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CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - a target for novel cancer therapy

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Abstract

Chemokines are proteins which induce chemotaxis, promote differentiation of immune cells, and cause tissue extravasation. Given these properties, their role in anti-tumor immune response in the cancer environment is of great interest. Although immunotherapy has shown clinical benefit for some cancer patients, other patients do not respond. One of the mechanisms of resistance to checkpoint inhibitors may be chemokine signaling. The CXCL9, -10, -11/CXCR3 axis regulates immune cell migration, differentiation, and activation, leading to tumor suppression (paracrine axis). However, there are some reports that show involvements of this axis in tumor growth and metastasis (autocrine axis). Thus, a better understanding of CXCL9, -10, -11/CXCR3 axis is necessary to develop effective cancer control. In this article, we summarize recent evidence regarding CXCL9, CXCL10, CXCL11/CXCR3 axis in the immune system and discuss their potential role in cancer treatment.

Keywords

CXCL9; CXCL10; CXCL11; CXCR3; cancer; immunotherapy

Introduction

Chemokines are small proteins (8–15 kD) which interact with a subset of G protein-coupled receptors. They play key roles to induce chemotaxis, promote differentiation and

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multiplication of leukocytes, and cause tissue extravasation.[1] In 1987, Yoshimura et al. first reported about CXCL8 (IL-8), which regulates neutrophil trafficking.[2] Since then, much attention has been devoted to understanding the functions and role of chemokines in immune response. The CXCL9, -10, -11/CXCR3 axis has been a major focus of research, since it regulates differentiation of naive T cells to T helper 1 (Th1) cells and leads migration of immune cells to their focal sites.[3] Due to this pivotal role, this axis is essential for immune system on command. Recent data has suggested its clinical significance, but little is known about clinical outcomes in patients with cancer.

The CXCL9, -10, -11/CXCR3 axis mainly regulates immune cell migration, differentiation, and activation. Immune reactivity occurs through this axis by recruitment of immune cells, such as cytotoxic lymphocytes (CTLs), natural killer (NK) cells, NKT cells, and macrophages. Furthermore, Th1 polarization by this axis also activates the immune cells in response to IFN-γ.[4] Tumor-infiltrating lymphocytes are a key for good clinical outcomes and prediction of the response to existing checkpoint inhibitors.[5, 6] However, in vivo studies suggest the axis plays a tumorigenic role as well by increasing tumor proliferation and metastasis.[7, 8] Thus, a better understanding of this axis in the tumor environment is necessary to discover its role as a potential target for immunotherapy or as a predictive indicator for existing cancer treatments.

In this review, we discuss the current evidence about the role of the CXCL9, -10, -11/CXCR3 axis in tumor environment (TME) and immune response, and discuss the opportunities for novel therapies.

The expression and implication of CXCL9, CXCL10, CXCL11 and CXCR3

Immune cells are regulated by many different cytokines (including chemokines) not only for differentiation, but also for promptly infiltrating focal tissues through chemotactic gradients. The selection of immune cells that respond to chemotaxes is based on their surface receptors. Therefore, discrimination of the chemotactic gradients must be affected by the complicated interactions between cytokines and their receptors. CXCL9, -10, -11 are selective ligands for CXCR3. The ligands are usually expressed at low levels in homeostatic conditions, but upregulated by cytokine stimulation. CXCL9, -10, -11 are mainly secreted by monocytes, endothelial cells, fibroblasts, and cancer cells in response to IFN-γ, which are synergistically enhanced by TNF-alpha.[9, 10] CXCR3 is a receptor preferentially expressed on the surface of monocytes, T cells, NK cells, dendritic cells, and cancer cells.[11, 12] CXC chemokines are classified into two groups with and without ELR (Glu-Leu-Arg) motif.[13] Those with the ELR motif can allow neutrophils to migrate and have an angiogenic effect, whereas those without the ELR motif primarily allow lymphocytic migration and inhibit angiogenesis. CXCL9, -10, -11 are ELR-negative CXC chemokines that generally attenuate angiogenesis, leading to an anti-tumor effect. Interestingly, some reports show that CXCL9, -10, -11 increase tumor proliferation and metastases.[7] This may be due to the different effects of the ligands on the variants of CXCR3 (CXCR3A, CXCR3B and CXCR3-alt). Previous studies have shown that these ligands have different temporal and spatial patterns of expression through different regulatory elements in distinct cell types. As far as

the CXCR3 receptor is concerned, there are three variants with different roles in tumorigenesis. The features of each protein are described below.

CXCL9, also known as monokine induced by gamma interferon (MIG), is located on human chromosome 4, and is induced by IFN-γ but not by IFN-α/β.[14] CXCL10 and CXCL11 are also located on human chromosome 4. CXCL9 predominantly mediates lymphocytic infiltration to the focal sites and suppresses tumor growth.[15] In vivo models by Gorbachev et al. showed that CXCL9-deficient cancer cells are more tumorigenic than cancer cells expressing both CXCL9 and CXCL10.[15] Menke et al. reported that both CXCR3 and CXCL9 deficient mice had fewer loss of kidney function than CXCL10 deficient mice, showing the mice had fewer intrarenal T cells and macrophages in immune-mediated nephritis.[16]

CXCL10, known as interferon γ-induced protein 10 (IP-10), is strongly induced by IFN-γ as well as by IFN-α/β[17] and weakly by TNFα.[10] In vitro, CXCL10 can also be induced by NF-kB, and has been shown to have an early role in hypoxia-induced inflammation.[18, 19] Activation of IFN-regulatory factor 3, toll-like receptors, retinoic acid-inducible gene (RIG)-I, and melanoma differentiation-associated gene (MDA)-5 work in synergy with IFNs for CXCL10 induction.[17, 20] Serum CXCL10 concentration, but not CXCL9, was reportedly correlated with the number of circulating lymphocytes in head and neck cancer with radiation therapy.[21] Ming-Fang et al. revealed that CXCL10-deficient mice had higher mortality rate with the dengue virus infection.[22]

CXCL11, also known as interferon-inducible T-cell alpha chemoattractant (I-TAC) or interferon-gamma-inducible protein 9 (IP-9), is induced by IFN-γ and IFN-β, and weakly by IFN-α.[23] The affinity of CXCL11 for CXCR3 is the highest of the three selective ligands, followed by CXCL10 and CXCL9.[24, 25] The binding domain of CXCL11 on CXCR3 is located at a different site from that of CXCL9 and CXCL10.[26] Furthermore, CXCL11 can bind to CXCR7, which is associated with invasiveness and reduces apoptosis of tumor cells. [27]

CXCR3, also known as G protein-coupled receptor 9 (GPR9) or CD183, is a 7 transmembrane domain G-protein coupled receptor, which was first reported in 1989.[28] Like CXCL9, -10, -11, CXCR3 is also predominantly driven by IFN-γ.[29] CXCR3 has two distinct intracellular domains for activation: one is a carboxy-terminal domain for CXCL9 and CXCL10, and another is in the third intracellular loop for CXCL11.[26] CXCR3 is heavily expressed on Th1 cells, CTLs, NK cells and NKT cells. CXCR3 is downregulated on naıve T cells, but is rapidly upregulated by antigen-presenting dendritic cells,[30] leading to Th1 polarization. After polarization, Th1 cells induce activation of CTLs, NK cells, and NKT cells through IFN-γ.[4] Biochemical studies have revealed that there are at least three CXCR3 variants; CXCR3A, CXCR3B and CXCR3-alt, with unique characteristics.[31] CXCR3A represents classical CXCR3 roles which include chemotaxis and cell proliferation in IFN-γ-inducible immune responses; CXCR3B, which is spliced at an extension of the N terminus by 52 amino acids, induces cell apoptosis and inhibits cell migration; CXCR3-alt, a 101-aminoacid-truncated version, mainly mediates CXCL11 function.[31–33] Importantly,

CXCR3B can also bind to CXCL4, which is released from activated platelets during platelet aggregation, in addition to CXCL9, -10, -11.[32]

The immune response for host disorders through CXCL9, -10, -11/CXCR3 axis appears to depend on both the ligands and the variants of its CXCR3 receptor. In addition, the ligands can act as antagonists for CCR3 which stimulates Th2 polarization, and only CXCL11 can bind to CXCR7, also known as atypical chemokine receptor 3 (ACKR3), which has tumorigenic potential.

CXCL9, CXCL10, CXCL11/CXCR3 axis for immune response

This axis works primarily for immune cell migration, differentiation, and activation. Immune reactivity for each disorder is dependent on the types of leukocytes infiltrating the focal sites. Therefore, it is critical to understand which immune cells are involved in migration, differentiation, and activation through this axis. The axis also acts directly on cancer cells and promotes cancer cell proliferation and metastasis. (Figure 1)

For immune cell migration, each of the CXCR3 ligands are equally effective on activated Th1 cells, CTLs, and NK cells in vivo models of cell recruitment. [34, 35] All three variants of CXCR3 are expressed on T cells, where CXCL9, -10, -11 collectively stimulate the loss of surface CXCR3 expression and elicit directional migration responses to the focal sites. [36] Chheda et al. demonstrated a critical role of CXCR3 for CTLs migration using CXCR3 knock-out mice in a syngeneic murine model of B16 melanoma, which revealed clear tumor growth and reduced survival.[37] Furthermore, CXCL9, -10, -11 attrac Th1 cells, and block the migration of Th2 cells in response to CCR3 ligands due to their ability to serve as antagonists for CCR3.[38] On the other hand, NK cell subsets, the anti-tumor effectors that express CXCR3, are also recruited to the site in a CXCR3-dependent manner.[35] Wende et al. reported that tumor-infiltrating NK cells significantly decreased in CXCR3 knock-out mice, where the CXCL10-controlled NK cell recruitment was not only correlated with tumor cell suppression, but with a good prognosis as well.[39] Furthermore, the accumulation of γδT cells, which shows an autoimmune response to infections or cancers, is reportedly governed by CXCL9/CXCR3 axis-dependent mechanisms.[40] Interestingly, although CXCL4 induces apoptotic signals through CXCL4/CXCR3B axis,[32] Korniejewska et al. showed that CXCL4 could not elicit T cell migration in spite of intracellular calcium mobilization as well as phosphorylation of Akt and ERK. It means CXCL4 may have other roles in T cell function.[36] Experimental studies in various disease models indicate that deficiency of the three ligands for CXCR3 significantly impairs cell-mediated immunity.[34, 35, 37, 39] However, some reports conversely show that the axis regulates immune suppression by inducing Treg migration to the focal sites.[41, 42]

For immune cell differentiation, some reports show that CXCL9, -10, -11 all lead to Th1 polarization through CXCR3, whereas other reports present different functions.[41, 43, 44] In vivo model by Zohar et al[44] showed that CXCL10, like CXCL9, drove increased transcription of T-bet and RORγ, leading to the polarization of Foxp3^{-} type 1 regulatory (Tr1) cells or T helper 17 (Th17) from naive T cells via STAT1, STAT4, and STAT5 phosphorylation. In contrast, CXCL11 decreased transcription of RORγ, but not T-bet,

leading to Tr1 or Th2 cells polarization from naive T cells via p70 kinase/mTOR pathways, similar to the mechanism involving TGFβ and IL-27.[45, 46] Unfortunately, these studies did not investigate variants of CXCR3. However, considering that CXCL11 has high affinity for CXCR3 and has such functions, CXCL11 might work to stimulate cancer growth. **S**everal studies have shown that tumor associated macrophages (TAMs) play modulatory activities in the TME, and the CXCL9, -10, -11/CXCR3 axis impacts TAMs polarization. The TAMs have opposite effects; M1 for anti-tumor activities, and M2 for pro-tumor activities. Interestingly, Oghumu et al clarified that CXCR3 deficient mice displayed increased IL-4 production and M2 polarization in a murine breast cancer model, and decreased innate and immune cell-mediated anti-tumor responses.[47] However, on the contrary, Liu et al. reported that CXCR3-positive B cells infiltrated to tumor site and operated in immunoglobulin G–dependent pathways to induce M2 polarization in hepatocellular carcinoma. This difference might be explained by the difference in the tissue background, the degree of inflammation, and the induced immune cells depending on organs and cancer types.

For immune cell activation, CXCL9, -10, -11 stimulate immune cells through Th1 polarization and activation. Th1 cells produce IFN-γ, TNF-α, IL-2 and enhance anti-tumor immunity by stimulating CTLs, NK cells, NKT cells, and macrophages.[4, 48] Furthermore, the IFN-γ-dependent immune activation loop also promotes CXCL9, -10, -11 release. Importantly, NK cells can display immune activity by modulating dendritic cell function, and also provide an early source for IFN-γ production.[35]

Naturally, immune cells, mainly Th1, CTLs, NK cells, and NKT cells, show anti-tumor effect against cancer cells through paracrine CXCL9, -10, -11/CXCR3 axis in tumor models.[15, 49, 50] However, the autocrine CXCL9, -10, -11/CXCR3 signaling in cancer cells increases cancer cell proliferation, angiogenesis, and metastasis. Past reports have already shown the possibility that cancer cells with CXCR3 have a propensity to metastasize due to autocrine signaling from the pre-metastatic niche in vitro and in vivo.[7, 8] The axis for metastases facilitates the migration of CXCR3 expressing cancer cells to ligand rich metastatic sites. As CXCR3-A plays a key role in metastasis,[51] treatment targeting only CXCR3A, not CXCR3B and CXCR3-alt, in the CXCL9, -10, -11/CXCR3 axis could be effective in metastatic disease.

The expression level of CXCR3 in clinical cancer samples is associated with metastatic potential and patients' prognosis.[52, 53] Hence, it is feasible to use this axis as a predictor for treatment efficacy or as a prognostic indicator. Although Wightman et al. identified the critical role of CXCL10/CXCR3 co-expression in increasing metastatic potential,[54] the relationship between the expression levels of three ligands and metastasis or prognosis are still controversial. The reduction of not only CXCR3, but also of CXCL9 and CXCL10 could suppress cancer metastatic frequencies in melanoma,[55] colon cancer,[7, 52, 56] and breast cancer models[57, 58]. There is a consensus among some groups about the association between CXCL9 [59, 60] and CXCL10 [54, 61] expression and poor prognosis or negative response to existing therapy, whereas others report that CXCL9 [62–64] and CXCL10 [65, 66] are related to opposite results. These differences in reports may be due to complex relationship between each ligand depending on the cancer types. Weisi et al.

reported the interesting strategy which systematically made a score using the expression levels of CXCL9, -10, -11 to predict the patients outcome.[61] In the future, we may have to consider the expression levels of CXCL9, -10, -11 to predict patient prognosis.

CXCL9, CXCL10, CXCL11/CXCR3 axis, a target for cancer treatment

The CXCL9, -10, -11/CXCR3 axis is a promising target for drug development by activating the paracrine axis, and inhibiting the autocrine axis. Agents that augment paracrine CXCL9, -10, -11 expression, and deactivate CXCR3 expression on cancer cells have shown anti-tumor activity in several tumor models. (Table 1)

The use of ligands that attract Th1 cells, CTLs, NK cells, NKT cells, and M1 macrophages into tumor sites can serve as an effective anti-tumor strategy. Zhang et al. reported that the combination of plasmid-borne CXCL9 plus cisplatin augmented colon and lung cancer reduction and CTLs activation.[67] In renal cell carcinoma tumor model, intratumoral CXCL9 and systemic IL-2 reduced tumor growth and angiogenesis through tumor-infiltrating CXCR3+ mononuclear cells.[68] Arenberg et al. reported that administration of CXCL10 by intratumor injections induced better survival of mice inoculated with lung carcinoma cells.[69] Using retroviral CXCL10 gene transduction, the usefulness of CXCL10 overexpression to inhibit tumor growth was reported in melanoma, sarcoma, and lung carcinoma models.[70, 71] Interestingly, Barash et al. showed promising results of a CXCL10–Ig fusion protein in a myeloma mouse model. This fusion protein is likely to have a longer half-life while maintaining the features of the recombinant protein, and inducing tumor infiltrating CTLs and NK cells into tumor sites.[72] Furthermore, a novel CXCL10-EGFRvIII fusion protein (IP10-scFv) with CTLs administration succeeded to induce tumor infiltrating lymphocytes and prolong survival, using a glioma mouse model.[73, 74] Since CXCL11 contributes to inducing Treg migration or promoting Tr1 and Th2 cells polarization, CXCL11-dependent therapy may be controversial as a new target for cancer therapy. In a mesothelioma mouse model, a tumor-selective oncolytic vaccinia virus with CXCL11 reportedly enhanced tumor-infiltrating CTLs and NK cells, but not CD4+ T cells, and prolonged survival.[75] In an autoimmune encephalomyelitis mouse model, the treatment with CXCL11-Ig fusion protein induced rapid disease remission through a downregulation of T cell migration and upregulation of Treg polarization,[44] suggesting the complexity of targeting CXCL11. Although these reports have not shown the role of CXCR3 variants, they might be targets of drug development by activating paracrine signaling.

The anti-CXCR3 therapy is promising. In murine models, pharmacological antagonism of CXCR3 reduced tumor growth and the development of metastasis. An antagonist for CXCR3, named AMG487, inhibited the implantation and growth of colon cancer and osteosarcoma cells in vitro, and suppressed lung metastasis in a vivo model.[7, 76] Interestingly, AMG487 could inhibit lung metastasis, but not local growth, in vivo in breast cancer.[8, 57] Cambien et al also showed that AMG487 could not suppress liver metastasis and the growth of metastatic tumor.[7] These findings indicate that anti-CXCR3 may specifically inhibit tumor metastasis while also adversely inhibiting anti-tumoral host response through paracrine CXCL9, -10, -11/CXCR3 axis. AMG 487 targets all variants of CXCR3, so suppression of paracrine axis may have a pro-tumor effect. Therefore,

administration of the combination of ligands for immune activation and pharmacological inhibition of CXCR3A to prevent metastasis may be a promising new approach.

CXCL9, CXCL10, CXCL11/CXCR3 axis, an enhancer for other immune pathways

Although the clinical relevance of the IFN-γ/CXCL9, -10, -11/CXCR3 axis is getting established, it is critical to understand how this pathway crosslinks with other immune consistent pathways. (Table 2)

The relationship between CXCL9, -10, -11/CXCR3 axis and the PDL-1/PD-1 axis is an important area of research. Programmed cell death-1 (PD-1) is heavily expressed on T cells at the tumor site than on T cells present in the peripheral blood,[77] and anti-PD-1 therapy can inhibit “immune escape” and strengthen the immune activation.[37, 77, 78] Peng et al. showed that anti-PD-1 could not only enhance T cell-mediated tumor regression but also increase the expression of IFN-γ and CXCL10, not CXCL9 and CXCL11 by bone marrow–derived cells.[77] Chheda et al. demonstrated a critical correlation between CXCR3-induced T cells homing to tumor site and anti-PD-1 treatment effect in a vivo model. Anti-PD-1 failed to shrink the tumor in CXCR3 knock out mice, suggesting that anti-PD-1 therapy is not effective without CXCL9, -10, -11/CXCR3 axis.[37] Blockade of the PDL-1/PD-1 axis in T cells may trigger a positive feedback loop at the tumor site through the CXCL9, -10/CXCR3 axis. Also using anti-CTLA4 antibody, this axis was significantly up-regulated in pretreatment melanoma lesions in patients with good clinical response after ipilimumab administration.[79] These results are in consensus and show the usefulness of tumor-infiltrating lymphocytes for anti-PD-1 therapy.

Recently, Barreira da Silva et al. showed that dipeptidyl peptidase 4, known as degradation of incretins, truncates the N-terminal of CXCL10 and limits lymphocyte migration to tumor sites. In vivo evidence showed that DPP4 inhibition enhanced tumor rejection by increasing lymphocytes homing into tumor sites through CXCL10/CXCR3 axis, which boosts the effect of immunotherapy.[80] Decalf et al. showed that DPP4 inhibition in humans can preserve the bioactive form of CXCL10, using a clinically approved DPP4 inhibitor.[81] Since DPP4 inhibitors are safe drugs with a few side effects, they are expected to be used in future therapeutic strategies.

The significance of CXCL9, -10/CXCR3 axis for cancer treatment is also further underscored by the observations that COX-inhibitors increase CXCL9, CXCL10 release from cancer cells in vitro, and promote anti-tumor effects in vivo.[63, 82] The expressions of COX2 and CXCL9 had an inverse correlation in human breast cancer tissue.[82] These reports support the important preclinical data that overexpression of both COX isoenzymes, COX-1 and COX-2, is significantly associated with a lower number of tumor-infiltrating lymphocytes and a worse prognosis in human cancers.[83–85] Furthermore, Li et al. demonstrated that the combination of COX-2 inhibitor and anti-PD1 through alginate hydrogel delivery system synergistically enhanced the presence of Th1 cells and CTLs, and increased the expression of CXCL9 and CXCL10 within the tumor.[86] Interestingly, these effects are accompanied with reduced Tregs and myeloid derived suppressor cells (MDSCs)

in the tumor microenvironment. Anti-PD-1 treatment alone could not reduce Treg and MDSCs within the tumor,[77] and therefore, effective drug combinations such as anti-PD-1 and anti CTLA4,[78, 87] or anti-PD1 and a COX inhibitor, may show increased efficacies.

Other existing treatments, such as lapatinib with doxorubicin,[88] all-trans retinoic acid (ATRA), [89] and existing chemotherapies[90, 91] have been reported to exert therapeutic effects through the CXCL9, -10, -11/CXCR3 axis, suggesting that activation of CXCL9, -10, -11/CXCR3 axis may increase efficacies of cytotoxic and targeted therapies. (Table 2)

CXCL9, -10, -11, and CCL5 have also been identified as candidate biomarkers of adoptive T cell transfer therapy in metastatic melanoma.[92] For MAGE-A3 cancer immunotherapy, Ulloa-Montoya et al. have suggested that the pretreatment expression of CXCL9, -10 reflects the clinical response of patients with melanoma or non-small cell lung cancer through gene expression signature analysis.[93, 94] In addition, the association of this axis with immunotherapy, such as dendritic cell vaccine therapy,[95] IL-2,[96] or IL-7[97, 98] administration therapy was reported and showed the importance of CXCL9, -10, -11/CXCR3 axis in the efficacy of immunotherapy. Although there are few reports showing epigenetic involvements in this axis, miR21, an oncogenic miRNA, was reported to be a regulator of CCL5 and CXCL10 in breast cancer cells.[99] (Table 2)

These new approaches targeting CXCL9, -10, -11/CXCR3 axis treatment highlight the role of synergy in cancer treatment. Further understanding of this pathway is warranted.

Concluding remarks

The current review paid attention to exploring the role of CXCL9, -10, -11/CXCR3 axis in TME and immune response. This axis plays a critical role in immune activation through paracrine signaling, impacting efficacy of cancer treatments. Based on pre-clinical data, the combination of pharmacological ligands and inhibition of CXCR3A may lead to new opportunities for more efficient immune therapies, and enhance the effectiveness of existing chemotherapies. Further understanding of the regulation of this pathway could provide a gateway to more effective strategies in the treatment of cancer.

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Abbreviations

Th1	T helper 1
CTLs	cytotoxic lymphocytes
NK	natural killer
TME	tumor environment

Tregs	regulatory T cells
Th2	T helper 2
Th17	T helper 17
Tr1	Foxp3^{-} type 1 regulatory

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Highlights

- Chemokines induce chemotaxis, promote differentiation of immune cells, and cause tissue extravasation.
- The CXCL9, -10, -11/CXCR3 axis regulates immune cell migration, differentiation, and activation through paracrine axis.
- The axis induces tumor growth and metastasis through autocrine axis.
- Preclinical researches are defining the axis as a promising target for cancer treatment.
- Other immune consistent pathways strongly crosslink with this axis.

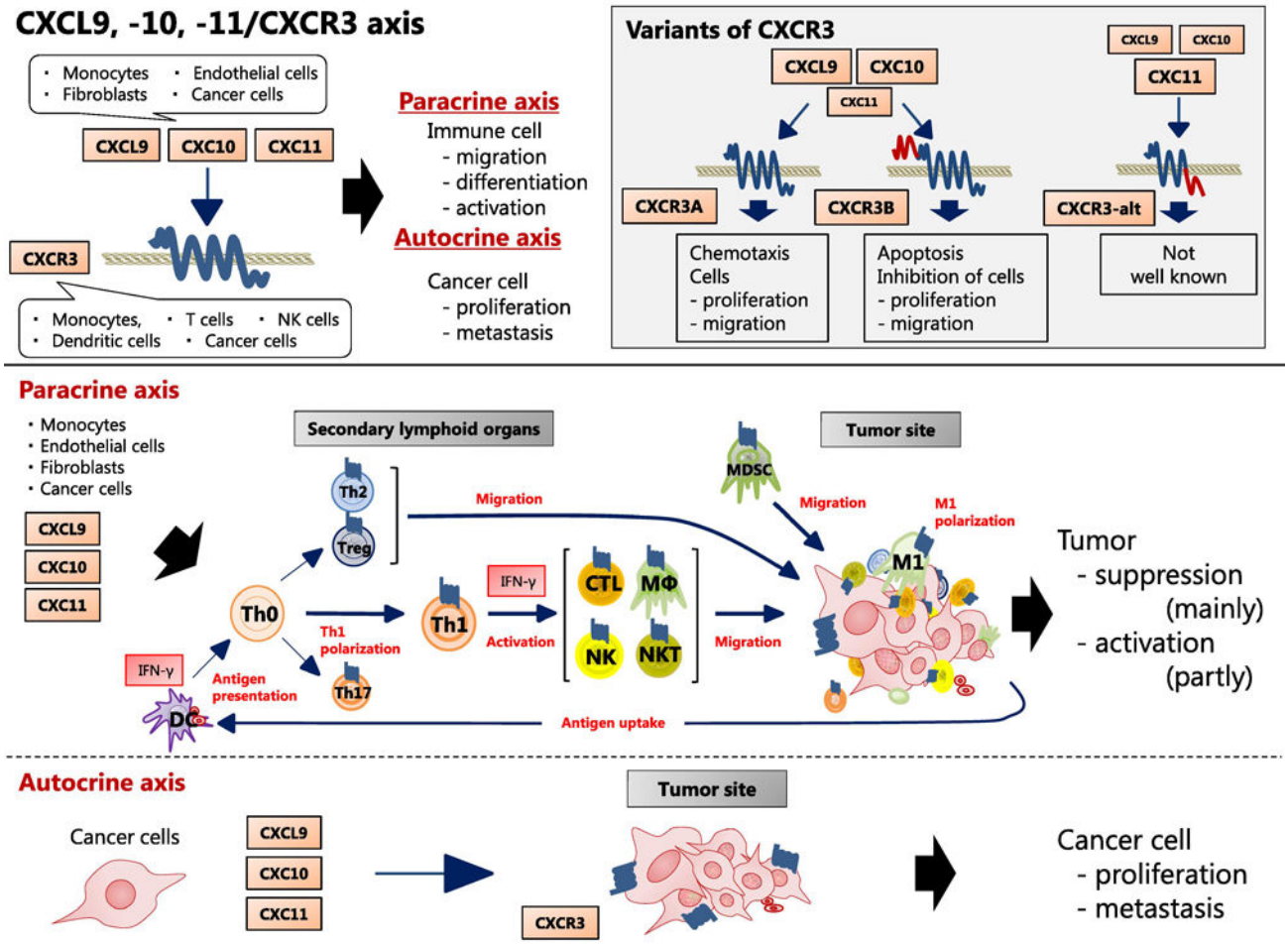

Figure 1.

CXCL9, -10, -11/CXCR3 axis in the tumor environment. CXCL9, -10, and -11 are mainly secreted by monocytes, endothelial cells, fibroblasts, and cancer cells in response to IFN-γ. The work of CXCL9, -10, -11/CXCR3 axis is mainly divided into two directions; paracrine signaling for immune activation and autocrine signaling for proliferation and metastasis of cancer cells. As for paracrine signal, this axis works primarily for immune cell migration, differentiation, and activation. Immune reactivity is occurred with recruitment of CTLs, NK cells, NKT cells, and macrophages through this axis, and Th1 polarization by this axis also activate the immune cells in response to IFN-γ. On the contrary, as for autocrine signal, cancer cells have a propensity to metastasize due to the tumor-derived ligands activity mainly through CXCR3A. Tumor-derived chemokines are also responsible for recruitment of Th2 cells, Tregs, and MDSCs, which play the role of creating a pro-tumoral microenvironment. Abbreviations: CTLs, cytotoxic lymphocytes; NK, natural killer; NKT, natural killer T; MΦ, macrophage; MDSCs, myeloid derived suppressor cells; Th0, naive T; Th1, T helper 1; Th2, T helper 2; Th17, T helper 17; Tregs, regulatory T cell

Table 1

Cancer treatment approaches using CXCL9, -10, -11./CXCR3 axis based on pre-clinical models

Target	Approach	Type of cancer	Works for the axis (in TME)	In vitro or in vivo	Outcome	Ref.
Immune activation						
CXCL9	Systemic plasmid-borne CXCL9 and low-dose cisplatin	Colon, lung	Induction of CTLs	in vivo	Reduction in tumor microvessel density and increasing apoptosis	[67]
	Intratumor recombinant CXCL9 and systemic IL-2	Kidney (RCC)	Induction of mononuclear cells	in vivo	Reduction in tumor growth and angiogenesis	[68]
CXCL10	Intratumor recombinant CXCL10	Lung (NSCLC)	–	in vivo	Reduction in tumor growth and angiogenesis, and inhibition of metastasis	[69]
	Retroviral CXCL10 gene transduction to tumor cells	Melanoma	(CXCL10 protein secretion)	in vitro and in vivo	Reduction in tumor microvessel density and tumor growth	[70]
	Retroviral CXCL10 gene transduction to tumor cells	Sarcoma, lung(LLC)	Macrophage infiltration and elevated IL-12	in vitro and in vivo	Reduction in tumor angiogenesis, mitotic activity, and tumor growth	[71]
	Recombinant CXCL10, CXCL10-Ig fusion protein	Myeloma	Induction of CTLs and NK cels	in vitro and in vivo	Reduction in tumor growth	[72]
	CXCL10-egfrviii fusion protein	Glioma	Induction of CTLs	in vivo	Reduction in tumor growth and improving prognosis	[73, 74]
CXCL11	CXCL11-Armed oncolytic poxvirus	Mesothelioma	Induction of CTLs (not only in TME but also in the spleen and lymph organs) and NK cells	in vivo	Reduction in tumor growth and improving prognosis	[75]
	(CXCL11-Ig fusion protein	Autoimmune encephalomyelitis	Reduction in T cell migration, and induction of regulatory T cells polarization	in vivo	)	[44]
Direct inhibitinon						
CXCR3	AMG487 (an antagonist of CXCR3)	Colon	–	in vitro and in vivo	Inhibition of lung metastasis, but not liver metastasis	[7]
		Breast	Induction of Th1 and CTLs in the peripheral blood	in vitro and in vivo	Inhibition of lung metastasis, but not local cancer growth	[8]
		Breast	–	in vitro and in vivo	Inhibition of lung metastasis, but not local cancer growth	[57]
		osteosarcoma	–	in vitro and in vivo	Inhibition of lung metastasis (in vivo), and cancer cell growth (in vitro)	[76]

Abrbreviations: CTLs, cytotoxic T lymphocytes; LLC, Lewis lung cancer; NK cells, natural killer cells; NSCLC, non small cell lung cancer; RCC, renal cell carcinoma; TME, tumor microenvironment,

Table 2

Prospective approaches related to CXCL9, -10, -11/CXCR3 axis for cancer treatment

Treatment	Target in the axis	Type of cancer	Works for the axis (in TME)	In vitro or in vivo	Outcome (for TME and host status)	Ref.
Existing drugs						
Anti-PD-1	CXCL9, -10	Breast, melanoma	Induction of CXCL9 and CXCL10	in vivo	An anti-PD-1 could not reduce the tumor growth in CXCR3 knock out mice.	[37]
	CXCL10, (IFN-γ)	Colon, melanoma	Induction of IFN-γ and CXCL10	in vivo	Induction of Tcells and reduction in tumor growth (with ACT therapy)	[77]
Anti-CTLA4	CXCL9, -10, -11	Melanoma	–	in vivo (human sample)	Induction of Tcells	[79]
Anti-DPP4	CXCL10	Colon, melanoma	Induction of CXCL10	in vivo	Improving the response to immunotherapy (anti-CTLA4)	[80]
	(CXCL10	Patients with chronic hepatitis C	Induction of bioactive CXCL10	in vivo (human sample)	Anti-DPP4 could presearve the bioactive form of CXCL10.)	[81]
COX-inhibitor	CXCL9, -10	Ovarian	Induction of CXCL9 and CXCL10	in vitro	Induction of CXCL9 and CXCL10 by unselective COX inhibition, and conversely reduction of that by COX-2-spedfic inhibition	[63]
	CXCL9, -10	Breast	Induction of CXCL9 and CXCL10	in vitro	Induction of CXCL9 and CXCL 10 by unselective COX inhibition, and conversely reduction of that by COX-2-spedfic inhibition	[82]
COX-inhibitor with anti- PD-1	CXCL9, -10	Breast, melanoma	Induction of CXCL9 and CXCL10	in vivo	In combination of celecoxib and anti-PD-1, induction of T cells and reduction of Tregs and MDSCs lead to inhibiting tumor growth	[86]
Lapatinib (with doxorubicin)	CXCL9, -10, -11	Breast	Induction of CXCL9,-10,-11 through STATI	in vivo	Induction of Tcells and reduction in tumor growth	[88]
ATRA	CXCL9, CXCL10	Melanoma	Induction of CXCL9 and CXCL10	in vitro	–	[89]
CMF	(CXCL9)	Breast	–	in vivo (human sample)	High expression levels of CXCL9 was associated with prolonged DFS	[90]
Dacarbazine, temozolomide, cisplatin	CXCL9, CXCL10	Melanoma	Induction of CXCL9 and CXCL10	in vitro and in vivo	Induction of Tcells and improving prognosis	[91]
Prospective drugs						
ACT therapy	CXCL9, -10, -11	Melanoma	–	in vitor and in vivo	The expressions of CXCL9,-10,-11 in pretreatment tumors were	[92]

Treatment	Target in the axis	Type of cancer	Works for the axis (in TME)	In vitro or in vivo	Outcome (for TME and host status)	Ref.
					associated with responsiveness to the treatment.	
Recombinant MAGE-A3 antigen	CXCL9, -10, -11	Melanoma	–	in vivo (human sample)	The expressions of CXCL9,-10,-11 in pretreatment tumors were associated with responsiveness to the treatment, (with an Immunostimulant, AS15 or AS02B)	[93, 94]
Dendritic cell vaccine therapy	CXCL9, -10, -11	Chronic lymphocytic leukemia	Induction of CXCL9,-10,-11	in vitro	Induction of NK cells and NKTcells	[95]
IL-2	CXCL9, -10, -11	Kidney (RCC)	Induction of CXCL9,-10,-11	in vivo (human sample)	–	[96]
IL-7 (IL-7/IL-7Rα-Fc)	CXCL9, CXCL10	Lung (LLC)	Induction of CXCL9 and CXCL10	in vitor and in vivo	Induction of Tcells and M1 macrophage, reduction in tumor growth and improving prognosis	[97]
IL-7 (DC-AdIL-7)	CXCL9, CXCL10	Bronchoalveolar cell carcinoma	Induction of CXCL9 and CXCL10	in vivo	Reduction in tumor growth	[98]
miR21	CXCL10	Breast	Reduction of CXCL10	in vitro	Reduction of lymphocyte migration	[99]

NOTE: DC-AdIL-7, adenovirus vector expressing interleukin (IL)-7; IL-7/IL-7Rα-Fc, chimeric γc homeostatic cytokine; lapatinib;dual, tyrosine kinase inhibitor which interrupts HER2/neu EGFR pathways.

Abrbreviations: ACT, adoptive cell transfer; ATRA, all-trans retinoic acid; CMF, cyclophosphamide, methotrexate and 5-fluorouracile; COX, cyclooxygenase; CTLA4, cytotoxic T-lymphocyte-associated protein 4; DFS, disease free survival; DPP4, dipeptidyl peptidase-4; LLC, Lewis lung cance; MAGE-A3, melanoma-associated antigen 3; MDSCs, myeloid derived suppressor cells; NK cells, natural killer cells; NKT cells, natural killer T; PD-1, programmed death-1; RCC, renal cell carcinoma; Tregs, regulatory T cells; TME, tumor microenvironment.