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Functions of the CXCL12-receptor ACKR3 / CXCR7 - What has been perceived and what has been overlooked

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Running Title: ACKR3 functions

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List of Abbreviations:

ACKR3, atypical chemokine receptor 3

ADM, adrenomedullin

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BAM22, bovine adrenal medulla 22

BRET, bioluminescence resonance energy transfer

FRET, Fluorescence resonance energy transfer

GnRH, gonadotropin-releasing hormone

GPCR, G-protein-coupled receptor

Grk, G protein-coupled receptor kinase

MIF, macrophage inhibitory factor

NC-IUPHAR, Nomenclature and Standards Committee of the International Union of Basic and Clinical Pharmacology

RGS, regulator of G protein signaling

SDF-1, stromal cell-derived factor 1

Abstract

The CXCL12 system is central to the development of many organs and is further crucially engaged in pathophysiological processes underlying cancer, inflammation, and cardiovascular disorders. This disease-associated role presently focuses major interest on the two CXCL12 receptors, CXCR4 and ACKR3 / CXCR7, as promising therapeutic targets. Major obstacles in these

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ongoing efforts are confusing reports on the differential use of either ACKR3 / CXCR7 and/or CXCR4 across various cells as well as on the specific function(s) of ACKR3 / CXCR7. While basically no doubts remain that CXCR4 represents a classical chemokine receptor, functions assigned to ACKR3 / CXCR7 range from those of a strictly silent scavenger receptor eventually modulating CXCR4-signaling to an active and independent signaling receptor. In this review, we depict a thorough analysis of our present knowledge on different modes of organization and functions of the cellular CXCL12 system. We further highlight the potential role of ACKR3 / CXCR7 as a "crosslinker" of different receptor systems. Finally, we discuss mechanisms with the potency to impinge on the cellular organization of the CXCL12 system and hence might represent additional future therapeutic targets.

Significance Statement

Delineating the recognized functions of ACKR3 and CXCR4 in CXCL12-signaling is central to the more detailed understanding of the role of the CXCL12-system in health and disease and will help to guide future research efforts.

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Introduction

A prominent feature of most chemokines and chemokine receptors is their high promiscuity, in terms that a given chemokine generally binds several chemokine receptors and *vice versa* a given chemokine receptor binds several chemokines. This polygamous feature most likely reflects the evolutionary necessity to generate rapid and powerful host immune responses (Zlotnik and Yoshie 2012). A long-hold exemption from this rule was CXCL12 (SDF-1) which was assumed to solely interact with CXCR4. CXCR4 represents a classical GPCR which preferentially binds $G\alpha_i$, but also $G\alpha_q$ and $G\alpha_{12/13}$ (Heuninck et al., 2019, for review), subsequently allowing for the activation of various signaling pathways / cascades (Mousavi, 2020, for a recent review). It has been further claimed that CXCR4 would additionally activate arrestin-dependent signaling (see Rigo et al., 2018 for a recent work). Notably, arrestins primarily represent adaptor proteins essential for desensitization and endocytosis of GPCRs. In addition, arrestins can allow for receptor signaling from endosomes by acting as scaffold proteins (Thomsen et al., 2018, for review). As a consequence of this manifold, knockout of arrestins was found to result in signal amplification, deletion or partial alteration of downstream signaling pathways (Luttrell et al., 2018). These remarkably divergent functions of the arrestin family seem to correlate with distinct binding sites at the GPCR, referred to as the C-terminal

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"tail" or the transmembranous "core" conformation (Cahill et al., 2017). Whereas previous work convincingly demonstrated that ligand-activated CXCR4 recruits ß-arrestin preferentially to its C-terminus (Luo et al., 2017), none of the available studies presented unequivocal evidence for arrestin-dependent signaling of CXCR4 by ruling out the potential involvement of other (dimerizing) receptor proteins.

The aforementioned intimacy between CXCL12 and CXCR4 ceased with the demonstration of CXCR7 as a second CXCL12 receptor (Burns et al., 2006; Balabanian et al., 2005). CXCR7 binds CXCL12 with distinctly higher affinity when compared to CXCR4 (K_d = 0.4 nM versus 3.6 nM) and uses CXCL11, macrophage inhibitory factor (MIF), adrenomedullin (ADM), opioid peptides, and the viral chemokine vCCL2/vMIP-II as additional ligands (Meyrath et al., 2020; Perpina-Viciano et al., 2020; Wang et al., 2018b for review). In 2014, the NC-IUPHAR subcommittee for the chemokine receptors considered CXCR7 as an atypical chemokine receptor and renamed it to ACKR3 (atypical chemokine receptor 3; Bachelerie et al., 2014). This step was spurred by several findings and observations:

(1) ACKR3 exhibits an altered DRYLAIV-motif (DRYLSIT) in the second intracellular loop, which is assumed to be essential for binding and activation of G proteins. It is, however, noteworthy that this dogma was challenged by the findings that alterations of the DRY-motif within XCR1 and CXCR6 - both considered as classical chemokine receptors - do not preclude activation of G-

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proteins and induction of cell migration (Fox et al., 2019; Chandrasekar et al., 2004).

(2) In several cell lines, such as CHO, MLE-12, CNE 2Z, MCF-7 cells, as well as primary vascular smooth muscle cells (VSMCs), ACKR3 does not induce calcium flux, considered as an indication that ACKR3 fails to activate G proteins (Del Molino Del Barrio et al., 2018; Chang et al., 2018; Qiao et al., 2016; Rajagopal et al., 2010; Burns et al., 2006). A single study demonstrated binding of $G_{\alpha I}$ to ACKR3 in HEK-293T cells by BRET-analysis, which however again does not allow for Ca^{2+}-responses (Levoye et al., 2009). Presently, the only known exceptions from this rule are primary astrocytes and distinct glioma cell(s) (lines). These cells respond to CXCL12-dependent activation of ACKR3 with increases in intracellular Ca^{2+} which can be reversed by pertussis toxin (Ödemis et al., 2012). Evidence that observed Ca^{2+}-responses are in fact directly linked to ACKR3 are further given by the observation that Ca^{2+}-increases likewise occur in $CXCR4^{-/-}$ astrocytes as well as glioma cells not harboring CXCR4 (Ödemis et al., 2012). It is noteworthy that disparate findings have been previously obtained with human glioblastoma U87 cells, in which CXCR4 or CXCR7 was stably over-expressed (Doijen et al., 2017).

(3) Finally, several groups established that ACKR3 acts as a scavenger of extracellular CXCL12 (and CXCL11) by constantly cycling between the plasma membrane and intracellular structures and hereby establishing a CXCL12-gradient (Koenen et al., 2019, for review). According to a recent study, the

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equilibrium between intracellular and membrane-bound ACKR3, and thus scavenging function, is tightly regulated by CXCL12-induced phosphorylation of the receptor protein and its subsequent protection from degradation (Lau et al., 2020).

These atypical functions, however, do not necessarily mean that ACKR3 represents a strictly silent CXCL12 receptor. In fact, an exhaustive body of literature shows active signaling of ACKR3 (see Tables 1 and 3), which is thought to depend on arrestin. This assumption is primarily based on the demonstration that ligand-dependent activation of CXCR7 results in the recruitment of arrestin to the receptor protein, which is followed by endocytosis and the activation of signaling pathways (Rajagopal et al., 2010; Luker et al., 2009). Furthermore, CXCR7-signaling is attenuated or prevented following either cellular arrestin depletion or truncation of the CXCR7 C-terminus (Min et al., 2020; Xu et al., 2019). In addition to the direct activation of signaling proteins / pathways by ACKR3 within the arrestin scaffold, indirect modes of activation seem to exist and involve the Src-kinase- and/or arrestin-dependent transactivation of EGFR (Xu et al., 2019; Salazar et al., 2014; McGinn et al., 2012). Of note, the concept of arrestin-dependent signaling of ACKR3 was recently challenged by the demonstration that arrestins fail to drive ERK-phosphorylation in the presence of fully inactivated G-proteins (Grundmann et al., 2018). Moreover, within endosomes arrestins, GPCR, and G-proteins can

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form so-called super-complexes, which allow for sustained GPCR-signaling (Thomsen et al., 2016). However, whether arrestins show similar interactions with (typical and/or atypical) chemokine receptors still needs to be seen.

As discussed in depth in the following chapters, signaling of CXCR4 and CXCR7 gets further complicated by formation of dimers/oligomers with other GPCR or non-GPCR. Special attention attracted the dimerization of CXCR4 with CXCR7 and resulting consequences for cell signaling (see following chapters). The formation of CXCR4/CXCR7 heteromers was previously demonstrated by BRET (Levoye et al., 2009), FRET (Del Molino Del Barrio et al., 2018), protein fragment complementation assay (Luker et al., 2009), and proximity ligation assay (Albee et al., 2017; Evans et al., 2016). Of note, only proximity ligation assay allows to demonstrate heterodimerization of native receptors whereas the other experimental approaches require the previous ectopic expression of appropriate tagged receptor proteins, which might behave differently. At present effects of receptor heteromerization on cell signaling have only been characterized in cells with either receptor overexpression or inhibited receptor expression, and hence altered levels of total cellular receptor proteins which could well bias “normal” CXCL12-signaling. An experimental approach, not biased by receptor expression levels, would include the possibility to interfere with the heteromerization process of CXCL12-receptors. Unfortunately, respective tools such as synthetic peptides preventing receptor

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heterodimerization are currently missing or lack sufficient efficacy (Evans et al., 2016).

On July 24th, 2020 the search term “CXCL12” retrieved 292 original publications for 2020 in PubMed. In almost 80% of these publications there is no mentioning of “ACKR3” or “CXCR7”. This shortfall reflects the still prevailing ignorance of ACKR3 as an active signaling receptor and spotlights the urgent need for the better awareness of the central role of ACKR3 in CXCL12-signaling. Recent review articles, which appeared in this journal, already give a comprehensive overview of the mechanistic base of CXCR4- and CXCR7-signaling and its principal modulation by receptor heteromerization/oligomerization, alternative ligands, and subcellular localization of receptors (Heunick et al., 2019; Fumagalli et al., 2019; Koenen et al., 2019). The present review intends to complete the picture by defining of how CXCR4 and ACKR3 are actually involved in CXCL12-signaling across cells. We start with an overview of the documented atypical functions of ACKR3. We then continue with a first time compilation of recognized molecular modes of cellular CXCL12 signaling and further discuss potential mechanisms involved in sculpting the CXCL12 system across cells and how ACKR3 might additionally link different receptor systems.

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Atypical functions of ACKR3 - ACKR3 as a CXCL12 scavenger

The (atypical) function of ACKR3 as a CXCL12 scavenger is central to the development of many organs and is crucial to the control and coordination of cell migration and positioning (Quinn et al. 2018, for review). Importantly, these atypical functions not solely depend on ACKR3, but require an intimate interplay between CXCL12, CXCR4, and ACKR3. As experimentally established in the zebrafish, ACKR3 enables CXCR4-dependent cell migration by continuous sequestration of CXCL12 and the subsequent formation of a CXCL12 gradient (see for example Dona et al., 2013). Interestingly, ACKR3 adjusts the CXCL12 gradient close to the dissociation constant of CXCR4 and, thus, ensures the optimal directional signal (Lau et al., 2020). Reflecting the crucial role of CXCL12 scavenging in many developmental processes, both ACKR3 mRNA and protein levels are high in embryonic organs / anlagen and decline into adulthood (Koenen et al. 2019, for review; Puchert et al., 2017). Nevertheless, low levels of ACKR3 in mature endothelial cells seem to be sufficient to form a sink, allowing to modulate CXCL12 plasma levels and, hence, tissue homeostasis (Berahovich et al., 2014). Along with ACKR3-dependent clearance of extracellular CXCL12, ACKR3 affects expression levels of CXCR4 by preventing its ligand-induced internalization and subsequent degradation, a mechanism essential to the formation of glomerular tufts in developing kidneys as well as during migration of interneurons (Haege et al.,

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2012; Sánchez-Alcañize et al., 2011). Importantly, the scavenging function of ACKR3 is not limited to physiological processes, but seems to be also active under pathological conditions. Indeed, studies using a mouse xenograft model of human breast cancer identified an intratumoral subpopulation of $ACKR3^+$-cells, which by sequestering CXCL12 favors proliferation of a subpopulation of $CXCR4^+$ cancer cells (Luker et al., 2012).

Is suppression of CXCR4-signaling another atypical function of ACKR3?

Soon after the identification of ACKR3 as a second CXCL12 receptor, it was claimed that ACKR3 might suppress or dampen CXCR4-signaling by forming ACKR3/CXCR4 heterodimers (Levoye et al., 2009; Fig. 1A). This claim was based on the demonstration that transient or stable overexpression of the receptor protein in HEK-293T cells attenuates CXCR4-induced rises in intracellular Ca^{2+}. More recently, a similar impairment of CXCR4-signaling was seen following ectopic overexpression of ACKR3 in U87 human glioma cells (Doijen et al., 2017). Despite these findings it remains still questionable whether such suppressive effects are a general feature of ACKR3 and would also apply for endogenous ACKR3. A pathophysiological condition typically associated with increased expression of (endogenous) CXCL12 and its receptors is hypoxia. Most intriguingly, hypoxia-induced increases of ACKR3 in primary hippocampal progenitor cells do not attenuate, but rather promote CXCL12-

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dependent chemotaxis, an effect reversed by ACKR3 shRNA interference (Liu et al., 2013b). A similar correlation between hypoxia-induced expression of ACKR3 and increased chemotactic responses was shown for several other types of cells, including renal cell carcinoma cell lines and colon cancer cells (Zhang et al., 2016; Romain et al., 2014). While not all of the potentiating effects of hypoxia seen with the various cell(s) / lines might be attributed to enhanced expression of ACKR3, but could also be due to the increased expression of CXCR4, an issue not tested in most studies, it is obvious that increases in endogenous ACKR3 do not necessarily dampen CXCR4 signaling. Nevertheless few examples exist in which depletion of endogenous ACKR3 seems to enhance CXCR4 function. In nasopharyngeal carcinoma cells in which ACKR3 expression is inhibited by RNA interference, CXCL12 induces a more rapid and slightly higher Ca^{2+}-response when compared to wild-type cells (Qiao et al., 2016). Similarly, knockdown of ACKR3 in the human prostate cancer cell line, LNCaP, enhances chemotactic responses to CXCL12 (Yu et al., 2019).

Active functions of ACKR3 - cell-specific use of ACKR3 and CXCR4 to control distinct cell functions

It is now indisputable that ACKR3 is capable to actively control the very same cell functions as CXCR4, including cell migration (chemotaxis) / transendothelial migration, and cell proliferation (see Table 1). Notwithstanding

the still ongoing dispute on the exact mode of activation (see above), it is further well documented - and not really surprising - that ACKR3 and CXCR4 induce a widely overlapping array of identical signaling molecules / pathways, including the ERK-, p38-, and PI3K/AKT signaling pathways (Mousavi et al., 2020; Heuninck et al., 2019; Wang et al., 2018b, for recent reviews). Interestingly, the available data do not point to the existence of a general link between a given CXCL12 receptor and a distinct cellular response (at least in differentiated cells), in terms that one CXCL12 receptor controls cell migration whereas the other one controls cell proliferation across various cell types. The findings rather argue for the cell-specific use of either CXCR4 or ACKR3 for controlling a given cellular function (Fig. 1B). Examples shown among others in Table 1 are the migration of melanocytes and decidual epithelial cells. Although (co)expressing both CXCL12 receptors, CXCL12-induced migration / chemotaxis of these cells either depends on ACKR3 or CXCR4, respectively (Zheng et al., 2018; Lee et al., 2013). Importantly, the differential use of either CXCR4 or ACKR3 to control a common cellular function likewise applies for tumor cells (see Table 1). This issue might be decisive for the therapeutical outcome of CXCR4 or ACKR3 antagonists applied to cancer patients.

We wish to note that we have deliberately not included studies to Table 1 in which the cellular function of CXCL12 was characterized by experimentally silencing either ACKR3 (see for example Wu et al., 2018) or CXCR4 (see for example Wang et al., 2018a). Although eligible, it is most evident that this

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experimental approach will not give an inherent view on the molecular function of the CXCL12 system in a given type of cells. This concern does not apply for studies in which the endogenous expression of either one of the CXCL12 receptors is not detectable (Luker et al., 2012).

It has been repeatedly argued that cell membrane expression (levels) of CXCR4 and ACKR3 would eventually determine which receptor mediates CXCL12 signaling. Especially ACKR3 was ruled out as the active CXCL12 receptor by many of these studies since in various cells the receptor protein prevails in intracellular structures, which was considered as a hint for its preferential interaction with CXCR4 (see for example, Hsiao et al., 2015; Tripathi et al., 2014; Hartmann et al., 2008). In this respect it is noteworthy that only a small portion of ACKR3 seems to cycle between the cell membrane and intracellular structures which might escape detection by immunofluorescence or cell sorting. In line with this view, Yan et al., (2012) demonstrated the crucial involvement of ACKR3 in the control of survival, adherence, and tube formation of human endothelial progenitor cells, although the receptor protein was almost exclusively detectable in intracellular structures by flow cytometry. Consequently, subcellular localization of CXCL12 receptors should be regarded as a rather poor indicator for their cellular use. An issue not yet experimentally tested is whether the differential use of CXCR4 and ACKR3 for controlling a common cell function in different types of cells is dictated by dimerization / oligomerization processes of either the ligand or the receptor itself. Indeed,

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secreted or soluble CXCL12 forms an equilibrium of monomers and dimers (Ray et al., 2012), which is modulated by several factors, such as pH and sulfate (Veldkamp et al., 2005). Monomeric CXCL12 seems to preferentially bind ACKR3 (Ray et al., 2012). Monomeric and dimeric CXCL12 on the other hand exhibit distinct differences in their interaction with CXCR4, allowing for different signaling responses (Ray et al., 2012; Drury et al., 2011). This could explain why monomeric CXCL12 more potently stimulates chemotaxis than its dimeric form (Chang et al., 2017; Drury et al., 2011). Like its ligand, CXCR4 exists as monomers or homodimers which both reside at the outer cell membrane as well as within intracellular structures (Wang et al., 2006). CXCR4 dimerization / oligomerization either depends on CXCL12 or occurs independently of the chemokine (see for example, Lao et al., 2017; Ge et al., 2017) and is further affected by CXCR4 expression levels (Lao et al., 2017). Interestingly, preventing CXCR4 dimerization abolishes CXCL12-induced chemotaxis of various cancer cell lines (Wang et al., 2006), and, thus, might represent a mechanism by which CXCR4 is either encompassed in or excluded from CXCL12-signaling. Further complexity of this process arises from recent demonstration that optimal CXCL12-responses require actin-dependent nanoclustering of CXCR4 (Martínez-Muñoz et al., 2018a). Although ACKR3 likewise exists as monomers and dimers (Levoye et al., 2009), currently no data are available to whether the different status of the receptor protein is associated with functional differences. As documented for differentiating monocytes,

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another twist could stem from the fact that receptor signaling and, thus, affected cell function might depend on receptor protein levels. Actually, monocyte differentiation is accompanied by an up-regulation of ACKR3 and the subsequent switch from ERK and AKT signaling to activation of JNK and p38 pathways (Ma et al., 2013).

It has to be further kept in mind that in addition to homodimerization, heterodimerization of CXCR4 or ACKR3 with other non-CXCL12 chemokine receptors (Martínez-Muñoz et al., 2018b), non-chemokine receptors, such as opioid receptors or adrenergic receptors (Albee et al., 2017; Nash and Meucci, 2014) or even non-receptor proteins (Fumagalli et al., 2019) might contribute to the sculpting of CXCL12-signaling. (The role of CXCR4 / ACKR3 heterodimers is discussed in the next chapter). In this respect, previous work suggested that heterodimerization of CXCR4 with CXCR3 prolongs CXCR4-signaling (Jin et al., 2018). Other currently known modulators of CXCL12 signaling, with the potency to impact the molecular organization of the CXCL12 system include regulators of G protein signaling (RGSs) as well as G protein-coupled receptor kinases (Grks) (see for example, Saaber et al., 2019; Karim et al., 2016; Lipfert et al., 2013a).

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Evidence that ACKR3 and CXCR4 form a functional receptor in distinct cell types

An interesting observation made by several studies using either primary cells or tumor cells is that blockade of either CXCR4 or ACKR3 results in the complete loss of CXCL12-dependent cellular responses (Table 2). These synergistic effects have been previously considered as an indication that CXCR4 and ACKR3 form a functional receptor complex in distinct cells (Lipfert et al., 2013b; Fig. 1C). In addition to homodimers, ACKR3 and CXCR4 equally form heterodimers (Levoye et al., 2009). Moreover, CXCR4/ACKR3 heterodimers seem to allow for enhanced recruitment of ß-arrestin when compared to receptor monomers/homodimers resulting in the potentiation of ß-arrestin-dependent cell signaling (Décaillot et al., 2011) and/or altered ERK-/AKT-phosphorylation kinetics (Del Molino del Barrio et al., 2018). However, despite these facts it has not yet been tested whether the proposed functional CXCR4-ACKR3 receptor complex present in some cells actually involves receptor heterodimers. In this respect it is noteworthy that combined fluorescence labeling of CXCR4 and ACKR3 in various tumor cell lines which require both CXCR4 and ACKR3 to respond to CXCL12 produced rather inconclusive results. Specifically, these experiments showed overlapping CXCR4 and ACKR3 staining in distinct cellular domains while other domains only showed staining for either CXCR4 or ACKR3 (Puchert et al., 2018). Finally, it has to be kept in mind that the

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requirement of CXCR4 and ACKR3 for CXCL12-dependent cellular responses does not necessarily reflect the involvement of CXCR4/ACKR3 heterodimers but could likewise be the result of converging downstream signaling events or the simultaneous activation of parallel signaling pathways.

Apart from the complete loss of CXCL12-induced cellular responses following blockade of either CXCR4 or ACKR3, few studies reported only partial losses of CXCL12-stimulated cell functions, hence, implying that in some instances the cellular effects of CXCR4 and ACKR3 are additive (Fig. 1D). Additive effects have been reported for the transendothelial migration of mature $CD14^+$-$CD16^+$-monocytes (Veenstra et al., 2017), migration of T lymphoblast (acute lymphoblastic leukemia) cell lines MOLT-4 and Jurcat (Melo et al., 2014), and tube formation of HUVEC cells (Zhang et al., 2017). The partial loss of cellular responses seen by these studies is not attributed to the incomplete blockade of receptor proteins since CXCR4 and ACKR3 antagonists (AMD3100, CCX771) were applied at effective concentrations (2.5 µM to 10 µM). It is, however, hard to judge whether in studies using RNA-interference (Melo et al., 2014) additive effects would arise from residual ACKR3 expression. Moreover, none of the studies excluded that additive effects are brought about by the presence of subpopulations of $CXCR4^+$, $ACKR3^+$ and $CXCR4^+$/$ACKR3^+$ cells. Whether existing additive effects result from the formation of CXCR4/ACKR3 heterodimers and subsequent potentiation of ß-

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arrestin-dependent cell signaling (Décaillot et al., 2011) has not been experimentally tested.

Within the same cell ACKR3 and CXCR4 control different cell functions

Our current knowledge on the cellular effects of CXCR4 and ACKR3 culminate in the demonstration that within some cells, CXCR4, ACKR3 and/or CXCR4/ACKR3 heteromers are in the control of different cell functions (Table 3; Fig. 1E). Notably, the relation of distinct CXCL12-receptors to distinct cell functions has so far been mostly reported for various types of stem and progenitor cells. Specifically, the available studies (Table 3) demonstrate that stem/progenitor cell migration regularly depends on CXCR4 (Table 3) with only few exemptions (Chen et al., 2015b). Provided parameters have been tested, the studies further see that survival of stem/progenitor cells depends on ACKR3 whereas transendothelial migration rather requires both CXCR4 and ACKR3. This organizational concept is also partly documented for $CD34^+$ hematopoetic stem cells, in which again CXCR4 controls cell migration, whereas CXCR4 in combination with ACKR3 affects cell adhesion (Hartmann et al., 2008). Whether this relation between receptors and cell functions applies to most stem/progenitor cells needs to be further evaluated. This future analysis should also include cancer stem cell, a clinically relevant subset of tumor cells which remains dormant, escapes chemotherapy and, hence, gives rise to tumor relapse

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(Atashzar et al., 2020; for a recent review). Likewise, it is presently unknown whether the relation persists in differentiated cells or whether the CXCL12 system is rearranged during differentiation processes. The occurrence of a rearrangement process is currently favored by the observed heterogeneity of the CXCL12 system across different cells.

Importantly, in addition to stem and progenitor cells, distinct links between CXCL12 receptors and affected cell functions seem to be also realized at least in some cancer cells. As previously shown (Puchert et al., 2018), CXCL12 controls proliferation of MDA-MB-231 breast cancer cells as well as of PC-3 prostate cancer cells via CXCR4 whereas CXCL12-dependent chemotaxis of both cell types requires ACKR3 and CXCR4 (Tables 1 and 2). Especially with respect to ongoing efforts to target CXCL12 receptors in cancer patients with receptor antibodies (Bobkov et al., 2019, for review) or receptor modulators (Adlere et al., 2019, for review), detailed knowledge of the organization of the CXCL12 system in distinct tumor cells emerges as a crucial prerequisite for successful therapy.

ACKR3 as a crosslinker of different receptor systems

As previously mentioned, additional recognized ligands for ACKR3 are CXCL11, MIF, ADM, BAM22, vCCL2/vMIP-II, and opioid peptides. So far active ACKR3 signaling has been shown to occur following binding of CXCL11 and MIF to ACKR3, but not of ADM, BAM22, vCCL2, and opioids implying

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that ACKR3 might function as a savenger for the latter ligands (Meyrath et al., 2020; Wang et al., 2018b, for review). Interestingly, CXCL11 binds CXCR3 as its prime receptor, which in turn forms heterodimers with CXCR4 and, thus, interweaves ACKR3 signaling with CXCR4 and CXCR3 signaling (Singh et al., 2013, for review). This meshwork gets further broadened by MIF, which in addition to ACKR3 binds CD74, CXCR2, and CXCR4 (Peperina-Viciano et al., 2020; Wang et al., 2018b). Moreover, CD74 heterodimerizes with ACKR3 (Alampour-Rajabi et al., 2015). Considering the intensive overlap of the various receptors surprisingly little is known if and how the respective receptors modulate each other. As both a proof of principle and a hint to the complexity of these interactions, previous work demonstrated that CXCL11 attenuates CXCL12-dependent chemotaxis of CT26.WT tumor cells (Rupertus et al., 2014) whereas pretreating HeLa cells with CXCL11 was found to facilitate CXCL12-induced chemotaxis (Miekus et al., 2010). Further work on this issue and subsequent insights into the therapeutic implications of these interactions is urgently needed, especially, as revealed by ongoing work in our lab, numerous cancer cells co-express for example ACKR3, CXCR4, and CXCR3 (Puchert et al., 2018, 2020).

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Concluding remarks

Findings on the function of ACKR3 obtained over the last decade reveal an unexpected heterogeneity of the molecular organization of the CXCL12 system across cells. In addition to its originally proposed silent function it now most evident that ACKR3 additionally acts as an active signaling receptor which depending on the cell (type) either controls CXCL12-dependent cell functions with similar efficacy as CXCR4 or interacts with CXCR4 in a fairly complex and diverse manner (Fig. 1). Although potential mechanisms capable of sculpting the cellular CXCL12 system such as ligand and receptor dimerization /oligomerization, receptor heteromerization, RGSs, and Grks emerged over recent years, the direct proof if and how these mechanisms dictate the molecular organization of the CXCL12 system in distinct cells still awaits experimental clarification. With the emerging role of ACKR3 and CXCR4 as therapeutic targets central to cancer, cardiovascular diseases, and inflammatory processes (Morein et al., 2020; Mousavi 2020; Wang et al., 2018b) knowledge of these sculpting mechanisms is becoming more and more eminent as they will allow for optimized therapeutic strategies. Finally, we hope that our present review will spark new interest into the function of ACKR3 which, as we feel, partially evaporated with its classification as an atypical chemokine receptor.

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Footnotes

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Figure Legends

Figure 1

Perceived possibilities of how ACKR3 and CXCR4 are involved in CXCL12-signaling

A detailed description of the various types of interaction is given in the text.

(A) ACKR3 dampens CXCR4-signaling by forming ACKR3/CXCR4 heterodimers. This interaction was originally described in cells ectopically overexpressing CXCR7, but has been rarely seen with endogenous CXCR7.

(B) As shown for numerous different cell types, a distinct cell function (e.g. migration, proliferation etc.) is either controlled by ACKR3 or CXCR4.

(C) Many examples exists in which CXCR4 and ACKR3 act synergistically, in terms that cellular effects of CXCL12 only occur when both receptors are present. Whether such synergistic effects depend on receptor heterodimerization is still unknown.

(D) In addition to synergistic effects, few studies demonstrated additive effects of ACKR3 and CXCR4 on a given cell function. Whether additive effects require receptor heterodimerization has again not yet been tested experimentally.

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(E) Within a given cell type, CXCR4, ACKR3, and CXCR4/ ACKR3 heterodimers control distinctly different cell functions. This seems to be the typical organization of the CXCL12-system in many types of stem and progenitor cells.

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Table 1

Studies having identified the CXCL12 receptor mediating distinct CXCL12 responses.

Only studies in which the involvement of the respective other CXCL12 receptor has been experimentally excluded are listed**.**

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Cell Type	**Cell Function**	**CXCL12-Receptor**	**Reference**
mouse M1 macrophages	migration	ACKR3	Zhang et al., 2020
human pulmonary adeno-carcinoma cell line (A549)	migration	ACKR3	Puchert et al., 2018; Choi et al., 2014
human colorectal adeno-carcinoma cell line (DLD-1)	migration	ACKR3	Puchert et al., 2018
human breast adenocarcinoma cell line (MDA-MB-231)	proliferation	CXCR4	Puchert et al., 2018
human prostate adenocarcinoma cell line (PC-3)	proliferation	CXCR4	Puchert et al., 2018
human decidual epithelial cells	migration	CXCR4	Zheng et al., 2018
mouse neural progenitor cells	proliferation	ACKR3	Wang et al., 2016
human acute myeloid leukemia cell lines (MO7e, U937)	migration	CXCR4	Kim et al., 2015
human non-small cell lung cancer cells	migration, transendothelial migration	CXCR4	Choi et al., 2014
bovine aortic endothelial cells	transendothelial electrical resistance (endothelial barrier enhancement)	CXCR4	Kobayashi et al., 2014
rhesus macaque chorioretinal cells (RF/6A)	migration, proliferation	CXCR4	Jin et al., 2013
human glioblastoma-derived endothelial cells	tube formation	CXCR4	Liu et al., 2013a
human macrophages	phagocytic activity	ACKR3	Ma et al., 2013
human melanocytes	migration	ACKR3	Lee et al., 2013
rat primary cortical astrocytes	proliferation, migration	ACKR3	Ödemis et al., 2012; 2010
human Burkitt's lymphoma cell line (NC-37)	transendothelial migration	ACKR3	Zabel et al., 2011

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Table 2

Cells proven to require both ACKR3 and CXCR4 to respond to CXCL12.

Cell type	**Cell function**	**Reference**
human cervical cancer cell line (C33A)	migration	Puchert et al., 2018
human breast adenocarcinoma cell line (MDA-MB-231)	migration	Puchert et al., 2018; Yang et al., 2019
human prostate adenocarcinoma cell line (PC-3)	migration	Puchert et al., 2018
human acute myeloid leukemia cell line (U937)	migration, homing	Melo et al., 2018
human endometrial cancer cell line (Ishikawa cells)	proliferation invasion	Huang et al., 2017 Long et al., 2016
human oral squamous cell carcinoma cell lines (SCC15, CAL27)	migration	Chen et al., 2016
human nasopharyngeal carcinoma cells	migration	Qiao et al., 2016
rat cardiac stem cells	migration	Chen et al., 2015a
mouse neural progenitor cells	migration	Chen et al., 2015b
human peripheral blood $CD34^+$ cells	proliferation	Torossian et al., 2014
rat primary microglia	migration, proliferation	Lipfert et al. 2013b
human glioblastoma cells (patient-derived)	migration , proliferation	Liu et al., 2013a

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Table 3

Cells in which CXCR4, ACKR3, and CXCR4/ACKR3 heterodimers control distinctly different cell functions.

Cell type	CXCR4	ACKR3	CXCR4/ ACKR3	Reference
human monocytes	migration	adhesion, survival	phagocytic activity, cell differentiation	Chatterjee et al., 2015
human adipose tissue-derived mesenchymal stem cells	migration	proliferation		Li et al., 2013
human endothelial progenitor cells	migration, proliferation, NO-production	survival	transendothelial migration, adhesion, tube formation	Yan et al., 2012
mouse embryonic fibroblasts	migration	proliferation		McGinn et al., 2012
rat endothelial progenitor cells	migration	survival	transendothelial migration, adhesion (on collagen and fibronectin), tube formation, proliferation	Dai et al., 2011
human renal progenitor cells	migration	adhesion, survival	recruitment, transendothelial migration	Mazzinghi et al., 2008
human hematopoetic stem cells	migration		adhesion	Hartmann et al., 2008

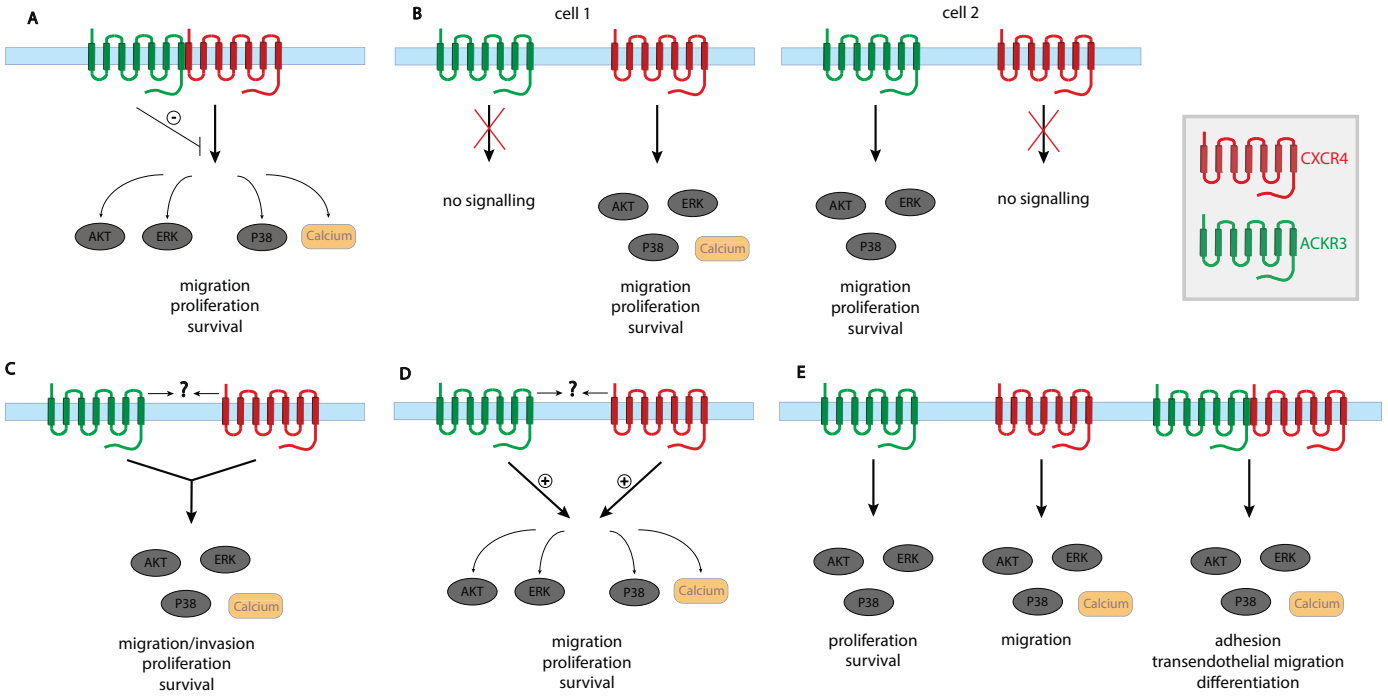

Figure 1