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Chemokine receptor trio: CXCR3, CXCR4 and CXCR7 crosstalk via CXCL11 and CXCL12

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Abstract

Although chemokines are well established to function in immunity and endothelial cell activation and proliferation, a rapidly growing literature suggests that CXC Chemokine receptors CXCR3, CXCR4 and CXCR7 are critical in the development and progression of solid tumors. The effect of these chemokine receptors in tumorigenesis is mediated via interactions with shared ligands I-TAC (CXCL11) and SDF-1 (CXCL12). Over the last decade, CXCR4 has been extensively reported to be overexpressed in most human solid tumors and has earned considerable attention toward elucidating its role in cancer metastasis. To enrich the existing armamentarium of anti-cancerous agents, many inhibitors of CXCL12–CXCR4 axis have emerged as additional or alternative agents for neoadjuvant treatments and even many of them are in preclinical and clinical stages of their development. However, the discovery of CXCR7 as another receptor for CXCL12 with rather high binding affinity and recent reports about its involvement in cancer progression, has questioned the potential of "selective blockade" of CXCR4 as cancer chemotherapeutics. Interestingly, CXCR7 can also bind another chemokine CXCL11, which is an established ligand for CXCR3. Recent reports have documented that CXCR3 and their ligands are overexpressed in different solid tumors and regulate tumor growth and metastasis. Therefore, it is important to consider the interactions and crosstalk between these three chemokine receptors and their ligand mediated signaling cascades for the development of effective anti-cancer therapies. Emerging evidence also indicates that these receptors are differentially expressed in tumor endothelial cells as well as in cancer stem cells, suggesting their direct role in regulating tumor angiogenesis and

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metastasis. In this review, we will focus on the signals mediated by this receptor trio via their shared ligands and their role in tumor growth and progression.

Keywords

CXC chemokines; Tumor growth; Angiogenesis; Metastasis

1. Introduction

Chemokines are a superfamily of small (7–16 kDa), proinflammatory chemoattractant cytokines, which were originally characterized by their ability to induce migration of leukocytes [1,2]. They were subsequently shown to play major roles in other pathophysiological conditions like embryogenesis, angiogenesis, hematopoiesis, atherosclerosis, HIV-infection and cancer [3,4]. The biological effects of these proteins are mediated through seven transmembrane domain G protein coupled receptors (GPCRs), known as chemokine receptors having an extracellular N-terminus and a cytoplasmic C-terminus. Classically, one of the intracellular loops of these receptors interacts with heterotrimeric, pertussis toxin sensitive G proteins called $G\alpha_i$ which initiate a cascade of signal transduction events in response to ligand binding [5,6]. In addition, chemokine receptors can also signal through non-G-protein-mediated pathways or even through other G-protein subtypes [7]. Over 50 chemokines and 20 chemokine receptors have been discovered so far and they are divided into 4 subgroups (CXC, CC, CX3C, and C) depending on the position of the conserved cysteine residues from the amino terminal end of these proteins [8]. Functionally, chemokines are classified into two groups: homeostatic and inflammatory chemokines. Homeostatic chemokines are expressed constitutively and play an important role in development and maintenance of immune system, whereas inflammatory chemokines are inducible in response to stimuli [9]. Furthermore, the CXC group is subdivided into ELR^+ and ELR^- chemokines depending on presence or absence of an ELR motif (tripeptide Glu-Leu-Arg) preceding the CXC domain. In general, the ELR^+ CXC chemokines such as Interleukin-8 or IL-8 (CXCL8) are angiogenic whereas ELR^- CXC chemokines such as Platelet Factor-4 or PF-4 (CXCL4) are angiostatic. However, stromal cell-derived factor-1 or SDF-1 (CXCL12) is an exception to this generalization; while being ELR^-, it mediates angiogenesis via interactions with its receptor CXCR4 [10,11].

To date, CXCR4 is one of the most studied chemokine receptors; it is over expressed in at least 20 different human cancers, including breast cancer, ovarian cancer, melanoma and prostate cancer [12]. Multiple inhibitors of the CXCL12–CXCR4 axis, such as AMD3100 or plerixafor, Nox-A12 have been shown to be effective in reducing tumor growth in preclinical studies involving animal tumor models [13]. However, the efficacies of these targeted inhibitors have been questioned after the discovery of CXCR7, another receptor for CXCL12 and recent reports indicating its critical involvement in cancer progression [14]. This ever-growing saga of chemokine biology has become even more complex with increasing evidence suggesting that pro-tumorigenic CXCR7 can also bind with another chemokine family member, interferon-inducible T cell alpha chemoattractant or I-TAC

(CXCL11). CXCL11 is a well established ligand for CXCR3 having pro- and anti-tumorigenic functional capabilities. For example, if CXCL11 interacts with CXCR3-A or CXCR7, it promotes proliferative signals, whereas, CXCL11–CXCR3-B binding results in growth inhibitory functions [15]. Therefore, chemokines and their receptors are not only highly promiscuous (multiple chemokines can bind to a single receptor) and pleiotropic (single chemokine can bind to multiple chemokine receptors), but the same ligand may function in an antagonistic manner depending on its binding to a receptor subtype on the target cell (Fig. 1). Although there are several paradigms regarding the feedback and regulatory loops of these chemokines, they seem to be essential for the physiological fine tuning of specific responses. Nevertheless, they also provide for challenges in regards to therapeutics.

In this review, we will focus our discussion on the CXC chemokine receptors CXCR4, CXCR7 and CXCR3 (receptor trio) and highlight the crosstalk between the receptor trio via their shared ligands in solid tumor development and progression. We will also emphasize how a single chemokine can function differentially in the context of tumor progression versus growth inhibition depending on the selective interaction with particular receptors. Finally, we will discuss the recent progress made in newly established signaling pathways mediated by this chemokine receptor trio and address limitations along with possible outcomes of therapeutic targeting of these receptor trio interactions.

2. Receptor trio (CXCR4, CXCR7, and CXCR3) in cancer

Although the reported association of CXCL12–CXCR4 in cancer is quite extensive, the novel involvement of CXCR7 as a high affinity binding partner of CXCL12 begs for reconsideration of the whole CXCR4 paradigm in the context of cancer biology. Moreover, the potential of CXCR7 interaction with CXCR3 via their shared ligand CXCL11 adds complexity to their functionality. Here, we initially describe the basic properties of each member of this receptor trio and we subsequently discuss their respective role(s) in cancer.

2.1. CXCR4

CXCR4 is an evolutionarily highly conserved, rhodopsin-like seven transmembrane GPCR that exclusively binds to CXCL12 [14,16]. Human CXCR4 was originally identified as a receptor for CXCL12 by screening chemokine receptor orphan genes for their ability to induce intracellular Ca^{++} in response to human CXCL12. The mouse CXCR4 receptor was subsequently found by cloning candidate chemokine receptors and comparing the amino acid sequence with the human cDNA [17]. The discovery that CXCR4 functions as a co-receptor for entry of T-tropic (X4) HIV viruses into $CD4^+$ T cells initiated a broad research effort to elucidate the function of this receptor [18]. CXCR4 is expressed at high levels by various immune cells including monocytes, B cells, and naive T cells in peripheral blood [19]. Differential expression of CXCR4 in $CD34^+$ progenitor cells is proposed to be involved in maintaining hematopoietic progenitor cells in the marrow and in the regulation of stem cell trafficking [20]. Studies have demonstrated that the CXCL12–CXCR4 axis plays an important role in development; CXCR4 knock-out mice are embryonically lethal due to failure in hematopoiesis, organ vascularization, and neuronal migration [21,22]. Further, *Cxcl12* and *Cxcr4* gene-deleted mice showed an identical, lethal phenotype,

suggesting a monogamous relationship between this receptor ligand pair [23,24]. CXCL12-stimulated chemotaxis occurs as a result of cytoskeletal rearrangements, actin polymerization, polarization, pseudopodia formation, and integrin-dependent adhesion to endothelial cells and other biologic substrates [25,26]. In recent years, the CXCL12–CXCR4 axis has been exploited as a target for therapeutics that blocks CXCL12–CXCR4 interactions or inhibits downstream intracellular signaling cascades [13,27].

2.2. CXCR4 in cancer

Although CXCR4 research initially focused on its role in HIV, its involvement in cancer was fueled by the observation that CXCR4 regulates organ specific metastasis of breast cancer cells [28–30]. After the publication of a seminal paper by Muller et al., CXCR4 has been reported to be overexpressed in several human cancers, and blockade of CXCR4–CXCL12 interactions has been extensively investigated as a potential cancer therapeutic. CXCR4 overexpression results in metastatic dissemination of breast cancer cells to the lungs and lymph nodes [28]. In contrast, CXCR4 contributes melanoma tumor cell dissemination selectively to the lungs but not to the lymph nodes. Hence, these studies clearly indicate that although CXCR4 contributes to tumor metastatic capacity, the same receptor expressed on different malignant cells directs tumor cells to different secondary sites. The mechanism underlying this tissue tropism has yet to be determined, but it may reflect differences in tumor cell survival at the secondary site as much as differences in initial deposition [29]. CXCR4 has also been found to be a prognostic biomarker in various types of cancer including leukemia, breast cancer and very recently in gliomas [31].

The upregulation of CXCR4 in malignant cells can occur through several mechanisms. Hypoxic conditions within solid tumors may induce CXCR4 expression via Hypoxia Inducible Factor (HIF) – 1α. Moreover, VEGF produced by tumor cells may induce CXCR4 expression on the tumor cell itself, and/or on tumor-associated endothelial cells that facilitate both angiogenesis and metastasis of primary tumor [32]. Duda et al. have suggested that this could account for the limited success of anti-VEGF therapy in most of the solid tumors, and they recommended combination therapy with anti VEGF and CXCR4 inhibitors as an optimal therapeutic option [13].

Recently, several studies have documented the existence of a small subset of cancer cells, that share the many characteristics of stem cells and thus, they have been collectively called cancer stem cells (CSCs) or tumor initiating cells (TICs). They constitute a reservoir of self-sustaining cells with the ability to maintain tumor growth and metastasis [33]. Interestingly, CXCR4 has been shown to be selectively present on CSCs derived from several solid tumors and they play a critical role in regulating carcinogenesis [34,35]. In glioma, $CXCR4^+$ tumor cells, and not $CXCR4^-$ cells, give rise to tumorospheres in serum-free medium, and the $CXCR4^+$ subpopulation have stronger tumorigenic capability, and are more resistant to chemotherapy and radiation therapy [36]. This observation signifies that $CXCR4^+$ glioma is rich in CSCs. Moreover, it has been reported that CXCR4 may serve as a biomarker of CSCs in pancreatic and prostate cancer [34,35].

2.3. CXCR7

Until recently, CXCL12 and CXCR4 were thought to be exclusive partners – a relative rarity in the redundant chemokine network. However, a second chemokine receptor, CXCR7/RDC1 was identified, which binds to CXCL12 with even higher affinity than CXCR4 [37,38]. Although this newest member of the seven CXCR family binds to two chemokines CXCL12 and CXCL11, classical GPCR mediated chemokine signaling could not be demonstrated [38]. Originally, the gene was cloned from a canine cDNA library (*R*eceptor *D*og *c*DNA) as a putative GPCR for the vasoactive intestinal peptide hormone VIP [39,40]. The human *cxcr7* gene is localized on the chromosome 2q37.3 and CXCR7 is highly conserved between mammalian species considering the sequence homologies observed in human, dog, mouse and rat. The *cxcr7* gene encodes only two exons, although the presence of other exons as well as alternative splicing on 5′ and 3′ ends has been proposed. Nevertheless, the translated coding region of CXCR7 is encoded solely by the last exon [41].

Chemokine receptor function classically regulates leukocyte trafficking [1,2], and a lot of effort has been given to characterize the expression and function of CXCR7 in leukocytes; nevertheless, its expression in hematopoietic cells remains controversial and challenged by different groups [42–44]. Earlier studies have shown that CXCR7 is highly expressed in monocytes and mature B cells and its expression inversely correlates with the activity of CXCR4 on B cells [44]. The latter is detectable at the cell surface during all stages of development, but is weakly responsive in mature B cells [45,46]. Survival and efficient differentiation of B cells into antibody producing cells correlate with CXCR7 levels at the plasma membrane [42]. Such findings are suggestive of a function that distinguishes CXCR7 from other chemokine receptors. Although several papers have reported the expression of CXCR7 in immune cells, Berahovich et al. recently questioned the antibody used to detect CXCR7 on these cell types, and ruled out its expression in immune cells [43,44]. Interestingly, their finding was further challenged very recently by Humpert et al., who used a proteomic approach to document the presence of CXCR7 on human B cells [47].

Despite having all the canonical features of GPCRs, CXCR7–ligand mediated activation of intracellular signals remain controversial, since unlike typical chemokine receptors, CXCR7 fails to activate heterotrimeric G proteins. A conserved DRYLAIV motif at the N-terminus of the second intracellular loop is unique among most of the chemokine receptors and it is assumed to be necessary but not sufficient for coupling to Gi-proteins. The sequence of CXCR7 is altered at two positions (A/S and V/T), but these modifications are also present in the lymphotactin receptor and CXCR6, both known to signal via G-proteins clearly indicating that these sequence alterations may not be the reason for this unusual signaling process [48–50]. Interestingly, it has been shown that CXCR7 has a dramatic effect on the signaling response resulting from CXCR4 activation. For example, CXCR7 can directly modulate CXCR4 signals via CXCR7–CXCR4 heterodimerization [51]. Growing evidence also indicates that CXCR7 may function as a “decoy” receptor or chemokine scavenger [52,53]. Internalization of CXCL11 or CXCL12 bound CXCR7 without any signaling would generate the gradient of chemokine necessary for an optimum CXCR4 migratory response [54]. Another function of this receptor other than “decoy” activity has recently been

described as CXCR7 also interacts with β-arrestin in a ligand-dependent manner. This interaction results in ERK1/2 phosphorylation and translocation via G-protein independent, β-arrestin mediated signal [52,55]. Very recently, it has been reported that serine/threonine residues present at the C-terminal of CXCR7 are essential for β-arrestin recruitment and has emerged as a potential factor during the initial signaling step after receptor activation [56,57].

2.4. CXCR7 in cancer

CXCR7, a dual specificity receptor is upregulated in many tumors and found to be involved in tumor cell growth, survival, and metastasis in several cancer types [14,38]. It is also expressed at high levels on tumor-associated vasculature and may have an important role in tumor neovascularization [58]. The role of CXCR7 in cell growth/survival was first suggested by the observation that CXCR7-transfected MDA-MB-435 cells expanded more rapidly in culture and they formed larger tumors than controls in an animal model [55,59]. In prostate cancer, CXCR7 is associated with a more aggressive disease exhibiting increased cell proliferation, adhesion and chemotaxis [60]. Interestingly, CXCL8 promotes CXCR7 expression, which in turn activates EGFR to facilitate prostate tumor cell proliferation in a ligand independent manner [61]. In an animal model of lung cancer, it has been shown that blockade of CXCR7 by its antagonist CCX754 results in the inhibition of tumor growth providing direct evidence of pro-tumorigenic properties of CXCR7 [38]. CXCR4 and CXCR7 were both identified in rhabdomyosarcoma (RMS) cells, where they play overlapping but distinct roles in regulating metastatic behavior. CXCR4 was highly expressed on the more metastatic alveolar RMS (ARMS) cell line whereas CXCR7 was restricted to non-metastatic cells [62]. Like CXCR4, hypoxia is a major inducer of CXCR7 in tumor cells and tumor-associated endothelial cells emphasizing its role in promoting tumor growth as well as angiogenesis in tumors [58].

2.5. CXCR3

CXCR3, an ELR$^-$, classic seven-transmembrane G protein coupled CXC chemokine receptor was originally cloned by Loetscher et al. and it has been reported to be expressed on activated T lymphocytes after *in vitro* stimulation but it is usually present on only a small fraction of resting T lymphocytes, B cells, monocytes, and granulocytes [63,64]. It has been initially observed to induce calcium flux and chemotaxis in response to Monokine-induced by human IFN-γ or Mig (CXCL9), IFN-γ-inducible 10-kDa protein or IP-10 (CXCL10), but not to other chemokines. Later, CXCL11 also called I-TAC, was observed to bind CXCR3 [65]. The CXCR3–ligand system has been widely studied in regulating host immunity especially Th1 type immune responses, allograft rejection, and angiogenesis [66–69]. More recently, it has been shown that CXCR3–ligand interactions result in diverse cellular functions including chemotactic migration and cell proliferation, or inhibition of migration, proliferation and even cell death depending on the cell type. This diversity of cell behavior is explained, in part, by the presence of at least two splice variants of CXCR3, CXCR3-A and CXCR3-B with reciprocal functional ability [15,70]. Although several reports suggested the presence of CXCR3 splice variants in human cells and tissues [15], their presence in mouse system is yet to be elucidated. However, the lack of reliable CXCR3-isoforms specific antibodies, limits further progress of this study. CXCR3-A, expressed in epithelial

cells, has been shown to promote cell proliferation, whereas CXCR3-B, primarily expressed on fibroblasts, endothelial, and epithelial cells, inhibits cell migration and apoptosis. Some studies have suggested that CXCR3-A and CXCR3-B play reciprocal roles through different G-protein coupling and trigger distinct signal transduction pathways [15,71]. Another splice variant called CXCR3-alt is characterized by a truncated C terminus with loss of the intact second and third extracellular loops, and was found to be co-expressed with CXCR3-A [72]. Cells expressing this variant are capable of migrating *in vitro* in response to stimulation with CXCL11. CXCL9, CXCL10, CXCL11 are known to interact with both the splice variants, whereas, CXCL4 selectively interacts with CXCR3-B [15,70].

2.6. CXCR3 in cancer

$CXCR3^+$ lymphocyte recruitment, directed by CXCL10, can promote spontaneous regression of melanoma, whereas CXCL11 increases tumor-infiltrating lymphocytes and inhibits tumor growth in both breast cancer and T-cell lymphoma [73–75]. Also, CXCR3–ligand interactions are strongly anti-angiogenic in nature as it inhibits endothelial cell proliferation *in vitro* and *in vivo* [76]. Therefore, CXCR3-mediated homing of immune cells as well as its strong angiostatic response classically represents a potential target for tumor therapy. However, emerging evidence now suggests that the CXCR3 signaling network can positively influence tumor cell growth, survival, and migration [70,77–83]. It is noteworthy that determination of the role of CXCR3 in tumorigenesis is complicated by the fact that many cells in the tumor microenvironment potentially express CXCR3 splice variants and their ligands. CXCR3 and its ligand CXCL10 are expressed in many human glioma cell lines, and CXCL10 treatment activates MAPK, stimulating cell proliferation [83]. Several breast cancer cell lines, such as, MCF-7, and MDA-MB-435 harbor CXCR3/ligand autocrine loops that influence cell proliferation [70]. CXCR3 has been associated with poor patient survival and promotion of metastasis; moreover, in a murine model, antagonism of CXCR3 by a small molecule inhibitor blocks pulmonary metastasis of a highly metastatic breast cancer [77,78]. In addition, CXCR3 has been found to be upregulated in human melanoma, where it correlates with tumor progression and contributes to the regulation of cell proliferation, survival, migration, and metastasis [79]. Furthermore, CXCL10/CXCR3 signaling has been reported to promote invasion-related properties as well as metastasis in human colorectal carcinoma cells [81]. Recent findings by Lo et al. suggest that aberrant CXCR3/ligand signaling promotes proliferative receptor isotypes [82,83]. Indeed, it has been reported that forced overexpression of CXCR3-A induces enhanced survival of human microvascular endothelial cells, whereas CXCR3-B overexpression dramatically reduces DNA synthesis and stimulates apoptosis in these cells [15]. These functional dichotomies of CXCR3–ligand mediated actions largely depend on the presence of two functionally opposite splice variants CXCR3-A and CXCR3-B on the cell surface. Angiostatic effect of CXCR3 ligands in normal *in vitro* and *in vivo* conditions could be explained due to selective expression of growth inhibitory CXCR3-B isoform on human microvascular endothelial cells.

3. Receptor trio crosstalk in cancer via shared ligands

Chemokine biology has been substantially evaluated with respect to its ability to modulate immune cell function. However, more recently, chemokine receptor–ligand interaction in non-hematopoietic cells, especially in tumor cells gained considerable attention due to their role in regulating organ specific metastasis of cancer [29,30]. Moreover, in pancreatic and prostate cancer, CXCR4 serves as one of the cancer stem cell markers [34,35]. As discussed above, CXCR4, CXCR7, CXCR3 and their ligands CXCL12, CXCL11, CXCL9, CXCL10, and CXCL4 are expressed in the tumor microenvironment including tumor epithelial, endothelial, and other cells but the ultimate biological effect of these ligand receptor interactions depend on the final outcome of their crosstalk at the level of receptor selection and the associated receptor-inducible intracellular signaling response.

3.1. Receptor trio crosstalk in tumor growth

As a GPCR, the mechanism of CXCR4 activation by CXCL12 is mediated by a heterotrimeric G-protein which is activated through the exchange of GTP in place of previously bound GDP on its $G\alpha_i$ subunit followed by dissociation of heterotrimeric $G\alpha\beta\gamma$ protein into activated $G\alpha_i$ and $G\beta\gamma$ subunit. $G\alpha_i$ activation results in release of calcium via PLCβ as well as inhibition of adenyl cyclase (AC) mediated cyclic adenosine monophosphate (cAMP) production (Fig. 2) [84]. CXCR4-induced tumor cell proliferation is mediated via PI3 kinase-AKT activation through both $G\alpha_i$ and $G\beta\gamma$ subunits. Also, activated $G\alpha_i$ promotes Ras–RAF–MEK–ERK and PI3K–AKT–NFkB signaling cascades to facilitate tumor cell survival and proliferation. By inactivating proapoptotic protein Bcl-2, CXCL12 promotes tumor cell proliferation via the activation of MEK–ERK and PI3K–AKT pathways [85]. Although it has been shown that β-arrestin is recruited downstream of GPCRs, including CXCR4, to control receptor trafficking and desensitization via G protein coupled Receptor Kinase (GRK), emerging evidence indicates a novel role of β-arrestin in CXCR7 mediated activation of MAP kinases (ERK1/2) resulting in significant induction of tumor cell proliferation and adhesion. CXCR7 acts primarily to modulate CXCR4 signaling and several contradictory reports have been put forward to explain the underlying role of CXCR7 in this process. It has been shown that CXCR7 may act as a scavenger of CXCL12, to generate its gradient that leads to differential CXCR4 signaling [86,87]. More interestingly, it has also been reported that CXCR7 heterodimerizes with CXCR4 to act as co-receptor based on over expression studies [51].

However, involvement of G-protein signaling after receptor heterodimerization is highly controversial as initial reports suggest that Gi-proteins are activated after dimerization but more recently, β-arrestin is reported to be constitutively recruited after dimerization instead of Gi proteins [52]. Hence, it is now clear that unlike other seven transmembrane receptors, such as CXCR4, which shows balanced or unbiased activity for signaling through β-arrestins or G protein pathways, CXCR7 acts in a biased agonism manner to selectively activate β arrestin upon ligand binding [7].

In addition to CXCL12, CXCR7 binds with low affinity to CXCL11 which can also bind to chemokine receptor CXCR3, precisely with both the splice variants namely CXCR3-A and CXCR3-B [15]. CXCR3-A–ligand (CXCL11/CXCL10/CXCL9) binding results in Ca^{++}

flux and AKT activation resulting in pro-proliferative response [15]. In contrast, CXCR3-B–ligand (CXCL11/CXCL10/CXCL9/CXCL4) interaction promotes dissociation of heterotrimeric Gs into $G\alpha_s$ and $G\beta\gamma$ subunits and activated $G\alpha_s$ subunit triggers AC-cAMP-p38 MAPK signaling cascade leading to growth arrest or cell death [88]. In breast cancer, we have reported that activation of Ras promotes CXCL10 production as well as CXCR3-B downregulation, which may subsequently facilitate the autocrine loop between CXCL10–CXCR3-A leading to tumor cell proliferation [70]. More recently, to dissect the mechanism of CXCR3-B mediated inhibitory signals; we have also demonstrated that CXCR3-B mediated downregulation of cell protective enzyme hemoxygenase-1 (HO-1) plays a critical role in renal tumor cell survival [89].

Therefore, the balance between CXCR4, CXCR7, and CXCR3-A mediated proliferative signals versus CXCR3-B mediated growth inhibitory actions would dictate the final outcome of any response in a particular tumor microenvironment. In this event, AKT and MAPK associated intracellular signaling crosstalk are the key regulatory factors for modulating either tumor cell proliferation or death.

3.2. Receptor trio crosstalk in metastasis

Upon ligand activation, CXCR4 mediates cell migration via different downstream signaling cascades. In one pathway, $G\alpha_i$/ $G\beta\gamma$ subunit mediated PI3K activation leads to stimulation of Cdc42, Rac and PAK proteins via phosphorylation of several adhesion components such as Pyk2, Paxillin and Crk. In another pathway, especially in metastatic tumor cells, CXCR4 mediated $G\beta\gamma$ subunit activates PLCβ to induce the degradation of inner membrane bound PIP2 into secondary messenger inositol triphosphate (IP3) and diacyl glycerol (DAG) (Fig. 2). In turn, IP3 induces the release of Ca^{++} from intracellular stores which is also positively correlated with cell migration via the phosphorylation of μ-calpain. Also, DAG activates ERK1/2 MAP kinase pathways via PKC and induces chemotaxis through activation of m-calpain [85,90,91]. In addition, CXCR4 signaling has been shown to induce tumor cell invasion by upregulating adhesion molecules (such as VLA-4) and matrix metalloproteinases (MMPs). Moreover, it has been shown that CXCL12 derived from breast cancer cells increases breast cancer cell invasiveness by up-regulating urokinase-type plasminogen activator receptor (uPAR). CXCL12 produced by normal breast cancer cells acts on CXCR4 and induces activation of the JNK pathway which in turn induces expression of uPAR. The up-regulation of this receptor was paralleled by urokinase-type plasminogen activator partitioning on the cell surface, which may account for the increased invasive properties of CXCL12-treated tumor cells [92].

The contribution of CXCR7 to the tumor microenvironment has introduced a new level of complexity to CXCL12 signaling in cancer metastasis. Although in glioma, CXCL12 and both its receptors are expressed in cancer cells, the CXCR4/CXCL12 axis is particularly prevalent in invading glioma cells. Supporting that notion, it was recently reported that CXCR4 is selectively expressed in glioma stem-like cells; whereas, expression of CXCR7 is more restricted to “differentiated” glioma cells [93]. Furthermore, Hernandez and colleagues delineate the role of CXCR4 and CXCR7 in tumor invasion and metastasis and convincingly demonstrate that co-expression of CXCR7 and CXCR4 results in inhibition of CXCL12-

mediated invasion, reduced intravasation of breast tumor cells into the vasculature, and fewer lung metastases compared to parental tumors [59]. In contrast, it has also been reported that CXCR4–CXCR7 heterodimer constitutively recruits β-arrestin to enhance cell migration [55]. In addition, a dynamic yin–yang interaction between CXCR4 and CXCR7 in breast cancer metastasis has been reported in which CXCR7 acts as a sink for monomeric CXCL12, leaving dimeric CXCL12 to bind CXCR4 and mediate signaling preferentially through the recruitment of β-arrestin, thus enhancing chemotaxis [94,95]. Therefore, one can postulate that when CXCR7 dimerizes with CXCR4, it may not only show its biasness to β-arrestin over Gi mediated signaling but also show its selectivity toward clathrin mediated endocytosis over ERK1/2 mediated signaling to precisely control cell migration.

The role of CXCR3 in metastasis is more complex due to the expression of functionally opposite isoforms CXCR3-A, and CXCR3-B. CXCR3-A activates Gi similar to CXCR4 and mediates it metastatic effects via either PI3K or PLC-β or by activating ERK1/2 pathways [96]. However, CXCR3-B shows a completely different signaling cascade by recruiting Gs instead of Gi and inhibits metastatic signals. Activated $G\alpha_s$ subunit further stimulates adenyl cyclase to increase cAMP concentration to activate its various targets such as PKA [15]. However increased intracellular cAMP concentration is inhibitory to ERK1/2 mediated signaling and cell migration (Fig. 2). Increase in prostate cancer cell migration and invasion is attributed not only through high CXCR3-A expression but also by downregulation of inhibitory signals via CXCR3-B [96]. Therefore, it can be hypothesized that chemokine CXCL11 may interact with CXCR3-A and activate its downstream signaling cascades which suppress the CXCR3-B expression either at post transcriptional or post translational level. This also provides a possibility of crosstalk between CXCR7 and CXCR3-A for the synergistic induction of tumor cell migration and invasion versus antagonistic role of CXCR3-B.

Therefore, relationship between CXCR4 and CXCR7 in the context of cancer metastasis is highly dynamic. In one hand, they could synergistically promote invasion via activating ERK1/2 MAPK pathway, on the other hand, they could also show reciprocal effects by CXCR7 mediated sinking of free CXCL12 leading to reduced CXCR4–PI3K/MAPK metastatic signals. In addition, CXCR3-A and CXCR3-B have shown to play opposite role in metastasis via activating two completely different G proteins (CXCR3-A–Gi/CXCR3-B–Gs) at their cytoplasmic domain just after ligand binding.

3.3. Receptor trio crosstalk in angiogenesis

Angiogenesis, which is basically the formation of new blood vessels from existing ones, is an integral part of many physiological as well as pathological processes including cancer [97]. Pathological angiogenesis is a very complex process provoked by disturbance of the delicate equilibrium between stimulatory and inhibitory signals. During the process of tumor growth this equilibrium is shifted to the direction where formation of stimulatory factors are favored and this process appears to be obligatory for sustained tumor growth as it helps in delivering oxygen and nutrients and removing waste products [4,98]. Several lines of evidence indicate that despite being ELR^-, CXCL12 promotes angiogenesis via endothelial cell (EC) proliferation, migration, as well as VEGF production [9–11]. Although both

CXCR4 and CXCR7 are shown to be moderately expressed in normal EC, the expression of CXCR7 on tumor endothelial cells is markedly upregulated and has recently been proposed as a marker of tumor vasculature in various tumors [58,93]. Hypoxic condition, abundant VEGF and CXCL8 in tumor microenvironment are thought to be the key regulators for the overexpression of CXCR7 in tumor vasculature.

Along with CXCR4 and CXCR7, CXCR3-B is also reported to be highly expressed in EC, whereas, expression of CXCR3-A is negligible or absent. Interestingly, vascular smooth muscle cells (VSMC) have been shown to preferably express CXCR3-A over CXCR3-B [15]. Therefore, in a similar condition, CXCL11/CXCL10 would result in VSMC proliferation and simultaneously inhibit EC growth due to the presence of different CXCR3 isoforms. CXCR3-B–CXCL10/CXCL4 interactions have shown to mediate robust angiostatic effects possibly due to the absence of counteracting CXCR3-A mediated signals. In contrast, CXCL12 is one of the strongest contenders for nullifying this anti-angiogenic effect of CXCL10/CXCL4 via interacting with highly expressed CXCR4 and CXCR7 on tumor associated endothelial cells.

4. Therapeutic potential and concluding remarks

The convergence to the CXCL12–CXCR4 axis in studies of multiple tumor types strongly suggests that tumors in different organs exploit this common pathway to spread and escape from therapy [13]. Importantly, the use of CXCR4/CXCL12 inhibitors in the treatment of solid tumors has produced some encouraging preclinical data [99]. However, CXCR4 inhibition with AMD3100 may not be sufficient to block the effects of CXCL12, which may also bind to CXCR7 on cancer or stromal cells. In fact, blockade of CXCR4 only partially inhibited responsiveness of tumor cells to CXCL12 gradients in several animal models [14]. Moreover, connection of CXCR7 with CXCR3 via their shared ligand CXCL11 and reciprocal functional capability of two CXCR3 splice variants have made this relationship more complex. Although individual inhibitors of CXCR4 (AMD3100), CXCR3 (AMG487), and CXCR7 (CCX2066) have shown to be partially effective in controlling solid tumor growth and metastasis but only a detailed mechanistic understanding of the action of CXCL12–CXCR4/CXCR7 pathway and their interrelationship with CXCR3 isoforms in solid tumors could provide novel insight into how to combine these inhibitors effectively and safely for improved cancer treatment. The opposing effects of these receptor trio interactions as generated by CXCR7 and CXCR3-B in response to their respective ligands should be carefully considered while designing combination therapies. Thus, biology-driven, rational design of new combination therapies will be critical for successfully pursuing these pathways as a novel target for sensitization to existing therapies. Overcoming these challenges would increase the chances of realizing the promise of receptor trio interactions and their selective inhibition as a potentially effective strategy to decrease the rates of local and distant failure after the use of currently available therapies for solid tumors.

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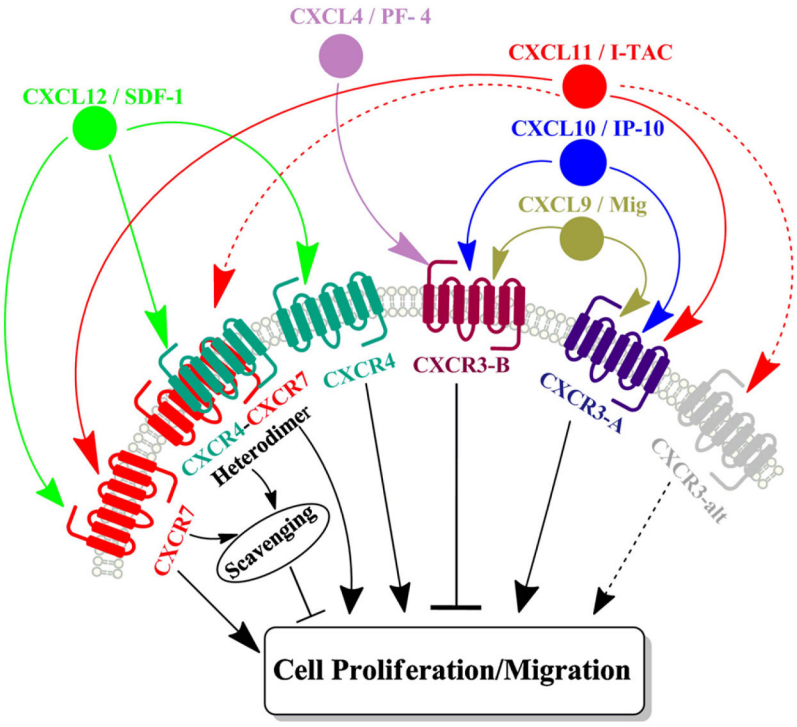

Fig. 1.

Schematic overview of CXC chemokine receptor trio (CXCR3, CXCR4, and CXCR7) interactions via respective ligands and their effects. Solid line indicates well established interactions whereas, dotted ones represent interactions supported with minimal evidence.

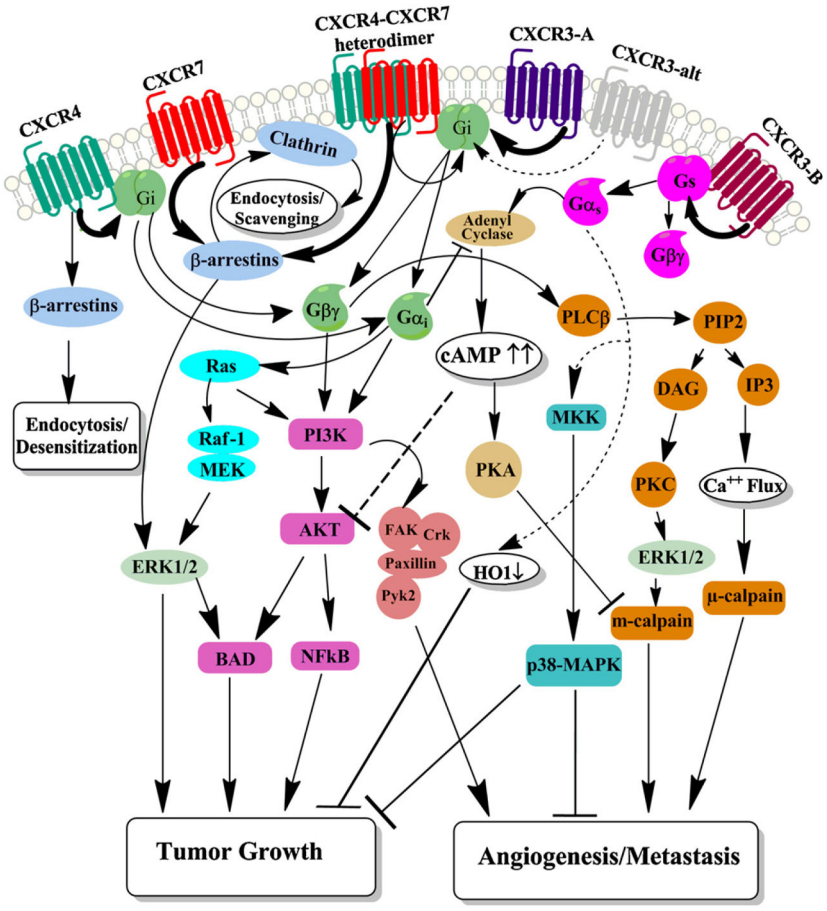

Fig. 2.

Schematic representation of CXC chemokine receptor trio (CXCR3, CXCR4, and CXCR7) intracellular signaling crosstalk. Bold arrows show predominant signals via receptor–ligand binding. Thin line corresponds to regular signals and dotted line signifies not so well established pathways.