

The significance of cancer cell expression of the chemokine receptor CXCR4

Fran Balkwill*

Translational Oncology Laboratory, Barts and The London, Queen Mary's Medical School, London EC1M 6BQ, UK

Abstract

Malignant cells from at least 23 different types of cancer express the chemokine receptor CXCR4 and respond to its ligand CXCL12. This receptor ligand pair appears to be involved in directed migration of cancer cells to sites of metastasis, increased survival of cancer cells in sub optimal conditions and establishment of a tumour promoting cytokine/chemokine network. Preliminary data from animal models suggest that CXCR4 may be an important therapeutic target in a range of cancers. However CXCR4 plays major roles in embryogenesis, homeostasis and inflammation. This raises questions concerning the specificity of CXCR4 antagonists in the treatment of cancer.

© 2003 Elsevier Ltd. All rights reserved.

Keywords: CXCR4; CXCL12; Metastasis; Invasion

1. Introduction

Chemokine gradients are central to the directed movement of cells in many normal and pathologic processes. Cancers have a complex chemokine network that may influence the leucocyte infiltrate and angiogenesis [1]. Malignant cells can also express chemokine receptors and respond to chemokine gradients and this may be related to the growth and spread of cancer. Different cancers express different CC and CXC chemokine receptors and the corresponding ligands are sometimes expressed at sites of tumour spread [1–3]. There is one chemokine receptor, however, that appears to be expressed by a majority of cancer types and this is CXCR4.

CXCR4 and its ligand CXCL12 are widely expressed in normal tissues and play a fundamental role in foetal development, mobilisation of haemopoietic stem cells, and trafficking of naïve lymphocytes [4]. CXCR4 is also a co-receptor for T-trophic HIV [5]. This review will discuss the significance of CXCR4 expression on cancer cells in relation to its roles in embryonic development, homeostasis and inflammatory disease.

2. CXCR4 and CXCL12 in embryonic development

Mice lacking *CXCR4* or its ligand develop normally for the first 13 days (E13) but by E17.5 a majority are dead,

showing reduced body size, generalised oedema and dysplasia of the ventricular septum. They have a range of defects in the haematopoietic system, heart, cerebellum and vasculature. These results suggest a critical role for CXCR4 in migration and patterning of a number of embryonic cell lineages and also that CXCL12 is the only known receptor for CXCR4 ([6,7] and references therein).

In zebrafish embryos the posterior lateral line, PLL, is a mechanosensory system comprising seven to eight sense organs aligned from head to tail along the flank of the animal. The PLL is formed by a primordium that originates from the cephalic placode [8]. A trail of a CXCL12-like chemokine is present along the pathway of the primordium, and a CXCR4-like receptor is expressed by the migrating cells.

CXCR4 and CXCL12 are also involved in the migration of primordial germ cells, PGCs, in fish and murine embryos. Germ cells are created at a distance from the future gonad and somatic tissues lining their migratory path provide attractive, repulsive and survival cues. Zebrafish PGCs are guided towards somatic cells producing high levels of a CXCL12-like molecule, following a dynamic pattern of CXCL12 expression [9]. There are two zebrafish homologues of CXCR4, *cxcr4a* and *cxcr4b*. Knocking down CXCL12 or CXCR4b mRNA with RNAi results in lack of directional PCG migration and ectopic localisation. PGCs with a mutated *cxcr4b* fail to undergo directed migration towards the target tissue, and exogenous CXCL12 can recruit germ cells to ectopic sites in the embryo [10]. Murine PGCs also express CXCR4, and in CXCL12^{-/-} embryos,

* Tel.: +44-20-7882-6108; fax: +44-20-7882-6110.

E-mail address: frances.balkwill@cancer.org.uk (F. Balkwill).

numbers of PGCs that arrive in the gonads are significantly reduced [11].

3. CXCR4 and CXCL12 in adult tissues

mRNA for CXCR4 is detected in a range of adult tissues. Functional receptor protein is found on peripheral blood lymphocytes and unprimed T cells [12], monocytes [13], pre B cells, plasma cells [14,15], CD16 negative NK cells [16], dendritic cells [17], mast cells [18], adult CD34+ bone marrow progenitor cells [19], vascular smooth muscle cells [20], endothelial cells [21,22], retinal pigment epithelial cells [23], intestinal [24] and alveolar epithelial cells [25], microglia, neurons and astrocytes [7]. CXCR4 expression can be regulated positively by cytokines such as TGF- β 1, VEGF, bFGF, and negatively by cytokines such as IL-5, IFN- α and γ . CXCL12 is constitutively expressed by stromal cells in many tissues and is generally considered to be a homeostatic chemokine [11].

CXCR4 and CXCL12 are involved in proliferation, survival, homing and retention of primitive haematopoietic CD34+ progenitor cells, HPC, in bone marrow [19,26]. Direct proof for a role for CXCR4/CXCL12 in CD34+ cell trafficking in humans comes from treatment of volunteers with the selective CXCR4 antagonist AMD3100 [27,28]. A single dose produces a rapid generalised leucocytosis associated with an increase in peripheral blood CD34+ cells. At the highest dose of AMD3100 the number of progenitors increases 15–20-fold.

Responsiveness to CXCL12 correlates with positioning of B lymphocytes within a secondary lymphoid organ and is regulated by the differentiation state of the cell and by B cell receptor engagement [29]. CXCR4/CXCL12 is also involved in the migration of antibody-secreting plasma blasts during the course of a memory immune response [15].

Some normal epithelial cells express CXCR4. There is a predominant apical distribution, for instance on enterocytes in normal human bowel [24,30] and when colon epithelial cells are stimulated by CXCL12, they are able to produce other CXC chemokines.

Different dendritic cell, DC, subsets, express a range of functional chemokine receptors such as CCR1, CCR2, CCR5, CCR6, CCR9 and CXCR4 and these are involved in extravasation and movement to peripheral sites [31]. After activation in the stroma, or contact with inflammatory cytokines, they change their chemokine receptor phenotype, upregulating CCR7 which allows DC to migrate to gradients of the chemokines that signal through CCR7 [31]. In this process, receptors such as CXCR4 are down regulated or become unresponsive to ligand binding.

3.1. Mechanisms of progenitor cell mobilisation via CXCR4

CXCR4 positive HPC are mobilised from the bone marrow by treatment with cytokines such as G-CSF. G-CSF

causes a reduction in bone marrow plasma CXCL12 levels, mainly due to degradation by neutrophil elastase [32,33]. G-CSF may also regulate CXCR4 expression although data are conflicting with evidence for cleavage of the CXCR4 receptor to an inactive form [33], or receptor upregulation [32]. Mobilised HPC are attracted to sites of increased CXCL12 expression at sites of tissue injury, e.g. the liver [34].

Thus, CXCR4 is expressed on a range of normal cells. Stimulation of the receptor can affect growth, survival and homing of those cells. The receptor and its ligand can be modulated by other cytokines and a further level of control may exist in terms of a receptor that is expressed but is not functional [31].

4. Intracellular signalling pathways activated by CXCL12

CXCR4, like all other chemokine receptors, is a seven transmembrane domain receptor that is associated with heterodimeric G proteins. Signal transduction by chemokine receptors leads to activation of G proteins and phospholipase C and the elevation of cytosolic free calcium [35]. Stimulation of chemokine receptors also results in activation of ERK-2 and PI 3-kinase leading to formation of PIP3 and activation of PKB/Akt. In contrast to other chemokine receptors, stimulation of CXCR4 can lead to prolonged activation of these two signalling pathways [36]. Signalling via CXCR4 also enhances tyrosine phosphorylation, association of components of focal adhesion complexes such as paxillin, and NF- κ B activity in nuclear extracts [35]. Optimal chemotactic response of T cells to CXCL12 requires activation of both class IA and class IB PI 3-kinases [37]. Chemokines, including CXCR4, activate integrins and Rap1 plays a pivotal role in this [38].

5. Splice variants and mutations of CXCR4 and CXCL12

Murine CXCR4 has two CXCL12-binding isoforms, which are differentially expressed in lymphoid tissues and brain [39]. There is also a report of an alternative less potent transcript for human CXCR4, CXCR4-lo [40] which can be detected in spleen, lungs, peripheral blood mononuclear cells and some cancer cell lines. CXCR4 shows considerable conformational heterogeneity [41] and the commonly used anti-CXCR4 antibody 12G5 is only able to recognise a sub population of CXCR4 molecules.

A truncating mutation in the cytoplasmic tail of *CXCR4* is associated with WHIM syndrome, an immunodeficiency disease characterised by peripheral neutropenia, hypogammaglobulinemia and extensive human papillomavirus infection [42]. Interestingly, patients with WHIM syndrome have abundant mature myeloid cells in their bone marrow.

6. CXCR4 and CXCL12 in inflammatory disease

Just as CXCR4/CXCL12 is involved in homing and retention of cells during embryogenesis and homeostasis of adult tissues, there is evidence for a similar role in inflammation. For instance, in inflammatory liver disease, CXCL12 is upregulated in biliary epithelial cells and plasma levels of CXCL12 are also higher than in normal controls [43].

In a murine model of airway inflammation, functional CXCR4 is expressed by primary bronchial epithelial cells and this is enhanced by IL-1 β [44]. IL-1 β also increased CXCR4 mRNA and enhanced CXCL12 signalling in human bronchial epithelial cells. Similar CXCR4 expression was found in the nasal tissue of patients with symptomatic allergic rhinitis. CXCL12 was detected in synoviocytes of the hyperplastic lining layer of the rheumatoid joint [45]. This CXCL12 accumulated in, and was immobilised by, heparan sulphate molecules on endothelial cells. CXCL12 may also be involved in the perivascular accumulation of T cells typical of rheumatoid arthritis [46]. Human synovial T cells have high levels of functional CXCR4 that is positively regulated by TGF- β 1 and mediates adhesion of T cells to fibronectin.

In a murine model of collagen-induced arthritis, exogenous CXCL12, injected in periarticular tissues, elicited an inflammatory response and the majority of leucocytes harvested from arthritic joints were Mac-1+ CXCR4+ [47]. The importance of CXCR4/CXCL12 was demonstrated by the beneficial effects of treating mice with the CXCR4 antagonist AMD3100 before the appearance of first symptoms.

Eosinophils constitutively express low levels of CXCR4 that can be upregulated by TNF- α , IFN- γ and TGF- β 1 and down regulated by the Th2 cytokines IL-5 and IL-4 [48]. In non-inflamed tissues, CXCR4 may be involved in the retention of eosinophils. The Th2 state may favour their liberation via a decrease in CXCR4 expression.

As described above, CXCR4 and CXCL12 are involved in neuronal cell migration and patterning [7]. There is recent evidence for their involvement in neurotransmission in adult brain and in neurotoxicity induced inflammation/infection [49]. CXCL12 is expressed at high levels in astrocytes and CXCR4 is found in glial cells and specific subsets of neurons. Stimulation of hippocampal slices of brain with CXCL12 induces, within seconds, the release of the neurotransmitter glutamate. CXCR4 stimulation induced rapid cleavage and release of membrane TNF- α . This TNF- α then initiates a rapid and complex chain of events leading to prostaglandin formation, astrocyte communication and cytokine release.

Thus, in inflammatory disease CXCR4 and CXCL12 can be modulated by cytokines present at inflammatory sites and this can lead to attraction of cells to the site of inflammation, retention, prevention of apoptosis, enhanced response to CXCL12 and generation of inflammatory mediators.

7. Malignant cells may respond to chemokine gradients

While restricted and specific CC and CXC chemokine receptor expression has been found on many cancer cells [1,2], the chemokine receptor most commonly found on tumour cells, both in human and experimental murine cancers, is CXCR4. Table 1 summarises and references data on CXCR4/CXCL12 expression in human cancer biopsies, freshly isolated cancer cells and in cancer cell lines. CXCR4 expression has been reported in at least 23 different epithelial, mesenchymal and haemopoietic cancers. However, not all cancer cells studied are CXCR4 positive. Some of the cell lines derived from ovarian cancer, acute myelogenous leukaemia, AML, anaplastic thyroid cancer and glioma are CXCR4 negative as are primary cells from acute myeloid AML, erythroid AML and undifferentiated AML. Moreover, within primary tumours such as ovary and non small cell lung cancer, only a sub population of cells express CXCR4. When it has been possible to study primary tumour cells (i.e. from leukaemias and cells isolated from ovarian cancer ascites), the CXCR4 receptor is functional with a variety of signalling pathways activated.

There is less information on CXCL12 in malignant tissues and cell lines (Table 1). The ligand is found in primary tumour sites in lymphoma, glioma, ovarian cancer and pancreatic cancer; at sites of metastasis in breast and thyroid cancer, neuroblastoma and haematologic malignancies. CXCL12 expression varies with individual cancer types in patterns that could be consistent with attraction of cancer cells to distant sites, or, conversely, to retention of cells in the primary tumour. In kidney cancer there is a report that CXCL12 mRNA levels are lower in the malignant tissue compared to adjacent normal areas. CXCL12 is not produced by cell lines derived from breast and ovarian cancer, but has been detected in cell lines derived from prostate and pancreatic cancer as well as glioma.

7.1. CXCR4/CXCL12 in cancer cell migration and invasion

In most of the studies summarised in Table 1, activation of CXCR4 stimulated directed migration of cancer cells, invasion through matrigel, endothelial cell, bone marrow stromal, or fibroblast monolayers, towards a CXCL12 gradient e.g. [50–59]. The concentrations of CXCL12 required to stimulate migration, invasion, calcium flux, proliferation and gene induction in malignant cells are comparable to those used to stimulate normal cells. Most commonly 100–1000 ng/ml CXCL12 gives an optimal response in HPC, monocytes, lymphocytes and endothelial cells and similar doses are effective on a range of cancer cell lines and primary cancer cells.

Further insight into the role of CXCR4/CXCL12 in invasion may come from experimental cancer models. In human ovarian cancer biopsies, CXCR4 was only expressed by a minority of cells in the primary tumour [60]. However, if CXCR4 is over expressed in ovarian cancer cells that

Table 1
Tumours that express CXCR4

Tumor type	CXCR4 expression	Functional receptor	Regulation of CXCR4	CXCL12 expression	References
B-CLL	Higher expression than on normal B cells	Yes	–	Plasma CXCL12 lower than in normals	[83,84]
AML	On myelomonocytic promyelocytic subtypes only	Yes	–	–	[85]
B-lineage ALL	Freshly isolated cancer cells. High CXCR4 expression predicted extramedullary organ infiltration	Yes	–	–	[85,86]
Intraocular lymphoma	Biopsies	–	–	Weak in retinal pigment epithelium	[87]
Non-Hodgkin lymphoma	Mainly in biopsies of anaplastic large cell lymphoma	–	–	–	[88]
Follicular center lymphoma	Freshly isolated cells	Yes, more active than on germinal center B cells	–	In lymph nodes and made by lymphoma cells	[59]
CML	Freshly isolated cancer cells	Yes, but less active than in normal counterpart	–	–	[89]
Multiple myeloma	Cell lines	Yes	–	–	[90]
Pancreatic cancer	Cancer cells and endothelial cells of large blood vessels	Yes	–	In cancer cells in tissues not cancer cell lines	[54]
Prostate	Cell lines and cancer cells in biopsies. Localised and metastatic cancers	Yes	–	Osteoblasts in bone marrow	[91]
Breast	Cell lines biopsies	Yes	Upregulated by VEGF	mRNA ↑↑ in metastatic tissue compared to normal tissue. Also in cancer cell lines mRNA at sites of metastasis Not in cell lines	[67] [2,72]
Ovary	Cell lines minority of cells in biopsies	Yes	Upregulated by TGF-β	mRNA and protein in biopsies, protein not in normal tissue or cell lines	[52,53]
Thyroid	Cell lines	Yes	–	mRNA in cell lines	[58]
Colorectal cancer	Cell lines	Yes	–	–	[78]
Oral squamous carcinoma	Cell lines biopsies	Yes	–	Protein in stromal cells adjacent to cancer cells in lymph node	[92]
Cervical cancer	Cell lines biopsies	–	–	–	Slettenaar and Wilson (personal communication)
Neuroblastoma	Cell lines	Yes	Downregulated by CXCL12	mRNA and protein cell lines	[55]
Kidney	Cell lines tumor biopsies (mRNA)	Yes	Upregulated in cancer tissues compared with normal	mRNA downregulated in malignant compared with normal tissues	[66]
Glioma	13/16 cell lines biopsies	Yes	–	mRNA and protein in cell lines and some tumor tissues	[65,68,81]
Astrocytoma	In biopsies increased with tumor grade	–	Upregulated in cancer compared to normal	Co-localised with CXCR4	[81]
Rhabdomyosarcoma	Cell lines	Yes	Upregulated by PAX3-FKHR gene	–	[50]
Small cell lung cancer	Cell lines	Yes	SCF and CXCL12 co-operated in activation	–	[70,93]
Melanoma	Cell lines and biopsies	Yes	–	–	[64,69,94]

Table showing data on expression of CXCR4 and CXCL12 in a range of tumours; (–) data not known.

normally have low levels of a weakly functional CXCR4 receptor, the transfected cells show increased migration and invasion in response to CXCL12 *in vitro*, increased adhesion to extracellular matrix and CXCL12-mediated survival under sub optimal growth conditions. When grown intraperitoneally in nude mice, the CXCR4-transfected cells are able to establish extensive metastases outside the peritoneum unlike wild type cells (Kulbe and Wilson, manuscript in preparation).

Subcutaneously injected prostate cancer cells transfected with CXCR4 grow larger tumours with increased blood vessel density and muscle invasion, compared with parental cells [61]. Intra-arterial injection of MDA-MB-231 human breast cancer cells in immunodeficient mice results in osteolytic bone metastases. Subpopulations of cells with enhanced metastatic abilities were isolated by *in vivo* selection [62]. A gene expression profile linked with the bone metastatic clones. It is of interest that one of four highly over expressed genes was CXCR4, along with IL-11, osteopontin and connective tissue-derived growth factor, CTGF. When overexpressed alone in parental cells, CXCR4 causes a limited but significant increase in bone metastases but triple transfectants with IL-11, osteopontin and CXCR4 or CTGF show a dramatic increase in both the rate and incidence of bone metastases. In other xenograft experiments, CXCR4 expression and CXCL12-mediated migration are increased in tumour cells growing in the mammary fat pad compared with parental cells grown in culture [63], and when non small cell lung cancer cells are grown in SCID mice only 35% cells in the primary tumour express CXCR4 compared with 99% of cells in metastases [30].

In the syngeneic B16 melanoma model, CXCR4-transfected cells show a 10-fold increase in lung metastases after *i.v.* injection and there is increased adhesion of these cells to dermal and pulmonary microvascular endothelial cells [64]. However, metastases to lymph node, liver and kidney were rare in the CXCR4-transfected B16 cells.

7.2. CXCR4/CXCL12 and cancer cell growth/survival

In some types of cancer, CXCL12 can also stimulate cancer cell proliferation or survival under suboptimal conditions, e.g. [53,65–70]. These chemokine actions may allow tumour cells to grow in distant and less favourable sites. However, the significance of high levels of tumor-derived CXCL12 at the site of the primary lesion is not understood. Although this may stimulate tumour growth and promote survival, by analogy with its role in bone marrow retention of HPC, it could also serve to retain the malignant cells, rather than encourage metastasis.

7.3. CXCR4/CXCL12 and malignant progression

CXCR4 expression and action may be linked to other factors that are involved in the processes of malignancy.

CXCL12 stimulation of ovarian cancer cell lines and primary cells isolated from ascitic disease caused production of the pro-inflammatory cytokine TNF- α [53]. This cytokine has been implicated in tumour/stromal communication in this disease, establishment of a tumour cytokine network and is also an endogenous tumour promoter in murine skin cancer [71].

As well as being an important regulator of angiogenesis, via its neuropilin-1 receptor, VEGF can have autocrine actions on breast cancer cells, including increased invasiveness. This is due to induction of CXCR4 [72]. In addition, CXCL12 is a target of estrogen action in ER alpha positive human ovarian and breast cancer cell lines [73]. The mitogenic effects of estradiol are neutralised by addition of CXCL12 antibody and the hormone upregulates CXCR4 expression. Also in breast cancer, cell motility was controlled by NF- κ B upregulation of CXCR4 [63].

Alveolar rhabdomyosarcoma is a highly aggressive tumour characterised by recurrent PAX3 and PAX7-FKHR gene fusions. Transfer of PAX3-FKHR into embryonal rhabdomyosarcoma cells activates CXCR4 expression [50].

CXCL12 production in primary tumours may also contribute to the local immune suppression that is linked to malignant progression. Tumours are also known to inhibit type I dendritic cell, DC1, migration and function, suppressing any specific immune response. In ovarian cancer, there is evidence that tumour cell production of CXCL12 weakens immunity by attracting and protecting CXCR4-expressing preDC2 cells. Tumor CXCL12 may also alter preDC1 distribution and stimulate fibrosis [74].

7.4. Signalling pathways activated in malignant cells by CXCL12

The same signalling pathways that are described above are reported to be activated in malignant cells. Indeed some of the CXCL12-mediated pathways were first defined in transformed lymphocytes, but it is not yet clear whether there are difference between normal and malignant cells in intensity of signal, kinetics, or affinity of chemokine for the receptor. There is one interesting report of cross talk between the BCR/ABL oncogenic tyrosine kinase and CXCR4 signalling [75]. In chronic myelogenous leukaemia, CML, BCR/ABL kinase phosphorylates, activates and dysregulates proliferation and survival pathways of progenitor cells in the bone marrow. Immature leukaemic cells leave the marrow and are found in large numbers in the blood and spleen. BCR/ABL strongly activates a CXCR4-dependent signalling component through the Src family tyrosine kinase, Lyn. Cross talk between BCR/ABL and CXCR4 signalling may allow the oncoprotein to couple to PI3-kinase and MAPK cascades and ‘take over’ the chemokine pathway. This could lead to disruption of chemotaxis and hence release of the transformed cells into the periphery.

Table 2
Other chemokine receptors expressed by cxcr4 positive tumor cells

Tumor type	Positive	Negative	References
Prostate	CXCR4, CCR9	–	[95]
Ovarian	CXCR4, CCR9	CCR1, 2, 3, 4, 5, 7, 8 CXCR 1, 2, 3, 5 CXCR3	[52] [96]
Breast	CXCR4, CCR7	CX3CR1, CXCR5, XCR1 CXCR1, CCR4, CCR3, CCR2, CCR1	[2]
Melanoma	CXCR4, CXCR3, CCR7, CCR10	–	[69,94]
Multiple myeloma	CXCR4, CCR1, CXCR3, CXCR6	–	[90]

(–) not studied.

7.5. Other chemokine receptors expressed together with CXCR4

In most, but not all, of the cancer cells studied, CXCR4 is co-expressed with other CC or CXC chemokine receptors, as summarised in Table 2. Expression of CCR7 and CCR10 on melanoma cells, for example, is linked to expression of ligands for these receptors at the two major sites of metastasis, skin and lymph nodes [2]. CCR7 is also found on gastric cancer cells and these cells respond to chemokine ligand by directed migration in vitro [76]. Stepwise regression analysis showed that the most important factor determining lymph node metastasis in gastric cancer was CCR7 expression in the primary tumour.

The repertoire of chemokine receptors on different tumour types may be related to patterns of spread but it is not clear whether chemokine receptors can act in a synergistic fashion on the malignant cells.

8. Inhibition of CXCR4/CXCL12 in murine cancer models

CXCR4 seems to be commonly expressed on cancer cells and plays a role in migration, invasion, proliferation, survival and other malignant processes. Is this receptor a therapeutic target? There are some data from experimental murine cancer models to support this idea. When cells are transfected with CXCL12 containing an ER retention sequence, the chemokine is able to bind CXCR4 in the endoplasmic reticulum and prevent its surface expression [77]. When T cell hybridoma cells are transfected with this construct and injected intravenously, they no longer metastasise to distant organs. When similar experiments are carried out with CT-26 colorectal cancer cells, lung and liver metastases are greatly reduced [78]. However, CXCR4-deficient CT-26 cells colonise the lungs to the same extent as control cells; they survive but do not proliferate.

Anti-CXCR4 antibodies inhibit spread of breast cancer xenografts to lymph node [2]. Pre-incubation of Namalwa lymphoblastoid cells with anti-CXCR4 or anti-CXCL12 antibodies delayed i.p. tumour growth in NOD/SCID mice and reduced tumour weight [79]. If cells preincubated

with CXCR4 antibodies are injected intravenously they are present in the circulation much longer than control cells, demonstrating a role for CXCR4 in extravasation. Tumour development is also inhibited if antibodies are administered to mice three days after Namalwa cell injection. Antibodies to CXCL12 inhibit organ metastases of non small cell lung cancer cells when they are given for eight weeks starting at the time of tumour cell injection [30].

Transfection of breast cancer cells with inducible small interfering CXCR4 RNA inhibits invasion in vitro [80] but no work has been done in animal models with such constructs as yet.

CXCR4 is expressed in glioblastoma multiforme and medulloblastoma [81] and the ligand is found in tumor-associated blood vessels. CXCL12 stimulates proliferation, chemotaxis and survival of glioblastoma cell lines [68]. Glioblastoma cells grow as intracranial xenografts and this growth is strongly blocked by systemic treatment with the CXCR4 antagonist AMD3100, with an increase in tumour cell apoptosis [82].

Hence, CXCR4 is a potential therapeutic target in human cancer but the studies are preliminary and more extensive studies using established tumours are required.

9. Conclusions

Functional CXCR4 is widely expressed by malignant cells. There is preliminary evidence that CXCR4 expression can confer an invasive phenotype in vivo. Moreover, the first animal experiments show that CXCR4 antibodies antagonists may inhibit cancer growth and spread. However, CXCR4 and its ligand are also widely expressed in a number of homeostatic and inflammatory situations and CXCR4 antagonists can mobilise stem cells from bone marrow. This raises the possibility that CXCR4 antagonists could have considerable side effects and even release CXCR4 positive cells from primary tumour sites with high levels of CXCL12. Relatively little is known about cross talk between CXCR4 signalling and the oncogenes and growth factors important in malignant disease. There are many other unanswered questions. By analogy with studies in zebrafish, do some cancer cells have mutations in *CXCR4*

which lead to random dispersal of cells at inappropriate sites? Does CXCL12 control directed migration of cells in a primary cancer? Is CXCR4 functional in primary cancers or is it inactive due to the inflammatory cytokine microenvironment or high levels of CXCL12 at the tumour site?

However, on the basis of the experiments reviewed in this article, it would seem that further understanding of the action of CXCR4 in normal and malignant disease could lead to exciting new therapeutic options in a range of malignancies.

References

- [1] Balkwill F. Chemokine biology in cancer. *Sem Immunol* 2003;15:49–55.
- [2] Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001;410:50–6.
- [3] Murphy PM. Chemokines and molecular basis of cancer metastasis. *N Engl J Med* 2001;354:833–5.
- [4] Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000;18:217–42.
- [5] 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;272:872–7.
- [6] Tachibana K, Hirota S, Lizasa H, Yoshida H, Kawabata K, Kataoka Y, et al. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature* 1998;393:591.
- [7] Zou Y-R, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature* 1998;393:595.
- [8] David NB, Sapede D, Saint-Etienne L, Thisse C, Thisse B, Dambly-Chaudiere C, et al. Molecular basis of cell migration in the fish lateral line: role of the chemokine receptor CXCR4 and of its ligand, SDF1. *PNAS* 2002;99:16297–302.
- [9] Doitsidou M, Reichman-Fried M, Stebler J, Kopranner M, Dorries J, Meyer D, et al. Guidance of primordial germ cell migration by the chemokine SDF-1. *Cell* 2002;111:647–59.
- [10] Knaut H, Werz C, Geisler R, Nusslein-Volhard C, Consortium TS, et al. A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. *Nature* 2003;421:279–82.
- [11] Ara T, Nakamura Y, Egawa T, Sugiyama T, Abe K, Kishimoto T, et al. Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). *PNAS* 2003;100:5319–23.
- [12] Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc Natl Acad Sci USA* 1997;94:1925–30.
- [13] Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 1996;382:829–33.
- [14] Nakayama T, Hieshima K, Izawa D, Tatsumi Y, Kanamaru A, Yoshie O. Profile of chemokine receptor expression on human plasma cells accounts for their efficient recruitment to target tissues. *J Immunol* 2003;170:1136–40.
- [15] Hauser AE, Debes GF, Arce S, Cassese G, Hamann A, Radbruch A, et al. Chemotactic responsiveness towards ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response. *J Immunol* 2002;169:1277–82.
- [16] Hanna J, Wald O, Goldman-Wohl D, Prus D., Markel G., Gazit R., et al. CXCL12 expression by invasive trophoblasts induces the specific migration of CD16 negative human natural killer cells. *Blood* 2003.
- [17] Zoeteij JP, Golding H, Mostowski H, Blauvelt A. Cytokines regulate expression and function of the HIV coreceptor CXCR4 on human mature dendritic cells. *J Immunol* 1998;161:3219–23.
- [18] Juremalm M, Hjertson M, Olsson N, Harvima I, Nilsson K, Nilsson G. The chemokine receptor CXCR4 is expressed within the mast cell lineage and its ligand stromal cell-derived factor-1a acts as a mast cell chemotaxin. *Eur J Immunol* 2000;30:3614–22.
- [19] Aiuti A, Webb IJ, Bleul C, Springer T, Guttierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilisation of CD34+ progenitors to peripheral blood. *J Exp Med* 1997;185:111–20.
- [20] Schecter AD, Berman AB, Taubman MB. Chemokine receptors in vascular smooth muscle. *Microcirculation* 2003;10:265–72.
- [21] Gupta SK, Lyso PG, Pillarisetti K, Ohlstein E, Stadel JM. Chemokine receptors in human endothelial cells. *J Biol Chem* 1998;273:4282–7.
- [22] Salcedo R, Wasserman K, Young HA, Grimm MC, Howard OMZ, Anver MR, et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells. *Am J Pathol* 1999;154:1125–35.
- [23] Crane IJ, Wallace CA, McKillop-Smith S, Forrester JV. CXCR4 receptor expression on human retinal pigment epithelial cells from the blood–retina barrier leads to chemokine secretion and migration in response to stromal cell-derived factor 1a. *J Immunol* 2000;165:4372–8.
- [24] Dwinell MB, Eckmann L, Leopard JD, Varki NM, Kagnoff MF, et al. Chemokine receptor expression by human intestinal epithelial cells. *Gastroenterology* 1999;117:359–67.
- [25] Murdoch C, Monk PN, Finn A. Functional expression of chemokine receptor CXCR4 on human epithelial cells. *Immunology* 1999;98:36–41.
- [26] Broxmeyer HE, Cooper S, Kohli L, Hangoc G, Lee Y, Mantel C, et al. Transgenic expression of stromal cell-derived factor-1/CXC chemokine ligand 12 enhances myeloid progenitor cell survival/antiapoptosis in vitro in response to growth factor withdrawal and enhances myelopoiesis in vivo. *J Immunol* 2003;170:421–9.
- [27] De Clercq E. The bicyclam AMD3100 story. *Nat Rev* 2003;2:581–7.
- [28] Liles WC, Broxmeyer HE, Rodger E, Wood B., Hubel K., Cooper S., et al. Mobilisation of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood* 2003.
- [29] Bleul CC, Schultze JL, Springer TA. B lymphocyte chemotaxis regulated in association with microanatomic localization, differentiation state, and B cell receptor engagement. *J Exp Med* 1998;187:753–62.
- [30] Jordan NJ, Kolios G, Abbot SE, Sinai MA, Thompson DA, Petraki K, et al. Expression of functional CXCR4 chemokine receptors on human colonic epithelial cells. *J Clin Invest* 1999;104:1061–9.
- [31] Allavena P, Sica A, Vecchi A, Locati M, Sozzani S, Mantovani A. The chemokine receptor switch paradigm and dendritic cell migration: its significance in tumor tissues. *Immunol Rev* 2000;177:141–9.
- [32] Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, et al. G-CSF induces stem cell mobilisation by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol* 2002;3:687–94.
- [33] Levesque J-P, Hendy J, Takamatsu Y, Simmons PJ, Bendall LJ, et al. Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilisation induced by G-CSF or cyclophosphamide. *J Clin Invest* 2003;111:187–96.
- [34] Kollet O, Shvitiel S, Chen YQ, Suriawinata J, Thung SN, Dabeva MD, et al. HGF, SDF-1, and MMP-9 are involved in stress induced CD34+ stem cell recruitment to the liver. *J Clin Invest* 2003;112:160–9.
- [35] Ganju RK, Brubaker SA, Meyer J, Dutt P, Yang Y, Qin S, et al. The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. *J Biol Chem* 1998;273:23169–75.

- [36] Tilton B, Ho L, Oberlin E, Loetscher P, Baleux F, Clarke-Lewis I, et al. Signal transduction by CXC chemokine receptor 4. Stromal cell-derived factor 1 stimulates prolonged protein kinase B and extracellular signal-regulated kinase 2 activation in T lymphocytes. *J Exp Med* 2000;192:313–24.
- [37] Curnock AP, Sotsios Y, Wright KL, Ward SG. Optimal chemotactic responses of leukemic T cells to stromal cell-derived factor-1 requires the activation of both class 1A and 1B phosphoinositide 3-kinases. *J Immunol* 2003;170:4021–30.
- [38] Shimonaka M, Katagiri K, Nakayama T, Fujita N, Tsuruo T, Yoshie O, et al. Rap1 translates chemokine signals to integrin activation, cell polarization, and motility across vascular endothelium under flow. *J Cell Biol* 2003;161:417–27.
- [39] Moepps B, Frodl R, Rodewald H-R, Baggiolini M, Gierschik P. Two murine homologues of the human chemokine receptor CXCR4 mediating stromal cell-derived factor 1a activation of G_{i2} are differentially expressed in vivo. *Eur J Immunol* 1997;27:2102–12.
- [40] Gupta SK, Pillarisetti K. CXCR4-Lo: molecular cloning and functional expression of a novel human CXCR4 splice variant. *J Immunol* 1999;163:2368–72.
- [41] Baribaud F, Edwards TG, Sharron M, Brelot A, Heveker N, Price K, et al. Antigenically distinct conformations of CXCR4. *J Virol* 2001;75:8957–67.
- [42] Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F, et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nat Genet* 2003;34:70–4.
- [43] Terada R, Yamamoto K, Hadoka T, Shimada N, Okano N, Baba N, et al. Stromal cell-derived factor-1 from biliary epithelial cells recruits CXCR4-positive cells: implications for inflammatory liver disease. *Lab Invest* 2003;83:665–72.
- [44] Eddleston J, Christiansen SC, Zuraw BL. Functional expression of the C-X-C chemokine receptor CXCR4 by human bronchial epithelial cells: regulation by proinflammatory mediators. *J Immunol* 2002;169:6445–51.
- [45] Pablos JL, Santiago B, Galindo M, Torres C, Brehmer MT, Blanco FJ, et al. Synovial cell-derived CXCL12 is displayed on endothelium and induces angiogenesis in rheumatoid arthritis. *J Immunol* 2003;170:2147–52.
- [46] Buckley CD, Amft N, Bradfield PF, Pilling D, Ross E, Arenzana-Seisdedos F, et al. Persistent induction of the chemokine receptor CXCR4 by TGF- β 1 on synovial T cells contributes to their accumulation within the rheumatoid synovium. *J Immunol* 2000;165:3423–9.
- [47] Matthys P, Hatse S, Vermeire K, Wuyts A, Bridger G, Henson GW, et al. AMD31000, a potent and specific antagonist of the stromal cell-derived factor-1 chemokine receptor CXCR4, inhibits autoimmune joint inflammation in IFN- γ receptor-deficient mice. *J Immunol* 2001;167:4686–92.
- [48] Nagase H, Miyamasu M, Yamaguchi M, Fujisawa T, Ohta K, Yamamoto K, et al. Expression of CXCR4 eosinophils: functional analyses and cytokine-mediated regulation. *J Immunol* 2000;164:5935–43.
- [49] Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, et al. CXCR4-activated astrocyte glutamate release via TNF α : amplification by microglia triggers neurotoxicity. *Nat Neurosci* 2001;4:702–10.
- [50] Libura J, Drukala J, Majka M, Tomescu O, Navenot JM, Kucia M, et al. CXCR4-SDF-1 signaling is active in rhabdomyosarcoma cells and regulates locomotion, chemotaxis, and adhesion. *Blood* 2002;100:2597–606.
- [51] Han Y, He T, Huang D, Pardo CA, Ransohoff RM. TNF- α mediates SDF-1 α -induced NF- κ B activation and cytotoxic effects in primary astrocytes. *J Clin Invest* 2001;108:425–35.
- [52] Scotton CJ, Wilson JL, Milliken D, Stamp G, Balkwill FR. Epithelial cancer cell migration: a role for chemokine receptors? *Cancer Res* 2001;61:4961–5.
- [53] Scotton CJ, Wilson JL, Scott K, Stamp G, Wilbanks GD, Fricker S, et al. Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res* 2002;62:5930–8.
- [54] Koshiba T, Hosotani R, Miyamoto Y, Ida J, Tsuji S, Nakajima S, 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;6:3530–5.
- [55] Geminder H, Sagi-Assif O, Goldberg L, Meshel T, Rechavi G, Witz IP, 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 and neuroblastoma. *J Immunol* 2001;167:4747–57.
- [56] Chen T, Triplett J, Dehner B, Hurst B, Colligan B, Pemberton J, et al. Transforming growth factor- β receptor type 1 gene is frequently mutated in ovarian carcinomas. *Cancer* 2001;61:4679–82.
- [57] Semizarov D, Frost L, Sarthy A, Kroeger P, Halbert DN, Fesik SW. Specificity of short interfering RNA determined through gene expression signatures. *PNAS* 2003;100:6347–52.
- [58] Hwang JH, Hwang JH, Chung HK, Kim DW, Hwang ES, Suh JM, et al. CXC chemokine receptor 4 expression and function in human anaplastic thyroid cancer cells. *J Clin Endocrinol Metab* 2003;88:408–16.
- [59] Corcione A, Ottonello L, Tortolina G, Facchetti P, Airoidi I, Gugliemino R, et al. Stromal cell-derived factor-1 as a chemoattractant for follicular center lymphoma B cells. *JNCI* 2000;92:628–35.
- [60] Scotton C, Milliken D, Wilson J, Raju S, Balkwill F. Analysis of CC chemokine and chemokine receptor expression in solid ovarian tumours. *Br J Cancer* 2001;85:891–7.
- [61] Darash-Yahana M, Pikarsky E, Karplus R, Kasem S, Pal B, Zeira E, et al. Human prostate cancer growth, vascularisation and metastasis is stimulated via high expression levels of the chemokine receptor CXCR4. *Proc AACR* 2003;44:789.
- [62] Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, et al. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 2003;3:537–49.
- [63] Helbig G, Christopherson KWN, Bhat-Nakshatri P, Kumar S, Kishimoto H, Miller KD, et al. NF- κ B promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. *J Biol Chem* 2003;278:21631–8.
- [64] Murakami T, Maki W, Cardones AR, Fang H, Kyi AT, Nestle FO, et al. Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. *Cancer Res* 2002;62:7328–34.
- [65] Zhou Y, Larsen PH, Hao C, Yong VW. CXCR4 is a major chemokine receptor on glioma cells and mediates their survival. *J Biol Chem* 2002;277:49481–7.
- [66] Schrader AJ, Lechner O, Templin M, Dittmar KEJ, Machtens S, Mengel M, et al. CXCR4/CXCL12 expression and signalling in kidney cancer. *Br J Cancer* 2002;86:1250–6.
- [67] Sun Y-X, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, et al. Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. *J Cell Biochem* 2003;89:462–73.
- [68] Barbero S, Bonavia R, Bajetto A, Porcile C, Pirani P, Ravetti JL, et al. Stromal cell-derived factor 1a stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt. *Cancer Res* 2003;63:1969–74.
- [69] Robledo MM, Bartolome RA, Longo N, Rodriguez-Frade JM, Melhado M, Longo I, et al. Expression of functional chemokine receptors CXCR3 and CXCR4 on human melanoma cells. *J Biol Chem* 2001;276:45098–105.
- [70] Kijima T, Maulik G, Ma PC, Tibaldi EV, Turner RE, Rollins B, et al. Regulation of cellular proliferation, cytoskeletal function, and signal transduction through CXCR4 and c-Kit in small cell lung cancer cells. *Cancer Res* 2002;62:6304–11.
- [71] Szlosarek P, Balkwill F. Tumour necrosis factor- α : a potential target in the therapy of solid tumours. *Lancet Oncol* 2003;4:565–73.

- [72] Bachelder RE, Wendt MA, Mercurio AM. Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. *Cancer Res* 2002;62:7203–6.
- [73] Hall JM, Korach KS. Stromal cell-derived factor 1, novel target of estrogen receptor action, mediates the mitogenic effects of estradiol in ovarian and breast cancer cells. *Mol Endocrinol* 2003;17:792–803.
- [74] Zou W, Machelon V, Coulomb-L'Hermin A, Borvak J, Nome F, Isaeva T, et al. Stromal-derived factor-1 in human tumours recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med* 2001;7:1339–46.
- [75] Ptasznik A, Urbanowska E, Chinta S, Costa MA, Katz BA, Stanislaus MA, et al. Crosstalk between BCR/ABL oncoprotein and CXCR4 signaling through a Src family kinase in human leukemia cells. *J Exp Med* 2002;196:667–78.
- [76] Mashino K, Sadanaga N, Yamaguchi H, Tanaka F, Ohto M, Shibuta K, et al. Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. *Cancer Res* 2002;62:2937–41.
- [77] Zeelenberg IS, Ruuls-Van Stalle L, Roos E. Retention of CXCR4 in the endoplasmic reticulum blocks dissemination of a cell hybridoma. *J Clin Invest* 2001;108:269–77.
- [78] Zeelenberg IS, Ruuls-Van Stalle L, Roos E. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. *Cancer Res* 2003;63:3833–9.
- [79] Bertolini F, Dell'Agnola C, Mancuso P, Rabascio C, Burlini A, Monestiroli S, et al. CXCR4 neutralization, a novel therapeutic approach for non-Hodgkin's lymphoma. *Cancer Res* 2002;62:3106–12.
- [80] Chen YC, Stamatoyannopoulos G, Song C-Z. Down-regulation of CXCR4 by inducible small interfering RNA inhibits breast cancer cell invasion in vitro. *Cancer Res* 2003;63:4801–4.
- [81] 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 Res* 2000;6:102–11.
- [82] Rubin JB, Kung AL, Klein RS, Chan JA, Sun Y, Schmidt K, et al. Systemic delivery of a small molecule inhibitor of CXCR4 blocks the growth of intracranial xenografts of glioblastoma multiforme. *Proc Am Assoc Cancer Res* 2003;44:544.
- [83] Mohle R, Failenschmid C, Bautz F, Kanz L. Overexpression of the chemokine receptor CXCR4 in B cell chronic lymphocytic leukemia is associated with increased functional response to stromal cell-derived factor-1 (SDF-1). *Leukemia* 1999;13:1954–9.
- [84] Barretina J, Junca J, Llano A, Gutierrez A., Flores A., Blanco J., et al. CXCR4 and SDF-1 expression in B-cell chronic lymphocytic leukemia and stage of the disease. *Ann Hematol* 2003;29.
- [85] Mohle R, Schittenhelm M, Failenschmid C, Bautz F, Kratz-Albers K, Serve H, et al. Functional response of leukaemic blasts to stromal cell-derived factor-1 correlates with preferential expression of the chemokine receptor CXCR4 in acute myelomonocytic and lymphoblastic leukaemia. *Br J Haematol* 2000;110:563–72.
- [86] Crazzolara R, Kreczy A, Mann G, Heitger A, Eibl G, Fink FM, et al. High expression of the chemokine receptor CXCR4 predicts extramedullary organ infiltration in childhood acute lymphoblastic leukaemia. *Br J Haematol* 2001;115:545–53.
- [87] Barbero S, Bajetto A, Bonavia R, Porcile C, Piccioli P, Pirani P, et al. Expression of the chemokine receptor CXCR4 and its ligand stromal cell-derived factor 1 in human brain tumours and their involvement in glial proliferation in vitro. *Ann N Y Acad Sci* 2002;973:60–9.
- [88] Weng AP, Shahsafaei A, Dorfman DM. CXCR4/CD184 immunoreactivity in T-cell non-Hodgkin lymphomas with an overall TH1–Th2+ immunophenotype. *Am J Clin Pathol* 2003;119:424–30.
- [89] Peled A, Hardan I, Trakhtenbrot L, et al. Immature leukemic CX34+CXCR4+ cells from CML patients have lower integrin-dependent migration and adhesion in response to the chemokine SDF-1. *Stem Cells* 2002;20:259–66.
- [90] Moller C, Stromberg T, Juremalm M, Nilsson G, Nilsson K. Expression and function of chemokine receptors in human multiple myeloma. *Leukemia* 2003;17:203–10.
- [91] 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;62:1832–7.
- [92] Uchida D, Begum N-M, Almofti A, Kawamata H, Nakashiro K-I, Tateishi Y, et al. Involvement of stromal cell derived factor-1/CXCR4 signaling in lymph node metastasis of oral squamous cell carcinoma. *Proc Am Assoc Cancer Res* 2003;44:452.
- [93] 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;167:1676–86.
- [94] Payne AS, Cornelius LA. The role of chemokines in melanoma tumor growth and metastasis. *J Invest Dermatol* 2002;118:915–22.
- [95] Singh S, Hannah PS, Singh UP, Grizzle WE, Lillard Jr JW. Differential CXCR4 and CCR9 expression by prostate cancer. *Proceedings of American Association for Cancer Res* 2003;44:772.
- [96] Hannah PS, Singh S, Singh UP, Partridge EE, Lillard Jr JW. Role of CXCR4 and CCR9 in ovarian cancer progression. *Proc Am Assoc Cancer Res* 2003;44:772.