

The transmembrane chemokines CXCL16 and CX3CL1 and their receptors are expressed in human meningiomas

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Abstract. Meningiomas are common slowly growing benign tumors, however, anaplastic meningiomas have an aggressive biological and clinical behavior associated with high rates of recurrence and unfavorable prognosis. Since the molecular mechanisms involved in progression of meningiomas are not yet fully understood and recent investigations have suggested a possible role of chemokines in tumor biology, the aim of the study was to investigate the expression of CX3CL1/CX3CR1 and CXCL16/CXCR6 on mRNA and protein level in human meningiomas. Quantitative reverse-transcription polymerase chain reaction, immunohistochemistry and double immunostaining techniques were used for the investigations. We showed that mRNA and protein expression of the chemokine/receptor pairs CX3CL1/CX3CR1 and CXCL16/CXCR6 were detectable in human meningioma samples. Double immunostaining revealed that the chemokines/receptors were predominantly expressed in the tumor cells themselves, in infiltrating microglia cells/macrophages and endothelial cells of blood vessels. Nevertheless, not all cells of different kinds were positive for different chemokine/receptors. Of note, in

comparison to more benign meningioma samples, CX3CR1 and CXCL16 were found to be expressed at lower levels in anaplastic variants. Moreover, a positive correlation between expression levels of ligands and corresponding receptors could be observed for some malignancy grades. Taken together, these results showed that chemokines and their receptors are involved in the pathogenesis of human meningiomas. Our results provide an interesting basis for further investigations that should be performed to characterize the functional roles of chemokines and their receptors in human meningiomas, and also enhance future therapeutic design.

Introduction

Meningiomas are neoplasms derived from arachnoidea cells and they can be found in the dura mater. Meningiomas are considered to be the second most common intracranial tumors and account for 13-26% of all primary brain tumors (1). Most (approximately 90%) of the meningiomas are slowly growing benign tumors, classified as World Health Organization (WHO) grade I (2). However, atypical meningiomas (WHO grade II), which are characterized by increased cellularity and mitotic activity, account for approximately 8% of this tumor type. Anaplastic variants (WHO grade III), which make up approximately 2%, show a more aggressive biological behavior and are associated with a high risk of local recurrence and a poor clinical outcome (1,3). Surgery is the mainstay of treatment, with the likelihood of recurrence being inversely related to the extent of resection (3,4). The recurrence rate for meningiomas is on average 10% after a complete resection and up to 50% after an incomplete resection (5).

In addition, recurrence rates are higher for the more aggressive histological variants, with 5-year recurrence rates of 38% for atypical meningiomas and 78% for malignant meningiomas (4). When patients were grouped by histological type of tumors, those with benign meningiomas had an overall 5-year survival rate of 70%, whereas the overall 5-year survival rates in patients with atypical and anaplastic meningiomas were 75 and 55%, respectively (6). Furthermore, accepted alternative therapies for patients who have failed surgical intervention are currently limited to radiotherapy which has been shown to delay recurrence (3,7). Currently, there are no pharmaceutical agents that are routinely used for adjuvant therapy.

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Abbreviations: C_T, cycle of threshold; EMA, epithelial membrane antigen; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; IHC, immunohistochemistry; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; vWF, von Willebrand factor protein; WHO, World Health Organization

Key words: chemokine, CXCL16/CXCR6, CX3CL1/CX3CR1, meningioma

Chemokines are 8- to 12-kDa peptides that bind to specific G-protein-coupled, seven-span transmembrane receptors on the plasma membrane of target cells. Most chemokines bind to multiple receptors, and the same receptor may bind to more than one chemokine (8,9). Chemokines are classified into four major subfamilies on the basis of the motif of the first two cysteine residues. They play an essential role in cellular migration and intracellular communication and were originally observed as inducible cytokines facilitating the recruitment of specific leukocyte subsets (10). They are also involved in many other physiological and pathological processes, including neoplasia, in which they play an important role through multiple mechanisms (11-16).

The chemokines CXCL16 (also termed SR-PSOX22) and CX3CL1 (also known as fractalkine or neurotactin) differ from the other known, approximately 50 human chemokines by their exceptional structure: They are synthesized as transmembrane multi-domain molecules consisting of a chemokine domain followed by a glycosylated mucin-like stalk and a single transmembrane helix (17). However, soluble forms of CXCL16 and CX3CL1 are generated by constitutive or induced cleavage (shedding) from the cell membranes through the action of the cell-surface proteases (14,18). As a result, by interacting with their receptors the released soluble forms of CX3CL1 and CXCL16 mainly function as leukocyte chemoattractants.

In non-malignant tissue, CXCL16 is produced by macrophages, dendritic, endothelial and smooth muscle cells. CX3CL1 is produced by IL-1-stimulated endothelial cells and has been shown to mediate migration of monocytes and T lymphocytes (19,20). Since CXCL16 and CX3CL1 are cytokine-inducible chemokines, their expression is associated with a number of human inflammatory diseases including rheumatoid arthritis, atherosclerosis, coronary artery disease and liver injury. However, high CXCL16 and CX3CL1 expression have been found in several types of cancer (15,21-29). CX3CR1 (also termed RBS11 or V28) is the highly specific receptor for CX3CL1 (30,31). In the periphery CX3CR1 is expressed on monocytes, dendritic cells, T cell subsets, CD16-positive natural killer cells and platelets (32). The CXCL16 receptor CXCR6 (also termed Bonzo, TYMSTR or STRL33) is expressed by CD4-positive effector memory T cell subsets, Va24-positive natural killer T cells, bone marrow plasma cells and myeloma cells (33-35).

Although a role for CXCL16/CXCR6 and CX3CL1/CX3CR1 in tumorigenesis has been established little is known about the expression of these chemokines and their receptors in human meningiomas. Therefore, the purpose of this study was to investigate the expression of CXCL16 and CX3CL1 on mRNA and protein level and to determine the expression of their receptors in meningiomas of different malignant grades.

Materials and methods

Tumor specimens. Meningioma samples were surgical dissected tissues from the Department of Neurosurgery (Kiel, Germany) and were obtained in accordance with the Helsinki Declaration of 1975 and with approval of the ethics committee of the University of Kiel. Tumors were classified according to the WHO criteria into the various subtypes of benign

meningiomas and atypical or anaplastic meningiomas (1). The diagnosis was established by a neuropathologist. In this study, samples of 10 WHO I meningiomas were included (9 meningothelial and 1 fibroblastic) from patients with a mean age of 59.8 ± 13.4 years (8 women, 2 men). Samples from 10 atypical meningiomas (WHO grade II) were from 6 females and 4 males with a mean age of 60.0 ± 9.4 years, and 7 anaplastic/malignant meningioma samples originated from 3 females and 4 males with a mean age of 61.3 ± 11.1 years, and one of these anaplastic meningiomas (male, 78 years) was a relapse from a previous meningioma affection. If possible (enough material available), for quantitative RT-PCR, immunohistochemistry and immunofluorescence double-stainings matched probes of individual tumour samples were used. However, due to small sample size and/or tissue integrity of cryosections this was not possible for all tested tissue samples.

Real-time RT-PCR. RNA was isolated with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), digested by DNase, cDNA synthesized and quantitative real-time RT-PCR (qRT-PCR) was performed (36) using TaqMan primer probes (Applied Biosystems, Foster City, CA, USA): *hGAPDH* (Hs99999905_m1), *hCX3CL1* (Hs00171086_m1), *hCX3CR1* (Hs00365842_m1), *hCXCL16* (Hs00222859_m1), *hCXCR6* (Hs00174843_m1). Ten meningiomas grade I, 10 meningiomas grade II, and 7 meningiomas grade III were analyzed by qRT-PCR. The reaction was carried out with the MyiQ™ Single Color Real-time PCR Detection System (Bio-Rad, Munich, Germany) and fluorescent data were converted into cycle threshold (C_T) measurements. ΔC_T values of each sample were calculated as $CT_{\text{gene of interest}} - CT_{\text{GAPDH}}$. A ΔC_T value of 3.33 corresponds to one magnitude lower gene expression compared to GAPDH (glyceraldehyde-3-phosphate-dehydrogenase). For each gene, logarithmic linear dependence of C_T -values from the numbers of copies was verified by using different amounts of cDNA.

Immunohistochemistry. For examination by immunohistochemistry (IHC), fresh-frozen meningioma tissues of different WHO grades were cut in a freezing cryostat into 6 μm sections. The sections were air dried and stored at -20°C until used. IHC was performed using the avidin-biotin-peroxidase complex (ABC) method. The sections were fixed with 4% para-formaldehyde in Tris-buffered saline (TBS) for 30 min at room temperature (RT). To block endogenous peroxidase and non-specific binding, 3% H_2O_2 in 0.3% Triton X-100/TBS (30 min at RT) and appropriate 10% normal blocking serum in TBS (60 min at RT; Jackson Immuno Research Laboratories, USA) were used, respectively. Sections were incubated with appropriate dilutions of primary antibodies overnight at 4°C (anti-CX3CL1, mouse monoclonal, 1:200, R&D Systems, Wiesbaden, Germany; anti-CX3CR1, rabbit polyclonal, 1:200, Santa Cruz, CA, USA; anti-CXCL16, goat polyclonal, 1:200, R&D Systems; anti-CXCR6, mouse monoclonal, 1:50, R&D Systems). The antibodies were diluted in 0.3% Triton X-100/TBS and appropriate 2% normal blocking serum in TBS. Primary antibodies were omitted for negative controls. Additionally, serum-control and isotype-controls (final concentrations similar to corresponding specific primary antibodies) were performed for each primary anti-

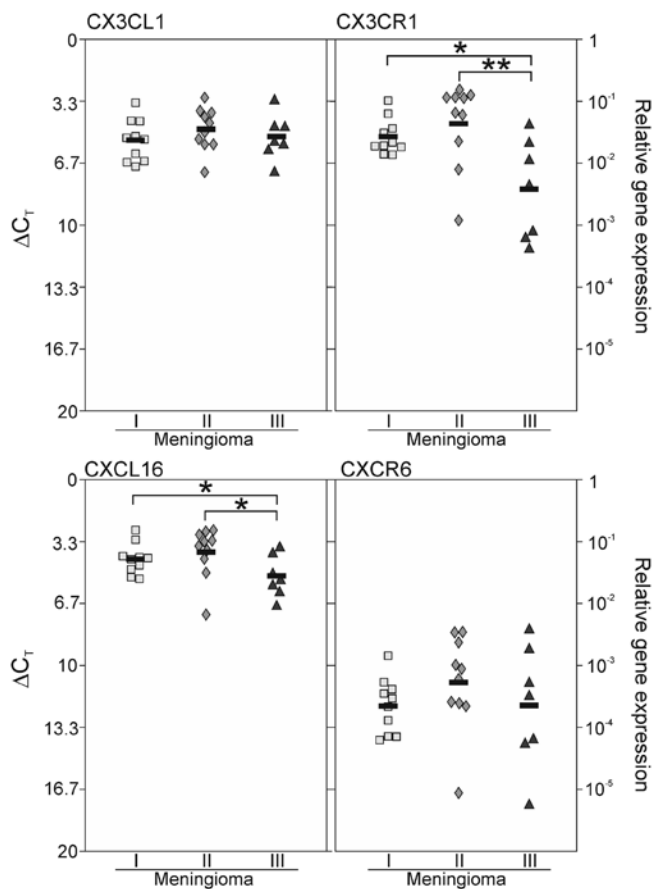


Figure 1. Expression of CX3CL1/CX3CR1 (upper lane) and CXCL16/CXCR6 (lower lane) in meningeiomas of different WHO grades evaluated by quantitative real-time RT-PCR (logarithmic scale, $\Delta C_t=3.3$ corresponds to a 10-fold difference). Single quadrates, rhombs and triangles indicate one tissue sample; bold lines indicate the mean value of the respective group. Undetectable values were not included in the mean calculation. Although a wide range between single samples occurred, with exception of CXCR6 high expression levels were detected for both chemokine/receptors in different meningeioma tissues. Asterisks indicate significant differences between tissues of meningeiomas with diverse malignant grades (* $p<0.05$, ** $p<0.01$).

body [normal mouse IgG, normal rabbit and normal goat IgG (R&D Systems)]. After washing steps with TBS (10 min for two times), the sections were incubated with corresponding secondary antibody diluted 1:200 in 1.5% blocking serum/TBS for 60 min at RT (biotinylated horse anti-mouse IgG for CX3CL1 and CXCR6, Vector Laboratories, Burlingame, CA, USA; biotinylated rabbit anti-goat IgG for CXCL16, Vector Laboratories; biotinylated donkey anti-rabbit for CX3CR1, Jackson Immuno Research). The signals were amplified using the ABC method with ABC Vectastain® kit (Vector Laboratories). The signal was visualized by incubation with 0.06% 3,3'-diaminobenzidine-tetrahydrochloride (Sigma-Aldrich, Munich, Germany) and 0.003% H_2O_2 in 0.1 M Tris-HCl (pH 7.6) for 3 min. Then, the slides were counterstained with Mayer's Hemalum (Carl Roth GmbH, Germany) for 60 sec. After washing with running tap water for 10 min, the slides were dehydrated step-wise with ethanol (concentration from 70%→80%→95%→95%→100%→100%, 1 min for each concentration). After clearing in RotiClear (Carl Roth GmbH, 10 min 3x), the slides were mounted with RotiMount® (Carl

Roth GmbH) quick-harden mounting medium and used for investigation. Brightfield microscopy with digital photography was performed using a Zeiss microscope and Zeiss camera (Zeiss, Oberkochen, Germany).

Immunofluorescence. Cryostat sections of different meningeioma tissues WHO grade I were fixed in acetone/methanol (1:1; 10 min) at -20°C , washed with Tris-buffered saline plus 0.1% Tween-20 (TBS-T, 3x, room temperature), washed with 20%, then 70% ethanol (each 2 min), blocked with Sudan black (1% in 70% ethanol) for 10 min, rinsed with 70% ethanol until dye free, then for 2 min with 20% ethanol, washed with TBS-T (3x), blocked with 0.1% bovine serum albumin and 0.2% glycine in TBS (1 h), then without washing incubated with primary antibodies in TBS-T at 4°C overnight. Primary antibodies were omitted for negative controls. After a washing step (3x TBS-T, 10 min) the first secondary antibody was incubated for 1 h at 37°C in darkness. The sections were washed with TBS-T (3x, 10 min). When both primary antibodies were obtained from mouse, an additional blocking step with goat anti-mouse FAB-fragments (1:1000 1 h, room temperature, from Dianova, Hamburg, Germany) was necessary. The second primary antibody was applied after another washing step (3x TBS-T) and incubated overnight at 4°C . Second primary antibodies were omitted for negative controls. The slides were washed again (3x TBS-T) and incubated with the second secondary antibody for 1 h at 37°C . After washing with TBS-T (1x 10 min), TBS (2x 10 min), nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI; Molecular Probes/Invitrogen, Life Technologies, Karlsruhe, Germany; 1:30000, 30 min room temperature), washed with TBS (3x) and finally distilled water. After embedding in Immu-Mount (Shandon, Pittsburgh, PA, USA) digital photography was performed using a Zeiss microscope and Zeiss camera (Zeiss).

In combination with anti-epithelial membrane antigen (EMA) (1:500, mouse monoclonal, Dako, Glostrup, Denmark), anti-von Willebrand factor protein (vWF) (1:1000, mouse monoclonal, Santa Cruz) and anti-CD11b (1:100, mouse monoclonal, Santa Cruz), the specific chemokine/receptor antibodies anti-CX3CL1 (1:100, mouse monoclonal, R&D Systems), anti-CX3CR1 (1:5000, rabbit polyclonal, Biomol, Hamburg, Germany), anti-CXCL16 (1:100, goat polyclonal, R&D Systems) and anti-CXCR6 (1:50, mouse monoclonal, R&D Systems) were always stained first with Alexa Fluor 555 coupled secondary antibodies (red, 1:1000, donkey anti-mouse IgG, donkey anti-rabbit IgG or donkey anti-goat IgG, Invitrogen), the secondary antibody detecting the cellular markers was donkey anti-mouse IgG Alexa Fluor 488 (green, 1:1000, Invitrogen).

Statistical analysis. Statistical methods of Student's t-test with independent samples and bivariate correlation analysis (Pearson correlation coefficients) were used. Significance levels ranged between $p<0.05$ and $p<0.01$.

Results

Expression of CX3CL1/CX3CR1 and CXCL16/CXCR6 in human meningeiomas. To evaluate mRNA and protein expression levels of the chemokine/receptor pairs CX3CL1/CX3CR1

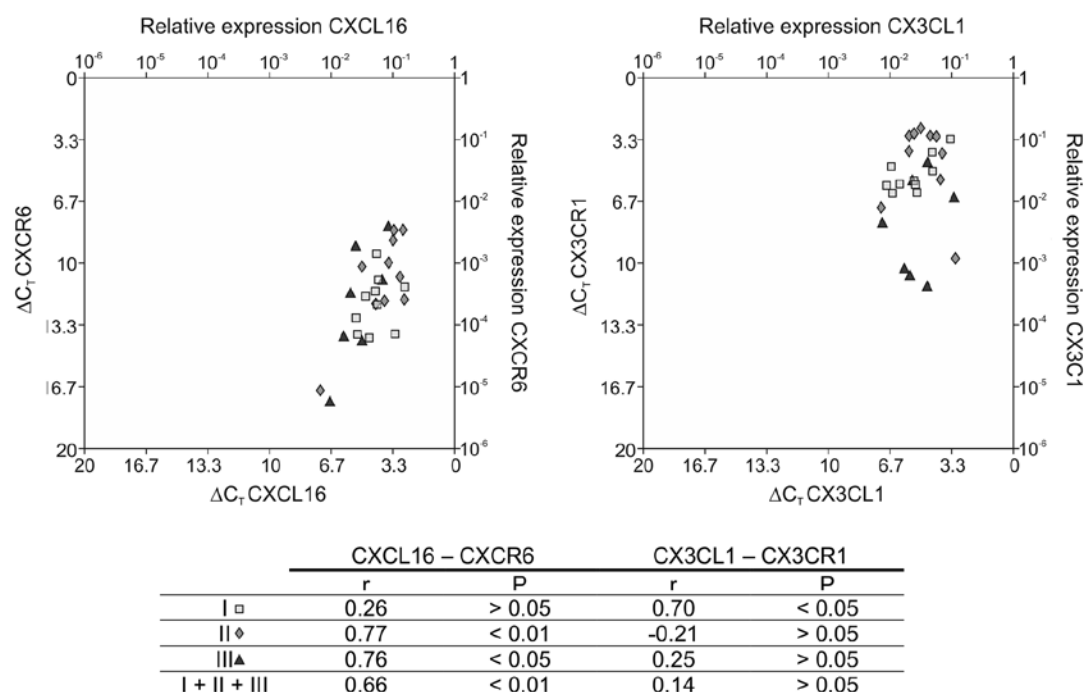


Figure 2. Bivariate correlation analysis of chemokine/receptor pairs. ΔC_T values of CX3CL1 or CXCL16 were plotted against their respective receptors, and results of bivariate Pearson's correlation analysis of different meningioma groups are shown. Positive correlation was obtained for CXCL16/CXCR6 in meningiomas grades II and III and when all WHO grades were included into the analysis. For CX3CL1/CX3CR1 positive correlation was observed for the meningioma WHO grade I group.

and CXCL16/CXCR6 in human meningiomas of different WHO grades qRT-PCR and immunohistochemistry of different tumors were performed. Results are shown in Figs. 1-3.

For the chemokine ligands CX3CL1 and CXCL16 high mRNA expression levels were detectable in all meningioma samples analysed. The normalized averaged ΔC_T values were 5.43, 4.84 and 5.23 for CX3CL1 and 4.28, 3.90 and 5.18 for CXCL16 in meningiomas of WHO grades I, II and III, respectively (Fig. 1). In case of CXCL16, expression in meningiomas of WHO grade III was significantly lower than for both other groups ($p < 0.05$). Although a wide range between single samples occurred, CX3CR1 was also found to be abundantly expressed in human meningiomas. The normalized averaged ΔC_T values were 5.23, 4.54 and 8.06 in meningiomas of WHO grades I, II and III, respectively (Fig. 1), and again, expression in WHO grade III meningiomas was significantly lower compared to grade I ($p < 0.05$) and II ($p < 0.01$) samples. In contrast, CXCR6 showed much lower expression in different tumor samples (averaged ΔC_T values were 12.18, 10.91 and 12.15 for meningiomas grades I-III, respectively).

To evaluate whether a correlation between the expression of CX3CL1 and its receptor CX3CR1 as well as of CXCL16 and its receptor CXCR6 in different meningioma samples occurred a bivariate correlation analysis (Pearson correlation coefficients) of qRT-PCR results was performed (Fig. 2). Statistically significant positive correlations between expression levels of CXCL16 and CXCR6 were found in meningiomas WHO grade II ($r = 0.77$, $p < 0.01$) and III ($r = 0.76$, $p < 0.05$) and if all WHO grades were taken together ($r = 0.66$, $p < 0.01$).

For CX3CL1/CX3CR1 a positive correlation was found only for meningioma grade I samples ($r = 0.70$, $p < 0.05$). As proven by immunohistochemistry, expression of the chemokine/

receptor pairs CX3CL1/CX3CR1 and CXCL16/CXCR6 were also detectable on protein levels in human meningiomas of different WHO grades (Fig. 3, positive stained regions/cells indicated by arrows). Additionally, in all analysed meningiomas positive stained regions / cells were separated by unstained ones (Fig. 3).

In summary, mRNA and protein expression of the chemokine/receptor pairs CX3CL1/CX3CR1 and CXCL16/CXCR6 were detectable in human meningioma samples, and in some cases a positive correlation between expression levels of ligands and corresponding receptors could be observed for some malignancy grades.

Cellular targets of CX3CL1/CX3CR1 and CXCL16/CXCR6 in human meningiomas. To investigate the expression of CX3CL1/CX3CR1 and CXCL16/CXCR6 in different cell subsets within the tumors, co-stainings with anti-epithelial membrane antigen (EMA), anti-von Willebrand factor protein (vWF), and anti-CD11b were performed and analysed by fluorescence microscopy. Meningiomas of WHO grade I were used for investigations. Results are shown in Fig. 4.

It should be mentioned that both staining intensities and amounts of positive regions differed among single samples and within the same sample. For all investigated meningioma samples both CX3CL1/CX3CR1 and CXCL16/CXCR6 co-stained with EMA (Fig. 4). EMA belongs to a heterogeneous population of human milk fat globule proteins which are also present in a variety of epithelial cells of normal and neoplastic types, and serves as a marker for meningiomas. Thus, meningioma cells expressed both CX3CL1/CX3CR1 and CXCL16/CXCR6 (examples for merged regions (yellow) are shown in Fig. 4). Additionally, cells which were solely

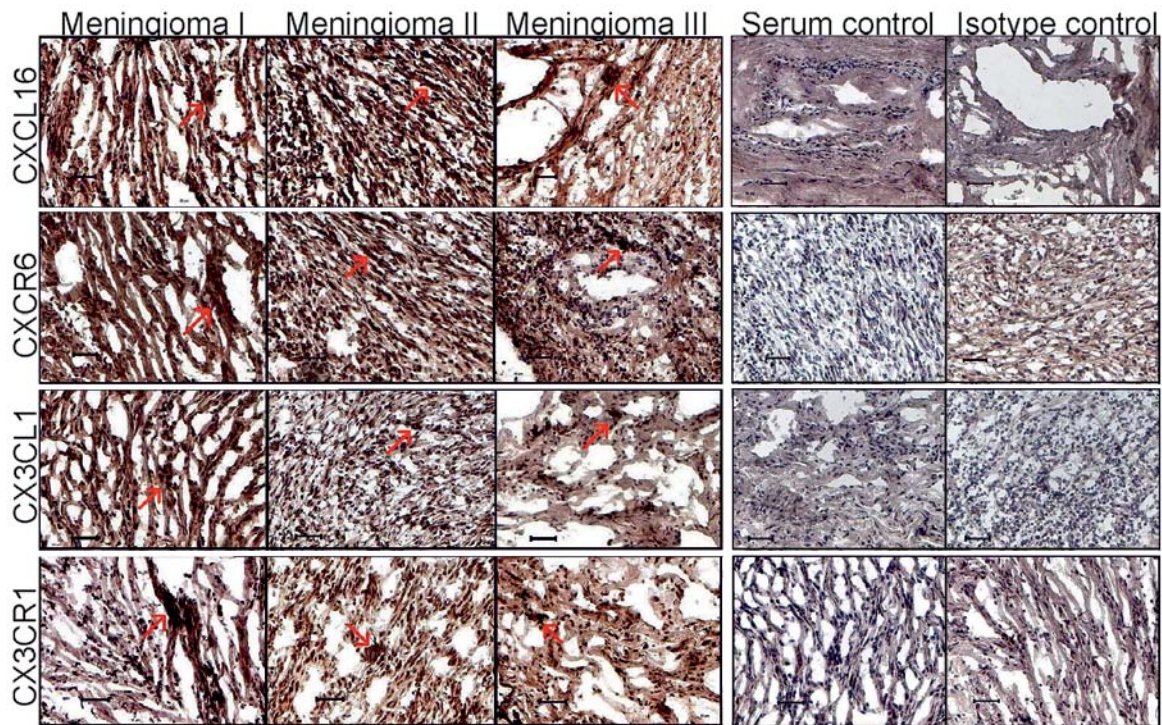


Figure 3. Protein expression of CX3CL1/CX3CR1 and CXCL16/CXCR6 in solid human meningiomas WHO grade I-III sections as shown by immunohistochemistry. In accordance with quantitative real-time RT-PCR results different tumor samples express the chemokines CX3CL1 and CXCL16 and their respective receptors on protein level as shown by exemplary images (arrows indicating positive stained cells/tumor regions). Notably, staining intensity differs between different tumor regions indicating the heterogeneity of the tumor tissues. Serum and isotype controls were performed for every tumor grade, representative examples are shown. Magnification x200 (bars indicate 50µm).

positive for EMA or the individual chemokine/receptor (or negative for both) existed within the tumor sections (Fig. 4). Expression of CX3CL1, CX3CR1 and CXCL16 was also found in combination with CD11b. No co-staining with CD11b was detectable in case of CXCR6. CD11b, also designated complement component receptor $\alpha 3$ chain or Integrin αM , is a cell adhesion molecule that acts as a receptor for cell surface ligands such as intracellular adhesion molecules. Integrin $\beta 2$ is important in the adherence of neutrophils and monocytes to the stimulated endothelium, which means that in meningioma section predominantly microglial cells/monocytes should be CD11b positive.

Thereby, in all meningioma sections analysed, investigated chemokines/receptors were found also in microglial cells/monocytes (Fig. 4; merged yellow regions). Nevertheless, also solely CD11b, CX3CL1/CX3CR1 and CXCL16/CXCR6 stained cells existed within the sections (Fig. 4). Immunofluorescence staining using vWF pointed to a co-staining of CX3CL1 and vWF, whereas other chemokines/receptors were not found in vWF-positive cells. Since vWF is a multimeric glycoprotein that is found in endothelial cells, plasma and platelets, and it is involved in the coagulation of blood at injury sites, for investigated chemokine/receptors only CX3CL1 was detectable in endothelial cells within human meningioma sections (Fig. 4).

Summarized, in human meningioma sections both CX3CL1/CX3CR1 and CXCL16/CXCR6 were found in EMA or, with exception of CXCR6, in CD11b positive cells, respectively, whereas vWF-positive cells were only CX3CL1-positive.

Discussion

Most meningiomas are slowly growing benign tumors, however, anaplastic meningiomas have an aggressive biological and clinical behavior associated with high rates of recurrence and unfavorable prognosis. The molecular mechanisms involved in initiation, proliferation and progression of meningiomas are not yet fully understood. Since recent investigations have suggested a possible role of chemokines in tumor growth, metastasis and host-tumor response (37), the aim of the study was to investigate the expression of CX3CL1/CX3CR1 and CXCL16/CXCR6 on mRNA and protein level in human meningiomas.

CX3CL1 is a unique CX3C chemokine acting as an adhesion molecule in its membrane form and behaving as a true chemokine in its soluble form. It is one of the most expressed chemokines in the brain (17,20,38,39). Beside neurons, astrocytes are major producers of CX3CL1, whereas its receptor CX3CR1 is predominantly found on microglial cells (40-42). Now we have shown for the first time that CX3CL1/CX3CR1 are also expressed in solid human meningioma samples, localized on the tumor cells themselves, infiltrating microglial cells/macrophages and (for CX3CL1) on endothelial cells. Additionally, CX3CR1 was found to be significantly lower expressed in WHO grade III. The downregulated expression of CX3CR1 mRNA and protein in poorly differentiated anaplastic meningiomas may be indicating the possibility that the CX3CL1/CX3CR1 axis was involved in malignant transformation of meningiomas. Furthermore, a significant

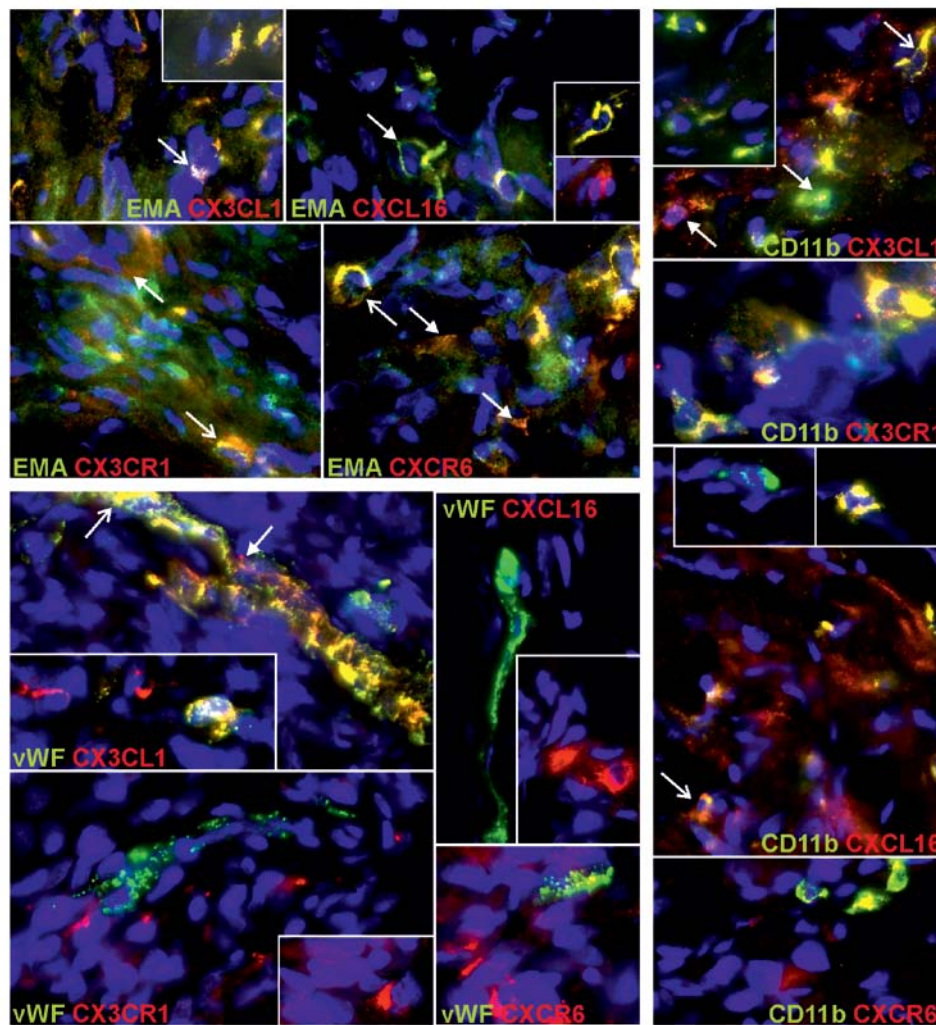


Figure 4. Examples of co-staining of different cellular markers EMA, vWF, CD11b and the chemokine/receptor pairs CX3CL1/CX3CR1 and CXCL16/CXCR6 in meningiomas WHO grade I sections determined by immuno-double-staining. The chemokine/receptor pairs CX3CL1/CX3CR1 and CXCL16/CXCR6 are partly co-stained with epithelial membrane antigen (EMA)-positive tumor cells, but not all tumor cells are positive for these molecules. Staining of CX3CL1/CX3CR1 and CXCL16 was also detected on CD11b-positive cells indicating microglial cells/monocytes. In contrast, the marker von Willebrand-factor (vWF) for endothelial cells did not merge with CX3CR1, CXCL16 or CXCR6 signals but was partly associated with CX3CL1. Shown is an exemplary choice of different tumor samples. Magnification, x630.

positive correlation between the expression of CX3CL1 and CX3CR1 was observed in meningiomas grade I, but not in grade II and III. Therefore, unbalanced expression of CX3CL1/CX3CR1 might be also associated with a malignant tendency of high-grade meningiomas.

Our results are in accordance with animal models of cancer vaccinations based on the overexpression of CX3CL1 in tumor cells which revealed as a consequence a strong antitumor response (43). The CX3CL1 gene was transduced into murine lung carcinoma cells and could induce antitumor immunity through chemoattraction and activation of T cells, dendritic cells and natural killer (NK) cells (43,44). Murine colon adenocarcinoma cells C26 and melanoma B16F10 cells showed that antitumor immune response induced by CX3CL1 gene transfer was depended on NK and T cells activation (28). However, Lavergne *et al* (45) reported that in an EL-4 lymphoma model, CX3CL1-mediated antitumor effects occurred only via NK cells but not T cells because this antitumor effect was observed only in T cell- and B cell-deficient Rag1^{-/-} mice, but was ablated

in NK cell-deficient beige mice. Therefore, transfer of the CX3CL1 gene into tumor cells could elicit a specific antitumor immunity through acting by its receptor CX3CR1 capable of inhibiting tumor growth which leads to increased survival of tumor-bearing hosts (46). In hepatocellular carcinoma patients, high expression of CX3CL1/CX3CR1 correlates with better prognosis and fewer recurrences, both local and distant (47).

CXCL16 is the second of the two known transmembrane chemokines, which is not only found on immune cells, but constitutive expression has also been described on fibroblasts, keratinocytes, endothelial cells and cancer cells of different tumor origin (13-15,17,21,48). Additionally, CXCL16 is expressed in the normal human brain at capillary endothelial cells and, pathologically, in malignant human glioma cells and reactive astrocytes *in situ* and *in vitro* (14,18). Furthermore, CXCL16 has been detected in human cerebrospinal fluid of patients with inflammatory brain diseases (49). Its receptor CXCR6 was reported to be expressed mainly on immune cells (34,50,51). In the present study we show that the chemokine/

receptor pair CXCL16/CXCR6 is expressed in solid human meningioma samples, especially localized on the tumor cells themselves and for CXCL16 on infiltrating microglial cells/macrophages. If comparing the expression levels of CXCL16 and CXCR6, CXCR6 is up to 100-fold lower expressed. Interestingly, CXCL16 was found to be significantly lower expressed in meningiomas WHO grade III. A positive correlation between the expression of CXCL16 and CXCR6 was observed especially in meningioma samples grade II and III. As indicated for CX3CL1/CX3CR1, these results support the hypothesis that also the CXCL16/CXCR6 axis might be associated with the pathogenesis of human malignant meningiomas.

The expression and functional role of CXCL16 and CXCR6 have been investigated in different types of tumor. Although CXCL16 and CXCR6 mostly seem to be overexpressed in different cancer cells, such as breast cancer tissue (14,52), prostate cancer tissues (53), colorectal cancer tissues (21), schwannomas (15) and pancreatic ductal adenocarcinoma (54,55), it was shown on the contrary that high CXCL16 expression correlated with good prognosis and increased levels of tumor-infiltrating lymphocytes (21). Additionally, CXCL16 was shown to inhibit proliferation in pancreatic carcinoma *in vitro* and *in vivo* (54,55). Moreover, an anti-migratory function of CXCL16 in renal cancer cells was demonstrated resulting in the patients with high CXCL16 expression showing significantly longer overall survival times (22). Noteworthy, in accordance with our results presented here for human meningiomas in human rectal cancer, downregulation of CXCL16 has been reported (13).

Summarized, the transmembrane chemokines CX3CL1 and CXCL16 and their corresponding receptors are clearly found to be abundantly expressed in human meningioma samples of different malignant grades. In view of these observations and our results, the CX3CL1/CX3CR1 and CXCL16/CXCR6 axis should be considered as potentially suitable targets for immunoprevention or gene therapy in human meningiomas, especially in anaplastic variants.

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