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Review

Vasculogenic Mimicry: Lessons from Melanocytic Tumors

KONSTANTINOS SPILIOPOULOS[1], DIMITRIOS PESCHOS[2], ANNA BATISTATOU[3], IOANNIS NTOUNTAS[4], NIKI AGNANTIS[2] and GEORGIOS KITSOS[1]

Departments of [1]Ophthalmology, [2]Physiology, and [3]Pathology, University Hospital of Ioannina, Ioannina, Greece; [4]First Department of Ophthalmology, 'G. Gennimatas' General Hospital of Athens, University of Athens, Athens, Greece

Abstract. *Tumor cell vasculogenic mimicry refers to the formation of tumor cell-lined vessels that contribute to tumor neovascularization and nutrient and oxygen supply. These tumor cells express many endothelial and stem cell markers, resulting in them having a unique phenotype. This phenomenon is observed in a variety of neoplasms, such as glioblastomas and sarcomas, as well as breast, ovarian, liver and lung carcinomas. It is also evident in melanocytic lesions, regardless of their benign or malignant nature. The biochemical and molecular events that regulate vasculogenic mimicry provide opportunities for development of novel forms of tumor-targeted treatments. Furthermore, the presence of this process in a tumor might have prognostic implications.*

Vasculogenic Mimicry: An Alternative Mode of Tumor Angiogenesis

Angiogenesis, from the Greek words angio (vessel) and genesis (birth), is the physiological process of new vessel formation from pre-existing vessels. Vasculogenesis is the process of blood vessel formation occurring by a *de novo* production of endothelial cells from recruited endothelial precursor cells. The development of neovasculature *via* angiogenesis is a vital component of many normal physiological processes and a number of disease states. Neovascularization is critical for the growth of solid malignant tumors and for the survival and development of metastases. Studies on tumor angiogenesis predominantly deal with malignant tumors, although examples for benign neoplastic counterparts can be found for almost every organ. Some of these benign variants might represent pre-malignant disease, *e.g.* colorectal adenomatous polyps. Benign proliferative lesions also require new vessel formation in order to grow. For many years there existed the notion that the only mechanism of tumor neovascularization was endothelial sprouting, which is an important angiogenic mechanism in normal growth and development, as well as in wound healing. However, scientists have recently recognized five modes of tumor angiogenesis: endothelial sprouting, intussusception (vessel splitting), co-option of existing vasculature, postnatal vasculogenesis by endothelial precursor cells, and vasculogenic mimicry (VM) (1-6) (Figures 1 and 2). For convenience, the term angiogenesis is used to describe all the aforementioned methods of blood vessel recruitment by tumors, although we know today that in at least two of the five modes, the actual process is or resembles vasculogenesis.

Correspondence to: Konstantinos Spiliopoulos, Department of Ophthalmology, University Hospital of Ioannina, Ioannina 45500, Greece. Mobile: +30 6909719915, e-mail: spiliopk@hotmail.com

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Definition of Vasculogenic Mimicry

VM is the *de novo* generation of vascular channels usually by aggressive or metastatic tumor cells and is not considered a strictly vasculogenic event because true vasculogenesis results in *de novo* formation of endothelial cell-lined vessels. Maniotis, Hendrix and colleagues were the first to observe and describe this phenomenon in 1999 and introduced the term vasculogenic mimicry to underscore the *de novo* formation of vascular structures and at the same time to differentiate it from true vasculogenesis (7). Since then, extended research on VM has been conducted. Maniotis *et al.* observed solid and hollow periodic acid-Schiff (PAS)-positive patterned microcirculatory (microvascular) networks of interconnected loops of extracellular matrix in histological sections of primary and metastatic areas of human uveal melanomas (7). It is noteworthy that 45% of primary uveal melanomas had areas of VM. By using light and electron microscopy, as well as melanocytic and endothelial cell markers (immunohistochemistry), they discovered that these microcirculatory networks or capillary-like structures are

composed of extracellular matrix and lined externally by melanoma cells, while endothelial cells are absent. Red blood cells were noted in the lumen of the channels. The inner layer of melanoma cell-lined microvascular structures is composed of extracellular matrix proteins such as laminin, collagens IV and VI, and heparan sulfate proteoglycans. Aggressive uveal and cutaneous melanoma cells were able to reconstitute patterned solid and hollow matrix channels *in vitro* without the presence of endothelial cells, fibroblasts or soluble growth factors in three-dimensional cultures containing Matrigel or dilute type-1 collagen (7-10). PAS stain as well as antibodies against endothelial cell markers CD31 or CD34 have been the gold-standard to characterize and study tumor cell VM. Of note, CD31 is a platelet endothelial cell adhesion molecule: PECAM1. VM channels are PAS-positive, CD31 (or CD34)-negative channels. VM networks were detected *in situ* in 34% of tumor primary sites in patients with human cutaneous melanoma, indicating that VM network prevalence in human cutaneous and uveal melanomas is probably similar (7, 11).

The four main characteristics of VM networks are a tumor cell-lined vasculature, the expression of a primitive phenotype by tumor cells with predominant stem cell and endothelial cell markers, a functional connection of VM networks to the endothelial cell-lined vessels, and the existence of antithrombotic agents that facilitate the blood flow in and out as well as through those networks, making them functional and important for tumor nutrient and oxygen supply.

VM has been found and studied in a variety of malignant tumor types, with malignant melanoma (both uveal and cutaneous) being the preferred tumor type for many researchers. The current review article focuses on studies of VM in malignant melanomas as well as in benign melanocytic lesions.

Functional Importance of VM

There has been a debate on whether VM networks are functional or are just an artifact of tumor cells positioned near blood vessels, or a replacement of endothelial cells by tumor cells when the latter invade blood vessels. Chang and colleagues observed mosaic vessels in which both endothelial and tumor cells lined the luminal surface of colon carcinoma xenografts and assumed that cancer cells might become exposed to the lumen upon shedding of existing endothelial cells, or that some endothelial cells might lose CD31 immunoreactivity (12, 13). The concept of mosaic vessels does not necessarily contradict the existence of VM and endothelial cell-lined vessels and tumor cell-lined vessels might actually be the two ends of a spectrum between which different percentages of tumor and endothelial cells line the vessel walls. Some authors report that VM predominates in early tumor growth and endothelial cell-lined vessels are the major pattern of microcirculation when the tumor size has considerably increased, with mosaic vessels predominating at the intermediate stage of tumor growth. Of note, at a very early stage, when tumor size is less than 2 mm, there is no need for neovascularization and a tumor can obtain nutrient and oxygen supply from the surrounding normal tissue simply through diffusion (14).

It has been shown with the use of tracers that VM networks can transfer fluid in aggressive uveal melanoma cell cultures *in vitro* (7, 15) and in murine xenografts of human cutaneous melanoma (9, 16). VM networks have also been shown not only to transfer (17) but also to circulate indocyanine green *in vivo* in patients with uveal melanoma (10), raising the possibility that these networks are functional and constitute an alternative route of tumor oxygen and nutrient supply. Moreover, *in vivo* Doppler ultrasound study with the use of microbubbles in a murine xenograft of human cutaneous melanoma revealed that there is a functional connection and physiological perfusion of blood between endothelium-lined mouse vessels and regions of human melanoma VM (18).

Research Models for VM

Hendrix and co-workers not only discovered the VM phenomenon but have also contributed much to VM research and the delineation of the molecular mechanisms that underlie it. A significant part of VM research has been conducted using *in vitro* systems. Specifically, three-dimensional collagen I gels are frequently used as substrate for the development of VM patterns from melanoma cells. The gels are produced as described by Hess *et al.* (19). Less often, three-dimensional Matrigel gels are used for the same purpose (7). The human cutaneous C8161 (aggressive) and C81-61 (poorly aggressive) melanoma cell lines and the human uveal MUM-2B (aggressive), C918 (aggressive) and MUM-2C (poorly aggressive) melanoma cell lines are the most frequently used cell lines in VM research. Murine allograft and xenograft models have also been used in VM studies with the immunodeficient NOD/SCID mouse, the immunocompetent C57BL/6 mouse and the albino BALB/c mouse being some of the most frequent animal models used. B16F10 murine melanoma cells are frequently used for the production of murine allograft models, while the aforementioned human melanoma cell lines are used for the production of murine xenograft models.

Characteristics of Cells Lining VM Channels

There are two dominant theories about the ontogeny of VM network-forming cells. The first argues that these cells are tumor cells that have undergone a de-differentiation process

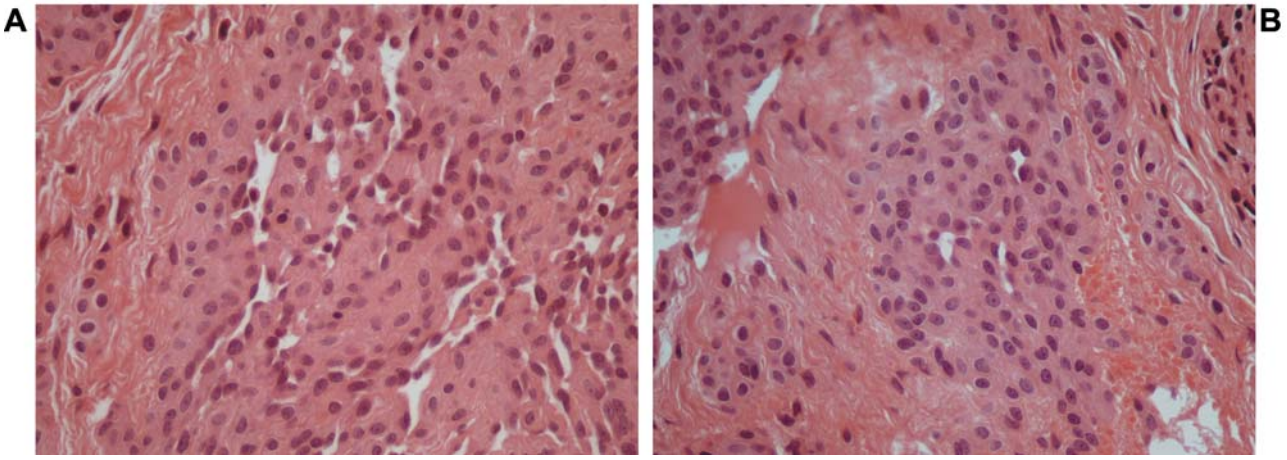

Figure 1. *Vasculogenic mimicry in benign dermal nevus. Note the vascular-like channels (A), also on cross section (B), formed by nevus cells (hematoxylin-eosin staining, magnification ×400).*

resulting in a primitive cell type which encompasses tumor cell, stem cell, endothelial cell and other cell-type characteristics. The second notion holds that VM network-forming cells arise from cancer stem cells (20-22).

Melanoma VM channel-forming cells resemble undifferentiated, primitive, embryonic-like stem cells based on their gene expression and molecular signature (20). Specifically these cells express genes associated with multiple cell lineages, including endothelial, epithelial, and hematopoietic cells, as well as neurons and myocytes (23). Furthermore, these cells express several genes associated with embryonic stem cells such as NODAL (24, 25). Melanoma cells expressing the ATP-binding cassette (ABC) member ABCB5 are considered to be 'melanoma stem cells' or 'malignant melanoma-initiating cells' and have VM-forming capability (26, 27). The ABC superfamily consists of active membrane transporters that are expressed on stem/primitive cells and mediate multi-drug resistance. CD133 (human prominin 1), a transmembrane pentaspan glycoprotein of unknown function found in stem and early progenitor cells, is colocalized with ABCB5 antigen, while CD133 knockdown down-regulated expression of ABCB5 and vascular endothelial (VE)-cadherin (also known as cadherin-5 or CD144) and abolished VM formation in murine xenografts of human melanoma (28). Uveal melanoma cells that are able to form VM channels express the tumor stem cell marker CD271 (also known as nerve growth factor receptor, NGFR or p75NTR) which has a role in cell survival and cell death. Non-VM-forming uveal melanoma cells did not express this marker (29).

Microarray analysis from over 30 human cutaneous melanoma cell lines found that many angiogenesis/vasculogenesis-related genes such as those encoding endothelial cell-specific vascular endothelial-cadherin (VE-cadherin), tyrosine kinase with Ig-like and epidermal growth factor like domains 1 (TIE1), and fibronectin-1 as well as many epithelial-associated genes such as those encoding ephrin type-A receptor 2 (EphA2) are overexpressed in aggressive melanoma cells in comparison to poorly aggressive cells (23). Moreover angiogenesis-related genes such as those for different ephrin ligands and vascular endothelial growth factor (VEGF) were found to have higher expression in vascular-like network areas *vs*. randomly arranged cell areas (nests) of aggressive cutaneous and uveal melanoma cell cultures (30).

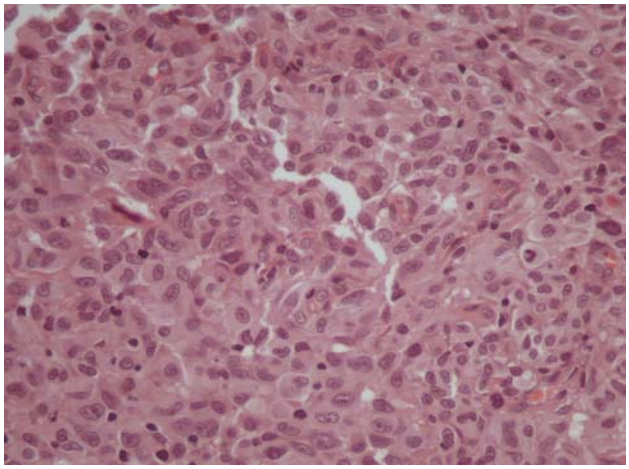

Figure 2. *Vasculogenic mimicry in malignant melanoma of skin. Note the vascular-like channel (center) formed by melanoma cells (hematoxylin-eosin, ×400).*

Molecules and Signaling Cascades Involved in VM

EphA2/VE-cadherin/focal adhesion kinase (FAK). Vascular signaling pathways have a predominant role in VM network formation and some molecules implicated in these pathways such as EphA2 and VE-cadherin constitute defining features of VM. The ephrin receptor family is the largest family of receptor tyrosine kinases, consisting of transmembrane proteins, and plays a role in embryonic vasculogenesis and adult angiogenesis. Microarray analysis revealed that aggressive melanoma tumor cells express many different ephrin receptors and ligands, including EphA2 and its ligand Ephrin-A1 (23, 30, 31). EphA2 is expressed in epithelial cells and plays an important role in adult angiogenesis and tumor neovascularization (32). It was found to be highly expressed and phosphorylated in aggressive, VM network-forming uveal melanoma tumor cells *in vitro* but not expressed in poorly-invasive melanoma cells, and its down-regulation abrogated VM formation (19). Inhibition of EphA2 expression in aggressive VM network-forming cutaneous melanoma cells *in vitro* led to limitation of their ability to engage in VM (33).

VE-cadherin, a transmembrane protein, is an endothelial-specific cell adhesion molecule that is important for embryonic vasculogenesis. It was found to be strongly expressed in aggressive cutaneous and uveal VM network-forming melanoma cells but not in poorly aggressive melanoma cells under culture, while its knockout abolished VM formation (34). Genistein, a isoflavone phytoestrogen, inhibits VM formation in uveal melanoma cell cultures and in mice xenografted with human uveal melanoma cells by down-regulating VE-cadherin expression (35). Similarly, lycorine hydrochloride, the main active component of the traditional Chinese medicinal herb Lycoris radiata, also inhibits VM formation in cutaneous melanoma cell cultures and in mice xenografted with human cutaneous melanoma cells by down-regulating VE-cadherin expression (36).

Both EphA2 and VE-cadherin co-localized in cell–cell adhesion junctions in human metastatic cutaneous and uveal melanoma cells in culture, as well as in uveal melanoma tissue sections. Knockout of VE-cadherin expression resulted in redistribution of EphA2 on the cell membrane, down-regulation of its phosphorylation, loss of EphA2 from cell–cell adhesion complexes and its concomitant appearance in the cytoplasm (37). Knockout of EphA2 expression did not alter VE-cadherin localization.

Downstream of EphA2 and VE-cadherin, there are some cytoplasmic kinases such as FAK and phosphoinositide-3-kinase (PI3K) that are phosphorylated during signal transduction. FAK is phosphorylated on its key tyrosine residues in aggressive cutaneous and uveal melanoma cells in culture but not in less aggressive cutaneous and uveal melanoma cells or normal melanocytes. FAK phosphorylation was also found in aggressive cutaneous and uveal melanoma *in situ*. FAK-related non-kinase (FRNK) which disrupts FAK signaling, inhibited VM formation in aggressive melanoma cell cultures by down-regulating extracellular signal-regulated kinases 1 and 2 (ERK1/2) and in turn urokinase activity. Of note, FRNK did not have any effect on membrane type 1 matrix metalloproteinase (MT1-MMP) and MMP2 activities. Direct ERK1/2 inhibition resulted in decreased urokinase and MT1-MMP and MMP2 activities, indicating that there might be an alternative signal transduction pathway independent of FAK signaling which is responsible for ERK1/2 phosphorylation, such as the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway (38, 39). The latter pathway is partially responsible for the inhibitive action of forskolin on VM formation in aggressive cutaneous and uveal melanoma cells *in vitro* (40). This organic compound, produced by the Indian Coleus plant, acts by raising the cellular cAMP level and also inhibits VM formation *via* PI3K inhibition, as well as *via* exchange protein directly activated by cAMP/Ras-related protein 1 (Epac/Rap1) activation (40). Curcumin, a natural phenol, inhibited VM formation in a murine allograft choroidal melanoma model through down-regulation of EphA2, PI3K and MMP2 (41).

Extracellular matrix and MMPs. The cascade initiated with EphA2 and VE-cadherin up-regulation and continued by phosphorylation of specific cytoplasmic kinases results in up-regulation of some MMPs, resulting in the cleavage of a specific laminin, with subsequent remodeling of the extracellular matrix which constitutes the tumor cell microenviroment.

Extracellular matrix is not a passive and inactive component of tissues, as previously regarded, but actually interacts with cells. It exerts its role on cells primarily *via* laminins which are basement membrane glycoproteins and consequently extracellular matrix components that bind to cell membranes through integrin receptors thus participating in and modulating multiple important cell functions such as cell attachment and differentiation. Conversely, cells are able to act on and modify the extracellular matrix. For example, poorly aggressive cutaneous melanoma cells seeded on collagen I matrices preconditioned by aggressive melanoma cells formed VM networks along the laminin 5 γ2 chain-enriched tracks deposited by the aggressive cells and expressed a gene panel characteristic of aggressive cells (42, 43). MMPs are zinc-dependent endopeptidases capable of degrading all kind of extracellular matrix proteins, as well as a number of bioactive molecules, and participate in many cell functions, such as cell proliferation, differentiation and migration.

It has been shown that levels of MT1-MMP (also known as MMP14), MMP2 and laminin 5 γ2 chain are elevated in aggressive cutaneous and uveal melanoma cells *in vitro* and

these proteins co-localize with VM areas, while their expression is decreased in poorly-aggressive cutaneous and uveal melanoma cells. Antibodies against MT1-MMP or MMP2, or inhibition of laminin 5 γ2 chain abrogated VM formation (42). Inhibition of PI3K led to decreased MT1-MMP, as well as decreased MMP2 activity, and blocked the cleavage of laminin 5 γ2 chain, resulting in decreased levels of γ2' and γ2x pro-migratory fragments in aggressive cutaneous and uveal melanoma cell cultures. It also inhibited VM formation by these cells (44). Thalidomide inhibited VM formation in a murine allograft melanoma model *in vivo*, through down-regulating MMP2 and VEGF (45). Doxycycline also reduced MMP2 expression and impaired VM formation in a murine allograft melanoma model (46). Chemically modified tetracycline-3 (CMT-3, also known as COL-3) inhibited the expression of MMP2 and VE-cadherin *in vitro* in both aggressive cutaneous and uveal melanoma cells, while it inhibited the expression of MT1-MMP and TIE1 in aggressive cutaneous and uveal melanoma cells, respectively, and impaired VM formation by both cutaneous and uveal aggressive cells. It also inhibited VM formation by poorly aggressive melanoma cells seeded onto an aggressive cell-preconditioned matrix by down-regulating laminin 5 γ2 chain pro-migratory fragment production (47). Vadimezan (DMXAA or AS1404), a small vascular-disrupting molecule, inhibited MMP2 expression and prevented VM formation in aggressive melanoma cell cultures, while the co-addition of SB203580, a selective inhibitor of p38 MAPK restored VM formation without influencing MMP2 expression (48).

Embryonic stem cell molecules. As previously mentioned, several stem cell molecules have been found to be overexpressed in tumor cells forming VM structures. Two important such molecules are Nodal and Notch. Nodal is a morphogen and regulator of stem cell fate which is expressed during embryogenesis and belongs to the transforming growth factor beta (TGFβ) superfamily. The Notch receptor family is also critical for the determination of embryonic stem cell fate and consists of 4 transmembrane receptors including Notch4, and 5 membrane-bound ligands. Notch4 is expressed in endothelial cells and participates in vascular formation and remodeling, and in tumor angiogenesis. Notch4 was exclusively expressed in aggressive melanoma cell lines *in vitro* as opposed to other members of the Notch family. Notch4 down-regulation reduced Nodal expression in aggressive melanoma cell lines. Inhibition of Notch4 activity impaired VM formation in aggressive melanoma cells, while the addition of exogenous Nodal protein to these cells restored VM formation. Nodal and VE-cadherin expression was also restored in these cells by exogenously expressed Notch4. This leads to the conclusion that Notch4 promotes VM formation by up-regulating Nodal expression (49). Nodal mRNA was restricted to melanoma cells within murine xenografts that formed VM networks (24). Human aggressive cutaneous melanoma cells treated with function–blocking antibodies against Nodal showed reduced VM-forming capacity (50).

Hypoxia, hypoxia-inducible factor-1alpha (HIF1α), reactive oxygen species (ROS) and VEGF. Tissue hypoxia plays a central role in tumor progression and VM network formation. Hypoxia-inducible factors are transcription factors that respond to changes in tissue oxygen concentration. Hypoxia stabilizes HIF1α, which in turn up-regulates several genes such as VEGF, to promote survival under low-oxygen conditions. Hypoxia induced the expression of HIF1α, MMP2 and VEGF, and promoted VM formation *in vivo*, as was shown by inoculation of mouse melanoma cells into mouse ischemic limbs and non-ischemic controls, leading eventually to equal size melanomas in ischemic and non-ischemic limbs despite the initial slower tumor size increase in ischemic limbs (51).

B-cell lymphoma-2 (BCL2) oncogene might also be implicated in VM network induction by hypoxia in melanoma cell cultures (52). Incubation of aggressive melanoma cells under hypoxic conditions increased ROS and HIF1α levels and revealed an enhanced VM network-forming capability in cell cultures, while inhibition of HIF-1α resulted in abrogation of VM network formation in cell cultures and mouse allografts. Furthermore, inhibition of ROS resulted in abrogation of VM network formation in mouse allografts (53). These findings might partially explain the failure, short-term efficacy, or even the pro-metastatic effect of antiangiogenic therapies which act by limiting tumor neovascularization and inducing tumor hypoxia (54-56). Antioxidants reduced ROS, VEGF, VEGFR1, VEGFR2 and abolished VM formation in cutaneous melanoma cells *in vitro* and also inhibited VM formation in mouse allografts (57).

Angiogenesis inhibitors anginex, TNP-470 and endostatin did not abolish VM network-forming capacity of aggressive cutaneous and uveal melanoma tumor cell cultures (58).

VEGFR1 has tyrosine protein kinase activity and is a transmembrane receptor of VEGFRA, with a critical physiological role in angiogenesis. VEGFR1 is highly expressed in ABCB5-positive melanoma cell subpopulations, which are malignant melanoma-initiating cells and are associated with VM. Knockdown of VEGFR1 inhibited VM formation in murine xenografts of human melanoma (26). VEGFR3 has tyrosine protein kinase activity and is a transmembrane receptor of VEGFRC and D, with an important role in lymphangiogenesis. VEGFR3 also seems to have a role in VM network formation *in vitro* by cutaneous melanoma cells (59). Endothelin-1–endothelin receptor B pathway participates in stromal–melanoma cell interactions promoting tumor growth, neovascularization, lymphangiogenesis and metastasis. There is cross talk

between endothelin receptor B and VEGFR3 (59). Endothelin-1, in combination with VEGFC, further increased VEGFR3 phosphorylation and VM network formation in comparison to isolated use of Endothelin-1 or VEGFC (59). Of note, human uveal melanomas metastasize exclusively through blood vessels, while in cutaneous melanomas, the lymphatic system aIso plays an important role.

Other molecules and signal transduction pathways. In order for VM channels to be functional, blood flow through them must be facilitated and it seems that this at least partially takes effect *via* inhibition of blood clotting. Tissue factor (TF), TF pathway inhibitor 1 (TFPI1) and 2 (TFPI2) are up-regulated in aggressive cutaneous and uveal melanoma cells *in vitro* compared to poorly-aggressive cutaneous and uveal melanoma cells. TF is the initiating cell surface receptor of the coagulation cascade (extrinsic coagulation pathway) and serves as a cofactor for VIIa. The procoagulant function of TF in highly aggressive melanoma cells is regulated by TFPI1, a reversible inhibitor of factor Xa, but not by TFPI2. This effect of TFPI1 might facilitate the fluid-conducting capability of VM networks. TFPI2 is important for VM formation through an alternative, coagulation-independent mechanism. Antibody to TFPI2 suppressed MMP2 activity and also completely suppressed VM network formation in aggressive cutaneous and uveal melanoma cell cultures (18).

Additional molecules such as extracellularly secreted proteins *e.g.* pigment epithelium-derived factor and bone morphogenic protein 4, or intracellular molecules *e.g.* inhibitor of DNA binding/differentiation protein 2 and caspace-3, have been found to participate in VM channel formation.

Pigment epithelium-derived factor is a secreted glycoprotein that belongs to the serin protease inhibitor superfamily. Pigment epithelium-derived factor does not have serin protease inhibitor action and probably binds to cell surface receptors to trigger various signaling cascades. It has anti-angiogenic and direct antitumor action. Its knockdown in poorly aggressive cutaneous melanoma cell lines augmented VM formation *in vitro* (60).

Another extracellular protein, bone morphogenic protein (BMP)4, promotes VM network formation. BMPs are secreted growth factors that belong to the TGFβ superfamily and which control many cell activities such as proliferation, differentiation, chemotaxis, motility and cell death in various cell types such as epithelial and mesenchymal cells. They are also embryonic morphogens and linked to tumor formation and progression. BMP4 inhibition resulted in down-regulation of EphA2 as well as VE-cadherin expression, and abrogated VM formation by aggressive cutaneous melanoma cells *in vitro* (61).

Intracellular proteins are also significant factors in VM network formation. Inhibitors of DNA binding/differentiation (Id) protein 2 is an example. Id proteins belong to a family of proteins that heterodimerize with basic helix-loop-helix transcription factors to inhibit their binding with DNA. Id proteins contain the helix-loop-helix-dimerization domain but lack the basic DNA-binding domain and thus negatively regulate basic helix-loop-helix transcription factors when they heterodimerize with them. Among other functions, they are associated with the differentiation of stem cells and tumor cells. Knockdown of Id2 in aggressive uveal melanoma cells *in vitro* resulted in decreased VE-cadherin expression and abrogated VM formation (62).

Caspase-3, another intracellular protein, is linked to VM network construction. Caspases (or cysteine-aspartic proteases or cysteine-dependent aspartate-directed proteases) are a family of cysteine proteases that participate in apoptotic cell death and several nonapoptotic cellular processes such as proliferation, differentiation and migration. Higher levels of active caspase-3 were detected in nonapoptotic cutaneous melanoma cells *in situ* that had formed VM networks compared to non-VM network-forming cells. Using low doses of caspase-3 inhibitor that reduced caspase-3 activity without affecting cell apoptosis resulted in decreased VM network formation in cutaneous melanoma cell cultures *in vitro*, indicating that caspase-3 has an important role in VM network formation, probably by cleaving pro-MMP2 to produce MMP2 (63). It seems that active caspase-3 also promotes VM network formation through its apoptotic effect. Apoptosis takes place before VM network formation. Active caspase-3 was initially undetectable, but appeared within a few hours and progressively accumulated in VM-forming melanoma cell cultures. The apoptotic index was higher in aggressive VM network-forming cutaneous melanoma cells *in vitro* than poorly aggressive non-VM network-forming cells (64). Blockage of caspase activity using a broad-range caspase inhibitor or a caspase-3 inhibitor blocked VM network formation (65).

Galectin-3, a lectin family member, is a β-galactoside-binding protein that is involved in cancer progression and metastasis. It is expressed in the nucleus, cytoplasm, cell surface and extracellular space. Galectin-3 knockout reduced VE-cadherin and fibronectin-1 expression and abolished VM formation by aggressive cutaneous melanoma cells *in vitro*, and reduced VE-cadherin and MMP2 expression in a murine xenograft (66).

Clinical Significance of VM and Future Prospects

The presence of VM patterns in patients with malignant tumors has been shown to be an independent negative prognostic factor in many cases. Patients with VM channel-forming melanomas, both uveal and cutaneous, had worse prognosis and shorter survival than their non-VM channel-forming counterparts (8, 11, 67-70). The same applies to other malignancies but not to all malignant tumors studied (70).

There is a paucity of studies on angiogenesis in benign tumors. Microvessel density is frequently used as an index to estimate the extent of angiogenesis in tumors. The microvessel density is higher in cutaneous malignant melanoma lesions compared to benign nevi (71), indicating that neovascularization is less pronounced in benign nevi. Indications of VM in benign nevi have been found in a few studies. Melanocyte-lined 'pseudovascular spaces' were observed in three specimens of benign pigmented nevus (72), in three specimens of benign melanocytic nevus (73) and in one of benign melanocytic nevus, while intralesional injection of local anesthetic failed to reproduce these spaces indicating that they are not an injection artifact (74).

Further research on benign, and particularly dysplastic, nevi will clarify whether VM networks exist in some of these lesions. This might help in defining which of these lesions harbor a potentially aggressive profile and are at higher risk for transformation to malignant melanoma (75). VM pattern identification could also be used in the future in conjunction with clinical and histopathological tumor features, as well as in aiding the rapidly advancing field of molecular genetic diagnostics for more accurate prognostication in both cutaneous and uveal melanomas (76-78) and other tumor types.

Without doubt, more research on VM needs to be conducted for further delineation of the molecular mechanisms and signal pathways that are implicated in this process. This will lead to the discovery and testing of drugs that disrupt these pathways. Targeting tumor blood supply provided through VM networks with some of the drugs that have been shown to have *in vitro* efficacy and were mentioned in this review in conjunction with anti-angiogenic agents suppressing endothelium-lined neovessels or other anticancer therapies available in our armamentarium might improve the efficacy of current cancer treatments.

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