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Chemokines in tumor angiogenesis and metastasis

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Abstract

Chemokines are a large group of low molecular weight cytokines that are known to selectively attract and activate different cell types. Although the primary function of chemokines is well recognized as leukocyte attractants, recent evidences indicate that they also play a role in number of tumor-related processes, such as growth, angiogenesis and metastasis. Chemokines activate cells through cell surface seven trans-membranes, G-protein-coupled receptors (GPCR). The role played by chemokines and their receptors in tumor pathophysiology is complex as some chemokines favor tumor growth and metastasis, while others may enhance anti-tumor immunity. These diverse functions of chemokines establish them as key mediators between the tumor cells and their microenvironment and play critical role in tumor progression and metastasis. In this review, we present some of the recent advances in chemokine research with special emphasis on its role in tumor angiogenesis and metastasis.

Keywords

Chemokines; Tumor growth; Angiogenesis; Metastasis; Tumor microenvironment

1 Introduction

The process of cancer metastasis consists of a series of sequential interrelated steps, each of which can be rate-limiting [1]. The major steps in the formation of a metastasis are as follows: (1) following initial transforming event, growth of neoplastic cells must be progressive, which requires extensive vascularization if a tumor mass is to exceed 2-mm in diameter; (2) local invasion of the host stroma by some tumor cells occurs as thin-walled venules, like lymphatic channels, offer very little resistance to penetration by tumor cells and provide the most common pathway for tumor cell entry into the circulation; (3) detachment and embolization of tumor cell aggregates occurs with subsequent arrest in the capillary beds of organs; (4) extravasation to secondary organ; and (5) proliferation and neovascularization within the distant organ parenchyma to produce detectable metastatic lesions. Intrinsic properties of the tumor cells, as well as their surrounding microenvironment, are crucial in defining the progression of cancer and the fate of metastases. Several factors have been identified that facilitate the interplay between tumor

cells and their microenvironment. Proliferation, neovascularization, invasion and migration of malignant cells to distinct organs are crucial steps for tumor progression and metastasis that can be regulated by chemokines (Fig. 1).

Over the last 20 years, it has been increasingly recognized that chemokines play an important part in regulation of the metastatic cascade, as they are known to be expressed by tumor cells, as well as by host cells in their proximity and at metastatic sites. A clear understanding of emerging roles of chemokines and the mechanisms of their actions in the processes of malignant progression and metastasis will open new doors for therapeutic interventions. In this review, we will highlight functional roles of the chemokines and their receptors, with a focus on angiogenesis and distant metastasis.

2 Chemokines and their receptors

The chemokine superfamily includes a large number of low molecular weight chemotactic proteins that regulate the trafficking of leukocytes to inflammatory sites [2–4]. Chemokines are generally 8–11 kDa in size and contain four conserved cysteine amino acid residues linked by disulfide bonds. Structurally, chemokines are classified into four (CXC, CC, C and CX₃C) subfamilies [3, 4]. There are more than 50 chemokines, the majority of which belong to the major CC and CXC chemokine subfamilies (Tables 1 and 2). According to a new classification, chemokine ligands/receptors are named ‘L’ or ‘R’, respectively [4]. Receptors are further grouped into four subfamilies, as each receptor binds to one of the four chemokine subfamilies (Tables 1 and 2). To avoid confusion, we have used the new designations and have listed their old and new names along with their respective receptors in Tables 1 and 2.

2.1 CXC chemokines

Members of the CXC (or α -chemokine) subfamily contain one non-conserved amino acid (X) between the first and second cysteine residues. Based on the presence or absence of a Glu-Leu-Arg (ELR) motif, the CXC chemokines can be further subdivided into two groups (ELR⁺ and ELR[−]) [3–5]. The ELR motif is located at the N-terminus immediately before the first cysteine amino acid residue [6]. Extensive investigations regarding the functions of the CXC subfamily have revealed that the presence/absence of the ELR motif determines the chemokine is angiogenic or angiostatic [7, 8]. The ELR⁺ chemokines are primarily chemotactic for neutrophils, and hence, are potent promoters of angiogenesis [7–10]. On the other hand, the main target(s) for ELR[−] members are T cells and B cells and they are potent inhibitors of angiogenesis [11]. The angiogenic CXC chemokine family members include CXCL1–3 and CXCL5–8 [7] whereas, angiostatic CXC chemokine members include CXCL4 (12) and CXCL9–11 [8, 11–13]. Although CXCL12, an ELR[−] chemokine, was originally described as a pre B-cell growth stimulating factor [14], it has also been shown to exhibit angiogenic activity [15–18]. Furthermore, ELR[−] CXC chemokines have been shown to inhibit neovascularization induced by classical angiogenic factors, such as basic fibroblast growth factor (FGF-2) and vascular endothelial cell growth factor (VEGF) [11].

2.2 CC chemokines

The CC chemokines (or β -chemokines) represent the largest family of chemokines and have adjacent cysteine residues. The known members of this family are CCL1–5, CCL7–8, CCL11 and CCL13–28, which are listed in Table 2. Members of this family exhibit the most diverse range of target cell specificities. They generally attract leukocytes, including monocytes, macrophages, T cells, B cells, basophils, eosinophils, dendritic cells, mast cells and natural killer cells [19–27]. So far, neutrophils have not been shown to respond to chemotactic stimuli from any of the CC chemokines.

2.3 C chemokines

Chemokines of the C subfamily (γ -chemokines) have only one of the cysteine residues and XCL1 (lymphotactin) [28] is the only member of this subfamily, with a molecular size of 16 kDa. This chemokine seems to be lymphocyte specific [28].

2.4 CX₃C chemokines

The CX₃C chemokine (δ -chemokines) have three non-conserved amino acids between the first two cysteines [29]. This family also has only one known member CX₃CL1 (Fractalkine), which has been shown to induce both the migration and the adhesion of leukocytes [30, 31]. In general, chemokines are secretory proteins, and CX₃CL1 is the only exception being a membrane-bound chemokine [32].

2.5 Chemokine receptors

All chemokines exert their biological function by binding to G-protein coupled receptors (GPCR) [3, 5, 33]. Chemokine receptors have an N-terminus outside the cell, three extracellular and three intracellular loops, and a C-terminus containing serine and threonine phosphorylation sites in the cytoplasm. In addition, they also possess seven hydrophobic trans-membrane domains, three extracellular and three intracellular loops. Currently, six CXC receptors, ten CC receptors, and one receptor each for C and CX₃C have been identified (Tables 1 and 2). Of the six CXC receptors, CXCR1 binds both CXCL8 and CXCL6 with high affinity [34, 35]. CXCR2, another receptor for CXCL8 has 78% identity with CXCR1 at the amino acid level. It has also been reported that CXCR2 can bind to all of the ELR⁺ CXC chemokines with high affinity [35–37]. The third receptor, CXCR3, has been shown to bind the ELR[−] CXC chemokines (CXCL9–11) [38–40]. CXCR4 has been shown to be a cofactor for human immunodeficiency virus (HIV) infection of T lymphocytes [41]. For CXCR4, the only known ligand is CXCL12, and this ELR[−] CXC chemokine can inhibit HIV infection by competing with lymphotropic HIV virus for binding of CXCR4 [42]. CXCR5, identified as a receptor on B lymphocytes, is responsible for B cell chemotaxis mediated by CXCL13 [43]. CXCR6 is a receptor for CXCL16, and was described previously as a fusion cofactor for HIV-1 and simian immunodeficiency virus (SIV) [44, 45].

Among the CC chemokine receptors, CCR1 is a receptor for CCL3, CCL5, CCL7–8, CCL13–16 and CCL23 [46–50]. CCR2 exists as two splice variants, CCR2a and CCR2b, among them CCR2b is the most studied and appears to bind at least CCL2, CCL7–8, CCL13, and CCL27 [51–53]. CCR3 binds to CCL5, along with CCL7–8, CCL11, CCL13,

NNY-CCL14 (CCL14 analogue), CCL15, CCL24 and CCL26–28 [26, 54–57]. CCR4 is a receptor for CCL17 and CCL22 [58, 59], whereas CCR5 is a major co-receptor for macrophage (M)-tropic HIV-1, HIV-2 and SIV strains [60]. CCR5 can bind to other CC chemokines (CCL2, CCL7–8 and CCL13) with decreased affinity [61]. CCR6 has been shown to be a receptor for CCL20 [62]. CCL19 and CCL21 bind to CCR7 [63] while CCR8 appears to bind CCL1 specifically [64]. CCR9 is a receptor for CCL25 [65]. Functional studies conducted on CCR10 indicate that it has high binding affinity for CCL2, CCL4, CCL27 and CCL28 [66, 67]. So far, one receptor each for C (XCR1) and CX₃C (CX₃CR1) chemokines have been identified (Table 1).

One of the remarkable features of the chemokine receptor superfamily is their promiscuity in ligand binding [68]. This suggests that the regulation of chemokine activities and the response in target cells are complex events. The intricate functional and regulatory nature of chemokine activities gives rise to diverse responses in normal homeostasis and pathological conditions, including angiogenesis and tumor metastasis.

3 Chemokines in angiogenesis

Angiogenesis is a biological process of new blood vessel formation from preexisting blood vessels and hence is also referred to as neovascularization. It is fundamental to many physiological as well as pathological processes in living organisms [69–72]. The process of angiogenesis is regulated by many angiogenic growth factors, enzymes, lipids and carbohydrates, including the members of the chemokine superfamily [8, 73]. Specific members of the chemokine superfamily can act as pro-angiogenic molecules and support the formation of new blood vessels, while others can antagonize these activities and therefore are angiostatic [9, 73].

Among all the chemokines, CXCL8 is extensively studied as a potent mediator of angiogenesis. The pro-angiogenic activity of CXCL8 *in vivo* was confirmed through the use of the rat mesenteric window assay, the rat and rabbit corneal assay, and a subcutaneous sponge model [74–77]. Human recombinant CXCL8 was shown to be angiogenic when implanted in the rat cornea and induced proliferation and chemotaxis of human umbilical vein endothelial cells [75]. In addition, the angiogenic properties of conditioned media from activated monocytes and macrophages were attenuated by CXCL8 anti-sense oligonucleotides [75]. Furthermore, it was shown that CXCL8 can act directly on vascular endothelial cells by promoting their survival [78]. Studies from our lab and other groups suggest that CXCL8 stimulates both endothelial proliferation and capillary tube formation *in vitro* in a dose dependent manner, and both of these effects can be blocked by monoclonal antibodies to CXCL8 [79, 80]. In addition, CXCL8 was shown to inhibit apoptosis of endothelial cells [81]. CXCL8 exerts its angiogenic activity by up-regulating MMP-2 and MMP-9 in tumor and endothelial cells [81–83]. Degradation of the extracellular matrix by MMPs is required for endothelial cell migration, organization, and, hence, angiogenesis [84]. It has been demonstrated by our group that CXCL8 directly enhances endothelial cell proliferation, survival, and MMP expression in CXCR1- and CXCR2-expressing endothelial cells, thus may be an important player in the process of angiogenesis [79].

Several investigators have suggested the angiogenic effect of CXCL8 is independent of its chemotactic and pro-inflammatory effects, since CXCL8 promotes angiogenesis in the absence of inflammatory cells [74, 77]. In addition, it has been reported that there is a direct correlation between high levels of CXCL8 and tumor angiogenesis, progression and metastasis in nude xenograft models of human cancer cells [83, 85]. In an experimental model of ovarian cancer, the expression of CXCL8 was directly correlated with neovascularization and poor survival [86]. CXCL8 may also play an important role in angiogenesis in prostate and breast cancer as serum levels of CXCL8 are elevated in patients with prostate and breast cancer, and correlate with disease stage [87–91]. The ability of CXCL8 to elicit angiogenic activity depends on the expression of its receptor by endothelial cells.

CXCL8 and its receptors, CXCR1 and CXCR2, have been observed on endothelial cells and have been shown to play a role in endothelial cell proliferation [75, 92, 93]. Recent studies indicate that CXCR1 is highly and CXCR2 is moderately expressed on human microvascular endothelial cells (HMEC), whereas human umbilical vein endothelial cells (HUVEC) show low levels of CXCR1 and CXCR2 expression [93]. Neutralizing antibodies to CXCR1 and CXCR2 abrogated CXCL8-induced migration of endothelial cells, indicating that these two receptors are critical for the CXCL8 angiogenic response [81, 93]. Of these two high affinity receptors for CXCL8, the importance of CXCR2 in mediating chemokine-induced angiogenesis was demonstrated to be fundamental to CXCL8-induced neovascularization [73, 94]. The role of CXCR2 in promoting tumor-associated angiogenesis has been confirmed in other tumor systems [95, 96].

In addition to CXCL8, other members of the chemokine family have been shown to play important roles in angiogenesis. Elevated levels of CXCL5 and CXCL8 correlated with the vascularity of non-small cell lung cancer (NSCLC) [73, 97]. In a severe combined immune-deficient (SCID) mouse model system, depletion of CXCL5 resulted in the attenuation of both tumor growth, angiogenesis and spontaneous metastasis [98]. In renal cell cancer, elevated levels of CXCL1, CXCL3, CXCL5 and CXCL8 were found to be expressed in the tumor tissue and detected in the plasma, and CXCR2 was found to be expressed on endothelial cells within the tumor biopsies [96]. Thus, multiple studies mentioned above demonstrate the important role of ELR⁺ CXC chemokines in tumor angiogenesis.

Despite of being a non-ELR, CXC chemokine, CXCL12 is of major interest in tumor angiogenesis. Several lines of evidence indicate that CXCL12 induces endothelial cell migration, proliferation, tube formation and increases in VEGF release by endothelial cells [99–101]. Blockade of CXCL12/CXCR4 results in decreased tumor growth *in vivo* due to inhibition of angiogenesis in a VEGF-independent manner [102]. The source of CXCL12 that drives angiogenesis is likely to be derived from specialized stromal cells and tumor cells [103]. In a recent study, it has been suggested that CXCL12 is partly responsible for the ability of breast carcinoma-associated fibroblasts to promote angiogenesis [16]. However, the effects of CXCL12 on angiogenesis cannot be generalized to all tumor systems, as inhibition of metastasis in a model of NSCLC by CXCL12 neutralization did not show reduction of tumor angiogenesis [104]. These observations suggest that the action of

CXCL12 may be tumor specific and/or may act in cooperation with other angiogenic proteins.

As mentioned above, the ELR⁻ CXC chemokines include angiostatic members that are known to inhibit neovascularization [9, 73, 101]. The angiostatic role of these chemokines has been demonstrated in several studies. For example, CXCL10 potently inhibited CXCL8- and FGF-2-induced angiogenesis [11]. Delivery of CXCL9 or CXCL10 into tumors by injection or by genetic manipulation to express the chemokines has been shown to suppress tumor angiogenesis [105–107]. In murine cancer models, intratumoral delivery of immunotherapeutic agents correlates with increased expression of CXCL9 and/or CXCL10 [108, 109]. Clinical studies of renal carcinoma patients have also indicated that intratumoral expression of CXCL9 and CXCL10 results in decreased tumor size [110]. It has been shown that CXCR3 is expressed on endothelial cells in a cell cycle-dependent manner, and its expression mediates the angiostatic activity of CXCL9–11 [111]. Recently, it has been suggested that overexpression of CXCL10 in human prostate LNCaP cells activates CXCR3 expression and inhibits cell proliferation [112]. These findings provide definitive evidence of CXCR3-mediated angiostatic activity by angiostatic ELR⁻ CXC chemokines. The presence of angiogenic and angiostatic regulators in the CXC chemokine family suggests that tumor angiogenesis may also be affected by the relative expression/activities of the different chemokines in the tumor microenvironment.

Recent reports demonstrate that CC chemokines can also participate in angiogenic activity in addition to members of CXC chemokine [113]. CCL2 has been added to the growing list of angiogenic modulators [113, 114]. Previous studies suggest that CCL2 indirectly stimulates angiogenesis [115, 116], however, recently it has been shown that CCL2 may also mediate angiogenic effects by acting directly on endothelial cells and increasing vascularity [114].

Fractalkine (FKN, CX₃CL1), a member of the CX₃C chemokine family, also belongs to the list of angiogenesis regulators [117–119]. Recent studies have suggested that the interaction of FKN and CX₃CR1 contributes to the pathogenesis of atherosclerosis [120, 121] and kidney diseases through the firm adhesion of leukocytes to endothelial cells [30, 122]. FKN has been also shown to participate in the pathogenesis of rheumatoid arthritis, probably by increasing the angiogenic process through endothelial cell activation [117–119]. The *in vivo* effect of FKN on angiogenesis has clearly shown that FKN plays a significant role in facilitating inflammatory angiogenesis by activating the GPCR [123].

Several lines of evidence suggest that a biological imbalance in the production of angiogenic and angiostatic factors, such as chemokines, contributes to the pathogenesis of several angiogenesis-dependent disorders, including cancer, rheumatoid arthritis, and psoriasis [11, 124–127]. However, the relative levels of these chemokines and their role in regulating tumor angiogenesis are not clear. More studies are needed to provide evidence that the imbalance in the expression of angiogenic or angiostatic chemokines regulates tumor angiogenesis. Based on published reports, one can predict that a shift in the balance of expression of these angiogenic and angiostatic chemokines dictates whether the tumor grows and develops metastasis or regresses. If this is correct, it will provide an opportunity to shift

this imbalance in favor of angiostasis by modulating the expression of the specific chemokine by pharmacological intervention to inhibit tumor growth and metastasis.

4 Chemokines and their receptors in tumor growth and metastasis

Cancer metastasis consists of multiple, complex interactions and interdependent steps [124, 128–131] where tumor cells exploit the host responses, which are a part of normal, physiological processes, in order to grow and metastasize, and chemokines play important roles in tumor-host interaction. To begin with, chemokines can provide chemo-attractive signaling that can be critical for cellular trafficking to distant organ sites. Different tumors express several chemokine receptors, and their corresponding ligands are expressed at the site of metastasis [132–134]. It has been shown that the site of metastasis depends on the characteristics of neoplastic cells and the specific microenvironment of the secondary organ [135]. Similar to leukocyte trafficking, the secondary organ of metastasis expresses constitutive levels of chemo-attractants that can provide signaling cues for malignant cell homing. The role of chemokines in organ specific metastasis was initially suggested in breast cancer. It was demonstrated that CCR7 and CXCR4 that are highly expressed in breast cancer cells determine the invasion and organ specificity of breast cancer metastasis [133]. The ligands CCL21 and CXCL12 for these receptors exhibit higher expression in the organs (lung and liver) that are preferred sites for breast cancer metastasis [133]. Recently, it has been suggested that osteoclasts may promote metastasis of lung cancer cells expressing CCR4 to the bone marrow by producing its ligand CCL22 [136]. Stimulation of osteoclast-like cells with CCR4 results in up-regulation of its ligand, CCL22. In addition, it has been demonstrated that a human lung cancer cell line, which expresses CCR4 metastasizes to bone when injected intravenously into NK cell-depleted SCID mice [136]. Together, the above data supports that the expression of chemokine ligands or their receptors in the organ environment or by malignant cells plays a major role in organ-specific metastasis.

CXCR4 appears to be the major chemokine receptor expressed on cancer cells. The expression of CXCR4 has been reported in more than 23 different types of cancer cell [137]. The accumulating evidence suggests that CXCR4 is an important regulator of breast cancer metastasis and can be predictive of lymph node metastasis [138–140]. In experimental studies, treatment of CXCR4-expressing breast cancer cells with neutralizing anti-CXCR4 antibody reduced metastasis to lung in a mouse model [133]. Gene array analysis of human breast cancer myoepithelial cells and myofibroblasts demonstrated up-regulation of both CXCR4 and CXCL12 which serve to enhance migration and invasion [141]. CXCR4 expression also mediates organ-specific metastasis of pancreatic cancer cells and a strong association of CXCR4 with advanced pancreatic cancer has also been suggested [142, 143]. *In vitro* stimulation of CXCR4-positive cancer cells with CXCL12 resulted in directed migration/invasion of ovarian cancer cells [144]. Stimulation with CXCL12 also up-regulated integrin expression and facilitated adhesion in lung cancer cells [145]. Furthermore, it has been shown that the expression of CXCR4 on human renal cells carcinoma (RCC) correlates with their metastatic ability in both heterotopic and orthotopic SCID mouse models [146]. Treatment with specific anti-CXCL12 antibodies markedly abrogated metastasis of RCC expressing high levels of CXCL12 to target organs in an orthotopic model of RCC, without significant changes in tumor cell proliferation, apoptosis,

or tumor-associated angiogenesis [146]. The above findings support the notion that the CXCL12/CXCR4 axis plays a critical role in regulating tumor growth and metastasis.

In addition, the clinical relevance of CXCR4 expression has been demonstrated in various types of cancers. In oesophageal cancer and melanoma, CXCR4 expression is associated with poor clinical outcome [147, 148]. In breast cancer, CXCR4 expression predicted lymph node metastasis [138]. Patients with high CXCR4 expression in NSCLC, colorectal or prostate cancer were more prone to metastasis [149–151]. The expression of CXCR4 in nasopharyngeal carcinoma and osteosarcoma is also associated with metastasis [152, 153]. In colorectal cancer, CXCR4 expression in primary tumors demonstrated significant association with recurrence, survival, and liver metastasis [154]. In contrast, a study of neuroblastoma patients found that, although neuroblastoma cells expressed CXCR4, this was not functional [155] and a study of approximately 300 breast cancer patients found that expression of CXCR4 was not associated with metastasis [156]. Above data strongly suggest the role of CXCR4 in regulating the progression of tumor cells to metastasize; however, additional investigations are needed to resolve the ambiguities in different studies.

CXCL8, a potent chemoattractant, has been demonstrated to contribute to human cancer progression through its potential function as a mitogenic and angiogenic factor. Elevated levels of CXCL8 have been detected in variety of tumors, such as ovarian carcinoma [157], NSCLC [158], metastatic melanoma [159], and colon carcinoma [160]. Studies from our laboratory and others suggest that the expression of CXCL8 correlates positively with disease progression [161–164].

A concomitant up-regulation of one of the two putative CXCL8 receptors has been reported in human melanoma specimens. Analysis of CXCR1 in human melanoma specimens from different Clark levels demonstrated that it was expressed ubiquitously in all Clark levels. In contrast, CXCR2 was expressed predominantly by higher grade melanoma tumors and metastases, suggesting an association between expression of CXCL8 and CXCR2 with vessel density in advanced lesions and metastasis [159]. In fact, the effect of CXCL8 can be mediated by CXCR1 and CXCR2, with CXCR1 being a selective receptor for CXCL8 [94]. Together, these data implicate that CXCL8 can directly modulate growth and the metastatic phenotype of cancer cells (Fig. 2).

CCR7 is another chemokine receptor highly expressed by several tumor types. CCR7 plays a critical role in lymphocyte and dendritic cell trafficking into and within lymph nodes. Human cancer cells from malignant breast, gastric, oesophageal, NSCLC, squamous cell carcinoma of the head and neck and colorectal carcinomas express functional CCR7 [133, 165–169]. Cancer cells that over-express CCR7 can migrate towards its ligand CCL21, which is strongly expressed in lymph nodes [167, 170]. It has been reported that when CCR7 was overexpressed in a murine melanoma cell line, metastasis to lymph nodes was increased *in vivo* [171]. Recently, it has been reported that CCR7 activation on thyroid carcinoma cell by CCL21 favors tissue invasion and cell proliferation, and therefore may promote thyroid carcinoma growth and lymph node metastasis [172]. CCR7 is a potential marker to predict metastasis in breast and colorectal cancer and is correlated with disease progression [138, 166]. In addition, patients with oral and oropharyngeal squamous cell

carcinomas that express CCR7 have lower survival rates than those with CCR7-negative tumors [173].

In addition, there are many more chemokines that have been detected in various cancers and are known to play role in metastasis. Recently, CCL2 was also shown to be extremely important in tumorigenesis and bone metastasis of several solid tumors [174]. The expression of CCR2 correlates with prostate cancer progression [175] and CCR3 in human renal cell carcinoma [176]. Co-expression of CCR4 and CCR10, the known pair of skin-homing chemokine receptors, may play an important role in adult T-cell leukemia/lymphoma (ATLL) invasion into the skin [177]. CCL5 and its receptor CCR5 has been detected on prostate cancer cell lines [178]. CCR6 and its ligand have been shown to be involved in colorectal cancer and its metastasis [179]; CCR9 on prostate and melanoma cells [180, 181]; and CCR10 on melanoma cells [133]. The expression of CCR10 and CCL27 in human melanomas increases the ability of neoplastic cells to grow, invade and disseminate to lymph node [182]. Expression of CXCR5 in carcinomas seems highly unlikely, but recently it has been shown that CXCR5 is expressed by colon carcinoma cells and promotes tumor growth and metastasis to liver [183]. In addition, prostate cancer cells express CX₃CR1 and stimulation with its ligand CX₃CL1 resulted in increased adhesion and migration [184].

It is possible that the cancer cell can exploit interferon-inducible chemokines for their own propagation, survival and metastasis. For example, CXCL9 and CXCL10 induce the migration and invasion of melanoma cell lines and multiple myeloma cells [185–187]. CXCR3, the receptor for CXCL9 and CXCL10 was shown to be expressed by cancer cells of solid tumors, such as breast tumor cells, renal carcinoma cells and melanoma cells [185, 188–190]. A direct role for CXCR3 in inducing tumor cell migration to metastatic sites was suggested by observations showing that the expression of this chemokine receptor by melanoma cells is causally involved in metastasis to lymph nodes [187]. Recently, it has also been demonstrated that CXCR3 plays a critical role in colon cancer cell metastasis to lymph nodes by inducing diverse cellular effects [191]. These data suggest that anti-malignant or pro-malignant behavior of these chemokines (such as CXCL9 and CXCL10) would be in part dictated by their receptor expression by the cancer cell.

Overall, the aberrant expression of chemokines and their receptors may be a common feature of various epithelial cancers. Although, the functional significance of some of these chemokines is yet to be elucidated, we can contemplate that the expression of certain chemokines and their receptors plays a key role in dictating the fate of developing tumors and their ability to metastasize to certain preferred organ sites.

5 Chemokine receptor signaling in tumor growth and metastasis

Despite the recent advances in our understanding of chemotaxis, the precise mechanisms through which tumor and endothelial cells respond to chemokines remains unclear. A conformational change in the GPCR trans-membrane domain is believed to be responsible for receptor activation. The initial events in chemokine-induced signal transduction determine the outcome of the response and must take place in the proximity of the receptor.

Here we will describe a simple schematic which is not meant to be complete, but to present the most evident effectors in chemokine receptor signaling. Activation of the receptor by a chemokine ligand induces the exchange in the α -subunit of the G-protein from GDP to the GTP bound state dissociating the α from β and γ from G-protein subunits. These subunits activate phospholipase (PL) C β 1 and C β 2, followed by hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP₂), which leads to formation of inositol triphosphate (IP₃) and diacylglycerol (DAG) with a subsequent increase in intracellular Ca²⁺ mobilization [33]. Although chemokine receptors lack tyrosine kinase activity, they can stimulate the phosphorylation of cytoskeletal proteins, p130 Cas and paxillin [192] and induce activation of the related focal adhesion kinases (FAKs) (also known as Pyk2 and CAK β) [193], mitogen activated protein kinases (MAPK) (ERK1/2, p38 and JNK) [193], phosphatidylinositol 3-kinase (PI3K) [193] and Janus kinase 2 (JAK2) [194, 195]. Extracellular signal-regulated kinases (ERK1 and ERK2) also termed p44/42 kinases, are important mediators of growth and other cell signaling [196, 197]. Because most of the G protein coupled receptors can activate a variety of effector pathways via various G protein subunits, considerable heterogeneity exists in signaling pathways leading to ERK1/2 phosphorylation and subsequent activation of transcription factors [33]. In addition, recent data suggests that signals dependent on G proteins mediated through *Rho* and *Rac* pathways result in actin polymerization, reconstitution of adhesion molecules and other cellular components leading to cell migration [198].

Chemokine-mediated signal transduction is more complex especially if one takes into account the cross-regulatory mechanism(s) of the integrated network [33, 199]. The analysis of signaling events further downstream from receptor activation is more complicated because of the potential contributions from various pathways since many pathways are shared by different receptor systems. For example, binding of CXCL8 to its receptors CXCR1 and CXCR2 can initiate many overlapping responses and might be mediated by diverse signal transduction mediators leading to distinct responses such as cell proliferation, survival and migration/invasion.

6 Conclusion and perspective

Overexpression of chemokines and chemokine receptors appears to be a hallmark of cancer. Furthermore, identifying the specific networks of chemokines and their receptors and understanding the effect of chemokines and their receptors expression on local immune response, angiogenesis and their interaction with other factors at the tumor milieu will reveal novel approaches for restricting tumor growth and metastasis.

The accumulating evidence resulting from the experimental studies points towards a critical role of chemokines and their receptors in cancer progression and metastasis. Many retrospective studies have now demonstrated that the expression of various chemokines and their receptors is associated with poor prognosis. This advocates that the measurement of chemokines and their receptor expression in tumor samples has the potential of becoming a biomarker of relative tumor aggressiveness. This will be helpful in selecting appropriate treatments for certain cancer patients. From the scientific perspective, we foresee that a better understanding of the biological and molecular mechanisms of chemokine-dependent

regulation of cellular phenotypes associated with tumor growth, angiogenesis and metastasis, will identify the cellular and molecular targets, which might be important in the distinct functional interactions of chemokines with their receptors on malignant cells and endothelial cells. An understanding of the mechanism(s) regulating modulation of chemokine-receptor pathways will be critical in prognosis and designing effective strategies for the development of novel targeted molecular therapeutics.

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Fig. 1.

The multifaceted role of chemokines in tumor growth, invasion and metastasis: Chemokines produced by tumor cells attract infiltrating leukocyte and/or promote proliferation and can also affect the microenvironment by promoting vascularization. Chemokines can stimulate their specific receptors that alter the adhesive capacity of tumor cells and their migration/invasion into circulation, and extravasation towards distant organs

Fig. 2. CXCL-8 in melanoma growth, angiogenesis and metastasis. CXCL-8 functions as an autocrine/paracrine growth factor for malignant melanoma cells [200–202], up-regulates expression of matrix metalloproteinase (MMP)-2 and MMP-9, regulates the migratory and invasive potential of melanoma cells and functions as a paracrine and autocrine angiogenic factor [200, 203–208]

Table 1Human CXC, C and CX₃C chemokines

Chemokines	Alternate name (<i>mouse protein</i>)	Known receptor(s)
CXC (alpha chemokines)		
CXCL1	GRO-α/SCYB-1/MGSA/GRO-1/NAP-3 (<i>MIP-2α/KC</i>)	CXCR1, CXCR2
CXCL2	GRO-β/SCYB-2/GRO-2/MIP-2 α (<i>MIP-2 β/KC</i>)	CXCR2
CXCL3	GRO-γ/SCYB-3/GRO-3/MIP-2 β (<i>KC</i>)	CXCR2
CXCL4	PF-4/SCYB-4	Unknown
CXCL5	ENA-78/SCYB-5 (<i>LIX</i>)	CXCR2
CXCL6	GCP-2/SCYB-6	CXCR1, CXCR2
CXCL7	NAP-2/SCYB-7/PBP/CTAP-III/β-TG	CXCR1, CXCR2
CXCL8	SCYB-8/GCP-1/NAP-1/MDNCF	CXCR1, CXCR2
CXCL9	MIG/SCYB-9	CXCR3
CXCL10	IP-10/SCYB-10	CXCR3, KSHV-GPCR
CXCL11	I-TAC/SCYB-11/β-R1/H174/IP-9	CXCR3
CXCL12	SDF-1/SCYB-12/PBSF	CXCR4
CXCL13	BCA-1/SCYB-13	CXCR5
CXCL14	BRAK/SCYB-14/Bolekine	unknown
CXCL16	Small inducible cytokine B6	CXCR6
C (gamma chemokines)		
XCL1	Lymphotactin/SCYC1/SCM-1α/Lymphotactin α	XCR1
XCL2	SCM-1b/SCYC2/ymphotactin β	XCR1
CX ₃ C (delta chemokines)		
CX ₃ CL1	Fractalkine/SCYD1	CX3CR1

Table 2

Human CC chemokines

Chemokines (beta chemokines)	Alternate name (mouse protein)	Known receptor(s)
CCL1	I-309/SCYA1 (<i>TCA-3</i>)	CCR8
CCL2	MCP-1/SCYA2/MCAF/HC11 (<i>JE</i>)	CCR2, CCR5, CCR10
CCL3	MIP-1 α /SCYA 3/LD78 α /SIS- α	CCR1, CCR5
CCL4	MIP-1 β /SCYA4/ACT-2/G-26/HC21/LAG-1/SIS- γ	CCR5, CCR10
CCL5	RANTES/SCYA5/SIS- δ	CCR1, CCR3, CCR5, CCR10
CCL7	MCP-3/SCYA7	CCR1, CCR2, CCR3 CCR5,
CCL8	MCP-2/SCYA8/HC14 (<i>MARC</i>)	CCR2, CCR3, CCR5 CCR1,
CCL11	Eotaxin/SCYA11	CCR3
CCL13	MCP-4/SCYA13/Ck β 10/NCC-1	CCR1, CCR2, CCR3, CCR5
CCL14	HCC-1/SCYA14/Ck β 1/MCIF/NCC-2/CC-1	CCR1
CCL15	MIP-1 δ /SCYA15/Lkn-1/HCC-2/MIP-5/NCC-3/CC-2	CCR1, CCR3
CCL16	HCC-4/SCYA16/Ck β 12/LEC/LCC-1/NCC-4/ILINCK/LMC/Mtn-1	CCR1
CCL17	TARC/SCYA17 (<i>ABCD-2</i>)	CCR4
CCL18	PARC/SCYA18/Ck β 7/DC-CK1/AMAC-1/MIP-4/DCtactin	unknown
CCL19	MIP-3 β /SCYA19/Ck β 11/ELC/Exodus-3	CCR7
CCL20	MIP-3 α /SCYA20/LARC/Exodus-1	CCR6
CCL21	6Ckine/SCYA21/Ck β 9/SLC/Exodus-2	CCR7
CCL22	MDC/SCYA22 (<i>ABCD-1</i>)	CCR4
CCL23	MPIF/SCYA23/Ck β 8/Ck β 8-1/MIP-3/MPIP-1	CCR1
CCL24	Eotaxin-2/SCYA24/Ck β 6/MPIF-2	CCR3
CCL25	TECK/SCYA25/Ck β 15	CCR9
CCL26	Eotaxin-3/SCYA26/MIP-4 α /TSC-1/IMA	CCR3
CCL27	CTACK/SCYA27/ESkine/Skinkine	CCR3, CCR2, CCR10
CCL28	CCL28/SCYA28/MEC	CCR10, CCR3