

# Gene Section

## Review

## PF4V1 (platelet factor 4 variant 1)

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### Abstract

Review on PF4V1, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

### Identity

**Other names:** CXCL4L1, CXCL4V1, PF4-ALT, PF4A, SCYB4V1

**HGNC (Hugo):** PF4V1

**Location:** 4q13.3

#### Note

The CXC chemokine CXCL4L1 is a nonallelic variant of the earlier identified platelet factor CXCL4. These rather atypical chemokines display a less prominent leukocyte chemoattractant activity, yet influence a large range of other processes. CXCL4L1 was characterized as an especially potent angiostatic chemokine (Struyf et al., 2004). Consequently, this platelet factor is an inhibitor of tumor growth and metastasis. The therapeutic potential of CXCL4L1 has been evidenced in preclinical B16 melanoma, Lewis lung carcinoma and A549 adenocarcinoma animal models, as it inhibited both tumor growth and metastasis by preventing tumor neovascularization (Struyf et al., 2007). Furthermore, the carboxy-terminal peptide CXCL4L1<sub>47-70</sub> retains its potential to suppress B16 melanoma growth in mice (Vandercappellen et al., 2010). Additionally, the recently highlighted impact of CXCL4L1 on lymphatic endothelial cells in vitro, corroborates a potential inhibitory effect on tumor dissemination in vivo (Prats et al., 2013; Van Raemdonck et al., 2014). Compared to CXCL4, the

affinity of CXCL4L1 for glycosaminoglycans (GAG) is rather moderate (Dubrac et al., 2010; Struyf et al., 2011), strongly increasing its in vivo half-life and diffusibility.

### DNA/RNA

#### Note

The gene and mRNA for CXCL4L1 are 1293 and 741 bp in length, respectively.

#### Description

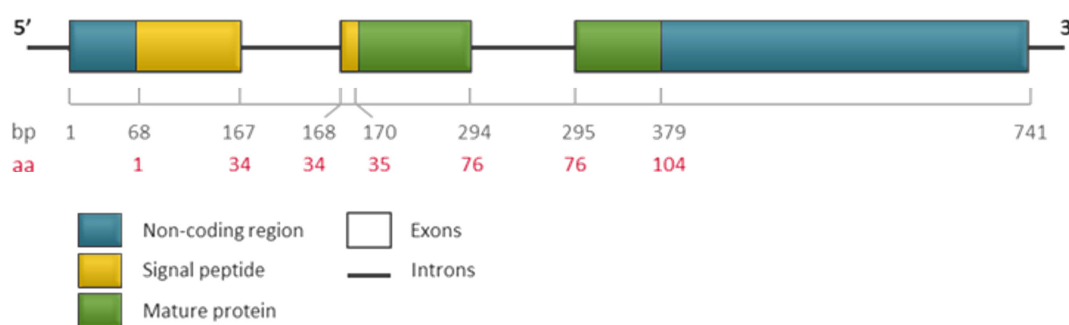
The CXCL4L1 mRNA is encoded by three exons. Duplication of the CXCL4 gene, giving rise to the homologous CXCL4L1 gene, is conserved in human and other primates including gorilla, chimpanzee, orangutan, gibbon and macaque.

#### Transcription

The existence of a CXCL4 variant was first evidenced by Eisman et al. (1990) and Green et al. (Eisman et al., 1990; Green et al., 1989). The CXCL4L1 mRNA is predominantly present in platelets, but has also been detected in vascular smooth muscle cells and to a lesser extent in T cells, monocytes and endothelial cells (Lasagni et al., 2007). CXCL4L1 mRNA detected in ovarian tissue has been attributed to macrophage CXCL4L1 expression (Furuya et al., 2012). CXCL4L1 expression was also observed in the HCT-8 colon adenocarcinoma cell line as evidenced by qPCR analysis (Verbeke et al., 2010).

#### Pseudogene

None.



**Figure 1. Structure of the human CXCL4L1 gene.** This figure schematically depicts the structure of the human CXCL4L1 gene as described in the NCBI database (NM\_002620). Lines represent the introns, whereas rectangular exons are coloured blue, yellow and green to represent the non-coding domains, the signal peptide and the mature protein, respectively. Grey numbers indicate the basepairs (bp) spanning the exons. Red numbers apply to the amino acids (aa) encoded.

## Protein

### Note

CXCL4L1 precursor: 104 amino acids (aa), 11553 Da; CXCL4L1 mature: 70 aa, 7805.8 Da.

### Description

CXCL4L1 is a member of the CXC chemokine family of chemoattractant cytokines.

CXCL4L1 is a non-ELR CXC chemokine, meaning that it lacks the sequence glutamic acid-leucine-arginine just in front of the two NH<sub>2</sub>-terminally located conserved cysteine residues.

### Expression

Blood platelets release both CXCL4 and CXCL4L1 after activation.

The exact location of CXCL4L1 inside the platelet is not yet determined, whereas platelet CXCL4 is stored in the alpha-granules.

In other cell types as well, CXCL4 is stored in secretory granules and released in response to protein kinase C activation, whereas CXCL4L1 is continuously synthesized and secreted through a constitutive pathway (Lasagni et al., 2007).

For instance, human aortic smooth muscle cells and human coronary smooth muscle cells constitutively release CXCL4L1.

Specific cancer cell lines have also been shown to produce CXCL4L1.

Secretion of CXCL4L1 in tumoral tissue was evidenced in vitro on stimulated osteosarcoma cells through the use of ELISA and further corroborated by immunohistochemical staining of different human sarcoma tissue sections (osteosarcoma, leiomyosarcoma and liposarcoma) (Vandercappellen et al., 2007).

Furthermore, CXCL4L1 was strongly detected in colorectal adenocarcinoma biopsy specimens (Verbeke et al., 2010).

### Localisation

Secreted.

### Function

CXCL4L1 has been described to be a strong inhibitor of angiogenesis. Together with its potential to chemoattract T cells, natural killer cells and immature dendritic cells, the vascular effects contribute to the antitumoral action of CXCL4L1 (Struyf et al., 2011). Struyf et al. (Struyf et al., 2007) indeed indicated the angiostatic platelet factor to exert an antitumoral effect by inhibiting branching of the vascular network and metastasis. Considering neutrophils and monocytes, CXCL4L1 as opposed to CXCL4 would not attract these pro-tumoral phagocytes (Vandercappellen et al., 2007). Lasagni et al. identified a splice variant of CXCR3, which was named CXCR3B, as a functional GPCR for CXCL4 (Lasagni et al., 2003). Currently, both CXCL4 and CXCL4L1 are known to activate CXCR3A, as well as CXCR3B (Mueller et al., 2008; Struyf et al., 2011; Van Raemdonck et al., 2014). In general, proliferative and positive migratory effects are supposed to be mediated by CXCR3A, whereas inhibition of chemotaxis, anti-proliferative and apoptotic effects are postulated to be provoked via CXCR3B (Lasagni et al., 2003). Besides endothelial cells and T cells, CXCR3 expressing cell types can be extended to fibroblasts, mesangial cells, airway epithelial and smooth muscle cells, pneumocytes and several sarcoma, carcinoma and myeloma cell types (Billottet et al., 2013).

CXCL4 exerts its action through many different mechanisms, including binding to GAG and heteromultimerisation with other chemokines and growth factors, whereas in the case of CXCL4L1 its distorted structure and unique protruding C-terminal helix are assumed to conflict with this mode of action. The open formation characteristic of CXCL4L1 decreases GAG-binding, however simultaneously enhancing anti-angiogenic and anti-tumoral effects (Dubrac et al., 2010; Kuo et al., 2013). Additionally, CXCL4L1 forms more stable homodimers due to a loss in positive charge.

This gained stability is likely to interfere with the ability to form heteromers which requires initial dissociation of the homomultimers (Kuo et al., 2013).

### Homology

CXCL4L1 is a non-allelic variant of CXCL4. Unlike CXCL4, its variant appears only in primates. In men, mature proteins only differ in 3 amino acids. On the other hand, the signal peptide of human CXCL4L1 displays 38% amino acid divergence compared to human CXCL4, affecting its subcellular localization and regulated secretion mechanism, as was described by Lasagni et al. (Lasagni et al., 2007).

## Implicated in

### Osteosarcoma

#### Disease

Secretion of CXCL4L1 in tumoral tissue was evidenced in vitro on stimulated osteosarcoma cells through the use of ELISA and further corroborated by immunohistochemical staining of different human sarcoma tissue sections (osteosarcoma, leiomyosarcoma and liposarcoma) (Vandercappellen et al., 2007). On the other hand, osteosarcoma cells also express the CXCR3 receptor guiding initial tumor dissemination to metastatic sites were CXCR3 ligands such as CXCL4L1 are expressed (Pradelli et al., 2009).

### Colorectal cancer

#### Disease

Study of CXCL4L1 expression in human epithelial tumors revealed a distinct presence of CXCL4L1 in colorectal cancer cells, whereas its expression in esophageal cancer was weak to undetectable (Verbeke et al., 2010). ELISA, qRT-PCR, immunocytochemistry as well as ex vivo immunohistochemistry support the hypothesis that CXCL4L1 is secreted by colorectal adenocarcinoma cells and may affect the complex process of tumor development. However, no correlation was found between the intensity or extent of CXCL4L1 staining of patient biopsies and the TNM stage. On the other hand, intratumorally administered CXCL4L1 has been shown to reduce tumor vascularization and, consequently, tumor growth and metastasis of A549 adenocarcinoma in mice, similar to its therapeutic benefit observed in preclinical studies on B16 melanoma and Lewis lung carcinoma (Struyf et al., 2007).

### Endometriosis-associated ovarian cancer (EAOC)

#### Oncogenesis

Both clear cell and endometrioid types of ovarian cancers occasionally develop on the bases of

endometriosis. CXCL4L1 is expressed in normal ovaries and especially during endometriosis (Furuya et al., 2012). However, CXCL4L1 mRNA levels were significantly lower in cancerous lesions. Endometriosis-associated ovarian cancers (EAOC) were reported to be infiltrated by CD68+ tumor-associated macrophages. CXCL4 and CXCL4L1 expression by those macrophages was studied at the protein level by Furuya and colleagues. However, antibodies not distinguishing CXCL4 from its variant were used. The tumor-associated macrophages displayed an impaired expression of either CXCL4, CXCL4L1 or possibly both. In conclusion, macrophage expression of the platelet factors appears to be associated with EAOC disease state and may prove to be a useful disease marker.

### Coronary artery disease

#### Prognosis

Recently, a possible prognostic significance for CXCL4L1 was evaluated in patients suffering from coronary artery disease (CAD) (De Sutter et al., 2012). Specifically in a selection of patients with stable CAD and preserved left ventricular function, CXCL4L1 levels significantly correlated to age, creatinine and circulating platelet number, as well as to N-terminal pro-B-type natriuretic peptide (NT-proBNP), a well validated prognostic marker in stable CAD. More importantly, CXCL4L1 showed an additional prognostic value on top of NT-proBNP as lower levels of CXCL4L1 predicted a higher event rate and worse outcome. Surprisingly, in these patients with stable CAD the prognostic value of CXCL4L1 is independent of NT-proBNP.

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