Editorial

Resistance mechanisms in glioblastoma stem cells: finding opportunities in challenges

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Glioblastoma remains the most aggressive and devastating form of primary brain tumor with universally dismal survival outcomes despite multi-modality management, essentially rendering it incurable in contemporary neuro-oncologic practice. It displays all the classical hallmarks of cancer including immune suppression, sustained proliferative signalling, inhibition of apoptosis, angiogenesis and invasion (1,2). Glioblastoma is biologically very heterogeneous; this intra-tumoral heterogeneity is explained by the stochastic model (2,3), wherein the tumor arises from a single clone of cells with further progression resulting from random accumulation of somatic mutations in genetically unstable cell population and sequential selection of malignant sub-clones through micro-environment cues. The current standard of care for newly-diagnosed glioblastoma comprising maximal safe resection followed by post-operative focal conformal radiotherapy to the tumor bed with concurrent and adjuvant temozolomide chemotherapy, results in a median survival of around 15 months and a 5-year overall survival rarely exceeding 8–10% (4).

Recent research suggests that glioblastomas may be organized hierarchically with a small number of progenitor cells (5-7), so called glioblastoma stem cells (GSCs) that have self-renewal capacity, multi-lineage potency, as well as properties of tumor initiation and propagation responsible for relapse, recurrence and/or progression in glioblastoma. It is widely believed, that GSCs reside preferentially within the hypoxic core of the glioblastoma tumour mass, while more differentiated cells are mainly localized along the peripheral and more vascularized part of the tumour (8). These GSCs have a selective tropism (9,10) for the subventricular zone (SVZ), the largest reservoir of neural stem cells in the adult brain (11). In addition to the established role of SVZ-derived cells towards olfactory function and neurogenesis for brain repair in response to injury, there is increasing evidence of its role in gliomagenesis (12,13). The identification of GSCs spawned a slew of experimental model systems to understand and characterize the relationship between SVZ and glioblastoma. The notion of SVZ as a potential oncogenic niche originated from an early study (14) suggesting that brain tumors in contact with ventricular walls might be arising from the embryonic rests present in the SVZ. This was followed by studies demonstrating mitoses occurring in the sub-ependymal layer of rodent and primate brain (15). In mice, it was initially shown that undifferentiated (precursor) cells are more easily transformed compared to cells that are terminally differentiated (16), thus corroborating the hypothesis that precursor or neural stem cells might represent the target of malignant transformation. In addition, another study (17) comparing cultures of astrocytes versus neurosphere precursor cells showed that dedifferentiation of astrocytes makes these cells susceptible to malignant transformation similarly to neural stem cells, by combining loss of critical tumor suppressors. Importantly, the phylogenetic relationship between SVZ and glioblastoma clearly establishes SVZ as a reservoir of GSCs (either early tumor clones or late-emergent clones) that are largely resistant to standard radiotherapy and chemotherapy, resulting in disease progression and treatment failure. In this context, GSCs isolated from SVZ could be used for

unravelling and understanding mechanisms of resistance in glioblastoma.

Apart from potential transformation of neural stem cells into GSCs, there is mounting evidence (10,18) suggesting considerable 'plasticity' of the stemness state i.e., acquisition of stem cell properties by tumor cells under the influence of a permissive microenvironment and/or aberrant transcriptional programming. This switch to stem cell program relies largely on epigenetic rather than genetic changes without the need of numerous cell generations for advantageous mutations to prevail. While the debate on origin of GSCs is not completely settled, there is growing appreciation of the role of microenvironment in driving cancer cell biology with an increasing number of preclinical studies underscoring its importance in carcinogenesis. The tumour microenvironment essentially consists of resident non-cancerous cells (stromal fibroblasts, endothelial cells and immune cells), connective tissue and extracellular matrix, supporting tumour structure, angiogenesis and growth. Chemokines, small pro-inflammatory chemoattractant cytokines that bind to specific G-protein coupled seven-span transmembrane receptors, are major regulators of cell trafficking and adhesion (19-21). The chemokine CXCL12 also known as stromal cell-derived factor-1 (SDF-1) and its receptor CXC receptor 4 (CXCR4) are two key factors in the cross-talk between cancer cells and their microenvironment. Binding of the ligand (CXCL12) to its receptor (CXCR4) induces intracellular signalling through several divergent pathways initiating signals related to chemotaxis, cell survival and/or proliferation, increase in intracellular calcium, and gene transcription (19-21). CXCR4 is expressed on multiple cell types including lymphocytes, hematopoietic stem cells, endothelial cells, epithelial cells, and cancer cells. The CXCL12/CXCR4 axis is involved in tumor progression, angiogenesis, vasculogenesis, and metastases. It has also been identified as a key mediator of mesenchymal activation in glioblastoma, a process involved in radiation resistance. Blocking the receptor-ligand interaction and/or inhibiting downstream signalling therefore presents an attractive therapeutic target.

Goffart and colleagues (22) had earlier demonstrated that adult mouse SVZ stimulates GSC-specific invasion through CXCL12/CXCR4 signalling. In further expansion of their seminal work, the group has now demonstrated the influence of SVZ-related factors in glioblastoma resistance to radiotherapy. Through a series of elegantly designed experiments reported in a recent issue of *Neuro-Oncology* (23), they demonstrate that (I) SVZ-nested GSCs are specifically resistant to irradiation *in vivo*; (II) these SVZ-nested GSCs display an enhanced mesenchymal signature known to be associated with radiation resistance compared to GSCs isolated from the tumor mass; (III) these mesenchymal traits are specifically upregulated by CXCL12 both *in vitro* and in the SVZ-environment; and (IV) blockade of the CXCL12/CXCR4 signalling via an antagonist (AMD3100) allows weakening of these mesenchymal roots and potentiates radiosensitivity both *in vivo* and *in vitro*.

It is widely known that anti-angiogenic therapy targeting the vascular endothelial growth factor (VEGF) pathway in glioblastoma decreases enhancement, but promotes invasion resulting in diffuse invasive recurrences. It is now postulated that anti-VEGF therapy upregulates CXCL12 and CXCR4 mRNA expression in tumor cells (21). Certain types of cytotoxic chemotherapy can also lead to specific enrichment of CXCR4-expressing chemo-resistant tumor cells (21), explaining the initial response to treatment, but ultimate recurrence and progression. In a mouse model of glioblastoma, local fractionated radiotherapy to the tumor mass resulted in hypoxia and consequent upregulation of CXCR4 and CXCL12 in the glioblastoma cells, promoting radiation resistance. Combining radiotherapy and CXCR4 inhibition with AMD3100 decreased vasculogenesis and abrogated tumor progression (21). Taken together, these data suggest that treatment with anti-angiogenic agents, cytotoxic chemotherapy, as well as radiotherapy can upregulate CXCL12/CXCR4 signalling resulting in enhanced invasive and metastatic potential, that may be countered through blockade of CXCL12/CXCR4 axis (20,21).

Given the presence of experimental evidence establishing the relationship between SVZ and glioblastoma, it would be reasonable to hypothesize that aggressive therapy directed at the SVZ might enhance glioblastoma control and prolong survival (2,24). However, clinical evidence for irradiating the stem cell niche in glioblastoma remains controversial with conflicting and contradictory results (2,24). While some reports suggest that increasing radiotherapy dose to the SVZ potentially influences outcomes, others have failed to find any significant correlation between SVZ dose and survival (2,24). However, all such studies have been retrospective in nature, included small number of patients, and suffer from inherent biases and methodological limitations such as variability in the delineation of the SVZ and clinical target volumes, radiotherapy dose-prescription (single phase *vs*. two-phase plan) and cut-off values of the

dose to the SVZ (2). An ongoing prospective randomized controlled trial (24) intentionally targeting the stem cell niche in newly-diagnosed glioblastoma should provide more definitive answers.

The interaction of cancer cells with their microenvironment that protects them from genotoxic stress represents an attractive target to improve anti-cancer treatment; disruption of such interaction may potentially increase the efficacy of conventional therapies. In this context, the implication of increased CXCL12/CXCR4 signalling in radiation resistance in glioblastoma opens up a window of newer therapeutic opportunities. Blockade of this signalling axis in combination with standard chemo-radiotherapy or in conjunction with increasing radiotherapy doses to the stem cell niche in the SVZ may be able to counter such resistance thereby improving outcomes. Given the near ubiquitous presence of the receptor and its ligand in a host of normal tissues, the challenge would lie in selective blockade of CXCL12/CXCR4 signalling in target tissues mitigating unwarranted systemic toxicity.

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Footnote

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