RESEARCH ARTICLE

Human glioma tumors express high levels of the chemokine receptor CX3CR1

Marco Locatelli[1], Leonardo Boiocchi[1,2], Stefano Ferrero[2], Filippo Martinelli Boneschi[3], Mario Zavanone[1,4], Samantha Pesce[5], Paola Allavena[5], Sergio Maria Gaini[1,4], Lorenzo Bello[1,4], Alberto Mantovani[5,6]

[1] Neurosurgery, Fondazione IRCCS, Ca'Granda Ospedale Maggiore Policlinico Mangiagalli e Regina Elena, Milan
[2] II Cattedra di Anatomia Patologica, Dipartimento di Medicina, Chirurgia e Odontoiatria, University of Milan, A.O. San Paolo Milan
[3] Institute of Experimental Neurology (INSPE), Scientific Institute San Raffaele, Milan
[4] Department of Neurological Sciences, University of Milan
[5] Istituto Clinico Humanitas IRCCS, Rozzano, Milan
[6] Department of Translational Medicine, University of Milan, Italy

Correspondence: Prof. A. Mantovani, Istituto Clinico Humanitas IRCCS, Via Manzoni 56, 20089 Rozzano, Milan, Italy
<Alberto.mantovani@humanitas.it>

ABSTRACT. The chemokine receptor CX3CR1 and its cognate ligand CX3CL1 (also known as fractalkine), are involved in central nervous system pathophysiology, in particular, in the cross-talk between neurons and microglia. It was therefore important to investigate the expression of CX3CR1 in gliomas, the most frequently occurring, malignant brain tumors. In a consecutive series of 70 patients with primary, central nervous glial tumors, CX3CR1 was highly expressed in tumor cells as assessed by RT-PCR mRNA and protein levels, and by immunohistochemistry, while the corresponding normal cells were negative. Receptor immuno-positivity did not correlate with histology, grade, chromosomal (1p,19q) deletion, or with methylation of the DNA repair gene promoter *MGMT* (O6-methylguanine-DNA methyltransferase). Thus, CX3CR1 expression is a frequent event in gliomas, irrespective of tumor classification and clinical severity. The molecular basis underlying CX3CR1 up-regulation and its functional biological significance remain to be determined.

Keywords: gliomas, chemokines, CX3CR1, CX3CL1, histochemistry

Diffuse gliomas are the most common malignant tumors of the brain. They include heterogeneous tumors that are classified, according to their pathological characteristics, as astrocytomas, oligodendrogliomas, and glioblastomas. The World Health Organization (WHO) has defined a malignancy scale: usually astrocytomas and oligodendrogliomas are grade II (or III in the anaplastic form), while glioblastomas are grade IV and are considered highly malignant [1-3]. In spite of optimal treatment, patients with glioblastomas survive less than one year and prognosis has not changed in the last two decades. These tumors have a rapidly expanding nature and invade the normal brain by active cell migration. The migratory ability of glioma cells has been investigated by electron microscopy, and it was shown that neoplastic cells easily adjust their shape and size to slip through the narrow extracellular brain spaces, a process that requires Cl$^-$ Channels [4].

It is now established that migrating malignant cells may exploit chemokine receptors to invade surrounding tissues and leading to distant metastasis [5, 6]. Chemokines are a large family of chemotactic factors inducing cell motility in several cell types [7, 8]. Chemokines have been mostly studied for their potent effect on the recruitment of leukocytes at sites of inflammation; however, it has become increasingly clear that tumors also express functional chemokine receptors [5, 6, 9, 10]. In addition to cell mobilization and metastatic ability, other important roles - relevant to tumor biology - have been attributed to the chemokine system, *e.g.* enhanced tumor cell proliferation, resistance to apoptosis and regulation of angiogenesis [5].

A number of studies investigated the expression of chemokine receptors in tumors, including gliomas. mRNA for receptors of the CXC subfamily have been reported, with CXCR4 being the most frequently expressed [5, 11-13]. Furthermore, the presence of CXCR4 has been associated with the most aggressive forms of gliomas and with poor patient survival [14, 15]. Cancer stem cells isolated from glioblastoma are positive for CXCR4 and treatment with the specific ligand CXCL12 stimulates their proliferation [15].

In this study, we have explored the expression of the chemokine receptor CX3CR1 in human gliomas. Physiologically, CX3CR1 is predominantly expressed by leukocytes such as monocytes, NK and Th1 lymphocytes, and

mediates adhesion and migration through the endo-thelium, the latter expressing the specific ligand CX3CL1 as a trans-membrane protein [16-19]. In the brain, CX3CR1 is expressed by the microglia, while neurons produce the ligand CX3CL1 (originally identi-fied as neurotactin or fractalkine) [20-22]. A few studies have documented an exception to this rule: in different species and conditions, neurons may also express the receptor [23, 24], while positivity for CX3CR1 in glial cells was more controversial [23-26].

The ligand CX3CL1 is one of the most expressed che-mokines in the brain [21, 22, 25]. Experimental evidence has established that the CX3CR1/CX3CL1 axis plays a major role in the neuron/microglia cross-talk, and in neuro-protection under conditions of inflammation/injury [22, 27-33].

We show in this study, involving a large case list of human gliomas, that neoplastic cells strongly express the CX3CR1. Receptor expression already occurs in low-grade tumors, suggesting that its up-regulation is an early event during malignant transformation.

METHODS AND MATERIALS

Patients

Seventy consecutive patients with primary CNS tumors, who attended the Neurosurgery Division of IRCCS Ospe-dale Maggiore Policlinico, Mangiagalli and Regina Elena, Milan, Italy, between 2005 and 2007 were enrolled in this study. Tumor specimens were diagnosed according to the last 2000 WHO classification: olidoden-drogliomas (n = 23); low-grade astrocytomas (n = 9), high-grade astrocytomas or anaplastic astrocytomas (n = 10), glioblastomas (n = 23), and oligoastrocytomas (n = 5). Informed consent was obtained from all patients.

Immunohistochemistry of glioblastoma samples for CX3CR1

All specimens were reviewed independently by two pathologists (SF and LB) blinded to the diagnosis and clinical data. From each block, three sections were selected, and deparaffinized in xylene. Antigen retrieval was performed using sodium citrate buffer (pH 6.0) in a microwave oven, three times for five minutes and sam-ples were stained (Genomix i-6000, BioGenex, San Ramon CA, USA) with rabbit polyclonal anti-human CX3CR1 antibody (Abcam, Cambridge, UK; 1:350 dilu-tion, overnight at 4°C). Reactions were revealed using NovoLink Polymer Detection System (Novocastra), according to the manufacturer's instructions. After a dia-minobenzidine reaction (DAB; Liquid DAB + Substrate Chromogen System, DakoCytomation), sections were counterstained with hematoxylin (Mayer, DIAPATH). We evaluated the percentage of positive tumor cells and the intensity of the staining. A semiquantitative four-grades scoring system was used for the evaluation of the percentage of positive neoplastic cells. Score 0: no immunoreactivity; score 1: < 10% of neoplastic cells were immunoreactive; score 2: immunoreactivity

between > 10% and < 50%; score 3: immunoreactiv-ity > 50%. Staining intensity was scored: 0: for no staining, 1: faint staining, 2: moderate and 3: strong. We multiplied these two scores (positive cells % × intensity) to obtain a final score with a continuous distribution.

RNA extraction and quantitative real-time RT-PCR (Q-PCR) for CX3CR1 mRNA

Total RNA was isolated from the following frozen tissue specimens: oligodendroglioma (n = 4); astrocytoma (n = 2); glioblastoma (n = 3). RNA was exctracted using TRI Reagent (Ambion), as previously described [34], and quantified using a Nanodrop Spectrophotometer ND-1000. Ten μg of RNA were treated with Turbo DNA-*free* (Ambion) to eliminate genomic DNA contamination. Two μg of total RNA were reverse-transcribed using the High Capacity cDNA Archive Kit (Applied Biosystems) according to the manufacturer's instruction. CX3XR1 mRNA expression was analyzed using SYBR green-based quantitative real time RT-PCR (Q-PCR) as previ-ously described [34]. 18S was used as an internal control to normalize samples. Specific primers were computer-designed:
18S:
Forward: 5′ CGC CGC TAG AGG TGA AAT TC 3′
Reverse: 5′ CTT TCG CTC TGG TCC GTC TT 3′
CX3CR1 :
Forward: 5′ GGG ACT GTG TTC CTG TCC AT 3′
Reverse: 5′ GAC ACT CTT GGG CTT CTT GC 3′
The amount of CX3CR1 mRNA relative to the house-keeping gene 18S was calculated as $2^{-\Delta Ct}$, where $\Delta Ct = Ct_{CX3CR1} - Ct_{18S}$. The threshold cycle Ct was auto-matically given by the SDS2.2 software package (Applied Biosystems).

Loss of heterozygosity at chromosomes 1p and 19q

Tumor DNA was extracted from paraffin-embedded tissues using the DNeasy Tissue Kit (QIAGEN, Inc. Milano, Italy) according to the manufacturer's protocol. Following DNA extraction, all tumor samples were sub-jected to control gene (PGK) amplification to assess DNA integrity. Constitutional DNA from peripheral blood leukocytes was isolated using the standard phenol/chloroform extraction method with ethanol pre-cipitation. Constitutional DNA/ tumor DNA pairs were evaluated by standard PCR-based LOH assays, as described. [35]

Methylation of the MGMT (O 6-methylguanine-DNA methyltransferase) promoter

The methylation status of the MGMT promoter gene was determined using methylation-specific PCR. Tumor DNA from paraffin embedded tissues (10 μm sections) was modified by sodium bisulfite, which converts unmethy-lated, but not methylated, cytosine to uracil, as described. [36, 37] Modified DNA was submitted to methylation-specific polymerase chain reaction (MSP) after a nested-polymerase chain reaction protocol. The following primers were used:

MGMT
Forward: 5′ GGATATGTTG GGATAGTT 3′; Reverse: 5′ CCAAAAACCCCAAACCC 3′.
The PCR products were separated on 4% agarose gels.

Statistical analysis

Statistical analysis was performed using the following non-parametric test: 1) the Kruskall-Wallis test and the Wilcoxon rank sum test for the comparison of the CX3CR1 scores respectively, in four categories of brain tumor severity (low-grade, low-grade recurrent, high-grade and high-grade recurrent), and in two categories based on the histopathological diagnosis (WHO grade II *versus* grade III); 2) The Wilcoxon rank sum test was used to compare the CX3CR1 score in brain biopsies with or without loss of heterozygosity (LOH) at chromosomes 1p and 19q, and with or without MGMT methylation. Moreover, a multivariate logistic regression model was built to assess the potential effect of demographic variables (age and sex), and brain biopsy-related variable (CX3CR1 score; presence of LOH; presence of MGMT methylation), on the risk of having a high-grade tumor. The coefficient of determination (Nagelkerke Pseudo R^2) was used as a measure of the percentage of the total variance explained by the different models. The strength of the association between predictors and the dependent variables was assessed by means of odds ratio (OR) and relative 95% confidence intervals (CI).

RESULTS

Seventy patients affected by cerebral tumors, including low-grade or type II WHO severity (oligodendroglioma; astrocytoma) and high grade or type III-IV WHO severity (glioblastoma and anaplastic oligodendroglioma) were involved in this study. Patients (40 males and 30 females) had a mean age of 42.9 years (SD 12.8 years). Thirty-nine out of the 70 (55.7%) were affected by low-grade tumors, and the remaining (44.3%) had high-grade tumors. Comparison of clinical characteristics between high- and low-grade tumors revealed that the male:female ratio was similar in the two groups (1.60 *versus* 1.56; Chi-square analysis: p = 0.95), while age was greater in high-grade tumors (49.5 *versus* 40.2; p = 0.003). Immunohistochemical evaluation of CX3CR1 was performed with a specific anti-CX3CR1 antibody on tumor sections obtained at surgery. The results are shown in *figure 1* and are summarized for all cases studied in *figure 2*. We found that immunoreactivity for CX3CR1 in normal brain was faint in scattered cells *(figure 1A)*, while tumor cells of each histological type showed a strong immunopositivity: *figure 1B* depicts a case of low-grade astrocytoma, while panels C-F show four cases of glioblastoma. Only a few samples of oligodendrogliomas showed a weak expression, although this was seen in more than 50% of cells. When the CX3CR1 score was compared in categories of tumor severity defined as low-grade, low-grade recurrent, high-grade and high-grade recurrent, no statistically significant difference

was found (p = 0.72). Similar results were obtained when low-grade tumors were stratified into oligodendroglioma (n = 32) and astrocytoma (n = 7) (p = 0.42). Overall, the marked expression of CX3CR1 was similar across low- and high-grade tumors based on the histopathological diagnosis (median value: 9.0; p = 0.30). Although no statistically significant difference was found, weak CX3CR1 scores were observed only in low-grade oligodendroglioma. Multivariate models, including age and gender as covariates, did not substantially changed the results.

To confirm receptor expression, CX3CR1 mRNA was studied in selected tumor tissues. *Figure 3* shows the levels of mRNA CX3CR1 in four cases of oligodendroglioma, two astrocytoma and three glioblastoma.

Ongoing efforts are aimed to identify biological and genetic alterations in brain tumors that may provide additional prognostic information, as well as guidance for making decisions about optimal therapy. We therefore considered other pathological variables reported to occur in malignant gliomas. Epigenetic silencing of the MGMT gene by promoter methylation has been associated with longer survival in glioblastoma patients receiving both radiotherapy and chemotherapy. Methylation of the *MGMT* promoter was detected in 77.6% of patients tested (45/62), and was less frequent in high-grade tumors (66.7% *versus* 85.3%; p = 0.09 Fisher's exact test).

Another biological variable currently analyzed in malignant gliomas is the loss of heterozygosity (LOH), at chromosomes 1p and 19q because of its correlation with histology and chemotherapy response, especially in oligodendrogliomas. [38, 39] LOH at either 1p or 19q was present in 53.3% of patients tested (32/60), and again was less frequent in high-grade than in low-grade tumors (34.8% *versus* 64.9%); p = 0.03 Fisher's exact test.

When all the variables were tested in a multivariate logistic regression model, the presence of chromosomal deletion at 1p or 19q was associated with a lower risk of high-grade tumor (OR: 0.2; 95% CI: 0.1-0.8; p = 0.02; *table 1*), while there was no apparent influence of either MGMT gene methylation or CX3CR1 expression *(table 1)*. The statistical model was able to explain 27.6% of the total variance of the dependent variable.

DISCUSSION

We show in this study that human gliomas have mRNA and immunopositivity for the chemokine receptor CX3CR1. Expression was evident even in low-grade tumors and was highest in glioblastomas. Expression of CX3CR1 by cancer cells has been poorly investigated: prostate tumors express the receptor, which is involved in metastasis to bone marrow [40, 41]. We have reported that human pancreatic tumors upregulate CX3CR1, while the normal pancreatic tissue is negative [34]. Recently, the involvement of the CX3CR1 receptor in the transmigration of neuroblastoma cells through bone-marrow endothelial cells has been reported [42]. Glial tumors have been investigated for the expression of several chemokine receptors, mainly CXCR4 [14, 15], but poorly studied for CX3CR1. Using a
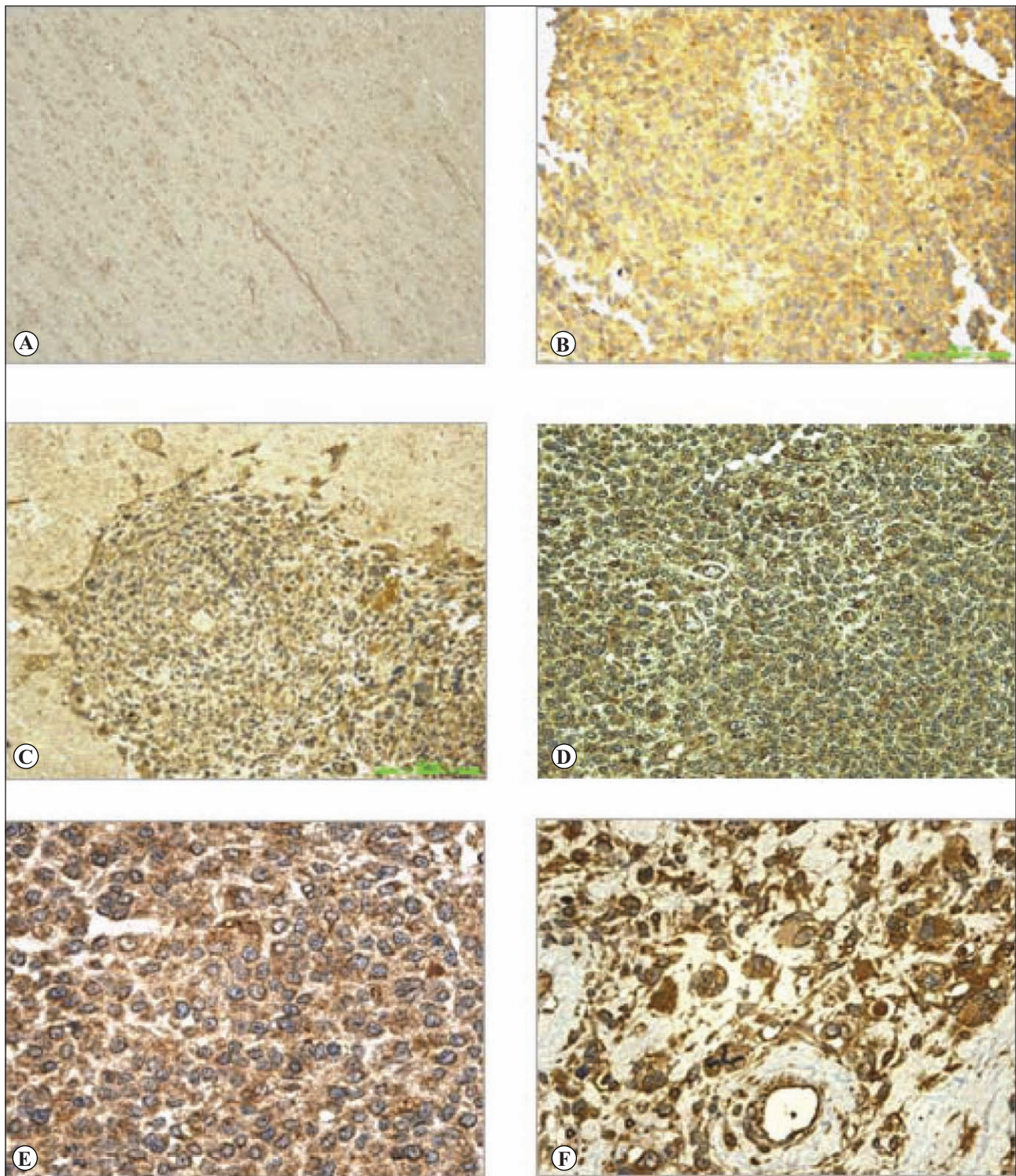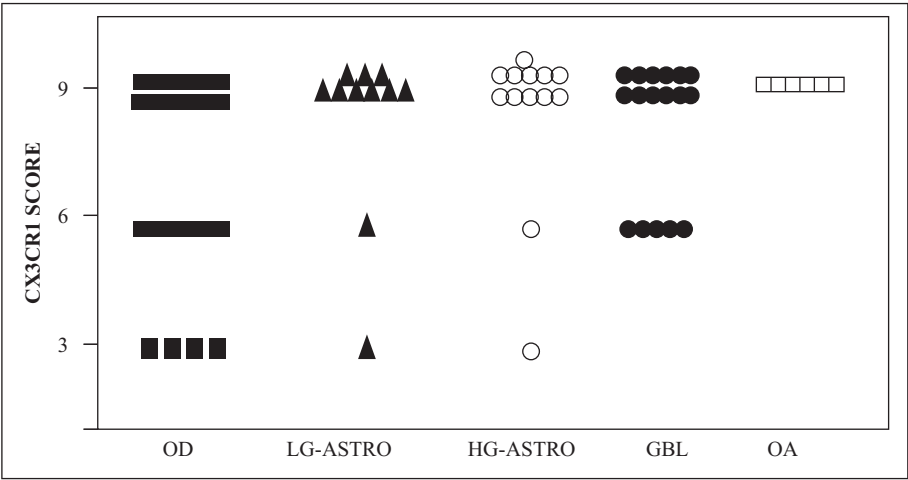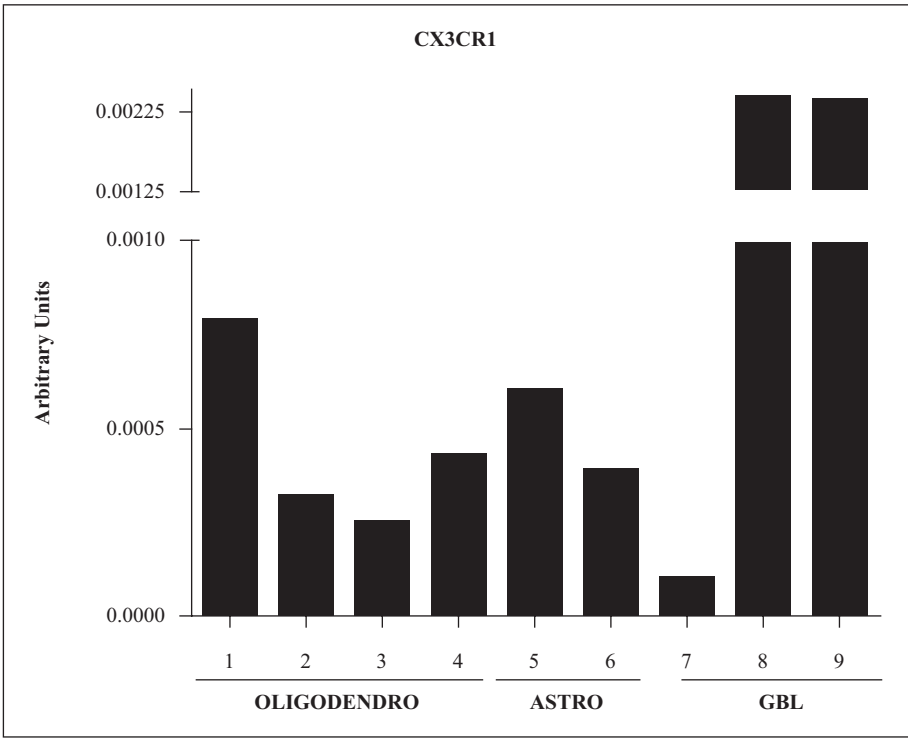
Figure 1
Glial tumors express the chemokine receptor CX3CR1. Immunohistochemistry of surgical samples of glial tumors stained with an anti-CXC3R1 antibody. **A)** Section of normal brain adjacent to a tumor tissue (magnification: 10 x). **B)** Low-grade astrocytoma (20 x). **C-F)** Glioblastomas (magnification: **C, D**: 10 x; **E**: 20 x; **F**: 40 x).

murine model of glioma obtained by intracranial injection of 3-methylcholantrene, Liu *et al.* showed a positive, *in situ* hybridization for CX3CR1 that corresponded however, to the localization of CD11b-positive microglia [43]. In human gliobastoma, Rodero *et al.* report a diffuse expression of CX3CR1, but mainly concentrate on the functional defects of polymorphic CX3CR1 receptor associated with infiltrating immune cells [44].

Increased expression of both CX3CR1 and its ligand occurs in different neuro-inflammatory conditions (e.g. infections, toxic insults and nerve injuries). Higher production and shedding from the membrane of CX3CL1 results in a higher density of CX3CR1+ inflammatory microglia recruited in the brain [30, 32, 45-47]. In experimental conditions, cytokines such as TNF and TGFβ, were responsible for the upregulation of both

Figure 2
Distribution of final scores for CX3CR1 (positive cells % x intensity) in the different tumor types. Every case is represented.
OD: oligodendrocytomas; LG-ASTRO: low-grade astrocytomas; HG-ASTRO: high-grade astrocytomas; GB: glioblastomas; OA: oligoastrocytomas.

Figure 3
CX3CR1 mRNA analysis of tumor tissues in surgical samples of oligodendrocytomas (1-4), astrocytomas (5, 6), glioblastomas (7-9). The relative amount of CX3CR1 mRNA was calculated with reference to the expression of the housekeeping gene 18S.

ligand and receptor in neurons and microglia, respectively [48]. These mediators are frequently present in tumors, including gliomas [9, 49-51], and may well be involved in the modulation of the CX3CL1/CX3CR1 axis in neoplastic conditions.

The biological significance of the up-regulation of CX3CR1 by glioma cells remains unclear. The constitutive expression, in the brain, of CX3CL1 and CX3CR1 has been the subject of intense investigation. Experimental evidence indicates a role for CX3CL1 in promoting neuronal survival in glutamate-mediated excitoxicity [32]. Enhanced neuron loss occurs in CX3CR1-

deficient mice after systemic lipopolysaccharide injection, in toxin-induced Parkinsonism, and in the SOD1^{G93A} transgenic mouse model of motor neuron disease [22]. In addition, CX3CL1 regulates microglia functions, inducing mobilization of intracellular Ca^{2+}, chemotaxis, inhibition of Fas-mediated apoptosis and of LPS-induced activation [28, 29, 31].

As CX3CL1 is a membrane-bound chemokine, the ligand/receptor axis can function as an adhesion molecule. CX3CR1 positive tumor cells may have enhanced adhesion to neurons expressing the ligand. In pancreatic cancer, we demonstrated that tumor samples with high

Table 1
Multivariate logistic regression analysis. Dependent variable: risk of having a WHO-type III tumor. Predictors: age; gender; CX3CR1 score; chromosomal 1p and 19q deletion; MGMT gene promoter methylation. OR: Odds ratio; 95% CI: 95% confidence intervals; P: p value according to the logistic regression analysis

Variables	OR	95% CI	P
Age	1.04	0.9-1.2	0.29
Gender: Female (Ref.)	1.0		
Male	0.70	0.2-2.5	0.58
CX3CR1 score	0.97	0.6-1.5	0.9
Chromosomal deletion 1p and/or 19q	0.20	0.1-0.8	0.02
MGMT gene promoter methylation	0.38	0.1-1.7	0.21

receptor scores have higher percentage of local nerve terminations infiltrated by cancer cells [34]. Tumor perineural tropism and dissemination along cerebral fibre tracts also occur in malignant glioblastoma [1-3].

In conclusion, the results reported here show that expression of the chemokine receptor CX3CR1 is a frequent event in human gliomas, irrespective of histology and grading. The molecular basis underlying CX3CR1 up-regulation and its functional biological significance remain to be determined.

REFERENCES

1. Schomas DA, Laack NN, Rao RD, et al. Intracranial Low-Grade Gliomas in Adults: 30-Year Experience With Long-term Follow-up at Mayo Clinic. *Neuro Oncol* 2009; 4: 437.

2. Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med* 2008; 359: 492.

3. Meyer MA. Malignant gliomas in adults. *N Engl J Med* 2008; 359: 1850 (author reply).

4. McCoy E, Sontheimer H. Expression and function of water channels (aquaporins) in migrating malignant astrocytes. *Glia* 2007; 55: 1034.

5. Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer* 2004; 4: 540.

6. Zlotnik A. Chemokines and cancer. *Int J Cancer* 2006; 119: 2026.

7. Luster AD. Chemokines:chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998; 338: 436.

8. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 2006; 354: 610.

9. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; 454: 436.

10. Sozzani S, Bonecchi R, D'Amico G, et al. In vitro and in vivo regulation of chemokine receptors. *Eur Cytokine Netw.* 2000; 11: 502-3.

11. Rempel SA, Dudas S, Ge S, Gutierrez JA. 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* 2000; 6: 102.

12. Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood* 2006; 107: 1761.

13. Bajetto A, Barbieri F, Dorcaratto A, 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.

14. Bian XW, Yang SX, Chen JH, et al. Preferential expression of chemokine receptor CXCR4 by highly malignant human gliomas and its association with poor patient survival. *Neurosurgery* 2007; 61: 570.

15. Ehtesham M, Mapara KY, Stevenson CB, Thompson RC. CXCR4 mediates the proliferation of glioblastoma progenitor cells. *Cancer Lett* 2009; 274: 305.

16. Imai T, Hieshima K, Haskell C, et al. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 1997; 91: 521-30.

17. Haskell CA, Cleary MD, Charo IF. Molecular uncoupling of fractalkine-mediated cell adhesion and signal transduction. Rapid flow arrest of CX3CR1-expressing cells is independent of G-protein activation. *J Biol Chem* 1999; 274: 10053.

18. Goda S, Imai T, Yoshie O, et al. CX3C-chemokine, fractalkine-enhanced adhesion of THP-1 cells to endothelial cells through integrin-dependent and -independent mechanisms. *J Immunol* 2000; 164: 4313.

19. Fraticelli P, Sironi M, Bianchi G, et al. Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses. *J Clin Invest* 2001; 107: 1173.

20. Pan Y, Lloyd C, Zhou H, et al. Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 1997; 387: 611.

21. Hulshof S, van Haastert ES, Kuipers HF, et al. CX3CL1 and CX3CR1 expression in human brain tissue: noninflammatory control versus multiple sclerosis. *J Neuropathol Exp Neurol* 2003; 62: 899.

22. Cardona AE, Pioro EP, Sasse ME, et al. Control of microglial neurotoxicity by the fractalkine receptor. *Nat Neurosci* 2006; 9: 917.

23. Meucci O, Fatatis A, Simen AA, Bushell TJ, Gray PW, Miller RJ. Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proc Natl Acad Sci USA* 1998; 95: 14500.

24. Meucci O, Fatatis A, Simen AA, Miller RJ. Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival. *Proc Natl Acad Sci USA* 2000; 97: 8075.

25. Hatori K, Nagai A, Heisel R, Ryu JK, Kim SU. Fractalkine and fractalkine receptors in human neurons and glial cells. *J Neurosci Res* 2002; 69: 418.

26. Maciejewski-Lenoir D, Chen S, Feng L, Maki R, Bacon KB. Characterization of fractalkine in rat brain cells: migratory and activation signals for CX3CR-1-expressing microglia. *J Immunol* 1999; 163: 1628.

27. Chen SH, Bacon KB, Li L, et al. In vivo inhibition of CC and CX3C chemokine-induced leukocyte infiltration and attenuation of glomerulonephritis in Wistar-Kyoto (WKY) rats by vMIP-II. *J Exp Med* 1998; 188: 193.

28. Zujovic V, Benavides J, Vige X, Carter C, Taupin V. Fractalkine modulates TNF-alpha secretion and neurotoxicity induced by microglial activation. *Glia* 2000; 29: 305.

29. Boehme SA, Lio FM, Maciejewski-Lenoir D, Bacon KB, Conlon PJ. The chemokine fractalkine inhibits Fas-mediated cell death of brain microglia. *J Immunol* 2000; 165: 397.

30. Hughes PM, Botham MS, Frentzel S, Mir A, Perry VH. Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, during acute and chronic inflammation in the rodent CNS. *Glia* 2002; 37: 314.

31. Mizuno T, Kawanokuchi J, Numata K, Suzumura A. Production and neuroprotective functions of fractalkine in the central nervous system. *Brain Res* 2003; 979: 65.

32. Limatola C, Lauro C, Catalano M, *et al.* Chemokine CX3CL1 protects rat hippocampal neurons against glutamate-mediated excitotoxicity. *J Neuroimmunol* 2005; 166: 19.

33. Miller RJ, Rostene W, Apartis E, *et al.* Chemokine action in the nervous system. *J Neurosci* 2008; 28: 11792.

34. Marchesi F, Piemonti L, Fedele G, *et al.* The chemokine receptor CX3CR1 is involved in the neural tropism and malignant behavior of pancreatic ductal adenocarcinoma. *Cancer Res* 2008; 68: 9060.

35. Barbashina V, Salazar P, Holland EC, Rosenblum MK, Ladanyi M. Allelic losses at 1p36 and 19q13 in gliomas: correlation with histologic classification, definition of a 150-kb minimal deleted region on 1p36, and evaluation of CAMTA1 as a candidate tumor suppressor gene. *Clin Cancer Res* 2005; 11: 1119.

36. Palmisano WA, Divine KK, Saccomanno G, *et al.* Predicting lung cancer by detecting aberrant promoter methylation in sputum. *Cancer Res* 2000; 60: 5954.

37. Hegi ME, Diserens AC, Gorlia T, *et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; 352: 997.

38. Smith JS, Alderete B, Minn Y, *et al.* Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. *Oncogene* 1999; 18: 4144.

39. Mason WP, Cairncross JG. Invited article: the expanding impact of molecular biology on the diagnosis and treatment of gliomas. *Neurology* 2008; 71: 365.

40. Shulby SA, Dolloff NG, Stearns ME, Meucci O, Fatatis A. CX3CR1-fractalkine expression regulates cellular mechanisms involved in adhesion, migration, and survival of human prostate cancer cells. *Cancer Res* 2004; 64: 4693.

41. Jamieson WL, Shimizu S, D'Ambrosio JA, Meucci O, Fatatis A. CX3CR1 is expressed by prostate epithelial cells and androgens regulate the levels of CX3CL1/fractalkine in the bone marrow: potential role in prostate cancer bone tropism. *Cancer Res* 2008; 68: 1715.

42. Nevo I, Sagi-Assif O, Meshel T, *et al.* The involvement of the fractalkine receptor in the transmigration of neuroblastoma cells through bone-marrow endothelial cells. *Cancer Lett* 2009; 273: 127.

43. Liu C, Luo D, Streit WJ, Harrison JK. CX3CL1 and CX3CR1 in the GL261 murine model of glioma: CX3CR1 deficiency does not impact tumor growth or infiltration of microglia and lymphocytes. *J Neuroimmunol* 2008; 198: 98.

44. Rodero M, Marie Y, Coudert M, *et al.* Polymorphism in the microglial cell-mobilizing CX3CR1 gene is associated with survival in patients with glioblastoma. *J Clin Oncol* 2008; 26: 59574.

45. Harrison JK, Jiang Y, Chen S, *et al.* Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. *Proc Natl Acad Sci USA* 1998; 95: 10896.

46. Tarozzo G, Campanella M, Ghiani M, Bulfone A, Beltramo M. Expression of fractalkine and its receptor, CX3CR1, in response to ischaemia-reperfusion brain injury in the rat. *Eur J Neurosci* 2002; 15: 1663.

47. Erichsen D, Lopez AL, Peng H, *et al.* Neuronal injury regulates fractalkine: relevance for HIV-1 associated dementia. *J Neuroimmunol* 2003; 138: 144.

48. Chen S, Luo D, Streit WJ, Harrison JK. TGF-beta1 upregulates CX3CR1 expression and inhibits fractalkine-stimulated signaling in rat microglia. *J Neuroimmunol* 2002; 133: 46.

49. Weller M, Fontana A. The failure of current immunotherapy for malignant glioma. Tumor-derived TGF-beta, T-cell apoptosis, and the immune privilege of the brain. *Brain Res Brain Res Rev* 1995; 21: 128.

50. Wick W, Platten M, Weller M. Glioma cell invasion: regulation of metalloproteinase activity by TGF-beta. *J Neurooncol* 2001; 53: 177.

51. Balkwill F. Tumour necrosis factor and cancer. *Nat Rev Cancer* 2009; 9: 361.