Inhibition of CXCR7 extends survival following irradiation of brain tumours in mice and rats

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Background: In experimental models of glioblastoma multiforme (GBM), irradiation (IR) induces local expression of the chemokine CXCL12/SDF-1, which promotes tumour recurrence. The role of CXCR7, the high-affinity receptor for CXCL12, in the tumour's response to IR has not been addressed.

Methods: We tested CXCR7 inhibitors for their effects on tumour growth and/or animal survival post IR in three rodent GBM models. We used immunohistochemistry to determine where CXCR7 protein is expressed in the tumours and in human GBM samples. We used neurosphere formation assays with human GBM xenografts to determine whether CXCR7 is required for cancer stem cell (CSC) activity in vitro.

Results: CXCR7 was detected on tumour cells and/or tumour-associated vasculature in the rodent models and in human GBM. In human GBM, CXCR7 expression increased with glioma grade and was spatially associated with CXCL12 and CXCL11/I-TAC. In the rodent GBM models, pharmacological inhibition of CXCR7 post IR caused tumour regression, blocked tumour recurrence, and/or substantially prolonged survival. CXCR7 expression levels on human GBM xenograft cells correlated with neurosphere-forming activity, and a CXCR7 inhibitor blocked sphere formation by sorted CSCs.

Conclusions: These results indicate that CXCR7 inhibitors could block GBM tumour recurrence after IR, perhaps by interfering with CSCs.

Radiotherapy has a major role in the treatment of glioblastoma multiformes (GBMs). However, despite the use of high radiation doses and the combination of anticancer agents and targeted therapies, the tumours invariably recur, leading to the demise of more than 75% of the patients by 2 years. Importantly, most of the recurrences occur within the radiation field (Hochberg and Pruitt, 1980; Liang et al, 1991; Sneed et al, 1994; McDonald et al, 2011). Thus, any method of improving local control of the primary tumour by radiotherapy would improve the curability of GBM patients.
One way to limit tumour growth after irradiation (IR) is blockade of revascularization. In animal models of cancer, doses of radiation in the therapeutic range block local angiogenesis (Ahn and Brown, 2008), forcing the tumour to generate new blood vessels (i.e., vasculogenesis) for its survival. In addition, IR induces the expression of CXCL12/SDF-1, a hypoxia-inducible chemokine (Kioi et al, 2010; Kozin et al, 2010). CXCL12 promotes vasculogenesis by recruiting CD11b+ monocytes, which express the CXCL12 receptor CXCR4, into the tumour (Kozin et al, 2010). The monocytes remodel the tumour matrix via proteases, such as MMP-9 (Ahn and Brown, 2008), presumably to allow the new vessels to spread through the matrix. Treatment with an anti-CD11b antibody (Ahn et al, 2010) or a CXCR4 antagonist (Kioi et al, 2010; Kozin et al, 2010) inhibits tumour revascularization and growth after IR, indicating that CXCL12:CXCR4-mediated recruitment of CD11b+ monocytes could be a target for post-IR control of GBM. In addition, as the monocytes themselves do not form the blood vessels (Kioi et al, 2010; Kozin et al, 2010), vasculogenesis could be blocked by therapies targeting the cell type that forms the new vessels, be it an endothelial cell, an endothelial precursor cell (EPC) (Asahara et al, 1997), a pericyte, and/or another cell type.

Another strategy to attenuate GBM tumour growth after IR is to target cancer stem cells (CSCs) within the tumour. CSCs are responsible for tumour propagation (Galli et al, 2004; Singh et al, 2004) and have an increased propensity to resist radiation (Bao et al, 2006) and chemotherapy (Liu et al, 2006). Interestingly, CSCs can differentiate into tumour endothelial cells (Ricci-Vitiani et al, 2010; Wang et al, 2010b). Although specific anti-CSC therapies are under development, strategies targeting CSCs in combination with conventional therapies have already shown promise in preclinical models (Wang et al, 2010a). CSCs express CXCR4, and addition of CXCL12 to these cells in vitro results in proliferative, pro-survival and self-renewal responses (Liu et al, 2006; Ehtesham et al, 2009; Gatti et al, 2013); moreover, these responses are blocked by CXCR4 antagonists (Gatti et al, 2013).

CXCR7 is, like CXCR4, a receptor for CXCL12/SDF-1, but with a 10-fold higher affinity towards it (Burns et al, 2010). CXCR7 is expressed on tumour cells, endothelial cells and pericytes in a wide variety of cancers, including GBM (Madden et al, 2004; Miao et al, 2007; Liu et al, 2010b). Although CXCR7 does not mediate chemotaxis of cells towards CXCL12 in vitro, CXCR7 facilitates binding to endothelial cells (Burns et al, 2006; Liu et al, 2010a; Dai et al, 2011; Yan et al, 2012) and regulates CXCL12-mediated transcytosis through the endothelium (Mazzinghi et al, 2008; Zabel et al, 2009; Dai et al, 2011; Yan et al, 2012). The role of CXCR7 in the glioma’s response to IR has, until now, not been addressed.

In the present study, we tested the hypothesis that blocking the interaction of CXCL12 with CXCR7 could improve tumour control by IR. First, we used the CXCR7 inhibitor CCX771 (Zabel et al, 2009; Cruz-Orenzano et al, 2011) in the U251 mouse GBM model to see if post-IR tumour recurrence could be blocked in the absence of CXCR4 antagonism. Second, we tested a novel CXCR7 inhibitor, CCX662, in what we believe is a highly realistic model of human brain cancer (Liu et al, 2013), autochthonous tumours in rats caused by exposure to the carcinogen ethylnitrosourea (ENU). Third, we tested whether CCX662 could prolong post-IR survival in the highly aggressive rat C6 GBM model. In the ENU and C6 models, we used immunohistochemistry to probe the tumours for CXCR7 expression. We also probed human glioma samples for the expression of CXCR7, CXCR4, CXCL12, the other CXCR7 ligand CXCL11 and the other CXCL11 receptor CXCR3. Finally, we determined whether CXCR7 is involved in the formation of neurospheres by human GBM xenograft CSCs in vitro. The predominance of the data suggests that CXCR7 inhibitors could be used in the post-IR treatment of GBM, by blocking the ability of CSCs to regrow the tumour.

**Materials and Methods**

**Animals and reagents.** All animal procedures were approved by Stanford’s Administrative Panel on Laboratory Animal Care or the ChemoCentryx Institutional Animal Care and Use Committee, and met the standards required by the United Kingdom Coordinating Committee on Cancer Research guidelines (Workman et al, 2010). Nude (nu/nu) mice and Sprague-Dawley rats (both from Charles River, Wilmington, MA, USA) were maintained in a germ-free environment and had access to food and water ad libitum. Animals were removed from tumour studies when body weights decreased 20% from baseline. CXCR7-specific inhibitors CCX771 and CCX662 were generated at ChemoCentryx, Inc. Multi-grade astrocytoma and glioblastoma AccuMax arrays were purchased from Accurate Chemicals (Westbury, NY, USA) or generated from biopsies at Stanford University Medical Center (Palo Alto, CA, USA) under an IRB-approved protocol. Anti-CXCR7 mAb 11G8 (catalogue no. MAB42273), anti-CXCL12 mAb (catalogue no. MAB350) and IgG1 isotype control mAb were purchased from R&D Systems (Minneapolis, MN, USA). Anti-CXCL11 Ab (catalogue no. ab9955) and anti-CXCR3 mAb (catalogue no. ab64714) were purchased from Abcam (Cambridge, MA, USA). Anti-CXCR4 Ab (catalogue no. C3116) was purchased from Sigma (St Louis, MO, USA). Rabbit IgG and biotinylated goat anti-rabbit IgG were purchased from Jackson Immunoresearch (West Grove, PA, USA).

**Mouse U251 model.** One million U251/pFB-Luc cells were injected intracranially into 24 6-week-old nude (nu/nu) mice and, after allowing 3 weeks for tumour establishment, 12 of the mice were given whole-brain IR (12 Gy), as previously described (Kawakami et al, 2005). Immediately thereafter, six mice from each group were injected subcutaneously with 30 mg kg⁻¹ CCX771, whereas the remaining six mice from each group received the vehicle, 10% Captisol (Captisol, La Jolla, CA, USA), once daily for 3 weeks. For periodic tumour imaging, the mice were anaesthetized and injected intraperitoneally with 150 mg kg⁻¹ of d-luciferin (PerkinElmer, Waltham, MA, USA); 5–30 min later, the mice were analysed with the IVIS system coupled to Living Image acquisition and analysis software (PerkinElmer). Photon flux was calculated for each mouse by using a rectangular region of interest encompassing the head of the mouse in a prone position. This value was scaled to a comparable background value (from a luciferin-injected mouse with no tumour cells), and then normalised to the value obtained immediately after tumour cell injection (day 0), so that all mice had an arbitrary starting bioluminescence index of 100.

**Rat ENU model.** Brain tumours were induced by intra-peritoneal injection of Sprague-Dawley rats (Taconic Farms, Germantown, NY, USA) at gestational day 18 with ENU (Sigma 50 mg kg⁻¹). During each experiment, at least one control pregnant rat received saline vehicle under identical conditions. Pups were born and weaned at normal age, after which they were monitored at least weekly for signs of illness. At 115 days of age, 12 rats were anaesthetised and placed in individual lead jigs with a cutout that allowed the brain to be irradiated tangentially with full shielding of the buccal cavity and the rest of the body. Whole-brain IR (20 Gy) was performed with a Phillips (Andover, MA, USA) X-ray unit operated at 200 kVp with a dose rate of 1.21 Gy min⁻¹ (20 mA with added filtration of 0.5 mm copper, distance from X-ray source to the target of 31 cm, and a half-value layer of 1.3 mm copper). To ensure the maximum uniformity of the dosage, the rats were turned 180° midway through the IR. Immediately after IR, each rat was implanted with a single Alzet 2004 infusion pump for delivery of 0.25 μl hr⁻¹ of CCX662 solution (six rats) or vehicle (six rats) for 4 weeks. For pump implantation, the rats were anaesthetised...
and a mid-scaphular incision was made to create a pocket in the subcutaneous tissue large enough to allow for some movement of the pump. The pump was then inserted into the pocket port-first to minimise the exposure of the compound to the incision. The mean plasma levels of CCX662 (386 ± 1/– 89 nM), sampled 9 and 16 days post pump implantation, were greater than the IC90 value of CCX662 for rat CXCR7 in 100% rat serum. Four rats receiving no IR or compound were included as controls.

**Rat C6 model.** One million C6 cells were injected intracranially into 24 6-week-old Sprague-Dawley rats, as previously described (Kubiatowski et al, 2001). After allowing 7 days for tumour establishment, 16 rats were irradiated (18 Gy) and given a subcutaneous Alzet pump exactly like the ENU-treated rats described above. The pumps delivered CCX662 (eight rats) or vehicle (eight rats) for 7 days, after which the pumps in the surviving rats were exchanged with freshly-loaded 7-day pumps. The rats surviving after these two pumps expired were maintained without compound. Eight rats receiving no IR or compound were included as controls. A second study was performed exactly as the first, containing seven IR + CCX662-treated rats, seven IR + vehicle-treated rats and 10 non-IR non-treated rats.

**Immunohistchemistry.** Tumour arrays were deparaffinized and hydrated by conventional methods, then subjected to antigen retrieval by immersion in 10 mM sodium citrate, 0.05% Tween-20 at 98°C for 20 min, followed by slow cooling at room temperature for 20 min. The tumour arrays were then treated with an endogenous biotin blocking kit (Vector Labs, Burlingame, CA, USA) and stained with anti-CXCR7, CXCR4, CXCR3, CXCL12 or CXCL11 Abs, or mouse IgG1 or rabbit IgG control Abs, using a catalysed signal amplification IHC kit from Dako (Dakopateria, CA, USA) and subsequently grown as spheres in complete neurobasal medium. For the neurosphere formation assay, spheres (10–100 cells in diameter) were counted. For the CSC analysis, T4121, T387, and T3832 GBM specimens were grown as previously described (Bao et al, 2006; Heddleston et al, 2009; Li et al, 2009; Eyler et al, 2011). Cells were maintained in mouse flank xenografts and processed using a papain dissociation kit (Vector Labs, Burlingame, CA, USA) and stained with anti-CXCR7, CXCR4, CXCR3, CXCL12 or CXCL11 Abs, or mouse IgG1 or rabbit IgG control Abs, using a catalysed signal amplification IHC kit from Dako (Dakopateria, CA, USA) and subsequently grown as spheres in complete neurobasal medium. For the neurosphere formation assay, spheres (10–100 cells in diameter) were counted.

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**Statistical analysis.** Statistical analyses were performed by the two-tailed Student’s *t*-test or one-way ANOVA to determine statistical significance. *P*-values of <0.05 were considered statistically significant. Kaplan–Meier curves and the log-rank test were used to compare survival times among the groups. All calculations were performed using Prism5 (GraphPad Software, La Jolla, CA, USA).

**RESULTS**

**Inhibition of CXCR7 with CCX771 post IR prevents recurrence of intracranial U251 GBM tumours in nude mice.** Immuno-deficient mice were injected intracranially with luciferase-expressing U251 human GBM cells. After tumour establishment, the mice were given whole-brain IR and then injected subcutaneously with CCX771 or its vehicle daily for 3 weeks. Whereas IR plus vehicle significantly slowed tumour growth, IR plus CCX771 resulted in rapid tumour regression (Figure 1). After the 3-week CCX771 treatment period ended, the tumours did not recur. CCX771 did not exhibit an anti-tumour effect in the absence of IR, suggesting that CXCR7 is involved in the tumour’s response to IR.

**Inhibition of CXCR7 with CCX662 post IR prolongs the survival of rats with ENU-induced brain tumours.** Pregnant rats were injected with the carcinogen ENU on day 17 of gestation. When the progeny were 115 days old, before their brain tumours were large enough to produce neurological symptoms, the rats were given whole-brain IR. The rats were immediately implanted with subcutaneous infusion pumps containing CCX662, a novel inhibitor of CXCR7 possessing high potency and selectivity for CXCR7 (Supplementary Figure 1), or its vehicle. The pumps provided 4 weeks’ worth of CCX662 at a concentration continually exceeding the compound’s serum IC90 value, that is, >90% CXCR7 inhibition. Whereas the group of non-irradiated rats had a median lifespan of 190 days and the group of vehicle-treated irradiated rats had a median lifespan of 205 days, the group of CCX662-treated irradiated rats had a median lifespan of 370 days (Figure 2). All control rats receiving IR but not ENU survived the 418 day study, indicating that the IR itself was not responsible for the rats’ mortality (data not shown). Similar effects were observed in the ENU tumour model with a different CXCR7 inhibitor and route of administration (Supplementary Figure 2).

![Figure 1. CCX771, in combination with IR, reduces tumour size and prevents tumour recurrence in mice containing U251 GBM tumours.](image-url)
Expression of CXCR7 in rat ENU and human GBM tumours. To determine whether tumour cells and/or tumour-associated vasculature in ENU-induced brain tumours expresses CXCR7, we performed immunohistochemistry on the tumours on killing with the anti-CXCR7 mAb clone 11G8. CXCR7 was detected in tumour cells in most tumours from both irradiated and non-irradiated rats (Figure 3). In addition, CXCR7 was detected in a subset of blood vessels, both in the tumour and in the non-tumoural tissue. In non-tumoural tissue, CXCR7 was also detected in rare neural cells (Figure 3). To examine CXCR7 expression in human gliomas, we performed immunohistochemistry with 11G8 on a multi-grade panel of gliomas, in which the incidence of CXCR7 expression on both tumour cells and tumour-associated vasculature increased with tumour grade, in agreement with prior studies (Madden et al, 2004; Hattermann et al, 2010; Liu et al, 2010). In rodent models of GBM, using xenografts from several human GBM specimens, first, we analysed neurosphere formation by CXCR7bright and CXCR7dim cells isolated from the xenografts. CXCR7bright cells exhibited a >10-fold higher ability to form spheres than CXCR7dim cells (Figure 8A). Second, we analysed the effect of CXCR7 inhibitor CCX771 on the ability of sorted xenograft CSCs to form neurospheres in culture. CCX771, but not its inactive analogue CCX704, inhibited sphere formation in a dose-dependent manner (Figure 8B). CCX771 was more potent than AMD3100, the CXCR4 antagonist, as 1 μM CCX771 reduced neurosphere formation to a greater extent than 1 μg ml⁻¹ (1.3 μM) AMD3100 (Figure 8B).

Inhibition of CXCR7 with CCX662 post IR prolongs the survival of rats with C6 GBM tumours. We sought to determine whether CXCR7 inhibition could delay post-IR growth of extremely aggressive GBM tumours. Towards this end, we used rat C6 GBM cells, which grow extremely quickly and cause death ~2 weeks post implantation. Rats were injected intracranially with C6 cells and, 7 days later, underwent whole-brain IR. Immediately after IR, the rats were infused with CCX662 or vehicle. Non-irradiated rats began succumbing to the tumours on day 10, and all rats in the non-irradiated group were dead by day 16 (Figure 6). Irradiated rats infused with vehicle began dying on day 11, and were all dead by day 18, indicating that IR does not prolong survival in this aggressive model. Remarkably, irradiated rats infused with CCX662 lived significantly longer, with median post-IR survival time increased from 6 (vehicle) to 9 days (CCX662) post IR.

Expression of CXCR7 in rat C6 GBM tumours. To assess CXCR7 protein expression in C6 GBM tumours, we performed immunohistochemistry on the tumours on killing with CXCR7 mAb 11G8. Although C6 GBM tumours are poorly vascularised, owing to the rapid growth of the tumour, CXCR7 was detected in a subset of blood vessels in the tumours (Figure 7). However, CXCR7 was not expressed by the tumour cells, reflecting the fact that the cell line is CXCR7⁻ (Supplementary Figure 3).

DISCUSSION

In these studies we show that CXCR7 inhibitors are effective at preventing tumour recurrence and prolonging survival after IR in rodent models of GBM. In a mouse model with intracranial tumours derived from human U251 GBM cells, IR was sufficient to block tumour growth for a number of weeks, but the tumours recurred. Daily treatment of the mice post IR with the CXCR7 antagonist, as 1 μM CCX771 reduced neurosphere formation to a greater extent than 1 μg ml⁻¹ (1.3 μM) AMD3100 (Figure 8B).
CXCR7 inhibitor exerted its anti-tumour activity by acting on the tumour vasculature or cells that form the vasculature.

To further explore the mechanism of action of the CXCR7 inhibitors, we tested them in neurosphere formation assays in vitro. Using xenografts from multiple human GBM specimens, we observed that a CXCR7 inhibitor reduced sphere formation from sorted CD133$^+$ CSCs in a dose-dependent manner. In separate experiments, we sorted CXCR7$^{bright}$ and CXCR7$^{dim}$ cells from total xenograft cells and found that the CXCR7$^{bright}$ cells were ≥10-fold more able than CXCR7$^{dim}$ cells to form neurospheres, indicating a correlation between CXCR7 expression level and stem activity. Although we were not able to assess CXCR7 function on CSCs in...
the ENU and C6 models, the human GBM xenograft results raise the possibility that the CSCs in these rat models were CXCR7$$^+$$ and impaired by the CXCR7 inhibitors.

Together, these results indicate that CXCR7 is required for GBM tumour regrowth after IR, perhaps by facilitating the repopulation of the tumour from CSCs. Interestingly, CSCs have been shown to differentiate into endothelial cells in vivo (Ricci-Vitiani et al., 2010; Wang et al., 2010b), raising the possibility that CXCR7 is involved in tumour vasculogenesis after IR. Vasculogenesis could also occur via CXCR7 independently of CSCs, as CXCR7 is expressed by EPCs and mediates their CXCL12-induced adhesion to, and transcytosis through, endothelium (Dai et al., 2011); perhaps EPCs in blood vessels in or near the radiation field enter the CXCL12-containing tumour parenchyma via CXCR7.

CXCR7 has also been shown to promote endothelial cell tube formation in vitro (Dai et al., 2011; Yan et al., 2012; Jin et al., 2013). Therefore, CXCR7 might facilitate vasculogenesis by mediating EPC trans-endothelial migration, CSC proliferation/differentiation, and/or vessel formation. CXCR7 probably does not directly participate in the IR-mediated recruitment of pro-vasculogenic CD11b$$^+$$ monocytes to the tumour, as these cells are CXCR7$$^-$$(Berahovich et al., 2010) and CXCR7 inhibitors do not block trans-endothelial migration of CXCR7$$^-$$(Zabel et al., 2009). However, CXCR7 inhibition raises the concentration of CXCL12 in the mouse bloodstream and blocks CD11b$$^+$$ cell migration into CXCL12-filled air pouches, presumably owing to a lessened

Figure 4. CXCR7 expression increases with glioma tumour grade. A multi-grade glioma array was stained by IHC with the CXCR7 mAb 11G8. (A) The incidence of 11G8 staining, on the tumour cells and/or tumour-associated vasculature, increased with tumour grade. (B) IHC staining of representative tumours with 11G8 (right column) or an isotype control mAb (left column). Note the staining of tumour cells (middle row) and tumour-associated vasculature (bottom row) in the Grade IV tumours by 11G8. Magnification x 200.
CXCL12 gradient (Berahovich et al., 2013), raising the possibility that CXCR7 inhibitors could block CD11b^+ monocyte migration into irradiated tumours in an indirect manner. Indeed, CXCR7 inhibitor-mediated elevations in plasma CXCL12 levels were noted in the ENU and C6 models (Walters and Ebsworth, unpublished).

Another way CXCR7 might help irradiated tumours is through its poorly defined ability to help cells grow in stressful conditions. For example, glial cells (Zhou et al., 2008), endothelial cells (Gambaryan et al., 2011; Costello et al., 2012), mesenchymal stem cells (Liu et al., 2010a) and tumour cells (Berahovich, unpublished) upregulate CXCR7 in response to hypoxia; glioma cells are protected from temozolomide-induced apoptosis via CXCR7 (Hattermann et al., 2010); CXCR7 promotes endothelial cell survival (Yan et al., 2012; Jin et al., 2013); and CXCR7-transfected...
but not parental tumour cells can be cultured in 1% serum (Burns et al., 2006). In an irradiated GBM tumour, CSCs, their progeny, or endothelial cells might use CXCR7 to survive in the presence of dangerous cellular debris.

As CXCR7 can bind to both CXCL12 and CXCL11, it is possible that CXCL11 may mediate some of the activities of CXCR7 in GBM. Although we were not able to ascertain whether CXCL11 is expressed in the rodent models, owing to the lack of a suitable CXCL11 antibody, we detected CXCL11 in all 52 human GBM samples we analysed. Like CXCR7 and CXCL12, CXCL11 was expressed by both tumour cells and tumour-associated vasculature. Although CXCL11 protein expression in human GBM has heretofore not been documented, CXCL11 mRNA levels were found to be higher in GBM samples than in normal brain or low-grade glioma samples (Calatozzolo et al., 2011). CXCL11 protein was detected on tumour cells and tumour-associated vasculature in meningioma (Wurth et al., 2011), and CXCL11 mRNA or protein has been detected in a number of other solid tumours (Furuya et al., 2007; Lo et al., 2010; Monnier et al., 2011; Xia et al., 2011). If CXCL11–CXCR7 interactions have a role in GBM, CXCR7 inhibitors will block these activities, as the inhibitors potently block CXCL11 binding to CXCR7.

In summary, we have used three orthotopic rodent models of GBM to show that CXCR7 inhibitors, in combination with IR, exhibit beneficial effects on tumour size/recurrence and animal survival. The fact that the effects of CXCR7 inhibition continued after the cessation of treatment suggests that IR induces a transient CXCR7-mediated event necessary for tumour regrowth. CXCR7 expression on tumour cells and/or tumour-associated vasculature in human GBM and the rodent models, coupled with inhibition of human GBM xenograft neurosphere formation by a CXCR7 inhibitor, suggest that this event could be vasculogenesis and/or proliferation/differentiation of CSCs. The results shown herein provide substantial evidence that CXCR7 is a promising target for the post-IR treatment of GBM.

| Table 1. Percentage of tumours expressing chemokines or receptors on the tumour cells or tumour-associated vasculature |
|---------------------------------------------------------------|
| Tumour cells | Vasculature |
| CXCR7 | 46 | 71 |
| CXCL12 | 27 | 57 |
| CXCL11 | 100 | 63 |
| CXCR4 | 97 | 10 |
| CXCR3 | 44 | 2 |

Figure 6. CCX662 increases post-IR survival time in rats containing C6 GBM tumours. C6 GBM cells were implanted intracranially into rats and, 7 days later, given 18 Gy whole-brain IR. Immediately following IR, rats were infused with CCX662 or its vehicle for 1 week. Surviving rats received another 1-week infusion of CCX662 or vehicle. CCX662 significantly increased post-IR median survival time (***P = 0.0025 compared with vehicle, log-rank test; n = 15–18 rats, compiled from two separate studies).

Figure 7. CXCR7 is expressed on the vasculature in C6 GBM tumours. (A) H&E staining of a representative tumour at × 20 magnification. (B) IHC staining of a representative tumour with the CXCR7 mAb 11G8 or an isotype control mAb at × 200 magnification.
Figure 8. CXCR7 is required for CSC activity in vitro. (A) The neurosphere formation assay using CXCR7bright and CXCR7dim sorted cells from GBM xenografts T3832, T387, and T4121. CXCR7bright cells exhibited a greater ability to form spheres than did CXCR7dim cells. (B) Neurosphere formation assay using sorted CD133CCX704, inhibited sphere formation. Inhibition of sphere formation was greater with 1 mAMD3100 than with 100 nM CCX771, but not its inactive chemical analogue CCX704, inhibited sphere formation. Inhibition of sphere formation was greater with 1 μM CCX771 than with 100 nM CCX771 or with 1 μg ml−1 (1.3 μM) AMD3100. Similar results were observed with GBM xenograft T3832 (not shown).

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CONFLICT OF INTEREST

MJW, KE, RDB, PZ, JPP, JCJ and TJS are employees of ChemoCentrx, Inc. S-CL, RAO, MK, SBC, DT, EEM-H, MS, RMR, JDL, JK, HEKK, MM, LR and JMB declare no conflict of interest.

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