Editorial

CXCL12/CXCR4 signaling in glioma stem cells—prospects for therapeutic intervention

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Glioblastomas (GBM) are highly invasive and angiogenic brain tumors that progress rapidly. The disease is intractable despite aggressive treatment, including postoperative radiotherapy and chemotherapy. Glioma stem cells (GSCs), which are phenotypically similar to neural stem cells and neural progenitor cells (NPCs), are more resistant to cancer therapies than differentiated GBM cells. One of the characteristics of GSCs is the utilization of the CXCL12/CXCR4 pathway in the tumor microenvironment.

CXCL12/CXCR4 signaling involves the chemokine, CXCL12, and its receptor, CXCR4, and is linked to the proliferation, survival, migration and homing of tumor cells. Many malignant tumors, such as breast, lung, kidney, prostate, melanoma, colon, pancreatic cancers and glioma, express the chemokine receptor, CXCR4 (1,2). For glioma, recent findings showed that CXCL12 and CXCR4 expression increases with tumor grade (3) and GSCs express CXCR4. CXCL12, also known as stromal cell-derived factor 1α (SDF-1α), attracts stem cells, immune cells and tumor cells via CXCL12/CXCR4 signaling (4). The brain subventricular zones (SVZs) are major sources of NPCs and attract GSCs through CXCL12/CXCR4 signaling (5). CXCL12 is also secreted from blood vessels and tumor cells at inflammatory or hypoxic tumor sites, and tumor cells, endothelial progenitor cells, macrophages and myeloid-derived suppressor cells (MDSCs) accumulate in these areas (6,7). CXCL12 influences the tumor microenvironment to promote tumor cell growth, escape from immune surveillance and maintenance of stem cell function.

Researchers have utilized CD133+ GSCs derived from GBM tissue (8) or classical sphere forming GBM cell lines in serum-free medium (9) as GSC models. However, transgenic GSC models derived from NPCs have also been established. Genetically engineered NPCs can form GSCs with pathogenic features similar to human GBM, *in vivo* (10). Calinescu *et al*. established a GSC model using the sleeping beauty transposase system by injecting recombinant oncogenic DNA vectors carrying NRAS and SV40-LgT into the lateral ventricles of mice (11). NPCs pooled in the SVZ while in contact with the lateral ventricles. Tumors arose that were histopathologically similar to human GBM, including multinucleated giant cell formation, atypical mitoses, vascular proliferation, pseudo-palisading necrosis, perivascular and diffuse invasion, and hemorrhages. The tumor cells isolated from the mouse model were intracranially transplanted into another mouse, and expression of stem cell markers, GBM formation and lethality within 30 days were confirmed. CD133 expression was maintained on the surface of sphere cells formed by culturing resected tumor cells in serum-free medium. These

data indicated that this transgenic GSC model retains strong stemness characteristics, both *in vitro* and *in vivo*. Importantly, Calinescu *et al*. also showed that CXCL12/CXCR4 signaling regulates the proliferation of CXCL12 expressing GSCs derived from NPCs (11).

Direct effects of CXCL12/CXCR4 signaling on GSCs *in vitro*

Previous studies suggested that CXCL12/CXCR4 signaling promotes GBM cell proliferation (4). Calinescu *et al*. demonstrated that AMD3100, an antagonist of CXCR4, inhibits the proliferation of GSC cell lines, especially after exposure for 4 days or longer. The addition of CXCL12 significantly reduced these inhibitory effects. This indicates that CXCL12/CXCR4 signaling drives the proliferation of GSCs.

HIF-1α controls the transcription of many genes that play pivotal roles in angiogenesis, cell survival, genomic stability, invasion and metastasis (12). HIF-1α expression under hypoxic conditions promotes the expansion of GSCs and inhibits the induction of GSC differentiation (13). A previous paper reported that radiation and hypoxia induced the HIF-1α dependent release of CXCL12. Induction of HIF-1α expression by hypoxia or radiation requires TGF-β (14). According to Calinescu *et al*., GSCs simultaneously increased the expression of cell-cycle regulators (CyclinD1, Cdk4, Cdk6) and CXCL12 regulatory molecules (Cxcl12, Hif1-α, Tgf-β) by day 4 in culture. AMD3100 suppressed the overexpression of all of these molecules except HIF-1α, and reduced the proliferation of GSCs. In our opinion, since GSCs form spheres in serum-free medium, GSCs sphere formation may worsen the nutrition and oxygen supply into inside of sphere, and may make hypoxia environment, thereby promoting the expression of HIF-1α. However, if AMD3100 also regulates the expression of these activated genes *in vivo*, the drug may be therapeutically effective against GSCs.

Previous studies mainly focused on CXCL12 and CXCR4 expression within the CXCL12/CXCR4 signaling cascade. Calinescu *et al*. expanded the analysis to relevant CXCL12/CXCR4 signaling target genes, including *Retinoblastoma* (*Rb*) and *HIF-1α*. After several days of culture, activation of the CXCL12/CXCR4 signaling loop increased in GSCs. AMD3100 reduced HIF-1α protein levels, but did not affect *HIF-1α* mRNA expression. These results suggest that CXCR4 signaling may control HIF-1α turnover via the ubiquitin proteasome pathway under hypoxia. Further research is required to verify this possibility, and determine whether HIF-1α protein levels are regulated in a similar way in other GBM cells. As CXCL12/CXCR4 signaling controls a wide range of cellular functions, DNA microarray and proteomic profiling will help to develop a more comprehensive picture of CXCL12/CXCR4 signaling in the future.

Indirect effects of CXCL12/CXCR4 signaling on GSCs *in vivo*

In the Calinescu model, GSCs derived from NPCs in the mouse brain produce CXCL12 and express nestin (a stem cell marker) as late as day 35 of tumorigenesis suggesting that stem cell characteristics are stably maintained. At the same time in this tumor model, there was a large amount of CD11b+ myeloid derived suppressor cell (MDSC) and macrophage infiltration into the tumor microenvironment (11). This phenomenon is consistent with our GSC study (15). MDSCs and macrophages attenuate immune cell function and provide a favorable environment for tumor growth, survival and migration (16). In addition, genomic profiling data suggest that macrophage-like gene expression signatures in GBM tissue are a poor prognostic feature (17), which may be an important consideration in the clinical setting. Indeed, the prognosis for mouse GBM was improved by preventing infiltration of monocytes using anti-CXCR4 antibody (18). CXCR4 blockade mediated inhibition of infiltrating monocytes may be more important than the cytostatic effect on GSCs for improving the prognosis of GBM patients.

The Calinescu study suggested that GSCs respond to autocrine and/or paracrine CXCL12/CXCR4 signaling. GSCs invade the brain parenchyma and are maintained within vascular niches (19). CXCL12 expressing GSCs facilitate CXCR4+ cell aggregation and the activation and maintenance of autocrine/paracrine CXCL12/CXCR4 signaling loops. Even for small or satellite GSC colonies, CXCL12/CXCR4 signaling promotes recurrence after aggressive treatment, similar to human GBM.

During tumor growth, rapid GSC proliferation generates hypoxic conditions and promotes angiogenesis. Even though the hypoxic environment is disadvantageous for tumor growth, VEGF secreted by GBM cells induces CXCL12 expression by tumor vessels. Release of CXCL12 is also regulated by TGF-β-dependent HIF-1α and TGF-β is secreted by tumor associated macrophages. Ultimately, large amounts of CXCL12 in the proliferative tumor microenvironment strongly promote the preferential survival and proliferation of GSCs over differentiated GBM cells.

Angiogenesis is prominent in the GBM microenvironment,

and angiogenic factors are therapeutic targets. VEGF/VEGFR signaling is the most important regulator of angiogenesis. Bevacizumab, an anti-VEGF antibody, inhibits tumor angiogenesis and tumor growth in GBM patients and GBM mouse models. Bevacizumab resistant GBM tissues express high levels of CXCR4, which is correlated with rapid tumor growth. This suggests that CXCR4 expressing GSCs are resistant to anti-angiogenic therapy. Combination therapy with bevacizumab and CXCR4 antagonists induces tumor cell apoptosis and prolongs the disease-free survival and overall survival of tumor engrafted mice (20). A Phase I study of AMD3100 and bevacizumab for the treatment of recurrent high-grade glioma that is currently underway (NCT01339039) will evaluate the significance of CXCL12/CXCR4 signaling and angiogenesis in GBM.

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