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REVIEW ARTICLE

Role of chemokines in the hepatocellular carcinoma microenvironment and their translational value in immunotherapy*

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Abstract

The difficulty of early diagnosis, high tumor heterogeneity, and high recurrence and metastasis rates lead to an unsatisfactory treatment status for hepatocellular carcinoma (HCC). HCC is a typical inflammation-driven tumor. Chronic inflammation allows nascent tumors to escape immunosurveillance. Chemokines are small, soluble, secreted proteins that can regulate the activation and trafficking of immune cells during inflammation. Several studies have shown that various chemokines with overarching functions disrupt the immune microenvironment during the initiation and progression of HCC. The dysregulated chemokine network in HCC contributes to multiple malignant processes, including angiogenesis, tumor proliferation, migration, invasion, tumor low response, and resistance to immune therapy. Here, we summarize the current studies focusing on the role of chemokines and their receptors in the HCC immune microenvironment, highlighting potential translational therapeutic uses for modulating the chemokine system in HCC.

Key words: hepatocellular carcinoma; chemokine; chemokine receptor; tumor microenvironment; immune therapy; therapeutic target

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Primary liver cancer is the sixth most common malignant tumor and the third leading cause of cancer-related mortality, with approximately 906,000 new cases and 830,000 deaths worldwide in 2020, according to latest data from the World Health Organization [1]. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for 75%-85% of cases [1]. HCC remains a global health challenge. Most patients are not diagnosed until the middle or late stages and therefore miss the optimal window for liver resection and transplantation. Hence, the importance of systemic therapies for HCC, including tyrosine kinase inhibitors (TKIs) and immune-checkpoint inhibitors (ICIs), cannot be overemphasized. However, despite the recent remarkable shift in the HCC treatment landscape, both TKIs and ICIs have limitations of limited drug response rates and development of drug resistance [2, 3].

The blood flow slows in the liver sinusoids, facilitating the execution of the immune response by increasing the detection and capture of circulating pathogens by liver-resident cells [4]. Multiple innate and adaptive immune cells are involved in this process, particularly Kupffer cells (KCs), natural killer (NK) cells, natural killer T (NKT) cells, $CD4^+$ T cells, and $CD8^+$ T cells. Tumor cells can alter the local immune tumor microenvironment (TME) and gain the ability to proliferate and migrate

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while resisting destruction by the host immune system. An increasing number of studies have reported the importance of chemokine signaling in this process [4]. Cancer cells and various stromal cells in the TME interact through chemokine networks to jointly shape an immunosuppressive TME and assist immune cells in evading immune surveillance. M2 tumor-associated macrophages (TAMs), regulatory T (Treg) cells, and myeloid-derived suppressor cells (MDSCs) are significant contributors to the immunosuppressive microenvironment. Further, multiple chemokines have shown dual roles in HCC development, including through a direct impact on tumor cells and through indirect remodeling of the TME. This review summarizes the role of chemokine signaling in different component cells in HCC and reviews the current treatments targeting chemokines or their receptors.

Chemokines and chemokine receptors in HCC

The chemokine system includes 48 chemokine ligands, 20 chemokine receptors, and 4 atypical chemokine receptors. This system participates in multiple tumor-related pathological processes, including angiogenesis, metastasis, vascularization, and distortion of the TME [5]. Chemokines are small, soluble, secreted proteins that regulate the activation and trafficking of immune cells during inflammation [5]. As the largest subfamily of cytokines, chemokines are classified into four main subtypes based on the number and location of N-terminal cysteine (C) residues in their protein sequence, as follows: CC chemokines, CXC chemokines, C chemokines, and CX3C chemokines [6]. Most chemokines, other than CX3CL1 and CXCL16, are secreted proteins. Tumor cells and stromal cells, including immune cells, secrete chemokines. Autocrine and paracrine chemokines are secreted and act on themselves or adjacent cells by binding to specific receptors [5]. CX3CL1 and CXCL16 can remain on the cell surface via a transmembrane mucin-like stalk [7].

The deregulation of chemokines and their receptors is closely associated with HCC pathogenesis (Figs. 1 and 2). Here, we will discuss typical dysregulated chemokine signaling in HCC and its correlation with clinical outcomes and the value of chemokines as prognostic and predictive markers. The detailed role of various chemokines in immune cells will be discussed in the next section.

CCL2 (also known as monocyte chemoattractant protein 1, MCP1) functions mainly in HCC through binding to CCR2 (CD192). CCL2 is a potent chemoattractant for monocytes, lymphocytes, NK cells, dendritic cells, and many other cell types. Therefore, the CCL2-CCR2 signaling pathway performs various

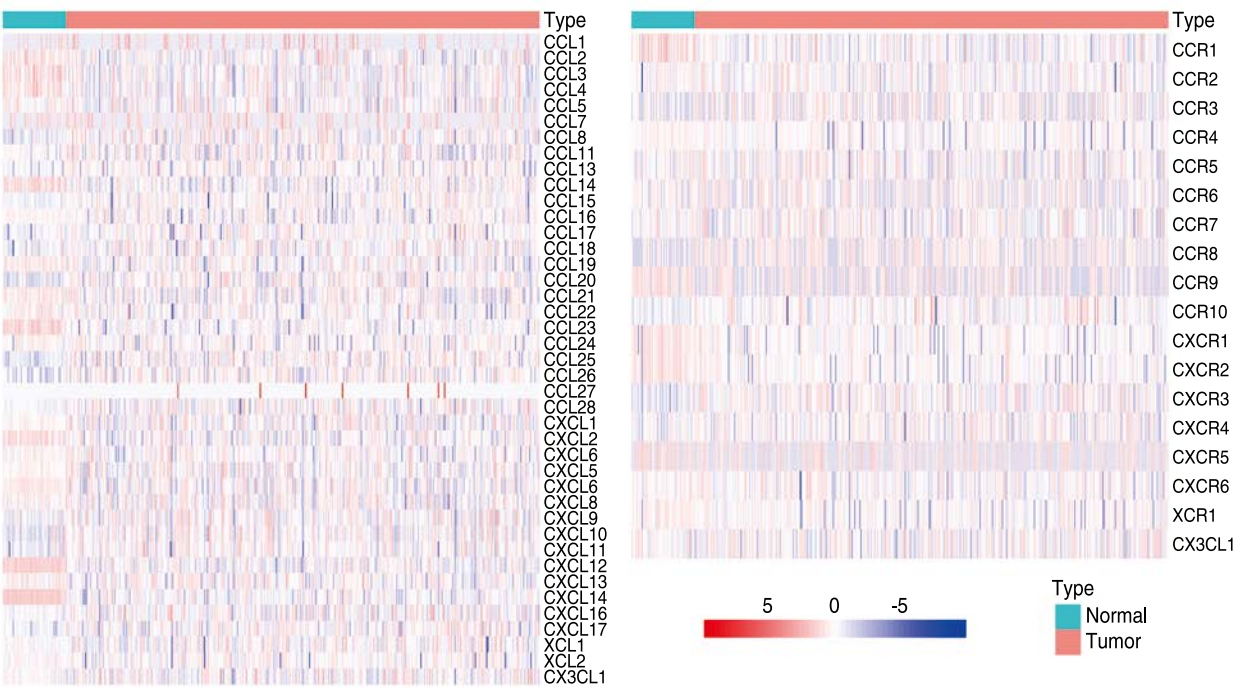

Fig. 1 Cytokine-chemokine profile in LIHC and normal liver tissues. The heat map shows cytokine and chemokine gene expression across TCGA-LIHC (tumor tissue, *n* = 371; normal tissue, *n* = 50). Datasets were analyzed using UCSC Xena (https:--xenabrowser.net-datapages-). LIHC: liver hepatocellular carcinoma; TCGA: The Cancer Genoma Atlas

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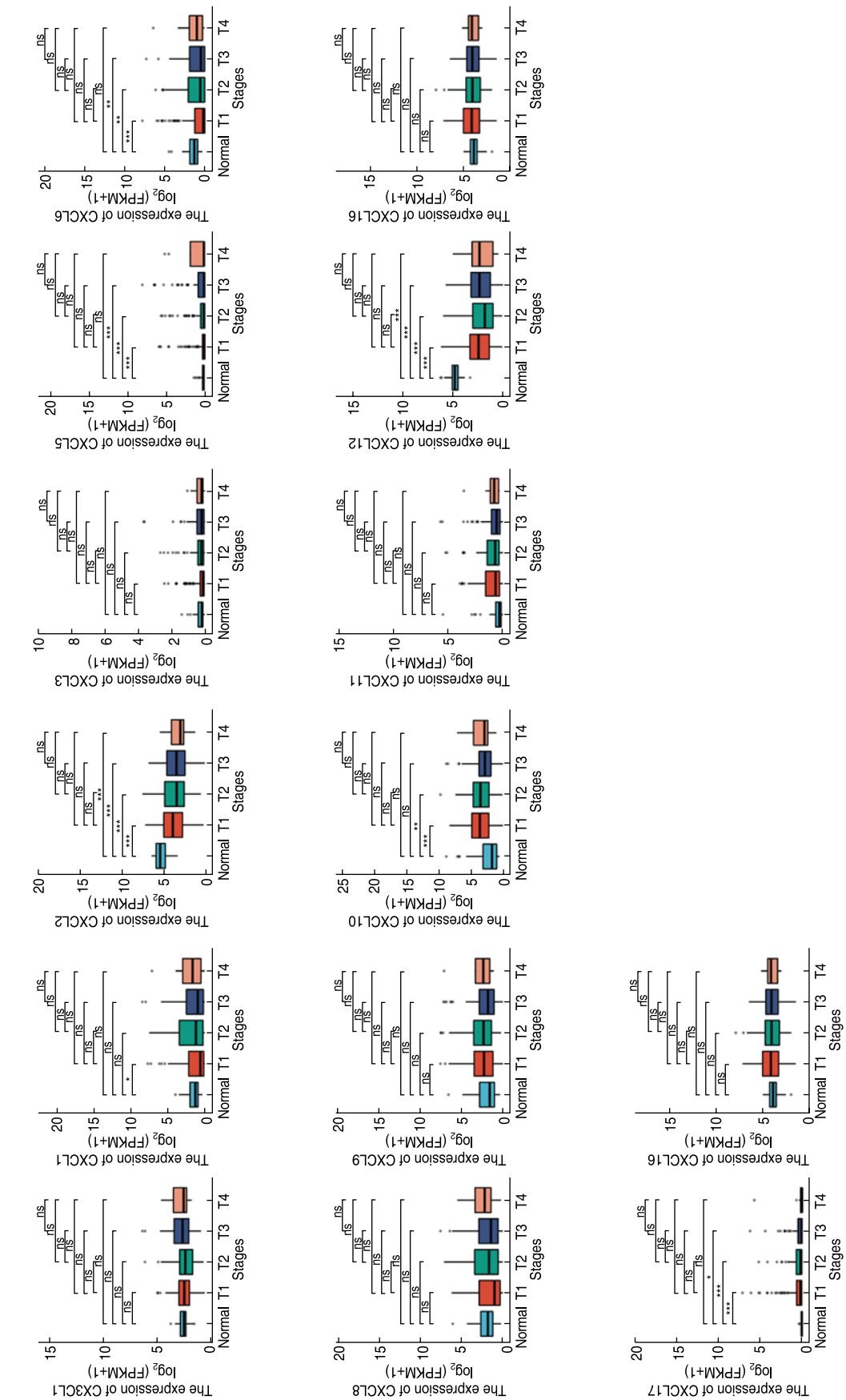
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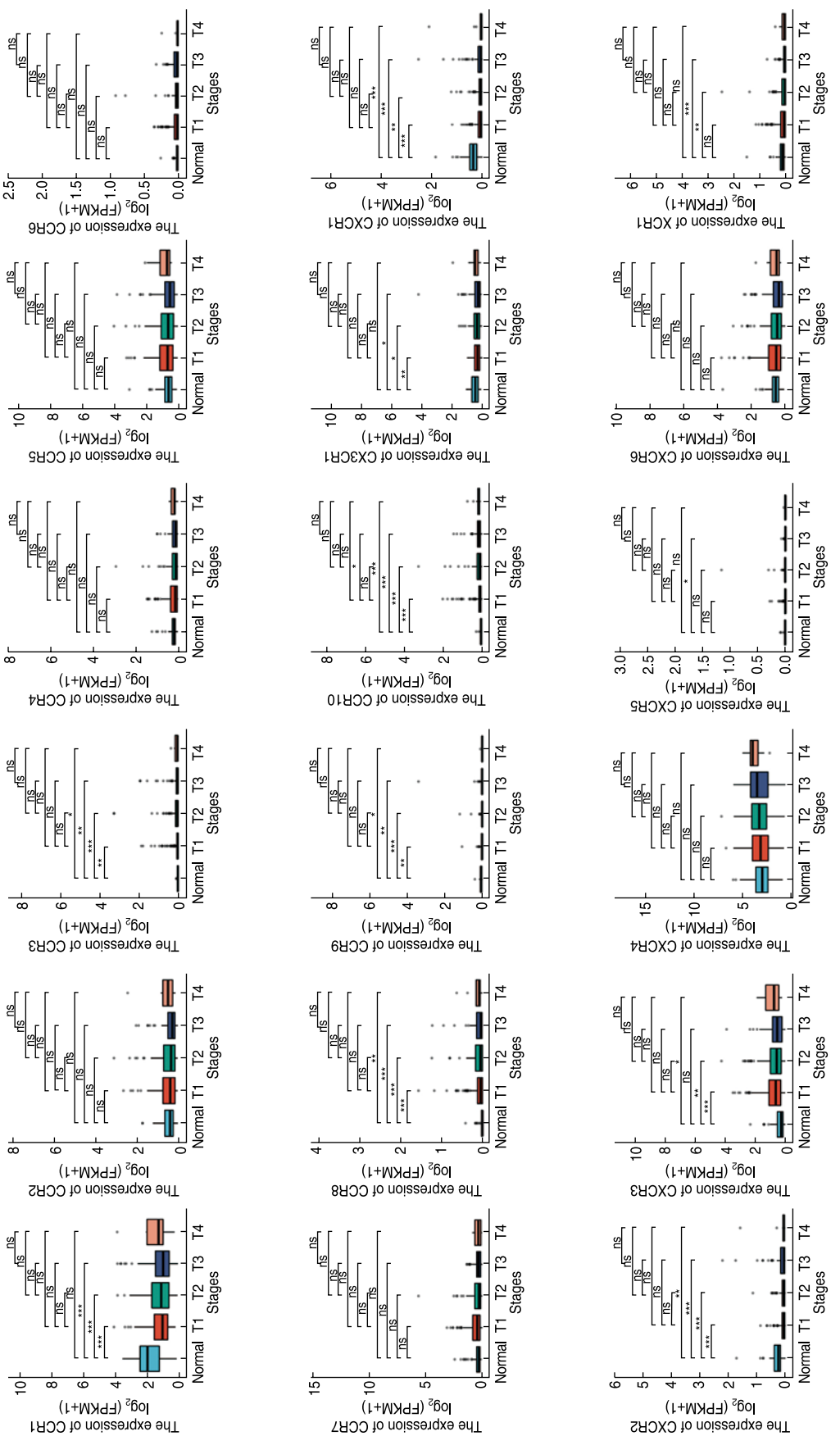

Fig. 2 The clinical correlation of chemokines and chemokine receptors with tumor stage in HCC. (a) Correlation between HCC tumor stage and CCL chemokines and XCL chemokines; (b) Correlation between the HCC tumor stage and CXCL chemokines; (c) Correlation between the HCC tumor stage and chemokine receptors. Datasets were obtained from TCGA-LIHC (tumor tissue, $n = 371$; normal tissue, $n = 50$; stage T1, $n = 183$; stage T2, $n = 95$; stage T3, $n = 80$; stage T4, $n = 13$). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. HCC: hepatocellular carcinoma; LIHC: liver hepatocellular carcinoma; TCGA: The Cancer Genoma Atlas

functions at different stages of HCC progression [8-10]. The composition of the immune TME may determine the anti- and protumorigenic effects of CCL2-CCR2 signaling. During early HCC, there are not enough $CCR2^+$ inhibitory immune cells in the TME (such as $CCR2^+$ Treg cells) to antagonize the interaction of CCL2 with CCR2 on $CD8^+$ T cells. Therefore, CCL2-CCR2 may protect against tumor initiation in the early stages of HCC. However, immunosuppressive cells in the TME of advanced HCC impair the recruitment of $CD8^+$ T cells, thus abolishing their antitumor effects [9]. CCL2 is highly expressed in HCC and is an independent prognostic factor of overall survival [11].

CCL5 (also known as regulated upon activation of normal T cell expressed and secreted factor, RANTES) may also play a dual role in HCC. CCL5 restores immune surveillance in β-catenin-driven HCC cells by recruiting $CD103^+$ dendritic cells and antigen-specific $CD8^+$ T cells [12]. Furthermore, CCL5 and CCL4 can attract γδ T cells to HCCs. γδ T cells have cytotoxic antitumor activity and regulate the infiltration and differentiation of $CD8^+$ T cells [13]. However, CCL5 is overexpressed in HCC compared to adjacent tissues and is associated with proliferation, migration, and epithelial-mesenchymal transition (EMT) in HCC [14]. Furthermore, CCL5 is overexpressed in circulating tumor cells (CTCs) and enhances the migration ability of CTCs by recruiting Treg cells [15].

CCL17 (also known as thymus and activation-regulated chemokine, TARC) and CCL22 (also known as macrophage-derived chemokine, MDC) share 37% homology in their amino acid sequences. CCR4 is a receptor for both CCL17 and CCL22 [16]. Both CCL17 and CCL22 are potent chemoattractants for trafficking Treg cells into the TME in HCC. Treg cells are involved in immune response disruption; therefore, these chemokines create a microenvironment conducive to metastasis [17, 18]. CCL17- and CCL22-recruited Treg cells also participate in the construction of an inhibitory immune environment for HBV-associated HCC [19].

CXCL8 (also known as interleukin-8, IL-8) is a pro-inflammatory chemokine with multiple protumorigenic roles in HCC. CXCL8 specifically binds to CXCR1 (IL-8 receptor [IL-8R] A or CD181) and CXCR2 (IL-8RB). Upstream NF-κB signaling promotes the production of CXCL8, which triggers activation of PI3K-MAPK signaling in HCC cells, thereby mediating proliferation, angiogenesis, and migration [20-22]. CXCL8 is overexpressed in HCCs and in highly metastatic HCC cell lines [20, 23]. Further, higher expression of CXCL8 may predict poor prognosis in HCC patients [23, 24].

CXCL9 (also known as monokine induced by γ interferon, MIG), CXCL10 (also known as interferon γ-induced protein 10, IP-10), and CXCL11 (also known as interferon-inducible T-cell alpha chemoattractant, ITAC) are all Th1-activating chemokines and selective ligands for CXCR3 [25]. These three chemokines are potent chemotaxis regulators of $CD8^+$ T cells and other effector immune cells by binding to CXCR3 in an autocrine or paracrine manner. They are secreted by tumor cells and $CD8^+$ T cells either dependent or independent of interferon-γ (IFN-γ) stimulation [26-28]. The expressions of CXCL9, CXCL10, and CXCL11 are closely associated with overall survival in HCC and sensitivity to immune therapy [29]. A detailed study of these three chemokines in HCC will be presented later.

The chemokine CXCL12 (also known as stromal cell-derived factor-1, SDF-12) binds primarily to CXC receptor 4 (CXCR4 or CD184) [30]. Hepatoma cells and hepatic stellate cells (HSCs) are the primary sources of CXCL12 [31, 32]. Some studies have noted CXCR4 expression in HCC tissue but not in normal liver tissue. In HCC, CXCR4 is expressed in multiple cell types, such as lymphocytes, HSCs, MDSCs, tumor cells, and other stromal cell types [31, 33, 34]. CXCL12-CXCR4 signaling in tumor cells promotes pathological angiogenesis, survival, invasion, and immune evasion surveillance [31]. Higher CXCR4 expression is positively associated with aggressive tumor behavior and poor prognosis [35].

It is worth mentioning that most chemokines showed tumor-promoting effects in most studies. Chemokines such as CCL14 (also known as hemofiltrate C-C chemokine-1) may be associated with tumor suppression. Zhu *et al*. observed that CCL14 is downregulated in HCC tissues, and low expression of CCL14 in HCC is associated with poor prognosis [36].

Role of chemokines and chemokine receptors in the HCC immune microenvironment

Chemokines mediate remodeling of the TME by recruiting immune cells and regulating their motility and function (Fig. 3). Here, we highlight the role of chemokines in immune cells in HCC.

Chemokines, chemokine receptors, and TAMs

Macrophages infiltrating the TME are called TAMs. Liver macrophages consist of liver-resident macrophages termed KCs and monocyte-derived macrophages recruited from the peripheral blood or bone marrow [37]. KCs originate from yolk sac-derived specific progenitor cells and seed in the liver. KCs have no migratory characteristics but do have phagocytic capacity and maintain liver homeostasis as a critical part of the innate immune system in the liver [38]. Multiple chemokines, as well as colony-stimulating factor 1 (SCF1), recruit peripheral monocytes into the TME and expand the macrophage pool during disease progression [39]. Of the

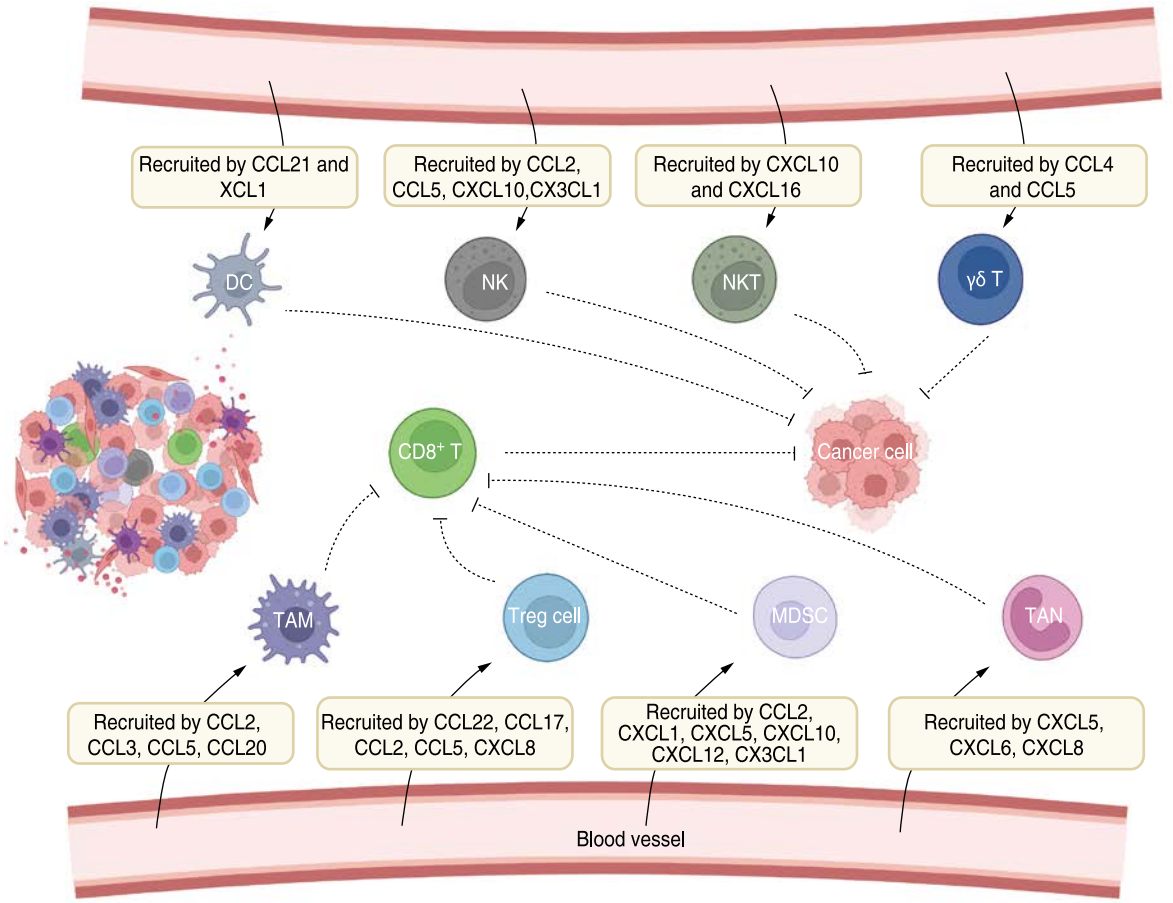

Fig. 3 Recruitment of immune cells or stromal cells by chemokines within the tumor microenvironment. DC: dendritic cell; NK: natural killer; NKT: natural killer T; TAM: tumor-associated macrophage; Treg: regulatory T; MDSC: myeloid-derived suppressor cells; TAN: tumor-associated neutrophil

chemokines involved in hepatocellular carcinogenesis, CCL2 has the greatest ability to recruit immature myeloid cells (iMCs), monocytes, macrophages, and TAMs [10, 40]. In pre-malignant liver tissue, senescent and injured hepatocytes secrete CCL2 and recruit CCR2+ monocytes and iMCs into the liver, which differentiate into pro-inflammatory macrophages and inhibit tumor initiation [8]. However, cytokine deregulation can block massive monocyte and iMC maturation, leading to a disordered TME. These abnormally differentiated cells lose their capacity for immune surveillance and instead exert an immunosuppressive effect, leading to immune escape [8]. CCL20 may be an additional chemoattractant signaling pathway that recruits TAMs via CCR6. CCR6+ monocytes-macrophages accumulate in the TME of HCC, and CCL20 expression is positively associated with intratumoral TAMs [41]. Furthermore, hypoxic cancer cells recruit monocytes by secreting CCL20, further stimulating indoleamine-2,3-dioxygenase 1 (IDO) expression in monocytes [42]. These inhibitory IDO+ monocyte-derived macrophages inhibit T cell responses and promote tumor antigen tolerance [42]. Interferon regulatory factor 8 (IRF8) can inhibit HCC by mediating IFN-γ and PD-1 signaling. IRF8 impedes TAM infiltration by inhibiting CCL20 secretion [43]. Further, CCL3-CCR1 signaling increases KC accumulation in N-nitrosodiethylamine (DEN)-induced hepatocarcinogenesis, suggesting a role for immune cells in this process.

Macrophages also secrete chemokines. Hou *et al.* noted that macrophages may be the primary source of CCL1. They demonstrated that the CCL1-CCR8 axis alters HCC intracellular signaling through epigenetic regulators and mediates crosstalk between HCC cells and macrophages [44]. KCs secrete CCL2 during miR-206-mediated KC M1 polarization. This KC-derived CCL2 promotes CD8+ T cell migration and expansion and impedes tumor progression in the early stage of HCC development [45]. CCL17 and CCL22 are cytokines that can recruit Treg cells and are secreted by M2 TAMs in sorafenib-treated HCC [46, 47], indicating that CCL2 mediates crosstalk between M2 TAMs and Treg cells, which contributes to an immunosuppressive microenvironment. M2 TAM-derived CCL22 can also directly target cancer cells and promote EMT in HCC [48]. Activated CD4+ T cells stimulate macrophages to produce

Table 1 The secreting and targeting cells of chemokines in HCC and their role within TME

Chemokine (Alternate names)	Receptor	Secretory cell	Target cell	Effect	References
CCL1 (I-309)	CCR8 (CD198)	Macrophage	Hepatoma cells	• Promotes the conversion of monocytes to macrophages and mediates the crosstalk between monocytes/ macrophages and HCC cells.	(1)
CCL2 (MCP1)	CCR2 (CD192)	Hepatoma cells KCs TANs NK cells	iMCs TAMs Treg cells MDSCs $CD8^+$ T cells NK cells	• Recruit CCR2+ iMCs into the vicinity of oncogene-induced senescent hepatocytes. • Acts tumor suppressive in early stages of liver tumorigenesis, while promotive during tumor progression. • Facilitates TAMs M1 polarization and increases CD8+ T cells infiltration during the initiation and early stage of HCC while educating the polarization of M2-type TAMs, MDSCs, and Treg cells in advance HCC.	(2-8)
CCL3 (MIP1α)	CCR1 CCR5 (CD195)	Monocytes Hepatoma cells (those stimulated by proinflammatory cytokines) NK cells	Hepatoma cells KCs	• Inhibits hepatoma cell lines proliferation. • Promotes angiogenesis through MMP9 in DEN-induced HCC. • Increases KCs infiltration in DEN-induced HCC.	(8-10)
CCL4 (MIP1β)	CCR1 CCR5	Hepatoma cells	γδ T cells	• Attracts γδ T cells from peripheral blood or peritumor regions into HCC. • Enhances anti-tumor immunity and improves HCC patients' prognosis.	(11)
CCL5 (RANTES)	CCR1 CCR4 (CD194) CCR5	Hepatoma cells CTCs MSCs CAFs NK cells	Treg cells γδ T cells Hepatoma cells NK cells	• Recruits Treg cells to prevent CTCs from immune clearance. • Attracts γδ T cells and NK cells from peripheral blood or peritumor regions into HCC. • Induces EMT and promotes the migration and invasion of HCC cells.	(7, 8, 11-14)
CCL14 (HCC-1)	CCR1 CCR3 CCR5	Not mentioned	Hepatoma cells	• Suppresses the proliferation of HCC cells and promotes their apoptosis.	(15)
CCL15 (LKN-1, MIP-5, HCC-2)	CCR1	Hepatoma cells (TNF-α and IFN-γ promote CCL15 production)	Monocytes Hepatoma cells MSCs	• Promotes HCC migration. • Recruits suppressive monocytes, impairing anti-tumor immunity and accelerating tumor proliferation and invasion. • Mediates the homing of MSCs into HCC, which are regarded as a promising delivery of therapeutic genes in anti-HCC therapy.	(16-18)
CCL16 (HCC-4, LEC)	CCR1 CCR8	Not mentioned	Hepatoma cells	• Mediate hepatoma cell adhesion and maximal migration at different concentration.	(19)
CCL17 (TARC)	CCR4	TANs Macrophages Hepatoma cells	Treg cells	• Promotes Treg cells intratumoral infiltration and facilitates HCC neovascularization and progression. • Contributes to sorafenib resistance.	(6, 20, 21)
CCL20 (LARC, MIP-3α)	CCR6 (CD196)	Hepatoma cells Myofibroblasts	TAMs Hepatoma cells $CD19^+$ $CD5^+$ B cells	• Enhances the migratory ability of macrophages and $CD19^+$ $CD5^+$ B cells. • Recruits Tregs and contributes to HCV-related HCC progression. • Enhances the capacity of tumor angiogenesis and migration through the responding B cells. • Promotes aerobic glycolysis in cancer cells. • Induces the expression of IDO of macrophage.	(22-26)

Chemokine (Alternate names)	Receptor	Secretory cell	Target cell	Effect	References
CCL21 (SLC, 6Ckine)	CCR7 (CD197)	Not mentioned	DCs	• Induces the maturation of DCs. • The intra-tumoral administration of CCL21 and anti-CD25 constitutes an anti-tumor environment in TME via altering the profiles of cytokines and immune cells.	(27)
CCL22 (MDC)	CCR4	Hepatoma cells M2-type TAM KCs	Treg cells	• Recruits Treg cells to facilitate immune escape. • Promotes HCC growth and enhances tumor invasiveness through EMT activation. • Promotes venous metastases and the development of portal vein tumor thrombus in HBV+ HCC. • Contribute to HBV-associated sorafenib resistance.	(21, 28-31)
CCL26 (eotaxin-3)	CCR3 (CD193)	CAFs	HSCs	• Recruits HSCs and exacerbates HCC initiation.	(32)
CXCL1 (GROα, MGSA)	CXCR1 (IL-8RA, CD181) CXCR2 (IL-8RB)	Hepatoma cells $CD133^+$ TICs	MDSCs	• Mediates the migration of MDSCs into HCC and subsequent immune escape. • Modulates tumorigenicity and self-renewal properties of CD133+ TICs	(33-35)
CXCL2 (GROβ, MIP2α)	CXCR1	TAMs IIC2s	Neutrophils	• Recruits and sustains the survival of neutrophils in HCC tumor milieus.	(36, 37)
CXCL5 (ENA-78, SCYB5)	CXCR2	Hepatoma cells	TANs Hepatoma cells MDSC	• Activates HCC cells EMT phenotype and promote HCC proliferation and lung metastasis. • Recruits immunosuppressive TANs and MDSCs into the tumor site of HCCs.	(38-41)
CXCL6 (GCP2)	CXCR1 CXCR2	Hepatoma cells	CAFs TANs	• Facilitates HCC cells' stem-like properties. • Activates ERK1/2 signaling in CAFs and mediates the crosstalk between CAFs and TAN, accelerating HCC progression.	(42)
CXCL8 (IL-8)	CXCR1 CXCR2	$CD133^+$TICs TAMs IIC2s Hepatoma cells CAF	Neutrophils Liver TICs Hepatoma cells LESCs M2-type macrophage	• Promotes tumorigenicity, angiogenesis, and self-renewal ability of liver TICs • Recruits neutrophils into HCC. • Induces M2-type macrophage polarization. • Enhances the permeability of LESCs via decreasing tight junctions between cells. • Enhances the capacity of LSECs to induce Treg cells. • Promotes HCC growth, migration, and invasion.	(35-37, 43-46)
CXCL9 (MIG)	CXCR3 (GPR9, CD183)	Hepatoma cells $CD8^+$ T cells	$CXCR3^+$ B cells $CD8^+$ T cells	• Promotes the recruitment of $CXCR3^+$ B cells. • Promotes $CD8^+$ T cells migration into HCC.	(47-49)
CXCL10 (IP10)	CXCR3	Hepatoma cells $CD8^+$ T cells TAMs	CXCR3+ B cells CD8+ T cells Treg cells NK cells MDSCs	• Promotes $CD8^+$ T cells migration into HCC. • Promotes the maturation of CXCR3+ B cells. • Recruits NK cells and NKT cells and enhances their anti-tumor efficiency through promoting IFN-γ secretion. • Recruits Treg cells and MDSCs, and mediates HCC growth and recurrence after liver transplantation.	(48-54)
CXCL11 (ITAC)	CXCR3 CXCR7 (GPR159)	Hepatoma cells $CD8^+$ T cells $\alpha2\delta1^+$ TICs HSCs CAFs	$CXCR3^+$ B cells $CD8^+$ T cells $\alpha2\delta1^+$ TICs Hepatoma cells	• Promotes the recruitment of CXCR3+ B cells and CXCR3+ T cells. • Promotes CD8+ T cells migration into HCC. • Promotes the stemness, proliferation and drug resistance of HCC TICs • Promotes HCC cells migration.	(48, 49, 55-57)

Chemokine (Alternate names)	Receptor	Secretory cell	Target cell	Effect	References
CXCL12 (SDF-1)	CXCR4 (CD184) CXCR7	OV6+ HCC cells Hepatoma cells HSCs	OV6+ HCC cells Hepatoma cells HSCs MDSCs	• Promotes OV6+ cell, a potential stem/progenitor-like cell, self-renewal and migration. • Promotes HCC cells migration and invasion. • Mediates HSCs differentiation to myofibroblasts in HCC and further fibrosis. • Increases Gr1+ myeloid cell infiltration in HCC after sorafenib treatment. • Modulates migration ability of MDSCs and endothelial cells.	(58-61)
CXCL16 (SRPSOX)	CXCR6 (CD186)	LSECs Hepatoma cells	NKT cells Hepatoma cells	• Mediates Simvastatin inhibition of HCC progression via recruiting NKT cells. • Contributes to HCC cell migration and invasion via an autocrine loop.	(62, 63)
CXCL17	Not mentioned	Hepatoma cells	TAMs	• Mediates TAMs polarization towards M2-type.	(64)
CX3CL1 (fractalkine)	CX3CR1 (GPR13)	Hepatoma cells	MDSCs NK cells	• Mediates MDSCs accumulation after CIK cell therapy, resulting in impaired anti-tumor activity. • Recruits NK cells that can function as robust effectors against HCC.	(64, 65)
XCL1	XCR1	NK cells CD8+ T cells	cDC1 cells	• Recruits cDC1 cells for tumor antigens presenting, attracting more CD8+ T cells to exert anti-tumor response.	(66)

CAFs: Cancer-associated fibroblasts; CTCs: circulating tumor cells; DEN: N-nitrosodiethylamine; EMT: Epithelial-mesenchymal transition; HSCs: Hepatic stellate cells; IFN-γ: Interferon-γ; IIC2s: G roup-2 innate lymphoid cells; iMCs: Immature myeloid cells; KCs: Kupffer cells; LESCs: Liver sinusoidal endothelial cells; MDSCs: Myeloid-derived suppressor cells; MSCs: mesenchymal stromal cells (MSC); NK cells: Natural killer cells; TAMs: Tumor-associated macrophages; TANs: Tumor-associated neutrophils; TICs: Tumor-initiating cells; TNF-α: Tumor necrosis factor; Treg cells: Regulatory T cells

CXCL10. After binding to CXCR3, CXCL10 stimulates B cells to transform into IgG-producing plasma cells, which produce IL-6, IL-10, and CCL20 [49]. Gut-derived IL-25 can also promote the secretion of CXCL10 from activated M2 TAMs, mediating the tumorigenesis of HCC [50]. Collectively, TAMs are attracted to tumor sites by chemokines and can communicate with surrounding cells by secreting chemokines to reshape the immune TME (Table 1).

Chemokines, chemokine receptors, and MDSCs

The disordered immune TME of HCC provides the necessary signals for the differentiation of immature myeloid cells into MDSCs with immunosuppressive activity. The levels of MDSCs are closely associated with overall survival, treatment efficacy, and tumor recurrence in HCC [51, 52]. Dysregulated chemokine signaling promotes the recruitment and activation of MDSCs during HCC development. MDSCs can be mobilized into the HCC tumor milieu through CXCL12-CXCR4 signaling [31]. In addition, tumor-associated fibroblast-derived CXCL12 can attract monocytes by binding to CXCR4, and their subsequent differentiation into MDSCs is mediated through leukocyte-derived IL-6-induced STAT3 signaling [53]. CCL2 from tumor cells can also direct MDSC homing in HCC, and CCR2 inhibition impedes MDSC accumulation [10]. CX3CL1-recruited MDSCs decrease the efficacy of cytokine-induced killer cell-based immunotherapy in advanced HCC [54]. The CXCL1-CXCR2 signaling pathway also contributes to the recruitment of MDSCs in HCC, and inhibiting CXCR2 reverses MDSC-mediated immunosuppression [55]. Further, psychological stress can affect tumor progression and clinical outcomes. A recent study indicated that chronic stress enhances MDSC mobilization and immunosuppressive proficiency via CXCL5-CXCR2-Erk signaling, revealing multiple roles for MDSCs in HCC [56].

Chemokines, chemokine receptors, and Treg cells

Treg cells are another group of cells that contribute significantly to the immunosuppressive TME. Identifying the complex signaling network among Treg cells and other immune, stromal, and tumor cells in the tumor milieu is of great therapeutic value.

Treg cells in HCC mainly express CCR4 and are recruited into the TME in response to CCL22 and CCL17. CCR4 is the only receptor for CCL17 [57]. Elevated CCL17 and CCL22 concentrations are associated with increased Treg cell infiltration in HCC [18, 58, 59]. Gao *et al.* noted that the chemokines CCL22 and CCL17 are upregulated by sorafenib, and CCR4+ Treg cells are the primary type of

Treg cells in HBV-associated HCC. These Treg cells are associated with sorafenib resistance and HBV load [19, 47]. HBV infection causes multiple pathological changes, including augmenting TGF-β signaling, which leads to the production of CCL22 and further recruitment of Treg cells [17]. CCL5-attracted Treg cells have also been reported to participate in immune evasion of CTCs by protecting them against immune clearance [15]. CXCL8-CXCR1 signaling provokes the polarization and accumulation of Treg cells to suppress antitumor immunity in HCC. Further, CXCL10, a typical chemoattractant for $CD8^+$ T cells and NKT cells, recruits Treg cells and MDSCs and mediates HCC growth and recurrence after liver transplantation [60, 61].

Chemokines, chemokine receptors, and cytotoxic T lymphocytes

$CD8^+$ T cells, or cytotoxic T lymphocytes, are a population of cytotoxic cells that can kill tumor cells by secreting high levels of IFN-γ, perforin, or protease granzyme B. They can also induce apoptosis via overexpression of FasL or tumor necrosis factor α (TNF-α) [62]. CXCL9, CXCL10, and CXCL11 are the main chemokines that attract $CD8^+$ T cells in HCC. CXCL10 is the most studied $CD8^+$ T cell chemoattractant in HCC. CXCL10 can activate tumor cells and promote IFN-γ secretion from NK cells and NKT cells, forming a positive feedback loop in the TME [26]. A recent study found that increased CXCL9/CXCL10 signaling may be responsible for the increased infiltration of $CD8^+$ T cells in HCC [29]. Kohei *et al*. determined that CXCL10 mediates increased $CD8^+$ T cell infiltration and provides a survival benefit in HCC patients treated with regorafenib and anti-PD-1 combination therapy [63]. In contrast, lower CXCL10 is associated with less $CD8^+$ T cell infiltration [64]. In addition, higher levels of CXCL9, CXCL10, and CXCL11 are associated with better response to PD-1 blockade [65]. Collectively, these findings suggest that the absence of CXCL9, CXCL10, and CXCL11 predisposes patients to HCC development.

Chemokines, chemokine receptors, and unconventional T cells

Unconventional T cells, such as γδ T and NKT cells, are also involved in tumor immunity in many cancers, although studies focusing on their role in HCC are relatively limited. γδ T cells and NKT cells are immune cells with cytotoxic activity, and their infiltration in HCC can enhance antitumor immunity and improve patient outcomes [13]. NKT cells are essential for antitumor immune surveillance in multiple tumor types, and their absence promotes tumor cancer development in HCC [66, 67]. CXCL10-CXCR3 and CXCL16-CXCR6 signaling can augment the migration of NKT cells into HCC. These NKT cells then regulate antitumor responses via the production of IFN-γ [26, 68]. CCL4 and CCL5 can promote the migration of γδ T cells into HCC through binding with CCR1 and CCR5, respectively, and both signaling pathways are associated with better overall survival and less aggressive tumors in HCC [13]. γδ T cells also express CCR2 and can be recruited to tumors by CCL2 [69]. However, intratumoral infiltration of γδ T cells is substantially impaired in HCC, which is partly mediated by Treg cells [70].

Chemokines, chemokine receptors, and NK cells

NK cells are innate immune system effector cells and play an indispensable role in tumor immune surveillance. The inflammatory cytokines TNF-α, IFN-γ, and Toll receptor-like ligands stimulate HCC cells and macrophages to secrete NK-trafficking chemokines that bind to receptors on NK cells, such as CXCL10, which binds to CXCR3 on NK cells [26]. Activated NK cells produce more IFN-γ, the best-characterized cytokine produced by NK cells. IFN-γ is a potent immune effector involved in multiple immune responses [71]. IFN-γ released by NK cells enhances the production of CXCL10, forming a positive feedback loop to block tumorigenesis [72]. CCL2, CCL5, and CX3CL1 can also chemoattract NK cells and enhance their cytotoxicity by binding to CCR2, CCR5, and CX3CR1, respectively [41, 72, 73]. Interestingly, human NK cells can secrete CCL2, CCL3, and CCL5

Table 2 Chemokine-targeted therapies in HCC

Target	Drug name	Combination strategy	Reference or Clinical trial number (disease)
CXCL12-CXCR4	AMD3100 (Plerixafo)		NCT01711073 (End-stage liver disease, excluding HCC)
		AMD3100+ Sorafenib	(104)
		AMD3100+ Sorafenib+ anti-PD-1 antibody	(111)
	RU486 (Metabolite)	RU486+ Sorafenib	(112)
CCL2-CCR2	RDC018		(11)
	BMS-813160	BMS-813160+ Nivolumab	NCT04123379 (NSCLC, HCC)
CXCL8-CXCR1-2	BMS-986253		NCT04123379 (NSCLC, HCC)

upon activation during the early stages of tumor growth. Therefore, these chemokines provide a mechanism to communicate between the innate immune response and the $CD8^+$ T cell-mediated immune response [74].

Chemokines, chemokine receptors, and tumor-associated neutrophils

Tumor-associated neutrophils (TANs) have emerged as essential players during tumorigenesis, mediating both pro- and antitumorigenic processes, depending on the composition of the TME. However, an increase in TANs tends to be associated with poor clinical outcomes in most cancers, including HCC [18, 75]. CXCL5 is the primary chemokine that mediates TAN infiltration in HCC [18, 76]. Sorafenib induces the recruitment of TANs via CXCL5 signaling. These TANs further increase the infiltration of macrophages and Treg cells by secreting CCL2 and CCL17, thereby mediating metastasis, neovascularization, and sorafenib resistance in HCC [18]. TANs can also augment the stem cell characteristics of HCC cells, which leads to higher levels of CXCL5, forming a positive feedback loop. Recently, CXCL16 was shown to contribute to the recruitment of TANs, facilitating tumor progression in middle -and late-stage HCC [77].

Treatment

Considering the crucial roles of chemokines and their receptors in HCC initiation and progression, efforts have been made to target chemokines in cancer. Although multiple preclinical studies have focused on chemokine signaling, few agents that directly target a single chemokine or chemokine receptor have been used clinically. This may due to the redundant nature of chemokine networks and cellular heterogeneity, which is not fully understood. Here, we summarize several single and combined strategies that focus on chemokine signaling in HCC.

CXCL12 and CXCR4 inhibition

The CXCL12-CXCR4 axis is one of the most studied chemokine axes and is regarded as a promising target in multiple cancer types [30, 108]. Sorafenib is the standard therapy for advanced HCC and was approved by the FDA in 2007 [109]. Although sorafenib prolongs survival in HCC, its efficacy is severely compromised because of the development of resistance. CXCL12 and CXCR4 may participate in sorafenib resistance [104]. Chen *et al*. observed that sorafenib-induced hypoxia increases the expression of CXCR4 and CXCL12, which increases the infiltration of immunosuppressive $Gr\text{-}1^+$ myeloid cells and HSCs [104]. AMD3100 (plerixafor) is a potent, specific antagonist of CXCR4 [110]. The combined administration of sorafenib and AMD3100 significantly slows murine tumor growth and alleviates hypoxia-induced tumor fibrosis, which decreases sorafenib resistance [104]. Chen *et al*. found that AMD3100 can also decrease the fraction of $F4\text{-}80^+$ TAMs and $CD4^+/CD25^+/FoxP3^+$ Treg cells in sorafenib-treated HCC murine models [111]. Furthermore, combination therapy of anti-PD-1 antibody, sorafenib, and AMD3100 increased $CD8^+$ T cell penetration and activation, ultimately delaying HCC progression better than the combinations of two of these drugs [111]. Zheng *et al*. found that metapristone (RU486 metabolite) reduces CXCR4 expression, which interrupts CXCL12-CXCR4 signaling and related downstream tumor-promoting signaling in HCC. Metapristone also enhances the anti-proliferative efficacy of sorafenib [112]. Moreover, to overcome the adverse side effects and poor pharmacokinetics of chemotherapy drugs, self-assembling nanocarriers to deliver sorafenib and metapristone into tumor tissue have been developed. The combined delivery of sorafenib and metapristone via CXCR4-targeted NPs significantly prolongs circulation time and enhances tumor absorption, leading to a stronger inhibitory effect of sorafenib [112].

CCL2-CCR2 inhibition

As discussed above, tumor cell-derived CCL2 recruits $CCR2^+$ immunosuppressive cells into the TME and induces TAM polarization toward the M2 phenotype. Li *et al*. validated that blocking CCL2-CCR2 signaling using the CCR2 antagonist RDC018 (US patent: US 8431590 B2) decreases the infiltration of TAMs, especially M2 TAMs, while enhancing peripheral $CD8^+$ T cells and cytotoxic $CD8^+$ TILs in a murine model [11]. Further, administration of a CCR2 antagonist significantly suppresses murine HCC growth and metastasis and prevents postsurgical recurrence in a $CD8^+$ T cell-dependent manner [11]. BMS-813160 is a potent and selective CCR2/5 dual antagonist. Several clinical trials are examining the combination of BMS-813160 and an anti-PD-1 antibody (nivolumab) in patients with non-small cell lung cancer (NSCLC), pancreatic ductal adenocarcinoma, and HCC (NCT04123379 and NCT03496662).

CXCL8-CXCR1/2 inhibition

The CXCL8-CXCR1/2 axis is involved in multiple malignant biological processes during HCC development, including tumor growth, angiogenesis, migration, and invasion [22, 91, 98]. CXCL8 and its receptors CXCR1/2 may be therapeutic targets in HCC. An anti-IL-8 mAb significantly increases overall survival and impairs angiogenesis in an HCC murine model [22]. Another study confirmed that IL-8 neutralizing antibodies can eliminate the pro-angiogenic and pro-tumorigenic activity of $CD133^+$ TICs in HCC [91]. A phase 2 clinical trial examining the administration of nivolumab and an anti-IL-8 monoclonal antibody (BMS-986253) in

patients with NSCLC or HCC is ongoing (NCT04123379). Reparixin, an investigational allosteric inhibitor of the IL-8 receptor CXCR1/2, is also being examined in clinical trials [113]. Reparixin inhibits HCC growth and metastasis by attenuating M2 polarization of TAMs and blocking EMT [98]. Reparixin also represses the stem cell features of HCC cells and enhances their sensitivity to sorafenib [114]. However, no clinical trials have examined reparixin in HCC.

Discussion

The HCC TME consists of carcinoma cells and multiple tumor-resident cells. These cells recruit various immune cells that express specific receptors from the peripheral blood or bone marrow by secreting cytokines [115]. Immune cells also secrete a variety of cytokines to reshape the TME. The process of tumorigenesis is modulated by cancer cells, tissue-resident cells, and immune cells. Chemokines, a specific subfamily of cytokines, act as messengers among these components. They shuttle between different or similar cells to regulate tumor initiation and development [5]. Because chemokines have tumor-promoting functions in HCC, including angiogenesis, invasion, migration, proliferation, and EMT, they are attractive therapeutic targets [116]. A series of preclinical experiments have examined the roles of chemokine inhibition in HCC. However, there are few studies examining chemokine inhibition as monotherapy. We posit two reasons for this. The first is the high degree of redundancy in chemokine signaling. When a specific chemokine signal is blocked, other chemokine signals can compensate to some extent and abolish the blocking effect. The second is that cellular functions are spatiotemporally heterogeneous. Cells of different subtypes and cells in various stages of tumor development display diverse responses to chemokine stimulation. This remains a blind spot to identifying the underlying mechanism of chemokine signaling. However, despite these limitations, chemokines are attractive therapeutic targets, and chemokine-targeted therapies will continue to be assessed clinically.

The application of ICIs has profoundly improved the treatment landscape of HCC. However, despite their success, primary or acquired resistance to ICIs has decreased their effectiveness in patients with HCC [117]. Multiple studies have demonstrated the contribution of chemokine signaling to ICI resistance [3]. Therefore, the combination of chemokine-targeted therapy and ICIs may be a therapeutic strategy. This combination has already been shown to achieve a meaningful clinical response. In addition, analysis of multi-dimensional "spatiotemporal" axes of HCC samples using single-cell techniques can further clarify the spatiotemporal heterogeneity during HCC development. Clarifying the various immune cellular subtypes and their responsiveness to chemokines will facilitate precise chemokine-targeted therapy.

In conclusion, chemokine-targeted therapy provides a transformative therapeutic avenue for HCC treatment. Future research should clarify the value of chemokine-targeted therapy in combination with other therapeutic options, including ICIs, and the functional heterogeneity of chemokines across time and different cell subsets.

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Conflicts of interest

The authors indicated no potential conflicts of interest.

Author contributions

Conceptualization, Y.W. and L.X.; writing - original draft preparation, Y.W.; writing - review and editing, Y.W. and L.X.; bioinformatics analysis, Y.W. and M.S.; visualization, TZ and Y.F; supervision, X.J., M.X., and D.L.; funding acquisition, L.X. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The datasets generated and analyzed during the current study are available from the China Drug Trials Repository (http://www.chinadrugtrials.org.cn/index). Chemokine expression information was obtained and analyzed using UCSC Xena (https://xenabrowser.net/datapages/).

Ethical approval

Not applicable.

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