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THE ROLE OF STROMAL DERIVED FACTOR-1 – CXCR7 AXIS IN DEVELOPMENT AND CANCER

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Abstract

Cancer metastasis is a major clinical problem that contributes to unsuccessful therapy. Augmenting evidence indicates that metastasizing cancer cells employ several mechanisms that are involved in developmental trafficking of normal stem cells. Stromal-derived factor-1 (SDF-1) is an important α -chemokine that binds to the G-protein-coupled seven-transmembrane span CXCR4. The SDF-1-CXCR4 axis regulates trafficking of normal and malignant cells. SDF-1 is an important chemoattractant for a variety of cells including hematopoietic stem/progenitor cells. For many years, it was believed that CXCR4 was the only receptor for SDF-1. However, several reports recently provided evidence that SDF-1 also binds to another seven-transmembrane span receptor called CXCR7, sharing this receptor with another chemokine family member called Interferon-inducible T-cell chemoattractant (I-TAC). Thus, with CXCR7 identified as a new receptor for SDF-1, the role of the SDF-1-CXCR4 axis in regulating several biological processes becomes more complex. Based on the available literature, this review addresses the biological significance of SDF-1's interaction with CXCR7, which may act as a kind of decoy or signaling receptor depending on cell type. Augmenting evidence suggests that CXCR7 is involved in several aspects of tumorigenesis and could become an important target for new anti-metastatic and anti-cancer drugs.

Keywords

SDF-1; CXCR7; CXCR4; cancer metastasis

1. Introduction

Augmenting evidence accumulates that several of G-protein linked receptors are playing a pivotal role in cancer metastasis, survival and proliferation. Thus, some of these receptors become attractive targets for pharmacological approaches. One of recently identified potential

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targets for anti-metastatic therapies is G_{αi}-protein linked receptor CXCR4 that binds α-chemokine stromal derived factor-1 (SDF-1).

Overall G-protein linked receptor family includes receptors for hormones, cytokines, neurotransmitters, visual light waves, and chemokines (Schier, 2003). Members of this receptor family are seven-transmembrane-spanning proteins residing predominantly in plasma membrane that transduce signals by coupling to guanine nucleotide-binding proteins (G-proteins). G-protein-coupled receptors regulate several aspects of cell biology with chemokine receptors being an important part of this family (Schier, 2003).

Chemokines, the small pro-inflammatory chemoattractant cytokines that bind to specific G-protein-coupled seven-transmembrane receptors present on the plasma membranes of target cells, are the major regulators of cell trafficking and adhesion (Zlotnik and Yoshie, 2000). Some chemokines are also reported to modulate cell survival and growth (Horuk, 2001). More than 50 different chemokines and 20 different chemokine receptors have been cloned so far (Zlotnik and Yoshie, 2000, Horuk, 2001).

Chemokines usually bind to multiple receptors and the same receptor may bind more than one chemokine. However, one exception to this rule was accepted for many years; the α-chemokine stromal-derived factor-1 (SDF-1) or CXCL12 binds exclusively to CXCR4 and has CXCR4 as its only receptor (Nagasawa et al., 1996, Ma et al., 1999, Bagri et al., 2002, Lazarini et al., 2003). This assumption was based on SDF-1 and CXCR4 murine knock-down (KD) data in which affected animals display similar phenotype. The concept that CXCR4 only binds SDF-1 suggested that the SDF-1-CXCR4 axis might play a uniquely important biological role among chemokine-chemokine receptors. This notion was also supported by the murine KD data, which also showed that SDF-1 secreted by bone marrow stromal cells during embryogenesis is critical for the colonization of marrow by fetal liver-derived hematopoietic stem/progenitor cells (David et al., 2002, Lapidot and Petit, 2002, Kortessidis et al., 2005). Furthermore, during adult life, SDF-1 has a pivotal role in the retention and homing of these cells into the bone marrow microenvironment (Aiuti et al., 1997, Kim et al., 1998, Lapidot and Petit, 2002, Guo et al., 2005). Thus, it is not surprising that perturbation of the SDF-1-CXCR4 axis (e.g., as seen after administration of mobilizing agents) is essential for the egress and mobilization of hematopoietic stem/progenitor cells from the bone marrow into peripheral blood (Devine et al., 2004, Lapidot et al., 2005, Papayannopoulou 2004, Pelus et al., 2008). On the other hand, proper functioning of the SDF-1-CXCR4 axis is crucial in directing homing and engraftment of hematopoietic stem cells into bone marrow after transplantation (Lapidot et al. 2005). Furthermore, the SDF-1-CXCR4 axis was also reported to be involved in proper development of brain, particularly the cerebellum (Zou et al.; 1998), as well as the ventricular septum in heart (Tachibana et al., 1998) and gastrointestinal vasculature (Nagasawa, 2001). In addition to hematopoietic stem/progenitor cells, SDF-1 was found to be an important developmental chemoattractant for several other types of organ/tissue-committed stem cells, including a population of pluripotent very small embryonic-like stem cells described by our team (Kucia et al., 2004). In the case of hematopoietic stem/progenitor cells, however, SDF-1 is the most important and pivotal chemoattractant so far (Aiuti et al., 1997, Nagasawa et al., 1996, Kucia et al., 2005).

SDF-1 becomes highly expressed in injured organs (e.g., heart infarct, stroke) and may chemoattract circulating CXCR4⁺ stem cells including very small embryonic like stem cells for tissue repair (Dalakas et al., 2005, Wojakowski et al., 2006, Ratajczak et al., 2006). In addition, mounting evidence suggests that the SDF-1-CXCR4 axis regulates the metastatic behavior of several malignancies including breast cancer, prostate cancer, lung cancer, and pediatric sarcomas (Libura et al., 2002, Kucia et al., 2005, Muller et al., 2001, Hartmann et al., 2005). In fact, cells from almost all cancer types were found to express CXCR4 and be

responsive to SDF-1 gradient (Yasuoka et al., 2008, Ratajczak et al., 2006). Expression of CXCR4 on anaplastic or undifferentiated tumor cells and their responsiveness to SDF-1 gradient seems to mimic a role of the SDF-1-CXCR4 axis in developmental migration of pluripotent and organ-committed stem cells during embryogenesis. Because its expression on tumor cells is correlated with a poor prognosis in cancer patients (Zagzag et al., 2005, Staller et al., 2003, Li et al., 2004, Darash et al., 2004, Laverdier et al., 2005, Russell et al., 2004, Scala et al., 2005, Katmi et al., 2005, Katayama et al., 2005), the CXCR4 become a potential target for developing new anti-metastatic drugs (Takenaga et al., 2004, Tsutsumi et al., 2007). Of note, the CXCR4 was also identified as a co-receptor for entry of T tropic human immunodeficiency virus (HIV) into cells (Feng et al., 1996, Oberlin et al., 1996, Ding et al., 2008). SDF-1 binding to CXCR4 may interfere with that HIV entry.

However, the concept of an exclusive interaction of SDF-1 with CXCR4 was questioned after it was noticed that murine fetal liver cells from CXCR4 KD mice still may bind SDF-1, for example (Burns et al., 2006). Furthermore, in several human cancer cell lines, some inconsistencies were observed between CXCR4 expression and SDF-1 binding (Burns et al., 2006). In addition, the small molecular inhibitors CCX451 and CCX751 and another chemokine called interferon-inducible T-cell chemoattractant (I-TAC) or CXCL11 were shown to partially block SDF-1 binding without interacting directly with the CXCR4 (Burns et al., 2006). All this suggested a presence of another SDF-1 binding receptor on the cell surface. This receptor was recently identified and named CXCR7 (Burns et al., 2006).

2. Identification of the CXCR7

From historical point of view, the CXCR7 was cloned approximately 20 years ago from a dog thyroid cDNA library and was initially named Receptor Dog cDNA 1 (RDC1), which is how it was known in the literature for many years (Libert et al., 1990). It was also known by other names such as GPCR 159, GPR159, GPRN159, chemokine orphan receptor 1 (CMKOR1), AW541270, and chemocentrin chemokine receptor 2 (CCX-CKR2; Melikian et al., 2004).

Chromosomal mapping located RDC1 close to sequences encoding other chemokine receptors such as CXCR1, CXCR2, and CXCR4 on murine chromosome 1 and human chromosome 2, respectively (Heesen et al., 1998, Shimizu et al., 2000). For many years, RDC1 was considered an orphan receptor or a scavenger-type receptor for certain chemokines (Law and Rosenzweig, 1994). More was understood on a potential ligand for this receptor when RDC1 was expressed in cells that previously did not bind SDF-1 as well as when these RDC1⁺CXCR4⁻ cells were demonstrated as binding with high affinity SDF-1, which confirmed that RDC1 may indeed be a new receptor for SDF-1 (Burns et al., 2006). Furthermore, from several other chemokines tested, these CXCR7⁺ cells were also found to bind I-TAC (Burns et al., 2006).

Thus, after demonstrating that SDF-1 and I-TAC bind with high affinity to RDC1, RDC1 was officially “deorphanized” and renamed CXCR7 as a seventh receptor belonging to the CXC class of the chemokine receptor family (Balabanian et al., 2005, Burns et al. 2006). However, what must be kept in mind, I-TAC which is another CXCR7 ligand can also bind to chemokine receptor CXCR3 (Cole et al., 1998). Thus, any biological effects of I-TAC assigned to specific activation of the CXCR7 should be excluded from the possibility of this chemokine also engaging the CXCR3 receptor, if this receptor is expressed on target cells. In addition, it was confirmed that I-TAC may compete with SDF-1 for binding to CXCR7 and thus perturb the SDF-1-CXCR7 interaction.

One of the common features of chemokine receptors is their association with G_{ai}-proteins (Zlotnik and Yoshie, 2000). However, because coupling of the CXCR7 to G-proteins is still controversial, the name CXCR7 is still somehow on hold despite common use of “CXCR” as a term describing members of the chemokine receptor family. The sub-committee on

chemokine receptor nomenclature of the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (www.iuphar.org/nciuphar.html) proposed that it shall remain unofficial until clear evidence of chemokine-specific signal transduction is provided. It is well known that chemokine receptors contain the so-called Asp-Arg-Tyr-Leu-Ala-Ile-Val (DRYLAIV) motif at the second intracellular loop, which is crucial for coupling a chemokine receptor to G_{ai}-signaling proteins (Haraldsen and Rot, 2006). However, in the case of CXCR7, this motif is slightly modified (Thelen and Thelen, 2008), which could potentially prevent its interaction with G_{ai}-signaling proteins. Nevertheless, more and more evidence accumulates that CXCR7 in several cell types is a signaling receptor as evidenced by phosphorylation of mitogen activated protein kinase (MAPK)p42/44 (Hartmann et al., 2008) or serine/threonine kinase Akt, for example (Wang et al., 2008). However, its sensitivity to pertussis toxin, which is a specific inhibitor of G_{ai}-proteins, requires further study (Hartmann et al., 2008). These experiments (e.g., receptor internalization or calcium flux studies) have to be performed in pertussis toxin-exposed and SDF-1- or I-TAC-stimulated CXCR4- and CXCR3-negative cells that express functional CXCR7.

3. Which biological SDF-1 effects are CXCR4- and which are CXCR7-mediated?

The recent evidence that SDF-1 may also bind to the CXCR7 raised several questions on the potential contribution of the SDF-1-CXCR7 axis to all these processes that were previously attributed solely to SDF-1-CXCR4 interactions. Considering this, however, one must realize several important factors that could affect biological responses after SDF-1 binding to one or another of its receptors (Fig. 1).

First, the responsiveness of cells to SDF-1 gradient via CXCR4 and/or CXCR7 depends on surface expression of both receptors. While CXCR4 is expressed at the protein level on several types of cells, expression of CXCR7 is more abundant on embryonic and neoplastic transformed cells (Burns et al., 2006, Goldmann et al., 2008, Wang et al., 2008, Schutyser et al., 2007, and Miao et al. 2007). For example, it is still not clear whether CXCR7 is expressed on the surface of most primitive hematopoietic stem cells, which is in contrast to CXCR4. Second, CXCR4 has only one ligand, SDF-1, while CXCR7 also responds to I-TAC in addition to SDF-1. Thus, in contrast to CXCR4, CXCR7 is able to interact with two chemokines, SDF-1 and I-TAC, which both could compete for binding to CXCR7 (Burns et al., 2006). As such, SDF-1-mediated responses could be potentially modulated by I-TAC. Third, there are some reports that CXCR7 does not activate signal transduction in some cell types that, in contrast, are responsive to SDF-1 stimulation, for example (Sierro et al., 2007). In this context and with the association of CXCR7 with G_{ai} proteins still in controversy, it is not clear at this point if the SDF-1-CXCR7 axis is similar to the SDF-1-CXCR4 axis in sensitivity to pertussis toxin (Hartman et al., 2008). Furthermore, signaling of G-protein-coupled chemokine receptors is regulated by small proteins known as regulators of G-protein signaling (RGS) (Gold et al., 1997), despite expression of both receptors on the cell surface. Therefore, SDF-1 could activate this receptor only if it is not associated and thus “not muted” by its specific RGS proteins expressed in the given cell type. However, a potential RGS proteins for CXCR7, in contrast to those for CXCR4, were not yet described.

Finally, since the tissue concentration of SDF-1 is regulated by several factors such as for example hypoxia (Schioppa et al., 2003), the level of SDF-1 expression and its affinity for binding to particular receptors may decide whether the SDF-1-CXCR4 axis or the SDF-1-CXCR7 axis would be responsible for final SDF-1-mediated biological effect. As reported, SDF-1 has approximately 10 times higher affinity to CXCR7 as compared to CXCR4. In this context, it had been also described that responsiveness of CXCR4 to SDF-1 gradient may be

positively modulated or primed by several small molecules related to inflammation, such as C3 complement cleavage fragments, for example (Wysoczynski et al., 2007, Reza et al., 2006). The molecular explanation for this phenomenon is observed in C3 cleavage fragments increasing incorporation of CXCR4 into membrane lipid rafts (Wysoczynski et al., 2005), which leads to dimerization and better association of CXCR4 incorporation into lipid rafts with downstream signaling molecules (Wysoczynski et al., 2007). It would be interesting to determine whether the responsiveness of CXCR7 to SDF-1 could be modulated by a similar phenomenon and if CXCR7 is a lipid raft-associated receptor.

All these possibilities regarding the biological responsiveness of CXCR7 versus CXCR4 to SDF-1 signaling (summarized in Fig. 1) must always be considered when the biological effects of respective axes are evaluated. Below, we review some of the biological effects of the SDF-1/ITAC-CXCR7 axis identified to date.

4. Biological effects of CXCR7 as chemotactic and signaling receptor for tumor cells

The biological effects of CXCR7 signaling in cancer cells are described more fully. This could be explained by higher expression of CXCR7 on neoplastically transformed cells as compared to their normal non-transformed counterparts (Burns et al., 2006). This also supports the postulation that the role of CXCR7 is more important during tumorigenesis. To support this notion, CXCR7 signaling increases proliferation of tumor cells on fibroblast, promotes tumor growth in nude mice (Rago et al., 2005), and exerts pro-survival (Mazzeinghi et al., 2008) and anti-apoptotic effects (Infantino et al., 2006). Thus, similarly to CXCR4, CXCR7 may promote expansion and metastasis of certain tumor types and could be a potential prognostic factor and a target for developing new anti-cancer and anti-metastatic drugs (Fig. 2).

Accordingly, it was reported that expression of CXCR7 on breast and lung cancer cells positively correlates with their proliferation, vascularization, and metastatic potential (Miao et al., 2007). CXCR7 was highly expressed on tumor-associated vessels, which is in contrast to endothelium unaffected by tumor tissues. While CXCR7-transduced murine human breast or lung cancer cell lines grow larger tumors in immunodeficient mice, downregulation of CXCR7 expression by siRNA resulted in formation of smaller tumors by these cells. CXCR7 over-expressing breast cancer cells also showed higher seeding efficiency to murine lungs in vivo (Miao et al., 2007).

In another recently published elegant study, CXCR7 was found to be highly expressed on human prostate cancer cells (Wang et al., 2008). Staining of high-density tissue microarrays demonstrated that CXCR7 expression at the protein level is higher on more aggressive tumors. Furthermore, studies on established prostate cancer cell lines revealed that CXCR7 regulates cell proliferation most likely because of enhanced cell survival, adhesion, and chemotaxis and, in addition, increases expression of proangiopoietic factors such as IL-8 and VEGF (Wang et al., 2008). Interestingly, the CXCR7 level was influenced in prostate cancer cells by CXCR4 activation. More importantly, evidence was also provided that CXCR7 signaling in prostate cancer cell lines results in phosphorylation of serine/threonine kinase Akt. Finally, prostate cancer cells overexpressing CXCR7 grew larger and better vascularized tumors in an immunodeficient mouse model (Wang et al., 2008).

With CXCR7 identified as a new receptor for SDF-1, our team became interested in a role of the SDF-1-CXCR7 axis in human RMS cells, which as previously described by us highly express CXCR4 and robustly respond to SDF-1 gradient and signaling (Libura et al., 2004). We found that CXCR7 was expressed at a high level by more primitive embryonic RMS cells lines (Libura et al., 2004). Overall, expression of CXCR7 was higher than CXCR4. More

importantly, the CXCR7 on RMS cell lines was functional after stimulation with ITAC and SDF-1 as evidenced by MAPKp42/44- and Akt-phosphorylation, chemotaxis, cell motility, and adhesion assays. We also noticed that CXCR7 undergoes rapid internalization after stimulation with SDF-1 and ITAC. However, similarly to CXCR4 (Libura et al., 2004), we also found that signaling from activated CXCR7 was not associated with increased RMS proliferation or cell survival. Thus, CXCR7 signaling is mostly involved in RMS cell adhesion and motility and does not directly affect cell proliferation.

In prostate cancer cells, CXCR7 was also shown to affect expression of several molecules involved in tumor invasiveness such as CD44, cadherin-11, IL-8, VEGF, and tumor growth factor-beta (TGF- β) (Wang et al., 2008). Because CXCR7 could be upregulated on endothelial cells by hypoxia (Bosco et al., 2006, Costello et al., 2008) and considering its high expression on tumor-associated vessels (Madden et al., 2004), CXCR7 involvement in neoangiogenesis and formation of tumor vasculature can be supported. SDF-1 is known as a strong chemoattractant for endothelial cells and this process has been assigned to stimulation of CXCR4 (Yamaguchi et al., 2003, Kucia et al., 2005). Thus, it is not clear at this point how important the interaction between SDF-1 and CXCR7 is in the chemoattraction of endothelial progenitors. In contrast to CXCR4, CXCR7 expression is relatively very low or undetectable on normal endothelial cells (Mazzeinghi et al., 2008). As such, it is also not currently known whether CXCR7 is expressed on hemangioblasts and endothelial progenitors.

Interestingly, some rearranged fusion genes that include the CXCR7 locus as a result of chromosomal translocations were reported as present in translocations of patients suffering from some benign tumors such as lipomas (Broberg et al., 2002) and tendosynovial giant cell tumors (TGCTs; Nilsson et al., 2005). It was also revealed that CXCR7 expression is crucial for proliferation of lymphoblastoid cell lines that are a result of Epstein-Barr virus (EBV) infection. KD of the CXCR7 decreased proliferation and survival of these cells (Lucchesi et al., 2008). However, more research is needed to evaluate the role of CXCR7 in pathogenesis of leukemias and lymphomas. These effects, however, have to be dissected from the role that the SDF-1-CXCR4 axis plays in all these processes (Devine et al., 2004, Alsayed et al., 2007, Burger and Peled 2009).

5. Expression of CXCR7 on embryonic and adult cells

Similarly to CXCR4, the CXCR7 is expressed in zebrafish and frog and is highly conserved in mammals (Heesen et al., 1998, Shimizu et al., 2000). In contrast, it has not yet been found in flies (Thelen and Thelen, 2008). It is obvious that studying the tissue distribution of this receptor on cells in different organs may give some hint of a biological significance and role of the SDF-1-CXCR7 and I-TAC-CXCR7 axes in various cellular systems.

Expression of CXCR7 was found in embryonic, juvenile, and adult tissues. However, while CXCR7 is poorly expressed on normal somatic cells, its expression was found to be higher on transformed cells and during embryonic development in both human and murine tissues (Thelen and Thelen, 2008). Accordingly, CXCR7 as a protein is highly expressed on the surface of fetal liver cells, term placentas, activated endothelium, and on several murine and human tumor cell lines (Madden et al., 2004, Miao et al., 2007, Burns et al., 2006, Schutyser et al., 2007, Goldmann et al., 2008, Wang et al., 2008). Interestingly, non-transformed tissues express little membrane CXCR7 protein. In contrast, CXCR7 is detectable much more frequently at the mRNA level by Northern Blot. Accordingly, high levels of CXCR7 mRNA were found in heart, brain, spleen, kidney, lung, bladder, skeletal muscles, Langerhans islets, cartilage, synovium, testes, and ovary (Autelitano 1998, Martinez et al. 2000, Jones et al., 2006, Gerrits et al. 2008).

In the hematopoietic system, CXCR7 was reported to be expressed on neutrophils, monocytes, and B-cells (Balabanian et al., 2005, Sierro et al., 2007, Infantino et al., 2006). In B-cells, CXCR7 surface expression correlates with efficient differentiation into plasmocytes producing antibodies (Sierro et al., 2007). Interestingly, in contrast to CXCR4, CXCR7 is very weakly expressed on megakaryocytes and platelets (Ratajczak, not published observations). Data are also still missing on whether CXCR7 is also expressed on the surface of most primitive hematopoietic stem cells. For example, it is not clear at this point whether CXCR7 positive cells sorted from hematopoietic organs protect similarly to CXCR4 positive cells in lethally irradiated animals or if they are able to grow in vitro hematopoietic colonies in short-term colony forming and long-term culture assays (Ratajczak 2008).

It is known that activated chemokine receptors are internalized and subsequently degraded in the endosomal compartment or recycle to the cell surface (Kucia et al., 2005). Recent investigations on T-lymphocytes revealed that the majority of CXCR7 is present in the intracellular compartment in so-called early endosomes (Hartman et al., 2008). Therefore, more CXCR7 protein was detected if cells were permeabilized before staining (Hartman et al., 2008). Thus, these CXCR7 expression observations reveal that CXCR7 is regulated at several steps. Accordingly, cells that even highly express mRNA for CXCR7 may regulate its surface expression: i) at the translational level by regulating the rate of protein synthesis or ii) at the post-translational level by modulating its intracellular transporting and incorporation into the cell membrane. Finally, as mentioned previously, the question of whether the CXCR7 is lipid raft associated and/or regulated requires further study.

CXCR7 is also highly expressed in brain. In situ hybridization studies in rats revealed high expression in the forebrain in neuronal, astroglial, and vascular cells (Schönemeier et al., 2008). This suggests that SDF-1 may signal through CXCR7 in large populations of neural cells in brain.

As previously mentioned, CXCR7 was also found to be expressed on several tumor cell types including breast, lung, cervical, lung cancers and sarcomas. Accordingly, CXCR7 is highly expressed on a number of cell lines including MCF-7 and 4T1 breast tumors, HeLa cervical carcinoma, BCL1 lymphoma, T98G glioma, and A549 lung carcinoma as well as in the PC3, C4-2B, and LWCaP prostate cancer cell lines (Burns et al., 2006, Wang et al., 2008). It was also detected on biopsies from patient samples including gliomas and colon-, lung-, breast-, and prostate cancer patients (Madden et al., 2004, Miao et al., 2007, Burns et al., 2006, Schutyser et al., 2007, Goldmann et al., 2008). Furthermore, it is also highly expressed on tumor-associated vasculature (Wang et al., 2008). Induced CXCR7 expression on tumor cells may facilitate tumor growth and survival and lead to enhanced metastatic potential of malignant cells (Burns et al., 2006).

6. The role of CXCR7 in development: lessons from CXCR7 KD animals

Important information about a role of CXCR7 in embryogenesis and development is derived from CXCR7 KD animals (Sierro et al. 2007, Gerrits et al. 2008). However, what must be emphasized SDF-1-CXCR4 knock-out data revealed that this axis has an important role in colonization of developing bone marrow by hematopoietic stem cells (Ratajczak et al., 2006) and in development of the central nervous system and gastrointestinal vessels (Ratajczak et al., 2006). In contrast, CXCR7 KD mice reveal normal hematopoiesis. Aside from heart defects, no major developmental abnormalities in other organs were observed (Sierro et al., 2007, Gerrits et al., 2008). Two CXCR7 KD data sets are reported so far (Sierro et al., 2007, Gerrits et al., 2008). Both point to an important role of the SDF-1-CXCR7 axis that is restricted to cardiac development only. Oddly, the observed cardiac phenotypes vary between those reports.

In the first report, the majority of CXCR7 KD mice die perinatally due to severe ventricular and atrial septal defects and semilunar heart valve malformation (Sierro et al., 2007). In contrast, the tricuspid and mitral valves appeared normal at all stages examined (Sierro et al., 2007). The defects in semilunar valve development are explained by decreased expression of proangiopoietic adrenomedullin and heparin-binding epidermal growth factor (HB-EGF) in neonatal valve developing region (Sierro et al., 2007). Upon closer examination, it was found that the valves in affected animals were severely calcified, thickened, or fused. In addition, the affected mice often displayed an overriding aorta. Since no aberrant changes were revealed in embryonic hearths examined at day 17.5 of development, this suggests later CXCR7 involvement in the development of cardiac tissues (Sierro et al., 2007). Interestingly however, SDF-1 as well as its receptors CXCR7 and CXCR4 were found to be co-expressed in developing hearths, while only CXCR4 and CXCR7 are detectable in the valve developing region. Therefore, SDF-1-CXCR7 signaling appears to be critical for valve formation (Sierro et al., 2007). Theoretically some effects could potentially be related to I-TAC-CXCR7 interaction. However, because C57BL/6 mice are normal mutants lacking the I-TAC protein that develop normally in the absence of functional I-TAC, the I-TAC-CXCR7 interaction is unlikely to play a non-redundant role in organogenesis including valve formation.

In contrast, the second KD paper on CXCR7 deficient mice displayed a different cardiac phenotype (Gerrits et al., 2008). In that particular study, 50% of animals presented at autopsy with degenerated and fibrotic myocardium (Gerrits et al., 2008). These changes were even observed in some CXCR7^{+/-} heterozygotes. However, despite 70% lethality in first week of life, the remainder of KD mice had normal lifespans (Gerrits et al., 2008). Furthermore, 25% of these mice had enlarged hearts. Interestingly, no defects in valve formation were observed in these animals (Gerrits et al., 2008). The discrepancies between this and the previously discussed report could be explained by the different genetic background of animals employed for KD experiments. This also suggests that developmental defects are subtle and a final phenotype is modulated by several other factors. Curiously, in this second report, high expression of CXCR7 was found in osteocytes. However, despite high expression of CXCR4 in these cells, no skeletal defects were noticed, even in adult animals after performing ovariectomy or orchidectomy, which could potentially exacerbate subtle bone defects.

The role of CXCR7 in development of the cardiovascular system may somehow support CXCR7 morpholino oligomer KD results in zebrafish (Boldajipour et al., 2008). These animals were found to possess defects in pericardium and vessel development. To support a potential role of CXCR7 in vasculogenesis, CXCR7 valve defects observed in CXCR7 KD mice were recapitulated after selective CXCR7 deletion in the endothelium using Tie2-Cre transgenic mice (Sierro et al., 2007).

In contrast to the described CXCR4 mutation in humans (as seen in WHIM syndrome), spontaneous CXCR7 human mutations have not been identified so far. However, based on the CXCR7 KD mice phenotype, potential CXCR7 mutations could be present in patients burdened with inherited cardiovascular disorders. Furthermore, since animal models revealed that CXCR7 could be involved in optimal migration of primordial germ cells (PGCs) (Boldajipour et al., 2008), CXCR7 could be potentially involved in some developmental pathologies of the reproductive system. This also requires further study.

It would be also interesting to see if CXCR7 is expressed on embryonic stem cells similarly to CXCR4 (Ratajczak et al., 2006) and whether these cells will respond to CXCR7 stimulation. Similarly, since CXCR4 expression was found on almost all types of tissue committed stem cells in the body (Ratajczak et al., 2006), more work is needed to determine if these cells also express CXCR7. So far, functional CXCR7 was identified on renal progenitor cells only (Mazzeinghi et al., 2007). This intriguing finding will be discussed in more detail later on.

7. Structural organization of human CXCR7 gene and regulation of its expression

The human CXCR7 gene is localized on the chromosome 2q37.3 and its DNA sequence is highly conserved among mammals (Heesen et al., 1998, Shimizu et al., 2000). The CXCR7 gene encodes two exons only (acc. no. NM_020311), although presence of other exons (acc. no. BC036661) as well as alternative splicing on 5' and 3' ends have been proposed (Lucchesi et al., 2008, Broberg et al., 2002). Nevertheless, the translated coding region of the CXCR7 is encoded solely by the last exon (Lucchesi et al., 2008). Upstream exon or exons that encode 5' untranslated regions (5'UTRs) are responsible for a presence of splice variants among CXCR7 mRNAs. CXCR7 being translated from one exon only has to be considered to exclude genomic DNA amplification when reverse transcriptase polymerase chain reaction (RT-PCR) primers are designed to detect mRNA for this receptor.

The 5' flanking sequence for the first two exons (untranslated and translated) possess features characteristic for promoter sequences, i.e., CpG islands and TATA-boxes. Our recent analysis of this putative CXCR7 promoter revealed that it contains several hypoxia inducible factor-1 alpha (HIF-1 α) sites as well as a canonical nuclear factor kappa B (NF- κ B) binding site. Our preliminary data also show an important role of this latter regulatory element in CXCR7 expression (manuscript in preparation). The presence of HIF-1 α binding sites -155, -1012, beginning and -1350 base pairs upstream at of the transcription initiation site suggests that its expression could be regulated by hypoxia. To support this notion, it was found that CXCR7 expression could be unregulated in rat brain during ischemia in regions even distant from the stroke area (Schönemeier et al., 2008). On the other hand, CXCR7 expression directly in the stroke region was relatively scarce and associated with blood vessels (Schönemeier et al., 2008).

In addition to hypoxia, CXCR7 expression is regulated positively by tumor necrosis factor alpha (TNF- α) and interleukin (IL)-1b (Burns et al., 2006). CXCR7 is also upregulated on endometrial stromal cells by progesterone (Okada et al., 2003). Interestingly, during human herpes virus (HHV)-8 Kaposi sarcoma-associated virus infection, CXCR7 is also unregulated by the viral K13 protein through strong induction of the NF- κ B pathway (Matta et al., 2007). This supports an important role of NF- κ B binding site regulation of CXCR7 expression. Surprisingly, CXCR7 expression was found to be regulated by the membrane level of CXCR4 (Wang et al., 2008). Furthermore, KD of CXCR4 may also distract the CXCR7 expression pattern implying functional interplay of both receptors (Dambly-Chauderie et al., 2007).

8. CXCR7 as signaling or non-signaling receptor

The biological function of CXCR7 depends on its tissue and organ expression as well as its ability to induce appropriate signaling responses (Fig. 1). As previously mentioned, in surprising contrast to other G-protein-coupled chemokine receptors, CXCR7 does not possess a canonical DRYLAIV sequence (Thelen et al., 2008), which is responsible for G_{ai} protein coupling and induction of calcium flux (Sierro et al., 2007, Burns et al., 2006). However, lack of this conservative motive does not impair CXCR7 internalization after SDF-1 or I-TAC binding in lymphocytes (Balbanian et al., 2005). Similarly, we have recently observed this phenomenon in established human rhabdomyosarcoma (RMS) cell lines (manuscript in preparation).

Generally but depending on cell type, CXCR7 was reported to either be a non-signaling or a signaling receptor. For example, it was postulated that CXCR7 is functional only when it dimerizes with other chemokine receptor partners (Mellado et al., 2001, Percherancier et al., 2005). To support this notion, the specific functionality was demonstrated by employing

Förster resonance energy transfer (FRET) analysis of SDF-1-mediated physical heterodimerization of CXCR7 and CXCR4 on HEK293 cells (Sierro et al., 2007). Furthermore, co-expression of CXCR4 and CXCR7 on the same cells resulted in stronger calcium flux and more robust phosphorylation of MAPKp42/44 in response to SDF-1 stimulation as compared to cells that express CXCR4 only (Sierro et al., 2007). This could be explained by the heterodimeric receptor potentially being able to activate a broader panel of intracellular pathways than activation of one receptor only (Mellado et al., 2001), or by heterodimerization being able to modulate more robust intracellular responses of receptor specific pathways. This was shown in the case of MAPKp42/44 phosphorylation (Sierro et al., 2007).

Below, we discuss the biological effects assigned to CXCR7 activation that are the result of its non-signaling and signaling responses. These demonstrate the complex nature of biological responses of the SDF-1-CXCR7 axis.

9. Biological effects of CXCR7 as a non-signaling receptor

Because SDF-1 binds with 10 times higher affinity to CXCR7 as compared to CXCR4, it was initially considered as a non-signaling decoy receptor or molecular sink for SDF-1 (Haraldsen et al., 2006, Boldajipour et al., 2008). This latter possibility was postulated based on migration of PGCs in zebrafish. It is well known that PGCs migrate to genital ridges in response to SDF-1 gradient (Doitsidou et al., 2002) and that PGCs highly express CXCR4 (Doitsidou et al., 2002). In contrast, CXCR7 was found to be expressed on cells in surrounding environments rather than on migrating PGCs (Boldajipour et al., 2008). In this context, CXCR7 expressed in somatic cells surrounding migrating PGCs acts as a molecular sink to sequester and internalize SDF-1, which provides the sharp SDF-1 gradient to facilitate proper chemotaxis and guide PGCs on their way to genital ridges (Boldajipour et al., 2008). To support this notion, CXCR7 KD zebrafish embryos exhibit a defect in PGCs migration to SDF-1 gradient. Thus, this model emphasizes a role of CXCR7 as a non-signaling receptor that indirectly regulates SDF-1-mediated chemotaxis of PGCs by regulating SDF-1 availability and optimal gradient for migrating cells.

Determining whether a similar phenomenon is more common in development and if it also plays a role in trafficking of other cells in adult organism requires further study. CXCR7 could behave like a member of the so-called interceptor family (Nibbs et al., 2003). Accordingly, interceptors possess high affinity of ligand binding, do not mediate signal transduction, and specialize in ligand scavenging through internalization. Thus, similarly to previously described interceptors such as Duffy blood group antigen/receptor for chemokines (DARC), D6 (Nibbs et al., 2003), and CCX-CKR (Comerford et al., 2006), CXCR7 may play a role as a molecular sink that controls the intercellular level of ligands and provides conditions facilitating the creation of sharp chemokine gradients. Molecular “sink activity” of CXCR7 could modulate or demarcate both SDF-1 and I-TAC gradients in several processes including: i) ischemia/reperfusion; ii) inflammation; iii) cell infiltration; and finally iv) proper cell migration during development.

Another interesting non-signaling biological effect of CXCR7 expression was described in human T lymphocytes and CD34⁺ cells, where an efficient availability of CXCR7 enhances SDF-1-induced- and CXCR4-mediated activation of cell surface integrins (Hartman et al., 2008). The authors postulate that CXCR7 may serve as a kind of adaptor protein for a subset of CXCR4 molecules specialized in transducing rapid SDF-1-mediated integrin activation. At the same time, CXCR7 is not essential for CXCR4 signaling that governs cell motility or survival. However, the molecular mechanism for this proposed CXCR7 and CXCR4 interaction is not completely clear at this point and requires further study. It is also not clear

whether this effect is specific only for hematopoietic cells, or if it also applies to other cell types.

10. Biological effects of CXCR7 as a chemotactic/signaling receptor in non-transformed cells

The physiological role of CXCR7 in adult tissues remains unclear. Dispute concerning direct signaling and typical chemokine responses after SDF-1 and I-TAC binding to CXCR7, including calcium flux and kinase phosphorylation leading to motility and chemotaxis, has yet to be settled. Furthermore, all these CXCR7-dependent signaling responses may vary with cell type.

The best and well-studied normal cell model so far are lymphocytes. However, reported data are contradictory. In an initial report, CXCR7 was described as a receptor that enhances SDF-1-dependent chemotaxis of human T lymphocytes together with CXCR4 (Balabanian et al., 2005). The chemotactic response of these cells to SDF-1 gradient was decreased if anti-CXCR7 antibodies blocked CXCR7. In the same study, it was also shown that T lymphocytes highly express CXCR7, which could be internalized after SDF-1 binding (Balabanian et al., 2005). Nevertheless, no data showing activation of intracellular signaling pathways were demonstrated at that point. In another study, these observations were not confirmed. First, CXCR7 was found to be expressed at very low levels on human T lymphocytes and no chemotactic responses of T lymphocytes or activation of MAPKp42/44 and Akt pathways were observed after CXCR7 activation by SDF-1 and/or I-TAC (Hartman et al., 2008). Different level of CXCR7 expression was reported by both groups. However, it could be explained by cells being fixed before staining in the first study, which also allowed the detection of intracellular CXCR7 (Hartman et al., 2008). In agreement with this notion and as mentioned above, CXCR7 was found to be mainly expressed intracellularly in lymphocytic cells, being enriched in the so-called sub-membrane area containing early endosomes that is accessible for antibodies after cellular permeabilization (Hartman et al., 2008). This was confirmed by showing co-localization of CXCR7 with the early endosomal marker EEA1 (Hartman et al., 2008). The intracellular localization of CXCR7 may at least account for differences in reported CXCR7 surface expression studies. However, discrepancies for functional chemotaxis data between both reports are not clear at this point.

In addition to T-lymphocytes, it was postulated that CXCR7 may play a role in B lymphopoiesis (Infantino et al., 2006). Accordingly, CXCR7, similarly to CXCR4, was found to be expressed by normal B lymphocytes and its expression seems to be tightly regulated during B-cell development and differentiation (Infantino et al., 2006). In addition, since CXCR7 expression in blood-derived switch memory B-cells correlates with differentiation of these cells after activation into immunoglobulin producing plasma cells, CXCR7 could be a marker for memory B-cells, which are precursors of antibody producing cells. Moreover, it was postulated that activated mature plasmacytoid dendritic cells produce unknown ligand for CXCR7 that could selectively downregulate expression of CXCR7 (Infantino et al., 2006). Again, no convincing signaling data in B-lymphocytes are presented so far to confirm these observations. Of note, despite CXCR7 being implicated in lymphopoiesis, no major developmental defects in lymphoid tissues were reported in CXCR7 KD animals (Sierro et al., 2007). This suggests that these effects, if present, could be rather subtle in nature.

An open question remains of whether CXCR7 is playing any role in homing, mobilization, proliferation, and survival of stem cells similarly to CXCR4. Lack of major hematopoietic defects in CXCR7 KD mice argues against this possibility. In addition, CXCR7 seems to be expressed at very low levels on CD34⁺ cells (Hartman et al., 2008). As mentioned, its expression and potential biological significance on most primitive hematopoietic stem cells

was not reported so far. Thus, a question of the importance of the SDF-1-CXCR7 axis in adult hematopoiesis remains unanswered and could be additionally addressed by appropriate CXCR7 inducible knock-out experiments in hematopoietic cells performed after birth in adult animals.

Interestingly, CXCR7 was found to be expressed together with the CXCR4 on a population of renal progenitor cells in human kidneys endowed with regenerative kidney potential as established in a model of immunodeficient mice (Mazzeinghi et al., 2007). Accordingly, blockade of either CXCR7 or CXCR4 abolished SDF-1-mediated engraftment of these cells in vivo in immunodeficient mice with acute renal failure. However, activity of both CXCR4 and CXCR7 was essential for transendothelial migration of renal progenitors. CXCR7 was found to be crucial for adhesion of these cells to endothelium, which is the first step for their migration into injured tissues. Similar pro-survival activity of CXCR7 was postulated for neural cells during ischemia related with stroke (Shonemeier et al., 2008). It is not clear at this point if neural progenitors express CXCR7, in contrast to mature cells in brain, and whether this receptor is functional. These results should also prompt other investigators to examine if CXCR7 is a marker of monopotent stem cells committed for various tissues (e.g., satellite-, oval-, neural-, or cardiac- stem cells) similarly to CXCR4 and whether it functions in the trafficking/circulation and biology of these cells.

CXCR7 may also play a role in homeostasis and pathology of connective tissues. As previously mentioned, CXCR7 is expressed on osteocytes in adult bones and chondrocytes in joints (Jones et al., 2006). It was reported that stimulation of CXCR7 promotes synthesis of matrix metalloproteinase (MMP)-1, MMP-13, and vascular endothelial growth factor (VEGF) and suppresses expression of collagen-2 and matrix synthesis. In addition and concurrently, CXCR7 stimulation up-regulates synthesis of collagen-10, IL-8, osteopontin, and osteocalcin. All this together suggests that CXCR7 may mediate early development of osteoarthritis and endochondral ossification (Jones et al., 2006).

Finally, as discussed previously, in addition to CXCR7 being an SDF-1 sink that decreases local SDF-1 concentration required for proper migration of primordium and lateral line (Boldajipour et al., 2008), some authors suggest active CXCR7 signaling during primordium migration. Inhibition of CXCR7 expression in zebrafish leads to stretching of the primordium, indicating that primordial migration is regulated by SDF-1 acting through two receptors, i.e., CXCR4 on the leading portion and CXCR7 on the trailing part of the migrating group. Furthermore, it is suggested that proper functioning of the SDF-1-CXCR4 and SDF-1-CXCR7 axes requires the presence of anosmin-1a (Boldajipour et al., 2008). Since depletion of both anosmin-1a and SDF-1 leads to defects in migration of the posterior lateral line of primordium and its disruption, anosmin-1a may take part in activation of signaling on both SDF-1-activated receptors.

11. The role of CXCR7 in HIV infection

CXCR4 is a well-known co-receptor for T-lymphotropic HIV (Orsini et al., 1999). CXCR7, similarly to CXCR4, was already shown to act as a co-receptor for several strains of HIV-1, HIV-2, and simian immunodeficiency virus (SIV), which infect both lymphocytes and brain cells (Shimizu et al., 2000). It has also been postulated that in HIV infected patients, CXCR7 most likely functions in development of Kaposi sarcoma (Poole et al., 2002, Raggo et al., 2005). Strong upregulation of CXCR7 expression (>500) was noticed in Kaposi sarcoma microvascular endothelial cells infected with HHV-8 (Raggo et al., 2005). Fibroblasts transfected with CXCR7 display a higher proliferation rate, tumor forming capacity, and anchorage independent growth (Raggo et al., 2005). Inhibition of CXCR7 in HHV-8-infected microvascular endothelial cells resulted in inhibition of cell proliferation and reversed

malignant phenotype of transformed cells. The potential role of CXCR7 in local reduction of immuno-survivance has not yet been shown, although infiltration of infected lymphocytes in tumor was presented (Hengge et al., 2002). However, the final infectability of cells by HIV via CXCR7 will depend on its overall surface expression. HIV potentially using CXCR7 as a co-entry receptor must be considered in all the strategies to develop therapeutic molecules (e.g., T140, AMD3100) that inhibit HIV interaction with the CXCR4 (Ray and Doms 2006, Harrison et al., 2008).

12. Blockage of CXCR7: a challenge for pharmacology

Involvement of CXCR7 in expansion and metastasis of several tumor types shows that blocking of CXCR7 could be employed as a therapeutic strategy (Fig. 2). Some small molecular inhibitors such as CCX733 or CCX266, siRNA, and blocking antibodies are already employed in experimental models in vitro and in vivo (Hartman et al., 2008). Furthermore, the ability of SDF-1 to activate CXCR7 in addition to CXCR4 sheds some doubts of whether the “selective blockage” of CXCR4 by T140 or AMD3100 postulated until recently will be therapeutically efficient without simultaneous blocking of CXCR7. This may only partially inhibit responsiveness to SDF-1.

In fact, blockage of CXCR4 only partially inhibited responsiveness of tumor cells to SDF-1 gradient in several animal models (Jankowski et al., 2003, Wysoczynski et al., 2007). Perhaps it would be more efficient to block some shared signaling molecules that are common for signaling from both receptors. Furthermore, since cancer metastasis could depend on the metastatic potential of a small fraction of cancer stem cells, it would be important to evaluate expression of both receptors on these rare cells responsible for tumor regrowth and spread after unsuccessful radio-chemotherapy therapy. It already known that CXCR4 is in fact expressed on several putative cancer stem cells (Hermann et al., 2007, Miki et al., 2007). Determining whether CXCR7 is also co-expressed on these cells requires further investigations.

13. Closing remarks

CXCR7 is a recently orphanized receptor for SDF-1 and I-TAC that is highly expressed at the protein level on the surface of malignant cells as compared to cells in normal adult tissues. CXCR7 binds SDF-1 with high affinity and exerts various biological effects depending on cell type as a result of initiation of signal transduction or its role as a scavenger-type receptor. In contrast to other classical chemokine receptors, CXCR7 probably does not induce G_{ai} -dependent calcium flux and receptor internalization. The data from CXCR7 KD mice differ between reports; however, they demonstrate its important exclusive role in the proper development of the heart, particularly cardiac valves. Similarly to CXCR4, CXCR7 also serves as a co-receptor for several HIV strains and SIV. In contrast to CXCR4 however, CXCR7 seems not to be crucial for trafficking of hematopoietic stem cells and its expression on CD34⁺ cells is very low.

In addition, CXCR7 was reported to be involved in tumor cell growth, survival, and metastasis in many tumor cell lines. It is highly expressed on tumor-associated vasculature and may have an important role in tumor neovascularization. Blockage of CXCR7 could be potentially employed along with CXCR4 blockage in the inhibition of SDF-1-dependent tumor progression and metastasis. This task remains a challenge for modern pharmacology.

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Figure 1. SDF-1 signaling through CXCR4 or CXCR7

Signaling of SDF-1 through both receptors depends on many factors, including: i) expression of both receptors on a population of target cells; ii) possible I-TAC competition with SDF-1 for receptor binding in CXCR7, iii) potential priming and sensitization of CXCR7 similarly to CXCR4 to SDF-1 stimulation by several small proinflammatory molecules (e.g., C3 complement cleavage fragments); and iv) possible CXCR4 signaling modulation by RGS proteins. Whether RGS proteins modulate CXCR7 signaling requires further study.

Figure 2. Different strategies to target the CXCR7-SDF-1/ITAC axis

Expression of CXCR7 and its responsiveness to the SDF-1/I-TAC gradient could be modulated at several levels. Accordingly, expression of CXCR4 could be inhibited at the transcriptional level siRNA or antisense oligodeoxynucleotides and at the protein receptor level by employing CXCR7 blocking antibodies or molecules (e.g., CCX451 and CCX751). Conversely, responsiveness of CXCR7 to an SDF-1 gradient could be affected similarly to CXCR4 by blocking CD26 by diprotin. It is not clear at this point whether CXCR7 is a lipid raft-associated receptor. If it is, then depletion of cell membrane cholesterol and thus inhibition of lipid raft formation (e.g., statins) would decrease responsiveness of CXCR7⁺ cells to an SDF-1 gradient.