Ltd. **B** | The unique ability of aggressive melanoma cells to form vasculogenic-like networks (arrows) in three-dimensional gels of collagen I *in vitro*. Networks begin to form by the end of day 1; they continue to mature into vasculogenic-like networks by day 3; and similar structures are perfusable (with a fluorescent dye) by day 14.

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endothelial cells. This transdifferentiation can be detected using multiple phenotype-specific markers.

In light of the results of recent trials of angiogenesis inhibitors[25], new information regarding effective mechanisms to destroy tumour-associated vasculature in humans is required. Recent *in vivo* and *in vitro* studies have shown that tumour vasculogenic mimicry is relatively unaffected by endostatin[26] (E. Sausville, personal communication). So, vasculogenic mimicry might be an important factor to consider in the design of anti-vascular therapies.

The vasculogenic phenotype in melanoma

Previous studies in patients with melanoma have shown a strong association between poor patient outcome and aggressive tumours that contain ECM-rich, PAS-positive, patterned, looping networks that resemble vasculogenic patterned networks[17–23]. In certain areas of aggressive melanomas that contain only small amounts of vasculature and no necrosis, these looping networks were originally thought to provide a unique microcirculation to the growing tumour mass. However, at the time that the concept of vasculogenic mimicry was introduced in 1999 (REF. 15), the biological function of these vessels was unclear and, therefore, great controversy arose over the interpretation of these findings[27].

Continuing research efforts focused on the differences between highly aggressive and poorly aggressive melanomas, in relation to vasculogenic mimicry, have produced several interesting findings. Aggressive melanoma cells, when seeded on three-dimensional matrices of collagen I, form ECM-rich patterned networks that surround clusters of tumour cells (FIG. 1B). These are similar to the patterned, looping networks that are observed in histological sections of tumours from patients[15,28]. Furthermore, microinjection of a fluorescent dye into these networks showed the ability of the structures to become perfused *in vitro* (FIG. 1B). By comparison, under the same culture conditions, poorly aggressive melanoma cells did not form patterned networks[2,15,28]. For a video showing the early *de novo* formation of networks, see http://www.anatomy.uiowa.edu/hendrix_nat_rev_cancer.

Gene-expression profiling of more than 45 human cutaneous and uveal melanoma-cell lines has produced unexpected findings regarding the phenotype of aggressive tumours cells[1–3] (TABLES 1,2). The level of expression of more than 6,000 genes was compared between highly aggressive and poorly aggressive melanoma cells isolated from the same patients[1–3]. The spectrum of upregulated genes reflected many cellular phenotypes, including those that are associated with several different types of progenitor cell. Some of these genes have been associated with the transcription profile of embryonic stem cells, and they require further investigation[29].

The expression of many melanoma-specific markers, by contrast, was downregulated in aggressive tumour cells — with the exception of melanoma-cell adhesion molecule (MCAM) (TABLE 1). For example, expression of microphthalmia-associated transcription factor (*MITF*) was downregulated by a factor of 34 in aggressive melanoma cells compared with poorly aggressive tumour cells[1] (TABLE 1). MITF activates expression of the gene encoding tyrosinase, an enzyme that is involved in MELANOCYTE differentiation[30]. The level of expression of the genes encoding tyrosinase and tyrosinase-related protein 1 (*TYRP1*) was also downregulated (by a factor of between 37 and more than 100) in the same aggressive tumour cells compared with poorly aggressive tumour cells (TABLE 1). Therefore, melanoma cells seem to de-differentiate as they become more aggressive, which might make them more difficult to identify using routine histopathology methods. It will be interesting to determine whether differentiated melanoma cells change into undifferentiated tumour cells, or whether subpopulations of differentiated and undifferentiated melanoma cells coexist.

Many of the genes that are upregulated by aggressive cancer cells include those that are involved in angiogenesis and vasculogenesis, such as the genes encoding vascular endothelial (VE)-cadherin (cadherin 5, *CDH5*; *CD144*), erythropoietin-producing hepatocellular carcinoma-A2 (*EPHA2*), and laminin 5 γ2-chain (*LAMC2*)[28,31,32] (TABLE 1). These molecules, with their binding partners, are a few of the factors that are required for the formation and maintenance of blood vessels[4–6,33]. They have also been shown to be required for vasculogenic mimicry in melanomas.

VE-cadherin is an adhesion protein — previously thought to be endothelial-cell specific — that belongs to the cadherin family of transmembrane proteins. These proteins promote homotypic cell–cell interactions[34–37]. EPHA2 is a receptor protein tyrosine kinase that is part of a large family of ephrin receptors[38]. Binding of its ligand, ephrin-A1, results in EPHA2 phosphorylation and signalling[39], and this pathway has been linked to tumour-cell proliferation[40]. High levels of expression of EPHA2 and ephrin-A1 have been associated with melanoma growth, tumour thickness and decreased patient survival[41].

Laminins are important components of basement membranes that are involved in regulating neurite outgrowth, tumour metastasis, cell attachment and migration, and angiogenesis[40,42,43]. Proteolytic cleavage of laminins — particularly of the laminin 5 γ2-chain — can alter the integrin-mediated migratory behaviour of certain cells[40,44–46], so laminin might be an environmental trigger facilitating melanoma progression.

At the protein level, VE-cadherin, EPHA2 and laminin 5 γ2-chain are expressed only by aggressive melanoma cells, and not by non-aggressive or poorly aggressive melanoma cells[28,31,32]. The biological relevance of these molecules in vasculogenic mimicry was further shown by independently reducing their levels of expression and measuring the consequences on the formation of vasculogenic-like networks *in vitro*. This approach showed that the downregulation of expression of VE-cadherin, EPHA2 or laminin 5 γ2-chain resulted in the complete inability of aggressive melanoma cells to form vasculogenic-like networks in 3D culture[28,31,32].

MELANOCYTE
A type of cell derived from the neural crest that is specialized to produce the pigment melanin. Melanocytes are commonly found in the skin and retina.

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Table 1 | **Altered expression of angiogenesis/vasculogenesis-related genes by melanoma cells**

Gene	Unigene	Function	Ratio of expression*
Melanocyte-specific markers			
Melan-A (*MLANA*)	Hs.154069	Melanoma surface antigen	0.044 (↓22)
Microphthalmia-associated transcription factor (*MITF*)	Hs.166017	Melanocyte-development transcription factor	0.029 (↓34)
Tyrosinase (*TYR*)	Hs.2053	Catalyses the conversion of tyrosine to melanin	0.027 (↓37)
Tyrosinase-related protein 1 (*TYRP1*)	Hs.75219	Catalyses the conversion of tyrosine to melanin	0.004 (↓>100)
Melanoma-cell adhesion molecule (*MCAM*)	Hs.211579	Cell-surface glycoprotein	27
Markers of other cellular phenotypes			
Tyrosine kinase receptor 1 (*TIE1*)‡	Hs.78824	Endothelial tyrosine kinase	25
Epithelial-cell kinase (*EPHA2*)‡	Hs.171596	Receptor tyrosine kinase	13
Vascular endothelial growth factor-C (*VEGFC*)‡	Hs.79141	FLT4 ligand	6.5
Neuropilin 1 (*NRP1*)	Hs.69285	VEGF receptor	5.3
Vascular endothelial (VE)-cadherin (*CDH5*)	Hs.76206	Cell–cell adhesion molecule	11
Selectin E (*SELE*)	Hs.89546	Adhesion molecule	6.6
Endoglin (*ENG*)‡	Hs.76753	TGF-β1 receptor	4.3
CD34‡	Hs.85289	Stem-cell marker, sialomucin	2.5
Hypoxia-inducible factor 1α (*HIF1A*)	Hs.197540	bHLH transcription factor	3.1
Tissue factor pathway inhibitor 1 (*TFPI1*)	Hs.170279	Coagulation inhibitor	4.0
TFPI2	Hs.78045	Coagulation inhibitor	8.5
Laminin 5 γ2 (*LAMC2*)	Hs.54451	Extracellular matrix	50
Fibronectin (*FN1*)	Hs.118162	Extracellular matrix	27
Collagen IV α2 (*COL4A2*)‡	Hs.75617	Extracellular matrix	3.6
Fibrillin 1 (*FBN1*)	Hs.750	Extracellular matrix	5.0
Endothelial differentiation receptor (*EDG1*)	Hs.154210	G-protein-coupled receptor	3.7
Endothelial cell-specific molecule (*ESM1*)	Hs.41716	Endothelium-specific signalling molecule	41
Endothelial differentiation-related factor 1 (*EDF1*)‡	Hs.174050	Endothelial-cell differentiation regulator	4.8
Plasminogen activator inhibitor 1 (*PAI1*)	Hs.82085	Serine protease inhibitor	31

*Change in expression level of aggressive compared with poorly aggressive melanoma cells. ‡Genes that are also associated with the transcriptional profile of embryonic stem cells. bHLH, basic helix-loop-helix; EPHA2, erythropoietin-producing hepatocellular carcinoma-A2; FLT4, fms-related tyrosine kinase 4; TGF-β1, transforming growth factor β1.

Additional experiments focused on the tumour-cell-associated ECM led to the discovery of the inductive potential of the microenvironment. ECM substrates 'pre-conditioned' or remodelled by aggressive melanoma cells were able to induce poorly aggressive melanoma cells to assume a vasculogenic and more-migratory phenotype[32]. This study indicated that cooperative interactions between laminin 5 γ2-chain and matrix metalloproteinase 2 (MMP2) and membrane type-1 matrix metalloproteinase (MT1-MMP) are required for vasculogenic mimicry of melanoma cells. Laminin was shown to co-localize with PAS-positive vasculogenic-mimicry networks *in vitro* and *in vivo*. These findings also indicated that highly aggressive melanoma cells 'deposit' molecular signals in their microenvironment. These signals are produced by cleavage of the laminin 5 γ2-chain by MT1-MMP and MMP2 into laminin 5 γ2′ and γ2x pro-migratory fragments (FIG. 2). These signals could induce a vasculogenic phenotype in poorly aggressive melanoma cells, resulting in the formation of vasculogenic-like networks and the concomitant expression of vascular-associated genes (such as those encoding VE-cadherin, EPHA2 and laminin 5 γ2-chain). This inductive potential of the tumour-cell microenvironment can be 'neutralized' by treatment with chemically modified tetracycline (CMT-3; COL-3)[47], which is a potent inhibitor of MMP activity.

The vasculogenic-mimicry signalling cascade

Researchers have recently learned a great deal about the signal-transduction pathways that regulate blood-vessel

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Table 2 | **Haematopoietic-associated markers in melanoma**

Gene	Unigene	Function	Ratio of expression*
Neutral endopeptidase (*CALLA*; *CD10*)	Hs.1298	Cell adhesion molecule	4.7
Haematopoietic lineage cell-specific protein 1 (*HCLS1*)	Hs.14601	LYN tyrosine kinase substrate	5.7
Activated leukocyte cell-adhesion molecule (*ALCAM*)	Hs.10247	CD6 ligand	17.5
Lymphocyte-specific protein 1 (*LSP1*)	Hs.56729	Signal transduction	2.9
Trophoblast–lymphocyte cross-reactive antigen (*MCP*; *CD46*)	Hs.83532	Membrane co-factor protein	3.9
5′ nucleotidase (*NT5*)	Hs.153952	Lymphocyte differentiation (CD73)	36
Macrophage maturation-associated protein (*MMA*)	Hs.79889	Differentiation molecule	4.5

*Change in expression level of aggressive compared with poorly aggressive melanoma cells. MCP, membrane co-factor protein.

BLOOD LAKES
Areas of haemorrhage generally lacking an endothelial-cell lining that are often seen in histological sections of high-grade neoplasms.

formation and stabilization during vasculogenesis and angiogenesis[5,48,49]. Similarly, an investigation of the signalling events that regulate melanoma vasculogenic mimicry is in progress[50]. Microarray analyses led to the identification of the receptor tyrosine kinase EPHA2 as a factor that is likely to be involved in melanoma vasculogenic mimicry[28]. Other signal-transduction molecules that seem to promote melanoma vasculogenic mimicry include VE-cadherin, focal adhesion kinase (FAK), and phosphatidylinositol 3-kinase (PI3K)[50]. Using various experimental approaches, a clearer understanding of the signalling pathways that facilitate melanoma vasculogenic mimicry is being developed. A model highlighting some of the molecules that are involved is shown in FIG. 2.

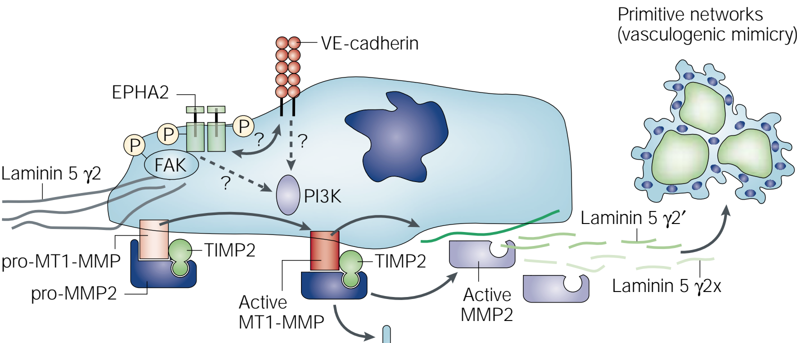

Figure 2 | **The vasculogenic-mimicry signalling cascade.** A model of the signal-transduction events that occur during melanoma tumour-cell vasculogenic mimicry. In this model, phosphorylated (P) erythropoietin-producing hepatocellular carcinoma-A2 (EPHA2) and vascular endothelial (VE)-cadherin are co-localized at the cell membrane. Phosphorylated EPHA2 subsequently interacts with phosphorylated focal adhesion kinase (FAK). The signal-transduction pathways activated by both EPHA2 and VE-cadherin converge to activate phosphatidylinositol 3-kinase (PI3K). Downstream, PI3K regulates the activity of membrane type-1 matrix metalloproteinase (MT1-MMP), which subsequently activates pro-MMP2 to an active MMP2 proteinase (through the formation of an MT1-MMP–TIMP2–pro-MMP2 ternary complex). Both MT1-MMP and MMP2 promote the cleavage of laminin 5 γ2-chain into pro-migratory γ2′ and γ2x fragments. The release of these fragments (molecular messages or signals) into the tumour microenvironment can increase the migration, invasion and, ultimately, vasculogenic mimicry of aggressive melanoma tumour cells. Based on an illustration by D. A. Kirschmann. TIMP2, tissue inhibitor of metalloproteinase 2.

As shown in this model, phosphorylated EPHA2 can co-localize with VE-cadherin, as well as with phosphorylated FAK, on the membrane of highly aggressive melanoma cells. Both VE-cadherin and EPHA2 have been shown to activate PI3K in other systems, so PI3K signalling is likely to occur downstream of EPHA2 and VE-cadherin signalling in this system. Recent studies have shown that the inhibition of PI3K activity can inhibit melanoma vasculogenic mimicry[50]. This could be because the inhibition of PI3K activity downregulates the expression and activity of MT1-MMP, together with the subsequent activation of MMP2. The activity of both of these proteinases was recently shown to be required for vasculogenic mimicry[32].

It is important to note that these molecules have also been shown to be involved in development (for a review of the subject, see REF. 51), which indicates that vasculogenic mimicry could be a recapitulation of an embryonic programme. Additional studies of the molecular pathways that regulate melanoma vasculogenic mimicry could lead to new strategies to target the aggressive melanoma phenotype.

Biological implications of vasculogenic mimicry

Several interpretations of vasculogenic mimicry have evolved, on the basis of various analyses of the original findings. One interpretation is simply a description of PAS-stained patterned networks in tumours. Another refers to cancer cells that line tumour spaces or channels, which also contain erythrocytes or BLOOD LAKES. Others have equated vasculogenic mimicry with tumour cells that express endothelium-specific genes. It is possible that any combination of these scenarios could explain the features of vasculogenic mimicry. The term 'vascular mimicry' has also been used synonymously with vasculogenic mimicry. However, vascular mimicry carries broader implications, as it includes other vascular cell-associated phenotypes, such as lymphocytes and macrophages (TABLE 2).

Studies to address the functional relevance of vasculogenic mimicry in human melanoma and in xenograft models are far more complex than those designed to investigate the *in vitro* cellular and molecular characteristics of vasculogenic mimicry. In fact, more questions than answers have been raised about the relevance of the *in vivo* studies. So far, two main questions regarding melanoma vasculogenic mimicry have been formulated. First, is there a morphological and functional connection between melanoma tumour-cell-lined networks and endothelium-lined vasculature? Second, is it possible for aggressive melanoma cells to form functional vessels when placed in an ischaemic, non-tumour microenvironment?

Tumour-vessel function. So, what are the biological and functional connections between the tumour-cell-lined, PAS-positive and laminin-positive matrix networks that form in aggressive melanomas and the normal endothelium-lined vasculature? Before the

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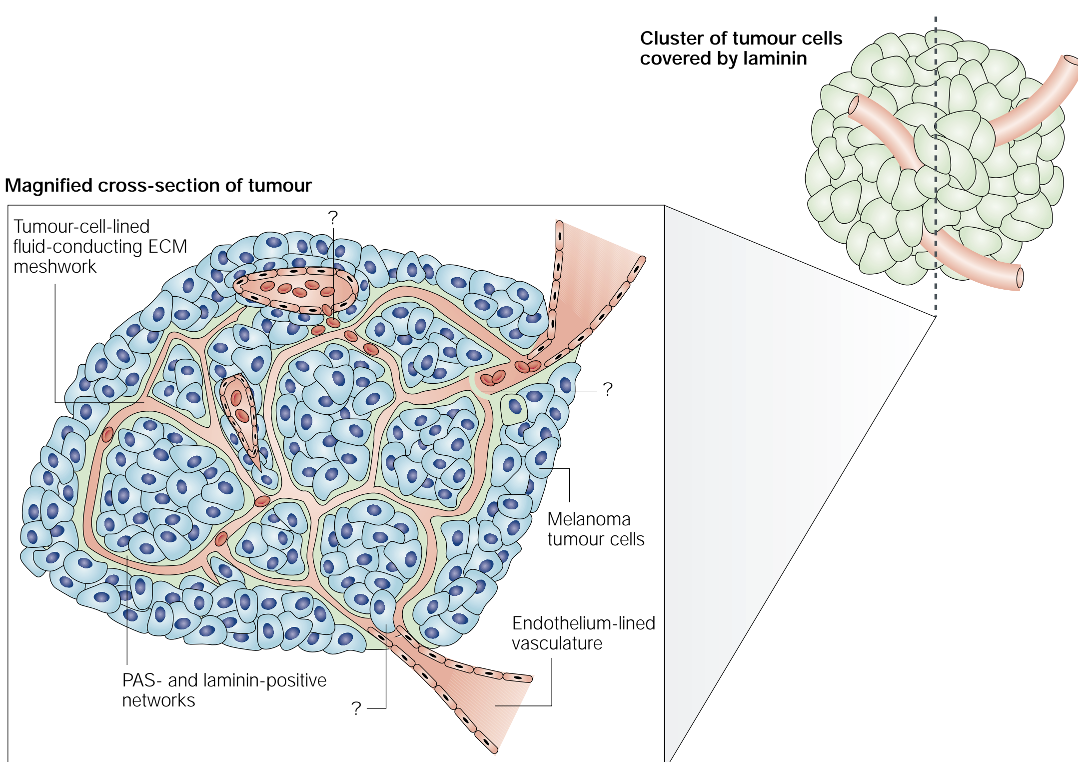

Figure 3 | **The melanoma fluid-conducting ECM meshwork.** The biological implications of melanoma vasculogenic mimicry have evolved to the description of a 'tumour-cell-lined fluid-conducting ECM meshwork' that corresponds to the previously described periodic acid Schiff (PAS)- and laminin-positive (green), patterned networks. This diagram shows the current interpretation of data from several studies[56–58,61] involving the use of tracers and perfusion analyses in mice with aggressive melanoma (blue cells). The endothelium-lined vasculature (pink) is closely apposed to the fluid-conducting meshwork formed from tumour cells. It is presumed that as the tumour remodels the vasculature, it becomes leaky, resulting in the extravascular accumulation of plasma and some erythrocytes (red). However, the biological implication(s) of this phenomenon and the relationship of the laminin-rich fluid-conducting meshwork to the endothelium-lined vasculature are still under investigation (as represented by question marks). Based on an illustration by D. A. Kirschmann. ECM, extracellular matrix.

discovery of vasculogenic mimicry, several previous studies of aggressive melanomas (and other tumour types) reported that tumour cells could line channels, lakes and sinuses, and come into contact with erythrocytes[52–54]. It was unclear, however, whether this had any relevance to the delivery of a blood supply to the growing tumour mass. In fact, the prevailing hypothesis was that any erythrocytes found in the extravascular spaces were probably the result of leaky blood vessels[55].

Morphological analysis showed that PAS-positive patterned networks, which were found in aggressive melanomas and were associated with poor clinical outcome[17–23], also seemed to converge with blood vessels[15,16]. So, it was proposed that some type of anastomosis occurs between the tumour-cell-lined networks and the endothelium-lined vasculature, which contributes to the accumulation of erythrocytes in the network infrastructure[15]. This led to the speculation that the tumour-cell-lined networks might provide a unique paracirculation that forms independently of, or simultaneously with, angiogenesis and/or vessel co-option. However, to prove such a complex phenomenon will require further detailed study. An orthotopic model of human uveal melanoma in immunocompromised mice has been developed to study further the generation of the unique network patterning that is characteristic of aggressive melanoma cells[56].

Studies have indicated the presence of a fluid-conducting ECM meshwork (FIG. 3) in xenograft models of human cutaneous and uveal melanoma that corresponds to the PAS- and laminin-positive patterned networks. These networks consist of arcs and back-to-back loops of matrix[57,58]. Studies involving a combination of intravenous tracers, together with confocal and immuno-electron microscopy, have shown that fluid can be conducted by the endothelium-lined vasculature, as well as extravascularly along the channel-like spaces created by the PAS- and laminin-positive patterned loops and networks that encase clusters of tumour cells[57–59]. Immunohistochemical studies have shown that this fluid-conducting meshwork contains fibrinogen. This indicates the presence of plasma surrounding the tumour-cell-lined clusters of tumour cells[58]. The plasma, in addition to the erythrocytes that have been observed in many PAS- and laminin-positive loops and networks of tumours, is likely to be derived from local tumour vessels that are leaky and undergoing remodelling.

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The functional relevance of the fluid-conducting ECM meshwork is still unclear, but there are several possible explanations. The fluid-conducting meshwork might provide a site for nutritional exchange for aggressive tumours, and might therefore prevent necrosis of the tumour. Alternatively, it might be analogous to an oedematous inflammatory response, in which increased blood pressure leads to the escape of fluid along connective-tissue pathways in intratissue spaces. The complex geometry of the laminin-containing ECM covering that encases the spheroidal clusters of tumour cells could also form a suppressive shield against immune surveillance.

There is a consensus that the microcirculation of aggressive tumours is complex, and depending on the time of observation, could consist of mosaic vessels (which consist of both tumour cells and endothelial cells)[60], co-opted vessels[61] and/or angiogenic vessels[7–14]. There is also strong evidence for the existence of an intratumoral, tumour-cell-lined, ECM-rich, patterned network that can provide an extravascular fluid pathway, now known as the fluid-conducting meshwork[57,58]. The entire microcirculation in aggressive tumours seems to be made up of a combination of these elements, and it is the result of destructive tumour growth and remodelling.

Subcutaneous injection of nude (immunodeficient) mice with fluorescently tagged human aggressive cutaneous melanoma cells allowed the study of the blood supply to primary tumours[62]. Perfusion of the mouse vasculature with a fluorescent tag and microbeads during tumour development, followed by confocal microscopy, revealed the close association of tumour-cell-lined networks with angiogenic mouse vessels at the human–mouse interface. The delivery of microbeads from the endothelium-lined mouse vasculature to the tumour-cell-lined networks indicated a possible physiological connection between the two compartments. Further destructive growth of melanoma into the vasculature led to the observation of erythrocytes and plasma (presumably due to leakage) in the tumour-cell-lined, fluid-conducting meshwork (FIG. 3). However, it is not clear whether a direct morphological and physiological anastomosis exists between the endothelium-lined vasculature and the tumour-cell-lined, fluid-conducting meshwork.

Attempts are underway to assess physiological blood flow of human melanoma xenografts using COLOUR DOPPLER IMAGING (CDI), and such studies have shown pulsatile turbulent flow at the mouse–human tissue interface (with mouse endothelium-lined neovasculature) and the central region of the tumour containing melanoma-cell-lined networks (video available at http://www.anatomy. uiowa.edu/hendrix_nat_rev_cancer). If a dynamic functional exchange of blood through a tumour-cell-lined meshwork (characterized by being rich in laminin) does occur, it is possible that blood flow is related to the high-level expression of the anti-coagulant factors tissue factor pathway inhibitor 1 (TFPI1) and TFPI2 by aggressive melanoma cells (TABLE 1). These tumour cells seem to have similar anti-coagulant properties to endothelial cells, which could contribute to the perfusability of the fluid-conducting meshwork. Although these preliminary findings are intriguing, a more detailed analysis is required to understand the precise sequence of events that are associated with the establishment of vascularization and the maintenance of blood flow during tumour growth and remodelling.

Alternatively, the PAS- and laminin-rich, fluid-conducting meshwork could be an early survival mechanism for nutrient exchange and the release of fluid pressure. This meshwork could eventually be replaced by endothelial cells from nearby angiogenic vessels or from the bone marrow[63]. This intriguing possibility would provide a different perspective on the vasculogenic phenotype of the melanoma cells that line these matrix meshworks and seem to disseminate through them, and it would require a higher standard to discriminate melanoma cells from endothelial cells unequivocally.

An additional enigmatic finding is the unexpected high-level expression by aggressive melanoma cells of vascular endothelial growth factor-C (VEGF-C) — a lymphangiogenesis-associated growth factor (TABLE 1). Interestingly, despite the fact that uveal melanomas lack traditional lymphatic vessels, overexpression of VEGF-C has been reported in these tumours by Clarijs and co-workers[64]. Lymphangiogenesis often accompanies angiogenesis, and this field of research is now gaining momentum with new research tools[65–67]. Interestingly, Ruoslahti and colleagues[68] have shown localization of lymphatic-vessel endothelial hyaluronan receptor 1 (LYVE1) in aggressive cutaneous melanoma. These results raise the intriguing possibility that the fluid-conducting meshwork could 'mimic' a lymphatic-like network.

Tumour-cell plasticity. But how 'plastic' are aggressive melanoma cells, and are they capable of forming vessels under experimental conditions? Investigators have placed aggressive melanoma cells in an ischaemic, non-tumour environment and then assessed whether they can participate in neovascularization. In this investigation[69], fluorescently labelled human cutaneous metastatic melanoma cells were injected into the ischaemic hindlimbs of nude mice. Five days later, the limb vasculature was reperfused. Histological cross-sections of the newly formed vasculature of reperfused limbs showed human melanoma cells adjacent to and overlapping with mouse endothelial cells in a linear arrangement, forming chimaeric vessels. This shows the influence of the microenvironment on the transendothelial differentiation of malignant melanoma cells during neovascularization and reperfusion.

NOTCH proteins have also been investigated for their ability to direct the differentiation of endothelial cells into vascular networks[70,71]. This family of receptors is involved in the cell-fate determination of stem cells — in particular, angioblasts — and non-terminally differentiated cell types[70,71]. Alterations in NOTCH expression and signalling have been implicated in the pathogenesis of human T-cell leukaemia[72], cervical carcinoma[73], mouse mammary carcinoma[74], prostatic tumour progression[75] and human RAS-transformed cells[76]. NOTCH4 was found to be highly expressed by malignant melanoma cells as they participated in neovascularization.

COLOUR DOPPLER IMAGING (CDI). An ultrasonographic method that allows simultaneous two-dimensional structural imaging and evaluation of blood flow. Originally developed to aid the analysis of cardiac function, tissue colour Doppler imaging is a technique in which the velocity of myocardial movement towards the transducer is displayed in a colour-coded form on myocardial images. This technology can be adapted to monitor the effects of antivascular therapies on the blood flow in a tumour.

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Box 1 | **Examples of vasculogenic mimicry**

- **Melanoma**[2,15,16,32,52–54,56,57,90]
- **Breast carcinoma**[24,79–81]
- **Prostatic carcinoma**[84,85]
- **Ovarian carcinoma**[82,83]
- **Lung carcinoma**[86]
- ***Drosophila* (large tumour suppressor gene, *lats*-negative) tumours**[89]
- **Synoviosarcoma**[87]
- **Rhabdomyosarcoma**[87]
- **Phaeochromocytoma**[88]
- **Cytotrophoblasts forming the placenta**[93,94]

Upregulation of expression of *NOTCH4* has also been detected in samples of invasive melanoma taken from patients (B. Nickoloff, personal communication), and interruption of NOTCH signalling induces apoptosis of malignant melanoma cells in culture[77]. Further investigation into NOTCH signalling mechanisms might offer clues to the regulation of the cell-fate decision-making pathways that are triggered in the undifferentiated tumour-cell phenotype.

These observations advance our understanding of the remarkable ability of the microenvironment to promote aggressive behaviour of tumour cells. This behaviour is associated with the upregulation of expression of vasculogenic, angiogenic and/or lymphangiogenic molecules, together with cell-fate determination proteins, leading to a transendothelial phenotype. These findings present new possibilities for therapeutic strategies and new perspectives on tumour-cell plasticity. They also emphasize the importance of early differentiation pathways, such as those involving NOTCH signalling, in cancer progression (for a review of the subject, see REF. 78).

Vasculogenic mimicry in non-melanoma tissues

There is a growing body of *in vivo* evidence that tumour cells can line channels, sinuses and vessel-like spaces (BOX 1). In addition, mosaic blood vessels have been observed in colon carcinomas and melanomas[54,58,60]. Ultrastructural studies are underway to map the tumour vasculature, on the basis of the heterogeneous expression of immunohistochemical markers[79]. Studies of aggressive breast carcinomas have reported vasculogenic mimicry, together with an absence of endothelial cells and lack of central necrosis in the tumour, indicating the presence of viable tissue without a traditional intra-tumoral vasculature[80]. Shirakawa and colleagues[24] have used dynamic micromagnetic resonance angiography analysis and LASER-CAPTURE MICRODISSECTION (LCM) to investigate the vascular connections between vasculogenic-mimicry networks in inflammatory breast-cancer xenografts and the systemic vasculature at the tumour margin. The use of LCM allowed the study of endothelium-associated gene expression in the inflammatory breast xenografts and provided evidence for the vascular phenotype of these tumour cells. However, it would be interesting to determine whether other phenotype-specific markers, such as lymphatic-specific molecules, are present also on the tumour cells. Other studies using breast-cancer tissue have indicated the presence of non-angiogenic, as well as angiogenic, pathways of tumour-cell dissemination[81].

Additional studies of ovarian carcinomas have provided *in vitro* and *in vivo* evidence that vasculogenic mimicry is associated with aggressive tumour cells and advanced-stage disease[82]. A follow-up blinded study that involved tissue analysis by two independent pathologists has shown a strong clinical correlation between the presence of tumour-cell-lined vasculature, advanced-stage disease and poor outcome[83]. However, only a small percentage (up to 15%) of the ovarian tumour vasculature is comprised of tumour cells, which might reflect the heterogeneity of a rapidly changing microenvironment and vascular supply. Recent reports on prostate cancer have indicated that vasculogenic mimicry occurs in aggressive tumour cells and is associated with high GLEASON GRADES[84]. In all of these studies, the functional relevance of the tumour-cell-lined vasculature and whether it occurs independently of the formation of laminin-rich, fluid-conducting meshworks are unclear.

Edgington and co-workers[85] have used a clever strategy of targeting prostate-specific membrane antigen, which is expressed by cancer cells that line tumour channels. This targeting results in the directed and selective thrombotic infarction of tumours. Lung carcinoma, synoviosarcoma, rhabdomyosarcoma and phaeochromocytoma have also shown evidence of vasculogenic mimicry[86–88]. In addition, studies of *Drosophila* tumours — which develop as a result of loss of the large tumour suppressor gene (*lats*) — have reported the formation of channel-like structures that surround spheroidal clusters of tumour cells. *Drosophila* have a duct system that is analogous to blood vessels[89]. These intriguing findings indicate a highly conserved mechanism for the perfusion of tumours, which develops independently of the need for blood circulation, in lower organisms.

Most noteworthy, however, is the range of various endothelium-associated genes that are expressed by different tumour types. For example, some aggressive melanoma-cell lines and tumours overexpress VE-cadherin[31], whereas aggressive breast, ovarian and prostatic tumour cells overexpress CD31 (REFS 82,84,90). All of these tumours have been shown to express CD34 (REFS 3,82,84,90–92). This potential difference in the level of expression of endothelium-associated genes by various tumour cells could be linked to the embryonic origin of the cell type(s) and their respective stages of transdifferentiation, a model that requires further investigation.

The formation of a microcirculation by cells other than endothelial cells has been reported in normal embryonic tissues. There is strong experimental evidence that human cytotrophoblasts can adopt an endothelial-cell phenotype as they participate in the establishment of the human placenta and primordial microcirculation.

LASER-CAPTURE MICRODISSECTION
(LCM). Energy from a low-power laser fitted to an inverted microscope is used to melt a thin vinyl film to precise locations on a tissue section to bind targeted cells. After the appropriate cells have been selected, the film and adherent cells are removed for gene-expression studies.

GLEASON GRADE
A grade of one (low grade) to five (high grade) is assigned to tumour-biopsy samples, based on how the cells look and how they are arranged. A lower Gleason grade indicates well-differentiated tumour cells, with a poor potential to spread. A higher Gleason grade indicates a poorly differentiated tumour, with a higher potential to spread.

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a Endothelial cells + endostatin

Angiogenesis is inhibited

b Melanoma cells + endostatin

Vasculogenic mimicry is unaffected

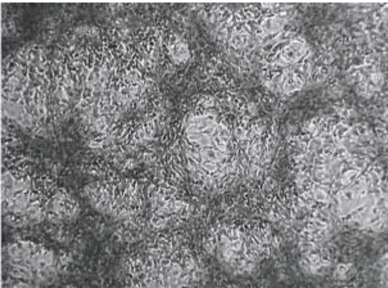

Figure 4 | **The differential effects of endostatin (an angiogenesis inhibitor) on endothelial cells compared with melanoma cells in three-dimensional gels of collagen I *in vitro*.** Phase-contrast microscopy of human microvascular endothelial cells (**a**) and human aggressive melanoma cells (**b**) treated with endostatin. Angiogenesis is inhibited in the human microvascular endothelial cells (**a**), as shown by the lack of vessel networks. Vasculogenic mimicry and network formation, however, are unaffected in the melanoma cells (**b**), as shown by the integrity of the morphologically distinctive, PAS-stained (black), vasculogenic-like networks. PAS, periodic acid Schiff.

This phenomenon has been termed 'pseudovasculogenesis'[93,94]. These studies indicate the existence of an intriguing link between the dysregulated tumour-cell phenotype and embryonic developmental programmes.

Clinical challenges and opportunities

Recent analyses of the molecular signatures of cancer cells and tissues[95] — in particular, of melanoma — have generated more questions than answers about the biological relevance of the phenotypes of aggressive tumour cells. The implications of these findings pose an important clinical challenge for detecting and targeting aggressive cancer cells, as these cells can 'look' like endothelial cells, angioblasts, leukocytes, macrophages or other cell types. However, great hope has been placed in the promise of microarray technology to improve the classification and clinical management of melanoma[1,96]. Without question, the 'plastic' phenotype of aggressive melanomas (and of other tumour types) confounds the fields of pathology and cancer biology, with respect to the proper evaluation of tumour blood supply and, in particular, microvascular density[91].

As aggressive cancer cells can phenotypically mimic other cell types, it is important that we improve the methods used to detect and identify melanoma cells, and to discriminate them from normal endothelial cells. For example, the use of two phenotype-specific detection markers is highly recommended when studying aggressive melanoma *in situ* — one marker should be tumour specific and the second marker could be endothelial-cell specific.

The existence of cancer stem cells adds to the challenge of cancer-cell detection[97]. Recent studies have challenged the previously held dogma regarding the tissue-restricted differentiation of postnatal stem cells, as evidence has shown the pluripotency of mesenchymal, neural and haematopoietic stem cells[29,98–100]. Transdifferentiation is emerging as an important phenomenon that adds a new level of complexity to developing rational therapeutic strategies[101–104]. It is interesting to note that during the development of Kaposi's sarcoma, endothelial cells transdifferentiate into tumour cells[105], whereas aggressive melanoma cells transdifferentiate to an endothelial-cell type. These observations raise the intriguing possibility that these two tumour-cell types could have a common origin or lineage. Although we have focused on vasculogenic mimicry in melanomas with respect to its potential to provide a perfusion pathway and dissemination route for aggressive tumours, mimicry of other cell phenotypes could provide other mechanisms to support tumour progression, such as immune evasion.

Vasculogenic mimicry is just one example of tumour-cell plasticity[106]. However, relatively little is known about the regulation of the molecular switch that controls this event. Our observations indicate the importance of VE-cadherin, EPHA2, laminin 5 γ2-chain, MMPs and NOTCH proteins as components of the vasculogenic switch, and strategies are being developed to inhibit the activity of these proteins. For example, monoclonal antibodies specific for VE-cadherin have been shown to inhibit endothelial-cell-driven angiogenesis in Lewis lung and epidermoid tumours[107], but it remains to be seen whether this therapeutic approach will have widespread use for other tumours. Blocking the activation of NOTCH4 triggers rapid apoptosis of melanoma cells, and interference with NOTCH signalling in melanoma cells results in down-regulation of the vasculogenic phenotype[77]. Targeting early angioblast-determination genes, such as those encoding NOTCH-family members, could have therapeutic potential for patients not only with melanoma, but also with several other aggressive cancers that are associated with overexpression of NOTCH proteins.

A great deal of intellectual capital has been devoted to targeting angiogenesis and lymphangiogenesis in patients with cancer[7–10,14,33,65–68,79,108–114]. The heterogeneity of the tumour vasculature presents an opportunity, as well as a clinical challenge, aside from issues of drug resistance [79,112,115,116]. Recent *in vitro* studies have shown that endostatin inhibits endothelial-cell-driven angiogenesis, but not the formation of melanoma-cell vascular networks[26] (FIG. 4). Similar *in vivo* observations have been reported (E. Sausville, personal communication). Gene-expression analysis of endothelial cells has shown that the level of expression of endothelium-specific genes, such as VE-cadherin and EPHA2, is reduced in endostatin-treated endothelial cells, but remains unaffected in melanoma cells[26]. This might indicate a differential drug response of two different vascular-cell phenotypes, and provides clues for the development of more effective anti-vascular drugs.

MMP inhibitors have also experienced challenges in clinical trials, but these proteinases are still worth consideration in the development of strategies to target the tumour microenvironment[47,117–119]. As we learn more about the pro-migratory inductive potential of proteolytically cleaved fragments of the ECM, it has become clear that these partially degraded molecules could be

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prime targets for therapeutic intervention — potentially for use in a combinatorial manner with other therapies (see the article by Raghu Kalluri on page 420 of this issue).

Successful management of malignant melanoma and other aggressive cancers could involve the targeting of one or more stages in the vasculogenic-mimicry signalling cascade. The identification of essential regulatory pathways of the undifferentiated, plastic tumour-cell phenotype that do not overlap with normal biological processes holds promise for the generation of new therapeutic strategies. The field of angiogenesis and anti-vascular targeting is filled with both challenges and hope as we continue to explore these intriguing scientific questions.

1. Bittner, M. *et al.* Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* **406**, 536–540 (2000).
This study reports the discovery of previously unrecognized subtypes of cutaneous melanoma, identified by mathematical analysis of differential gene-expression profiles.
2. Seftor, E. A. *et al.* Molecular determinants of human uveal melanoma invasion and metastasis. *Clin. Exp. Metastasis* **19**, 233–246 (2002).
3. Seftor, E. A. *et al.* Expression of multiple molecular phenotypes by aggressive melanoma tumor cells: role in vasculogenic mimicry. *Crit. Rev. Oncol. Hematol.* **44**, 17–27 (2002).
4. Risau, W. Mechanisms of angiogenesis. *Nature* **386**, 671–674 (1997).
5. Carmeliet, P. Mechanisms of angiogenesis and arteriogenesis. *Nature Med.* **6**, 389–395 (2000).
6. *Assembly of the Vasculature and Its Regulation* (ed. Tomanek, R. J.) (Birkhauser, Boston, 2002).
7. Folkman, J. Seminars in Medicine of the Beth Israel Hospital, Boston. Clinical applications of research on angiogenesis. *N. Engl. J. Med.* **333**, 1757–1763 (1995).
This paper describes the rationale and promise of targeting angiogenesis as a strategy for inhibiting tumour growth.
8. Rak, J. & Kerbel, R. S. Treating cancer by inhibiting angiogenesis: new hopes and potential pitfalls. *Cancer Metastasis Rev.* **15**, 231–236 (1996).
9. Kumar, R. & Fidler, I. J. Angiogenic molecules and cancer metastasis. *In Vivo* **12**, 27–34 (1998).
10. Carmeliet, P. & Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **407**, 249–257 (2000).
An excellent review of pathological angiogenesis in cancer and various ischaemic and inflammatory diseases.
11. Gullino, P. M. Angiogenesis and oncogenesis. *J. Natl Cancer Inst.* **61**, 639–643 (1978).
12. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
13. Bouck, N., Stellmach, V. & Hsu, S. C. How tumors become angiogenic. *Adv. Cancer Res.* **69**, 135–174 (1996).
14. Kerbel, R. S. Tumor angiogenesis: past, present and the near future. *Carcinogenesis* **21**, 505–515 (2000).
15. Maniotis, A. J. *et al.* Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry. *Am. J. Pathol.* **155**, 739–752 (1999).
The first paper to report the concept of vasculogenic mimicry by aggressive human melanoma cells.
16. Folberg, R., Hendrix, M. J. C. & Maniotis, A. J. Vasculogenic mimicry and tumor angiogenesis. *Am. J. Pathol.* **156**, 361–381 (2000).
17. Folberg, R. *et al.* The prognostic value of tumor blood vessel morphology in primary uveal melanoma. *Ophthalmology* **100**, 1389–1398 (1993).
18. Makitie, T., Summanen, P., Tarkkanen, A. & Kivela, T. Microvascular loops and networks as prognostic indicators in choroidal and ciliary body melanomas. *J. Natl Cancer Inst.* **91**, 359–367 (1999).
19. Sakamoto, T. *et al.* Histologic findings and prognosis of uveal malignant melanoma in Japanese patients. *Am. J. Ophthalmol.* **121**, 276–283 (1996).
20. Seregard, S., Spangberg, B., Juul, C. & Oskarsson, M. Prognostic accuracy of the mean of the largest nucleoli, vascular patterns, and PC-10 in posterior uveal melanoma. *Ophthalmology* **105**, 485–491 (1998).
21. Thies, A., Mangold, U., Moll, I. & Schumacher, U. PAS-positive loops and networks as a prognostic indicator in cutaneous malignant melanoma. *J. Pathol.* **195**, 537–542 (2001).
22. Warso, M. A. *et al.* Prognostic significance of periodic acid-Schiff-positive patterns in primary cutaneous melanoma. *Clin. Cancer Res.* **7**, 473–477 (2001).
23. Rummelt, V. *et al.* Microcirculation architecture of metastases from primary ciliary body and choroidal melanomas. *Am. J. Ophthalmol.* **126**, 303–305 (1998).
24. Shirakawa, K. *et al.* Hemodynamics in vasculogenic mimicry and angiogenesis of inflammatory breast cancer xenograft. *Cancer Res.* **62**, 560–566 (2002).
This study reports a haemodynamic connection between vasculogenic mimicry in inflammatory breast cancer and angiogenesis.
25. Garber, K. Angiogenesis inhibitors suffer new setback. *Nature Biotech.* **20**, 1067–1068 (2002).
26. van der Schaft, D. W. J. *et al.* The differential effects of angiogenesis inhibitors on vascular network formation by endothelial cells versus aggressive melanoma tumor cells. *Proc. Am. Assoc. Cancer Res.* **44**, 696A (2003).
27. McDonald, D. M., Munn, L. & Jain, R. K. Vasculogenic mimicry: how convincing, how novel, and how significant? *Am. J. Pathol.* **156**, 383–388 (2000).
28. Hess, A. R. *et al.* Molecular regulation of tumor cell vasculogenic mimicry by tyrosine phosphorylation: role of epithelial cell kinase (Eck/EphA2). *Cancer Res.* **61**, 3250–3255 (2001).
29. Ramalho-Santos, M. *et al.* 'Stemness': transcriptional profiling of embryonic and adult stem cells. *Science* **298**, 597–600 (2002).
30. Tachibana, M. *et al.* Ectopic expression of *MITF*, a gene for Waardenburg syndrome type 2, converts fibroblasts to cells with melanocytes characteristics. *Nature Genet.* **14**, 50–54 (1996).
31. Hendrix, M. J. C. *et al.* Expression and functional significance of VE-cadherin in aggressive human melanoma cells: role in vasculogenic mimicry. *Proc. Natl Acad. Sci. USA* **98**, 8018–8023 (2001).
This study provides direct evidence for the importance of vascular endothelial (VE)-cadherin expression by aggressive melanoma cells involved in vasculogenic mimicry.
32. Seftor, R. E. B. *et al.* Cooperative interactions of laminin 5 $\gamma 2$ chain, matrix metalloproteinase-2, and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. *Cancer Res.* **61**, 6322–6327 (2001).
33. Hynes, R. O., Bader, B. L. & Hodivala-Dilke, K. Integrins in vascular development. *Braz. J. Med. Biol. Res.* **32**, 501–510 (1999).
34. Hynes, R. O. Specificity of cell adhesion in development: the cadherin superfamily. *Curr. Opin. Genet. Dev.* **2**, 621–624 (1992).
35. Kemler, R. Classical cadherins. *Semin. Cell Biol.* **3**, 149–155 (1992).
36. Lampugnani, M. G. A novel endothelial-specific membrane protein is a marker of cell–cell contacts. *J. Cell Biol.* **118**, 1511–1522 (1992).
37. Gumbiner, B. M. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell* **4**, 345–357 (1996).
38. Pasquale, E. B. The Eph family of receptors. *Curr. Opin. Cell Biol.* **9**, 608–615 (1997).
39. Rosenburg, I. M., Goke, M., Kanai, M., Reinecker, H. C. & Podolsky, D. K. Epithelial cell kinase-B-61: an autocrine loop modulating intestinal epithelial migration and barrier function. *Am. J. Physiol.* **273**, G824–G832 (1997).
40. Straume, O. & Akslen, L. A. Importance of vascular phenotype by basic fibroblast growth factor, and influence of the angiogenic factors basic fibroblast growth factor/fibroblast growth factor receptor-1 and Ephrin-A1/EphA2 on melanoma progression. *Am. J. Pathol.* **160**, 1009–1019 (2001).
41. Easty, D. J. *et al.* Up-regulation of ephrin-A1 during melanoma progression. *Int. J. Cancer* **84**, 494–501 (1999).
42. Malinda, K. M. & Kleinman, H. K. The laminins. *Int. J. Biochem. Cell Biol.* **28**, 957–959 (1996).
43. Colognato, H. & Yurchenco, P. D. Form and function: the laminin family of heterotrimers. *Dev. Dynamics* **218**, 213–234 (2000).
44. Malinda, K. M. *et al.* Identification of laminin $\alpha 1$ and $\beta 1$ chain peptides active for endothelial cell adhesion, tube formation, and aortic sprouting. *FASEB J.* **13**, 53–62 (1999).
45. Koshikawa, N., Giannelli, G., Cirulli, V., Miyazaki, K. & Quaranta, V. Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. *J. Cell Biol.* **148**, 615–624 (2000).
46. Giannelli, G., Falk-Marzillier, J., Schiraldi, O., Stetler-Stevenson, W. G. & Quaranta, V. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. *Science* **277**, 225–228 (1997).
47. Seftor, R. E. B., Seftor, E. A., Kirschmann, D. A. & Hendrix, M. J. C. Targeting the tumor microenvironment with chemically modified tetracyclines: inhibition of laminin 5 $\gamma 2$ chain promigratory fragments and vasculogenic mimicry. *Mol. Cancer Ther.* **1**, 1173–1179 (2002).
This article shows the effectiveness of inhibiting the formation of pro-migratory signals in the tumour microenvironment, which results in the abrogation of melanoma vasculogenic mimicry.
48. Korpelainen, E. I. & Alitalo, K. Signaling angiogenesis and lymphangiogenesis. *Curr. Opin. Cell Biol.* **10**, 159–164 (1998).
49. Yancopoulos, G. D. *et al.* Vascular-specific growth factors and blood vessel formation. *Nature* **407**, 242–248 (2000).
50. Hess, A. R., Seftor, E. A., Seftor, R. E. B. & Hendrix, M. J. C. Phosphoinositide 3-kinase acts downstream of EphA2 to regulate the membrane-type 1 matrix metalloproteinase (MT1-MMP) and matrix metalloproteinase-2 (MMP-2) promoting vasculogenic mimicry *in vitro*. *Mol. Biol. Cell* **13**, 210A (2002).
51. *Annual Review of Cell and Developmental Biology* (eds Schekman, R., Goldstein, L., McKnight, S. L. & Rossant, J.) (Annual Reviews, Palo Alto, California, 2001).
52. Shubik, P. & Warren, B. A. Additional literature on 'vasculogenic mimicry' not cited. *Am. J. Pathol.* **156**, 736 (2000).
53. Warren, B. A. & Shubik, P. The growth of the blood supply to melanoma transplants in the hamster cheek pouch. *Lab. Invest.* **15**, 464–478 (1966).
54. Tímár, J. & Tóth, J. Tumor sinuses — vascular channels. *Pathol. Oncol. Res.* **6**, 83–86 (2000).
55. Hashizuma, H. *et al.* Openings between defective endothelial cells explain tumor vessel leakiness. *Am. J. Pathol.* **156**, 1363–1380 (2000).
56. Mueller, A. J. *et al.* An orthotopic model for human uveal melanoma in SCID mice. *Microvasc. Res.* **64**, 207–213 (2002).
57. Clarijs, R., Otte-Holler, I., Ruiter, D. J. & de Waal, R. M. W. Presence of a fluid-conducting meshwork in xenografted cutaneous and primary human uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* **43**, 912–918 (2002).
The first report of an extracellular-matrix, fluid-conducting meshwork in xenografts of human cutaneous and uveal melanoma.
58. Maniotis, A. J. *et al.* Control of melanoma morphogenesis, endothelial survival, and perfusion by extracellular matrix. *Lab. Invest.* **82**, 1031–1043 (2002).
This study provides evidence of plasma in the laminin-positive, fluid-conducting meshwork in aggressive melanoma, which indicates the presence of an extracellular-matrix conducting system.
59. Potgens, A. J. G., van Altena, M. C., Lubsen, N. H., Ruiter, D. J. & de Waal, R. M. W. Analysis of the tumor vasculature and metastatic behavior of xenografts of human melanoma cell lines transfected with vascular permeability factor. *Am. J. Pathol.* **148**, 1203–1217 (1996).
60. Chang, Y. S. *et al.* Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. *Proc. Natl Acad. Sci. USA* **97**, 14608–14613 (2000).
61. Döme, B., Paku, S., Somlai, B. & Tímár, J. Vascularization of cutaneous melanoma involves vessel co-option and has clinical significance. *J. Pathol.* **197**, 355–362 (2002).
62. Hendrix, M. J. C. *et al.* The plasticity of aggressive melanoma tumor cells: recapitulation of an embryonic stem cell program. *Rec. Adv. Res. Updates* **3**, 191–200 (2002).
63. Hattori, H. *et al.* Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1^{+} stem cells from bone-marrow microenvironment. *Nature Med.* **8**, 841–849 (2002).

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64. Clarijs, R., Schalkwijk, L., Ruiter, D. J. & de Waal, R. M. W. Lack of lymphangiogenesis despite coexpression of VEGF-C and its receptor Flt-4 in uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* **42**, 1422–1428 (2001).
65. Witte, M. H., Bernas, M. J., Martin, C. P. & Witte, C. L. Lymphangiogenesis and lymphangiodysplasia: from molecular to clinical lymphology. *Microscopy Res. Tech.* **55**, 122–145 (2001).
66. Alitalo, K. & Carmeliet, P. Molecular mechanisms of lymphangiogenesis in health and disease. *Cancer Cell* **1**, 219–227 (2002).
67. Padera, T. P. *et al.* Lymphatic metastasis in the absence of functional intratumor lymphatics. *Science* **296**, 1883–1886 (2002).
68. Laakkonen, P., Porkka, K., Hoffman, J. A. & Ruoslahti, E. A tumor-homing peptide with a targeting specificity related to lymphatic vessels. *Nature Med.* **8**, 751–755 (2002).
This paper provides evidence that tumour lymphatics express specific markers that can be targeted and used effectively to destroy lymphatic vessels.
69. Hendrix, M. J. C. *et al.* Transendothelial function of human metastatic melanoma cells: role of the microenvironment in cell-fate determination. *Cancer Res.* **62**, 665–668 (2002).
This study shows that human metastatic melanoma cells can carry out a transendothelial function and participate in the neovascularization of ischaemic tissue.
70. Uyttendaele, H., Ho, J., Rossant, J. & Kitajewski, J. Vascular patterning defects associated with expression of activated Notch4 in embryonic endothelium. *Proc. Natl Acad. Sci. USA* **98**, 5643–5648 (2001).
71. Gridley, T. Notch signaling during vascular development. *Proc. Natl Acad. Sci. USA* **98**, 10733–10738 (2001).
72. Ellisen, L. W. *et al.* TAN-1, the human homolog of the *Drosophila* notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**, 649–661 (1991).
73. Zagouras, P., Stifani, S., Blaumueller, C. M., Carcangiu, M. L. & Artavanis-Tsakonas, S. Alterations in Notch signaling in neoplastic lesions of the human cervix. *Proc. Natl Acad. Sci. USA* **92**, 6414–6418 (1995).
74. Robbins, J., Blonel, B. J., Gallahan, D. & Callahan, R. Mouse mammary tumor gene Int-3: a member of the Notch gene family transforms mammary epithelial cells. *J. Virol.* **66**, 2594–2599 (1992).
75. Shou, J., Ross, S., Koeppen, H., de Sauvage, F. J. & Gao, W.-Q. Dynamics of Notch expression during murine prostate development and tumorigenesis. *Cancer Res.* **61**, 7291–7297 (2001).
76. Weijzen, S. *et al.* Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. *Nature Med.* **8**, 979–986 (2002).
77. Qin, J.-Z. *et al.* Interrupting activated Notch signaling triggers apoptosis in melanoma cells. *Proc. J. Invest. Dermatol.* **185A**, 78 (2003).
78. Allenspach, E. J., Maillard, I., Aster, J. C. & Pear, W. S. Notch signaling in cancer. *Cancer Biol. Ther.* **1**, 466–476 (2002).
79. Pasqualini, R., Arap, W. & McDonald, D. M. Probing the structural and molecular diversity of tumor vasculature. *Trends Mol. Med.* **8**, 563–571 (2002).
80. Shirakawa, K. *et al.* Absence of endothelial cells, central necrosis, and fibrosis are associated with aggressive inflammatory breast cancer. *Cancer Res.* **61**, 445–451 (2001).
81. Pezzella, F. P. *et al.* Evidence for novel non-angiogenic pathway in breast-cancer metastasis. *Lancet* **355**, 1787–1788 (2000).
82. Sood, A. K. *et al.* Molecular determinants of ovarian cancer plasticity. *Am. J. Pathol.* **158**, 1279–1288 (2001).
83. Sood, A. K. *et al.* The clinical significance of tumor cell-lined vasculature in ovarian carcinoma: implications for anti-vasculogenic therapy. *Cancer Biol. Ther.* **1**, 511–517 (2002).
84. Sharma, N. *et al.* Prostatic tumor cell plasticity involves cooperative interactions of distinct phenotypic subpopulations: role in vasculogenic mimicry. *Prostate* **50**, 189–201 (2002).
85. Liu, C. *et al.* Prostate-specific membrane antigen directed selective thrombotic infarction of tumors. *Cancer Res.* **62**, 5470–5475 (2002).
This study reports a successful prostatic tumour-ablation strategy directed against prostate-specific membrane antigen-positive tumour cells that partially line the microvasculature, in combination with doxorubicin.
86. Passalidou, E. *et al.* Vascular phenotype in angiogenic and non-angiogenic lung non-small cell carcinomas. *Br. J. Cancer* **86**, 244–249 (2002).
87. Hao, X., Sun, B., Zhang, S. & Zhao, X. Microarray study of vasculogenic mimicry in bi-directional differentiation malignant tumor. *Zhonghua Yi Xue Za Zhi* **82**, 1298–1302 (2002) (in Chinese).
88. Favier, J., Plouin, P. F., Corvol, P. & Gasc, J. M. Angiogenesis and vascular architecture in pheochromocytomas: distinctive traits in malignant tumors. *Am. J. Pathol.* **161**, 1235–1246 (2002).
89. Potter, C. J., Turenchalk, G. S. & Xu, T. *Drosophila* in cancer research: an expanding role. *Trends Genet.* **16**, 33–39 (2000).
90. Hendrix, M. J. C., Seftor, E. A., Kirschmann, D. A. & Seftor, R. E. B. Molecular biology of breast cancer metastasis. Molecular expression of vascular markers by aggressive breast cancer cells. *Breast Cancer Res.* **2**, 417–422 (2000).
91. Chen, X., Maniotis, A. J., Majumdar, D., Pe'er, J. & Folberg, R. Uveal melanoma cells staining for CD34 and assessment of tumor vascularity. *Invest. Ophthalmol. Vis. Sci.* **43**, 2533–2539 (2002).
92. Hoang, M. P., Selim, M. A., Bentley, R. C., Burchette, J. L. & Shea, C. R. CD34 expression in desmoplastic melanoma. *J. Cutan. Pathol.* **28**, 508–512 (2001).
93. Zhou, Y. *et al.* Human cytotrophoblasts adopt a vascular phenotype as they differentiate: a strategy for successful endovascular invasion? *J. Clin. Invest.* **99**, 2139–2151 (1997).
94. Zhou, Y., Damsky, C. H. & Fisher, S. J. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype: one cause of defective endovascular invasion in this syndrome? *J. Clin. Invest.* **99**, 2152–2164 (1997).
95. Klausner, R. D. The fabric of cancer cell biology — weaving together the strands. *Cancer Cell* **1**, 3–10 (2002).
This article provides a global perspective of the many strands of cancer research and the future of intervention strategies.
96. Kim, C. J., Reintgen, D. S. & Yeatman, T. J. The promise of microarray technology in melanoma care. *Cancer Control* **9**, 49–53 (2002).
97. Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. Stem cells, cancer, and cancer stem cells. *Nature* **414**, 105–111 (2001).
98. Anderson, D. J., Gage, F. H. & Weissman, L. Can stem cells cross lineage boundaries? *Nature Med.* **7**, 393–395 (2001).
99. Weissman, I. L. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* **100**, 157–168 (2000).
100. Ivanova, N. B. *et al.* A stem cell molecular signature. *Science* **298**, 601–604 (2002).
101. Stocum, D. L. A tail of transdifferentiation. *Science* **298**, 1901–1903 (2002).
102. LaBarge, M. A. & Blau, H. M. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* **111**, 589–601 (2002).
103. Kon, K. & Fujiwara, T. Transformation of fibroblasts into endothelial cells during angiogenesis. *Cell Tissue Res.* **278**, 625–628 (1994).
104. Condorelli, G. *et al.* Cardiomyocytes induce endothelial cells to transdifferentiate into cardiac muscle: implications for myocardium regeneration. *Proc. Natl Acad. Sci. USA* **98**, 10733–10738 (2001).
105. Nickoloff, B. J. & Foreman, K. E. Etiology and pathogenesis of Kaposi's sarcoma. *Recent Results Cancer Res.* **160**, 332–342 (2002).
106. Bissell, M. J. & Radisky, D. Putting tumours in context. *Nature Rev. Cancer* **1**, 46–54 (2001).
An excellent overview of the interactions of tumour cells with their microenvironment, providing insights into new therapeutic strategies.
107. Liao, F. *et al.* Monoclonal antibody to vascular endothelial-cadherin is a potent inhibitor of angiogenesis, tumor growth, and metastasis. *Cancer Res.* **60**, 6805–6810 (2000).
108. Jain, R. K. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nature Med.* **7**, 987–989 (2001).
109. O'Reilly, M. S. Vessel maneuvers: vaccine targets tumor vasculature. *Nature Med.* **8**, 1352–1354 (2002).
110. Miller, K. D., Sweeney, C. J. & Sledge, G. W. Jr. The Snark is a Boojum: the continuing problem of drug resistance in the antiangiogenic era. *Ann. Oncol.* **14**, 20–28 (2003).
111. Scappaticci, F. A. Mechanisms and future directions for angiogenesis-based cancer therapies. *J. Clin. Oncol.* **20**, 3906–3927 (2002).
112. Gee, M. S. *et al.* Tumor vessel development and maturation impose limits on the effectiveness of anti-vascular therapy. *Am. J. Pathol.* **162**, 183–193 (2003).
113. Goetz, D. E., Yu, J. L., Kerbel, R. S., Burns, P. N. & Foster, F. S. High-frequency Doppler ultrasound monitors the effects of antivascular therapy on tumor blood flow. *Cancer Res.* **62**, 6371–6375 (2002).
114. Hood, J. D. *et al.* Tumor regression by targeted gene delivery to the neovasculature. *Science* **296**, 2404–2407 (2002).
115. Grossman, D. & Altieri, D. C. Drug resistance in melanoma: mechanisms, apoptosis, and new potential therapeutic targets. *Cancer Metastasis Rev.* **20**, 3–11 (2001).
116. Eberhard, A. *et al.* Heterogeneity of angiogenesis and blood vessel maturation in human tumors: implications for antiangiogenic tumor therapies. *Cancer Res.* **60**, 1388–1393 (2000).
117. Coussens, L. M., Fingleton, B. & Matrisian, L. M. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* **295**, 2387–2392 (2002).
118. Egeblad, M. & Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nature Rev. Cancer* **2**, 161–174 (2002).
A review of new functions associated with matrix metalloproteinases in cancer progression, with special emphasis on translational opportunities and strategies.
119. Liotta, L. A. & Kohn, E. C. The microenvironment of the tumour–host interface. *Nature* **411**, 375–379 (2001).

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Online links

DATABASES

The following terms in this article are linked online to:

Cancer.gov: http://www.cancer.gov/search/
melanoma

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
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FURTHER INFORMATION

Blood flow in aggressive human melanoma xenografts:
http://www.anatomy.uiowa.edu/hendrix_nat_rev_cancer/

ScienceDaily — never-before-seen look deep inside cancerous tumors: http://www.sciencedaily.com/releases/2001/07/010706075857.htm

The University of Illinois at Chicago Uveal Melanoma Research Program:
http://www.uic.edu/depts/mcpt/eyepath/resmel.htm

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