

Chemokine/chemokine receptor pair CCL20/CCR6 in human colorectal malignancy: An overview

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Abstract

Chemokines belong to a superfamily of small, cytokine-like proteins, which induce multiple physiological functions, particularly cytoskeletal rearrangement and compartment-specific migration through their interaction with G-protein-coupled receptors. Chemokines and their receptors have been widely acknowledged as essential and selective mediators in leukocyte migration in inflammatory response. It is now established that the chemokine/chemokine receptor system is also used by cancer cells to direct lymphatic and haematogenous spreading and additionally has an impact on the site of metastatic growth of different tumours. In recent years an increasing number of studies have drawn attention to CC-chemokine cysteine motif chemokine ligand 20 (CCL20) and its physiological sole receptor CCR6 to play a role in the onset, development and metastatic spread of various gastrointestinal cancer entities. Among various cancer types CCR6 was also demonstrated to be significantly overexpressed in colorectal cancer (CRC) and stimulation by its physiological ligand CCL20 has been reported to promote CRC cell proliferation and migration *in vitro*. Further, the CCL20/CCR6 system apparently plays a role in the organ-selective liver metastasis of CRC. Here we review the literature on expression patterns of CCL20 and CCR6 and their physiological interactions as well as the currently presumed role of CCL20 and CCR6 in the formation of CRC and the development of liver metastasis, providing a potential basis for novel treatment strategies.

Key words: Chemokine/chemokine receptor pair; CCR6; Chemokine ligand 20; Colorectal cancer; Metastasis; Liver

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Core tip: Here we review the current literature published with respect to the expression pattern of chemokine/chemokine receptor pair cysteine motif chemokine ligand 20 (CCL20)/CCR6 and their physiological interactions as well as the currently presumed role of the CCL20/CCR6 system in the onset and development of colorectal cancer (CRC) and its apparent role in the organ-selective metastatic spread of CRC cells to the liver. Disrupting the chemokine/chemokine receptor interaction of CCL20/CCR6 may therefore be a promising novel treatment strategy in CRC and metastasis.

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INTRODUCTION

Chemokines constitute a superfamily of small structurally related chemotactic cytokines that direct the migration of leukocytes throughout the body, both under physiological and inflammatory conditions^[1,2]. Furthermore, they play a central role in many biological events, such as embryonic development, wound healing, angiogenesis, T-helper (Th)1/Th2 development, leukocyte homeostasis, lymphatic organ development, inflammatory diseases, tumour growth and metastasis. Chemokines exert their various biological functions by activating 7-transmembrane-domain G-protein coupled receptors on their target cells^[3].

Since 1987, when CXCL8 [interleukin (IL)-8] was isolated as the first chemokine, remarkable progress was made in the field of chemokine research^[4,5]. Until now, more than 50 different chemokines and 20 chemokine receptors have been discovered.

According to the presence and the relative position of the NH₂-terminal Cystein (C) residues, chemokines are structurally grouped into the CC, CXC, CX₃C and C chemokines. Alternatively, chemokines may be subdivided according to their function into inflammatory/inducible or homeostatic/constitutive chemokines^[2,6]. However, there are some members in the chemokine family which possess both inflammatory and homeostatic functions (Table 1).

To date, 20 different chemokine receptors have been characterized, which share many common structural features. They are composed of approximately 350 amino acids that are divided into a short and acidic N-terminal end, seven helical transmembrane domains with three intracellular and three extracellular hydrophilic loops and an intracellular C-terminus containing serine and threonine residues that act as phosphorylation sites during receptor regulation^[7].

The interactions between chemokines and their receptors are often not perfectly specific. However, every chemokine receptor binds only one group of chemokines. Thus, on the basis of their binding-properties, chemokine receptors are divided into different families, CXC chemokine receptors, CC chemokine receptors, CX₃C chemokine receptors and XC chemokine receptors that correspond to the four distinct subfamilies of the chemokines they bind.

While most chemokine receptors bind to multiple chemokines, providing a certain redundancy to the system. CCR6, however, is unique with respect to the fact that this receptor is found to bind only a single chemokine ligand, the homeostatic and inflammatory chemokine ligand 20 (CCL20). The selectivity of the CCR6/CCL20 ligand receptor interaction in contrast to the other chemokine receptor binding properties already suggests tightly regulated functional roles.

PHYSIOLOGICAL FUNCTIONS OF CCL20 AND CCR6

The cysteine-cysteine motif CCL20 - also known as liver-and activation-regulated chemokine (LARC), macrophage inflammatory protein-3a (MIP-3a), and exodus-1 - was discovered independently by three research groups using bioinformatic techniques^[8-10].

CCL20 is expressed in a variety of human tissues and by different types of immune cells. While CCL20 expression was predominantly observed in mucosa associated lymphatic tissue (MALT), other lymphatic tissues, lung and liver tissues^[9-11], its expression is virtually not detectable in spleen or bone marrow^[12,13]. CCL20 expression has further been demonstrated in inflammation related cells such as endothelial cells^[14,15], neutrophils^[16], natural killer (NK) cells^[17], Th17 cells^[18], B-cells^[19] and a variety of other immune cells^[13,20] as well as in normal tissue of the colon, pancreas, stomach, prostate, testis, uterine cervix and skin^[11].

The chemokine receptor CCR6 was originally described to be constitutively expressed in both lymphatic as well as in non-lymphatic tissue: predominantly in spleen, lymph nodes, appendix and pancreas and to a lesser extent in thymus, colon, small intestine, foetal liver and testis^[11]. CCR6 is further expressed on various leukocyte subsets, including immature dendritic cells (iDCs), B-cells, T-cells (pro-inflammatory Th17 cells, regulatory Treg cells), NKT cells and neutrophils^[11,21,22]. Early after its discovery, CCR6 was found to function in part as a key mediator linking iDCs to adaptive immune responses. In particular, it mediates the accurate positioning of iDCs in tissue^[23], a critical early step in the afferent part of adaptive immune induction. As iDCs take up antigen, mature and become activated, they down-regulate CCR6 and up-regulate CCR7. This "chemokine receptor switch" detaches the cell from tissue and enables its

Table 1 Nomenclature of selected chemokines, coding chromosomal location, major function and interacting receptor

	Synonymes	Location	Major function	Receptor
CC				
Chemokine				
CCL3	MIP-1 α /LD78 α	17q11.2	Inflammation	CCR1
CCL4	MIP-1 β	17q12	Inflammation	CCR1 CCR5
CCL5	RANTES	17q12	Inflammation	CCR5
CCL19	MIP-3 β /ELC/ Exodus-3	9p13.3	Homing	CCR7
CCL20	MIP-3 α /LARC/ Exodus-1	2q36.3	Homing, Inflammation	CCR6
CCL21	6Ckine/SLC/ Exodus-2	9p13.3	Homing	CCR7
CCL25	TECK/Ck β 15	19p13.2	Homing	CCR9
CXC				
Chemokine				
CXCL8	IL-8	4q13.3	Inflammation, Angiogenesis ¹	CXCR1 CXCR2
CXCL9	MIG	4q21.1	Inflammation, Angiogenesis ²	CXCR3
CXCL10	IP-10	4q21.1	Inflammation, Angiogenesis ²	CXCR3
CXCL11	I-TAC	4q21.1	Inflammation, Angiogenesis ²	CXCR3 CXCR7
CXCL12	SDF-1 α / β	10q11.21	Homing	CXCR4 CXCR7
CXCL13	BLC/BCA-1	4q21.1	Homing	CXCR5
Cx ₃ C				
Chemokine				
Cx ₃ CL1	Fractalkine	16q13	Inflammation	CX3CR1
C-Chemokine				
XCL1	Lymphotactin/ SCM-1 α	1q24.2	Homing	XCR1
XCL2	SCM-1 β	1q24.2	Homing	XCR1

¹Promotor of angiogenesis; ²Inhibitor of angiogenesis.

migration to draining lymph nodes in response to the CCR7 ligands CCL19 and CCL21 expressed on lymphatic endothelial cells^[24,25]. To date, the exact role of the CCL20/CCR6 axis in steady-state immune dynamics still has to be elucidated. However, the fact that opposing cell subtypes like pro-inflammatory Th17 and regulatory Treg cells express and respond to CCL20 alludes to a potential regulatory balance between immune activation and suppression and implies an intriguing feedback loop^[26].

Yamazaki *et al.*^[18] reported that lack of CCR6 in Th17 cells inhibits their own as well as Treg recruitment into inflammatory tissues, reasoning that CCR6 deficiency in T cells decreases the susceptibility to autoimmune diseases.

Among many other functions, the CCL20 and CCR6 system also plays an important and again tightly regulated physiological role in the colonic mucosa. Typically, CCL20 is weakly expressed in normal colonic mucosa. Yet, in response to an inflammatory stimulus CCL20 is strongly up-regulated. If mucosa cells grow in a polarized fashion, CCL20 secretion is located predominantly at the basolateral side of the cell and a pro-inflammatory stimulus through tumour

necrosis factor alpha (TNF- α) or interleukin 1 beta (IL-1 β) induces an increase in CCL20 secretion^[27-29]. The chemokine/chemokine receptor pair CCL20/CCR6 presumably plays a role in combating infectious microorganisms, as chemoattraction of CCR6 bearing dendritic cells *via* CCL20 may contribute to the qualitative differences between systemic and mucosal immunity as shown *in vitro* and *in vivo* by Cook *et al.*^[30] and Kucharzik *et al.*^[31]. CCR6 expression is also found in the normal colon mucosa^[32-35], but in contrast to CCL20, CCR6 expression is polarized predominantly to the apical side, thus, not accessible by CCL20 which is released from the basolateral side. Moreover, in contrast to CCL20 expression CCR6 expression is not influenced by inflammatory disease. The co-expression of ligand and receptor in the same cell opens up the possibility of autocrine and/or paracrine signalling, and consequently, as self-perpetuating cycle of recruitment within the intestinal epithelial cells^[36].

CCL20 AND CCR6 EXPRESSION IN COLORECTAL CANCER

For clarity, the literature describing the CCL20 and CCR6 expression in colorectal cancer (CRC) is summarized in Table 2.

In sharp contrast to mucosa cells, CRC cells express both CCL20 and its corresponding receptor CCR6 in a non-polarized fashion, providing a basis for efficient autocrine and paracrine loops. Compared to the low CCR6 expression rates in normal colon mucosa tissue and normal liver tissue, CCR6 expression is significantly up-regulated in colorectal malignancies such as CRC^[35-39] and colorectal liver metastasis^[39,40]. Thus, Hu *et al.*^[38] described high expression rates of CCR6 in CRC which were significantly associated with metachronous metastasis to liver or lung. However, these high CCR6 expression rates were not organ-specific, thus allowing no differentiation between metastasis to liver or lung.

Accordingly, CCL20 expression in CRC^[39-41] and colorectal liver metastasis^[37,40] is also significantly increased compared to the corresponding normal tissue, respectively. Functional assays demonstrated that CCL20 stimulation of CRC cells led to increased proliferation and migration of CRC cells *in vitro* as well as to phosphorylation of p130^{cas}, an adaptor/scaffolding protein associated with cytoskeletal and other focal adhesion proteins involved in adhesion and migration^[32,33]. Moreover, stimulation with CCL20 led to activation of the ERK-MAP kinase and Act pathways^[33]. To date, a large number of literature provides evidence that the expression of microRNAs (miRNAs) is dysregulated in cancer while it is yet unknown if this directly influences the carcinogenic process. In one of our studies we have outlined a functional interaction of miRNA-21 (miR-21) with the 3'UTR of CC-chemokine ligand CCL20. Further, we have demonstrated that

Table 2 CCL20 and CCR6 data in colorectal cancer available in the literature

Author	Year	Cell-lines	Clinical samples	Animal model	CCL20 determination	CCR6 determination	Functional assays	Ref.
Rossi	1997	Yes	No	No	SB, NB	No	No	[8]
Izadpanah	2001	Yes	Yes	No	IHC, PCR, ELISA	IHC, PCR	No	[27]
Fujiie	2001	Yes	No	No	ELISA, PCR	No	No	[28]
Kwon	2002	Yes	No	No	ELISA, PCR	No	No	[29]
Dellacasagrande	2003	No	Yes	Yes	No	PCR	AA	[43]
Yang	2005	Yes	No	No	No	FC, CM	cAMP, PP, CF	[32]
Brand	2006	Yes	Yes	No	PCR, IHC	PCR, IHC	PA, WA, APA	[33]
Ghadjar	2006	No	Yes	No	No	IHC	No	[34]
Rubie	2006	No	Yes	No	PCR, ELISA	PCR, WB	No	[35]
Liu	2011	Yes	No	Yes	IF, WB, PCR	FC, IF	No	[51]
Vicinus	2012	Yes	No	No	PCR, ELISA		miR, LUC	[42]
Hu	2013	No	Yes	No	No	IHC	No	[38]
Frick	2013	No	Yes	No	PCR, ELISA, IHC	PCR, IHC	No	[37]
Vicinus	2013	No	Yes	No	PCR, ELISA, IHC	No	No	[41]
Nandi	2014	Yes	Yes	Yes	ELISA, IHC	IHC, PCR, WB	No	[39]

SB: Southern blot; NB: Northern blot; PCR: Polymerase chain reaction; IHC: Immunohistochemistry; PA: Proliferation assay; ELISA: Enzyme-linked immunosorbent assay; AA: Actin assay; FC: Flow-cytometry; CM: Confocal microscopy; cAMP: cAMP assay; PP: Protein phosphorylation; CF: Calcium flux; WA: Wounding assay; APA: Apoptosis assay; WB: Western blot; IF: Immunofluorescence staining; miR: miRNA assay; LUC: Dual luciferase reporter assay.

miR-21 down-regulates CCL20 gene expression in three miR-21 transfected CRC cell lines, namely CaCo, SW480 and SW620^[42].

A study performed by Dellacasagrande *et al.*^[43] demonstrated that small CRC liver metastases express higher amounts of CCR6 compared to the surrounding tissue hypothesizing a role for CCR6 in the development of liver metastasis. CCR6 expression was also shown to be lower in large established liver metastases compared to the corresponding primary CRC tumours, which could be due to the fact that CCR6 expression may not be necessary for CRC cells that have already formed large established metastases^[33,34].

While the connection between inflammation and tumorigenesis is well established, the exact mechanisms linking these conditions have remained elusive. Successful evasion of the host's immune response is thought to be the main mechanism responsible for cancer development^[44]. Furthermore, communication between tumour cells and their microenvironment is widely thought to be crucial for tumour growth. Particularly, the interactions between tumour cells and infiltrating lymphocytes represent a powerful relationship that influences disease progression and patient prognosis^[45]. Therefore, the types of tumour-infiltrating lymphocytes are believed to affect the prognosis of CRC^[46]. Accumulating evidence indicates that although cancer patients exhibit a generalized immunosuppressive status, the inflammatory reaction at tumour site can foster tumour growth and progression. The perpetuation of chronic inflammation is largely achieved through positive feedback loops, which include inflammatory cells producing cytokines that induce chemokine synthesis in malignant and stromal cells leading to prolonged recruitment of inflammatory cells into

the tumour environment^[47]. The newly described IL-17 secreting subset of CD4⁺ T helper cells (Th17) are one of the most critical immune cell subsets in this respect and thus have tumour-promoting effect. In patients with hepatocellular carcinoma^[48], esophageal carcinoma^[49], prostate cancer^[50] and CRC^[51] high levels of intratumoural Th17 cells were found to be positively associated with poor prognosis. Also it has been suggested by Liu *et al.*^[51] that the expression of IL-17 in Th17 cells and macrophages is involved in VEGF production and angiogenesis and is associated with poor survival in patients with colorectal carcinoma. Chen *et al.*^[52] demonstrated that the distribution of helper T-lymphocytes is significantly different between colorectal tumour tissues and the peritumoural tissues. They reported that the percentage of infiltrating regulatory Th1 cells was significantly decreased, while the percentage of infiltrating suppressive Tregs-, type 1 regulatory T (Tr1)-, and IL-17-positive cells were significantly increased in tumour tissues compared to peritumoural tissues. Likewise the ratio of suppressive T-helper (Tregs-, Tr1-, IL-17-positive cells) to regulatory Th1 cells was significantly higher in tumour tissues than in peritumoural tissues. It is well known, that the migration of T cells is tightly regulated by chemokine/chemokine receptor interaction^[6]. Previous studies showed that recruitment of Th17 cells is governed by multiple pathways, including CCR2/CCL2, CCR4/CCL17/CCL22 and CCR6/CCL20^[53-56]. In a recent study Yu *et al.*^[57] showed that the CCR6/CCL20 pathway is the preferential chemoattractant for the trafficking of circulating Th17 cells into tumour tissue of cervical cancer.

Chin *et al.*^[58] demonstrated *in vitro* that IL-17 treatment induces an increase of CCR6 expression in CRC HCT-116 cells and that IL-17 induced cell migration is mediated through the ERK and p38

intracellular cascades and through the transcription factor NF- κ B.

It is also accepted that tumour-associated macrophages (TAMs) contribute to the increased production of CCL20 that recruits CCR6⁺ regulatory Tregs cells and promotes CRC in mice^[59]. Another study demonstrated in a CCR6 mice knock out model that intestinal tumorigenesis driven by CCL20/CCR6 interactions may be driven by macrophage recruitment into the intestine as well as proliferation of neoplastic epithelial cells^[39].

It is also well known that immune cell subsets, when chronically activated, directly foster tumour development and promote cancer progression^[60,61]. Kryczek *et al.*^[62] focused on the interaction between IL-22 secreting (IL-22⁺) immune cells and cancer (stem) cells. They demonstrated that IL-22⁺ CD4⁺ T cells promote CRC stemness *via* STAT3 transkription factor activation and induction of the methyltransferase DOT1L and that is relevant for outcome in patients with CRC.

CCL20 AND CCR6 IN TUMOUR METASTASIS

The morbidity and mortality of patients with cancer is mainly attributed to distant tumour metastasis^[63]. For instance, the 5-year survival rate for patients with tumours restricted only to the colon decreases dramatically from 90% to 10% in the presence of distant metastasis^[64,65]. The current understanding of the metastatic process is far from sufficient, but various experimental and clinical findings support the thesis that metastatic dissemination is a result of a complex multistep molecular machinery which does not occur randomly, but as a result of different coordinated organ-specific processes^[66]. Thus, some organs, such as liver, lung, brain and bones are frequently involved in tumour metastasis, while other organs like muscle and mucosal membranes of the gastrointestinal tract are seldomly affected by metastasis. Such differences cannot solely be explained by anatomic differences in blood and lymphatic supply.

In the last decades different concepts were postulated trying to explain the metastatic behaviour of different tumour entities. The most central of these theories is the "seed and soil" theory of metastasis, first proposed in 1889 by Stephen Paget^[67]. Paget predicted that cancer cells (the "seed") can survive and proliferate only in secondary sites (the "soil") that produce growth factors appropriate to that type of cell, and this theory has largely withstood the test of time^[68]. According to the "adhesion theory" cancer cell extravasation is triggered by certain adhesion molecules that are expressed on endothelial cells in an organ specific manner^[69]. Another theory gaining popularity in recent years suggests that epithelial-mesenchymal transition (EMT) may contribute to the

metastatic process. The EMT phenotype in cancer has been associated with a decrease in tumour growth, increased resistance to apoptosis, increased motility and invasiveness and enhanced metastatic ability^[70]. As these phenotypic transitions are reversible it is hypothesized that tumour cells may transform back into an epithelial phenotype once they have reached their destination thus facilitating tumour growth in the secondary site^[71].

The "homing theory" proposes that different organs produce chemotactic factors which can attract the corresponding chemokine receptor bearing cancers cells so that they migrate into distinct organs towards the chemokine gradient^[72-74]. Thus, various publications support the involvement of chemokine/chemokine receptor interactions in tumour metastasis^[75-81] and demonstrated that blockade of the respective chemokine/chemokine receptor interaction leads to a reduction of metastasis development *in vivo*^[82-84].

CRC is a tumour with a high propensity for metastatic spread, mostly affecting liver and lungs. Liver metastases develop in approximately 50% of CRC patients at some point in the course of their disease and worsen the prognosis for patient survival dramatically^[64]. With respect to colorectal liver metastasis CCR6 is of special importance as well as its unique chemokine ligand CCL20, which is predominantly expressed in mucosa-associated and lymphoid tissues and in the liver^[11]. Within the liver, the CCL20 expression profile is not random, but predominantly limited to the periportal area. Such local designation may be explained by the fact, that the periportal area is the physiological entry site of the blood draining from most of the lower gastrointestinal tract. Also potential invading microorganisms are likely to use this mode of entry. Therefore, the periportal expression of CCL20 may be important for recruiting CCR6-expressing dendritic cells as a response to encounter with microorganisms. In addition, the periportal area is also the presumed initial entry site for cancer cells, which originate from cancers of the colon or proximal rectum, which have gained access to the portal bloodstream^[10,11,20].

Recent studies explored the question, if there was any physiological example of CCL20/CCR6 interaction resulting in recruitment of CCR6-expressing cells to the liver. Indeed, CCL20 is weakly expressed in normal liver, but after an inflammatory stimulus CCL20 is strongly up-regulated and this results in the chemoattraction of CCR6 bearing T-lymphocytes into the liver *in vitro* and *in vivo*^[20,85].

If you assemble all these pieces of evidence, you may hypothesize, that the expression of CCL20 within the periportal area of the liver may be "the soil" for CCR6 expressing CRC cells, which detached from the primary tumour, "the seed". If micrometastases are present, the autocrine mechanism of CCL20/CCR6 interactions might contribute to enhanced tumour growth. On the other hand, the tumour itself may provide support for tumour growth in a paracrine

manner by triggering CCL20 production in the surrounding liver tissue.

Two independent studies performed by Ghadjar *et al.*^[34] and Hu *et al.*^[38] demonstrated in concordance with the hypothesis stated above that increased CCR6 expression on primary CRCs are an independent risk factor for distant metastasis. Furthermore, multivariate analysis showed that along with the expression of CCR6 also CXCR2 expression and the preoperative serum carcinoembryonic antigen (CEA) level were the major independent factors affecting distant metastasis^[38].

Moreover, Ghadjar *et al.*^[34] demonstrated a significant up-regulation of CCR6 in CRC compared to the colonic mucosa. Interestingly, the CCR6 expression in liver metastatic tissue was significantly down-regulated as compared to the primary tumour. In a parallel study, we were able to confirm the finding by Ghadjar *et al.*^[36] namely the up-regulation of CCR6 from colonic mucosa to CRC. Further, we could demonstrate that patients with CRC who experienced liver metastasis, express significantly higher amounts of CCL20 in their liver compared to controls without metastases, suggesting an association between CCL20/CCR6 expression in human CRC and the promotion of colorectal liver metastasis. Moreover we demonstrated that CCL20 expression correlates clinicopathologically with the transition of an inflammatory disease to the adenoma and adenocarcinoma sequence. Likely, serum CCL20 was recently suggested as an independent predictive factor for liver metastasis correlating high levels of serum CCL20 with poor prognosis^[86].

Taken together, these data strongly support the hypothesis that up-regulation of CCR6 expression and high amounts of CCL20 in the organ of metastatic spread, are correlated with liver metastasis, suggesting that the chemokine/chemokine receptor CCL20/CCR6 system plays a central role in tumour progression and metastasis.

However, this hypothesis needs to be further validated by functional studies.

The identification of key targets promoting metastasis is important for the development of new treatments and inhibiting metastasis development by interfering with the chemokine/chemokine receptor is a promising strategy for adjuvant treatments^[87,88].

Moreover, it would be important to investigate if a neutralizing antibody against CCR6 can block the influence of CCL20 on proliferation and migration *in vitro*. Subsequently, the concept of liver metastasis facilitated by CCL20/CCR6 interactions should also be validated *in vivo* by knock-out animal models.

CONCLUSION

The chemokine/chemokine receptor pair CCL20/CCR6 is involved in CRC leading to proliferation and migration *via* autocrine and/or paracrine mechanisms.

Moreover, the formation of colorectal liver metastasis might be advantaged by CCL20/CCR6 interactions.

The identification of key targets promoting disease progression and metastasis is of great interest for the development of specific treatment strategies. Attempts to inhibit metastasis by interfering with chemokine/chemokine receptor interactions is a promising new therapeutic strategy. Various small-molecule chemokine receptor antagonists compounds are currently undergoing development in phase I to III studies in infectious and autoimmune diseases and more recently also in cancer.

REFERENCES

- 1 **Yoshie O**, Imai T, Nomiya H. Chemokines in immunity. *Adv Immunol* 2001; **78**: 57-110 [PMID: 11432208]
- 2 **Zlotnik A**, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* 2000; **12**: 121-127 [PMID: 10714678]
- 3 **Murphy PM**. The molecular biology of leukocyte chemoattractant receptors. *Annu Rev Immunol* 1994; **12**: 593-633 [PMID: 8011292]
- 4 **Yoshimura T**, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc Natl Acad Sci USA* 1987; **84**: 9233-9237 [PMID: 3480540]
- 5 **Walz A**, Peveri P, Aschauer H, Baggiolini M. Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. *Biochem Biophys Res Commun* 1987; **149**: 755-761 [PMID: 3322281]
- 6 **Moser B**, Loetscher P. Lymphocyte traffic control by chemokines. *Nat Immunol* 2001; **2**: 123-128 [PMID: 11175804]
- 7 **Allen SJ**, Crown SE, Handel TM. Chemokine: receptor structure, interactions, and antagonism. *Annu Rev Immunol* 2007; **25**: 787-820 [PMID: 17291188]
- 8 **Rossi DL**, Vicari AP, Franz-Bacon K, McClanahan TK, Zlotnik A. Identification through bioinformatics of two new macrophage proinflammatory human chemokines: MIP-3alpha and MIP-3beta. *J Immunol* 1997; **158**: 1033-1036 [PMID: 9013939]
- 9 **Hromas R**, Gray PW, Chantry D, Godiska R, Krathwohl M, Fife K, Bell GI, Takeda J, Aronica S, Gordon M, Cooper S, Broxmeyer HE, Klemsz MJ. Cloning and characterization of exodus, a novel beta-chemokine. *Blood* 1997; **89**: 3315-3322 [PMID: 9129037]
- 10 **Hieshima K**, Imai T, Opendakker G, Van Damme J, Kusuda J, Tei H, Sakaki Y, Takatsuki K, Miura R, Yoshie O, Nomiya H. Molecular cloning of a novel human CC chemokine liver and activation-regulated chemokine (LARC) expressed in liver. Chemotactic activity for lymphocytes and gene localization on chromosome 2. *J Biol Chem* 1997; **272**: 5846-5853 [PMID: 9038201]
- 11 **Schutysse E**, Struyf S, Van Damme J. The CC chemokine CCL20 and its receptor CCR6. *Cytokine Growth Factor Rev* 2003; **14**: 409-426 [PMID: 12948524]
- 12 **Yoshie O**, Imai T, Nomiya H. Novel lymphocyte-specific CC chemokines and their receptors. *J Leukoc Biol* 1997; **62**: 634-644 [PMID: 9365118]
- 13 **Hromas R**, Kim CH, Klemsz M, Krathwohl M, Fife K, Cooper S, Schnizlein-Bick C, Broxmeyer HE. Isolation and characterization of Exodus-2, a novel C-C chemokine with a unique 37-amino acid carboxyl-terminal extension. *J Immunol* 1997; **159**: 2554-2558 [PMID: 9300671]
- 14 **Meissner A**, Zilles O, Varona R, Jozefowski K, Ritter U, Marquez G, Hallmann R, Korner H. CC chemokine ligand 20 partially controls adhesion of naive B cells to activated endothelial cells under shear stress. *Blood* 2003; **102**: 2724-2727 [PMID: 12816871]
- 15 **Kriehuber E**, Breiteneder-Geleff S, Groeger M, Soleiman A, Schoppmann SF, Stingl G, Kerjaschki D, Maurer D. Isolation

- and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. *J Exp Med* 2001; **194**: 797-808 [PMID: 11560995 DOI: 10.1084/jem.194.6.797]
- 16 **Scapini P**, Laudanna C, Pinardi C, Allavena P, Mantovani A, Sozzani S, Cassatella MA. Neutrophils produce biologically active macrophage inflammatory protein-3alpha (MIP-3alpha)/CCL20 and MIP-3beta/CCL19. *Eur J Immunol* 2001; **31**: 1981-1988 [PMID: 11449350]
 - 17 **Cella M**, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, Doherty JM, Mills JC, Colonna M. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* 2009; **457**: 722-725 [PMID: 18978771 DOI: 10.1038/nature07537]
 - 18 **Yamazaki T**, Yang XO, Chung Y, Fukunaga A, Nurieva R, Pappu B, Martin-Orozco N, Kang HS, Ma L, Panopoulos AD, Craig S, Watowich SS, Jetten AM, Tian Q, Dong C. CCR6 regulates the migration of inflammatory and regulatory T cells. *J Immunol* 2008; **181**: 8391-8401 [PMID: 19050256]
 - 19 **Bowman EP**, Campbell JJ, Soler D, Dong Z, Manlongat N, Picarella D, Hardy RR, Butcher EC. Developmental switches in chemokine response profiles during B cell differentiation and maturation. *J Exp Med* 2000; **191**: 1303-1318 [PMID: 10770798]
 - 20 **Shimizu Y**, Murata H, Kashi Y, Hirano K, Kunitani H, Higuchi K, Watanabe A. CC-chemokine receptor 6 and its ligand macrophage inflammatory protein 3alpha might be involved in the amplification of local necroinflammatory response in the liver. *Hepatology* 2001; **34**: 311-319 [PMID: 11481616]
 - 21 **Ito T**, Carson WF, Cavassani KA, Connett JM, Kunkel SL. CCR6 as a mediator of immunity in the lung and gut. *Exp Cell Res* 2011; **317**: 613-619 [PMID: 21376174 DOI: 10.1016/j.yexcr.2010.12.018]
 - 22 **Lee AY**, Eri R, Lyons AB, Grimm MC, Korner H. CC Chemokine Ligand 20 and Its Cognate Receptor CCR6 in Mucosal T Cell Immunology and Inflammatory Bowel Disease: Odd Couple or Axis of Evil? *Front Immunol* 2013; **4**: 194 [PMID: 23874340 DOI: 10.3389/fimmu.2013.00194]
 - 23 **Dieu MC**, Vanbervliet B, Vicari A, Bridon JM, Oldham E, Ait-Yahia S, Briere F, Zlotnik A, Lebecque S, Caux C. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* 1998; **188**: 373-386 [PMID: 9670049]
 - 24 **Sallusto F**, Schaerli P, Loetscher P, Schaniel C, Lenig D, Mackay CR, Qin S, Lanzavecchia A. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol* 1998; **28**: 2760-2769 [PMID: 9754563]
 - 25 **Caux C**, Ait-Yahia S, Chemin K, de Bouteiller O, Dieu-Nosjean MC, Homey B, Massacrier C, Vanbervliet B, Zlotnik A, Vicari A. Dendritic cell biology and regulation of dendritic cell trafficking by chemokines. *Springer Semin Immunopathol* 2000; **22**: 345-369 [PMID: 11155441]
 - 26 **Comerford I**, Bunting M, Fenix K, Haylock-Jacobs S, Litchfield W, Harata-Lee Y, Turvey M, Brazzatti J, Gregor C, Nguyen P, Kara E, McColl SR. An immune paradox: how can the same chemokine axis regulate both immune tolerance and activation?: CCR6/CCL20: a chemokine axis balancing immunological tolerance and inflammation in autoimmune disease. *Bioessays* 2010; **32**: 1067-1076 [PMID: 20954179 DOI: 10.1002/bies.201000063]
 - 27 **Izadpanah A**, Dwinell MB, Eckmann L, Varki NM, Kagnoff MF. Regulated MIP-3alpha/CCL20 production by human intestinal epithelium: mechanism for modulating mucosal immunity. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G710-G719 [PMID: 11254498]
 - 28 **Fujiie S**, Hieshima K, Izawa D, Nakayama T, Fujisawa R, Ohyanagi H, Yoshie O. Proinflammatory cytokines induce liver and activation-regulated chemokine/macrophage inflammatory protein-3alpha/CCL20 in mucosal epithelial cells through NF-kappaB [correction of NK-kappaB]. *Int Immunol* 2001; **13**: 1255-1263 [PMID: 11581170]
 - 29 **Kwon JH**, Keates S, Bassani L, Mayer LF, Keates AC. Colonic epithelial cells are a major site of macrophage inflammatory protein 3alpha (MIP-3alpha) production in normal colon and inflammatory bowel disease. *Gut* 2002; **51**: 818-826 [PMID: 12427784]
 - 30 **Cook DN**, Prosser DM, Forster R, Zhang J, Kuklin NA, Abbondanzo SJ, Niu XD, Chen SC, Manfra DJ, Wiekowski MT, Sullivan LM, Smith SR, Greenberg HB, Narula SK, Lipp M, Lira SA. CCR6 mediates dendritic cell localization, lymphocyte homeostasis, and immune responses in mucosal tissue. *Immunity* 2000; **12**: 495-503 [PMID: 10843382]
 - 31 **Kucharzik T**, Hudson JT, Waikel RL, Martin WD, Williams IR. CCR6 expression distinguishes mouse myeloid and lymphoid dendritic cell subsets: demonstration using a CCR6 EGFP knock-in mouse. *Eur J Immunol* 2002; **32**: 104-112 [PMID: 11754009]
 - 32 **Yang CC**, Ogawa H, Dwinell MB, McCole DF, Eckmann L, Kagnoff MF. Chemokine receptor CCR6 transduces signals that activate p130Cas and alter cAMP-stimulated ion transport in human intestinal epithelial cells. *Am J Physiol Cell Physiol* 2005; **288**: C321-C328 [PMID: 15483227]
 - 33 **Brand S**, Olszak T, Beigel F, Diebold J, Otte JM, Eichhorst ST, Göke B, Dambacher J. Cell differentiation dependent expressed CCR6 mediates ERK-1/2, SAPK/JNK, and Akt signaling resulting in proliferation and migration of colorectal cancer cells. *J Cell Biochem* 2006; **97**: 709-723 [PMID: 16215992]
 - 34 **Ghadjar P**, Coupland SE, Na IK, Noutsias M, Letsch A, Stroux A, Bauer S, Buhr HJ, Thiel E, Scheibenbogen C, Keilholz U. Chemokine receptor CCR6 expression level and liver metastases in colorectal cancer. *J Clin Oncol* 2006; **24**: 1910-1916 [PMID: 16622267]
 - 35 **Rubie C**, Oliveira V, Kempf K, Wagner M, Tilton B, Rau B, Kruse B, König J, Schilling M. Involvement of chemokine receptor CCR6 in colorectal cancer metastasis. *Tumour Biol* 2006; **27**: 166-174 [PMID: 16641550]
 - 36 **Ghadjar P**, Rubie C, Aebersold DM, Keilholz U. The chemokine CCL20 and its receptor CCR6 in human malignancy with focus on colorectal cancer. *Int J Cancer* 2009; **125**: 741-745 [PMID: 19480006 DOI: 10.1002/ijc.24468]
 - 37 **Rubie C**, Frick VO, Ghadjar P, Wagner M, Justinger C, Graeber S, Sperling J, Kollmar O, Schilling MK. Effect of preoperative FOLFOX chemotherapy on CCL20/CCR6 expression in colorectal liver metastases. *World J Gastroenterol* 2011; **17**: 3109-3116 [PMID: 21912453 DOI: 10.3748/wjg.v17.i26.3109]
 - 38 **Hu D**, Du C, Xue W, Dou F, Yao Y, Gu J. The expression of chemokine receptors CCR6, CXCR2 and CXCR4 is not organ-specific for distant metastasis in colorectal cancer: a comparative study. *Histopathology* 2013; **63**: 167-173 [PMID: 23758411 DOI: 10.1111/his.12127]
 - 39 **Nandi B**, Pai C, Huang Q, Prabhala RH, Munshi NC, Gold JS. CCR6, the sole receptor for the chemokine CCL20, promotes spontaneous intestinal tumorigenesis. *PLoS One* 2014; **9**: e97566 [PMID: 24866282 DOI: 10.1371/journal.pone.0097566]
 - 40 **Frick VO**, Rubie C, Kölsch K, Wagner M, Ghadjar P, Graeber S, Glanemann M. CCR6/CCL20 chemokine expression profile in distinct colorectal malignancies. *Scand J Immunol* 2013; **78**: 298-305 [PMID: 23790181 DOI: 10.1111/sji.12087]
 - 41 **Vicinus B**, Rubie C, Stegmaier N, Frick VO, Kölsch K, Kauffels A, Ghadjar P, Wagner M, Glanemann M. miR-21 and its target gene CCL20 are both highly overexpressed in the microenvironment of colorectal tumors: significance of their regulation. *Oncol Rep* 2013; **30**: 1285-1292 [PMID: 23817679 DOI: 10.3892/or.2013.2580]
 - 42 **Vicinus B**, Rubie C, Faust SK, Frick VO, Ghadjar P, Wagner M, Graeber S, Schilling MK. miR-21 functionally interacts with the 3' UTR of chemokine CCL20 and down-regulates CCL20 expression in miR-21 transfected colorectal cancer cells. *Cancer Lett* 2012; **316**: 105-112 [PMID: 22099878 DOI: 10.1016/j.canlet.2011.10.031]
 - 43 **Dellacasagrande J**, Schreurs OJ, Hofgaard PO, Omholt H, Steinsvoll S, Schenck K, Bogen B, Dembic Z. Liver metastasis of cancer facilitated by chemokine receptor CCR6. *Scand J Immunol* 2003; **57**: 534-544 [PMID: 12791091]
 - 44 **Swann JB**, Smyth MJ. Immune surveillance of tumors. *J Clin Invest* 2007; **117**: 1137-1146 [PMID: 17476343]
 - 45 **Quail DF**, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med* 2013; **19**: 1423-1437 [PMID:

- 24202395 DOI: 10.1038/nm.3394]
- 46 **de la Cruz-Merino L**, Henaó Carrasco F, Vicente Baz D, Nogales Fernández E, Reina Zoilo JJ, Codes Manuel de Villena M, Pulido EG. Immune microenvironment in colorectal cancer: a new hallmark to change old paradigms. *Clin Dev Immunol* 2011; **2011**: 174149 [PMID: 22162710 DOI: 10.1155/2011/174149]
 - 47 **Grivennikov SI**, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; **140**: 883-899 [PMID: 20303878 DOI: 10.1016/j.cell.2010.01.025]
 - 48 **Liao R**, Sun J, Wu H, Yi Y, Wang JX, He HW, Cai XY, Zhou J, Cheng YF, Fan J, Qiu SJ. High expression of IL-17 and IL-17RE associate with poor prognosis of hepatocellular carcinoma. *J Exp Clin Cancer Res* 2013; **32**: 3 [PMID: 23305119 DOI: 10.1186/1756-9966-32-3]
 - 49 **Chen D**, Jiang R, Mao C, Shi L, Wang S, Yu L, Hu Q, Dai D, Xu H. Chemokine/chemokine receptor interactions contribute to the accumulation of Th17 cells in patients with esophageal squamous cell carcinoma. *Hum Immunol* 2012; **73**: 1068-1072 [PMID: 22863447 DOI: 10.1016/j.humimm.2012.07.333]
 - 50 **Zhang Q**, Liu S, Ge D, Zhang Q, Xue Y, Xiong Z, Abdel-Mageed AB, Myers L, Hill SM, Rowan BG, Sartor O, Melamed J, Chen Z, You Z. Interleukin-17 promotes formation and growth of prostate adenocarcinoma in mouse models. *Cancer Res* 2012; **72**: 2589-2599 [PMID: 22461511 DOI: 10.1158/0008-5472.CAN-11-3795]
 - 51 **Liu J**, Duan Y, Cheng X, Chen X, Xie W, Long H, Lin Z, Zhu B. IL-17 is associated with poor prognosis and promotes angiogenesis via stimulating VEGF production of cancer cells in colorectal carcinoma. *Biochem Biophys Res Commun* 2011; **407**: 348-354 [PMID: 21396350 DOI: 10.1016/j.bbrc.2011.03.021]
 - 52 **Chen J**, Chen Z. The effect of immune microenvironment on the progression and prognosis of colorectal cancer. *Med Oncol* 2014; **31**: 82 [PMID: 25034363 DOI: 10.1007/s12032-014-0082-9]
 - 53 **Sato W**, Aranami T, Yamamura T. Cutting edge: Human Th17 cells are identified as bearing CCR2+CCR5- phenotype. *J Immunol* 2007; **178**: 7525-7529 [PMID: 17548586]
 - 54 **Acosta-Rodriguez EV**, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, Sallusto F, Napolitani G. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat Immunol* 2007; **8**: 639-646 [PMID: 17486092]
 - 55 **Hirata T**, Osuga Y, Takamura M, Kodama A, Hirota Y, Koga K, Yoshino O, Harada M, Takemura Y, Yano T, Taketani Y. Recruitment of CCR6-expressing Th17 cells by CCL 20 secreted from IL-1 beta-, TNF-alpha-, and IL-17A-stimulated endometrial stromal cells. *Endocrinology* 2010; **151**: 5468-5476 [PMID: 20881253 DOI: 10.1210/en.2010-0398]
 - 56 **Hirota K**, Yoshitomi H, Hashimoto M, Maeda S, Teradaira S, Sugimoto N, Yamaguchi T, Nomura T, Ito H, Nakamura T, Sakaguchi N, Sakaguchi S. Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J Exp Med* 2007; **204**: 2803-2812 [PMID: 18025126]
 - 57 **Yu Q**, Lou XM, He Y. Preferential recruitment of Th17 cells to cervical cancer via CCR6-CCL20 pathway. *PLoS One* 2015; **10**: e0120855 [PMID: 25768730 DOI: 10.1371/journal.pone.0120855]
 - 58 **Chin CC**, Chen CN, Kuo HC, Shi CS, Hsieh MC, Kuo YH, Tung SY, Lee KF, Huang WS. Interleukin-17 induces CC chemokine receptor 6 expression and cell migration in colorectal cancer cells. *J Cell Physiol* 2015; **230**: 1430-1437 [PMID: 25201147 DOI: 10.1002/jcp.24796]
 - 59 **Liu J**, Zhang N, Li Q, Zhang W, Ke F, Leng Q, Wang H, Chen J, Wang H. Tumor-associated macrophages recruit CCR6+ regulatory T cells and promote the development of colorectal cancer via enhancing CCL20 production in mice. *PLoS One* 2011; **6**: e19495 [PMID: 21559338 DOI: 10.1371/journal.pone.0019495]
 - 60 **Ben-Neriah Y**, Karin M. Inflammation meets cancer, with NF- κ B as the matchmaker. *Nat Immunol* 2011; **12**: 715-723 [PMID: 2172280 DOI: 10.1038/ni.2060]
 - 61 **Coussens LM**, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation: a magic bullet? *Science* 2013; **339**: 286-291 [PMID: 23329041 DOI: 10.1126/science.1232227]
 - 62 **Kryczek I**, Lin Y, Nagarsheth N, Peng D, Zhao L, Zhao E, Vatan L, Szeliga W, Dou Y, Owens S, Zgodzinski W, Majewski M, Wallner G, Fang J, Huang E, Zou W. IL-22(+)CD4(+) T cells promote colorectal cancer stemness via STAT3 transcription factor activation and induction of the methyltransferase DOT1L. *Immunity* 2014; **40**: 772-784 [PMID: 24816405 DOI: 10.1016/j.immuni.2014.03.010]
 - 63 **Murphy PM**. Chemokines and the molecular basis of cancer metastasis. *N Engl J Med* 2001; **345**: 833-835 [PMID: 11556308]
 - 64 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96 [PMID: 18287387 DOI: 10.3322/CA.2007.0010]
 - 65 **Booth RA**. Minimally invasive biomarkers for detection and staging of colorectal cancer. *Cancer Lett* 2007; **249**: 87-96 [PMID: 17275174]
 - 66 **Nicolson GL**. Cancer progression and growth: relationship of paracrine and autocrine growth mechanisms to organ preference of metastasis. *Exp Cell Res* 1993; **204**: 171-180 [PMID: 8382620]
 - 67 **Paget S**. The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev* 1989; **8**: 98-101 [PMID: 2673568]
 - 68 **Fidler IJ**. Seed and soil revisited: contribution of the organ microenvironment to cancer metastasis. *Surg Oncol Clin N Am* 2001; **10**: 257-269, vii-viii [PMID: 11382586]
 - 69 **Qian F**, Hanahan D, Weissman IL. L-selectin can facilitate metastasis to lymph nodes in a transgenic mouse model of carcinogenesis. *Proc Natl Acad Sci USA* 2001; **98**: 3976-3981 [PMID: 11274419]
 - 70 **Kang Y**, Massagué J. Epithelial-mesenchymal transitions: twist in development and metastasis. *Cell* 2004; **118**: 277-279 [PMID: 15294153]
 - 71 **Stover DG**, Bierie B, Moses HL. A delicate balance: TGF-beta and the tumor microenvironment. *J Cell Biochem* 2007; **101**: 851-861 [PMID: 17486574]
 - 72 **Homey B**, Müller A, Zlotnik A. Chemokines: agents for the immunotherapy of cancer? *Nat Rev Immunol* 2002; **2**: 175-184 [PMID: 11913068]
 - 73 **Ratajczak MZ**, Zuba-Surma E, Kucia M, Reza R, Wojakowski W, Ratajczak J. The pleiotropic effects of the SDF-1-CXCR4 axis in organogenesis, regeneration and tumorigenesis. *Leukemia* 2006; **20**: 1915-1924 [PMID: 16900209]
 - 74 **Kucia M**, Reza R, Miekus K, Wanzeck J, Wojakowski W, Janowska-Wieczorek A, Ratajczak J, Ratajczak MZ. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. *Stem Cells* 2005; **23**: 879-894 [PMID: 15888687]
 - 75 **Müller A**, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verástegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001; **410**: 50-56 [PMID: 11242036]
 - 76 **Wiley HE**, Gonzalez EB, Maki W, Wu MT, Hwang ST. Expression of CC chemokine receptor-7 and regional lymph node metastasis of B16 murine melanoma. *J Natl Cancer Inst* 2001; **93**: 1638-1643 [PMID: 11698568]
 - 77 **Murakami T**, Maki W, Cardones AR, Fang H, Tun Kyi A, Nestle FO, Hwang ST. Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. *Cancer Res* 2002; **62**: 7328-7334 [PMID: 12499276]
 - 78 **Mashino K**, Sadanaga N, Yamaguchi H, Tanaka F, Ohta M, Shibuta K, Inoue H, Mori M. Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. *Cancer Res* 2002; **62**: 2937-2941 [PMID: 12019175]
 - 79 **Ding Y**, Shimada Y, Maeda M, Kawabe A, Kaganai J, Komoto I, Hashimoto Y, Miyake M, Hashida H, Imamura M. Association of CC chemokine receptor 7 with lymph node metastasis of esophageal squamous cell carcinoma. *Clin Cancer Res* 2003; **9**: 3406-3412 [PMID: 12960129]
 - 80 **Taichman RS**, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res* 2002;

- 62: 1832-1837 [PMID: 11912162]
- 81 **Letsch A**, Keilholz U, Schadendorf D, Assfalg G, Asemisen AM, Thiel E, Scheibenbogen C. Functional CCR9 expression is associated with small intestinal metastasis. *J Invest Dermatol* 2004; **122**: 685-690 [PMID: 15086554]
- 82 **Takenaga M**, Tamamura H, Hiramatsu K, Nakamura N, Yamaguchi Y, Kitagawa A, Kawai S, Nakashima H, Fujii N, Igarashi R. A single treatment with microcapsules containing a CXCR4 antagonist suppresses pulmonary metastasis of murine melanoma. *Biochem Biophys Res Commun* 2004; **320**: 226-232 [PMID: 15207725]
- 83 **Tamamura H**, Hori A, Kanzaki N, Hiramatsu K, Mizumoto M, Nakashima H, Yamamoto N, Otaka A, Fujii N. T140 analogs as CXCR4 antagonists identified as anti-metastatic agents in the treatment of breast cancer. *FEBS Lett* 2003; **550**: 79-83 [PMID: 12935890]
- 84 **Zeelenberg IS**, Ruuls-Van Stalle L, Roos E. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. *Cancer Res* 2003; **63**: 3833-3839 [PMID: 12839981]
- 85 **Varona R**, Cadenas V, Gómez L, Martínez-A C, Márquez G. CCR6 regulates CD4+ T-cell-mediated acute graft-versus-host disease responses. *Blood* 2005; **106**: 18-26 [PMID: 15774622]
- 86 **Iwata T**, Tanaka K, Inoue Y, Toiyama Y, Hiro J, Fujikawa H, Okugawa Y, Uchida K, Mohri Y, Kusunoki M. Macrophage inflammatory protein-3 alpha (MIP-3a) is a novel serum prognostic marker in patients with colorectal cancer. *J Surg Oncol* 2013; **107**: 160-166 [PMID: 22926691 DOI: 10.1002/jso.23247]
- 87 **Cambien B**, Karimjee BF, Richard-Fiardo P, Bziouech H, Barthel R, Millet MA, Martini V, Birnbaum D, Scoazec JY, Abello J, Al Saati T, Johnson MG, Sullivan TJ, Medina JC, Collins TL, Schmid-Alliana A, Schmid-Antomarchi H. Organ-specific inhibition of metastatic colon carcinoma by CXCR3 antagonism. *Br J Cancer* 2009; **100**: 1755-1764 [PMID: 19436305 DOI: 10.1038/sj.bjc.6605078]
- 88 **Varney ML**, Singh S, Li A, Mayer-Ezell R, Bond R, Singh RK. Small molecule antagonists for CXCR2 and CXCR1 inhibit human colon cancer liver metastases. *Cancer Lett* 2011; **300**: 180-188 [PMID: 21035946 DOI: 10.1016/j.canlet.2010.10.004]

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