RESEARCH ARTICLE

Expression of the CXCL12/SDF-1 Chemokine Receptor CXCR7 in Human Brain Tumours

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Abstract

Purpose: Receptor 7 (CXCR7) has recently been characterized as a novel receptor for CXCL12/SDF-1 (stromal cell derived factor-1). Given the demonstrated importance of CXCL12/SDF-1 in angiogenesis and tumour metastasis, we hypothesized that CXCR7 may also play a role in tumour pathogenesis. Located in the limited space of the intracranial cavity, any brain tumours can be inherently serious and life-threatening. However, the expression of CXCR7 in pituitary adenoma, neurilemmoma or hemangioblastoma remains to be elucidated. Therefore, we aimed to determine the potential contribution of CXCR7 in the development of brain tumours. Methods: In this study we examined and quantified the mRNA expression of CXCR7 in four different human brain tumours - 27 patients with neurilemmoma (8 patients), pituitary adenoma (7 patients), hemangioblastoma (6 patients), or meningioma (6 patients) undergoing surgical resection in the West China Hospital of Sichuan University. There were 15 females and 12 males aged from 28 to 70 years old. Total RNA was isolated and mRNA was measured by quantitative real-time RT-PCR. One-way analysis of variance (ANOVA) was performed using SPSS 11.0 statistical software to compare the mRNA levels of CXCR7 among four groups. Results: We found that CXCR7 mRNA was detected in all tumour samples. Quantitative results showed that the levels of CXCR7 mRNA in brain tissues from patients with neurilemmoma or meningioma were significantly higher than those with pituitary adenoma or hemangioblastoma. Conclusions: The results suggest that the CXCR7 may play a role in progression, metastasis and angiogenesis of brain tumours.

Keywords: CXCR7 - chemokine receptors - neurilemmoma - pituitary adenoma - hemangioblastoma

Asian Pacific J Cancer Prev, **13 (10)**, 5281-5286

Introduction

Brain neoplasms include constitutional brain neoplasms and metastatic tumour. Neurilemmoma, also known as Schwann cell tumours, is formed in peripheral nerve by Schwann sheath. Pituitary adenoma originates in the pituitary gland and accounts for 8%~15% of intracranial tumour. Hemangioblastomas, originating in the brain and spinal cord, are highly vascular differentiated benign tumour. Angioreticuloma, also known as vasogenic hemangioblastoma. Located in the limited space of the intracranial cavity, brain tumours can be inherently serious and life-threatening.

Chemokines are key factors in central nervous system physiology and pathology, especially in tumour development (Bajetto et al., 2001). Among various chemokines, CXCL12/ Chemokine receptor 4 (CXCR4) engagement plays an important and unique role in the regulation of stem/progenitor-cell trafficking. High levels of CXCL12 in the common destination organs of metastasis, such as the lymph nodes, lungs, liver, and bones, may attract CXCR4-positive metastatic cancer cells (Shim et al., 2006). In the brain tumours, glioma tumour stem-like cells promote tumour angiogenesis and vasculogenesis via the CXCL12/CXCR4 pathway (Terasaki et al., 2011). CXCL12/CXCR4 interaction has recently been found to be present in lymphomas of the primary central nervous system (Terasaki et al., 2011). Besides, CXCL12/CXCR4 has a role in other brain tumours, such as pituitary adenoma and mengioma (Barbieri et al., 2006; do Carmo et al., 2010). CXCL12/CXCR4 interaction was assumed to have potential roles in brain tumour development through the activation of ERK1/2 and the Ca^{2+}-dependent, cytosolic tyrosine kinase Pyk2 (Massa et al., 2006).

More recently, CXCR7 was shown to be the novel receptor of CXCL12. The particular responsiveness to CXCL12 via CXCR4 and/or CXCR7 depends on expression of surface expression of its receptors, and the binding affinity to receptor (Burns et al., 2006; Goldmann et al., 2008). CXCR7 was more frequently expressed in a series of embryonic cells, neoplastic transformed cells, and cancer cells, but was not detected in many normal non-transformed counterparts (Miao et al., 2007; Schutyser et

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al., 2007). For instance, CXCR7 expression was detected in MCF-7 breast cancer cells, A764 human glioma cells, CT26 colon and KEP1 mammary carcinoma cells (Meijer et al., 2008; Meincke et al., 2011). It was shown that CXCL12 has approximately 10 times higher affinity to CXCR7 than to CXCR4 (Hou et al., 2010). In transitional cells and in embryos, CXCR7 becomes a more obvious factor. For example, CXCR7 promotes tumor growth and metastasis in breast cancer and other malignancies (Luker et al., 2010) and CXCR7 is required in the trailing cells of the primordium in zebrafish (Perlin et al., 2007). So it appears that CXCL12/CXCR7 signalling pathway has an important function in a variety of cell types, especially embryonic cells, transitional cells, neoplastic transformed cells, and cancer cells. High-flux tissue microarray and quantitative analysis have shown that CXCR7 is stained in weak positive in normal prostate epithelium and intraepithelial neoplastic tissue, while it is strongly positive in the metastasis of prostate cancer, suggesting that the expression of CXCR7 has certain correlation with the degree of malignancy (Wang et al., 2008). However this conclusion is still under debate. Moreover, expression of CXCR7 in breast cancer cells enhances the ability of these cells to seed and proliferate in lung metastases (Miao et al., 2007). This can be explained by activation of serine/threonine protein kinase AKT signalling pathway. The CXCR7-positive cell lines of prostate cancer, mouse colon cancer, pancreatic cancer are proliferation induced and apoptosis inhibited. Furthermore, CXCR7 increases expression levels of proangiopoietic factors, such as interleukin-8 (IL-8) and vascular endothelial growth factor (VEGF) (Wang et al., 2008). The IL-8 and VEGF are generated by blood vessels in solid tumours and have a significant effect on vascularization. Contrarily, downregulation of CXCR7 results in reduced proliferation rate and increased apoptosis (Singh et al., 2011). Importantly, cancer-associated fibroblasts, but not normal fibroblasts, stimulate tumour progression through CXCL12 secretion. And the effect of CXCL12 on proliferation of CT26 colon tumour cells is mediated by CXCR7 (Meijer et al., 2008). Similar results were reported in angiogenesis experiments in breast and lung cancer cells (Miao et al., 2007; Goldmann et al., 2008). Macrophage migration inhibitory factor (MIF), which is secreted by rhabdomyosarcoma (RMS), is an autocrine/paracrine factor that interacts with CXCR4 as well as with CXCR7 to enhance the adhesiveness of RMS cells (Tarnowski et al., 2010). EPCs may offer a possible biomarker for efficient of treatment and prognosis (Morita et al., 2011). Specifically, CXCR7 makes it easier for EPC promoters to stay on the endothelium, to penetrate the area of angiogenesis, and to enhance the stability of the extracellular matrix. It is postulated that interaction between CXCL12 and CXCR7 contributes to cancer cell proliferation, invasion, and metastasis by recruiting EPCs from the bone marrow (Wang et al., 2008; Dai et al., 2011). It was reported that CXCR7 was expressed in meningioma, growth hormone (GH)-producing adenomas, and prolactin (PRL)-producing adenomas (Yoshida et al., 2009; Würth et al., 2011). However, the expression of CXCR7 in pituitary adenoma, neurilemmoma or hemangioblastoma remains to be elucidated. To further define the role of CXCR7 in brain tumours, our present study examined the mRNA expression of CXCR7 in four typical human brain tumours, neurilemmoma, pituitary adenoma, hemangioblastoma, and meningioma, via real-time quantitative RT-PCR.

Materials and Methods

Tissue preparation

Brain tissue samples were obtained from 27 patients (15 females and 12 males (49.0±20.0 years)) with neurilemmoma (8 patients), pituitary adenoma (7 patients), hemangioblastoma (6 patients) and meningioma (6 patients) undergoing surgical resection of the tumour in the West China Hospital of Sichuan University. This study was approved by the Ethics Committee of West China Hospital of Sichuan University. All tumour samples used for RNA extraction were taken within the tumour. Only tissues with anatomic pathology features that allow a matching diagnosis with the MRI and the pathology report were used.

Design of primers to identify human CXCR7

We designed two pairs of primers for PCR amplification according to the NCBI Genebank of human (ACCESSION: NM_001047841). The primers were synthesized by Shanghai SAGON. The primers for human CXCR7 were as follows: forward primer 5'-CACTGCTACATCTTGAACCT -3' and reverse primer 5'- GTTGATGGAGAAGATGAGGTGT -3' (144 bp). The primers for actin beta (ACTB) were as follows: forward primer 5'- GAAGATCAAGATCATTGCTCCT -3' and reverse primer 5'- TACTCCTGCTTGCTGATCCA 3' (111bp).

RNA isolation and reverse transcription

Total RNA from the tissues was isolated using Trizol reagent (USA Invitrogen). The RNA was reverse transcribed into cDNA using the Revert Aid™ First Strand cDNA Synthesis Kit (MBI Fermentas Inc.) with addition of random hexamer primers. A total of 5 μl of purified RNA, 1 μl M-MLV reverse transcriptase (200 U), 1 μl of random hexamer primer, 4 μl of 5× reaction buffer, 2 μl of 1× hexamer (Roche), 2 μl of dNTP mix (10 mM each), and 6 μl of RNase-free water were used for cDNA synthesis. The reverse transcriptase was inactivated at 70 °C for 10 min after incubation. The cDNAs were stored at –20 °C until further analysis.

Real-time quantitative PCR

Real-time PCR was used to determine the gene expression profiles of the CXCR7. The cDNAs were amplified by real-time PCR using an FTC-2000 (Funglyn, Canada). Each analysis was performed in a total volume of 30 μl reaction mixture containing 2 μl cDNA sample, 2 μl gene-specific forward and reverse primers(10 μM each), and 1 μl SYBR GreenI. Housekeeping gene ACTB was included to normalize the data. Amplifications were performed as follows, 45 cycles at 94ºC for 2 min, 94ºC for 20 sec and 54ºC for 20 sec, with a final extension at

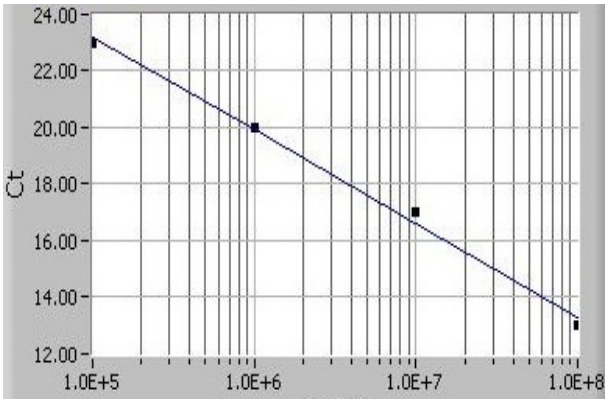

Figure 1. The Standard Curve for the CXCR7 Set Generated from Ct Values and Plotted Against the Input Standard Genomic DNA Molecules. Using serial dilutions of the standard cDNA, the standard curve ranged from 105 to 108 copies was generated on the basis of the linear relationship between the existing Ct and the logarithm of the copy number

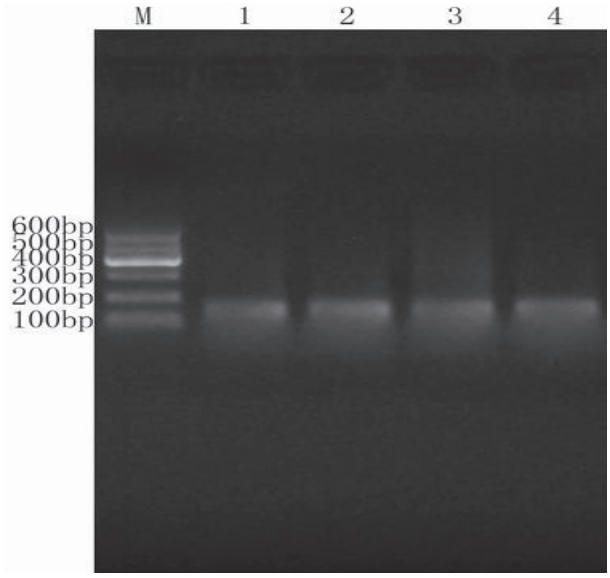

Figure 2. Ethidium Bromide Staining of PCR Products of CXCR2 on a 2% Agarose Gel. Meningioma cDNA of the encephaloma was used as a positive control for CXCR7 PCR primers (lane 1). Total RNA preparation from neurolemmoma (lane 2), pituitary adenoma (lane 3), and hemangioblastoma (lane 4) after reverse transcription was subjected to 45 cycles of PCR. Amplification of CXCR7 cDNA is a fragment of 144 bp, and amplification of ACTB cDNA is a fragment of 111 bp. M indicates the marker. Blank (Negative control), in which we added water, showed no any cDNA reaction

60ºC for 30 sec. The cycle threshold (Ct) number, defined as the number of PCR amplification cycles required to reach fluorescent intensity above the threshold, was determined for each gene and each developmental time point analyzed. Using serial dilutions of the test sample cDNA, the standard curve was generated on the basis of the linear relationship of existing Ct and the logarithm of the copy number. The slope of the curve was shown to be -3.30 and a strong linear relationship was demonstrated (R2 = 1.00; Figure 1).

Analysis

Results were normalized internally using the Ct

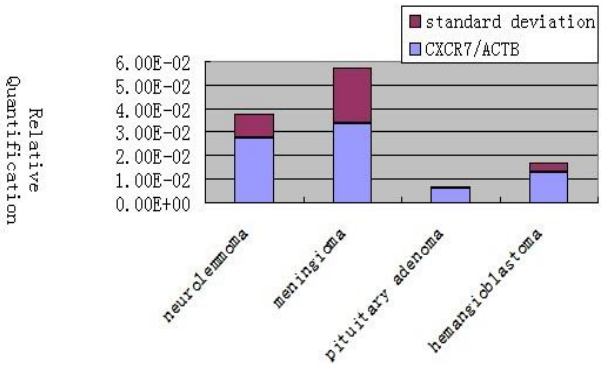

Figure 3. The Quantitative mRNA Expression of CXCR7 in Neurilemmoma, Meningioma, Pituitary Adenoma, and Hemangioblastoma by Real-time RT-PCR. The expression of CXCR7 in neurilemmoma was normalized to the expression of CXCR7 in meningioma (positive control), which was arbitrarily set to a value of 1. The quantitative results showed that the levels of CXCR7 mRNA in brain tissues from patients with neurilemmoma or meningioma were significantly higher than those with pituitary adenoma or hemangioblastoma

number of the housekeeping gene ACTB as follows: ΔCt (sample) = (Ct sample) - (Ct ACTB). The mean Ct of CXCR7 RNA from the blank was set to a relative quantity (RQ) value of 1 using the ΔΔCt, calculated as follows: ΔΔCt (sample) = ΔCt (sample) - ΔCt (blank), and RQ = $2^{-\Delta\Delta Ct}$. The data is reported as means ± standard errors (SE). The one-way analysis of variance (ANOVA) was performed using SPSS 11.0 statistical software. P values of less than 0.05 were considered to be statistically significant.

Results

We have demonstrated that the standard curve was generated on the basis of the linear relationship between the existing Ct and the logarithm of the CXCR7 copy number (Figure 1). The mRNA of CXCR7 was detected in all tumour samples examined in the current study (Figure 2 and 3). The quantitative results showed that the levels of CXCR7 mRNA in brain tissues from patients with meningioma and neurilemmoma were significantly higher compared to those with adenoma and hemangioblastoma ($P < 0.05$) (Figure 3). Blank (Negative control), in which we did not add cDNA samples, showed no positive reaction. It indicated that there was no contamination of sample or reagent.

Discussion

In this study, real-time quantitative RT-PCR was used to determine CXCR7 expression in neurilemmoma, pituitary adenoma, and hemangioblastoma. Meningioma, which was previously shown to express CXCR7, was used as positive controls. PCR is a rapid and powerful technique for in vitro amplification of DNA. Being specific and sensitive, real-time quantitative RT-PCR has been used widely to measure mRNA expression.

Previous findings suggest that CXCR7 is involved

in tumour proliferation and apoptosis. With tumour progression and metastasis appearance, CXCR7 expression may increase accordingly in certain tumours, such as pancreatic adenocarcinoma (Gebauer et al., 2011). Evidence has shown that CXCL12 binds to the CXCR7 and/or CXCR4 in brain tumours (Tseng et al., 2011). However this raises a concern about the potential contribution of the CXCL12/CXCR7 pathway other than CXCL12/CXCR4 pathway in all the processes that were previously attributed to CXCL12/CXCR4 signalling in brain tumours. In the present study, the expression of CXCR7 in neurilemmoma was investigated in vivo. We showed that CXCR7 was expressed in neurilemmoma, which was not reported previously. However, contribution of CXCL12/CXCR7 pathway to tumour development remains largely unclear. According to previous findings, CXCR7 is an active component of CXCL12 signalling in Schwann cells (Calatozzolo et al., 2011). Cultured cortical astrocytes and peripheral nerve schwann cells exhibit comparable total and cell-surface expression levels of CXCR7 (Odemis et al., 2010). In Schwann cells, RNA interference-mediated depletion of CXCR7 silences CXCL12 signalling (Odemis et al., 2010). Our findings support the presumption that CXCL12/CXCR7 pathway influences the Schwann cell proliferation or it even has certain function in peripheral nerve, with or without contribution of CXCR4.

In this study, pituitary adenoma appears to be CXCR7-negative. The role of CXCL12/CXCR7 interaction in modulating the hypothalamic-pituitary axis and its possible involvement in the development of pituitary adenomas were previously reported in AtT20 mouse pituitary tumour cells (Dai et al., 2011). It was reported that prominent expression of CXCR7 was observed in GH-producing adenomas, PRL-producing adenomas, and macroadenomas (Dai et al., 2011). However, our studies showed the opposite results. One of the possibilities for such discrepancy may be due to the fact that our tissue samples, which are from multipled cell clones, contain PRL -positive, PRL-weakly positive, thyroid stimulating hormone (TSH)-positive, TSH-negative, GH-positive, GH-negative, adrenocorticotropic hormone (ACTH)-positive, and ACTH-negative components. Pituitary adenomas produce the chemokine stromal CXCL12 and its receptor CXCR4. The CXCR4 antagonist AMD3100 suppressest hypoxia-mediated growth hormone production in GH3 rat pituitary adenoma cells (Yoshida et al., 2010). It is speculated that CXCR7 is not prominent in adrenocorticotrophic or TSH-producing adenomas. In addition, pituitary adenoma lacks protein matrix and reticuloprotein. It was recently suggested that CXCL12 plays a critical role as a chemo-attractant in cancer development possibly at the level of the tumor niche (Begley et al., 2005). Low expression of CXCR7 in pituitary adenoma may be because CXCL12/CXCR7 mainly contributes to maintain stability in ground substance, but not pituitary adenoma cell itself.

Hemangioblastoma is highly vascular differentiated benign tumour. Originating in the perivascular mesenchymal tissue, hemangioblastoma contains two components, capillary network and intervascular reticuloendothelial cells. The mRNA expression levels of CXCR4 were found significantly higher in hemangioblastomas compared with the normal cerebellar tissues (Liang et al., 2007). Previous study suggested that CXCR7 also plays a significant role in blood vessel formation (Zheng et al., 2010). In rat brain ischemia model, CXCR7 expression was significantly increased (Schönemeier et al., 2008). In situ hybridization demonstrated that CXCR7 mRNA was confined to the vascular endothelium at the depths of glial cell tumours, where CXCL12 was concentrated (Odemis et al., 2012). Furthermore, CXCR7 plays an important role in human cord blood derived EPCs in response to CXCL12 by inducing EPCs adhesion to active umbilical vein endothelial cells and trans-endothelial migration (Burns et al., 2006; Morita et al., 2011; Tseng et al., 2011). In the present study, CXCR7 mRNA was detected in hemangioblastoma, but significantly lower than that in brain tissues from patients with neurilemmoma or meningioma, which was not found before. Out results suggest that CXCR7 does not appear to play a significant role in the formation of hemangioblastoma. It can be inferred that CXCR7 promotes metastasis only partially related to its role in angiogenesis. And CXCR7 significantly increase cellular adhesions (Raggo et al., 2005; Burns et al., 2006; Miao et al., 2007). That is to say, CXCR7 influence cancer metastasis through mechanisms of cell adhesion. For instance, it has been shown that high levels of CXCR7 in breast cancer facilitates the ability of cancer cells pass through the hemato-encephalic barrierleading to brain metastasis (Salmaggi et al., 2009). Hemangioblastoma, as a benign neoplasm, does not show strong tendency to perform metastasis. So, CXCR7 was not highly expressed in hemangioblastoma. Alternatively, we hypothesize that in hemangioblastoma, the effects of CXCL12 are mainly CXCR4-mediated, as increased expression of CXCR4 was detected in more active hemangioblastomas and there are several small molecules such as the third complement component (C3), which positively modulates responsiveness of CXCR4 to CXCL12 (Reca et al., 2006; Wojakowski et al., 2006; Begley et al., 2007; Liang et al., 2007).

In this study, we found that CXCR7 mRNA was detected in all tumour samples, and the quantitative results showed that the levels of CXCR7 mRNA in brain tissues from patients with neurilemmoma or meningioma were significantly higher than those with pituitary adenoma or hemangioblastoma, which was not reported previously. CXCR7 in brain tumours may induce neurilemmoma, as CXCR7 is an active component in schwann cell proliferation. CXCR7 may also co-work with CXCL12 in brain tumour angiogenesis by recruiting EPCs (Wang et al., 2008). Or CXCL12/CXCR7 interaction results in phosphorylation of AKT and affects on carcinogenesis and the neovascularisation linked to tumour progression (Proost et al., 2007; Kumar et al., 2012). In this way, cancer cells are proliferation-induced and apoptosis-inhibited, with increased IL-8 and VEGF produced. In conclusion, the results suggest that CXCR7 may play a role in progression, metastasis and angiogenesis of brain tumours.

Acknowledgements

This project was supported by the National Innovative Experimental Projects of Sichuan University, 2012 (201210610114) and the Natural Science Foundation of China (30770749). We would like to thank Mr. Zengliang Xia and Mr. Faqiang Zhang in the west China laboratory of molecular genetics for their excellent technical assistance. The author(s) declare that they have no competing interests.

References

Bajetto A, Bonavia R, Barbero S, Florio T, Schettini G (2001). Chemokines and their receptors in the central nervous system. *Front Neuroendocrinol*, **22**, 147-84.

Barbieri F, Bajetto A, Porcile C, et al (2006). CXC receptor and chemokine expression in human meningioma: SDF1/CXCR4 signaling activates ERK1/2 and stimulates meningioma cell proliferation. *Ann N Y Acad Sci*, **1090**, 332-43.

Begley LA, MacDonald JW, Day ML, Macoska JA (2007). CXCL12 activates a robust transcriptional response in human prostate epithelial cells. *J Biol Chem,* **282**, 26767-74.

Begley L, Monteleon C, Shah RB, Macdonald JW, Macoska JA (2005). CXCL12 over expression and secretion by aging fibroblasts enhance human prostate epithelial proliferation in vitro. *Aging Cell*, **4**, 291-8.

Burns JM, Summers BC, Wang Y, et al (2006). A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J Exp Med,* **203**, 2201-13.

Calatozzolo C, Canazza A, Pollo B, et al (2011). Expression of the new CXCL12 receptor, CXCR7, in gliomas. *Cancer Biol Ther*, **11**, 242-53.

Dai X, Tan Y, Cai S, et al (2011). The role of CXCR7 on the adhesion, proliferation and angiogenesis of endothelial progenitor cells. *J Cell Mol Med*, **15**, 1299-309.

do Carmo A, Patricio I, Cruz MT, et al (2010). CXCL12/CXCR4 promotes motility and proliferation of glioma cells. *Cancer Biol Ther*, **9**, 56-65.

Gebauer F, Tachezy M, Effenberger K, et al (2011). Prognostic impact of CXCR4 and CXCR7 expression in pancreatic adenocarcinoma. *J Surg Oncol*, **104**, 140-5.

Goldmann T, Drömann D, Radtke J, et al (2008). CXCR7 transcription in human non-small cell lung cancer and tumor-free lung tissues; possible regulation upon chemotherapy. *Virchows Arch*, **452**, 347-8.

Kumar R, Tripathi V, Ahmad M, et al (2012). CXCR7 mediated Giα independent activation of ERK and Akt promotes cell survival and chemotaxis in T cells. *Cell Immunol*, **272**, 230-41.

Liang X, Shen D, Huang Y, et al (2007). Molecular pathology and CXCR4 expression in surgically excised retinal hemangioblastomas associated with von Hippel-Lindau disease. *Ophthalmology*, **114**, 147-56.

Luker KE, Steele JM, Mihalko LA, Ray P, Luker GD (2010). Constitutive and chemokine-dependent internalization and recycling of CXCR7 in breast cancer cells to degrade chemokine ligands. *Oncogene*, **29**, 4599-610.

Massa A, Casagrande S, Bajetto A, et al (2006). SDF-1 controls pituitary cell proliferation through the activation of ERK1/2 and the Ca^{2+}-dependent, cytosolic tyrosine kinase Pyk2. *Ann N Y Acad Sci*, **1090**, 385-98.

Meijer J, Ogink J, Roos E (2008). Effect of the chemokine receptor CXCR7 on proliferation of carcinoma cells in vitro and in vivo. *Br J Cancer*, **99**, 1493-501.

Meincke M, Tiwari S, Hattermann K, Kalthoff H, Mentlein R (2011). Near-infrared molecular imaging of tumors via chemokine receptors CXCR4 and CXCR7. *Clin Exp Metastasis*, **28**, 713-20.

Miao Z, Luker KE, Summers BC, et al (2007). CXCR7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. *Proc Natl Acad Sci U S A*, **104**, 15735-40.

Morita R, Sato K, Nakano M, et al (2011). Endothelial progenitor cells are associated with response to chemotherapy in human non-small-cell lung cancer. *J Cancer Res Clin Oncol,* **137**, 1849-57.

Odemis V, Boosmann K, Heinen A, Küry P, Engele J (2010). CXCR7 is an active component of SDF-1 signalling in astrocytes and Schwann cells. *J Cell Sci*, **123**, 1081-8.

Odemis V, Lipfert J, Kraft R, et al (2012). The presumed atypical chemokine receptor CXCR7 signals through G(i/o) proteins in primary rodent astrocytes and human glioma cells. *Glia*, **60**, 372-81.

Perlin JR, Talbot WS (2007). Signals on the move: chemokine receptors and organogenesis in zebrafish. *Sci STKE*, **2007**, 45.

Proost P, Mortier A, Loos T, et al (2007). Proteolytic processing of CXCL11 by CD13/aminopeptidase N impairs CXCR3 and CXCR7 binding and signaling and reduces lymphocyte and endothelial cell migration. *Blood*, **110**, 37-44.

Raggo C, Ruhl R, McAllister S, et al (2005). Novel cellular genes essential for transformation of endothelial cells by kaposi's sarcoma-associated herpesvirus. *Cancer Res*, **65**, 5084-95.

Reca R, Wysoczynski M, Yan J, Lambris JD, Ratajczak MZ (2006). The role of third complement component (C3) in homing of hematopoietic stem/progenitor cells into bone marrow. *Adv Exp Med Biol*, **586**, 35-51.

Salmaggi A, Maderna E, Calatozzolo C, et al (2009). CXCL12, CXCR4 and CXCR7 expression in brain metastases. *Cancer Biol Ther*, **8**, 1608-14.

Schönemeier B, Schulz S, Hoellt V, Stumm R (2008). Enhanced expression of the CXCL12/SDF-1 chemokine receptor CXCR7 after cerebral ischemia in the rat brain. *J Neuroimmunol*, **198**, 39-45.

Schutyser E, Su Y, Yu Y, et al (2007). Hypoxia enhances CXCR4 expression in human microvascular endothelial cells and human melanoma cells. *Eur Cytokine Netw*, **18**, 59-70.

Shim H, Lau SK, Devi S, et al (2006). Lower expression of CXCR4 in lymph node metastases than in primary breast cancers: potential regulation by ligand-dependent degradation and HIF-1alpha. *Biochem Biophys Res Commun*, **346**, 252-8.

Singh RK, Lokeshwar BL (2011). The IL-8-regulated chemokine receptor CXCR7 stimulates EGFR signaling to promote prostate cancer growth. *Cancer Res*, **71**, 3268-77.

Sun X, Cheng G, Hao M, et al (2010). CXCL12 / CXCR4 / CXCR7 chemokine axis and cancer progression. *Cancer Metastasis Rev*, **29**, 709-22.

Tarnowski M, Grymula K, Liu R, et al (2010). Macrophage migration inhibitory factor is secreted by rhabdomyosarcoma cells, modulates tumor metastasis by binding to CXCR4 and CXCR7 receptors and inhibits recruitment of cancer-associated fibroblasts. *Mol Cancer Res*, **8**, 1328-43.

Terasaki M, Sugita Y, Arakawa F, et al (2011). CXCL12/CXCR4 signaling in malignant brain tumors: a potential pharmacological therapeutic target. *Brain Tumor Pathol*, **28**, 89-97.

Tseng D, Vasquez-Medrano DA, Brown JM (2011). Targeting SDF-1/CXCR4 to inhibit tumour vasculature for treatment of glioblastomas. *Br J Cancer*, **104**, 1805-9.

Wang J, Shiozawa Y, Wang J, et al (2008). The role of CXCR7/

RDC1 as a chemokine receptor for CXCL12/SDF-1 in prostate cancer. *J Biol Chem*, **283**, 4283-94.

Wojakowski W, Tendera M, Zebzda A, et al (2006). Mobilization of CD34(+), CD117(+), CXCR4(+), c-met(+) stem cells is correlated with left ventricular ejection fraction and plasma NT-proBNP levels in patients with acute myocardial infarction. *Eur Heart J*, **27**, 283-9.

Yoshida D, Nomura R, Teramoto A (2009). Signalling pathway mediated by CXCR7, an alternative chemokine receptor for stromal-cell derived factor-1α, in AtT20 mouse adrenocorticotrophic hormone-secreting pituitary adenoma cells. *J Neuroendocrinol*, **21**, 481-8.

Yoshida D, Koketshu K, Nomura R, Teramoto A (2010). The CXCR4 antagonist AMD3100 suppresses hypoxia-mediated growth hormone production in GH3 rat pituitary adenoma cells. *J Neurooncol*, **100**, 51-64.

Zheng K, Li HY, Su XL, et al (2010). Chemokine receptor CXCR7 regulates the invasion, angiogenesis and tumor growth of human hepatocellular carcinoma cells. *J Exp Clin Cancer Res*, **29**, 31.