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Regulated Shedding of Syndecan Ectodomains by Chemokines

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Chemokines are chemotactic cytokines that govern multiple aspects of host defense[1]. Interest in chemokines has increased as a result of their emerging role in immune and inflammatory responses, hematopoeisis, HIV infection[2,3,4], cancer cell proliferation, and migration[5]. Chemokines mediate biological activities through activation of G-protein-coupled receptors (GPCRs)[6]; however, they also bind to glycosaminoglycans (GAGs)[7,8,9], especially heparan sulfate (HS)[10]. GAGs are long, linear, and heterogeneous sulfated polysaccharides that are generally closely associated with or immobilized on cell surfaces by attachment to protein cores, forming proteoglycans (PGs)[11]. Four chemokine subgroups have been named C, CC, CXC, or CX_3C according to the number and spacing of cysteine residues[1]. Stromal cell-derived factor-1 (SDF-1/CXCL12), a CXC chemokine, constitutively expressed in a wide variety of tissues, binds to the GPCR CXCR4[6,12,13]. CXCR4 also functions as a coreceptor for X4 HIV isolates[14] and SDF-1/CXCL12 blocks cellular entry of these isolates[4,14]. Optimal inhibition requires SDF-1 interaction with cell surface HS[15]. However, the SDF-1/CXCL12-CXCR4 axis is involved in other pathological processes: angiogenesis, invasiveness, migration, and proliferation of cancer cells[16,17]. Nevertheless, the pathophysiological roles of chemokine-GAG interactions have not yet been elucidated.

In one of our recent works published in *Glycobiology*, we characterized the cell PG that binds to SDF-1/CXCL12[18]. The syndecans are a PG family, which together with the lipid-linked glypicans, are the major source of cell surface HS[11]. By way of their HS chains, syndecans bind a wide variety of ligands[11]. These PGs were also identified as HIV attachment receptors[19]. In this context, we showed that SDF-1/CXCL12 forms complexes on the human epidermoid carcinoma HeLa cell line, and on human primary lymphocytes and monocyte-derived macrophages (MDM), which comprise CXCR4 as expected and syndecan-4 (SDC-4), but not other PGs, syndecan-1 (SDC-1), CD44, nor betaglycan[18]. We also demonstrated that while SDC-4 and CXCR4 form a heteromeric complex on these cells, SDF-1/CXCL12 directly binds SDC-4 in a GAG-dependent manner. This suggests that SDF-1/CXCL12 may specifically bind a GAG structure present on SDC-4. However, this does not exclude that SDF-1/CXCL12 may also interact with some domains of the protein core of SDC-4. Moreover, in another recent paper published in *FEBS Journal*, we showed that SDC-4 behaves as a specific SDF-1/CXCL12 receptor, involved in SDF-1/CXCL12–induced transduction pathways. By specifically reducing SDC-4 expression using RNA

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interference, we demonstrated that Erk1/2 and JNK/SAPK MAPK activation by SDF-1/CXCL12 requires SDC-4 expression. Moreover, SDF-1/CXCL12 induces the phosphorylation of its GPCR, CXCR4, and also that of SDC-4[20].

Shed syndecan ectodomains are newly defined mediators of inflammation that may be involved in cell proliferation and in several regulatory processes[21,22]. It was shown that matrix metalloproteinases (MMPs) are involved in syndecan shedding and catabolic processes of syndecans[23,24]. MMPs are secreted as latent enzymes and require proteolytic cleavage for activation. Chemokine stimulation of cell MMPs have been described. For instance, SDF-1/CXCL12 stimulates the production of MMP-9 by murine RAW cells[25]. We have consequently investigated whether SDF-1/CXCL12 accelerates the shedding of PG ectodomains from human cell lines and primary cells, and tried to elucidate which transduction pathways and protease(s) are involved and whether SDF-1/CXCL12 forms complexes with the shed ectodomains of PGs.

In a recent study published in *Glycobiology*[26], we demonstrated that SDF-1/CXCL12 accelerates the shedding of SDC-4 and, to a lesser extent, that of SDC-1 from HeLa cells and MDM (Fig. 1). Syndecan shedding accelerated by SDF-1/CXCL12 depends on cell surface HS chains, since it was abolished in heparitinase-treated cells, but does not depend on CXCR4 expression, as CXCR4 silencing by RNA interference had no effect. However, this SDF-1/CXCL12–induced shedding depends on a PKC transduction pathway because it was inhibited by a PKC inhibitor. In this context, we showed that SDF-1/CXCL12 increases MMP-9 mRNA level and MMP-9 activity in HeLa cells, and that MMP-9 silencing by RNA interference strongly decreases SDC-1 and SDC–4 ectodomain shedding accelerated by SDF-1/CXCL12. Moreover, the shedding of SDC-1 and SDC–4 accelerated by SDF-1/CXCL12 from MDM was significantly inhibited by anti-MMP-9 antibodies. Therefore, MMP-9 is involved in the accelerated shedding of syndecans induced by SDF-1 in both a tumoral cell line and in human primary macrophages. However, the fact that the accelerated shedding of SDC-1 was not decreased in MMP-9–deficient mice during allergic lung infection[27] suggests that different shedding sites may be used in this PG according to the chemokine, in a tissue-dependent manner, and also possibly in a disease-specific manner. Strikingly, immunoprecipitation experiments showed us that while SDF-1/CXCL12 binds to membrane-anchored SDC-4, it does not bind to SDC-1 or SDC-4 ectodomains shed from HeLa cells. Therefore, membrane association of SDC-4 may well be critical for its interaction with SDF-1/CXCL12. In addition, the high density of SDC-4 and cell surface association may induce particular GAG chain orientation leading to optimal SDF-1/CXCL12 binding.

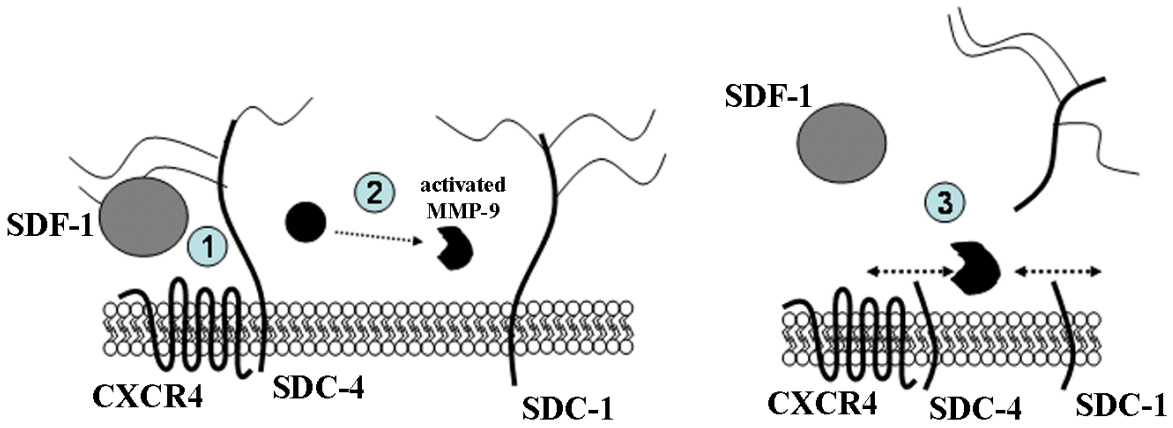

FIGURE 1. The shedding of SDC-4 and SDC-1 from HeLa cells and human primary macrophages is accelerated by SDF-1/CXCL12 and mediated by MMP-9. (1) SDF-1 binding to SDC-4 facilitates its presentation to CXCR4; (2) SDF-1 activates MMP-9, which is involved in syndecan shedding; (3) decreased membrane expression of syndecans down-regulates SDF-1 binding to the cells.

In summary, our data strongly indicate that the shedding of SDC-4 and SDC-1 accelerated by SDF-1/CXCL12 from human epidermoid carcinoma cells and from human primary macrophages is mediated by MMP-9. Considering that in our experiments, syndecan shedding accelerated by SDF-1/CXCL12 is associated with decreased syndecan membrane expression, as assessed by cytofluorimetric analysis after specific labeling, and that SDF-1/CXCL12 does not bind to soluble syndecan ectodomain, the role of syndecan shedding in the biological effect of SDF-1 may then be related to changes in the target cell surface phenotype rather than to a competitive inhibition in the binding of the chemokine to the target cell surface. In fact, one can speculate that SDC-4 shedding induced by SDF-1 /CXCL12 and mediated by MMP-9 could be part of an autoregulatory/down-regulation cycle: (1) SDF-1/CXCL12 binding to SDC-4 facilitates its presentation to CXCR4; (2) SDC-4 is a signaling molecule for SDF-1/CXCL12; (3) while SDF-1 accelerates SDC-4 ectodomain shedding, this chemokine decreases SDC-4 membrane expression. Therefore, SDF-1/CXCL12 down-regulates the cell plasma membrane expression of its coreceptor, the SDC-4. Interestingly, we recently demonstrated that RANTES/CCL5 (1) accelerates the shedding of SDC-1 and SDC-4 from HeLa cells, which depends on CCR5 and on both Erk1/2 MAPK and PKC transduction pathways; and (2) forms GAG-dependent complexes with the shed ectodomains of these PGs[28]. In addition, Xu et al. showed that SDC-1 ectodomains bind to CCL-7, -11, and -17 during lung inflammation[27]. Therefore, the respective molecular events involved in the acceleration of shedding of PGs induced by RANTES/CCL5 or SDF-1/CXCL12 differ according to the chemokine. Moreover, the binding capability of chemokines to shed syndecan ectodomains differs. For instance, RANTES/CCL5 and SDF-1/CXCL12, respectively, CC- and CXC-chemokines, are quite different. They show different GAG-binding epitopes and different quaternary structure (dimeric and oligomeric states)[29]. They also exhibit different expression patterns. RANTES is preferentially secreted during inflammatory process, whereas SDF-1/CXCL12 is constitutively expressed in various cell types and may play a “homeostatic” role. Therefore, SDC-1 and SDC-4 shedding accelerated by RANTES/CCL5 could be expected to occur during inflammatory and wound repair processes, whereas PG shedding accelerated by SDF-1/CXCL12 could occur even in the absence of any tissue injury. This accelerated shedding by chemokines may, therefore, represent fine regulatory mechanisms of chemokine activity in physiology and diseases.

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