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Vascular Mimicry: A Novel Neovascularization Mechanism Driving Anti-Angiogenic Therapy (AAT) Resistance in Glioblastoma

Kartik Angara, Thaiz F. Borin and Ali S. Arbab

Laboratory of Tumor Angiogenesis, Georgia Cancer Center, Department of Biochemistry and Molecular Biology, Augusta University, Augusta, GA, USA

Abstract

Glioblastoma (GBM) is a hypervascular neoplasia of the central nervous system with an extremely high rate of mortality. Owing to its hypervascularity, anti-angiogenic therapies (AAT) have been used as an adjuvant to the traditional surgical resection, chemotherapy, and radiation. The benefits of AAT have been transient and the tumors were shown to relapse faster and demonstrated particularly high rates of AAT therapy resistance. Alternative neovascularization mechanisms were shown to be at work in these resilient tumors to counter the AAT therapy insult. Vascular Mimicry (VM) is the uncanny ability of tumor cells to acquire endothelial-like properties and lay down vascular patterned networks reminiscent of host endothelial blood vessels. The VM channels served as an irrigation system for the tumors to meet with the increasing metabolic and nutrient demands of the tumor in the event of the ensuing hypoxia resulting from AAT. In our previous studies, we have demonstrated that AAT accelerates VM in GBM. In this review, we will focus on the origins of VM, visualizing VM in AAT-treated tumors and the development of VM as a resistance mechanism to AAT.

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Introduction

Tumor growth and metastasis have been long associated with the processes of angiogenesis and vasculogenesis assuming a pathological form. This dogma was rendered obsolete when a novel non-angiogenic dependent pathway was introduced in 1999 by Maniotis et al. Vascular Mimicry (VM) was described as a completely neoteric blood supply system feeding tumor cells in malignant melanoma, which utilizes the uncanny ability of aggressive melanoma cells to transdifferentiate into stem cell-like state to subsequently assume an endothelial-like phenotype [1]. VM enables the tumors to form matrix-embedded vascular structures containing plasma and blood cells to meet with the increasing nutrient and metabolic demands of the neoplastic tissues. Since the channels are formed without the contribution of pre-existing host endothelial cells, the process is vasculogenic in nature. The ensuing vascular-like structures are not true blood vessels but merely mimic the function of vessels, thereby clearly defining the phenomenon of VM [1–4]. VM vessels are matrix rich structures rich in laminin, positive for PAS staining and are often found encapsulating nests or lobules of tumor cells as closed loops. The formation of extracellular patterned matrices rich in laminin, proteoglycans, heparan sulfate and collagens IV and VI as a part of their basement membranes visualized by the aforementioned PAS staining has already been established as a crucial histopathological evidence of VM in hypervascular tumors [2–8]. After the initial discovery of VM as a novel neovascularization mechanism in aggressive melanomas, VM was also reported in many other non-melanoma neoplastic malignancies such as breast [9], ovarian [10,11], prostate [12], lung [13] and also in glioblastoma (GBM) [14]. However, the phenomenon of VM has been a subject of intense controversy and many studies have in fact questioned the validity of VM [15,16]. Though the occurrence of VM structures is rare in tumors, the presence of these patterned matrices rich in ECM is positively correlated with the increased risk of metastasis, poor clinical

Address all correspondence to: Ali S. Arbab, MD, PhD, Georgia Cancer Center, Augusta University, 1410 Laney Walker Blvd, CN3141, Augusta, GA 30912.
E-mail: aarbab@augusta.edu
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outcome, and bleak survival time after initial diagnosis in patients [7,17]. In this review, we will describe the possible mechanisms of neovascularization in GBM including VM, antiangiogenic therapy (AAT)-induced VM, different subtypes of VM, visualization of VM, and possible therapy to target VM.

Mechanisms of Neovascularization in Glioblastoma

Tumor blood vessels are radically different from the normal host blood vessels, both morphologically and physiologically. The host endothelial vascular structures are comprised of endothelial cells (ECs) and supporting cells such as pericytes and astrocytes to form an intact highly regulated membranous Blood Brain Barrier (BBB). On the other hand, GBM vessels are tortuous, disorganized, highly permeable, destabilized structures with abnormal endothelial and pericyte coverage [18,19]. The classical tumor development was attributed, hitherto, to two main mechanisms of vascularization, the classical sprouting angiogenesis, in which the pre-existing ECs proliferate and migrate to form neovessels, and vasculogenesis, in which the cells from the bone marrow are recruited to the tumor sites to contribute to the formation of neovascular structures [20,21]. Several studies have also reported the role of endothelial progenitor cells (EPCs) in contributing to the tumor vascular endothelium. These EPCs are recruited to the tumor microenvironment (TME) in a VEGF and SDF-1 dependent manner. However, this concept has not been riddled with controversies [22–25]. As tumors grow in size, they need an increased supply of blood vessels to keep them viable. Avascular tumors that are unable to acquire new blood vessels to meet their metabolic and nutrient demands eventually regress [26–28]. Co-option of the pre-existing vessels in the neighboring tissue is one of the most critical steps utilized by the tumor to keep itself viable and growing [29]. During the early formative stages of tumor development, angiopoietin-2 (Ang-2) is expressed on the co-opted tumor vasculature, and as the tumors grow in size, there is a widespread increase in the expression of Ang-2 on both the endothelial cells and the tumor cells. The over-expression of Ang-2 causes destabilization of blood vessels caused by the disengagement of pericytes and loss of endothelial barrier integrity to promote tumor hypoxia and necrosis [18,20,29]. The initiation of hypoxic conditions in the tumor prompt the translocation of hypoxia inducible factor-1α (HIF-1α) into the nuclei of cells and thereby up-regulating the expression of HIF-1α dependent expression of pro-angiogenic factors such as matrix metalloproteinases (MMP-2 and -9), VEGF, stromal cell derived factor-1α (SDF-1α) and inteleukin-8 (IL-8). The combined action of these causes the degradation of ECM scaffold and basement membrane to facilitate the creation of new areas in and around tumors, where neovessels can be established. In addition, these alterations of the ECM scaffolds in the TME bolster the metastatic capability of tumor cells by synergizing the migration and extravasation of tumor cells [30–32]. VEGF can subsequently up-regulate the expression of VEGFR2 on the ECs and facilitate sprouting angiogenesis and vascular remodeling in conjunction with Ang-2. Many studies have already established the role of Ang-2 in initiation of vessel sprouting and pericyte loss to promote neovessel formation in the tumors [20,33–37]. Delta like-4 (Dll4)-Notch-1 signaling and Ephrin B2 regulate the sprouting and branching processes in the endothelial tip cells in response to VEGF. Moreover, greater expression of Dll4 is associated with a high-grade glioma, where it facilitates vascular function by thwarting non-functional angiogenesis in mouse models of glioma and other tumors [38–43]. Several pro-angiogenic growth factors and chemokines such as VEGF, SDF-1α, platelet-derived growth factor (PDGF), IL8, fibroblast growth factor (FGF2) and angiopoietin-1 create an environment conducive to EC recruitment, proliferation, and migration [18]. Integrin complexes such as αvβ3, αvβ5, and α5β1 are crucial to the tumor angiogenesis [18,44] with αvβ3 integrin mediating FGF2-dependent angiogenesis and αvβ5 integrin being responsible for VEGF-dependent angiogenesis [45]. The corresponding expression of these integrins on the tumor cells, usually in high-grade gliomas, positively correlates with poor prognosis and an aggressive phenotype [46–48].

Anti-Angiogenic Therapy Resistance in Glioblastomas

Ide et al. in 1939 made the first observation of tumor growth being juxtaposed with formation of new blood vessels in a process known as angiogenesis [49]. Folkman et al. demonstrated that injecting tumor cells into isolated perfused organs did not facilitate tumor growth, but when the same tumor cells were transplanted into syngeneic mice, these tumors grew beyond few millimeters owing to rapid revascularization. This groundbreaking experiment proved beyond doubt that tumors depended on blood supply and neovascularization for growth. Following these experiments was the idea that since tumor growth was angiogenesis-dependent, inhibition of this process could lead to therapeutic benefits. Thus the idea of "anti-angiogenesis" came into existence, both as a concept and in to clinical practice, so much so that tumor angiogenesis has become one of the "hallmarks of cancer" [26,50,51]. Owing to the hypervascular nature of the GBM tumors, anti-angiogenic therapies (AAT) including, vatalanib, sunitinib, and cediranib were used to control the abnormal angiogenesis and also to normalize the tumor vasculature [52–57]. It was believed that since the endothelial cells have comparatively lower genetic instability than tumor cells, a more viable option would be to target the VEGF-VEGFR pathways to counter angiogenesis without imposing drug resistance. Regrettably, the benefits offered by AAT are transitory, and the tumors showed higher rates of relapse and potent refractoriness [58]. Previous studies in our lab have already shown that targeting VEGFR2 using vatalanib (PTK787) significantly increased the tumor size as visualized using DCE-MRI [59]. Vatalanib treatment induced hypoxia and was associated with the increased expression of VEGF, SDF-1α, HIF-1α, FGF-1, FGF-2, ephrin-A1, ephrin-A2, angiopoietin-1, VEGFR2, VEGFR3, and EGFR at the peripheral part of the tumors compared to that of central part of tumors [60]. Cediranib and sunitinib, inhibitors of VEGFR, PDGFR and c-kit receptors, have been associated with limited therapeutic efficacy and offered transient benefits with high toxicity rates despite showing promising results in pre-clinical trials [61–65]. Similarly, vatalanib did not offer any considerable benefits in clinical trials [64]. In addition, another study has reported that there was an increase in the plasma concentration of FGF-2 in colon cancer and GBM patients following AAT [66]. Vatalanib (PTK787, Novartis Pharmaceuticals Corporation, East Hanover, NJ) is a pan-VEGFR, PDGFR, and c-Kit tyrosine kinase inhibitor (TKI) and its utility in recurrent GBM has been studied both as monotherapy [67] or in combination with temozolomide and lomustine [68]. Vatalanib also significantly increased CD68+ myeloid cells, and CD133+, CD34+, and Tie2+ endothelial cell signatures demonstrated in a recent study from our lab [91]. By preventing the mobilization of BMDCs and interaction of CXCR4-SDF-1 using whole body irradiation and AMD3100, respectively, paradoxical growth of tumor following Vatalanib treatment was controlled [69]. Bevacizumab, a humanized monoclonal antibody against VEGF-165, the predominant isoform

of VEGF-A was the first angiogenesis inhibitor that got the approval of the Food and Drug Administration (FDA) in 2004. It also was the first commercially successful anti-angiogenic drug marketed [70]. Bevacizumab in combination with cytotoxic chemotherapy produced some dramatic tumor size reduction as seen on radiographic images with prolonged progression free survival (PFS) and also reduced the

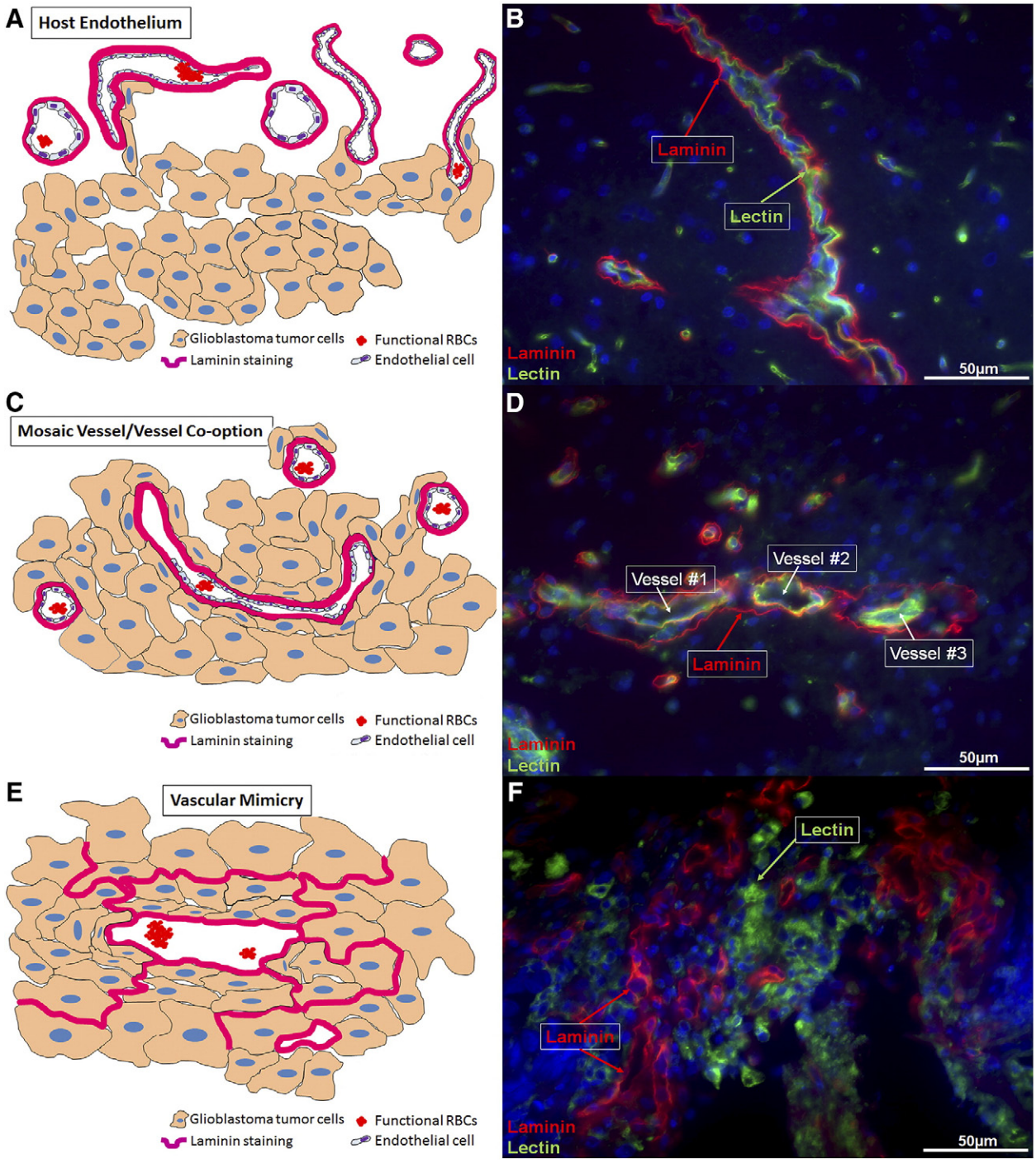


Figure 1. Schematic of vascular mimicry development driving anti-angiogenic therapy (AAT) resistance in glioblastoma. (A) Host endothelial-dependent blood vessel formation. (B) Host endothelial vessels visualized outside the tumor area. In the host tissue, the Laminin staining is present on the outside and the Lectin staining is present on the inside. (C) Formation of mosaic vessels in the tumor periphery. (D) The formation of a mosaic vessels can be evidenced here. The three host endothelial vessels are being encapsulated by the tumor cells (in red, Laminin). However, the tumors can be seen trying to form a physiological connection with the host endothelial-cell dependent vessels as observed in the vessels #1 and #2. The laminin-positive loops are seen to be attached or incorporated into host endothelial vessels and thus present as a super-imposed yellow colored structures (green from host endothelium and red from the tumor derived laminin). (E) Vascular Mimicry-Tube like vascular structures in the tumor center. Here the laminin based patterned networks are independent of the host endothelial cell contribution. (F) Vascular mimicry like patterned networks can be seen in the tumor center. In the tumor center, the Laminin staining is present independently of the Lectin staining because the tumor has begun the process of VM. The Lectin now depicts the tumor cells acquiring endothelial like properties, reminiscent of the host endothelial cells (Magnification 40×).

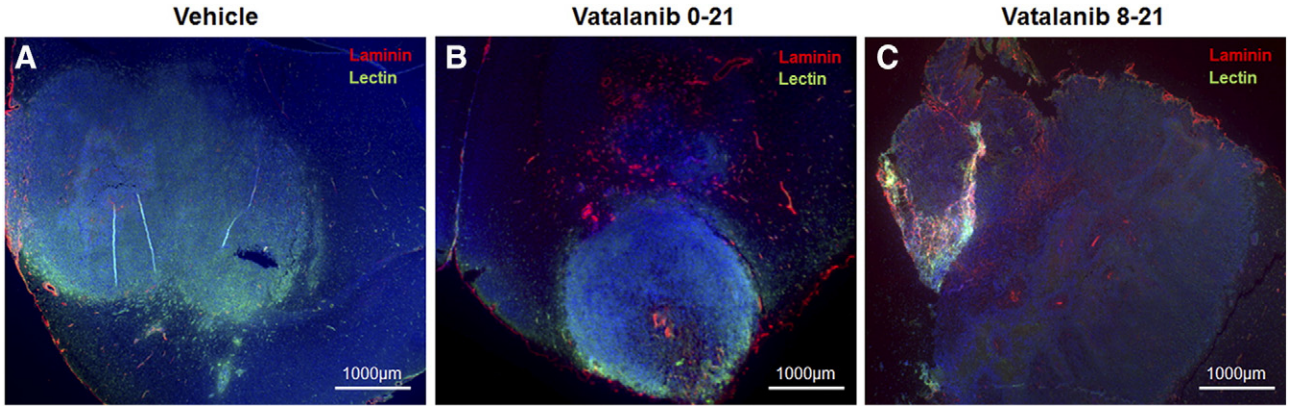


Figure 2. Accelerated VM in AAT-treated tumors in orthotopic human GBM mouse model. (B and C) Vatalanib-treated tumors show significantly higher number of laminin-positive areas that are spread over the entire tumor when compared to the vehicle-treated tumors (A). The images have been taken at a magnification of 2.5× to ease the depiction of tumor size in each treated animal of the respective group.

need for corticosteroids [71]. However, prolonged use of bevacizumab resulted in deteriorated clinical outcome with patients developing resistance and eventually succumbing to the disease owing to ineffective therapies [71–73]. The development of resistance to AAT is thought to be due to activation of alternative pathways of neovascularization [21]. One of the mechanisms is VM.

VM in gliomas is associated with extremely poor prognosis and positively correlates with high grades of tumor malignancy and invasiveness [74,75]. In the light of these evidences, it becomes imperative to understand VM and its molecular signaling mechanisms to develop novel therapeutics to target tumor growth and metastasis.

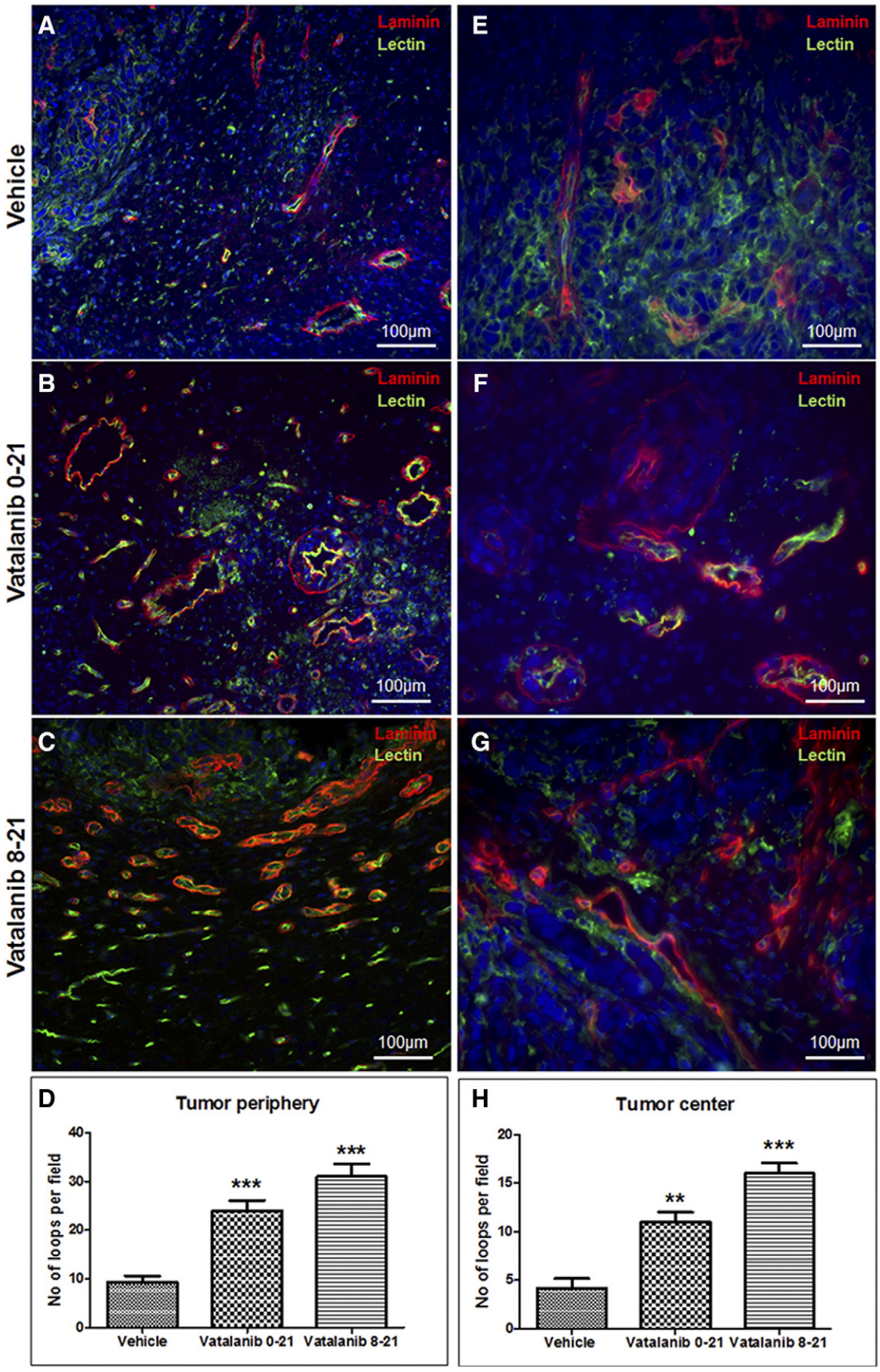
Alternative Neovascularization Pathways and Vasculogenic Mimicry (VM)

Anti-angiogenic therapies act through different pathways in concert to delay tumor progression and prolong survival in patients. Tumor starvation was achieved by the coordinated mechanisms of action such as the induction of apoptosis of ECs, preventing the migration of EPCs, inhibition of formation of new blood vessels, obliteration of existing microvessels, decrease tumor vascular irrigation and thus impeding the oxygenation and nutrient supply to the tumor [49]. Evolutionary biology has always promoted natural selection of survival by favoring the development or acquisition of novel adaptive dynamics that produce a resilient system. Since AAT works as a selective pressure upon cancer cells, the surviving tumor cells develop adaptive mechanisms that ensure a survival advantage for these cells in the TME. Cancer cells are notorious for being genetically instable and heterogeneous. The hypoxia that ensues following AAT paves way for initiation of alternative pathways of tumor adaptation, activates pro-angiogenic, invasive and metastatic gene signatures in tumors cells and ECs, up-regulates a variety of growth factors, chemokines and cytokines necessary for recruitment of ECs, EPCs, vasculogenic leukocytes, angiogenic myeloid cells to cope with the demands of the compromised microenvironment [93–98]. In the event of a therapeutic attack with AAT, the tumors resort to various neovascularization mechanisms in addition to the regularly documented angiogenesis and vasculogenesis. VM is one such novel neovascularization mechanism that is slowly gaining attention in the scientific field due to its widely accepted existence across several tumors and validated evidences. Glioblastoma stem-like cells (GSCs) and transdifferentiated tumor cells with endothelial properties are the cellular source of tumor blood vessels.

In the burgeoning tumor, the neoplastic cells are hyper proliferative and their metabolic and nutrient demands grow exponentially. This surpasses the rate of formation of new blood vessels and simulates a scenario of a tumor undergoing AAT. To meet with the increasing demands, the tumor has to resort to a non-endothelial system of nutrient supply and waste disposal. A novel yet interesting phenomenon that encompasses an irrigational system of perfusing tumors is speculated to be VM. VM describes the uncanny ability of tumor cells to transdifferentiate into endothelial-like cells and thereby acquire the capability of forming tube like structures mimicking blood vessels independent of the ECs [1,76]. These transformed endothelial-like cells secrete matrix proteins such as collagens IV and VI, proteoglycans, heparan sulfate and laminin that aid the formation of tubular networks within the tumors that are anatomically in stark contrast to the regular traditional blood vessels, where the basal lamina is present behind the ECs. These VM like tubular networks have basal lamina towards the lumen and concomitantly have polysaccharides as markers of VM demonstrating strong positive staining for periodic Acid-Schiff (PAS) [2]. The plasticity of tumor cells and their corresponding heterogeneity favors the acquisition of endothelial markers such as CD31, VEGFR2 and VE-Cadherin and as such enables the tumor cells to incorporate into host endothelial tubular structures and form a connection with them [1]. Similar to the contribution of the trophoblasts to the formation of non-endothelial vascular structures in the maternal uterine walls [77], the vascular structures formed by the endothelial-like transformed cells function as a percolative system controlling the oxygen and nutrient supply to meet with the metabolic needs as well as to discharge the cellular secretion. Eventually, the tumor-derived vasculature merges with the endothelial-derived vasculature to form a "mosaic vasculature", an anatomically hybrid structure engineered by the tumor as a fusion of both the endothelial and non-endothelial cellular sources [2,78–80].

Types of Vasculogenic Mimicry (VM)

There are two distinct types of VM reported in literature, the tubular type [81] and the classical patterned matrices type [2,14]. The tumor cells


Vehicle
Vatalanib 0-21
Vatalanib 8-21
A
B
C
E
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Lectin
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associated with the VM structures revert to an undifferentiated, embryonic like phenotype and acquire the characteristics of endothelial cells. This conversion of the tumor cells into an embryonic like state empowers the cells to form blood vessel like structures reminiscent of the embryonic vasculogenic structures [1,82,83].

In our previous study [84], we have established histopathological confirmation of the existence of different forms of VM, including mosaic vessels, sustenance of vascular mimicry (as patterned matrices), and VM like structures. Figure 1*A* depicts a pictographical representation of a host endothelial-cell derived blood vessel. Figure 1*B* shows the host endothelial vessel outside the tumor area. Upon observation, it is clearly evident that the laminin staining is present on the outer side of the vessel lumen and the lectin staining is present on the inside of the lumen. This characteristic differential localization of laminin (outside) and lectin (inside) is specific to the host endothelium. Figure 1*C* is a pictographical depiction of tumor vessel co-option and formation of a mosaic vessel at the tumor periphery. Since mosaic vessels are the hybrid structures formed by the tumor and host endothelial cell contribution, the laminin staining (in pictographical depiction) comes from the tumor cells and host endothelial cells. However, the host endothelial lectin staining differentiates the tumor from the host blood vessels. Figure 1*D* shows tumor encapsulation of the existing host endothelial structures in an attempt to form mosaic vessels. The figure clearly demonstrates the tumor cells attempting to incorporate into the host endothelial vascular structures as represented by the laminin and lectin co-localization. The cartoon in Figure 1*E* depicts the VM like structures in the tumor center. These structures are laminin positive and are devoid of any host endothelial contribution. Figure 1*F* shows the VM in the tumor center. Laminin-positive loops characteristic of VM are the most standard hallmarks of this form of neovascularization. These laminin loops are devoid of any lectin staining but are believed to acquire lectin staining once the tumors transdifferentiate into endothelial phenotypes.

AAT-Induced VM in Glioblastomas

Investigators have reported increased number of VM in therapy resistant tumors. Our recently published works also indicated higher number of VM in glioblastomas following AAT [84]. We noticed accelerated VM both in the center and in the peripheral part of the AAT-treated tumors. Figure 2 shows the example of VM following AAT. Figure 2 represents the tumor areas co-stained with laminin and lectin. The figure shows that the vatalanib-treated tumors have comparatively higher laminin-positive areas than the vehicle-treated group. We have quantified the laminin-positive loop like structures indicative of the VM or mosaic vessels (laminin co-localized with lectin) both at the tumor periphery as in Figure 3, *A–D* and in the tumor center as in Figure 3, *E–H*. In our *in vitro* studies, mCherry U251 GBM cells were co-cultured with HUVEC cells on matrigel. After incubation for 6 h, we found that the GBM cells had incorporated into the endothelial (HUVEC)-dependent vascular tube like networks as shown in Figure 4, *A–C*, *E–G*. We analyzed the number of complete tube like networks without any breaks or disintegration and also analyzed the incomplete tube like-structures in the control (PBS) and AAT (vatalanib and avastin (bevacizumab))-treated groups as shown in Figure 4, *D* and *H*. We found that the number of complete tube-like networks were significantly higher in AAT-treated wells compared to the control group (Figure 4*D*). This clearly demonstrates the fact that following AAT, tumors resort to aggressive neovascularization mechanisms to cope with the therapeutic insult and thereby adopt VM as a novel neovascularization mechanism to counter the ensuing hypoxic environment within the tumor.

A Focus on the Cancer Stem Cells (CSCs) and Glioblastoma Stem Like Cells (GSCs) in VM

It is already known beyond doubt that the cancer cells exhibiting high degree of plasticity acquire endothelial-like phenotypes owing to their multipotent nature. These multipotent cells undergo sequential transformations from being epithelial to mesenchymal to endothelial phenotypes, resembling embryonic stem cells [2]. Though specific criteria for establishing GSCs capable of VM are lacking, CD133 and Nestin have been established as biomarkers common to GSC and VM-initiating stem cells [85,86]. Anti-CD133 antibodies can be used to isolate and enrich cancer stem cells (CSCs) or CSCs can also be obtained by the generation of neurospheres in culture conditions using serum-free media containing epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) [87]. The transdifferentiation of tumor cells into endothelial like cells (termed tumor derived endothelial cells, TDECs), which were CD45-CD31+ CD34+ was proposed as a critical switch in functional phenotype of tumor cells to promote VM in GBM models [88]. Yet another study highlighted the role of glioblastoma stem-like cells of CD133+ CD144+ phenotype that promote the VM in GBM tumors. The role of CD133+ CD144- cells in generating the endothelial progenitors (CD133+ CD144+) in the TME due to the tumor-secreted angiogenic factors was conclusively demonstrated with the help of efficient *in vitro* and *in vivo* studies [89]. Previous studies from our lab and others have also reported the endothelial progenitor like characteristics of CD133+ glioma stem like cells or glioma initiating cells [90–92]. Hypoxia is a critical factor that governs VM and hypoxia induced up-regulation of CD144 (VE-Cadherin) has been cited as the key factor driving VM in GBM [93]. A recent study demonstrated that a subpopulation of melanoma cells expressing the vascular cell adhesion molecule PECAM1 but not VEGFR2 is capable of undergoing VM and forming vascular tube like networks. At the mechanistic level, activating enhancer binding protein-2α (AP-2α) was found to be the crucial transcription factor governing the expression of PECAM-1 [94]. The formation of CSCs in general and GSCs in particular is a process that encompasses two closely related yet different mechanisms of (i) multistep mutagenic insults to the normal stem cells and (ii) sequential

Figure 3. Mosaic vessels and Vascular Mimicry like-structures in the AAT-treated tumors. Tumor periphery showing laminin loops present in conjunction with the host-endothelial structures as visible with the green lectin staining in vehicle- (A) and vatalanib (B and C)-treated tumors. The mosaic vessels are also present at the tumor periphery, which are evident as the red-laminin and green-lectin superimposed yellow loop like structures. (D) Quantification of laminin-positive loop like-structures in the tumor periphery. In the central areas, Vatalanib-treated (F and G) tumors show significantly higher number of laminin-positive loop like structures indicative of VM, compared to the vehicle-treated tumors (E). These laminin loops are present independently of the host-endothelial structures. These laminin-positive patterned matrices are formed by the tumor cells alone, without any contribution from the host endothelial cells. (H) The quantification of the laminin-positive loops in the tumor center. Images have been taken from four different areas of the tumor. One representative image from one animal in the respective group has been shown here. The laminin-positive loops have been counted using Image J software and the statistical analyses were performed using Student's *t* test and $P < .05$ was considered significant.

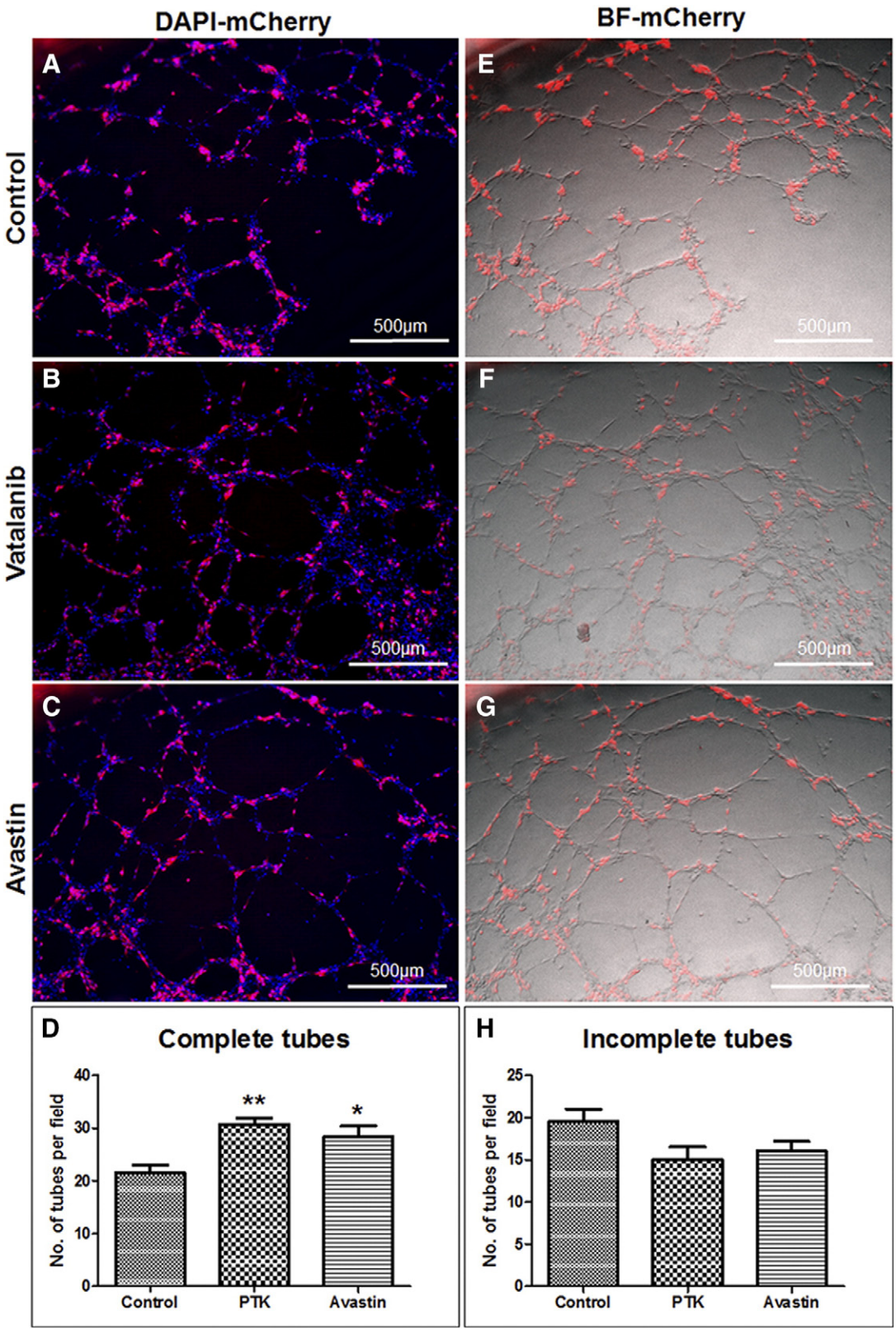

Figure 4. Post-treated glioblastoma cells incorporate into host endothelial blood vessels. Panels A–C represent the super-imposition of red fluorescent (mCherry U251 cells) and the DAPI images. (D) Quantification of complete tubes in AAT-treated groups. Panels E–G represent the superimposition of red fluorescent (mCherry U251 cells) image over the bright field (BF) image (HUVEC cells). (H) Quantification of incomplete tubes in AAT-treated groups. mCherry U251 GBM cells and HUVEC cells were seeded in 1:2 ratio, treated with AAT post seeding and incubated for 6 h. One representative image from each treatment group has been shown here. Statistical analyses were performed using Student's *t* test and $P < .05$ was considered significant.

de-differentiation of epithelial cells into mesenchymal phenotypes termed epithelial-to-mesenchymal transition (EMT), a process that sets the stage for the acquisition of dedifferentiated phenotypes with mesenchymal features reminiscent of the embryonic stem cells. The generation of GSCs is therefore a bridge that connects VM and EMT, thus explaining why EMT is crucial to the formation of VM in tumors.

Evidence in Clinical Settings, Clinical Implications, and Future Prospects of Therapy in VM

In a meta-analysis of 22 clinical studies which had 3062 patients across 15 different types of cancers enrolled for a study, the 5-year overall survival of the VM+ cancer patients was 31% whereas the overall survival for VM– cancer patients was found to be around 56%. Also, the relative risk of failure of the VM+ patient surviving for five years was calculated to the significantly higher that the VM– cancer patients [95]. Of all the cancer types observed, studied and analyzed to date, VM is reported to be present in less than 50% of the cases and therefore may be indicative or characteristic of a common, aggressive phenotype. The presence of VM was detected in around 22.7% (15 of 66) tumors in osteoblastic-type osteosarcomas [96], 43% (52 of 120) carcinomas analyzed in ovarian tumors [97], 22% (40 of 173) patients with gastric adenocarcinoma and 35% of triple-negative breast cancers (TNBC) as opposed to 17.8% in non-TNBC cases [98]. In yet another study of analyzed ovarian carcinomas, 43% cases were reported to be VM+. These VM+ patients displayed significantly higher pathological grade, histologic type and had overall poor survival [99]. A recent study implicated the role of mTOR in the VM as observed in 26.8% (34 of 127) glioma cases [100]. 20.5% (31 of 151) hepatocellular carcinoma (HCC) cases exhibited evidence of patterned matrix VM and the presence of VM was associated with larger tumors, vascular invasion, high-grade HCC, and late-stage HCC. Also, the VM+ cases had poorer overall survival and disease-free survival compared to the VM– cases [101]. 21.67% (44 of 203) cases were shown to have both VM and endothelium dependent vessel in laryngeal squamous cell carcinoma (LSCC). The presence of VM in these LSCC cases contributed to progression of LSCC by promoting lymph node metastasis and served as an independent indicator of poor prognosis [102].

Future Direction

Since the process of VM involved the transdifferentiation of tumor cells into endothelial-like phenotypes, the genes associated with angiogenesis/vascular signaling (VE-Cadherin, VEGFR1 and 2, EphA2), embryonic signaling (Nodal, Notch, Snail, Slug) and hypoxia-related signaling (HIF-1α, Twist1) pathways have been implicated for VM as well [103]. Hypoxia seems to be one of the crucial inducers of VM [1,104]. In the light of several experimental evidences coming from *in-vitro* studies, many studies have shown VM to occur in normoxia as well [105], including the results published from our lab in this manuscript. Several pro-angiogenic molecules such as VEGF and its cognate receptor VEGFR2 have been implicated in VM [106,107]. In our studies, we have demonstrated that AAT accelerated VM. This led us to further probe into the mechanistic details of VM to search for better therapeutic targets. Studies from our lab have previously shown that HET0016, a selective inhibitor of 20-HETE synthesis, decreases pro-angiogenic factors and inhibits growth of TNBC in mice [108]. 20-HETE regulates the angiogenic functions of EPC *in vitro* and EPC-mediated angiogenesis *in vivo* and using a 20-HETE antagonist, 20-HEDGE, the proliferation of EPCs was negated [109]. In our very recent study, we found HET0016 as a novel therapeutic drug capable of inhibiting VM *in vivo* [84]. In the light of these interesting evidences from our lab, we are excited to look into the prospect of developing HET0016 as a novel therapeutic for VM and study the mechanistic details of the action of HET0016 in inhibiting VM at the molecular level. Since VM serves as an alternative neovascularization mechanism capable of providing vascular irrigation to tumors in the event of an AAT therapy attack, understanding the molecular mechanisms and delineating the process of VM at the cellular level is the need of the hour. Identifying VM in the growing tumors and focusing scientific inquiries into designing novel therapies to counter VM is the current exigency.

Acknowledgement

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