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REVIEW

Progress in tumor vascular normalization for anticancer therapy: challenges and perspectives

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Abstract **Antitumor angiogenic therapy has been shown promising in the treatment of several advanced cancers since the approval of the first antiangiogenic drug Avastin in 2004. Although the current antiangiogenic drugs reduce the density of tumor blood vessels and result in tumor shrinkage at the early stage of treatment, recent studies have shown that antiangiogenic therapy has transient and insufficient efficacy, resulting in tumor recurrence in patients after several months of treatment. Blockage of blood and oxygen supplies creates a hypoxic and acidic microenvironment in the tumor tissues, which fosters tumor cells to become more aggressive and metastatic. In 2001, Jain proposed tumor vascular normalization as an alternative approach to treating cancers based on the pioneering work on tumor blood vessels by several other researchers. At present, normalizing the disorganized tumor vasculature, rather than disrupting or blocking them, has emerged as a new option for anticancer therapy. Preclinical and clinical data have shown that tumor vascular normalization using monoclonal antibodies, proteins, peptides, small molecules, and pericytes resulted in decreased tumor size and reduced metastasis. However, current tumor vascular normalizing drugs display moderate anticancer efficacy. Accumulated data have shown that a variety of vasculogenic/angiogenic tumor cells and genes play important roles in tumor neovascularization, growth, and metastasis. Therefore, multiple-targeting of vasculogenic tumor cells and genes may improve the efficacy of tumor vascular normalization. To this end, the combination of antiangiogenic drugs with tumor vascular normalizing therapeutics, as well as the integration of Western medicine with traditional Chinese medicine, may provide a good opportunity for discovering novel tumor vascular normalizing drugs for an effective anticancer therapy.**

Keywords angiogenesis; vasculogenesis; neovascularization; tumor; vasculature; normalization; traditional Chinese medicine

Introduction

The link between the expansion, invasion, and metastasis of malignant tumors and the ability of the tumor to acquire an adequate vascular supply has now been well established [1]. Since Folkman [2] introduced a new concept of angiogenesis and proposed a novel antiangiogenic strategy for anticancer therapy in 1971, five antiangiogenic drugs have been used in a clinical setting to treat colon cancer, non-small cell lung cancer, and several other cancers. Avastin, one of the best antiangiogenic therapeutics, prolonged cancer patient life for 4 to 6 months [3–5]. While people were encouraged by this promising anti-antigenic therapy for a few cancers, recent clinical data have shown that these drugs exhibit transient and insufficient anticancer efficacy, resulting in tumor recurrence in patients after several months on the antiangiogenic drug [6,7]. One of the fundamental reasons for the moderate efficacy of antiangiogenic therapy is that current antiangiogenic drugs reduce blood and oxygen supplies, creating a hypoxic and acidic tumor microenvironment in tumor tissues that induces tumor cells to become more aggressive and metastatic, resulting in relapse in the cancer patients [8,9]. Hence, new strategies and approaches for antitumor neovascularization are highly desired.

Tumor vasculature is disorganized. In 2001, Jain [10] proposed a new concept of normalization of disorganized tumor vasculature, rather than blockage or disruption of tumor blood vessels for anticancer therapy, and the concept was re-enforced in 2005 [11]. Preclinical and clinical data have shown that normalization of the disorganized tumor

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vasculature using monoclonal antibodies, proteins, peptides, small molecules, and pericytes reduced the tumor size and metastasis [12,13]. Tumor vascular normalization has recently emerged as an alternative approach to antitumor neovascularization and anticancer therapy [14].

In the current paper, recent progress in tumor vascular normalization will first be reviewed, the challenges in current tumor vascular normalization therapy will be discussed, and new strategies and approaches for novel tumor vascular normalizing drug discovery will be addressed. Finally, the perspectives on tumor vascular normalization for anticancer therapy will be discussed.

Morphological and molecular bases of tumor vascular normalization

When a tumor grows bigger than 2 mm, the cells in the middle of the tumor tissue live in a hypoxic microenvironment because oxygen diffusion in the tissue is only 1 mm in diameter. Hypoxia in the tumor profoundly changes the gene expression profile of tumor cells and initiates tumor angiogenesis and vasculogenesis [8,9]. As a consequence, the tumor tissue forms disorganized tumor vasculature with morphological and molecular characteristics that are distinct from normal blood vessels.

Morphological characteristics of tumor blood vessels

Tumor blood vessels are heterogeneous and exhibit unique morphological and functional characteristics compared with normal vasculature. Normal blood vessels are organized in a hierarchy of evenly distributed arteries, capillaries, and veins. The vessels are covered by pericytes to maintain vascular integrity. By contrast, tumor blood vessels consist of irregular branches with arteriovenous shunts, which are absent in pericyte coverage, and are highly permeable to plasma and plasma proteins (Fig. 1). Consequently, the tumor tissue usually suffers from local edema, bleeding, poor blood circulation, and insufficient clearance of carbon dioxide and other metabolites. Indeed, tumor cells live in a hypoxic and acidic microenvironment, which induces the overexpression of genes responsible for tumor angiogenesis, vasculogenesis, growth, and metastasis [8,9]. In addition, tumor blood vessels lack functional lymphatic vessels inside the tumor, resulting in the elevation of interstitial fluid pressure in the tumor and the formation of a physiologic barrier to the delivery of therapeutic agents to the tumor [13]. These unique characteristics of the disorganized tumor vasculature provide the rationale for tumor vascular normalization [15].

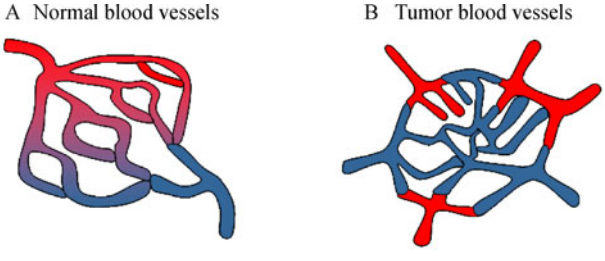

Fig. 1 Comparison of normal vasculature with tumor vasculature. (A) Normal blood vessels are organized in a hierarchy of evenly distributed arteries, capillaries, and veins. The vessels are covered by pericytes to maintain the integrity of the vessels. (B) Tumor blood vessels are heterogeneous and consist of irregular branches with arteriovenous shunts.

Cell types and resources of tumor blood vessels

Tumor atavism has been recognized as a common phenomenon in tumor development for quite some time, and aggressive tumor cells usually exhibit the properties of embryonic stem/progenitor cells. Accumulated data have indicated that malignant tumors undergo embryonic vasculogenesis and angiogenesis to generate unique tumor vasculature in six distinct ways, namely, angiogenesis, vasculogenesis, intussusception, vessel co-option, vascular mimicry, and through cancer stem cell-differentiated tumor endothelial cells [16–19]. Increasing evidence has shown that aside from the well-known vascular endothelial growth factor (VEGF) and endothelial cell-mediated tumor angiogenesis, aggressive tumor cells and many other cells also play an important role in tumor neovascularization by undergoing vasculogenesis and angiogenesis. These vasculogenic cells include precancerous stem cells [20–22], cancer stem cells [23–29], bone marrow-derived endothelial progenitors (EPCs), blood monocytes, mast cells [30–35], mesenchymal stem cells (MSCs) [36,37], and tumor-associated macrophages (TAMs) [38,39]. In particular, a variety of vasculogenic tumor cells can form tumor blood vessels through either vasculogenic mimicry [40–48] or trans-differentiation into tumor endothelial cells [20–29]. Tumor endothelial cells exhibit tumor cell genetic, morphologic, and functional characteristics that are different from normal endothelial cells. Notably, recent studies revealed that more than 60% of the tumor blood vessels in glioma patients consisted of tumor endothelial cells trans-differentiated from glioma stem cells [25,26].

A total of 62 human tumor cell lines were recently examined using an *in vitro* tube formation system; 25 tumor cell lines from various tumors were found to form capillary tubes on Matrigel, with most of these tumor cells actively participating in tumor angiogenesis and vasculogenesis *in vivo* (unpublished observation). On the other hand, the cellular and molecular mechanisms of tumor cell-predominant neovascularization remain to be elucidated. Collectively, vasculogenic tumor cells play a critical role in tumor neovascularization. These emerging data provide new insight into the disorganized tumor vasculature, broadening the prospects on tumor vascular normalization.

Molecular characteristics of tumor blood vessels

Malignant tumors can form new blood vessels via a complex process involving neovascularization, which is now believed to result from two distinct mechanisms: (1) induced *angiogenesis*, by which neovascularization is achieved through tumor cell-produced angiogenic factor induced ingrowth of the tumor vasculature supply from preexisting blood vessels [2,18,19], and (2) v*asculogenesis*, by which the tumor cells and several types of bone marrow cells directly develop into the blood vessels required for neovascularization [40–48]. However, the molecular mechanisms underlying the formation of tumor blood vessels remain to be determined.

Initial tumor neovascularization is driven by both tumor cell intrinsic factors and hypoxia. Tumor cell intrinsic factors play basic roles in the initiation and development of tumor blood vessels, whereas hypoxia triggers tumor vascularity. Genomic instability and epigenetic abnormality may cause chromosome translocations, gene deletions, insertions, and mutations, resulting in the formation of various oncogenes or inactivation of tumor suppressive genes that are closely associated with tumor neovascularization [49,50]. Several oncogenes and tumor suppressors have been implicated in tumor neovascularization, such as Von Hippel/Linda (VHL) [51], Piwi-like protein 2 (Piwil2) [52], and fibronectin extra domain B (ED-B) [53]. In brief, mutant VHL diminishes the degradation of the hypoxia-inducible factor (HIF) and promotes HIF-mediated gene transcription, resulting in tumor vascularization [53]. A splicing error causes intron sequence retention in fibronectin mRNA to produce fibronectin ED-B in a variety of tumors, and fibronectin ED-B functions as an extracellular matrix protein and contributes to tumor vasculogenesis [53]. Our previous study [52] revealed that overexpression of a 60 kDa Piwil2 in tumor cells promoted tumor vasculogenesis and tumorigenesis [54]. In short, these data suggest that tumor intrinsic factors are critical to tumor neovascularization. Of note, current understanding of tumor intrinsic factors in tumor neovascularization are very shallow; hence, identification of additional intrinsic factors responsible for tumor angiogenesis and vasculogenesis will not only help in understanding the mechanisms of tumor vasculature generation, but will also provide new targets for novel tumor vascular normalizing drug discovery.

Hypoxia induces the overexpression of HIF-1, HIF-2, and many other vasculogenic and angiogenic genes and is an indication of tumor neovascularization. HIF-1 and HIF-2 consist of oxygen-dependent α subunits and constitutive β subunits and play important roles in the initiation and development of tumor vasculature [8,9]. Under normoxia, the active HIF heterodimer is not abundantly present in the tumor cells because its formation is tightly regulated by a master HIF regulatory protein, prolyl hydroxylase domain-containing protein 2 (PHD2). PHD2 promotes hydroxylation and proteasomal degradation of HIF α subunits in an oxygen-dependent manner, leading to the inactivation of HIF activity. However, under hypoxial circumstances, PHD2 is in an inactivated state, and the HIF-1 α subunits are stabilized to form an active heterodimer with the HIF-1 β subunits. The active HIFs go to the cell nucleus, bind to the gene promoter region containing hypoxia-response elements, and activate the transcription of hundreds of hypoxia-response genes, including a variety of key vasculogenic and angiogenic genes such as VEGF, VEGF receptor-2, EphrinB2, Eph receptor B2 (EphB2), EphB4, vascular endothelial cadherin (VE-cadherin), cluster of differentiation 31 (CD31), semaphorin 4D (Sema4D) and its receptor plexin-B1, integrins, extra cellular matrix proteins (ECMs), and metal matrix proteases (MMPs) [8,9].

Several hypoxia- and HIF-induced genes play an important role in tumor neovascularization. For example, hypoxia switches tumor cells on to express embryonic vasculogenic genes such as EphrinB2 and its receptor EphB4, and both of the proteins locate at the cell membrane surface and contribute to tumor vasculogenesis [55,56]. Hypoxia also actuates the overexpression of MMPs, which degrade ECMs and promote the migration of tumor cells to the surrounding normal tissue and enhance tumor cell invasion and metastasis [57]. HIF-induced VE-cadherin and EphB2 in various tumor cells function as important adhesive molecules that connect tumor cells to one another to form capillary-like structures [58]. HIF induces the overexpression of Sema4D and its receptor plexin-B1 in tumors and promotes tumor neovascularization and enhances tumor invasive growth and metastasis [59].

Taken together, tumor cell intrinsic factors are critical to tumor neovascularization. A hypoxial and acidic environment triggers tumor vascularity by inducing tumor cells to express a variety of vasculogenic and angiogenic genes, which enable the tumor cells to form blood vessels. On the other hand, tumor cell-released growth factors, cytokines, and chemokines recruit bone marrow and blood cells into the tumor tissue to form a tumor microenvironment that fosters tumor neovascularization. Apparently, tumor cells play a pivotal role in tumor vascular initiation and development. Hitherto, targeting vasculogenic tumor cells and impeding the expression of vasculogenic and angiogenic genes in tumors has become a reasonable strategy for disorganized tumor vascular normalization.

Tumor vascular normalization for anticancer therapy

Malignant tumors require blood vessels for growth and metastasis. Based on the tumor angiogenesis theory of Folkman [2], a tumor produces its new blood vessel branches from preexisting blood vessels. Antitumor angiogenic therapy has mainly targeted VEGF and vascular endothelial cell-mediated angiogenesis during the past three decades, and

the widely held view is that antiangiogenic therapy should inhibit or block tumor blood vessels, thereby depriving the supply of oxygen and nutrients in the tumors [2,18,19]. Traditional antiangiogenic strategies attempt to reduce the tumor vascular supply, but their success at an early stage is hampered by insufficient efficacy and the subsequent development of resistance [6,7]. Based on pioneering research studies [60–63], Jain [10] first proposed the normalization of tumor blood vessels as a new paradigm for anticancer therapy in 2001. The new concept of tumor vascular normalization encompasses an increase in tumor vascular cell tight connection and pericyte coverage, as well as vascular integrity to remedy the disorganized tumor vasculature and improve the blood circulation in tumor tissue. Emerging preclinical and initial clinical evidence has shown that normalization of disorganized tumor vasculature using therapeutics, rather than the blockage or disruption of tumor blood vessels, reduces tumor hypoxia, interstitial fluid pressure, and hyperpermeability and facilitates the delivery of exogenous therapeutics, the efficacy of radiotherapy, and the immunity of immune cells. Meanwhile, the coverage of tumor blood vessels by pericytes reduces the migration of metastatic tumor cells into circulation, curtailing tumor metastasis. Tumor vascular normalization has become a complementary therapeutic paradigm for cancer and other vascular disorders [10–15]. Several types of tumor vascular normalizing agents have shown anticancer potential, including anti-VEGF signaling agents, PHD2 agonists, VE-cadherin functional inhibitory proteins and antibodies, various small molecules such as traditional Chinese herbal drugs, and potential therapeutics to increase pericyte vascular coverage.

Current anti-VEGF signaling agents in tumor vascular normalization

Under the guidance of classic angiogenesis theory, several antiangiogenic drugs that mainly target VEGF signaling and endothelial cell-mediated angiogenesis have been used in clinical or preclinical assessments for the anticancer therapy, including the humanized VEGF monoclonal antibody bevacizumab (Avastin), the anti-VEGFR2 antibody IMC-1121b, the small molecule VEGFR2 inhibitor vatalanib, the VEGF receptor chimeric protein afliberecept, the VEGFR2 inhibitor peptide CT-322, and a VEGFR downstream protein kinase inhibitor Sutent [3–5]. VEGF is undoubtedly a critical growth factor for vascular endothelial cell-mediated angiogenesis, and anti-VEGF therapy has become promising in the treatment of advanced colon and non-small cell lung cancers. The angiostatic effects of current anti-VEGF signaling drugs have been attributed to the inhibition of endothelial cell-mediated angiogenesis [64,65] because VEGF receptors have traditionally been thought to be limited to vascular endothelial cells. However, increasing evidence has shown that VEGFR2 is also expressed in perivascular cells, monocytes/macrophages, dendritic cells, hematopoietic stem cells, and bone marrow-derived circulating cells [9], suggesting that these cells may be affected by VEGF-VEGFR signaling inhibitors. The lack of a close association between the levels of VEGF in cancer patients and the antiangiogenic efficacy of the anti-VEGF signaling drugs has been a puzzle for researchers and clinical doctors for quite some time. VEGF can not be regarded as a biomarker of anti-VEGF and antiangiogenic therapies [66], suggesting that other mechanisms may be contributing to the angiostatic function of Avastin and other VEGF inhibitors.

The production and release of VEGF by tumor cells has been well established. On the one hand, VEGF induces endothelial cell migration to the tumor tissue and the formation of tumor blood vessels [1,2]. On the other hand, it reduces pericyte responsiveness to the related growth factor, platelet-derived growth factor (PDGF), resulting in small pericyte coverage in tumor vasculature [67]. Increasing evidence has indicated that anti-VEGF signaling drugs have dramatic effects on mural and perivascular cells in the tumors that increase pericyte coverage of the tumor blood vessels, resulting in tumor vascular normalization [68,69]. Thus, normalization of disorganized tumor vasculature through an increase in pericyte coverage is one of the important mechanisms of the anti-VEGF drug activity.

Although several current antiangiogenic drugs inhibit VEGF signaling and produce tumor vascular normalization effects, these drugs are notably still insufficient in curtailing tumor growth and metastasis [6–9], indicating that more potent tumor vascular normalizing agents are needed to achieve high efficacy of anticancer therapies.

PHD2 and its agonists in tumor vascular normalization

PHD enzymes belong to the iron(II)-2-oxoglutarate-dependent dioxygenase families and have potential in regulating the HIF level and activity in cells. Among the PHD enzymes, PHD2 is the most important in controlling the levels of HIF-1α and HIF activity under normoxial conditions [70]. Increasing data have shown that PHD2 functions as a tumor suppressor gene through the degradation of the HIF-1α unit and the decline in HIF activity. Convincingly, mutation of the PHD2 gene or loss of PHD2 activity under hypoxial conditions affects tumor angiogenesis and tumor development [70–72]. Accordingly, PHD2 has been targeted for tumor vascular normalizing drug discovery.

Choi *et al*. [73] screened a molecular library consisting of more than 600 small molecules using PHD2 as the target and found a potent PHD2 activator called KRH102053 [2-amino-4-methylsulphanyl-butylic acid-4-methoxy-6-(4-methoxy-benzylamino)-2,2-dimethyl-chroman-3-yl ester], which acted as a PHD2 agonist to decrease the levels of HIF-1α and HIF activity. As a consequence, the expression of HIF-regulated downstream vasculogenic target genes decreased. KRH102053 also inhibited human umbilical vein endothelial cell-mediated tube formation and HIF-related migration and

invasion of human osteosarcoma cells [73]. Moreover, another PHD2 activator, KRH102140, effectively inhibited HIF-1α activity and decreased angiogenesis [74]. *In vivo* studies of these PHD2 activators in tumor vascular normalization, growth, and metastasis are needed to evaluate the antitumor efficacy and toxicity of these compounds.

By contrast, several studies have revealed that a haplodeficiency of PHD2 in mice resulted in tumor vascular normalization [71,72]. Carmeliet *et al*. [71] found the vascular normalizing effect of endothelial PHD2 haplodeficiency. In mice, the haplodeficiency of PHD2 promotes vessel maturation and normalization, resulting in improved tumor perfusion and oxygenation and the inhibition of tumor cell invasion, migration, and metastasis [71,72]. In view of the discrepancy between PHD2 agonists and PHD2 haplodeficiency in tumor vascular normalization, the neat effect of PHD2 on blood vessel normalization needs to be verified.

Targeting VE-cadherin for tumor vascular normalization

VE-cadherin is located at the surface of vascular endothelial cells and functions as a bridge that connects vascular endothelial cells [61]. Knockout of the VE-cadherin gene in mice resulted in early embryonic death of mice because of failure to generate primitive blood vessels, indicating that VE-cadherin plays a pivotal role during early embryonic vasculogenesis and development. In a normal human body after birth, VE-cadherin only expresses in vascular endothelial cells and not in other normal tissues and cells; it is a major player in the maintenance of vascular integrity and function [58,75–77]. VE-cadherin was also found in the osteosarcoma cell line MG63, which could form a tube-like capillary structure *in vitro*, convincingly silencing VE-cadherin expression in MG63 cells via SiRNA-hindered angiogenic cell function [78]. Increasing evidence has shown that the expression of VE-cadherin in tumor cells is implicated in tumor vasculogenesis and progression [79,80]. More recently, VE-cadherin was found overexpressed in the human ovarian cancer cell line Hey1B and 22 other tumor cell lines. Interestingly, most of these tumor cells gained the ability to form tube-like capillary structures *in vitro* in Matrigel (unpublished observation), suggesting that VE-cadherin plays a key role in tumor cell-mediated vasculogenesis. Hence, tumor cell VE-cadherin may be a good target for novel antitumor vasculogenic drug discovery. The mechanisms of tumor cell-dominant vasculogenesis are still not clear; vasculogenic genes and biomarkers in these tumor cells are currently being actively identified to come up with new strategies and approaches to normalize disorganized tumor vasculature.

For that reason, several VE-cadherin-functional inhibitory proteins and monoclonal antibodies have been tested to suppress tumor angiogenesis. T2-tryptophany-tRNA synthetase (T2-TrpRS) effectively inhibited retinal angiogenesis in a mouse model, but it did not affect or disrupt normal vasculature. Interestingly, T2-TrpRS promoted the regeneration of normal blood vessels in retinopathy but thwarted pathological retinol vasculature [81,82], suggesting that T2-TrpRS especially normalizes angiogenic blood vessels.

T2-TrpRS has recently been found to block tumor VE-cadherin, and particularly suppress tumor endothelial cell-mediated angiogenesis, although it did not affect normal endothelial cell-mediated angiogenesis [83]. More recently, our mechanism study revealed that T2-TrpRS bound to the two critical amino acids Trp2 and Trp4 in VE-cadherin extra cellular domain 1 and prohibited VE-cadherin to form dimers on the tumor cell surface. As a result, tumor endothelial cells with unengaged VE-cadherin failed to connect to each other to form capillary tubes [84], suggesting that T2-TrpRS can be a specific tumor vascular normalizing agent.

In addition, monoclonal antibodies against VE-cadherin have also exhibited specific antitumor effects. Actinium-225, an alpha-emitting antibody construct (E4G10), specifically bound to the unengaged form of VE-cadherin and showed potent and selective killing of tumor neovascular endothelium and EPCs in the bone marrow; however, no specific normal-tissue uptake of E4G10 was seen via imaging or post-mortem biodistribution studies in mice [85].

Taken together, the unengaged form of VE-cadherin in tumor cells, as well as the tumor neovascular endothelium and bone marrow-derived EPCs can be targeted for special tumor vascular normalizing drug discovery.

Targeting tumor-associated macrophages for tumor vascular normalization

During tumor development, several types of bone marrow-derived cells, including EPCs, TAMs, and MSCs, are recruited from the blood to the tumor tissues [38]. Increasing evidence has shown that TAMs contribute to tumor angiogenesis and vasculogenesis, and TAMs with proangiogenic and immune-suppressive (M2-like) phenotypes are hallmarks of malignancy [38,39]. Rolny *et al*. [86] reported that histidine-rich glycoprotein (HRG) inhibited tumor growth and metastasis through the enhancement of vessel normalization and antitumor immune responses. Mechanism studies indicated that HRG suppressed the expression of placental growth factor and induced the polarization of tumor-associated macrophages, resulting in tumor vascular normalization and enhancement of immunity [86]. This finding suggests that the modulation of TAMs may be another way to curb tumor neovascularization.

Traditional Chinese herbal drugs in tumor vascular normalization

Historically, traditional Chinese medicine has been widely used in China for thousands of years and has shown promising results in the prevention and treatment of cancers. For example, the Realgar-*Indigo naturalis* formula has been

proven very effective in the treatment of human acute promyelocytic leukemia [87]. One type of traditional Chinese medicinal drug named the "activating blood circulation to dissipate blood stasis" (活血化瘀) has been used in China to prevent and treat both cardiac vascular diseases and tumors for many years [88–98]. Accumulated data have shown that "activating blood circulation to dissipate blood stasis" drugs can increase blood microcirculation, facilitate oxygen and nutrition supply, and promote the removal of waste materials from tissues, resulting in an improvement of tissue blood circulation and vascular function [88]. So far, various "activating blood circulation to dissipate blood stasis" herbal extracts and purified active compounds, including celastrol [89], morelloflavone [90], gambogic acid [91], acetyl-11-keto-β-boswellic acid [92,93], 1′-acetoxychavicol acetate [94], cudratricusxanthone G [95], *Salvia miltiorrhiza* Bunge [96], *Erigeron breviscapus* Hand-Mazz [97], and *Hedyotis diffusea* Willd [98], have been used to improve tissue blood circulation and treat malignant tumors. In particular, the combination of the "activating blood circulation to dissipate blood stasis" drugs with current anticancer chemotherapeutics exerted a better effect as a collective anti-tumor treatment [99].

Manipulation of the pericytes in tumor tissues for vascular normalization

Tumor vasculature is characterized by dilated and tortuous vessels, thickened basement membranes, small pericyte coverage, immaturity, and high permeability. The mechanisms of the generation of the disorganized tumor vasculature are under investigation. One possibility is that tumor blood vessels can be lined by tumor cells through vasculogenesis. Although tumor cells gain vasculogenic capability, the cells clearly have not acquired the entire function of normal vascular endothelial cells, instead generating the disorganized tumor vasculature, as previously mentioned [16–30,41–48,100]. Another possibility is that tumor tissues have high VEGF levels, which impede pericyte vascular coverage.

The inhibition of VEGF and its receptor can have dramatic and unexpected effects on mural and perivascular cell functions in the tumor tissue, resulting in vascular smooth muscle cell and pericyte activation as well as blood vessel normalization/maturation [63–66,101,102]. Angiopoietin-1 (Ang1) has been shown to act as an angiogenic promoter in embryonic angiogenesis by promoting vascular branching, pericyte recruitment, and endothelial survival. Increasing experimental data suggest that Ang1-stimulated association of mural cells with endothelial cells leads to the stabilization of newly formed blood vessels. Ang1 can be used, alone or in combination with VEGF, to promote therapeutic angiogenesis [101,102]. In addition, inhibition of angiogenesis with an antibody DC101 that blocks VEGFR2 significantly promotes pericyte recruitment and increases the number of pericyte-covered vessels [71,72,103]. Increasing data have shown that normalization of tumor blood vessels can lead to an improvement in the metabolic profile of the tumor microenvironment, as well as in the delivery and efficacy of exogenously administered therapeutics, efficacy of radiotherapy and of effective immune cells, and a reduction in the number of metastatic cells shed by tumors into circulation [10–14,71,72,101–104]. Therefore, manipulation of pericytes in tumor tissues has opened another option to normalize the disorganized tumor vasculature.

Challenges and perspectives

Since the new concept of the normalization of disorganized tumor vasculature for anticancer therapy was raised by Jain in 2001, sound progress has been made in preclinical and initial clinical trials. Normalization of disorganized tumor blood vessels results in an increase in tumor tissue blood and oxygen supply, improvement of the distribution and efficacy of other anticancer drugs, and reduction in tumor growth and metastasis [10–14]. However, moderate antitumor effects of current tumor vascular normalizing drugs have shadowed the prospect of vascular normalization strategies in anticancer therapy. In the subsequent section, with the development of new strategies and approaches to uncover potent tumor vascular normalizing agents and correct the disorganized tumor vasculature for an effective anticancer therapy will be discussed.

Targeting vasculogenic cells in tumors for tumor vascular normalization

Current antitumor angiogenesis and vascular normalization therapies have mainly focused on anti-VEGF and endothelial cell-predominant angiogenesis, overlooking tumor vasculogenesis mediated by tumor cells and BM-derived cells. As previously mentioned, aside from endothelial cells, various other cells, including tumor cells, EPCs, MSCs, TAMs, and pericytes, play an important role in tumor neovascularization through vasculogenesis [1,18,19]. In particular, a variety of tumor cells, including pre-cancer stem cells, cancer stem cells, tumor endothelial cells, and various other aggressive tumor cells, participate in the formation of tumor blood vessels through at least three ways [18]: (1) blood vessel co-option between tumor cells and vascular endothelial cells, (2) tumor cell-predominant vascular mimicry (independent of vascular endothelial cells), and (3) cancer stem cell transdifferentiation into tumor endothelial cells (Fig. 2). Tumor cell-consisting blood vessels have unique morphological characteristics that differ from normal vasculature, as previously stated [8,9,13,15], and account for poor blood circulation in the tumor tissues. Accumulated data have shown that vasculogenic tumor cells play critical roles in tumor neovascularization, growth, and metastasis [16–30,41–48,100]. Hence, vasculogenic tumor cells should be targeted for the normalization of disorganized tumor vasculature.

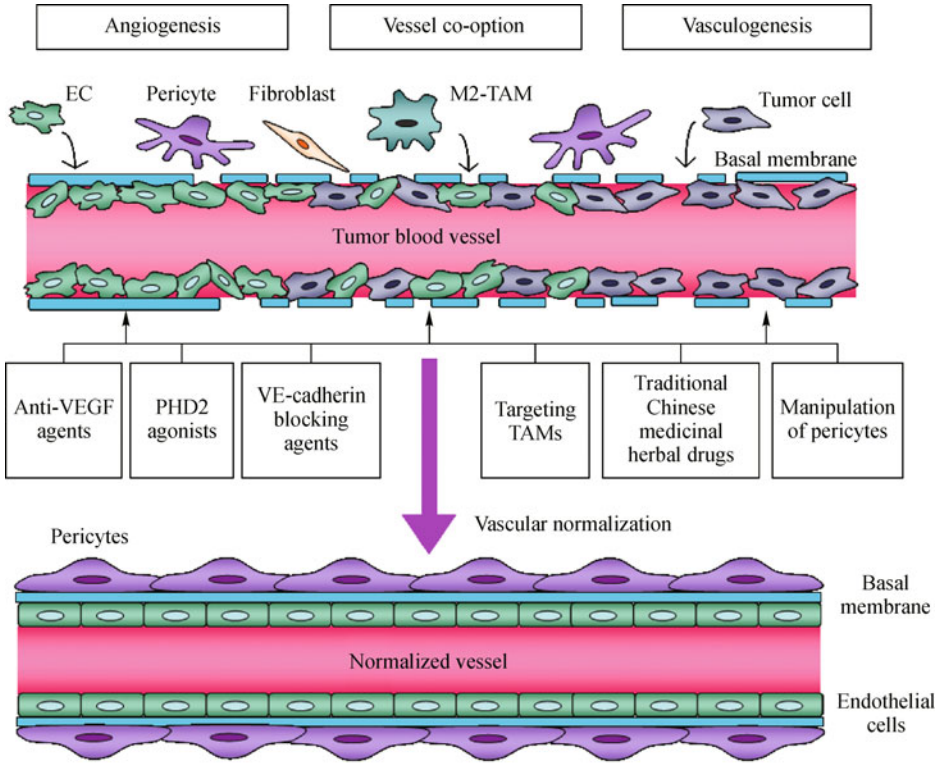

Fig. 2 Normalization of disorganized tumor blood vessels for anticancer therapy. Malignant tumors may make their own blood vessels via a complex process of neovascularization through (1) VEGF and endothelial cell-mediated angiogenesis, (2) vessel co-option between endothelial cells and tumor cells, and (3) tumor cell-predominant vasculogenesis. The disorganized tumor blood vessels could be normalized using several options, including (1) anti-VEGF therapeutics, (2) PHD2 agonists, (3) VE-cadherin blocking agents, (4) targeting tumor-associated macrophages, (5) traditional Chinese medicinal herbal drugs, and (6) manipulation of pericytes.

Vasculogenic and angiogenic tumor cells must be targeted for effective normalization of the disorganized tumor vasculature because of two reasons. First, tumor cells can directly generate blood vessels via vasculogenesis. Second, tumor cells secrete a variety of angiogenic factors that initiate and promote tumor angiogenesis. Incredibly, most of the aggressive tumor cells have vasculogenic and/or angiogenic capabilities; therefore, targeting vasculogenic and angiogenic tumor cells may be a "one stone hits two birds" approach to impeding tumor neovascularization and hindering tumor cell-mediated tumorigenesis. In addition, targeting vasculogenic tumor cells and genes may provide a new opportunity for novel antitumor vasculogenic drug discovery. Moreover, the combination of antitumor vasculogenic agents with anti-angiogenic drugs may exhibit synergistic effects in anticancer therapy.

Identification of biomarkers for tumor vascular normalization

VEGF, which is an important angiogenic factor, is over-expressed in a variety of tumors and has been targeted for antiangiogenic therapy. However, clinical investigation has indicated that VEGF levels in cancer patients are generally poorly associated with the outcome of antiangiogenic therapy, and that VEGF is not a good biomarker for antiangiogenesis and anticancer therapies [69]. Although five antiangiogenic drugs have been utilized for anticancer therapy in clinical settings, no validated biomarkers for monitoring and evaluating antitumor angiogenic therapy has been found at present. Ironically, hypertension, a toxic side effect of antiangiogenic therapy, has been reported to be closely associated with a better outcome of antiangiogenic therapy [6,7,69]. Therefore, identifying tumor angiogenic and vasculogenic biomarkers beyond VEGF signaling is highly desirable.

Sorensen *et al*. [105] have recently reported a "vascular normalization index," which measured the changes in the Ktrans, microvessel volume, and circulating collagen IV in recurrent glioblastoma patients, as a biomarker for tumor vascular normalization. They showed that the vascular normalization index was closely correlated to the duration of overall survival and/or to the progression-free survival ($P < 0.05$) of the patients. Determining whether the vascular

normalization index is a universal biomarker for tumor vascular normalization in other types of tumor would be worthwhile.

The interstitial fluid pressure is usually more than 10-fold higher in a variety of tumor tissues compared with those of normal tissues. Tumor vasculature is highly permeable, and intravascular fluids and plasma proteins are accumulated in the tumor tissues [13]. Hence, interstitial fluid pressure and intravascular plasma proteins in the tumor may be considered as biomarker candidates for tumor vascular normalization. Accordingly, appropriate techniques will be required to measure these biomarkers. In this context, new, noninvasive imaging technologies, including noninvasive variable-magnification *in vivo*-fluorescence imaging and fluorescence tomography [106], magnetic resonance imaging [107], and acoustic radiation force-induced optical spectroscopy, would be very useful. Further clinical validation of these biomarkers and new technologies is needed to evaluate the anticancer efficacy of tumor vascular normalization drugs and improve anticancer therapy.

Multiple-targeting of tumor blood vessels for tumor vascular normalization

Tumor tissues consist of cancer cells and non-malignant cells, which include endothelial cells, pericytes, fibroblasts, macrophages, lymphocytes, dendritic cells, mast cells, and many other types of cells. Tumor cells orchestrate with other cells to form a disorganized tumor vasculature and a hostile tumor microenvironment that promotes tumor growth, invasion, metastasis, and resistance to various therapies. In addition, various vasculogenic and angiogenic genes are overexpressed in tumor vascular cells and contribute to tumor neovascularization. Thus, the multiple-targeting of vasculogenic tumor cells, bone marrow-derived cells, and other vasculogenic cells in a tumor microenvironment, as well as the retardation of vasculogenic and angiogenic gene overexpression, may improve the efficacy of tumor vascular normalization.

In addition, the combination of tumor vascular normalization agents with current antiangiogenic and cytotoxic drugs may achieve a synergistic effect during tumor vascular normalization. Furthermore, many traditional Chinese herbal drugs display antiangiogenic effects and enhance tumor vascular normalization. Therefore, the integration of Western medicine with traditional Chinese medicine may provide a good opportunity for achieving a high efficacy of tumor vascular normalization.

Although tumor vascular normalization has only been studied for 10 years, the sound progress in tumor vascular normalization indicates a bright prospect for this approach and warrants further investigation. Success in the identification of specific genes, cells, and biomarkers in disorganized tumor vasculature is predicted, and multiple-targeting of key vasculogenic cells and genes in tumor blood vessels, as well as the integration of Western medicine with traditional Chinese medicine, will bring a breakthrough in tumor vascular normalization for an effective anticancer therapy.

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