Biochemical and Biophysical Research Communications 429 (2012) 168–172

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Elevated chemokine CC-motif receptor-like 2 (CCRL2) promotes cell migration and invasion in glioblastoma

Fengqiong Yin [a,b], Zhenhua Xu [c], Zifeng Wang [d], Hong Yao [c], Zan Shen [e], Fang Yu [f], Yiping Tang [b], Dengli Fu [b], Sheng Lin [g], Gang Lu [d], Hsiang-fu Kung [c], Wai Sang Poon [d], Yunchao Huang [a,*], Marie Chia-mi Lin [a,d,*]

[a] Department of Cardiothoracic Surgery, The Third Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China
[b] Department of Neurology and Oncology, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China
[c] School of Biomedical Science, Center for Emerging Infectious Disease, The Chinese University of Hong Kong, Shatin, Hong Kong, China
[d] Brain Tumor Centre and Division of Neurosurgery, Department of Surgery, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China
[e] Department of Oncology, Affiliated 6th People's Hospital, Shanghai Jiaotong University, Shanghai 200233, PR China
[f] Department of Pathology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China
[g] Laboratory of Medical Genetics, Shenzhen Research Institute of Population and Family Planning, Shenzhen, China

ARTICLE INFO

Article history:
Received 26 October 2012
Available online 6 November 2012

Keywords:
CCRL2
Glioblastoma
Tumor migration
Tumor invasion
Glioma dissemination

ABSTRACT

Chemokine CC-motif receptor-like 2 (CCRL2) is a 7-transmembrane G protein-coupled receptor which plays a key role in lung dendritic cell trafficking to peripheral lymph nodes. The function and expression of CCRL2 in cancer is not understood at present. Here we report that CCRL2 expression level is elevated in human glioma patient samples and cell lines. The magnitude of increase is positively associated with increasing tumor grade, with the highest level observed in grade IV glioblastoma. By gain-of-function and loss-of-function studies, we further showed that CCRL2 did not regulate the growth of human glioblatoma U87 and U373 cells. Importantly, we demonstrated that over-expression of CCRL2 significantly enhanced the migration rate and invasiveness of the glioblastoma cells. Taken together, these results suggest for the first time that elevated CCRL2 in glioma promotes cell migration and invasion. The potential roles of CCRL2 as a novel therapeutic target and biomarker warrant further investigations.

1. Introduction

Chemokine receptors are cell-surface receptors belonging to a superfamily of rhodopsin-like G-protein coupled 7-transmembrane receptors (GPCRs) [1]. Chemokine receptors together with their interacting small secreted chemotactic chemokines constitute the chemokine systems [2]. Chemokine systems control nearly all classes of leukocytes trafficking in the immune system [1], and many types of mammalian cells including neurons and endothelial cells express the chemokines and chemokine receptors [3]. So far, 50 chemokines and 18 chemokine receptors have been discovered in various human cells [4]. Recently, large scale clinical studies have demonstrated that chemokines and their receptors are often up-regulated in a wide variety of human cancers [5,6] and the expression levels of several chemokine receptors are often positively associated with tumor progression [7–9]. It has been demonstrated that overexpression of chemokine receptors CXCR4 or CCR7 alone is sufficient to promote the metastatic ability of melanoma cancer cells [10–12], and knock-down of CXCR4 effectively inhibits the metastasis of breast cancer cells [13,14]. These studies further suggest the important roles of chemokine systems in cancer development.

Glioblastoma multiforme (GBM, WHO grade IV), the most severe form of glioma (tumor of the central nervous system), is locally very invasive. With no effective treatment, it is the primary cause of glioma-related death [15]. The median survival of patients with GBM is 12–15 months, and only 2% survived beyond 3 years [16]. CXCR4 is the only chemokine receptor which has been demonstrated to play important roles in modulating immune response and regulating cell growth, migration, and angiogenesis in the malignant progression of GBM [17–21]. These observations raised the possibility that other chemokines and chemokine receptors may also play pivotal roles in GBM.

Chemokine CC-motif receptor-like 2 (CCRL2) is first cloned from a polymorphonuclear neutrophils (PMN) cDNA library, and has been found in almost all hemopoietic cells [22,23]. CCRL2 is located

* Corresponding authors. Address: Brain Tumor Center and Division of Neurosurgery, Department of Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China (M.C.-m. Lin). Fax: +852 26377974.
E-mail addresses: huangych2001@yahoo.com.cn (Y. Huang), mcllin@surgery.cuhk.edu.hk (M.Chia-mi Lin).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.10.120

on chromosome 3p21 in close proximity to chemokine receptors CCR5, CCR2, CX3CR1, CCR3 and CCR8 [24,25]. Hydrophobicity and homology analysis of CCRL2 amino acid sequence indicated that CCRL2 codes for a putative GPCR [22], and it has been reported that CCRL2 is activated by chemokines CCL2, CCL5, CCL7, and CCL8 [26–28]. A recent knockout mice study further indicated that CCRL2 plays a non-redundant role in lung dendritic cell trafficking to peripheral lymph nodes [29]. To date, the role of CCRL2 in human cancers is unknown. In this study, we demonstrated for the first time that CCRL2 expression level is significantly elevated in both low grade and high grade human glioma patients and cell lines. We further demonstrated that overexpression of CCRL2 significantly enhanced GBM cell migration and invasion. These results provide new insights into the novel roles of CCRL2 in glioma carcinogenesis, and suggest CCRL2 as a potential therapeutic target and biomarker for GBM.

2. Materials and methods

2.1. Quantitation of CCRL2 mRNA levels in human glioma patient tissues and cell lines

Total RNA was isolated from glioma cell lines and frozen glioma patient tissues (from the Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong) using TRizol Reagent (Invitrogen Corp). Aliquots (2 μg) of total RNA were reverse transcribed to cDNA using SuperScript™ II Reverse Transcriptase (Invitrogen Corp). The expression level of CCRL2 was determined by real time RT-PCR using the Brilliant® II SYBR Green qPCR master mix (Stratagene Corp) by Applied Biosystems 7500 real time PCR system (Applied Biosystems Corp). Housekeeping gene GAPDH was used as an internal control. The following primers were used: CCRL2 specific primers (forward, 5′-ACA AGT ATG ACG CCC AGG CAC T-3′, reverse, 5′-CCA GGA TAA GCA CAA CCA GGA GA-3′) and GAPDH specific primers (forward, 5′-GAA TCT ACT GGC GTC TTC ACC-3′, reverse, 5′-GTC ATG AGC CCT TCC ACG ATG C-3′).

2.2. CCRL2 expression plasmid and CCRL2 knockdown siRNA

To construct the CCRL2 expression plasmid, human CCRL2 open reading frame (ORF) was amplified by RT-PCR using cDNA from human glioblastoma patient tissues and a pair of CCRL2 ORF primers (forward, 5′-AAA TGC TAG CAC CAT GAT CTA CAC CCG TTT CTT AAA AGG-3′, reverse, 5′-GGA TCT CGA GTT ACA CTT CGG TGG AAT GGT CAG-3′). The PCR product was purified and digested with *Nhel* and *Xhol*, then inserted into the pcDNA3.1(+) vector (Invitrogen Corp). The sequence and orientation were confirmed by sequencing. CCRL2 knockdown siRNA si-CCRL2 were designed and purchased from Santa Cruz (sc-77982, Santa Cruz).

2.3. Cell culture

Human GBM cell lines U87 MG and U373 MG were obtained from ATCC. Both cell lines were cultured in Dulbecco's modified eagles medium (DMEM, Gibco Corp) supplemented with 10% fetal bovine serum (FBS, Gibco Corp), in a humidified atmosphere with 5% CO_2 at 37 °C.

2.4. MTT assay for cell viability

GBM cells were seeded in 96-well plates with initial density of 3000 cells per well. After 24 h, cells were transfected with CCRL2 expression plasmids (pcDNA-CCRL2) or control plasmids (pcDNA-GFP) using FuGENE® HD (Roche Corp) transfection reagent. In different experiments, cells were transfected with CCRL2 knockdown siRNA (si-CCRL2) or the negative control siRNA (siRNA-NC) using Lipofectamine™ RNAiMAX (Invitrogen Corp) transfection reagent. At Days 1, 2, 3, 4 and 5, after transfection, the mediums were removed and the cells were incubated with 20 μl of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT, Sigma–Alrich) stock solution at 37 °C for 4 h, and the absorbencies were determined by a microplate reader (Thermo Electron Multiskan Ex Corp) at detection wavelength 570 nm (Abs_{570}) following manufacture instructions. The relative cell viabilities were calculated in accordance with the equation: Cell viability $[\%] = \text{test } Abs_{570}/\text{control } Abs_{570} \times 100\%$.

2.5. Wound healing assay

GBM cells were plated onto a 6-well plate at 3×10^5 cells per well and transfected with pcDNA-GFP or pcDNA-CCRL2. At 70% confluency, the monolayer cells were scratched by a sterile pipette tip. The wounded cell layers were washed twice and then incubated in complete culture medium. The wound healing processes were monitored at 0 h, 10 h, and 24 h using inverted microscope (Olympus IX50) and the migrated distances determined. The cell migration distances were calculated as a percentage of the original total distance calculated at 0 h (as 100%). Each experiment was repeated three times.

2.6. Matrigel transwell assay

GBM cells were transfected with pcDNA-GFP or pcDNA-CCRL2 and suspended in a medium containing 1% FBS medium. Equal numbers (3×10^4) of cells were then seeded into a 24-well BD BioCoat™ Matrigel™ Invasion Chamber (BD Biosciences Corp). Complete culture medium was added to the lower chamber as a chemoattractant. After the chambers were incubated for 22 h in culture incubator, the non-invading cells were removed from the upper surface of the membrane by "scrubbing". Cells on the lower surface of the membrane were fixed and stained with 100% methanol and 1% Toluidine blue, respectively. Each experiment was performed three times in triplicates. Invading cells were photographed under the microscope. Numbers of migrated cells in five random selected fields were counted, and the average number calculated. The ratio of cells migrated into the lower chamber (migration ratio) were calculated as the percentage of the total cells originally seeded onto the upper compartment.

2.7. Statistics analysis

The data were analyzed by unpaired two-tailed Student's *t* test and expressed as mean ± standard deviation (SD). Differences were considered statistically significant at $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$; $^{***}p \leqslant 0.001$.

3. Results

3.1. Elevated CCRL2 mRNA levels in glioma patient tissue samples and glioma cell lines

We performed quantitative Real Time RT-PCR to determine the expression levels of CCRL2 mRNA in human glioma cell lines and patient tissues. As shown in Fig. 1A, the expression level of CCRL2 mRNA in the high-grade (HG, WHO grade IV) GBM cell lines U87 MG, U373 MG and U118 MG were significantly higher than that of the low-grade (LG, grade II and III) glioma cell line SW1088, and the lowest CCRL2 mRNA level was observed in human medulloblastoma cell line PFSK1. Furthermore, as compared to normal brain tissues, CCRL2 mRNA levels in 24 high-grade GBM patient tis-

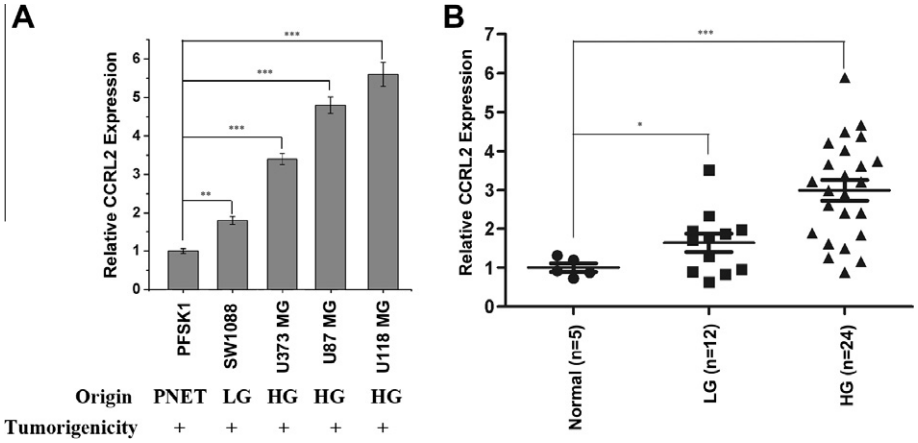

Fig. 1. Elevated CCRL2 expressions in glioma. The relative expression levels of CCRL2 mRNA in (A) five glioma cell lines and (B) 24 high-grade GBM tissues, 12 low-grade glioma tissues and 5 normal brain tissues. LG, low-grade glioma; HG, high-grade glioma. $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$; $^{***}p \leqslant 0.001$.

sues and 12 low-grade glioma patient tissues were 2.99 ($p < 0.001$) and 1.64 ($p < 0.05$) folds higher, respectively (Fig. 1B).

3.2. Over-expression of CCRL2 did not change cell proliferation rate in GBM

Many chemokines and their receptors have been shown to promote tumor malignant progression *via* promoting cell proliferation [18,30]. Hence, we first investigated the ability of CCRL2 in regulating cells proliferation by gain of function of study, *via* over-expressing CCRL2 in GBM cells. As shown in Fig. 2, U87 (2A, upper panel) and U373 (2B, upper panel) cells transfected with a plasmid encoding CCRL2 (pcDNA-CCRL2) showed significantly increased CCRL2 mRNA expressions as compared to the control cells transfected with a plasmid encoding GFP (pcDNA-GFP). However, result from MTT assay indicated that both CCRL2 over-expressing cells exhibited similar cell proliferation rate as compared to control cells (Fig. 2A and B, lower panels). In addition, knockdown of CCRL2 by siRNA targeting CCRL2 significantly decreased CCRL2 expression level in U87 cells (Fig. 2C, upper panel), but did not cause any change in cell proliferation rate (Fig. 2C, lower panel). Taken together, these results suggested that CCRL2 did not regulate the proliferation rate of GBM cells.

3.3. Over-expression of CCRL2 promoted cell migration in GBM cells

As a highly invasive tumor, GBM exhibited extraordinarily high migration ability which greatly enhances its malignant progression [31]. Therefore, characterizing the factors driving cell migration is very important. CCRL2 has been shown to drive lung dendritic cell migration [29]. As tumor cell migration shares many similarities with leukocyte trafficking [10], hence, we propose that CCRL2 might play roles in GBM cell migration. To test this hypothesis, wound healing assay was performed. As shown in Fig. 3A, CCRL2-overexpressing cells showed a significantly faster migration rate than that of the control cells. At 10 and 24 h post wound creation, cells had migrated to cover 60% and 100% of the total wounded distance, respectively (Fig. 3B). In contrast, the control pcDNA-GFP and mock transfected cells only migrated to cover 30% to 50% of the total wounded distance, respectively.

3.4. Over-expression of CCRL2 promoted cell invasion in GBM cells

Invasion into surrounding tissues is the key process for tumor dissemination [32]. We proposed that elevated CCRL2 promoted the invasiveness of GBM cells. To test this hypothesis, *in vitro* transwell Matrigel assay was performed in U87 and U373 cells.

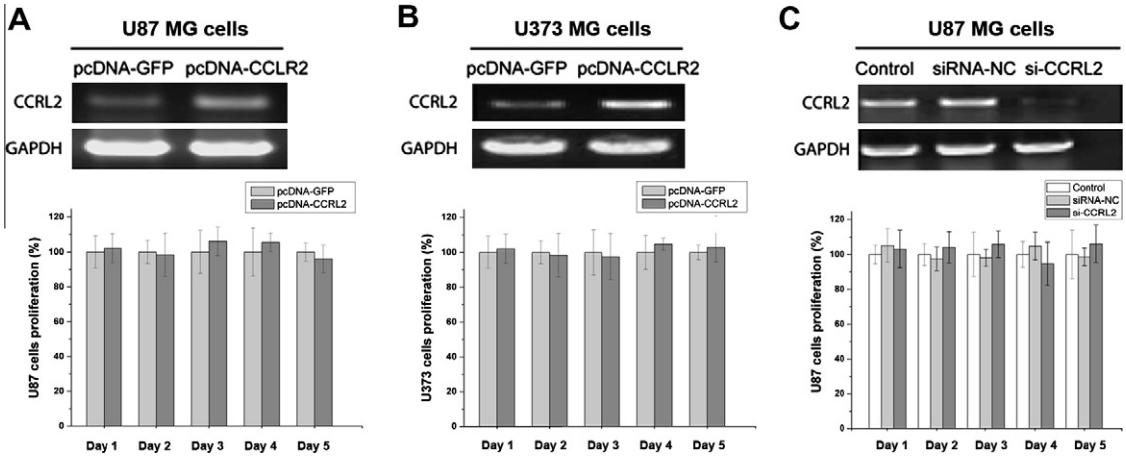

Fig. 2. Over-expression or knockdown of CCRL2 had no effect in cell proliferation ratio in GBM. (A) U87 MG and (B) U373 MG cells were transfected with control pcDNA-GFP or pcDNA-CCRL2 plasmid. The expression level of CCRL2 mRNA was determined by RT-PCR. The cell proliferation rate was measured by MTT assay. (C) U87 MG cells were mock-transfected or transfected with scrambled siRNA (siRNA-NC) or siRNA-CCRL2. The expression level of CCRL2 mRNA was determined by RT-PCR. The cell proliferation rate was measured by MTT assay.

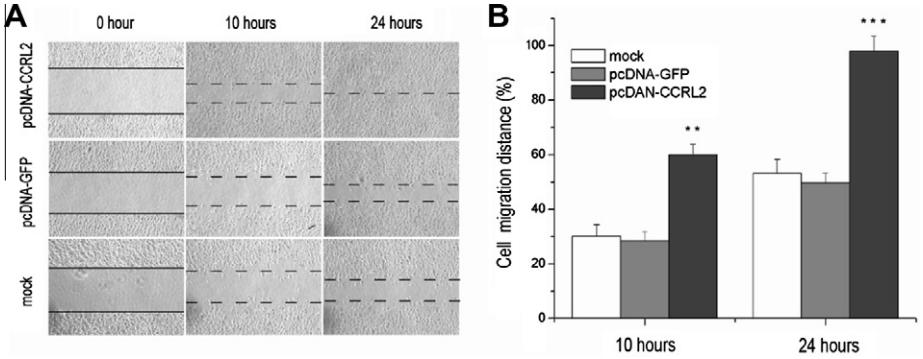

Fig. 3. Over-expression of CCRL2 promoted cell migration in GBM cells. (A) U373 MG cells were transfected with control pcDNA-GFP or pcDNA-CCRL2. A single wound was induced across the well. At 10 and 24 h after wound induction, phase-contrast images of the cell were photographed digitally under an inverted microscope. (B) The distance of the wound areas was measured, and the cell migration distance was calculated as described in Section 2. $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$; $^{***}p \leqslant 0.001$.

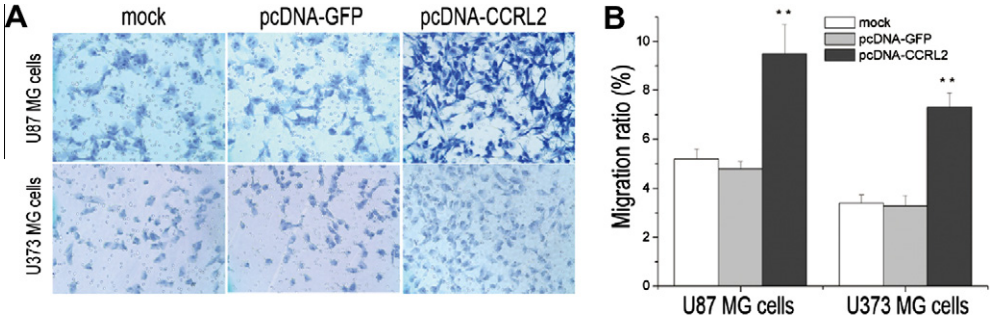

Fig. 4. Over-expression of CCRL2 promoted cell invasion in GBM cells. (A) U87 MG and U373 MG cells were transfected with PBS (Mock), pcDNA-GFP or pcDNA-CCRL2. The invasive penetrated cells were photographed under the microscope. (B) The average number of invasive penetrated cells on the lower surface of the membrane were quantified as described in the Section 2. $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$; $^{***}p \leqslant 0.001$.

As shown in Fig. 4A, as compared to control, CCRL2-overexpressing cells significantly increased the number of cells that penetrated the membrane at 22 h after the seeding of cells. In control mock and pcDNA-GFP transfected cells, only 5% and 3.5% of the total cells migrated to the lower chamber in U87 and U373 GBM, respectively. In contrast, the percentage of migrated cells in CCRL2 over-expressing U87 and U373 cells was 9.5% and 7.5%, respectively (Fig. 4B). These results indicated that elevated CCRL2 promoted cell invasion.

4. Discussion

GBM is the most frequent and malignant brain tumor of the central nervous system in adults [16]. Extensive invasion and dissemination of tumor cells into adjacent non-neoplastic brain tissue prevents complete surgical resection and leads to a poor prognosis. Understanding the molecular mechanisms underlying GBM migration and invasion are important for the development of new and effective drugs for GBM. In recent years, a variety of molecules involved in tumor migration and invasion have been identified, including growth factors, adhesion molecules, proteases and extracellular matrix components [33,34]. Increasing attention has been drawn to the chemokine systems. Here we demonstrated significantly elevated expressions of chemokine receptor CCRL2 in glioma and glioblastoma in Hong Kong patients samples. Furthermore, we showed for the first time that elevated CCRL2 promoted glioma cell migration and invasion.

It has been proposed that migration mechanisms used by tumor cells were similar, if not identical, to those that occur in immune-cell trafficking [35]. Indeed, many chemokines and chemokine receptors initially identified for their ability to control leukocytes trafficking, have been subsequently demonstrated to promote tumor cells migration and invasion. The two most well-studied chemokine/receptor pairs, the CXCL12/CXCR4 and CCL21/CCR7 have been shown to mediate cancer cells metastasis to the lymph nodes in multiple cancers [8]. Emerging evidence has suggested chemokine systems are promising therapeutic targets. For example, CXCR4 has been shown to be an effective target to inhibit breast cancer metastasis [13]. He we demonstrated that CCRL2 could be a new therapeutic target for GBM invasion and dissemination. CCRL2 inhibitors in combination with radiotherapy and chemotherapy drugs are potentially effective therapeutics for GBM. Further *in vivo* and *in vitro* studies in preclinical animal models are needed.

At present, it is not known how CCRL2 expression is up-regulated in glioma. The ligands of CCRL2 also remain elusive. Biber and colleagues have previously suggested that chemokines CCL2, CCL5, CCL7 and CCL8 might be the ligands of L-CCR in mice (equivalent to human CCRL2) [28]. Therefore, we analyzed the expression levels of human CCL2, CCL5 and CCL7 in glioma according to GEO Profile (GDS1813). Our analysis showed that CCL2 expression pattern is very similar to that of CCRL2 (Table 1), as its expressions are significantly increased with increasing glioma tumor grade. In contrast, CCL5 expression level is decreased with increasing glioma tumor grade. The potential interactions between CCRL2 and CCL2 warrant further investigations.

The downstream target and signaling molecules of CCRL2 are also unknown. Members of the rhodopsin-like G-protein coupled 7-transmembrane receptors (GPCRs) often possess a DRYLAIV motif, which is a conserved (Asp-Arg-Tyr-Leu-Ala-Ile-Val) motif in the second intracellular loop domain and crucial for chemokine GPCR

Table 1
The expression levels of potential CCRL2 ligands in normal brain tissues and glioma with different grades according to GEO Profile (GDS1813).

	Normal brain tissues ($n = 4$)	Oligodendroglioma WHO grade II ($n = 8$)		Anaplastic oligoastrocytoma WHO grade III ($n = 6$)		Glioblastoma WHO grade IV ($n = 30$)	
	Average	Average	*p Value	Average	*p Value	Average	*p Value
CCL2	−2.0058	−0.0738	0.0001	0.0333	0.0034	2.0070	0.0000
CCL5	1.6968	0.6905	0.0002	0.2938	0.0003	0.4024	0.0000
CCL7	−0.9820	0.1406	0.2683	−0.0767	0.3670	1.7018	0.0601

* All the p value in each group was calculated as compared to the expression levels in normal brain tissues by unpaired two-tailed Student's t test.

signaling and function [36]. However, CCRL2 presents a non-canonical DRYLAIV motif, which is Gln-Arg-Tyr-Leu-Val-Phe-Leu (QRYLVFL) in huCCRL2 and Gln-Arg-Tyr-Arg-Val-Ser-Phe (QRYRVSF) in mCCRL2 [37]. Thereafter, the intracellular signaling molecule(s) of this CCRL2 may be atypical and different from that of other receptors, and warrant further investigations.

In summary, we demonstrated for the first time an elevated expression of chemokine receptor CCRL2 in glioma. More importantly, we showed that over-expression of CCRL2 alone is sufficient to increase the migration and invasion of glioma tumor cells. The regulations and the ligand, downstream signaling pathway require further studies, and the potential applications of CCRL2 as therapeutic target or a biomarker in GBM and other chancers warrant further investigations.

Acknowledgments

We thank Dr. Yvonne Chan for language editing. This work was supported by the National Basic Research Program of China (973 Program) Grants 2010CB529400 and 2010CB912800; National Natural Science Foundation of China PC: 81001023 and 81201641, Research Grants Council of Hong Kong Grant 467109, and Yunnan Provincial Application Basic Research Foundation (Yunnan Province, China), project number: 2011FB191.

References

[1] T. Kakinuma, S.T. Hwang, Chemokines, chemokine receptors, and cancer metastasis, J. Leukoc. Biol. 79 (2006) 639–651.
[2] M.E. DeVries, A.A. Kelvin, L. Xu, et al., Defining the origins and evolution of the chemokine/chemokine receptor system, J. Immunol. 176 (2006) 401–415.
[3] B.J. Rollins, Chemokines, Blood 90 (1997) 909–928.
[4] A. Zlotnik, O. Yoshie, Chemokines: a new classification system and their role in immunity, Immunity 12 (2000) 121–127.
[5] A. Zlotnik, Chemokines and cancer, in. Ernst Schering Res Found Workshop, (2004) pp. 53–58.
[6] B. Homey, A. Muller, A. Zlotnik, Chemokines: agents for the immunotherapy of cancer?, Nat Rev. Immunol. 2 (2002) 175–184.
[7] I. Takanami, Overexpression of CCR7 mRNA in nonsmall cell lung cancer: correlation with lymph node metastasis, Int. J. Cancer 105 (2003) 186–189.
[8] C.C. Schimanski, S. Schwald, N. Simiantonaki, et al., Effect of chemokine receptors CXCR4 and CCR7 on the metastatic behavior of human colorectal cancer, Clin. Cancer Res. 11 (2005) 1743–1750.
[9] I.M. Ghobrial, N.D. Bone, M.J. Stenson, et al., Expression of the chemokine receptors CXCR4 and CCR7 and disease progression in B-cell chronic lymphocytic leukemia/ small lymphocytic lymphoma, Mayo Clin. Proc. 79 (2004) 318–325.
[10] A. Muller, B. Homey, H. Soto, et al., Involvement of chemokine receptors in breast cancer metastasis, Nature 410 (2001) 50–56.
[11] H.E. Wiley, E.B. Gonzalez, W. Maki, et al., Expression of CC chemokine receptor-7 and regional lymph node metastasis of B16 murine melanoma, J. Natl. Cancer Inst. 93 (2001) 1638–1643.
[12] T. Murakami, W. Maki, A.R. Cardones, et al., Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells, Cancer Res. 62 (2002) 7328–7334.
[13] Z.X. Liang, T. Wu, H. Lou, et al., Inhibition of breast cancer metastasis by selective synthetic polypeptide against CXCR4, Cancer Res. 64 (2004) 4302–4308.
[14] M.C.P. Smith, K.E. Luker, J.R. Garbow, et al., CXCR4 regulates growth of both primary and metastatic breast cancer, Cancer Res. 64 (2004) 8604–8612.
[15] H. Xia, Y. Qi, S.S. Ng, et al., microRNA-146b inhibits glioma cell migration and invasion by targeting MMPs, Brain Res. 1269 (2009) 158–165.
[16] D.N. Louis, H. Ohgaki, O.D. Wiestler, et al., The 2007 WHO classification of tumours of the central nervous system, Acta Neuropathol. 114 (2007) 97–109.
[17] S.X. Yang, J.H. Chen, X.F. Jiang, et al., Activation of chemokine receptor CXCR4 in malignant glioma cells promotes the production of vascular endothelial growth factor (vol 335, pg 523, 2005), Biochem. Biophys. Res. Commun. 337 (2005) 411.
[18] A. Sehgal, C. Keener, A.L. Boynton, et al., CXCR-4, a chemokine receptor, is overexpressed in and required for proliferation of glioblastoma tumor cells, J. Surg. Oncol. 69 (1998) 99–104.
[19] S.A. Rempel, S. Dudas, S.G. Ge, et al., Identification and localization of the cytokine SDF1 and its receptor, CXC chemokine receptor 4, to regions of necrosis and angiogenesis in human glioblastoma, Clin. Cancer Res. 6 (2000) 102–111.
[20] Y. Zhou, P.H. Larsen, C.H. Hao, et al., CXCRA is a major chemokine receptor on glioma cells and mediates their survival, J. Biol. Chem. 277 (2002) 49481–49487.
[21] A. Bajetto, F. Barbieri, A. Dorcaratto, 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. 49 (2006) 423–432.
[22] P. Fan, H. Kyaw, K. Su, et al., Cloning and characterization of a novel human chemokine receptor, Biochem. Biophys. Res. Commun. 243 (1998) 264–268.
[23] I. Migeotte, J.D. Franssen, S. Goriely, et al., Distribution and regulation of expression of the putative human chemokine receptor HCR in leukocyte populations, Eur. J. Immunol. 32 (2002) 494–501.
[24] C.J. Raport, J. Gosling, V.L. Schweickart, et al., Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1 beta, and MIP-1 alpha, J. Biol. Chem. 271 (1996) 17161–17166.
[25] A. Maho, A. Bensimon, G. Vassart, et al., Mapping of the CCXCR1, CX3CR1, CCBP2 and CCR9 genes to the CCR cluster within the 3p21.3 region of the human genome, Cytogenet. Cell Genet. 87 (1999) 265–268.
[26] C.L. Galligan, W. Matsuyama, A. Matsukawa, et al., Up-regulated expression and activation of the orphan chemokine receptor, CCRL2, in rheumatoid arthritis, Arthritis Rheum. 50 (2004) 1806–1814.
[27] M.W. Zuurman, J. Heeroma, N. Brouwer, et al., LPS-induced expression of a novel chemokine receptor in (L-CCR) mouse glial cells in vitro and in vivo, Glia 41 (2003) 327–336.
[28] K. Biber, M.W. Zuurman, H. Homan, et al., Expression of L-CCR in HEK 293 cells reveals functional responses to CCL2 CCL5 CCL7 and CCL8, J. Leukoc. Biol. 74 (2003) 243–251.
[29] K. Otero, A. Vecchi, E. Hirsch, et al., Nonredundant role of CCRL2 in lung dendritic cell trafficking, Blood 116 (2010) 2942–2949.
[30] M. Ehtesham, K.Y. Mapara, C.B. Stevenson, et al., CXCR4 mediates the proliferation of glioblastoma progenitor cells, Cancer Lett. 274 (2009) 305–312.
[31] P.A. Agudelo-Garcia, J.K. De Jesus, S.P. Williams, et al., Glioma cell migration on three-dimensional nanofiber scaffolds is regulated by substrate topography and abolished by inhibition of STAT3 signaling, Neoplasia 13 (2011) U831–U896.
[32] M. Onishi, T. Ichikawa, K. Kurozumi, et al., Angiogenesis and invasion in glioma, Brain Tumor Pathol. 28 (2011) 13–24.
[33] B.B. Tysnes, R. Mahesparan, Biological mechanisms of glioma invasion and potential therapeutic targets, J. Neurooncol. 53 (2001) 129–147.
[34] A. Kwiatkowska, M. Symons, Signaling determinants of glioma cell invasion, Adv. Exp. Med. Biol. 986 (2013) 121–141.
[35] P. Friedl, K. Wolf, Tumour-cell invasion and migration: diversity and escape mechanisms, Nat. Rev. Cancer 3 (2003) 362–374.
[36] S.G. Ward, K. Bacon, J. Westwick, Chemokines and T lymphocytes: more than an attraction, Immunity 9 (1998) 1–11.
[37] B.A. Zabel, S. Nakae, L. Zuniga, et al., Mast cell-expressed orphan receptor CCRL2 binds chemerin and is required for optimal induction of IgE-mediated passive cutaneous anaphylaxis, J. Exp. Med. 205 (2008) 2207–2220.