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CXCR4 Expression is Elevated in Glioblastoma Multiforme and Correlates with an Increase in Intensity and Extent of Peritumoral T2-weighted Magnetic Resonance Imaging Signal Abnormalities

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Abstract

Objective—With the objective of investigating the utility of CXCR4, a chemokine receptor known to mediate glioma cell invasiveness, as a molecular marker for peritumoral disease extent in high-grade gliomas, we sought to characterize the expression profile of CXCR4 in a large panel of tumor samples and determine whether CXCR4 expression levels within glioblastoma multiforme might correlate with radiological evidence of a more extensive disease process.

Methods—Freshly resected tumor tissue samples were processed for immunohistochemical and quantitative polymerase chain reaction analyses to identify and quantify expression levels of CXCR4 and its corresponding ligand CXCL12. T1 postcontrast and T2-weighted magnetic resonance imaging brain scans were used to generate voxel signal intensity histograms that were quantitatively analyzed to determine the extent and intensity of peritumoral signal abnormality as a marker of disseminated disease in the brain.

Results—CXCR4 expression was markedly elevated in Grade III and IV tumors compared with Grade II gliomas. Significantly, when patients with glioblastoma multiforme were segregated into two groups based on CXCR4 expression level, we observed a statistically significant increase in the intensity and extent of peritumoral magnetic resonance imaging signal abnormalities associated with CXCR4 high-expressing gliomas.

Conclusion—Our data confirm that high-grade gliomas robustly express CXCR4 and demonstrate a correlative relationship between expression levels of the CXCR4 receptor and the magnetic resonance imaging-based finding of a diffuse and more extensive disease process in the brain. CXCR4 expression status may, therefore, prove useful as a marker of disseminated disease in patients with glioblastoma multiforme.

Keywords

Brain tumor; CXCR4; Glioma

Gliomas are the most common type of primary brain tumor and, according to the World Health Organization convention, are divided into four clinical grades (I–IV) on the basis of tumor histopathology (11). Grade I tumors represent a distinct subset of neoplasms occurring predominantly in children and young adults and are characterized as relatively benign lesions with well-demarcated margins and lending themselves to surgical cure. In contrast to this, Grades II, III, and IV gliomas are generally tumors of adulthood and reflect a spectrum of increasingly infiltrative and aggressive disease. These tumors have poorly defined margins and invade directly adjacent brain parenchyma, thereby precluding complete surgical resection. Despite recent advances in adjuvant radio- and chemotherapeutic regimens, current clinical treatment protocols fail to eliminate residual infiltrative tumor foci in their entirety. Additionally, invasive nests of tumor cells are associated with the initiation of neovascular angiogenesis and disruption of the blood-brain barrier via the release of vasomodulatory cytokines. Taken together, these factors contribute to a rapid dissemination of the disease process beyond the confines of the original radiologically identifiable tumor mass, eventually resulting in near-universal tumor recurrence and, ultimately, death. Whereas patients with Grade II tumors can survive for 7 to 15 years, a diagnosis of a Grade IV glioma, or glioblastoma multiforme (GBM), carries a median survival prognosis of approximately 9 months (17). GBM also represents the most infiltrative spectrum of this disease and is frequently associated with significant involvement of peritumoral parenchyma as evidenced by the presence of extensive T2 signal abnormalities on magnetic resonance imaging (MRI). Indeed, postmortem analyses in GBM-affected brains have demonstrated the presence of tumor cells deep in the contralateral hemisphere (15). Current therapeutic regimens do not adequately address the disseminated disease burden associated with infiltrative gliomas, and there is, therefore, an urgent need to develop novel treatment approaches to specifically target the invasive capacity of these tumors.

The clinical evaluation of patients with high-grade gliomas centers on the use of T1-weighted MRI sequences obtained after the administration of a gadolinium-based contrast agent to demarcate the boundaries of what is generally considered to be the surgically targetable main tumor mass. Additionally, T2-weighted imaging is used to assess the extent of the peritumoral disease process. T2-weighted signal abnormalities surrounding the T1-weighted postgadolinium-enhancing lesion are attributable to multiple facets of a progressively disseminating disease process that is inexorably extending its effects throughout the brain. In this context, our definition of glioma-associated disseminated disease would include the presence of microscopic infiltrative disease (8), foci of tumor-induced neovascularization, and generalized vasogenic edema resulting from cytokines elaborated by invasive glioma cells. As such, the extent and intensity of T2-weighted signal abnormalities can serve as a quantifiable reflection of a disseminated neoplastic disease process in the brain.

Fundamental to the objective of developing effective translational therapies for this disease are the requirements for a better understanding of the biological mechanisms that govern glioma cell dispersal. Concurrently, it is critical to develop clinical imaging tools to link the presence and activity of specific biological mechanisms that drive tumor invasion and angiogenesis with an evaluation of the extent of neoplastic disease in patients with high-grade gliomas. In this context, we previously characterized the cell surface chemokine receptor CXCR4 as an important mediator of glioma cell invasiveness in vitro and in vivo (5), whereas others have reported its role in GBM neovascularization (1). In this report, we now detail an extensive analysis of CXCR4 expression in a large set of human glioma tissue samples and report a strong correlation between increasing histological grade and CXCR4 expression levels. Furthermore, we report a novel association within GBM between CXCR4 expression levels and an increased extent and intensity of preoperative T2-weighted peritumoral MRI signal abnormalities. In conjunction with our earlier report detailing the mechanistic role of CXCR4 in mediating glioma cell dispersal, these findings underscore the clinical relevance of CXCR4 based on its association with increasing invasive tumor grade and imaging-related evidence of a more disseminated disease process in the brain. Our study indicates that, in addition to further supporting the previously described roles for CXCR4 as a mediator of invasion and neovascularization, tumor CXCR4 expression levels can be used to segregate patient sets into distinct groups based on evidence of extended peritumoral disease process on T2-weighted MRI. This provides a valuable link between a routinely used clinical evaluation tool and a signaling mechanism that governs the biological aggressiveness of these tumors.

Materials and Methods

Sample Collection and Processing

Tumor and nonneoplastic (epilepsy) brain samples were obtained intraoperatively from patients undergoing open craniotomy for resection of intracranial lesions. Patients provided consent through a Vanderbilt Institutional Review Board-approved protocol. For putative tumor specimens, tissue was sampled intraoperatively from within what was clinically judged to be the main tumor lesion (which in the case of suspected GBM comprised the T1 postcontrast-enhancing mass). After intraoperative frozen-section pathology analysis confirming a likely diagnosis of glioma, tissue was sampled from radiographically confirmed postcontrast-enhancing regions of the brain and transported on ice to the research laboratory. For each patient, several (2–10, depending on the amount of resected sample available) tumor fragments measuring roughly between 25 and 250 mm³ each were pooled together to average out for operative sampling heterogeneity and processed collectively for ribonucleic acid (RNA) extraction, as previously described (5). Similarly, multiple tissue fragments were embedded in paraffin for subsequent histological and immunohistochemical analyses. Grossly necrotic tissue was not used for either RNA isolation or histological analysis. Final tissue diagnoses were confirmed using hematoxylin and eosin stains on paraffin-embedded specimens.

Quantitative Polymerase Chain Reaction

Isolated RNA was processed for complementary deoxyribonucleic acid generation as described (5) and then subjected to triplicate multiplex real-time quantitative polymerase chain reaction amplification for CXCR4 and CXCL12 nucleotide sequences using predesigned *Taqman* probe and primer sets (Applied Biosystems, Norwalk, CT) in an ABI 7300 thermal cycler (Applied Biosystems). 18s ribosomal RNA was used as an internal control amplification target. Threshold cycles were determined for each amplification run as described (5) and normalized against sequence-specific plasmid-generated standard curves. Run-to-run (i.e., interassay) variability was eliminated by including, within each run, a constant calibrator sample. Data from four temporal lobe samples from patients undergoing surgery for intractable epilepsy

were averaged and used as nonneoplastic controls against which expression levels for all patient samples were expressed.

Immunohistochemistry

Paraffin-embedded tissue sample blocks were sectioned using a microtome, and, after routine antigen retrieval processing, slide-mounted sections were immunoprobed using primary antibodies against CXCR4 and CXCL12 (R&D Systems, Minneapolis, MN) as well as factor VIII (Dako, Carpinteria, CA) and neuron-specific enolase (Chemicon, Temecula, CA). Slides were then either probed with fluorescent secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) or processed for colorimetric development (Vector Laboratories, Burlingame, CA) per standard methodology.

Imaging Methodology and Analyses

Data were obtained from preoperative scans acquired using a General Electric Genesis Signa (Waukesha, WI) or a Philips Intera Achieva (Cleveland, OH) system. All analyzed scans were obtained 1 to 4 days before initial tumor resection from patients who were treatment- (i.e., radio- and chemotherapy) naive. Both scanner magnets from which images were derived were rated at 1.5 T. The General Electric fast spin echo scan used the following parameters: repetition time = 4000 milliseconds, echo time = 106.3 milliseconds, slice thickness/gap = 3/3 mm, field of view = 240 mm, matrix = 256×256 . The Philips spin echo sequence was: repetition time = 6715 milliseconds, echo time = 110 milliseconds, slice thickness/gap = 3/3 mm, field of view = 230 mm, matrix = 512×512 . Preoperative scans were performed in the axial plane after administration of contrast.

T1- and T2-weighted images for 22 patients were analyzed using an automated process with the Matlab software package as described below so a correlation with CXCR4 expression levels could be made. First, we demarcated the boundaries of contrast enhancement on postcontrast T1-weighted images. Once these images were aligned to their respective T2-weighted maps, the area corresponding to T1 enhancement was subtracted from the T2 images. To account for scaling differences over patients, normalization of the signal intensities was performed. We divided the intensity value for each voxel by the maximum signal intensity value located in the cerebrospinal fluid in the non-tumor hemisphere to complete this normalization. Histograms were then formed from the tumor-bearing hemisphere of the T2-weighted image using bins sized to contain 1% of the normalized signal intensity for each patient. For each of 100 bins, there existed a count of the number of voxels for the given signal intensity range. All histograms (i.e., those generated for diseased as well as non-tumor-bearing hemispheres) were independently normalized for differing patient-to-patient brain volumes by dividing the number of voxels at any particular signal intensity by the total number of voxels within that specific analyzed hemisphere. Using this methodology, the distribution of voxel signal intensities across each histogram ceases to be a function of hemisphere volume as the area under each independent histogram is forced to a uniform value (arbitrarily designated as 1 in our calculations). Finally, histograms were averaged across groups.

Results

CXCR4 is significantly overexpressed in human high-grade gliomas. Our earlier work identified CXCR4 overexpression as a key mediator of glioma cell invasiveness in a panel of primary and cell line-based glioma cultures and in a rodent brain tumor model (5). With the aim of placing these findings in a clinical context, we surveyed 80 primary human gliomas spanning World Health Organization Grades II, III, and IV for CXCR4 expression levels. Using quantitative real-time polymerase chain reaction, we calculated the CXCR4 message level for each tumor relative to the average CXCR4 expression level determined from four

nonneoplastic temporal lobe samples that were surgically resected from patients with intractable epilepsy. As illustrated in Figure 1, we found that CXCR4 expression in Grade II gliomas ($n = 18$) was very close to that found in control samples ($n = 4$): 1.05 ± 0.2 fold, average $\pm$ standard error of the mean (SEM). In contrast, Grade III tumors ($n = 24$) and GBMs ($n = 38$) expressed significantly more CXCR4 message: 2.54 ± 0.47 fold ($P = 0.021$; Wilcoxon rank-sum test) and 4.85 ± 1.51 fold ($P = 0.002$), respectively. These results clearly indicate the significant overexpression of CXCR4 within human high-grade gliomas and, in particular, GBMs.

GBMs demonstrate neoplastic cell expression of CXCR4 and neuronal production of CXCL12. After confirming that GBMs expressed markedly elevated levels of CXCR4 transcript, we wished to confirm expression at the protein level. To this end, we immunohistochemically probed a panel of five human GBM specimens and observed robust expression of CXCR4 protein in neoplastic cells. The pattern of localization was predominantly nuclear in all analyzed samples (representative fields are shown in Fig. 2, A and B), a pattern of CXCR4 staining in paraffin-embedded tissue that has been reported previously in nonsmall cell lung cancer (25). In a random sampling of five high-power fields from five slide-mounted hematoxylin-counterstained GBM specimens (also selected randomly from our patient pool), we observed that $66 \pm 11\%$ of visible nuclei stained positive for CXCR4. CXCR4 expression in a subset of Grade III gliomas ($n = 4$) and GBMs ($n = 6$) was also confirmed by Western immunoblot (not shown). Given that we and others have previously identified CXCL12 as a potent activator of CXCR4-mediated glioma cell proliferation (3) and invasiveness (5), we sought to identify cell populations within GBMs that expressed CXCL12, as the endogenous presence of ligand would provide a strong mechanistic context to the markedly elevated CXCR4 expression profile that we observed. Indeed, immunohistochemical profiling of GBM tissues from the same five samples previously analyzed for CXCR4 expression did reveal strong expression of CXCL12 in these same tumors. This was found to be localized predominantly within endothelial cells (representative panels are shown in Fig. 2, C and E) as well as in overrun neurons (Fig. 2, D and F).

MRI scans of patients with CXCR4-overexpressing GBMs demonstrated a marked increase in intensity and extent of peritumoral T2 signal abnormality. In preclinical in vitro and in vivo experimental models, we previously demonstrated that CXCR4 mediates the adoption of an invasive phenotype by GBM tumor cells (5). Given our subsequent observation of significantly elevated CXCR4 transcript in a large number of human GBM specimens, we sought to ascertain whether CXCR4 expression levels might correlate with clinical evidence of increased tumor dissemination. For this purpose, we examined postgadolinium T1- and T2-weighted preoperative MRI scans of the patients from whom we had collected tumor tissue samples for quantitative CXCR4 expression level analysis. Of the 38 GBM specimens that we had collected, we identified 22 patients as being treatment-naïve (i.e., the tissue sample that we obtained was harvested before the initiation of radiation or chemotherapy in these patients), whereas the remaining 16 patients represented tumor recurrences with a history of adjuvant treatments. For our imaging analyses, we excluded the patients who had received previous radiation and/or chemotherapy given the expected nonspecific changes that are known to occur in brain MRI after such treatments. The remaining 22 patients were arranged in ascending order of CXCR4 expression level (as determined by quantitative polymerase chain reaction). We observed a continuous distribution of CXCR4 expression level in these samples (Table 1), and in the absence of a clear bimodal data set, we chose to arithmetically divide the patients into two equal groups of 11 patients each, with the higher CXCR4 expressors denoted as the CXCR4-high group and the lower CXCR4-expressing patients designated as the CXCR4-low group.

The average CXCR4 expression in the CXCR4-high group was 10.99 ± 4.72 fold (average $\pm$ SEM) versus nonneoplastic sample controls (as detailed in Materials and Methods), whereas expression in the CXCR4-low group was 1.17 ± 0.18 fold (Fig. 3A). This difference was highly significant ($P < 0.001$; Mann-Whitney test). Before further analysis, we confirmed that, with the exception of one patient for whom data were not available, all patients received similar summative doses of corticosteroid therapy as routinely used in patients diagnosed with intracranial space-occupying lesions. (For detailed corticosteroid dose information, see Table 2.) In the CXCR4-low patient group, corticosteroid administration ranged from 0 to 40 mg/d over a course of 3 to 12 days before preoperative MRI scanning. In the CXCR4-high group, doses ranged from 0 to 40 mg/d over a course of 3 to 18 days before preoperative MRI scanning. The average cumulative corticosteroid dose administered before MRI scanning was 127.3 ± 29.3 mg in the CXCR4-low group (average $\pm$ SEM) versus 122.4 ± 28.9 mg in the CXCR4-high group ($P = 0.91$; t test). We also confirmed that the corticosteroid dose bore no correlation with CXCR4 expression level ($P = 0.69$; Spearman rank correlation test).

Postgadolinium T1-weighted scans were then used to calculate the volume of the contrast-enhancing lesion for each patient. As illustrated in Figure 3B, this revealed nearly identical average T1-weighted contrast-enhancing tumor volumes in the CXCR4-high (79.04 ± 18.17 cm³ [average $\pm$ SEM]) and CXCR4-low (76.88 ± 15.59 cm³) patient groups (Fig. 3B). As described in Materials and Methods, an analysis of voxel (i.e., three-dimensional pixel) signal intensity was then performed for each patient using the T2-weighted axial scan series for that same patient. Signal intensity was normalized within each patient by assigning a value of 1.0 unit to the brightest voxel located within any of the axial slices analyzed for that patient. This voxel was confirmed to lie within cerebrospinal fluid in each case. All remaining voxels in each patient's entire T2-weighted axial series were assigned decreasing signal intensity values (with a resolution of 0.01 unit) in relation to the identified 1.0-unit control voxel. After this, the three-dimensional volume correlating with tissue that demonstrated post-gadolinium enhancement on corresponding T1-weighted MRI was digitally removed from the rasterized T2-weighted MRI scan. The resulting map then consisted of an entire axial T2-weighted MRI series of the brain minus the T1-weighted post-contrast-enhancing lesion. In this manner, we restricted our analysis to peritumoral signal abnormalities and removed signal contamination emanating from what would be considered the main (and surgically targetable) tumor mass.

These modified T2-weighted MRI scans (comprising the entire axial series for the ipsilateral diseased cerebral hemisphere) for each patient were used to generate histograms of voxel signal intensity versus the number of voxels normalized for hemisphere size to account for differences in brain volumes across patients. The average profiles generated from the CXCR4-high and CXCR4-low patient groups demonstrated statistically significant differences. For control purposes, we generated histograms from the non-tumor-bearing cerebral hemispheres of three CXCR4-high and three CXCR4-low patients. As demonstrated in Figure 3C, the diseased cerebral hemispheres from both CXCR4-high and CXCR4-low patients displayed significantly decreased peak height compared with nondiseased hemispheres on voxel signal intensity histograms. These peaks all fell within an intensity range of 0.3 to 0.4 unit, which correlated with nondiseased brain parenchyma (Fig. 4). Although we thought that using contralateral non-tumor-involved brain from our own patient sample represented the optimal age- and condition-matched control group, we also analyzed T2 histograms from an additional control group comprising brain magnetic resonance images from 11 otherwise healthy student volunteers (data not shown). For analytic purposes, it should be clarified that the presence of fewer voxels at peak intensity is reflective of less normal tissue, whereas a greater area under the curve represents more abnormal tissue. Although this descriptive methodology can appear counterintuitive, when analyzing our voxel-intensity histograms, we chose to remain consistent with the conventions of the imaging literature.

Significantly, the histograms from CXCR4-high and CXCR4-low patients also demonstrated significantly depressed peak height compared with this additional control group, and there was no statistical difference between the histogram plots derived from the nondiseased contralateral hemispheres of our glioma patients and those obtained from healthy volunteers. These results indicated that depressed peak height from tumor-bearing cerebral hemispheres (from both CXCR4-high and CXCR4-low patients) represented a marked decrease in the amount of normal tissue present in the analyzed hemispheres from these patients. Significantly, we observed a striking difference in peak height between CXCR4-high and CXCR4-low patients (2.88 versus 3.85% of normalized total voxels, $P = 0.02$; Wilcoxon test) (Fig. 3C). This indicated that, in GBM patients, the number of normal peritumoral voxels in CXCR4-high patients was significantly lower than that in CXCR4-low patients, thereby indicating that CXCR4-high tumors were affecting/involving brain tissue (which fell outside the confines of the T1-weighted postgadolinium-enhancing lesion) to a more significant extent than CXCR4-low tumors.

To further elucidate this, we focused on a specific portion of the histogram signal intensity plot between the values of 0.38 and 0.65 unit of normalized signal intensity. This range was confirmed as predominantly covering the spectrum of signal between normal nondiseased brain parenchyma and cerebrospinal fluid. This analysis revealed that, within this signal intensity range, CXCR4-low GBM-bearing patient brain hemispheres contained a similar number of voxels as control nondiseased patient brain hemispheres (28.9 and 27.1%, respectively; $P = 0.14$; Wilcoxon test) (Fig. 3D). Significantly, however, CXCR4-high patients displayed a markedly greater number of voxels (37.7%) within this signal intensity range, clearly indicating that CXCR4-high GBMs were creating high-signal voxel abnormalities within peritumoral brain parenchyma to a much greater extent than CXCR4-low tumors ($P = 0.036$; Wilcoxon test) (Fig. 3D). Based on these quantitative and highly sensitive analytic techniques, we concluded that CXCR4-high GBMs were associated with an increased extent and intensity of peritumoral T2-weighted signal abnormalities compared with CXCR4-low tumors.

To further illustrate these results, we generated visual representations wherein axial T2-weighted MRI slices were overlaid with a colorimetric voxel signal intensity gradient (with a resolution of 0.1 unit per color change). Figure 4 displays representative single slices from a CXCR4-high patient and a CXCR4-low patient. Evidence of diffuse increases in peritumoral T2-signal abnormalities, as revealed by our quantitative analysis described above, could be readily visualized on analysis of colorimetrically graded axial MRI scans.

Discussion

CXCR4 is a seven-domain transmembrane G protein-coupled receptor and was initially characterized on the basis of its ability to stimulate the differentiation and chemotaxis of hematopoietic progenitor cells and as a coreceptor for human immunodeficiency virus entry into CD4⁺ T lymphocytes (19). In recent years, significant evidence has elucidated additional roles for CXCR4, and its secreted protein ligand CXCL12, in directing stem cell differentiation and migration in multiple organ systems (19). This is clearly demonstrated by the observation that CXCR4 knockout mice are embryonic lethal with massive defects in neural tube formation, cardiogenesis, and gastrointestinal development (13,16). Additionally, the role of CXCR4 in cancer has also received increasing attention. Numerous reports detail the expression and functional mediation of tumor cell proliferation and migration by CXCR4 in models of breast (14), colon (31), prostate (6), pancreatic (24), and lung (26) cancer. In the context of brain tumors, we (5) and others (3,22,32) have detailed the ability of CXCR4 to affect glioma growth and invasiveness in vitro and in vivo. Rubin et al. (22) described the use of a small-molecule CXCR4-specific inhibitor to significantly impair the growth of human U87 glioblastomas in nude mice. Recently, Redjal et al. (20) reported that CXCR4 inhibition markedly sensitizes

glioma cell lines to in vitro chemotherapy. In conjunction with additional evidence establishing a role for the CXCL12/CXCR4 axis in glioma neovascularization (1,23,30) and a demonstration by our group of CXCR4-mediated regulation of cancer stem cell-like cell proliferation in human GBM-derived cell cultures (unpublished data), these developments readily establish the clinical relevance of targeting CXCR4 in patients with malignant glioma. Furthermore, our growing understanding of CXCR4 as a potentiator of GBM dissemination in the brain may allow us to use CXCR4 expression quantitation as a marker for the assessment of patient-specific disease behavior and ultimately prognosis. Such analyses in patients with osteosarcoma (12), colon cancer (10), and melanoma (9) have revealed a clear correlation between CXCR4 expression and clinical outcome. In this study, our limited sample size of 22 treatment-naïve GBM patients did not permit us to investigate a link between pretherapeutic tumor CXCR4 expression levels and patient prognosis. However, our results do establish a robust connection between CXCR4 expression and radiographic evidence of increased disease dissemination as observed on T2-weighted MRI, a routinely employed and readily available imaging modality used in the management of patients with brain tumors.

Although there is considerable excitement about, and movement toward, the implementation of multiparametric imaging modalities to better identify and characterize neoplastic disease in the brain, in this study, we chose to use traditional T2-weighted MRI in combination with a highly quantitative analysis of whole-hemisphere voxel signal intensities. As detailed under Materials and Methods, this involves the use of an automated software platform to digitally assign normalized intensity values to each analyzed voxel and then plot these data into averaged histograms. Such histogram-based assessment has been used previously for whole-brain or hemisphere-based analysis in patients with multiple sclerosis to quantitate sizable yet subtle signal abnormalities (27,28). In addition, comparisons of the peak height on these histograms have demonstrated decreases in mean signal diffusivity using scans obtained from cases of patients with migraine headaches (21), subtle early changes in the brain parenchyma of patients with acute lymphoblastic leukemia (29), and diffusion and magnetization transfer changes in cases of Alzheimer's disease (4). Overall, in addition to being objective and user-independent, histogram generation and analysis are fast and simple to implement (28).

The generation of peritumoral T2 signal abnormalities associated with high-grade gliomas is attributable to multiple mechanisms. Key among these is the presence of invading tumor cells and associated foci of neovascularization. Additionally, the elaboration of multiple cytokines by these infiltrative nests of tumor and endothelial cells contributes to the generation of vasogenic edema. As such, we believe that our use of quantitative peritumoral T2 signal abnormality analysis was a well-suited method for investigating whether CXCR4 expression levels in GBM patients could be indicative of increased extent and intensity of peritumoral disease involvement in the brain. Indeed, the presence of increased T2-weighted peritumoral signal abnormalities portends a worse clinical prognosis in patients with GBM (7,18). In relation to analysis of T2 signal variability, Aghi et al. (2) previously reported an operator-dependent analytic methodology for interpretation of peritumoral imaging changes in patients with GBM. This involved manual assignment of a border sharpness coefficient value based on an observer's on-screen assessment of the T2 signal at the margin of a visualized tumor. In contrast, our data use an operator-independent and fully quantitative methodology based on the automated assignment of voxel signal intensities using a wide dynamic range of 100 units spanning the extent from no signal to brightest signal.

Conclusion

In this study, we have now identified expression levels of the CXCR4 receptor, known to control activity of a key cellular signaling mechanism mediating multiple aspects of glioma biology, as a correlative marker for disseminated disease in the brains of GBM patients. These

findings provide a critical bridge between currently used diagnostic tools and the molecular biology of these tumors. In conjunction with our earlier mechanistic descriptions (5) of CXCR4 signaling in gliomas and those of others (1,3,22,32), these current results further support the use of CXCR4 inhibitors in the clinical treatment of malignant glioma. More specifically, our findings argue strongly for such therapy in cases in which quantitative analysis of peritumoral tissue using T2-weighted MRI reveals increased diffuse signal abnormalities, as this is highly likely to correlate with CXCR4 overexpression and therefore potentially treatment-responsive tumors.

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Abbreviations

GBM	glioblastoma multiforme
MRI	magnetic resonance imaging
RNA	ribonucleic acid
SEM	standard error of the mean

FIGURE 1.

Graphic illustration of CXCR4 expression levels as determined by quantitative polymerase chain reaction in a survey of 80 human gliomas. *NS*, not significant; *GBM*, glioblastoma multiforme.

FIGURE 2.

Immunohistochemistry revealing the presence and localization of CXCR4 and CXCL12 expression in human glioblastoma multiforme (GBM). *A* and *B*, immunoprobings of paraffin-embedded GBM tumor tissue sections revealing robust expression of CXCR4 protein. *C* and *D*, CXCL12 protein also readily detectable in GBM tissue specimens and observed both within vascular endothelium and pyramidal neurons. *E*, CXCL12 (*green*) colocalization with factor VIII-expressing endothelial cells (*red*) confirmed using immunofluorescence. *F*, fluorescent staining with neuron-specific enolase (*red*) in combination with CXCL12 (*green*), confirming the presence of CXCL12-expressing neurons within GBM parenchyma. (*A*, *C*, and *E*, original magnification, $\times 200$; *B*, *D*, and *F*, original magnification, $\times 400$.)

FIGURE 3.

Quantitative histogram analysis of T2-weighted magnetic resonance imaging (MRI), revealing significantly increased intensity and extent of peritumoral signal in CXCR4-high GBM patients. **A**, study patients divided into CXCR4-high and CXCR4-low groups based on quantitative polymerase chain reaction-measured levels of receptor expression. **B**, both groups demonstrate nearly equivalent gross tumor volumes as measured on T1 postgadolinium MRI scans. **C** and **D**, quantitative histogram analysis of T2-weighted MRI scans from the same patients (after digital subtraction of T1 postgadolinium-enhancing lesions), revealing marked increases in both intensity (indicated by decreased peak height in **C**) and extent (indicated by increased area under the curve in **D**) of T2 signal abnormality in CXCR4-high GBM patients

compared with CXCR4-low patients or control subjects. *NON DZ. HEM*, non-tumor-involved contralateral hemisphere from three CXCR4-high and three CXCR4-low patients. *Asterisks* indicate significance.

FIGURE 4.

CXCR4 expression level correlated with the extent and intensity of T2-weighted magnetic resonance imaging signal. As illustrated by scans from a representative patient from each group (CXCR4-high and CXCR4-low), despite similar T1 postcontrast tumor volumes, marked increases in T2-weighted signal abnormalities in patients whose tumor tissue revealed elevated CXCR4 expression can be observed.

TABLE 1

Details of measured CXCR4 levels, tumor volume, and tumor location in glioblastoma multiforme patients analyzed by magnetic resonance imaging^a

Analyzed GBM group and patient no.	CXCR4 level	Volume (cm ³) of T1 postcontrast-enhancing lesion	Location
CXCR4-low (avg)		76.88 ± 15.59	
1	0.29	41.98	Frontal
2	0.31	105.80	Frontal
3	0.43	7.23	Posterior frontal
4	1.07	39.30	Inferior frontal
5	1.1	72.00	Frontotemporal
6	1.17	164.56	Temporal
7	1.3	90.77	Parieto-occipital
8	1.66	196.63	Frontal
9	1.73	41.32	Temporal
10	1.82	12.70	Posterior frontal
11	1.95	97.20	Parietal
CXCR4-high (avg)		79.04 ± 18.17	
12	2.01	156.06	Frontal
13	2.52	52.02	Inferior frontal
14	3.17	135.68	Parietal
15	3.27	9.20	Parieto-occipital
16	3.47	112.66	Frontal
17	3.57	87.41	Temporal
18	5.56	128.12	Frontal
19	6.39	35.10	Temporal
20	8.49	77.57	Temporal
21	31.5	5.20	Temporal
22	50.89	46.66	Temporal

^a GBM, glioblastoma multiforme.

Details of preimaging corticosteroid administration in glioblastoma multiforme patients analyzed by magnetic resonance imaging^a

TABLE 2

Corticosteroids

Analyzed GBM group and patient no.	Use (yes/no)	Type	Dose (mg/d)	Length of use (no. of days before MRI)	Cumulative preimaging dose (mg)	CXCR4 level	Area under the curve (0.38–0.65)	P value (t test)
CXCR4-low (avg)								
1	Y	Dexamethasone	40	3	127.3 ± 29.3 ^b	0.29	0.2576	
2	Y	Dexamethasone	24	4	96	0.31	0.2491	
3	Y	Dexamethasone	10	8	80	0.43	0.2489	
4	Y	Dexamethasone	24	4	96	1.07	0.2385	
5	Y	Dexamethasone	8	0	0	1.1	0.2768	
6	Y	Dexamethasone	16	12	192	1.17	0.2469	
7	Y	Dexamethasone	16	7	112	1.3	0.2768	
8	Y	Dexamethasone	40	8	320	1.66	0.2549	
9	N	Dexamethasone	16	9	144	1.73	0.2996	
10	Y	Dexamethasone	24	10	240	1.82	0.2894	
11	Y	Dexamethasone	24	10	240	1.95	0.2339	
CXCR4-high (avg)								
12	Y	Dexamethasone	24	10	122.4 ± 28.9	2.01	0.4262	0.91
13	Y	Dexamethasone	16	5	80	2.52	0.2702	
14	N	Dexamethasone	24	3	0	3.17	0.2756	
15	Y	Dexamethasone	24	4	72	3.27	0.4382	
16	Y	Dexamethasone	24	4	96	3.47	0.4774	
17	Y	Dexamethasone	No data	18	No data	3.57	0.2883	
18	Y	Dexamethasone	40	4	160	5.56	0.2857	
19	Y	Dexamethasone	24	8	192	6.39	0.3217	
20	Y	Dexamethasone	40	3	120	8.49	0.3893	
21	Y	Dexamethasone	24	11	264	31.5	0.4514	
22	N	Dexamethasone	24	11	0	50.89	0.2368	

^aIn individual patients, corticosteroid use bore no correlation with CXCR4 expression level ($P = 0.69$; Spearman rank correlation test). GBM, glioblastoma multiforme; Y, yes; N, no; MRI, magnetic resonance imaging.

^bStandard error of the mean.