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CXCL4 and CXCL4L1 in cancer

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ABSTRACT

Chemokines regulate leukocyte migration during physiological and pathological conditions. It is currently accepted that these chemotactic cytokines are also important in the development and progression of cancer. CXCL4 and its non-allelic variant CXCL4L1 are two platelet-associated chemokines that have been attributed anti-tumoral activity as a result of their angiostatic potential and the chemotactic activity for anti-tumoral leukocytes. Here we review the role of CXCL4 and CXCL4L1 in cancer, the use of both chemokines as cancer biomarkers and discuss some possible therapeutic opportunities.

1. Introduction

Chemokines or chemotactic cytokines are a superfamily of low molecular weight (8–14 kDa) proteins that orchestrate leukocyte activation, trafficking and recruitment. In addition to being immune regulators, chemokines are also involved in many physiological processes including embryogenesis and hematopoiesis [1]. A functional distinction is made between inflammatory and homeostatic chemokines. However, more frequently chemokines are structurally subdivided based on the arrangement of the first two of four conserved cysteine residues, into C, CC, CXC and CX_3C chemokines [2]. The CXC and CC subfamilies constitute the majority of chemokines, whereas the number of C and CX_3C chemokines is limited. The CXC chemokine ligands are further classified based on the presence or absence of the tripeptide Glu-Leu-Arg (the "ELR motif"), preceding the first conserved cysteine. Chemokines exert their biological activities through binding to G protein-coupled receptors (GPCRs) [3]. These seven-transmembrane domain GPCRs are classified according to the subclass of chemokines recognized (CXCR, CCR, CX_3CR, XCR) [4]. Many chemokines and chemokine receptors have been detected in neoplastic tissues [5]. In the tumor microenvironment, chemokines and chemokine receptors are expressed by the tumor cells themselves and stromal cells such as endothelial cells and fibroblasts. Chemokines can directly affect tumor development by modulating tumor transformation, growth, invasion and metastasis and indirectly by controlling tumor-leukocyte interactions and angiogenesis. It has become evident that some chemokines may favor tumor progression, whereas other display tumor-suppressive capabilities. Here we will focus on the chemokine CXCL4 and its non-allelic variant CXCL4L1.

The platelet-derived ELR$^-$ CXC chemokine CXCL4, alternatively known as PF-4 (platelet factor-4), is the oldest member of the chemokine family [6]. CXCL4 is stored in secretory granules of blood platelets and is released in response to protein kinase C [7] and Rac1 activation [8]. Platelets are considered to be the major cellular source of CXCL4. In the α-granules of blood platelets CXCL4 is kept as a tetramer bound to two molecules of chondroitin sulphate. Besides blood platelets, CXCL4 is also localized in mast cells as shown by immunohistochemistry [9] and produced by cultured microglia [10], monocytes [11,12] and activated T-cells [13]. Additionally, CXCL4 was found to be expressed in the prostate cancer cell lines DU-145 and PC3 and the colorectal cancer cell line HCT-8 [14]. CXCL4 is also highly upregulated in plasmacytoid dendritic cells (pDCs) in systemic sclerosis [15] and dendritic cells (DCs) after severe trauma [16].

The first and major biological function described for CXCL4 is the moderation of the effects of heparin-like molecules on the endothelial cell surface of blood vessels, thereby inhibiting local antithrombin III activity, which results in a procoagulant role of CXCL4. As such, CXCL4 was predicted to play a role in wound repair. On the other hand, CXCL4 stimulates the generation of activated protein C by thrombomodulin binding, mediating an anticoagulant effect. Furthermore, CXCL4 has been recognized to play a role in hematopoiesis and immune cell modulation [17]. Indeed CXCL4 has been shown to modulate the proliferation, phenotype and function of immune cells. For instance, CXCL4 has been reported to promote monocyte survival and macrophage activation [18]. Gleissner *et al.* even found that the exposure of monocyte-derived macrophages to CXCL4 induces a unique transcriptome different from M1 and M2 macrophages, and named these M4 macrophages [19]. Besides having an effect on monocytes, CXCL4

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inhibited the proliferation of activated T cells [20]. Additionally, it was found that CXCL4 inhibited proliferation of $CD4^+$ $CD25^-$ T cells, but stimulated $CD4^+$ $CD25^+$ Treg cell proliferation [21]. Further, it has been demonstrated that CXCL4 affected the production of cytokines by human Th1 and Th2 cells [22]. Recently, it was shown that CXCL4 induces the differentiation of monocytes into dendritic cells with a more mature phenotype and an increased responsiveness to TLR ligands. Moreover, these CXCL4 monocyte-derived dendritic cells were more potent at inducing proliferation of autologous $CD4^+$ T cells and $CD8^+$ T cells [23]. In addition, CXCL4 is implicated in tumor biology mainly through its ability to inhibit endothelial cell proliferation, migration and angiogenesis.

Since long, glycosaminoglycans (GAGs) were known to be crucial for CXCL4's biological function [24]. In addition, Lasagni et al. identified a splice variant of CXCR3, which was designated CXCR3B, to be a functional GPCR for CXCL4 [25]. Human CXCR3 has three splice variants: CXCR3A, CXCR3B and CXCR3-alt [26]. Besides CXCL4 and CXCL4L1, the CXCR3A receptor binds to CXCL9, CXCL10 and CXCL11 as well [25,27–30]. Interestingly, the CXCR3A and the CXCR3B variants induce opposite physiological functions. In general CXCR3A mediates proliferation, survival and cell migration, while CXCR3B appears to mediate apoptosis and to inhibit proliferation and migration. The CXCR3-alt variant, which differs extremely from the classic seven transmembrane CXCR3A structure, mediates a modest calcium increase and chemotactic response only upon stimulation with CXCL11 [29]. Currently, CXCL4 is known to activate both CXCR3A and CXCR3B splice variants [25,31]. The role of the CXCR3 receptor in cancer has already been investigated. Expression of CXCR3 was found upregulated in many primary and metastatic tumors and has been correlated with poor prognosis in melanoma [32], breast [33], colon [34] and renal cancer patients [35]. More recent studies have revealed that the growth-promoting CXCR3A variant is up-regulated and the more apoptotic CXCR3B variant is down-regulated in those cancer cell types [36,37].

The *CXCL4L1* gene, the non-allelic variant of CXCL4 was discovered in 1989 by Green et al. [38] and cloned shortly thereafter [39]. The protein of the CXCL4L1 isoform was not isolated from thrombin-stimulated platelets until 2004 [40]. Both isoforms are secreted as 70 amino-acid long mature proteins, differing only in 3 amino acids at the carboxy-terminal side ($Pro^{58} \rightarrow Leu$, $Lys^{66} \rightarrow Glu$, $Leu^{67} \rightarrow His$; CXCL4 → CXCL4L1). CXCL4L1 seems to be constitutively released by platelets, smooth muscle cells, human aortic and coronary smooth muscle cells [7,41] and is inducible in monocytes, endothelial and osteosarcoma cells by inflammatory mediators [12]. *In vivo,* weak to strong expression of CXCL4L1 was detected in sarcoma tissue by immunohistochemistry [12]. CXCL4L1, as well as CXCL4, functions as a chemoattractant for activated T cells, NK cells and immature dendritic cells [30]. CXCL4L1 was found to be a more potent angiostatic chemokine compared to CXCL4. The single amino acid change ($Leu^{67} \rightarrow$ His) at the carboxy-terminal end is associated with an alteration of the 3D structure and a lower heparin-binding capacity [42]. Compared to CXCL4, CXCL4L1 is less likely to form heterodimers with fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF) or to compete for GAG-binding [40,43,44]. Therefore the angiostatic effect is presumed to be the result of the interaction of CXCL4L1 with the CXCR3B receptor. In addition to the earlier described effect of CXCL4 on macrophages, we previously reported that CXCL4 and CXCL4L1 exert a direct effect on monocytes and immature monocyte-derived dendritic cells, resulting in different phenotypes [45].

In this study, the role of CXCL4 and CXCL4L1 in cancer is reviewed as well as the potential clinical applications of both chemokines in diagnosis, prognosis and cancer treatment.

2. CXCL4 and CXCL4L1 in hematologic malignancies

Megakaryocytes (MK), the cells giving rise to blood platelets in the bone marrow, are located adjacent to the bone marrow sinusoids and are associated with the vascular niche [46]. Several studies showed that cytokines and growth factors produced by MK are involved in the regulation and protection of bone marrow niches [47]. More specifically, CXCL4 is considered as an important component released by MK that influences hematopoietic stem cell (HSC) maintenance and quiescence by limiting the proliferation of HSCs [48]. Altered CXCL4 levels have been shown in a number of hematological malignancies but the number of studies on its role in disease severity and prognosis is limited.

Shi *et al.* found that serum levels of CXCL4 were decreased in patients with pediatric acute lymphoblastic leukemia (ALL) [49]. A possible role for CXCL4 in ALL tumorigenesis was already supported by the identification of the translocation 4q21, 11q23 in a subclass of patients with ALL [50]. Griffin *et al.* showed that the breakpoint in 4q21 was distal from the *CXCL4* gene [51].

CXCL4, together with another platelet-derived chemokine CXCL7 was found to be decreased in serum of patients with myelodysplastic syndrome (MDS) progressing to acute myeloid leukemia (AML) [52]. Bai *et al.* confirmed that CXCL4 was downregulated in newly diagnosed AML patients and that CXCL4 levels increased after complete remission [53]. Also, Kim *et al.* showed that CXCL4 protein levels are indicative for the recovery of the blood count in complete remission of AML [54].

In a more recent study granulocyte/macrophage progenitor (GMP) behavior in the bone marrow niche was investigated. Two functional states of GMPs were identified, with self-renewing GMPs building GMP clusters, and differentiating GMPs producing mature myeloid cells. The transient formation of regenerating GMP clusters is controlled by the timed release of important bone marrow niche signals such as CXCL4. In a murine model of leukemia it was shown that the lack of termination signals such as CXCL4 drives a constant GMP cluster formation and granulocyte production [55]. The molecular mechanism by which CXCL4 instigates HSC quiescence is still unknown but may involve binding with cell surface chondroitin sulfate and increased HSC adhesion to stromal cells, which results in cell cycle arrest and induction of quiescence [56–58].

More recently, CXCL4 was also found to be deregulated in patients with chronic myelomonocytic leukemia (CMML). In a subgroup of CMML patients, which were resistant to decitabine (a DNA methyltransferase inhibitor) treatment, CXCL4 and CXCL7 were found to be upregulated. Moreover, treatment of primary CMML cells with these platelet-derived chemokines blocked decitabine effects, suggesting that their upregulation contributes to primary decitabine resistance [59].

Finally, in multiple myeloma (MM) Cheng *et al.* observed that the *CXCL4* gene was silenced by promoter hypermethylation in 15 out of 28 patients, thereby suggesting a tumor suppressive role for CXCL4 [60]. In a follow-up study by the same group, CXCL4 was found to suppress myeloma-induced angiogenesis, to inhibit myeloma cell growth and to induce apoptosis [61].

3. CXCL4 and CXCL4L1 in solid tumors

As already mentioned, CXCL4 inhibits endothelial cell proliferation and migration, leading to suppression of angiogenesis. The formation of new blood vessels is a critical process, since cancer cells depend on an adequate supply of oxygen and nutrients and removal of waste products for intensive proliferation. The impact of CXCL4 in the tumor microenvironment depends on the expression of CXCR3 splice variants on leukocytes, endothelial cells and tumor cells. Depending on the CXCR3A and CXCR3B isoform expression, CXCL4 can stimulate tumor cell proliferation and migration or inhibit these processes. Furthermore, CXCL4 interacts with a variety of immune cells like monocytes [18], dendritic cells [30,41,45] and lymphocytes [31]. In the following part, the involvement of CXCL4 and CXCL4L1 in solid tumors is described and summerized in Table 1.