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Abstract

Chemokines were originally identified as mediators of the inflammatory process and regulators of leukocyte trafficking. Subsequent studies revealed their essential roles in leukocyte physiology and pathology. Moreover, chemokines have profound effects on other types of cells associated with the inflammatory response, such as endothelial cells and fibroblasts. Thus, chemokines are crucial for cancer-related inflammation, which can promote tumor development and progression. Increasing evidence points to the vital effects of several chemokines on the proliferative and invasive properties of tumor cells. The wide range of activities of chemokines in tumorigenesis highlights their roles in tumor development and progression.

Keywords; invasion, inflammation, neovascularization, leukocyte, fibroblast, metastasis.

Introduction

Chemokines are a superfamily of heparin-binding proteins and they are characterized by the presence of 4 cysteine residues inconserved positions [1]. Two intramolecular disulfide bonds are formed between the first and third cysteines, and between the second and fourth cysteines, and these bonds result in the formation of triple-stranded β-sheet structures [2]. The carboxy-terminal region forms an α-helix and has the capacity to bind heparin. Through the carboxy-terminal region, chemokines bind to proteoglycans on the vascular endothelium and other cell surfaces, and may be immobilized by proteoglycans on endothelial cells and in the extracellular matrix proteins. All chemokine receptors are 7-span transmembrane proteins that signal mainly through receptor-coupled trimeric G proteins.

Chemokines are divided into 4 subgroups, namely, CXC, CC, CX_3C, and C [1]. The first 2 cysteines are separated by 1 and 3 amino acids in CXC and CX_3C chemokines, respectively, while the first 2 cysteines are adjacent in CC chemokines. The single C chemokine lacks the second and fourth cysteines [1]. Systematic chemokine nomenclature is based on their cysteine subclass roots, followed by “L” for “ligand” [3]. The numbers correspond to the number used in the corresponding gene nomenclature. The CXC chemokines are further grouped based on the presence or absence of a 3-amino acid sequence, glutamic acid-leucine-arginine (the “ELR” motif), immediately preceding the CXC sequence [4]. Because most chemokine receptors can bind to a single chemokine subclass, the nomenclature system of chemokine receptors is rooted by the chemokine subclass specificity, followed by “R” for “receptor” and the corresponding number [3] (Table).

The human and mouse chemokine families differ in gene number, and the orthologous relationship between them remains ambiguous [5]. A notable difference between species has been found in one of the major chemokine, CXCL8, and its receptors, CXCR1 and CXCR2. Mice and rats do not possess a homolog of the *CXCL8/IL-8* gene, which is present in other species, including humans [5]. Moreover,

the *CXCR1* and *CXCR2* genes encode functional receptor proteins in humans, whereas no functional CXCR1 has been identified in mice or rats [6]. These findings should be taken into consideration when observations obtained with mouse models are extrapolated to human situations.

In the present review, we discuss the potential roles of chemokines in tumor development and progression by focusing on their effects on components of tumor tissues, including cancer cells, leukocytes, endothelial cells, and fibroblasts.

Direct effects on cancer cells

The tyrosine kinase, RET, which is a prototypic transforming oncogene in human papillary thyroid carcinoma induces the expression of CCL2, CCL20, ELR-positive CXC chemokines, and CXCL12 and its receptor, CXCR4 [7]. Moreover, components of the Ras-Raf signaling pathway induce the production of CXCL8 by activating NF-κB [8]. Constitutive activation of NF-κB induces the expression of chemokines, including CXCL1, CXCL8, and CCL2 in various types of tumors [9]. The *CXCR4* gene can further be transactivated by the activation of hypoxia-inducible factor (HIF)-1α, arising from either loss of the von-Hippel-Lindau tumor suppressor (VHL) or due to the hypoxic conditions observed frequently in tumor tissues [10]. NF-κB and HIF are also activated in inflammation, which is frequently associated with tumorigenesis [11]. Thus, even in the absence of direct oncogene activation, the expression of chemokines and their receptors can be augmented in tumor sites.

The term “cellular senescence” has been used to denote a stable and long-term loss of proliferative capacity, despite continued viability and metabolic activity [12]. Cellular senescence can occur in response to the activation of an oncogene. Oncogene-induced senescence (OIS) serves as a potent barrier against oncogenic transformation by suppressing the unscheduled proliferation of early neoplastic cells [12]. Two independent groups reported that cells undergoing OIS secrete CXCR2-binding chemokines and IL-6 through the activation of 2 pro-inflammatory transcription factors, C/EBP-β and NF-κB [13, 14]. In human colon adenomas,

CXCL8/IL-8 specifically colocalizes with arrested $p16^{INK4A}$-positive epithelium [13]. Furthermore, the reduction of CXCR2 expression alleviates OIS and diminishes the DNA-damage response, while ectopic expression of CXCR2 results in premature senescence via a p53-dependent mechanism [14]. Thus, CXCR2-binding chemokines can promote the arrest of cellular growth and eventually delay the early phase of tumorigenesis (Figure 1).

Epithelial-mesenchymal transition (EMT) is a physiological process that occurs during embryogenesis [15]. During EMT, epithelial cells lose the expression of components of cell polarity, such as E-cadherin, while expressing mesenchymal components of the cytoskeleton and acquiring motility and scattering properties. A closely related phenotypic conversion is detected in cancer and is associated with the capacity of tumor cells to invade and metastasize [15]. In response to prolonged exposure to transforming growth factor (TGF)-β, rat hepatoma cells exhibit a mesenchymal phenotype, and a higher migratory and invasive capacity, coincident with increased CXCR4 expression [16]. The invasive capacity of TGF-β-exposed cells is reduced by a CXCR4 antagonist [16]. Likewise, the expression of CXCL8 and its receptor, CXCR1, is induced in a human colon cancer cell line undergoing TGF-β-driven EMT [17]. Increasing lines of evidence indicate that EMT in cancer cells is initiated by overexpression of several transcription factors, including Twist and Snail [15]. Overexpression of a T-box transcription factor, Brachyury, also induces EMT in human pancreatic cancer cell lines, along with enhanced expression of several chemokines, including CXCL8, CCL5, and CXCL1 [18]. The inhibition of CXCL8 signaling pathway also abrogates Brachyury-induced EMT phenotypes and invasive capacity of cells [18]. These observations suggest that several chemokines may play important roles in EMT, which is a necessary step for the malignant progression of cancer (Figure 1).

Chemokine receptor engagement can also activate the mitogen-activated protein (MAP)/Erk kinase pathway [19], leading to gene expression and cell proliferation. CXCL8 can induce human gastric cancer cell lines, which possess CXCR1 and

CXCR2, to express epidermal growth factor (EGF) receptor, matrix metalloproteinase (MMP)-9, and vascular endothelial growth factor (VEGF) [20]. The same pathway is utilized to promote proliferation of esophageal cancer cells [21] and melanoma cells [22]. Accumulating evidence also implicates CXCR4 in the proliferation of various cancer cells including ovarian, glioma, melanoma, lung, renal, and thyroid cancer cells [23, 24]. The CXCL12/CXCR4 axis delivers surviving signals to hepatoma, ovarian, and chronic leukemia cells and CXCR4 blockade induces the apoptosis of these malignant cells [16, 25, 26] (Figure 1). CXCL12 expression correlates well with lower apoptosis in human myelodysplastic syndrome [27]. Likewise, CCR6 and CXCR6 can promote the proliferation of colorectal cancer cells [28] and prostate cancer cells [29], respectively. Furthermore, phosphatidylinositol-3-kinase (PI3K)-mediated protection of tumor cells from apoptosis has been reported after CCR10 activation in melanoma [30] and CCR7 activation in squamous cell carcinoma of the head and neck [31] (Figure 1). In contrast, CCR5 blockade can enhance the proliferation of breast cancer cells, which express wild-type p53 [32]. This is supported by the observation that disease-free survival is shorter in breast cancer patients bearing the CCR5Δ32 allele with a premature stop codon, than in CCR5 wild-type patients; this holds true only in wild-type p53-expressing tumors [32]. Likewise, human hepatocellular carcinoma (HCC) cells express CCR1 and its ligands can reduce the proliferation of human HCC cell lines [33].

Circulating tumor cells (CTCs) are presumed to be a source of metastasizing tumor cells, but they can also colonize tumors at their original site. This process, which is called tumor self-seeding, can accelerate tumor growth and angiogenesis. CTCs produce ELR-positive CXC chemokines including CXCL8 and CXCL1, and these chemokines eventually promote self-seeding [34].

Adult T cell leukemia (ATL) cells are characterized by frequent expression of CCR4 and can migrate *in vitro* to CCL17 and CCL22, which are ligands for CCR4 [35]. The CCL17/CCL22-CCR4 axis may account for the frequent infiltration of ATL into skin and lymph nodes, where CCL17 and CCL22 are abundantly expressed.

Humanized monoclonal antibody to CCR4 has been proven to be effective against ATL [36].

CXCR4 is the most commonly detected chemokine receptor in tumor cells, and CXCR4-expressing cells can migrate *in vitro* towards CXCL12 [26, 27]. CCR7, CCR9, CXCR1, and CXCR2 are also detected in tumor cells and their ligands can induce the chemotaxis of the corresponding receptor-expressing cells [37-40]. These chemokines can serve as inducers of invasion within the primary tumor and dissemination to distant organs (Figure 1).

Several models have been proposed to explain the molecular mechanisms underlying the enhancement and regulation of metastasis by these chemokines. Some research suggested that specific chemokine receptor-expressing tumor cells migrate to organs with high expression levels of the respective chemokines along a concentration gradient [37]. This hypothesis may explain the tissue tropism observed in certain types of cancer, but there is little evidence to indicate the presence of chemokine concentration gradients between primary and metastatic sites. Shields et al. demonstrated that a transcellular CCR7 ligand gradient is created when cancer cells produce CCR7 ligands under flow conditions and that this resultant gradient is the basis of lymphatic metastasis [41]. These observations would indicate that cancer cells themselves actively promote their own metastasis and tropism by producing chemokines. Another plausible explanation is that the arrival of tumor cells in a specific organ is passive and that chemokine receptor expression provides tumor cells with an advantage to survive and grow in a different ligand-rich metastatic microenvironment [42]. Moreover, CXCL12 and a CCR7 ligand, CCL21, can reduce the sensitivity of cancer cells to anoikis, which is believed to be one of the major blocks in the metastatic spread of various types of cancer, by regulating pro-apoptotic Bmf and anti-apoptotic Bcl-xL proteins [43]. Thus, chemokines may accelerate metastasis by promoting tumor cell proliferation or preventing tumor cell death.

These findings collectively suggest that chemokines can prevent tumorigenesis in the early phase by inducing cellular senescence and can also promote invasion and metastasis by enhancing the motility and survival of tumor cells (Figure 1).

Leukocytes

It is widely acknowledged that leukocytes are localized in both the tumor-supporting stroma and the tumor areas and might account for up to 50% of the tumor mass, the most predominant subset being macrophages [22, 44]. Tumor-associated macrophages (TAMs) are derived mostly from circulating monocytes which are attracted into tumor sites, by locally produced chemotactic factors, such as CCL2, CCL5, CCL7, CCL8, CXCL12, and macrophage colony stimulating factor (M-CSF) [22].

Several lines of evidence indicate that CCL2 plays an important role in TAM recruitment among these chemotactic factors [22, 44]. Combined treatment with azoxymethane and repeated dextran sodium sulfate solution ingestion causes the development of multiple tumors in murine colons, together with a massive infiltration of monocytes/macrophages expressing cyclooxygenase (COX)-2, an enzyme crucially involved in colon carcinogenesis [45]. Abundant CCL2 is detected in colon tissues, and it induces CCR2-positive COX-2 expressing monocytes/macrophages to infiltrate colon tissues, thereby promoting colon carcinoma development and progression [45]. Moreover, CCL2 recruits monocytes to pulmonary metastatic sites of murine breast cancer. As a consequence, infiltrated monocytes promote the extravasation of tumor cells, a necessary step for metastasis, in a process that requires monocyte-derived VEGF [46].

TAMs produce various growth factors such as TGF-β and fibroblast growth factor (FGF) in addition to VEGF and prostaglandin [22, 44]. Moreover, TAMs exhibit the properties of M2-polarized macrophages and express scavenger receptors and the mannose receptor [47]. Furthermore, they produce immunosuppressive molecules including IL-10, TGF-β, and arginase [47]. These properties endow TAMs with an

immunosuppressive capacity. Thus, TAMs can promote tumor progression through the production of growth factors and the suppression of adaptive anti-tumor immunity (Figure 2).

Tumor tissues contain additional types of cells that can suppress adaptive immunity. One prominent example is myeloid-derived suppressor cells (MDSCs), which are characterized by the co-expression of the myeloid-cell lineage differentiation antigen Gr-1 and CD11b in the mouse [48]. In humans, MDSCs are defined as $CD14^{-}CD11b^{+}$ cells or as cells that express the common myeloid marker CD33 but lack the expression of mature myeloid and lymphoid markers [48]. MDSCs express the immunosuppressive enzymes, arginase 1 and inducible NO synthetase (iNOS), and produce immunosuppressive cytokines such as TGF-β1 and IL-10, thereby inhibiting the T cell response [48]. CCL2 recruits MDSCs in several types of mouse cancer, including Lewis lung carcinoma, methA sarcoma, melanoma and lymphoma [49]. However, CCR2 deficiency results in the conversion of the MDSC phenotype to neutrophil lineage without affecting tumor growth [50]. CXCL5 and CXCL12 also induce MDSC infiltration in mouse mammary adenocarcinoma [51] (Figure 2). However, the roles of chemokines in MDSC recruitment in human are still poorly defined.

Regulatory T (Treg) cells are physiologically engaged in the maintenance of immunological self-tolerance [52]. A large number of Treg cells often infiltrate into tumors and systemic removal of Treg cells enhances natural as well as vaccine-induced anti-tumor T cell immunity. Treg cells express CCR4, and its ligand, CCL22, regulates intratumoral Treg infiltration in various tumors [52]. Hypoxia induces the expression of another chemokine, CCL28, in tumor sites. CCL28 promotes angiogenesis and recruits Treg cells, thereby also propagating immune tolerance [53] (Figure 2).

Anti-tumor responses are attributable to several types of cells infiltrating into a tumor site, such as tumor infiltrating lymphocytes (TIL) and dendritic cells [54]. An abundance of TILs is a favorable prognostic sign in various human cancers, especially

in colorectal cancer [55]. TILs express the chemokine receptor, CXCR3, and the corresponding ligands, CXCL9 and CXCL10, can elicit anti-tumor responses, which are accompanied by increased infiltration of CD4- and CD8-positive lymphocytes [56]. Another chemokine, CXCL16, can also recruit TIL and tumors expressing high levels of CXCL16 have slower progression with infiltration of CD4- and CD8-positive lymphocytes [57] (Figure 2). The appearance of apoptotic cells induces the migration of dendritic cells to the draining lymph nodes and eventually generates a specific cytotoxic T lymphocyte population by utilizing the CCL3-CCR5/CCR1 axis [58].

CCL2 transduction in tumor cells does not enhance but rather retards tumor growth *in vivo* by activating natural killer (NK) cell activity [59]. Transduction of *CCL2* may result in its expression to a larger extent compared with endogenous production by tumor cells, and may thus modulate the functions of NK cells. Thus, chemokines may act on different types of cells present in tumor sites and may have different effects on tumorigenesis, in a context-dependent manner.

Neovascularization

Neovascularization is crucial for tumor growth, progression, and metastasis [60]. Chemokines have critical roles in tumor neovascularization [61]. The ELR-positive CXC chemokines, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 directly promote the migration and proliferation of endothelial cells and eventually neovascularization, mainly by interacting with CXCR2 but not with CXCR1 [62]. Although CXCL12 is not an ELR-positive CXC chemokine, it also has potent angiogenic effects [36]. In addition, 3 CC chemokines, CCL2, CCL11, and CCL16 have also been implicated in tumor neovascularization [63-65]. CCR2, a specific receptor for CCL2, is expressed by endothelial cells and CCL2 exerts its angiogenic activity in a membrane type 1 (MT1)-MMP-dependent manner [63] (Figure 2).

CXCL4 and interferon-inducible ELR-negative CXC chemokines such as CXCL9, CXCL10, and CXCL11 inhibit the angiogenesis induced by ELR-positive CXC

chemokines, VEGF, and bFGF [66, 67]. The anti-angiogenic effects of these chemokines are mediated by a common receptor, CXCR3 (Figure 2). Moreover, the Duffy antigen, which can sequester the ELR motif-positive CXC chemokines without eliciting any intracellular signals (Table 1), has been shown to suppress the angiogenic effects of the ELR- positive CXC chemokines [68]. Thus, the balance between pro-angiogenic and anti-angiogenic chemokines may determine the degree of tumor neovascularization.

Chemokines have indirect roles in tumor angiogenesis by regulating the trafficking of several types of myeloid-derived cells including TAMs and MDSCs [22, 44]. These cells are recruited to tumor sites mainly by CCL2, and they promote angiogenesis by producing a wide variety of angiogenic factors such as VEGF, TGF-β, CXCL8, platelet-derived growth factor (PDGF), and MMPs such as MMP-2 and MMP-9. Moreover, recruited TAMs and MDSCs may acquire endothelial cell phenotypes and are incorporated into the newly formed vascular structure [69]. Endothelial cell-derived ELR-positive CXC chemokines, especially CXCL6, induce angiogenesis in gastrointestinal cancer by recruiting neutrophils [70].

Fibroblasts

Fibroblasts present in tumor tissues are designated as cancer-associated fibroblasts (CAFs). These fibroblasts are attracting increasing attention as producers of tumor-promoting molecules such as TGF-β, FGF, hepatocyte growth factor (HGF), and EGF [71]. The proposed cellular sources of CAFs include locally resident fibroblasts, cells undergoing EMT, cells undergoing endothelial-mesenchymal transition (EndMT), or bone marrow (BM)-derived mesenchymal stem cells (MSCs) [72]. In a mouse gastric cancer model, it is estimated that BM-derived MSCs can contribute as much as 25 % to the CAF population [73]. Conversely, in a mouse lung metastasis model, BM-derived cells do not significantly contribute to fibroblast accumulation in lungs [74].

In a mouse gastric cancer model, CXCL12 expression was found to be enhanced together with enhanced fibrosis in tumor sites [73]. Because MSCs express CXCR4 and CXCR7, and migrate to their ligand, CXCL12, the CXC12-CXCR4/CXCR7 axis may regulate the accumulation of fibroblasts and consequent fibrosis development [72]. Moreover, CAFs present in various types of cancer produce abundant CXCL12, which in turn promotes the angiogenic, proliferative, and migratory properties of tumor cells. CAFs produce CCL2, CCL5, CCL7, CXCL8, and CXCL14 and these chemokines promote tumor progression mainly by enhancing the motility of tumor cells [72].

In a mouse lung metastasis model, HGF-expressing fibroblasts were found to be increased in the lungs [74]. Lung fibroblasts express CCR5, and genetic deletion of CCR5 or its ligand, CCL3, attenuates intrapulmonary lung metastasis formation together with reduced fibroblast accumulation and HGF expression [74] (Figure 2).

When activated, hepatic stellate cells (HSCs) express α-smooth muscle actin (α-SMA), a marker of myelofibroblasts. α-SMA-positive HSCs secrete CXCL12, just as CAFs do [75]. Moreover, in a mouse liver metastasis model, tumor-derived CCL2 was found to induce the accumulation of α-SMA-positive HSCs to accumulate at tumor sites and the expression of MMP-2. Genetic deletion of CCR2 markedly attenuates tumor formation with reduced HSC accumulation and MMP-2 expression [76]. Thus, CCR2-mediated signals may regulate the trafficking and functions of HSCs in tumorigenesis.

Concluding Remarks

Cancer-related inflammation denotes the association of inflammation with tumor development and progression. Inflammatory responses consist of leukocyte infiltration, neovascularization, and fibrosis. Chemokines were initially identified as mediators of the inflammatory response, capable of regulating the trafficking of leukocytes, but subsequent investigations revealed that chemokines also have profound effects on endothelial cells and fibroblasts. Thus, chemokines play crucial

roles in cancer-related inflammation by affecting the major components of the inflammatory response, namely leukocytes, endothelial cells, and fibroblasts. Moreover, several chemokines have direct effects on the proliferative and invasive properties of tumor cells. In view of the multifactorial roles of chemokines in tumorigenesis, the elucidation of their roles will further advance our understanding of the pathophysiological processes of tumor development and progression.

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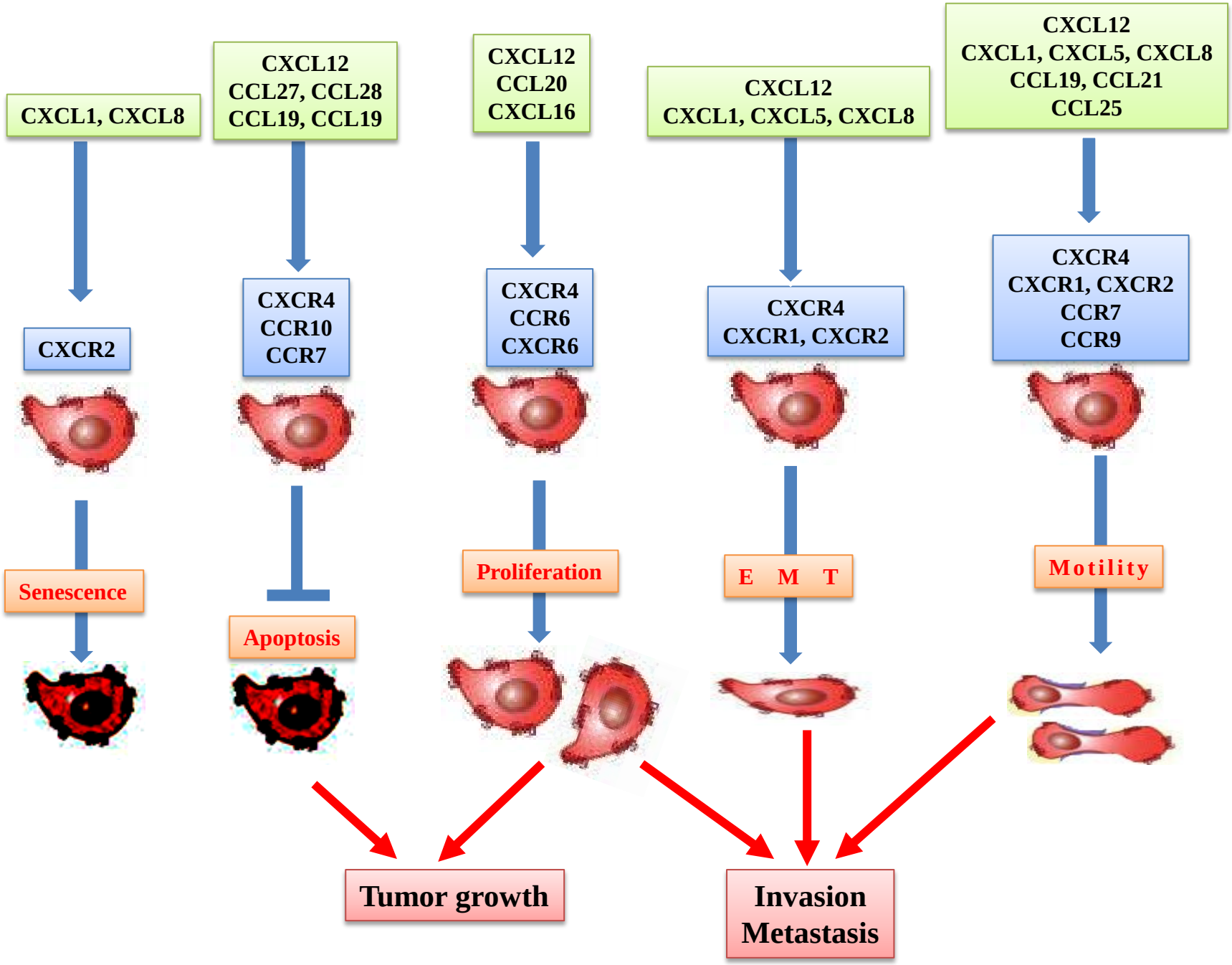
Chemokine Receptor	Chemokines	Receptor Expression in			
		Leukocytes	Epithelium	Endothelium	CAF
CXCR1	CXCL6, 8	PMN	+	-	-
CXCR2	CXCL1, 2, 3, 5, 6, 7, 8	PMN	+	+	-
CXCR3	CXCL4, 9, 10, 11	Th1, NK	-	+	-
CXCR4	CXCL12	Widespread	+	+	-
CXCR5	CXCL13	B	-	-	-
CXCR6	CXCL16	activated T	+	-	+
CXCR7	CXCL12, CXCL11	Widespread	+	+	-
Unknown	CXCL14 (acts on monocytes)				
CCR1	CCL3, 4, 5, 7, 14, 15, 16, 23	Mo, Mϕ, iDC, NK	+	+	-
CCR2	CCL2, 7, 8, 12, 13	Mo, Mϕ, iDC, NK activated T, B	+	+	-
CCR3	CCL5, 7, 11, 13, 15, 24, 26, 28	Eo, Ba, Th2	-	+	-
CCR4	CCL2, 3, 5, 7, 22	iDC, Th2, NK, T, Mϕ	-	-	-
CCR5	CCL3, 4, 5, 8	Mo, Mϕ, NK, Th1 activated T	+	-	+
CCR6	CCL20	iDC, activated T, B	+	-	-
CCR7	CCL19, 21	mDC, Mϕ, naïve T activated T	+	-	-
CCR8	CCL1, 4, 17	Mo, iDC, Th2, Treg	-	-	-
CCR9	CCL25	T	+	-	-
CCR10	CCL27, 28	activated T, Treg	+	-	-
Unknown	CCL18 (acts on mDC and naïve T)				
CX3CR1	CX3CL1	Mo, iDC, NK, Th1	+	-	-
XCR1	XCL1, 2	T, NK	-	-	-
Miscellaneous					
Duffy antigen	CCL2, 5, 11, 13, 14, CXCL1, 2, 3, 7, 8				
D6	CCL2, 3, 4, 5, 7, 8, 12, 13, 14, 17, 22				

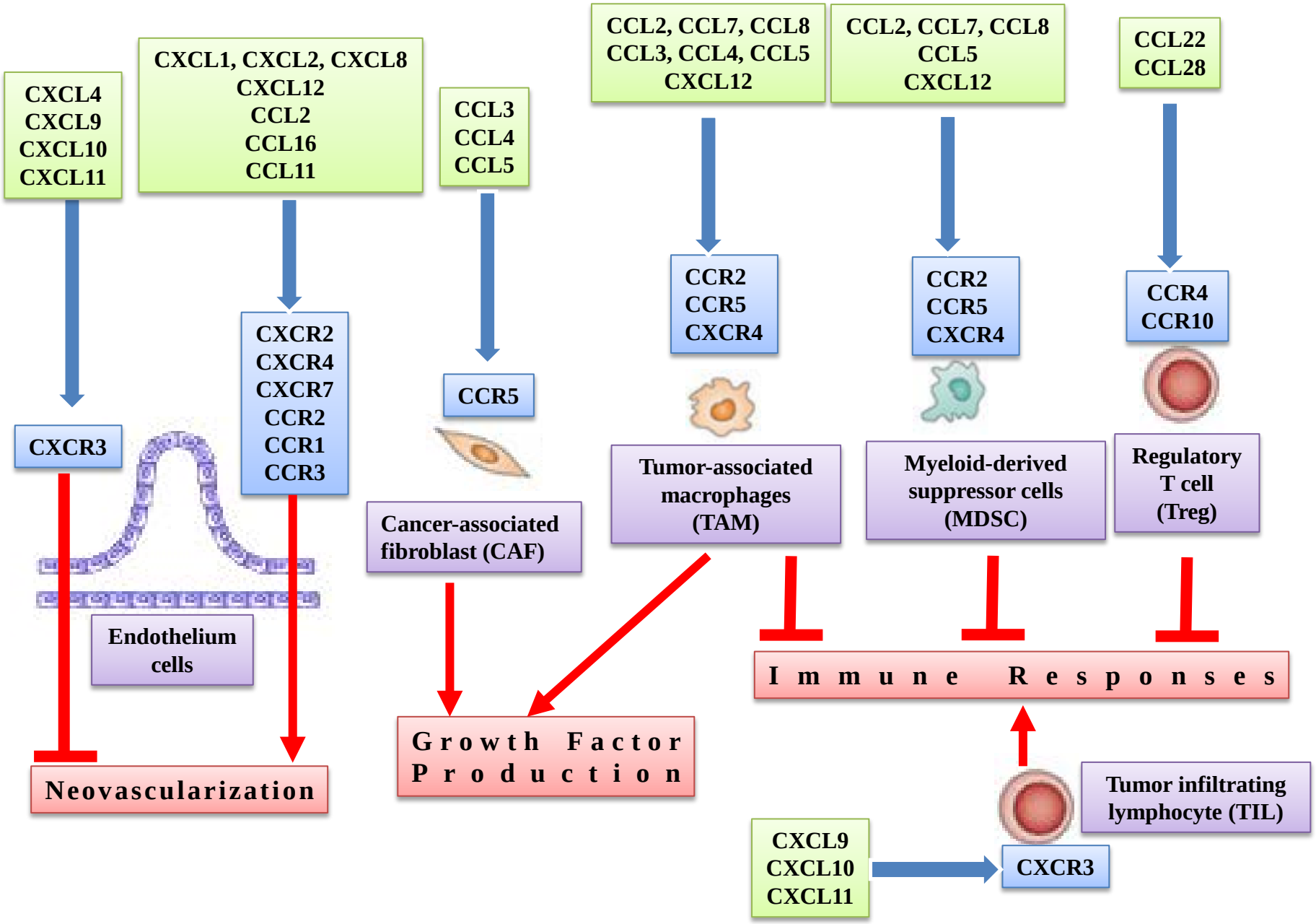
Table 1. The human chemokine system. Leukocyte anonyms are as follows: Ba, basophil; Eo, eosinophil; iDC, immature dendritic cell; mDC, mature dendritic cell; Mo, monocyte; Mϕ, macrophage; NK, natural killer cell; Th1, type I helper T cell; Th2, type II helper T cell; Treg, regulatory T cell. CAF, cancer-associated fibroblast.

Legend to Figure

Figure 1. Effects of chemokines on tumor cells.

Figure 2. Chemokine-mediated interaction between tumor cells and stromal cells.


CXCL1, CXCL8
CXCR2
Senescence
CXCL12
CCL27, CCL28
CCL19, CCL19
CXCR4
CCR10
CCR7
Apoptosis
CXCL12
CCL20
CXCL16
CXCR4
CCR6
CXCR6
Proliferation
CXCL12
CXCL1, CXCL5, CXCL8
CXCR4
CXCR1, CXCR2
E M T
CXCL12
CXCL1, CXCL5, CXCL8
CCL19, CCL21
CCL25
CXCR4
CXCR1, CXCR2
CCR7
CCR9
Motility
Tumor growth
Invasion
Metastasis


CXCL4
CXCL9
CXCL10
CXCL11
CXCL1, CXCL2, CXCL8
CXCL12
CCL2
CCL16
CCL11
CCL3
CCL4
CCL5
CCL2, CCL7, CCL8
CCL3, CCL4, CCL5
CXCL12
CCL2, CCL7, CCL8
CCL5
CXCL12
CCL22
CCL28
CXCR3
CXCR2
CXCR4
CXCR7
CCR2
CCR1
CCR3
CCR5
CCR2
CCR5
CXCR4
CCR2
CCR5
CXCR4
CCR4
CCR10
Endothelium cells
Cancer-associated fibroblast (CAF)
Tumor-associated macrophages (TAM)
Myeloid-derived suppressor cells (MDSC)
Regulatory T cell (Treg)
Neovascularization
Growth Factor Production
Immune Responses
Tumor infiltrating lymphocyte (TIL)
CXCL9
CXCL10
CXCL11
CXCR3