Biochimica et Biophysica Acta 1790 (2009) 1643–1650

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Glycosaminoglycans and syndecan-4 are involved in SDF-1/CXCL12-mediated invasion of human epitheloid carcinoma HeLa cells

Severine Brule [a,*], Véronique Friand [a], Angela Sutton [a,b], Françoise Baleux [c], Liliane Gattegno [a,b], Nathalie Charnaux [a,b]

[a] INSERM U698, Université Paris 13, Bobigny, France
[b] Laboratoire de Biochimie, Hôpital Jean Verdier, AP-HP, Bondy, France
[c] Institut Pasteur, Unité de Chimie des Biomolécules CNRS URA2128, France

ARTICLE INFO

Article history:
Received 22 April 2009
Received in revised form 3 August 2009
Accepted 7 August 2009
Available online 17 August 2009

Keywords:
Chemokine
Invasion
SDF-1/CXCL12
Glycosaminoglycan
Proteoglycan
Syndecan

ABSTRACT

Background: In addition to their physiologic effects in inflammation and angiogenesis, chemokines are involved in cancer pathology. The CXC-chemokine stromal cell-derived factor-1 (SDF-1)/CXCL12 mediates its biological activities through activation of G protein-coupled receptor CXCR4 and binds to glycosaminoglycans (GAGs).
Methods: Using Bio-coat cell migration chambers, specific antagonists, flow cytometry and RNA interference, we evaluate the involvement of heparan sulfate proteoglycans (HSPG) in the SDF-1/CXCL12-induced invasion of human cervix epitheloid carcinoma HeLa cells.
Results: The SDF-1/CXCL12-induced cell invasion is dependent on CXCR4. Furthermore, Protein Kinase C delta (PKC δ) and c-jun NH2-terminal kinase/stress-activated protein kinase (JNK/SAPK) are implicated in this event, but not extracellular signal-regulated kinase (ERK) 1/2. Moreover, the invasion of HeLa cells induced by SDF-1/CXCL12 was dependent on matrix metalloproteinase-9 (MMP-9). The pre-incubation of HeLa cells with heparin or with anti-heparan sulfate antibodies or with β-D-xyloside inhibited SDF-1/CXCL12-mediated cell invasion. Furthermore, the down-regulation of syndecan-4, a heparan sulfate proteoglycan, decreased SDF-1/CXCL12-mediated HeLa cell invasion. GAGs, probably on syndecan-4, are involved in SDF-1/CXCL12-mediated cell chemotaxis.
General significance: These data suggest that targeting the glycosaminoglycan/chemokine interaction could be a new therapeutic approach for carcinomas in which SDF-1/CXCL12 is involved.

1. Introduction

Chemokines are chemoattractant cytokines for leukocytes and their receptors belong to a family of specific G protein-coupled receptors (GPCRs). They recruit various types of leukocytes, including monocytes/macrophages, lymphocytes, and dendritic cells, thereby modulating host responses to tumors [1]. Moreover, several chemokines can control angiogenesis, a process essential for tumor growth, or guide the growth and the mobility of tumor cells, thereby affecting the process of tumor progression [2,3].

Stromal cell-derived factor-1 (SDF-1)/chemokine (CXC motif) ligand 12 (CXCL12), a CXC chemokine is a homeostatic chemokine that signals through chemokine (CXC motif) receptor 4 (CXCR4) [4], a G protein-coupled receptor, which in turn plays an important role in hematopoiesis, development and organization of the immune system [5]. However, like other chemokines, this chemokine binds to glycosaminoglycans (GAGs) [6]. Several studies have indicated that SDF-1/CXCL12 is expressed in some cancer cells (malignant ovarian and breast cancer cell lines) and is involved in tumor cell migration and metastasis [7].

The syndecans (SDCs) are a family of proteoglycans, which, together with the lipid-linked glypicans, are the major source of heparan sulfate chains at cell surface [8,9]. By the way of their heparan sulfate chains, SDCs bind a wide variety of soluble and insoluble ligands, such as HIV-1 [9,10]. We previously demonstrated that SDF-1/CXCL12 forms complexes on HeLa cells and human primary lymphocytes or macrophages, which comprise CXCR4 and SDC-4 [4]. We also showed the occurrence of a heteromeric complex between SDC-4 and CXCR4 at the plasma membrane of these cells. Moreover, our data demonstrated that SDF-1/CXCL12 binds directly to SDC-4, which may be a signalling molecule for the chemokine [11].

The aims of this present study were 1—to determine whether SDF-1/CXCL12 induces invasion of human epitheloid carcinoma HeLa cells and 2—to elucidate the molecular mechanisms of these effects, including the involvement of CXCR4 and the role played by GAGs and proteoglycans.

* Corresponding author. Tel.: +331 40 25 75 43; fax: +331 48 02 65 03.
E-mail address: severinebrule@yahoo.fr (S. Brule).

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doi:10.1016/j.bbagen.2009.08.001

2. Materials and methods

2.1. Materials

SDF-1/CXCL12 and mutated SDF-1 (3/6) were a gift from F. Baleux. SDF-1 (3/6) was generated by combined substitution of the basic cluster of residues Lys24, His25 and Lys27 by Ser. SDF-1 (3/6) conserves the global native structure and functional properties of SDF-1/CXCL12, but is unable to interact with heparin [12].

2.2. Cell culture conditions

HeLa cells were cultured as previously described [13]. For glycosaminoglycan biosynthesis inhibition, cells were incubated with 1 mM 4-Nitrophenyl-β -D-xylopyranoside (β-D-xyloside) (Sigma-Aldrich) for 72 h [14].

2.3. Cell viability assay

Cell viability was measured using the reduction of 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich). Cells (5×10^3) were treated for 24 h at 37 °C with AMD3100 (1.2 to 120 μM), PD98059 (0.1 to 100 μM), rottlerin (1 to 10 μM) or SP600125 (1 to 100 μM; all from Sigma-Aldrich, France). Cells were then incubated with 0.5 mg/ml MTT for 1 h at 37 °C. After MTT withdrawal, the resulting blue formazan cristae were solubilized in DMSO (Merck, Fontenay-sous-Bois, France). Absorbance was read at 595 nm.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

HeLa cells were incubated, or not, with 50 nM SDF-1/CXCL12 for 16 h. Human syndecan-1- (SDC-1), syndecan-4- (SDC-4) and glyceraldehyde 3-phosphodehydrogenase (GAPDH) mRNAs were amplified by semi-quantitative RT-PCR. Specific primers were designed as follows: SDC-1 (NM_002997): 5′-TCT GAC AAC TTC TCC GGC TC-3′ (forward) and 5′-CCA CTT CTG GCA GGA CTA CA-3′ (reverse); SDC-4 (NM_002999): 5′-CGA GAG ACT GAG GTC ATC GAC-3′ (forward) and 5′-CGC GTA GAA CTC ATT GGT GG-3′ (reverse); GAPDH (NM_002046): 5′-CTG AAC GGG AAG CTC ACT GG-3′ (forward) and 5′-TGA GGT CCA CCA CCC TGT TG-3′ (reverse). In experiments, optimum semi-quantitative RT-PCR conditions were established to remain in the linear phase of amplification curve [15].

2.5. Immunofluorescence staining and FACscan analysis of the cells

5×10^5 HeLa cells were permeabilized with Dulbecco's Modified Eagle Medium (DMEM)/BSA 2%/saponin 0.3% (Sigma-Aldrich) and then incubated in this medium for 15 min at 20 °C with anti-SDC-1 or anti-SDC-4 goat antibodies (Abs) (10 μg/ml; goat IgG) corresponding to the C-terminal domain of human SDC-1 or SDC-4; clone C-20 or D-16 respectively (Santa Cruz Biotechnology) or their isotypes (BD Bioscience Pharmingen, San Diego, USA). After washing, cells were incubated for 30 min at 20 °C in 300 μl PBS/BSA 2% supplemented with fluorescein isothiocyanate-labeled (FITC)-mouse anti-goat IgG Abs (10 μg/ml, Pharmingen), fixed in 1% PFA and analysed by flow cytometry. β-D-xyloside cell treatment efficiency was assayed by flow cytometry using anti-heparan sulfate monoclonal Abs (mAbs) (10 μg/ml, clone 10E4, Seikagaku Corporation, Tokyo, Japan) and the isotype IgM as described [15].

2.6. Cell invasion

HeLa cell invasion was performed using Bio-coat cell migration chambers (Becton Dickinson). Inserts containing 8 μm pore size filters were coated with Matrigel (320 μg/ml, BD Pharmingen) for invasion assay. After filter hydration with DMEM for one night at 37 °C, 2×10^5 cells in DMEM/BSA 0.1% were added. Wild-type (Wt) SDF-1/CXCL12 or mutated SDF-1 (3/6) was added to 500 μl of DMEM supplemented with BSA 1% in the lower chamber. In parallel, cells were pre-incubated for 2 h at 37 °C with AMD3100 (12 μM), with anti-SDC-1 mAbs (10 μg/ml clone DL101, mouse IgG1), anti-SDC-4 mAbs (10 μg/ml clone 5G9, mouse IgG2a), anti-matrix metalloproteinase-9 (MMP-9) mAbs (10 μg/ml; clone 2C3, IgG1), anti-CXCR4 (10 μg/ml, clone 12G5, mouse IgG2a) mAbs (all from Santa Cruz Biotechnology Inc.), anti-heparan sulfate (HS) (10 μg/ml, clone 10E4) mAbs , anti-CXCR4 (10 μg/ml, clone G19, goat IgG, Santa Cruz Biotechnology Inc.) or the isotypes IgG1, IgG2a, IgM or IgG goat (10 μg/ml, BD Bioscience Pharmingen), or with pharmacological signalling inhibitors: PD98059 (1 μM), SP600125 (1 μM), rottlerin (5 μM) (all from Sigma-Aldrich).

Alternatively, cells were treated with β-D-xyloside (for 48 h and for each insert 2×10^5 cells in DMEM/BSA 0.1% were further incubated with β-D-xyloside for a 24 h invasion assay. After 24 h, cells that have migrated through the filter pores were fixed with methanol, stained with hematoxylin and counted. In some experiments, cells were treated with β-D-xyloside (for 48 h, then incubated with heparin (100 ng/ml, 1 μg/ml, 10 μg/ml, 100 μg/ml) for 16 h and allowed to invade towards SDF-1/CXCL12 through Matrigel.

The percentage of inhibition was $[(D1-D2)/D1]\times100$; D1 was the difference between the number of untreated cells that migrated toward SDF-1/CXCL12 and that of untreated cells that migrated toward the culture medium without SDF-1/CXCL12; D2 was the difference between the number of treated cells that migrated toward SDF-1/CXCL12 and that of treated cells that migrated toward culture medium.

In some case, SDF-1/CXCL12 was pre-incubated for 2 h at 20 °C with heparin (low molecular weight H3149 from Sigma-Aldrich, 100 μg/ml). Heparin alone or SDF-1/CXCL12 pre-incubated with heparin was added in the lower chamber of culture. The percentage of inhibition was $[(D1-D3)/D1]\times100$ where D3 was the difference between the number of cells that migrated toward SDF-1/CXCL12 pre-incubated with heparin and the number of cells that migrated toward heparin alone.

2.7. RNA interference

SDC-4 gene-specific sense and antisense 21 nt single-stranded RNAs with symmetric 2 nt 3′(2′-deoxy) thymidine overhangs were designed as described [16], chemically synthesized and HPLC purified (Eurogentec, Seraing, Belgium). RNA sequences corresponding to SDC-4 double-stranded RNA (SDC-4 dsRNA) were: sense 3′-GUU GUC CAU CCC UUG GUG CdTdT-5′; antisense 5′-GCA CCA AGG GAU GGA CAA CdTdT dTdT-3′. For RNA interference experiments, dsRNAs were generated by mixing equimolar amounts (50 μM) of sense and antisense single-stranded RNAs in annealing buffer (50 mM Tris, pH 7.5–8.0, 100 mM NaCl in DEPC-treated water) for 1 min at 94 °C, following by 60 min incubation at room temperature. HeLa cells were transfected with 320 nM dsRNA in serum-free medium using Jetsi transfectant reagent (Eurogentec) following the manufacturer's instructions. Mock cells were cultured in parallel and transfected with the transfection mixture lacking dsRNA. Cells transfected were used 3 days after transfection. In each experiment a negative control snc-RNA (Eurogentec) was used [11].

2.8. Statistical analysis

For the determination of statistical significance, ANOVA test was done with the Statview software. $P<0.05$ was used as the criteria of statistical significance.