

Atypical Chemokine Receptors in Cancer

Parisa SARMADI¹, [PhD],
Gurcan TUNALI¹, [MSc],
Gunes ESENDAGLI^{1*}, [PhD],

¹ Department of Basic Oncology, Hacettepe University Cancer Institute, Ankara, Turkey.

* Corresponding Author: Gunes Esendagli, PhD, Department of Basic Oncology, Hacettepe University Cancer Institute, 06100 Sıhhiye, Ankara – Turkey

e-mail: gunes.esendagli@lycos.com

Received: 19 October 2016; Accepted: 29 December 2016;
Published on line: 30 December 2016

ABSTRACT

Chemokines or chemoattractant cytokines are a superfamily of small (8-12 kDa) secreted proteins. They primarily promote and regulate the directional migration and trafficking of the cells. Approximately 50 chemokine ligands and 20 chemokine receptors have been identified in humans. These molecules constitute a complex network of ligand-receptor interactions. Generally, upon binding a chemokine receptor to its cognate ligand, a signaling pathway is induced via G protein coupling to the receptor. However, because of the modified DRYLAIV motif in the second intracellular loop, atypical chemokine receptors do not couple with G proteins and do not induce signal transduction. Up to date, five decoy chemokine receptors have been identified; namely, DARC, D6, CXCR7, CCX-CKR, and CCRL2. Except CXCR7, other decoy chemokine receptors regulate cell migration by sequestering their cognate chemokine ligands. This review focuses on atypical chemokine receptors that play important roles in cancer.

Key words: Atypical chemokine receptors, chemokines, cancer

Introduction

Chemokines or chemoattractant cytokines are a superfamily of small (8-12 kDa) secreted proteins which primarily promote and regulate the directional migration and trafficking of leukocytes, endothelial cells, and epithelial cells. Approximately 50 chemokine ligands and 20 chemokine receptors have been identified in humans [1]. This system of molecules displays a promiscuous network, in which chemokine receptors interact with different chemokines with variable affinities and multiple chemokines bind to the same receptor [2]. This feature might be important in fine-tuning of chemokine-associated responses. Chemokines and chemokine receptors are divided into four categories on the basis of the sequence around the first two conserved N-terminus cysteine residues namely XC, CC, CXC and CX3C (where C is the amino acid Cysteine and X refers to any amino acid) [3, 4].

Chemokines can also be categorized under two sub-groups based on their functions: homeostatic/developmental chemokines and inflammatory chemokines [1, 5]. Homeostatic chemokines are constitutively expressed and have crucial roles in

embryonic development and organogenesis, stem cell migration, lymphoid organogenesis, and immune surveillance [5]. The expression of inflammatory chemokines can be induced by stimuli such as tumor necrosis factor (TNF), interferon- γ (IFN- γ), microbial products or trauma; and play a vital role in maintenance of innate and adaptive immunity to tissue damage, infection, and other physiological abnormalities [6]. Their expression is temporary and ceases with the resolution of the inflammation. Some chemokines can function in both groups depending on the biological status or on the pathological circumstances [7].

Chemokine receptors are of seven transmembrane G protein-coupled receptor (GPCR) family [1, 8]. CC-chemokines bind to receptors CCR1 to CCR11, CXC-chemokines bind to CXCR1 to CXCR7, and XCR1 is the receptor for lymphotactin, and CX3CR1 is the receptor for fractalkine [4, 9]. Upon specific ligand binding, a cascade of downstream signals, including calcium mobilization and the activation of mitogen-activated protein kinase (MAPK), phospholipase-C (PLC), phosphatidylinositol 3-kinase (PI3K), RAS, the RHO family of GTPases, and NF- κ B, begins [10].

Table 1. Atypical chemokine receptors and their ligands.

Atypical Chemokine Receptors	Proposed or Specific Ligands
DARC (ACKR1)	CCL2, CCL5, CCL7, CCL11, CCL13, CCL14, CCL17, CXCL1, CXCL2, CXCL5, CXCL6, CXCL7, CXCL8, CXCL11
D6 (ACKR2)	CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL14
CXCR7 (ACKR3)	CXCL12, CXCL11
CCRL1 (ACKR4)	CCL19, CCL21, CCL25, CXCL13
CCRL2 (ACKR5)	CCL5, CCL19, Chemerin

Atypical Chemokine Receptors

The sophisticated system of chemokines and chemokine receptors is adjusted by the group of atypical or decoy chemokine receptors including Duffy Antigen Receptor for Chemokines (DARC), D6, Chemocentryx Chemokine Receptor (CCX-CKR, CCRL1), CXCR7, and C-C Chemokine Receptor-Like 2 (CCRL2, Chemokine Receptor on Activated Macrophages, CRAM) [11]. Recently, these families of atypical chemokine receptors have been adapted to a new nomenclature. In this naming system DARC was renamed as ACKR1, D6 as ACKR2, CXCR7 as ACKR3, CCRL1 as ACKR4, and CCRL2 as ACKR5 (Table 1) [12].

These receptors structurally resemble to the seven transmembrane G protein-coupled receptors. However, they do not elicit signal transduction, because they contain a modified DRYLAIV motif in their second intracellular loop that prevents them

from coupling with G proteins (e.g. DKYLEIV instead of DRYLAIV amino acid sequence in D6) [11]. Atypical chemokine receptors bind their cognate chemokine ligands with high affinity and effectively internalize them; thus, they act as scavengers [11]. Internalized chemokines become degraded by lysosomal enzymes in endocytic vesicles (Figure 1). This decoy function of atypical chemokine receptors regulates and modifies the chemokine gradient and bioavailability in the microenvironment. In addition, this function indirectly changes the surface expression level of the specific receptor. After the ligands are degraded in the vesicle, the receptor recirculates to the cell membrane [11].

The presence of atypical chemokine receptors have been determined in different types of cancers. For example, the expression and function of DARC were studied in lung, breast, and prostate cancers, D6 in lung and breast cancers, CXCR7 in different cancer

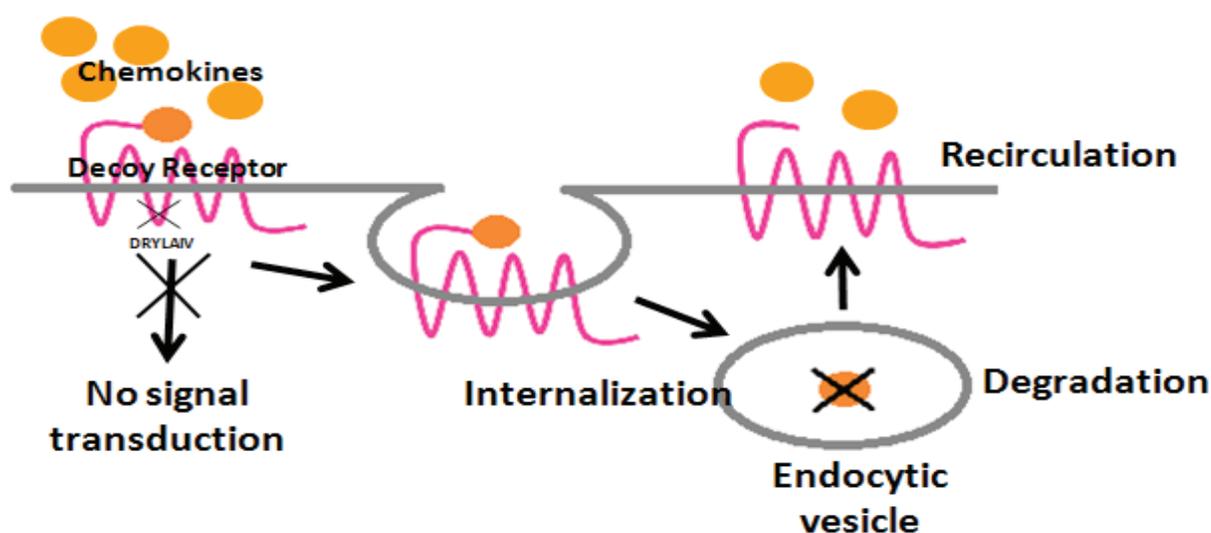


Figure 1. Schematic representation for decoy function of atypical chemokine receptors. Atypical chemokine receptors do not induce signal transduction after binding their cognate chemokines. Internalized chemokines can be either degraded or released by exocytosis in a delayed fashion following recirculation.

types, including glioma, lung, breast, prostate, and liver cancers, CCRL1 in breast and cervix cancers, and CCRL2 in glioblastoma and B-cell chronic lymphocytic leukemia (CLL).

DARC

DARC was primarily identified as Duffy blood group antigen, the erythrocyte receptor for malaria parasite *Plasmodium vivax*; and later, it was described as an erythrocyte receptor for CXCL8. Other than erythrocytes of the Duffy antigen positive individuals, DARC is also found on the vascular endothelial cells lining post-capillary venules, collective venules and small veins in different tissues such as spleen and lymph nodes, on the epithelial cells of kidneys and lungs, and expressed by neurons in discrete anatomic sites of the brain including Purkinje cells [13]. DARC does not contain intracellular signaling motifs and does not induce signaling or migration cascades. This silent receptor has important functions in chemokine transcytosis, clearing chemokines from the blood stream and leukocyte tethering to the endothelium, thus regulating plasma chemokine concentration [14]. DARC expression is upregulated during inflammation and it binds both CC (e.g. CCL2, CCL5) and CXC (e.g. CXCL1, CXCL3, CXCL5, CXCL6, CXCL8) the pro-inflammatory families of chemokines, but not the homeostatic chemokines [15]. DARC is the only mammalian chemokine receptor that binds ligands from more than one chemokine subfamily [16].

D6

D6 is the second best characterized member of atypical chemokine receptors. It is expressed in placenta, on lymphatic endothelial cells (in gut, skin, lung, and liver) and on immune cells. This atypical receptor binds many inflammatory CC chemokines (CCL2, 3L1, 4, 5, 7, 8, 11-14, and 22) and weakly CCL17. Upon ligation, D6 undergoes rapid internalization and the internalized ligand becomes degraded while the receptor recycles onto the cell surface. The lack of D6 expression has been correlated with impaired chemokine clearance, uncontrolled and continuous inflammation in various inflammatory models such as *Mycobacterium tuberculosis* infection, phorbol ester-induced cutaneous inflammation, and inflammatory bowel disease. In addition to lymphatic endothelial cells, different populations of immune cells including dendritic cells (DCs), macrophages and B cells express D6. It has been reported that in contrast to its classical function on lymphatic

endothelium, when expressed on the leukocytes D6 can also promote inflammatory reactions in certain experimental models [17].

CXCR7

Coupling of CXCR7 with G proteins is still controversial [18]. In several cell types, CXCR7 functions as a signaling receptor as evidenced by phosphorylation of MAPKs or serine/threonine kinase Akt [18]. Recently, CXCR7 has also been classified as a member of the atypical chemokine receptor family [12]. The ligands of CXCR7 are CXCL12 (stromal cell-derived factor 1, SDF-1) and CXCL11 (interferon-inducible T cell α chemoattractant, I-TAC). CXCR7 expression is found on T cells, differentiated neurons, activated tumor-associated endothelial cells, and in many types of tumors. It has been shown to be important for proliferation and survival of the tumor cells [19, 20]. CXCR7 has a nearly 10 times higher binding affinity for CXCL12 as compared with CXCR4 [21]. In contrast to CXCR4, CXCR7 does not activate intracellular signaling upon interaction with CXCL12 or CXCL11 [22]. Other than competing for CXCL12 binding, CXCR7 can modulate CXCR4-mediated processes directly by crosstalking and forming heterodimers with CXCR4 [23-25]. Alternatively, it is interesting that CXCR7 was shown to induce signals that influence the cell proliferation and migration in different types of tumor cells, primary neurons, primary rodent astrocytes, and human glioma cells [21, 26, 27].

CCX-CKR

CCX-CKR (Chemocentryx Chemokine Receptor, Chemokine CC motif Receptor-Like 1, CCRL1) is predominantly expressed on the epithelial cells of heart and lung [28]. CCX-CKR binds the homeostatic chemokines CCL19, CCL21, CCL25, and weakly CXCL13 [29]. It is involved in the regulation of homeostatic lymphocyte trafficking and of immune responses [30]. By efficiently binding CCL19 and CCL21, CCX-CKR especially regulates the CCR7/CCL19/CCL21 axis. Binding of CCL19 to CCR7 ends with its internalization and degradation, then the receptor becomes desensitized. On the other hand, following CCL21 ligation, CCR7 remains stable at the cell surface but its signaling capacity is limited [31]. In the absence of CCX-CKR, CCL19 and CCL21 levels rise in tissues and lymph nodes [32]. In mice lacking CCX-CKR, DC migration to lymph nodes is impaired and immune responses become weakened in the

draining lymph nodes. Following immunization for experimental autoimmune encephalomyelitis (EAE), *Ccr1*^{-/-} mice show earlier disease onset and irregular type 17 helper T (Th17) responses. Herein, high levels of CCL21 induce IL-23 synthesis in DCs that promote the pathogenic differentiation [33]. Thus, a complicated relationship exists between CCX-CKR and CCR7 axis in the peripheral immune system. CCX-CKR is also expressed in the thymus and affects thymic stroma, thymic chemokine localization, and negative selection of thymocytes indicating its role in the maintenance of tolerance [34].

CCRL2

CCRL2 (Chemokine CC motif Receptor-like 2) was first cloned from a polymorphonuclear leukocyte (PMN) cDNA library as an orphan receptor called human chemokine receptor (HCR) [35]. The human CCRL2 gene is transcribed into two variants deriving from alternative splicing named chemokine receptor on activated macrophages (CRAM)-A and a 36 base pair shorter, more common variant, CRAM-B. CRAM-B is also known as human chemokine receptor, HCR [36, 37]. The protein sequence of CRAM-A is twelve amino acid longer at the N-terminus than that of CRAM-B [35].

Like other members of the atypical chemokine receptor family, CCRL2 holds a modified DRYLAIV motif, with a glutamine (Q) at position 127 instead of aspartic acid (D), replacing an acidic residue with a neutral one preventing coupling to the G_i protein. Therefore, interaction of CCRL2 with its ligands does not induce any classical signaling response. Also, it has been shown that CCRL2 expression was associated with reduced MAPK activity [31].

CCRL2 is expressed on almost all human hematopoietic cells including monocytes, macrophages, basophils, mast cells, PMNs, CD4⁺ and CD8⁺ T cells, pro- and pre-B cells (depending on the maturation stage), DCs, NK cells, and CD34⁺ progenitor cells [38]. At mRNA level, CCRL2 expression is up-regulated on human PMNs stimulated with LPS or TNF; on human monocyte-derived iDCs in response to LPS+IFN- γ and CD40L; and, on human T cells upon stimulation with anti-CD3 or IL-2 [39]. In addition, CCRL2 mRNA is also detected in human pre-B acute lymphoblastoid leukemia cell lines and in mouse astrocytes and microglia cells [45]. It has been reported that CCRL2 expression is induced during DC maturation [40].

CCRL2 displays a narrow binding spectrum.

This atypical receptor couples with chemerin, an adipokine and chemotactic factor agonist for chemokine-like receptor 1 (CMKLR1) (also known as ChemR23) and G protein-coupled receptor 1 (GPR1). Binding of chemerin to CMKLR1 triggers calcium mobilization, receptor and ligand internalization, and cell migration. Whereas, coupling of chemerin with CCRL2 does not induce this classical receptor activation. On the other hand, CCRL2 concentrates chemerin on the cell surface and presents it to cells in the vicinity. Thus, CCRL2 can regulate chemerin bioavailability [41].

It has been shown that CCRL2 possesses the capacity to alter the levels of CCL5. Thus CCL5 is suggested to be a ligand for CCRL2. Furthermore, the expression of CCRL2 can be induced via MAPK activation upon the cells' exposure to CCL5. On the other hand, no calcium response or migration towards CCL5 was detected in Nalm6 cells, a CCRL2⁺ pre-B acute lymphoblastic leukemia cell line [36]. It has been reported that mCCRL2/HEK293 transfected cells respond functionally to chemokine ligands CCL2, CCL5, CCL7, and CCL8 through intracellular calcium mobilization and transwell chemotaxis [42]. On the other hand, in a study done by Zabel et al. to investigate possible functional roles for CCRL2 and to identify CCRL2 ligands, the chemokines CCL2, CCL5, CCL7, and CCL8 were applied on mCCRL2-transfected L1.2 cells, the mouse pre-B lymphoma cell line. These chemokines did not induce cell migration in transwell chemotaxis assays [43].

CCL19 is a specific ligand for CCRL2. Upon binding CCL19, CCRL2 internalization occurs but the receptor constitutively recycles back to cell surface. These properties suggest a regulatory role for CCRL2 in immune responses and homing processes [37]. Leick et al. reported that beside CCX-CKR, CRAM-B is another atypical chemokine receptor that has some relevance to the CCR7 axis, since it can bind and internalize CCL19 [37, 32]. CCRL2 shows a constitutive cycling activity, which results in CCL19 internalization. Therefore, it is an effective modulator of CCR7 functions [31]. In the beginning, CCRL2 might compete with CCR7 to bind CCL19, but later it may favor CCL19 presentation to CCR7-expressing cells of the B lymphocyte origin [31].

Atypical Chemokine Receptors and Cancer

Chemokines and chemokine receptors are key players in the cancer-related inflammation [44]. Unresolved infections and chronic inflammation are closely

related with cancer development. These fundamental molecules can be produced by malignant and stromal cells in the tumor microenvironment. In the progressive way of carcinogenesis, chemokines and their receptors become factors mediating the recruitment of tumor-promoting cells such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes (TILs). Chemokine systems welcome directional migration and metastasis of tumor cells together with leukocytes to specific target tissues. They can mediate the leukocytes' effects on cancer cell survival, metastasis, and regulation of angiogenesis [44]. Chemokines and chemokine receptors are upregulated in different types of cancers. Type and amount of the secreted chemokines specify character and quantity of immune cell infiltration in the tumor microenvironment [45].

DARC and D6, the two best characterized members of atypical chemokine receptors may serve as a systemic barrier to metastasis of cancer cells through the two main spreading (hematogenous and lymphatic) ways. DARC is broadly expressed on erythrocytes and vascular endothelial cells and D6 is expressed on lymphatic endothelial cells [46]. Experimental studies have demonstrated that DARC expression has a negative influence on tumor metastasis and angiogenesis [47]. DARC is the essential modulator of prostate cancer progression by clearing angiogenic chemokines from the tumor microenvironment and reducing angiogenesis. It has been shown that in humans who lack erythroid DARC, prostate cancer progression and mortality was increased [4]. In breast cancer, D6 expression attenuates lymph node metastasis and is negatively correlated with clinical tumor stage (Figure 2) [48]. D6 expression is significantly decreased in colon tumors compared to non-tumor tissue from the same individual. Moreover, D6 expression was lower in advanced tumors. Hence, tumor cells can benefit D6 downregulation as a mechanism to shape the regional chemokine network to favor tumorigenesis and spread (Figure 2) [16].

CXCR7 expression is higher in transformed cells as compared to their normal non-transformed counterparts [18]. CXCL12-CXCR7 or CXCL12-CXCR4 interactions mediate recruitment of endothelial progenitor cells and sustain neo-angiogenesis in tumors [49]. CXCR7 is high on breast and lung cancer cells. Hypoxia induces its expression on endothelial and tumor-associated vessels. This

correlates with cell proliferation, vascularization, and metastatic potential. CXCR7 has been found to be upregulated on prostate cancer cells and on aggressive tumors. This correlates with cell proliferation, survival, adhesion, chemotaxis and increases the expression of pro-angiogenic factors such as IL-8, vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)- β . CXCR7 expression level is suppressed by CXCR4 activation in prostate cancer cells. Thus, CXCR7 expression on tumor cells can be an important factor in promoting tumor cell proliferation and angiogenesis (Figure 2) [50].

In breast cancer, CCX-CKR overexpression restricts cell proliferation and invasion, *in vitro*; and tumor progression and metastasis, *in vivo* (Figure 2). In contrast, low levels of CCX-CKR expression are correlated with poor prognosis in breast cancer patients [16]. Recent studies on breast and gastric cancer tissues have shown that co-expression of DARC, D6, and CCX-CKR is significantly lower in invasive breast cancer compared to non-invasive specimens and healthy breast tissues. The presence of these markers is indicative of relapse-free and improved overall survival both in breast and gastric cancers [16, 51].

The role of CCRL2 in human cancers is not fully determined and understood. There are limited studies on the relationship between CCRL2 and cancer. One of these studies demonstrated a substantial CCRL2 expression in mice breast cancer tissue and human cell lines. CCRL2 attenuated breast cancer cell migration and invasion by blocking CCL2 activity (Figure 2) [52]. In B-CLL, CCL19-CCR7 interaction motivates the formation and maintenance of germinal center-like proliferative foci. By scavenging CCL19, CCRL2 modifies CCL19's availability and regulates function of CCR7 [31]. Breast cancer cells express high amount of CCRL2. Its expression is related with high immune infiltration in the tumor tissue. Especially CRAM-A variant is upregulated in the presence of IFN- γ [53]. Thus, CCRL2 may modulate cancer biology and immunology.

Conclusion

The relationship between atypical chemokine receptors and cancer has been described in various experimental models and human cancer types. Upon binding to their cognate chemokine ligands, in contrast to specific functional chemokine receptors, atypical receptors reduce or regulate chemokine gradients. Chemokine-mediated behaviors of cancer

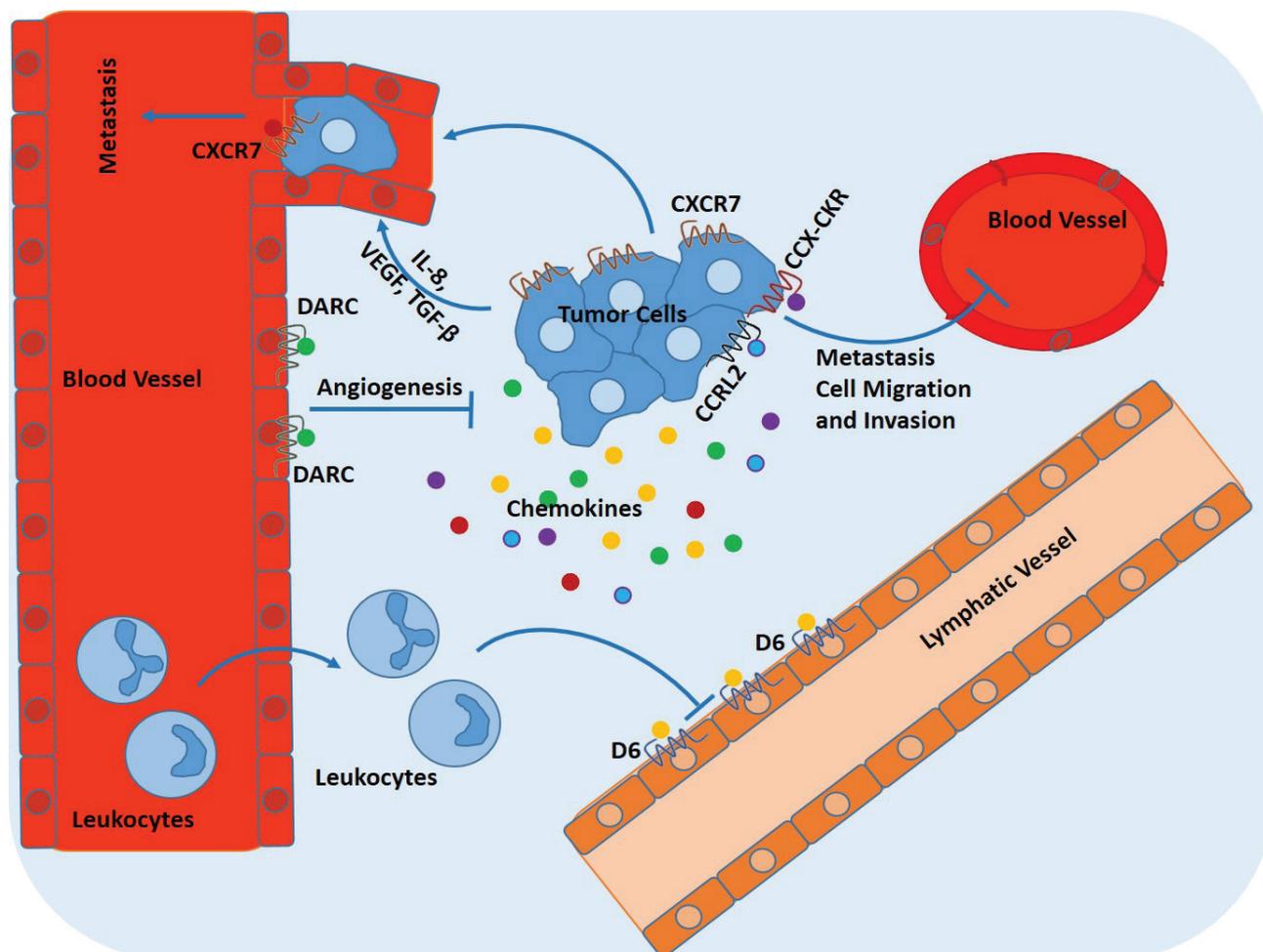


Figure 2. Role of atypical chemokine receptors in cancer. Atypical chemokine receptors regulate tumorigenic process. DARC is primarily expressed on the surface of blood endothelial cells and reduces angiogenesis by clearing angiogenic chemokines. D6 is found on lymphatic endothelial cells and prevents metastasis through lymphatics. CXCR7 is expressed by many tumor cells. This receptor regulates recruitment of endothelial progenitor cells, increases expression of pro-angiogenic factors in tumor cells and mediates metastasis. CCX-CKR blocks invasion and metastasis. CCRL2 is found on tumor cells and suppresses cell migration and invasion.

cells such as proliferation, survival, migration, invasion, angiogenesis and metastasis can be modulated through atypical chemokine receptors.

Therefore, this new family of chemokine receptors may have important implications in cancer biology and immunology research.

REFERENCES

- [1] Sarvaiya PJ, Guo D, Ulasov I, Gabikian P, Lesniak MS. Chemokines in tumor progression and metastasis. *Oncotarget* 2013; 4(12): 2171-2185.
- [2] Mantovani A. The chemokine system: redundancy for robust outputs. *Immunol Today* 1999; 20(6): 254-257.
- [3] Murphy JW, Yuan H, Kong Y, Xiong Y, Lolis EJ. Heterologous quaternary structure of CXCL12 and its relationship to the CC chemokine family. *Proteins* 2010; 78(5): 1331-1337.
- [4] Ali S, Lazennec G. Chemokines: novel targets for breast cancer metastasis. *Cancer Metastasis Rev* 2007; 26(3-4): 401-420.
- [5] Zlotnik A, Burkhardt AM, Homey B. Homeostatic chemokine receptors and organ-specific metastasis. *Nat Rev Immunol* 2011; 11(9): 597-606.
- [6] Raman D, Sobolik-Delmaire T, Richmond A. Chemokines in health and disease. *Exp Cell Res* 2011; 317(5): 575-589.
- [7] Allen SJ, Crown SE, Handel TM. Chemokine: receptor structure, interactions, and antagonism. *Annu Rev Immunol* 2007; 25: 787-820.
- [8] Nomiyama H, Osada N, Yoshie O. Systematic classification of vertebrate chemokines based on conserved synteny and evolutionary history. *Genes Cells* 2013; 18(1): 1-16.
- [9] Murdoch C, Finn A. Chemokine receptors and their role in inflammation and infectious diseases. *Blood* 2000; 95(10): 3032-3043.
- [10] Comerford I, McColl SR. Mini-review series: focus on chemokines. *Immunol Cell Biol* 2011; 89(2): 183-184.

- [11] **Feng LY, Ou ZL, Wu FY, Shen ZZ, Shao ZM.** Involvement of a novel chemokine decoy receptor CCX-CKR in breast cancer growth, metastasis and patient survival. *Clin Cancer Res* 2009; 15(9): 2962-2970.
- [12] **Nibbs RJ, Graham GJ.** Immune regulation by atypical chemokine receptors. *Nat Rev Immunol* 2013; 13(11): 815-829.
- [13] **Graham GJ, Locati M, Mantovani A, Rot A, Thelen M.** The biochemistry and biology of the atypical chemokine receptors. *Immunol Lett* 2012; 145(1-2): 30-38.
- [14] **Mantovani A, Locati M, Vecchi A, Sozzani S, Allavena P.** Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. *Trends Immunol* 2001; 22(6): 328-336.
- [15] **Borroni EM, Bonocchi R.** Shaping the gradient by nonchemotactic chemokine receptors. *Cell Adh Migr* 2009; 3(2): 146-147.
- [16] **Langenes V, Svensson H, Borjesson L, Gustavsson B, Bemark M, Sjoling A, Quiding-Jarbrink M.** Expression of the chemokine decoy receptor D6 is decreased in colon adenocarcinomas. *Cancer Immunol Immunother* 2013; 62(11): 1687-1695.
- [17] **Hajrasouliha AR, Sadrai Z, Lee HK, Chauhan SK, Dana R.** Expression of the chemokine decoy receptor D6 mediates dendritic cell function and promotes corneal allograft rejection. *Mol Vis* 2013; 19: 2517-2525.
- [18] **Maksym RB, Tarnowski M, Grymula K, Tarnowska J, Wysoczynski M, Liu R, Czerny B, Ratajczak J, Kucia M, Ratajczak MZ.** The role of stromal-derived factor-1—CXCR7 axis in development and cancer. *Eur J Pharmacol* 2009; 625(1-3): 31-40.
- [19] **Shimizu S, Brown M, Sengupta R, Penfold ME, Meucci O.** CXCR7 protein expression in human adult brain and differentiated neurons. *PLoS One* 2011; 6(5): e20680.
- [20] **Miao Z, Luker KE, Summers BC, Berahovich R, Bhojani MS, Rehemtulla A, Kleer CG, Essner JJ, Nasevicius A, Luker GD et al.** CXCR7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. *Proc Natl Acad Sci U S A* 2007; 104(40): 15735-15740.
- [21] **Odemis V, Lipfert J, Kraft R, Hajek P, Abraham G, Hattermann K, Mentlein R, Engele J.** The presumed atypical chemokine receptor CXCR7 signals through G(i/o) proteins in primary rodent astrocytes and human glioma cells. *Glia* 2012; 60(3): 372-381.
- [22] **Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, Penfold ME, Sunshine MJ, Littman DR, Kuo CJ et al.** A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J Exp Med* 2006; 203(9): 2201-2213.
- [23] **Boldajipour B, Mahabaleswar H, Kardash E, Reichman-Fried M, Blaser H, Minina S, Wilson D, Xu Q, Raz E.** Control of chemokine-guided cell migration by ligand sequestration. *Cell* 2008; 132(3): 463-473.
- [24] **Hartmann TN, Grabovsky V, Pasvolosky R, Shulman Z, Buss EC, Spiegel A, Nagler A, Lapidot T, Thelen M, Alon R.** A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells. *J Leukoc Biol* 200; 84(4): 1130-1140.
- [25] **Levoye A, Balabanian K, Baleux F, Bachelier F, Lagane B.** CXCR7 heterodimerizes with CXCR4 and regulates CXCL12-mediated G protein signaling. *Blood* 2009; 113(24): 6085-6093.
- [26] **Grymula K, Tarnowski M, Wysoczynski M, Drukala J, Barr FG, Ratajczak J, Kucia M, Ratajczak MZ.** Overlapping and distinct role of CXCR7-SDF-1/ITAC and CXCR4-SDF-1 axes in regulating metastatic behavior of human rhabdomyosarcomas. *Int J Cancer* 2010; 127(11): 2554-2568.
- [27] **Wang Y, Li G, Stanco A, Long JE, Crawford D, Potter GB, Pleasure SJ, Behrens T, Rubenstein JL.** CXCR4 and CXCR7 have distinct functions in regulating interneuron migration. *Neuron* 2011; 69(1): 61-76.
- [28] **Hou T, Liang D, Xu L, Huang X, Huang Y, Zhang Y.** Atypical chemokine receptors predict lymph node metastasis and prognosis in patients with cervical squamous cell cancer. *Gynecol Oncol* 2013; 130(1): 181-187.
- [29] **Comerford I, Litchfield W, Harata-Lee Y, Nibbs RJ, McColl SR.** Regulation of chemotactic networks by 'atypical' receptors. *Bioessays* 2007; 29(3): 237-247.
- [30] **Comerford I, Milasta S, Morrow V, Milligan G, Nibbs R.** The chemokine receptor CCX-CKR mediates effective scavenging of CCL19 in vitro. *Eur J Immunol* 2006; 36(7): 1904-1916.
- [31] **Catusse J, Leick M, Groch M, Clark DJ, Buchner MV, Zirlik K, Burger M.** Role of the atypical chemoattractant receptor CRAM in regulating CCL19 induced CCR7 responses in B-cell chronic lymphocytic leukemia. *Mol Cancer* 2010; 9: 297.
- [32] **Comerford I, Harata-Lee Y, Bunting MD, Gregor C, Kara EE, McColl SR.** A myriad of functions and complex regulation of the CCR7/CCL19/CCL21 chemokine axis in the adaptive immune system. *Cytokine Growth Factor Rev* 2013; 24(3): 269-283.
- [33] **Comerford I, Nibbs RJ, Litchfield W, Bunting M, Harata-Lee Y, Haylock-Jacobs S, Forrow S, Korner H, McColl SR.** The atypical chemokine receptor CCX-CKR scavenges homeostatic chemokines in circulation and tissues and suppresses Th17 responses. *Blood* 2010; 116(20): 4130-4140.
- [34] **Bunting MD, Comerford I, Seach N, Hammett MV, Asquith DL, Korner H, Boyd RL, Nibbs RJ, McColl SR.** CCX-CKR deficiency alters thymic stroma impairing thymocyte development and promoting autoimmunity. *Blood* 2013; 121(1): 118-128.
- [35] **Del Prete A, Bonocchi R, Vecchi A, Mantovani A, Sozzani S.** CCRL2, a fringe member of the atypical chemoattractant receptor family. *Eur J Immunol* 2013; 43(6): 1418-1422.
- [36] **Hartmann TN, Leick M, Ewers S, Diefenbacher A, Schraufstatter I, Honczarenko M, Burger M.** Human B cells express the orphan chemokine receptor CRAM-A/B in a maturation-stage-dependent and CCL5-modulated manner. *Immunology* 2008; 125(2): 252-262.
- [37] **Leick M, Catusse J, Follo M, Nibbs RJ, Hartmann TN, Veelken H, Burger M.** CCL19 is a specific ligand of the constitutively recycling atypical human chemokine receptor CRAM-B. *Immunology* 2010; 129(4): 536-546.
- [38] **Migeotte I, Franssen JD, Goriely S, Willems F, Parmentier M.** Distribution and regulation of expression of the putative human chemokine receptor HCR in leukocyte populations. *Eur J Immunol* 2002; 32(2): 494-501.

- [39] **Yoshimura T, Oppenheim JJ.** Chemokine-like receptor 1 (CMKLR1) and chemokine (C-C motif) receptor-like 2 (CCRL2); two multifunctional receptors with unusual properties. *Exp Cell Res* 2011; 317(5): 674-684.
- [40] **Otero K, Vecchi A, Hirsch E, Kearley J, Vermi W, Del Prete A, Gonzalvo-Feo S, Garlanda C, Azzolino O, Salogni L et al.** Nonredundant role of CCRL2 in lung dendritic cell trafficking. *Blood* 2010; 116(16): 2942-2949.
- [41] **Monnier J, Lewen S, O'Hara E, Huang K, Tu H, Butcher EC, Zabel BA.** Expression, regulation, and function of atypical chemerin receptor CCRL2 on endothelial cells. *J Immunol* 2012; 189(2): 956-967.
- [42] **Biber K, Zuurman MW, Homan H, Boddeke HW.** Expression of L-CCR in HEK 293 cells reveals functional responses to CCL2, CCL5, CCL7, and CCL8. *J Leukoc Biol* 2003; 74(2): 243-251.
- [43] **Zabel BA, Nakae S, Zuniga L, Kim JY, Ohyama T, Alt C, Pan J, Suto H, Soler D, Allen SJ et al.** Mast cell-expressed orphan receptor CCRL2 binds chemerin and is required for optimal induction of IgE-mediated passive cutaneous anaphylaxis. *J Exp Med* 2008; 205(10): 2207-2220.
- [44] **Allavena P, Germano G, Marchesi F, Mantovani A.** Chemokines in cancer related inflammation. *Exp Cell Res* 2011; 317(5): 664-673.
- [45] **Lazennec G, Richmond A.** Chemokines and chemokine receptors: new insights into cancer-related inflammation. *Trends Mol Med* 2010; 16(3): 133-144.
- [46] **Yang C, Yu KD, Xu WH, Chen AX, Fan L, Ou ZL, Shao ZM.** Effect of genetic variants in two chemokine decoy receptor genes, DARC and CCBP2, on metastatic potential of breast cancer. *PLoS One* 2013; 8(11): e78901.
- [47] **Shen H, Schuster R, Stringer KF, Waltz SE, Lentsch AB.** The Duffy antigen/receptor for chemokines (DARC) regulates prostate tumor growth. *Faseb J* 2006; 20(1): 59-64.
- [48] **Wu FY, Ou ZL, Feng LY, Luo JM, Wang LP, Shen ZZ, Shao ZM.** Chemokine decoy receptor d6 plays a negative role in human breast cancer. *Mol Cancer Res* 2008; 6(8): 1276-1288.
- [49] **Anders HJ, Romagnani P, Mantovani A.** Pathomechanisms: homeostatic chemokines in health, tissue regeneration, and progressive diseases. *Trends Mol Med* 2014.
- [50] **Wang J, Shiozawa Y, Wang Y, Jung Y, Pienta KJ, Mehra R, Loberg R, Taichman RS.** The role of CXCR7/RDC1 as a chemokine receptor for CXCL12/SDF-1 in prostate cancer. *J Biol Chem* 2008; 283(7): 4283-4294.
- [51] **Zhu Z, Sun Z, Wang Z, Guo P, Zheng X, Xu H.** Prognostic impact of atypical chemokine receptor expression in patients with gastric cancer. *J Surg Res* 2013; 183(1): 177-183.
- [52] **Wang LP, Cao J, Zhang J, Wang BY, Hu XC, Shao ZM, Wang ZH, Ou ZL.** The human chemokine receptor CCRL2 suppresses chemotaxis and invasion by blocking CCL2-induced phosphorylation of p38 MAPK in human breast cancer cells. *Med Oncol.* 2015; 32(11): 254.
- [53] **Sarmadi P, Tunali G, Esendagli-Yilmaz G, Yilmaz KB, Esendagli G.** CRAM-A indicates IFN- γ -associated inflammatory response in breast cancer. *Molecular Immunology* 2015; 68: 692-698.

