A target for antiangiogenic therapy: Vascular endothelium derived from glioblastoma

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Glioblastoma is the most frequent primary brain tumor in the adult, accounting for 53.8% of all gliomas (http://www.cbtrus.org), and it is one of the most deadly among all human tumors. Despite aggressive treatment at diagnosis, consisting of resection followed by radiation with concurrent and subsequent adjuvant chemotherapy with temozolomide, the tumor almost invariably recurs or progresses, with a patient median survival of 14.6 mo (1). The hallmark of glioblastoma that distinguishes it from all of the other glial tumors is microvascular proliferation in conjunction with necrosis. Therefore, treatment with antiangiogenic agents holds great promise to block the growth of this most vascularized tumor. The best-known antiangiogenic agents are inhibitors of VEGF-A, an indispensable angiogenic factor during developmental organogenesis and growth of numerous tumors. However, treatment with bevacizumab, a neutralizing antibody to VEGF-A, at relapse only confers transient benefit and a marginal increase in survival, indicating at tumor progression a VEGF-independent angiogenic mechanism of glioblastoma resistance (2). Several mechanisms have been implicated in angiogenesis. One is the sprouting of capillaries from preexisting blood vessels by endothelial proliferation (3). Another is the cooption of preexisting blood vessels by tumor cells, leading to expression of angiopoietin-2 by those vessels' endothelial cells and tumor cell proliferation, followed later by involution of preexisting vessels in the core of the tumor, massive tumor cell apoptosis, organization of remaining tumor cells into pseudopalisading that resides around areas of necrosis, and tumor rescue at the margins by angiogenesis (4, 5). Expression of HIF-1α and up-regulation of VEGF-A have been identified in hypoxic perinecrotic pseudopalisading tumor cells (4–6). Hypoxia induces elevated levels of VEGF-A (6) and VEGF-A receptors that appear up-regulated in tumor endothelial cells but not in normal brain (7). Another mechanism is the release of angiogenic factors by the tumor that recruit bone marrow-derived endothelial progenitors, hematopoietic stem and progenitor cells that participate in vessel formation (8–10). In PNAS, Soda, Verma, and colleagues reveal a new paradigm for glioblastoma angiogenesis whose main contribution is transdifferentiation of glioblastoma cells into endothelial cells (11) (Fig. 1). Notably, these tumor-derived endothelial cells (TDECs) are refractory to inhibition of both VEGF-A and basic fibroblast growth factor (bFGF, FGF-2) pathways. By mapping GFP^+ p53-deficient glioblastoma established in glial-specific Cre mice (GFAP-Cre) (12), Soda et al. find that tumor cells can directly transdifferentiate into $CD31^+CD34^+$ endothelial cells that lack VEGF-A receptors (VEGFR), constituting over 20% of total $CD31^+CD34^+$ tumor endothelial population. These TDECs are capable of forming patent vessels. Moreover, further analysis by hypoxyprobe unraveled their preferential localization in deep hypoxic areas of the tumors. The hypoxic-associated distribution of TDECs and elevated expression of HIF-1α, a hypoxia-induced transcription factor, indicate the role of hypoxia as the key determinant in forcing putative glioma cells to differentiate into endothelial-like cells.

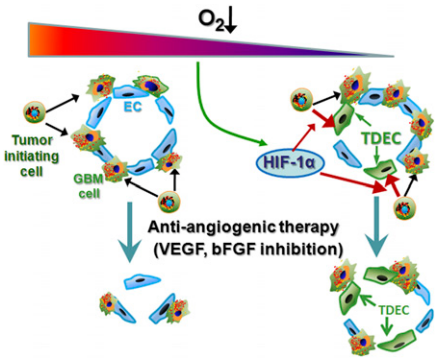

Fig. 1. Model of glioblastoma cells transdifferentiating into endothelial cells. Glioblastoma are a source of TDECs. The formation of TDECs is enhanced by HIF-1α induced by hypoxia and independent of VEGF and FGF inhibition. EC, endothelial cell; GBM, glioblastoma.

Although TDECs share certain common endothelial markers such as CD31, CD34, vWF, and CD144, their special feature is demonstrated by the lacking expression of VEGFR2, the major tyrosine kinase receptor of VEGF-A. The absence of VEGFR is further illustrated by the negligible effect conferred by inhibition of all VEGF receptors in either EC-dependent tube formation in Matrigel or in vivo tumor growth. Notably, although TDECs exhibit high expression of FGFR1, the main endothelial receptor of bFGF, dual inhibition of both VEGFR and FGFR1 failed to cause substantial effects in inhibiting tube formation of TDECs. Therefore, TDECs have a unique VEGF-A, bFGF-independent angiogeneic mechanism that potentially accounts for the resistance to anti-VEGF-A therapy in glioblastoma treatment.

Furthermore, cord formation by TDECs continues to occur under hypoxia conditions, when VEGF-A autocrine function is blocked by neutralizing antibody or when autophosphorylation of VEGFR tyrosine kinase is inhibited by an antagonist, suggesting that transdifferentiation of tumor cells into endothelial cells is VEGF-A-independent. This observation is confirmed in vivo by showing that survival does not change for the animals treated with this antagonist. In the treated animals, TDECs increase, more at the tumor margins than in deep areas, supporting a role for TDECs in offering resistance to anti-VEGF treatment of glioblastoma. TDECs were also found in human glioblastoma specimens. These TDECs express FGFR1 but do not express VEGFR1, VEGFR2, or VEGFR3. The lack of VEGF receptors is a plausible explanation for resistance of TDECs to antiangiogenesis treatment. Are there other alternate pathways of resistance to anti-VEGF-A treatment that implicates TDECs? Tumor stromal cells including myeloid cells and bone marrow-derived cells are known to contribute to tumor angiogenesis by rendering the tumor refractory to antiangiogenic treatment (13). We can speculate that glioblastoma-derived endothelial cells participate in the recruitment of stromal cells that generate a VEGF-A-independent pathway of tumor resistance to antiangiogenic treatment. In fact, there is a growing concept that endothelial cells are not merely

Author contributions: A.H., B.-S.D., and S.R. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 4274.

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passive conduits but have an instructive role, producing angiocrine factors, inflammatory and protumorigenic at the vascular niche, leading to mobilization of other cells, promoting tumor growth and modulation of response to treatment (14).

The concept that tumor cells could generate vascular channels was previously introduced (15). Tube formation can be produced by other nonendothelial cells, in a process known as vasculogenic mimicry. Endothelial-like cells derived from tumors such as melanoma lack the full angiogenic repertoire of the endothelial cells. Recently, a tubular form of vasculogenic mimicry was recognized for glioblastoma, as $CD133^+$ glioblastoma stem-like cells were found capable of transdifferentiating into tubular vascular smooth muscle-like cells forming structures deprived of endothelial cells, despite immunoreactivity for collagen-IV, a component of vessel basement membrane (16). In vitro, the $CD133^+$ cells from glioblastoma that contain tumor cell-lined vessels were capable of expressing endothelium-associated genes, such as *ephrin* receptor, *neuropilin-2*, and *laminin5γ2*, but not *CD31* and *CD34* (16). However, CD31, CD34, and vWF can also be expressed by hematopoietic cells. During embryogenesis and brain development, the VEGF receptors are present in endothelial cells and up-regulation of VEGFR1 and VEGFR2 is detected in glioblastoma endothelial cells. It is also known that VEGFR2 signaling is necessary for endothelial cell regeneration after myelosuppression (17). If these receptors are fundamental to the vascular compartment of the brain and glioblastoma tumors, and absent on TDECs, can a true functional endothelium be defined without a major endothelial marker? Strictly speaking probably not, but rather refer to TDECs that do not express either VEGFR1 or VEGFR2 as vascular mimicry. The new study also shows that in human glioblastoma, EGFR amplification found in glioblastoma cells is also detected in some endothelial cells in the tumor but is absent in the endothelial cells in the normal brain. One might consider that EGFR amplification is a characteristic of tumor cells, and that it does not belong to the molecular signature of endothelial cells. Alternatively, they might give indirect evidence for a shared common lineage for a subset of tumor-derived endothelial cells and glioblastoma cells. Two other recent studies showed that glioblastoma stem-like cells and a subset of tumor-derived endothelial cells harbor the same genomic mutations, and $CD144^+$ or VE-cadherin and VEGFR2 are expressed by the emergent endothelium, suggesting a link between the endothelium and neural compartments of a glioblastoma (18, 19).

Soda, Verma, and colleagues reveal a new paradigm for glioblastoma angiogenesis.

What are the signaling molecules that participate in the production of TDECs? What are the specific genes downstream of HIF-1α that are up-regulated with hypoxia and are responsible for transdifferentiation? Elucidating the intermediates in this cell differentiation, the angiocrine modulators between tumor cells and endothelial cells, and whether the canonical or noncanonical HIF-1 pathway leads to up-regulation of endothelial-specific genes under hypoxia will be crucial to reveal mechanisms of tumor resistance to antiangiogenic agents.

Are there other alternate pathways of resistance to anti-VEGF-A treatment that implicates TDECs? Tumor stromal cells including myeloid cells and bone marrow-derived cells are known to contribute to tumor angiogenesis by rendering the tumor refractory to antiangiogenic treatment (13). In particular, upon activation of the Id pathway, endothelial progenitor cells (EPCs) mobilized from bone marrow can initiate angiogenesis through release of paracrine factors rather than structurally incorporating into vessel wall (20). In addition, CXCR4 activation by stromal-derived factor 1 has been shown to play an essential role in the mobilization and recruitment of EPCs, which also might explain the VEGF-A, bFGF-independent angiogenic effects in glioblastoma. We can speculate that glioblastoma-derived endothelial cells participate in the recruitment of bone marrow-derived EPCs that generate a VEGF-A-independent pathway of tumor resistance to antiangiogenic treatment. Is this mechanism of VEGF-A resistance uniform to all glioblastoma or are there alternate pathways used for different subsets of glioblastoma? It is conceivable that combinations of antiangiogenic agents in conjunction with other conventional therapies will provide an ideal approach to improve progression-free survival in glioblastoma patients while diminishing toxicity.

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