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Emerging roles of atypical chemokine receptor 3 (ACKR3) in normal development and physiology

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ABSTRACT

The discovery that atypical chemokine receptors (ACKRs) can initiate alternative signaling pathways rather than classical G-protein coupled receptor (GPCR) signaling has changed the paradigm of chemokine receptors and their roles in modulating chemotactic responses. The ACKR family has grown over the years, with discovery of new functions and roles in a variety of pathophysiological conditions. However, the extent to which these receptors regulate normal physiology is still continuously expanding. In particular, atypical chemokine receptor 3 (ACKR3) has proven to be an important receptor in mediating normal biological functions, including cardiac development and migration of cortical neurons. In this review, we illustrate the versatile and intriguing role of ACKR3 in physiology.

1. Atypical chemokine receptor family

Originally characterized as decoy or silent chemokine receptors, atypical chemokine receptors (ACKR) are major regulators of chemokine internalization, degradation, and transcytosis [1–3]. The term "atypical" stems from the observation that ACKRs either lack, or have alterations in the canonical DRYLAIV motif; this motif is found in the second intracellular loop, and is typically required for most G-protein activation and signaling [4–9]. Instead, these silent receptors elicit their biological effects through modulation of extracellular ligands, and although they do not directly mediate chemotaxis, they participate in chemotactic events through chemokine scavenging and degradation. The family consists of five major receptors: ACKR1/DARC, ACKR2/D6, ACKR3/CXCR7, ACKR4/CCX-CKR, and ACKR5/CCRL2, and includes one provisional addition, ACKR6/PITPNM3. Like most chemokine receptors, the ACKRs can also bind to a variety of different ligands to elicit their biological effects. Initially described as regulating innate and adaptive immune responses and leukocyte recruitment, the expansion of ACKR research in recent years has led to alternative roles for ACKRs in physiology, including contributions to cardiovascular and lymphatic vessel growth, embryonic development, and central nervous system function [9–13]. Several recent reviews highlight ACKRs concerning disease mechanisms, including their roles in cell migration and proliferation [14–18]. Here, we focus our efforts on emphasizing the diverse physiological ligands and roles of ACKR3 beyond participating in chemotaxis (Fig. 1).

2. ACKR3 and ligands

2.1. CXCL12 signaling mediated by ACKR3

Initially considered an orphan receptor, the discovery that ACKR3 (originally named RDC1 and CXCR7) could bind to C-X-C motif chemokine ligand 12 (CXCL12) challenged the previous notion that CXCL12 exerted all of its biological functions solely from binding to CXCR4 (Fig. 1) [19]. Interestingly, although ACKR3 possesses the common G-protein coupling DRY motif, it binds CXCL12 using a unique N-terminal binding site located in extracellular loops two and three [20–22]. Although CXCL12 can form homodimers under physiological conditions, ACKR3 preferentially interacts with CXCL12 monomers with a 10-fold higher affinity compared to CXCR4 [23,24]. Because this interaction activates β-arrestin recruitment rather than classic $G_{\alpha i}$-protein signaling, one can categorize ACKR3 as a β-arrestin-biased receptor that promotes CXCL12 internalization and early endosome degradation [6]. The receptor will also undergo constitutive rapid recycling back to the cell surface, which is necessary for continued membrane localization and activation [25]. Furthermore, recent *in vivo* studies have shown that ACKR3 inhibition causes an increase in CXCL12 plasma levels, implicating ACKR3 as an important regulator of CXCL12 concentration [19,26–28]. In zebrafish embryos, CXCL12 sequestration by ACKR3 is critical for the primordium to deposit cell clusters across the trunk and tail, facilitating sensory cues for water flow [29]. Finally, CXCL12 gradient regulation by ACKR3 promotes neural progenitor cell survival [30,31]. Extensive research focusing on

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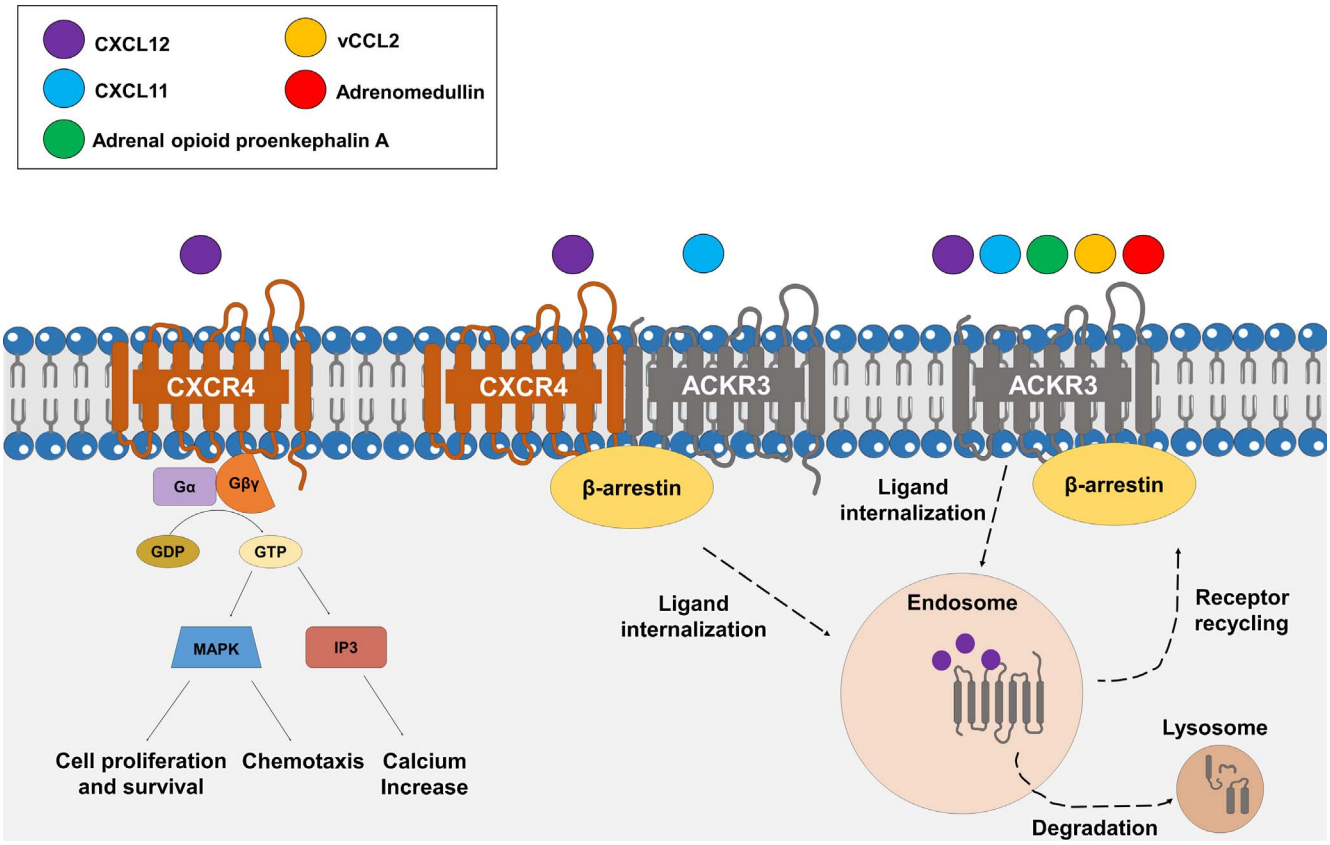

Fig. 1. Distinct signaling pathways for atypical chemokine receptor 3 (ACKR3). Typically, C-X-C chemokine ligand 12 (CXCL12, purple circle) binds to CXCR4 and activates classical GPCR signaling events such as cell proliferation, chemotaxis and calcium influx. ACKR3 can heterodimerize with CXCR4, causing conformational rearrangements in G-protein complexes and partiality to β-arrestin rather than classical GPCR signaling in response to CXCL12 binding. This heterodimer effect is reduced by CXCL11 (blue circle) binding to ACKR3/CXCR4. ACKR3 can also sequester CXCL12, CXCL11, adrenomedullin (red circle), adrenal opioid proenkephalin A (green circle), and vCCL2 (yellow circle) ligands, leading to possible ligand internalization and degradation through β-arrestin recruitment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chemotactic properties of ACKR3:CXCL12 interaction has demonstrated successful therapeutic avenues for the treatment of several cancers [32]. However, because previous research primarily focused on the CXCR4:CXCL12 signaling axis, the full breadth of ACKR3:CXCL12 regulation of CXCL12 beyond contributing to chemotaxis is still warranted.

ACKR3 regulation of CXCL12 availability is complicated further by its ability to heterodimerize with CXCR4. ACKR3 and CXCR4 co-immunoprecipitate in HEK293 cells and co-localize in Neuro2A cells and in several tissues [33]. Expression of ACKR3 induces conformational rearrangements within $G_{\alpha i}$ protein complexes of CXCR4, thus impairing $G_{\alpha i}$ protein activation. This modulation of downstream signaling is partially attributed to ACKR3 β-arrestin signaling. When both CXCR4 and ACKR3 are co-transfected in HEK293 cells there is a concomitant increase in CXCL12-induced β-arrestin co-immunoprecipitation with ACKR3 [33]. Unlike CXCR4 signaling alone, this heterodimeric effect increases ligand-stimulated and membrane recruitment of β-arrestin and causes sustained activation of ERK1/2 and p38 MAPK signaling pathways [33,34]. CXCR4:ACKR3 heteromeric complexes have proven to be critical in valve formation in the heart, and integrin activation in T-cells [35–37]. The significance of CXCR4:ACKR3 heterodimers and alteration of CXCL12 signaling downstream of $G_{\alpha i}$ may be physiologically relevant and remains to be further explored.

2.2. ACKR3 is a low affinity receptor for CXCL11

Interestingly, CXCL11 (also known as ITAC) can reduce the heterodimer effects of CXCR4:ACKR3 by modulating β-arrestin recruitment [33]. Treatment with CXCL11 in CXCR4/ACKR3 co-expressed glioblastoma cells increased cAMP production, leading to the assumption that CXCL11 can rescue CXCL12 signaling inhibition induced by the CXCR4:ACKR3 heterodimeric complex. However, more research is necessary to determine how exactly CXCL11 acts as an allosteric modulator of CXCR4:ACKR3 dimer complexes [33].

Although extensive research has determined the biological implications of ACKR3 on CXCL12 signaling, few studies have focused on ACKR3:CXCL11 pathway beyond mediating chemokine scavenging and degradation. Originally presumed to bind only to CXCR3, CXCL11 also binds to ACKR3 with low affinity. For this reason in radioligand binding assays, the affinity of the ^{125}I-CXCL11 tracer is so low that competition assays are performed in a heterologous system with ^{125}I-CXCL12 as the tracer. In this system, CXCL11 inhibits ^{125}I-CXCL12 binding to ACKR3 with an IC50 of 9 nM, whereas CXCL12 inhibits ^{125}I-CXCL12 binding with an IC50 of 1.3 nM [38–40]. More recent reports have indicated a 10-fold difference in binding affinity for CXCL11 vs CXCL12, 4 nM and 0.4 nM, respectively [41]. As with CXCR3, CXCL11 binding to ACKR3 depends on acidic residues of the N-terminus [40]. Not only are high ACKR3 expression levels required for CXCL11 scavenging and degradation, but CXCL11 internalizes ACKR3 faster than CXCL12 and delays recycling [42]. This may be attributed to differences in affinity and dependence on β-arrestin 2 recruitment or specific intracellular transport properties for CXCL11 [26,40,42]. Most studies on CXCL11 have primarily focused on inflammatory pathways, due to its characteristics as an inflammatory chemokine, with an increase in activation following interferon stimulation [38]. Research efforts focusing on how CXCL11 modulates ACKR3 will be critical for determining physiologically relevant disease mechanisms.