Editorial

Subventricular zone microenvironment protects glioblastoma cells from radiotherapy cytotoxicity: role of the chemokine CXCL12

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Glioblastoma (GBM) is the most aggressive primary glial tumor in adults and, notwithstanding all the novel treatment approaches to date developed, the outcome remains frustrating poor (1). Indeed, in most cases GBM rapidly relapses also after aggressive multimodal therapy (surgical resection, followed by radiotherapy with concomitant and adjuvant chemotherapy with temozolomide), and the limited benefit of the available treatments at relapse are not able to improve GBM patients' median overall survival (OS) beyond 15 months (2). However, although current therapeutic approaches have not improved patients' life expectance, in the recent years significant progresses were obtained in defining the biological features of GBM cells. In particular, in last few years a new paradigm of tumorigenesis has been proposed, in which a small cell population within the tumor mass, the so-called cancer stem cells (CSC), was identified as the main determinant of tumor development, progression, and recurrence (3) and, due to the intrinsic high resistance to chemo- and radio-therapy of these cells, it is now evident that efficient suppression of CSC in GBM [i.e., glioma stem cell (GSC)] represents an absolutely required goal to eradicate the tumor and prevent relapses (4). Therefore, the identification of the molecular determinants involved in the pharmacological resistance of GSC is urgently expected as a new potential targets for innovative and more efficacious therapeutic interventions.

In this light, as possible specific target to impair GSC functioning, several studies addressed the role of the CXCL12-CXCR4 axis. This ligand-receptor couple, belonging to CXC chemokine family, has been involved in the development and dissemination of several human benign and malignant tumors (5-8), including GBM (9,10). Interestingly, most of the effects observed after CXCR4 activation in tumor cells resembled those described for GSC and a direct modulation by CXCR4 ligand CXCL12 of this GBM subpopulation was indeed formally demonstrated. GSCs express high levels of CXCR4 (11,12) and its binding by both exogenous or autocrine CXCL12 (often produced by GSCs themselves) represents a master signal in the regulation of self-renewal, proliferation, migration, neoangiogenesis, and chemo-and radio-resistance of GSC (12-17). Importantly, CXCL12-CXCR4 system supports GSC persistence and activity not only directly acting on tumor cells, and on GSC in particular, but also regulating cell-to-cell interactions, motility and functioning in the microenvironment of the CSC niches (18).

These observations suggest that the blockade of CXCL12-CXCR4 signaling represents a potential valid target for neo-adjuvant treatments towards GBM. Nevertheless, a deeper knowledge of the role of this pathway in GBM microenvironment, including GSCs, tumor bulk, and surrounding brain tissue, is needed to define the best therapeutic strategy.

In a recent publication in *Neuro-Oncology* (19), Nicolas Goffart and colleagues addressed this topic in particular focusing on the mechanisms by which CXCL12 mediates GBM resistance to radiotherapy. Their research is based on previous observations, in which GSCs show a specific tropism for the adult subventricular zone (SVZ) in both human and mouse brains (20,21). According to the "seed and soil" theory, the SVZ represents a feeder niche for neural stem cells, promoting self-renewal and preventing

spontaneous differentiation until this pathway is induced according to the organism's requirements. Because it was reported that, like normal stem cells, GSCs require a dedicated environment in order to maintain their biological features (in particular self-renewal), it was proposed that SVZ niche might represent a potential reservoir of GSCs in which, away from the primary tumor, SVZ-nested cells are resilient to therapy. In support to this view it was demonstrated that the extension of the areas of irradiation to SVZ improved progression-free survival (PFS) and OS in GBM patients (22,23). Thus, in the Goffart's study (19), the possibility that GSC migration toward SVZ could represent a mechanism of protection of GBM cells from therapy, allowing their persistence in the brain and determining disease relapse, was addressed at mechanistic level. In particular, they analyzed the role of CXCL12-CXCR4 in light of (I) the high levels of CXCR4 in GSC and the observation that the expression of this receptor represents a radioresistance biomarker (24); and (II) the observation that CXCL12, the ligand for CXCR4, is secreted by SVZ cells, allowing not only the migration of CXCR4-expressing cells in this radioprotected niche, but also directly activating mechanisms responsible of the extrinsic resistance to irradiation.

After having confirmed the persistence of human primary GBM cells in mouse SVZ after irradiation, adopting an *in vitro* protocol of radiotherapy, they show that GBM cell survival (analyzed using both human GBM primary cultures and the U87 cell line) was dependent on soluble components released from SVZ cells, and identify CXCL12 as key mediator of this process. In particular, adding a specific anti-CXCL12 antibody to human (or mouse) SVZ cell conditioned media, they obtained the reacquisition of the sensitivity of GBM cells to the cytotoxicity induced by irradiation, while the administration of exogenous CXCL12 provided a similar, although incomplete, protection than SVZ conditioned medium. In fact, proteomic studies identified the presence in SVZ conditioned medium of other potential molecules endowed with radio-protectant activity (CCL20, CXCL8, etc.), besides CXCL12. Thus, it was hypotesized that the concomitant activity of all these molecules was responsible of the more complete radioprotection observed when the conditioned medium was used instead of purified CXCL12. In agreement with these observations, previous studies reported that GBM cells isolated from SVZ show higher drug resistance than cells derived from the primary mass of the same tumor (25).

Moreover, another important achievement in this study was the identification of the induction of epithelial-to-mesenchymal transition (EMT) upon CXCL12 stimulation of SVZ-nested GBM cells, as demonstrated by higher expression level of mesenchymal markers, such as vimentin and N-cadherin and crucial EMT transcription factors (ESR1, FOXC2, SOX10, and ZEB2). Interestingly, CXCR4 blockade using the specific antagonist AMD3100, led to a decrease of the tumor's mesenchymal signature of CXCL12-treated GBM cells, and, *in vivo*, resensitized the SVZ-nested GBM cells to irradiation. Altogether, these data not only highlight that SVZ-released CXCL12 confers radioprotection to GBM cells *in vivo*, but also underlie that the activation of EMT could represent a molecular correlate for such an effect. EMT is a process that promotes the ability of solid tumors to invade local environment and metastasize at distant sites. This process, typically recognized in CSCs, also favors the transition of solid malignancies from low to high-grades, worsening the prognosis. During EMT, tumor cells undergo a developmental switch causing epithelial-like cells to lose adhesion and polarity, while increasing proliferation, motility and invasiveness. Although with different features from epithelial tumors (the absence of membrane basement, the rarely detected expression of E-cadherin), this process, was also described in GBM and is considered a typical feature of GSC (26). Thus the possibility to revert the EMT-dependent phenotype using CXCR4 antagonists is an extremely relevant observation in this paper.

To date several approaches are under development to block CXCL12-CXCR4 axis, mainly focusing on CSC activity (27). Thus the observation that GSC persistence after irradiation is dependent on specific microenvironment signaling within SVZ, in which a CXCL12-dependent activation of EMT contribute to GSC survival, can open the way to significant innovative therapeutic approaches. In particular, most studies are currently directed to pharmacologically blunt CXCR4 activity, but possibly also the inhibition of the activity of other cyto/chemokines locally produced, could contribute to improve the prognosis of GBM patients. Of course, the bench to bed translation is a long and difficult process, but, as suggested by the authors (19), a further and deeper knowledge on the role of CXCL12 in GBM resistance to therapy will validate the potential therapeutic benefits of CXCL12 (or others chemokines) inhibitors pushing their translation to the clinic as pharmacological approach in combination with radiotherapy.

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