

REVIEW ARTICLE

Vasculogenic mimicry and tumor metastasis

Jingxin Zhang^{1*}, Lili Qiao^{2*}, Ning Liang³, Jian Xie³, Hui Luo¹, Guodong Deng², Jiandong Zhang³

¹Department of Graduate, Weifang Medical College, Weifang, Shandong, China, ²Department of Oncology, Shandong University of Medicine, Jinan, Shandong, China, ³Department of Radiation Oncology, Qianfoshan Hospital Affiliated to Shandong University, Jinan, Shandong, China.

*These authors contributed equally to this work and are considered co-first authors

Summary

Vasculogenic mimicry (VM), a microvascular channel made up of nonendothelial cells, has been accepted as a new model of neovascularization in aggressive tumors, owing to the specific capacity of malignant cells to form vessel-like networks which provide sufficient blood supply for tumor growth. Multiple molecular mechanisms, especially vascular endothelial (VE)-cadherin, erythropoietin-producing hepatocellular receptor A2 (EphA2), phosphatidylinositol 3-kinase (PI3K), matrix metalloproteinases (MMPs), vascular endothelial growth factor receptor (VEGFR1), and hypoxia inducible factor (HIF)-1 α , have been reported to par-

ticipate in VM formation which is associated with tumor migration and invasion. In addition, hypoxia, cancer stem cells (CSCs) and epithelial-mesenchymal transition (EMT) are regarded as significant factors in VM formation and tumor metastasis. Due to the important effects of VM on tumor progression, a review was carried out in the present study, to synthetically analyze the relationship between VM and tumor metastasis.

Key words: clinical significance, molecular mechanisms, tumor metastasis, vasculogenic mimicry

Introduction

Malignant tumors possess the capabilities of survival, growth, invasion, and metastasis. Two models of forming blood vessels were shown to be implicated in tumor progression: vasculogenesis [1], reorganizing randomly distributed incorporation of cells implanting into a blood vessel network, and angiogenesis [2], a form of new vessels from preexisting vasculature because of external chemical stimulation. Numerous studies have paid much attention to the roles of angiogenesis, showing that new blood vessels are recruited by tumor cells from the existing vasculature [3] and the factors secreted by tumor cells [4,5], or the surrounding stromal cells [6]. Besides traditional tumor angiogenesis and vasculogenesis, VM, which was first elaborated in 1999, is a new phenomenon about fluid-conducting channels formed

by highly aggressive melanoma cells [1]. In VM microcirculatory channels lined by nonendothelial cells are generated by pluripotent embryonic stem cells, highly invasive tumor cells and the extra-cellular matrix in aggressive primary and metastatic tumors. VM mimics the function of blood vessels that allows red blood cells (RBCs) to flow through them, and provides an alternative mechanism to supply malignant tumors with adequate blood [7]. When the growth of blood vessels depending on endothelial cells is unable to keep pace with the tumor tissues' growth, some tumor cells change their original functions to imitate those of endothelial cells. These findings demonstrated that VM plays a significant role in blood supply in malignant tumors [1,2,7].

From then on, VM has been found in many

malignant tumors such as breast cancer, liver cancer, glioma, ovarian cancer, melanoma, prostate cancer, and bidirectional differentiated malignant tumors, and the existence of VM was confirmed through many advanced technologies such as laser scanning confocal angiography, electron microscopy, and three-dimensional (3D) cell culture. Previous studies mainly aimed at analyzing the important roles of VM in tumor growth, but no systematic elucidation regarding the relationship between VM and tumor metastasis exists. Therefore, in the present study, a review was made to provide an updated study on VM in tumor metastasis, further discussing the origin of VM, current status of studies on VM in tumors, molecular mechanisms of VM, factors affecting VM formation in tumor metastasis, and clinical significance of VM formation in tumor metastasis.

The origin of vasculogenic mimicry

A great deal of studies in pathology described a high degree of plasticity related to aggressive cancer. However, researchers did not have enough tools to clarify the pathogenesis or the biological significance of tumor cell plasticity. In 1999, the American Journal of Pathology published an article presenting a new interpretation of previous findings. The new interpretation described cancer cells lined by nonendothelial vascular channels within a tumor mass that contained RBC's [8]. The article "Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry" written by Maniotis et al. [1], evoked a bouncing debate for several years. Subsequently, a positive and controversial commentary about the significance of this article was published. "Tumor plasticity allows vasculogenic mimicry, a novel form of angiogenic switch" reported by Bissell [9] was further in favor of the implications of the original article with respect to the efficacy of angiogenesis inhibitors by a highlight note in Science [10]. The controversial commentary entitled "Vasculogenic mimicry: how convincing, how novel, and how significant?" [11] presented several questions regarding the original VM report. For example, the VM structures are like blood vessels and could they contribute to blood supply significantly? Or in blood vessels is there a connection between endothelial cells and tumor cells? Afterwards, these questions were discussed in different meetings or reports. Using molecular tools that have become available, researchers made great efforts to explore the molecular determinants driving aggressive cells to express an

underlying angiogenic program forming the early vascular channel. Several key molecules including MMP, VE-cadherin, PI3K, Akt, MUM-2, have been demonstrated to be involved in VM [12]. And evidence regarding a novel occurrence of tumor vascularity has been published, which suggests that tumor cells themselves composed the channels because their structures are lined by cells lacking the endothelial phenotype and markers [13-15], indicating that the pattern of tumor vascularization appears to be VM.

The definition of VM was first put forward to depict the unique ability of forming capillary-like structures and matrix-rich patterned network in 3D culture that imitates embryonic vasculogenic network via highly aggressive melanoma cells [1]. The hypothesis was put forth at the time, accounting to the transport of injected fluorescent dye throughout VM networks in 3D culture, and VM was reported to serve as a selective advantage for fast growing tumors by giving a perfusion pathway, transporting fluid from vessels in leak, and relating with traditional vasculature lined endothelial cells. The VM pattern is involved in vessels lined with tumor cells which mimic the endothelial cells' existence and function [1,7], and derive from the tumor cells.

Current studies on VM in tumors

Now it is suggested that tumor stem cells can successfully differentiate into endothelial cells which line up to form a lumen, then the new lumen connects with mosaic vessels or endothelium-dependent vessels, and the new vessels become stable when blood flow becomes smooth. There are many studies supporting this theory. Tumor stem cells possess the capacity for self-renewal and differentiation, which aroused researchers' great interest in redefining tumor vascularization [16]. The genes' expression associated with vascular cells in tumor cells could be explained via the plasticity of tumor stem cells [17,18]. Meanwhile, the organization of tubular structures by tumor cells has been explained by tumor stem cells [1]. A human renal cell carcinoma study observed that a subset of tumor-initiating cells expressing CD105, the mesenchymal stem cell marker, and displaying stem cell properties but lacking of differentiative epithelial markers, can generate epithelial and endothelial cells *in vitro* [19]. A study *in vivo* also revealed that both tumor epithelial and endothelial cells can be differentiated by tumor stem cells [20]. Recently, it has been reported that in neuroblastomas, tumor stem cells had the

capacity of differentiating into endothelial cells [21,22]. Further studies have shown that tumor stem cells co-expressing CD133 and CD144 [22], or Oct4 and tenascin C [23] had the potential of becoming tumor vasculature. Selective targeting of tumor-derived endothelium in mouse xenografts led to tumor reduction and degeneration, showing a relevant role of vasculogenesis derived from tumor stem cell [21].

Molecular mechanisms of VM

Expression of high levels of matrix metalloproteinase substances by tumor cells participates in vascular signaling pathways

Compared with highly aggressive melanoma cells, microarray analyses of non aggressive melanoma cells revealed a differential expression pattern of candidate genes, associated with the ECM and cell-ECM interactions, which could be involved in and facilitate VM. Specifically, highly aggressive melanoma cells can overexpress the level of matrix metalloproteinases (MMP-1, 2, 9, and 14) and the $\gamma 2$ chain (compared with $\alpha 3$ or $\beta 3$ chains) of the laminin 5 (laminin-332; Ln5), an heterotrimeric basement membrane glycoprotein, compared to less aggressive melanoma cells [24,25]. This high level of expression of MMPs and the presence of the laminin receptor on tumor cells' surface can promote cells to attract more laminin. Laminin can be cleaved into several short chains by activated MMPs, which eventually promote the formation of VM. PI3K is a lipid kinase that acts through phosphorylation of its substrates, mainly including phosphatidylinositol or its derivatives. The PI3K signaling pathway has been shown to be inevitable in normal cell processes such as proliferation, differentiation, survival, metabolism, and motility [26]. Akt, known as protein kinase B (protein kinaseB, PKB), is a serine/threonine protein kinase that plays an integral role in the PI3K signaling pathway. Like PI-3, 4-P2 and PI-3, 4, 5-P3, the PI3K's products after activation, could combine with Akt's pleckstrin homology (PH) domain which leads to Akt's translocation from the cytoplasm to the cell membrane and its conformational change further promotes its activation. It has been shown that the PI3K/Akt signaling pathway can regulate the function of MMP-14 (MT1-MMP), which activates MMP-2 with the help of the tissue inhibitor of MMP-2 (TIMP2), and the activated MMP-2 then cleaves $5\gamma 2$ chain into $\gamma 2'$ and $\gamma 2x$ chains [27]. MMP overexpression in human ovarian cancers has been

shown to contribute to the formation of a vascular system lined by tumor cells [28]. Furthermore, it has been found that Ln5- $\gamma 2$ chain, MMP-14 and MMP-2 could co-locate with VM tubular networks formed in a 3D type I collagen matrix by aggressive (but not non aggressive) melanoma cells, and resembled laminin networks in tumor sections of aggressive melanoma, as well as in human melanoma xenografts in nude mice. The findings demonstrated that PI3K/Akt, MMPs, and Ln-5 $\gamma 2$ chain contribute to extra-cellular matrix remodeling and VM formation, which implies an important target for anticancer therapy via inhibiting the PI3K/Akt signaling pathway and the MMP activation.

Secretion of adhesion molecules by tumor cells enhances the adherence of the VM wall and participates in vascular signaling pathways

VE-cadherin has been considered as an adherent protein restricted in endothelial cells. It is a transmembrane protein of the cadherin family and plays a key role in forming tight connections among endothelial cells [29]. VE-cadherin closely related with the virtual channels, formed only in the presence of highly aggressive tumors. Highly aggressive melanoma cells have been reported to express VE-cadherin, however, no expression was observed in less aggressive melanoma cells. It is also proved that downregulating of VE-cadherin expression can inhibit VM formation [18].

Two of the first proteins identified to play an important role in mediating VM in melanoma were VE-cadherin, a cell-cell adhesion molecule associated with endothelial cells, and EPHA2, a member of the ephrin-receptor family of PTKs expressed in melanoma cells with a metastatic phenotype, both of which are crucial to angiogenesis [18,30]. Studies designed to detect the function of these proteins in promoting VM formation of melanoma revealed that downregulation of either VE-cadherin or EPHA2 inhibited VM. EPHA2 knockdown could make cells lose their VM-forming abilities and lead to EPHA2's redistribution in the cell membrane, but it did not affect the VE-cadherin's position in cell-cell adhesion [31]. Furthermore, VE-cadherin has been proved to modulate the location and level of EPHA2 phosphorylation, providing the first evidence that signal transduction from the plasma membrane is necessary for melanoma VM [32].

In addition, FAK is a cytoplasmic tyrosine kinase associated with focal adhesion [32]. Relevant studies showed that FAK could regulate VM

migration, invasion, and formation in malignant tumors. When FAK is positioned on a membrane, it can activate extracellularly signal-regulated kinase 1 and 2 (ERK1/2). ERK1/2's phosphorylation further mediates MT1- MMP and MMP-2 through the PI3K signal pathway, and is involved in the extracellular matrix plasticity, migration, invasion, and VM formation [33,34].

Dedifferentiation of tumor cells is vital to formate VM channels

Many studies have demonstrated that VM can be only found in highly aggressive melanomas. There is a cDNA microarray study in a patient with poorly and highly aggressive melanoma cells, which revealed that highly aggressive tumor cells expressed genes related to multiple cellular phenotypes of endothelial and hematopoietic stem cells [18,35,36]. It has been found that two clones could be discovered by the transplanted tumors of the human melanoma cell line MUM-2: MUM-2B and MUM-2C [32]. MUM-2B, owing to an epithelioid and mesenchymal phenotype, has high aggressiveness, and VM is found in tumor tissues. However, MUM-2C, showing a mesenchymal phenotype, has low aggressiveness, and VM is seldom observed in these tumor tissues.

Part of tumor cells possess the capability of self-renewal and another part of tumor cells called CSCs have the ability of multiple potential differentiation, which were observed in many tumors, including breast cancer [37], glioblastoma [38], colon cancer [39], melanoma [40,41], ovarian cancer [42,43], prostate cancer [44], and pancreatic cancer [45]. Recently, many studies implicated the effect of CSCs on VM formation. For example, adherent bone marrow stromal cells (BMSCs) derived from CD133+/CD34+ stem cells via secreting higher level of insulin growth factor-1 (IGF-1) and SDF-1 alpha resulted in forming capillary-like structures (VM) on Matrigel [46]. Ricci-Vitiani et al. found that in glioblastoma, the vessels in tumor xenografts generated by orthotopic or subcutaneous injection of glioblastoma stem-like cells in immunocompromised mice were made up of human endothelial cells, which indicated the CSCs' differentiation potential along the endothelial lineage and their involvement in VM formation [21].

Other relevant factors

Tissue factor (TF), a transmembrane protein, is expressed in many cell types, including smooth

muscle cells, endothelial cells, macrophages, and solid tumors [47,49-51], and is related to vascular system development [51,52]. TF pathway 1 (TFPI1) and TF pathway 2 (TFPI2) are two coagulation pathway inhibitors, playing an important role in maintaining coagulation and anticoagulation system balances. Recently, a study demonstrated that TF, TFPI-1, and TFPI-2 were overexpressed in human invasive melanoma cells [53]. TFPI-1 is associated with perfusion of VM by its anticoagulant function, and TFPI2 through the interaction with MMP-2 is involved in endothelial cell matrix remodeling and VM formation.

Almost all tumor cells secrete vascular endothelial growth factor (VEGF)-A that belongs to the angiogenic growth factor family associated with tumor angiogenesis. Binding VEGF-A to its ligand results in dedifferentiation of endothelial cells into its precursors, stimulating vascular channel proliferation and formation in tumors, especially in avascular regions. These findings showed that, in melanoma, VM and angiogenesis were mediated by VEGF-A [54]. VEGF-A upregulates VE-cadherin, EphA2 and MMPs expressions [55], and VEGFR2 expression contributes to the formation of capillary-like structures (VM) [56,57].

Hypoxia promotes VM formation by inducing EMT [58]. HIF-1 α activates the expression of VEGF, and the latter is related to VM formation [59-61]. The expression of VE-cadherin is mediated by Gal-3 and MMP-2 which have been confirmed to promote VM formation [62]. Silencing of Gal-3 results in the inhibition of VE-cadherin and IL-8 promoter activities. The increase of cyclic adenosine monophosphate (cAMP), a second messenger regulating cell growth and differentiation, results in inhibition of VM formation through activation of Epac/Rap1 pathway and inhibition of MMP-2 and MT1-MMP expression. Nodal is a member of one transformation growth factor β (TGF- β) superfamily and plays a vital role in maintaining tumorigenicity and melanoma progression. Activation of Nodal contributes to VM formation by increasing VE-cad expression [63]. And inhibition of VM formation could be inhibited via the activation of Nodal signal mediated by cAMP [64]. Cyclooxygenase (COX), a necessary enzyme in prostaglandins synthesis, consists of the isoenzymes COX-1 and COX-2. COX-2 results in upregulation of VEGF expression by activating PKC, and PGE-2 expression, thereby promoting VM formation [65].

Inhibitors of DNA binding 2 (Id2), migration-inducing protein 7 (Mig-7), caspase-3, en-

dothelin (ET)-1, bone morphogenetic protein 4 (BMP4), and human chorionic gonadotropin (hCG) are all associated with the induction VM formation.

Factors affecting VM formation in tumor metastasis

Hypoxia promotes VM formation and increases tumor metastasis

Hypoxia, either persistent or transient, is a distinguishing feature of most solid tumors and can regulate pathways in cellular differentiation, induction or maintenance of stem-like cell characteristics, tumor progression, angiogenesis, and VM, all of which are markers of poor prognosis in cancer patients. The HIF complex, including HIF-1 β and one HIF- α subunit (HIF-1 α , HIF-2 α , or HIF-3 α) is a vital regulator of oxygen homeostasis in both physiological and pathological environments. Under low oxygen availability, HIF-1 α – after protein stabilization and translocation – goes into the nucleus, where it binds to gene regulatory regions containing hypoxia response elements and activates transcription of hypoxia-target genes [66,67].

Especially, hypoxia and subsequent HIF overexpression in tumor cells induce the expression of gene products that are associated with angiogenesis (eg, VEGF), which is important for cell viability, tumor survival, and metastasis. Meanwhile, it was demonstrated that hypoxia can induce VM in hepatocellular carcinoma, Ewing's sarcoma, and melanoma. Furthermore, hypoxia can induce a dedifferentiated phenotype in breast carcinoma [68].

Related to VM, hypoxia can directly modulate VEGF-A, VEGFR1, EphA2, Twist, Nodal osteopontin, COX-2, VE-cadherin, TF, and PEDF expression [69]. Furthermore, hypoxia can regulate the expression of Notch-responsive genes by HIF-1 α stabilization of the Notch intracellular domain protein and subsequent activate genes with Notch-responsive promoters. The interaction between HIF-1 α and Notch signaling pathways is thought to promote an undifferentiated cell state, which illuminates the possible etiology of tumor cell plasticity underlying VM. Another mechanism is through the generation of mitochondrial reactive oxygen species, by which hypoxia can promote VM. It is proved that Redox-dependent stabilization of HIF-1 α and induction of VM are true in melanoma [70,71]. These studies demon-

strated that hypoxia-induced VM plays a critical role in tumor progression. Treatment with some antiangiogenic agents inhibiting tumor perfusion and increasing intratumoral hypoxia, has demonstrated increased metastatic potential and VM [33,72,73].

Invasive CSCs are crucial in tumor invasion and metastasis

Recently, a increasing number of studies indicated that CSCs are implicated in VM formation. There is evidence showing that adherent BMSCs derived from CD133+/CD34+ stem cells from acute leukemia patients were able to secrete higher levels of insulin growth factor-1 (IGF-1) and SDF-1 alpha, and could result in the formation the capillary-like structures (VM) on Matrigel [21]. Furthermore, it is shown that in glioblastoma, the vessels in tumor xenografts generated by orthotopic or subcutaneous injection of glioblastoma stem-like cells in immunocompromised mice were composed of human endothelial cells, which indicated CSCs' differentiation potential along endothelial lineage and their involvement in VM formation [21]. In addition, it is demonstrated that in oral squamous cell carcinoma (SCC), TRA-1-60+/beta6+ stem cells were capable of producing vascular-like structures *in vivo*.

Several authors have reported that CSCs are able of differentiating towards tumor and endothelial lineages [21,74]. In addition, CSCs are associated with tumor invasion and metastasis [75], and many CSC markers were involved in these processes, such as ALDH1 [76], FRMD4A [77], and CD44 [78]. Hermann et al. showed that CSCs phenotypes of CD133/CXCR4 in pancreatic cancer are related to tumor metastasis and tumorigenesis, and CSCs are classified into two types: invasive CSCs, crucial in tumor invasion and metastasis, and stationary CSCs associated with tumorigenesis [45].

Epithelial-mesenchymal transition (EMT) involved in tumor invasion and metastasis

EMT is a dedifferentiation process that plays an integral role in tumor progression. Via epithelial cells transitioning into mesenchymal cells, EMT acquires mesenchymal features and loses epithelial phenotypes, mainly including epithelial marker downregulation, mesenchymal marker upregulation, and cell polarity loss. An increasing amount of evidence shows that EMT is associat-

ed with tumor invasion and metastasis. Tumor cells generate oncogenic metabolism, which can create an acidic tumor microenvironment to promote EMT and overexpress tumor cell stemness. CSCs interact with various other cells in the niche via adhesion molecules. Molecular signals are exchanged among these cells, which can maintain the specific features of stem cells and increase metastatic capabilities [79,80]. Recent studies show that transcription factors related to EMT are upregulated in VM-forming tumor cells. As a main EMT-mediated process regulator, Twist reportedly promotes breast cancer metastasis into distant regions [81]. In colorectal carcinoma (CRC), Liu et al. showed that in VM-positive samples ZEB1 expression was upregulated compared with VM-negative samples, while it occurred concurrently with EMT traits. Furthermore, ZEB1 knockdown in tumor cells abolished VM formation, resulted in epithelial phenotype restoration, and evidently repressed tumor migration and invasion [82]. Moreover, decreasing expression of ZEB1 led to decreased VE-cadherin expression and Flk-1, which went against VM formation. All of these mean that EMT contributes to VM formation, while contributes to tumor metastasis.

Clinical significance of VM formation in tumor metastasis

The special feature of VM channels' structure plays a critical role in hematogeneous metastasis of tumor cells. Tumor cells line the VM channels' inner surface, and are directly exposed to blood flow. Tumor cells can migrate through the bloodstream to finish leaking out and metastasize to other organs and tissues. Moreover, tumor cells which line the VM channels possess high aggressiveness, poor differentiation and high plasticity. The cells can secrete proteins mediating tumor invasion and metastasis, which can degrade adjacent connective tissues and penetrate the basement membrane of blood vessels.

Several studies have demonstrated that VM is implicated in poor patient clinical prognosis [1,83,84]. We all know that antiangiogenic treatment is widely accepted as an effective anti-cancer therapy. Traditional antiangiogenesis drugs, like angiostatin and endostatin, mainly exert important effects on inhibiting cancer growth and metastasis via reducing endothelial cell proliferation or inducing endothelial cell apoptosis. But they have little effect on vessel-like structures lined by tumor cells because of the absence of endothelial cells. When the number of blood vessels

is reduced as a result of antiangiogenic therapy, it may lead to hypoxia. Subsequently, oxygen and nutrient deficiency as a compensatory stimulus will contribute to VM formation and indirectly promote tumor progression. There are many arguments to support this viewpoint. For example, in breast cancer, the preclinical and clinical results are likely due to the development of a hypoxic microenvironment within the tumor, resulting in the proliferation of CSCs, a cell type with the greatest degree of plasticity and ability to metastasize [85]. In addition, there is evidence suggesting that a hypoxic microenvironment within a tumor may promote the development of tumor-derived endothelial cells in glioblastoma [86].

Considering the diverse nature of vascular perfusion pathways in tumors, it may be prudent to detect the efficacy of currently available angiogenesis inhibitors on tumor cell VM, in addition to endothelial cell-driven angiogenesis. Increasing evidence demonstrated that curcumin, imatinib, and thalidomide have all been shown to inhibit melanoma VM, concomitant with decreases in EPHA2, VE-cadherin, PI3K, VEGF, HIF-1, MMP-2, and MMP-9 expression and/or activity, suggesting that these compounds can affect several different aspects about the signaling mechanisms mediating VM [87-89].

Therefore, with further studies and a large number of clinical trials, VM inhibitors combined with antiangiogenic therapies appear to be a promising therapeutic target in antitumor therapy.

Conclusion

VM, being a new pattern of blood supply, has attracted the attention of many researchers. However, many unique abilities and functions to VM channel formation remain to be elucidated. Now it is common knowledge that VM describes the functional abilities of aggressive cancer cells to express a multipotent, stem cell-like phenotype and form ECM-rich and patterned vasculogenic-like networks to provide adequate blood supply for tumor growth in a 3D matrix. To be a unique perfusion way, VM has been found in a variety of aggressive tumors. Many molecular mechanisms, especially VE-cadherin, EPHA2, PI3K, MMPs, VEGFR1, and HIF-1 α , are involved in tumor migration, invasion, and VM formation. In addition, hypoxia, CSCs and EMT are considered as significant factors in the relationship between VM and tumor metastasis. Due to the significantly different structures from endothelium-dependent ves-

sels, traditional antivasculature therapies aiming at endothelial cells play no effective role in malignant tumors with VM. Now it is time to contribute to a promising therapeutic target in anti-tumor therapy by combining of VM inhibitors with antiangiogenic therapies.

Acknowledgement

This study was supported by the Natural Science Foundation of Shandong Province (Grant No. ZR2012HM095).

Conflict of interests

The authors declare no conflict of interests.

References

- Maniotis AJ, Folberg R, Hess A et al. Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. *Am J Pathol* 1999; 155:739-752.
- Folberg R, Maniotis AJ. Vasculogenic mimicry. *APMIS* 2004;112:508-525.
- Risau W. Mechanisms of angiogenesis. *Nature* 1997;386:671-674.
- Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; 285:1182-1186.
- Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996;86:353-364.
- Fukumura D, Xavier R, Sugiura T et al. Tumor induction of VEGF promoter activity in stromal cells. *Cell* 1998;94:715-725.
- Folberg R, Hendrix MJ, Maniotis AJ. Vasculogenic mimicry and tumor angiogenesis. *Am J Pathol* 2000;156:361-381.
- Seftor RE, Hess AR, Seftor EA et al. Tumor cell vasculogenic mimicry: from controversy to therapeutic promise. *Am J Pathol* 2012;181:1115-1125.
- Bissell MJ. Tumor plasticity allows vasculogenic mimicry, a novel form of angiogenic switch. A rose by any other name? *Am J Pathol* 1999;155:675-679.
- Barinaga M. New type of blood vessel found in tumors. *Science* 1999;285:1475.
- McDonald DM, Munn L, Jain RK. Vasculogenic mimicry: how convincing, how novel, and how significant? *Am J Pathol* 2000;156:383-388.
- Zhang S, Zhang D, Sun B. Vasculogenic mimicry: current status and future prospects. *Cancer Lett* 2007;254:157-164.
- Pezzolo A, Parodi F, Corrias MV et al. Tumor origin of endothelial cells in human neuroblastoma. *J Clin Oncol* 2007;25:376-383.
- Gunsilius E, Duba HC, Petzer AL. Evidence from a leukaemia model for maintenance of vascular endothelium by bone-marrow-derived endothelial cells. *Lancet* 2000;355:1688-1691.
- Rigolin GM, Fraulini C, Ciccone M. Neoplastic circulating endothelial cells in multiple myeloma with 13q14 deletion. *Blood* 2006;107:2531-2535.
- Gao JX. Cancer stem cells: the lessons from precancerous stem cells. *J Cell Mol Med* 2007;58:267-284.
- El Hallani S, Boisselier B, Peglion F et al. A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry. *Brain* 2010;133:973-982.
- Seftor EA, Meltzer PS, Schatteman GC et al. Expression of multiple molecular phenotypes by aggressive melanoma tumor cells: role in vasculogenic mimicry. *Crit Rev Oncol Hematol* 2002;44:17-27.
- Bussolati B, Bruno S, Grange C et al. Identification of a tumor-initiating stem cell population in human renal carcinomas. *FASEB J* 2008;22:3696-3705.
- Bussolati B, Grange C, Sapino A et al. Endothelial cell differentiation of human breast tumour stem/progenitor cells. *J Cell Mol Med* 2009;13:309-319.
- Ricci-Vitiani L, Pallini R, Biffoni M et al. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature* 2010;468:824-828.
- Wang R, Chadalavada K, Wilshire J et al. Glioblastoma stem-like cells give rise to tumour endothelium. *Nature* 2010; 468: 829-833.
- Pezzolo A, Parodi F, Marimpietri D et al. Oct-4(+)/Tenascin C(+)neuroblastoma cells serve as progenitors of tumor derived endothelial cells. *Cell Res* 2011;21:1470-1486.
- Seftor EA, Koshikawa N, Meltzer PS et al. Cooperative interactions of laminin 5c2 chain, matrix metalloproteinase-2, and membrane type-1-matrix/ metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. *Cancer Res* 2011;71:6322-6337.
- Seftor EA, Kirschmann DA, Hendrix MX. Targeting the tumor microenvironment with chemically modified tetracyclines: inhibition of laminin 5c2 chain promigratory fragments and vasculogenic mimicry. *Mol Cancer Ther* 2002;1:1173-1179.
- Link W, Rosado A, Fominaya J et al. Membrane localization of all class I PI 3-kinase isoforms suppresses c-Myc-induced apoptosis in Rat1 fibroblasts via Akt. *J Cell Biochem* 2005;95:979-989.

27. Hess AR, Seftor EA, Hendrix MJ. Phosphoinositide 3-kinase regulates membrane Type 1-matrix metalloproteinase (MMP) and MMP-2 activity during melanoma cell vasculogenic mimicry. *Cancer Res* 2003;63:4757-4762.
28. Sood AK, Fletcher MS, Coffin JE et al. Functional role of matrix metalloproteinases in ovarian tumor cell plasticity. *Am J Obstet Gynecol* 2004;190:899-909.
29. Hendrix MJ, Kirschmann DA, Quaranta V et al. Remodeling of the microenvironment by aggressive melanoma tumor cells. *Ann NY Acad Sci* 2003;995:151-161.
30. Hess AR, Seftor EA, Gardner LM et al. Molecular regulation of tumor cell vasculogenic mimicry by tyrosine phosphorylation: role of epithelial cell kinase (Eck/EphA2). *Cancer Res* 2001;61:3250-3255.
31. Fan YZ, Sun W. Molecular regulation of vasculogenic mimicry in tumors and potential tumor-target therapy. *World J Gastrointest Surg* 2010;2:117-127.
32. Hess AR, Seftor EA, Gruman LM. VE-cadherin regulates EphA2 in aggressive melanoma cells through a novel signaling pathway: implications for vasculogenic mimicry. *Cancer Biol Ther* 2006;5:228-233.
33. Hess AR, Hendrix MJ. Focal adhesion kinase signaling and the aggressive melanoma phenotype. *Cell Cycle* 2006;5:478-480.
34. Hess AR, Postovit LM, Margaryan NV et al. Focal adhesion kinase promotes the aggressive melanoma phenotype. *Cancer Res* 2005;65:9851-9860.
35. Seftor EA, Kirschmann DA, Margaryan NV et al. The epigenetic reprogramming of poorly aggressive melanoma cells by a metastatic microenvironment. *J Cell Mol Med* 2006;10:174-196.
36. Seftor EA, Kirschmann DA, Pe'er J et al. Molecular determinants of human uveal melanoma invasion and metastasis. *Clin Exp Metastasis* 2002;19: 233-246.
37. Al Hajj M, Wicha MS, Benito Hernandez A et al. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;100:3983-3988.
38. Singh SK, Hawkins C, Clarke ID et al. Identification of human brain tumour initiating cells. *Nature* 2004;432:396-401.
39. O'Brien CA, Pollett A, Gallinger S et al. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007;445:106-110.
40. Fang D, Nguyen TK, Leishear K et al. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005;65:9328-9337.
41. Schatton T, Murphy GF, Frank NY et al. Identification of cells initiating human melanomas. *Nature* 2008;451:345-349.
42. Bapat SA, Mali AM, Koppikar CB et al. Stem and progenitor like cells contribute to the aggressive behavior of human epithelial ovarian cancer. *Cancer Res* 2005;65:3025-3029.
43. Alvero AB, Chen R, Fu HH et al. Molecular phenotyping of human ovarian cancer stem cells unravel the mechanisms for repair and chemo resistance. *Cell Cycle* 2009;8:158-166.
44. Collins AT, Berry PA, Hyde C et al. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005;65:10946-10951.
45. Hermann P, Huber S, Herrler T et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell* 2007;1:313-323.
46. Mirshahi P, Rafii A, Vincent L et al. Vasculogenic mimicry of acute leukemic bone marrow stromal cells. *Leukemia* 2009;23:1039-1048.
47. Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler Thromb Vasc Biol* 2004;24:1015-1022.
48. Gale AJ, Gordon SG. Update on tumor cell procoagulant factors. *Acta Haematol* 2001;106:25-32.
49. Abdulkadir SA, Carvalhal GF, Kaleem Z et al. Tissue factor expression and angiogenesis in human prostate carcinoma. *Hum Pathol* 2000;31:443-447.
50. Ueda C, Hirohata Y, Kihara Y et al. Pancreatic cancer complicated by disseminated intravascular coagulation associated with production of tissue factor. *J Gastroenterol* 2001;36:848-850.
51. Carmeliet P, Mackman N, Moons L et al. Role of tissue factor in embryonic blood vessel development. *Nature* 1996;383:73-75.
52. Contrino J, Hair G, Kreutzer DL et al. In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. *Nat Med* 1996;2:209-215.
53. Ruf W, Seftor EA, Petrovan RJ et al. Differential role of tissue factor pathway inhibitors 1 and 2 (TFPI-1 and 2) in melanoma vasculogenic mimicry. *Cancer Res* 2003;63:5381-5389.
54. Vartanian AA, Burova OS, Stepanova EV et al. The involvement of apoptosis in melanoma vasculogenic mimicry. *Melanoma Res* 2007;17:1-8.
55. Wang JY, Sun T, Zhao XL et al. Functional significance of VEGF-A in human ovarian carcinoma: role in vasculogenic mimicry. *Cancer Biol Ther* 2008;7:758-766.
56. Tallquist MD, Soriano P, Kinghoffer RA. Growth factor signaling pathways in vascular development. *Oncogene* 1999;18:7917-7932.
57. Sharma PS, Sharma R, Tyagi T. VEGF/VEGFR pathway inhibitors as anti-angiogenic agents: present and future. *Curr Cancer Drug Targets* 2011;11:624-653.
58. Du J, Sun B, Zhao X et al. Hypoxia promotes vasculogenic mimicry formation by inducing epithelial-mesenchymal transition in ovarian carcinoma. *Gynecol Oncol* 2014;133:575-583.
59. Su M, Feng YJ, Yao LQ et al. Plasticity of ovarian cancer cell SKOV3ip and vasculogenic mimicry in vivo. *Int J Gynecol Cancer* 2008;18:476-486.
60. Semenza GL. Expression of hypoxia-(2000) inducible factor 1: mechanisms and consequences. *Biochem Pharmacol* 2000;59:47-53.
61. Jones MK, Szabó IL, Kawanaka H et al. von Hippel Lindau tumor suppressor and HIF-1 α : new targets of NSAIDs inhibition of hypoxia-induced angiogenesis. *FASEB J* 2002;16:264-266.
62. Mourad-Zeidan AA, Melnikova VO, Wang H et al. Expression profiling of Galectin-3-depleted melanoma cells reveals its major role in melanoma cell

- plasticity and vasculogenic mimicry. *Am J Pathol* 2008;173:1839-1852.
63. Topczewska JM, Postovit LM, Margaryan NV et al. Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat Med* 2006;12:925-932.
 64. Lissitzky JC, Parriaux D, Ristorcelli E et al. Cyclic AMP signaling as a mediator of vasculogenic mimicry in aggressive human melanoma cells in vitro. *Cancer Res* 2009;69:802-809.
 65. Luo H, Chen Z, Jin H et al. Cyclooxygenase-2 up-regulates vascular endothelial growth factor via a protein kinase C pathway in non-small cell lung cancer. *J Exp Clin Oncol* 2011;30:6-10.
 66. De Bock K, Mazzone M, Carmeliet P. Antiangiogenic therapy, hypoxia, and metastasis: risky liaisons, or not? *Nat Rev Clin Oncol* 2011;8:393-404.
 67. Benizri E, Ginouvès A, Berra E. The magic of the hypoxia-signaling cascade. *Cell Mol Life Sci* 2008;65:1133-1149.
 68. Kirschmann DA, Seftor EA, Hardy KM et al. Molecular pathways: vasculogenic mimicry in tumor cells: diagnostic and therapeutic implications. *Clin Cancer Res* 2012;18:2726-2732.
 69. Fernández-Barral A, Orgaz JL, Gomez V et al. Hypoxia negatively regulates antimetastatic PEDF in melanoma cells by a hypoxia inducible factor-independent, autophagy dependent mechanisms. *PLoS One* 2012;7:e32989.
 70. Vartanian A, Stepanova E, Grigorieva I et al. VEGFR1 and PKC α signaling control melanoma vasculogenic mimicry in a VEGFR2 kinase-independent manner. *Melanoma Res* 2011;21:91-98.
 71. Comito G, Calvani M, Giannoni E et al. HIF-1 stabilization by mitochondrial ROS promotes Met-dependent invasive growth and vasculogenic mimicry in melanoma cells. *Free Radic Biol Med* 2011;51:893-904.
 72. Xu Y, Li Q, Li XY et al. Short-term anti-vascular endothelial growth factor treatment elicits vasculogenic mimicry formation of tumors to accelerate metastasis. *J Exp Clin Oncol* 2012;31:16.
 73. Ebos JM, Lee CR, Cruz-Munoz W. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer Cell* 2009;15:232-239.
 74. Wang R, Chadalavada K, Wilshire J et al. Glioblastoma stem like cells give rise to tumour endothelium. *Nature* 2010;468:829-833.
 75. Shiozawa Y, Nie B, Pienta KJ et al. Cancer stem cells and their role in metastasis. *Pharmacol Ther* 2013;138:285-293.
 76. Charafe Jauffret E, Ginestier C, Iovino F et al. Aldehyde dehydrogenase 1 positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. *Clin Cancer Res* 2010;16:45-55.
 77. Goldie SJ, Mulder KW, Tan DW et al. FRMD4A upregulation in human squamous cell carcinoma promotes tumor growth and metastasis and is associated with poor prognosis. *Cancer Res* 2012;72:3424-3436.
 78. Davis SJ, Divi V, Owen JH et al. Metastatic potential of cancer stem cells in head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg* 2010;136:1260-1266.
 79. Corselli M, Chin CJ, Parekh C et al. Perivascular support of human hematopoietic stem/progenitor cells. *Blood* 2013;121:2891-2901.
 80. Lai CY, Schwartz BE, Hsu MY. CD133+ melanoma subpopulations contribute to perivascular niche morphogenesis and tumorigenicity through vasculogenic mimicry. *Cancer Res* 2012;72:5111-5118.
 81. Yang J, Mani SA, Donaher JL et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004;117:927-939.
 82. Liu Z, Sun B, Qi L et al. Zinc finger E box binding homeobox 1 promotes vasculogenic mimicry in colorectal cancer through induction of epithelial to mesenchymal transition. *Cancer Sci* 2012;103:813-820.
 83. Sun BZS, Zhao X, Zhang W. Vasculogenic mimicry is associated with poor survival in patients with mesothelial sarcomas and alveolar rhabdomyosarcomas. *Int J Oncol* 2004;25:1609-1614.
 84. Hao XSB, Zhang S, Zhao X. Microarray study of vasculogenic mimicry in bi-directional differentiation malignant tumor. *Zhonghua YiXue Za Zhi* 2002;82:1298-1302.
 85. Conley SJ, Gheordunescu E, Kakarala P. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. *Proc Natl Acad Sci USA* 2012;109:2784-2789.
 86. Soda Y, Marumoto T, Friedmann-Morvinski D et al. Transdifferentiation of glioblastoma cells into vascular endothelial cells. *Proc Natl Acad Sci USA* 2011;108:4274-4280.
 87. Zhang S, Li M, Gu Y. Thalidomide influences growth and vasculogenic mimicry channel formation in melanoma. *J Exp Clin Oncol* 2008;27:60.
 88. Liu Z, Li Y, Zhao W. Demonstration of vasculogenic mimicry in astrocytomas and effects of Endostar on U251 cells. *Pathol Res Pract* 2011;207:645-651.
 89. Chen LX, He YJ, Zhao SZ. Inhibition of tumor growth and vasculogenic mimicry by curcumin through down-regulation of the EphA2/PI3K/MMP pathway in a murine choroidal melanoma model. *Cancer Biol Ther* 2011;11:229-235.