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CXCR1 and CXCR2 and Ligands

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Synonyms

IL-8Ra, IL-8Rb, CD181, CD182; Receptors for CXCL8; Receptors for interleukin-8 (IL-8)

Definition

The CXC chemokine receptors CXCR1 and CXCR2 belong to the family of seven transmembrane-spanning, G protein-coupled receptors (GPCRs) that are activated by different so-called inflammatory CXC chemokines: with interleukin 8 (IL-8, CXCL8), one of the first characterized and most prominent. Both CXCR1 and CXCR2 play a pivotal role in innate immune responses by regulating the recruitment in particular of neutrophils, but also of monocytes, dendritic cells, natural killer cells, and mast cells to sites of infected and damaged and/or inflamed tissue. Furthermore, the two receptors are found on nonimmune cells, such as endothelial and epithelial cells, and cells of the central nervous system. Accumulating evidence implicates CXCR1 and CXCR2 in tumor cell function, regulating tumor cell proliferation, tumor angiogenesis, and metastasis of malignant tumor cells.

Characteristics

CC and CXC Chemokines

Chemokines constitute a family of structurally related, low molecular mass (8–12 kDa) cytokines with chemotactic activities. Based on the relative position of conserved cysteines that form intramolecular disulfide bonds, chemokines are subdivided into four subfamilies: CC, CXC, CX_3C, and C chemokines. Chemokines orchestrate leukocyte trafficking by controlling chemotaxis, adhesion, and transendothelial migration, and thus play a fundamental role as regulators of hematopoiesis, and immune surveillance. They also regulate functions of nonhematopoietic cells, such as cells of the central nervous system, endothelial and epithelial cells, and progenitor cells, and hence are involved in neurogenesis, angiogenesis, and embryogenesis. Furthermore, chemokines act as mediators of tumor cell growth and metastasis. More than 50 chemokines have been identified to date, mainly belonging to the CC- and CXC-chemokine subfamilies. According to their functions, members of both main subfamilies are further subdivided into inflammatory chemokines (e.g., CXCL8) expressed upon induction by inflammatory stimuli, homeostatic constitutively expressed

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chemokines (e.g., CXCL12), and chemokines that fulfill dual functions (e.g., CXCL2). CXC chemokines are further subgrouped into chemokines containing a conserved Glu-Leu-Arg-motif (ELR^+) proceeding the first conserved cysteine, and chemokines lacking the ELR motif (ELR^-). ELR^+ CXC chemokines like CXCL8 show angiogenic properties and predominantly act on neutrophils, while ERL^- chemokines like CXCL10 show angiostatic properties and preferentially act on T lymphocytes. Transmembrane signaling of chemokines is mediated by chemokine receptors, which are, in most cases, coupled to activation of pertussis toxin-sensitive heterotrimeric G proteins (for excellent reviews see Bachelerie et al. 2014; Stillie et al. 2009).

CXCR1 and CXCR2 and Their Ligands

According to their interaction with CXC chemokines, the two human CXCL8-binding receptors were designated CXCR1 and CXCR2. The cDNAs encoding the two receptors were cloned in 1991 side by side by the groups of Murphy and Tiffany, and Holmes et al., from human neutrophil-like HL-60 cells, and from human neutrophils, respectively (Holmes et al. 1991; Murphy and Tiffany 1991). The encoding receptor proteins were shown to bind CXCL8 with high affinity and to function as main regulators of innate immune responses by controlling the recruitment and extravasation of neutrophils to sites of infected and damaged and/or inflamed tissue (Bachelerie et al. 2014; Stillie et al. 2009). Both CXCR1 and CXCR2 belong to the family of seven transmembrane-spanning, G protein-coupled receptors (GPCRs) and share considerable amino acid sequence identity (77 %). Amino acid sequence identity is high within the seven transmembrane domains and connecting loops. However, the amino terminal and carboxyl terminal regions of CXCR1 and CXCR2 show significant differences. Analysis depicting the underlying structural requirements of CXCL8/CXCR1 and/or CXCL8/CXCR2 interactions by alanine scanning mutagenesis and NMR spectroscopy identified the amino terminal domain of CXCR1 and CXCR2 as the main part for binding to CXCL8 (Park et al. 2011). Accordingly, ligand specificity of the two receptors CXCR1 and CXCR2 was described to reside within this amino terminal domain (Park et al. 2011). CXCR1 binds CXCL8 with high affinity, and to a lesser extent CXCL6 (also known as granulocyte chemoattractant protein-2; GCP-2). In contrast, CXCR2 shows far more promiscuity and interacts with several CXC chemokines including CXCL1 through 3 (also known as growth-related oncogenes, GROα, GROβ, and GROγ), CXCL5 (also known as epithelial-derived neutrophil attractant-78; ENA-78), CXCL6, CXCL7 (also known as neutrophil-activating peptide-2, NAP-2), and as aforementioned CXCL8. All these CXC chemokines belong to the group of inflammatory ELR^+ chemokines with known proangiogenic properties (Bachelerie et al. 2014; Stillie et al. 2009). Several studies have indicated that CXCL8 like other chemokines reversibly exists as monomer and dimer and that both forms activate the receptors, albeit with different affinities and potencies (Nasser et al. 2009). Binding to glycosaminoglycans (GAGs) on the cell surface is thought to influence the equilibrium between the two chemokine forms. Chemokine/GAG interaction in particular on the cell surface of endothelial cells promotes the formation of chemokine gradients that direct migration of immune cells during inflammation (Handel et al. 2005). Interestingly, ERL^+ CXC chemokines, like CXCL8 and CXCL6, additionally act through posttranslational modified truncated forms, e.g., amino terminally and/or carboxyl terminally processed proteolytic products (Mortier et al. 2012). Many of these modified CXC chemokines display higher affinity at the receptors and/or changes in the interaction with GAGs when compared to the wild type proteins (Mortier et al. 2012).

Orthologs of the human CXCR1 and CXCR2 receptors and their activating ligands have been identified in several other species, including monkeys, cattle, chicken, rabbit, and zebrafish (Zlotnik et al. 2006). Surprisingly, an ortholog of CXCL8 is missing in mouse and rat, and the CXCR1 proteins identified in these species strongly differ in the amino terminal portion of

the proteins when compared to human CXCR1 (Bachelerie et al. 2014; Stillie et al. 2009). Recent findings indicate that the mouse CXCR1 receptor is activated by the murine orthologs of human CXCL1 and CXCL6 and by human CXCL8 (Stillie et al. 2009). However, CXCR1-deficient mice show no impairment in innate immune cell function, and the functional relevance of the murine CXCR1 protein in neutrophils is so far unknown. In contrast, the role of mouse CXCR2 in regulating neutrophil function is well established. Murine CXCR2 binds ERL^+ chemokines, including the murine CXCL1 (also known as KC) and murine CXCL2 (also known as macrophage inflammatory protein-2; MIP-2), and the receptor responds to human CXCL1 through 3 and CXCL8. Due to impaired neutrophil migration and altered leukocyte rolling capacity, CXCR2-deficient mice display reduced clearing of pathogens in murine models of bacterial and parasitic infection (Bachelerie et al. 2014; Cacalano et al. 1994; Chapman et al. 2009; Stillie et al. 2009). In addition to CXCR1 and CXCR2, the atypical chemokine receptor ACKR1, formerly known as Duffy antigen receptor, was reported to promiscuously bind CXC chemokines, including ERL^+ chemokines like CXCL8 (Comerford and Nibbs 2005). ACKR1 has been proposed to act as scavenger that downmodulate, e.g., ERL^+-induced angiogenesis in tumors by internalizing chemokines (Bachelerie et al. 2014; Horton et al. 2005). Of interest, the viral CXC chemokine homolog, vCXCL1, encoded by the human cytomegalovirus (HCMV) acts as agonist at CXCR1 and CXCR2. The viral CXCL1 is produced by HCMV-infected endothelial cells and attracts neutrophils to the infected cells. The recruited neutrophils have been proposed to serve as vehicles to carry the virus to uninfected endothelial cells, thus supporting viral spread and maintaining a pool of HCMV-infected cells (Bachelerie et al. 2014; Lüttichau 2010). Besides CXC chemokines, binding of nonchemokine ligands to human CXCR1 and/or CXCR2 have been reported, including HIV-1 matrix protein p17, the collagen fragment *N*-acetyl-prolin-glycin-prolin (*N*-acetyl-PGP), and macrophage inhibitory factor (MIF) (Bachelerie et al. 2014; Chapman et al. 2009). Interestingly, *N*-acetyl-PGP has been implicated with increased neutrophil recruitment in chronic lung diseases such as COPD and cystic fibrosis by either directly or indirectly acting as neutrophil chemoattractant (Bachelerie et al. 2014). MIF have been shown to display chemokine-like function by binding to CXCR2 and promoting monocyte and T cell recruitment to inflamed tissue, in particular in atherosclerosis (Chapman et al. 2009).

Cellular Signaling of CXCR1 and CXCR2

Several cellular functions of immune and nonimmune cells are regulated by either CXCR1, or CXCR2, or both, depending on the cell type (e.g., neutrophils versus endothelial cells), cellular environment (e.g., peripheral blood versus central nervous system), cellular context (e.g., acute versus chronic inflammation), and/or activating ligand (for excellent reviews see Bachelerie et al. 2014; Stillie et al. 2009; Waugh and Willson 2008). Binding of chemokine ligands, for example, CXCL8 or CXCL1, induces conformational changes in the receptors that initiate coupling to pertussis toxin (PTX)-sensitive and -insensitive G proteins. The Gα- and/or Gβγ-subunits of the activated G proteins regulate the activity of a series of downstream effector proteins, including adenylylcyclases, phospholipase C (PLC) and phospholipase D (PLD) isoenzyme(s), small GTPases, phosphatidylinositol-3 kinase (PI3K), mitogen-activated protein kinases (MAPK, e.g., ERK and p38), and protein tyrosine kinases (src kinases, focal adhesion kinase) (Bachelerie et al. 2014; Waugh and Willson 2008). As a result, changes in second messenger concentrations (cAMP, Ca^{2+}, and diacylglycerol) and in the activation of typical and atypical protein kinase C (PKC) isoforms, protein kinase B (PKB/Akt), and various transcription factors are induced. Transcription factors that are activated include activator protein-1 (AP-1), nuclear factor kappa B (NF-κB), hypoxia-inducible factor-1α (HIF-1α), and signal transducer and activator of transcription 3 (STAT3). In addition, CXCR1 and CXCR2 are able to form complexes with

β-arrestins to initiate G protein-independent signaling, and/or desensitization and internalization of the receptors. Furthermore, different chemokine ligands may activate different intracellular G protein-dependent and/or independent intracellular pathways although binding to the same receptor, a function referred to as biased signaling that so far has not been explored for CXCR2 (Zweemer et al. 2014). The cellular responses generated by CXCR1 and/or CXCR2 through activation of one or the other signaling pathway go from cell adhesion, cell polarization and cell migration, to cell proliferation and cell growth, and cell survival (Bachelerie et al. 2014; Stillie et al. 2009; Waugh and Willson 2008).

Activity of ligand-stimulated CXCR1 and CXCR2 receptors is regulated by phosphorylation, desensitization, and internalization. Distinct amino acid sequence motifs and phosphorylation sites present in the variable carboxyl terminal portions of the two receptors are of major importance for this regulation (Bachelerie et al. 2014; Stillie et al. 2009). When internalized through clathrin/β-arrestin-dependent pathways, the receptors are either degraded or recycled to the cell membrane surface. Interestingly, CXCR1 slowly internalizes only at high ligand concentrations, and CXCR2 rapidly internalizes already at low chemokine concentrations, but recovers faster. Also of note, only CXCR1 has been reported to heterologously desensitize other neutrophil chemotactic receptors, like receptors activated by C5a and formylated peptides in transfected rat basophilic leukemia cells. The differences in the regulation of CXCR1/CXCR2 activity potentially contribute to the fine tuning of neutrophil function in acute and chronic inflammation (Bachelerie et al. 2014; Nasser et al. 2009; Stillie et al. 2009).

The functional complexity of CXCR1/CXCR2 signaling is further increased by the possibility of the receptors to form homo- and heterodimers (Stillie et al. 2009). The dynamic equilibrium between homo- and heterodimers of CXCR1 and CXCR2 is thought to influence receptor ligand interaction, to regulate coupling to distinct G proteins and activation of specific signaling pathways, and to control receptor internalization (Bachelerie et al. 2014; Stillie et al. 2009). Interestingly, formation of heterodimers of CXCR2, e.g., with δ-opioid receptors, and AMPA-type GluR1 receptors has been reported, with CXCR2 modulating the signaling of the heterodimeric partner in cotransfected HEK293 cells (Stillie et al. 2009). To add to the complexity of CXCR2 signaling, activation of the receptor by CXCL8 has been shown to transactivate the epidermal growth factor receptor (EGFR) thereby enhancing the mitogenic effect of CXCL8 on tumor cells in ovarian cancer (Waugh and Willson 2008).

Expression and Function of CXCR1 and CXCR2

Both human CXCR1 and CXCR2 receptors are predominantly expressed by neutrophils. In addition, CXCR1 expression has been described for monocytes, subsets of T cells, for natural killer cells, mast cells, basophils, and endothelial cells. CXCR2 expression has been reported for monocytes, T cells, natural killer cells, mast cells, eosinophils, smooth muscle cells, endothelial and epithelial cells, and a variety of cells of the central nervous system (Bachelerie et al. 2014; Stillie et al. 2009). Furthermore, many cancer cells express CXCR1 and/or CXCR2 (Bachelerie et al. 2014; Campbell et al. 2013; Zhou et al. 2014).

Neutrophils are major players in host defense responses to infection, tissue injury, and inflammation. Neutrophil recruitment to sites of infection and inflammation and clearance of pathogens or cellular debris by neutrophils is regulated by CXCR1 and/or CXCR2. Stimulation of neutrophils by CXCL8 triggers antibacterial and cytotoxic responses of neutrophils including the respiratory burst with the production and release of reactive oxygen species, phagocytosis of microbes and/or tissue debris, and the release of proteolytic enzymes stored in preformed granules either into phagosomes or to the extracellular surrounding by degranulation. Both the CXCR1 and CXCR2 were shown to induce changes in intracellular Ca^{2+} concentrations, to initiate degranulation, and to contribute to directed migration of neutrophils (Bachelerie et al. 2014; Stillie et al. 2009). However, phospholipase

D activation and generation of the respiratory burst were attributed to the activation of CXCR1 at high CXCL8 concentrations, whereas migration of neutrophils was found predominantly mediated by activation of CXCR2 at least at low CXCL8 concentrations (Bachelerie et al. 2014; Stillie et al. 2009). Similarly, the murine CXCR2 was shown to preferentially regulate neutrophil trafficking under various inflammatory conditions in mice (Bachelerie et al. 2014; Chapman et al. 2009). Also of note, activation of CXCR2, but not of CXCR1, has been described to participate in the control of neutrophil release from the bone marrow to the circulation (Eash et al. 2010).

Expression of CXCR1 and/or CXCR2 in other immune and in nonimmune cells correlates with additional functions in innate and adaptive immunity, in wound healing, in angiogenesis, and in tumorigenesis, as well as functions of the central nervous system (Bachelerie et al. 2014; Chapman et al. 2009, 2013; Stillie et al. 2009). Thus, a role of CXCR2 for the recruitment of monocytes/macrophages into atherosclerotic lesions has been shown (Chapman et al. 2009). Expression of CXCR2 by endothelial cells and activation by ERL^+ CXC chemokines has been correlated with angiogenesis in particular in tumorigenesis, and with leukocyte recruitment and transendothelial migration in inflammation (Chapman et al. 2009).

The complex process of cutaneous wound healing with the three phases known as inflammation, proliferation, and tissue remodeling requires a coordinated interplay between cells, soluble mediators, and extracellular matrix proteins. CXC chemokines and CXCR2 signaling have been implicated in all phases of wound healing (Raman et al. 2011). Upregulation of CXCL1 and CXCR2 expression has been reported in human burn wounds during wound healing. In mice, expression of CXCR2 has been shown for infiltrating neutrophils, migrating and proliferating keratinocytes, and for endothelial cells in the area of the wounded tissue (Raman et al. 2011). In this line, inactivation of the murine CXCR2 by targeted deletion results in reduced neutrophil recruitment into cutaneous wounds during the phase of inflammation. The CXCR2-deficient mice show reduced proliferation of keratinocytes during the phase of wound epithelialization, and reduced neovascularization and delayed wound closure in the phase of tissue remodeling (Raman et al. 2011).

CXCR2 is expressed in neurons of several regions in the brain and spinal cord, and signaling of the receptor contributes to neuronal electrical activity, neurotransmitter release, and synaptic plasticity (Ragozzino 2002; Semple et al. 2010). Furthermore, ligands of CXCR2 have been reported to modulate proliferation and cell survival of cells of the central nervous system (Chapman et al. 2009; Ragozzino 2002). Constitutive expression of CXCR2 in oligodendrocyte precursors of mice has been shown to be of major importance during spinal cord patterning in the embryo (Chapman et al. 2009; Semple et al. 2010). Major sources of CXCR2 activating ligands, such as CXCL1, CXCL2, and CXCL8, include activated microglia, astrocytes, endothelial cells, and recruited neutrophils in the brain (Semple et al. 2010).

Uncontrolled recruitment and activation of immune cells can lead to tissue damage. Hence, expression and secretion of inflammatory ERL^+ CXC chemokines, and CXCR1 and/or CXCR2 expression, is not only regulated in a spatiotemporal and tissue-specific manner, but also tightly controlled under inflammatory conditions (Bachelerie et al. 2014). Factors that regulate the expression include bacterial lipopolysaccharides (LPS), pathogen-recognition Toll-like receptor (TLR) agonists, reactive oxygen species, and proinflammatory cytokines (e.g., tumor necrosis factor α; TNF-α, interleukin-1). Expression of CXC chemokines/receptors is also induced as a result of environmental stress, like hypoxia (Bachelerie et al. 2014; Campbell et al. 2013). In tumor cells, activation of the transcription factors NF-κB and hypoxia-inducible factor-1α (HIF-1α) increases CXC chemokine and CXCR1/CXCR2 expression (Campbell et al. 2013).

Role of CXCR1 and CXCR2 in Infectious, Inflammatory, and Autoimmune Diseases

The physiological function of CXCR1 and CXCR2 on neutrophils is associated with host immune defense against acute infection, leading to clearance of pathogens. A role of CXCR2 in clearance of bacterial and fungal infection in mouse models is well documented (Chapman et al. 2009). Pathologically reduced CXCR1/CXCR2 receptor expression and/or receptor function in neutrophils results in decreased recruitment of neutrophils to sites of infection and decreased bacterial clearance (Martynowicz et al. 2014). Of interest, a correlation between genetic variations (polymorphisms) of the CXCR1/CXCR2 encoding genes leading to reduced receptor expression and increased susceptibility to infection has been reported, for example, for patients with cutaneous and mucocutaneous leishmaniasis in Brazil and northeast India (Mehrotra et al. 2011).

Under physiological conditions, neutrophils and/or monocytes maintain innate immune surveillance. However, excessive recruitment and activation of these leukocytes can result in tissue damage and can amplify acute and chronic inflammation (Chapman et al. 2009; Simpson et al. 2009). Accordingly, CXCR1 and CXCR2 and their activating ligands have been implicated in a variety of pro- and inflammatory acute and chronic diseases that are characterized by persistent neutrophil infiltration and/or a high neutrophil tissue load. Examples include various diseases of the lung, and autoimmune diseases such as inflammatory bowel disease and rheumatoid arthritis (Chapman et al. 2009; Simpson et al. 2009). Neutrophilic inflammation is a characteristic of acute lung diseases, like acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), and of chronic lung diseases, like neutrophil-type of asthma bronchiale and chronic obstructive pulmonary diseases (COPD). Increased expression and secretion of ELR^+ CXC chemokines, in particular CXCL8 by airway resident stromal cells, smooth muscle cells, infiltrating neutrophils, and/or possibly endothelial cells significantly contribute to the initiation of neutrophilic inflammation. CXCR2 expression was found in accumulating neutrophils, and resident endothelial cells, fibroblasts, and smooth muscle cells (Chapman et al. 2009; Simpson et al. 2009). Due to reduced apoptosis of neutrophils and reduced phagocytosis of apoptotic cells by macrophages the clearance of tissue neutrophils is impaired in neutrophilic inflammation. Persistent release of metalloproteases, elastase, and CXCL8 by activated neutrophils fuels the activated immune system and results in further damage of the airway system (Chapman et al. 2009; Simpson et al. 2009). Of note, CXCR2 antagonists now have reached clinical trials, e.g., for the treatment of COPD (Campbell et al. 2013; Stillie et al. 2009).

Neutrophils have been shown to contribute to the pathology of inflammatory autoimmune diseases, like rheumatoid arthritis (RA). RA is characterized by chronic inflammation in the synovial membranes leading to cartilage and joint destruction (Raman et al. 2011; Wright et al. 2014). Synovial macrophages, and probably synovial lining fibroblasts and endothelial cells, secrete the ERL^+ chemokines CXCL8, CXCL5, and CXCL1 (Raman et al. 2011). The number of neutrophils is high in synovial tissue and joint fluid, and their cytotoxic and immunomodulatory effects have been shown to contribute to destruction of cartilage. There is accumulating evidence that neutrophils may also provide a source of autoantigens (Wright et al. 2014).

By promoting macrophage and neutrophil infiltration into the brain, the chemokines CXCL1 and CXCL8 and CXCR2 have been implicated in the development of various neuroinflammatory and neurodegenerative disorders, including trauma, ischemic injury, and multiple sclerosis (Bachelerie et al. 2014; Semple et al. 2010). Increased CXCL8 expression and subsequently functional signaling of CXCR1/CXCR2 on endothelial and/or smooth muscle cells of the vascular bed has been correlated to angiotensin II mediated regulation of blood pressure in rats (Martynowicz et al. 2014). Accordingly, interfering with CXCR2 signaling by small inhibitory RNA or application of the small molecule CXCR2 antagonist reparixin caused reduction in blood pressure in a rat model of

hypertension (Martynowicz et al. 2014). Last, a role of CXCR1- and/or CXCR2-expressing immune and/or nonimmune cells in the pathology of hypoxia and reperfusion injury, and sepsis, and in other (pro)inflammatory disease conditions has been proposed (Bachelerie et al. 2014; Stadtmann and Zarbock 2012).

Role of CXCR1 and CXCR2 in Cancer

It is well appreciated that chemokines and their receptors, including CXCR1 and CXCR2, play important roles in tumorigenesis (Bachelerie et al. 2014; Chapman et al. 2009, 2013; Raman et al. 2011). CXCR1 and CXCR2 receptors were found expressed on a variety of cancer cells, including lung, ovarian, prostate, pancreatic, colon cancer cells, and malignant melanoma tumor cells (Murphy and Tiffany 1991; Zhou et al. 2014). Additionally, cells of the tumor microenvironment, such as endothelial cells, stromal fibroblasts, mesenchymal stem cells, tumor-associated macrophages, and neutrophils, were described to express CXCR1 and CXCR2 (Campbell et al. 2013; Zhou et al. 2014). Constitutive autocrine and paracrine production in particular of CXCL1 and CXCL8 by tumor cells and inflammatory cells were shown to contribute to tumor-specific immune responses, to the composition of the tumor microenvironment, to tumor cell proliferation and survival, to angiogenesis, and to metastasis of various malignant tumors (Campbell et al. 2013; Zhou et al. 2014).

Mitogenic effects of CXCL1 and/or CXCL8 on cancer cells have been reported for a variety of cancers, including malignant melanoma, lung cancer, pancreatic carcinoma, breast cancer, ovarian cancer, and colon carcinoma (Campbell et al. 2013; Waugh and Willson 2008). In malignant melanoma and prostate cancer cells dysregulation of NF-κB signaling drives constitutive CXCL1 and/or CXCL8 production (Campbell et al. 2013). A mutation in the CXCL8 encoding gene leading to an increase in CXCL8 production and secretion appears to be more prevalent in patients with metastatic prostate cancer (Campbell et al. 2013; Zhou et al. 2014). Also of note, an increased production of tumor promoting inflammatory cytokines, including CXCL1 and CXCL8, has been reported in tumors with loss of the functional tumor suppressor gene PTEN and in tumors with ras oncogene mutations (Campbell et al. 2013).

It is now widely accepted that unresolved chronic inflammation is one tumor hallmark involved in the induction of tumorigenesis. Infiltration of immune cells including neutrophils and macrophages into the tumor microenvironment contributes to the development and progression of many human cancers. Tumor-infiltrating neutrophils (TINs) (also known as tumor-associated neutrophils; TANs) have been shown to elicit tumor specific immune responses and to contribute to tumor angiogenesis, growth, and metastasis (Campbell et al. 2013; Fridlender and Albelda 2012; Raman et al. 2011). Of note, in the early stage of tumorigenesis cytotoxic effects of neutrophils might be protective (Bachelerie et al. 2014; Fridlender and Albelda 2012). However, ERL^{+} CXC chemokine-stimulated TINs secrete proangiogenic factors, like vascular endothelial growth factor (VEGF), and induce intratumoral secretion of cellular matrix degrading metalloproteases (MMPs), like MMP-9 (Fridlender and Albelda 2012; Vandercappellen et al. 2008). Clinical studies correlated the presence of intratumoral neutrophils with poor prognosis in multiple cancers, including malignant melanoma, colorectal cancer, and renal cell carcinoma (Campbell et al. 2013; Zhou et al. 2014). Furthermore, inhibition of neutrophil recruitment by blocking CXCL8/CXCR1/2 interaction has been shown to reduce tumor growth in vivo (Campbell et al. 2013).

CXCL8 signaling promotes tumor cell survival by driving antiapoptotic gene expression in tumor cells (Campbell et al. 2013). Chemoresistance of certain cancers to chemotherapy agents (e.g., oxaliplatin) has been implicated with an increase in CXCL8 and CXCR1/2 receptor expression that is induced by DNA-damaged hypoxic cells in response to the chemotherapeutic intervention. Increased expression is regulated by NF-κB activity and probably involves a CXCR1/CXCR2-regulated autocrine loop. NF-κB activation consecutively drives

upregulation of antiapoptotic genes. In this line, coadministration of the CXCR2 antagonist DF2162 and oxaliplatin has been shown to attenuate NF-κB activation leading to downregulation of antiapoptotic gene expression and increased cytotoxicity of oxaliplatin (Campbell et al. 2013).

Neovascularization is another hallmark of solid malignant tumors, ensuring the supply of nutrients and oxygen for the growing tumor, and promoting dissemination and metastasis (Campbell et al. 2013). CXCL8 was the first described angiogenic ERL^+ chemokine, and as aforementioned both CXCL-8 binding receptors were found to be expressed on endothelial cells (Bachelerie et al. 2014; Raman et al. 2011; Stillie et al. 2009). Activation of CXCR2 on endothelial cells by ERL^+ chemokines, in particular CXCL1 and CXCL8, enhances endothelial cell survival, endothelial cell proliferation and MMP production, regulates chemotaxis of endothelial cells, and controls the organization and maturation of capillary-like structures in tumor tissue (Campbell et al. 2013; Raman et al. 2011). Furthermore, the proangiogenic CXC chemokines indirectly contribute to endothelial cell angiogenesis, by increasing vascular endothelial growth factor (VEGF) expression via activation of CXCR2 as shown for ovarian tumor cells (Yang et al. 2010).

Chemokines and their receptors regulate metastasis by directing metastasizing cancer cells toward chemokine-producing secondary organs. There is convincing evidence that CXCR2 signaling contributes to metastasis of a variety of solid tumors (Campbell et al. 2013; Raman et al. 2011; Zhou et al. 2014). For example, CXCR2 activity was found associated with melanoma metastasis to the lung in mice (Richmond et al. 2009). Downregulation of CXCR2 has been reported to reduce the capacity for invasion and metastasis in vitro and in vivo of a breast cancer tumor cell line (Nannuru et al. 2011), and inhibition of CXCR1/CXCR2 activity by an antagonist has been shown to inhibit human liver metastasis of a colon cancer cell line in nude mice (Campbell et al. 2013; Zhou et al. 2014).

Therapeutic Targeting of CXCR1 and CXCR2 and Their Ligands

Due to their important role in infectious, inflammatory, and autoimmune diseases, and in cancer, CXCR1 and CXCR2 and their activating ligands are potential therapeutic targets. Small molecule antagonist, neutralizing antibodies, and peptide-derived inhibitors have been developed to directly target ERL^+ CXC chemokine-induced signaling of CXCR1 and/or CXCR2. The inhibitory activities of these therapeutics have been tested preclinically in a variety of models of inflammatory and autoimmune diseases, and in cancer, and some showed promising results (for excellent review see Bachelerie et al. 2014; Campbell et al. 2013; Stillie et al. 2009).

The group of developed small molecule antagonists include the acylmethanesulfonamide derivative reparixin (also known as repartaxin; Dome, Milan, Italy) and related compounds (e.g., DF2162), the cyclobutenedione compound, SCH527123 (Schering-Plough, Kenilworth, NJ), the phenol-containing diaryurea SB656933 (GlaxoSmithKline, London, UK), and a pyrimidine-based CXCR2-selective antagonist AZD5069 (AstraZeneca, London, UK). These antagonists have shown promising results in preclinical or clinical studies such as adjuvant-induced polyarthritis, transplantation of insulin-producing islets, bleomycin- and ozone-induced pulmonary inflammation, lung fibrosis, and COPD. The CXCR2-selective antagonist AZD5069 has been reported to reduce tumor growth and microvessel density in a preclinical xenograft model of colorectal cancer (Bachelerie et al. 2014; Campbell et al. 2013; Stillie et al. 2009). Neutralizing humanized antibodies to CXCL8 have been used to inhibit melanoma tumor growth, angiogenesis, and metastasis (Richmond et al. 2009), and CXCR1 antibodies have been applied to inhibit CXCL8-induced tumor proliferation of small-cell lung cancer (Campbell et al. 2013). So far none of these antibodies have reached clinical trials. Examples for peptide-derived inhibitors include molecules based on the *N*-Acetyl-PGP peptide, synthetic chemokine derivates (e.g., G31P), and pepducins (Campbell et al. 2013; Stillie et al. 2009). The

latter are small peptide-derived inhibitors that have lipophilic properties and that intracellularly block the receptor-induced signal transduction (Campbell et al. 2013). The peptide-derived inhibitors were shown for example to improve chronic neutrophilic lung disease, to act anti-inflammatory in arthritis mouse models, and to inhibit growth of human prostate cancer cells in nude mice (Bachelerie et al. 2014; Campbell et al. 2013; Stillie et al. 2009).

The development of some CXCR1/CXCR2 inhibitors has been declined due to lack of effectiveness or suboptimal pharmacokinetic parameters. Other therapeutics are in preclinical studies or have reached clinical trials addressing inflammatory diseases, including asthma, COPD, and cystic fibrosis, and cancer, like HER-2-negative early breast cancer (Campbell et al. 2013). The future will show whether, or not, these therapeutic agents will reach the pharmaceutical market.

Summary

The CXC chemokine receptors CXCR1 and CXCR2 play a pivotal role in innate immune responses by regulating the recruitment in particular of neutrophils to sites of infected and damaged and/or inflamed tissue. In addition, both receptors are found on other immune cells, such as monocytes, dendritic cells, natural killer cells, and mast cells, and on nonimmune cells, such as endothelial and epithelial cells and cells of the central nervous system. CXCR1 and CXCR2 are activated by members of the inflammatory ERL^+-CXC chemokines such as CXCL1 through CXCL3, CXCL5 through CXCL7, and CXCL8, as well as by proteolytically modified products of these CXC chemokines. The cellular functions of immune and nonimmune cells regulated by either CXCR1, or CXCR2, or both, through the activation of a series of downstream effector proteins, include cell adhesion, cell polarization and cell migration, as well as cell proliferation and cell growth, and cell survival. CXCR1 and CXCR2 have been implicated in a variety of pro- and inflammatory acute and chronic diseases such as lung diseases, inflammatory bowel disease, atherosclerosis, rheumatoid arthritis, and neuroinflammatory and neurodegenerative disorders. In addition, CXCR1 and CXCR2 signaling has been attributed to tumor cell function, regulating tumor cell proliferation, tumor angiogenesis, and metastasis of malignant tumor cells. Due to their important role in infectious, inflammatory, and autoimmune diseases, and in cancer, CXCR1 and CXCR2 and their activating ligands are interesting therapeutic targets, and some receptor antagonists already reached clinical trials.

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