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Chemoattractant receptors as pharmacological targets for elimination of glioma stem-like cells

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Abstract

Malignant tumors are thought to be initiated by a small population of cells that display stem cell properties, including the capacity of self-renewal, multipotent differentiation, initiation of tumor tissues and resistance to therapy. Cancer stem cells (CSCs) have also been identified in gliomas in which they are named as glioma stem-like cells (GSLCs), or glioma stem cells. In xenograft transplantation models, GSLCs propagate tumor and promote tumor progression. The tumorigenesis of GSLCs depends not only on their autonomous proliferation but also on interaction with microenvironment components. Among these components, G protein coupled chemoattractant receptors (GPCRs) and their agonists have attracted much attention for their capacity to mediate leukocyte infiltration, angiogenesis, tumor invasion and metastasis. Chemoattractant GPCRs are widely expressed by tumor cells and stromal cells and recognize agonists present in the tumor microenvironment. Such GPCRs have been found to be expressed also by CSCs including GSLCs. In this brief review, we will summarize the recent development in the studies of the function, regulation and signal transduction of chemoattractant GPCRs in GSLCs in hope to promote a better understanding of the mechanistic basis of the progression of gliomas and the identification of molecular targets for the novel anti-glioma therapy.

Keywords

glioma; cancer stem cells; chemoattractant receptors; chemokine receptors

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1. Introduction

Gliomas are the most common tumor type in the central nervous system (CNS) and range from slowly growing low-grade tumors to rapidly growing high-grade tumors, such as anaplastic astrocytoma and glioblastoma (GBM). The majority of gliomas are malignant and the high mortality rate of patients convert this relatively infrequent malignancy into the third leading cause of cancer-related death among men and women of 15–54 year old. The mortality of GBM is especially high with a median survival time of 9–12 months despite multiple therapeutic regimens designed to optimize surgery, radiation and chemotherapy. Like most malignancies, the cause of glioma is not clear. However, a large body of evidence suggests that malignant glioma cells exhibit a unique capability to recognize growth promoting signals present in the microenvironment and the tumor cells produce growth and angiogenic factors in their own favor. Studies using human glioma cell lines and cells derived from primary tumors reveal that most of these cells express higher level of surface receptors important for cell survival, invasion and angiogenesis including epidermal growth factor receptor (EGFR) and basic fibroblast growth factor receptor (bFGFR) that form paracrine and autocrine growth stimulation loop for gliomas.

Malignant glioma cells also express G protein coupled chemoattractant receptors (GPCRs), which transduce extracellular signals into intracellular effector pathways through the activation of heterotrimeric G proteins. This receptor superfamily includes GPCRs for classical chemoattractants such as formyl peptides (fMLF) produced by Gram negative bacteria and host cell mitochondria, the complement cleavage components, leukotriene B₄ (LTB₄), and platelet activating factor (PAF) as well as GPCRs for chemokines [1]. A number of chemoattractant GPCRs are expressed by cells in the CNS and participate in physiological process as mediators of cell migration, tissue patterning, neural survival, neurotransmission and cell-cell communication [2]. In fact, GPCRs have been described as the third major transmitter system in the brain [3].

Studies have shown that glioma cells express chemoattractant GPCRs which exert complex connections to the development and progression of gliomas. For instance, highly motile malignant glioma cells utilize chemoattractant GPCRs to invade surrounding tissues and yield poor prognosis of the tumor bearing hosts [4, 5]. In addition to cell migration, chemoattractant GPCRs enhance glioma cell proliferation and contribute to tumor angiogenesis by promoting the production of angiogenic factors by tumor and stromal cells. A number of chemoattractant GPCRs have been detected in glioma cells including the classical chemoattractant receptor FPR1, and chemokine GPCRs CCR3, CCR5, CX3CR1, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5 and CXCR7 [6–14]. Highly malignant GBM cells utilize the otherwise physiological function of chemoattractant GPCRs to sense cognate ligands produced in the microenvironment that exacerbate the malignant behavior of the tumor cells [8, 15–17].

Many malignant tumors contain a small fraction of stem-like cells with the potential of self-renewal, multipotent differentiation, and initiating tumor growth. These cancer stem cells (CSCs) are also resistant to chemo-therapeutic agents and irradiation. In malignant gliomas, such stem like cells have also been identified and isolated. These cells are termed glioma stem-like cells (GSLCs), or glioma stem cells, and express chemokine GPCRs CXCR4 and CX3CR1 as well as a classical chemoattractant GPCR FPR1 (originally named FPR). These GPCRs recognize agonists present in the glioma microenvironment and contribute to the tumor progression and angiogenesis initiated by GSLCs [18–22] (Table 1). Given the important features of chemoattractant GPCRs in GSLCs, studies of the function, regulation and signal transduction pathways of chemoattractant GPCRs in GSLCs will not only promote a better understanding of the mechanistic basis of the progression of the tumor, but

also the identification of potential molecular targets for the novel anti-glioma therapy aiming at eliminating GSLCs.

2. The identification of glioma stem-like cells (GSLCs)

In 1855, Virchow proposed that a cancer might originate from embryonic-like cells [23]. In 1994, Lapidot et al. found that a population of cells in acute myeloid leukemia (AML) displayed stem cell like properties and reconstituted AML when implanted into NOD/SCID mice [24]. Subsequent studies revealed the existence of stem-like subsets, now called CSCs, in a variety of solid tumors, including tumors of the brain, colon, breast, prostate and pancreas [25–29]. CSCs are defined based on some characteristics resembling normal stem cells: the expression of stem cell markers, the capacity of self-renewal, and differentiation into certain system lineages.

CSCs have been identified and isolated from human glioblastoma (GBM) based on the expression of CD133⁺, nestin, Oct4 and Notch. CD133⁺ GSLCs form floating spheres in fetal calf serum (FCS)-free medium. In the presence of FCS, they grow in adherence and are capable of differentiating into astrocytic, neuronal, and oligodendrocytic lineages. When injected into immunocompromised mice, as few as one hundred GSLCs were sufficient to form a lethal tumor, whereas the injection of ten thousand CD133[−] GBM cells failed to form a tumor over the same period of observation [21, 25, 30]. Thus, CD133⁺ GSLCs are highly tumorigenic and tumors formed by GSLCs maintain the phenotypic heterogeneity present in primary tumors, indicating that this subpopulation within GBM is responsible for the formation of tumors and the establishment of neoplastic heterogeneity to recapitulate the characteristics of GBM [21]. In addition to CD133, more surface markers were identified and used to isolate GSLCs from cell lines and primary tumor specimens. CD15/SSEA-1, L1CAM, A2B5 and integrin $\alpha 6$ have been utilized to enrich stem-cell populations within gliomas [31–34]. Serum-free neurosphere medium has been used to generate tumor spheroids that are enriched in self-renewing tumorigenic stem cells. The presence of serum in culture drives the differentiation of subpopulations from the spheres.

3. The expression of chemoattractant GPCRs in GSLCs

A key issue in CSC study is to determine which pathways CSCs use to maintain their stemness phenotype. CSCs only represent a subpopulation of cells in a growing tumor but are capable of initiating metastasis and promoting tumor vascularization. CSCs are difficult to be eradicated by conventional treatments such as chemo- and radiotherapy and they can regroup (or function as seeds) to form new tumors after unsuccessful treatment. CSCs express a number of cell surface molecules which enable their interaction with tumor microenvironment cues for propagation and tumor initiation [18, 21, 22, 35, 36]. We have shown that CD133⁺ GSLCs express higher levels of the chemoattractant GPCR FPR1 than their counterpart CD133[−] cells. FPR1 expressed by GSLCs is functional, since stimulation of FPR1 with bacteria or host derived chemotactic agonists promotes GSLC chemotaxis and the production of VEGF [21]. GSLCs growing as floating spheroids also highly expressed the chemokine GPCR CXCR4 and upon differentiation in the presence of FCS, the expression of CXCR4 diminished, suggesting a preferential expression of CXCR4 by tumor initiating cells [14]. This is supported by the observation in primary human gliomas in which CXCR4 expression is higher in CD133⁺ cells than CD133[−] cells. CXCR4 in GSLCs isolated from glioma cell lines or primary human tumors is activated by the CXCR4 chemokine agonist CXCL12 to show intracellular calcium mobilization, an indicator of GPCR signaling, and to promote the production of VEGF [19, 20]. CXCL12 also induces a significant proliferative response in GSLCs but not in differentiated tumor cells [37]. This is similar to observation with a mouse Lewis lung cancer cell line (LLC) in which CXCR4⁺

LLC cells isolated by magnetic sorting, form spheres in serum-free medium and showed lower sensitivity to chemotherapeutic agents than CXCR4⁺ LLC cells [38]. Another chemokine GPCR CX3CR1 and its agonist CX3CL1 have also been reported to play roles in GSLC initiated tumor progression. CX3CL1 is one of the most expressed chemokines in the brain [39] and is a unique member of the chemokine family by mediating both chemotaxis and adhesion of inflammatory cells via its highly selective receptor CX3CR1. CX3CR1 is overexpressed in gliomas, regardless of tumor classification and clinical severity [40], while the expression of the ligand CX3CL1 is correlated with glioma grade and overall patient survival. The increased expression of CX3CL1 in higher grade gliomas implies the involvement of CX3CL1 and its receptor CX3CR1 in tumor progression, possibly in two aspects: 1. by affecting host defense mediated by immune cells and 2. by directly promoting tumor cell growth. GSLCs isolated from GBM specimens express both CX3CR1 and CX3CL1, indicating that the expression of CX3CR1 and CX3CL1 axis is an early event in the tumor initiation process. Confocal microscopy revealed that CX3CL1 was primarily expressed in the cells of the outer layer of the neurospheres formed by GSLCs, whereas CX3CR1 was located in both the outer and inner cells, suggesting the ligand and receptor may function in an autocrine or paracrine loop [40]. Thus, GSLCs express multiple chemoattractant GPCRs, which may actively participate in tumor formation initiated by GSLCs (Table 1).

4. The role of chemoattractant GPCRs in the invasiveness and metastasis of GSLCs

In human pancreatic cancer tissue, CSCs expressing CD133 are tumorigenic and highly resistant to standard chemotherapy. In the invasive front of pancreatic tumors, a distinct subpopulation of CD133⁺/CXCR4⁺ CSCs was identified that appears to determine the metastatic behavior of an individual tumor [41, 42]. Depletion of this CXCR4⁺ CSC subpopulation from the entire CSC pool effectively abrogated the metastatic capacity of pancreatic cancer cells without affecting their tumorigenicity [41, 42], suggesting that the expression of CXCR4 defines a metastatic subpopulation within the CSCs of pancreatic tumors and CXCR4 may likely be a mediator of metastasis. In primary human GBM, CXCR4⁺ cells were observed at the invasion front consisting of a small population of tumor cells and stem-like cells [19, 20]. In vitro, spheroids derived from GBM cell lines were composed of a large number of GSLCs, which expressed a higher level of CXCR4 and showed more potent chemotactic response to the agonist CXCL12 [19, 20].

The invasion process of GBM requires the detachment of invading cells from a tumor mass, attachment of these motile cells to ECM components, ECM degradation, and subsequent cell infiltration into surrounding brain tissues. As reported, the major biologic effects of CXCR4/CXCL12 axis are to induce tumor cell migration, adhesion and secretion of MMPs. Attachment of tumor cells to ECM components is an essential phase of invasion mediated by integrins that are overexpressed on both glioma cells and tumor vasculature. It is known that CXCR4/CXCL12 promotes the adhesion of tumor cell to VCAM-1, ICAM-1 and fibronectin on vascular surface by activating integrins, including $\alpha 2$, $\alpha 4$, $\alpha 5$, and $\beta 1$ [43, 44]. Inhibition of integrin function disrupts GBM cell migration. In vitro, interference of CXCR4 with the urokinase-receptor (uPAR) reduces the adhesion of CXCL12-mediated CXCR4⁺ GBM cells to collagen, the main component of ECM [45]. Other factors that mediate tumor invasion include MMPs that degrade ECM. CXCR4 mediated glioma invasion in vivo was associated with its capacity to activate MMPs [45]. It has been reported that CXCR4 and its downstream ERK and NF- κ B signaling pathways increase MMPs including MMP9 in glioma cells. Recent studies showed that the expression of MMP9 mRNA in CXCR4⁺ LLC, which can form tumor spheres in vitro, was higher than in CXCR4⁻ LLC [38]. Another chemokine GPCR, which mediates GSLC migration in primary tumors is CX3CR1.

Confocal microscopy detects the distribution of CX3CR1 in the outer layer cells of the spheres formed by GSLCs [22]. Activation of CX3CR1 in GSLCs causes the release of MMP9 to promote matrix degradation and activates adhesion molecules on GSLCs. Thus, chemoattractant GPCRs play an important role in GSLC adhesion and ECM degradation that are crucial procedures resulting in tumor invasion.

5. Contribution of chemoattractant GPCRs to epithelial-mesenchymal transition (EMT)

Epithelial-mesenchymal transition (EMT) is a major event linked to tumor metastasis processes. It is believed that epithelial tumor cells have the capacity to activate the primal development program, thus converting differentiated epithelial tumor cells into dedifferentiated status exhibiting mesenchymal features [47]. Indeed, induction of EMT in immortalized human mammary epithelial cells resulted in the expression of stem cell markers, gain of mesenchymal behavior, and phenotypes associated with CSCs [48]. These findings support a direct link between EMT and the acquirement of properties of migratory stem cells [49]. The EMT process is reversible, since when tumor cells reach the metastatic sites, they transform back into an epithelial phenotype followed by formation of secondary (metastatic) lesions. In glioma, tumor invasion into adjacent normal brain is associated with the acquirement of mesenchymal features by GBM cells, indicating the importance of mesenchymal transition in the malignant behavior of GBMs cells and GSLCs [50]. More recently, aldehyde dehydrogenase1 (ALDH1), which is a marker of normal and malignant human epithelial stem cells, was found to be also an important marker and regulator for epithelial-mesenchymal transitions, which is crucial for tumor cell to acquire a more migratory and proliferative phenotype [51].

It is interesting to note that EMT is associated with the expression of chemoattractant GPCRs [47]. In an EMT model of colon carcinoma, upregulation of the chemokine GPCR CXCR1 and its cognate ligand CXCL8 has been observed. CXCL8 is a chemokine mediating neutrophil infiltration in inflammation and promoting angiogenesis in tumors by activating vascular endothelial cell. However, in the colon cancer EMT model, CXCL8 and CXCR1 mediate the migration of colon carcinoma cells undergoing EMT [52]. CXCL12/CXCR4 pair also induces EMT of oral squamous cell carcinoma [53] and the C-terminal domain of CXCR4 seems to play a critical role in receptor function during EMT [54, 55]. In breast cell line MCF-7, the C-terminal truncation mutation in CXCR4 causes a higher growth rate and altered phenotype that resembles EMT. Thus, chemoattractant GPCRs are involved in EMT of CSCs including GSLCs that are endowed with a highly invasive property [49].

6. GPCRs in GSLCs trigger angiogenic switch

GSLCs play an important role in promoting the formation of tumor vasculature resulted from angiogenesis and vasculogenesis. GSLCs have been shown to promote tumor vasculature formation through increased production of VEGF, since the anti-VEGF antibody, bevacizumab (Avastin), that neutralizes GSLC-released VEGF inhibited tumor growth in xenograft models [56, 57]. In fact, bevacizumab has been approved by FDA for clinical trials in combination with other standard chemotherapeutic agents in newly diagnosed and recurrent GBM patients [58]. In primary tumors, GSLCs frequently accumulate within highly vascularized areas and in xenograft, GSLC-enriched CD133⁺ fraction of human glioma cells consistently form more highly vascularized and rapidly growing tumors which contain high levels of VEGF [18, 19, 20, 56]. Therefore, GSLCs may provide signals necessary to initiate the “angiogenic switch” in the early phase of primary or metastatic tumors [59]. In our studies of the potential for GSLCs to trigger tumor

angiogenesis, we confirmed that CD133⁺ GSLCs from a human GBM cell line, U87, express chemoattractant GPCRs FPR1 and CXCR4 [19, 20, 21], which, upon activation by their agonists, promote the production of VEGF and angiogenesis. Moreover, in human glioma specimens, CD133⁺ cells adjacent to tumor microvessels expressed higher levels of CXCR4 and the corresponding tumor areas are densely vascularized. Interestingly, treatment with a CXCR4 antagonist AMD3100 decreased the microvessel density in tumors formed by GSLCs from a rat glioma cell line C6. It is important to note that necrotic GBM cells and GSLCs release a FPR1 agonist Annexin1 [60] and the CXCR4 agonist CXCL12 is produced by stromal cells as well as GBM cells *per se*. Therefore, constitutively expressed chemoattractant GPCRs, such as FPR1 and CXCR4, may allow the participation of GSLCs in glioma angiogenesis by stimulating their release of VEGF in response to agonists in the tumor microenvironment.

Angiogenesis is a process of pre-existing blood vessels sprouting into cancer cell clusters to form new vessels. On the other hand, vasculogenesis involves the mobilization and recruitment of endothelial progenitor cells (EPCs) from bone marrow (BM) into tumor tissue to initiate neovascularization. EPCs from BM enter the circulation in response to host and tumor-derived factors then are recruited into tumors where they incorporate into nascent vessels [61–63]. GSLC-rich C6 glioma contains markedly higher level of EPCs which are believed to be recruited by GSLC-derived factors [63]. This notion was supported by subsequent observations showing that the mobilization and recruitment of EPCs were reduced by an anti-VEGFR2 monoclonal antibody DC101 and by the CXCR4 antagonist AMD3100, suggesting that CXCR4 and VEGFR2 synergistically mediate EPC recruitment into the tumor to favor angiogenesis. GSLCs also mediate vasculogenesis during tumor recurrence following radiation therapy. GSLC subsets are enriched in the original tumor area following radiotherapy and the recurrence mostly occurs within the irradiated regions [64]. Since normal vessels surrounding the tumor might be deprived of the capacity to generate new extensions, GSLC-mediated recruitment of EPC into the recurrent tumor after irradiation becomes an important alternative for neovascularization through a CXCR4/CXCL12 pathway. Orthotopic mouse brain implantation of human U251 GBM cells resulted in progressively growing tumors, which were partially inhibited by irradiation yet were also followed by rapid recurrence [64]. Administration of the CXCR4 antagonist AMD3100 prevented the recruitment of BMDCs into tumor sites and the subsequent formation of vessel structures. Therefore, blockade of CXCR4 effectively inhibited the growth of recurrent tumors, indicating a prominent role of CXCR4 in mediating vasculogenesis by EPCs in cooperation with GSLCs.

In addition to CXCR4 and FPR1, another chemoattractant GPCR FPR2 (originally termed FPRL1) has also been shown to mediate angiogenesis in a human ovarian tumor xenograft model [65]. FPR2 is a variant of FPR1 and interacts with a variety of host derived agonists in addition to the bacterial peptide fMLF, which is also an agonist for FPR1. In human ovarian cancer xenograft model, tumor cells were found to produce high levels of an FPR2 specific agonist peptide LL-37, which is responsible for the recruitment of transplanted human BM mesenchymal stem cells to form pericyte layers in tumor vasculature, a crucial step for neovascularization [66]. Blockade of LL-37 interaction with mesenchymal stem cells abrogated tumor growth. These observations strengthen the notion for the active participation of chemoattractant GPCRs in tumor angiogenesis mediated by interaction with CSCs and mesenchymal stem cells.

7. Chemoattractant GPCRs mediate the interaction between GSLCs and the microenvironment niche

CSCs are thought to be similar to normal stem cells in a number of features and they are located in specific physical and functional microenvironment niches that are essential for the maintenance of their stemness. GSLCs in brain tumors have been found to reside in niches that contribute directly to cell renewal and tumor growth [57]. The niches are formed by brain tumor microvasculature and other stromal cells. Disruption of the niches may diminish GSLC population and halt tumor growth [57]. In comparison to differentiated tumor cells, GSLCs exhibit remarkable plasticity suggesting that the cells may change phenotype depending on the microenvironment content. EC-derived factors are found to maintain GSLCs in a stem cell-like state and promote their tumorsphere forming ability [58, 67]. Orthotopic glioblastoma xenograft model suggests that GSLCs from brain tumors secrete angiogenic factors that increase the recruitment and formation of tumor blood vessels [56], indicating a close interaction between GSLCs and their surrounding niches.

It is noteworthy that CXCR4/CXCL12 has been shown to mediate the homing of bone marrow progenitor cells which form niches at the sites of irradiated GBM [64]. These bone marrow EC formed niches in turn are required for the homing and retention of GSLCs [58]. In addition to ECs, a niche also contains tumor-associated fibroblasts and immune cells. Fibroblasts from breast cancer patients, but not normal fibroblasts, were found to promote the self-renewal of CD44⁺ CD24⁻ breast CSCs (BCSCs) and the tumorigenicity of BCSCs in NOD/SCID mice through a CXCR4/CXCL12 mediated pathway [68]. CXCL12 secreted by stromal fibroblasts activates CXCR4-expressing BCSCs to undergo proliferation. The CXCR4 antagonist AMD3100 markedly reduced the formation of mammosphere and the expression of BCSC markers in secondary mammosphere cells. Thus, CXCL12/CXCR4 signaling participates in the cross-talk of CSCs from various tumors with the niches. Other chemokine GPCRs, such as CXCR1 and CXCR2 also mediate the interaction of the niche with CSCs from breast cancer [69]. Co-culturing BCSCs with human bone marrow-derived mesenchymal stem cells expand the proportion of BCSCs without affecting total cell numbers, suggesting that co-culture results in an increase in the CSC self-renewal. Further studies showed that the level of some chemokines such as CXCL7 and CXCL8 were significantly increased in the coculture which expands the BCSCs population. This is consistent with previous findings that CSCs expressed the CXCL7 receptor CXCR2 and the CXCL8 receptor CXCR1. These observations provide strong evidence that chemoattractant GPCRs mediated the interaction between the microenvironment and CSCs including GSLCs.

8. Conclusions

Little progress has been achieved in the treatment of malignant gliomas particularly in regard to the high-grade gliomas, such as GBM. The resistance of malignant gliomas to therapy is attributed not only to their rapid growth and invasion, but also to the presence of a small population of GSLCs within the heterogeneous tumor mass. GSLCs have been shown to utilize a variety of mechanisms to evade therapies. It is now believed that a more efficient way to improve GBM treatment resides in the development of therapies that may selectively eliminate GSLCs. Successful therapeutic strategies to targeting GSLCs require better understanding of the mechanisms that regulate the self-renewal and differentiation of GSLCs into offspring tumor cells. There is now mounting evidence showing that chemoattractant GPCRs play multiple roles in the generation and maintenance of GSLCs in tumor niches by promoting their self-renewal, invasion and angiogenesis [Fig. 1]. However, chemoattractant GPCRs may not be the sole driving force of the contribution of GSLCs to tumor development. Further molecular epidemiologic and genetic studies are required to

obtain a whole picture of the mechanisms utilized by GSLCs to promote tumor progression. It is also important not to single out a given receptor to study GSLCs biology, but rather, studies should consider the complex host environment in which many factors may enable the aberrant expression of chemoattractant GPCRs and ligands. In addition, the interaction of chemoattractant GPCRs such as CXCR4 and FPR1 with other growth factor receptors has been reported [70]. In fact, different receptors may cooperate to exacerbate the progression of malignant gliomas. Therefore, recognition of the multifaceted role of chemoattractant GPCRs in GSLCs is fundamental for the development of novel therapeutic agents that may efficiently target GSLCs.

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References

1. Le Y, Murphy PM, Wang JM. Formyl-peptide receptors revisited. *Trends Immunol.* 2002; 23:41–8.
2. Ambrosini E, Aloisi F. Chemokines and glial cells: a complex network in the central nervous system. *Neurochem Res.* 2004; 29:1017–38. [PubMed: 15139300]
3. Adler MW, Rogers TJ. Are chemokines the third major system in the brain? *J Leukoc Biol.* 2005; 78:1204–09. [PubMed: 16204637]
4. Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood.* 2006; 107:1761–67. [PubMed: 16269611]
5. Huang J, Chen K, Chen J, Gong W, Dunlop NM, Howard OM, et al. The G-protein-coupled formylpeptide receptor FPR confers a more invasive phenotype on human glioblastoma cells. *Br J Cancer.* 2010; 102:1052–60. [PubMed: 20197768]
6. Liang Y, Bollen AW, Gupta N. CC chemokine receptor-2A is frequently overexpressed in glioblastoma. *J Neurooncol.* 2008; 86:153–163. [PubMed: 17703277]
7. Kouno J, Nagai H, Nagahata T, Onda M, Yamaguchi H, Adachi K, et al. Up-regulation of CC chemokine, CCL3L1, and receptors, CCR3, CCR5 in human glioblastoma that promotes cell growth. *J Neurooncol.* 2004; 70:301–7. [PubMed: 15662971]
8. Huang J, Chen K, Gong W, Dunlop NM, Wang JM. G-protein coupled chemoattractant receptors and cancer. *Front Biosci.* 2008; 13:3352–63. [PubMed: 18508437]
9. Bajetto A, Barbieri F, Dorcaratto A, Barbero S, Daga A, Porcile C, et al. Expression of CXC chemokine receptors 1–5 and their ligands in human glioma tissues: role of CXCR4 and SDF1 in glioma cell proliferation and migration. *Neurochem Int.* 2006; 49:423–32. [PubMed: 16621164]
10. Maru SV, Holloway KA, Flynn G, Lancashire CL, Loughlin AJ, Male DK, et al. Chemokine production and chemokine receptor expression by human glioma cells: role of CXCL10 in tumour cell proliferation. *J Neuroimmunol.* 2008; 199:35–45. [PubMed: 18538864]
11. Brat DJ, Bellail AC, Van Meir EG. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro Oncol.* 2005; 7:122–33. [PubMed: 15831231]
12. Liu C, Luo D, Reynolds BA, Meher G, Katritzky AR, Lu B, et al. Chemokine receptor CXCR3 promotes growth of glioma. *Carcinogenesis.* 2010; 32:129–37. [PubMed: 21051441]
13. Raychaudhuri B, Vogelbaum MA. IL-8 is a mediator of NF- κ B induced invasion by gliomas. *J Neurooncol.* 2011; 101:227–35. [PubMed: 20577780]
14. Hattermann K, Held-Feindt J, Lucius R, Mürköster SS, Penfold ME, Schall TJ, et al. The chemokine receptor CXCR7 is highly expressed in human glioma cells and mediates antiapoptotic effects. *Cancer Res.* 2010; 70:3299–308. [PubMed: 20388803]

15. Yao XH, Ping YF, Chen JH, Chen DL, Xu CP, Zheng J, et al. Production of angiogenic factors by human glioblastoma cells following activation of the G-protein coupled formylpeptide receptor FPR. *J Neurooncol.* 2008; 86:47–53. [PubMed: 17611713]
16. Ping YF, Yao XH, Chen JH, Liu H, Chen DL, Zhou XD, et al. The anti-cancer compound Nordy inhibits CXCR4-mediated production of IL-8 and VEGF by malignant human glioma cells. *J Neurooncol.* 2007; 84:21–9. [PubMed: 17415525]
17. Huang J, Chen K, Gong W, Zhou Y, Le Y, Bian X, et al. Receptor “hijacking” by malignant glioma cells: a tactic for tumor progression. *Cancer Lett.* 2008; 267:254–61. [PubMed: 18433988]
18. Ping YF, Yao XH, Bian XW, et al. Activation of CXCR4 in human glioma stem cells promotes tumor angiogenesis. *Zhonghua Bing Li Xue Za Zhi.* 2007; 36:179–183. in Chinese. [PubMed: 17535685]
19. Ping YF, Yao XH, Jiang JY, Zhao LT, Yu SC, Jiang T, et al. The chemokine CXCL12 and its receptor CXCR4 promote glioma stem cell-mediated VEGF production and tumor angiogenesis via PI3K/AKT signaling. *J Pathol.* 2011; 224:344–54. [PubMed: 21618540]
20. Ping YF, Bian XW. Contribution of cancer stem cells to neovascularization. *Stem Cells.* 2011; 29:888–94. [PubMed: 21557392]
21. Yao XH, Ping YF, Chen JH, Xu CP, Chen DL, Zhang R, et al. Glioblastoma stem cells produce vascular endothelial growth factor by activation of a G-protein coupled formylpeptide receptor FPR. *J Pathol.* 2008; 215:369–76. [PubMed: 18523971]
22. Erreni M, Solinas G, Brescia P, Osti D, Zunino F, Colombo P, et al. Human glioblastoma tumours and neural cancer stem cells express the chemokine CX3CL1 and its receptor CX3CR1. *Eur J Cancer.* 2010; 46:3383–92. [PubMed: 20728344]
23. Virchow R. Editorial. *Virchows Arch Pathol Anat Physiol Klin Med.* 1855; 3:23.
24. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukemia after transplantation into SCID mice. *Nature.* 1994; 367:645–48. [PubMed: 7509044]
25. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. *Nature.* 2004; 432:396–401. [PubMed: 15549107]
26. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA.* 2003; 100:3983–88. [PubMed: 12629218]
27. Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, et al. Highly purified CD44⁺ prostate cancer cells from xenografts human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene.* 2006; 25:1696–1708. [PubMed: 16449977]
28. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland MJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res.* 2005; 65:10946–51. [PubMed: 16322242]
29. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. *Cancer Res.* 2007; 67:1030–37. [PubMed: 17283135]
30. Yi L, Zhou ZH, Ping YF, Chen JH, Yao XH, Lu JY, Feng H, Wang JM, Bian XW. Isolation and characterization of stem cell-like precursor cells from primary human anaplastic oligoastrocytoma. *Mod Pathol.* 2007; 20:1061–68. [PubMed: 17660801]
31. Bao S, Wu Q, Li Z, Sathornsumetee S, Wang H, McLendon RE, et al. Targeting cancer stem cells through L1CAM suppresses glioma growth. *Cancer Res.* 2008; 68:6043–48. [PubMed: 18676824]
32. Lathia JD, Gallagher J, Heddleston JM, Wang J, Eyler CE, Macswords J, et al. Integrin alpha 6 regulates glioblastoma stem cells. *Cell Stem Cell.* 2010; 6:421–32. [PubMed: 20452317]
33. Ogden AT, Waziri AE, Lochhead RA, Fusco D, Lopez K, Ellis JA, et al. Identification of A2B5/CD133- tumor-initiating cells in adult human gliomas. *Neurosurgery.* 2008; 62:505–14. discussion 514–15. [PubMed: 18382330]
34. Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. *Cell Stem Cell.* 2009; 4:440–52. [PubMed: 19427293]
35. Viswanathan A, Painter RG, Lanson NA Jr, Wang G. Functional expression of N-formyl peptide receptors in human bone marrow-derived mesenchymal stem cells. *Stem Cells.* 2007; 25:1263–69. [PubMed: 17234990]

36. Kunwar PS, Lehmann R. Developmental biology: Germ-cell attraction. *Nature*. 2003; 421:226–7. [PubMed: 12529629]
37. Ehtesham M. CXCR4 mediates the proliferation of glioblastoma progenitor cells. *Cancer Lett*. 2009; 274:305–12. [PubMed: 19008040]
38. Nian WQ, Chen FL, Ao XJ, Chen ZT. CXCR4 positive cells from Lewis lung carcinoma cell line have cancer metastatic stem cell characteristics. *Mol Cell Biochem*. 2011 [Epub ahead of print].
39. Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, et al. A new class of membrane-bound chemokine with a CX3C motif. *Nature*. 1997; 385:640–44. [PubMed: 9024663]
40. Locatelli M, Boiocchi L, Ferrero S, Martinelli Boneschi F, Zavanone M, Pesce S, et al. Human glioma tumors express high levels of the chemokine receptor CX3CR1. *Eur Cytokine Netw*. 2010; 21:27–33. [PubMed: 20146987]
41. Singh S, Srivastava SK, Bhardwaj A, Owen LB, Singh AP. CXCL12-CXCR4 signalling axis confers gemcitabine resistance to pancreatic cancer cells: a novel target for therapy. *Br J Cancer*. 2010; 103:1671–79. [PubMed: 21045835]
42. Moriyama T, Ohuchida K, Mizumoto K, Cui L, Ikenaga N, Sato N, et al. Enhanced cell migration and invasion of CD133⁺ pancreatic cancer cells cocultured with pancreatic stromal cells. *Cancer*. 2010; 116:3357–68. [PubMed: 20564084]
43. Hartmann TN, Burger M, Burger JA. The role of adhesion molecules and chemokine receptor CXCR4 (CD184) in small cell lung cancer. *J Biol Regul Homeost Agents*. 2004; 18:126–30. [PubMed: 15471215]
44. Hartmann TN, Burger JA, Glodek A, Fujii N, Burger M. CXCR4 chemokine receptor and integrin signaling co-operate in mediating adhesion and chemoresistance in small cell lung cancer (SCLC) cells. *Oncogene*. 2005; 24:4462–71. [PubMed: 15806155]
45. Montuori N, Bifulco K, Carriero MV, La Penna C, Visconte V, Alfano D, et al. The cross-talk between the urokinase receptor and fMLP receptors regulates the activity of the CXCR4 chemokine receptor. *Cell Mol Life Sci*. 2010; 1007/s00018-010-0564-7
46. Kryczek I, Wei S, Keller E, Liu R, Zou W. Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis. *Am J Physiol Cell Physiol*. 2007; 292:C987–C95. [PubMed: 16943240]
47. Eccles SA, Welch DR. Metastasis: recent discoveries and novel treatment strategies. *Lancet*. 2007; 369:1742–57. [PubMed: 17512859]
48. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008; 133:704–15. [PubMed: 18485877]
49. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell*. 2009; 139:871–90. [PubMed: 19945376]
50. Mikheeva SA, Mikheev AM, Petit A, Beyer R, Oxford RG, Khorasani L, et al. TWIST1 promotes invasion through mesenchymal change in human glioblastoma. *Mol Cancer*. 2010; 9:194. [PubMed: 20646316]
51. Biddle A, Liang X, Gammon L, Fazil B, Harper LJ, Emich H, et al. Cancer stem cells in squamous cell carcinoma switch between two distinct phenotypes that are preferentially migratory or proliferative. *Cancer Res*. 2011 [Epub ahead of print].
52. Bates RC, DeLeo MJ, Mercurio AM. The epithelial-mesenchymal transition of colon carcinoma involves expression of IL-8 and CXCR-1-mediated chemotaxis. *Exp Cell Res*. 2004; 299:315–24. [PubMed: 15350531]
53. Onoue T, Uchida D, Begum NM, Tomizuka Y, Yoshida H, Sato M. Epithelial-mesenchymal transition induced by the stromal cell-derived factor-1/CXCR4 system in oral squamous cell carcinoma cells. *Int J Oncol*. 2006; 29:1133–38. [PubMed: 17016644]
54. Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F, et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nat Genet*. 2003; 34:70–4. [PubMed: 12692554]
55. Ueda Y, Neel NF, Schutyser E, Raman D, Richmond A. Deletion of the COOH-terminal domain of CXC chemokine receptor 4 leads to the down-regulation of cell-to-cell contact, enhanced motility and proliferation in breast carcinoma cells. *Cancer Res*. 2006; 66:5665–75. [PubMed: 16740704]

56. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res.* 2006; 66:7843–48. [PubMed: 16912155]
57. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. *Cancer Cell.* 2007; 11:69–82. [PubMed: 17222791]
58. Chamberlain MC. Emerging clinical principles on the use of bevacizumab for the treatment of malignant gliomas. *Cancer.* 2010; 116:3988–99. [PubMed: 20564141]
59. Ping YF, Bian XW. Cancer stem cells switch on tumor neovascularization. *Curr Mol Med.* 2011; 11:69–75. [PubMed: 21189116]
60. Yang Y, Liu Y, Yao XH, Ping YF, Jiang T, Liu Q, et al. Annexin 1 released by necrotic human glioblastoma cells stimulates tumor cell growth through the formyl peptide receptor 1. *Am J Pathol.* 2011 in press.
61. Garcia-Barros M, Paris F, Cordon-Cardo C, Lyden D, Rafii S, Haimovitz-Friedman A, et al. Tumor response to radiotherapy regulated by endothelial cell apoptosis. *Science.* 2003; 300:1155–59. [PubMed: 12750523]
62. Duda DG, Cohen KS, Kozin SV, Perentes JY, Fukumura D, Scadden DT, et al. Evidence for incorporation of bone marrow-derived endothelial cells into perfused blood vessels in tumors. *Blood.* 2006; 107:2774–76. [PubMed: 16339405]
63. Folkins C, Shaked Y, Man S, Tang T, Lee CR, Zhu Z, et al. Glioma tumor stem-like cells promote tumor angiogenesis and vasculogenesis via vascular endothelial growth factor and stromal-derived factor 1. *Cancer Res.* 2009; 69:7243–51. [PubMed: 19738068]
64. Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest.* 2010; 120:694–705. [PubMed: 20179352]
65. Coffelt SB, Tomchuck SL, Zwezdaryk KJ, Danka ES, Scandurro AB. Leucine leucine-37 uses formyl peptide receptor-like 1 to activate signal transduction pathways, stimulate oncogenic gene expression, and enhance the invasiveness of ovarian cancer cells. *Mol Cancer Res.* 2009; 7:907–15. [PubMed: 19491199]
66. Coffelt SB, Marini FC, Watson K, Zwezdaryk KJ, Dembinski JL, LaMarca HL, et al. The pro-inflammatory peptide LL-37 promotes ovarian tumor progression through recruitment of multipotent mesenchymal stromal cells. *Proc Natl Acad Sci.* 2009; 106:3806–11. [PubMed: 19234121]
67. Folkins C, Man S, Xu P, Shaked Y, Hicklin DJ, Kerbel RS. Anticancer therapies combining antiangiogenic and tumor cell cytotoxic effects reduce the tumor stem-like cell fraction in glioma xenograft tumors. *Cancer Res.* 2007; 67:3560–64. [PubMed: 17440065]
68. Huang M, Li Y, Zhang H, Nan F. Breast cancer stromal fibroblasts promote the generation of CD44⁺CD24[−] cells through SDF-1/CXCR4 interaction. *J Exp Clin Cancer Res.* 2010; 29:80. [PubMed: 20569497]
69. Liu S, Ginestier C, Ou SJ, Clouthier SG, Patel SH, Monville F, et al. Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. *Cancer Res.* 2011; 71:614–24. [PubMed: 21224357]
70. Huang J, Hu J, Bian X, Chen K, Gong W, Dunlop NM, et al. Transactivation of the epidermal growth factor receptor by formylpeptide receptor exacerbates the malignant behavior of human glioblastoma cells. *Cancer Res.* 2007; 67:5906–13. [PubMed: 17575160]

Highlights

Glioma stem-like cells (GSLCs) possess the capacity of self-renewal, multi-potent differentiation, and tumor initiation. GSLCs contribute to tumor initiation and progression by interaction with stromal factors through aberrantly expressed cell surface receptors. G protein coupled chemoattractant receptors (GPCRs) have been found to be one group of receptors over-expressed by GSLCs. In this review, we summarize recent development of studies of GPCRs in GSLCs and propose their potential as targets for eliminating glioma initiating cells.

The contribution of chemoattractant GPCRs to glioma stem-like cell mediated tumorigenesis, invasion and angiogenesis express various GPCRs. Interaction of GPCRs with agonists produced by tumor and stromal cells elicit signaling cascades that are crucial for GSLC self-renewal, invasion, angiogenesis, recruitment of BMDC and EMT transition. In addition, the GPCRs function in GSLCs is mediated in part by crosstalk with EGFR through a Src kinase pathway.

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Table 1

The expression and function of chemoattractant GPCRs in glioma stem like cells (GSLCs)

GPCRs	Ligand (sources)	Major functions on GSLCs	References
FPR1	fMLF (bacteria); Mitochondrial peptides (ruptured cells) Annexin1(necrotic tumor cells and GSLCs)	Angiogenesis; chemotaxis	[21] [60]
CXCR1	CXCL8 (tumor cells; mesenchymal stem cells)	EMT [*] ; Metastasis; Self- renewal	[52] [69]
CXCR2	CXCL7 (mesenchymal stem cells)	Self-renewal	[69]
CXCR4	CXCL12 (tumor cells, GSLCs and stromal cells)	Proliferation; Angiogenesis; Vasculogenesis; Metastasis; EMT [*] ; Niche formation	[18] [19]
CX3CR1	CX3CL1 (tumor cells and GSLCs)	Metastasis	[40]

* EMT: epithelial-mesenchymal transition