Tumor Immunotherapy by Utilizing a Double-Edged Sword, Chemokines

Tumor immunotherapy by utilizing a double-edged sword, chemokines

Naofumi Mukaida,* So-ichiro Sasaki, and Tomohisa Baba

Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University

Kakuma-machi, Kanazawa 920-1192, Japan

N. Mukaida (✉) ・S. Sasaki・T. Baba

* To whom all correspondence should be addressed to: Naofumi Mukaida, MD, PhD, Division of Molecular Bioregualtion, Cancer Research Institute, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan.

eTel: +81-76-264-6735

Fax: +81-76-234-4520

E-mail: mukaida@staff.kanazawa-u.ac.jp

Abstract

Both innate and adaptive immune responses have an essential role in protection against tumor cells. Various types of immune cells such as dendritic cells and lymphocytes contribute to the establishment of immune responses to tumor cells. Chemokines, a family consisting of more than 40 related chemoattractant proteins, have a crucial role in the control of the recruitment of immune cells needed for the induction and activation of tumor immunity. Based on these properties, several chemokines have been utilized in pre-clinical models to augment tumor immunity by enhancing the migration and activation of immune cells. Paradoxically, tumor tissues use chemokines to evade immunosurveillance by attracting immune suppressive cells. Moreover, chemokines can mediate survival and migration of tumor cells, and promote new blood vessel formation, thereby leading to tumor progression and metastasis. Thus, a number of therapeutic strategies have been proposed to target chemokines, in order to reduce tumor progression and metastasis, although these strategies have not yet be translated to clinical situations. Here, we will briefly summarize the pre-clinical results obtained by using and/or targeting chemokines to combat tumors and discuss the potential efficacy of these methods.

Introduction

Chemokines are heparin-binding proteins characterized by the presence of 4 cysteine residues in the conserved positions [1](Moser 2004). Two intermolecular disulfide bonds are formed between the first and third cysteines, and between the second and fourth cysteines, and these bonds result in the formation of triple-stranded β-sheet structures, while the carboxyl-terminal region forms an α-helix form [2](Fernandez 2002). Thus, although overall sequence similarities are not high among chemokines, they exhibit a similar three-dimensional structure. Chemokines exert their biological activities by binding their cognate receptors, which belong to G-protein coupled receptor (GPCR) with 7-span transmembrane portions [1]. Thus, the target cell specificity of each chemokine is determined by the expression pattern of its corresponding receptor. At their high concentrations, chemokines tend to dimerize by forming hydrogen bonds between their β-sheet structures (Jansma 2009)[3]. The current consensus is that monomeric forms of chemokines are sufficient for receptor binding to induce cell migration. It still remains elusive on the functions of dimerized chemokines, although the dimer is assumed to be associated with other complex functional roles (Jansma 2009)[3]. Moreover, through the carboxyl-terminal region with the capacity to bind heparin, chemokines can bind to proteoglycans and glycosaminoglycans with a high avidity. Consequently, most chemokines are produced as a secretory proteins, but upon their secretion, they can be immobilized on endothelium cells and in extracellular matrix by interacting with proteoglycans and glycosaminoglycans (Fernandez 2002)[2]. The immobilization facilitates the generation of a concentration gradient, which is crucial for inducing the target cells to migrate in a directed way.

Based on their structure, chemokines are classified into 4 subgroups, namely, CXC, CC, CX_3C and C (Moser 2004) [1] (Table 1). The first 2 cysteines are separated by 1 and 3 amino acids in CXC and CX_3C chemokines, respectively, while the first 2 cysteines are adjacent in CC chemokine. The C chemokine lacks the second and the fourth cysteines. Systematic chemokine nomenclature is based on their cysteine subclass roots, followed by "L" for "ligand" (Zlotnik 2000)[4]. The numbers correspond generally to the same number

used in the corresponding gene nomenclature. Because most chemokine receptors can bind to a single chemokine subclass, the nomenclature system of chemokine receptors is rooted by the chemokine subclass specificity, followed by "R" for "receptor" and the number (Zlotnik 2000)[4] (Table 1). The CXC chemokines are further grouped based on the presence or the absence of a 3-amino acid sequence, glutamic acid-leucine-arginine (the "ELR" motif), immediately preceding the CXC sequence (Vandercappellen 2008)[5]. In general, CXC chemokines with the ELR motif can bind CXCR1 and/or CXCR2, and exhibit an angiogenic and a neutrophil chemotactic activity (Vandercappellen 2008)[5].

Chemokines can be classified as inflammatory, homeostatic, or both, based on their expression pattern (Mantovani 2006)[6]. Various types of inflammatory stimuli induce the expression of inflammatory chemokines, which have a crucial role in the infiltration of inflammatory cells including granulocytes and monocytes/macrophages. Representative inflammatory chemokines are CXC chemokines with ELR motif and CCL2. On the contrary, homeostatic chemokines are expressed constitutively in specific tissues or cells. They are involved in organogenesis of various organs including lymph nodes, as they have key roles in stem cell migration. Moreover, most homeostatic chemokines can regulate the trafficking of immune cells such as lymphocytes and dendritic cells, and eventually adaptive immunity.

The human and mouse genomes contain over 44 and 38 different chemokine genes, respectively (Nomiyama 2010)[7]. There is a difference in gene numbers with some ambiguities of orthologous relationship between the human and mouse chemokine family. These observations would indicate that the chemokine gene family has been rapidly evolving, resulting in species-specific expansions and contractions. A notable difference has been found in one of the major chemokine, CXCL8, and its receptors, CXCR1 and CXCR2. Mice and rats do not possess a homolog of the *CXCL8/IL-8* gene, which is present in other species including humans, rabbits, cats, and dogs (Nomiyama 2010)[7]. Moreover, the *CXCR1* and *CXCR2* genes encode functional receptor proteins in humans, whereas there still remains a question on the presence of functional CXCR1 in mice or rats (Moepps 2006)[8]. Different expression patterns between humans and mice were observed also on

other chemokine receptors such as CCR1 (Su 1996)[9]. These observations should be taken into consideration when the findings obtained with mouse models are extrapolated to human conditions.

Chemokine receptor signaling (Figure 1)

Approximately 20 signaling chemokine receptors have been identified as well as several non-signaling receptors (Table 1) (Allen 2007)[10]. The presence of a DRY motif in the second transmembrane region is responsible for the ability of chemokine receptors to signal upon ligand binding, and non-singaling signaling receptors lack this motif. Chemokine receptors are coupled with heterotrimeric Gαβγ proteins bound to intracellular loops. The Gα subunit contains a GTPase domain involved in binding and hydrolysis of GTP. In the inactive state, the Gα subunit binds GDP, and interacts directly with the intracellular loop of chemokine receptors and with Gβ subunit, which in turn forms a tight complex with Gγ subunit. A two-step model has been proposed for activation of the receptor (Fernandez 2002)[2]. In the first step, a chemokine specifically recognizes and binds the receptor. Consequently, the amino-terminus of the chemokine interacts with the receptor, leading to the activation of the receptor. Simultaneously, ligand binding induces internalization of the chemokine receptor by using the clarthrin-mediated pathway or the lipid rafts/caveole-dependent internalization routes (Neel 2005)[11]. Internalized receptors are recycled and reappear on the cell surface quickly. However, it still remains controversial on the necessity of internalization and recycling for chemokine-mediated signaling and chemotaxis.

The activation induces dissociation of GDP from Gα and replacement of GTP. Gα-GTP eventually dissociates from the receptor and the Gβγ heterodimer, and both complexes activate a series of downstream effectors (Figure 1). Generated Gβγ heterodimer recruits and activates phosphatidylinosinol 3-kinase-γ (PI3K-γ), which in turn generates phosphatidylinositol 3,4,5-trisphosphate (PIP_3) (Servant 2000)[12]. PIP_3 activates protein kinase B (Akt) as well as small GTPase such as Rac and Rho (Figure 1). In addition, active Gα and Gβγ facilitate the polarization of the cells with the leading edge (pseudopodium) in

the front and the formation of a trailing tail (uropod) at the back. PI3K and Rac accumulate at the leading edge to induce actin polymerization and F-actin formation (Ridley 2003)[13]. Simultaneously, Rho and its effector molecules accumulate at the trailing edge to facilitate actomyosin contraction and tail retraction, thereby leading to the migration of the cells.

GPCR-mediated signals can be down-regulated by regulators of G protein signaling (RGS) proteins. RGS proteins are a family consisting of 20 members and can activate GTPase activities. RGS proteins directly interact with GTP-bound Gα subunit to catalyze GTP hydrolysis and G protein downregulation and eventually decrease the half-life of the active GTP-bound state of Gα. RGS1, RGS3, and RGS4 attenuated CXCL8-mediated signals in neutrophils (Druey 1996)[14] while RGS1 and RGS13 reduces CXCL12- and CXCL13-mediated signals in B cells (Shi 2002, Le 2005)[15, 16].

The binding of a chemokine to its corresponding receptor exposes the tyrosine residue in DRY motif in the second transmembrane region (Mellado 1998, Rodríguez-Frade 2001)[17, 18]. This exposure allows access of Janus kinase, which activates the receptor by tyrosine phosphorylation. Simultaneous activation of Janus kinase leads to the recruitment of STAT (signal transducers and activators of transcription) and eventually STAT-mediated expression of the target genes [17, 18](Mellado 1998, Rodríguez-Frade 2001) (Figure 1). Moreover, this pathway requires ligand-induced homodimerization of chemokine receptors, as observed on other GPCRs that can frequently exist as dimers and/or high-order oligomers (Breitwieser 2004)[19]. In the case of CCR5, Ile52 in transmembrane region-1 (TM1) and Val150 in TM4 are key residues in the interaction surface between CCR5 molecules (Hernanz-Falcon 2004)[20]. Moreover, mutation in these residues generates nonfunctional receptors that cannot dimerize or trigger signaling. Similar regions in CCR2 receptor are required for CCL2-induced homodimerization and subsequent activation (Rodríguez-Frade 1999)[18].

It is widely accepted that even distantly related GPCRs can form heterodimers (Breitwieser 2004)[19]. Indeed, heterodimerization isare also observed among several chemokine receptors including CCR2, CCR5, CXCR2, and CXCR4 (Rodríguez-Frade 2001)[21]. For example, the heterodimerization of CCR2 with CCR5 cooperates to trigger

calcium influx at concentrations 10- to 100-folde lower than the threshold for either CCL2, a ligand for CCR2, and or CCL5, a ligand for CCR5 (Mellado 2001)[22]. However, it recruits a dissimilar signaling pathway such as $G_{\alpha/11}$ association and delays activation of PI3-K. The consequences are triggering of cell adhesion rather than chemotaxis. In the case of CCR2/CXCR4 heterodimers, specific antagonists of one receptor inhibit the binding of chemokines to other receptor both in recombinant cell lines and primary leukocytes (Sohy 2007)[23]. This results in a significant functional cross-inhibition in terms of calcium mobilization and chemotaxis. Thus, chemokine receptor antagonists can regulate allosterically the functions of receptors, which they do not directly bind. These observations may have important implications for the effects of these antagonists.

Ras and its downstream signaling pathway, mitogen activated protein kinase (MAPK)/Erk kinase pathway, can be activated by several chemokine receptors including CXCR1, CXCR2 (Knall 1996)[24], and CXCR4 (Barbero 2003)[25] (Figure 1). The activation is frequently observed in tumor cells and leads to gene expression and cell proliferation. Moreover, activation of CXCR4 stimulates ovarian cancer cell growth through transactivation of the epidermal growth factor receptor (Porcile 2005)[26]. The activation of these signaling pathways may favor tumor cell proliferation.

Effector cells in tumor immunity and chemokines

Accumulating evidence indicates the presence of cytotoxic T lymphocytes (CTLs) that can specifically recognize tumor-associated antigens (TAA) and attack tumor cells in humans as well as in mice (Knutson 2005)[27] (Figure 2). In this immunological approach to cancer, antigen-presenting cells can deliver TAAs and prime TAA-specific T cells. Dendritic cells (DCs) are professional antigen-presenting cells and can express on their cell surface major histocompatibility complex (MHC) class I and II molecules, and as well as co-stimulatory molecules, all of which assist in T cells activation (Palucka 2012)[28]. DCs are widely distributed over peripheral tissues, and DCs in peripheral tissues are in an immature state and have a high capacity to endocytose various materials (Sozaani 2005)[29]. In periphery, DCs capture exogenous and endogenous antigens including tumor

cell-derived antigens (Figure 2). When DCs capture antigens in the absence of inflammatory cues such as Toll-like receptor-mediated signals, they failed to increase the expression of co-stimualatory molecules and to present antigens efficiently. On the contrary, when DCs capture antigens in the presence of inflammatory stimuli, they change to a mature state with a loss of endocytosis ability and start to migrate into the T cell areas of regional lymph nodes via afferent lymphatic venules under the guidance of chemokines (Figures 2 and 3). Mature DCs process the antigens into the peptides presented on MHC molecules, exhibit enhanced expression of co-stimulatory molecules, and induce primary immune responses through antigen presentation to T cells in the regional lymph node (Sozaani 2005)[29] (Figure 3). Immature DCs in peripheral tissues express various chemokine receptors including CCR1, CCR2, CCR4, CCR5, CCR6, CCR8, and CXCR4, whereas mature DCs express a limited set of chemokine receptors, CCR7 and CXCR4 (Sozaani 2005) [29] (Figure 3).

CCR7 and its ligands, CCL19 and CCL21, have a pivotal role in DC migration to lymph nodes in both steady state and inflammatory conditions (Förster, 1999)[30] although the contribution of another chemokine receptor, CCR8, cannot be excluded (Qu 2004)[31]. Antigen-pulsed $CCR7^{+/+}$ but not $CCR7^{-/-}$ DCs migrate efficiently to the draining lymph nodes when an antigen is injected intravenously (Martin-Fontecha 2003)[32]. Moreover, DC migration is markedly enhanced when intranodal CCL21 expression is augmented by pretreatment with interleukin (IL)-1 or tumor necrosis factor (TNF). Furthermore, the magnitude and quality of T cell response is proportional to the number of antigen-carrying DCs in the lymph nodes (Martin-Fontecha 2003)[32]. Furthermore, DCs can produce the chemokines which affect the trafficking and functions of natural killer (NK) cells, a main executor of innate immunity-mediated tumor cell killing (Sozaani 2005) [29].

Once generated in the regional lymph nodes, TAA-specific CTLs should migrate to tumor sites to kill tumor cells (Figure 2). Numerous clinical studies have indicated that the presence of $CD3^{+}$ or $CD8^{+}$ tumor-infiltrating lymphocytes (TILs) has a positive prognostic influence on survival (Gooden 2011)[33]. Most TILs are deemed to possess cytotoxic activities against tumor cells. Evidence is accumulating to indicate that several

chemokines regulate the migration of CTLs into tumor sites. CXCR3 is deemed to be a major chemokine receptor expressed by TILs. In a mouse model, increased expression of ligands for CXCR3, CXCL9 and CXCL10, can elicit antitumor response accompanied with an enhanced infiltration of $CD4^+$ and $CD8^+$ lymphocytes (Pan 2006)[34]. In line with this observation, in human gastric and colorectal cancer, TILs express CXCR3 (Musha 2005, Ohtani 2009, Muthuswamy 2012).[35, 36, 37]. Moreover, high levels of CXCL9 and CXCL10, ligands for CXCR3, are produced by stromal cells, mainly macrophages (Ohtani 2009).[36]. $CD8^+$ TILs also express CCR5 (Musha 2005, Muthuswamy 2012)[35, 37]. Concomitantly, $CD8^+$ TIL numbers correlate well with the expression of CCL5, a ligand for CCR5, by tumor tissues (Muthuswamy 2012)[37]. TILs express other chemokine receptors, CX3CR1 and the expression of its ligand, CX3CL3, is elevated in tumor cells in colorectal cancer tissues (Ohta 2005)[38]. Furthermore, the expression level of CXCL16 also correlates with $CD4^+$ and $CD8^+$ TIL numbers with a better prognosis although cells expressing CXCR6, a receptor for CXCL16, are not identified (Hojo 2007)[39]. Thus, CXCL9, CXCL10, CXCL16, CCL5, and CX3CL1 can be used to efficiently mobilize CTLs from regional lymph nodes to tumor tissues with an objective to enhance CTL-mediated tumor destruction.

NK cells are unconventional lymphocytes and were initially identified as a leukocyte to kill tumor cells without any antigen stimulation (Vivier 2008)[40]. Mouse and human NK cells can in vitro kill a broad range of tumor cells of both hematopoietic and non-hematopoietic origin by utilizing perforin and secreting interferon (IFN)-γ (Vivier 2008)[40]. Moreover, in vivo, mouse NK cells can eliminate many transplantable and spontaneous tumors. Distinct sets of chemokine receptors are utilized for NK cell trafficking (Table 1). NK cells migrate to lymph nodes mainly by utilizing CXCR3 and CCR7, while their migration to the inflamed tissues including tumor sites involves CCR1, CCR2, CCR5, CXCR3, and CX3CR1 (Walzer 2011)[41]. Thus, the ligands for these receptors can regulate NK cell trafficking and augment their functions. However, in colorectal tumor tissues, NK cells are scarce despite a significant lymphocyte infiltration, even in the presence of high levels of CXCL9, CXL10, CCL3, CCL4, CCL5, and CX3CL1

(Halama 2011)[42]. These observations suggest that NK cell migration into tumor tissues is impaired early during tumor development by the mechanism that do not affect TIL trafficking.

Chemokine-mediated enhancement in tumor immunity

As discussed above, the establishment of tumor immunity is a process consisting of multiple steps; migration of DCs to tumor sites, capture of tumor antigens by DCs, migration of DCs to regional lymph nodes, antigen presentation to effector cells by DCs in regional lymph nodes, and migration of effector cells to tumor sites (Figure 2). Chemokines have profound effects on tumor immunity, particularly migration steps.

The appearance of apoptotic cells induces the migration of immature dendritic cells to the tumor tissues. Accumulated immature dendritic cells capture TAAs and migrate to draining lymph nodes, where DCs present antigens to induce specific CTLs (Figure 2). Tumor-infiltrating DCs expressed CCR1 and CCR5, and a ligand for these receptors, CCL3, was abundantly detected in mouse bearing hepatocellular carcinoma (HCC) (Iida 2008)[43]. Moreover, DCs in tumor sites and lymph nodes, and subsequent cytotoxicity generation were reduced in CCR1-, CCR5-, or CCL3-deficient mice (Iida 2008)[43]. These observations may mirror the capacity of CCL3 to mobilize immature DCs to peripheral blood from bone marrow by interacting with CCR1 or CCR5 (Zhang 2004)[44]. Actually, systemic administration of CCL3 increased the numbers of DCs in peripheral blood and tumor tissues, and concomitantly augmented antitumor effects after radiofrequency ablation of murine HCCs (Iida 2010)[45]. These observations suggest that CCL3 may be effective to enhance tumor immunity by inducing the migration of immature DCs through peripheral blood to dying tumor cells.

The interaction between CCR7 and its ligands, CCL19 and CCL21, regulates DC migration to lymph nodes for antigen presentation to naïve T cells, which also utilize CCR7-mediated mechanisms to enter T cell zone (Förster, 1999)[30]. Moreover, CCL19 and CCL21 can attract NK cells to the lymph node. These observations suggest the potency of these chemokines to enhance acquired and innate immunity against various antigens

including TAAs. Indeed, when CCL21 was injected into a regional lymph node of SV40-transgenic mice that developed bilateral multifocal lung adenocarcinomas, it increased $CD4^+$ and $CD8^+$ lymphocytes as well as DCs at lymph nodes and tumor sites, and eventually led to a marked reduction in tumor burdens with enhanced survival (Sharma 2001)[46]. Similar results were also obtained when CCL19 was injected intranodally into SV40-transgenic mice (Hillinger 2006)[47].

Ex vivo generated DC have a very limited capacity to move from the injected sites to locally draining lymph nodes (Chang 2002)[48]. This limitation may account for a clinical weakness in DC-based vaccines. The capacity of CCL19 and CCL21 to effectively induce DC migration prompted the use of these chemokines to modify *ex vivo* generated DCs. Intratumoral injection of *CCL21* gene-modified DCs resulted in tumor growth inhibition that was significantly better than unmodified control DCs (Kirk 2001a)[49], together with intratumoral accumulation of DCs and T cells (Kirk 2001b)[50]. Moreover, even when *CCL21* gene-modified DCs were pulsed with tumor lysates and subsequennetly injected subcutaneously to tumor-free sites in tumor-bearing mice, it elicited an antitumor response (Kirk 2001a)[49]. These promising preclinical results have led to ongoing phase I clinical trials (Baratelli 2008)[51].

Intratumoral administration of *CCL21* gene-modified DCs reduced tumor burden in spontaneous murine lung carcinoma, accompanied with extensive T cell infiltration, and the enhanced elaboration of IFN-γ, IL-12, CXCL9, and CXCL10 (Yang 2004)[52]. Moreover, *in vivo* depletion of either CXCL9 or CXCL10 significantly reduced the antitumor efficacy of *CCL21* gene-modified DCs. This may mirror the fact that CXCR3 is highly expressed by activated effector $CD8^+$ T cells and Th1-type $CD4^+$ T cells (Groom 2011)[53]. CXCL10 gene transduction into tumor cells had few effects on *in vitro* tumor cell proliferation but in vivo elicited a potent T cell-dependent antitumor response (Luster 1993)[54]. Likewise, tumor cells expressing CXCL10, induced the infiltration of tumor-specific cytotoxic T cells into the tumor site (Yang 2006)[55]. Moreover, tumor cells induced these cytotoxic T cells to proliferate and to produce high level of IFN-γ, while CXCL10 expanded these tumor-specific T cells. Gene transduction of another ligand for

CXCR3, CXCL11, into tumor cells, also retarded *in vivo* tumor growth accompanied by intratumoral infiltration of $CD8^+$ cells (Hensbergen 2005)[56]. As T cells rapidly acquire CXCR3 expression upon activation with IL-2 (Groom 2011)[53], combined strategy of systemic IL-2 with intratumor CXCL9 administration was proven to be more efficacious than either cytokine alone, for augmenting tumor-associated immunity (Pan 2006)[34]. Thus, CXCR3-binding chemokines can be utilized to redirect the migration of effector T cells to tumor sites.

Muthuswamy observed that colorectal tumors with reduced accumulation of $CD8^+$ effector cells express low levels of CXCL10 and CCL5, the chemokine with potent chemoattractant activities for $CD8^+$ effector cells (2012)[37]. They demonstrated that a combination of IFN-α and a TLR3 ligand, poly-I:C, can uniformly enhance the production of CXCL10 and CCL5. Moreover, these effects can be optimized by the further addition of cyclooxygenase (COX)-2 inhibitors. Of interest is that this triple combination also consistently suppresses the production of a ligand for CCR4, CCL22, a chemokine associated with Treg infiltration. Thus, this strategy can enhance the intratumoral trafficking of $CD8^+$ effector T cells and can simultaneously reduce that of Treg cells, thereby augmenting local tumor immunity.

CCL2 protein was initially isolated as a factor which can augment monocyte-mediated tumor cytostatic activity and can exhibit monocyte chemotactic activity (Matsushima 1989)[57]. Indeed, tumor formation was suppressed *in vivo* but not *in vitro* when the tumor was genetically engineered to express *CCL2* gene (Rollins 1991)[58]. CCL2-expressing cells elicited a predominantly monocytic infiltrate at the site of injection, suggesting the roles of infiltrating monocytes in tumor rejection process (Rollins 1991)[58]. In addition to monocytes/macrophages, a receptor for CCL2, CCR2, is expressed by additional types of leukocytes such as NK cells (Table 1). *CCL2* gene transduction into tumor cells retardeds tumor growth *in vivo* by inducing NK infiltration into tumor sites (Nokihara 2000)[59]. Moreover, NK cell infiltration was associated with elevated Th1 response in tumor sites (Tsuchiyama 2007)[60], suggesting that CCL2 can regulate the

infiltration and activation of Th1 cells in tumor sites through NK cell recruitment and activation.

Tumor formation was also suppressed *in vivo* when mouse lymphoma cell lines were transduced with the gene of another chemokine, CX3CL1 (Lavergne 2003)[61]. This antitumor response was abolished in NK cell-deficient beige mice but not in T- and B-cell-deficient $Rag1^{-/-}$ mice, indicating the indispensable roles of NK but not T cells. Gene therapy using *CX3CL1* gene could activate T cells as well as NK cells to exert its antitumor responses (Tang 2007, Zeng 2007)[62, 63]. Moreover, intratumoral injection of a DNA plasmid coding for a chimeric immunoglobulin presenting CX3CL1 chemokine domain provided strong antitumor activity (Lavergne 2003)[64]. The administration of this fusion protein with tumor antigens, induced a strong *in vivo* antigen-specific T cell proliferation and effector function, accompanied with myeoloid DC accumulation (Iga 2007)[64]. Thus, CX3CL1 can redirect T cells and DCs as well as NK cells, thereby augmenting adaptive immunity to tumor antigens.

In order to enhance the capacity to move to tumor sites by utilizing the chemokine(s) produced by tumor cells, several groups genetically engineered T cells to express the corresponding chemokine receptor. The Reed-Sternberg cells of Hodgkin lymphoma predominantly produce CCL17 and CCL22, which preferentially attract CCR4-expressing Th2 and Treg cells (van den Berg 1999)[65]. On the contrary, effector $CD8^+$ T cells lack CCR4. When $CD8^+$ cells were forced to express CCR4, these cells migrated more efficiently to Hodgkin lymphoma site. Moreover, tumor formation was more effectively inhibited by the administration of T lymphocytes expressing CCR4 and a chimeric antigen receptor directed to the Hodgkin lymphoma-associated antigen CD30 (di Stasi 2009)[66]. Similarly, CCL2 was highly secreted by malignant pleural mesothelioma cells, but CCR2 was minimally expressed on activated human T cells transduced with a chimeric antibody receptor (CAR) directed to mesothelioma tumor antigen, mesothelin (mesoCAR T cells) (Moon 2011)[67]. *CCR2* gene-transduced mesoCAR T cells exhibited enhanced antitumor responses accompanied with augmented T cell infiltration into tumor sites, when they were given intravenously [67]. This novel gene therapy technology using a

chemokine receptor can effectively enhance the migration of adoptively transferred T cells into tumor sites, where a corresponding chemokine is expressed abundantly.

Reversal of suppressor cell-mediated immune suppression by targeting chemokines

Tumor immunity can frequently induce immune suppressive mechanisms to dampen the "immunity to self". Thus, tumor immunity can be reduced by the action of several negative immunoregulatory receptors such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and the programmed death receptor-1 (PD-1)-PD ligand-1 (PD-L1) axis. Indeed, evidence is accumulating to indicate that the antagonizing monoclonal antibodies to CTLA-4, PD-1, or PD-L1, are effective against various types of cancer even at advanced stages (Sarnaik 2009, Ribas 2012)[68, 69]. These observations indicate that targeting tumor-induced immune suppression can be effective to enhance tumor immunity.

Tumor tissues contain the leukocytes that can diminish tumor immunity. The most predominant subset is tumor-associated macrophages (TAMs) (Sica 2008)[70]. Circulating monocytes are mostly the precursor of these TAMs and are attracted into tumor sites, by chemotactic factors including CCL2, CCL5, CCL7, CCL8, CXCL12, and macrophage colony stimulating factor (M-CSF), which are produced in tumor tissues (Sica 2008)[70] (Figure 4). In human colorectal cancer tissues, macrophage accumulation increases with tumor stages and correlates with CCL2 expression in tumor sites (Bailey 2007)[71]. Thus, CCL2-induced TAM infiltration can have a pro-tumorigenic activity.

Hypoxia in tumor microenvironment induces TAMs to produce abundantly vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), two potent angiogenic factors (Sica 2008)[70]. Moreover, a fraction of TAMs can be incorporated into tumor vasculature (Kim 2009)[72]. TAMs are frequently polarized into M2 phenotypes under the influence of various factors present in tumor microenvironment, such as IL-4, IL-10, and prostaglandins (PGs) (Ruffell 2012) [73]. M2 phenotype is characterized by the expression of arginniase (Arg)-1 and inducible NO synthase (iNOS), the enzymes responsible for the generation of reactive oxygen species (ROS), which can inhibit CTL proliferation [73](Ruffle 2012). TAMs can additionally produce IL-10 and

TGF-β to promote the generation of another immunosuppressive cells, regulatory T cells (Treg) (Sica 2008)[70], while they can also produce CCL22 to induce intratumoral Treg migration (Curiel 2004)[74]. Moreover, a fraction of TAMs express B7-H4 on their surface to inhibit CTL proliferation (Kryczek 2006)[75]. These properties endow TAMs with an immunosuppressive capacity. Thus, TAMs can promote tumor progression by inducing angiogenesis and suppression of adaptive and innate anti-tumor immunity (Figure 4).

Systemic delivery of neutralizing anti-CCL2 antibody attenuated tumor burdens in human prostate cancer-bearing mice although its effects of TAMs have not been examined (Loberg 2007)[76]. Combined treatment of azoxymethane and repeated dextran sodium sulfate solution ingestion caused multiple tumors in murine colons, together with a massive infiltration of monocytes/macrophages expressing COX-2, an enzyme crucially involved in colon carcinogenesis (Popivanova 2009)[77]. CCL2 was abundantly detected in colon tissues and induced CCR2-positive COX-2 expressing monocytes/macrophages to infiltrate colon tissues and blocking CCL2 retarded tumor progression with reduced macrophage infiltration (Popivanova 2009)[77]. CCL2 also recruited monocytes to pulmonary metastatic sites of murine breast cancer [78]. As a consequence, infiltrated monocytes promoted the extravasation of tumor cells, a prerequisite step for metastasis, in a process that required monocyte-derived VEGF and CCL2 blockade markedly reduced lung metastasis (Qian 2011).

Myeloid-derived suppressor cells (MDSCs) is are an additional type of cells characterized by a strong ability to suppress various T cell functions (Condamine 2011)[79]. MDSCs represent a heterogenic population of immature myeloid cells that consists of precursors of macrophages, granulocytes, and dendritic cells. In mice, MDSCs are characterized by the co-expression of two distinct myeloid-cell lineage differentiation antigens, Gr-1 and CD11b in mouse (Condamine 2011)[79]. In humans, MDSCs are defined as $CD14^{-}CD11b^{+}$ cells or as cells that express the common myeloid marker CD33 but lack the expression markers of mature myeloid and lymphoid markers. Similarly as TAMs do, MDSCs express Arg-1 and iNOS, and produce immunosuppressive cytokines such as TGF-β1 and IL-10, thereby inhibiting T cell response (Condamine 2011)[79]

(Figure 4). CCL2 recruits MDSCs in several types of mouse cancer including Lewis lung carcinoma, MethA sarcoma, melanoma and lymphoma (Huang 2007)[80]. Moreover, CCL2-mediated MDSC accumulation can negatively regulate the entry of adoptively transferred activated $CD8^+$ cells into tumor sites (Lesokhin 2012)[81]. However, CCR2 deficiency caused conversion of the MDSC phenotype to neutrophil lineage without affecting tumor growth (Sawanobori 2008)[82], probably because MDSC contains a subset of immature neutrophils (Brandau 2011)[83]. CXCL5 and CXCL12 also induced MDSC infiltration in mouse mammary adenocarcinoma (Yang 2008)[84]. In ascites isolated from human ovarian cancer patients, PGE_2 induced CXCL12 production and the expression of its receptor, CXCR4, and the CXCL12-CXCR4 axis subsequently induced the accumulation of MDSCs (Obermajer 2011)[85]. Due to the heterogeneity of MDSCs (Condamine 2011)[79], it remains elusive on the relevance of this observation.

Treg cells are characterized by the expression of CD4 and CD25 on their cell surface with the expression of a transcription factor, Foxp3 (Nishikawa 2010)[86]. Treg cells are polarized from $CD4^+$ naïve T cells in thymus or periphery, and are physiologically engaged in the maintenance of immunological self-tolerance. A large number of Treg cells often infiltrate into tumors and systemic removal of Treg cells enhances natural as well as vaccine-induced anti-tumor T cell immunity [86](Nishikawa 2010). Intratumoral $CD8^+/Foxp3^+$ ratio but not absolute $Foxp3^+$ cell numbers correlated inversely with survival (Gooden 2011)[33]. Thus, the relative ratio of Treg to $CD8^+$ CTL but not absolute Treg number can have impacts on immune tolerance to tumor cells.

Treg cells express CCR4 and its ligand, CCL22, mainly regulates intratumoral Treg infiltration in various tumors [86] (Nishikawa 2010) (Figure 4). Indeed, intratumoral CCL22 expression correlated well with Foxp3 expression in colorectal carcinoma tissues (Muthuswamy 2012)[37]. Hypoxia induced the expression of another chemokine, CCL28, in colorectal tumor cells (Facciabene, 2011)[87]. CCL28 seemed to utilize mainly CCR10 to induce Treg migration into tumor sites (Figure 4) although CCL28 was reported to utilize both CCR3 and CCR10 as its receptors (Table 1). Moreover, infiltrating Treg cells can produce VEGF to promote tumor neovascularization (Facciabene, 2011)[87].

Furthermore, anti-CCL2 antibody augmented cancer immunotherapy against non-small cell lung cancer in mice when it was administered in combination with a tumor vaccine (Fridelender 2010)[88]. This enhanced tumor immunity was associated with reduced intratumoral Tregs and increased numbers of intratumoral $CD8^+$ cells that are more activated and more antitumor antigen-specific. These observations illustrate that targeting these chemokines can reduce intratumoral Treg cells, resulting in the enhancement of tumor immunity.

Adult T cell leukemia (ATL) cells are also characterized by robust expression of CCR4 and can migrate *in vitro* to CCL17 and CCL22, ligands for CCR4 [89] (Yoshie 2002). By using genetic engineering methods, humanized monoclonal antibody to CCR4 has been defucosylated to exert more potent antibody-dependent cytotoxicity (ADCC) (Ishida 2011)[90]. The resultant antibody is capable of removing CCR4-expressing ATL cells in peripheral blood and bone marrow mainly by ADCC. Thus, this antibody may also be effective to reduce intratumoral Treg cell numbers in solid tumors, thereby augmenting T cell-mediated cytotoxicity against tumor cells.

Recently, CCR1-expressing $CD34^+$ immature myeloid cells have been detected in murine intestinal tumors with SMAD4 deficiency (Kitamura 2007)[91]. These cells expressed abundantly MMP9MMP-9 and MMP2MMP-2, and were involved in invasion. Moreover, a CCR1 antagonist suppressed colon cancer liver metastasis by blocking accumulation of this $CD34^+$ immature myeloid cells (Kitamura 2010)[92].

Other strategies of antitumor therapy targeting chemokines

Chemokines were originally identified as factors affecting leukocyte migration and activation (Oppenheim 1990)[93]. Subsequent studies revealed that chemokines have effects on non-leukocytic cells including tumor cells and endothelial cells (Figure 5). Indeed, several chemokines can directly induce cancer cells to express pro-tumorigenic genes and to proliferate. CXCL8 can induce the proliferation of human gastric cancer cells (Kitadai 2000)[94], esophageal cancer cells (Wang 2006)[95], and melanoma cells (Singh 2009)[96]. CXCR4 activation also caused the proliferation of various cancer cells including

ovarian, glioma, melanoma, lung, renal, and thyroid cancer cells (Teicher 2010)[97]. Likewise, CCR6 and CXCR6 can promote the proliferation of colorectal cancer cells (Ghadjar 2009)[98] and prostate cancer cells (Darsh-Yahana 2009)[99], respectively. Furthermore, the activation of CXCR4, CCR10, or CCR7 axis delivered surviving signals to various types of malignant cells (Murakami 2003, Wang 2008, Bertran 2009, Righi 2011, Messmer 2011)[100, 101, 102, 103, 104]. Thus, the inhibition of these chemokines may directly reduce *in vivo* tumor cell proliferation.

Metastasis is a complicated process wherein cancer cells extravasate from the original tissues, move inside bloodstream and/or lymphatics, invade to and grow in distant organs. The first step of metastasis, extravasation from the original tissues, requires epithelial-mesenchymal transition (EMT) (Bertran 2009)[102]. Accumulating evidence indicates the crucial roles of CXCL12 [102](Bertran 2009) and CXCL8 in EMT (Fernando 2011)[105]. Moreover, when tumor cells enter circulation, tumor cells are prone to anoikis, which is a form of cell death arising from the lack of the support from extracellular matrix and is a major block in the metastatic spread of various types of cancer cells. CXCL12 and a CCR7 ligand, CCL21, can reduce the sensitivity of cancer cells to anoikis by regulating pro-apoptotic Bmf and anti-apoptotic Bcl-xL proteins (Kochetkova 2009)[106].

CXCR4, CCR7, CCR9, CXCR1, and CXCR2 were detected in tumor cells and their ligands induced the chemotaxis of the corresponding receptor-expressing cells (Mùller 2001, Buonamici 2009, Amersi 2008, Waugh 2008, Messner 2011, Zhang 2012)[107, 108, 109, 110, 111]. Specific chemokine receptor-expressing tumor cells may migrate to organs with high expression levels of respective chemokines along a concentration gradient (Mùller 2001)[107]. However, there remains a question on the presence of a concentration gradient between primary and metastatic sites. Alternatively, cancer cells themselves are actively promoting their own metastasis and tropism by producing chemokines (Shields 2007)[112]. Moreover, the arrival of tumor cells in a specific organ is passive and chemokine receptor expression provides tumor cells with an advantage to survive and grow in another ligand-rich metastatic microenvironment (Zhang 2009)[113]. Nevertheless,

several chemokines can serve as inducers of metastasis to distant organs and therefore, may be a good target for controlling metastasis.

Neovascularization is crucial for tumor growth, progression, and metastasis (Fidler 1994)[114]. The ELR motif-positive CXC chemokines, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 can directly promote the migration and proliferation of endothelial cells and eventually neovascularization, mainly interacting with CXCR2, but not CXCR1 (Keeley 2011)[115] (Figure 5). Indeed, the administration of anti-CXCL8 reduced the tumor sizes of human non-small cell lung cancer cells which are injected into severe combined immune deficient (SCID) mice in advance (Arenberg 1996)[116]. The reduction in tumor size was associated with a decline in tumor-associated vascular density and was accompanied by a decrease in spontaneous lung metastasis.

CXCL12 is not an ELR-positive CXC chemokine but exhibits potent angiogenic effects (Kryczek 2007)[117]. In addition, three CC chemokines, CCL2, CCL11, and CCL16 have also been implicated in tumor neovascularization (Gâlvez 2005, Salcedo 2001, Strasly 2004)[118, 119, 120]. Indeed, CCR2, a specific receptor for CCL2, was expressed by endothelial cells and CCL2 exerted its angiogenic activity in a membrane type 1 (MT1)-MMP-dependent manner (Gâlvez 2005) [118] (Figure 5). TAMs and MDSCs are recruited at tumor sites mainly by CCL2 and promote angiogenesis by producing a wide variety of angiogenic factors such as VEGF, TGF-β, CXCL8, platelet-derived growth factor (PDGF), and MMP such as MMP-2MMP-2 and MMP-9MMP-9. Moreover, recruited TAMs and MDSCs may acquire endothelial cell phenotypes and can be incorporated into the newly formed vascular structure (Rehman 2003)[121]. Thus, targeting these chemokines may be effective to control tumor neovascularization.

CXCL4 and interferon-inducible ELR motif-negative CXC chemokines such as CXCL9, CXCL10, and CXCL11 inhibit the angiogenesis induced by ELR motif-positive CXC chemokines, VEGF, and bFGF (Maione 1990, Romagnani 2001)[122, 123]. The anti-angiogenic effects of these chemokines are mediated by a common receptor, CXCR3 (Figure 5) and targeted expression of CXCL9 or intratumoral CXCL9 administration retarded *in vivo* tumor growth by inhibiting tumor-derived angiogenesis (Addison 2000,

Pan 2006)[34, 124]. Thus, these chemokines can be effective for tumor therapy by inhibiting neovascularization as well as inducing CXCR3-expressing cytotoxic T cell infiltration.

Perspective

Chemokines regulate the trafficking of leukocytes including immune cells in the presence of a concentration gradient and. Chemokines have a crucial role in the control of the recruitment of immune cells needed for the induction and activation of tumor immunity. As we described above, based on these properties, several chemokines have been utilized in pre-clinical models to augment tumor immunity by enhancing the migration and activation of immune cells. Most of these trials, however, have not yet been translated into clinical trials. However, trafficking of a particular type of immune cells is regulated simultaneously by several distinct chemokines in a redundant manner (Table 1). Thus, it still remains to be investigated which chemokine(s) is the most suitable for inducing the trafficking of the targeted immune cells, to exert efficient immune response to tumors. ~~Chemokines regulate the trafficking of leukocytes including immune cells in the presence of a concentration gradient.~~ In order to obtain a local high concentration, gene therapy techniques were used in most trials and this may preclude the translation into clinical trials. Thus, it is mandatory to develop a drug delivery system to supply efficiently a chemokine protein into the targeting site.

Moreover, iIt is embarrassing that the same chemokine can induce tumor progression as well as protection against a tumor. One representative chemokine is CCL2, which can destroy tumor tissues when administered to tumor tissues by using gene therapy technology. It, however, exhibits a wide variety of actions involved in promotion of tumor progression and metastasis, and targeting CCL2 was proven to be effective for reducing tumor burdens and metastasis in several murine models. This paradox may be explained by the assumption that endogenously produced CCL2 can act on the cells present in tumor tissues but cannot cause a concentration gradient sufficient to attract immune cells from

outside of the tumor tissues. OtherwiseAlternatively, the responsiveness of immune effector cells to CCL2 may be much lower than that of immune suppressive cells, endothelial cells, and tumor cells. This may favor endogenous chemokine-mediated generation of pro-tumorigenic microenvironments rather than antitumor immune response. Thus, we should also clarify the local concentration of the chemokine, which is required for the responsiveness of immune effector cells but not that of immune suppressive cells, endothelial cells, and tumor cells. Based on the information, we should devise a method to sustain a local chemokine concentration sufficient to attract immune effector cells to elicit immune response to tumor. Alternatively, genetic modification of immune effector cells with a chemokine receptor gene can confer a capacity to respond more efficiently to a chemokine on immune effector cells. Thus, this may be an attractive maneuver to change chemokine-mediated pro-tumorigenic environments, where a particular chemokine is present abundantly, into an effective immune surveillance situationsystem, where the abundantly expressed chemokine can attract immune effector cells to exert immune responses..

References

1. Moser B et al, Wolf M, Walz A, Loetscher P (2004) Chemokines: multiple levels of leukocyte migration control. Trends Immunol 25(2):75-84
2. Fernandez EJ, Lolis E (2002) Structure, function, and inhibition of chemokines. Annu Rev Pharmacol Toxicol 42:469-499
3. Jansma A, Handel TM, Hamel DJ (2009) Homo- and hetero-oligomerization of chemokines. Methods Enzymol 461:31-50
4. Zlotnik A, Yoshie O (2000) Chemokines: a new classification system and their role in immunity. Immunity 12(2):121-127
5. Vandercappellen J, Van Damme J, Struyf S (2008) The role of CXC chemokines and their receptors in caner. Cancer Lett 267(2):226-244
6. Mantovani A, Bonecchi R, Locati M (2006) Tuning inflammation and immunity by chemokine sequestration: decoys and more. Nat Rev Immunol 6(12):907-918
7. Nomiyama H, Osada N, Yoshie O (2010) The evolution of mammalian chemokine genes. Cytokine Growth Fact Rev 21(4):253-262
8. Moepps B et al (2006) A homolog of the human chemokine receptor CXCR1 is expressed in mouse. Mol Immunol 43(7):897-914
9. Su S-B et al (1996) Preparation of specific antagonizing polyclonal antibodies to a C-C chemokine receptor, CCR1 and determination of its distribution of various types of leukocytes. J Leukocyte Biol 60(5):658-666
10. Allen SJ, Crown SE, Handel TM (2007) Chemokine: receptor structure, interactions, and antagonism. Annu Rev Immunol 25:787-820
11. Neel NF et al (2005) Chemokine receptor internalization and intracellular trafficking. Cytokine Growth Factor Rev 16(6):637-658
12. Servant G et al (2000) Polarizattion of chemoattractant receptor signaling during neutrophil chemotaxis. Science 287(5455):1037-1040
13. Ridley AJ et al (2003) Cell migration: integrating signals from front to back. Science 302(5651):1704-1709

Druey KM et al (1996) Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family. Nature 379(6567):742-746

14.

15. Shi GX et al (2002) RGS13 regulates germinal center B lymphocytes responsiveness to CXC chemokine ligand (CXCL)12 and CXCL13. J Immunol 169(5):2507-2515
16. Le Y et al (2005) CXC chemokine lignd 12-induced focal adhesion kinase activation and segregation into membrane domains is modulated by regulator of G protein signaling 1 in pro-B cells. J Immunol 174(5):2582-2590
17. Mellado M et al (1998) The chemokine MCP-1 triggers tyrosine phosphorylation of the CCR2B receptor and the JAK2/STAT3 pathway. J Immunol 161(2):805-813
18. Rodríguez-Frade JM et al (1999) The chemokine monocyte chemoattractant protein-1 induces functional responses through dimerization of its receptor CCR2. Proc Natl Acad Sci USA 96(7):3628-3633
19. Breitwieser GE (2004) G protein-coupled receptor oligomerization: implications for G protein activation and cell signaling. Circ Res 94(1):17-27
20. Hernanz-Falcón P et al (2004) Identification of amino acid residues crucial for chemokine receptor dimerization. Nature Immunol 5(2):216-223
21. Rodríguez-Frade JM, Mellado M, Martínez-A C (2001) Chemokine receptor dimerization: two are better than one. Trends Immunol 22(11): 612-617
22. Mellado M et al (2001) Chemokine receptor homo- or heterodimerization activates distinct signaling pathways. EMBO J 20(10):2497-2507
23. Sohy D, Parmentier M, Springael JY (2007) Allosteric transinhibition by specific antagonists in CCR2/CXCR4 heterodimers. J Biol Chem 282(41): 30062-30069
24. Knall C et al (1996) Interleukin-8 regulation of the Ras/Raf/mitogen-activated protein kinase pathway in human neutrophils. J Biol Chem 271(5): 2832-2838
25. Barbero S et al (2003) Stromal cell-derived factor 1α stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt**.** Cancer Res 63(8):1969-1974

26. Porcile C et al (2005) Stromal cell-derived factor-1α (SDF-1α/CXCL12) stimulates ovarian cancer cell growth through the EGF receptor transactivation. Exp Cell Res 308(2):241-53

27. Knutson KL, Disis ML (2005) Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. Cancer Immunol Immunother 54(8): 721-728

1.28. Palucka K, Banchereau J (2012) Cancer immunotherapy via dendritic cells. Nat Rev Cancer 12(4):265-277. doi: 10.1038/nrc3258.

29. Sozzani S (2005) Dendritic cell trafficking: more than just chemokines. Cytokine Growth Factor Rev 16(6):581-592

30. Förster R et al (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 99(1):23-33

31. Qu C et al (2004) Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes. J Exp Med 200(10):1231-1241

32. Martin-Fontecha A et al (2003) Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J Exp Med 198(4):615-621

33. Gooden MJ et al (2011) The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. Br J Cancer 105(1):93-103. doi: 10.1038/bjc.2011.189

34. Pan J et al (2006) CXCR3/CXCR3 ligand biological axis impairs RENCA tumor growth by a mechanism of immunoangiostasis. J Immunol 176(3):1456-1464

35. Musha H et al (2005) Selective infiltration of CCR5(+)CXCR3(+) T lymphocytes in human colorectal carcinoma. Int J Cancer 116(6):949-956

Ohtani H et al. (2009) Abundant expression of CXCL9 (Mig) by stromal cells that include dendritic cells and accumulation of $CXCR3^+$ T cells in lymphocyte-rich gastric cancer. J Pathol 217(1):21-31.

36.

37. Muthuswamy R et al (2012) NF-κB hyperactivation in tumor tissues allows tumor-selective reprogramming of the chemokine microenvironment to enhance the recruitment of cytolytic T effector cells. Cancer Res 72(15):3735-3743

38. Ohta M et al (2005) The high expression of fractalkine results in a better prognosis in colorectal cancer patients. Int J Oncol 26(1):41-47
39. Hojo S et al (2007) High level expression of chemokine CXCL16 by tumor cells correlates with a good prognosis and increased tumor-infiltrating lymphocytes in colorectal cancer. Cancer Res 67(10):4725-4731
40. Vivier E et al (2008) Functions of natural killer cells. Nat Immunol 9(5):503-510
41. Walzer T, Vivier E (2011) G-protein-coupled receptors in control of natural killer cell migration. Trends Immunol 32(10):486-492
42. Halama N et al (2011) Natural killer cells are scarce in colorectal carcinoma tissue despite high levels of chemokines and cytokines. Clin Cancer Res 17(4):678-689
43. Iida N et al (2008) Tumor cells apoptosis induces tumor-specific immunity in a CC chemokine receptor 1- and 5-dependent manner in mice. J Leukoc Biol 84(4):1001-1010
44. Zhang Y et al (2004) Mobilization of dendritic cell precursors into the circulation by administration of MIP-1α in mice. J Natl Cancer Inst 96(3):201-209
45. Iida N et al (2010) Antitumor effect after radiofrequency ablation of murine hepatoma is augmented by an active variant of CC chemokine ligand 3/macrophage inflammatory proein-1α. Cancer Res 70(16):6556-6566
46. Sharma S et al (2001) Secondary lymphoid organ chemokine reduces pulmonary tumor burden in spontaneous murine bronchoalveolar cell carcinoma. Cancer Res 61(17):6406-6412
47. Hillinger S et al (2006) CCL19 reduces tumour burden in a model of advanced lung cancer. Br J Cancer 94(7):1029-1034
48. Chang AE et al (2002) A phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. Clin Cancer Res 8(4):1021-32
49. Kirk CJ et al (2001) T cell-dependent antitumor immunity mediated by secondary lymphoid tissue chemokine: augmentation of dendritic cell-based immunotherapy. Cancer Res 61(5):2062-2070

50. Kirk CJ, Hartigan-O'Connor D, Mulé JJ (2001b) The dynamics of the T-cell antitumor response: chemokine-secreting dendritic cells can prime tumor-reactive T cells extranodally. Cancer Res 61(24):8794-8802
51. Baratelli F, Takedatsu H, Hazra S, Peebles K, Luo J, Kurimoto PS, Zeng G, Batra RK, Sharma S, Dubinett SM, Lee JM (2008) Pre-clinical characterization of GMP grade CCL21-gene modified dendritic cells for application in a phase I trial in non-small cell lung cancer. J Transl Med 6:38
52. Yang SC et al (2004) Intratumoral administration of dendritic cells overexpressing CCL21 generates systemic antitumor responses and confers tumor immunity. Clin Cancer Res 10(8):2891-2901
53. Groom JR, Luster AD (2011) CXCR3 in T cell function. Exp Cell Res 317(5):620-631
54. Luster AD, Leder P (1993) IP-10, a -C-X-C- chemokine, elicits a potent thymus-dependent antitumor response in vivo J Exp Med 178(3):1057-1065
55. Yang X et al (2006) Targeted in vivo expression of IFN-γ-inducible protein 10 induces specific antitumor activity. J Leukoc Biol 80(6):1434-1444
56. Hensbergen PJ et al (2005) The CXCR3 targeting chemokine CXCL11 has potent antitumor activity in vivo involving attraction of CD8+ T lymphocytes but not inhibition of angiogenesis. J Immunother 28(4):343-351
57. Matsushima K et al (1989) Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. J Exp Med 169(4):1485-1490
58. Rollins BJ, Sunday ME (1991) Suppression of tumor formation in vivo by expression of the JE gene in malignant cells. Mol Cell Biol 11(6):3125-3131
59. Nokihara H et al, Yanagawa H, Nishioka Y, Yano S, Mukaida N, Matsushima K, Sone S (2000) Natural killer cell-dependent suppression of systemic spread of human lung adenocarcinoma cells by monocyte chemoattractant protein-1 gene transfection in severe combined immunodeficient mice. Cancer Res 60(24):7002-7007
60. Tsuchiyama T, Nakamoto Y, Sakai Y, Marukawa Y, Kitahara M, Mukaida N, Kaneko S (2007) Prolonged, NK cell-mediated antitumor effects of suicide gene therapy

combined with monocyte chemoattractant protein-1 against hepatocellular carcinoma. J Immunol 178(1):574-583

61. Lavergne E et al (2003) Fractalkine mediates natural killer-dependent antitumor responses in vivo. Cancer Res 63(21):7468-7474
62. Tang L et al (2007) Gene therapy with CX3CL1/Fractalkine induces antitumor immunity to regress effectively mouse hepatocellular carcinoma. Gene Ther 14(16):1226-1234
63. Zeng Y et al (2007) Fractalkine (CX3CL1)- and interleukin-2-enriched neuroblastoma microenvironment induces eradication of metastases mediated by T cells and natural killer cells. Cancer Res 67(5):2331-2338
64. Iga M et al (2007) Single CX3CL1-Ig DNA administration enhances T cell priming in vivo. Vaccine 25(23):4554-4563
65. van den Berg A, Visser L, Poppema S (1999) High expression of the CC chemokine TARC in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltratein Hodgkin's lymphoma. Am J Pathol 154(6):1685-1691
66. Di Stasi A et al (2009) T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood 113(25):6392-6402
67. Moon EK et al (2011) Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. Clin Cancer Res 17(14):4719-1430
68. Sarnaik AA, Weber JS (2009) Recent advances using anti-CTLA-4 for the treatment of melanoma. Cancer J 15(3):169-173
69. Ribas A (2012) Tumor immunotherapy directed at PD-1. New Engl J Med 366(26): 2517-2519
70. Sica A, Allavena P, Mantovani A (2008) Caner related inflammation: The macrophage connection. Cancer Lett 267(2):204-215

71. Bailey C et al (2007) Chemokine expression is associated with the accumulation of tumour associated macrophages (TAMs) and progression in human colorectal cancer. Clin Exp Metastasis 24(2):121-130

72. Kim SJ et al (2009) Circulating monocytes expressing CD31: implications for acute and chronic angiogenesis. Am J Pathol 174(5):1972-1980

73. Ruffell B, Affar NI, Coussens LM (2012) Differential macrophage programming in the tumor microenvironment. Trends Immunol 33(3): 119-126

74. Curiel TJ et al (2004) Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 10(9):942-949

75. Kryczek I et al (2006) B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. J Exp Med 203(4):871-881

76. Loberg RD et al (2007) Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor progression *in vivo*. Cancer Res 67(19):9417-9424

77. Popivanova BK et al (2009) Blockade of a chemokine, CCL2, reduces chronic colitis-associated carcinogenesis in mice. Cancer Res 69(19):7884-7892

2.78. Qian B-Z et al, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, Kaiser EA, Snyder LA, Pollard JW (2011) CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature 475(7355):222-226. doi: 10.1038/nature10138

79. Condamine T, Gabrilovich DI (2011) Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. Trends Immunol 32(1):19-25

80. Huang B et al (2007) CCL2/CCR2 pathway mediates recruitment of myeloid suppressor cells to cancers. Cancer Lett 252(1):86-92

81. Lesokhin AM et al (2012) Monocytic CCR2^{+} myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment. Cancer Res 72(4):876-886

82. Sawanobori Y (2008) Chemokine-mediated rapid turnover of myeloid-derived suppressor cells in tumor-bearing mice. Blood 111(12):5457-5466

83. Brandau S et al (2011) Myeloid-derived suppressor cells in the peripheral blood of cancer patients contain a subset of immature neutrophils with impaired migratory properties. J Leukoc Biol 89(2):311-317
84. Yang L et al (2008) Abrogation of TGF β signaling in mammary carcinomas recruits Gr-$1^{+}$$CD11b^{+}$ myeloid cells that promote metastasis. Cancer Cell 13(1):23-35
85. Obermajer N et al (2011) PGE2-induced CXCL12 production and CXCR4 expression controls the accumulation of human MDSCs in ovarian cancer environment. Cancer Res 71(24):7463-7470
86. Nishikawa H, Sakaguchi S (2010) Regulatory T cells in tumor immunity. Int J Cancer 127(4):759-767
87. Facciabene A et al (2011) Tumour hypoxia promotes tolerance and antigogenesis via CCL28 and Treg cells. Nature 475(7355):226-230. doi: 10.1038/nature10169
88. Fridlender ZG et al (2010) CCL2 blockade augments caner cancer immunotherapy. Cancer Res 70(1):109-118
89. Yoshie O et al (2002) Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T cells. Blood 99(5):1505-1511
90. Ishida T, Ueda R (2011) Antibody therapy for Adult T-cell leukemia–lymphoma. Int J Hematol 94(5):443-452
91. Kitamura T et al (2007) SMAD4-deficient intestinal tumors recruit $CCR1^{+}$ myeloid cells that promote invasion. Nature Genet 39(4):467-475
92. Kitamura T et al (2010) Inactivation of chemokine (C-C motif) receptor 1 (CCR1) suppresses colon cancer liver metastasis by blocking accumulation of immature myeloid cells in a mouse model. Proc Natl Acad Sci USA 107(29):13063-13068
93. Oppenheim JJ et al (1991) Properties of the novel proinflammatory supergene "intercrine" cytokine family. Annu Rev Immunol 9:617-648
94. Kitadai Y et al (2000) Regulation of disease-progression genes in human gastric carcinoma cells by interleukin-8. Clin Cancer Res 6(7):2735-2740

95. Wang B et al , Hendricks DT, Wamunyokoli F, Parker MI (2006) A growth-related oncogene/CXC chemokine receptor 2 autocrine loop contributes to cellular proliferation in esophageal cancer. Cancer Res 66(6):3071-3077

96. Singh S et al (2009) CXCR1 and CXCR2 enhances human melanoma tumourigenesis, growth and invasion, Brit J Cancer 100(10):1638-1646

97. Teicher BA, Fricker SP (2010) CXCL12 (SDF-1)/CXCR4 pathway in cancer. Clin Cancer Res 16(11):2927-2931

98. Ghadjar P et al (2009) The chemokine CCL20 and its receptor CCR6 in human malignancy with focus on colorectal cancer. Int J Cancer 125(4):741-745

99. Darash-Yahana M et al (2009) The chemokine CXCL16 and its receptor, CXCR6, as markers and promoters of inflammation-associated cancers, PlosOne 4(8):e6695

100. Murakami T et al (2003) Immune evasion by murine melanoma mediated through CC chemokine receptor-10. J Exp Med 198(9):1337-1347

101. Wang J et al (2008) Autocrine and paracrine chemokine receptor 7 activation in head and neck cancer: implications for therapy. J Natl Cancer Inst 100(7):502-512

102. Bertran E et al, Caja L, Navarro E, Sancho P, Mainez J, Murillo MM, Vinyals A, Fabra A, Fabregat I (2009) Role of CXCR4/SDF-1α in the migratory phenotype of hepatoma cells that have undergone epithelial-mesenchymal transition in response to the transforming growth factor-β. Cell Signal 21(11):1595-1606

103. Righi E et al (2011) CXCL12/CXCR4 blockade induces multimodal antitumor effects that prolong survival in an immunocompetent mouse model of ovarian cancer, Cancer Res 71(16):522-5534

104. Messmer D et al (2011) Chronic lymphocytic leukemia cells receive RAF-dependent survival signals in response to CXCL12 that are sensitive to inhibition by sorafenib. Blood 117(3):882-889

105. Fernando RI et al (2011) IL-8 signaling plays a critical role in the epithelial-mesenchymal transition of human carcinoma cells., Cancer Res 71(15):5296-5306

106. Kochetkova M, Kumar S, McColl SR (2009) Chemokine receptors CXCR4 and CCR7 promote metastasis by preventing anoikis in cancer cells. Cell Death Differ 16(5):664-673

107. Müller A et al (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410(6824):50-56

108. Buonamici S et al (2009) CCR7 signaling as an essential regulator of CNS infiltration of T-cell leukaemia. Nature 459(7249):1000-1004

109. Amersi FF et al (2008) Activation of CCR9/CCL25 in cutaneous melanoma mediates preferential metastasis to the small intestine. Clin Cancer Res 14(3):638-645

110. Waugh DJ, Wilson C (2008) The interleukin-8 pathway in cancer. Clin Cancer Res 14(21):6735-6741

111. Zhang Y et al (2012) SDF-1/CXCR4 axis in myelodysplastic syndromes: correlation with angiogenesis and apoptosis. Leukemia Res 36(3):281-286

112. Shields JD et al (2007) Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling. Cancer Cell 11(6):526-538

113. Zhang XH et al (2009) Latent bone metastasis in breast cancer tied to Src-dependent survival signals, Cancer Cell 16(1):67-78

114. Fidler IJ, Ellis EM (1994) The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell 79(2):185-188

115. Keeley EC, Mehrad B, Strieter RM (2011) Chemokines as mediators of tumor angiogenesis and neovascularization. Exp Cell Res 317(5):685-690

116. Arenberg DA et al (1996) Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J Clin Invest 97(12):2792-2802

117. Kryczek I et al (2007) Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis. Am J Physiol Cell Physiol 292(3):C987-995

118. Gálvez BG et al (2005) Membrane type 1-matrix metalloproteinase is regulated by chemokines monocyte-chemoattractant protein-1/ccl2 and interleukin-8/CXCL8 in endothelial cells during angiogenesis. J Biol Chem 280(2):1292-1298

119. Salcedo R et al (2001) Eotaxin (CCL11) induces in vivo angiogenic responses by human $CCR3^+$ endothelial cells. J Immunol 166(12):7571-7578

120. Strasly M et al (2004) CCL16 activates an angiogenic program in vascular endothelial cells. Blood 103(1):40-49

121. Rehman J et al (2003) Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation 107(8):1164-1169

122. Maione TE, Gray GS, Petro J, Hunt AJ, Donner AL, Bauer SI, Carson HF, Sharpe RJ (1990) Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science 247(4938):77-79

123. Romagnani P et al (2001) Cell-cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. J Clin Invest 107(1):53-63

124. Addison CL et al, Arenberg DA, Morris SB, Xue YY, Burdick MD, Mulligan MS, Iannettoni MD, Strieter RM (2000) The CXC chemokine, monokine induced by interferon-gamma, inhibits non-small cell lung carcinoma tumor growth and metastasis. Hum Gene Ther 11(2):247-61.

Allen SJ, Crown SE, Handel TM. (2007) Chemokine: receptor structure, interactions, and antagonism. Annu Rev Immunol 25:787-820.

Amersi FF, Terando AM, Goto Y, Scolyer RA, Thompson JF, Tran AN, Faries MB, Morton DL, Hoon DS (2008) Activation of CCR9/CCL25 in cutaneous melanoma mediates preferential metastasis to the small intestine. Clin Cancer Res 14(3):638-645.

Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM (1996) Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J Clin Invest 97(12):2792-2802.

Bailey C, Negus R, Morris A, Ziprin P, Goldin R, Allavena P, Peck D, Darzi A (2007) Chemokine expression is associated with the accumulation of tumour associated macrophages (TAMs)9 and progression in human colorectal cancer. Clin Exp Metastasis 24(2):121-130.

Baratelli F, Takedatsu H, Hazra S, Peebles K, Luo J, Kurimoto PS, Zeng G, Batra RK, Sharma S, Dubinett SM, Lee JM (2008) Pre-clinical characterization of GMP grade CCL21-gene modified dendritic cells for application in a phase I trial in non-small cell lung cancer. J Transl Med 6:38.

Barbero S, Bonavia R, Bajetto A, Porcile C, Pirani P, Ravetti JL, Zona GL, Spaziante R, Florio T, Schettini G (2003) Stromal cell-derived factor 1α stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt**.** Cancer Res 63(8):1969-1974.

~~Bertran E, Caja L, Navarro E, Sancho P, Mainez J, Murillo MM, Vinyals A, Fabra A, Fabregat I (2009) Role of CXCR4/SDF-1α in the migratory phenotype of hepatoma cells that have undergone epithelial-mesenchymal transition in response to the transforming growth factor-β. Cell Signal 21:1595-1606.~~

Bindea G, Mlecnik B, Fridman WH, Pagès F, Galon J (2010) Natural immunity to cancer in humans. Curr Opin Immunol. 22:215-222.

Brandau S, Trellakis S, Bruderek K, Schmaltz D, Steller G, Elian M, Suttmann H, Schenck M, Welling J, Zabel P, Lang S (2011) Myeloid-derived suppressor cells in the peripheral blood of cancer patients contain a subset of immature neutrophils with impaired migratory properties. J Leukoc Biol 89:311-317.

Breitwieser GE (2004) G protein-coupled receptor oligomerization: implications for G protein activation and cell signaling. Circ Res 94:17-27.

Buonamici S, Trimarchi T, Ruocco MG, Reavie L, Cathelin S, Mar BG, Klinakis A, Lukyanov Y, Tseng JC, Sen F, Gehrie E, Li M, Newcomb E, Zavadil J, Meruelo D, Lipp M, Ibrahim S, Efstratiadis A, Zagzag D, Bromberg JS, Dustin ML, Aifantis I (2009) CCR7 signaling as an essential regulator of CNS infiltration of T-cell leukaemia. Nature 459:1000-1004.

Chang AE, Redman BG, Whitfield JR, Nickoloff BJ, Braun TM, Lee PP, Geiger JD, Mulé JJ. (2002) A phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. Clin Cancer Res 8:1021-32.

~~Condamine T, Gabrilovich DI (2011) Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. Trends Immunol 32:19-25~~.

Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W (2004) Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 10:942-949.

Darash-Yahana M, Gillespie JW, Hewitt SM, Chen YY, Maeda S, Stein I, Singh SP, Bedolla RB, Peled A, Troyer DA, Pikarsky E, Karin M, Farber JM (2009) The chemokine CXCL16 and its receptor, CXCR6, as markers and promoters of inflammation-associated cancers, PlosOne 4:e6695.

Di Stasi A, De Angelis B, Rooney CM, Zhang L, Mahendravada A, Foster AE, Heslop HE, Brenner MK, Dotti G, Savoldo B (2009) T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. Blood 113:6392-6402.

Druey KM, Blumer KJ, Kang VH, Kehrl JH (1996) Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family. Nature 379:742-746.

Facciabene A, Peng X, Hagemann IS, Balint K, Barchetti A, Wang LP, Gimotty PA, Gilks CB, Lal P, Zhang L, Coukos G (2011) Tumour hypoxia promotes tolerance and antigogenesis via CCL28 and Treg cells. Nature 475:226-230.

Fernando RI, Castillo MD, Litzinger M, Hamilton DH, PalenaC (2011) IL-8 signaling plays a critical role in the epithelial-mesenchymal transition of human carcinoma cells, Cancer Res 71:5296-5306.

Fernandez EJ, Lolis E (2002) Structure, function, and inhibition of chemokines. Annu Rev Pharmacol Toxicol 42:469-499.

Fidler IJ, Ellis EM (1994) The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell 79:185-188.

Förster R, Schubel A, Breitfeld D, Kremmer E, Renner-Müller I, Wolf E, Lipp M (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 99:23-33.

Fridlender ZG, Buchlis G, Kapoor V, Cheng G, Sun J, Singhal S, Crisanti MC, Wang LC, Heitjan D, Snyder LA, Albelda SM. (2010) CCL2 blockade augments caner cancer immunotherapy. Cancer Res 70:109-118.

Gálvez BG, Genis L, Matias-Román S, Oblander SA, Tryggvason K, Apte SS, Arroyo AG (2005) Membrane type 1-matrix metalloproteinase is regulated by chemokines monocyte-chemoattractant protein-1/ccl2 and interleukin-8/CXCL8 in endothelial cells during angiogenesis. J Biol Chem 280:1292-1298.

Ghadjar P, Rubie C, Aebersold DM, Keilholz U (2009) The chemokine CCL20 and its receptor CCR6 in human malignancy with focus on colorectal cancer. Int J Cancer 125:741-745.

Gooden MJ, de Bock GH, Leffers N, Daemen T, Nijman HW (2011) The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. Br J Cancer 105:93-103.

Groom JR, Luster AD (2011) CXCR3 in T cell function. Exp Cell Res 317:620-631.

Halama N, Braun M, Kahlert C, Spille A, Quack C, Rahbari N, Koch M, Weitz J, Kloor M, Zoernig I, Schirmacher P, Brand K, Grabe N, Falk CS. (2011) Natural killer cells are scarce in colorectal carcinoma tissue despite high levels of chemokines and cytokines. Clin Cancer Res 17:678-689.

Hensbergen PJ, Wijnands PG, Schreurs MW, Scheper RJ, Willemze R, Tensen CP (2005) The CXCR3 targeting chemokine CXCL11 has potent antitumor activity in vivo involving attraction of CD8+ T lymphocytes but not inhibition of angiogenesis. J Immunother 28:343-351.

Hernanz-Falcón P, Rodríguez-Frade JM, Serrano A, Juan D, del Sol A, Soriano SF, Roncal F, Gómez L, Valencia A, Martínez-A C, Mellado M (2004) Identification of amino acid residues crucial for chemokine receptor dimerization. Nature Immunol 5:216-223.

Hillinger S, Yang SC, Batra RK, Strieter RM, Weder W, Dubinett SM, Sharma S (2006) CCL19 reduces tumour burden in a model of advanced lung cancer. Br J Cancer 94:1029-1034.

Hojo S, Koizumi K, Tsuneyama K, Arita Y, Cui Z, Shinohara K, Minami T, Hashimoto I, Nakayama T, Sakurai H, Takano Y, Yoshie O, Tsukada K, Saiki I. (2007) High level expression of chemokine CXCL16 by tumor cells correlates with a good prognosis and increased tumor-infiltrating lymphocytes in colorectal cancer. Cancer Res 67:4725-4731.

Huang B, Lei Z, Zhao J Gong W, Liu J, Chen Z, Liu Y, Li D, Yuan Y, Zhang G-M, Feng Z-H (2007) CCL2/CCR2 pathway mediates recruitment of myeloid suppressor cells to cancers. Cancer Lett 252:86-92.

Iga M, Boissonnas A, Mahé B, Bonduelle O, Combadière C, Combadière B (2007) Single CX3CL1-Ig DNA administration enhances T cell priming in vivo. Vaccine. 25:4554-4563.

Iida N, Nakamoto Y, Baba T, Kakinoki K, Li Y-Y, Wu Y, Matsushima K, Kaneko S, Mukaida N (2008) Tumor cells apoptosis induces tumor-specific immunity in a CC chemokine receptor 1- and 5-dependent manner in mice. J Leukocyte Biol 84:1001-1010.

Iida N, Nakamoto Y, Baba T, Nakagawa H, Mizukoshi E, Naito M, Mukaida N, Kaneko S (2010) Antitumor effect after radiofrequency ablation of murine hepatoma is augmented by an active variant of CC chemokine ligand 3/macrophage inflammatory proein-1α. Cancer Res 70:6556-6566.

Ishida T, Ueda R (2011) Antibody therapy for Adult T-cell leukemia–lymphoma. Int J Hematol 94:443-452.

Jansma A, Handel TM, Hamel DJ. (2009) Homo- and hetero-oligomerization of chemokines. Methods Enzymol 461:31-50.

Keeley EC, Mehrad B, Strieter RM (2011) Chemokines as mediators of tumor angiogenesis and neovascularization. Exp Cell Res 317:685-690.

Kim SJ, Kim JS, Papadopoulos J, Wook Kim S, Maya M, Zhang F, He J, Fan D, Langley R, Fidler IJ. (2009) Circulating monocytes expressing CD31: implications for acute and chronic angiogenesis. Am J Pathol. 174:1972-1980.

Kirk CJ, Hartigan-O'Connor D, Nickoloff BJ, Chamberlain JS, Giedlin M, Aukerman L, Mule JJ (2001a) T cell-dependent antitumor immunity mediated by secondary lymphoid tissue chemokine: augmentation of dendritic cell-based immunotherapy. Cancer Res 61:2062-2070.

Kirk CJ, Hartigan-O'Connor D, Mulé JJ (2001b) The dynamics of the T-cell antitumor response: chemokine-secreting dendritic cells can prime tumor-reactive T cells extranodally. Cancer Res 61:8794-8802.

Kitadai Y, Haruma K, Mukaida N, Ohmoto Y, Matsutani N, Yasui W, Yamamoto S, Sumii K, Kajiyama G, Fidler IJ, Tahara E (2000) Regulation of disease-progression genes in human gastric carcinoma cells by interleukin-8, Clin Cancer Res 6:2735-2740.

Kitamura T, Kometani K, Hashida H, Matsunaga A, Miyoshi H, Hosogi H, Aoki M, Oshima M, Hattori M, Takabayashi A, Minato N, Taketo MM (2007) SMAD4-deficient intestinal tumors recruit $CCR1^+$ myeloid cells that promote invasion. Nature Genet 39:467-475.

Kitamura T, Fujishita T, Loetscher P, Revesz L, Hashida H, Kizaka-Kondoh S, Aoki M, Taketo MM (2010) Inactivation of chemokine (C-C motif) receptor 1 (CCR1) suppresses colon cancer liver metastasis by blocking accumulation of immature myeloid cells in a mouse model. Proc Natl Acad Sci USA 107:13063-13068.

Knall C, Young S, Nick JA, Buhl AM, Worthen GS, Johnson GL (1996) Interleukin-8 regulation of the Ras/Raf/mitogen-activated protein kinase pathway in human neutrophils. J Biol Chem 271: 2832-2838.

Knutson KL, Disis ML (2005) Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. Cancer Immunol Immunother 54: 721-728.

Kochetkova M, Kumar S, McColl SR (2009) Chemokine receptors CXCR4 and CCR7 promote metastasis by preventing anoikis in cancer cells. Cell Death Differ 16:664-673.

Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, Mottram P, Brumlik M, Cheng P, Curiel T, Myers L, Lackner A, Alvarez X, Ochoa A, Chen L, Zou W. (2006) B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. J Exp Med 203:871-881.

Kryczek I, Wei S, Keller E, Liu R, Zou W (2007) Stroma-derived factor (SDF-1/CXCL12) and human tumor pathogenesis. Am J Physiol Cell Physiol 292:C987-995.

Lavergne E, Combadière B, Bonduelle O, Iga M, Gao JL, Maho M, Boissonnas A, Murphy PM, Debré P, Combadière C (2003) Fractalkine mediates natural killer-dependent antitumor responses in vivo. Cancer Res 63:7468-7474.

Le Y, Honczarenko M, Glodek AM, Ho DK, Silberstein LE (2005) CXC chemokine lignd 12-induced focal adhesion kinase activation and segregation into membrane domains is modulated by regulator of G protein signaling 1 in pro-B cells. J Immunol 174:2582-2590.

Lesokhin AM, Hohl TM, Kitano S, Cortez C, Hirschhorn-Cymerman D, Avogadri F, Rizzuto GA, Lazarus JJ, Pamer EG, Houghton AN, Merghoub T, Wolchok JD. (2012) Monocytic $CCR2^{+}$ myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment. Cancer Res 72:876-886.

Loberg RD, Ying C, Craig M, Day LL, Sargent E, Neeley C, Wojno K, Snyder LA, Yan L, Pienta KJ. (2007) Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor progression *in vivo*. Cancer Res 67:9417-9424.

Luster AD, Leder P (1993) IP-10, a -C-X-C- chemokine, elicits a potent thymus-dependent antitumor response in vivo J Exp Med 178:1057-1065.

Maione TE, Gray GS, Petro J, Hunt AJ, Donner AL, Bauer SI, Carson HF, Sharpe RJ (1990) Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science 247:77-79.

Mantovani A, Bonecchi R, Locati M. (2006) Tuning inflammation and immunity by chemokine sequestration: decoys and more. Nat Rev Immunol 6:907-918.

Martin-Fontecha A, Sebastiani S, Höpken UE, Uguccioni M, Lipp M, Lanzavecchia A, Sallusto F (2003) Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J Exp Med 198:615-621.

Matsushima K, Larsen CG, DuBois GC, Oppenheim JJ (1989) Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. J Exp Med 169:1485-1490.

Mellado M, Rodriguez-Frade JM, Aragay AM, del Real G, Vila-Coro AJ, , de Ana AM, Serrano A, Mayor FJR, Martiez-A C (1998) The chemokine MCP-1 triggers tyrosine phosphorylation of the CCR2B receptor and the JAK2/STAT3 pathway. J Immunol 161:805-813.

Mellado M, Rodríguez-Frade JM, Vila-Coro AJ, Fernández S, Martín de Ana A, Jones DR, Torán JL, Martínez-A C. (2001) Chemokine receptor homo- or heterodimerization activates distinct signaling pathways. EMBO J 20:2497-2507.

Messmer D, Fecteau J-F, O'Hayre M, Bharati IS, Handel TM, Kipps TJ (2011), Chronic lymphocytic leukemia cells receive RAF-dependent survival signals in response to CXCL12 that are sensitive to inhibition by sorafenib. Blood 117:882-889.

Moepps B, Nuesseler E, Braun P, Gierschick P. (2006) A homolog of the human chemokine receptor CXCR1 is expressed in mouse. Mol Immunol 43:897-914.

Moser B, Wolf M, Walz A, Loetscher P (2004) Chemokines: multiple levels of leukocyte migration control. Trends Immunol 25:75-84.

Moon EK, Carpenito C, Sun J, Wang LC, Kapoor V, Predina J, Powell DJ Jr, Riley JL, June CH, Albelda SM (2011) Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. Clin Cancer Res 17:4719-1430.

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 (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50-56.

Murakami T, Cardones AR, Finkelstein SE, Restifo NP, Klaunberg BA, Nestle FO, Castillo SS, Dennis PA, Hwang ST (2003), Immune evasion by murine melanoma mediated through CC chemokine receptor-10. J Exp Med 198:1337-1347.

Musha H, Ohtani H, Mizoi T, Kinouchi M, Nakayama T, Shiiba K, Miyagawa K, Nagura H, Yoshie O, Sasaki I. (2005) Int J Cancer. 2005 Oct 10;116(6):949-56.

Muthuswamy R, Berk E, Junecko BF, Zeh HJ, Zureikat AH, Normolle D, Luong TM, Reinhart TA, Bartlett DL, Kalinski P. (2012) NF-κB hyperactivation in tumor tissues allows tumor-selective reprogramming of the chemokine microenvironment to enhance the recruitment of cytolytic T effector cells. Cancer Res 72:3735-3743.

Neel NF, Schutyser E, Sai J Fan G-H, Richmond A. (2005) Chemokine receptor internalization and intracellular trafficking. Cytokine Growth Factor Rev 16:637-658.

Nishikawa H, Sakaguchi S (2010) Regulatory T cells in tumor immunity. Int J Cancer 127:759-767.

Nomiyama H, Osada N, Yoshie O (2010) The evolution of mammalian chemokine genes. Cytokine Growth Fact Rev 21:253-262.

~~Nokihara H, Yanagawa H, Nishioka Y, Yano S, Mukaida N, Matsushima K, Sone S (2000) Natural killer cell-dependent suppression of systemic spread of human lung adenocareinoma cells by monocyte chemoattractant protein-1 gene transfection in severe combined immunodeficient mice. Cancer Res 60:7002-7007~~.

Obermajer N, Muthuswamy R, Odunsi K, Edwards RP, Kalinski P (2011) PGE2-induced CXCL12 production and CXCR4 expression controls the accumulation of human MDSCs in ovarian cancer environment. Cancer Res 71:7463-7470.

Ohta M, Tanaka F, Yamaguchi H, Sadanaga N, Inoue H, Mori M. (2005) The high expression of fractalkine results in a better prognosis in colorectal cancer patients. Int J Oncol 26:41-47Ohtani H, Jin Z, Takegawa S, Nakayama T, Yoshie O. (2009) Abundant expression of CXCL9 (Mig) by stromal cells that include dendritic cells and accumulation of CXCR3$^{\pm}$ T cells in lymphocyte-rich gastric cancer. J Pathol 217:21-31.

.

Oppenheim JJ, Zachariae COC, Mukaida N, Matsushima K (1991) Properties of the novel proinflammatory supergene "intercrine" cytokine family. Annu Rev Immunol 9:617-648.

Ohta M, Tanaka F, Yamaguchi H, Sadanaga N, Inoue H, Mori M (2005) The high expression of fractalkine results in a better prognosis for colorectal cancer patients. Int J Oncol 26:41-47.

~~Ohtani H, Jin Z, Takegawa S, Nakayama T, Yoshie O. (2009) Abundant expression of CXCL9 (Mig) by stromal cells that include dendritic cells and accumulation of CXCR3^{+} T cells in lymphocyte-rich gastric cancer. J Pathol 217:21-31.~~

~~Palucka K, Banchereau J (2012) Cancer immunotherapy via dendritic cells. Nat Rev Cancer 12:265-277.~~

Pan J, Burdick MD, Belperio JA, Xue YY, Gerard C, Sharma S, Dubinett SM, Strieter RM (2006) CXCR3/CXCR3 ligand biological axis impairs RENCA tumor growth by a mechanism of immunoangiostasis. J Immunol 176(3):1456-1464.

Porcile C, Bajetto A, Barbieri F, Barbero S, Bonavia R, Biglieri M, Pirani P, Florio T, Schettini G (2005) Stromal cell-derived factor-1α (SDF-1α/CXCL12) stimulates ovarian cancer cell growth through the EGF receptor transactivation. Exp Cell Res 308(2):241-53.

Popivanova BK, Kostadinova FI, Furuichi K, Shamekh MM, Kondo T, Wada T, K. Egashira K, Mukaida N (2009) Blockade of a chemokine, CCL2, reduces chronic colitis-associated carcinogenesis in mice. Cancer Res 69(19):7884-7892.

~~Qian B-Z, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, Kaiser EA, Snyder LA, Pollard JW (2011) CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature 475(7355):222-226. doi: 10.1038/nature10~~

Qu C, Edwards EW, Tacke F, Angeli V, Llodrá J, Sanchez-Schmitz G, Garin A, Haque NS, Peters W, van Rooijen N, Sanchez-Torres C, Bromberg J, Charo IF, Jung S, Lira SA, Randolph GJ. (2004) Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes. J Exp Med 200(10):1231-1241.

Rehman J, Li J, Orschell CM, March KL (2003) Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation 107(8):1164-1169.

Ribas A (2012) Tumor immunotherapy directed at PD-1. New Engl J Med 366(26): 2517-2519.

Ridley AjAJ, Schwartz MA, Burrige K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR (2003) Cell migration: integrating signals from front to back. Science 302(5651):1704-1709.

Righi E, Kashiwagi S, Yuan J, Santosuosso M, Leblanc P, Ingraham R, Forbes B, Edelblute B, Collette B, Xing D, Kowalski M, Mingari MC, Vianello F, Birrer M, Orsulic S, Dranoff G, Poznanasky MC (2011) CXCL12/CXCR4 blockade induces multimodal antitumor effects that prolong survival in an immunocompetent mouse model of ovarian cancer, Cancer Res 71(16):522-5534.

Rodríguez-Frade JM, Vila-Coro AJ, de Ana AM, Albar JP, Martínez-A C, Mellado M (1999) The chemokine monocyte chemoattractant protein-1 induces functional responses through dimerization of its receptor CCR2. Proc Natl Acad Sci USA 96(7):3628-3633.

Rodríguez-Frade JM, Mellado M, Martínez-A C (2001) Chemokine receptor dimerization: two are better than one. Trends Immunol 22(11): 612-617.

Rollins BJ, Sunday ME (1991) Suppression of tumor formation in vivo by expression of the JE gene in malignant cells. Mol Cell Biol 11(6):3125-3131.

Romagnani P, Annunziato F, Lasagni L, Lazzeri E, Beltrame C, Francalanci M, Uguccioni M, Galli G, Cosmi L, Maurenzig L, Baggiolini M, Maggi E, Romagnani S, Serio M (2001) Cell-cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. J Clin Invest 107(1):53-63.

~~Ruffell B, Affar NI, Coussens LM (2012) Differential macrophage programming in the tumor microenvironment. Trends Immunol 33(3): 119-126~~.

Salcedo R, Young HA, Ponce ML, Ward JM, Kleinman HK, Murphy WJ, Oppenheim JJ (2001) Eotaxin (CCL11) induces in vivo angiogenic responses by human $CCR3^{+}$ endothelial cells. J Immunol 166(12) :7571-7578.

Sarnaik AA, Weber JS (2009) Recent advances using anti-CTLA-4 for the treatment of melanoma. Cancer J 15(3):169-173.

Sawanobori Y, Ueha S, Kurachi M, Shimaoka T, Talmadge JE, Abe J, Shono Y, Kitabatake M, Kakimi K, Mukaida N, Matsushima K (2008) Chemokine-mediated rapid turnover of myeloid-derived suppressor cells in tumor-bearing mice. Blood 111(12):5457-5466.

Servant G, Weiner OD, Hezmark P, Balla T, Sedat JW, Bourne HR (2000) Polarizattion of chemoattractant receptor signaling during neutrophil chemotaxis. Science 287(5455):1037-1040.

Sharma S, Stolina M, Zhu L, Lin Y, Batra R, Huang M, Strieter R, Dubinett SM (2001) Secondary lymphoid organ chemokine reduces pulmonary tumor burden in spontaneous murine bronchoalveolar cell carcinoma. Cancer Res 61(17):6406-6412.

Shi GX, Harrison K, Wilson GL, Moratz C, Kehrl JH (2002) RGS13 regulates germinal center B lymphocytes responsiveness to CXC chemokine ligand (CXCL)12 and CXCL13. J Immunol 169(5):2507-2515.

Shields JD, Fleury ME, Yong C, Tomei AA, Randolph GJ, Swartz MA (2007) Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling. Cancer Cell 11(6):526-538.

Sica A, Allavena P, Mantovani A (2008) Caner related inflammation: The macrophage connection. Cancer Lett 267(2)64:204-215.

Singh S, Nannuru KC, Sadanandam A, Varney ML, Singh RK (2009) CXCR1 and CXCR2 enhances human melanoma tumourigenesis, growth and invasion, Brit J Cancer 100(10):1638-1646.

Sohy D, Parmentier M, Springael JY (2007) Allosteric transinhibition by specific antagonists in CCR2/CXCR4 heterodimers. J Biol Chem 282(41): 30062-30069.

Sozzani S (2005) Dendritic cell trafficking: more than just chemokines. Cytokine Growth Factor Rev 16(6):581-592.

Strasly M, Doronzo G, Cappello P, Valdembri D, Arese M, Mitola S, Moore P, Alessandri G, Giovarelli M, Bussolino F (2004) CCL16 activates an angiogenic program in vascular endothelial cells. Blood 103(1):40-49.

Su S-B, Mukaida N, Wang J-B, Nomura H, Matsushima K. (1996) Preparation of specific antagonizing polyclonal antibodies to a C-C chemokine receptor, CCR1 and determination of its distribution of various types of leukocytes. J Leukocyte Biol 60(5):658-666.

Tang L, Hu HD, Hu P, Lan YH, Peng ML, Chen M, Ren H (2007) Gene therapy with CX3CL1/Fractalkine induces antitumor immunity to regress effectively mouse hepatocellular carcinoma. Gene Ther 14(16):1226-1234.

Teicher BA, Fricker SP (2010) CXCL12 (SDF-1)/CXCR4 pathway in cancer. Clin Cancer Res 16(11):2927-2931.

Tsuchiyama T, Nakamoto Y, Sakai Y, Marukawa Y, Kitahara M, Mukaida N, Kaneko S (2007) Prolonged, NK cell-mediated antitumor effects of suicide gene therapy combined with monocyte chemoattractant protein-1 against hepatocellular carcinoma. J Immunol 178(1):574-583.

van den Berg A, Visser L, Poppema S (1999) High expression of the CC chemokine TARC in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltratein Hodgkin's lymphoma. Am J Pathol 154(6):1685-1691.

Vandercappellen J, Van Damme J, Struyf S (2008) The role of CXC chemokines and their receptors in caner. Cancer Lett 267(2):226-244.

Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S (2008) Functions of natural killer cells. Nat Immunol 9(5):503-510.

Walzer T, Vivier E (2011) G-protein-coupled receptors in control of natural killer cell migration. Trends Immunol 32(10):486-492.

~~Wang B, Hendricks DT, Wamunyokoli F, Parker MI (2006) A growth-related oncogene/CXC chemokine receptor 2 autocrine loop contributes to cellular proliferation in esophageal cancer. Cancer Res 66(6):3071-3077~~.

Wang J, Seethala RR, Zhang Q, Gooding W, van Waes C, Hasegawa H, Ferris RL (2008) Autocrine and paracrine chemokine receptor 7 activation in head and neck cancer: implications for therapy. J Natl Cancer Inst 100(7):502-512.

Waugh DJ, Wilson C (2008) The interleukin-8 pathway in cancer. Clin Cancer Res 14(21):6735-6741.

Yang L, Huang J, Ren X, Gorska AE, Chytill A, Aakre M, Carbone DP, Matrisian LM, Richmond A, Lin PC, Moses HL (2008) Abrogation of TGF β signaling in mammary carcinomas recruits Gr-1^{+}CD11b^{+} myeloid cells that promote metastasis. Cancer Cell 13(1):23-35.

Yang SC, Hillinger S, Riedl K, Zhang L, Zhu L, Huang M, Atianzar K, Kuo BY, Gardner B, Batra RK, Strieter RM, Dubinett SM, Sharma S (2004) Intratumoral administration of dendritic cells overexpressing CCL21 generates systemic antitumor responses and confers tumor immunity. Clin Cancer Res 10(8):2891-2901.

Yang X, Chu Y, Wang Y, Zhang R, Xiong S (2006) Targeted in vivo expression of IFN-γ-inducible protein 10 induces specific antitumor activity. J Leukoc Biol 80(6):1434-1444.

Yoshie O, Fujisawa R, Nakayama T, Harasawa H, Tago H, Izawa D, Hieshima K, Tatsumi, Matsushima K, Hasegawa H, A. Kanamaru A, S. Kamihira S, Y. Yamada Y (2002) Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T cells. Blood 99(5):1505-1511.

Zeng Y, Huebener N, Fest S, Weixler S, Schroeder U, Gaedicke G, Xiang R, Schramm A, Eggert A, Reisfeld RA, Lode HN (2007) Fractalkine (CX3CL1)- and interleukin-2-enriched neuroblastoma microenvironment induces eradication of metastases mediated by T cells and natural killer cells. Cancer Res 67(5):2331-2338.

Zhang XH, Wang Q, Gerald W, Hudis CA, Norton L, Smid M, Foekens JA, Massagué J (2009) Latent bone metastasis in breast cancer tied to Src-dependent survival signals, Cancer Cell 16(1):67-78.

Zhang Y, Yoneyama H, Wang Y, Ishikawa S, Hashimoto S, Gao JL, Murphy P, Matsushima K (2004) Mobilization of dendritic cell precursors into the circulation by administration of MIP-1α in mice. J Natl Cancer Inst 96(3):201-209.

Zhang Y, Zhao H, Zhao D, Sun L, Zhi Y, Wu X, Huang W, Da W (2012) SDF-1/CXCR4 axis in myelodysplastic syndromes: correlation with angiogenesis and apoptosis. Leukemia Res 36(3):281-286.

Zlotnik A, O. Yoshie O (2000) Chemokines: a new classification system and their role in immunity. Immunity 12(2): 121-127.

Chemokine Receptor	Chemokines	Receptor Expression in		
		Leukocytes	Epithelium	Endothelium
CXCR1	CXCL6, 8	PMN	+	-
CXCR2	CXCL1, 2, 3, 5, 6, 7, 8	PMN	+	+
CXCR3	CXCL4, 9, 10, 11	Th1, NK	-	+
CXCR4	CXCL12	Widespread	+	+
CXCR5	CXCL13	B	-	-
CXCR6	CXCL16	activated T	+	-
CXCR7	CXCL12, CXCL11	Widespread	+	+
Unknown	CXCL14 (acts on monocytes)			
CCR1	CCL3, 4, 5, 7, 14, 15, 16, 23	Mo, Mϕ, iDC, NK	+	+
CCR2	CCL2, 7, 8, 12, 13	Mo, Mϕ, iDC, NK activated T, B	+	+
CCR3	CCL5, 7, 11, 13, 15, 24, 26, 28	Eo, Ba, Th2	-	+
CCR4	CCL2, 3, 5, 7, 22	iDC, Th2, NK, T, Mϕ	-	-
CCR5	CCL3, 4, 5, 8	Mo, Mϕ, NK, Th1 activated T	+	-
CCR6	CCL20	iDC, activated T, B	+	-
CCR7	CCL19, 21	mDC, Mϕ, naïve T activated T	+	-
CCR8	CCL1, 4, 17	Mo, iDC, Th2, Treg	-	-
CCR9	CCL25	T	+	-
CCR10	CCL27, 28	activated T, Treg	+	-
Unknown	CCL18 (acts on mDC and naïve T)			
CX3CR1	CX3CL1	Mo, iDC, NK, Th1	+	-
XCR1	XCL1, 2	T, NK	-	-
Miscellaneous----------scavenger receptors for chemokines				
Duffy antigen	CCL2, 5, 11, 13, 14 CXCL1, 2, 3, 7, 8			
D6	CCL2, 3, 4, 5, 7, 8, 12 CCL13, 14, 17, 22			

Table 1. The human chemokine system. Leukocyte anonyms are as follows: Ba, basophil; Eo, eosinophil; iDC, immature dendritic cell; mDC, mature dendritic cell; Mo, monocyte; Mϕ, macrophage; NK, natural killer cell; Th1, type I helper T cell; Th2, type II helper T cell; Treg, regulatory T cell.

Legends to Figures

Figure 1. Intracellular signaling pathway of chemokines.

Figure 2. Tumor immunity generation.

Figure 3. Maturation stages of dendritic cells.

Figure 4. Biological effects of chemokines on suppressive leukocytes in tumors

Figure 5. Biological effects of chemokines on tumor and endothelial cells.

Figure 1 Mukaida

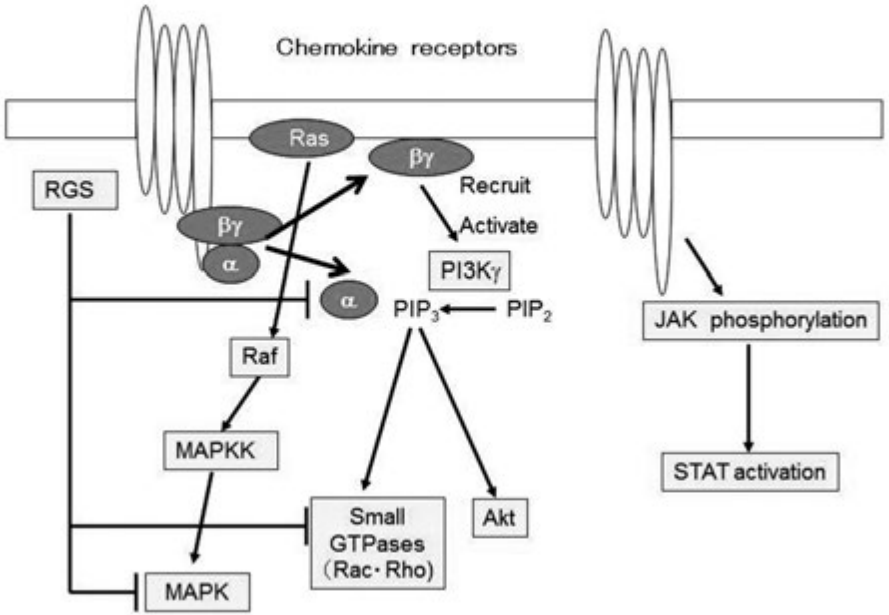


Figure 2 Mukaida

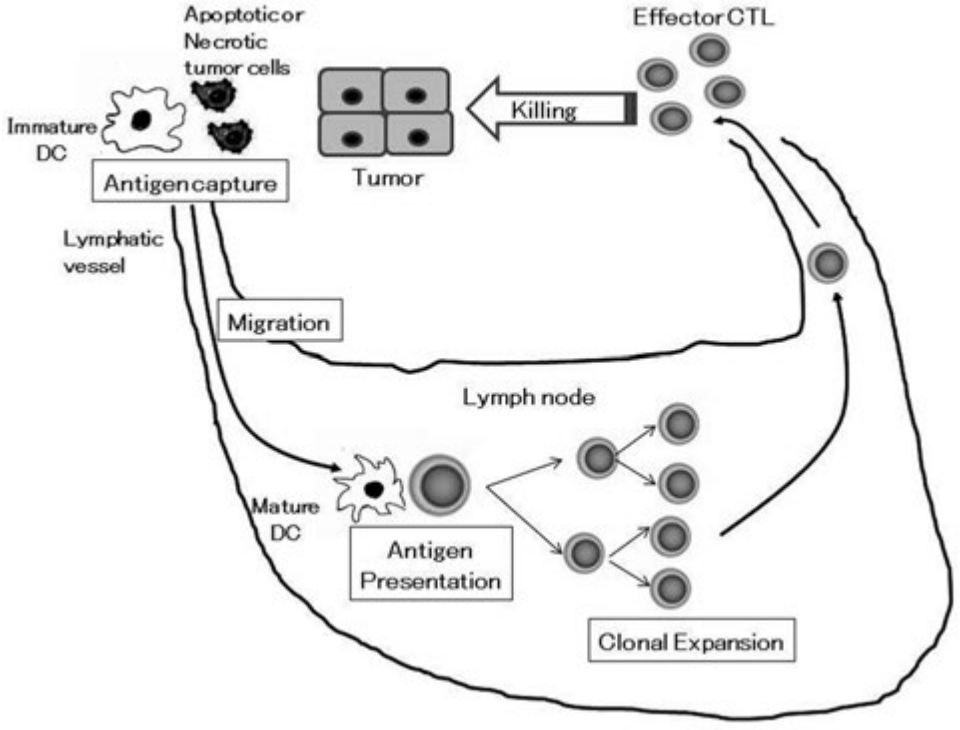
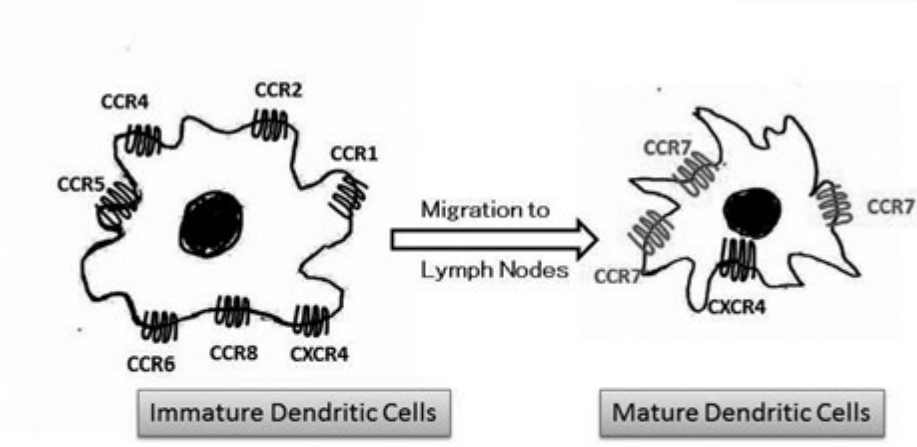


Figure 3 Mukaida



Antigen Capture Capacity	**High**	**Low**
Antigen Presentation Capacity	**Low**	**High**
MHC class II Expression	**Low**	**High**
Co-stimulatory Molecule Expression	**Low**	**High**

Figure 4 Mukaida

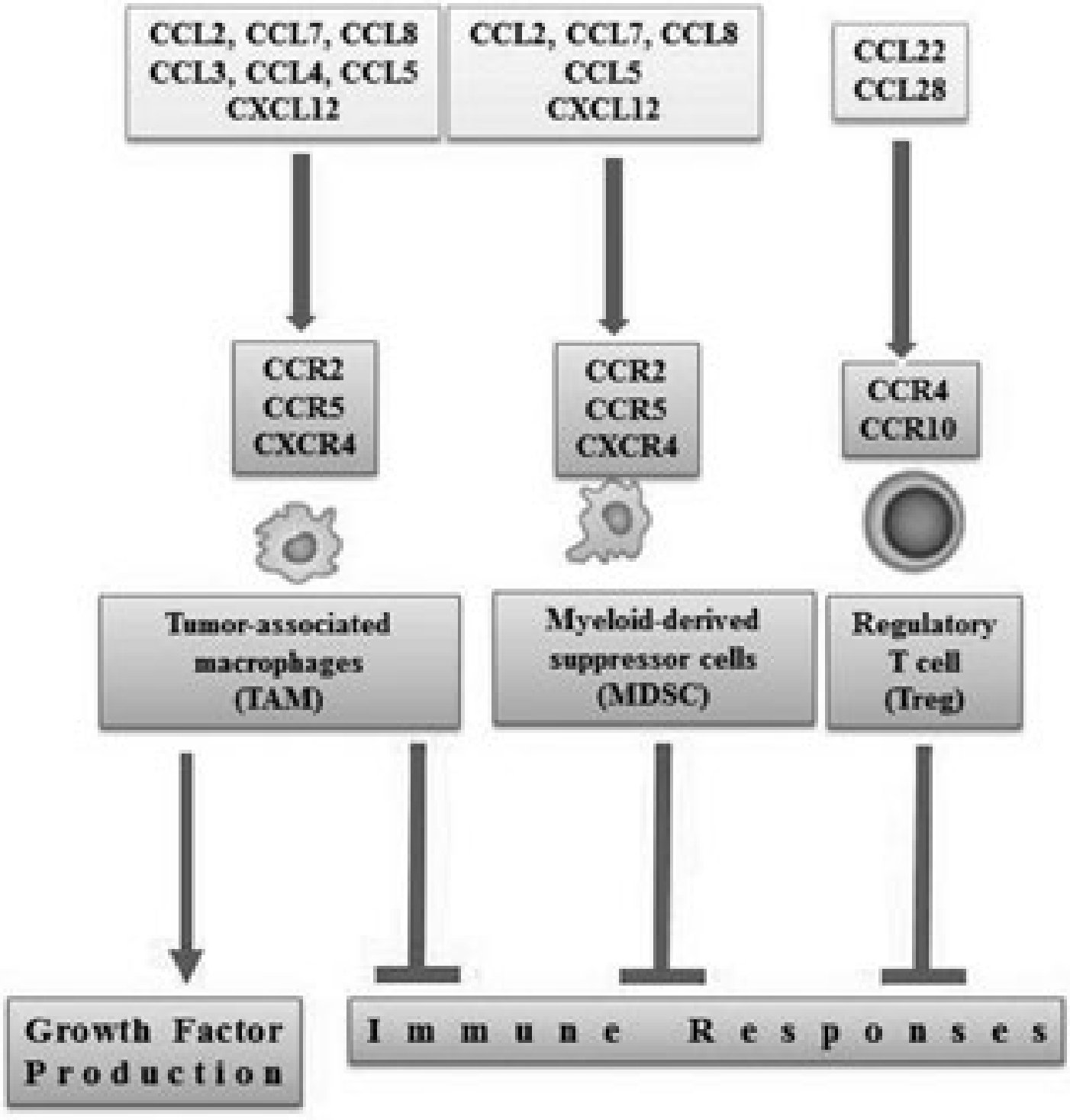


Figure 5 Mukaida