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Expression of the chemokines CXCL12 and CX3CL1 and their receptors in human nerve sheath tumors

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Summary. Peripheral nerve sheath tumors are in most cases slowly growing neoplasms that can be adequately cured by surgical resection. However, facing the risk of a neurosurgical intervention and the trend of multiple relapses of nerve sheath tumors the development of additional therapy strategies seems to be favourable, and therefore substantiated knowledge of molecular and cellular mechanisms in nerve sheath tumors should be achieved.

Here, we firstly describe the expression of the chemokines CXCL12 (SDF-1) and CX3CL1 (fractalkine) and their respective receptors CXCR4, CXCR7 and CX3CR1 in different entities of human nerve sheath tumors and normal control tissues. Both ligands and their receptors are expressed in high to moderate levels on mRNA and protein level in benign and malignant nerve sheath tumors. While CXCL12 was mainly found in schwannoma cells ($S100^+$) *in situ*, its receptor CXCR4 is also partly found on CD11b-positive macrophages / microglia and its alternative receptor CXCR7 is also expressed by endothelial cells and macrophages. CX3CL1 is expressed by parts of the schwannoma and endothelial cells, whereas its receptor CX3CR1 is expressed by nearly all tumor cells and macrophages, but not by endothelial cells.

Taken together, we could show the presence of CXCL12 and CX3CL1 and their respective receptors in benign and malignant human nerve sheath tumors. Further investigations may show their functional role in health and disease.

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Introduction

Primary tumors of the peripheral nerve sheath are neoplasms derived from cells within the nerve sheath or from cells differentiating towards a corresponding cell type. Most peripheral nerve sheath tumors in human are benign schwannomas. They are slow growing, mostly encapsulated and show the molecular and ultrastructural characteristics of Schwann cells (Scheithauer et al., 1997). They typically express the calcium-binding protein S100 (Weiss et al., 1983). Based on their localization, schwannomas can be subdivided into intracranial, spinal, peripheral and visceral schwannomas (Greenberg, 2006). Intracranial schwannomas account for about 8% of all intracranial tumors and predominantly originate from the Schwann cells accompanying the vestibular branch of the 8th intracranial nerve (Tos and Thomsen, 1984). Despite their benign character these vestibular schwannomas (VS) can cause clinical symptoms by compression of the nerve and neighbored brain structures ranging from unilateral hearing loss and vertigo to severe compression syndromes of the brainstem. Spinal schwannomas (SPS)

Abbreviations. IHC, immunohistochemistry; FNCLCC, Federation-Nationale-des-Centres-de-Lutte-Contre-le-Cancer; MPNST, malignant peripheral nerve sheath tumor; NCN, normal cranial nerve; NSN, normal spinal nerve; RT-PCR, reverse-transcription polymerase chain reaction; SPS, spinal schwannoma; VS, vestibular schwannoma; WHO, World Health Organization

represent about 25% of all primary intradural neoplasms of the spinal cord (Dorsi and Belzberg, 2004; Celli et al., 2005). Depending on size and localization of the tumors clinical symptoms range from mild back pain to severe neurological deficiencies. Schwannomas can occur sporadically or in the course of genetic disorders e.g. neurofibromatosis (NF) type 1 and 2 (Halliday et al., 1991). Studies on NF2 patients revealed mutations of the tumor suppressor NF2 gene which encodes for the schwannomin / merlin protein, and a lack of intact schwannomin / merlin protein was also observed in about 50% of sporadic VS (Roche et al., 2008).

Most peripheral nerve sheath tumors are benign according to their histopathological characteristics. However, malignant peripheral nerve sheath tumors (MPNST) form a group of highly malignant soft tissue sarcomas originating from or differentiating to nerve sheath cells (Gupta et al., 2008). They grow within and along nerves, may invade surrounding tissue, tend to multiple recurrences and may eventually cause metastasis by haematogenous and perineural spreading (Patil et al., 2007). MPNST can arise spontaneously in adult patients, but up to 40% of all MPNST are associated to neurofibromatosis type 1 (Evans et al., 2002), and rare cases of malignant progression of benign nerve sheath tumors have been described (e.g. Woodruff et al., 1994). The formation of MPNST is often associated with mutations / deletions of the *p53* and *Ink4a* genes and deregulation of signaling pathways (Menon et al., 1990; Kourea et al., 1999).

However, as described for many types of tumors tumorigenesis of peripheral nerve sheath tumors seems to be massively influenced by deregulation of intracellular signaling pathways. The signaling substances include growth factors (e.g. Ammoun et al., 2012; Badache and DeVreis, 1998), cytokines (Weerda et al., 1998) and also chemokines (Mori et al., 2004; Held-Feindt et al., 2008).

Chemokines play an important role in immunological as well as developmental processes, and they are sometimes referred to as the third mayor signaling system in the brain - apart from neurotransmitters and neuropeptides (Adler et al., 2006).

The chemokine family is comprised of about 50 small mostly secreted peptide mediators (about 8 - 12 kDa) and is subdivided into four subgroups depending on conserved cysteine motifs. Chemokines bind to a class of 7TMD G-protein coupled receptors, and remarkably, in most cases one chemokine can bind to more than one receptor, and most chemokine receptors can also be activated by several chemokines (Murphy, 2002). They were initially described as chemotactic cytokines attracting different subsets of leukocytes to sites of inflammation, but soon were proved to influence a broad spectrum of physiological processes, including angiogenesis (Strieter et al., 1995), haematopoiesis (Broxmeyer and Kim 1999) and development (Dambly-Chaudiere et al., 2007; Hattermann et al., 2008). However, chemokines can also play important roles in pathologies, and among these in tumor initiation, survival and progression (O'Hayre et al., 2008; Vandercappelen et al., 2008).

In particular, the chemokine CXCL12 (also termed SDF-1, *stromal cell-derived factor-1*) and its two receptors CXCR4 and CXCR7 seem to play an important role for tumor survival and metastasis. The first described functional role of CXCL12 in tumor progression was to drive metastasis of CXCR4-positive breast cancer cells to sides of high CXCL12 expression (Müller et al. 2001). This report was followed by numerous studies in further tumor models e.g. prostate cancer and glioblastomas (reviewed by Zlotnik et al., 2011). The chemokine receptor CXCR7, which was later discovered to be an additional receptor for CXCL12 (Balabanian et al., 2005; Burns et al., 2006) has also been described to essentially promote progression and survival of multiple tumor types, e.g. breast and lung tumors (Miao et al., 2007), prostate cancer (Wang et al., 2008) and glioblastomas (Hattermann et al., 2010, 2012) by activation of intracellular pathways, modulation of cell adhesion and cell migration and protection from chemically induced apoptosis.

Apart from direct effects that chemokines may exert on tumor cells, they may also influence the tumor stroma by attracting or stimulating non-tumor cells like invading leukocytes and endothelial cells. One example is CX3CL1 / fractalkine / neurotactin attracting and activating different subsets of leukocytes in the tumor, e.g. T cells in hepatocellular carcinomas (Tang et al., 2007) and macrophages / microglia in glioblastomas (Held-Feindt et al., 2010). Among the chemokines, CX3CL1 has a unique multi-domain structure composed of the chemokine domain, a mucin-like stalk region, a transmembrane domain and an intracellular tail. It can be constitutively or inducibly released by shedding processes mediated by distinct matrix-metalloproteinases (Ludwig und Mentlein, 2008). The chemokine domain binds to the chemokine receptor CX3CR1 that is expressed by monocytes, dendritic cells and lymphocyte subsets (reviewed by Ludwig and Weber, 2007). Apart from cytokine-induced CX3CL1 expression and release in inflammatory diseases like atherosclerosis and colitis, the CX3CL1 / CX3CR1 axis has been described for different tumor types, including epithelial ovarian cancer (Gaudin et al., 2011), prostate cancer (Jamieson et al., 2008) and pancreatic adenocarcinoma (Marchesi et al., 2008).

The role of CXCL12 and its two receptors CXCR4 and CXCR7, and CX3CL1 and its only receptor CX3CR1 for tumor formation, progression and survival has been elucidated for many types of tumors yielding promising targets for therapy strategies. However, in benign and malignant tumors of the nerve sheath little is known about the expression and role of chemokines, in particular about CXCL12 and CX3CL1 and their

respective receptors. The purpose of this study was to describe the mRNA and protein expression of CXCL12 / CXCR4 / CXCR7 and CX3CL1 / CX3CR1 in human benign and malignant nerve sheath tumors and to characterize the ligand and / or receptor expressing cells *in situ*.

Materials and methods

Tumor specimens

Tumor samples were obtained from 18 patients who underwent surgery in the Department of Neurosurgery, Kiel, Germany. In the operating room, the samples were snap-frozen within 15 min and permanently stored in liquid nitrogen or at -80°C until usage. As the Department of Neurosurgery Kiel is certificated by a quality management system (DIN EN ISO 9001:2008), the procedures of tissue sampling and storage are strictly controlled and the quality of the samples should be optimal. In total, 6 vestibular schwannomas (VS; 3 females 47.7±5.7 y, 3 males 53.7±19.2 y), 6 spinal periphery schwannomas (SPS; 3 females 55.7±15.9 y, 3 males 56.3±18.8 y) and 6 malignant peripheral nerve sheath tumors (MPNST; 1 female 58 y, 5 males 62.8±10.5 y) as diagnosed by a neuro-pathologist were included in this study. All tumors were diagnosed according to the WHO classification of tumors of the central nervous system (Louis et al., 2007), and included tumors were not of special types. For the diagnosis of malignant peripheral nerve sheath tumors (MPNSTs) rhabdomyosarcoma, leiomyosarcoma and synovial sarcoma were carefully ruled out. All MPNSTs were sporadic, 4 of them were FNCLCC grade II (WHO grade III), and 2 of them were FNCLCC grade III (WHO grade IV).

Control tissue from 3 normal spinal nerves (NSN) and 2 normal 8th cranial nerves (NCN) were obtained from autopsies in the Department of Pathology. All samples were obtained in accordance with approved ethical standards of the ethics committee of the University of Kiel and the Helsinki Declaration of 1975. If enough material was available matched probes were used for different experiments.

Real-time RT-PCR

Tumor tissue and normal tissue samples were homogenized in TRIZOL Reagent (Invitrogen, Carlsbad, CA), and RNA was isolated following the manufacturer's instructions. Genomic DNA was digested by RNase-free DNase (1 U/μl, Promega, Madison, WI), and cDNA was synthesized using random hexamer primers, dNTP mix and RevertAid™ H Minus M-MuLV Reverse Transcriptase (200 U/μl, all Fermentas, St. Leon-Rot, Germany). Quantitative PCR was performed in triplicate in a total volume of 20 μl comprising 10 μl 2x TaqMan Universal Master Mix (Applied Biosystems, Forster City, CA), 7 μl sterile DNase-free H_2O bidest, 1 μl of 20x Assay-on-Demand™ Gene Expression Assay Mix (Applied Biosystems) and 10 ng or 100 ng template cDNA in 2 μl. Identification numbers of Gene Expression Assays were: GAPDH Hs99999905_m1; CXCL12 Hs00171022_m1; CXCR4 Hs00607978_s1; CXCR7 Hs00602736_s1; CX3CL1 Hs00171086_m1; CX3CR1 Hs00365842. Thermal cycling protocol (2 min 50°C, 10 min 95°C, 40 cycles: 15 s 95°C, 1 min 60°C) was performed with the MyiQ™ Single Color Real-Time PCR Detection System (Bio-Rad, Munich, Germany) and fluorescence data were collected. Transcription of the respective gene of interest was normalized to Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) yielding ΔC_T values: $\Delta C_T = C_T$ (gene) - C_T (GAPDH). Due to logarithmic amplification, an increase of the ΔC_T value by 3.3 (3.3 cycles later exceeding the fluorescence threshold) corresponds to a 10-fold lower gene expression.

Immunohistochemistry

For immunohistochemistry, 10 μm sections of fresh-frozen tissues were fixed for 30 min with 4% paraformaldehyde (PFA), Tris-buffered saline was used as rinsing buffer. Sections were blocked for unspecific peroxidase activity with 3% H_2O_2 in 0.3% Triton-X100/TBS and for unspecific protein recognition with appropriate 10% normal blocking serum (Jackson ImmunoReseach Laboratories, Suffolk, UK). Primary antibodies were diluted in 0.3% Triton-X100/TBS with appropriate 2% normal serum, applied to the sections and incubated at 4°C over night: anti-CXCL12 (rabbit polyclonal sc-28876, 2 μg/ml, Santa Cruz Biotechnology, Santa Cruz, CA), anti-CXCR4 (rabbit polyclonal ab7199, 3 μg/ml, Abcam, Cambridge, MA), anti-CXCR7 (mouse monoclonal 11G8, 3 μg/ml, generously provided by M.E.T. Penfold, Chemocentryx, Mountain View, CA), anti-CX3CL1 (mouse monoclonal MAB3651, 2.5 μg/ml, R&D Systems, Wiesbaden, Germany), anti-CX3CR1 (rabbit polyclonal sc-30030, 1 μg/ml, Santa Cruz). For secondary antibody controls, isotype controls of respective species were used. Biotinylated secondary antibodies (Horse anti-mouse, 0.75 μg/ml, Vector Labs, Burlingame, CA; Donkey anti-rabbit, 0.75 μg/ml, Jackson ImmunoResearch Laboratories) were diluted in 1.5% normal blocking serum in TBS and applied to the sections for 60 min at room temperature. Amplification of the signal was achieved with the ABC Vectastain® Kit (Vector Labs), and peroxidase activity was detected by incubation with 0.06% 3,3' diaminobenzidine-tetrahydrochloride (DAB, Sigma-Aldrich, Munich, Germany) in 0.003% H_2O_2/ 0.1 M Tris for 3 min. Negative controls were performed by omitting the primary antibodies, and serum-controls and isotype-controls were included for each antibody using concentrations corresponding to secondary antibody concentrations. The sections were counterstained with

Meyer's Hemalum (Carl Roth GmbH, Karlsruhe, Germany) for 60 s and rinsed with tap water for 10 min. Afterwards, sections were dehydrated, cleared in RotiClear and mounted with RotiMount (both Carl Roth GmbH). For immunohistochemical stainings, tissue slides were employed that were obtained in the range of up to 100 μm (10 slides distinct) from the respective hematoxylin-eosin stained slides. Slides were viewed and images were taken with a Zeiss microscope and Zeiss digital camera (Zeiss, Oberkochen, Germany).

Immunofluorescence

Cryostat sections from fresh-frozen vestibular and spinal schwannoma and control nerve specimens were fixed with an icecold mixture of acetone / methanol (1:1) for 10 min, rinsed with washing buffer Tris-buffered saline plus 0.1% Tween 20 (TBS-T, 3x), incubated in 20%, then 70% ethanol (2 min each) and blocked with 1% Sudan black (in 70% ethanol) for 10 min to block lipid autofluorescence. Slides were washed dye-free in 70% ethanol, then in 20 % ethanol (2 min). Unspecific bindings were blocked with 0.1% bovine serum albumin (BSA) / 0.2% glycine in TBS for 60 min, then primary antibodies were applied and incubated over night at 4°C. Primary antibodies for cellular markers were anti-S100 (rabbit polyclonal, 1:5000, Sigma-Aldrich s2644), anti-von-Willebrand factor (vWF; mouse monoclonal, 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) and anti-CD11b (mouse monoclonal, 1:100, Santa Cruz), antibodies against chemokines and receptors as described above except for anti-CX3CR1 (rabbit polyclonal, 1:5000, Biomol, Hamburg, Germany). For secondary antibody controls, primary antibodies were omitted.

After washing, secondary antibodies were applied in TBS-T for 60 min at 37°C. If co-stainings were performed with two antibodies from the same species, incubations were performed successively with an additional blocking step with FAB fragments (1:1000, Dianova, Hamburg, Germany) in between. Secondary antibody for cellular markers was donkey anti-mouse Alexa Fluor 488 (green, 1:1000; Invitrogen, Karlsruhe, Germany) and for chemokines donkey anti-mouse or anti-rabbit Alexa Fluor 555 (red, 1:1000; Invitrogen). Nuclei were counterstained with 4´,6-diamidino-2-phenylindole (DAPI, 1:30 000; Invitrogen) for 30 min at room temperature and slides were embedded using Immu-Mount (Shandon, Pittsburgh, PA, USA).

Statistical analysis

For statistical analysis, Student's t-test with independent samples (comparison of each tumor entity to its respective control tissue) and bivariate correlation analysis (Pearson correlation coefficients) were performed. Significance was indicated by asterisk (* $p<0.05$ and ** $p<0.01$).

Results

Expression of CXCL12 / CXCR4 / CXCR7 and CX3CL1 / CX3CR1 in human nerve sheath tumors and control nerve tissue

Initially, we investigated the transcription of chemokine - receptor axes CXCL12 - CXCR4 and CXCR7 and CX3CL1 - CX3CR1 in homogenates of different human nerve sheath tumors, malignant peripheral nerve sheath tumors (MPNST) and spinal schwannomas (SPS) in comparison to normal spinal nerve (NSN) tissue representing almost exclusively Schwann cells. Vestibular schwannomas (VS) were compared to tissue of the normal 8th cranial nerve (NCN). As shown in Fig. 1, both ligands CXCL12 and CX3CL1 are transcribed in moderate to high levels in nerve sheath tumors, the mean ΔC_T values for CXCL12 were 1.16 (SPS), -2.21 (MPNST) and 1.93 (VS) reaching nearly the transcription level of GAPDH, and for CX3CL1 6.15 (SPS), 3.60 (MPNST) and 7.15. Remarkably, in our sample collection CXCL12 and CX3CL1 mRNA levels were even around 10 to 100-fold higher in normal spinal nerves (NSN), with ΔC_T values of -3.99 and -0.26, respectively. In normal cranial nerves (NCN), CXCL12 was only slightly lower (ΔC_T 3.96) than in VS, whereas CX3CL1 mRNA was more highly expressed (ΔC_T 4.16) than in VS. Comparing both schwannoma entities SPS and VS, expression levels are about equal. Regarding the CXCL12 receptor CXCR4, mRNA levels differed little between tumor specimen and respective control tissue. However, transcription was moderate with ΔC_T values of 4.01 (NSN), 4.74 (SPS), 5.48 (MPNST), and 6.84 (NCN) and 5.29 (VS). In contrast, the CXCR7 mRNA level was altered resembling the expression level of its ligand CXCL12. In SPS (ΔC_T 0.40) and MPNST (ΔC_T 0.27) CXCR7 was approximately 10-fold lower transcribed than in normal control tissue (NSN, ΔC_T -2.51), and in VS significantly higher (ΔC_T 1.81) than in NCN (ΔC_T 3.57), and again SPS and VS had almost the same expression level.

The CX3CL1 receptor CX3CR1 was expressed at moderate levels, and with mean expression levels of peripheral tumors (SPS, ΔC_T 1.04; MPNST, ΔC_T 5.59) not significantly different from normal control tissue (NSN, 2.49). However, variation within the sample groups was quite substantial. In VS, CX3CR1 expression was approximately 10-fold higher (ΔC_T 1.56) than in control tissue (NCN, ΔC_T 5.01), contrasting the above described significantly lower transcription of the ligand CX3CL1.

To discover correlations between the alterations of ligands and their respective receptors we performed a bivariate Pearson correlation analysis. Each ligand was compared to its respective receptor(s) regarding each tumor entity and the group of all nerve sheath tumors. Graphics and resulting correlation coeffients are shown in Fig. 2. Positive correlation was shown for CXCL12

and CXCR4 in vestibular schwannomas (VS), and negative correlation for CXCL12 and CXCR7 in VS and for CX3CL1 and CX3CR1 in all investigated tumor specimens.

To confirm mRNA data at protein level we analysed tumor and normal tissue entities by immunohistochemistry and light microscopy. As shown in Fig. 3, all tumor specimens showed immunoreactivity for CXCL12 and CX3CL1 and their respective receptors CXCR4 and CXCR7, and CX3CR1. Red arrows mark positive stained tumor cells or tumor cell aggregates (in case of MPNST, SPS and VS), or comparable non-malignant Schwann cell regions (in control nerve tissues). To allow for better structural interpretation, DAB stainings are shown together with respective isotype and serum controls. Each sample was inspected in comparison to hematoxylin-eosin stainings (examples in the bottom row). Remarkably, some regions were more intensely

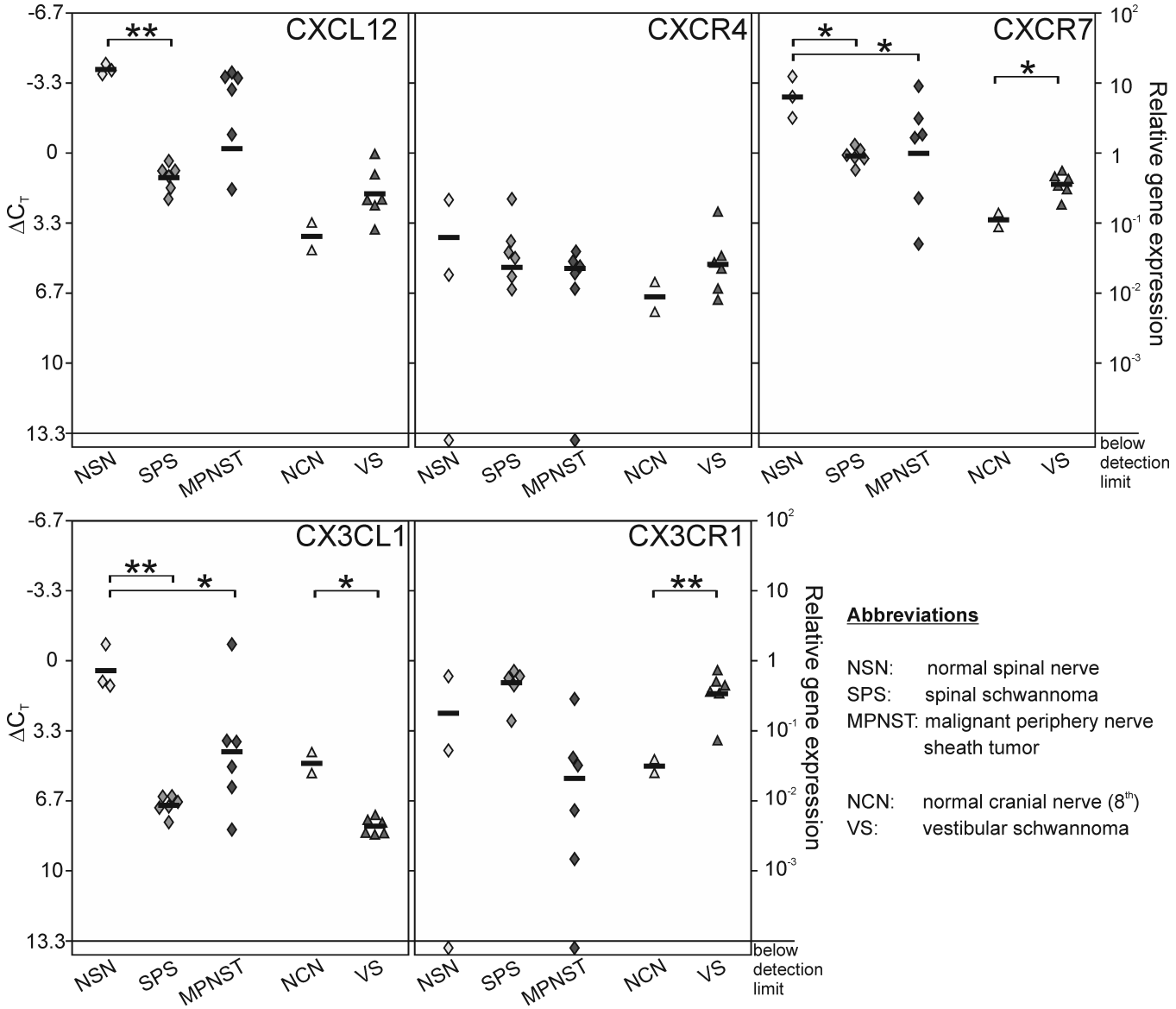

Fig. 1. Transcription of CXCL12 and its receptors CXCR4 and CXCR7 (upper row) and CX3CL1 and CX3CR1 (lower row) in human nerve sheath tumors and corresponding normal tissue. By quantitative RT-PCR mRNA levels of tumor and normal tissue samples were analysed and normalized to GAPDH yielding ΔC_T values. Single diamonds 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. Due to logarithmic amplification, differences between ΔC_T values of 3.33 correspond to one magnitude. Medium to high expression levels were detected for both chemokines and their receptors in normal as well as in tumor tissue. Asterisks indicate significant differences between normal and tumor tissue (* < 0.05, ** < 0.01).

stained than others, hinting at heterogeneity within tumor and also normal tissue.

In situ expression of CXCL12 and CX3CL1 and their receptors by cell entities

To investigate the expression of the chemokines CXCL12 and CX3CL1 and their receptors on distinct cell subsets within the nerve sheaths and tumors we performed fluorescence immune-doublestainings of the chemokines / receptors and cellular markers for schwannoma cells (S100), endothelial cells (von Willebrand-factor, vWF) and macrophages / microglia (CD11b) in different vestibular and spinal schwannoma tissue sections and peripheral nerves. As shown in Fig. 4 (first row), CXCL12 was co-localized with the schwannoma marker S100 expressed by tumor cells (although not all tumor cells are immunopositive for S100), but not produced by endothelial cells. CD11b positive microglia showed infrequent immunoreactivity for CXCL12, which may depict small portions of receptor-bound or secreted peptide. However, S100 positive schwannoma cells seem to be the major source for CXCL12. In normal nerve sheaths, CXCL12 was comparably expressed by Schwann cells (indicated by co-localization with the S100) but not expressed by endothelial cells (Fig. 4, lower panel). In contrast to schwannoma tissue, macrophages / microglia (indicated by CD11b) were hardly found in the normal tissue samples. Lumina of endothelial formations could rarely be recognized due to the use of cryostat sections (lumina often collapsed). Staining of CXCL12 sometimes occurred quite diffusely which is due to the fact that CXCL12 is secreted and may dispense throughout the extracellular space. Its receptors CXCR4 and CXCR7 were also expressed by schwannoma cells and partly by CD11b positive macrophages, but not all CD11b expressing cells were also receptor-positive. Tumor-associated endothelial cells marked by vWF were negative for CXCR4, but some of them were immunopositive for CXCR7 (Fig. 4, second row CXCR4, third row CXCR7). Likewise, in normal peripheral nerves, CXCR4 and CXCR7 were both found on Schwann cells, whereas endothelial cells partly expressed CXCR7 but not CXCR4 (Fig. 4, lower panel).

Immuno-doublestainings of the chemokine CX3CL1 and its receptor CX3CR1 and cellular markers revealed that most vestibular and spinal schwannoma cells identified by S100 are co-stained for CX3CL1, although its signal was partly weak, probably due to shedding processes (Fig. 5, first row). Virtually all tumor cells also expressed the receptor CX3CR1 (Fig. 5, second row). While the ligand CX3CL1 was also co-localized with the endothelial marker vWF, these endothelial cells were negative for the receptor, although CX3CR1 expression was remarkably high in proximity to blood capillaries. Macrophages / microglia cells that were stained with the surface marker CD11b were mostly negative for CX3CL1, but positive for its receptor CX3CR1. However, not all CX3CR1 positive cells co-expressed CD11b. In normal peripheral nerves, CX3CL1 expression was found in Schwann cell areas, although sometimes no clear merge was observed (probably due to different cellular localization; CX3CL1 membrane-bound or secreted, S100 intracellular). Like in schwannomas, CX3CL1 was co-localized with vWF indicating endothelial cells, and the receptor CX3CR1 was found on S100-positive Schwann cells but not on endothelial cells (Fig. 5, lower panel).

Taken together, we could observe mRNA and protein expression of CXCL12 and its two receptors CXCR4 and CXCR7, as well as CX3CL1 and its unique receptor CX3CR1 in human nerve sheaths and benign and

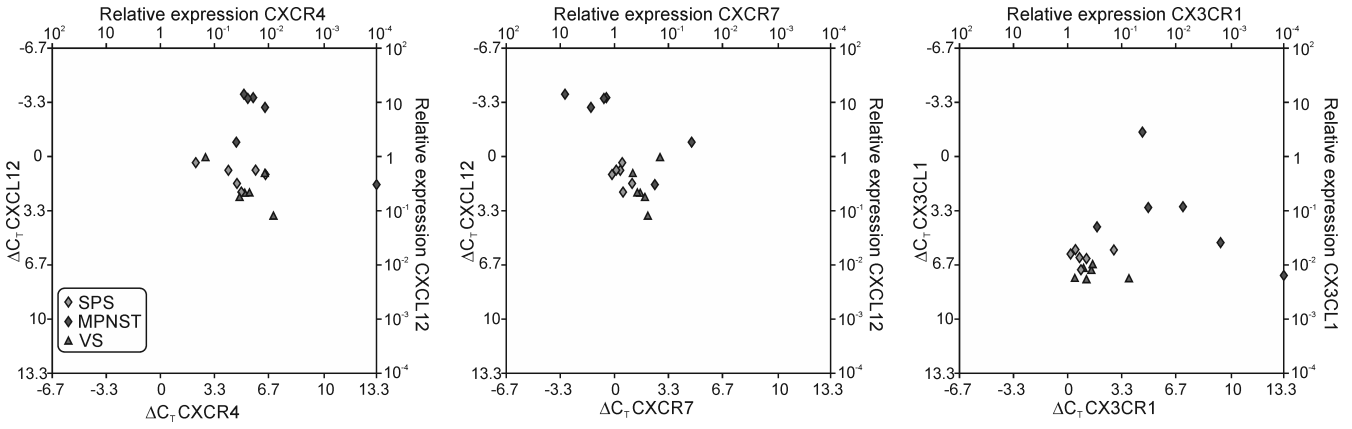

Fig. 2. Bivariate correlation analysis of chemokine / receptor pairs. ΔC_T values of CXCL12 or CX3CL1 were plotted against their respective receptors, and results of bivariate Pearson's correlation analysis of tumor groups and all nerve sheath tumors are shown. Positive correlation was obtained for CXCL12 / CXCR4 in vestibular schwannoma (VS), while negative correlation was observed for CXCL12 / CXCR7 in VS and for CX3CL1 / CX3CR1 in the complete tumor group.

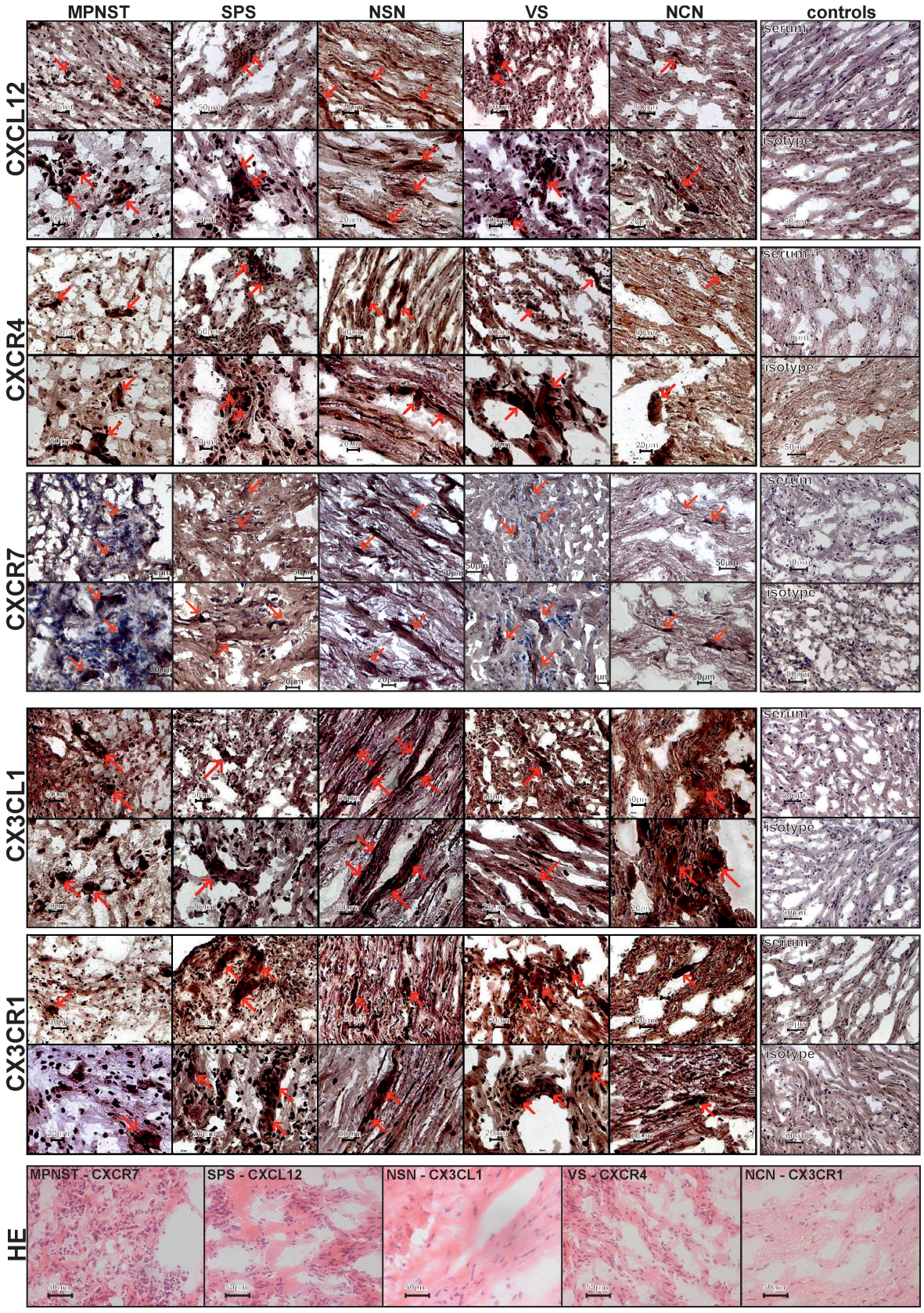

Fig. 3. Protein expression of chemokines and receptors as shown by immunohistochemistry. Nerve sheath tumors and peripheral nerves express the chemokines CXCL12 and CX3CL1 and their respective receptors at protein level, as shown by sample images of several different tumors. Arrows indicate positive stainings of tumor cells (MPNST, SPS, VS) or Schwann cells (NSN, NCN). Remarkably, staining intensity differs between different tumor regions indicating the heterogeneity of the tumor tissue. To verify that shown immunopositive regions were typical tumor or normal nerve sheath regions, sections were inspected in comparison to hematoxylin-eosin stainings (samples shown in the bottom row). Original magnifications are 200x for the upper rows and 400x for the lower rows of each chemokine or receptor and 200x for negative controls (serum and isotype controls) and HE stainings.

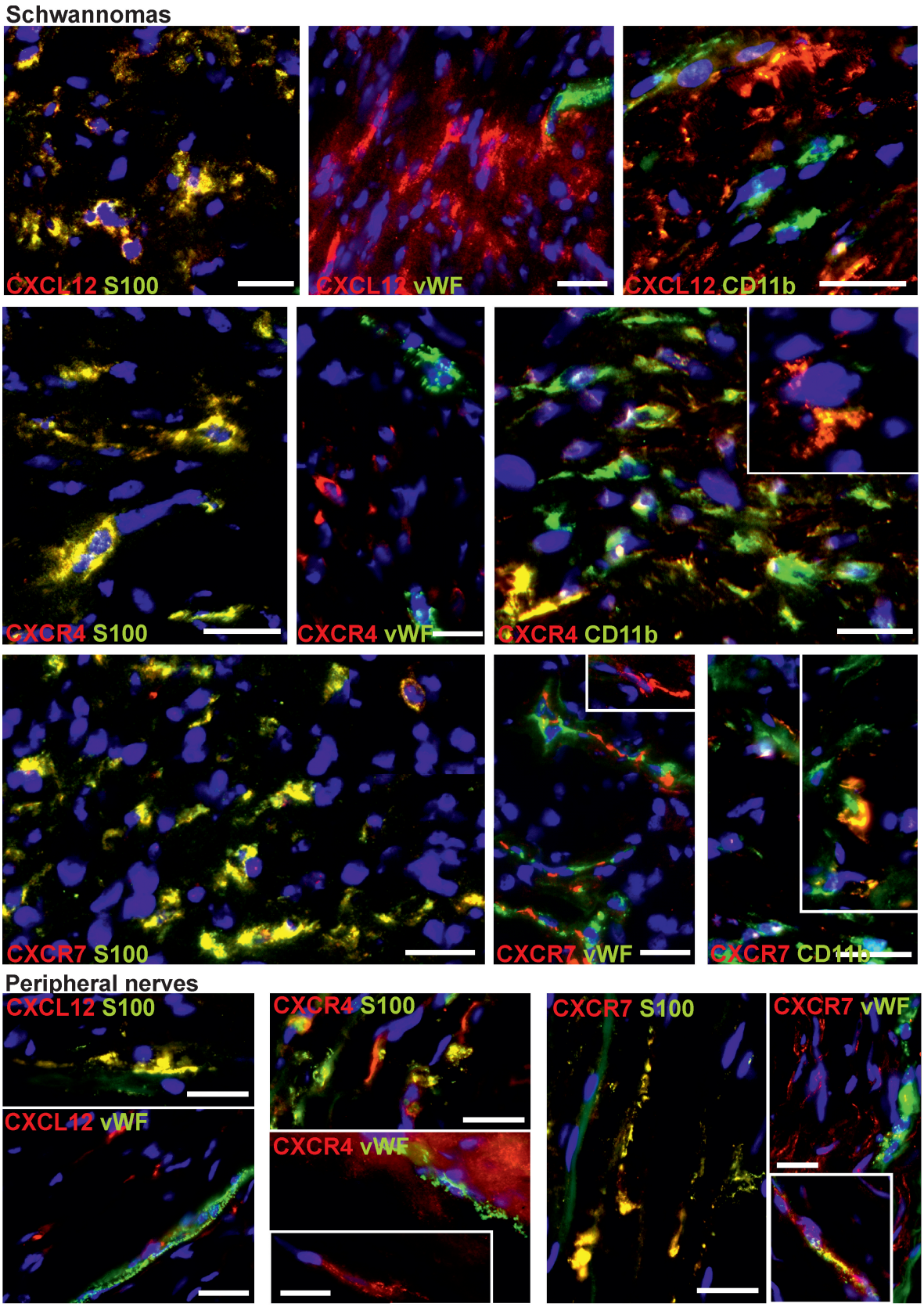

Fig. 4. Immuno-double staining of CXCL12 and its receptors CXCR4 and CXCR7 with cellular markers S100, vWF and CD11b in vestibular and spinal schwannomas and peripheral nerves. S100-positive tumor and Schwann cells were immunopositive for CXCL12 as well as for both its receptors, CXCR4 and CXCR7. The marker von Willebrand-factor (vWF) for endothelial cells did not coincide with CXCL12 or CXCR4 signals but was partly associated with CXCR7. CD11b positive macrophages/microglia were infrequently co-stained with CXCL12, but were positive for CXCR4 and partly for CXCR7. In peripheral nerve tissue, microglia cells were hardly observed (not shown). Shown is a choice of different tumor and nerve samples in original 630x magnification, scale bars indicate 20 μm. Inserts show clear co-stainings or clear single stainings to give a better impression of part co-localization.

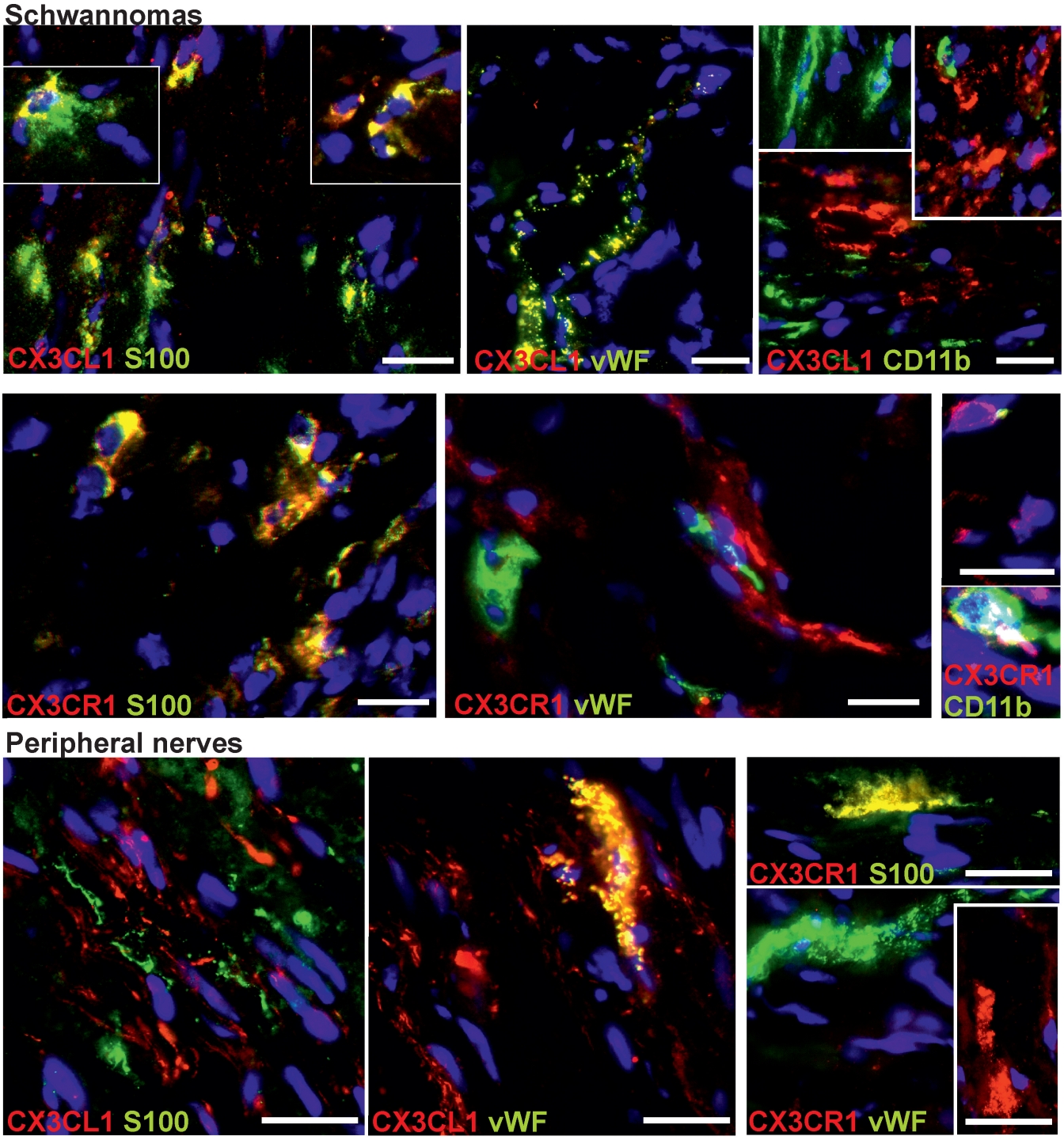

Fig. 5. Immuno-double staining of CX3CL1 and its receptor CX3CR1 with cellular markers S100, vWF and CD11b in vestibular and spinal schwannomas and peripheral nerves. The chemokine CX3CL1 is partly co-localized with the schwannoma marker S100, but not all tumor cells are positive for this ligand, while in peripheral nerves CX3CL1 was massively found in Schwann cell areas. However, most tumor cells and some Schwann cells express the receptor CX3CR1. Endothelial cells as indicated by immunoreactivity for vWF are positive for CX3CL1 but not for its receptor in tumors and in control tissue. In contrast, CD11b positive macrophages / microglia are mostly co-stained for CX3CR1, but not for the ligand CX3CL1; they were not observed in normal nerve tissues. Again, sample images in 630x magnification are shown with scale bars representing 20 μm. Inserts show clear co-stainings or clear single stainings to give a better impression of part co-localization.

malignant nerve sheath tumors. In some cases, ligand and receptor expression showed positive or negative correlation in distinct tumor entities. While S100-positive Schwann cells and tumor cells expressed both chemokines, CXCL12 and CX3CL1, and their respective receptors, tumor-associated endothelial cells and macrophages / microglia showed distinct expression patterns of ligands and / or receptors.

Discussion

In the nervous system, chemokines and their receptors are involved in many processes in health and disease. In our study, we firstly show the expression of CXCL12 and its receptors CXCR4 and CXCR7 at moderate to high levels in different nerve sheath tumors, as well as in normal control tissue samples. In some cases there were remarkably large variations between the individual tumor samples of one sample group, which might be due to different localization and, in case of MPNST, also from the different genesis of the individual tumors. However, whereas CXCR4 expression seemed to be quite constant, CXCL12 and CXCR7 expressions were reduced in peripheral nerve sheath tumors in comparison to NSN. Comparing VS with NCN, expression levels are slightly elevated, but in general the results of VS are comparable to those of SPS. Surprisingly, in NCN CXCL12 and CXCR7 levels are remarkably lower than in NSN. Thus, the mean CXCL12 and CXCR7 expression levels of all investigated nerve sheath tumors (MPNST, SPS, VS) are almost equal. However, the question if the difference between NCN and NSN is due to micro environmental conditions, or a random phenomenon of the limited sample number of these raw tissue samples, cannot be answered. Regarding cellular localization, Schwann cells / schwannoma cells are the major source of CXCL12 in nerve sheaths and their tumors, and they also express the receptors CXCR4 and CXCR7, probably enabling auto / parakrine effects. In contrast to many other chemokines that are strongly upregulated in inflammatory conditions, CXCL12 and its receptors CXCR4 and CXCR7 are constitutively expressed in various tissues, including the adult brain (Stumm et al., 2002; Schönemeier et al., 2008). Regarding nerve sheath building cells, the CXCL12 / CXCR4 pair has been shown to contribute to oligodendrocyte differentiation and remyelination processes in a murine demyelination model (Patel et al., 2010), and the receptor CXCR4 is regulated in Schwann cells by tumor necrosis factor-alpha (Küry et al., 2003). These interesting findings point to a functional role of CXCL12 and its receptors in inflammation and regeneration processes. In many types of tumors, including intracranial / nervous system tumors, CXCL12 can exert pro-tumoral effects, e.g. rescue from apoptosis in glioblastomas (Hattermann et al., 2010, 2012), proliferation in pituitary adenomas (Barbieri et al., 2008) and meningiomas (Barbieri et al., 2006). On the other hand, for less malignant low-grade astrocytomas, downregulation of CXCL12 by promotor hypermethylation has been reported (Zhou et al., 2008). To make it even more complex, stimulation of cultured Schwann cells can activate different pathways via CXCR4 and CXCR7, and both receptors are thought to mediate different as well as common cellular effects (Ödemis et al., 2010). Thus, by overexpression of one receptor, the effects of the other receptor may be diminished as both receptors compete for CXCL12. Indeed, scavenging CXCL12 to block CXCR4 activation was initially supposed to be the main function of CXCR7 until CXCR7-signalling was doubtlessly proved.

So the CXCL12 / CXCR4 / CXCR7 axis which underlies complex regulatory mechanisms probably contributes to building and / or maintenance of peripheral nerve sheaths and may be (partly) imbalanced in benign and malignant nerve sheath neoplasms. This hypothesis needs thorough evaluation by functional investigations especially as specific antagonists for the receptors CXCR4 (e.g. AMD3100, reviewed by De Clerq, 2003) and agonists and antagonists for CXCR7 (by Chemocentryx, Mountain View, CA) are available.

Apart from the expression of CXCL12 and its receptors, we firstly show the expression of CX3CL1 and its receptor CX3CR1 in human nerve sheath tumors. Regarding our sample collection, the chemokine CX3CL1 is moderately expressed in all nerve sheath tumor entities. However, expression is remarkably reduced compared to control tissue. Compared to CXCL12, CX3CL1 expression again is remarkably lower in NCN than in NSN, and expression levels in both schwannoma groups are almost equal. But here, in contrast to CXCL12, there is a significant decrease of CX3CL1 expression in neoplasms in intracranial as well as extracranial samples. The receptor CX3CR1 is slightly to significantly increased in benign schwannomas but slightly decreased in MPNST. However, the great variation among MPNST samples in CX3CL1 and CX3CR1 expression is again probably due to different genesis and origin. *In situ*, schwannoma cells express both ligand and receptor; endothelial cells only produce CX3CL1 whereas on macrophages / microglia only the receptor CX3CR1 is detectable. Whereas the expression of CX3CL1 by endothelial cells (Bazan et al., 1997) and of CX3CR1 by macrophages / microglia (Hughes et al., 2002; Held-Feindt et al., 2010) has been reported earlier, to our knowledge, this is the first report on CX3CL1 and CX3CR1 expression in Schwann cells and schwannoma cells that may influence nerves in health and disease. CX3CL1 is known to have neuroprotective effects by inhibition of microglia activation in animal models of different neural diseases, e.g. Parkinson's disease and stroke (Cipriani et al., 2011; Pabon et al., 2011). In tumorigenesis, it is described to be a mediator for perineural spreading of tumor cells (Marchesi et al., 2008). This effect might be interesting to study in the invasion of MPNST as these have

decreased CX3CL1 (and CX3CR1) levels but also infiltrate perineurium and epineurium as well as the surrounding tissues. In glioblastomas, CX3CL1 has been reported to attract CX3CR1-expressing macrophages / microglia *in vitro* (Held-Feindt et al., 2010). Here we show that CX3CR1-expressing macrophages / microglia are present in nerve sheath tumor entities whereas this leukocyte population is very rare in normal control tissues. Although the bulk of Schwann cells / schwannoma cells are also CX3CR1-positive, these invaded macrophages / microglia may contribute to elevated CX3CR1 levels in nerve sheath neoplasms. However, whether the attraction of leukocytes to the tumor is favourable or not regarding tumor growth and progression surely depends on the tumor type and is still under investigation. Additionally, recent investigations have revealed a further relevance of CX3CL1 and its receptor in tumor-stroma-interaction by promotion of angiogenesis in hepatocellular carcinoma cells *in vitro* and *in vivo* (Li et al., 2010).

The fact that CX3CL1 is expressed as a membrane-bound molecule that can be shed by matrix-metalloproteinases, especially ADAM10 and ADAM17 (Garton et al., 2001; Hundhausen et al., 2003), reveals a powerful regulation mechanism apart from transcriptional regulation upon inflammatory cytokine stimulation. By proteolytic cleavage, the transmembrane CX3CL1 loses its function as an adhesion molecule, and the released soluble form may act as chemoattractant or as para / autocrine stimulation factor.

Regarding these effects on leukocyte attraction, angiogenesis and adhesion, further functional studies are needed to reveal the role of the CX3CL1 / CX3CR1 axis in healthy nerve sheaths and its deregulation in nerve sheath tumors. Helpful tools may be neutralizing or receptor-blocking antibodies, truncated CX3CL1 analogs and non-specific antagonists (reviewed by D'Haese et al., 2010) or inhibitors of CX3CL1 proteolytic cleavage (Ludwig et al., 2005).

Taken together, the comparably high expression levels of the chemokines CXCL12 and CX3CL1 and their respective receptors hint at a functional role in the physiology of human nerve sheaths. In benign and malignant nerve sheath tumors, the imbalanced regulation of these chemokines may influence the neoplastic cells and also contribute to tumor-stroma interaction. Thus, CXCL12 and CX3CL1 and their respective receptors may be interesting targets for further functional studies on their role in the growth, progression and environment interaction of human nerve sheath tumors.

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References

Adler M.W., Geller E.B., Chen X. and Rogers T.J. (2006). Viewing chemokines as a third major system of communication in the brain. AAPS J. 7, E865-870.

Ammoun S., Schmid M.C., Zhou L., Ristic N., Ercolano E., Hilton D.A., Perks C.M. and Hanemann C.O. (2012). Insulin-like growth factor-binding protein-1 (IGFBP-1) regulates human schwannoma proliferation, adhesion and survival. Oncogene 31, 1710-1722.

Badache A. and DeVreis G.H. (1998). Neurofibrosarcoma-derived Schwann cells overexpress platelet-derived growth factor (PDGF) receptors and are induced to proliferate by PDGF BB. J. Cell. Physiol. 177, 334-342.

Balabanian K., Lagane B., Infantino S., Chow K.Y., Harriague J., Moepps B., Arenzana-Seisdedos F., Thelen M. and Bachelerie F. (2005). The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. J. Biol. Chem. 280, 35760-35766.

Barbieri F., Bajetto A., Porcile C., Pattarozzi A., Massa A., Lunardi G., Zona G., Dorcaratto A., Ravetti J.L., Spaziante R., Schettini G. and Florio T. (2006). CXC receptor and chemokine expression in human meningioma: SDF1/CXCR4 signaling activates ERK1/2 and stimulates meningioma cell proliferation. Ann. N Y Acad. Sci. 1090, 332-343.

Barbieri F., Bajetto A., Stumm R., Pattarozzi A., Porcile C., Zona G., Dorcaratto A., Ravetti J.L., Minuto F., Spaziante R., Schettini G., Ferone D. and Florio T. (2008). Overexpression of stromal cell-derived factor 1 and its receptor CXCR4 induces autocrine/paracrine cell proliferation in human pituitary adenomas. Clin. Cancer Res. 14, 5022-5032.

Bazan J.F., Bacon K.B., Hardiman G., Wang W., Soo K., Rossi D., Greaves D.R., Zlotnik A. and Schall T.J. (1997). A new class of membrane-bound chemokine with a CX3C motif. Nature 385, 640-4.

Broxmeyer H.E. and Kim C.H. (1999). Regulation of hematopoiesis in a sea of chemokine family members with a plethora of redundant activities. Exp. Hematol. 27, 1113-1123.

Burns J.M., Summers B.C., Wang Y., Melikian A., Berahovich R., Miao Z., Penfold M.E., Sunshine M.J., Littman D.R., Kuo C.J., Wei K., McMaster B.E., Wright K., Howard M.C.and Schall T.J. (2006). A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J. Exp. Med. 203, 2201-2213.

Celli P., Trillo G. and Ferrante L. (2005). Spinal extradural schwannoma. J. Neurosurg. Spine 2, 447-456.

Cipriani R., Villa P., Chece G., Lauro C., Paladini A., Micotti E., Perego C., De Simoni M.G., Fredholm B.B., Eusebi F. and Limatola C. (2011). CX3CL1 is neuroprotective in permanent focal cerebral ischemia in rodents. J. Neurosci. 31, 16327-16335.

Dambly-Chaudiere C., Cubedo N. and Ghysen A. (2007). Control of cell migration in the development of the posterior lateral line: antagonistic interactions between the chemokine receptors CXCR4 and CXCR7/RDC1. BMC Dev. Biol. 7, 23.

De Clerq E. (2003). The bicyclam AMD3100 story. Nat. Rev. Drug Discov. 2, 581-587.

D'Haese J.G., Demir I.E., Friess H. and Ceyhan G.O. (2010). Fractalkine/CX3CR1: why a single chemokine-receptor duo bears a major and unique therapeutic potential. Expert Opin. Ther. Targets 14, 207-219.

Dorsi M.J. and Belzberg A.J. (2004). Paraspinal nerve sheath tumors.

Neurosurg. Clin. N. Am. 15, 217-222.

Evans D.G., Baser M.E., McGaughran J., Sharif S., Howard E. and Moran A. (2002). Malignant peripheral nerve sheath tumors in neurofibromatosis 1. J. Med. Genet. 39, 311-314.

Garton K.J., Gough P.J., Blobel C.P., Murphy G., Greaves D.R., Dempsey P.J. and Raines E.W. (2001). Tumor necrosis factor-alpha-converting enzyme (ADAM17) mediates the cleavage and shedding of fractalkine (CX3CL1). J. Biol. Chem. 276, 37993-38001.

Gaudin F., Nasreddine S., Donnadieu A.C., Emilie D., Combadière C., Prévot S., Machelon V. and Balabanian K. (2011). Identification of the chemokine CX3CL1 as a new regulator of malignant cell proliferation in epithelial ovarian cancer. PLoS One 6, e21546.

Greenberg M.S. (2006). Handbook of neurosurgery, 6th ed. Thieme Medical Publishers. New York, USA.

Gupta G., Mammis A. and Maniker A. (2008). Malignant peripheral nerve sheath tumors. Neurosurg. Clin. N. Am. 19, 533-543.

Halliday A.L., Sobel R.A. and Martuza R.L. (1991). Benign spinal nerve sheath tumors: Their occurrence sporiadically or in neurofibromatosis type 1 and 2. J. Neurosurg. 74, 248-253.

Hattermann K., Ludwig A., Gieselmann V., Held-Feindt J. and Mentlein R. (2008). The chemokine CXCL16 induces migration and invasion of glial precursor cells via its receptor CXCR6. Mol. Cell. Neurosci. 39, 133-141.

Hattermann K., Held-Feindt J., Lucius R., Sebens Müerköster S., Penfold M.E., Schall T.J. and Mentlein R. (2010). The chemokine receptor CXCR7 is highly expressed in human glioma cells and mediates anti-apoptotic effects. Cancer Res. 70, 3299-3308.

Hattermann K., Held-Feindt J. and Mentlein R. (2012). CXCL12 mediates apoptosis resistance in rat C6 glioma cells. Oncol. Rep. 27, 1348-1352.

Held-Feindt J., Hattermann K., Muerkoster S., Wedderkopp H., Knerlich-Lukoschus F., Ungefroren H., Mehdorn H.M. and Mentlein R. (2010). CX3CR1 promotes recruitment of human glioma-infiltrating microglia/macrophages (GIMs). Exp. Cell. Res. 316, 1553-1566.

Hughes P.M., Botham M.S., Frentzel S., Mir A. and Perry V.H. (2002). Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, during acute and chronic inflammation in the rodent CNS. Glia 37, 314-27.

Hundhausen C., Misztela D., Berkhout T.A., Broadway N., Saftig P., Reiss K., Hartmann D., Fahrenholz F., Postina R., Matthews V., Kallen K.J., Rose-John S. and Ludwig A. (2003). The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX3CL1 (fractalkine) and regulates CX3CL1-mediated cell-cell adhesion. Blood 102, 1186-1195.

Jamieson W.L., Shimizu S., D'Ambrosio J.A., Meucci O. and Fatatis A. (2008). 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. 68, 1715-1722.

Kourea H.P., Orlow I., Scheithauer B.W., Cordon-Cardo C. and Woodruff J.M. (1999). Deletions of INK4A gene occur in malignant peripheral nerve sheath tumors but not in neurofibromas. Am. J. Pathol. 155, 1860-1866.

Küry P., Köller H., Hamacher M., Cornely C., Hasse B. and Müller H.W. (2003). Cyclic AMP and tumor necrosis factor-alpha regulate CXCR4 gene expression in Schwann cells. Mol. Cell. Neurosci. 24,1-9.

Li F., Wang Z., Liu Y. and Li J. (2010). Down-regulation of fractalkine inhibits the *in vitro* and *in vivo* angiogenesis of the hepatocellular carcinoma HepG2 cells. Oncol. Rep. 24, 669-675.

Louis D.N., Ohgaki H., Wiestler O.D. and Cavenee W.K. (eds) (2007) WHO Classification of tumours of the central nervous system. IARC Press. Lyon, France.

Ludwig A. and Weber C. (2007). Transmembrane chemokines: versatile 'special agents' in vascular inflammation. Thromb. Haemost. 97, 694-703.

Ludwig A. and Mentlein R. (2008). Glial cross-talk by transmembrane chemokines CX3CL1 and CXCL16. J. Neuroimmunol. 198, 92-97.

Ludwig A., Hundhausen C., Lambert M.H., Broadway N., Andrews R.C., Bickett D.M., Leesnitzer M.A. and Becherer J.D. (2005). Metalloproteinase inhibitors for the disintegrin-like metalloproteinases ADAM10 and ADAM17 that differentially block constitutive and phorbol ester-inducible shedding of cell surface molecules. Comb. Chem. High Throughput Screen 8, 161-71.

Marchesi F., Piemonti L., Fedele G., Destro A., Roncalli M., Albarello L., Doglioni C., Anselmo A., Doni A., Bianchi P., Laghi L., Malesci A., Cervo L., Malosio M., Reni M., Zerbi A., Di Carlo V., Mantovani A. and Allavena P. (2008). The chemokine receptor CX3CR1 is involved in the neural tropism and malignant behavior of pancreatic ductal adenocarcinoma. Cancer Res. 68, 9060-9069.

Menon A.G., Anderson K.M., Riccardi V.M., Chung R.Y., Whaley J.M., Yandell D.W., Farmer G.E., Freiman R.N., Lee J.K., Li F.P., Barker D.F., Ledbetter D.H., Kleider A., Martuza R.L., Gusella J.F. and Seizinger B.R. (1990). Chromosome 17p deletions and p53 gene mutations associated with the formation of malignant neurofibrosarcomas in von Recklinghausen neurofibromatosis. Proc. Natl. Acad. Sci. USA 87, 5435-5439.

Miao Z., Luker K.E., Summers B.C., Berahovich R., Bhojani M.S., Rehemtulla A., Kleer C.G., Essner J.J., Nasevicius A., Luker G.D., Howard M.C. and Schall T.J. (2007). CXCR7 (RDC1) promotes breast and lung tumor growth *in vivo* and is expressed on tumor-associated vasculature. Proc. Natl. Acad. Sci. USA 104, 15735-40.

Mori K., Chano T., Yamamoto K., Matsusue Y. and Okabe H. (2004). Expression of macrophage inflammatory protein-1alpha in Schwann cell tumors. Neuropathology 24, 131-135.

Müller A., Homey B., Soto H., Ge N., Catron D., Buchanan M.E., McClanahan T., Murphy E., Yuan W., Wagner S.N., Barrera J.L., Mohar A., Verástegui E. and Zlotnik A. (2001). Involvement of chemokine receptors in breast cancer metastasis. Nature 410, 50-6.

Murphy P.M. (2002). International Union of Pharmacology. XXX. Update on chemokine receptor nomenclature. Pharmacol. Rev. 54, 227-229.

O'Hayre M., Salanga C.L., Handel T.M. and Allen S.J. (2008). Chemokines and cancer: migration, intracellular signaling, and intercellular communication in the microenvironment. Biochem. J. 409, 635-649.

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

Pabon M.M., Bachstetter A.D., Hudson C.E., Gemma C. and Bickford P.C. (2011). CX3CL1 reduces neurotoxicity and microglial activation in a rat model of Parkinson's disease. J. Neuroinflammation 8, 9.

Patel J.R., McCandless E.E., Dorsey D. and Klein R.S. (2010). CXCR4 promotes differentiation of oligodendrocyte progenitors and remyelination. Proc. Natl. Acad. Sci. USA 107,11062-7.

Patil K., Mahima V.G. and Ambika L. (2007). Malignant peripheral nerve sheath tumour: an elusive diagnosis. Indian J. Dent. Res. 18, 19-22.

Roche P.H., Bouvier C., Chinot O. and Figarella-Branger D. (2008). Genesis and biology of vestibular Schwannomas. Prog. Neurol.

Surg. 21, 24-31.

Scheithauer B.W., Woodruff J.R. and Erlandson J. (1997). Tumors of the peripheral nervous system: Atlas of tumor pathologies. 3rd series. Fascicle 24. Washington, D.C., Armed Forces Institute of Pathology.

Schönemeier B., Kolodziej A., Schulz S., Jacobs S., Hoellt V. and Stumm R. (2008). Regional and cellular localization of the CXCL12/SDF-1 chemokine receptor CXCR7 in the developing and adult rat brain. J. Comp. Neurol. 10, 207-220.

Strieter R.M., Polverini P.J., Kunkel S.L., Arensberg D.A., Burdick M.D., Kasper J., Dzuiba J., Damme J.V., Walz A., Marriot D., Chan S.Y., Roczniak S. and Shanafelt A.B. (1995). The functional role of the ERL-motif in CXC chemokine-mediated angiogenesis. J. Biol. Chem. 270, 27348-27357.

Stumm R.K., Rummel J., Junker V., Culmsee C., Pfeiffer M., Krieglstein J., Höllt V. and Schulz S. (2002). A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia. J. Neurosci. 22, 5865-5878.

Tang L., Hu H.D., Hu P., Lan Y.H., Peng M.L., Chen M. and Ren H. (2007). Gene therapy with CX3CL1/Fractalkine induces antitumor immunity to regress effectively mouse hepatocellular carcinoma. Gene Ther. 14, 1226-1234.

Tos M. and Thomsen J. (1984). Epidemiology of acoustic neuromas. J. Laryngol. Otol. 98, 685-692.

Vandercappellen J., Van Damme J. and Struyf S. (2008). The role of CXC chemokines and their receptors in cancer. Cancer Lett. 28, 226-44.

Wang J., Shiozawa Y., Wang J., Wang Y., Jung Y., Pienta K.J., Mehra R., Loberg R. and Taichman R.S. (2008). The role of CXCR7 / RDC1 as a chemokine receptor for CXCL12 / SDF-1 in prostate cancer. J. Biol. Chem. 283, 4283-4294.

Weerda H.G., Gamberger T.I., Siegner A., Gjuric M. and Tamm E.R. (1998). Effects of transforming growth factor-beta1 and basic fibroblast growth factor on proliferation of cell cultures derived from human vestibular nerve schwannoma. Acta Otolaryngol. 118, 337-343.

Weiss S.W., Langloss J.M. and Enzinger F.M. (1983). Value of S-100 protein in the diagnosis of soft tissue tumors with particular reference to benign and malignant Schwann cell tumors. Lab. Invest. 49, 299-308.

Woodruff J.M., Selig A.M., Crowley K. and Allen P.W. (1994). Schwannoma (neurilemoma) with malignant transformation. A rare, distinctive peripheral nerve tumor. Am. J. Surg. Pathol. 18, 882-895.

Zhou W., Jiang Z., Song X., Liu Y., Wen P., Guo Y., Xu F., Kong L., Zhang P., Han A. and Yu J. (2008). Promoter hypermethylation-mediated down-regulation of CXCL12 in human astrocytoma. J. Neurosci. Res. 86, 3002-3010.

Zlotnik A., Burkhardt A.M. and Homey B. (2011). Homeostatic chemokine receptors and organ-specific metastasis. Nat. Rev. Immunol. 11, 597-606.

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