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The chemokine network in cancer - much more than directing cell movement

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ABSTRACT Cytokine and chemokine gradients are central to the directed movement of cells in both homeostatic and pathological processes. Most cancers have a complex chemokine network which can influence immune responses to the tumor, direct the extent and cellular composition of the leukocyte infiltrate and also play a role in angiogenesis. Tumor cells can also hijack the chemokine system and gain expression of certain chemokine receptors and respond to specific chemokine gradients. Chemokine receptor expression and activation on malignant cells may be central to the growth, survival and migration of cancer cells from the primary tumor. Chemokine receptors, both CC and CXC have been detected on malignant cells and the relevant ligands are sometimes expressed at the tumor site and at sites of tumor spread, suggesting a role for the chemokine family in malignant growth and metastasis.

KEY WORDS: *chemokine, CXCL12, CXCR4, metastasis*

Introduction

Chemokines are a large subfamily of chemoattractant cytokines which are classified into 4 highly conserved groups: CXC, CC, C and CX$_3$C, based on the position of the first two cysteines adjacent to the amino terminus. The CXC chemokines act predominantly on neutrophils and T-lymphocytes, while the CC chemokines are active on various cell types, including monocytes and lymphocytes.

Chemokines selectively regulate the recruitment and trafficking of leukocyte subsets to inflammatory sites through chemoattraction and by activating leukocyte integrins to bind their adhesion receptors on endothelial cells (Ebnet and Vestweber, 1999). Chemokines exert their effects by binding to seven transmembrane domain G protein-coupled receptors. Currently 11 receptors for the CC chemokines (CCR1-11) and six receptors for the CXC chemokines have been identified in man (Mackay, 2001). Their ligands bind to the extracellular N-terminus, leading to phosphorylation of serine/threonine residues on the cytoplasmic C-terminus, signalling and receptor desensitisation (Thelen, 2001). There is apparent redundancy in the system; each receptor can respond to more than one chemokine; most chemokines can use more than one receptor and each leukocyte subset may express several receptors.

Recently there have been many reports of malignant cells expressing chemokine receptors and utilizing these to facilitate tumor cell survival, growth and movement. This review will describe the chemokine network in tumors, the expression of chemokine receptors by tumor cells and give mechanistic examples of how this large family of ligands and receptors can contribute to tumor cell spread.

Chemokine receptor expression profiles of cancer cells

The directed migration of tumor cells to distant organs via lymphatics and blood resembles chemokine-directed lymphocyte migration. Recent papers suggest that tumor cells may express restricted and specific patterns of chemokine receptors and 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 (Balkwill, 2004). For example tumor cells from breast, prostate, pancreatic, lung and ovarian carcinomas, neuroblastoma and glioblastoma, all express CXCR4 (Koshiba *et al.*, 2000; Rempel *et al.*, 2000; Geminder *et al.*, 2001; Muller *et al.*, 2001; Scotton *et al.*, 2001, Schrader *et al.*, 2002; Taichman *et al.*, 2002; Burger *et al.*, 2003; Hwang *et al.*, 2003;

Abbreviations used in this paper: ATLL, adult T-cell leukaemia/lymphoma; CML, chronic myeloid leukaemia; CTCL, cutaneous T-cell lymphoma; ER endoplasmic reticulum; HIF-1alpha, hypoxia inducible factor; MMP matrix metalloprotease; NSCLC non small cell lung carcinoma; SCLC small cell lung carcinoma; VEGF vascular endothelial growth factor; VHL, von Hippel Lindau.

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Zeelenberg *et al.*, 2003). 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 (Muller *et al.*, 2001). Functional CCR7 is also found on gastric carcinoma cells (Mashino *et al.*, 2002) and esophageal carcinoma (Ding Y *et al.*, 2003). Melanoma cells are reported to express CCR7 and CCR10 (Muller *et al.*, 2001) and in another study to co-express CXCR4 and CXCR3 (Robledo *et al.*, 2001). Leukaemic and lymphoma cells express a wider repertoire of chemokine receptors, probably reflecting their haematopoetic origin, adult T cell leukaemia/lymphoma (ATLL) cells frequently express CCR4 (Ishida *et al.*, 2003), cutaneous T cell lymphoma (CTCL) cells express functional CCR3 (Kleinhans *et al.*, 2003) and B cell lymphomas are reported to express CXCR3 and CXCR5 (Jones *et al.*, 2000).

CXCR4 and CXCL12 in normal development

Below we will describe how the repertoire of chemokine receptors on different tumor types may be related to patterns of spread.

It must be noted that CXCR4 is not a tumor specific marker and not all cancers express this receptor. Furthermore, both receptor and ligand, CXCL12 (previously SDF1-α), are widely expressed in normal tissues and play a critical role in fetal development, mobilization of haemopoietic stem cells and trafficking of naïve lymphocytes (Rossi and Zlotnik, 2000)**.** Despite the apparent redundancy within the chemokine system this particular pair are mutually exclusive, CXCR4 is the only receptor for CXCL12 and cannot bind any other ligand. The importance of this interaction is highlighted by the fact that deletion of either *CXCR4* or *CXCL12* is embryonic lethal. Mice lacking either this ligand or receptor do not develop normally after E13. Defects can be found in the haematopoietic system, heart, cerebellum and vasculature (Zou *et al.*, 1998) and (Tachibana *et al.*, 1998). The critical role for CXCR4 in embryogenesis has been studied in zebrafish where formation of the mechanosensory posterior lateral line and migration of primordial germ cells (PGC) is controlled by CXCR4 expressing cells which respond to a CXCL12-like molecule (David *et al.*, 2002) and (Doitsidou *et al.*, 2002). Migration of PGC in fish and murine embryos is also controlled via CXCR4-CXCL12 interactions, deletion or inhibition of CXCR4 or CXCL12 results in reduced migration and ectopic location of PGCs (Ara *et al.*, 2003; Knaut *et al.*, 2003).

Within primary tumors of the ovary (and possibly other primary cancers) only a sub-population of malignant cells express CXCR4 (1 - 20% in the case of ovarian cancer express CXCR4) (Scotton *et al.*, 2001). Furthermore, when non small cell lung cancer cells (NSCLC) are grown in SCID mice only 35% cells in the primary tumor express CXCR4 compared with 99% of cells in metastases (Balkwill, 2004). With the knowledge of the role of CXCR4 in stem cell biology (Youn *et al.*, 2000) and the current interest in cancer stem cells (Singh *et al.*, 2003) these observations raise the intriguing possibility that CXCR4 may be a marker for cancer stem cells. Further investigation is needed to determine whether the CXCR4 expressing sub-population within a primary tumor are those that migrate and survive to form metastatic deposits.

Chemokine expression in the tumor microenvironment

Within most cancers there is an extensive network of chemokines and chemokine receptors (Vicari and Caux, 2002) (Balkwill and Mantovani, 2001), often tumor production of chemokines is disregulated and receptor expression and signalling may be abnormal (Dhawan and Richmond, 2002; Skinnider *et al.*, 2002). 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 (Zhang *et al.*, 2003), 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 (Balkwill and Mantovani, 2001). CCL2 stimulation of monocytes promoted tumor formation of melanoma cells (Nesbit *et al.*, 2001). $CD4^+$ T cells were reported to enhance invasion and disease progression in an experimental model of skin carcinogenesis (Daniel *et al.*, 2003).

The composition of the leukocyte infiltrate in many carcinomas is related, in particular, to tumor and stromal cell production of CC chemokines (Balkwill and Mantovani, 2001). There are limited 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 (TARC), CCL11 (eotaxin), CCL22 (MDC) that attract Th2 lymphocytes and the Th1-attracting chemokines CXCL10 (IP-10), CXCL9 (Mig-1), CCL2 (MCP-1), CCL3 (MIP-1α), CCL5 (RANTES) and CXCL1 (GROα) (Skinnider and Mak, 2002). 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 (Negus *et al.*, 1995; Negus *et al.*, 1997). CCL3, CCL4 (MIP-1β) 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 (Negus *et al.*, 1997). In ovarian cancer ascites, mRNA and pico to nanomolar levels of protein for CCL2, CCL3, CCL4, CCL5, CCL8 (MCP-2) and CCL22 were detected. Variable numbers of macrophages and $CD3^+$ T lymphocytes (predominantly $CD4^+$) were present in ascites and a direct correlation between CCL5 concentration in ascitic fluid and infiltrating $CD3^+$ T cells was reported (Milliken *et al.*, 2002). 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 (Luboshits *et al.*, 1999; Saji *et al.*, 2001). CCL5 levels correlated with breast cancer progression whereas benign breast disease had minimal chemokine expression (Azenshtein *et al.*, 2002). In esophageal squamous cell carcinomas CCL2 expression was significantly associated with the extent of macrophage infiltration, tumor cell invasion and tumor vascularity (Ohta, *et al.*, 2002). 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 (Lin *et al.*, 2001).

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 (Monti *et al.*, 2003). Likewise, CCL5 expression by tumor cells in NSCLC patients was associated with an 'active lymphocyte response' and was a positive predictor of survival (Moran *et al.*, 2002).

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 (Fig. 1A). In the following section we will discuss whether similar chemokine-receptor networks can control tumor cell movement out of a cancerous tissue.

Chemokine gradients and the spread of malignant cells

Chemokines control the directional migration of leukocytes and it seems that mechanisms utilised for leukocyte trafficking may also used by tumor cells (Fig. 1B). We described in section 3, how certain cancer cells can have restricted and specific expression of chemokine receptors, in particular CXCR4 and CCR7 and this may be one factor in the development of site specific metastasis. For chemokine receptor expression by a cancer cell to be advantageous a chemokine gradient is required/needs to be established and in breast, prostate and ovarian cancer, neuroblastoma, melanoma and some forms of leukaemia, the respective ligand is strongly expressed at sites of tumor spread. Melanoma cells express functional CCR7 and CCR10 and high expression of ligands for these receptors are reported at the two major sites of metastasis, skin and lymph nodes (Muller *et al.*, 2001). CCR7 and CXCR4 are found on human breast cancer cells and the ligand for CCR7, CCL21, (SLC) is highly expressed in lymph nodes and strong expression of the CXCL12 was reported in the target organs for breast cancer metastases (Muller *et al.*, 2001). Moreover, breast cancer cells migrated towards tissue extracts from these target organs and chemotaxis could be partially abrogated by neutralising antibodies to CXCR4 (Muller *et al.*, 2001).

Data from 600 prostate cancer patients revealed that CXCR4 protein expression was significantly elevated in localised and metastatic prostate cancer compared to normal or benign prostate tissue and CXCL12 protein levels were higher in metastatic, compared to normal, prostate tissue (Sun *et al.*, 2003). Expression of CCR7 by gastric cancer cell lines results in directed migration *in vitro* (Mashino *et al.*, 2002). Studies using clinical biopsies suggested that the most important factor determining lymph node metastasis in gastric cancer was CCR7 expression in the primary tumor as there was a significant difference in both lymph node metastasis and lymphatic invasion between CCR7 positive and negative cases. CCR7 expression was also reported in chronic lymphocytic leukaemia (CLL) and the specific ligands, CCL21 and CCL19, were located in high endothelial venules (Till *et al.*, 2002). Two chemokine receptors, CCR3 and CCR4 have been implicated in skin homing of lymphoma cells. In CTCL, CCR3 and CCL11 were detected in tumor cells in skin lesions (Kleinhans *et al.*, 2003). Whereas expression of CCL17 by cutaneous endothelia results in accumulation of CCR4 expressing ATLL cells in the skin (Ishida *et al.*, 2003). CCR4 was significantly associated with skin involvement in this disease and was an unfavourable prognostic factor (Ishida *et al.*, 2003).

Experimental murine cancer models provide some experimental proof that cancer cells may harness chemokine-directed trafficking mechanisms to home to specific sites. When the chemokine receptor CCR7 was introduced into B16 melanoma cells metastasis to lymph nodes was increased (Wiley *et al.*, 2001). However, when the same cells were transfected CXCR4 spread to the lymph nodes was rare but B16-CXCR4 had a marked increase in the ability to form lung metastases (Murakami *et al.*, 2002). In most *in vitro* studies with CXCR4 expressing cancer cell lines, activation of the receptor with CXCL12 stimulates specific migration of cancer cells or invasion through matrigel or through monolayers of endothelial cells, fibroblasts or bone marrow stromal cells (Koshiba *et al.*, 2000; Geminder *et al.*, 2001; Scotton *et al.*, 2001; Scotton *et al.*, 2002; Hwang *et al.*, 2003). However, we feel that this may be an oversimplification, that tumor cell expression of CXCR4 is not simply a migrational cue.

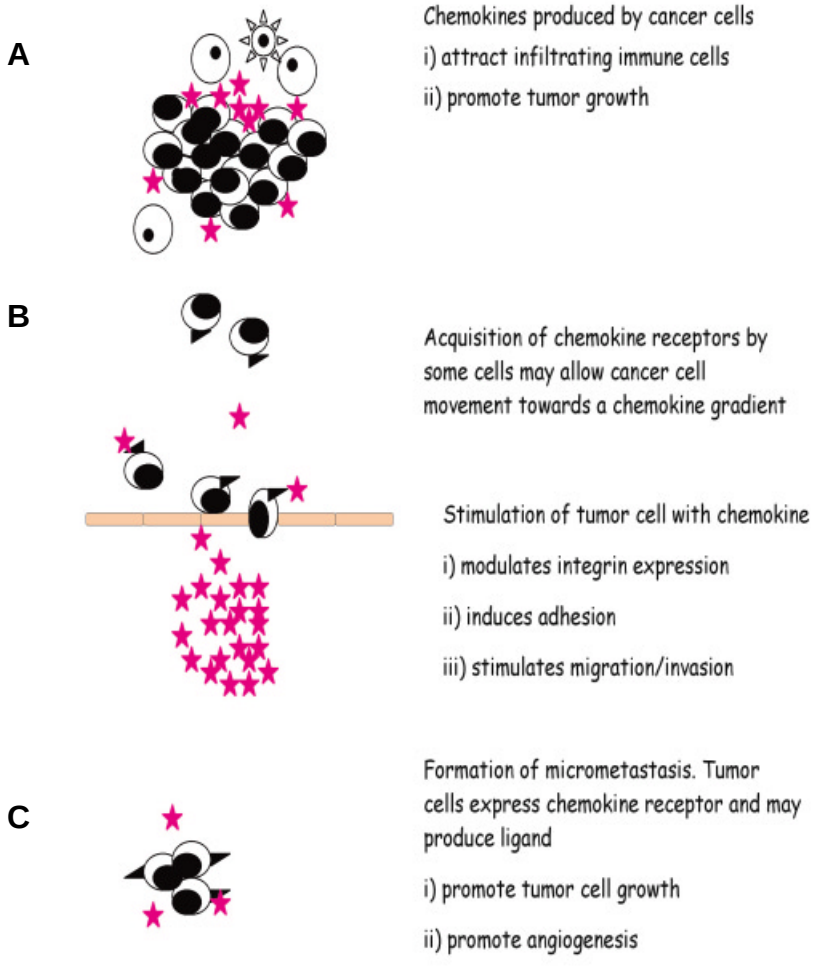

Fig. 1. Role of chemokines in tumor progression/invasion/outgrowth. (A) *Chemokines (stars) produced by tumor cells within a solid tumor, attract a leukocyte infiltrate and/or promote proliferation of cancer cells.* **(B)** *Cancer cells which acquire expression of chemokine receptors may migrate towards a chemokine gradient. Chemokine stimulation of receptors may also alter the adhesive capacity of tumor cells.* **(C)** *Within a metastatic deposit, chemokine production may stimulate tumor growth, but also effect the microenvironment by promoting angiogenesis.*

Many studies have not examined the expression of CXCL12 in malignant tissue, although it is found in primary tumor sites in lymphoma, glioma, ovarian and pancreatic cancer, but, in other cancers, such as breast, thyroid, neuroblastoma and certain haematological cancers, CXCL12 is found at sites of metastasis (Balkwill, 2004). 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 tumor. A similar contradiction is apparent *in vitro*, CXCR4 expressing breast and ovarian cancer cell lines do not express CXCL12 although glioma, prostate and pancreatic cancer cell lines do express this ligand. Indeed, our work with ovarian cancer demonstrated that nanogram quantities of CXCL12 were present in ascitic fluid and CXCR4 was expressed more strongly expressed by tumor cells in ascites. Our initial conclusion was that this chemokine receptor/ligand interaction could be involved in peritoneal spread of this cancer (Scotton *et al.*, 2001). However, we then found that CXCL12, was also strongly expressed by tumor cells in the solid ovarian cancer biopsies (Scotton *et al.*, 2002) and had many more functions beside stimulating migration. Thus far, the significance of high levels of tumor-derived CXCL12 at the site of the primary lesion is not fully understood. However, the process of metastasis is complex multi-step process and there are several stages at which the interaction between tumor cell chemokine receptors and their ligands could be important. We will discuss evidence to suggest that, in addition to tumor cell movement to a gradient, chemokines play a role in tumor cell adhesion, invasion, survival, growth and angiogenesis.

Intracellular signaling pathways activated by chemokine receptors on mailgnant cells

All chemokine receptors are seven transmembrane domain receptors; signal transduction through these leads to activation of G proteins and phospholipase C and the elevation of cytosolic free calcium (Thelen, 2001). 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 signaling pathways (Tilton *et al.*, 2000). Signaling via CXCR4 also enhances tyrosine phosphorylation, association of components of focal adhesion complexes such as paxillin and NF-kB activity in nuclear extracts (Ganju *et al.*, 1998).

There is one interesting report of cross talk between the BCR/ABL oncogenic tyrosine kinase and CXCR4 signalling (Ptasznik *et al.*, 2002). In CML, BCR/ABL kinase phosphorylates, activates and disregulates 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.

In a metastatic breast cancer cell line, NF-κB directly regulates the CXCR4 promoter and can upregulate expression of CXCR4, facilitating increased responses to CXCL12 (Helbig *et al.*, 2003). Also in breast cancer lines, CXCL12 also induces phosphorylation of the molecules FAK, Pyk2, the cytoskeletal proteins paxillin and Crk, the tyrosine phosphatase SHP2 and the adaptor protein Cbl (Fernandis *et al.*, 2004).

Chemokines, integrins, adhesion and invasion

Signalling via chemokine receptors can modulate tumor cell expression of integrins which can then facilitate adhesion of cancer cells to and/or invasion through the extracellular matrix (Fig. 1B). Work in our laboratory, has demonstrated that CXCL12 stimulation of different ovarian cancer cell lines upregulates the expression of β1 integrin (Scotton *et al.*, 2001) and this integrin modulation correlates with increased tumor cell adhesion to fibronectin (Kulbe *et al.,* manuscript in preparation). Ovarian cancer cell lines invade through matrigel to CXCL12 and this is abrogated by the broad spectrum matrix metalloprotease (MMP) inhibitor marimistat (Scotton *et al.*, 2002). β1 integrins have also been reported to regulate both the formation of and adhesion within, ovarian cancer spheroids (Casey and Skubitz, 2000). In small cell lung cancer cells (SCLC) CXCL12 stimulation induced firm adhesion to marrow stromal cells via activation of α4β1 integrin and also induced SCLC cell invasion into the extracellular matrix (Burger *et al.*, 2003). Overexpression of the signalling molecule Akt2 in both breast and ovarian cancer cell lines resulted in cell lines which exhibited increased adhesion to and invasion through collagen IV due to upregulation of β1 integrins and these cell lines were also more metastatic than control lines *in vivo* (Arboleda *et al.*, 2003). In lymphocytes, the chemokines CXCL12 and CCL21 activate adhesion, mediated by LFA-1 and VLA-4, and also transendothelial migration where the small GTPase, RAP1, serves to increase the adhesive capacity of these adhesion molecules (Shimonaka *et al.*, 2003). In both normal and transformed lymphocytes CXCL12 augments α4β7 mediated adhesion via activation of the small GTPase RhoA (Wright *et al.*, 2002). Adhesion mechanisms can also impact on chemokine receptor expression, e.g. in non-transformed lymphocytes, activation of L-selectin (by antibody cross-linking or specific ligands) mobilises intracellular stores of CXCR4 to increase cell surface expression (Ding Z *et al.*, 2003). It would be interesting to also investigate these interactions of chemokines and adhesion on tumor cells under flow conditions.

In the B16 melanoma experimental metastasis model, CXCR4 transfected cells show enhanced adhesion to dermal and pulmonary microvascular endothelial cells (Murakami *et al.*, 2002). Under flow conditions, these transfected cell lines showed no evidence of rolling before arrest and adhesion was dependent on β1 integrin expression and adhesion of transfectants to endothelial cells both *in vitro* and *in vivo* was inhibited by anti-β1 antibodies (Cardones *et al.*, 2003). Unpublished data from our laboratory suggest that transfection of ovarian cancer cells with CXCR4 results in a concomitant upregulation of various α and β integrin subunits including αv (Kulbe *et al.*, manuscript in preparation). Data from the study of metastatic breast cancer cells suggest that the expression of αvβ3 integrin strongly promotes breast cancer metastasis via a mechanism where αvβ3 cooperates with MMP-9 to facilitate migration (Rolli *et al.*, 2003).

Growth / survival of tumor cells by chemokines

Deregulated chemokine production by tumors may contribute directly to transformation of tumor cells by acting as growth and

survival factors, in an autocrine and/or paracrine manner (Fig. 1C). Melanoma cells express elevated levels of the CXCR2 receptor and also constitutively produce CXCL1 and CXCL8 (IL-8) allowing autocrine stimulation by these chemokines which enhances survival, proliferation and tumor cell migration (Schadendorf *et al.*, 1993; Luan *et al.*, 1997; Dhawan and Richmond, 2002). The chemokines CXCL1 and CXCL8 also stimulate growth of pancreatic tumor cell lines (Takamori *et al.*, 2000). Pancreatic cancer samples also express CCR6 and proliferate in response to the CC chemokine CCL20 (MIP-3α) (Kleeff *et al.*, 1999).

In many cancers interactions between CXCL12 and CXCR4 stimulate proliferation. Examples include, prostate cancer cells (Sun *et al.*, 2003), glioblastoma cells (Zhou *et al.*, 2002; Barbero *et al.*, 2003) and malignant plasma cells of multiple myeloma (Hideshima *et al.*, 2002).

In other cancers, CXCL12 can also stimulate cancer cell proliferation or survival under suboptimal conditions e.g. epithelial ovarian cancer where CXCL12 stimulation enhances tumor cell proliferation in low serum conditions (Scotton *et al.*, 2002). The CXCR4-CXCL12 interaction and downstream signalling, often partially regulated by phosphorylation of Akt, may promote growth/survival of tumor cells to allow cells to grow in distant and less favourable sites (Kikima *et al.*, 2002; Schrader *et al.*, 2002; Zhou *et al.*, 2002; Barbero *et al.*, 2003; Sun *et al.*, 2003).

Role of chemokines in malignant progression

Chemokines may also regulate angiogenesis within both the primary tumor and metastatic deposits (Fig. 1C). CXC chemokines containing the 3 amino acid (ELR) motif glutamine-leucine-arginine, such as CXCL8, CXCL1, CXCL5 (ENA-78), CXCL6 (GCP-2) and CXCL7 (NAP-2), promote angiogenesis whereas those lacking this motif, such as CXCL9 and CXCL10 (IP-10), are often anti-angiogenic (Belperio *et al.*, 2000). Examples include, levels of CXCL10 in human lung cancers which were inversely related to tumor progression (Belperio *et al.*, 2000) and elevated levels of CXCL5 which were found in NSCLC and correlated with the vascularity of the tumors and angiogenesis (Arenberg *et al.*, 1998).

An elegant study demonstrated that CXCR4 surface expression could be prevented by transfecting cell lines with a CXCL12 construct containing an endoplasmic reticulum (ER) retention sequence, this binds CXCR4 in the ER retains it there (Zeelenberg *et al.*, 2001). When T cell hybridoma cells were transfected with this construct and injected i.v. there was a reduction in the number of metastases to distant organs (Zeelenberg *et al.*, 2001). Transfection of colorectal tumor cells with this construct again resulted in reduced metastasis to lungs and liver. However, closer investigation revealed that these cells colonised the lungs to a similar extent as the parental cells but these micrometastases, although they survive, fail to proliferate (Zeelenberg *et al.*, 2003). Unpublished work from our laboratory has also shown a role for CXCR4 in malignant progression, over expression of CXCR4 in ovarian cancer cells leads to increased migration, invasion, adhesion and survival in response to CXCL12 *in vitro*. When grown intraperitoneally in nude mice, the CXCR4 transfected cells are able to establish extensive metastases outside the peritoneum unlike parental cells (Kulbe *et al.,* manuscript in preparation). Furthermore, when subpopulations of breast cancer cells with enhanced metastatic abilities were isolated by *in vivo* selection, gene expression profiles detected four highly over expressed genes: CXCR4, IL-11, osteopontin and connective tissue-derived growth factor, CTGF. Transfection studies demonstrated that 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 (Kang *et al.*, 2003).

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 (Scotton *et al.*, 2002). Production of TNF-α in this disease has been implicated in tumor/stromal communication and tumor progression (Szlosarek and Balkwill, 2003). There are also links between CXCR4 and vascular endothelial growth factor (VEGF). In breast cancer cell lines, VEGF was demonstrated to have an autocrine action and induce expression of CXCR4 expression which promoted migration/invasion towards CXCL12 (Bachelder *et al.*, 2002). VEGF and basic fibroblast growth factor also induce expression of CXCR4 on human endothelial cells (Salcedo *et al.*, 1999) which again highlights the reciprocal links between chemokines and angiogenic factors.

Hormones have also been linked with chemokine action which maybe particularly relevant in gynaecological cancers. It was reported that CXCL12 is a target of estrogen action in estrogen receptor-alpha positive human ovarian and breast cancer cell lines (Hall and Korach, 2003). Estradiol treatment induces expression of CXCL12 by estrogen receptor+ cell lines and promotes cell proliferation, proliferation can be blocked with either addition of anti-CXCL12 antibody or ER antagonists demonstrating another chemokine-mediated paracrine pathway to promote cancer cell proliferation. Regions of hypoxia are common in solid tumours due to the chaotic and intermittent blood supply and the high metabolic rate of tumour cells (Vaupel *et al.*, 1998). Two recent studies have demonstrated an interaction between hypoxia and CXCR4 expression. (Schioppa *et al.,* 2003) demonstrated that low levels of oxygen induce high expression of CXCR4 on monocytes, macrophages, endothelial cells and tumor cells. Hypoxia inducible factor-1α (Hif-1α) activation is involved in CXCR4 upregulation, as is an increase in the stability of CXCR4 mRNA (Schioppa *et al.*, 2003). Under normoxic conditions the von Hippel-Lindau tumor suppresser protein (pVHL) negatively regulates CXCR4 expression as it targets HIF-1α for degradation, however under hypoxic conditions this program is suppressed which results in HIF-1α-dependent CXCR4 upregulation. These results suggest the first mechanism of how CXCR4 becomes expressed by tumor cells and also how it could confer a survival advantage to the cells that acquire such expression (Staller *et al.*, 2003). A study of mutations of the VHL gene in renal cell carcinoma revealed that mutations are associated with high CXCR4 expression and poor patient prognosis (Staller *et al.*, 2003).

Concluding remarks

Recent studies have demonstrated that chemokine-receptor interactions, in particular those relating to CXCR4-CXCL12, can impact at any stage of tumor biology from tumor development to

stimulation of cell movement, adhesion, invasion, proliferation and malignant progression.

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References

ARA, T., NAKAMURA, Y., EGAWA, T., SUGIYAMA, T., ABE, K., KISHIMOTO, T., MATSUI, Y. and NAGASAWA, T., (2003). Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). *Proc Natl Acad Sci U S A*, 100: 5319-5323.

ARBOLEDA, M. J., LYONS, J. F., KABBINAVAR, F. F., BRAY, M. R., SNOW, B. E., AYALA, R., DANINO, M., KARLAN, B. Y. and SLAMON, D. J., (2003). Overexpression of AKT2/protein kinase Bbeta leads to up-regulation of beta1 integrins, increased invasion and metastasis of human breast and ovarian cancer cells. *Cancer Res*, 63: 196-206.

ARENBERG, D. A., KEANE, M. P., DIGIOVINE, B., KUNKEL, S. L., MORRIS, S. B., XUE, Y. Y., BURDICK, M. D., GLASS, M. C., IANNETTONI, M. D. and STRIETER, R. M., (1998). Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. *J Clin Invest*, 102: 465-472.

AZENSHTEIN, E., LUBOSHITS, G., SHINA, S., NEUMARK, E., SHAHBAZIAN, D., WEIL, M., WIGLER, N., KEYDAR, I. and BEN-BARUCH, A., (2002). The CC chemokine RANTES in breast carcinoma progression: regulation of expression and potential mechanisms of promalignant activity. *Cancer Res*, 62: 1093-1102.

BACHELDER, R. E., WENDT, M. A. and MERCURIO, A. M., (2002). Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. *Cancer Res*, 62: 7203-7206.

BALKWILL, F., (2004). The significance of cancer cell expression of the chemokine receptor CXCR4. *Nature Cancer Reviews*, in press.

BALKWILL, F. and MANTOVANI, A., (2001). Inflammation and cancer: back to Virchow? *Lancet*, 357: 539-545.

BARBERO, S., BONAVIA, R., BAJETTO, A., PORCILE, C., PIRANI, P., RAVETTI, J. L., ZONA, G. L., SPAZIANTE, R., FLORIO, T. and SCHETTINI, G., (2003). Stromal cell-derived factor 1alpha stimulates human glioblastoma cell growth through the activation of both extracellular signal-regulated kinases 1/2 and Akt. *Cancer Res*, 63: 1969-1974.

BELPERIO, J. A., KEANE, M. P., ARENBERG, D. A., ADDISON, C. L., EHLERT, J. E., BURDICK, M. D. and STRIETER, R. M., (2000). CXC chemokines in angiogenesis. *J Leukoc Biol*, 68: 1-8.

BURGER, M., GLODEK, A., HARTMANN, T., SCHMITT-GRAFF, A., SILBERSTEIN, L. E., FUJII, N., KIPPS, T. J. and BURGER, J. A., (2003). Functional expression of CXCR4 (CD184) on small-cell lung cancer cells mediates migration, integrin activation and adhesion to stromal cells. *Oncogene*, 22: 8093-8101.

CARDONES, A. R., MURAKAMI, T. and HWANG, S. T., (2003). CXCR4 enhances adhesion of B16 tumor cells to endothelial cells *in vitro* and *in vivo* via beta(1) integrin. *Cancer Res*, 63: 6751-6757.

CASEY, R. C. and SKUBITZ, A. P., (2000). CD44 and beta1 integrins mediate ovarian carcinoma cell migration toward extracellular matrix proteins. *Clin Exp Metastasis*, 18: 67-75.

DANIEL, D., MEYER-MORSE, N., BERGSLAND, E. K., DEHNE, K., COUSSENS, L. M. and HANAHAN, D., (2003). Immune enhancement of skin carcinogenesis by CD4+ T cells. *J Exp Med*, 197: 1017-1028.

DAVID, N. B., SAPEDE, D., SAINT-ETIENNE, L., THISSE, C., THISSE, B., DAMBLY-CHAUDIERE, C., ROSA, F. M. and GHYSEN, A., (2002). Molecular basis of cell migration in the fish lateral line: role of the chemokine receptor CXCR4 and of its ligand, SDF1. *Proc Natl Acad Sci USA*, 99: 16297-16302.

DHAWAN, P. and RICHMOND, A., (2002). Role of CXCL1 in tumorigenesis of melanoma. *J Leukoc Biol*, 72: 9-18.

DING, Y., SHIMADA, Y., MAEDA, M., KAWABE, A., KAGANOI, J., KOMOTO, I., HASHIMOTO, Y., MIYAKE, M., HASHIDA, H. and IMAMURA, M., (2003). Association of CC chemokine receptor 7 with lymph node metastasis of esophageal squamous cell carcinoma. *Clin Cancer Res*, 9: 3406-3412.

DING, Z., ISSEKUTZ, T. B., DOWNEY, G. P. and WADDELL, T. K., (2003). L-selectin stimulation enhances functional expression of surface CXCR4 in lymphocytes: implications for cellular activation during adhesion and migration. *Blood* 101: 4245-4252.

DOITSIDOU, M., REICHMAN-FRIED, M., STEBLER, J., KOPRUNNER, M., DORRIES, J., MEYER, D., ESGUERRA, C. V., LEUNG, T. and RAZ, E., (2002). Guidance of primordial germ cell migration by the chemokine SDF-1. *Cell*, 111: 647-659.

EBNET, K. and VESTWEBER, D., (1999). Molecular mechanisms that control leukocyte extravasation: the selectins and the chemokines. *Histochem Cell Biol*, 112: 1-23.

FERNANDIS, A. Z., PRASAD, A., BAND, H., KLOSEL, R. and GANJU, R. K., (2004). Regulation of CXCR4-mediated chemotaxis and chemoinvasion of breast cancer cells. *Oncogene*, 23: 157-167.

GANJU, R. K., BRUBAKER, S. A., MEYER, J., DUTT, P., YANG, Y., QIN, S., NEWMAN, W. and GROOPMAN, J. E., (1998). 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*, 273: 23169-23175.

GEMINDER, H., SAGI-ASSIF, O., GOLDBERG, L., MESHEL, T., RECHAVI, G., WITZ, I. P. and BEN-BARUCH, A., (2001). 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*, 167: 4747-4757.

HALL, J. M. and KORACH, K. S., (2003). 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*, 17: 792-803.

HELBIG, G., CHRISTOPHERSON, K. W., 2ND, BHAT-NAKSHATRI, P., KUMAR, S., KISHIMOTO, H., MILLER, K. D., BROXMEYER, H. E. and NAKSHATRI, H., (2003). NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. *J Biol Chem*, 278: 21631-21638.

HIDESHIMA, T., CHAUHAN, D., HAYASHI, T., PODAR, K., AKIYAMA, M., GUPTA, D., RICHARDSON, P., MUNSHI, N. and ANDERSON, K. C., (2002). The biological sequelae of stromal cell-derived factor-1alpha in multiple myeloma. *Mol Cancer Ther*, 1: 539-544.

HWANG, J. H., CHUNG, H. K., KIM, D. W., HWANG, E. S., SUH, J. M., KIM, H., YOU, K. H., KWON, O. Y., RO, H. K., JO, D. Y. and SHONG, M., (2003). CXC chemokine receptor 4 expression and function in human anaplastic thyroid cancer cells. *J Clin Endocrinol Metab*, 88: 408-416.

ISHIDA, T., UTSUNOMIYA, A., IIDA, S., INAGAKI, H., TAKATSUKA, Y., KUSUMOTO, S., TAKEUCHI, G., SHIMIZU, S., ITO, M., KOMATSU, H., WAKITA, A., EIMOTO, T., MATSUSHIMA, K. and UEDA, R., (2003). Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. *Clin Cancer Res*, 9: 3625-3634.

JONES, D., BENJAMIN, R. J., SHAHSAFAEI, A. and DORFMAN, D. M., (2000). The chemokine receptor CXCR3 is expressed in a subset of B-cell lymphomas and is a marker of B-cell chronic lymphocytic leukemia. *Blood* 95: 627-632.

KANG, Y., SIEGEL, P. M., SHU, W., DROBNJAK, M., KAKONEN, S. M., CORDON-CARDO, C., GUISE, T. A. and MASSAGUE, J., (2003). A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell*, 3: 537-549.

KIJIMA, T., MAULIK, G., MA, P. C., TIBALDI, E. V., TURNER, R. E., ROLLINS, B., SATTLER, M., JOHNSON, B. E. and SALGIA, R., (2002). Regulation of cellular proliferation, cytoskeletal function and signal transduction through CXCR4 and c-Kit in small cell lung cancer cells. *Cancer Res*, 62: 6304-6311.

KLEEFF, J., KUSAMA, T., ROSSI, D. L., ISHIWATA, T., MARUYAMA, H., FRIESS, H., BUCHLER, M. W., ZLOTNIK, A. and KORC, M., (1999). Detection and localization of Mip-3alpha/LARC/Exodus, a macrophage proinflammatory chemokine and its CCR6 receptor in human pancreatic cancer. *Int J Cancer*, 81: 650-657.

KLEINHANS, M., TUN-KYI, A., GILLIET, M., KADIN, M. E., DUMMER, R., BURG, G. and NESTLE, F. O., (2003). Functional expression of the eotaxin receptor CCR3 in CD30+ cutaneous T-cell lymphoma. *Blood* 101: 1487-1493.

KNAUT, H., WERZ, C., GEISLER, R. and NUSSLEIN-VOLHARD, C., (2003). A zebrafish homologue of the chemokine receptor Cxcr4 is a germ-cell guidance receptor. *Nature*, 421: 279-282.

KOSHIBA, T., HOSOTANI, R., MIYAMOTO, Y., IDA, J., TSUJI, S., NAKAJIMA, S., KAWAGUCHI, M., KOBAYASHI, H., DOI, R., HORI, T., FUJII, N. and IMAMURA, M., (2000). Expression of stromal cell-derived factor 1 and CXCR4 ligand receptor system in pancreatic cancer: a possible role for tumor progression. *Clin Cancer Res*, 6: 3530-3535.

LIN, E. Y., NGUYEN, A. V., RUSSELL, R. G. and POLLARD, J. W., (2001). Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med*, 193: 727-740.

LUAN, J., SHATTUCK-BRANDT, R., HAGHNEGAHDAR, H., OWEN, J. D., STRIETER, R., BURDICK, M., NIRODI, C., BEAUCHAMP, D., JOHNSON, K. N. and RICHMOND, A., (1997). Mechanism and biological significance of constitutive expression of MGSA/GRO chemokines in malignant melanoma tumor progression. *J Leukoc Biol*, 62: 588-597.

LUBOSHITS, G., SHINA, S., KAPLAN, O., ENGELBERG, S., NASS, D., LIFSHITZ-MERCER, B., CHAITCHIK, S., KEYDAR, I. and BEN-BARUCH, A., (1999). Elevated expression of the CC chemokine regulated on activation, normal T cell expressed and secreted (RANTES) in advanced breast carcinoma. *Cancer Res*, 59: 4681-4687.

MACKAY, C. R., (2001). Chemokines: immunology's high impact factors. *Nat Immunol*, 2: 95-101.

MASHINO, K., SADANAGA, N., YAMAGUCHI, H., TANAKA, F., OHTA, M., SHIBUTA, K., INOUE, H. and MORI, M., (2002). Expression of chemokine receptor CCR7 is associated with lymph node metastasis of gastric carcinoma. *Cancer Res*, 62: 2937-2941.

MILLIKEN, D., SCOTTON, C., RAJU, S., BALKWILL, F. and WILSON, J., (2002). Analysis of chemokines and chemokine receptor expression in ovarian cancer ascites. *Clin Cancer Res*, 8: 1108-1114.

MONTI, P., LEONE, B. E., MARCHESI, F., BALZANO, G., ZERBI, A., SCALTRINI, F., PASQUALI, C., CALORI, G., PESSI, F., SPERTI, C., DI CARLO, V., ALLAVENA, P. and PIEMONTI, L., (2003). The CC chemokine MCP-1/CCL2 in pancreatic cancer progression: regulation of expression and potential mechanisms of antimalignant activity. *Cancer Res*, 63: 7451-7461.

MORAN, C. J., ARENBERG, D. A., HUANG, C. C., GIORDANO, T. J., THOMAS, D. G., MISEK, D. E., CHEN, G., IANNETTONI, M. D., ORRINGER, M. B., HANASH, S. and BEER, D. G., (2002). RANTES expression is a predictor of survival in stage I lung adenocarcinoma. *Clin Cancer Res*, 8: 3803-3812.

MULLER, A., HOMEY, B., SOTO, H., GE, N., CATRON, D., BUCHANAN, M. E., MCCLANAHAN, T., MURPHY, E., YUAN, W., WAGNER, S. N., BARRERA, J. L., MOHAR, A., VERASTEGUI, E. and ZLOTNIK, A., (2001). Involvement of chemokine receptors in breast cancer metastasis. *Nature*, 410: 50-56.

MURAKAMI, T., MAKI, W., CARDONES, A. R., FANG, H., TUN KYI, A., NESTLE, F. O. and HWANG, S. T., (2002). Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. *Cancer Res*, 62: 7328-7334.

NEGUS, R. P., STAMP, G. W., HADLEY, J. and BALKWILL, F. R., (1997). Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. *Am J Pathol*, 150: 1723-1734.

NEGUS, R. P., STAMP, G. W., RELF, M. G., BURKE, F., MALIK, S. T., BERNASCONI, S., ALLAVENA, P., SOZZANI, S., MANTOVANI, A. and BALKWILL, F. R., (1995). The detection and localization of monocyte chemoattractant protein-1 (MCP-1) in human ovarian cancer. *J. Clin. Invest.* 95: 2391-2396.

NESBIT, M., SCHAIDER, H., MILLER, T. H. and HERLYN, M., (2001). Low-level monocyte chemoattractant protein-1 stimulation of monocytes leads to tumor formation in nontumorigenic melanoma cells. *J Immunol*, 166: 6483-6490.

OHTA, M., KITADAI, Y., TANAKA, S., YOSHIHARA, M., YASUI, W., MUKAIDA, N., HARUMA, K. and CHAYAMA, K., (2002). Monocyte chemoattractant protein-1 expression correlates with macrophage infiltration and tumor vascularity in human esophageal squamous cell carcinomas. *Int J Cancer*, 102: 220-224.

PTASZNIK, A., URBANOWSKA, E., CHINTA, S., COSTA, M. A., KATZ, B. A., STANISLAUS, M. A., DEMIR, G., LINNEKIN, D., PAN, Z. K. and GEWIRTZ, A. M., (2002). Crosstalk between BCR/ABL oncoprotein and CXCR4 signaling through a Src family kinase in human leukemia cells. *J Exp Med*, 196: 667-678.

REMPEL, S. A., DUDAS, S., GE, S. and GUTIERREZ, J. A., (2000). 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*, 6: 102-111.

ROBLEDO, M. M., BARTOLOME, R. A., LONGO, N., RODRIGUEZ-FRADE, J. M., MELLADO, M., LONGO, I., VAN MUIJEN, G. N., SANCHEZ-MATEOS, P. and TEIXIDO, J., (2001). Expression of functional chemokine receptors CXCR3 and CXCR4 on human melanoma cells. *J Biol Chem*, 24: 24.

ROLLI, M., FRANSVEA, E., PILCH, J., SAVEN, A. and FELDING-HABERMANN, B., (2003). Activated integrin alphavbeta3 cooperates with metalloproteinase MMP-9 in regulating migration of metastatic breast cancer cells. *Proc Natl Acad Sci USA*, 100: 9482-9487.

ROSSI, D. and ZLOTNIK, A., (2000). The biology of chemokines and their receptors. *Annu Rev Immunol*, 18: 217-242.

SAJI, H., KOIKE, M., YAMORI, T., SAJI, S., SEIKI, M., MATSUSHIMA, K. and TOI, M., (2001). Significant correlation of monocyte chemoattractant protein-1 expression with neovascularization and progression of breast carcinoma. *Cancer*, 92: 1085-1091.

SALCEDO, R., WASSERMAN, K., YOUNG, H. A., GRIMM, M. C., HOWARD, O. M., ANVER, M. R., KLEINMAN, H. K., MURPHY, W. J. and OPPENHEIM, J. J., (1999). 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-1alpha. *Am J Pathol*, 154: 1125-1135.

SCHADENDORF, D., MOLLER, A., ALGERMISSEN, B., WORM, M., STICHERLING, M. and CZARNETZKI, B. M., (1993). IL-8 produced by human malignant melanoma cells *in vitro* is an essential autocrine growth factor. *J Immunol*, 151: 2667-2675.

SCHIOPPA, T., URANCHIMEG, B., SACCANI, A., BISWAS, S. K., DONI, A., RAPISARDA, A., BERNASCONI, S., SACCANI, S., NEBULONI, M., VAGO, L., MANTOVANI, A., MELILLO, G. and SICA, A., (2003). Regulation of the chemokine receptor CXCR4 by hypoxia. *J Exp Med*, 198: 1391-1402.

SCHRADER, A. J., LECHNER, O., TEMPLIN, M., DITTMAR, K. E., MACHTENS, S., MENGEL, M., PROBST-KEPPER, M., FRANZKE, A., WOLLENSAK, T., GATZLAFF, P., ATZPODIEN, J., BUER, J. and LAUBER, J., (2002). CXCR4/CXCL12 expression and signalling in kidney cancer. *Br J Cancer*, 86: 1250-1256.

SCOTTON, C. J., WILSON, J. L., MILLIKEN, D., STAMP, G. and BALKWILL, F. R., (2001). Epithelial cancer cell migration: a role for chemokine receptors? *Cancer Res*, 61: 4961-4965.

SCOTTON, C. J., WILSON, J. L., SCOTT, K., STAMP, G., WILBANKS, G. D., FRICKER, S., BRIDGER, G. and BALKWILL, F. R., (2002). Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res*, 62: 5930-5938.

SHIMONAKA, M., KATAGIRI, K., NAKAYAMA, T., FUJITA, N., TSURUO, T., YOSHIE, O. and KINASHI, T., (2003). Rap1 translates chemokine signals to integrin activation, cell polarization and motility across vascular endothelium under flow. *J Cell Biol*, 161: 417-427.

SINGH, S. K., CLARKE, I. D., TERASAKI, M., BONN, V. E., HAWKINS, C., SQUIRE, J. and DIRKS, P. B., (2003). Identification of a cancer stem cell in human brain tumors. *Cancer Res*, 63: 5821-5828.

SKINNIDER, B. F., ELIA, A. J., GASCOYNE, R. D., PATTERSON, B., TRUMPER, L., KAPP, U. and MAK, T. W., (2002). Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood* 99: 618-626.

SKINNIDER, B. F. and MAK, T. W., (2002). The role of cytokines in classical Hodgkin lymphoma. *Blood* 99: 4283-4297.

STALLER, P., SULITKOVA, J., LISZTWAN, J., MOCH, H., OAKELEY, E. J. and KREK, W., (2003). Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature*, 425: 307-311.

SUN, Y. X., WANG, J., SHELBURNE, C. E., LOPATIN, D. E., CHINNAIYAN, A. M., RUBIN, M. A., PIENTA, K. J. and TAICHMAN, R. S., (2003). Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) *in vivo*. *J Cell Biochem*, 89: 462-473.

SZLOSAREK, P. W. and BALKWILL, F. R., (2003). Tumour necrosis factor alpha: a potential target for the therapy of solid tumours. *Lancet Oncol*, 4: 565-573.

TACHIBANA, K., HIROTA, S., IIZASA, H., YOSHIDA, H., KAWABATA, K., KATAOKA, Y., KITAMURA, Y., MATSUSHIMA, K., YOSHIDA, N., NISHIKAWA, S., KISHIMOTO, T. and NAGASAWA, T., (1998). The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature*, 393: 591-594.

TAICHMAN, R. S., COOPER, C., KELLER, E. T., PIENTA, K. J., TAICHMAN, N. S. and MCCAULEY, L. K., (2002). Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res*, 62: 1832-1837.

TAKAMORI, H., OADES, Z. G., HOCH, O. C., BURGER, M. and SCHRAUFSTATTER, I. U., (2000). Autocrine growth effect of IL-8 and GROalpha on a human pancreatic cancer cell line, Capan-1. *Pancreas*, 21: 52-56.

THELEN, M., (2001). Dancing to the tune of chemokines. *Nat Immunol*, 2: 129-134.

TILL, K. J., LIN, K., ZUZEL, M. and CAWLEY, J. C., (2002). The chemokine receptor CCR7 and alpha4 integrin are important for migration of chronic lymphocytic leukemia cells into lymph nodes. *Blood* 99: 2977-2984.

TILTON, B., HO, L., OBERLIN, E., LOETSCHER, P., BALEUX, F., CLARK-LEWIS, I. and THELEN, M., (2000). 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*, 192: 313-324.

VAUPEL, P., THEWS, O., KELLEHER, D. K. and HOECKEL, M., (1998). Current status of knowledge and critical issues in tumor oxygenation. Results from 25 years research in tumor pathophysiology. *Adv Exp Med Biol*, 454: 591-602.

VICARI, A. P. and CAUX, C., (2002). Chemokines in cancer. *Cytokine Growth Factor Rev*, 13: 143-154.

WILEY, H. E., GONZALEZ, E. B., MAKI, W., WU MT, M. T. and HWANG, S. T., (2001). Expression of CC Chemokine Receptor-7 and Regional Lymph Node Metastasis of B16 Murine Melanoma. *J Natl Cancer Inst*, 93: 1638-1643.

WRIGHT, N., HIDALGO, A., RODRIGUEZ-FRADE, J. M., SORIANO, S. F., MELLADO, M., PARMO-CABANAS, M., BRISKIN, M. J. and TEIXIDO, J., (2002). The chemokine stromal cell-derived factor-1 alpha modulates alpha 4 beta 7 integrin-mediated lymphocyte adhesion to mucosal addressin cell adhesion molecule-1 and fibronectin. *J Immunol*, 168: 5268-5277.

YOUN, B. S., MANTEL, C. and BROXMEYER, H. E., (2000). Chemokines, chemokine receptors and hematopoiesis. *Immunol Rev*, 177: 150-174.

ZEELENBERG, I. S., RUULS-VAN STALLE, L. and ROOS, E., (2001). Retention of CXCR4 in the endoplasmic reticulum blocks dissemination of a T cell hybridoma. *J Clin Invest*, 108: 269-277.

ZEELENBERG, I. S., RUULS-VAN STALLE, L. and ROOS, E., (2003). The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. *Cancer Res*, 63: 3833-3839.

ZHANG, L., CONEJO-GARCIA, J. R., KATSAROS, D., GIMOTTY, P. A., MASSOBRIO, M., REGNANI, G., MAKRIGIANNAKIS, A., GRAY, H., SCHLIENGER, K., LIEBMAN, M. N., RUBIN, S. C. and COUKOS, G., (2003). Intratumoral T cells, recurrence and survival in epithelial ovarian cancer. *N Engl J Med*, 348: 203-213.

ZHOU, Y., LARSEN, P. H., HAO, C. and YONG, V. W., (2002). CXCR4 is a major chemokine receptor on glioma cells and mediates their survival. *J Biol Chem*, 277: 49481-49487.

ZOU, Y. R., KOTTMANN, A. H., KURODA, M., TANIUCHI, I. and LITTMAN, D. R., (1998). Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. *Nature*, 393: 595-599.