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Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy

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Abstract

The tumour microenvironment is the primary location in which tumour cells and the host immune system interact. Different immune cell subsets are recruited into the tumour microenvironment via interactions between chemokines and chemokine receptors, and these populations have distinct effects on tumour progression and therapeutic outcomes. In this Review, we focus on the main chemokines that are found in the human tumour microenvironment; we elaborate on their patterns of expression, their regulation and their roles in immune cell recruitment and in cancer and stromal cell biology, and we consider how they affect cancer immunity and tumorigenesis. We also discuss the potential of targeting chemokine networks, in combination with other immunotherapies, for the treatment of cancer.

Chemokines are small, secreted proteins that are best known for their roles in mediating immune cell trafficking and lymphoid tissue development[1,2]. The chemokines are the largest subfamily of cytokines and can be further subdivided into four main classes depending on the location of the first two cysteine (C) residues in their protein sequence: namely, the CC-chemokines, the CXC-chemokines, C-chemokines and CX_3C-chemokines[2]. There is an important degree of redundancy in the chemokine superfamily, with many ligands binding different receptors and vice versa[2] (FIG. 1). In the tumour microenvironment, chemokines can be expressed by tumour cells and other cells, including immune cells and stromal cells. In response to specific chemokines, different immune cell subsets migrate into the tumour microenvironment and regulate tumour immune responses in a spatiotemporal manner. In addition, chemokines can directly target non-immune cells — including tumour cells and vascular endothelial cells — in the tumour microenvironment, and they have been shown to

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regulate tumour cell proliferation, cancer stem-like cell properties, cancer invasiveness and meta stasis. Therefore, chemokines directly and indirectly affect tumour immunity; shape tumour immune and biological phenotypes; and influence cancer progression, therapy and patient outcomes[3–10] (FIG. 1). In this Review, we describe the expression patterns and regulation of the main chemokines that are found in the human cancer microenvironment, and their effects on immune cells and non-immune cells. There has recently been a huge amount of research on cancer immunology and immunotherapy[10,11], and here we discuss whether selectively targeting chemokine–chemokine receptor signalling could complement and increase the efficacy of the immunotherapies that are currently being used in cancer treatment[3,4,10,12].

Immune cell tumour trafficking

Different lymphocytes traffic into the tumour microenvironment, and they can modulate tumour immune responses in both primary tumours and metastatic sites. Here, we discuss several key chemokine networks that regulate lymphocyte recruitment into the tumour microenvironment, and discuss how the recruited lymphocyte subsets regulate tumour immunity and tumorigenesis.

The recruitment of effector T cells and natural killer cells

$CD8^+$ T cells that are specific for tumour-associated antigens (TAAs) can engage tumour cells in an antigen-specific manner, and they drive antitumour immunity by secreting effector cytokines, releasing cytotoxic molecules (such as granzyme B and perforin) and inducing apoptosis in tumour cells. In addition to $CD8^+$ T cells, interferon-γ (IFNγ)-expressing T helper 1 (T_H1) cells and natural killer (NK) cells have potent antitumour effects in the tumour microenvironment. Effector $CD8^+$ T cells, T_H1 cells and NK cells express CXC-chemokine receptor 3 (CXCR3), which is the receptor for the T_H1-type chemokines CXC-chemokine ligand 9 (CXCL9) and CXCL10, and they can migrate into tumours in response to these chemokines (FIG. 2). Increased levels of CXCL9 and CXCL10 are associated with increased numbers of tumour-infiltrating $CD8^+$ T cells, and correlate with decreased levels of cancer metastasis and improved survival in patients with ovarian cancer and colon cancer[13–18]. Recent studies have demonstrated that tumour-infiltrating $CD8^+$ T cells and intratumoural T_H1-type chemokines are associated with positive responses to therapeutic blockade of the immune checkpoint molecules programmed cell death protein 1 (PD1) and PD1 ligand 1 (PDL1; also known as B7-H1)[10]. Interestingly, $CD8^+$ T cells in the tumour microenvironment were shown recently to regulate the metabolism of the chemotherapeutic agent cisplatin by fibroblasts in ovarian cancer[19]. In this study, $CD8^+$ T cell-derived IFNγ altered glutathione and cysteine metabolism in fibroblasts, and abolished their resistance to platinum-based chemotherapy[19], suggesting that $CD8^+$ T cells can also affect tumour cell fate in a TAA-independent manner. Therefore, T_H1-type chemokines can recruit effector immune cells into the tumour microenvironment, and these immune cells can subsequently shape tumour immunity and therapeutic responses through both TAA-specific and TAA-independent mechanisms.

The recruitment of T_H17 cells

Human T_H17 cells express high levels of CC-chemokine receptor 6 (CCR6), CXCR4, multiple CD49 integrins and the C-type lectin-like receptor CD161 (REFS 17,20–22). These homing molecules may be associated with T_H17 cell migration and retention within inflammatory tissues and tumours[17,21,23,24]. For example, high levels of CXCL12 (also known as SDF1; the ligand for CXCR4)[25,26] and CC-chemokine ligand 20 (CCL20; the ligand for CCR6)[27] are found in human tumour microenvironments. This chemokine profile may facilitate the trafficking of T_H17 cells into tumours. T_H17 cells do not express CD62 ligand (CD62L; also known as L-selectin) or CCR7 (REF. 17), which promote lymphocyte homing to lymph nodes, and this suggests that their potential to home to lymphoid tissues is limited. In both humans and mice, tumour- infiltrating T_H17 cells are polyfunctional[17,22,28], have stem-like properties and mediate potent antitumour immunity[8,17,22,24,28]. T_H17 cells do not secrete cytotoxic molecules such as granzyme B and perforin, but instead mediate antitumour activity by recruiting $CD8^+$ T cells[17,22], NK cells[29] and dendritic cells (DCs)[24] into the tumour microenvironment. Of note, interleukin-17 (IL-17) has been shown to target the tumour stroma and also to promote tumour angiogenesis in mouse models, an effect that may promote tumour growth[8]. Nevertheless, the chemokine-driven recruitment of polyfunctional T_H17 cells into the tumour micro environment may be beneficial for patients with cancer[8].

The recruitment of T_H22 cells

T_H22 cells are found in the microenvironment of several types of human cancer, including colon cancer, pancreatic cancer and hepatocellular carcinoma[30–33]. These cells express CCR6, migrate towards the CCR6 ligand CCL20 in the colon cancer microenvironment, and have been shown to promote and support tumorigenesis[30]. T_H22 cell-derived IL-22 acts on cancer cells to promote the activation of the transcription factor signal transducer and activator of transcription 3 (STAT3), increase the expression of the histone H3 lysine 79 (H3K79) methyltransferase DOT1L[30], and upregulate the expression of the H3K27 methyltransferase Polycomb repressive complex 2 (PRC2), particularly the enhancer of zeste homologue 2 (EZH2) subunit[34]. The DOT1L complex induces the expression of the core stem cell genes *NANOG*, *SOX2* (which encodes SRY-box 2) and *POU5F1* (which encodes POU class 5 homeobox 1), resulting in increased cancer stemness and tumorigenic potential[30], whereas increased expression of EZH2 has been shown to support the proliferation of colon cancer cells[34]. A pro-tumour role of IL-22 has been supported by studies in two mouse models of colon cancer. In a bacteria-induced colon cancer model, IL-22-expressing colonic innate lymphoid cells (ILCs) accumulate in the tumour tissues, and their depletion blocks the development of invasive colon cancer[35]. In a colon tumour model that is induced by azoxymethane and dextran sulfate sodium, the downregulation of IL-22-binding protein (IL-22BP) expression increases the ratio of IL-22 to IL-22BP and promotes tumorigenesis[36]. Thus, the recruitment of T_H22 cells into the tumour microenvironment via the CCL20–CCR6 axis may promote tumorigenesis[37].

The recruitment of regulatory cells

Another way in which chemokines may promote tumorigenesis is by mediating the recruitment of regulatory T (T_{reg}) cells into the tumour microenvironment. T_{reg} cells express CCR4 and are recruited into the tumour micro environment in response to CCL22, which is produced mainly by macrophages and tumour cells[38]. T_{reg} cells suppress spontaneous and therapy-induced T cell antitumour immunity, leading to tumour growth and poor patient outcomes[5,38]. In addition to the CCL22–CCR4 signalling pathway, T_{reg} cells express CCR10 and migrate in response to the CCL28 that is found in hypoxic regions of the tumour microenvironment[39].

The bone marrow is a common site of tumour metastasis in humans, suggesting that the bone marrow may provide an immunosuppressive microenvironment that supports tumour retention and growth[40]. In line with this notion, high frequencies of T_{reg} cells are found in the bone marrow[41]. Bone marrow T_{reg} cells exhibit a memory phenotype and express functional CXCR4 (REF. 41). T_{reg} cells can be mobilized from the bone marrow into the periphery by granulocyte colony-stimulating factor (G-CSF), which promotes the degradation of CXCL12 in the bone marrow[41]. High numbers of T_{reg} cells in the bone marrow may provide an immune ‘shield’ that facilitates tumour metastasis to this site. This may explain why cancers often metastasize to the bone marrow[6,42]. In further support of this possibility, the numbers of T_{reg} cells are further increased in the bone marrow of patients with prostate cancer who show bone metastasis[42]. These T_{reg} cell populations are recruited into the bone marrow via the CXCL12–CXCR4 signalling pathway and are expanded by DCs via the receptor activator of NF-κB (RANK)–RANK ligand (RANKL) signalling pathway (also known as the TNFRSF11A–TNFSF11 signalling pathway)[42].

T_{reg} cells may express inflammatory cytokines — including CXCL8 (also known as IL-8)[43] and IL-17 (REF. 44) — in the human colon cancer microenvironment. Interestingly, $CXCL8^+$ and $IL\text{-}17^+$ T_{reg} cells not only mediate T cell suppression but also promote inflammation in the cancer microenvironment. Thus, the chemokine-mediated recruitment of T_{reg} cells into the tumour microenvironment and their presence at pre-metastatic sites supports tumour initiation, progression and metastasis (FIG. 3).

The recruitment of NKT cells

Type I NKT cells, which are defined by their expression of an invariant T cell receptor (TCR) — namely, $V\alpha14J\alpha18^+$ in mice and $V\alpha24J\alpha18^+$ in humans — mainly have antitumour immune activities, as they produce IFNγ to activate NK cells and $CD8^+$ T cells, and they activate DCs to produce IL-12 (REF. 45). By contrast, type II NKT cells, which are characterized by the expression of more diverse TCRs that recognize lipids presented by CD1d, primarily inhibit tumour immunity[45]. Most NKT cells express non-lymphoid-homing or inflammation- related chemokine receptors including CCR2, CCR5 and CXCR3 (REF. 46). CCL2 mediates the trafficking of type I NKT cells into neuroblastomas[47,48]. NKT cell trafficking into other types of tumour is poorly studied.

The recruitment of B cells

Human tumour-infiltrating B cells have been less well-studied than have effector T cells[49]. B cells express CXCR4 and may be recruited by CXCL12 into the cancer microenvironment. High levels of tumour-infiltrating B cells are associated with a survival advantage in breast cancer[50], high-grade serous ovarian cancer[51] and cervical cancer[52]. B cells may also be present in tumour-associated tertiary lymphoid structures[53] and increase T cell responses by releasing cytokines and chemokines, by serving as antigen-presenting cells (APCs) and by producing antibodies. However, mouse studies indicate that B cells may negatively regulate tumour immunity and promote tumour progression via IL-10 and transforming growth factor-β (TGFβ) expression[54–56]. Furthermore, by activating Fcγ receptors (FcγRs) on myeloid cells and mast cells, B cells can promote tumour angiogenesis and the recruitment of tumour-promoting immune cells[54–56]. There may be different subsets of B cells, including regulatory B cells[57]; however, it is unknown whether different B cell subsets are recruited into the tumour microenvironment by different chemokines, and whether they have differential roles in human tumour immunity and tumorigenesis.

In summary, different lymphocyte subsets are recruited into the tumour microenvironment by distinct chemokine–chemokine receptor signalling pathways. Effector T cells, NK cells and perhaps NKT cells may mediate an antitumour immunity, whereas T_{reg} cells, T_H22 cells and perhaps B cells may promote tumorigenesis.

Chemokines and tumour-associated APCs

APCs — including DCs, macrophages, B cells (discussed above) and perhaps myeloid-derived suppressor cells (MDSCs) — are recruited into the tumour microenvironment; they regulate antitumour immunity by interacting with T cells, and affect tumorigenesis by interacting with tumour (stem-like) cells and stromal cells.

The recruitment of myeloid DCs to tumours

Mature myeloid DCs can drive potent antitumour immune responses by priming and activating TAA-specific T cells[58]. By contrast, immature myeloid DCs are poor mediators of T cell activation and can induce T_H2-type immune responses[27,59], which may support tumour progression. Immature myeloid DCs, but not mature myeloid DCs, are found in breast cancer and the cancer stroma[27,60]. Immature DCs express CCR6 and are recruited into tumours in response to tumour-derived CCL20 (REFS 27,60). However, in experimental mouse models, the overexpression of CCL20 (REF. 61) and CXCL14 (REF. 62) can attract myeloid DCs to the tumour, and promote DC maturation and inhibit tumour growth. The trafficking patterns of myeloid DCs in other types of human cancer are poorly defined.

The recruitment of plasmacytoid DCs

Plasmacytoid DCs are found in the human tumour microenvironment. Tumour and stromal cells produce CXCL12 (REFS 25,26,63), and plasmacytoid DCs express integrin α5 (also known as VLA5) and CXCR4, which are the key molecules that mediate plasmacytoid DC trafficking to tumours[25]. CXCL12 also protects plasmacytoid DCs in tumours from undergoing apoptosis[26]. *In vitro* studies have shown that plasmacytoid DCs isolated from

human tumour tissues can respond to viral infection by producing high levels of type I IFN[25]. However, plasmacytoid DCs can also induce the development of IL-10-producing regulatory $CD8^+$ T cells that suppress the ability of myeloid DCs to activate TAA-specific effector T cells[25,64]. Furthermore, these regulatory $CD8^+$ T cells express CCR7, and may home to the draining lymph nodes and suppress TAA-specific T cell priming[64]. In addition, plasmacytoid DCs can promote tumour angiogenesis[65]. Thus, the recruitment of plasma cytoid DCs into the human tumour micro environment by CXCL12 may support the development of an immunosuppressive site that is permissive for tumour progression.

The recruitment of macrophages

Macrophages can be recruited into the tumour microenvironment by CCL2–CCR2 signalling[66]. CCL2 expression by tumours correlates with the numbers of tumour-associated macro phages (TAMs) in many tumours and is associated with poor patient prognosis in some cancers, including breast cancer[67]. TAMs may inhibit TAA-specific T cell activation via the expression of inhibitory B7 family members, including PDL1 and B7-H4 (also known as VTCN1), and through the induction of the galectin 9–T cell immunoglobulin mucin 3 (TIM3) pathway[68–71]. Furthermore, TAMs support chemo resistance[72], and promote cancer stemness and meta stasis[67,73]. CCL2 can also activate metastasis-associated macrophages to secrete CCL3, which further promotes macrophage retention in the tumour and tumour meta static sites[74]. The CCL5–CCR5 pathway may be an additional chemo attractant signalling axis that affects macrophages in breast cancer. Increased CCL5 expression correlates with more advanced stages of breast cancer[75,76]. Thus, the recruitment of macrophages into the tumour micro environment may promote tumour progression. However, different subsets of macrophages[77] and macrophages in different maturation stages may have diverse functions in tumours[71,78,79]. For example, $CD68^+$ macrophages are associated with improved survival among patients with colon cancer[79]. $CD169^+$ macro phages can mediate TAA-specific T cell cross-priming in tumour-draining lymph nodes, and initiate and promote tumour immunity in a mouse model[80]. Furthermore, macrophages can either increase or antagonize the antitumour efficacy of cytotoxic chemotherapy, cancer-cell targeting antibodies and immunotherapeutic agents[81]. Thus, targeting macrophages by the manipulation of chemokine–chemokine receptor signalling as a therapeutic approach may need to take these effects into account.

The recruitment of MDSCs

MDSCs represent a heterogeneous population of myeloid cells that includes monocytic and granulocytic cells[82]. Monocytic MDSCs are macrophages in different maturation stages. Granulocytic MDSCs are mostly neutrophils in different maturation stages. The immune-suppressive effects of MDSCs are relatively well-studied in mouse tumour models[82–85] and in patients with cancer[73,86–88]. Interestingly, recent studies demonstrate that MDSCs endow cancer cells with stem cell-like properties and are linked with cancer stemness[73,86–88]. Monocytic MDSCs (macrophages) can be recruited into the tumour microenvironment by CCL2 (REFS 74,89). The CXCL5–CXCR2 and CXCL12–CXCR4 signalling pathways are also reported to be involved in MDSC trafficking in a breast tumour mouse model[90]. CXCL8 regulates granulocytic MDSC migration and degranulation via CXCR1 and CXCR2 signalling[91]. Tumour cells and myeloid cells express CXCL8, and recruit neutrophils into

the tumour microenvironment[91]. Certain subsets of T_{reg} cells in the human cancer microenvironment express CXCL8 and promote neutrophil migration into tumours[43]. Neutrophils secrete various molecules that support and promote tumour angiogenesis. Thus, the recruitment of neutrophils by CXCL8 is generally thought to promote tumour progression and metastasis[91,92].

In summary, distinct chemokines mediate the recruitment of different APC subsets into the tumour microenvironment, and these APCs differentially regulate tumour immunity and cancer progression. In addition to targeting immune cells, chemokines can also affect tumorigenesis by directly targeting tumour cells and tumour stromal cells.

Effects of chemokines on tumour cells

Chemokines can directly and indirectly target tumour stem-like cells and stromal cells in tumours. Below, we discuss how different chemokine–chemokine receptor signalling pathways affect tumour cell proliferation, stemness and angiogenesis to ultimately alter tumour metastasis and disease outcomes in patients (TABLE 1).

Direct pro-tumour effects of chemokines

CCL2, CCL3 and CCL5 can promote tumour invasion and meta stasis. CCL2 targets vascular endothelial cells via the Janus kinase 2 (JAK2)–STAT5 and p38 mitogen-activated protein kinase pathways[93], and affects tumour vas cularization[94–96] and tumour metastasis[93]. CCL2, CCL3 and CCL5 can induce matrix metalloproteinase 9 (MMP9) secretion by monocytes[76,97]; MMP9, by degrading the matrix, allows for tumour cell extravasation[98]. Furthermore, CCL2 and CCL5 can promote cancer cell proliferation, survival, motility[99], epithelial–mesenchymal transition (EMT) and stemness[100–103]. In addition, these chemokines recruit MDSCs and macrophages into the tumour microenvironment, and in turn, promote and sustain human cancer stemness[73,86,88,104].

CCL18 can directly influence tumour cells by, for example, promoting invasion, metastasis and EMT in breast cancer, pancreatic cancer, ovarian cancer and prostate cancer[105–109]. However, the effect of CCL18 seems to depend on the cancer type, as high levels of CCL18 are a good prognostic factor in gastric cancer[110]. CCL18 inhibits cutaneous T cell proliferation[111]. In some cancers, such as breast cancer, the main source of CCL18 in the tumour is TAMs, whereas ovarian cancer cells can overexpress CCL18 (REFS 105,106). Although not shown in cancer, CCL18 has an immunosuppressive effect on DCs and macrophages[112–115]. CCL18-conditioned APCs may induce the differentiation of T_{reg} cells, leading to immunosuppression. This CCL18-driven immunosuppression might exist in the tumour microenvironment[112–115].

The receptor for CCL25, CCR9, is highly expressed in many cancers. CCR9 signalling in tumour cells increases their resistance to chemotherapy[116,117] and their expression of MMPs, which promote cancer invasion and metastasis[118–122]. CCL25 can also promote metastasis by recruiting $CCR9^+$ cancer cells into CCL25-expressing tissues, such as the small intestine. For example, cutaneous melanoma cells, and possibly adult lymphoblastic

leukaemia cells, preferentially metastasize to the small intestine owing to signalling via the CCR9–CCL25 axis[123–125].

CXCL8 targets vascular endothelial cells and regulates angiogenesis by promoting endothelial cell survival[126]. CXCL8 targets cancer cells; promotes cancer invasion and migration[127]; induces tumour premature senescence[128]; contributes to hypoxia-induced tumour apoptosis resistance[129]; and promotes EMT[130] and cancer stemness[131–135]. Thus, CXCL8 signalling is important in cancer cell biology.

CXCL12 targets vascular endothelial cells and synergizes with vascular endothelial growth factor (VEGF) to promote tumour angiogenesis[26,136]. CXCL12 can also promote tumour cell proliferation and survival[63,137]. Furthermore, the CXCL12–CXCR4 signalling pathway promotes cancer cell invasion and metastasis[138–143]. It has been suggested that $CXCR4^{+}$ tumour cells may have stem-like properties, have a high metastatic potential and show radiation resistance[144– 146]. Thus, the CXCL12–CXCR4 signalling pathway has a role in tumour proliferation, metastasis and stemness. CXCL12 can also bind to CXCR7 (REFS 147,148). Although considered a decoy and scavenger receptor[147–149], CXCR7 can signal through non-G-protein-mediated mechanisms in cancer cells and endothelial cells, including tumour-associated endothelial cells[150,151]. Its role as a stand-alone G protein-coupled receptor is still under debate, but it can bind to CXCR4 and mediate signalling through intracellular CXCR4 signalling molecules, a process implicated in the chemotaxis of T cells[147,152]. As mentioned above, cancer cells express CXCR7, which can promote the adhesion, invasion, survival and growth of prostate cancer[153,154], breast cancer[148,150], and lung cancer cells[150]. CXCR7 signalling can also indirectly contribute to angiogenesis by increasing the expression of CXCL8 and VEGF in prostate cancer cells[153].

CXCL14 (also known as BRAK) has been reported to be involved in tumorigenesis. Interestingly, the effect of CXCL14 in different cancers varies. Cancers such as those of pancreas[155] and prostate[156] show increased CXCL14 expression, whereas other types of cancer — including breast cancer, kidney cancer, cervical cancer, and head and neck cancer — consistently lose expression of CXCL14 (REFS 157–160). In line with this, the overexpression of CXCL14 in breast tumours that lack CXCL14 or even in tumour myoepithelial cells leads to reduced tumour growth, metastasis and invasion[158,161]. Even though the receptor for CXCL14 is still unknown, *in vivo* loss of CXCL14 is correlated with reduced DC loss in a head and neck squamous cell carcinoma mouse model[62].

CXCL17 is highly expressed in various cancer cells, recruits granulocytic MDSCs into the tumour, and increases tumour growth partially by increasing angiogenesis[162,163]. Indeed, CXCL17 induces VEGF expression in monocytes and endothelial cells[163,164]. As CXCL17 is highly expressed in many cancers, including colon cancer, it is likely to be an important chemokine for mucosal tumours and mucosal immunity[165].

Direct antitumour effects of chemokines

Dying cancer cells can be immunogenic and can direct the antitumour immune response. CXCL8, for example, can increase the immunogenicity of dying cancer cells by translocating calreticulin to the cell surface[166]. Calreticulin exposure on the cell surface

increases the immunogenicity of the cell, and thus promotes the phagocytosis of these cells and antitumour immune responses to the tumour[167]. CXCL9 and CXCL10 are endogenous tumour angiogenesis inhibitors[168,169]. CXCL10 prevents both CXCL8-induced and basic fibroblast growth factor-induced angiogenesis *in vivo* and *in vitro*[170,171].

Regulation of chemokine expression in tumours

Chemokine expression is regulated by cancer-intrinsic genetic and epigenetic mechanisms and by environmental cues in the tumour microenvironment.

Epigenetic and oncogenic regulation of chemokine expression

The role of oncogenic genetic and epigenetic pathways is extensively studied in cancer biology. Interestingly, recent studies have shown that in human ovarian cancer and colon cancer, the PRC2 complex, the H3K27me3 demethylase JMJD3 (also known as KDM6B) and DNA methylation repress the expression of T_H1-type chemokines in tumours and prevent the trafficking of effector T cells into the tumour micro environment[172,173]. Pharmacological and genetic interventions that increase T_H1-type chemokine production lead to increased effector T cell trafficking into tumours, and improve the therapeutic efficacy of PDL1 blockade and T cell transfusion in preclinical models[172,173]. *CXCL14* expression is also reported to be repressed in lung cancer cells by DNA methylation, and forced expression of CXCL14 leads to reduced tumour growth[174]. Furthermore, human melanoma tissues that show little T cell infiltration display active β-catenin signalling[175]. In a genetically engineered mouse melanoma model, β-catenin activation results in poor expression of CCL4 — a chemokine that is essential for $CD103^+$ DC migration — and subsequently limits DC-mediated effector T cell activation and expansion within the tumour[175]. Another epigenetic repressor, histone deacetylase 1 (HDAC1) can interact with the nuclear factor-κB (NF-κB) subunit p65 (also known as RELA) and repress *CXCL8* expression[176,177].

Genetic regulation, mainly mutations, can influence chemokine receptor expression and function. A point mutation has been identified in *CXCR4* (G574A) in a melanoma cell line and a colon cancer cell line[178]. This mutation is functionally active, and the mutant receptor signals and traffics in response to CXCL12, but when tumours expressing this mutant receptor were allowed to grow *in vivo*, tumour growth was delayed[178]. Indirectly, a common gene fusion of *PAX3* (which encodes paired box 3) and *FKHR* (which encodes forkhead homologue in rhabdomyosarcoma; also known as *FOXO1*) in rhabdomyosarcoma is associated with higher CXCR4 expression[179]. Transfer of this gene fusion leads to higher CXCR4 expression in embryonic rhabdomyosarcoma cells and increases invasion *in vitro*[179]. Both of these studies indicate the diverse roles of the chemokine receptor CXCR4. Thus, tumour-intrinsic oncogenic[175] and epigenetic[172,173] pathways control chemokine expression, and influence immune cell activation in and/or migration into the tumour microenvironment.

Both PRC2-mediated epigenetic silencing[180] and β-catenin signalling are tumour-intrinsic tumorigenic mechanisms, and are associated with cancer EMT and a stem cell-like biological phenotype. Interestingly, these tumour-intrinsic mechanisms can regulate

chemokine expression and control immune cell infiltration into tumours. Based on having either relatively high or low immune cell infiltration, tumours may be immunologically classified into ‘hot’ (inflamed) or ‘cold’ (non-inflamed) phenotypes, respectively. Thus, oncogenic genetic and epigenetic pathways simultaneously define the biological and immunological phenotypes of the tumour, affect tumour progression, and alter spontaneous and therapy-induced tumour-specific T cell immunity (FIG. 4). The manipulation of these tumour-intrinsic pathways may promote the infiltration of T cells into tumours, alter tumour immune phenotype and ultimately lead to tumour regression.

Hypoxia and chemokine expression in tumours

Hypoxia is a general phenomenon in the cancer microenvironment. The transcription factor hypoxia-inducible factor 1 (HIF1; which comprises HIF1α and HIF1β) is the central mediator of the cellular response to hypoxia[181]. Hypoxia triggers CXCL12 expression in primary human ovarian tumour cells[26], fibroblasts[182] and haematopoietic stem cells (HSCs)[183]. In the promoter region of the *CXCL12* gene, there are two potential HIF1-binding sites (HBSs) termed HBS1 and HBS2 (REF. 183). The HBS1 region may be responsible for the HIF1-dependent induction of CXCL12 synthesis[183]. Hypoxia also promotes CXCR4 expression in TAMs and tumour cells[184,185]. In renal cell carcinoma, the mechanism of CXCR4 upregulation involves mutation of the tumour-suppressor gene *VHL* (which encodes von Hippel–Lindau protein)[185]. Increased CXCR4 expression and migration towards CXCL12 are dependent on HIF1α activation and *CXCR4* transcript stabilization[184,185]. As well as inducing the expression of CXCR4 and CXCL12, hypoxia can induce CXCR7 expression in rhabdomyosarcoma cells[186]. In addition, CCL2 also has HBSs in its promoter, and hypoxia has been found to induce CCL2 expression in human astrocytes[187] and CXCL8 expression in ovarian cancer cells[188]. Thus, hypoxia can affect tumour immunity and biology by regulating the expression of several chemokines and chemokine receptors.

Metabolic regulation of chemokine expression in the tumour microenvironment

Aerobic glycolysis is a feature of cancer cell metabolism. In aerobic glycolysis, cancer cells produce lactic acid, which activates NF-κB and induces CXCL8 expression in vascular endothelial cells, resulting in angiogenesis in breast and colon cancer[189]. In breast cancer cells, reactive oxygen species can upregulate *CXCL14* expression through the transcription factor activator protein 1 (AP-1), thus increasing cell invasion and motility[190]. Hormones can also regulate chemokine expression. In breast cancer cells, oestrogen can upregulate the expression of CXCR4 and CXCL12 and downregulate that of CXCR7 (REF. 191). Further studies will determine how cancer metabolism affects the expression of different chemokines in the tumour microenvironment.

The microbiota and tumour chemokine expression

Different bacteria can negatively and positively influence tumour growth. The microbiota and its by- products can modulate the tumour immune response[192,193]. These bacteria can recruit specific immune cell subsets, thus shaping tumour growth. For example, *Fusobacterium nucleatum* accelerates intestinal tumours, in part by increasing the infiltration of myeloid cells that suppress T cell activity into the tumours[194]. The binding of short-chain

fatty acids from microorganisms to G protein-coupled receptor 43 (GPR43) leads to inflammation resolution in mouse models. GPR43-deficient mice have high levels of inflammation and immune cell recruitment, and an exacerbated immune response[195]. Another bacterium, *Faecalibacterium prausnitzii*, is an anti-inflammatory commensal bacterium that decreases in abundance in patients with Crohn's disease[196,197]. Its metabolites block NF-κB activation and CXCL8 production[196,197]. Thus, although there is no direct evidence of microbiota-induced regulation of chemokine expression in tumours, the microbiota and its by-products are presumably involved in tumour immune responses in specific types of human cancers such as colon cancer.

Chemokines and cancer immunotherapy

Given that chemokines and their receptors have crucial roles in inflammatory human diseases, efforts have been made to target chemokine networks in patients with autoimmune diseases and chronic inflammation. Drugs that target CCR5 (namely, maraviroc) and CXCR4 (namely, plerixafor; also known as AMD3100 and marketed as Mozobil by Genzyme) have been approved for use in HIV infection and for the mobilization of HSCs for transplantation, respectively. However, the targeting of chemokines and chemokine receptors has so far failed to yield any viable anti-inflammatory drugs. As discussed above, the chemokines CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10 and CXCL12 are relatively well studied in human cancer. Below, we focus on the potential of targeting these chemokines and their receptors to promote antitumour immune responses in patients with cancer, and we discuss the possibility of combining chemokine-based therapies with current cancer immunotherapies.

CXCL9 and CXCL10

Poor tumour infiltration by T cells has been attributed to potent epigenetic silencing of the genes encoding the T_H1-type chemokines CXCL9 and CXCL10 in tumours[172,173]. As discussed above, these chemokines promote the migration of effector T cells and NK cells into tumours. Studies have shown that improved therapeutic responses to cancer immunotherapy and chemotherapy are associated with increased levels of T_H1-type chemokines and increased numbers of effector T cells in the tumour microenvironment[10,198]. Thus, cancer epigenetic reprogramming may remove the epigenetic repression of genes encoding T_H1-type chemokines, thus promoting effector T cell trafficking into the tumour microenvironment and improving the therapeutic efficacy of immunotherapy. In support of this, treatment with cancer epigenetic re programming drugs — including EZH2 inhibitors, DZNep[199], a selective inhibitor of EZH2 methyltransferase activity (GSK126)[200] or a DNMT inhibitor (5-aza-2′-deoxycytidine) — increases tumour T_H1-type chemokine production and T cell trafficking into tumours[172,173], and augments the therapeutic effects of PDL1 blockade and T cell therapy in a preclinical model[172]. Furthermore, treatment with azacitidine upregulates the expression of IFN signature genes in several human cancer cell lines[201,202]. 5-aza-2′-deoxycytidine treatment increases the expression of the cancer and germline TAA NY-ESO-1 (also known as cancer/testis antigen 1) in human ovarian cancer cells[203], and promotes chemokine expression and T cell tumour trafficking in a mouse ovarian cancer model[198]. Thus, epigenetic re-programming can de-

repress the repressed T_H1-type chemokine-encoding genes, and promote the expression of IFN signature genes and TAAs, and may thus promote T cell infiltration into tumours and ultimately potentiate PDL1 and PD1 blockade therapy[172,173,198].

CXCL12 and CXCR4

CXCL12–CXCR4 signalling is implicated in immune cell tumour trafficking and tumour cell biology. CXCL12–CXCR4 signalling mediates plasmacytoid DC trafficking into tumours[25] and T_{reg} cell homing to the bone marrow microenvironment[41,42], and is involved in tumour cell proliferation[63], metastasis[138] and tumour vascularization[26]. AMD3100 is a CXCR4 antagonist and has been used in human clinical trials for the treatment of HIV infection[204]. The blockade of CXCR4–CXCL12 signalling may reduce tumour angiogenesis, invasiveness and tumour-induced immunosuppression. Indeed, anti-CXCR4 and anti-CXCL12 antibodies each prevented metastasis, reduced tumour weight and prevented tumour extravasation in preclinical models[138,205–208]. Thus, it is tempting to speculate that the administration of antagonists of CXCR4–CXCL12 signalling could be therapeutically beneficial in combination with current immunotherapies. A clinical trial is now underway to evaluate the safety of combinatorial immunotherapy with the CXCR4 peptide antagonist LY2510924 and the anti-PDL1 antibody durvalumab[209].

CXCL8 and CXCR1

CXCL8–CXCR1 signalling is involved in tumour angiogenesis, tumour stemness and inflammatory immune cell trafficking into the tumour microenvironment[91]. Strategies that aim to interfere with this chemokine regulatory loop may represent a strategy for targeting the cancer microenvironment. Repertaxin (also known as reparixin) is a noncompetitive allosteric inhibitor of CXCR1 and CXCR2. Repertaxin was originally developed to block CXCL8 activity, and thus reduce tissue damage after myocardial infarction or stroke[210]. A phase I clinical trial has demonstrated that repertaxin is well tolerated in healthy volunteers[211]. Further clinical trials are needed to determine the safety and efficacy of repertaxin in combination with current immunotherapies in patients with cancer.

CCL2, CCL3 and CCL5

These chemokines are implicated in macrophage and neutrophil recruitment into the tumour microenvironment. CCL5 promotes ovarian cancer stem-like properties[101,102]. CCL2, CCL3 and CCL5 may bind to CCR1, CCR2, CCR3 and CCR5. Targeting these chemokine receptors may prevent the accumulation of immunosuppressive myeloid cell in tumours. Indeed, targeting CCL2, CCL3 or CCL5 signalling inhibits metastasis and angiogenesis in mouse models of breast cancer, lung cancer and ovarian cancer[66,74,96,101]. However, the cessation of CCL2 neutralization monotherapy leads to increased metastasis and rapid death in mouse models of breast cancer[96]. Thus, CCL2 blockade may need to be combined with other immunotherapies to increase the antitumour response and avoid the potential detrimental effect of single-chemokine blockade. For example, CCL2 blockade synergistically improves the cancer vaccine response in mouse models of lung cancer and mesothelioma[212]. Macrophage depletion increases the therapeutic efficacy of anti-cytotoxic T lymphocyte antigen 4 (CTLA4) and anti-PD1 antibodies in mouse pancreatic cancer models[213]. Given that different subsets of macrophages may be functionally different,

clinical studies are needed to determine whether macrophage depletion can yield an antitumour response. Indeed, a phase II clinical trial has been conducted using MLN1202, an anti-CCR2 monoclonal antibody, in patients with cancer bone metastasis[214]. In addition, Chemocentryx recently initiated a phase Ib trial of a CCR2 antagonist (CCX872) in patients with non-resectable pancreatic cancer[215]. These clinical studies will provide the most-needed information on the safety and potential therapeutic efficacy of CCR2 signalling blockade in patients with cancer.

Concluding remarks

Chemokines and chemokine receptors mediate immune cell trafficking into the tumour micro environment. Different immune cell subsets differentially contribute to cancer progression and therapy. The genes that encode T_H1-type chemokines are repressed by epigenetic mechanisms in cancer, and this affects the numbers of antitumour effector immune cells that are present within tumours, determines the cancer immune phenotype, and shapes the therapeutic efficacy of immune checkpoint blockade, adoptive T cell therapy and conventional therapy[10,172,173]. By contrast, the chemokines associated with the tumour trafficking of myeloid cells, T_{reg} cells and T_H22 cells can directly and indirectly influence the biological phenotype of a tumour (for example, whether it is a stem/EMT-type or non-stem/EMT-type tumour)[30,34,43,73,86]. Thus, direct and indirect manipulation of chemokine–chemokine receptor signalling pathways may reshape the immune and biological phenotypes of a tumour in a manner that increases the therapeutic efficacy of immunotherapy. Of note, clinical trials of agents that directly target a single chemokine or chemokine receptor have not yielded impressive therapeutic efficacy in patients with chronic inflammatory diseases such as AIDS, diabetes and rheumatoid arthritis. One reason for this is that chemokines generally bind to multiple receptors. These ligands may then activate alternative receptors, abrogating the effect of the single antagonist or blocker. Furthermore, therapies that target specific chemokines or chemokine receptors can affect the trafficking of different immune cell subsets into tumours and alter the biological activities of non-immune cells in the tumour microenvironment. Hence, similarly to what is seen in chronic inflammatory diseases, a therapeutic strategy of directly targeting a single chemokine or chemokine receptor may not achieve a meaningful clinical response. Based on current findings and the above discussion, it is predicted that directly targeting both pro-tumour and antitumour chemokine– chemokine receptor signalling pathways[10,172,173] in combination with other immunotherapies could achieve clinical benefits in patients with cancer. Further studies in preclinical models and patients are required to bring this combination approach into clinical application.

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Glossary

Cancer stem-like cell

A cell that can self-propagate, is less-differentiated and can give rise to other tumour cells. These properties enable these cells to be potentially key players in tumour initiation, metastasis, and treatment resistance and/or cancer relapse

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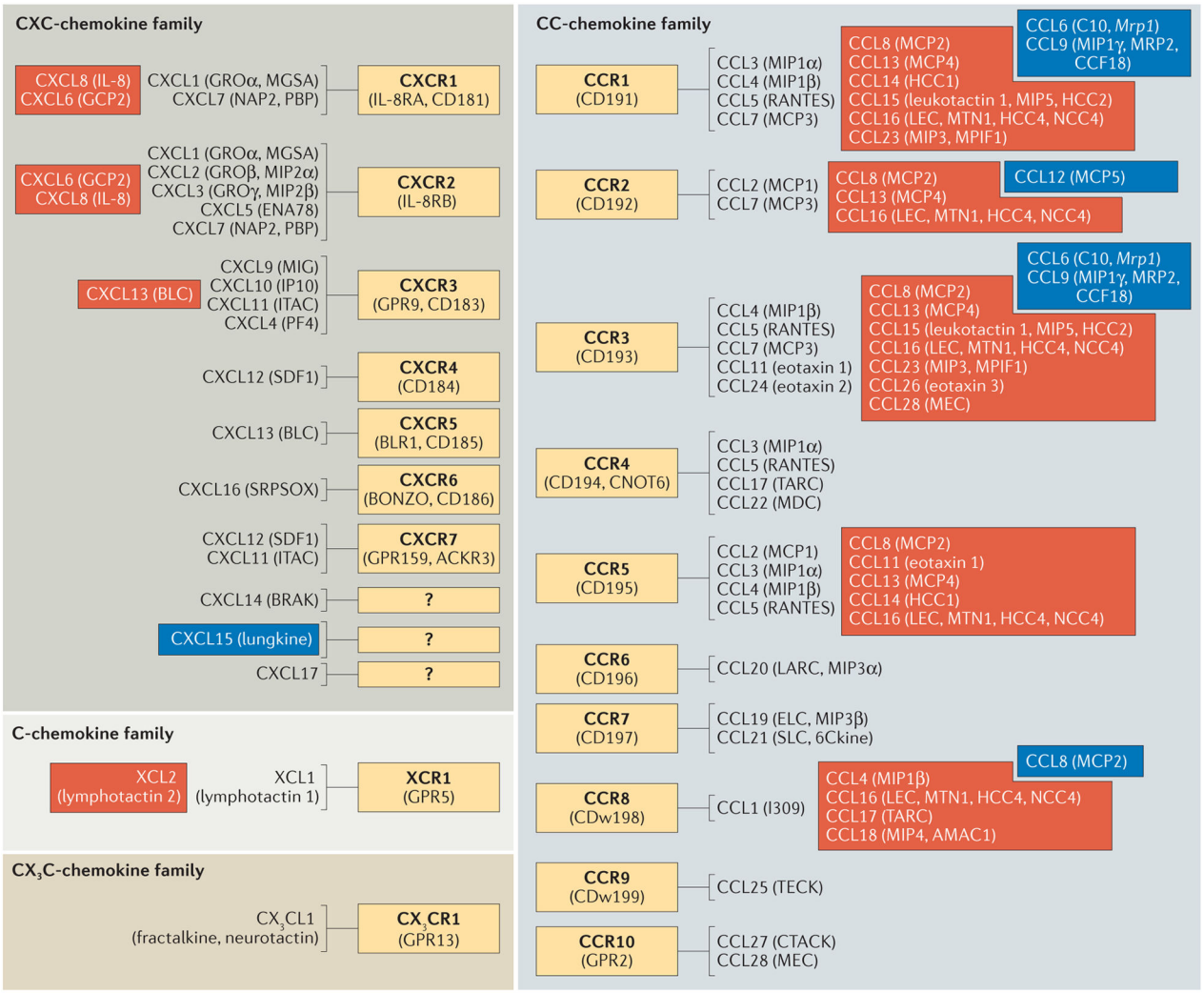


Figure 1. Chemokine receptor and ligand pairings

The chemokine receptors and ligands that belong to each of the main chemokine families (namely, the C-, CC-, CXC- and CX_3C-chemokine families) are shown. Blue and red boxes represent chemokine–chemokine receptor interactions that occur in mice and humans, respectively, and the non-boxed interactions occur in both humans and mice. Abbreviations enclosed in parentheses indicate alternative names for the preceding chemokine or chemokine receptor. Question marks indicate that the respective chemokine receptor is currently unknown.

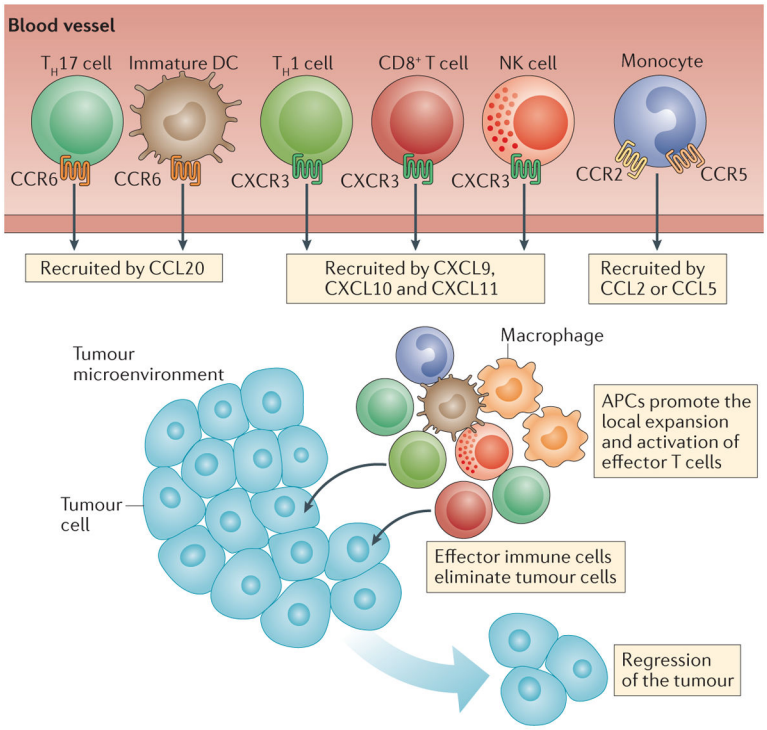


Figure 2. The promotion of tumour immunity by chemokines

Immune cells with antitumour effects — such as $CD8^+$ T cells, T helper 1 (T_H1) cells, polyfunctional T_H17 cells and natural killer (NK) cells — are recruited to the tumour microenvironment through chemokine–chemokine receptor signalling pathways. CXC-chemokine receptor 3 (CXCR3) and its ligands CXC-chemokine ligand 9 (CXCL9) and CXCL10 have a key role in driving the trafficking of T_H1 cells, $CD8^+$ T cells and NK cells into the tumour microenvironment, whereas CC-chemokine ligand 20 (CCL20) signalling through CC-chemokine receptor 6 (CCR6) promotes the recruitment of T_H17 cells. Antigen-presenting cells (APCs) such as macrophages and dendritic cells are also recruited into the tumour microenvironment, and they can activate and expand the local effector immune cells, thereby promoting tumour regression.

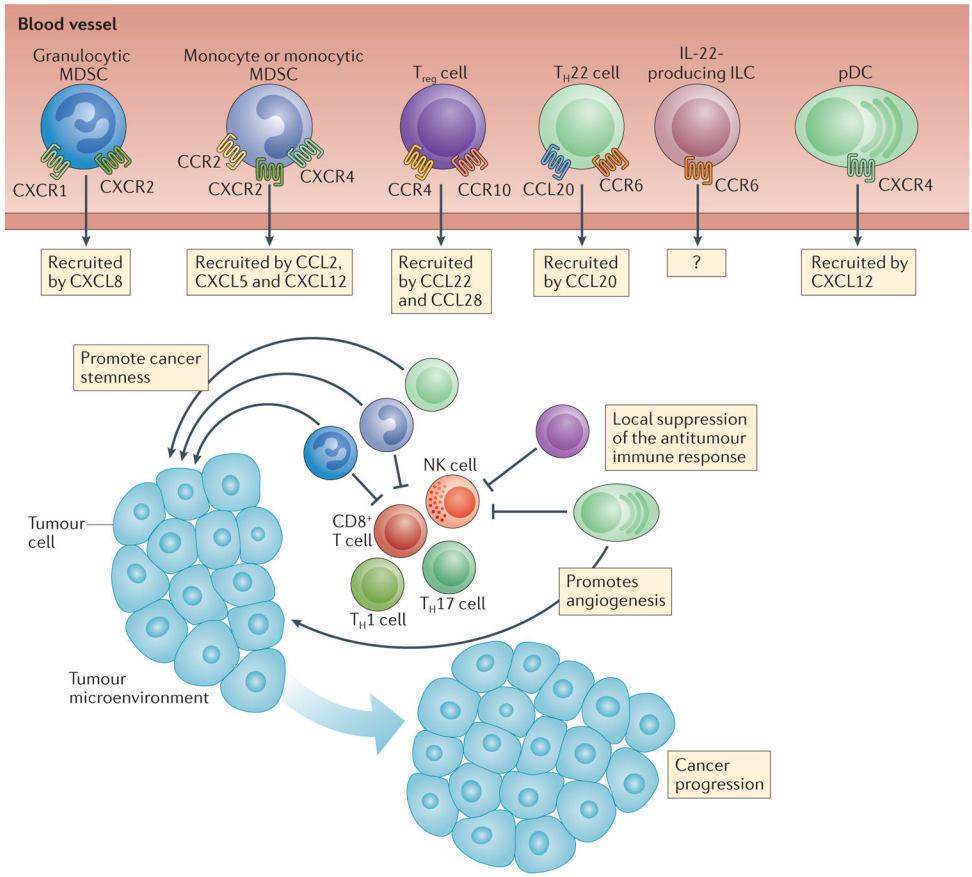
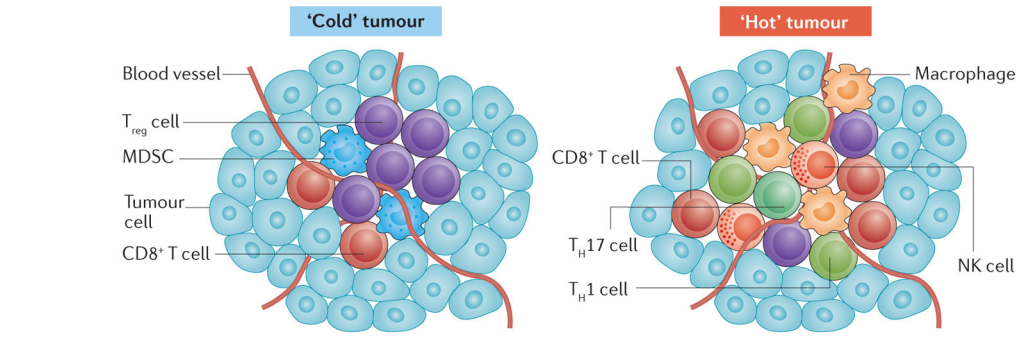


Figure 3. Pro-tumour effects of chemokines

Immune cell populations such as granulocytic and monocytic myeloid-derived suppressor cells (MDSCs), regulatory T (T_{reg}) cells, IL-22^+CD4^+ T helper 22 (T_H22) cells, IL-22^+ innate lymphoid cells (ILCs) and plasmacytoid dendritic cells (pDCs) can promote tumour growth. These cells are recruited to the tumour microenvironment in response to different chemokines that are expressed in the tumour microenvironment (the relevant receptors and ligands are shown). Pro-tumour immune cells may inhibit antitumour immune responses, and may also promote and maintain cancer stemness and angiogenesis, leading to cancer progression. CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor.



Biological characteristics	• Epigenetic silencing • Active β-catenin signalling • Mesenchymal-like cells • Stem cell-like cells • Less-differentiated cells	• Epigenetic reprogramming • Suppressed β-catenin signalling • Epithelial cells • Highly differentiated cells • High PDL1 expression
Immunological characteristics	• Enriched in immunosuppressive cytokines • High numbers of T_{reg} cells and MDSCs • Few T_H1 cells, NK cells and $CD8^+$ T cells • Few functional APCs	• Enriched in T_H1-type chemokines • High numbers of effector immune cells (T_H1 cells, NK cells and $CD8^+$ T cells) • High numbers of functional APCs

Figure 4. The relationship between, and mechanisms that underlie, tumour immune phenotype and biological phenotype

Active tumour β-catenin signalling inhibits CC-chemokine ligand 4 (CCL4) expression, and limits $CD103^+$ dendritic cell (DC) recruitment and $CD8^+$ T cell activation and expansion. The expression of the genes encoding the T helper 1 (T_H1)-type chemokines CXC-chemokine ligand 9 (CXCL9) and CXCL10 is repressed by the histone-lysine *N*-methyltransferase enhancer of zeste homologue 2 (EZH2) and DNA methyltransferase (DNMT)-mediated epigenetic silencing. Consequently, $CD8^+$ T cells poorly infiltrate the tumour, and the tumour is immunologically 'cold' (left). High levels of tumour β-catenin, EZH2 and DNMTs endow cancer stemness, which can be further promoted and maintained by pro-tumour immune cells. Thus, the immunologically cold tumour is biologically prone to have a more stem-like phenotype. Reversing this mechanism by epigenetic reprogramming and the suppression of β-catenin signalling may make the tumour immunologically 'hot' and promote the recruitment of effector immune cells with antitumour functions (including T_H1 cells, natural killer (NK) cells, $CD8^+$ T cells, polyfunctional T_H17 cells and functional antigen-presenting cells (APCs)), thereby driving tumour regression. MDSC, myeloid-derived suppressor cell; PDL1, programmed cell death protein 1 ligand 1; T_{reg} cell, regulatory T cell.

Table 1

Chemokine functions in the tumour microenvironment

Chemokine	Effects on immune cell trafficking into the tumour or bone marrow	Direct effects on tumour cells	Indirect effects on tumour cells	Refs
Chemokines with pro-tumour roles				
CCL2	Recruitment of monocytes, NKT cells and monocytic MDSCs	Promotes tumour cell proliferation, stemness and survival	Promotes tumour vascularization, and cancer extravasation and metastasis	93–97,99,100
CCL3	Recruitment of monocytes and macrophages	ND	Promotes cancer extravasation	97
CCL5	Recruitment of monocytes and macrophages	Drives metastasis	Promotes cancer invasion	76,97,103
CCL18	ND	Promotes invasion and metastasis	ND	105–109
CCL25	ND	Promotes chemoresistance, and tumour invasion and metastasis	ND	116–120,122
CXCL8	Recruitment of neutrophils and granulocytic MDSCs	Promotes stemness, invasion and migration; apoptosis; resistance to hypoxia; and premature senescence	Promotes angiogenesis	126–129,132–135
CXCL12	Recruitment of B cells and pDCs; and recruitment of T_{reg} cells into the bone marrow	Promotes proliferation and survival; drives invasion and metastasis; and promotes stemness	Promotes angiogenesis	For CXCR4: 26,136,139–144,146 For CXCR7: 148,150,153,154
CXCL14	Recruitment of DCs	Promotes invasion and motility	ND	190
CXCL17	Recruitment of granulocytic MDSCs	ND	Promotes angiogenesis	163,164,216
Chemokines with antitumour roles				
CXCL8	Recruitment of neutrophils and granulocytic MDSCs	Increases the immunogenicity of the tumour	ND	166
CXCL9 and CXCL10	Recruitment of T cells and NK cells	ND	Angiogenesis inhibitors	169–171
CXCL14	Recruitment of DCs	Inhibits proliferation, invasion and metastasis; increases apoptosis	ND	158,161,174

CCL, CC-chemokine ligand; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; DC, dendritic cell; MDSC, myeloid-derived suppressor cell; ND, not defined; NK, natural killer; NKT, natural killer T; pDC, plasmacytoid DC; T_{reg} cell, regulatory T cell.