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Supplementary Materials for

Heparan sulfate differentially controls CXCL12α- and CXCL12γ-mediated cell migration through differential presentation to their receptor CXCR4

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Methods

Synthesis and characterization of CXCR4 N-terminal and CXCL12γ C-terminal peptides

The peptides (fig. S2, A to C) were synthesized by the Merrifield solid-phase method on an Applied 433 peptide synthesizer using fluorenylmethyloxycarbonyl (Fmoc) chemistry. The CXCR4 N-terminal peptides (amino acid residues 1 to 29; fig. S2, A and B) were synthesized on a H-Phe-2-ClTrt-PS-resin (Rapp Polymere). Fmoc-Tyr-(SO3.NnBu4)-OH (Novabiochem) was used to introduce sulfotyrosines at positions 7, 12, and 21 of the sulfated peptide. Stepwise elongation of the peptide chain was performed with 10 equivalents of Fmoc amino acids using HATU activation. Peptides were released from the resin by treatment with trifluoroacetic acid (TFA):triisopropylsilane (TIS):H_2O (95:2.5:2.5) for 90 min at room temperature for the nonsulfated peptideand for 30 min at 4°C in an ice bath followed by a 1-hour return to room temperature for the sulfated peptide. The crude peptides were isolated by cold diethyl ether precipitation and rapidly dissolved in 100 mM ammonium hydrogen carbonate buffer. Peptides were purified by preparative C18 RP-HPLC with100 mM triethylamine acetate buffer/acetonitrile gradients, and final purity was controlled by analytical C18 RP-HPLC.

The CXCL12γ C-terminal peptide (amino acid residues 69 to 98; fig. S2C) was synthesized on a Fmoc-Asn(Tri)-Wang resin with chain elongation as described earlier. An extra cysteine was introduced at the Nterminus to introduce a biotin to the purified peptide throughan EZ-link-maleimide-PEG2-Biotin reagent (Pierce 21901). The peptide was released from the resin by treatment with TFA:TIS:H_2O:EDT (94.5:0.5:2.5:2.5) for 4 hours at room temperature. The crude peptide, isolated after precipitation with cold diethyl ether, was purified by a C18 RP-HPLC withan 0.08% aqueous TFA/acetonitrile gradient.

Isolated peptides were carefully quantified by amino acid analysis on a Hitachi L-8800 amino acid analyzer after 20 hours of hydrolysis in 6 N HCl, 0.2% phenol in the presence of a known amount of norleucine as an internal standard. The molecular masses of the CXCR4 N-terminal peptides were measured by ion-spray mass spectrometry (Waters Q-Toff Micro, negative mode) with cone and collision voltages set to 5V to minimize sulfate loss. The N-ter Biot-PEG2-CXCL12γ C-terminal peptide was analyzed withthe positive mode (table S1).

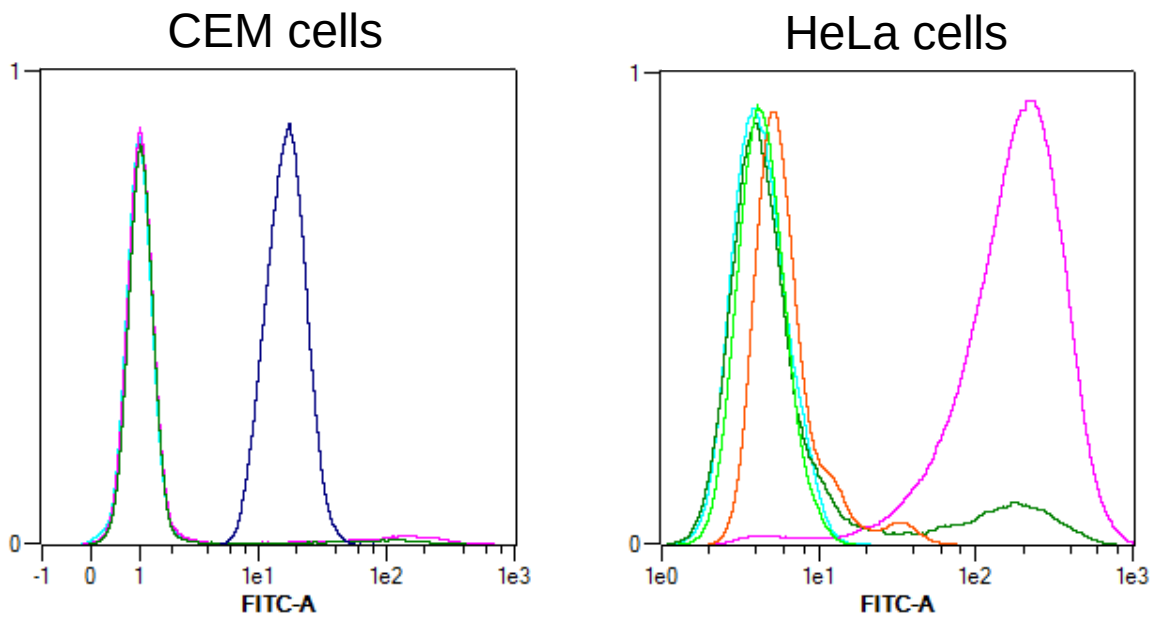

Fig. S1. Analysis of CXCR4 and GAG abundances on the surface of CEM cells. Cells were left untreated or were incubated with a mixtureof heparinase 1 and heparinase 3 (each at 100 mU/ml) and chondroitinase ABC (1 U/ml) for 90 min at 37°C.The abundanceof GAGs on the cell surface wasquantified by flow cytometric analysis with antibodies specific forHS (clone 10E4; 10 µg/ml) or chondroitin sulfate (CS, clone CS-56; 1:100 dilution). Because CEM cells naturally have little or no surface GAGs, the activities of the GAG lyases were analyzed in parallel underthe same conditions with HeLa cells (right), which synthesize HS and CS. The presence of CXCR4 on the surface of CEM cells was also assessed by flow cytometric analysis with 12G5 (5 µg/ml), an antibody that binds to aconformation-dependent extracellular epitope of CXCR4. Data are representative of three experiments.

A MEGISIYTSDNYTEEMGSGDYDSMKEPAF

B MEGISIY$_{SO3}$TSDNY$_{SO3}$TEEMGSGDY$_{SO3}$DSMKEPAF

C Biotin-PEG2-CGRREEKVGKKEKIGKKKRQKKRKAAQKRKN

Fig. S2. Sequences of the CXCR4 N-terminal and CXCL12γ C-terminal peptides. (**A**) Sequence of the CXCR4 N-terminal peptide. (**B**) Sequence of the CXCR4 N-terminal peptide containingsulfotyrosine residues at positions 7, 12, and 21 (red). (**C**) Sequence of the CXCL12γ C-terminal peptide. The extra cysteineresidue towhich a biotin group was coupled is shown in red.

Table S1. Mass spectrometric analysis of the CXCR4 N-terminal and CXCL12γ C-terminal peptides. The table shows the chemical formula of each peptide forwhich the expected molecular mass (Expected MM) was calculated and the actual molecular mass (Found MM) wasdetermined by ion-spray MS analysis. Molecular masses are given in Daltons.

Peptide	**Chemical formula**	**Expected MM**	**Found MM**
N-ter CXCR4	$C_{139}H_{203}N_{31}O_{54}S_3$	3268.5154	3268.1011
N-ter CXCR4 sulfated	$C_{139}H_{203}N_{31}O_{63}S_6$	3508.7080	3508.8533
N-ter Biot-PEG2-(C-ter CXCL12γ)	$C_{180}H_{328}N_{66}O_{48}S_2$	4249.1324	4249.5537