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Review

Molecular pathways of platelet factor 4/CXCL4 signaling

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ABSTRACT

The platelet-derived chemokine CXCL4 takes a specific and unique position within the family of chemotactic cytokines. Today, much attention is directed to CXCL4's capacity to inhibit angiogenesis and to promote innate immune responses, which makes this chemokine an interesting tool and target for potential intervention in tumor growth and inflammation. However, such attempts demand a comprehensive knowledge on the molecular mechanisms and pathways underlying the corresponding cellular functions. At least two structurally different receptors, CXCR3-B and a chondroitin sulfate proteoglycan, are capable of binding CXCL4 and to induce a specific intracellular signaling machinery. While signaling mediated by CXCR3-B involves Gs proteins, elevated cAMP levels, and p38 MAP kinase, signaling via proteoglycans appears to be more complicated and varies strongly between the cell types analyzed. In CXCL4-activated neutrophils and monocytes, tyrosine kinases of the Src family and Syk as well as monomeric GTPases and members of the MAP kinase family have been identified as essential intracellular signals. Most intriguingly, signaling does not proceed in a linear sequence of events but in a repeated activation of certain transducing elements like Rac2 or sphingosine kinase 1. Depending on the downstream targets, such biphasic kinetics either leads to a redundant and prolonged activation of a single pathway or to a timely separated initiation of disparate signals and functions. Results of the studies reviewed here help to understand the molecular basis of CXCL4's functional diversity and provide insights into integrated signaling processes in general.

Introduction

Platelet factor 4 (PF-4/CXCL4), discovered in 1977 (Deuel et al., 1977), was the first member of a large group of cytokines, which, based on their common property to induce a directed cellular migration, were later subsumed to form the family of chemokines. One essential reason for the enormous progress in chemokine research and the almost exploding increase of newly discovered chemokines and chemokine receptors during the last 20 years was simply founded on the fact that these molecules are so closely related in structure and function. This circumstance did not only enable the rapid identification of so far uncharacterized peptides as chemokines but, moreover, allowed the prediction of the structures of their corresponding receptors, their major intracellular signaling events and finally, by their common capacity to induce chemotaxis, of at least one major cellular function.

Today, the family of chemokines contains more than 50 members, showing sequence identities between 20 and 70% and a conserved secondary and tertiary structure, which is, except in lymphotactin, stabilized by two disulfide-bridges. Except for CXCR3B, all of the 19 different chemokine receptors which have been identified so far, belong to the group of Gi protein-coupled seven transmembrane domain (7-TMD) receptors, mediating transient calcium fluxes and the activation of a limited set of kinases and phospholipases (reviewed in: Mantovani et al., 2010; Ransohoff, 2009; Zlotnik et al., 1999; Thelen, 2001).

CXCL4 was originally described to induce at rather high concentrations a chemotactic response in neutrophils and monocytes (Deuel et al., 1981; Bebawy et al., 1986). However, these findings could not be confirmed by later studies (Walz et al., 1989; Clark-Lewis et al., 1993). Today it is clear that highly purified CXCL4 lacks chemotactic activity for neutrophils and monocytes (Petersen et al., 1996; Fleischer et al., 2002; Pervushina et al., 2004). Some controversial results reported in the past may be explained by small contaminations with other chemokines, identified later on as RANTES (CCL5) and NAP-2 (CXCL7) (Kameyoshi et al., 1992; Brandt et al., 1989; Walz and Baggiolini, 1989), found in CXCL4 preparations at that time, when purification methods were much less sophisticated than nowadays. Due to these erroneous results, CXCL4, without after all being a true chemotaxin itself, had a cru-

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Table 1
CXCL4-mediated biological functions on different cell types.

Target cell	Biological functions	References
Neutrophils	Increase of adherence to EC Induction of exocytosis (in the presence of TNF or integrins)	Petersen et al. (1996, 1999a)
NK cells	Secretion of IL-8	Marti et al. (2002)
T cells	Inhibition of cell proliferation Reduced release of IL-2 and TH1 cytokines (IFN-γ) Increased release of TH2 cytokines (IL-4, IL-5, IL-13)	Fleischer et al. (2002), Liu et al. (2005), Romagnani et al. (2005)
Monocytes	Generation of reactive oxygen intermediates Increased phagocytosis Increased release of TNF Inhibition of spontaneous apoptosis Monocyte-differentiation into macrophages Monocyte-differentiation into APC (in the presence of IL-4)	Pervushina et al. (2004), Scheuerer et al. (2000), Fricke et al. (2004)
Endothelial cells	Inhibition of cell proliferation and angiogenesis	Maione et al. (1990), Gengrinovitch et al. (1995)
Fibroblasts	Inhibition of cell proliferation	Watson et al. (1994)
Hematopoietic stem cells	Increased survival	Han et al. (1997)
Megakaryopoietic lineage cells	Inhibition of cell differentiation	Han et al. (1990), Xi et al. (1996)
Eosinophils	Induction of adherence	Hayashi et al. (1994)

cial impact on the classification and naming of the whole family of chemokines. Although CXCL4 has been under intense investigation for more than 30 years, its cellular functions, receptors, and their corresponding signaling pathways are still not fully understood. In this review we will outline the recent progress in CXCL4 research with regard to novel cellular functions, receptors and receptor-mediated intracellular signals.

Sources and biological functions

Megakaryocytes and platelets until recently have been described as the exclusive source of CXCL4 which in fact becomes released in high amounts from platelet α-granules within minutes upon platelet activation. As was reported for CXCL7, normal CXCL4 plasma levels are below 1 nM while normal serum contents reach more than a thousand-fold higher concentrations (1–2.5 μM) (Brandt et al., 2000; Files et al., 1981; Levine and Krentz, 1977). Today, also other cell types like smooth muscle cells, microglia, macrophages, or T cells (Schaffner et al., 2005; Lasagni et al., 2007; de Jong et al., 2008) have been recognized to express CXCL4. However, since the amounts released by these cells are substantially lower than those derived from platelets, the physiological role of "non-platelet CXCL4" remains unclear and may be limited to local functions of this chemokine.

Beside CXCL4, the product of a nonallelic gene variant termed PF4alt (CXCL4L1) (Green et al., 1989; Eisman et al., 1990) with only three amino acid substitutions in the C-terminal α-helix has been isolated from platelets and other cells (Struyf et al., 2004; Lasagni et al., 2007). Interestingly, due to divergences in the signal peptide region this variant shows a distinct subcellular localization. In contrast to CXCL4 which is stored and released upon activation, CXCL4L1 appears to be continuously synthesized and secreted through a constitutive pathway (Lasagni et al., 2007). Both variants are active on the same target cells but differ strongly with regard to their biological efficacy and time of half-life as well as to their affinity for glycosaminoglycans (Dubrac et al., 2010).

Although not being chemotactic for monocytes and neutrophils, CXCL4 affects practically all nucleated cells of the vasculature (Table 1). Different to most other chemokines, CXCL4 is involved in the control of many long-term regulatory processes like apoptosis, cell differentiation, survival, and proliferation. CXCL4 supports the survival of hematopoietic stem cells as well as of progenitor cells (Han et al., 1997) and suppresses the development and maturation of cells from the megakaryopoietic lineage (Han et al., 1990). Moreover, several authors reported an anti-proliferative activity of this chemokine on endothelial cells and fibroblasts as well as an anti-angiogenic activity (Watson et al., 1994; Luster et al., 1995; Maione et al., 1990; Tanaka et al., 1997). We could show that CXCL4 acts as a potent suppressor of T cell function in terms of reducing lymphoproliferation and inhibiting the release of IL-2 and IFN-γ (Fleischer et al., 2002).

Concerning mononuclear phagocytes, CXCL4 can be seen as a first and second line mediator in the host defense against microbial invaders. During an initial phase, the chemokine induces the generation of oxygen radicals and phagocytosis (Pervushina et al., 2004). Thereafter, CXCL4 initiates a cellular program, which prevents monocytes from undergoing spontaneous apoptosis and mediates their differentiation into a specific subtype of macrophages (Scheuerer et al., 2000). Phenotype and functions of these CXCL4-derived macrophages appear to be rather unusual and differ remarkably from those induced by M-CSF or GM-CSF. Most intriguingly, macrophages differentiated by CXCL4 are characterized by a lack of surface-expressed HLA-DR antigen (Scheuerer et al., 2000). The development of a high capacity for uptake and killing of microorganisms and endothelial cells (Pervushina et al., 2004; Woller et al., 2008) accompanied by a substantial release of TNF and proinflammatory chemokines like CXCL8 (interleukin 8; IL-8), CCL3 (macrophage inflammatory protein 1α; MIP-1α), or CCL4 (MIP-1β) (Scheuerer et al., 2000; Kasper et al., 2007), indicates that these cells are highly specialized for inducing and maintaining an innate immune response.

Several cellular functions of CXCL4 are only observed in the presence of appropriate costimuli. Neutrophils undergo a rapid and firm adhesion in response to the chemokine alone, however, full activation in terms of exocytosis requires costimulation by defined soluble (TNF, LPS, fMLP) or immobilized (ICAM-1) activators (Petersen et al., 1999a; Kasper et al., 2004; Fig. 1). Moreover, treatment of monocytes with CXCL4 alone results in the development of activated macrophages while a combination of CXCL4 and IL-4 induces differentiation into professional antigen-presenting cells (APCs) which are clearly distinct from conventional dendritic cells and macrophages (Fricke et al., 2004).

CXCL4 receptors

In spite of the broad spectrum of different biological activities which has been described for CXCL4 during the years, CXCL4 receptors are still mysterious and could only be partially identified. In 2003, Lasagni and coworkers discovered an alternative splice variant of the CXCR3 (termed CXCR3-B) as a receptor for CXCL4 in endothelial cells (Lasagni et al., 2003). In cells transfected with either variant the authors could show that CXCL9 (monokine induced by γ-interferon; Mig), CXCL10 (interferon γ-induced protein 10; IP10), and CXCL11 (interferon-inducible T-cell