When tumor cells make blood vessels: implications for glioblastoma therapy

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Summary of methods & results
Based on recent findings in other cancers, Soda et al. speculated that glioblastoma multiforme (GBM) cells might transdifferentiate into endothelial cells (ECs) and thus contribute to GBM blood vessel formation [1]. To test for the possible presence of tumor-derived ECs (TDECs) in the tumor vasculature, the authors used their previously developed mouse GBM model [2]. In this model, loxP lentiviral vectors encoding the oncogenes H-Ras and Akt and expressing green fluorescence protein (GFP) are injected into the brains of GFAP-Cre-p53 mice to generate tumors possessing characteristics of GBM. GFAP- cells and tumors express H-Ras, Akt and GFP, and show loss of p53. Using microscopy and flow cytometry, the authors investigated the vasculature of tumors generated in these mice and found that between 2 and 37.8% of endothelial cells in the tumor vasculature were GFP- (i.e., they were derived from tumor cells). Moreover, they showed the presence of TDECs in clinical samples of GBM patients. In this case, TDECs were identified by the expression of EGF receptor, which was also present in the tumor cells but not in the normal ECs. Most TDEC-forming vessels in mice were functional and exhibited blood flow. Since it has been previously described that normal neural stem/progenitor cells can differentiate into endothelial lineages [3], TDECs could also arise from neural stem/progenitor cells transduced with oncogene-expressing vectors. To show that this is not the only mechanism of TDEC formation, the authors demonstrated the presence of TDECs in the vasculature of GFP-labeled transplanted tumors derived from GBM tumor cell clones and subclones. To exclude the possibility that TDECs might result from the fusion of tumor cells and ECs, the authors applied confocal microscopy to examine the GFP tumor transplants in mice ubiquitously expressing red fluorescein protein (dsRed) and found that no GFP+ cells were dsRed+, and vice versa, which excluded the possibility of cell fusion. In vitro studies revealed that cultured GBM-initiating cells could transdifferentiate into ECs. Both activation of hypoxia-inducible factor (HIF)-1 in the presence of the iron chelator deferoxamine, and low concentrations of oxygen enhanced the transition of tumor cells to an endothelial-like morphology and the formation of tube-like structures when cells were put into a 3D culturing system. Additional evidence of the important role of hypoxia in TDEC formation came from the observation that in experimental tumors, most TDECs were located in the deep part of the tumors, which are more hypoxic, rather than on the tumor surface. Because HIF-1 is known to promote the formation of blood vessels through upregulation of VEGF, it would be
expected that anti-VEGF neutralizing antibodies or an anti-VEGF receptor (VEGFR)-specific small-molecule inhibitor would result in a lower number of TDECs or reduced tube formation, but no such effects were observed. The lack of these effects was explained by the finding that the majority of TDECs did not express VEGFR \[^4\]. While the number of regular ECs decreased in mice treated with a VEGFR inhibitor, their loss was compensated by the expanding TDECs that appeared to be resistant to anti-VEGF therapy. Altogether, the study shows that GBM tumor cells can transdifferentiate into vascular endothelial cells and give rise to functional tumor blood vessels that are insensitive to VEGFR inhibition. This finding provides a new potential mechanism of resistance of GBM to anti-VEGF therapy.

**Discussion**

The existence of cancer stem cells consisting of a relatively small cell subpopulation within the tumor that is responsible for tumor progression and self-renewal has been proposed \[^5\]. Cancer cells and normal stem cells share many features, such as the expression of similar markers indicating an undifferentiated state and utilization of similar signaling pathways that may regulate self-renewal in stem cells and cancer cells. Previously, the plasticity (i.e., the ability to differentiate into many cell types) was considered to be more of an attribute of normal stem cells, while the level of tumor malignancy was inversely linked to their level of differentiation. More recently, accumulating evidence suggests that cancer stem cells also possess considerable plasticity. The ability of tumor cells to differentiate into ECs and the so-called vasculogenic mimicry (the formation of fluid-conducting channels by tumor cells) has been suggested in various malignancies \[^6\]–\[^13\]. Because GBM is one of the most vascular-rich tumors, antiangiogenic therapies are currently being actively pursued in clinical trials. These therapies that mostly target VEGF/VEGFR signaling frequently fail due to the development of resistance after initial responsiveness. The results presented in this article describe one new possible mechanism of anti-VEGF resistance of human GBM. They stipulate that TDECs that contribute to GBM vessels cannot be targeted by anti-VEGF therapies. The substitution of the regular ECs with the TDECs may account for the observed transient effects of antiangiogenic therapies, as well as for the increased aggressiveness of the tumors in the post-treatment period. In support of the authors’ findings, two other recently published papers have demonstrated the formation of tumor vessels from tumor cells. Ricci-Vitiani and coauthors showed that 20–90% of endothelial cells in GBM carry the same genomic alterations as tumor cells, indicating that a significant portion of vascular endothelium has a neoplastic origin \[^14\]. Wang and coauthors demonstrated that the stem cell-like CD133\(^{+}\) fraction of GBM includes a subset of vascular endothelial-cadherin (CD144\(^{+}\))-expressing cells with the
characteristics of endothelial progenitors that are capable of maturation into endothelial cells [15]. They further showed that this subpopulation is multipotent and capable of differentiation along tumor and endothelial lineages, possibly via intermediate CD133+/CD144+ progenitor cells. However, according to their data, blocking VEGF or silencing VEGFR2 inhibits the maturation of tumor endothelial progenitors into endothelium, but not the differentiation of CD133+ cells into endothelial progenitors.

**Future perspective**

The plasticity of tumor cells and their capacity to generate tumor vasculature are novel findings that provide not only new insights into the biology of gliomas [15], but also have a number of significant implications for future strategies of anticancer therapy. These findings imply that antiangiogenic tumor therapies have to target both regular ECs and TDECs to be successful. Soda et al. suggest that while regular ECs respond to anti-VEGF therapies, TDECs are dependent on hypoxia and HIF-1 and might therefore respond to anti-HIF-1 therapies [1]. However, the study does not go far enough to directly implicate HIF-1 in TDEC formation, and this latter point requires a more rigorous and direct experimental confirmation. Nonetheless, it will be important to systematically investigate the differences and similarities between ECs and TDECs in order to be able to develop common or combination therapies that target both of them. Alternatively, strategies that prevent the transdifferentiation of tumor cells into TDECs could theoretically also have therapeutic value. The findings of the Soda et al. manuscript also raise a number of questions with potential clinical implications: are TDECs more or less resistant to the currently used chemo- and radio-therapy compared with cells within the main tumor mass and with normal ECs? Is the fraction of TDECs in a tumor variable, and if so, does it vary according to tumor grade and does it affect clinical prognosis? Could strategies aimed at inhibiting or inducing specific pathways of differentiation of cancer cells be beneficial for clinical practice? These and other important issues will require intensive research to better understand the processes of tumor cell transdifferentiation and its potential prognostic and therapeutic implications.

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No writing assistance was utilized in the production of this manuscript.

**Bibliography**

Papers of special interest have been highlighted as:

- of interest


- One of the most comprehensive reviews that discusses the concept of ‘cancer stem cells’.


- Along with [15], presents data on the differentiation of human glioblastoma multiforme cells into vascular endothelial cells.


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