ABSTRACT

The chemokine system is a fundamental component of cancer-related inflammation involved in all stages of cancer development. It controls not only leukocyte infiltration in primary tumors but also angiogenesis, cancer cell proliferation, and migration to metastatic sites.

Atypical chemokine receptors are a new, emerging class of regulators of the chemokine system. They control chemokine bioavailability by scavenging, transporting, or storing chemokines. They can also regulate the activity of canonical chemokine receptors with which they share the ligands by forming heterodimers or by modulating their expression levels or signaling activity. Here, we summarize recent results about the role of these receptors (atypical chemokine receptor 1/Duffy antigen receptor for chemokine, atypical chemokine receptor 2/D6, atypical chemokine receptor 3/CC-chemokine receptor 7, and atypical chemokine receptor 4/CC-chemokine receptor-like 1) on the tumorigenesis process, indicating that their effects are strictly dependent on the cell type on which they are expressed and on their coexpression with other chemokine receptors. Indeed, atypical chemokine receptors inhibit tumor growth and progression through their activity as negative regulators of chemokine bioavailability, whereas, on the contrary, they can promote tumorigenesis when they regulate the signaling of other chemokine receptors, such as CC-chemokine receptor 4. Thus, atypical chemokine receptors are key components of the regulatory network of inflammation and immunity in cancer and may have a major effect on anti-inflammatory and immunotherapeutic strategies. J. Leukoc. Biol. 99: 927–933; 2016.

Introduction

Chemokines are a superfamily of cytokines regulating the leukocyte traffic. They act by binding a cognate family of 7 transmembrane-spanning G protein-coupled receptors, triggering intracellular signaling events that induce directional cell migration [1]. Besides these canonical chemokine receptors, a distinct group of receptors have been identified and recently named ACKRs [2–4]. They share structural features with canonical chemokine receptors and bind with high-affinity chemokines, but they are unable to induce cell migration. On the contrary, ACKRs modulate chemokines concentration and bioavailability by their internalization and transport to intracellular degradative or storage compartments or, in the case of polarized cells, transporting them to the opposite site of the cell monolayer. They also control the chemokine system through regulation of the signaling of other chemokine receptors [5]. Four molecules have been recently included in this group as follows: ACKR1, previously called DARC; ACKR2, also known as D6 or CCBP2; ACKR3, also called CXCR7 or RDC1; and ACKR4, previously called CCR1 and also known as CCX-CKR. Two other molecules tentatively included in the ACKR family will not be covered by this review because they are awaiting functional confirmation [4].

ROLES OF CHEMOKINES AND CHEMOKINE RECEPTORS IN CANCER DEVELOPMENT

Chemokines are involved in almost all pathologies, and they have also a central role in cancer [6]. Inflammatory response is a key component of the tumor microenvironment and has been recognized as a constitutive hallmark of cancer [7, 8]. Two pathways link inflammation to cancer: the extrinsic pathway, whereby preexisting chronic inflammatory conditions increase the risk to develop cancer, and the intrinsic pathway, characterized by genetic events that cause cancer and at the same time orchestrate the constitution of an inflammatory microenvironment [7]. Chemokines are important in both pathways linking inflammation to cancer. Indeed, chemokines are key mediators of chronic inflammatory processes, and their expression is often regulated by oncogenic pathways and transcription factors deregulated in the pathogenesis of cancer [6].

Chemokines control several aspects of tumor biology, including immune infiltrate at the primary tumor site, the...
angiogenesis process, cancer cell proliferation, and migration to metastatic sites. Chemokines can be functionally classified as inflammatory or homeostatic, depending on the induced or constitutive mechanism of production [2]. Inflammatory CC (e.g., CCL2, CCL3, CCL5 acting on CCR1, CCR2, and CCR5) and ELR+ CXC (e.g., CXCL8 acting on CXCR2) chemokines recruit at the tumor site both immature cells of myeloid origin, called myeloid-derived suppressor cells, and more mature cells, such as monocytes and neutrophils, which then differentiate into tumor-associated macrophages or tumor-associated neutrophils. All these myeloid cells are subverted in their function and help tumor growth, inducing neoangiogenesis and inhibiting the development of anti-tumor T cell responses [9]. Furthermore, CCL17 and CCL22 acting on CCR4 can directly recruit T<sub>reg</sub> and Th2 lymphocytes that inhibit antitumor responses contributing to tumor survival [10]. On the other hand, chemokines acting on CXCR3, CXCR6, and CX3CR1 attract NK cells and T lymphocytes, which can elicit antitumoral responses [6].

Chemokines are also important regulators of the angiogenesis process that sustains cancer cell proliferation and metastatic spread. The CXC chemokine family includes both angiogenic and angiostatic chemokines. ELR+ CXC chemokines and CXCL12, acting on CXCR2 and CXCR4, respectively, are potent promoters of angiogenesis, stimulating proliferation and inhibiting apoptosis of endothelial cells [11]. On the contrary, the ELR- CXC chemokines CXCL9, CXCL10, and CXCL11 exert direct angiostatic effect acting on endothelial CXCR3. ELR- CXC chemokines also orchestrate immunoangiostasis, which is the inhibition of angiogenesis by CXCR3<sup>+</sup> mononuclear cells (Th1 lymphocytes and NK cells) recruited at the tumor site, where they promote type 1, cytokine-dependent, cell-mediated immunity and, concurrently, inhibit tumor-associated angiogenesis [12–14].

Starting from early observations on melanomas [15], it is now well documented in several tumor settings that chemokine receptors expressed on cancer cells affect different aspects of their behavior and that chemokines can serve as cues for their secondary localization [16, 17]. The main chemokine receptor involved in the metastatic process is CXCR4, whose expression is up-regulated in several different tumor types. CXCR4<sup>+</sup> cells preferentially metastasize to organs secreting high levels of the ligand CXCL12, such as lung, liver, bone, and lymph nodes. Blocking the CXCR4/CXCL12 axis results in reduced metastatic spreading in different tumor types in animal models [18, 19]. The CCR7/CCL21 axis represents a second chemokine axis used by some tumor cells to metastasize to lymph nodes, in the same way dendritic cells migrate from peripheral tissues to lymph nodes after antigen uptake [17]. Finally, the expression of chemokine receptors by tumor cells not only endows them with the ability to metastasize but also affects their proliferation and survival, activating the PI3K-AKT-NFκB axis and also via MEK1/2 and Erk1/2 [20, 21].

**ROLES OF ACKR IN CANCER DEVELOPMENT**

Both preclinical observations obtained in ACKRs gene-targeted mice and clinical data from patient samples provide evidence that the regulation exerted by ACKRs on the chemokine system has an important role in cancer biology (Fig. 1).

**ACKR1/DARC**

ACKR1, previously known as DARC, is a promiscuous, atypical chemokine receptor for >20 inflammatory chemokines belonging to the CC and CXC families. ACKR1 is constitutively expressed on red blood cells and endothelial cells of venules and small veins [22, 23]. ACKR1 expressed by red blood cells is an important regulator of chemokine bioavailability by acting as a depot for chemokines, reducing their concentration in the circulation. This was demonstrated in preclinical models [24] and by studies conducted with African individuals lacking ACKR1 expression on erythrocytes (Duffy null or negative) that have higher levels of circulating chemokines [25]. Although ACKR1 completely lacks the highly conserved motif for G-protein coupling found in GPCRs at the boundary between the third transmembrane domain and the second intracellular loop (DNYLAIV motif), the possibility that it can signal through G-protein-independent pathways is suggested by experiments performed with endothelial cells. Indeed, ACKR1 expressed in these cells mediates chemokine internalization [22] and chemokine transcytosis helping in the generation of chemokine gradients that guide leukocytes from blood to inflamed tissues [5, 23, 26].

The role of ACKR1 in cancer was initially studied in prostate cancer because the Duffy null polymorphism was supposed to be one of the causes of the greater incidence of prostate cancer in African people. Using the transgenic adenocarcinoma of the mouse prostate model, ACKR1<sup>+/−</sup> mice showed enhanced tumor growth because of increased levels of angiogenic CXC chemokines, such as CXCL8 and CXCL2 [27]. Despite this preclinical, encouraging result, few studies on African American people have been conducted, and they showed no correlation between Duffy null phenotype and prostate cancer incidence in this population [28]. Preclinical studies were also performed by overexpressing ACKR1 in breast cancer [29] and NSCLC cells [30]. In both models, ACKR1 inhibited tumor angiogenesis and metastasis, despite having different effects on the primary tumor. The protective role of ACKR1 was also proven with transgenic mice overexpressing the receptor on endothelial cells; these mice, s.c. injected with human melanoma cell line Mela, displayed reduced growth and angiogenesis [31].

In addition, other studies indicated that ACKR1 expressed by endothelial cells can inhibit metastasis with a mechanism unrelated to its chemokine control activity. Indeed, ACKR1 interaction with the tetraspan CD82/KAI expressed by tumor cells, induces their senescence and inhibits lung metastasis, increasing p21 levels [32], which block CXCL8-mediated extravasation [33]. A protective role of ACKR1 in cancer is also suggested by data obtained with different human tumors. In breast [29, 34, 35], thyroid [36], colorectal [37], and laryngeal squamous cell [38] tumors, ACKR1 expression was positively correlated with a better outcome, even if the cell types expressing ACKR1 in these samples were not characterized. In summary, these results indicate ACKR1 as a negative regulator of tumor growth. It inhibits tumor angiogenesis and metastasis by scavenging angiogenic chemokines. Moreover, ACKR1 expressed by endothelial cells inhibits metastasis because its interaction with CD82 induces senescence and blocks extravasation of tumor cells.
ACKR2/D6

ACKR2, also known as D6 or CCBP2, is a scavenger receptor that binds, internalizes, and leads to degradation of a broad panel of inflammatory CC chemokines acting as agonists for receptors from CCR1 to CCR5 [39]. This receptor is highly expressed in the placenta by the syncytiotrophoblast layer, in lymphatic endothelial cells, and by some leukocyte populations, including innate-like B cells and alveolar macrophages [40–42]. ACKR2 is a constitutively recycling receptor with a scavenger function by transporting chemokines in intracellular compartments for degradation. It was initially supposed to be a silent receptor (not inducing any intracellular signaling pathway after ligand engagement) because it is not able to induce cell migration. Recently, it became clear that, after chemokine binding, ACKR2 activates β-arrestin–dependent signaling, which increases receptor recycling and plasma membrane localization to adapt its function to chemokine extracellular concentrations [43, 44].

Through its chemokine-scavenging ability, ACKR2 has a protective role in different pathologies [45–48], and it was also described having a protective role in different tumor contexts. The first model used was TPA/DMBA skin carcinogenesis, in which ACKR2-deficient mice showed earlier and increased disease severity [49]. ACKR2/−/− mice were also more susceptible to developing colon cancer using the AOM/DSS model [50]. On the contrary, there is no evidence of a protective role of ACKR2 in DEN-induced hepatocellular carcinoma model, although liver of ACKR2/−/− mice had greater macrophage infiltrates [51]. Referring to colon cancer, Langenes et al. [52] found that ACKR2 expression by lymphatic endothelial and mononuclear cells was decreased in human colon adenocarcinoma samples compared with healthy tissues, and this decrease correlated with greater lymphatic vessel density and increased CCL22 levels.

Because ACKR2 is expressed by lymphatic endothelial cells, its role was also studied in vascular tumors. ACKR2 is highly expressed by different types of vascular tumors, including Kaposi sarcoma spindle cells [53]. In this latter tumor, using in vitro and in vivo models, we have described a protective role of ACKR2 in tumor progression through inhibition of the inflammatory chemokines CCL2, CCL5, and CCL3 and reduced macrophage infiltration and angiogenesis. In patients with Kaposi sarcoma ACKR2 is down-regulated in more-aggressive forms through the oncogenic pathway KRas/BRaf/MEK/MAPK [54].

ACKR2 is expressed by breast cancer cells, and in this tumor, it has a protective role, even if it is still unclear whether normal epithelial cells express the receptor [55]. Indeed ACKR2 overexpression in a human breast cell line decreases levels of inflammatory chemokines and tumor aggressiveness; in human breast cancer samples, ACKR2 expression is inversely correlated to lymph node metastasis, as well as to clinical stage, and positively correlated to disease-free survival rate [56]. Moreover, ACKR2 is down-regulated during breast cancer progression, and the down-regulation, together with the other 2 atypical chemokine receptors, ACKR1 and ACKR4, correlates with a malignant outcome in the pathology [34, 35]. Interestingly, the host
genotype of ACKR2 and ACKR1 is important for the clinical response in patients with breast cancer. Indeed, two minor single-nucleotide polymorphisms in the coding sequence of these receptors increase their chemokine scavenger activity and are correlated with increased relapse-free survival [57, 58]. Expression of ACKR2, ACKR1, and ACKR4 was correlated with a better outcome in cervical squamous cell cancer and in gastric cancer as well [59, 60]. The protective role of ACKR2 was also described in human lung cancer. ACKR2 overexpression in the human lung cancer cell line A549 inhibits in vitro cancer cell proliferation and in vivo tumorigenesis because of an enhanced clearance of inflammatory chemokines [61]. Collectively, these results indicate that ACKR2 inhibits cancer proliferation through its chemokine scavenger activity, which prevents the recruitment of protumoral leukocytes that sustain and enhance tumor growth.

ACKR3/CXCR7

ACKR3, previously identified as CXCR7 or RDC-1, binds with high-affinity CXCL12 and with lower-affinity CXCL11, which are, respectively, CXCR4 and CXCR3 ligands. It is expressed by some hematopoietic cells, mesenchymal cells, activated endothelial cells, and neurons. ACKR3 is a β-arrestin–biased receptor (mainly signaling through β-arrestin pathways) and transduces signaling pathways that activate ERKs or protein kinase B (PKB or Akt) [62]. ACKR3 is constitutively ubiquitinated; after agonist binding, it is deubiquitinated, binds β-arrestin, and undergoes internalization. It then releases chemokines in early endosomes for degradation, releases β-arrestin, and undergoes recycling. The functional role of ACKR3 is not only the modulation of CXCL12 bioavailability by scavenging but also the modulation of CXCR4 expression and function. Indeed, ACKR3 can form heterodimers with CXCR4 and can enhance or inhibit CXCL12-mediated G protein signaling [67, 68]. The interaction of ACKR3 with CXCL11 and its canonical receptor CXCR4 have been less well investigated and is complicated by this chemokine being able to induce either proliferative or growth-inhibitory signals, depending on the CXCR3 variant it binds [69].

In the cancer context, ACKR3 was initially found expressed on many tumor cell lines and in endothelial cells associated with tumors [70]. ACKR3 is also expressed by different tumor types [65] and is often found up-regulated in tumor tissues compared with their healthy counterparts. Some mechanisms of ACKR3 regulation have been described, such as hypoxia, DNA methylation of the tumor suppressor gene hypermethylated in cancer 1, microRNA-430 and microRNA-101 [65]. Different from the other ACKRs, ACKR3 expression by cancer cells, in most of the cases, promotes the tumorigenesis process affecting tumor cell growth, survival, and metastasis [71].

ACKR3 promotes cancer cell growth, inducing ERK1/2 phosphorylation and inhibiting apoptosis in breast cancer [72]. In breast and prostate cancer, ACKR3 forms heterodimers with EGFR and stimulates its signaling promoting tumor-cell proliferation in a ligand-independent manner [73–75]. In lung cancer, the use of an ACKR3 antagonist inhibits tumor growth [70]. It was also found that in lung adenocarcinoma TGF-β1 increases ACKR3 expression that promote tumor growth, and its expression correlates with decreased survival rates in patients [76]. ACKR3 expression, together with CXCR4, is also correlated with poor prognosis in renal cell carcinoma; in this tumor, ACKR3 promotes tumor growth, activating the mTOR pathway [77].

Referring to the metastasis process, contrasting results have been published. ACKR3 can promote metastasis in a breast cancer model [78] and in hepatocellular carcinoma through up-regulation of osteopontin [79]. ACKR3 may also regulate CXCR4-mediated transendothelial migration of human tumor cells toward a CXCL12 source [80]. On the contrary, Hernandez et al. [81] reported that ACKR3 could inhibit breast tumor metastasis by decreasing CXCR4-mediated effects, such as metalloproteinase-12 production and impairing CXCL12-stimulated matrix degradation and invasion. In addition, in rhabdomyosarcoma, CXCR7 expression was correlated with a less-metastatic phenotype compared with CXCR4 [82].

ACKR3 also has an important role in tumor angiogenesis, as revealed by more-recent studies. ACKR3 expressed by endothelial progenitors and tumor endothelial cells has a proangiogenic role inducing endothelial progenitor transendothelial migration [83] and survival [84]. An opposite role for endothelial ACKR3 was found by the use of conditional knockout mice with selective depletion of the receptor in vascular endothelial cells. Indeed, ACKR3 protects mice from lung metastasis in breast cancer after orthotopic injection of the AT-3 cell line, by decreasing CXCL12 plasma levels [85]. Also in patients with glioblastoma, ACKR3 expression by endothelial cells was correlated with a better prognosis [86].

These results indicate that ACKR3 has multiple roles in tumor biology, not only regulating CXCL12 bioavailability and CXCR4 signaling but also directly activating intracellular G-protein–independent pathways that promote cell growth and survival. Despite the expression of ACKR3 by tumor cells being, in most of the cases, correlated with an increase in tumor growth, contrasting results were published on the role of ACKR3 expression by endothelial cells in the tumor context. Indeed, the receptor can have a proangiogenic role, but at the same time, it can protect from metastatic dissemination. Finally, ACKR3 could promote tumor growth through scavenging of CXCL11, one of the ligands of CXCR3, a chemokine receptor required for an efficient immune response and that can have direct and indirect angiostatic effects.

ACKR4/CCRL1

ACKR4, also known as CCRL1 or CCX-CKR, is a scavenger receptor for the homeostatic chemokines CCL19, CCL21, CCL25, and CXCL13 [87]. ACKR4 is expressed by thymic epithelial cells, bronchial cells, and keratinocytes [88, 89]. It is a constitutively internalizing receptor, and after chemokine binding, it recruits β-arrestin 2, but it is not known whether it activates signaling through β-arrestin pathways [90].

Few results have been published about the role of ACKR4 in cancer biology. Overexpression of ACKR4 inhibited the in vitro proliferation of breast cancer cell lines MDA-MB-425 and MDA-MB-231 [91] and hepatocellular carcinoma cell lines MHCC97L.
and HCCLM3 [92]. In vivo, beside inhibition of tumor growth, there is also reduced metastasis with concomitant decreased levels of ACKR4 ligands. Interestingly, in colon cancer, ACKR4 overexpression reduces cell migration to serum and matrigel invasion and inhibits the expression of CCR7, CCR9, CXCR5, and CXCR4 [93]. Opposite results were found using the breast cancer cell line 4T1.2 overexpressing ACKR4, which displayed increased lung metastasis from enhanced EMT and TGF-β1 expression [94].

Supporting the protective role of ACKR4 in tumor growth and dissemination, there are data from human breast [91], hepato-cellular [92], and colon [93] cancer samples in which down-regulation of ACKR4 is correlated with a worse outcome.

Collectively, these results indicate that ACKR4 expression inhibits tumor growth and metastasis by scavenging its ligands. Because a recent publication indicates that ACKR4 could also have a prometastatic role, inducing EMT of breast cancer cells, it is evident that more data are required to understand the role of this receptor beyond its chemokine-scavenging activities.

CONCLUDING REMARKS

Chemokines are central components of cancer-related inflammation because they are mediators of the chronic inflammatory process and are transcriptionally regulated by oncogenes and deregulated transcription factors [6]. They have multiple effects on tumor growth, and it is emerging that, during tumor development, they can have both tumor-promoting and tumor-suppressive capabilities [6].

ACKRs are an important level of regulation of the chemokine system, and their function is also important in the tumorigenesis process. ACKR1, 2, and 4 act as negative regulators of the cancer system, and their function is also important in the tumorigenesis suppression capabilities [6].

ACKRs are an important level of regulation of the chemokine system, and their function is also important in the tumorigenesis process. ACKR1, 2, and 4 act as negative regulators of the cancer process. When they are expressed by cancer cells, they inhibit tumor growth through scavenging of angiogenic chemokines with concomitant inhibition of tumor vascularization (ACKR1), scavenging of inflammatory chemokines that results in inhibition of myeloid cell infiltrate (ACKR2), or scavenging of homeostatic chemokines, which inhibits tumor growth and metastasis (ACKR4). Their role as tumor suppressors is also supported by evidence from patient samples in which these 3 receptors are down-regulated by tumor cells during progression [55]. We have found that, in Kaposi sarcoma, ACKR2 is a target of the oncogenic pathway KRas/BrCaf/MEK/MAPK [54]; however, it is unknown which is the mechanism of down-regulation for ACKR1 and ACKR4.

A completely different picture was found for ACKR3, which, when expressed by cancer cells, acts as a tumor promoter by directly inducing an intracellular signaling of proliferation and survival or modulating the signaling of CXCR4, the receptor with which it shares the ligand CXCL12. Contrary to the other ACKRs, ACKR3 is up-regulated on cancer cells and on tumor endothelial cells compared with healthy tissues, and its expression correlates with disease progression [65].

Interestingly, in addition to the regulation of chemokine system, new and unexpected functions of ACKRs are emerging that can affect tumor growth. ACKRs can interact with molecules other from chemokines; for example, ACKR1 interacts with a tetraspanin, inducing tumor senescence and inhibiting metastasis [32], whereas ACKR3 interacts with EGFR, promoting cell proliferation [73]. Furthermore, ACKRs, despite not being chemotactic receptors, are able to transduce intracellular signaling that can modulate different biologic functions. For example, ACKR3 transduces proliferation and survival signaling and promotes EMT [65].

Further work is required to understand the role of ACKRs expressed by tumor stromal cells. Indeed, most of the preclinical data are derived from full knockout mice, in which it is difficult to extrapolate the role of ACKRs on different cell types composing the tumor mass. In the case of ACKR3, the use of a conditional knockout mouse, lacking the receptor only on endothelial cells, has revealed an unexpected phenotype of metastasis protection [85]. Moreover, the biologic consequences of the interaction of ACKRs with canonical chemokine receptors need to be better understood.

Therefore, further study of the differential protumor and antitumor activities of ACKRs and their contribution to the overall outcome of a tumor is warranted to develop more-effective therapies against cancer.

AUTHORSHIP


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The authors declare no conflicts of interest.

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Atypical chemokine receptors in cancer: friends or foes?

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