Targeting glioblastoma stem-cells: a recurrent challenge in neuro-oncology

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Comment on: Yan H, Romero-López M, Benitez LI, *et al.* 3D Mathematical Modeling of Glioblastoma Suggests That Transdifferentiated Vascular Endothelial Cells Mediate Resistance to Current Standard-of-Care Therapy. Cancer Res 2017;77:4171-84.

Glioblastoma (GBM) is the most common and most aggressive primary brain tumor in adults. To date, this tumor remains rapidly fatal despite treatment, median overall survival not exceeding 15 months from diagnosis (1). The resistance of GBM to the current standard of care is thought to derive, at least in part, from cancer stem-cells located in intratumoral niches (2). Glioblastoma stem-cells (GSC) have in fact the potential to differentiate into committed tumor cells, replacing the cells depleted by cytotoxic treatments and leading to tumor recurrence (2).

In their article (3), Yan and colleagues proposed a sophisticated three-dimensional mathematical model simulating the dynamics of growth and evolution of human GBM. This model accounted for the proliferation, apoptosis, motility and differentiation of different types of tumor cells, including GCS. The dynamic interactions between cell subpopulations, occurring upon intercellular signaling, were recapitulated. The supply of oxygen and nutrients to different tumor areas were estimated based on blood vessel density, substrate concentration and diffusivity. The process of neoangiogenesis induced by hypoxic signals was integrated in the model, together with the interactions between newly-formed vessels and existing vasculature. The authors also entered in their model the transdifferentiation of GCS into endothelial cells. Therefore, this model accounted for all the main features of GBM, including intense proliferation, invasiveness, necrosis and neovascularization, as well as for their reciprocal interactions.

The model was then used to simulate the response of human GBM to different antineoplastic treatments, administered alone or in combination. This model predicted that cytotoxic therapies alone are bound to fail in controlling tumor growth, since additional therapies targeting GSC are mandatory to achieve durable tumor response. Based on these results, the authors ultimately proposed a treatment combination based on cytotoxic compounds, antiangiogenics, differentiating agents, and drugs targeting transdifferentiated GCS to test in clinical practice. The assumption of the authors is that administering a treatment combination active on all the subsets of tumor cells, for an appropriate amount of time, could potentially lead to GBM eradication.

GSC were first described over 15 years ago (4,5). Similarly to other cancer stem-cells, GSC are defined by functional characteristics such tumor initiation upon secondary transplantation, persistent proliferation, and sustained self-renewal (2). In some circumstances, GSC can also transdifferentiate, giving rise to committed stromal cells (2), pericytes (6), or even endothelial cells (7-9). This accounts for the plasticity of GBM, its remarkable capacity for adaptation and self-sustenance.

GSC reside in protective niches localized in close proximity to blood vessels within the hypoxic core, along perivascular spaces, and at tumor margins (10-12). Each niche has its own microenvironment, supporting GSC

and modulating their activity (13). GSC niches have specific functions that include, but are not limited to, GSC maintenance (11,12). The perivascular niche is responsible for tumor neoangiogenesis, which is promoted by proangiogenic factors secreted by resident GSC (10,11,14). The hypoxic niche, located in GBM necrotic core, is the main reservoir of GSC, whose survival and stemness is promoted by hypoxia signaling pathways (10,11). The invasive niche, located at tumor margins, is responsible for the invasion of surrounding tissue carried on by GSC following mesenchymal transition (10,11).

Therefore, GSC are involved into tumor initiation, progression and neoangiogenesis, and participate to GBM resistance to antineoplastic agents. Being intrinsically resistant to radiochemotherapy (15,16), GSC will survive cytotoxic treatments and will eventually drive tumor recurrence by differentiating into committed tumor progenitors. It has also been proposed that GSC may be responsible for GBM resistance to antiangiogenics by transdifferentiating into endothelial cells to form new blood vessels (17).

Endothelial transdifferentiation is clearly operant in xenograft models, where human GSC differentiate into bona fide blood vessels to supply tumor growth (7-9). However, this is an artificial model, and studies conducted on fresh GBM samples suggest that, in normal conditions, the phenomenon rarely occurs (18,19). Endothelial transdifferentiation may instead be more frequent at recurrence, operating as a mechanism of resistance to antiangiogenics. The same has been suggested for vascular mimicry, which has been documented in a patient presenting with tumor recurrence after antiangiogenic therapy (20). Indeed, we still do not know to which extent endothelial transdifferentiation may be involved in secondary resistance to antiangiogenics, since the number of patients undergoing surgery after bevacizumab is very limited. In addition, several other mechanisms of resistance to antiangiogenics have been reported (21,22), and their relative contribution in determining treatment resistance is still unclear.

Since GSC are possibly involved in the resistance to both cytotoxic and antiangiogenic agents, specifically targeting these cells seems a rational treatment strategy to counteract the escape of GBM to the current standard of care. In the neuro-oncological community, efforts are being made to identify therapies capable of inhibiting the stemness and self-renewal of GSC. Although several compounds are being investigated as pro-differentiating agents, current data are limited to pre-clinical models and there is still no evidence of efficacy in humans.

Indeed, how sophisticated a mathematical model may be, it is always an oversimplification, and results should therefore be taken with caution. The model adopted by Yan *et al.* (3) is based on *a priori* assumptions that may not entirely reflect biological complexity, as discussed above for endothelial transdifferentiation. In addition, the hierarchical model adopted here postulates the existence of distinct types of tumor cells, only a subset of which has the ability to initiate tumor growth: GSC can give rise to committed cells but committed cells should not dedifferentiate into GSC. In fact, this last point remains controversial. *In vitro* experiments have shown that the induction of few transcription factors (POU3F2, SOX2, SALL2, OLIG2) is sufficient through epigenetic changes to reprogram differentiated GBM into stem-like cells capable of *in vivo* tumor propagation (23). Whether this bidirectional plasticity between GSC and differentiated tumor cell works *in vivo*, and to which extent, remains to be investigated. In this case, the specific targeting of GSC would be less relevant. Lastly, the model by Yan *et al.* (3) operates under the assumption that treatments can actually eradicate the totality of targeted cells and does not account for acquired drug resistance.

Despite these limitations, the mathematical model of human GBM proposed by Yan and colleagues (3) remains an appealing and elegant tool for predicting the effects of novel agents and for orienting treatment strategies, at the condition that it is implemented with the most accurate assumptions.

Footnote

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