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Mechanisms of Neovascularization and Resistance to Anti-angiogenic Therapies in Glioblastoma Multiforme

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Abstract

Glioblastoma Multiforme (GBM) is the most malignant brain tumor and highly resistant to intensive combination therapies. GBM is one of the most vascularized tumors and vascular endothelial growth factor (VEGF) produced by tumor cells is a major factor regulating angiogenesis. Successful results of preclinical studies of anti-angiogenic therapies using xenograft mouse models of human GBM cell lines encouraged clinical studies of anti-angiogenic drugs such as bevacizumab (Avastin), an anti-VEGF antibody. However, these clinical studies have shown that most patients become resistant to anti-VEGF therapy after an initial response. Recent studies have revealed some resistance mechanisms against anti-VEGF therapies involved in several types of cancer. In this review, we address mechanisms of angiogenesis, including unique features in GBMs, and resistance to anti-VEGF therapies frequently observed in GBM. Enhanced invasiveness is one such resistance mechanism, and recent works report the contribution of activated MET signaling induced by inhibition of VEGF signaling. On the other hand, tumor cell-originated neovascularization including tumor-derived endothelial cell-induced angiogenesis and vasculogenic mimicry has been suggested to be involved in the resistance to anti-VEGF therapy. Therefore, these mechanisms should be targeted in addition to anti-angiogenic therapies to achieve better results for patients with GBM.

Keywords

glioblastoma; angiogenesis; invasion; MET; alternative neovascularization; vasculogenic mimicry; endothelial differentiation

Introduction

Despite optimal treatment and evolving standard of care, the median survival of patients diagnosed with GBM is only 12 to 15 months [1]. GBMs are highly vascularized tumors and higher microvessel density in glioma is significantly correlated with worse prognosis [2]. In contrast, low grade astrocytoma is not angiogenic, grows along pre-existing vessels [3, 4] and has better survival outcome. Blood vessels in GBMs have unique features, including aggressive proliferation of endothelial cells (ECs), sometimes exhibiting “glomeruloid tufts”. The proliferation of ECs is observed frequently and exclusively in mesenchymal type GBMs, correlating with poor prognosis [5, 6]. In this review, we discuss the mechanisms of GBM angiogenesis and the role of VEGF and other pro-angiogenic factors. Furthermore,

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potential resistance mechanisms, including enhanced tumor invasion and alternative angiogenesis will be described in the context of anti-angiogenic therapy for GBM.

Tumor blood vessels in glioblastoma

Tumor vessels in GBMs are different from normal blood vessels morphologically and functionally [6]. In normal brain, the vasculature consists of ECs, pericytes and astrocytes, which collectively form the blood-brain barrier (BBB). BBB function is maintained by several mechanisms including tight junctions between ECs, preventing entry of high molecular weight substances into the brain and active transport of substances from the brain by efflux pumps such as P-glycoprotein. In contrast to the highly organized normal brain vasculature, tumor vessels in GBM are tortuous, disorganized, highly permeable, and have abnormal endothelial walls, pericyte coverage and basement membrane structure, resulting in the loss of BBB integrity. The impaired BBB results in brain edema, which often causes serious symptoms in GBM patients, and VEGF produced by tumor cells contributes significantly to this process by increasing permeability of blood vessels. However, in contrast to the tumor center, the BBB is still functional near the infiltrating edges of the tumor, making delivery of drugs to these areas challenging [7].

Formation of tumor vessels

During the initial development of tumors, tumor cells grow along pre-existing blood vessels (known as co-option), and depending on tumor development, neovascularization occurs by several mechanisms [4, 8]. Traditionally, tumor vessel formation occurs through angiogenesis, which is mediated by proliferation and migration of local ECs [9]. On the other hand, vasculogenesis, which originates from bone marrow-derived cells, has been reported recently as an important mechanism in tumor vessel formation [10]. During the formation of tumor blood vessels, circulating endothelial precursor cells (EPCs), which express VEGF receptor 2 (VEGFR2, also called as KDR or Flk1), are recruited from the bone marrow by VEGF and then differentiate and incorporate into new tumor vessels. However, a number of controversial papers report that EPC-derived vessels are a rare population in tumor angiogenesis [11–14]. This discordance may be explained by different expression levels of stromal-derived factor-1 (SDF-1) in tumor cells. In orthotopic mouse glioma models, SDF-1-expressing tumor cells initiated vasculogenesis while SDF-1-negative cells did not [15]. Furthermore, the vasculogenesis was enhanced by VEGF, though SDF-1 did not mobilize EPCs from the bone marrow, suggesting an intratumoral local role of SDF-1. More recently, we and other groups reported that numerous tumor vessel ECs are transdifferentiated from GBM cells in mouse models and human tumor samples [16–18]. Details about this newly proposed mechanism will be reviewed in later sections.

Mechanisms of angiogenesis in glioblastoma

In the early stage of tumor development, angiopoietin-2 (Ang-2) is expressed in co-opted blood vessels [19]. As the tumors grow, they disrupt the pre-existing blood vessels. ECs and tumor cells express Ang-2 which disrupts EC junctions and decreases surrounding pericytes, resulting in destabilization of blood vessels [4, 19, 20]. This process results in tumor hypoxia and necrosis [6]. In the early steps of angiogenesis, degradation of basement membrane and extracellular matrix by matrix metalloproteinase (MMPs) is also an important process to promote invasion of ECs. Hypoxia induces expression of MMP-2 and MMP-9 in tumor cells and ECs, and expression levels of these MMPs is correlated with the histological grade of glioma [21, 22]. Hypoxic tumor cells produce angiogenic factors, and the most predominant hypoxia-induced angiogenic factor is VEGF. Hypoxia also induces nuclear accumulation of the hypoxia-inducible factor (HIF) alpha and beta complex resulting in transcriptional activation of VEGF [23], and can upregulate expression of

VEGFR2 in ECs. VEGF signaling may then promote vascular remodeling and sprouting in conjunction with Ang-2 [3, 4].

In the sprouting and branching process, delta-like 4 (Dll4) -Notch1 signaling and ephrin B2 are involved in tip cell formation in response to VEGF [24, 25]. It has been reported that frequent expression of Dll4 in ECs of tumor vessels correlates with higher grade in glioma [26, 27], and Dll4 facilitates tumor growth by blocking non-functional angiogenesis and improving vascular function in mouse models of glioma and other tumors [26, 28, 29]. In addition, angiopoietin 1, fibroblast growth factor 2 (FGF2), platelet-derived growth factor (PDGF), SDF-1 and interleukin 8 (IL-8) can promote angiogenesis by stimulating EC proliferation and/or migration [6]. Expression of the αvβ3, αvβ5 and α5β1 integrin complexes is also important in tumor angiogenesis [6, 30]. FGF2-dependent angiogenesis requires αvβ3 integrin while VEGF-dependent angiogenesis is dependent on αvβ5 integrin [31]. In particular, 7agr;vβ3 integrin is required for the survival of ECs and maturation of new blood vessels [32, 33]. Interestingly, these integrins are expressed not only in blood vessel ECs but also in tumor cells, particularly in higher grade glioma where their expression correlates with an aggressive phenotype [34–37].

Tumor cell invasion as a consequence of anti-angiogenesis therapy

GBM is one of the most highly vascularized tumors and produces large amounts of VEGF [38–40]. Furthermore, higher VEGF expression in glioma patients correlates with the number of lesions and higher histological grade, although plasma VEGF levels do not correlate with survival [41, 42]. These findings provide a rationale for the development of inhibitors of angiogenic signaling as a means to control tumor growth. Given the importance of VEGF in tumor angiogenesis, several different approaches to suppress VEGF signaling have been developed, including monoclonal antibodies and small molecule inhibitors [43]. The most well characterized anti-angiogenic therapy currently used in the treatment of human GBM is bevacizumab, a humanized monoclonal antibody which binds to circulating VEGF and prevents its interaction with the VEGFRs, thereby suppressing VEGF signaling. In a mouse xenograft model of human stem-like glioma cells, bevacizumab significantly inhibited tumor growth and angiogenesis [44].

Bevacizumab is currently approved by the US Food and Drug Administration for the treatment of metastatic colorectal cancer, non-small cell lung cancer, metastatic kidney cancer and GBM under specific conditions. For GBM, bevacizumab is approved for single-agent use when frontline therapy consisting of surgical resection followed by radiotherapy and temozolomide have failed [45]. Unfortunately, while bevacizumab treatment prolongs progression-free survival (PFS) in a subset of patients, only minimal improvements in overall survival (OS) are observed and patients invariably relapse [46–51]. Several small molecule inhibitors targeting VEGFR tyrosine kinase activity, including cediranib, sunitinib and imatinib have also been tested in GBM patients. Similar to results with bevacizumab, as single agents these inhibitors are either ineffective or prolong PFS with little effect on OS [52–56]. Understanding why GBM patients ultimately relapse during anti-angiogenic therapy will be crucial to improving this therapeutic approach. Emerging evidence from both the clinic and the laboratory suggests that one adaptive mechanism to anti-angiogenic therapy in GBM is the transition to a pro-invasive tumor phenotype.

Enhanced tumor invasion following anti-angiogenesis therapy in glioblastoma patients

As with other targeted cancer therapies, inhibition of tumor angiogenesis leads to an adaptive tumor response. One particularly problematic adaptive response documented during the treatment of GBM patients with bevacizumab is the transition to a more invasive tumor phenotype [39]. An early retrospective analysis demonstrated a trend towards

enhanced infiltrative disease in bevacizumab treated glioma patients [57]. When comparing those patients who had a radiographic response to bevacizumab with those who did not, a statistically significant increase in infiltrative tumor progression was identified in bevacizumab responders. This suggests that enhanced tumor invasion may be a direct consequence of the inhibition of VEGF signaling. A study by Narayana et al. of 61 patients with recurrent high grade glioma treated with bevacizumab and either irinotecan or carboplatin found that 30% of patients relapsed with diffuse, infiltrative disease characterized by the up-regulation of mesenchymal markers within the tumor [58]. In another study, following discontinuation of bevacizumab treatment due to tumor progression in 37 patients with GBM, 46% of the patients had an enhancing local recurrence, while 35% displayed non-enhancing tumor progression and 16% developed a multifocal enhancing tumor in a location distinct from the primary tumor [59]. The development of a non-enhancing tumor, indicative of infiltrative disease, was correlated with poorer OS. Immunohisto-chemical analysis of the tumors from these bevacizumab treated patients demonstrated up-regulation of HIF-1α and the hypoxia marker carbonic anhydrase-9. Such increases in infiltrative disease and tumor hypoxia following bevacizumab treatment in human GBM patients have been identified in others studies [60].

Recent clinical trials are also assessing bevacizumab as a frontline therapy for newly diagnosed GBM, as opposed to recurrent disease. Results from one of these studies supports the enhanced infiltrative relapse following bevacizumab treatment, with 57% of patients who relapsed demonstrating diffuse infiltrative disease [61]. It must be noted, however, that while the incidence of infiltrative disease in these studies is higher than many historical controls, the notion that anti-angiogenesis therapy for GBM promotes a pro-invasive phenotype remains controversial [62]. Valid criticisms of several of the studies supporting this idea are a lack of proper control arms in clinical trials, variable definitions of tumor recurrence patterns and a lack of pre-bevacizumab disease progression analysis. However, collectively these findings suggest that bevacizumab alters the pattern of tumor recurrence, leading to infiltrative tumor spread. Further studies will be required to clarify the incidence and clinical relevance of this phenomenon in human GBM patients.

Anti-angiogenesis therapy elicits tumor cell invasion in mouse models of glioma

Data from mouse models of glioma substantiate the clinical observations that suppression of VEGF signaling promotes an invasive tumor phenotype. Initial studies demonstrated that loss of HIF-1α, a major transcriptional activator of VEGF, generated more invasive tumors in an orthotopic astrocytoma mouse model [63]. The invasive behavior of these HIF-1α deficient astrocytomas could be abolished by tumor-specific expression of VEGF [64]. These findings provided direct evidence that VEGF can act as a negative regulator of tumor cell invasion. Similar results have been found in rodent models of GBM following treatment with bevacizumab or other VEGF blocking antibodies. For example, xenografts of human U87 cells become more invasive following treatment of the mice with bevacizumab [60] or with VEGF Trap, a decoy VEGF receptor [65]. Human GBM G55 cells also become more invasive and form satellite tumors in nude rats treated with an anti-VEGF antibody [66]. In orthotopic mouse models of GBM, VEGFA ablation or pharmacologic inhibition of VEGF signaling results in increased perivascular tumor cell invasion with little change in overall animal survival [67]. In the same study, suppression of VEGF signaling also promoted a more invasive phenotype in the RIP-Tag2 model of pancreatic neuroendocrine cancer (PNET), demonstrating that such effects are not limited to GBM.

Until recently, the molecular mechanisms regulating enhanced tumor cell invasion following anti-angiogenesis therapy remained unknown. Several studies have now identified the receptor tyrosine kinase MET, the cellular receptor for hepatocyte growth factor, as a key mediator of tumor invasion following angiogenesis inhibition. Aberrant MET activation

occurs frequently in cancer and is a major driver of tumor invasion and metastasis [68]. In PNET, the enhanced invasive tumor phenotype following VEGFR inhibition can be blocked by combined inhibition of MET signaling [69]. The addition of MET inhibitors to suppress tumor invasion following VEGF inhibition has also been demonstrated in both xenograft and orthotopic mouse models of GBM [70]. Lu et al. demonstrated that VEGF signaling blocks activation of the MET receptor. This occurs through an interaction between VEGFR2 and MET, wherein VEGF signaling stimulates recruitment of the non-receptor protein tyrosine phosphatase 1B (PTP1B) to the VEGFR2/MET complex, resulting in MET dephosphorylation. Thus, when VEGF signaling is suppressed, MET is activated and the tumors become more invasive. These findings provide the first molecular mechanism to explain the increased invasive behavior of cells experiencing inhibition of VEGF signaling. Interestingly, this mechanism supports the observations of one clinical trial wherein a statistically significant enhancement of tumor cell invasion following bevacizumab treatment only occurred in patients who had a radiographic response to bevacizumab [57]. Thus, it appears that and actual response to anti-VEGF therapy (i.e. VEGF signaling inhibition) is required to activate MET and promote invasive disease. These findings suggest that the combination of angiogenesis inhibitors with therapies targeting the invasive component of the tumor, such as MET, may produce a more efficacious therapeutic effect in GBM.

Alternative neovascularization

In addition to the conventional neovascularization mechanisms of angiogenesis and vasculogenesis, alternative neovascularization mechanisms have been documented. One model for alternative neovascularization is vasculogenic mimicry (VM) and the other model is transdifferentiation of tumor cells into ECs. In both mechanisms, tumor cells, particularly glioma stem-like cells (GSCs), are the source of new tumor vessels. As with tumor cell invasion, these alternative neovascularization mechanisms may contribute to resistance to anti-angiogenic therapies in GBM.

Vasculogenic Mimicry

Tumors experiencing a reduction in blood flow and associated nutrients may adapt by re-organization of tumor tissue in order to patch damaged existing blood vessels with tumor cells (mosaic vessels). Moreover, tumors can generate vessel-like networks composed almost entirely of tumor cells to act as artificial channels. Both of these processes are forms of VM. VM was first reported in melanoma, where the degree of VM correlates with poor prognosis [71–73]. Several groups have also reported VM in mouse models of GBM [74, 75] as well as in human GBM, where epidermal growth factor receptor (EGFR) amplified cells, which do not express EC markers, line vascular channels of the tumor [76].

Some of the initial evidence of VM was found using a three dimensional co-culture system of glioma cells with ECs [74]. Here, glioma cells incorporated into the vascular structure with ECs. Although these glioma cells did not express EC markers, they did express CD133, supporting the role of GSCs in VM. Another supportive study cultured tumor spheres expressing CD133 isolated from tumors that either exhibited VM or did not [77]. When re-injected as xenografts, only cells from primary tumors displaying VM re-exhibited mimicry. In contrast to VM in other tumors, which can sometimes express EC markers, VM in GBM have not been reported to express EC antigens [76, 78, 79]. Immuno-histochemically, tumors identified as high VM have decreased overall vascular densities, supporting the idea of an alternative vascularization mechanism at work. These patients tend to have higher grade and more aggressive gliomas, with shorter survival than those without evidence of VM [79]. Other studies have suggested a link between VM in GBM and radio-resistance, which may have clinical implications [8, 74].

Mechanisms of Vascular Mimicry

The process of VM in GBM is believed to be mediated by GSC. Several reports demonstrate markers for GSCs being present adjacent to the tumor vasculature within GBM [80–82]. This perivascular localization, while regulating GSC maintenance and self-renewal, may also be important for the contribution of GSCs to VM since this process is intimately associated with the tumor vasculature. Major components of the GSC niche which guide GSC fate include integrins and laminins. For example, GSC self-renewal and *in vivo* tumorigenicity are controlled through GSC-specific expression of integrin-α6 [81]. Recently laminin-α2, a ligand for integrin-α6, was shown to be a negative prognostic marker in GBM and to be associated with the mesenchymal and classical subtypes of GBM [83]. Moreover, laminin-α2 expressed by both ECs and GSCs contributes to the *in vivo* tumorigenicity of GSCs. In addition to laminin-α2, laminin-8 expression also correlates with glioma patient survival [84].

Beyond the mechanisms regulating GSCs in the perivascular niche, the molecular details of how GBM cells contribute to VM are not understood, although VEGFR2 has been implicated in the process [78]. A model of VM in melanoma suggests phosphorylated ephrin type-A receptor 2 (EPHA2) associated with focal adhesion kinase (FAK) can signal through phosphatidylinositol 3-kinase (PI3K) to regulate activity of MMPs. Here, MMPs promoted cleavage of laminin into fragments ultimately resulting in increased migration, invasion and VM [85–89]. In addition, melanomas with high VM also have increased HIF-1α, VEGF, and MMP expression levels [90]. However, whether a similar mechanism regulating VM exists in GBM is unknown.

Transdifferentiation of tumor cells to ECs

The newest concept of tumor neovascularization is transdifferentiation of tumor cells into ECs. We recently reported identification of tumor-derived ECs (TDECs) in our mouse GBM model generated by stereotaxic injection of Cre-loxP-controlled lenti-vectors encoding oncogenes and GFP into the brains of GFAP-Cre mice [18, 91]. Immunofluorescence assays revealed blood vessels within the tumor expressing both endothelial antigens and the tumor marker GFP (Fig. 1) [18]. In addition, GFP–transduced human GBM cells transplanted into the brains of NOD-SCID mice generate both GFP^- and GFP^+ ECs, demonstrating that both mouse and human GBMs are capable of forming TDECs.

Similar reports support this finding with confirmation of TDECs in human GBMs [16, 17]. Ricci-Vitiani et al. found a significant number of ECs ($CD31^+/CD144^+$) that appeared to originate from the tumor due to their shared chromosomal aberrations with the associated tumor [16]. Concurrently, Wang et al., also published evidence for the generation of ECs from $CD133^+$ tumor cells purified from human GBMs [17]. ECs fractionated from the tumor shared the same chromosomal aberrations as the tumor cells, supporting the idea that these ECs transdifferentiated from tumor cells.

Mechanisms of TDEC formation

The expression of nestin in mouse GBM TDECs and CD133 in human TDECs suggests these cells likely arise from GSCs [17, 18]. This is supported by the finding that normal neural stem cells can also differentiate into vascular ECs [92]. Evidence suggests a requirement for activated VEGFR2 in GSC transdifferentiation [17, 93]. Using human GSCs, it has been shown that bevacizumab can block the later stages of differentiation of GBM cells into mature ECs [17]. Several mechanisms exist to activate VEGFR2, including both VEGF-dependent and –independent mechanisms. Classical VEGF-dependent activation of VEGFR2 can be enhanced via extracellular matrix proteins, such as fibronectins, by promoting the interaction of integrin αvβ1 and VEGFR2 [94]. In ECs,

MMP-1 can also enhance classical VEGF-dependent VEGFR2 signaling by upregulating VEGFR2 expression [95]. Once VEGFR2 activity is enhanced, traditional angiogenic signaling is mimicked through activated mitogen-activated protein kinase MAPK ERK1/2 [94]. Given that GSC can express VEGFR2 [96], this enhanced VEFGR2 signaling may play a role in the transdifferentiation of GSCs into TDECs. VEGF-independent activation of VEGFR2 has also been reported [97]. Future experiment will be required to determine the role of VEGF-dependent and -independent VEGFR2 activation in TDEC differentiation.

We reported that the majority of mouse GBM TDECs lack VEGFR2 expression and therefore a VEGFR2-independent mechanism for TDEC formation may also exist (Fig. 2). One possible mechanism for VEGFR2-independent TDEC formation could be through Notch signaling, due to its importance in regulating GSCs in GBM angiogenesis [17, 98]. Wang et al., found that Notch-1 silencing or treatment with γ-secretase inhibitors blocked the early stages of GBM cell differentiation to an EC intermediate [17]. However, in this system, VEGFR2 signaling was also required since bevacizumab blocked the differentiation of the EC intermediate to a mature EC, but did not affect the early stage of differentiation. These results demonstrate that Notch and VEGFR2 signaling may mediate distinct steps in the generation of TDECs, however, the requirement of VEGFR2 in GSC differentiation to ECs requires further clarification in future studies.

Hypoxia appears to be another important factor regulating TDEC formation. In mouse models of GBM, TDECs are observed more frequently in the hypoxic, deep area of the tumor compared to surface [18]. Furthermore, culturing tumor cells in the presence of deferoxamine (to mimic hypoxia) or at low O_2 concentrations enhances tube formation and the expression of EC markers. Ricci-Vitiani et al. also reported that there were more tumor-derived $CD144^+$ ECs in the core of the human GBM xenograft tumors compared to the surface [16]. These findings support the role of hypoxia in endothelial differentiation of GBM cells, although a direct role for HIF-1 or HIF-2 in the generation of TDECs has yet to be reported. However, the HIFs are known to function in normal and cancer stem cell (CSC) biology [99, 100] and therefore it would not be surprising to find they play a role in differentiation of GBM cells to ECs. Furthermore, an interplay between HIF-1 and Notch signaling also exists. HIF-1α and Notch-1 can directly interact, and HIF-1α can enhance Notch signaling [101]. In CSCs from hematological malignancies, HIF-1α supports CSC maintenance and tumorigenicity by suppressing a negative feedback loop in the Notch pathway [102]. Thus, given the role of Notch-1 in GBM differentiation to ECs [17], it is plausible that hypoxia-driven Notch signaling mediates differentiation of GBM cells to ECs.

Conclusions and perspectives

To date, anti-angiogenic therapy for GBM has not produced the therapeutic effect initially envisioned. Emerging evidence has detailed several mechanisms, including enhanced invasion and alternative angiogenesis, by which GBM adapts to and circumvents anti-angiogenic therapy. We have recently demonstrated that terminally differentiated astrocytes and neurons can be a cell-of-origin for mouse GBM [103]. Following oncogenic insult, these cells de-differentiate to produce tumor initiating neuro-progenitor cells which can generate all the lineages of cells present in the tumor, including astrocytes, neurons and oligodendrocytes (Fig. 3). These tumor initiating neuro-progenitor cells can also trans-differentiate into TDECs, potentially contributing to resistance to anti-angiogenic therapy.

GBM is very invasive and the inability to remove infiltrating tumor cells during surgical resection is the primary cause of relapse. Therefore, development of specific inhibitors of GBM invasion should be a high priority. Importantly, these inhibitors may synergize with current anti-angiogenic agents. Despite progress in characterizing tumor invasion following

angiogenesis inhibition in GBM, many questions remain. For example, does anti-angiogenesis therapy potentiate a pre-existing infiltrative program or does this response represent an alternative mechanism of invasion? Is activation of MET during anti-angiogenesis therapy the primary driver of GBM invasion? Moreover, what will be the therapeutic effect of targeting alternative neovascularization (such as inhibition of Notch signaling) alone or in combination with suppression of classical angiogenesis? It seems likely that novel mechanisms driving baseline and anti-angiogenesis induced GBM invasion, as well as alternative neovascularization, will be discovered and the challenge will be to translate these findings into novel therapeutics for the treatment of this devastating disease.

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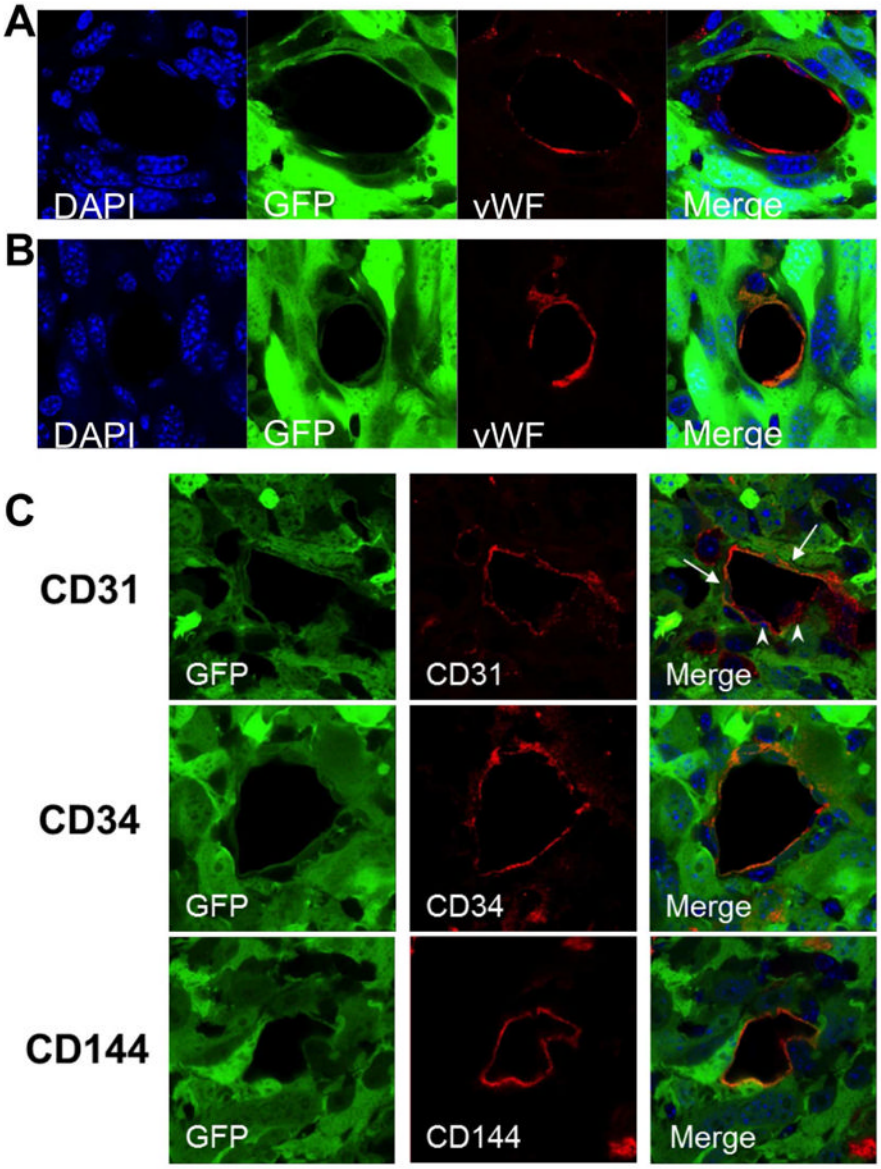

Figure 1. Tumor derived endothelial cells (TDECs)

Confocal microscope images of vascular endothelial cells (ECs) in mouse GBM. (A) Regular ECs line the vessel lumen and express EC marker Von Willebrand factor (vWF) but not tumor marker GFP. (B) In contrast, TDECs express both vWF and GFP. DAPI was used as a nuclear marker. (C) Expression of EC antigens CD31 (upper panels), CD34 (middle panels) and CD144 (lower panels) in TDECs. Some GFP^+ ECs (arrows in upper right panel) form vessels with GFP^- regular ECs (arrowheads). DAPI was used as nuclear marker and the image was incorporated in the merge panels. All confocal pictures are single slice images at Airy factor of 1.0. Original magnification: x63 with 3x electrical zoom (189x total magnification). These images were reprinted from Ref. 18 and reorganized.

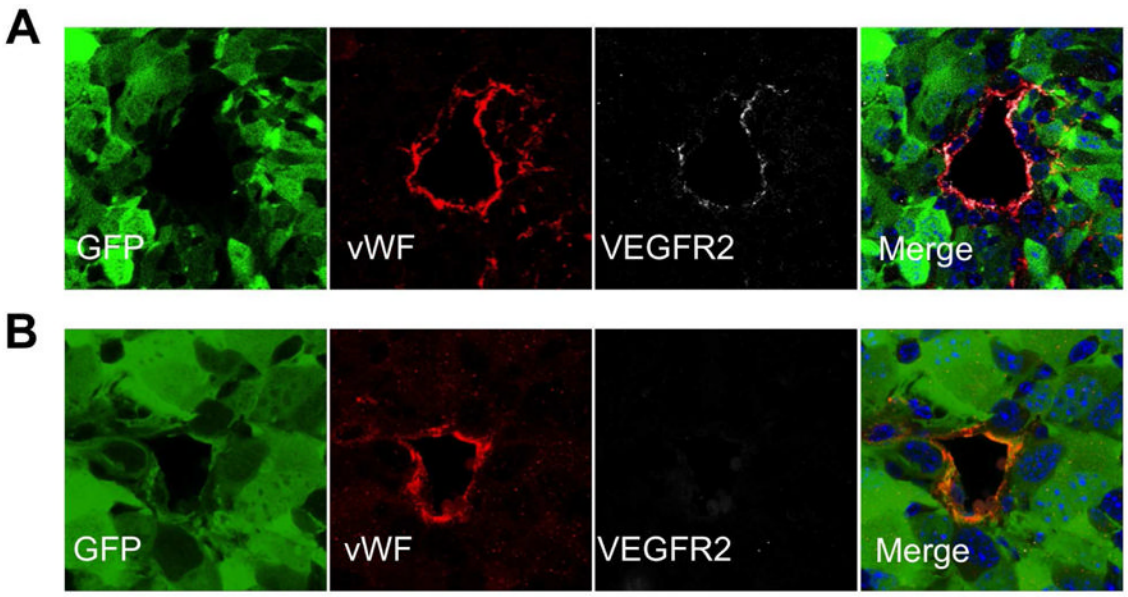

Figure 2. Characterization of VEGFR2 in TDECs

Confocal microscope images of TDECs in mouse GBM. (A) Regular tumor ECs express VEGFR2 (upper panels) but (B) TDECs did not express VEGF-R2 (lower panels). DAPI was used as nuclear marker and the image was incorporated in the merge panels. All confocal pictures are single slice images at Airy factor of 1.0. Original magnification: x63 with 3x electrical zoom (189x total magnification). These images were reprinted from Ref. 18 and reorganized.

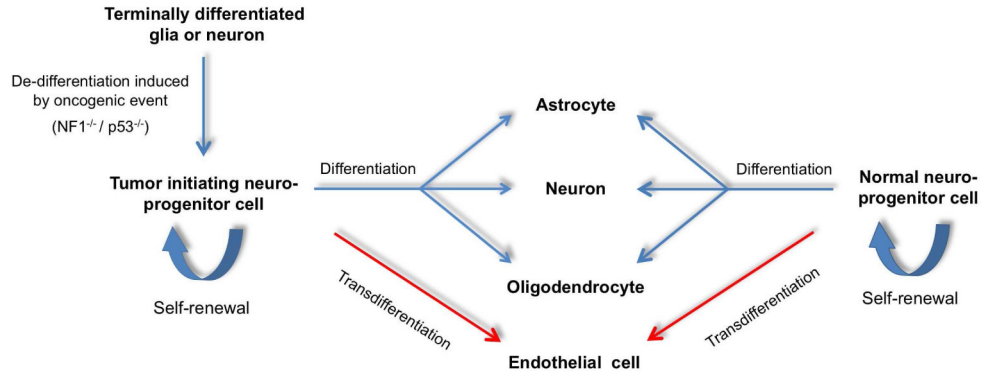

Figure 3. Transdifferentiation of tumor initiating neuro-progenitor cells into endothelial cells

Upon oncogenic insult, such as loss of NF1 and p53, terminally differentiated glia or neurons can de-differentiate into tumor initiating neuro-progenitor cells. These cells can self-renew and also differentiate into astrocytes, neurons and oligodendrocytes. Tumor initiating neuro-progenitor cells can also transdifferentiate into endothelial cells. In a similar fashion, normal neuro-progenitor cells can also differentiate into astrocytes, neurons and oligodendrocytes and transdifferentiate into endothelial cells.