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Invited review

An Infernal Trio: The chemokine CXCL12 and its receptors CXCR4 and CXCR7 in tumor biology

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SUMMARY

Chemokines are small peptide mediators that play a role in many physiological and pathological processes. Apart from their initially discovered function in trafficking of leukocytes, they also influence migration, proliferation, survival and gene expression of a variety of cell types in their respective microenvironment. Chemokines can exert these effects via their respective G protein-coupled receptor. Over the recent decade, the involvement of chemokines and their respective receptors in tumor biology has been successively elucidated. This review will focus on the signaling and effects of the widespread chemokine CXCL12 and its long known G protein-coupled receptor CXCR4 and the recently discovered non-G protein-coupled receptor CXCR7 with a detailed reflection on glioma biology.

Contents

1. The chemokine family and their receptors

The human chemokine superfamily is comprised of about 50 small soluble peptides at a size range of 8–12 kDa (Rollins, 1997). They were initially discovered as *chemo*tactic cyto*kines* exerting chemotactic effects on different subsets of leukocytes. Beyond their function in recruitment and activation of leukocytes, the involvement of chemokines in a variety of processes in development, homeostasis and also pathology of vertebrates has been described during the last two decades. The chemokine family is divided into four subfamilies based on two conserved N-terminal cysteine residues that built disulfide bonds to two other cysteine residues within the peptide. These conserved cysteine residues are separated by one amino acid (CXC- or α-chemokines), directly adjacent (CC- or β-chemokines) or separated by three amino acids (CX3C- or δ-chemokine). Lymphotactin, the only known human member of the XC- or γ-chemokine family, harbors only one N-terminal cysteine residue. Several members of the CXC-chemokines share a conserved ELR-motif, and these ELR$^+$ chemokines can exert angiogenic effects via their common receptor CXCR2, while at least some ELR$^-$ chemokines seem to have angiostatic properties (Strieter et al., 2005).

Beyond this structurally based classification, chemokines can also be distinguished in homeostatic and inflammatory chemokines, and those that can have both properties depending on the environmental conditions. Being constitutively expressed,

Abbreviations: AP-1, activator protein 1; cAMP, cyclic adenosine monophosphate; CNS, central nervous system; CREB, cAMP response element-binding; DPP, dipeptidyl peptidase; ECM, extracellular matrix; ELK-1, ETS-like transcription factor 1; ERK, extracellular-signal related kinase; GBM, Glioblastomamultiforme; hBD, human beta defensin; HGF/SF, hepatocyte growth factor/scatter factor; HIV, human immunodeficiency virus; IFNγ, interferon-γ; IL, interleukin; ITAC, interferon-inducible T cell alpha chemoattractant XCL11; JAK, Janus kinase; MAPK, mitogen-activated proteinkinase; MIF, macrophage migration inhibitory factor; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; PBSF, pre-B-cell growth-stimulating factor; PI3-kinase, phosphoinositide 3-kinase; SDF-1, stromal cell-derived factor 1 XCL12; STAT, signal transducer and activator of transcription; TEM, transendothelial migration; TFF2, trefoil factor 2; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

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homeostatic chemokines regulate physiological migration processes of leukocytes and their precursors, e.g. trafficking of lymphocytes to distinct positions in lymphoid organs and thus supporting their maturation (Yoshie et al., 1997). Apart from the immune system, homeostatic chemokines can contribute to the development and maintenance of tissues and organs, for example in the brain, where migration and patterning processes as well as differentiation to different lineages is influenced by chemokines (Reiss et al., 2002; Padovani-Claudio et al., 2006; Hattermann et al., 2008). Additionally, there are good hints that chemokines function as a third communication system in the brain (beside neurotransmitters and neuropeptides) mediating and modulating the cross-talk of neurons and glial cell types under physiological conditions, but also in diseases like neuroinflammation and neuropathic pain (Adler et al., 2005; Rostene et al., 2011) which leads over to the group of pro-inflammatory chemokines. Pro-inflammatory chemokines are mostly inducible by pro-inflammatory cytokines, e.g. induction of CCL2 and CXCL8 by interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α, Kasahara et al., 1991), as well as induction of CXCL10 by interferon-γ (IFNγ, Narumi and Hamilton, 1991). They contribute to inflammatory processes by recruiting leukocytes to inflammatory regions and facilitating the trespass of the endothelial barrier (Smith et al., 1991; Ebisawa et al., 1994; van Golen et al., 2008). Additionally, ELR$^+$ CXC-chemokines (e.g. CXCL8) promote angiogenesis supporting the wound healing but also the neovascularization of tumors (for review: Keeley et al., 2011).

Chemokines can exert their functional effects by binding to G protein-coupled 7 transmembrane domain receptors of the chemokine receptor family. To date, about 20 human chemokine receptors have been identified, and while some ligands exclusively bind to one receptor, others may interact with several members of the respective receptor family. This redundancy is assumed to allow for fine tuning of (immune) responses, but is also the reason why mutations and deletions of single receptors can be compensated. Shortly after their initial identifications in the early 1990s (e.g. Loetscher et al., 1994), some chemokines receptors gained attention as they proved to be co-receptors for the human immunodeficiency virus (HIV) entry (Deng et al., 1996; Feng et al., 1996).

Though lacking a general signature, the members of the chemokine receptor family possess several common motifs facilitating binding of their ligands and downstream signaling, e.g. the DRYLAIVHA motif in the second intracellular loop that was thought to be crucial in signaling for a long time (Murphy et al., 2000). The intracellular C-terminal tail harbors serine and threonine residues that can be phosphorylated upon ligand binding eliciting intracellular signaling like G protein-recruitment, elevated calcium levels and generation of inositol phosphate (Haribabu et al., 1997). Additionally, β-arrestin binds to distinct serine residues at the C-terminus determining the G protein-coupling and initializing the internalization of the receptor (and the bound ligand) which is an important feature in regulation of chemokine functions (Barlic et al., 1999; Orsini et al., 1999).

Beyond their influence in several inflammatory diseases, chemokines and their receptors were shown to play diverse roles in tumor biology affecting leukocyte recruitment, neovascularization and tumor progression as nicely reviewed before (O'Hayre et al., 2008; Vandercappellen et al., 2008). In particular, the chemokine CXCL12/SDF-1 (stromal cell-derived factor 1) and its receptor CXCR4 have gained attention when their significant influence on the metastasis of breast cancer cells was reported (Müller et al., 2001).

CXCL12 was initially described as soluble pre-B-cell growth-stimulating factor (PBSF) secreted by a stromal cell line and supporting bone marrow B cell progenitor proliferation (Nagasawa et al., 1994). About the same time, its receptor CXCR4/LESTR/Fusin was independently cloned as a cytokine/chemokine receptor from a monocyte library (Loetscher et al., 1994) and as an HIV-entry cofactor (Feng et al., 1996), and functional interaction of CXCL12 and CXCR4 was initially shown (Bleul et al., 1996). Further investigations soon revealed that CXCL12 is a main factor to home CXCR4 expressing hematopoietic progenitor cells to the bone marrow (Aiuti et al., 1997), but also stem and progenitor cells from a variety of other tissues express CXCR4 and seem to be guided to their niche or their final distribution by CXCL12 gradients (e.g. Reiss et al., 2002). Accordingly, CXCR4 as well as CXCL12 knockout mice showed severely impaired hematopoiesis and CNS development (Ma et al., 1998), and also vascular defects (Tachibana et al., 1998; Takabatake et al., 2009). For a long time, CXCR4 was thought to be the only receptor for CXCL12, with CXCL12 being its only (physiological) ligand. However, based on structural similarities to other chemokine receptors, the orphan receptor RDC-1 was shown to bind and internalize CXCL12 on T lymphocytes (Balabanian et al., 2005). This report was soon supported by routinely performed binding assays with liver cells from CXCR4-deficient mice revealing persistent affinity for radio-labeled CXCL12 which also led to the identification of the former orphan receptor RDC-1/CXCR7 as alternative receptor for CXCL12, and also CXCL11 with about 10-fold lower affinity (Burns et al., 2006). In the following, the expression and function of CXCL12 and its two receptors CXCR4 and CXCR7 will be described focusing on their cellular effects and their role in tumor biology with special focus on gliomas.

2. Cellular localization and signaling of CXCL12 and its receptors CXCR4 and CXCR7

In different tumor cell types, depending on differentiation status and environment, the CXCL12 receptors CXCR4 and CXCR7 may be expressed uniquely or in combination. For example, CXCR7, but not CXCR4, is expressed by most investigated human glioblastoma cell lines, small cell lung cancer cell lines transcribe CXCR4, but not CXCR7, and mixed expression of both receptors occurs in some mamma carcinoma cell lines (Hattermann et al., 2010; Holzenburg et al., unpublished data). When co-expressed, CXCR4 and CXCR7 may form homo- and heterodimers, and especially heterodimerization seems to play an important role in the modulation of the down-stream signaling (Levoye et al., 2009; Luker et al., 2009; Decaillot et al., 2011). The opportunities of fine tuning include availability and completion of ligands, receptor interaction, recruitment of G proteins and/or β-arrestin, receptor (co-)internalization and activation of specific intracellular signaling pathways as described in Fig. 1 and the following text section.

Early investigations on CXCR4 signaling revealed G protein-coupling followed by inhibition of the adenylylcyclase by the $G_{i\alpha}$ subunit yielding lower cAMP levels, and by IP_3 dependent mobilization of intracellular calcium (Gupta et al., 1998). In contrast, CXCR7 is lacks the specific DRYLAIV motif that is necessary for G protein-recruitment, and consequently fails to mobilize calcium after ligand binding (Burns et al., 2006). Therefore, CXCR7 was initially regarded as a decoy receptor scavenging CXCL12 to prevent CXCR4 signaling and effects (Naumann et al., 2010) until further investigations revealed that CXCL12 mediated activation of CXCR7 alone may evoke intracellular signaling events, e.g. activation of the MAP-kinase pathway (Hattermann et al., 2010; Ödemis et al., 2010). This signaling is dependent on the recruitment of β-arrestin (Rajagopal et al., 2010; Decaillot et al., 2011) that commonly binds to phosphorylated serine residues of activated 7 transmembrane span receptors initializing the internalization of the ligand–receptor complex which leads to desensitization of the cell. However, additional recruitment of G proteins by to date unknown mechanisms can also occur (Ödemis et al., 2012). Aside

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