Review Article

Chemokines in Homeostasis and Cancers

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Abstract

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Chemokines are a small group of related chemoattractant peptides that play an essential role in the development and homeostatic maintenance of the immune system. They control the recruitment of cells needed for the induction and activation of innate and adaptive immune responses. Stromal cell-derived factor 1(SDF-1) and its receptor (CXCR4) have role in regulation of trafficking of normal hematopoietic stem cells (HSCs) in their homing/ retention in bone marrow, also they control lymphocyte trafficking, angiogenesis, cell adherent or migration. In addition, chemokines and their receptors involved in several auto- immune diseases such as inflammation, HIV. In fact, chemokines are involved directly or indirectly in almost every aspect of tumorigenesis. They mediate survival and metastatic spread of tumors, promote new blood vessel formation (neovascularization). SDF- 1/ CXCR4 axis for migration, enhanced resistance to apoptosis and an increased capacity for drug resistance. .A number of therapeutic strategies have been proposed to target almost every step of the chemokine or chemokine receptor involvement in tumors. Yet, despite occasional success stories, most of them appear to be ineffective or impractical,. The strategy would only be effective if it also promoted anti-tumor activity and more study is needed to clear the tumor relapse mechanism..

Keywords: Chemokines, chemokine receptors, SDF-1, CXCR4

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Introduction

Chemokines, originally known as chmotactic cytokines, and it is well understood that they are secreted and related 8-15 KDa peptides with 70-125 amino acids (except CXC3CL1) with very simple structures. To date, the human chemokine system currently consist of approximately 50 members that base on their structure, the first two conserved cysteins are arranged and they are classified into four groups, designed CC, CXC, C and CX3C by different classification (Table 1) (1,2).

Table1: Chemokine classification

Alpha beta classification	Classic classification
1) Class α or CXC	1) Class I or XC
2) Class β or CC	2) Class II or CC
3) Class γ or XC	3) Class III or CXC
4) Class δ or CX3C	4) Class IV or CX3C

CXC: Cys- any amino acid- Cys

All chemokines are structurally similar and most of them also have at least four cystein in conserved positions. In human the genes encoding CXC chemokine proteins cluster at chromosome 14q12-21 (except for stromal derived factor {SDF}-1a/ CXC ligand 12 whose gene maps to chromosome 10 and genes encoding CC chemokine proteins cluster at chromosome 17q11.2-12 (except for macrophage inflammatory protein [MIP]-3b whose gene maps to chromosome 9 and MIP-3a which maps to chromosome 2), lymphotactin is a strucyurally related chemokine having only one cystein and its gene located in chromosome 1q23. The CX3C chemokin also called "fractalkine" or "neurotactin", its gene located at chromosome 16 (3).

Some chemokines have role in homeostatic that are produced and secreted constitutively in discrete microenvironments and they are involved in maintaining the physiological trafficking of immune cells (4). Such chemokines are involved in localization of lymphocytes with antigen in lymphatic organs, immune surveillance, stem cell and lymphocyte trafficking. The simplistic view of chemokines as recruiters of immune cells and regulators of the directional migration (chemotaxic) is changing owing to important role they play in the innate and adaptive immune responses (5). Other chemokines are only produced by cells during infection (inducible) or a pro-inflammatory stimulus and prompt the migration of leukocytes to an injured or infected site. This inflammatory chemokines can also active cell to raise an immune response and commence the wound healing process (Table 2). Also they play a crucial role in development, inflammatory, autoimmune diseases, HIV infection, tumor associated angiogenesis as well as tumor progression, migration and recruitment of various subsets of immune cells and even malignant cells (Fig 1) (6-8).

Table 2: Chemokines

Chemokines	-ELR-	H/I	Synonyms	Major target cells showing chemotaxis
CC chemokines				
CCL1	NA	I	I-309, TCA3, P500	monocytes, T cells
CCL2	NA	I	MCP-1, MCAF (mouse; JE)	monocytes, T cells, basophils, NK cells, progenitors
CCL3	NA	I	LD78α, LD78β, MIP-1α	monocytes, T cells, NK cells, basophils, eosinophils, dendritic cells, hematopoietic progenitors
CCL4	NA	I	Act-2, G-26, HC21, H400, MIP-1β, LAG-1,, SISγ, MAD-5	monocytes, T cells, dendritic cells, NK cells,
CCL5	NA	I	RANTES progenitors	progenitors
CCL6	NA	I	C10 (mouse), MRP-1 (mouse)	T cells, eosinophils, basophils, NK cells, dendritic cells macrophages,
CCL7	NA	I	MCP-3 monocytes,	T cells, eosinophils, basophils, NK cells, dendritic cells
CCL8	NA	I	MCP-2, HC14	monocytes, T cells, eosinophils, basophils, NK cells
CCL9	NA	I	MRP-2 (mouse), MIP-1γ (mouse)	T cells
CCL10	NA	I	CCF18	T cells
CCL11	NA	I	eotaxin	eosinophils, T cells
CCL12	NA	I	MCP-5 (mouse)	monocytes, T cells, eosinophils
CCL13	NA	I	MCP-4, NCC-1, CKβ10	monocytes, T cells, eosinophils
CCL14	NA	I	HCC-1, HCC-3, NCC-2, CKβ1, MCIF	monocytes, hematopoietic progenitors
CCL15	NA	I	HCC-2, NCC-3, MIP-5, Lkn-1, MIP-1	monocytes, T cells, eosinophils
CCL16	NA	I	NCC-4, LEC, HCC-4, LMC, LCC-1, CKβ12	T cells, neutrophils
CCL17	NA	H	TARC	T cells
CCL18	NA	H?	DC-CK1, PARC, MIP-4, CKβ7, DCCK1	naïve T cells
CCL19	NA	H	ELC, MIP-3β, exodus-3, CKβ11	T cells, B cells, dendritic cells, activated NK cells
CCL20	NA	H	MIP-3, LARC, exodus-1, ST38, CKβ4	T cells, B cells
CCL21	NA	H	SLC, 6CKine, exodus-2, TCA4, CKβ9	T cells, B cells, dendritic cells, activated NK cells, macrophage progenitors
CCL22	NA	H	MDC, STCP-1, DC/B-CK	T cells, eosinophils
CCL23	NA	H	MIP-3, MPIF-1, CKβ8	dendritic cells, osteoclasts
CCL24	NA	I	MPIF-2, CKβ6, eotaxin-2	effector Th2 cells
CCL25	NA	H	TECK, CK15	memory T cells, B cells, immature thymocytes
CCL26	NA	I	eotaxin-3, IMAC, MIP-4α, TSC-1	eosinophils, T cells
CCL27	NA	H	ALP, skinkine, ILC, ESkine, PESKY, CTAK	CLA+ T cells
CCL28	NA	H	MEC, CCK1	T cells
CXC chemokines				
CXCL1	ELR+	I	GROα, MGSA-α, NAP-3 (mouse/rat; KC, MIP-2, CINC-2β)	neutrophils, endothelial cells
CXCL2	ELR+	I	GROα, MIP-2α, MGSA-β, CINC-2α	neutrophils, endothelial cells
CXCL3	ELR+	I	GROγ, MIP-2α, CINC-2β	neutrophils
CXCL4	ELR+	I	PF4 fibroblasts	endothelial cells
CXCL5	ELR+	I	ENA-78	neutrophils
CXCL6	ELR+	I	GCP-2	neutrophils
CXCL7	ELR+	I	CTAPIII, NAP-2, LA-PF4, MDGF, LDGF,,β-TG	Fibroblasts
CXCL8	ELR+	I	IL-8, NAP-1	neutrophils, T cells, basophils, endothelial cells
CXCL9	ELR-	I	Mig	T cells, progenitors
CXCL10	ELR-	I	IP-10	T cells
CXCL11	ELR-	I	I-TAC	T cells
CXCL12	ELR-	I	SDF-1, SDF-1, PBSF	monocytes, B cells, hematopoietic progenitors, non-hematopoietic cells
CXCL13	ELR-	H	BLC, BCA-1	B cells
CXCL14	ELR-	I	BRAK, bolekine, MIP-2, BMAC, KS1	neutrophils, NK cells, B cells?
CXCL15	ELR-	H	lungkine	airspace neutrophils
CXCL16	ELR-	?	SR-PSOX, SEXCKINE	dendritic cells
C chemokines				
XCL1	NA	I	lymphotactin, SCM-1, ATAC	B cells, T cells, NK cells, neutrophils
XCL2	NA	I	SCM-1	B cells, T cells, NK cells, neutrophils
CX3C chemokine				

H, homeostatic chemokine; I, inflammatory chemokine; NA, not applicable. For definitions of the various synonyms (10), ELR, The glutamic acid-leucine-argenine sequnce in front of CXC group wich promote angiogenesis.

Table 3: Chemokine receptors

Receptors	Synonyms	Chemokine ligands	Receptor-expressing cells
CCCR			
CCR1	CKR1, CC CKR1, CMKBR1	CCL3,5,7,8,13,14,15,16,23	monocytes, immature DCs, T cells, PMNs, eosinophils, esangial cells, platelets
CCL3, 5, 7, 8, 13, 14, 15, 16, 23	CKR2, CC CKR2, CMKBR2	CCL2,7,8,12,13	monocytes, immature DCs, basophils, PMNs, T cells, NK cells, endothelial cells, fibroblasts
CCR3	CKR3, CC CKR3, Eot R CMKBR3	CCL5,7,8,11,13,14,15,24,26	eosinophils, basophils, T cells (Th2>Th1), DCs, latelets, mast cells
CCR4	CKR4, CC CKR4, CMKBR4,K5-5	CCL17,22	immature DCs, basophils, T cells (Th2>Th1), platelets
CCR5	CKR5, CC CKR5, ChemR1	CCL3,4,5,8,11,13,14,20	Th1 cells, immature DCs, monocytes,NK cells, CMKBR5 hymocytes
CCR6	GPR-CY4, CKR-L3, STRL22, CRY-6, DCR2, CMKBR6	CCL20	immature DCs, T cells, B cells
CCR7	BLR-2, CMKBR7	CCL19,21	mature DCs, T cells, B cells
CCR8	TER1, CKR-L1, GPR-CY6, ChemR1, CMKBR8	CCL1,4,1	monocytes, B cells, T cells, thymocytes
CCR9	GPR9-6	CCL25	T cells, thymocytes, DCs, macrophages
CCR10	GPR2	CCL27,28	T cells, melanocytes, dermal endothelia dermal fibroblasts, Langerhans cells,
CCR11	PPR1	CCL2,8,13,19,21,25	astrocytes
CXCR			
CXCR1	IL-8RA, IL-8R-I, IL-8R	CXCL2,3,5,6,7,8	PMNs, monocytes, astrocytes, endothelia, mast cells
CXCR2	IL-8RB, IL-8R-II, IL-8R	CXCL1,2,3,5,6,7,8	PMNs, monocytes, eosinophils, endothelia, mast cells
CXCR3	IP10/MigR, GPR9	CXCL9,10,11	T cells (Th1>Th2), B cells, NK cells, mesangial cells, smooth muscle cells, endothelia
CXCR4	HUMSTSR fusin, LESTR,HM89	CXCL12	hematopoietic progenitors, T cells, immature DCs, monocytes, B cells, PMNs, platelets, astrocyte, endothelia
CXCR5	BLR-I, MDRI5	CXCL13	T cells, B cells, astrocytes
CXCR6	Bonzo, STRL33, TYMSTR	CXCL16	memory T cells
XCR			
XCR1	GPR5	XCL1, XCL2	T cells
CX3CR			
CX3CR1	GPR13, V28, CMKBRL1	X3CL1	PMNs, monocytes, NK cells, T cells, astrocytes
Duffy	DARC	CXCL1,7,8, CCL1,5	red blood cells, endothelia
D6 C	CR9,10, JAB61	CCL2,4,5,8,13,14,15	B cells

NK cell, natural killer cell; DC, dendritic cell; PMN, polymorphonuclear granulocyte.

The story of cell migration from endothelial vessels barrier to tissue is interesting. The rolling cells express chemokine receptor and chemokines on the luminal endothelial surface can active their receptors of the rolling cells, thus triggering integrin activation. This results in arrest, firm adhesion and transendothelial migration into tissues towards chemokine gradiants. (9)

Chemokine Receptors

Chemokines transmit their signaling via binding with cell-surface G protein –coupled seven-transmembrane receptors, so-called chemokine receptors (Fig 2), designated CXCR1-8, CCR1-11, CXCR1 and CX3CR1 (11). Most of chemokines recognize one or more cell surface receptors and most of receptors have more than one ligand (Table 3).

Chemokine receptors share 25-80% homology in 340-370 amino acid residues with an acidic N-terminus, conserved ten amino acid sequence in the second intracellular loop, and one cysteine in each of the four extracellular domains. The chemokin binding site is complex and it located in N-terminal segment (12).

Major biological function of chemokines and receptors

Hemostastasis and development

Studies show the important roles for some chemokines in hematopoiesis and organ development. Hematopoiesis is an active progression controlled by a variety of cytokines. It is well clear that the chemokine family plays an critical role in this regulatory system. At least 25 chemokines of the CC, CXC and C subgroups have been found to suppress the in vitro proliferation of myeloid progenitor cells (12, 13). However, the in vivo evidence based on knockout mice studies provides data of contrasting hematopoietic effect for only few of the chemokines and their receptors. For example, CCL3 (MIP-1α) arrests cell cycling and reduces the bone marrow progenitor cells number in mice (14). Mice depleted of CCR1, as CCL3 receptor, display enhanced lineage-committed myeloproliferation and leukocyte mobilization into the blood stream (15). CXCL12 (SDF-1) is constitutively expressed by stromal cells in bone marrow, promotes proliferation of B cell progenitors (16), and recruit hematopoietic precursors to the bone marrow in embryogenesis time (17). Mice deficient in CXCL12 (SDF-1) or its receptor (CXCR4) die before birth with in B lymphopoiesis and myelopoiesis deficiency and also with an incomplete development of the cardiac septums and cerebellum development, it show the involvement of CXCL12 /CXCR4 pair in a number of vital developmental processes (18-20). The thymus is organ for T cell development and it expresses mRNA for several chemokines with lymphocyte-attracting properties, that well described (21,22). Some chemokines control the development and organization of secondary lymphoid organs such as peripheral lymph nodes and Peyer's patches (23,24).

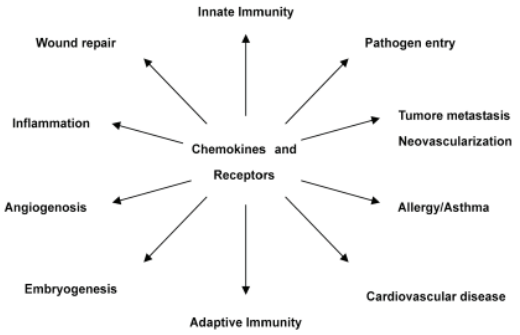

Fig 1: The role of chemokines and receptors in patho-physiologyic conditions

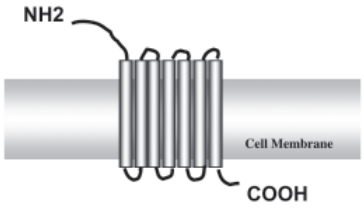

Fig 2: Schematics shame of Chemokines receptors which their genes were reularly on chromosomes 14 and 17 in human

Leukocyte trafficking and homing

Chemokines control lymphocyte trafficking in immune system homeostasis., Lymphocyte homing to lymphoid and non-lymphoid tissues and recirculation between secondary lymphoid organs critically depend on the chemokines present in different sites. For example, CCL19 and CCL21 (which bind to CCR7), and CXCL13 (which binds to CXCR5), are expressed in the lymphatic vessels, high endothelial venules (HEVs) and secondary lymphoid organs, to promote the entry of antigen-presenting cells (APCs), T cells and B cells into these organs (25). Immune cell, DC precursors in peripheral tissues phagocytose microorganisms or cell debris and are activated by pathogens or antigens then start to maturation and CCR7expression which enables them to migrate in response to CCR7 ligands into the draining lymph nodes via the lymphatic vessels, and to infiltrate the T-cell zones where they present processed antigen epitopes to T cells (26). In contrast to DCs, B cells and naïve T cells enter lymph nodes through HEVs. The CCR7 ligands CCL19 and CCL21 by the endothelial cells of HEVs are transcytosed to the luminal surface and induce lymphocyte extravasation to the T-cell zones of the lymph nodes (27). CCL19 produced by mature, inter digitating DCs facilitates the "scanning" of DCs by naïve T cells in the lymphoid organs in search of their cognate antigens (26).

B cells express CXCR5/CXCL13 is produced by follicular stromal cells in lymph nodes. B cells proliferate in the follicles, giving rise to germinal centers (GC) after activated by T cells. Activated T cells expressing CXCR5 may also enter the follicles to participate in the T-B interaction. In addition, CCL19 and CCL21 are responsible for the proper positioning of lymphocytes within distinct microenvironments of lymphoid organs. For instance, CCL19 and CCL21, expressed by DCs and stromal cells retain T cells within the T-cell zones of secondary lymphoid organs. On the other hand, CXCL13 expressed by follicular DCs and stromal cells in follicles attracts B cells and some of the T cell subsets into the B-cell areas. Furthermore, the capacity of B cells to respond to CCR7 as well as CXCR5 ligands controls the position of B cells at the boundary of the follicles and T-cell zones in the spleen, where naïve, mature B cells interact with T cells that are newly activated in the adjacent zones (28,29). Non-activated B cells and T cells then leave

Angiogenesis

Angiogenesis occurs rapidly but transiently and is tightly regulated physiologically, but unbalanced production of CXC subgroup of chemokines which act as positive and negative regulators, results pathological angiogenesis that seen during chronic inflammation and tumor growth. CXC containing ELR motif (10), CXCL8 (IL-8), CXCL5 (ENA78), and CXCL1, 2, 3 (GRO-α, β, γ) induce vessel formation in rabbit cornea (15, 16). In contrast, ELR negative CXC chemokines CXCL4 (PF4), CXCL10 (IP-10) and CXCL9 (MIG) abrogate the angiogenesis induced by ELR+ CXC chemokines (30,31).

One exception is CXCL12 (SDF-1), which despite the absence of the ELR motif, acts as an angiogenic factor both in vitro and in vivo had reported (32).
Angiogenesis is crucial for tumor growth. The ELR positive CXC chemokine CXCL8 promotes neovascularization and tumorigenesis of ovarian carcinoma (33). Treatment of the mice bearing CXCL8 producing tumors with anti-CXCL8 antibodies or with angiostatic chemokine CXCL10 (IP-10) inhibits tumor growth and metastasis (34, 35). These results confirm the proposals to utilize selected chemokines or their inhibitors to control angiogenesis in tumor and wound healing.
It should be noted that most of the results regarding the activity of chemokines in angiogenesis and angiostasis had achieved from in vitro or specifically designed experiments in animals. However more information should be reached by using knockout models. In this way, mice missing its SDF-1 or its exclusive receptor show defects in cardiovascular development and provide clear evidence of an important role for this chemokine and receptor in angiogenesis during development.

Inflammation
It has known the key role of chemokines in inflammation event. chemokines participate in and control the process of a number of acute and chronic inflammatory conditions by promoting the infiltration and activation of inflammatory cells into injured or infected tissues and wound repair.
Some of CC chemokines including CCL3 and CCL5 (RANTES) are expressed in sepsis and exert proinflammatory effects by mediating organ specific leukocyte influx and activation. Members of the CXC chemokines are also implicated in the pathogenesis of systemic inflammatory response (36- 39).
Asthma, the submucosa of small airways is infiltrated by mononuclear, eosinophil and mast cells causing mucous gland hyperplasia and subepithelial fibrosis. Asthmatic patients and animal models of allergic airway inflammation confirm a key role for chemokines in regulating lung inflammation. Chronic obstructive pulmonary disease (COPD) is characterized by progressive development of airflow limitation caused by chronic inflammation with increased recruitment of neutrophils, macrophages and IFN-γ-producing by CD8+ T cells in the lung, the levels of CXCL8 and CXCL10 are increased in COPD and it correlate with the degree of infiltration by neutrophils and CD8+ T cells (40).

Atherosclerosis is widely accepted as an inflammatory disease, that chemokines play a means role in leukocyte recruitment, angiogenesis, and more interestingly in the proliferation of vascular smooth muscle cells and their migration into plaques (41). Many factors known to promote atherosclerosis such as plasma cholesterol, hypertension and diabetes, stimulate chemokine release by atheromatous lesions. Atherosclerotic lesions express a number of chemokines including CCL2, CCL3, CCL4, CCL5, CCL11 and CXCL8. The cellular sources of chemokines within atherosclerotic lesion are multiple and include support the involvement of CCL2/CCR2 chemokine receptor pair in atherosclerosis. CCL2 is essential for monocyte recruitment, has angiogenic activity and also causes smooth muscle cell proliferation and migration. Adhesion of leukocytes to endothelial cells also increases chemokine release in the pathogenic process of atherosclerosis. Therefore, chemokines and receptors become important molecular targets for avoiding the formation and development of atherosclerotic lesions (26, 42). This affords an excellent event of the importance of a functional chemokine receptor in contributing to the progression of atherosclerosis.
Rheumatoid arthritis (RA) is characterized by a mixed Th1-type inflammatory cell infiltration (Th1, neutrophils, monocytes) in synovial space of the joints, in association with cartilage destruction and bone remodeling. Chemokines produced in the inflamed joints attract leukocytes across the endothelial barrier to initiate and maintain active RA. Among CXC chemokines, high concentrations of CXCL8, CXCL5, and CXCL1 are detected in the sera, synovial fluids, and synovial tissues of RA patients. These chemokines attract neutrophils and promote angiogenesis. High production of CC chemokines CCL2, CCL3 and CCL5 which attract mainly monocytes is also found in RA (43, 44). CXCL12 expressed in the rheumatoid synovium, recruits CD4 memory T cells, which express increased levels of CXCR4, at the RA site. CXCL12 also blocks T cells from undergoing activation-induced apoptosis, thus further increasing the accumulation of T cells in the rheumatoid synovium. Interestingly, CXCL12 may induce the migration of DCs from blood stream into the rheumatoid area, implying its potential role in amplifying a detrimental autoimmune response. (45)
Multiple sclerosis (MS) as a chronic inflammatory demyelinating disorder of the central nervous system (CNS) is thought to be caused by an autoimmune response directed against self-myelin-associated antigens. The immune cells infiltrate in CNS lesions of MS patients consist of CD4, CD8 T cells and macrophages (46). Many chemokines are detected in active lesions in the CNS of MS patients and the cerebrospinal fluids of relapsing patients contain elevated levels of CCL3. In MS, infiltrating macrophages express CCR2 and CCR5, while T cells and reactive astrocytes in active lesions express CXCR3 and CCR5 (47- 50). Similar chemokine expression patterns are found in experimental autoimmune encephalomyelitis (EAE), an animal model more related to MS. In EAE, increased expression of CCL2, CCL3, CCL4, CCL5 and CXCL10 correlates with the severity of the disease (78). Neutralizing antibodies to selected chemokines either inhibit the onset or reduce the severity of the EAE (51, 52). A more definitive correlation between chemokines and EAE was established by experiments with CCR1- and CCR2-deficient mice, in which a reduction in disease incidence and severity were clearly documented (53, 54).

Chemokines and Malignanacy

Chemokine receptor expression profiles of cancer cells

Recent papers suggest that tumor cells may express restricted and specific patterns of chemokine receptors that responses to chemokine gradients may contribute to metastatic spread. There is one chemokine receptor that appears to be expressed by a majority of cancer types, namely CXCR4, which is expressed by 23 different types of cancer, including cancers of epithelial, mesenchymal and haematopoeitic origin (55). For example tumor cells from breast, prostate, pancreatic, lung and ovarian carcinomas, neuroblastoma and glioblastoma, all express CXCR4 (56-65). In other cancer cells studied, CXCR4 may be co-expressed with other CC or CXC chemokine receptors or less commonly, other receptors are present without expression of CXCR4. Human breast cancer cells express CXCR4 and CCR7 (59). Functional CCR7 is also found on gastric carcinoma cells (66) and esophageal carcinoma 67). Melanoma cells are reported to express CCR7 and CCR10 (59) and in another study to co-express CXCR4 and CXCR3 (68). Leukaemic and lymphoma cells express a wider range of chemokine receptors, probably reflecting their haematopoetic origin, adult T cell leukaemia/ lymphoma (ATLL) cells frequently express CCR4 (69), cutaneous T cell lymphoma (CTCL) cells express functional CCR3 (70) and B cell lymphomas are reported to express CXCR3 and CXCR5 (71).

Chemokines expression in the tumor microenvironment

Within most cancers there is an extensive network of chemokines and chemokine receptors (55, 72), often tumor production of chemokines is disregulated and receptor expression and signalling may be abnormal (73). Solid tumors comprise a mixture of malignant and host stromal cells. Initially, stromal cells have to be recruited into the tumor tissue by the cancer cells and although there are some reports of infiltrating immune cells controlling tumor growth (74), it is possibly more likely that a tumor attracts stromal cells which are advantageous for tumor growth. For example, infiltrating macrophages produce growth factors, angiogenic factors, inflammatory cytokines and chemokines (75). CCL2 stimulation of monocytes promoted tumor formation of melanoma cells (76). CD4+ T cells were reported to enhance invasion and disease progression in an experimental model of skin carcinogenesis (77).

The composition of the leukocyte infiltrate in many carcinomas is related, in particular, to tumor and stromal cell production of CC chemokines (75). There are few examples of tumors where the complex chemokine network has been fully characterised and then related to infiltrating leukocytes. Examples include Hodgkin's disease which expresses CCL17, CCL11, CCL22 that attract Th2 lymphocytes and the Th1-attracting chemokines CXCL10, CXCL9, CCL2, CCL3, CCL5 and CXCL1 (78). Ovarian cancer is characterised by the presence of infiltrating macrophages and CD8+ T lymphocytes. CCL2 localised to epithelial areas of the tumor and correlated with the extent of lymphocyte and macrophage infiltration. CCL3, CCL4 and CCL5 were also present in solid ovarian tumors and localised to tumor infiltrating leukocytes. CCL5 expression also correlated with the extent of the CD8+ T lymphocyte infiltrate (79). In ovarian cancer ascites, mRNA and pico to nanomolar levels of protein for CCL2, CCL3, CCL4, CCL5, CCL8 and CCL22 were detected (80).

Breast cancer cells have been reported to produce CCL2 and CCL5 and there is a positive correlation between macrophages, lymph node metastasis and clinical aggressiveness (81). CCL5 levels correlated with breast cancer progression whereas benign breast disease had minimal chemokine expression (82).In an experimental murine breast cancer model, overexpression of the cytokine CSF-1 (M-CSF) increased infiltration of macrophages and accelerated tumor growth, invasion and metastasis (83).

In esophageal squamous cell carcinomas CCL2 expression was significantly associated with the extent of macrophage infiltration, tumor cell invasion and tumor vascularity (84).

However, there are conflicting data on the association of CCL2 and CCL5 expression, the extent of the leukocyte infiltrate and tumor progression. For instance, high serum levels of CCL2 in pancreatic cancer patients correlated with the extent of macrophage infiltration

into the tumor but was associated with good patient prognosis (85). It is clear that the tumor microenvironment contains an extensive and varied mix of chemokines, both CC and CXC chemokines and that this 'network' may control the leukocyte infiltrate into the tumor. In the following section we will discuss whether similar chemokine-receptor networks can control tumor cell movement out of a cancerous tissue.

SDF-1 (CXCL12) and human tumore pathogenosis

Expression and regulation of SDF-1 in tumor

Classically, two alternatively spliced isoforms of SDF have been identified. SDF-1α is an 89 amino acid protein that is the predominantly expressed form of SDF-1 while SDF-1β contains a four amino acid extension at the carboxyl terminus, CXCL12 was initially cloned from bone marrow stromal cells (86). Strikingly, CXCL12 is widely expressed in various organs including heart, liver, brain, kidney, skeletal muscle, and lymphoid organs. Vascular endothelial cells, stromal fibroblasts, and osteoblasts are the major cellular source for CXCL12 in these organs (87-91). Interestingly, high levels of functional CXCL12 were first reported in human ovarian cancer in 2001 (92-94). Subsequent studies documented a strong correlation between CXCL12 expression and bone marrow and lymph node metastasis of breast (95) and prostate cancer (96). Interest in the role of CXCL12/CXCR4 in tumor pathology was provoked by these studies. In addition to ovarian cancer, CXCL12 expression is reported in breast

cancer (97), glioblastoma (98), pancreatic cancer (99),

prostate cancer (100), thyroid cancer (51), and many other human tumors.
Tumor stroma is an active element of tumor microenvironment. Recently, it was shown that in breast cancer, activated stroma fibroblasts produce CXCL12 and contribute to tumor vascularization by endothelial stem cell attraction (101). It also has been suggested that CXCL12 is involved in prostate epithelial cell transformation induced by aging fibroblasts (102). Although CXCL12 does not directly induce transformation, CXCL12 may provide conditions supportive of a transforming event. Therefore, stroma and cancer cells, two main components of tumor microenvironment, can produce CXCL12. Strikingly, regulation of CXCL12 expression in the tumor microenvironment has been poorly studied. It has been reported that estradiol activates estrogen receptor and induces the production of CXCL12 by tumor cells (103). It observed that hypoxia triggers CXCL12 expression by primary human ovarian tumor cells (104) and prostate tumor cell lines.

SDF-1 tumor proliferation and survival
There is evidence to demonstrate that CXCL12 can modulate tumor cell proliferation and survival (105) provided the first evidence for mitotic CXCL12 activity in human tumors, CXCL12-dependent proliferation correlated with the activation of ERK1/2 and AKT pathways. Both these pathways are known to be involved with the transduction of proliferative signals in normal and tumor cells (106).
CXCL12 can induce proliferation of several tumor cell lines, including ovarian carcinoma, small cell lung cancer, prostate cancer, neck squamous cell carcinoma, and pancreatic cancer. mechanistically, CXCL12-dependent cell proliferation is linked to ERK activation (107, 108).
CXCL12/CXCR4-mediated tumor cell proliferation may be regulated through estrogen signaling (109). About 60% of human ovarian and breast cancers are hormone dependent and over express the progesterone and/or estrogen receptors (110-111). It was demonstrated that CXCL12 was required for estrogen-induced proliferation of both breast and ovarian cancers. (109)
CXCL12 also can regulate tumor cell apoptosis. CXCL12 activates NF-kB (Abroun S. submitted data). Moreover, activation of NF-kB can sensitize cancer cells to CXCL12 stimulation through upregulation of CXCR4 expression, which in turn inhibits radiation-induced tumor necrosis factor alpha (TNF-α) production and tumor apoptosis (112).
Many chemotherapeutic drugs exert their effects by inducing apoptosis in the targeted cell population. CXCL12 can protect tumor cells from drug-induced apoptosis directly through the activation of antiapoptotic pathways but also indirectly by modulating the adherence of cancer cells. For example, CXCL12 mediates adhesion of small-cell lung cancer cells (SCLC) to marrow stroma cells and protects SCLC against etoposide-induced apoptosis. The protective effect could be antagonized by CXCR4-specific inhibitors as well as by blocking integrin _4 (113-114). Similar observations are found in myeloma (115), glioma cells (116), and head and neck cancer (117). Thus CXCL12 signals may be implicated in tumor cell proliferation and survival.

SDF-1 and tumor Vascularization
CXCL12 exhibits angiogenic activity. Initially, the angiogenic role of CXCL12 was observed in mice lacking CXCL12 or CXCR4 (118, 119).
These mice had defective formation of large vessels supplying the gastrointestinal tract. Subsequent in vitro studies suggested a potential effect of CXCL12 on blood vessel formation. For example, CXCL12 stimulates the formation of capillary-like structures with human vascular endothelial cells (120,121). Interestingly, although high concentrations of CXCL12 are able to induce angiogenesis in vivo (122), but. in presence of low concentrations of vascular endothelial growth factor (VEGF) (104), revealing profound synergistic effects between CXCL12 and VEGF. CXCL12 synergizes with soluble factors, including fibroblast growth factor (FGF) family members and VEGF (123), and coordinates with immune cells, including plasmacytoid DCs (124), to induce potent vascularization in vivo.
Migration, expansion, and survival of vascular endothelial cells form the essential functional network of angiogenesis. Vascular endothelial cell migration is strongly dependent on CXCL12 (125, 126). In support of this, neutralizing antibodies against CXCL12 inhibit endothelial cell invasion into subcutaneously injected Matrigel (127). Hypoxia simultaneously stimulates CXCR4 expression (128, 129) and CXCL12 (104) production. Therefore, it is reasoned that hypoxia would promote vascular endothelial cell migration toward CXCL12 and induce tumor vascularization in a CXCL12-dependent manner

SDF-1 and tumor metastasis
Tumor metastasis was once viewed as a passive consequence of a single tumor cell simply "escaping" from a primary tumor and traveling great distances through draining lymph nodes and blood, lodging in small blood vessels and thereby forming micrometastases (130). Recent data, however, have demonstrated that tumor metastasis is an active process employing multiple molecular and cellular mechanisms. The interaction between tumor cells and stroma is crucial for tumor metastasis (131-132).

Multiple myeloma.
Multiple myeloma (MM) is a monoclonal plasma cell anomaly and this is second hematopoeitic malignancy in word. During B-cell differentiation into plasma cells, plasma cells undergo a coordinated change in chemokine responsiveness. As B cells differentiate into plasma cells, they become increasingly sensitive to CXCL12 while losing responsiveness to B- and T-zone chemokines (CXCL13, CCL19, CCL21) through respective down-regulation of CXCR5 and CCR7 (133-134). In addition to CXCR4, plasma cells express functional CXCR6,

CCR10, and CCR3 chemokine receptors. (135) recently was demonstrated a stage-specific homing of the earliest B-cell precursors (pre–pro-B cells) and plasma cells to the same marrow niches in which stromal cells secrete high levels of CXCL12, suggesting that CXCL12 maintains immature and terminally differentiated B-cell types in the marrow microenvironment. However, we founded that myeloma cell derived from MM patients express CXCR4 at different level as myeloma cell lines (Abroun S, unpublished data) but neither primary myeloma cell nor myeloma cell lines express SDF-1 (136). SDF-1 induced primary myeloma cells or myeloma cell lines proliferation in serum free condition by NF-KB and ERK pathways activation in-vitro. These result show the role of stromal cell in bone marrow to support myeloma cell survival and proliferation by SDF-1 secretion.(137).

Multiple myeloma cells home to the BM where they adhere to marrow stromal cells and extracellular matrix (ECM) proteins in the marrow microenvironment through VLA-4 integrins to stromal fibronectin. In addition, myeloma cells display functional CXCR4 chemokine receptors that cooperate with VLA-4 integrins in myeloma cell adhesion and migration..This mechanism may allow myeloma cells to home to the marrow microenvironment, where adhesive interactions promote growth, survival, and confer cell adhesion-mediated drug resistance (CAM-DR) (138).

SDF-1 and tumor cell adhesion or migration

Cancer dissemination can be viewed as a tissue remodeling process that involves proteolytic degradation of extracellular matrix. Metalloproteases (MMPs) are a family of enzymes involved in the degradation of extracellular matrix in the surrounding normal tissue and known to mediate cancer invasion and metastases (139). Activation of MMPs breaks down the physical barriers of metastasis, thus promoting invasion by cancer cells (140). Several studies have documented that CXCL12 induces MMP synthesis in different cell types (141) and facilitates tumor cell adhesion and colonization. CXCL12 also modulates the expression and function of cell surface integrin molecules and, in turn, promotes tumor cell adhesion.

The CXCL12/CXCR4 pathway is involved in the "homing" of lymphocytes. It was hypothesized that chemokines and chemokine receptors including CXCL12/CXCR4 might mediate cancer cells to "home" to specific secondary sites, thereby promoting organ-specific metastasis. Blocking of CXCR4 expression on the cell surface greatly reduced the ability of colon cancer cells to metastasize to the liver and lungs (142). These studies demonstrate the pivotal role of CXCL12/CXCR4 in tumor metastasis..

Several papers published and shown hunman cancer cells including neuroblastoma (143), glioblastoma (98), ovarian (94), breast (3, 57), colon (54), pancreas (144), and prostate (100) express CXCL12. It is reasoned that endogenous CXCL12, together with CXCR4 on tumor cells, should keep cancer cells within the primary tumor environment, rather than facilitate metastasis over a long distance. Nonetheless, the effects of CXCL12/ CXCR4 on tumor metastasis may be explained by multiple factors in the tumor environment. In summary, although other factors need to be considered, it is evident that the CXCL12/CXCR4 pathway is implicated in the mechanistic process of tumor metastasis, including tumor cell adhesion and migration.

CXCL12 and Therapeutic Applications

Strong evidence demonstrates that CXCL12/CXCR4 signal is implicated in tumor proliferation, survival, vascularization, metastasis, and immunosuppression. The in vivo blockade of this pathway reduces tumor growth and metastasis in mouse models (142). Statistical studies suggest a possible negative association between high levels of CXCR4 expression and patient outcome in certain human tumors (97-144). Targeting CXCL12/CXCR4 pathway is a logic strategy in treating cancer patients. CXCR4 is one of the co-receptors for human immunodeficiency virus (HIV). AMD3100 is a CXCR4 antagonist and has been used in human clinical trials for treatment of HIV infection (145,146). AMD3100. Phase I pharmacokinetic studies demonstrated the feasibility of intravenous dosing and showed that AMD3100 was well tolerated by the healthy volunteers (47). Our results clarified that AMD3100 suppressed myeloma cell proliferation which stimulated by SDF-1 in- vitro, (abroun S. submitted data). AMD3100 also mobilized CD34 negative cells from the bone marrow into the peripheral blood of healthy volunteers as well as cancer patients (147,148). Although these studies did not test AMD3100 as an anti-cancer intervention, the observations suggest that CXCL12/CXCR4 inhibitors would be potentially used in clinical trials in treating cancer patients. On the other hand, thousands of patients worldwide have received treatment with angiogenesis inhibitors or antagonists. Bevacizumab, a monoclonal antibody against VEGF, is one of them (149). Although administration of bevacizumab results in increased patient survival with certain cancers (150,151), the clinical efficacy needs significant improvement. CXCL12 and VEGF synergistically induce tumor vascularization (104). Thus it is expected that combination of anti-VEGF and anti-CXCL12 may be more effective. It is possible that targeting CXCR4/CXCL12 pathway may yield unexpected clinical effects. it is evident that CXCR4/CXCL12 pathway is actively implicated in tumor pathogenesis and plays a significant role in tumor immunopathogenesis. Therefore, manipulation of this pathway will establish new strategy for cancer treatment (151, 152). Thus it need to allow in mind that although targeting CXCR4/CXCL12 is an attractive option in treating human tumors, it is highly likely that to achieve effective, reliable, and consistent clinical efficacy, a complicated combinatorial therapeutic regimen may be warranted.

Conclusion

Chemokines are distinct from other cytokines in their structure, cell surface receptors and unique pattern of activities and they have been considering in physiological and pathophysiological conditions. Metastasis is a lethal yet entirely inefficient process, metastatic cells share many similarities with normal stem cells, including an unlimited capacity for self renewal; the requirement for a specific 'niche' or microenvironment to grow; use of the SDF-1/CXCR4 axis for migration; enhanced resistance to apoptosis; and an increased capacity for drug resistance. The experiments with gene depletion, using specific antibody neutralization and use siRNA suggest each chemokine and receptor may have a special position on the stage of orchestrated biological and pathophysiological responses. Chemokine research is a new field and the brief information contained in this review reflects only the tip of an ice burg whose full identity remains to be explored. It is predictable that the importance of chemokines and receptors will be further appreciated with the enthusiastic participation in the research by scientists from multi-disciplinary backgrounds and the development of new therapeutic agents directed against chemokines or receptors with proven effectiveness in circumventing human diseases.

References

1. Bacon K, Baggiolini M, Broxmeyer H, et al. Chemokine/chemokine receptor nomenclature. J Interferon Cytokine Res 2002;22:1067-1068
2. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity. 2000; 12: 121-127
3. Rossi D, Zlotnik A. The biology of chemokines and their recveptor. Annu Rev Immunol, 2000; 8: 217-242
4. Moser B, Wolf M, Walz A, Loetscher P. Chemokines: multiple levels of leukocyte migration control. Trends Immunol. 2004; 25: 75-84
5. Ebert LM, Schaerli P, Moser B. Chemokine-mediated control of T cell traffic in lymphoid and peripheral tissues. Mol Immunol 2005; 42: 799-8096
7. Ishida T, Iida S, Akatsuka Y, et al. The CC chemokine receptor 4 as a novel specific molecular target for immunotherapy in adult T-cell leukemia/lymphoma. Clin Cancer Res 2004; 10: 7529-7539
8. Feng Y, Broder CC, Kennedy PE, Berger EA. HIV-1 entry cofactor, functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 1996; 272: 872-877
9. He J, Chen Y, Farzan M, et al. CCR3 and CCR5 are coreceptors for HIV-1 infection of microglia. Nature 1997; 385: 645-649
10. Murphy PM, Baggiolini M, Charo IF, et al. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol Rev. 2000; 52: 145-176
11. Thelen M. Dancing to the tune of chemokines. Nat Immunol, 2001; 2: 129-134
12. Broxmeyer HE, Kim CH. Regulation of hematopoiesis in a sea of chemokine family members with a plethora of redundant activities. Exp Hematol. 1999; 27: 1113-1123
13. Broxmeyer HE, Kim CH, Cooper SH, Hangoc G, Hromas R, Pelus LM. Effects of CC, CXC, C, and CX3C chemokines on proliferation of myeloid progenitor cells, and insights into SDF-1-induced chemotaxis of progenitors. Ann NY Acad Sci. 1999; 872: 142-162
14. Cooper S, Mantel C, Broxmeyer HE. Myelosuppressive effects in vivo with very low dosages of monomeric recombinant murine macrophage inflammatory protein-1 alpha. Exp Hematol. 1994; 22: 186-193
15. Broxmeyer HE, Cooper S, Hangoc G, Gao JL, Murphy PM. Dominant myelopoietic effector functions mediated bychemokine receptor CCR1. J Exp Med. 1999; 189: 1987-1992
16. D'Apuzzo M, Rolink A, Loetscher M, et al. The chemokine SDF-1, stromal cell-derived factor 1, attracts early stage B cell precursors via the chemokine receptor CXCR4. Eur J Immunol. 1997; 27: 1788-1793
17. Aiuti A, Tavian M, Cipponi A, et al. Expression of CXCR4, the receptor for stromal cell-derived factor-1 on fetal and adult human lympho-hematopoietic progenitors. Eur J Immunol. 1999; 29: 1823-1831
18. Tachibana K, Hirota S, Iizasa H, et al. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature. 1998; 393: 591-594
19. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature. 1998; 393: 595-599
20. Ma Q, Jones D, Borghesani PR, et al. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc Natl Acad Sci USA. 1998; 95: 9448-9453
21. Bleul CC, Boehm T. Chemokines define distinct microenvironments in the developing thymus. Eur J Immunol. 2000; 30: 3371-3379
22. Vicari AP, Figueroa DJ, Hedrick JA, et al. TECK: a novel CC chemokine specifically expressed by thymic dendritic cells and potentially involved in T cell development. Immunity. 1997; 7: 291-301
23. Forster R, Mattis AE, Kremmer E, Wolf E, Brem G, Lipp M. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. Cell. 1996; 87: 1037-1047
24. Ansel KM, Ngo VN, Hyman PL, et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature. 2000; 406: 309-314
25. Luster AD. Chemokines chemotactic cytokines that mediate inflammation. N Engl J Med. 1998; 338: 436-445
26. Yingying Le, Ye Zhou1, Pablo Iribarren and Ji Ming Wang. Chemokines and Chemokine Receptors: Their Manifold Roles in Homeostasis and Disease. Cellular and Molecular Immunology. 2004; 2: 95-104
27. Baekkevold ES, Yamanaka T, Palframan RT, et al. The CCR7 ligand elc (CCL19) is transcytosed in high endothelial venules and mediates T cell recruitment. J Exp Med. 2001; 193: 1105-1112
28. Reif K, Ekland EH, Ohl L, et al. Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. Nature. 2002;416:94-99.
29. Muller G, Hopken UE, Lipp M. The impact of CCR7 and CXCR5 on lymphoid organ development and systemic immunity. Immunol Rev. 2003; 195: 117-135
30. Strieter RM, Kunkel SL, Arenberg DA, Burdick MD, Polverini PJ. Interferon γ-inducible protein 10 (IP-10), member of the C-X-C chemokine family, is an inhibitor of angiogenesis. Biochem Biophys Res Commun. 1995; 210: 51-57
31. Strieter RM, Polverini PJ, Kunkel SL, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem. 1995; 270: 27348-27357
32. Salcedo R, Wasserman K, Young HA, et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: In vivo neovascularization induced by stromal-derived factor-1-alpha. Am J Pathol. 1999; 154: 1125-1135
33. Yoneda J, Kuniyasu H, Crispens MA, Price JE, Bucana CD, Fidler IJ. Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. J Natl Cancer Inst. 1998; 90: 447-454
34. Arenberg DA, Polverini PJ, Kunkel SL, et al. The role of

CXC chemokines in the regulation of angiogenesis in non-small cell lung cancer. J Leukoc Biol. 1997; 62: 554-562
35. Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J Clin Invest. 1996; 97: 2792-2802
36. Standiford TJ, Kunkel SL, Lukacs NW, et al. Macrophage inflammatory protein-1 alpha mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. J Immunol. 1995; 155: 1515-1524
37. VanOtteren GM, Strieter RM, Kunkel SL, et al. Compartmentalized expression of RANTES in a murine model of endotoxemia. J Immunol. 1995; 154: 1900-1908
38. Standiford TJ, Strieter RM, Lukacs NW, Kunkel SL. Neutralization of IL-10 increases lethality in endotoxemia. Cooperative effects of macrophage inflammatory protein-2 and tumor necrosis factor. J Immunol. 1995; 155: 2222-2229
39. Van Zee KJ, DeForge LE, Fischer E, et al. IL-8 in septic shock, endotoxemia, and after IL-1 administration. J Immunol. 1991; 146: 3478-3482
40. D'Ambrosio D, Mariani M, Panina-Bordignon P, Sinigaglia F. Chemokines and their receptors guiding T lymphocyte recruitment in lung inflammation. Am J Respir Crit Care Med. 2001; 164: 1266-1275
41. Burke-Gaffney A, Brooks AV, Bogle RG. Regulation of chemokine expression in atherosclerosis. Vascul Pharmacol. 2002; 38: 283-292
42. Quyyumi AA, Murphy PM. Association between polymorphism in the chemokine receptor CX3CR1 and coronary vascular endothelial dysfunction and atherosclerosis. Circ Res. yingying 2001; 89: 401-407
43. Szekanecz Z, Koch AE. Chemokines and angiogenesis. Curr Opin Rheumatol. 2001; 13: 202-208
44. Nanki T, Hayashida K, El-Gabalawy HS, et al. Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+T cell accumulation in rheumatoid arthritis synovium. J Immunol. 2000; 165: 6590-6598
45. Nanki T, Hayashida K, El-Gabalawy HS, et al. Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. J Immunol. 2000; 165: 6590-6598
46. Traugott U, Reinherz EL, Raine CS. Multiple sclerosis: distribution of T cell subsets within active chronic lesions. Science. 1983; 219: 308-310
47. McManus C, Berman JW, Brett FM, Staunton H, Farrell M, Brosnan CF. MCP-1, MCP-2 and MCP-3 expression in multiple sclerosis lesions: an immunohistochemical and in situ hybridization study. J Neuroimmunol. 998; 86: 20-29
48. Sorensen TL, Sellebjerg F. Distinct chemokine receptor and cytokine expression profile in secondary progressive MS. Neurology. 2001; 57: 1371-1376
49. Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1α and IP-10 are expressed in demyelinating brain lesions. Proc Natl Acad Sci U S A. 1999; 96: 6873-6878.
50. Simpson J, Rezaie P, Newcombe J, Cuzner ML, Male D, Woodroofe MN. Expression of the β-chemokine receptors CCR2, CCR3 and CCR5 in multiple sclerosis central nervous system tissue. J Neuroimmunol. 2000; 108: 192-200
51. Karpus WJ, Kennedy KJ. MIP-1alpha and MCP-1 differentially regulate acute and relapsing autoimmune encephalomyelitis as well as Th1/Th2 lymphocyte differentiation. J Leukoc Biol. 1997; 62: 681-687
52. Liu MT, Keirstead HS, Lane TE. Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. J Immunol. 2001; 167: 4091-4097
53. Izikson L, Klein RS, Charo IF, Weiner HL, Luster AD. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. J Exp Med. 2000; 192: 1075-1080
54. Rottman JB, Slavin AJ, Silva R, Weiner HL, Gerard CG, Hancock WW. Leukocyte recruitment during onset of experimental allergic encephalomyelitis is CCR1 dependent. Eur J Immunol. 2000; 30: 2372-2377
55. Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. Semin Cancer Biol. 2004; 14(3): 171-179
56. Koshiba T, Hosotani R, Miyamoto Y, et al. Expression of stromal cell-derived factor 1 and CXCR4 ligand receptor system in pancreatic cancer: a possible role for tumor progression. Clin Cancer Res, 2000; 6: 3530-3535
57. Rempl SA, Dudas S, Ge S, Gutierrez JA, Identification and localization of the cytokine SDF1 and its receptor, CXC chemokine receptor 4, to regions of necrosis and angiogenesis in human glioblastoma. Clin Cancer Res, 2000; 6: 102-111
58. Geminder H, Sagi-Agl-Assif O, Goldberg L, et al. A Possible Role for CXCR4 and Its Ligand, the CXC Chemokine Stromal Cell-Derived Factor-1, in the Development of Bone Marrow Metastases in Neuroblastoma. J Immunol, 2001; 167: 4747-4757
59. Muller A, Homy B, Soto H, Ge N, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature, 2001; 410: 50-56
60. Scotton CJ, Wilson JL, Milliken D, Stamp G, Balkwill FR. Epithelial cancer cell migration: a role for chemokine receptors? Cancer Res, 2001; 61: 4961-4965
61. Scharder AJ, Lechner O, Templin M, et al. CXCR4/CXCL12 expression and signalling in kidney cancer. Br J Cancer, 2002 ;86: 1250-1256
62. Taichman RS, Cooper C, Keller ET, Pinta KJ, Taichman NS, Mccauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res, 2002; 62: 1832-1837
63. Burger M, Glodek A, Hartmann T, et al. Functional expression of CXCR4 (CD184) on small-cell lung cancer cells mediates migration, integrin activation and adhesion to stromal cells. Oncogene, 2003; 22: 8093-8101
64. Hwang JH, Chung HK, Kim DW, et al. CXC chemokine receptor 4 expression and function in human anaplastic thyroid cancer cells. J Clin Endocrinol Metab, 2003; 88: 408-416
65. Zeelenberg IS, Ruuls SL, Roos E. Retention of CXCR4 in the endoplasmic reticulum blocks dissemination of a T cell hybridoma.. J Clin Invest, 2001; 108: 269-277
66. Mashino K, Sadanaga N, Yamaguchi H, et al. Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. Cancer Res, 2002; 62: 2937-2941
67. Ding Y, Shimada Y, Maeda M, et al. J Association of CC chemokine receptor 7 with lymph node metastasis of esophageal squamous cell carcinoma. Clin Cancer Res, 2003; 9: 3406-3412
68. Robledo MM, Bartolome RA, Longo N, et al. Expression of functional chemokine receptors CXCR3 and CXCR4 on human melanoma cells. J Biol Chem, 2001; 24: 24
69. Ishida T, Utsunomiya A, Iida S, et al. Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. Clin Cancer Res, 2003; 9: 3625-3634
70. Kleihans M, Tun-Kyi A, Gilliet M, et al. Functional expression of the eotaxin receptor CCR3 in CD30+ cutaneous T-cell lymphoma. Blood. 2003; 101: 1487-1493
71. Jones D, Benjamin RJ, Shahsafi A, Dorfman DM. The chemokine receptor CXCR3 is expressed in a subset of B-cell lymphomas and is a marker of B-cell chronic lymphocytic leukemia. Blood. 2000; 95: 627-632
72. Vicari AP, Caux C. Chemokines in cancer. Cytokine Growth Factor Rev. 2002; 13: 143-154
73. Dhawan P, Richmond A. Role of CXCL1 in tumorigenesis of melanoma. J Leukoc Biol, 2002; 72: 9-18
74. Zhang L, Conejo JR, Katsaos D. Intratumoral T cells, re-

currence and survival in epithelial ovarian cancer. N Engl J Med, 2003; 348: 203-213
75. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet, 2001; 357: 539-545
76. Nesbit M, Schader H, Miller TH, Herlyn M, Low-level monocyte chemoattractant protein-1 stimulation of monocytes leads to tumor formation in nontumorigenic melanoma cells. J Immunol, 2001; 166: 6483-6490
77. Danel D, Meyer MN, Bergsland EK, Dehne K, Coussens LM, Hanahan D. Immune enhancement of skin carcinogenesis by CD4+ T cells. J Exp Med, 2003;197: 1017-1028
78. Skinnider BF, Elia AJ, Gascoyane RD, et al.. Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. Blood 2002; 99: 618-626
79. Negus RP, Stamp GW, Relef MG, et al. The detection and localization of monocyte chemoattractant protein-1 (MCP-1) in human ovarian cancer. J. Clin. Invest. 1995; 95: 2391-2396
80. Milliken D, Scotton C, Rajij S, Balkwill F, Wilson J. Analysis of chemokines and chemokine receptor expression in ovarian cancer ascites. Clin Cancer Res, 2002; 8: 1108-1114
81. Luboshits G, Shina S, Kaplan O, et al. Elevated expression of the CC chemokine regulated on activation, normal T cell expressed and secreted (RANTES) in advanced breast carcinoma. Cancer Res, 1999; 59: 4681-4687
82. Azenshtein E, Luboshits G, Shina S, et al. The CC chemokine RANTES in breast carcinoma progression: regulation of expression and potential mechanisms of promalignant activity. Cancer Res, 2002; 62: 1093-1102
83. Lin EY, Nguyen AV, Russell RG, Pollard JW. Colonystimulating factor 1 promotes progression of mammary tumors to malignancy.J Exp Med, 2001; 193: 727-740
84. Ohta M, Kitadi Y, Tanaka S, et al. Monocyte chemoattractant protein-1 expression correlates with macrophage infiltration and tumor vascularity in human esophageal squamous cell carcinomas. Int J Cancer, 2002; 102: 220-224
85. Monti P, Leone BE, Marchesi, et al. The CC chemokine MCP-1/CCL2 in pancreatic cancer progression: regulation of expression and potential mechanisms of antimalignant activity. Cancer Res, 2003; 63: 7451-7461
86. Tashiro K, Tada H, Heilker R, Shirozu M, Nakano T, Honjo T. Signal sequence trap: a cloning strategy for secreted proteins and type Imembrane proteins. Science, 1993; 261: 600-603
87. Grunewald M, Avraham I, Dor Y, et al. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell, 2006; 124: 175-189
88. Katayama Y, Battista M, Kao WM, et al.. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell,2006; 124: 407-421
89. Petit I, Szyper-Kravitz M, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immuno, 2002; 3: 687-694
90. Ponomaryov T, Peled A, Petit I, et al. Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. J Clin Inves,t 2000; 106: 1331-1339
91. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature,1998;. 393: 595-599
92. Kryczek I, Grybos M, Dlubek D, Klimczak A, Rabczynski J, Lange A. Accumulation of CD45RO_ cells in peritoneal carcinomatous fluid favours survival of ovarian carcinoma patients. Cancer Immunol Immunother, 2002;. 51: 513-519
93. Scotton CJ, Wilson JL, Milliken D, Stamp G, Balkwill FR. Epithelial cancer cell migration: a role for chemokine receptors? Cancer Res. 2001; 61: 4961-4965
94. Zou W, Machelon V, Coulomb, et al. Stromal derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. Nat Me, 2001;7: 1339-1346
95. Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature, 2001; 410: 50-56
96. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res. 2002; 62: 1832-1837
97. Kang H, Watkins G, Parr C, et al. Stromal cell derived factor-1: its influence on invasiveness and migration of breast cancer cells in vitro, and its association with prognosis and survival in human breast cancer. Breast Cancer Res, 2005; 7: R402-R410
98. Rempel SA, Dudas S, Ge S, Gutierrez JA. Identification and localization of the cytokine SDF1 and its receptor, CXC chemokine receptor 4, to regions of necrosis and angiogenesis in human glioblastoma. Clin Cancer Res2000; 6: 102-111
99. Marchesi F, Monti P, Leone BE, et al. Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4. Cancer Res 2004 ; 64: 8420-8427
100. Sun YX, Wang J, Shelburne CE, et al. Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. J Cell Biochem 2003; 89: 462-473
101. Orimo A, Gupta PB, Sgroi DC, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005; 121: 335-348
102. Begley L, Monteleon C, Shah RB, Macdonald JW, Macoska JA. CXCL12 overexpression and secretion by aging fibroblasts enhance human prostate epithelial proliferation in vitro. Aging Cell. 2005; 4: 291-298--,.
103. Hall JM, Korach KS. Stromal cell-derived factor 1, a novel target of estrogen receptor action, mediates the mitogenic effects of estradiol in ovarian and breast cancer cells. Mol Endocrinol2 003; 17: 792-803
104. Kryczek I, Lange A, Mottram P, et al. CXCL12 and vascular endothelial growth factor synergistically induce neoangiogenesis in human ovarian cancers. Cancer Res 2005; 65: 465-472
105. Sehgal A, Keener C, Boynton AL, Warrick J, Murphy GP. CXCR-4 ,a chemokine receptor, is overexpressed in and required for proliferation of lioblastoma tumor cells. J Surg Oncol, 1998; 69: 99-104
106. Sonoda Y, Ozawa T, Aldape KD, Deen DF, Berger MS, Pieper RO. Akt pathway activation converts anaplastic astrocytoma to glioblastoma multiforme in a human astrocyte model of glioma. Cancer Res, 2001; 61: 6674-6678
107. Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM. The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. Am J Respir Crit Care Med, 2003; 167: 1676-1686
108. Scotton CJ, Wilson JL, Scott K, et al. Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. Cancer Res 2002; 62: 5930-5938
109. Hall JM, Korach KS. Stromal cell-derived factor 1, a novel target of estrogen receptor action, mediates the mitogenic effects of estradiol in ovarian and breast cancer cells. Mol Endocrinol, 2003; 17: 792-803
110. Jordan VC, Morrow M. Tamoxifen, raloxifene, and the prevention of breast cancer. Endocr Rev, 1999; 20: 253-278
111. Lindgren P, Backstrom T, Mahlck CG, Ridderheim M, Cajander S. Steroid receptors and hormones in relation to cell proliferation and apoptosis in poorly differentiated epithelial ovarian tumors. Int J Oncol, 2001; 19: 31-38
112. Wang CY, Mayo MW, Baldwin AS Jr. TNF and cancer therapyinduced apoptosis: potentiation by inhibition of NF- B. Science, 1996; 274: 784-787
113. Hartmann TN, Burger JA, Glodek A, Fujii N, Burger M. CXCR4 chemokine receptor and integrin signaling co-operate

in mediating adhesion and chemoresistance in small cell lung cancer (SCLC) cells. Oncogene, 2005; 24: 4462-4471
114. Sethi T, Rintoul RC, Moore SM, et al. Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: a mechanism for small cell lung cancer growth and drug resistance in vivo. Nat Med, 199; 5: 662-668
115. Hazlehurst LA, Damiano JS, Buyuksal I, Pledger WJ, Dalton WS. Adhesion to fibronectin via _1 integrins regulates p27kip1 levels and contributes to cell adhesion mediated drug resistance (CAM-DR). Oncogene, 2000; 19: 4319-4327
116. Uhm JH, Dooley NP, Kyritsis AP, Rao JS, Gladson CL. Vitronectin, a glioma-derived extracellular matrix protein, protects tumor cells from apoptotic death. Clin Cancer Res,1999; 5: 1587-1594,
117. Muller A, Sonkoly E, Eulert C, et al. Chemokine receptors in head and neck cancer: association with metastatic spread and regulation during chemotherapy. Int J Cancer, 2006; 118: 2147-2157
118. Ma Q, Jones D, Borghesani PR, et al. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc Natl Acad Sci USA, 1999; 5: 9448-9453
119. Tachibana K, Hirota S, Iizasa H, et al. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature, 1998; 393: 591-594
120. Molino M, Woolkalis MJ, Prevost N, et al. CXCR4 on human endothelial cells can serve as both a mediator of biological responses and as a receptor for HIV-2. Biochim Biophys Acta. 2000; 1500: 227-240
121. Salcedo R, Wasserman K, Young HA, et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: In vivo neovascularization induced by stromal-derived factor-1. Am J Pathol. 1999; 154: 1125-1135
122. Mirshahi F, Pourtau J, Li H, Muraine M, et al. SDF-1 activity on microvascular endothelial cells: consequences on angiogenesis in in vitro and in vivo models. Thromb Res. 2000; 99: 587-594
123. Murdoch C. CXCR4: chemokine receptor extraordinaire. Immunol Rev 2000; 177: 175-184
124. Curiel TJ, Cheng P, Mottram P, et al. Dendritic cell subsets differentially regulate angiogenesis in human ovarian cancer. Cancer Res, 2004; 64: 5535-5538
125. Gupta SK, Lysko PG, Pillarisetti K, Ohlstein E, Stadel JM. Chemokine receptors in human endothelial cells. Functional expression of CXCR4 and its transcriptional regulation by inflammatory cytokines. J Biol Chem. 1998; 273: 4282-4287
126. Salcedo R, Wasserman K, Young HA, et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: In vivo neovascularization induced by stromal-derived factor-1. Am J Pathol. 1999; 154: 1125-1135
127. Salvucci O, Yao L, Villalba S, Sajewicz A, Pittaluga S, Tosato G. Regulation of endothelial cell branching morphogenesis by endogenous chemokine stromal-derived factor-1. Blood. 2002; 99: 2703-2711
128. Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med. 2004; 10: 858-864
129. Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W. Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. Nature. 2003; 425: 307-311
130. Zlotnik A. Chemokines in neoplastic progression. Semin Cancer Biol. 2004; 14: 181-185
131. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer. 2002; 2: 563–572
132. Chung LW. Prostate carcinoma bone-stroma interaction and its biologic and therapeutic implications. Cancer, 2003; 97: 772-778
133. Hargreaves DC, Hyman PL, Lu TT, et al. A coordinated change in chemokine responsiveness guides plasma cell movements. J Exp Med. 2001; 194: 45-56
134. Cyster JG. Homing of antibody secreting cells. Immunol Rev. 2003; 194: 48-60
135. Nakayama T, Hieshima K, Izawa D, Tatsumi Y, Kanamaru A, Yoshie O. Cutting edge: profile of chemokine receptor expression on human plasma cells accounts for their efficient recruitment to target tissues. J Immunol. 2003; 170: 1136-1140
136. Abroun S, Otsuyama KI, Shamsasenjan K, et al. Galectin-1 supports the survival of CD45RA(-) primary myeloma cells in vitro. Br.J Hematology. 2008 in press
137. Islam A, Otsuyama K, Abroun S, et al. SDF-1 Is Responsible for the Constitutively High NF-kB Activity in Human Myeloma Cells. Blood ASH Annual Meeting Abstracts 108, Issue 11
138. Damiano JS, Cress AE, Hazlehurst LA, Shtil AA, Dalton WS. Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. Blood. 1999; 93:1658-1667
139. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer. 2002; 2: 161-174
140. Hojilla CV, Mohammed FF, Khokha R. Matrix metalloproteinases and their tissue inhibitors direct cell fate during cancer development. Br J Cancer. 2003;.89: 1817–1821,
141-Yu X, Collin-Osdoby P, Osdoby P. SDF-1 increases recruitment of osteoclast precursors by upregulation of matrix metalloproteinase-9 activity. Connect Tissue Res. 2003; 44 Suppl 1: 79-84
142. Zeelenberg IS, Ruuls-Van Stalle L, Roos E. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. Cancer Res, 2003; 63: 3833-3839
143. Geminder H, Sagi-Assif O, Goldberg L, et al. A possible role for CXCR4 and its ligand, the CXC chemokine stromal cell-derived factor-1, in the development of bone marrow metastases in neuroblastoma. J Immunol, 2001; 167: 4747-4757
144. Koshiba T, Hosotani R, Miyamoto Y, et al. Expression of stromal cell-derived factor 1 and CXCR4 ligand receptor system in pancreatic cancer: a possible role for tumor progression. Clin Cancer Res. 2000; 6: 3530-3535
145. De Clercq E. The bicyclam AMD3100 story. Nat Rev Drug Discov, 2003; 2: 581-587
146. Hendrix CW, Collier AC, Lederman MM, et al. Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. J Acquir Immune Defic Syndr. 2004; 37: 1253-1262
147. Devine SM, Flomenberg N, Vesole DH, et al. Rapid mobilization of CD34- cells following administration of the CXCR4 antagonist AMD3100 to patients
with multiple myeloma and non-Hodgkin's lymphoma. J Clin Oncol, 2004; 22: 1095-1102
148. Liles WC, Broxmeyer HE, Rodger E, et al. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. Blood, 2003; 102: 2728-2730
149. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science, 2005; 307: 58-62
150. Cobleigh MA, Langmuir VK, Sledge GW, et al. A phase I/II ose-escalation trial of bevacizumab in previously treated metastatic breast cancer. Semin Oncol. 2003; 30: 117-124
151. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan,fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350: 2335-2342
152. Kabbinavar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol, 2003; 21: 60-65