CX3CL1 Signaling in the Tumor Microenvironment

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Abstract

CX3CL1 (Fractalkine) is a multifunctional inflammatory chemokine with a single receptor CX3CR1. The biological effects elicited by CX3CL1 on surrounding cells vary depending on a number of factors including its structure, the expression pattern of CX3CR1, and the cell type. For instance, the transmembrane form of CX3CL1 primarily serves as an adhesion molecule, but when cleaved to a soluble form, CX3CL1 predominantly functions as a chemotactic cytokine (Fig. 1.1). However, the biological functions of CX3CL1 also extend to immune cell survival and retention. The pro-inflammatory nature of CX3CR1-expressing immune cells place the CX3CL1:CX3CR1 axis as a central player in multiple inflammatory disorders and position this chemokine pathway as a potential therapeutic target. However, the emerging role of this chemokine pathway in the maintenance of effector memory cytotoxic T cell populations implicates it as a key chemokine in anti-viral and anti-tumor immunity, and therefore an unsuitable therapeutic target in inflammation. The reported role of CX3CL1 as a key regulator of cytotoxic T cell-mediated immunity is supported by several studies that demonstrate CX3CL1 as an important TIL-recruiting chemokine and a positive prognostic factor in colorectal, breast, and lung cancer. Such reports are conflicting with an overwhelming number of studies demonstrating a pro-tumorigenic and pro-metastatic role of CX3CL1 across multiple blood and solid malignancies.

This chapter will review the unique structure, function, and biology of CX3CL1 and address the diversity of its biological effects in the immune system and the tumor microenvironment. Overall, this chapter highlights how we have just scratched the surface of CX3CL1's capabilities and suggests that further in-depth and mechanistic studies incorporating all CX3CL1 interactions must be performed to fully appreciate its role in cancer and its potential as a therapeutic target.

Keywords

Chemokines · CX3CL1 · CX3CR1 · Cancer · Inflammation · T cells · Natural killer (NK) cells · Tumor-associated macrophages (TAMs) · Metastasis · Tumor microenvironment · Cell adhesion · Migration

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1.1 The CX3CL1:CX3CR1 Axis

C-X3-C motif chemokine ligand 1 (CX3CL1) is a unique chemokine functioning in both a transmembrane and soluble form, unlike other chemokines which are solely expressed as soluble proteins [1]. It is a protein of 373 amino acids forming three main domains: chemokine, mucin-like stalk, and transmembrane [1]. In its transmembrane form CX3CL1 functions in immune cell adhesion in an integrin-independent manner (Fig. 1.1) [2, 3]. The mucin-like stalk facilitates cleavage of CX3CL1 by metalloproteases: ADAM metallopeptidase domain 10 (ADAM10) under homeostatic conditions and ADAM metallopeptidase domain 17 (ADAM17) under inflammatory conditions [4, 5]. Such cleavage releases the soluble glycoprotein form of CX3CL1, in which it primarily functions as a chemotactic cytokine [1]. CX3CL1 has also been shown to mediate immune cell survival and has been recently described in the maintenance of memory populations of cytotoxic T cells [6–11]. The distinct biological effects of CX3CL1 are mediated through its sole receptor CX3CR1, which is expressed by $CD8^+$ T cells, natural killer (NK) cells, B cells, monocytes, macrophages, neurons, microglia, smooth muscle cells, and even tumor cells in malignancies such as pancreatic cancer, multiple myeloma, B-chronic lymphocytic leukemia (B-CLL), and metastatic

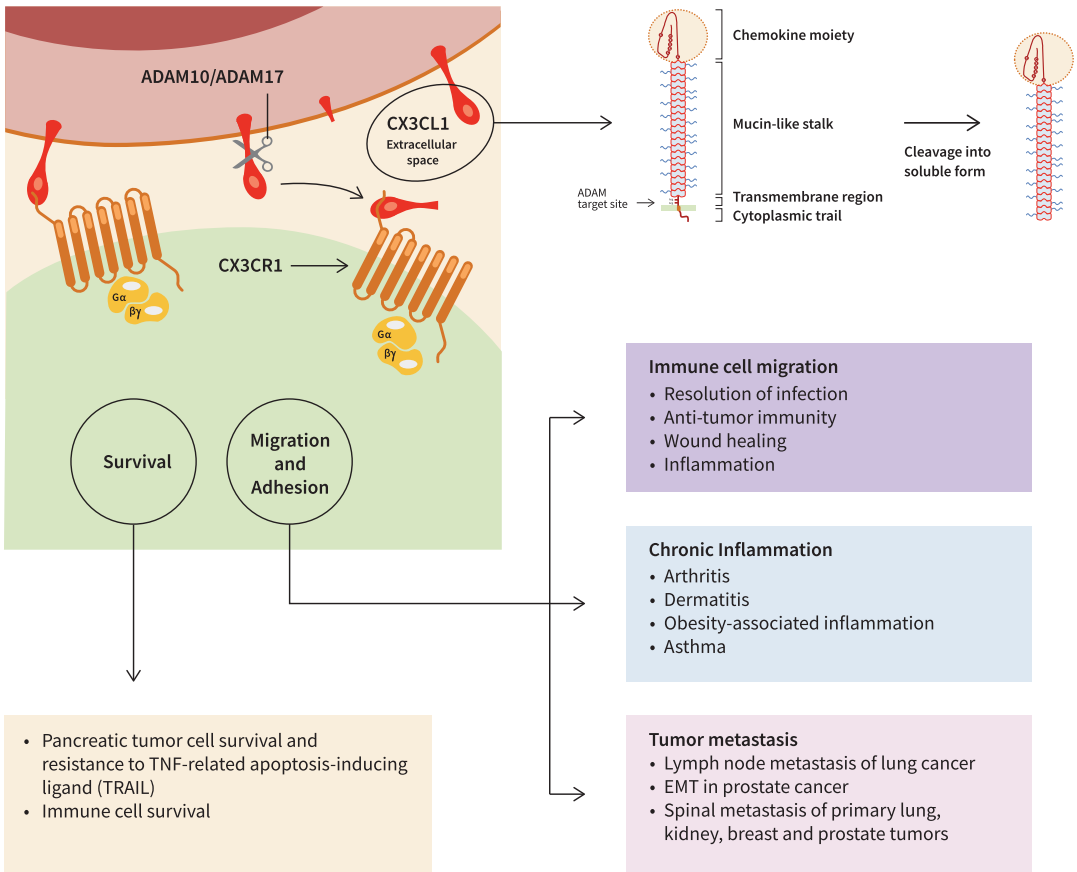

Fig. 1.1 The diverse biological functions of CX3CL1. CX3CL1 is expressed as a transmembrane protein which can be cleaved into a soluble form and secreted into the extracellular space. In its transmembrane form, it predominantly functions in adhesion. In a soluble form, it functions as a survival factor and a chemotactic cytokine. Its biological effects on cell migration can enable crucial immune responses but have also been implicated in chronic inflammation and tumor metastasis [1–5, 8, 9, 11, 16, 24, 27, 30, 50–54]

prostate cancer [1, 3, 12–17]. The biological effects of CX3CL1 are also determined by the differential expression of CX3CR1, which can be epigenetically regulated by immune cells [10]. Specifically, higher CX3CR1 expression by IL-7Rαl^{ow} effector memory (EM) $CD8^+$ T cells is accompanied by higher levels of DNA methylation of the CX3CR1 gene promoter and this confers increased migratory capacity toward CX3CL1, in contrast to the functionally distinct IL-7Rα^{high} EM $CD8^+$ T cell subset [10]. Therefore, since IL-7Rα^{low} EM $CD8^+$ T cells have higher levels of perforin, it is likely that they have greater cytotoxic potential than IL-7Rα^{high} EM $CD8^+$ T cells and this implicates CX3CL1 as a pivotal player in anti-tumor and anti-viral immunity [10]. Moreover, CX3CL1 has been shown to differentiate $CX3CR1^+$ pro-inflammatory macrophages in the liver implicating it in macrophage-mediated hepatic inflammation [18].

The redundancy of the chemokine system is an essential fail-safe for the functionality of the immune system and may present challenges for effectively therapeutically targeting specific pathways. For a long time, it was believed that the CX3CL1:CX3CR1 axis lacked this redundancy and comprised an exclusive ligand and receptor pair, which increased the attractiveness of this pathway for therapeutics [3]. However, in 2010 Nakayama et al. unexpectedly discovered that the chemokine CCL26 is a functional agonist for CX3CR1 [3, 12]. CCL26 was already established as an antagonist for chemokine receptors CCR1, CCR2, and CCR5, and a low-affinity agonist for CCR3 with 10-fold less affinity than CCL11 [19–21]. Therefore, CCL26 is regarded as an interesting and widely interacting chemokine, albeit a low-affinity chemokine; it has dual agonist and triple antagonist capabilities. CCL26 is classically regarded as a pivotal player in allergy and its ability to recruit CX3CR1-expressing cells adds another layer of complexity to the CX3CL1:CX3CR1 axis. In fact, it has been shown to work together with CX3CL1 to recruit NK cells to allergic nasal tissue [22]. This highlights the need for consideration of CCL26 in any future studies scrutinizing the role of CX3CL1 in cancer.

While the CX3CL1:CX3CR1 pathway is important in the recruitment of key anti-tumor immune cells such as NK cells and T cells, it has been implicated in the progression and metastasis of both hematological and solid malignancies [1–3, 13–16, 23–31]. Here, we review key studies uncovering this chemokine pathway to evaluate whether CX3CL1 is a friend or foe in tumorigenesis.

1.2 Pro- and Anti-Tumor Immune Roles of CX3CL1

There are a number of conflicting studies on the role of CX3CL1 in anti-tumor immunity. Numerous reports implicate CX3CL1 as a potent recruiter of NK cells and T cells into the tumor microenvironment and a crucial promoter of strong anti-tumor activity [25, 32–35]. Recent studies have identified CX3CR1 as a novel marker of effector memory $CD8^+$ T cells and a potential marker of PD-1 therapy-responsive and chemotherapy-resistant $CD8^+$ T cell subsets, implicating the importance of this chemokine pathway in successful anti-tumor immune responses and response to immunotherapy [7, 36]. In a case study of 158 patients with T2/T3 stage gastric adenocarcinoma, higher CX3CL1 levels were shown to correlate with higher infiltrations of $CD8^+$ T cells and NK cells and were identified as independent prognostic factors for disease-free survival [37]. Similar results were reported in a cohort of 80 colorectal cancer patients in which intratumoral CX3CL1 positively correlated with better prognosis and higher density of tumor-infiltrating lymphocytes (TILs) [38]. Similarly in 204 invasive breast carcinoma patients, intratumoral CX3CL1 levels positively correlated with higher tumor infiltrations of NK cells, T cells, and dendritic cells (DCs) and were identified as independent prognostic factors for disease-free and overall survival [39]. Interestingly, high intratumoral CX3CL1 expression was associated with smaller tumor size and lower tumor stage regardless of receptor status, i.e., estrogen receptor (ER^+ and ER^-), progesterone receptor (PR^+ and PR^-), and human epidermal

growth factor receptor 2 ($HER2^+$ and $HER2^-$) [39]. Moreover, high intratumoral CX3CL1 was more prevalent in patients without lymph node metastasis further supporting a positive role for this chemokine in controlling breast cancer progression [39]. Unsurprisingly, intratumoral CX3CL1 positively correlated with higher tumor-infiltrating lymphocytes (TILs) in a second breast cancer study [40]. However in contrast to the Park et al. study, this study revealed that high CX3CL1 levels observed in 252 of the 757 patients corresponded with reduced survival and poorer clinical outcomes such as lymph node involvement, high Ki67, α-B crystallin expression, and luminal B (worse prognosis luminal cancers) subtype [40]. Such adverse clinical outcomes were mostly observed in the patients with the lowest TILs suggesting that the complex and multifaceted functions of CX3CL1 in breast carcinoma requires further scrutiny and more in-depth immune profiling among multiple cohorts using comparable measures of high and low CX3CL1 expression before definitive conclusions can be drawn [40]. As we will discuss later, other studies have focused on the pro-tumorigenic role of CX3CL1 in breast cancer, which may negate any positive effects this chemokine has on anti-tumor immunity [26, 27, 31, 40].

To address the concept of CX3CL1 as a pro-tumor chemokine, it must first be noted that several studies have identified it as a key recruiter of tumor-associated macrophages (TAMs) into the tumor microenvironment and recent reports have uncovered soluble and molecular regulators of these processes [23, 41]. Zhou et al. reported that CX3CL1 correlates with increasing tumor stage in a cohort of 38 lung cancer patients, comprising 23 cases of squamous cell carcinoma and 15 cases of adenocarcinoma ranging in stage from I to IV. Schmall et al. demonstrated that this could potentially be mediated via the recruitment of TAMs into non-small cell lung carcinomas by CX3CL1 and CCL2. These data revealed that macrophage-derived IL-10 within the tumor microenvironment mediated the upregulation of CX3CR1, while tumor-derived CX3CL1 and CCL2 expression was driven by CCL1, granulocyte colony-stimulating factor (G-CSF), and CCL3 [41]. Furthermore, genetic ablation of CX3CL1 and CCL2 inhibited tumor growth and metastasis, shifted TAMs toward an anti-tumor M1 phenotype, and suppressed tumor vessel growth suggesting that blockade of these chemokine pathways might have therapeutic potential to block TAM-mediated immune suppression in lung cancer [41]. Furthermore, this study demonstrated that the prevalence of intratumoral $CCR2^+$ TAMs correlated with tumor stage in a cohort of 72 non-small cell lung cancer patients providing further evidence that CX3CL1 and CCL2 contribute to lung tumor progression [41]. While the previous studies provide insights into CX3CL1's role in lung cancer progression, a larger study examining datasets for 2443 patients across France, Sweden, Canada, and the USA was reported using data from The Gene Expression Omnibus database and The Cancer Genome Atlas and concluded that higher levels of intratumoral CX3CL1 mRNA was associated with improved overall survival in lung adenocarcinoma but not lung squamous cell carcinoma [42]. Such CX3CL1 expression was associated with genes regulating cell adhesion, leukocyte migration, T cell activation, and NK cell-mediated cytotoxicity in lung adenocarcinoma [42]. These reports present conflicting conclusions on the immune repertoires recruited to CX3CL1-expressing lung tumors and its divergent implications on patient outcomes might be impacted by other chemokines in the tumor microenvironment. For instance, CX3CL1 alone might promote anti-tumor immune responses against lung tumors, but in the presence of CCL2, it may enhance intratumoral TAM accumulation. In a testicular germ cell cancer study, Batool et al. demonstrated that the miRNA miR-125b is epigenetically repressed and that its restoration can reduce CX3CR1 expression and TAM recruitment in vivo, suggesting a possible means through which TAM accumulation in the tumor microenvironment might be regulated [23]. However, the role of miRNA miR-125b in the recruitment of $CX3CR1^+$ $CD8^+$ T cells and NK cells would need to be assessed before this could be considered therapeutically.

CX3CL1-mediated recruitment of myeloid derived suppressor cells (MDSCs) into the tumor

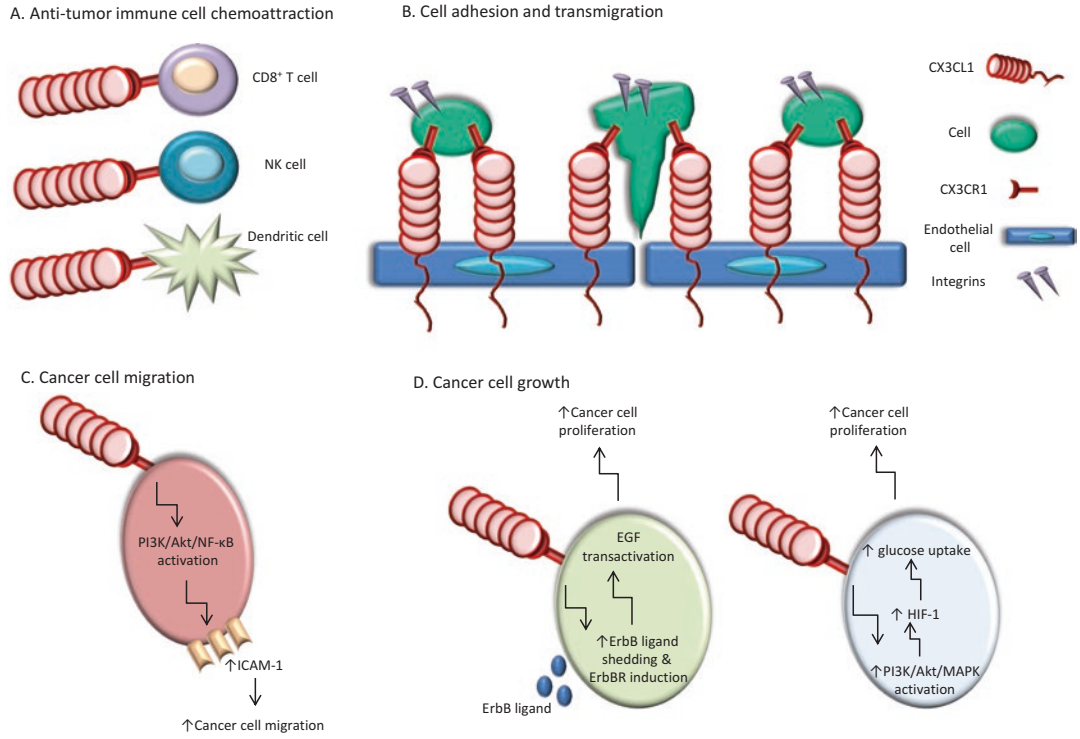

Fig. 1.2 The anti-tumor and pro-tumor roles of CX3CL1. Schematic illustrating the biological effects of CX3CL1 exerted on immune cells and tumor cells. (**a**) CX3CL1 facilitates anti-tumor immune responses via chemoattraction of $CD8^+$ T cells, natural killer (NK) cells, and dendritic cells (DC). (**b**) In its transmembrane form, CX3CL1 facilitates cell adhesion and transendothelial migration. (**c**, **d**) CX3CL1 has been shown to promote osteosarcoma cell migration via intercellular adhesion molecule-1 (ICAM-1) upregulation, breast cancer cell growth via transactivation of the epidermal growth factor (EGF) pathway, and pancreatic cancer cell growth via hypoxia-inducible factor (HIF)-1α [2, 3, 31, 39, 48]

microenvironment is another means through which this chemokine compromises anti-tumor immunity and facilitates tumor progression (Fig. 1.2) [43]. A recent study has pinpointed the mechanisms through which CX3CR1 expression on MDSC is regulated and a potential means of attenuating their CX3CL1-mediated recruitment to tumor [43]. In brief, the cyclin-dependent kinase (CDK) inhibitors p16INK4 and p21Cip1/Waf1 are highly expressed by monocytic MDSCs (Mo-MDSCs) and stimulate CX3CR1 chemokine receptor expression by preventing CDK-mediated phosphorylation and inactivation of SMAD3 [43]. This study demonstrated that deletion of p16INK4 and p21Cip1/Waf1 reduces CX3CR1 expression on Mo-MDSC and significantly reduces their recruitment to CX3CL1-expressing tumors, thus suppressing tumor advancement and offering therapeutic potential to prevent MDSC-mediated immune suppression in the tumor microenvironment [43]. It must be noted that other chemokines such as CCL2, CXCL12, and CXCL8 have also been shown to enhance MDSC recruitment to the tumor microenvironment in gastric and ovarian cancers, and therefore they may offer additional targets for use with CX3CL1-targeted therapy to block migration of tumor-promoting immune cells [44, 45]. Furthermore, prostaglandin E2 (PGE2) has been shown to modulate production of some of these chemokines, which presents further therapeutic opportunities through antagonism of the PGE2 G-protein coupled receptors [45].

With a diverse range of reported immune profiles and clinical outcomes accompanying high intratumoral CX3CL1, it appears that the biological ramifications are dependent on several

factors including the tumor type and the conditions within the tumor microenvironment. In the absence of thorough immune profiling studies and at a time when all biological functions of CX3CL1 are not completely understood, it is difficult to define the role of CX3CL1 in cancer and future studies must consider a wider array of factors to truly uncover whether this multifaceted chemokine is an enemy or ally of the anti-tumor immune response [23, 39, 40].

1.3 CX3CL1 Exerts Direct Biological Effects on Tumor Progression

As previously described, CX3CL1 is a potent driver of NK cell and $CD8^+$ T cell migration and this in itself should make it a formidable component in the immune system's arsenal against tumor growth. While several studies have demonstrated that high intratumoral CX3CL1 levels correspond to high TIL prevalence, there is emerging evidence that this chemokine also plays a role in shaping the immunosuppressive features of the tumor microenvironment [23, 25, 37–39, 41]. Aside from governing aspects of the immune control of tumor growth, CX3CL1 has also been shown to elicit direct pro-tumorigenic effects on tumor cells through its receptor CX3CR1 and these will be discussed in this section.

Pancreatic ductal adenocarcinoma (PDAC) is a malignancy plagued with dismal survival rates of ~5% and high levels of treatment resistance. There is emerging evidence that high expression of CX3CR1 and CX3CL1 correlates with poor prognosis in PDAC and that CX3CL1 can directly and indirectly promote PDAC tumor growth [17, 29, 46]. This has been the focus of several studies; a study by Huang et al. revealed significantly elevated CX3CL1 and CX3CR1 expression in PDAC tumors with highest levels associated with metastasis and severity of disease [17]. Further analysis demonstrated that CX3CL1 could directly enhance both growth and migration of PDAC cells and that such growth was regulated through the activation of the JAK/STAT signaling pathway [17]. In support of this, a more recent study has demonstrated that CX3CL1 can promote motility, invasion, and contact-independent growth of PDAC cells and that this can be reduced by the CX3CR1 inhibitor JMS-17-2 in an AKT-dependent manner [29]. These data suggest that the CX3CL1:CX3CR1 axis might be therapeutically targeted to limit PDAC tumor cell growth and metastasis [29]. Recent evidence demonstrates that CX3CL1 also promotes PDAC tumor cell survival and resistance to TNF-related apoptosis-inducing ligand (TRAIL) in an indirect manner via the recruitment of inflammatory cells, which in turn induces apoptosis resistance in PDAC cells, which is mediated at least in part by a RelA-CX3CL1 paracrine pathway [24]. This study showed that TRAIL-resistant pancreatic cancer cell lines exhibit enhanced NF-κB activity compared to sensitive cell lines, and TRAIL treatment induces CX3CL1 expression in these cells via the RelA-centered NF-κB signaling pathway [24]. In summary, these data reveal that the augmented migration of inflammatory cells conferring TRAIL resistance to PDAC tumors is facilitated by TRAIL-mediated activation of RelA/NF-κB and subsequent CX3CL1 expression in such tumors (Fig. 1.1) [24].

CX3CL1-elicited effects via NF-κB activity is not confined to TRAIL resistance and has been shown to govern tumor progression and metastasis in osteosarcoma through the regulation of intercellular adhesion molecule-1 (ICAM-1) [47]. This study showed that CX3CL1-induced human osteosarcoma cell migration, via upregulation of intercellular adhesion molecule-1 (ICAM-1) expression, was regulated through the CX3CR1/PI3K/Akt/NF-κB pathway (Fig. 1.2) [47]. Furthermore, intratumoral levels of CX3CL1 and ICAM-1 were associated with tumor stage in osteosarcoma patients and further in vivo studies revealed that lung metastases of osteosarcomas are governed at least in part by CX3CL1 [47]. Interestingly, such CX3CL1-mediated regulation of ICAM-1 was not observed in a separate study on circulating $CD8^+$ T cells suggesting that the effects of CX3CL1 are dependent on the cell type [6].

It is well established that tumor cells must alter their metabolic processes to survive in the hypoxic and nutrient-depleted tumor microenvironment. A study by Ren et al. has revealed that CX3CL1 stimulated Hypoxia-Inducible Factor (HIF)-1α expression in PDAC through the PI3K/Akt and MAPK pathways subsequently facilitating enhanced glucose uptake, thus identifying CX3CL1 as a promoter of PDAC tumor cell growth and survival in the challenging tumor microenvironment (Fig. 1.2) [48].

While the last section highlighted CX3CL1-mediated effects on breast cancer progression via its cross talk with the immune system, this complex chemokine has also been shown to directly exert biological effects on breast cancer cells. For instance, a recent study demonstrated that CX3CL1 transactivates ErbB receptors in human breast cancer cells and triggered proliferation of such cells through the proteolytic shedding of an ErbB ligand (Fig. 1.2) [31]. The group concluded that it is likely that CX3CL1 acts as a specific tumor promoter for ErbB2-expressing mammary carcinomas [31].

The CX3CL1-mediated promotion of tumor progression is not limited to solid malignancies and has been shown in several hematological malignancies. It is well established that CXCR4 and its ligand CXCL12 are players in the migration of myeloma cells to bone marrow and studies are uncovering a role for the CX3CL1:CX3CR1 axis in cross talk between multiple myelomas and their bone microenvironments [14]. In *B*-cell *chronic lymphocytic leukemia* (B-CLL), a study by Corcione et al. reported that CX3CL1 binding to CX3CR1 on B-CLL cells leads to the upregulation of CXCR4 and increased migration of B-CLL cells to monocyte-derived nurse like cells (NLCs) expressing CXCL12 in the tumor microenvironment [15].

It is clear that CX3CL1 elicits biological effects outside its defined function of an immune cell adhesion and chemotactic protein and that its role within the tumor microenvironment is shaped by complex multicellular and molecular cross talk.

1.4 CX3CL1 in the Metastatic Niche

Emerging evidence suggests that CX3CL1 governs spinal metastasis of primary lung, kidney, breast, and prostate tumors [16, 27]. In a study by Liu et al., a lack of CX3CR1 expression in such metastatic tumors was reported and this is likely due to sustained reduction of the receptor on the cell surface following ligand binding [6, 27]. So far, such CX3CL1-mediated internalization of CX3CR1 has only been reported on $CD8^+$ T cells and further work is warranted to determine whether this also occurs in cancer cells and to confirm the biological ramifications [6]. However, others have shown that both CX3CL1 and CX3CR1 expression is higher in prostate metastatic tumors in spine compared to the primary tumor site and that CX3CR1 overexpression leads to more spinal metastasis in mice [16, 27]. Further analyses revealed that CX3CL1 elicited its effects on prostate tumor metastasis via activation of the Src/FAK pathway in an epidermal growth factor receptor (EGFR)-dependent manner [16]. The inhibitors of these kinases repressed the cell migration induced by CX3CL1 or CX3CR1 overexpression and therefore may represent potential targets for preventing spinal metastasis in prostate cancer [16]. Others have also implicated CX3CL1-mediated spinal metastasis of breast tumors via the Src/FAK pathway and have demonstrated that the Src inhibitor Bosutinib and FAK inhibitor PF-00562271 can reduce CX3CL1-driven migration further, indicating that these are targetable pathways in the prevention of spinal metastasis of solid tumors [26]. In PDAC, tumor perineural dissemination is a common mechanism through which tumor recurrence occurs following surgery and the CX3CL1:CX3CR1 pathway is believed to drive the migration of the PDAC cells to local peripheral nerves [28]. Of course, CX3CL1-mediated metastasis is not restricted to the spine and the chemokine has also been implicated in lymph node involvement [40, 49]. Lymph node metastasis was promoted by CX3CL1 via activation of JNK and MMP2/MMP9 activity in lung cancer

cells [49]. Furthermore CX3CL1 was shown to promote epithelial-to-mesenchymal transition through the TACE/TGF-α/EGFR pathway and subsequent upregulation of Slug expression in prostate cancer cells [30].

Overall, it appears that the CX3CL1 can govern cancer metastasis, either via CX3CR1 overexpression by metastatic tumor cells to facilitate spread to the CX3CL1-enriched microenvironments or via direct effects on signaling pathways affecting the EMT pathways of cancer cells [30, 42].

1.5 CX3CL1 in Inflammation-Driven and Obesity-Associated Cancer

As a key regulator of pro-inflammatory cell migration, it is now well established that CX3CL1 plays a central role in multiple inflammatory conditions such as asthma, obesity, dermatitis, diabetes, and neuropathic pain [6, 9, 50–53]. In neuroinflammation, CX3CL1 mediates its effects via activation of CX3CR1 on microglial cells and subsequent phosphorylation of p38 MAPK [50]. Furthermore, CX3CL1-mediated activation of satellite glial cells leads to TNFα, IL-1β, and prostanoid expression to maintain the inflammatory-associated pain [52]. In airway inflammation, CX3CL1 serves to promote survival of pro-inflammatory T cells in the inflamed lungs of asthma patients [9]. The involvement of CX3CL1 in inflammatory disorders is further facilitated through its induction by inflammatory cytokines such as TNF-α and IFN-γ which are often expressed by the CX3CL1-responsive lymphocytes and myeloid cells, and this can provide a pathological feedback loop in maintaining the chronicity of the inflammation and in the exacerbation of disease [54]. For instance, the inflammatory subtype of monocytes/macrophages expressing intermediate levels of CX3CR1 ($CX3CR1^{INT}$) and producing TNF-α, IL-1 IL-6, and CCL2 are enriched in inflamed colon, where they undoubtedly contribute to tissue inflammation and further recruitment of inflammatory cells [55].

Inflammation-driven and obesity-associated malignancies represent a unique challenge for the immune system in that the desired immune response should facilitate tumor eradication without exacerbating tumorigenic inflammation derived from obese adipose tissue, and this presents challenges for cancer immunotherapy. Given the involvement of CX3CL1 in a plethora of inflammatory disorders, one would assume that it plays a role in the pathogenesis of inflammation-driven malignancies [9, 50–53, 56]. However, studies have shown that CX3CL1 is associated with better outcomes in the inflammatory-driven and obesity-associated colorectal cancer [38]. Moreover, a study by Erreni et al. indicates that this may be facilitated by the adhesive and retention functions of CX3CL1, which prevents migration of primary tumor cells and limits metastatic progression [57]. This is in striking contrast to the pro-metastatic role of CX3CL1 in breast, lung, kidney, pancreatic, bone, and prostate cancer described earlier.

While CX3CL1 has been identified as a key player in adipose tissue inflammation in obesity, its role in the tumorigenesis of obesity-associated cancers such as oesophageal adenocarcinoma (OAC) is poorly understood. OAC is an exemplar model of inflammation-driven cancer and has one of the strongest associations with obesity of all malignancies [58]. Several studies have revealed that the immune system is dysregulated and several chemokine pathways are altered in OAC patients and this undoubtedly contributes to chronic inflammation and compromised anti-tumor immunity in this cohort [6, 59–62]. One study by Conroy et al. has identified that CX3CL1 is a key regulator of $CD8^+$ T cells in OAC and that its abundance in the visceral adipose tissue (VAT) strongly correlates with markers of meta-inflammation such as CRP in these patients [6]. This is not surprising since there is an abundance of T cells expressing CX3CL1-inducing cytokines IFN-γ and TNF-α in these tissues [59, 60]. However, the role of CX3CL1 in anti-tumor immunity at the tumor site and its effects on tumor metastasis and patient outcomes warrant further investigation in these patients. It is known that CX3CL1 can alter the CX3CR1 and

L-selectin expression of cytotoxic T cells in OAC and this is likely to have significant ramifications for their anti-tumor activities in this cohort [6]. Inflammation-triggered changes in CX3CR1 expression by monocytes/macrophages have also been observed in inflamed colon leading to the accumulation of the most inflammatory $CX3CR1^{INT}$ populations of these cells in inflamed tissue, in comparison to the $CX3CR1^{HI}$ phenotype observed in normal colon [55]. Other phenotypic and transcriptional alterations observed in this study suggested that this altered CX3CR1 expression represents impaired monocyte-to-macrophage differentiation in inflamed colon, but the role of CX3CL1 in this cross talk was not elucidated [55]. However, given its regulation of CX3CR1 expression by T cells, CX3CL1 is a likely candidate in the inflammatory shift of gut-homing monocytes in intestinal inflammation [6, 55].

At a time when inflammation-driven and obesity-associated malignancies such as CRC and OAC are increasing in prevalence, it is becoming more urgent to delineate the role of CX3CL1 in the chronic inflammation and anti-tumor immune responses of such immunologically complex patients.

1.6 Challenges of Therapeutically Targeting the CX3CL1:CX3CR1 Pathway

The complex functionality of CX3CL1 as a regulator of migration, adhesion, and survival of both anti-tumor and pro-tumor immune cells and a facilitator of cancer growth and metastasis cloud its potential as an anti-cancer therapeutic. While the blockade of the CX3CL1:CX3CR1 chemokine pathway might limit tumor progression, it is also likely to attenuate NK cell and cytotoxic T cell recruitment to the tumor microenvironment, potentially disrupting effective anti-tumor immunity. Moreover, CX3CL1's reported role in limiting metastasis of colorectal tumors and in the maintenance of crucial T cell populations would also be compromised if its signaling through CX3CR1 was antagonized. While its biological effects vary between cancer types and cell types, the ability of CX3CL1 to recruit NK cells and T cells remains constant which should be taken into account when considering its suitability as an immunotherapeutic target. While the CX3CR1 antagonist JMS-17-2 shows great promise to disrupt breast cancer metastasis and might present a feasible approach in other malignancies, such antagonism might have negative effects on CX3CL1's governance of T cells and NK cells and this must be heavily scrutinized before such therapeutics are considered [6, 7, 25, 32, 63]. The multifaceted functionality of CX3CL1 in the context of the immune system alone is fascinating and complex, from a recruiter of MDSCs and TAMs to a potent orchestrator of anti-tumor immune cell migration and phenotype, and a driver of pathological inflammation [6, 17, 23, 25, 32, 43, 51, 56]. However, the doubled-edged nature of CX3CL1's biological effects present it as a more challenging therapeutic target and therefore more in-depth in vivo studies are warranted to gain a deeper understanding of its role in the progression of cancer and governance of the immune system (Table 1.1).

Table 1.1 Pro- and anti-tumorigenic functions of CX3CL1

Cancer type	Pro-tumorigenic functions	Anti-tumorigenic functions	Reference
Breast	CX3CL1 governs spinal metastasis of breast tumors via the Src/FAK pathway. CX3CL1 induces ErbB receptors in human breast cancer cells and triggered proliferation of such cells through the proteolytic shedding of an ErbB ligand and transactivates EGF pathway.	High intratumoral CX3CL1 associated with higher TILs, less lymph node involvement, lower tumor stage and size.	[26, 31, 39]

(continued)

Table 1.1 (continued)

Cancer type	Pro-tumorigenic functions	Anti-tumorigenic functions	Reference
Pancreatic	High CX3CL1 and CX3CR1 associated with metastasis and disease severity. CX3CL1 promotes invasion and growth of pancreatic cancer cells and drives their migration to local peripheral nerves. TRAIL resistance in pancreatic cancer is conferred via RelA-CX3CL1 paracrine pathway. CX3CL1 stimulates HIF-1α expression via PI3K/Akt and MAPK pathways facilitating enhanced glucose uptake and pancreatic tumor cell growth.		[17, 24, 28, 29, 48]
Colorectal		High CX3CL1 correlates with higher TILs.	[38]
Gastric		High CX3CL1 correlates with higher TILs.	[37]
Lung	CX3CL1 correlates with increasing tumor stage and contributes to TAM accumulation in lung cancer. CX3CL1 promoted lymph node metastasis via JNK and MMP2/MMP9 pathway in lung cancer cells.	High CX3CL1 expression associated with leukocyte migration, T cell activation and NK cell-mediated cytotoxicity and better prognosis in lung adenocarcinoma.	[41, 42, 49]
Osteosarcoma	CX3CL1 induces osteosarcoma cell migration, by enhancing ICAM-1 via the CX3CR1 /PI3K/Akt/NF-κB pathway. CX3CL1 governs lung metastasis.		[47]
B-CLL	CX3CL1 enhances B-CLL cell migration.		[15]
Multiple Myeloma (MM)	CX3CL1:CX3CR1 axis plays a role in MM cell migration to their bone microenvironment.		[14]
Prostate	CX3CL1 and CX3CR1 govern spinal metastasis of prostate tumors via the Src/FAK pathway. CX3CL1 promotes EMT transition in prostate tumors via TACE/TGF-α/EGFR and upregulation of Slug.		[16, 27, 30]

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