[Frontiers in Bioscience S3, 1569-1582, June 1, 2011]

Chemokines: key players in cancer progression and metastasis

Rajesh Singh[1], James W. Lilladr, Jr. [1], Shailesh Singh[1]

[1]*Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, GA 30310*

TABLE OF CONTENTS

1. ABSTRACT

Instructed cell migration is a fundamental component of various biological systems and is critical to the pathogenesis of many diseases including cancer. Role of chemokines in providing navigational cues to migrating cancer cells bearing specific receptors is well established. However, functional mechanisms of chemokine are not well implicit, which is crucial for designing new therapeutics to control tumor growth and metastasis. Multiple functions and mode of actions have been advocated for chemokines and their receptors in the progression of primary and secondary tumors. In this review, we have discussed current advances in understanding the role of the chemokines and their corresponding receptor in tumor progression and metastasis.

2. INTRODUCTION

Malignancy involves group of diseases that share a characteristic of uncontrolled growth. In most tissues and organs, a balance between cell proliferation and cell death maintains homeostasis. Occasionally, cells neglect the skill to acknowledge the standard growth machineries and clones of cells arise, which can grow to a considerable size producing tumors or neoplasm. Most malignant tumors are able to metastasize, a process of new tumor formation and growth at distant sites, which is responsible for 90% cancer associated deaths (1). At the tumor–host interface, a wide range of host factors including growth factors, adhesion molecules, cytokines and chemo attractants support tumor cell proliferation, survival and migration (2). Recently, members of chemo attractant cytokines, named

chemokines, which were primarily known as key regulators of the immune system, have been implicated in malignant transformation and tumor progression (3-5). Most importantly, a growing body of experimental evidence both *in vitro* and *in vivo* suggests that members of the chemokine family together with their cognate receptors play a key role in the metastatic progression of numerous tumor types (6). Depending on number and spacing between 4 chemokine subfamilies have been defined (Figure 1). The largest group of chemokines has the first 2 cysteines in an adjacent position (CC chemokines). Most of these molecules, products of a large multigenic cluster on chromosome 17q11.2, act on monocytes, whereas other CC chemokines, products of different chromosomal loci, are active on different cell types (Figure 1). Group of chemokines that has the first 2 of 4 total cysteines separated by an intervening amino acid are called CXC chemokines. Two large multigenic clusters code most of these molecules. The first, located on chromosome 4q12-q13, includes CXC chemokines containing an ELR-conserved amino acid sequence on the N-terminus (ELR^+CXC chemokines) that act on neutrophils. The second located on 4q21.21, includes CXC chemokines lacking the ELR sequence (ELR^- CXC chemokines) that act mainly on T lymphocytes (Figure 1).

The chemokines, a super family of low molecular weight chemotactic cytokines are key regulators of leukocyte trafficking and other biological activities such as development, angiogenesis and haematopoiesis. Chemokine mediates many cellular responses (i.e. cytoskeleton rearrangement, cell polarization and integrin dependent adhesion) in leukocytes through activation of G proteins and subsequent downstream effectors kinases, which are essential for cell migration (7, 8), (Figure 2). Nonetheless, it has become evident that they also participate in the activities of other cells types and play crucial role in pathological conditions including cancer (4, 7, 9). The expression of chemokines has been reported in a variety of human tumors and they have been identified as important mediators of tumor progression (3, 4). In the tumor microenvironment, specific chemokines have been shown to be responsible for the recruitment of leukocytes into the tumor sites. However, chemokines may also play a role in the functionality of tumor cells and other cells in tumor microenvironment, which together regulate the process of tumor development and progression (6). Indeed, a number of evidence demonstrates the contribution of chemokines to multiple steps of tumor progression, including tumourigenesis, growth/survival, angiogenesis, invasion and metastasis (5, 10).

3. CHEMOKINES AND THE TUMOR IMMUNE RESPONSE

Tumor microenvironment is composed of tumor cells as well as various types of stromal cells such as fibroblasts and endothelial cells. It is apparent that several types of inflammatory cells including neutrophils, macrophages and lymphocytes are recruited to the tumor and play both positive and negative roles in the cancer progression (10-14). Accumulating data indicate that the infiltration of the inflammatory cells is regulated by a variety of biologically active molecules in the tumor microenvironment and the chemokines, in particular, play an important role in this process (10). Such as, ovarian cancer cells secrete CCL2 (14) and CCL2 produced in epithelial areas of the tumor positively correlates with number of epithelial cells expressing CCL2 and the total number of $CD8^+$ T lymphocytes and macrophages in tumor vicinity (14). In esophageal carcinoma, the expression of CCL2 is positively correlated with the number of macrophage infiltration, which correlates with tumor angiogenesis (15). CCL2 also induces the production of monocyte matrix metalloproteinases (MMPs), the matrix-degrading enzymes that are required for the extravasations of leukocytes through basement membrane into the tissue (16). Similar to CCL2, the chemokine CCL5 is localized to areas containing tumor-infiltrating leukocytes and is responsible for the selective infiltration of T lymphocytes in human colorectal carcinoma (14). Consequently, as demonstrated in numerous studies, different types of leukocytes recruited to tumor sites such as Th2 lymphocytes, immature dendritic cells, T regulatory cells and macrophages act in different manners to suppress or promote tumourigenesis (12).

4. CHEMOKINES IN TUMOR DEVELOPMENT, GROWTH AND ANGIOGENESIS

Mounting evidence suggests that chemokine receptors are highly expressed by the tumor cells and their corresponding ligands (chemokines) are produced within the tumor microenvironment. Furthermore, interaction of chemokines with their corresponding liagd play crucial role in tumor progression. CXCR2, which is receptor for multiple inflammatory chemokines such as CXCL1 and CXCL8, demonstrates a high degree of homology to the GPCR encoded by Kaposi's sarcoma-associated herpesvirus-8, called KSHV-GPCR. A point mutation of CXCR2 results in the constitutive signaling of the receptors and cellular transformation similar to KSHV-GPCR (17, 18). Over expression of CXCL2 and CXCL3 the ligands for CXCR2, in melanocytes also increase tumourigenicity *in vitro* and in nude mice (19). A proliferative effect of chemokines on tumor cells has also been demonstrated. CXCL1 and CXCL8, ligands for CXCR2 have been identified as autocrine growth factors of melanoma cells, which constitutively express CXCR2. Blocking CXCL1 or CXCR2 with specific antibodies inhibits melanoma cell growth (20-22). In addition, some chemokines may support tumor growth by providing anti-apoptotic signals. For example CXCL12 and CXCL9 enhance tumor cell survival, expressing their cognate receptors CXCR4 and CXCR3, respectively, in serum-free conditions (23, 24). Chemokines also affect tumor growth by regulating the formation of new vascular networks around the tumor and CXC chemokines, containing an ELR (Glu-Leu-Arg) motif at the NH2-terminus play significant role in this process. The ELR-positive chemokine (i.e. CXCL8) elicits an angiogenic effect on prostate tumors. CXCL8 is highly elevated in prostate cancer (PCa) patients, compared to healthy subjects or patients with benign tumors (25, 26). The administration of CXCL8 neutralizing antibody

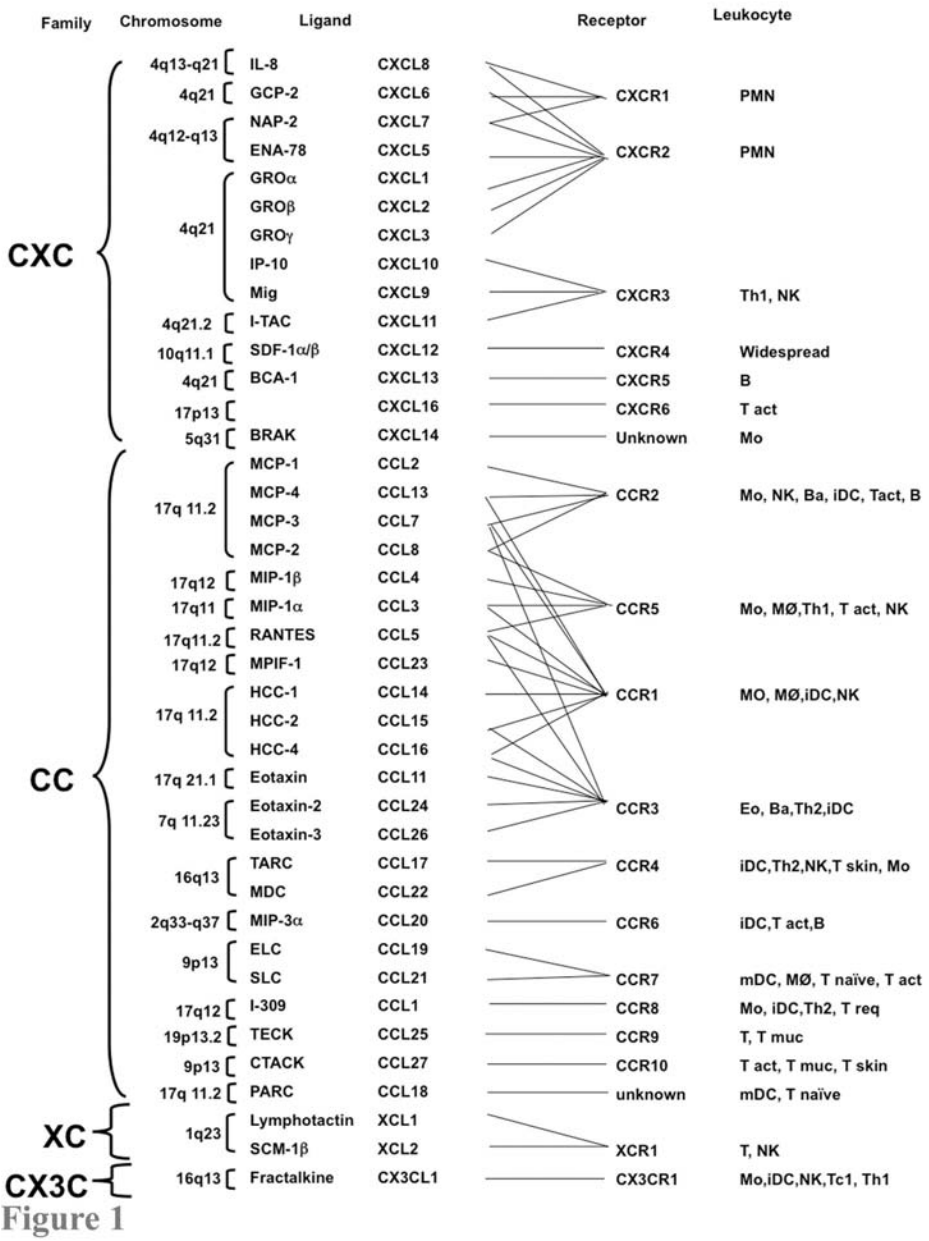

Figure 1. The chemokine system: Chemokines, their receptors, and predominant receptor repertoires in different leukocyte populations are listed. The selected ligands are identified with one old acronym and the new nomenclature, the first part of the name identifies the family and L stands for "ligand," followed by a progressive number. Chemokine acronyms are as follows: BCA, B-cell activating chemokine; BRAK, breast and kidney chemokine; CTACK, cutaneous T-cell–attracting chemokine; ELC, Epstein-Barr virus–induced receptor ligand chemokine; ENA-78, epithelial cell–derived neutrophil-activating factor (78 amino acids); GCP, granulocyte chemo attractant protein; GRO, growth-related oncogene; HCC, hemofiltrate CC chemokine; IP, interferon-inducible protein; I-TAC, interferon-inducible T-cell A chemo attractant; MCP, monocyte chemo attractant protein; MDC, macrophage-derived chemokine; Mig, monokine induced by γ interferon; MIP, macrophage inflammatory protein; MPIF, myeloid progenitor inhibitory factor; NAP, neutrophil-activating protein; PARC, pulmonary and activation-regulated chemokine; RANTES, regulated upon activation normal T cell–expressed and secreted; SCM, single C motif; SDF, stromal cell–derived factor; SLC, secondary lymphoid tissue chemokine; TARC, thymus and activation-related chemokine; TECK, thymus-expressed chemokine. Ba, basophils; CC, chemokine with the first 2 cysteines in adjacent positions; Eo, eosinophils; iDC, immature dendritic cells; MC, mast cells; mDCs, mature dendritic cells; Mo, monocytes; Mø, macrophages; NK, natural killer cells; PMN, neutrophils; T act, activated T cells; T naive, naive T cells; T muc, mucosal-homing T cells; Treg, regulatory T cells; T skin, skin-homing T cells.

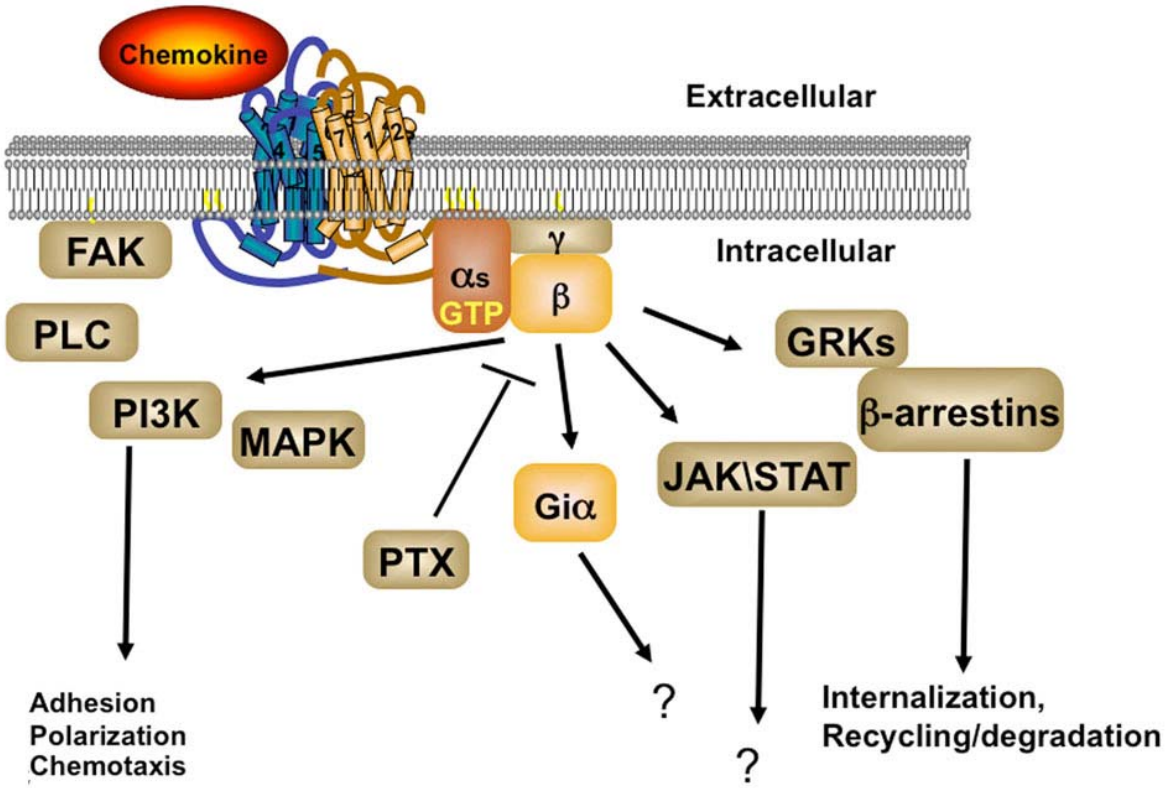

Figure 2. Summary of major signal transduction pathways downstream of chemokine receptors. This simplified figure depicts signaling molecules activated following chemokine receptor activation. The binding of chemokines to their respective receptors results in the activation of three main downstream signaling pathways to various cellular responses. The classical G-protein pathway activates various downstream systems including PLC, PI3K, FAK and MAPK, leading to cell adhesion, polarization and chemotaxis. Inactivating Giα using PTX can be inhibite this pathway. The activation of the JAK/STAT pathway being initiated following the tyrosine phosphorylation of activated chemokine receptors has been documented. The signaling pathways initiated by phosphorylation at serine/threonine residues on the COOH-terminus region involve the activities of GRKs and β-arrestins. This pathway is responsible for internalization and recycling/degradation of the activated receptors. Arrows indicate ‘‘activated downstream’’. Question marks indicate unclear downstream signaling pathways. FAK focal adhesion kinase, PLC phospholipase C, PI3K phosphatidylinositol 3-kinase, MAPK mitogen-activated protein kinase, PTX pertussis toxin, JAK/STAT Janus kinase/Signal transducer and activator of transcription, GRKs G-protein-coupled receptor kinases.

inhibits tumor growth and angiogenesis in PCa mouse model, which constitutively produce CXCL8 (27). Other ELR-positive CXC chemokines such as CXCL1, CXCL2 and CXCL3 also show angiogenic properties and experimental evidences suggest that over expression of these chemokines in non-tumorigenic mouse melanocytes promotes formation of highly vascular tumors in mice (19, 28). In contrast, ELR-negative CXC chemokines such as CXCL4, CXCL9 and CXCL10 show angiostatic activity (29-31). In SCID mice, the intra-tumoral injection of CXCL10 attenuates the growth and neovascularization of non-small cell lung cancer, whereas blocking the function of CXCL10 by the administration of CXCL10 neutralizing antibody promote tumor growth and angiogenesis (32). However, the ELR-negative CXC chemokine CXCL12 appears to exhibit angiogenic ability both *in vitro* and *in vivo* (33). Overall, balances between the activities of angiogenic and angiostatic factors, including certain CXC chemokines are required for the formation of neovascular network in tumors (30, 31).

5. CHEMOKINES IN TUMOR INVASION AND METASTASIS

Chemokines and their receptors have gained much attention in recent years due to their significant roles in advance stages of cancer. The function of these molecules in cancer has been inferred from the physiological activities of leukocytes, especially the chemotaxis, which is tightly regulated by chemokines. In a seminal study by Muller *et al.* (34) demonstrates that metastasizing tumor cells migrate towards chemokine-producing secondary organs similar to the directional migration of leukocytes to the sites of inflammation. Numerous studies have demonstrated the involvement of various chemokines and chemokine receptors in metastasis and invasion of cancer (10). Expression of CXCL8 as well as its corresponding receptors, CXCR1 and CXCR2, increases the invasiveness of human melanoma cells and correlates with the metastatic potential of the cells in mice (35, 36). Furthermore, over expression of CXCL8 also

results in an increased invasion and metastasis of PCa, both *in vitro* and *in vivo* (37). CCR7, the receptor for CCL19 and CCL21, has also been implicated in melanoma metastasis (38).

The functional expression of CCR7 enhances the metastasis of B16 murine melanoma cells and CCL21 neutralizing antibody inhibits metastasis (38). A number of chemokines promote the invasive and metastatic potential of breast cancer cells. A wide range of ligands including CCL3, CCL4, CCL2, CCL5, CXCL10, CXCL7, CXCL1 and CXCL2 have been shown to induce the migration of human breast cancer cells (39). The expression of CXCL8 has also been correlated with the metastatic potential of breast cancer cell lines (40).

Muller and colleagues has provided first experimental evidence revealing the molecular mechanism involved in chemokine-mediated organ-specific metastasis of breast cancer (41). Amongst all known chemokines receptors, the human breast cancer cell lines and malignant breast tumors predominantly express CXCR4 and CCR7. The functionality of CXCR4 and CCR7 was demonstrated by *in vitro* experiments showing that the stimulation of human breast cancer cells with CXCL12 and CCL21 mediates actin polymerization, chemotaxis and invasion. A panel of normal human organs was screened for the respective ligands: CXCL12 for CXCR4 and CCL21 for CCR7. Interestingly, expression of CXCL12 was higher in all target organs for breast cancer metastasis (i.e. lung, liver, bone marrow, and lymph nodes) and low at other organs (41). On the other hand, CCL21 was expressed selectively in lymph nodes. Protein extracts of the target organs exerted chemotactic activity on breast cancer cells. Blocking CXCR4-CXCL12 interaction with CXCR4 neutralization antibody in metastatic breast cancer model MDA-MB-231 cells attenuated metastasis to the regional lymph node and lung (41). This study indicates a significant involvement of CXCR4/CXCL12 and CCR7/CCL21 in invasion and metastasis of breast cancer. Studies by other investigators have also shown that down regulation of CXCR4 receptors by inducible small-interfering RNA (siRNA) inhibits the metastasis of breast cancer cells both *in vitro* and *in vivo* (42, 43) and blocking CXCR4 activity using a specific antagonist delays the growth of breast cancer cells in the lung (44). In addition to breast cancer CXCR4/CXCL12 axis has been shown to play important role in prostate, melanoma, pancreatic and ovarian cancers metastasis (45, 46). Hecnce, CXCR4/CXCL12 axis is currently considered as one of the major determinants of metastatic progression of cancer. A number of recent reports have extensively investigated the biological functions of CXCR4/CXCL12 associated with the metastatic potential of cancer cells and several mechanisms underlying these processes (10). First, the activation of CXCR4 may trigger the arrest of circulating cancer cells on endothelial cells by promoting the interaction between adhesion molecules on both cell types. In B16 murine melanoma cells, the activation of CXCR4 rapidly increases the affinity of β1 integrin on the cells for vascular cell adhesion molecule-1 (VCAM-1). Under shear stress conditions, over expression of CXCR4 in B16 cells resulted in greater than ten-fold increase in adhesion to lung endothelial cells expressing VCAM-1 in response to TNF-α (47). In addition to these studies, it has also been shown that stimulation of CXCR4 expressing melanoma cell, glioma cells and ovarian cancer cells with CXCL12 facilitate cell proliferation and survival (48-51). CXCR4/CXCL12 signal transduction leading to cell survival is likely to be mediated by the activation of PI3Ks and their downstream PKB/Akt (51). Furthermore, it has been recently documented that the malignant transformation and the function of CXCR4 and CCR7 chemokine receptors are interconnected and most importantly, are interdependent in breast cancer cells (52).

5. CROSS TALK BETWEEN CHEMOKINES AND GROWTH FACTORS IN CANCER PROGRESSION

Tumor progression is influenced by homeostatic factors released locally and systemically, such as adhesion molecules, immune components and growth factors (2, 53, 54). At tumor–stroma interface, a tumor cell can be simultaneously exposed to variety of humoral factors produced by both tumor and host cells. It is now well accepted that the families of G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) play important roles in mediating the signals from extra cellular stimuli such as hormones, growth factors and cytokines involved in a variety of physiological and pathological conditions (10, 55). More importantly, recent studies have indicated that the signal transduction cascades mediated by these two receptors share downstream signaling pathways and may modulate each other to transduce the signals (56, 57). Cross talk between these receptors adds a significant complexity to their downstream signal transduction networks, which translate multiple signals from extra cellular stimulus into cellular activities and this may affect the patho-physiological conditions mediated by the two types of receptors. As mentioned above, several growth factors i.e. EGF, VEGF and IGF families play a crucial roles in cancer development and have been identified as therapeutic targets for cancer (55). One of the well-established growth factor systems, the IGF family, has been documented to contribute to the processes of transformation, proliferation, survival, and lesser defined invasion and metastasis, therefore controlling the accomplishment of malignant potential of tumor (58-61). Numerous molecular studies indicate that the activation of IGF tyrosine kinase receptor and CXCR4 receptor by their respective ligands, i.e. IGFs and CXCL12 respectively, triggers signal transduction pathways in involved in cell migration - a cellular activity mandatory for metastasis and invasion of cancer cells (58, 62). We have recently shown that PCa cell lines and primary prostate tumors express CXCR5, which correlates with the tumor grade. In this study, we present the first evidence that CXCL13, the only ligand for CXCR5 and IL-6 were significantly elevated in the serum of PCa patients compared to normal healthy donors with benign prostatic hyperplasia (BPH), or high-grade prostatic intraepithelial neoplasia (HGPIN) as well as normal healthy (NHD). Serum CXCL13 levels significantly ($p<0.0001$) correlated with serum prostate-specific antigen (PSA), whereas serum IL-6 levels significantly ($p<0.0003$)

correlated with CXCL13 serum levels. CXCL13 was found to be a better predictor of PCa than PSA. CXCL13 was highly expressed by human bone marrow endothelial (HBME) cells and osteoblasts (OBs), but not osteoclasts (OCs), following treatment with physiologically relevant levels of interleukin-6 (IL-6). We further demonstrate that CXCL13, produced by IL-6-treated HBME cells, was able to induce PCa cell invasion in a CXCR5-dependent manner. CXCL13-mediated PCa cell adhesion to HBME cells and $\alpha_v\beta_3$-integrin clustering was abrogated by CXCR5 blockade. These results demonstrate that the CXCL13-CXCR5 axis is significantly associated with PCa progression (63).

7. EXPRESSION OF CHEMOKINE RECEPTORS AND CANCER METASTASIS

The expression of chemokine receptors CXCR4 and CCR7 in particular, on tumor cells has recently received a great attention due to their direct involvement in promoting cancer metastasis. Expression of these chemokine receptors has been reported on a number of cancer cell lines, tissues and primary tumors and interaction with their specific ligands has been demonstrated to stimulate migration of cancer cells *in vitro* and *in vivo*. Retrospective studies in clinical samples positively correlates with chemokine expression and clinical out come. Presence of CCR7 in non-small cell lung cancer (NSCLC) cells has been directly associated with the development of lymph node metastasis (64). More specifically, CCR7 mRNA was expressed at higher levels in cancer tissue and not in the neighboring normal lung tissues. Furthermore, the expression of CCR7 mRNA correlated with the stage of lymphatic invasion, suggesting that CCR7 may participate in the emigration of cancer cells from peripheral tissue to lymph nodes via the lymphatics. As a consequence, it was proposed that CCR7 expression could be used as a potential diagnostic tool for predicting lymph node metastasis before surgery and may improve therapeutic out come of NSCLC. Since CXCR4 is the most common chemokine receptor expressed by tumor cells, being implicated in over 25 different epithelial, mesenchymal and haemopoietic human cancers (45, 46). CXCR4 expression has been extensively studied variety of tumor cells and tissues. Notably, not all cancer cells studied are CXCR4-positive; the cell lines derived from ovarian cancer, acute myelogenous leukemia (AML), anaplastic thyroid cancer and glioma are CXCR4-negative (45, 46). Moreover, within primary tumors such as ovary and NSCLC, only a subpopulation of cells expresses CXCR4. More importantly, CXCR4 expression has been linked in promoting the production of other factors that are involved in tumor development. For example, stimulation of ovarian cancer cells and primary cells isolated from ascitic disease produce more pro-inflammatory cytokine (i.e. TNF-α) after CXCL12 stimulation (65), which has been implicated in tumor/stromal communication in this disease.

The majority of these studies have focused on positively correlating receptor expression with the metastatic disease. However, conflicting evidence shows that the expression of CXCR4 on cancer cells is not simply confined to tumor cells exhibiting metastatic abilities. Schmid *et al* have shown that CXCR4 expression is initiated at a very early point in the transition from normal to a transformed phenotype in breast epithelium and highly expressed in ductal carcinoma *in situ* (DCIS) (66). Moreover, high levels of CXCR4 were detected in 94% of studied cases of atypical ductal hyperplasia, potentially the first clonal pre-neoplastic expansion of ductal epithelial cells, representing an early stage in tumourigenic transformation (66). Therefore, these observations raise the possibility that expression of CXCR4 and CCR7 receptors implicated in metastasis and not restricted to the malignant forms of breast cancer.

In view of these contrasting data, the precise relationship of chemokine receptor expression in cancer cells and their potential role in metastasis is still not clear and requires considerable clarification. To establish if the level of chemokine receptor expression relates to the disease stage of breast cancer, the analysis of CXCR4 and CCR7 expression in breast cancer cells was performed *in vitro* in a panel of cell lines ranging from non-transformed immortalized breast epithelial cells to highly aggressive breast cancer cell lines (67). The examination of CXCR4 and CCR7 expression revealed significant levels of expression throughout the panel of breast epithelial cancer cell lines. CXCR4 and CCR7 expression were not restricted to the metastatic cell types, since a non-transformed immortalized breast cancer cell line (MCF-10A), exhibits similar levels to that observed in highly invasive transformed breast cancer cell line, MDA-MB-231. These data challenge previous reports and strongly suggest that CXCR4 and CCR7 expression alone is not an indicator of aggressive breast cancer and therefore its application as a prognostic marker of metastasis requires further clarification. We have shown that CCR9 is highly expressed by the PCa cells. Neutralization of CCL25-CCR9 interactions impaired the migration and invasion potential of the LNCaP and PC3 cell lines towards chemotactic gradient of CCL25. CCL25 differentially modulated the expression of collagenase-1 or matrix metalloproteinase (MMP)-1, collagenase-3 (MMP-13), stromalysin-2 (MMP-10), stromalysin-3 (MMP-11), and gelatinase-A (MMP-2), but not MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, or MMP-14 in PCa cells. These studies suggest that the expression and activation of CCR9 affects cancer cell migration, invasion, and MMP expression, which together may affect PCa metastasis (68).

8. ROLE OF CHEMOKINE AND THEIR RECEPTORS IN CANCERS

8.1. Breast Cancer

The normal breast expresses a set of chemokines, albeit at low levels. Indeed, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8 have been detected in human milk (69, 70). Primary cultures of normal breast epithitelial cells secrete CXCL8 in addition to TNFα and IL-6 (70). In many cases, levels of chemokines are higher in cancer tissues compared to normal tissue. A global gene expression profiling comparing normal epithelial cells and *in situ*, invasive and metastatic breast carcinomas using

serial analysis of gene expression (SAGE) analysis revealed that many chemokines are highly expressed in normal breast and absent in carcinoma, including CXCL1, CXCL2, CXCL5, CXCL6, CXCL8, CXCL20, CX3CL1, CCL2 and CCL7 (71). Most studied chemokine in breast cancer are CXCL8, CCL2, CCL4 and CCL5.

CXCL8 has been shown to play an important role in various pathological conditions and in many types of cancers (72). CXCL8 is over expressed in breast cancer compared to normal tissues both at the protein levels and the RNA levels in primary tumors (73) and in the sera of patients (74, 75). In addition to CXCL8, CCL2 and CCL4 also highly expressed by the malignant tissue compared to normal (73). To our knowledge, only one study performed on a limited number of samples did not report any differences in CXCL8 levels between infiltrating mammary carcinoma and normal breast (76). CCL5 is highly expressed by the tumor cells and rarely in normal breast biopsies (77). Similarly, serum CCL5 levels in patients with high-grade breast tumors are higher, compared to low-grade tumors (78). In addition to these facts, CCL5 levels are also elevated in primary tumors, as well as at metastatic sites (78).

Potential role of chemokine and their corresponding receptor interaction in breast cancer progression is now well accepted. Most studies chemokine in breast cancer are CXCR4 and CCR7, which bind CXCL12 and CCL19/CCL21 respectively (41). CCL21 is mainly expressed in lymph node, which could explain the migration of breast tumor cells to the lymph node (41). On the other hand, CXCL12 is present at high levels in lymph node, bone marrow, liver and lung, which could also account for the migration of breast cancer cells to the sentinel lymph node and subsequently to distant metastasis organs (3, 41). It is important to note that the levels of chemokine receptors might not be sufficient to determine whether a particular cell will be responsive to its natural ligand. Indeed, Holland *et al.* have shown that by analyzing a panel of breast cancer cell lines that expression of CXCR4 is relatively uniform between the different cells lines both at RNA and protein levels, but only metastatic cell lines displayed a functional receptor (52).

8.2. Prostate Cancer

Besides the role of chemokines and their receptors in angiogenesis, they also seem to be involved in the process of tumor cell migration, invasion, and metastasis. Metastasis is a multistep process in which tumor cells gain access to the vasculature in the primary tumor, survive in circulation, arrest in the microvasculature of the target organ, exit from the microvasculature, and proliferate in the target tissues. It has been shown that greater numbers of tumor vessels increase the opportunity for tumor cells to enter the circulation (79). In prostate carcinoma (PCa), a histological analysis has shown direct positive correlation between microvessels density of invasive prostate carcinoma and the incidence of metastases (80). However, directional migration of cancer cells, which critical step in metastasis, is determined by the chemokine receptors expressed by the PCa cells and expression of corresponding ligand (chemokine) at the target organs. In this regard, it has been shown that different human PCa cell lines from the same cancer type use distinct angiogenic CXC chemokines (81). Since, PCa has the tendency to metastasize to the bone, there has been more focus on bone-associated chemokines and their receptors expressed by the PCa cells. Recent reports indicate that the CXCL12/CXCR4 chemokine axis is involved in PCa metastasis (82, 83). Expression of CXCR4 in PCa cells has been implicated in ‘‘homing’’ to microenvironments of higher concentrations CXCL12 (83), such as bone (84). During this process, the circulating PCa cell mimics hematopoetic and immune cells in terms of localizing to the high CXCL12-expressing sites, firm adhesion to endothelial cells, transmigration across the blood vessel wall and migration toward the chemokine (85). Moreover, it has demonstrated that CXCL12-CXCR4 is key factor for metastatic PCa (86, 87). It has also been shown that androgen receptor negatively regulates CXCR4 (3), suggesting that loss of androgen receptor enhances PCa migration. Another mechanism through which CXCR4 could be regulated to promote metastasis is through NF-κB. NF-κB has been shown to up-regulate chemotaxis of breast cancer cell by inducing CXCR4 expression (88). However, inhibition of CXCR4 *in vivo* only partially blocks the metastatic behavior of PCa, suggesting that other factors may play a role in controlling tissue-specific migration (89). Our laboratory also reported the cellular and molecular mechanisms of CXCR4/CXCL12-mediated PCa cell migration and invasion (90). In addition to highly studied CXCL12-CXCR4 axis, we have recently reported that CXCR5 a chemokine receptor for CXCL13 is highly expressed by the PCa cell and tissues (91). Additionally, we have also shown clinical and biological significance of CXCL13-CXCR5 in PCa (91). A recent report indicates that CXCR7, another receptor for CXCL12 is expressed in PCa. *In vitro* and *in vivo* studies with PCa cells suggest that alterations in CXCR7 expression are associated with enhanced adhesive and invasive activities in addition to a survival advantage (86, 87). It has been shown that CXCR7 also regulates the expression of the pro-angiogenic factors CXCL8 or VEGF, suggesting a role in the regulation of tumor angiogenesis. Furthermore, studies have shown that signaling through or expression of CXCR4 alters CXCR7 levels, while CXCR7 expression is not directly linked to CXCR4 expression (86, 87). CXCR7 forms functional heterodimers with CXCR4 that potentiates CXCL12 signaling (92). A previous report supports that CXCR7 mediates many biological and pathological processes, including cell growth/survival and adhesion, as well as the promotion of tumor growth (93). However, it was suggested that the CXCR7 signaling pathway might be distinct from the typical G-protein-coupled receptor mechanism. Other than CXCR4, CXCR5 and CXCR7 a significant positive correlations between expression of CXCL16 and CXCR6 vs. the stage and grade of PCa has been established very recently. In this study authors have also shown that CXCL16 can stimulate the growth of PCa cell lines transfected to express CXCR6 (94). Hence, multiple chemokies and their corresponding receptors are involved in etiopathogenesis of PCa.

8.3. Ovarian Cancer

It is now well established that complex biology of both normal and malignant ovarian cells are regulated by the chemokine (95,96). Chemokines expressed by the cells of tumor microenvironment can affect the types and the degree of the immune infiltrate in the tumor. The CC chemokine subfamily, particularly CCL2 is the most often expressed in ovarian cancer histotypes (97) and particularly involved in macrophage recruitment. Negus *et al.* (86) reported the expression of CCL2 and CCL5 in epithelial ovarian cancer cells and demonstrated the direct relationship between the levels of CCL2 with degree of the immune cell infiltration. More recently, the analysis of ovarian cancer ascitic fluid and ascite cells showed significant expression of CCL2, CCL3, CCL 4, CCL5, CCL8, and CCL22, and their corresponding receptors (namely, CCR1, CCR2a, CCR2b, CCR3, CCR4, CCR5, and CCR8), at mRNA and protein level (98). However, a definite correlation between this expression pattern and the total cell counts in ascites or the stage of the disease still unclear. Indeed, tumors appear to utilize the same molecular mechanisms used by the normal immune system to eliminate malignant cells. In this regard, influence of chemokines on anti-tumor immune response has been described in a study that strongly supports the view that tumor-associated regulatory T cells (mediators of the immune tolerance by suppressing auto reactive T cells directed towards tumor antigens) impair the function of T effector cells in tumor bearing patients (99). Tumor tissue and ascites from ovarian cancer patients contains high levels regulatory T cells. These cells migrate into the tumor microenvironment in response to CCL22 are capable of suppressing antitumor responses. This specific recruitment of regulatory T cells represents a mechanism by which tumors may develop immune advantages and, as a consequence, the suggested inhibition of regulatory T cell migration or function using antibodies against CCL22 may represent a novel anti-tumor approach.

One of the main features of all solid tumors is their dependence on neovascularization. Cancer cells recruit endothelial cells by producing several chemokines, cytokines, and growth factors. Angiogenesis is also critical for ascites development and metastasis in ovarian cancer. The role of chemokines in tumor angiogenesis is well known and mainly controlled by the CXC chemokines family in a negative (angiostatic chemokines, ELR^-) or a positive manner (angiogenic, ELR^+) (100). In particular, CXCL8/IL-8 and CXCL1-3/GROα, -β and -γ, and CXCL5/ENA-78 induce angiogenesis through the activation of direct mechanisms on endothelial cells (101). A direct relationship between the expression of angiogenic molecules and the pathological behavior of five different human ovarian cancers xenografted indicates that the expression of CXCL8/IL-8 is associated with neovascularization and inversely correlated to survival (102). Moreover, CXCL8-overexpressing cells when xenotransplanted in mice display an increased cell growth, micro vessel density, and tumourigenic rate (103). Similarly, *in vitro*, IL-6 and CXCL8/IL-8 accelerate the proliferation rate of several EOC cell lines (104).

The fate of metastatic tumors cells at new homing site is determined by its microenvironment, which supports the homing as well as growth of the tumor cells. Several observations indicate that the tumor microenvironment at metastatic sites is enriched with chemokines and tumor cells expressing the cognate receptors migrate and adhere in response to these chemokines. An important role for the CXCL12/CXCR4 axis in metastasis of ovarian tumor is well established (105). However, it is clear that CXCL12 alone is not responsible in determining the fates of metastatic cells. In particular, the role of CCL11/eotaxin-1 in proliferation and invasion of ovarian cancer cells was analyzed. EOC over expressed CCR2, -3, -5, and the cognate receptors of CCL11, with a strong positive correlation between tumor grade and the levels of each of these receptors. Interestingly, the inhibition of CXCL11 activity by neutralizing antibodies significantly increases cis-platinum response in ovarian carcinoma cells (106,107). Higher efficacy of cis-platinum after CXCL11 neutralization suggests that chemokines also play significant role in up regulating anti-apoptotic molecules.

9. MOLECULAR MECHANISMS OF THE FUNCTIONAL ON-SWITCH OF THE CHEMOKINE RECEPTORS IN METASTATIC CANCER CELLS

It is now very clear that a large number of downstream effectors molecules that are regulated by chemokines are highly involved in the pathobiology of tumors. However, role of various effectors of chemokine receptors in primary and metastatic tumors are not well established. Moreover, chemokine signaling has been predominantly investigated in leukocytes and very little evidence is available on signaling cascades mediated by the chemokines in other cell types.

Although at the present, relatively little is known regarding the signaling pathways activated by chemokines in cancer cells, preliminary data show that CXCR4 and CCR7 are capable of activating a number of different intracellular events such as chemotaxis, invasion and adhesion, which are essential for cancer cells to achieve metastatic goal (108, 109).

In non-metastatic breast cancer cells, actin polymerization and chemotactic responses are not induced, when CXCR4 and CXCR7 is stimulated with their ligands. Therefore it is possible that the signaling intermediates further upstream of these events are disrupted. Chemokine signaling pathways are mediated through G-protein coupled receptors (GPCRs) and generally use the Gi subclass of G-proteins (3) that results in the activation of the Gαi subunit, which mediates the inhibition of adenyl cyclase-mediated cAMP production and the mobilization of intracellular calcium (110). Treatment with CXCL12 or CCL19 inhibits forskolin-induced adenyl cyclase-mediated cAMP in metastatic breast cancer cell lines but not in non-metastatic cells. Furthermore, chemokines induce Gαi-dependent intracellular calcium mobilization in metastatic breast cancer cells are inhibited by Gαi inhibitors and pertussis toxin.

In leucocytes, the βγ subunit of the G-protein complex is responsible for the activation of numerous kinase cascades downstream of GPCRs (111). In metastatic breast cancer cells, a rapid and sustained activation of ERK1/2, IkBα, JNK, Akt, p38MAPK and GSK-3 α/β are observed in response to CXCR4 and CCR7 ligands, where as little or no activation detected in the non-metastatic cell lines. Several of these effector molecules play a significant role during the migration of leukocytes, these data imply that breast cancer cells use similar chemokine mediated mechanisms to regulate migration, proliferation and survival required for cancer progression and metastasis. Moreover, Gβγ-dependent signaling is not activated in the non-metastatic cell types, suggesting the blocked in functional signaling in further upstream of these signaling intermediates. Overall, the analysis of chemokine-mediated signaling events downstream of the G-protein α and βγ subunits in breast cancer cells suggests that the blockade in CXCR4 and CCR7 function in non-metastatic cells occurs at the level of G protein activation.

The heterotrimeric G proteins act as molecular switches in signaling pathways by coupling the activation of heptahelical receptors at the cell surface to intracellular responses. This role depends on the ability of the Gα subunit to cycle between a resting conformation primed for interaction with an activated receptor and a signaling conformation capable of modulating the activity of downstream effector proteins (112). In the resting state, the Gα subunit binds GDP and Gβγ. Receptors activate G proteins by catalysing GTP for GDP exchange on the Gα subunit, leading to a conformational change in the Gα and Gβγ subunits that allows their dissociation and activation of a variety of downstream effector proteins. The G protein returns to the resting conformation following GTP hydrolysis and subunit re-association.

Analysis of the chemokine receptor and G-protein coupling in resting and chemokine-activated metastatic and non-metastatic breast cancer cells demonstrated that CXCR4 and CCR7 forms complexes with the Gαi subunit constitutively in both cell types. However, following chemokine stimulation, dissociation of Gαi from the receptor takes place only in the metastatic cells. In parallel, the Gβ subunit associated with chemokine receptors in both non-invasive and metastatic breast cancer cells; however, the dissociation of Gβ from the receptor upon ligand stimulation occurred only in the metastatic cells. Further investigations revealed that the formation of the heterotrimeric G-protein complex could only be detected in the invasive cell types that express functional chemokine receptors. The differences in Gαi and Gβ binding observed throughout the panel of breast cancer cells lines were not due to the absence of Gβ protein since all cells examined expressed Gβ subunits. Therefore, this novel finding indicates that in non-invasive cells with non-functional CXCR4 or CCR7, Gαi and Gβγ do not form the functional heterotrimeric complex, which is critical for GDP to GTP transfer and activation of signaling pathways downstream of G proteins (113).

Furthermore, these findings strongly suggest the existence of specific regulatory mechanisms that may be switched on or off during the metastatic progression and acquisition of an invasive phenotype. The hypothetical functional ‘‘on-switch’’ for chemokine receptors expressed in breast cancer cells is controlled at the level of the chemokine receptor (i.e. CXCR4 and/or CCR7) and G-protein subunit interactions. The lack of G-protein heterotrimeric complex formation in the non-invasive cells may be due to the expression of ‘‘incompatible’’ α and βγ subunits. It is noteworthy that the family of heterotrimeric G proteins consists of 27α, 5β and 14γ subunits, which may leads to a very high number of possible αβγ subunit combinations of varying affinity for a multitude of GPCRs (51). Another plausible explanation for the inability of Gα and Gβγ subunits to form stable complexes in selective cell lines may be due to the expression of one or more inhibitory molecules. Many aspects of G-protein-mediated signaling remain to be elucidated, and to gain a broader understanding of the functional roles of chemokine receptor signaling pathways in human breast and other cancer cells. Additional studies are necessary to delineate the regulation of G-proteins downstream of CXCR4 and CCR7. A detailed understanding of the physiological and pathophysiological role of G-protein-mediated signaling in normal and transformed cells will allow the full exploitation of this multifaceted signaling system as a target for pharmacological interventions.

10. CONCLUSION AND PERSPECTIVE

Cancer research has emphasized the importance of intrinsic properties of tumors as well as the host homeostatic systems activated during cancer progression. Accumulating evidence suggest that chemokies play significant role in cancer progression and metastasis. Importantly, chemokines in the tumor microenvironment and potentially interact with growth factors to regulate the process of invasion and metastasis of cancer. As thoroughly reviewed, molecular analyses have indicated that the activation of chemokine receptors by chemokines triggers the signal transduction networks leading to cell migration, which is mandatory for cancer cells to accomplish metastatic goal. While different types of cross talk between GPCR and RTK signal transduction are increasingly documented, it has been clearly demonstrated that the migrational signaling downstream of chemokine receptors can be transactivated by growth factors as is the case for the cross talk between CXCR4/CXCL12 and IGF-1R/IGF-I in metastatic breast cancer cells. Despite the existence of cross talk between the two types of receptors being documented, many important aspects require further investigation. Of importance, a variety of normal cells, diseased cells and tissues, including cancer cells other than those derived from breast cancer, should be examined for similar cross talk. Different factors involved in establishing the CXCR4/IGF-1R signaling complex must be identified in order to elucidate the precise mechanism of the cross talk. The role of this cross talk in breast cancer metastasis and invasion would also need to be studied in *in-vivo* models to develop specific approaches to inhibit the cross talk. Currently, cross talk between different types of

molecules exerting their biological effects on tumor cell is being increasingly studied and this aspect is essential for understanding the molecular pathogenesis of cancer and lead to the development of effective cancer therapy.

11. REFERENCES

1. M. B. Sporn: The war on cancer. *Lancet*, 347(9012), 1377-81 (1996)

2. G. L. Nicolson: Cancer progression and growth: relationship of paracrine and autocrine growth mechanisms to organ preference of metastasis. *Exp Cell Res*, 204(2), 171-80 (1993)

3. A. Zlotnik: Involvement of chemokine receptors in organ-specific metastasis. *Contrib Microbiol*, 13, 191-9 (2006)

4. D. Uchida, N. M. Begum, Y. Tomizuka, T. Bando, A. Almofti, H. Yoshida and M. Sato: Acquisition of lymph node, but not distant metastatic potentials, by the overexpression of CXCR4 in human oral squamous cell carcinoma. *Lab Invest*, 84(12), 1538-46 (2004)

5. A. S. Payne and L. A. Cornelius: The role of chemokines in melanoma tumor growth and metastasis. *J Invest Dermatol*, 118(6), 915-22 (2002)

6. T. Kakinuma and S. T. Hwang: Chemokines, chemokine receptors, and cancer metastasis. *J Leukoc Biol*, 79(4), 639-51 (2006)

7. Y. X. Sun, J. Wang, C. E. Shelburne, D. E. Lopatin, A. M. Chinnaiyan, M. A. Rubin, K. J. Pienta and R. S. Taichman: Expression of CXCR4 and CXCL12 (SDF-1) in human PCas (PCa) *in vivo*. *J Cell Biochem*, 89(3), 462-73 (2003)

8. P. Mitra, K. Shibuta, J. Mathai, K. Shimoda, B. F. Banner, M. Mori and G. F. Barnard: CXCR4 mRNA expression in colon, esophageal and gastric cancers and hepatitis C infected liver. *Int J Oncol*, 14(5), 917-25 (1999)

9. P. Staller, J. Sulitkova, J. Lisztwan, H. Moch, E. J. Oakeley and W. Krek: Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature*, 425(6955), 307-11 (2003)

10. A. Spiegel, O. Kollet, A. Peled, L. Abel, A. Nagler, B. Bielorai, G. Rechavi, J. Vormoor and T. Lapidot: Unique SDF-1-induced activation of human precursor-B ALL cells as a result of altered CXCR4 expression and signaling. *Blood*, 103(8), 2900-7 (2004)

11. T. Nagasawa, K. Tachibana and T. Kishimoto: A novel CXC chemokine PBSF/SDF-1 and its receptor CXCR4: their functions in development, hematopoiesis and HIV infection. *Semin Immunol*, 10(3), 179-85 (1998)

12. A. T. Collins and N. J. Maitland: Prostate cancer stem cells. *Eur J Cancer*, 42(9), 1213-8 (2006)

13. M. Burger, A. Glodek, T. Hartmann, A. Schmitt-Graff, L. E. Silberstein, N. Fujii, T. J. Kipps and J. A. Burger: Functional expression of CXCR4 (CD184) on small-cell lung cancer cells mediates migration, integrin activation, and adhesion to stromal cells. *Oncogene*, 22(50), 8093-101 (2003)

14. T. N. Hartmann, M. Burger and J. A. Burger: The role of adhesion molecules and chemokine receptor CXCR4 (CD184) in small cell lung cancer. *J Biol Regul Homeost Agents*, 18(2), 126-30 (2004)

15. H. Kulbe, T. Hagemann, P. W. Szlosarek, F. R. Balkwill and J. L. Wilson: The inflammatory cytokine tumor necrosis factor-alpha regulates chemokine receptor expression on ovarian cancer cells. *Cancer Res*, 65(22), 10355-62 (2005)

16. A. J. Schrader, O. Lechner, M. Templin, K. E. Dittmar, S. Machtens, M. Mengel, M. Probst-Kepper, A. Franzke, T. Wollensak, P. Gatzlaff, J. Atzpodien, J. Buer and J. Lauber: CXCR4/CXCL12 expression and signalling in kidney cancer. *Br J Cancer*, 86(8), 1250-6 (2002)

17. M. Kucia, R. Reca, K. Miekus, J. Wanzeck, W. Wojakowski, A. Janowska-Wieczorek, J. Ratajczak and M. Z. Ratajczak: Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. *Stem Cells*, 23(7), 879-94 (2005)

18. P. M. Murphy: The molecular biology of leukocyte chemoattractant receptors. *Annu Rev Immunol*, 12, 593-633 (1994)

19. I. Kryczek, A. Lange, P. Mottram, X. Alvarez, P. Cheng, M. Hogan, L. Moons, S. Wei, L. Zou, V. Machelon, D. Emilie, M. Terrassa, A. Lackner, T. J. Curiel, P. Carmeliet and W. Zou: CXCL12 and vascular endothelial growth factor synergistically induce neoangiogenesis in human ovarian cancers. *Cancer Res*, 65(2), 465-72 (2005)

20. N. Cabioglu, J. Summy, C. Miller, N. U. Parikh, A. A. Sahin, S. Tuzlali, K. Pumiglia, G. E. Gallick and J. E. Price: CXCL-12/stromal cell-derived factor-1alpha transactivates HER2-neu in breast cancer cells by a novel pathway involving Src kinase activation. *Cancer Res*, 65(15), 6493-7 (2005)

21. Y. Oda, Y. Ohishi, Y. Basaki, H. Kobayashi, T. Hirakawa, N. Wake, M. Ono, K. Nishio, M. Kuwano and M. Tsuneyoshi: Prognostic implications of the nuclear localization of Y-box-binding protein-1 and CXCR4 expression in ovarian cancer: their correlation with activated Akt, LRP/MVP and P-glycoprotein expression. *Cancer Sci*, 98(7), 1020-6 (2007)

22. J. B. Rubin, A. L. Kung, R. S. Klein, J. A. Chan, Y. Sun, K. Schmidt, M. W. Kieran, A. D. Luster and R. A. Segal: A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. *Proc Natl Acad Sci U S A*, 100(23), 13513-8 (2003)

23. J. J. Campbell, J. Hedrick, A. Zlotnik, M. A. Siani, D. A. Thompson and E. C. Butcher: Chemokines and the arrest of lymphocytes rolling under flow conditions. *Science*, 279(5349), 381-4 (1998)

24. G. Monaco, J. W. Belmont, M. Konopleva, M. Andreeff, S. Tavor, I. Petit, O. Kollet and T. Lapidot: Correlation between CXCR4 and homing or engraftment of acute myelogenous leukemia. *Cancer Res*, 64(18), 6832 author reply 6832-3 (2004)

25. I. Petit, M. Szyper-Kravitz, A. Nagler, M. Lahav, A. Peled, L. Habler, T. Ponomaryov, R. S. Taichman, F. Arenzana-Seisdedos, N. Fujii, J. Sandbank, D. Zipori and T. Lapidot: G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol*, 3(7), 687-94 (2002)

26. E. Schutyser, Y. Su, Y. Yu, M. Gouwy, S. Zaja-Milatovic, J. Van Damme and A. Richmond: Hypoxia enhances CXCR4 expression in human microvascular endothelial cells and human melanoma cells. *Eur Cytokine Netw*, 18(2), 59-70 (2007)

27. J. L. Benovic and A. Marchese: A new key in breast cancer metastasis. *Cancer Cell*, 6(5), 429-30 (2004)

28. P. A. Hernandez, R. J. Gorlin, J. N. Lukens, S. Taniuchi, J. Bohinjec, F. Francois, M. E. Klotman and G. A. Diaz: Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nat Genet*, 34(1), 70-4 (2003)

29. M. Baggiolini: Chemokines and leukocyte traffic. *Nature*, 392(6676), 565-8 (1998)

30. Y. Feng, C. C. Broder, P. E. Kennedy and E. A. Berger: HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science*, 272(5263), 872-7 (1996)

31. I. Gockel, C. C. Schimanski, C. Heinrich, T. Wehler, K. Frerichs, D. Drescher, C. von Langsdorff, M. Domeyer, S. Biesterfeld, P. R. Galle, T. Junginger and M. Moehler: Expression of chemokine receptor CXCR4 in esophageal squamous cell and adenocarcinoma. *BMC Cancer*, 6, 290 (2006)

32. B. Moser and P. Loetscher: Lymphocyte traffic control by chemokines. *Nat Immunol*, 2(2), 123-8 (2001)

33. G. Helbig, K. W. Christopherson, 2nd, P. Bhat-Nakshatri, S. Kumar, H. Kishimoto, K. D. Miller, H. E. Broxmeyer and H. Nakshatri: NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. *J Biol Chem*, 278(24), 21631-8 (2003)

34. A. Orimo, P. B. Gupta, D. C. Sgroi, F. Arenzana-Seisdedos, T. Delaunay, R. Naeem, V. J. Carey, A. L. Richardson and R. A. Weinberg: Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell*, 121(3), 335-48 (2005)

35. R. K. Singh, M. Gutman, R. Radinsky, C. D. Bucana and I. J. Fidler: Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. *Cancer Res*, 54(12), 3242-7 (1994)

36. M. L. Varney, A. Li, B. J. Dave, C. D. Bucana, S. L. Johansson and R. K. Singh: Expression of CXCR1 and CXCR2 receptors in malignant melanoma with different metastatic potential and their role in interleukin-8 (CXCL-8)-mediated modulation of metastatic phenotype. *Clin Exp Metastasis*, 20(8), 723-31 (2003)

37. K. Inoue, J. W. Slaton, B. Y. Eve, S. J. Kim, P. Perrotte, M. D. Balbay, S. Yano, M. Bar-Eli, R. Radinsky, C. A. Pettaway and C. P. Dinney: Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. *Clin Cancer Res*, 6(5), 2104-19 (2000)

38. H. E. Wiley, E. B. Gonzalez, W. Maki, M. T. Wu and S. T. Hwang: Expression of CC chemokine receptor-7 and regional lymph node metastasis of B16 murine melanoma. *J Natl Cancer Inst*, 93(21), 1638-43 (2001)

39. S. J. Youngs, S. A. Ali, D. D. Taub and R. C. Rees: Chemokines induce migrational responses in human breast carcinoma cell lines. *Int J Cancer*, 71(2), 257-66 (1997)

40. J. E. De Larco, B. R. Wuertz, K. A. Rosner, S. A. Erickson, D. E. Gamache, J. C. Manivel and L. T. Furcht: A potential role for interleukin-8 in the metastatic phenotype of breast carcinoma cells. *Am J Pathol*, 158(2), 639-46 (2001)

41. A. Muller, B. Homey, H. Soto, N. Ge, D. Catron, M. E. Buchanan, T. McClanahan, E. Murphy, W. Yuan, S. N. Wagner, J. L. Barrera, A. Mohar, E. Verastegui and A. Zlotnik: Involvement of chemokine receptors in breast cancer metastasis. *Nature*, 410(6824), 50-6 (2001)

42. Y. Chen, G. Stamatoyannopoulos and C. Z. Song: Down-regulation of CXCR4 by inducible small interfering RNA inhibits breast cancer cell invasion *in vitro*. *Cancer Res*, 63(16), 4801-4 (2003)

43. Z. Liang, Y. Yoon, J. Votaw, M. M. Goodman, L. Williams and H. Shim: Silencing of CXCR4 blocks breast cancer metastasis. *Cancer Res*, 65(3), 967-71 (2005)

44. M. C. Smith, K. E. Luker, J. R. Garbow, J. L. Prior, E. Jackson, D. Piwnica-Worms and G. D. Luker: CXCR4 regulates growth of both primary and metastatic breast cancer. *Cancer Res*, 64(23), 8604-12 (2004)

45. F. Balkwill: Cancer and the chemokine network. *Nat Rev Cancer*, 4(7), 540-50 (2004)

46. F. Balkwill: The significance of cancer cell expression of the chemokine receptor CXCR4. *Semin Cancer Biol*, 14(3), 171-9 (2004)

47. A. R. Cardones, T. Murakami and S. T. Hwang: CXCR4 enhances adhesion of B16 tumor cells to endothelial cells *in vitro* and *in vivo* via beta(1) integrin. *Cancer Res*, 63(20), 6751-7 (2003)

48. G. S. Chen, H. S. Yu, C. C. Lan, K. C. Chow, T. Y. Lin, L. F. Kok, M. P. Lu, C. H. Liu and M. T. Wu: CXC chemokine receptor CXCR4 expression enhances tumorigenesis and angiogenesis of basal cell carcinoma. *Br J Dermatol*, 154(5), 910-8 (2006)

49. T. Murakami, W. Maki, A. R. Cardones, H. Fang, A. Tun Kyi, F. O. Nestle and S. T. Hwang: Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. *Cancer Res*, 62(24), 7328-34 (2002)

50. C. J. Scotton, J. L. Wilson, K. Scott, G. Stamp, G. D. Wilbanks, S. Fricker, G. Bridger and F. R. Balkwill: Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res*, 62(20), 5930-8 (2002)

51. Y. Zhou, P. H. Larsen, C. Hao and V. W. Yong: CXCR4 is a major chemokine receptor on glioma cells and mediates their survival. *J Biol Chem*, 277(51), 49481-7 (2002)

52. J. D. Holland, M. Kochetkova, C. Akekawatchai, M. Dottore, A. Lopez and S. R. McColl: Differential functional activation of chemokine receptor CXCR4 is mediated by G proteins in breast cancer cells. *Cancer Res*, 66(8), 4117-24 (2006)

53. L. A. Liotta and E. C. Kohn: The microenvironment of the tumour-host interface. *Nature*, 411(6835), 375-9 (2001)

54. C. F. Singer, E. Kubista, F. Garmroudi and K. J. Cullen: Local feedback mechanisms in human breast cancer. *Breast Cancer Res Treat*, 63(2), 95-104 (2000)

55. D. S. Krause and R. A. Van Etten: Tyrosine kinases as targets for cancer therapy. *N Engl J Med*, 353(2), 172-87 (2005)

56. L. M. Luttrell, Y. Daaka and R. J. Lefkowitz: Regulation of tyrosine kinase cascades by G-protein-coupled receptors. *Curr Opin Cell Biol*, 11(2), 177-83 (1999)

57. C. Waters, S. Pyne and N. J. Pyne: The role of G-protein coupled receptors and associated proteins in receptor tyrosine kinase signal transduction. *Semin Cell Dev Biol*, 15(3), 309-23 (2004)

58. E. Foulstone, S. Prince, O. Zaccheo, J. L. Burns, J. Harper, C. Jacobs, D. Church and A. B. Hassan: Insulin-like growth factor ligands, receptors, and binding proteins in cancer. *J Pathol*, 205(2), 145-53 (2005)

59. J. M. Gross and D. Yee: The type-1 insulin-like growth factor receptor tyrosine kinase and breast cancer: biology and therapeutic relevance. *Cancer Metastasis Rev*, 22(4), 327-36 (2003)

60. D. Sachdev and D. Yee: The IGF system and breast cancer. *Endocr Relat Cancer*, 8(3), 197-209 (2001)

61. E. Surmacz, M. A. Guvakova, M. K. Nolan, R. F. Nicosia and L. Sciacca: Type I insulin-like growth factor receptor function in breast cancer. *Breast Cancer Res Treat*, 47(3), 255-67 (1998)

62. M. Mellado, J. M. Rodriguez-Frade, S. Manes and A. C. Martinez: Chemokine signaling and functional responses: the role of receptor dimerization and TK pathway activation. *Annu Rev Immunol*, 19, 397-421 (2001)

63. S. Singh, R. Singh, P. K. Sharma, U. P. Singh, S. N. Rai, L. W. K. Chung, C. R. Cooper, K. R. Novakovic, W. E. Grizzle and J. W. Lillard, Jr.: Serum CXCL13 positively correlates with prostatic disease, prostate-specific antigen and mediates prostate cancer cell invasion, integrin clustering and cell adhesion. *Cancer Letters*, 283(1), 29-35 (2009)

64. I. Takanami: Overexpression of CCR7 mRNA in nonsmall cell lung cancer: correlation with lymph node metastasis. *Int J Cancer*, 105(2), 186-9 (2003)

65. Y. F. Ping, X. H. Yao, J. H. Chen, H. Liu, D. L. Chen, X. D. Zhou, J. M. Wang and X. W. Bian: The anti-cancer compound Nordy inhibits CXCR4-mediated production of IL-8 and VEGF by malignant human glioma cells. *J Neurooncol*, 84(1), 21-9 (2007)

66. B. C. Schmid, M. Rudas, G. A. Rezniczek, S. Leodolter and R. Zeillinger: CXCR4 is expressed in ductal carcinoma *in situ* of the breast and in atypical ductal hyperplasia. *Breast Cancer Res Treat*, 84(3), 247-50 (2004)

67. O. Tomescu, S. J. Xia, D. Strezlecki, J. L. Bennicelli, J. Ginsberg, B. Pawel and F. G. Barr: Inducible short-term and stable long-term cell culture systems reveal that the PAX3-FKHR fusion oncoprotein regulates CXCR4, PAX3, and PAX7 expression. *Lab Invest*, 84(8), 1060-70 (2004)

68. S. Singh, U. P. Singh, J. K. Stiles, W. E. Grizzle and J. W. Lillard: Expression and Functional Role of CCR9 in Prostate Cancer Cell Migration and Invasion. *Clinical Cancer Research*, 10(24), 8743-8750 (2004)

69. A. Maheshwari, R. D. Christensen and D. A. Calhoun: ELR+ CXC chemokines in human milk. *Cytokine*, 24(3), 91-102 (2003)

70. F. Basolo, P. G. Conaldi, L. Fiore, S. Calvo and A. Toniolo: Normal breast epithelial cells produce interleukins 6 and 8 together with tumor-necrosis factor: defective IL6 expression in mammary carcinoma. *Int J Cancer*, 55(6), 926-30 (1993)

71. D. A. Porter, I. E. Krop, S. Nasser, D. Sgroi, C. M. Kaelin, J. R. Marks, G. Riggins and K. Polyak: A SAGE

(serial analysis of gene expression) view of breast tumor progression. *Cancer Res*, 61(15), 5697-702 (2001)

72. K. Xie: Interleukin-8 and human cancer biology. *Cytokine Growth Factor Rev*, 12(4), 375-91 (2001)

73. C. Chavey, F. Bibeau, S. Gourgou-Bourgade, S. Burlinchon, F. Boissiere, D. Laune, S. Roques and G. Lazennec: Oestrogen receptor negative breast cancers exhibit high cytokine content. *Breast Cancer Res*, 9(1), R15 (2007)

74. L. Kozlowski, I. Zakrzewska, P. Tokajuk and M. Z. Wojtukiewicz: Concentration of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in blood serum of breast cancer patients. *Rocz Akad Med Bialymst*, 48, 82-4 (2003)

75. I. H. Benoy, R. Salgado, P. Van Dam, K. Geboers, E. Van Marck, S. Scharpe, P. B. Vermeulen and L. Y. Dirix: Increased serum interleukin-8 in patients with early and metastatic breast cancer correlates with early dissemination and survival. *Clin Cancer Res*, 10(21), 7157-62 (2004)

76. F. Basolo, S. Calvo, L. Fiore, P. G. Conaldi, V. Falcone and A. Toniolo: Growth-stimulating activity of interleukin 6 on human mammary epithelial cells transfected with the int-2 gene. *Cancer Res*, 53(13), 2957-60 (1993)

77. G. Luboshits, S. Shina, O. Kaplan, S. Engelberg, D. Nass, B. Lifshitz-Mercer, S. Chaitchik, I. Keydar and A. Ben-Baruch: Elevated expression of the CC chemokine regulated on activation, normal T cell expressed and secreted (RANTES) in advanced breast carcinoma. *Cancer Res*, 59(18), 4681-7 (1999)

78. Y. Niwa, H. Akamatsu, H. Niwa, H. Sumi, Y. Ozaki and A. Abe: Correlation of tissue and plasma RANTES levels with disease course in patients with breast or cervical cancer. *Clin Cancer Res*, 7(2), 285-9 (2001)

79. S. Ali, J. Kaur and K. D. Patel: Intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and regulated on activation normal T cell expressed and secreted are expressed by human breast carcinoma cells and support eosinophil adhesion and activation. *Am J Pathol*, 157(1), 313-21 (2000)

80. H. P. Dong, M. B. Elstrand, A. Holth, I. Silins, A. Berner, C. G. Trope, B. Davidson and B. Risberg: NK- and B-cell infiltration correlates with worse outcome in metastatic ovarian carcinoma. *Am J Clin Pathol*, 125(3), 451-8 (2006)

81. A. Mantovani, W. J. Ming, C. Balotta, B. Abdeljalil and B. Bottazzi: Origin and regulation of tumor-associated macrophages: the role of tumor-derived chemotactic factor. *Biochim Biophys Acta*, 865(1), 59-67 (1986)

82. A. Mantovani, P. Allavena and A. Sica: Tumour-associated macrophages as a prototypic type II polarised phagocyte population: role in tumour progression. *Eur J Cancer*, 40(11), 1660-7 (2004)

83. K. Okita, T. Furuhata, Y. Kimura, M. Kawakami, K. Yamaguchi, T. Tsuruma, H. Zembutsu and K. Hirata: The interplay between gastric cancer cell lines and PBMCs mediated by the CC chemokine RANTES plays an important role in tumor progression. *J Exp Clin Cancer Res*, 24(3), 439-46 (2005)

84. M. R. Hussein: Tumour-infiltrating lymphocytes and melanoma tumorigenesis: an insight. *Br J Dermatol*, 153(1), 18-21 (2005)

85. R. Fukui, H. Nishimori, F. Hata, T. Yasoshima, K. Ohno, H. Nomura, Y. Yanai, H. Tanaka, K. Kamiguchi, R. Denno, N. Sato and K. Hirata: Metastases-related genes in the classification of liver and peritoneal metastasis in human gastric cancer. *J Surg Res*, 129(1), 94-100 (2005)

86. R. P. Negus, G. W. Stamp, J. Hadley and F. R. Balkwill: Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. *Am J Pathol*, 150(5), 1723-34 (1997)

87. I. Simonitsch and G. Krupitza: Autocrine self-elimination of cultured ovarian cancer cells by tumour necrosis factor alpha (TNF-alpha). *Br J Cancer*, 78(7), 862-70 (1998)

88. S. A. Eissa, S. A. Zaki, S. M. El-Maghraby and D. Y. Kadry: Importance of serum IL-18 and RANTES as markers for breast carcinoma progression. *J Egypt Natl Canc Inst*, 17(1), 51-5 (2005)

89. K. B. Hahm, Y. J. Song, T. Y. Oh, J. S. Lee, Y. J. Surh, Y. B. Kim, B. M. Yoo, J. H. Kim, S. U. Han, K. T. Nahm, M. W. Kim, D. Y. Kim and S. W. Cho: Chemoprevention of Helicobacter pylori-associated gastric carcinogenesis in a mouse model: is it possible? *J Biochem Mol Biol*, 36(1), 82-94 (2003)

90. S. Singh, U. P. Singh, W. E. Grizzle and J. W. Lillard: CXCL12-CXCR4 interactions modulate prostate cancer cell migration, metalloproteinase expression and invasion. *Lab Invest*, 84(12), 1666 (2004)

91. S. Singh, R. Singh, U. P. Singh, S. N. Rai, K. R. Novakovic, L. W. K. Chung, P. J. Didier, W. E. Grizzle and J. James W. Lillard: Clinical and biological significance of CXCR5 expressed by prostate cancer specimens and prostate cancer cell lines. *International Journal of Cancer.*, In Press (2009)

92. E. Padovan, L. Terracciano, U. Certa, B. Jacobs, A. Reschner, M. Bolli, G. C. Spagnoli, E. C. Borden and M. Heberer: Interferon stimulated gene 15 constitutively produced by melanoma cells induces e-cadherin expression on human dendritic cells. *Cancer Res*, 62(12), 3453-8 (2002)

93. F. Sallusto, C. R. Mackay and A. Lanzavecchia: The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol*, 18, 593-620 (2000)

94. M. Darash-Yahana, J. W. Gillespie, S. M. Hewitt, Y. Y. Chen, S. Maeda, I. Stein, S. P. Singh, R. B. Bedolla, A. Peled, D. A. Troyer, E. Pikarsky, M. Karin and J. M. Farber: The chemokine CXCL16 and its receptor, CXCR6, as markers and promoters of inflammation-associated cancers. *PLoS One*, 4(8), e6695 (2009)

95. F. Burke and F. R. Balkwill: Cytokines in animal models of cancer. *Biotherapy*, 8(3-4), 229-41 (1996)

96. M. A. Nash, G. Ferrandina, M. Gordinier, A. Loercher and R. S. Freedman: The role of cytokines in both the normal and malignant ovary. *Endocr Relat Cancer*, 6(1), 93-107 (1999)

97. A. Mantovani, P. Allavena, S. Sozzani, A. Vecchi, M. Locati and A. Sica: Chemokines in the recruitment and shaping of the leukocyte infiltrate of tumors. *Semin Cancer Biol*, 14(3), 155-60 (2004)

98. D. Milliken, C. Scotton, S. Raju, F. Balkwill and J. Wilson: Analysis of chemokines and chemokine receptor expression in ovarian cancer ascites. *Clin Cancer Res*, 8(4), 1108-14 (2002)

99. T. J. Curiel, G. Coukos, L. Zou, X. Alvarez, P. Cheng, P. Mottram, M. Evdemon-Hogan, J. R. Conejo-Garcia, L. Zhang, M. Burow, Y. Zhu, S. Wei, I. Kryczek, B. Daniel, A. Gordon, L. Myers, A. Lackner, M. L. Disis, K. L. Knutson, L. Chen and W. Zou: Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med*, 10(9), 942-9 (2004)

100. R. M. Strieter, J. A. Belperio, R. J. Phillips and M. P. Keane: CXC chemokines in angiogenesis of cancer. *Semin Cancer Biol*, 14(3), 195-200 (2004)

101. B. Homey, A. Muller and A. Zlotnik: Chemokines: agents for the immunotherapy of cancer? *Nat Rev Immunol*, 2(3), 175-84 (2002)

102. J. Yoneda, H. Kuniyasu, M. A. Crispens, J. E. Price, C. D. Bucana and I. J. Fidler: Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. *J Natl Cancer Inst*, 90(6), 447-54 (1998)

103. L. Xu and I. J. Fidler: Interleukin 8: an autocrine growth factor for human ovarian cancer. *Oncol Res*, 12(2), 97-106 (2000)

104. Y. Wang, J. Yang, Y. Gao, Y. Du, L. Bao, W. Niu and Z. Yao: Regulatory effect of e2, IL-6 and IL-8 on the growth of epithelial ovarian cancer cells. *Cell Mol Immunol*, 2(5), 365-72 (2005)

105. H. Kajiyama, K. Shibata, M. Terauchi, K. Ino, A. Nawa and F. Kikkawa: Involvement of SDF-1alpha/CXCR4 axis in the enhanced peritoneal metastasis of epithelial ovarian carcinoma. *Int J Cancer*, 122(1), 91-9 (2008)

106. R. S. Freedman, Q. Ma, E. Wang, S. T. Gallardo, I. O. Gordon, J. W. Shin, P. Jin, D. Stroncek and F. M. Marincola: Migration deficit in monocyte-macrophages in human ovarian cancer. *Cancer Immunol Immunother*, 57(5), 635-45 (2008)

107. V. Levina, B. M. Nolen, A. M. Marrangoni, P. Cheng, J. R. Marks, M. J. Szczepanski, M. E. Szajnik, E. Gorelik and A. E. Lokshin: Role of eotaxin-1 signaling in ovarian cancer. *Clin Cancer Res*, 15(8), 2647-56 (2009)

108. R. K. Ganju, S. A. Brubaker, J. Meyer, P. Dutt, Y. Yang, S. Qin, W. Newman and J. E. Groopman: 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(36), 23169-75 (1998)

109. M. M. Robledo, R. A. Bartolome, N. Longo, J. M. Rodriguez-Frade, M. Mellado, I. Longo, G. N. van Muijen, P. Sanchez-Mateos and J. Teixido: Expression of functional chemokine receptors CXCR3 and CXCR4 on human melanoma cells. *J Biol Chem*, 276(48), 45098-105 (2001)

110. S. R. Neves, P. T. Ram and R. Iyengar: G protein pathways. *Science*, 296(5573), 1636-9 (2002)

111. M. Lopez-Ilasaca: Signaling from G-protein-coupled receptors to mitogen-activated protein (MAP)-kinase cascades. *Biochem Pharmacol*, 56(3), 269-77 (1998)

112. J. E. Celis, P. Gromov, T. Cabezon, J. M. Moreira, N. Ambartsumian, K. Sandelin, F. Rank and I. Gromova: Proteomic characterization of the interstitial fluid perfusing the breast tumor microenvironment: a novel resource for biomarker and therapeutic target discovery. *Mol Cell Proteomics*, 3(4), 327-44 (2004)

113. M. Rahmatullah and J. D. Robishaw: Direct interaction of the alpha and gamma subunits of the G proteins. Purification and analysis by limited proteolysis. *J Biol Chem*, 269(5), 3574-80 (1994)

Key Words: Chemokine, Cancer, Metastasis, Prostate, Breast, Ovary

Send correspondence to: Shailesh Singh, Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, 720 Westview Drive, Hugh Gloster Building, Room 341B, Atlanta, GA 30310, Tel: 404-756-5784, Fax: 404-752-1179, E-mail: shsingh@msm.edu

http://www.bioscience.org/current/vol3S.htm