Synthesis of heparan sulfate with cyclophilin B-binding properties is determined by cell-type specific expression of sulfotransferases

Audrey Deligny[1], Agnès Denys[1], Adeline Marcant[1], Aurélie Melchior[1], Joël Mazurier[1], Toin H. van Kuppevelt[2], and Fabrice Allain[1]

From the [1]Unité de Glycobiologie Structurale et Fonctionnelle, Unité Mixte de Recherche No. 8576 du CNRS, Institut de Recherche Fédératif No. 147, Université des Sciences et Technologies de Lille, Villeneuve d'Ascq, France, and the [2]Department of Matrix Biochemistry, Nijmegen Center for Molecular Life Sciences, Nijmegen, The Netherlands.

SUPPLEMENTAL DATA

TABLE I
Characteristics of specific anti-HS antibodies

Antibody	V_H CDR3 sequence	V_H family	DP gene	Class of GAG	Modifications involved in binding	Modification which may reduce binding	References
HS4C3	GRRLKD	3	38	HS	*N*-sulfation 2-*O*-sulfation 6-*O*-sulfation 3-*O*-sulfation		(30,33,34)
HS4E4	HAPLRNTRTNT	3	38	HS	*N*-acetylation *N*-sulfation C5-epimerization	2-*O*-sulfation[a] 6-*O*-sulfation	(31,33)
AO4B08	SLRMNGWRAHQ	3	47	HS	*N*-sulfation C5-epimerization 2-*O*-sulfation[b] 6-*O*-sulfation		(31,33)
RB4Ea12	RRYALDY	3	32	HS	*N*-acetylation *N*-sulfation 6-*O*-sulfation		(31,33)
IO3H10	AKRLDW	1	7	CS			(32,33)
MPB49	WRNDRQ	3	38	- (control)	-		

Given are the antibody name, amino acid sequence of the V_H complementary determining region 3 (CDR3), V_H germ line gene family, DP gene number, the class of GAG with which the antibody reacts, and essential/inhibitory modifications required for antibody binding. [a]Antibody HS4E4 requires essential but unspecified *O*-sulfated residues. [b]Antibody AO4B08 requires an internal 2-*O*-sulfated IdoUA residue.
HS, heparan sulfate; CS, chondroitin sulfate.

TABLE II
Primers used for real-time RT-PCR analysis

Gene	RefSeq	PCR primer sequences	Product size (bp)
NDST1	NM_001543	Forward: 5'-ACCACAGCCAGACTGAACGTTTGTG-3' Reverse: 5'-ACAGGGAAATGTCCAGTCTGTCTCC-3'	443
NDST2	NM_003635	Forward: 5'-TTCCAGTCTGGGCTGATGTCCCAGC-3' Reverse: 5'-GTAGATCCCACTGCCTGGCTAAAGG-3'	426
2-OST	NM_012262	Forward: 5'-CGAAGTCCGAGAAATTGAGC-3' Reverse: 5'-AATGAAGTGCTTGCCGTTTT-3'	123
3-OST1	NM_005114	Forward: 5'-TTATCTCCTCGGCGATTCCTTG-3' Reverse: 5'-TAGCCAGTCACTAACTGCTTCTCCAT-3'	117
3-OST3A 3-OST3B	NM_006042 NM_006041	Forward: 5'-CCATCCAGATCGGCATCTACGC-3' Reverse: 5'-TGCTTGTCCGTGATGATCCTCTTG-3'	166
3-OST5	NM_153612	Forward: 5'-TGGGAGCTTGGATAGGCTACA-3' Reverse: 5'-TGGAGGCGAACCTGCTCC-3'	159
HPRT	NM_000194	Forward: 5'-GACCAGTCAACAGGGGACAT-3' Reverse: 5'-AACACTTCGTGGGGTCCTTTTC-3'	195

TABLE III
Synthetic siRNA used for RNA interference experiments

Name	Target mRNA Sequence	Position
siNDST1	5'-CCUCCGACUUCUACUUUGA(dTdT)-3'	2508-2526
siNDST2	5'-GGACCUUAGUUCCCAACUU(dTdT)-3'	1591-1609
si2-OST	5'-GCGCUUUGUAAAGAAUAUA(dTdT)-3'	749-767
si3-OST3	5'-CGGACAAGCACUUCUACUU(dTdT)-3'	1808-1826 (3A) 1295-1313 (3B)
siGFP	5′-GAACGGCAUCAAGGUGAACTT(dTdT)-3	

Negative controls with two-bases changes were designed from the sequences of specific siRNA. Modified nucleotides are underlined in target mRNA sequences. Changes were as follows: U → A and A → U.

LEGEND TO SUPPLEMENTAL FIGURES

FIGURE S1. **Inhibition of the expression of mRNAs encoding NDST1, NDST2, 2-OST and 3-OST3 by RNA interference.** Synthetic siRNAs (siNDST1, siNDST2, si2-OST, si3-OST3) were used to specifically inhibit the expression of mRNAs encoding NDST1, NDST2, 2-OST and 3-OST3 in Jurkat T cells. Negative control siRNAs, in which two nucleotides have been changed from the target sequences, were used to demonstrate the specificity of silencing. A synthetic siRNA targeting GFP transcript (siGFP) was used as irrelevant siRNA. (**A**) The expression of mRNAs encoding each sulfotransferases was quantified at 24 h, 48 h and 72 h in treated Jurkat T cells by real-time RT-PCR. HPRT mRNA was used to normalize the expression of the genes of interest. (**B**) In order to check for the absence of any cross-reactivity, the levels of mRNA encoding NDST1, NDST2, 2-OST and 3-OST3 were quantified after 48 h of cell treatment with each specific siRNA. Results are presented as percentages of mRNA expression of HS sulfotransferases in cells treated with specific or negative control siRNAs relative to the maximal values obtained with irrelevant siRNA, which were set at 100 %. Each bar of the histograms represents mean ± SD of triplicates from three separate experiments.

FIGURE S2. **Effect of siRNA targeting HS sulfotransferases on the pattern of sulfation of cell surface HS.** The effect of siRNA treatment on cell surface HS was analyzed by measuring the reactivity of anti-HS antibodies. Jurkat T cells were treated with siRNA that specifically target NDST1, NDST2, 2-OST and 3-OST3 or respective negative control siRNA. A synthetic siRNA targeting GFP transcript (siGFP) was used as irrelevant siRNA. After 48 h of treatment, cells were stained for HS with HS4E4, HS4C3, RB4Ea12 and AO4B08, or for chondroitin sulfate with IO3H10. For rescue experiments, cells were first treated with specific siRNA or corresponding negative control for 24 h. Thereafter, cells were transfected with plasmids expressing sequences refractory to siRNA and stained 24 h later with anti-HS antibodies. Cell surface staining was analyzed by flow cytofluorimetry. Data are presented as ΔFMV % and are means ± SD of three separate experiments.

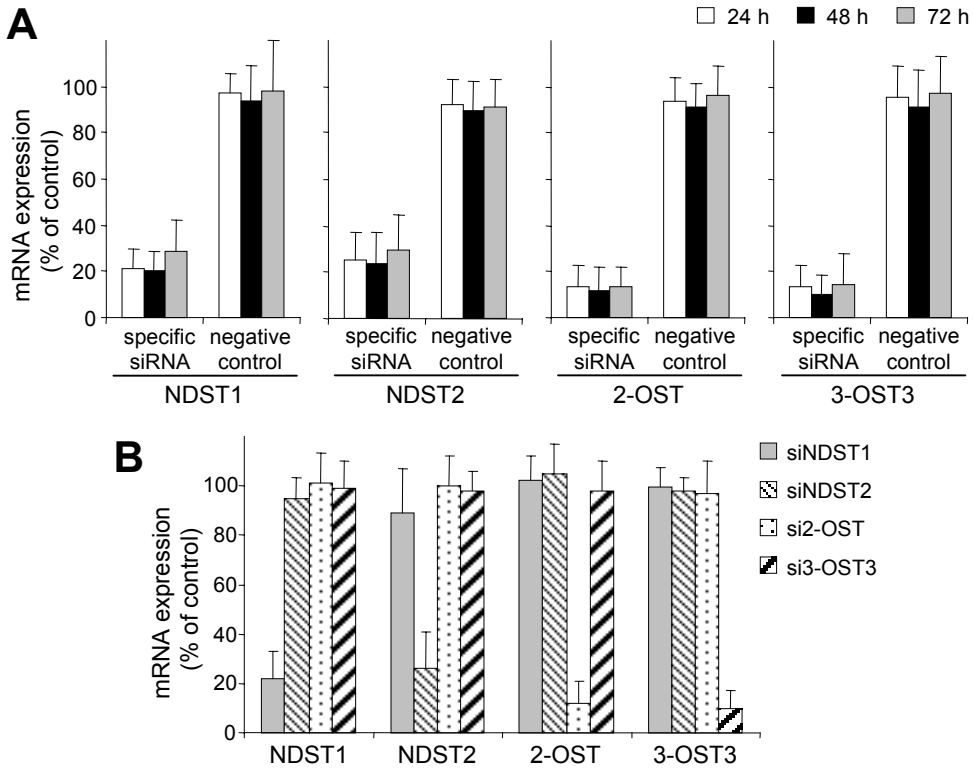
A
24 h
48 h
72 h
mRNA expression (% of control)
0
20
40
60
80
100
specific siRNA
negative control
NDST1
NDST2
2-OST
3-OST3
B
siNDST1
siNDST2
si2-OST
si3-OST3
mRNA expression (% of control)
0
20
40
60
80
100
NDST1
NDST2
2-OST
3-OST3

Figure S1

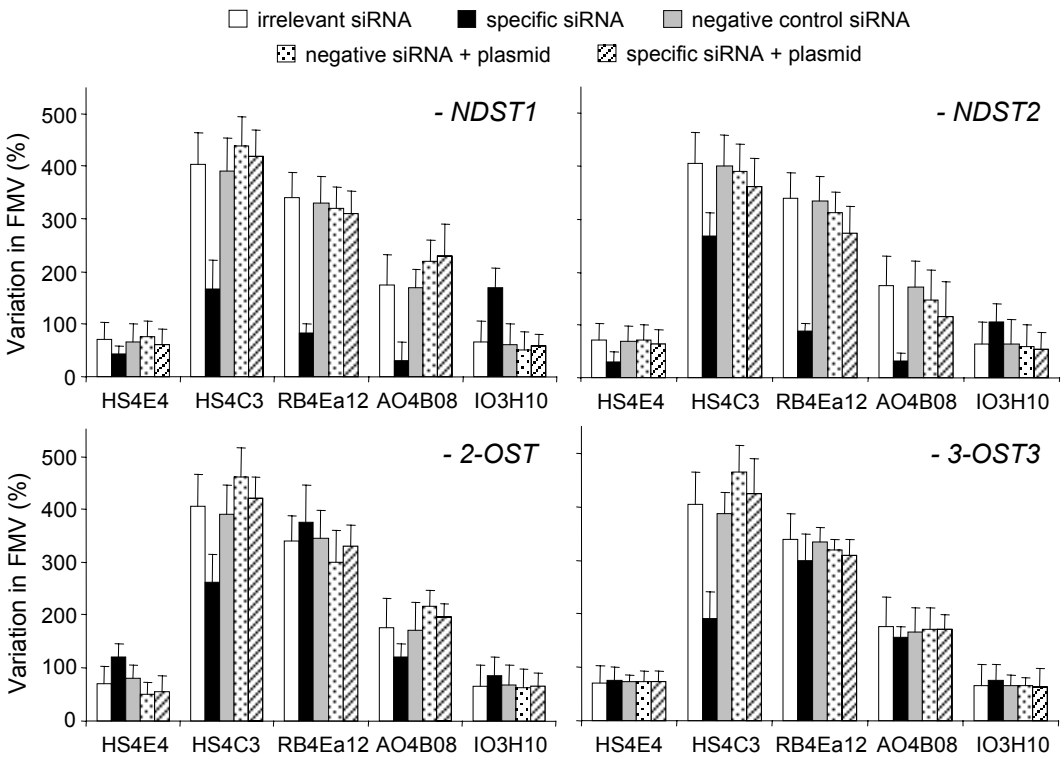
irrelevant siRNA
specific siRNA
negative control siRNA
negative siRNA + plasmid
specific siRNA + plasmid
- NDST1
- NDST2
- 2-OST
- 3-OST3
Variation in FMV (%)
0
100
200
300
400
500
HS4E4
HS4C3
RB4Ea12
AO4B08
IO3H10

Figure S2