Heparan-Sulfate 6-*O*-Sulfotransferase 1-3 (HS6ST1-3)

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Naoko Nagai and Koji Kimata

Contents

Introduction

The functions of heparan sulfate (HS) are involved in various cellular processes such as proliferation, differentiation, adhesion, migration, morphology, and maintenance of stem cells (Habuchi et al. 2004; Bishop et al. 2007; Lindahl and Li 2009; Buresh et al. 2010; Shah et al. 2011; Buresh-Stiemke et al. 2012). It has been shown that HS plays some roles in various physiological phenomena such as inflammation,

N. Nagai (✉)
Institute for Molecular Science of Medicine, Aichi Medical University, Nagakute, Aichi, Japan
e-mail: nnaoko@aichi-med-u.ac.jp

K. Kimata
Advanced Medical Research Center, Aichi Medical University, Nagakute, Aichi, Japan
e-mail: kimata@aichi-med-u.ac.jp

N. Taniguchi et al. (eds.), *Handbook of Glycosyltransferases and Related Genes*,
DOI 10.1007/978-4-431-54240-7_68,

blood coagulation, tumor cell invasion, and malignancy. Moreover, infections of host cells with pathogens such as viruses, bacteria, and parasites have been shown to occur through the interactions with cell surface HS on host cells (Mettenleiter et al. 1990; Trybala et al. 1996; Liu and Thorp 2002). 6-*O*-Sulfate residues in HS are greatly involved in the above various biological and pathological processes primarily by modulating various signal transduction pathways such as fibroblast growth factors (FGFs), vascular endothelial growth factors (VEGFs), Wnts, and hedgehog. HS 6-*O*-sulfotransferases (HS6STs) transfer sulfate to position 6 of the *N*-sulfoglucosamine/*N*-acetylglucosamine residue in heparin/HS. Three isoforms are identified in mice and humans. All HS6STs are type II transmembrane proteins with short cytoplasmic domain at the N-terminus, followed by transmembrane and luminal domain which have 3′-phosphoadenosine 5′-phosphosulfate (PAPS) binding site and sulfotransferase activity localized at the Golgi apparatus.

Databanks

NC-IUBMB number: EC 2.8.2.

Brookhaven Protein Data Bank accession number: not available

Sequence accession numbers/codes for DNA and amino acid sequences, with binomial names of organisms are listed below.

Heparan-sulfate 6-*O*-sulfotransferase 1-3 (HS6ST1-3)

Species	Gene symbol	Gene symbol	NCBI reference number (protein)	UniprotKB ID
HS6ST1				
Homo sapiens (human)	*HS6ST1*	NM_004807.2	NP_004798.3	O60243
Mus musculus (mouse)	*Hs6st1*	NM_015818.2	NP_056633.2	Q9QYK5
Rattus norvegicus (rat)	*Hs6st1*	NM_001108210.1	NP_001101680.1	D4A6E6
Gallus gallus (chicken)	*HS6ST1*	NM_204482.1	NP_989813.1	Q76KB2
Danio rerio (zebrafish)	*hs6st1a*	NM_001020508.2	NP_001018344.2	Q56UJ5
HS6ST2				
Homo sapiens (human)	*HS6ST2*	NM_001077188.1	NP_001070656.1	Q96MM7
		NM_147175.3	NP_671704.3	
Mus musculus (mouse)	*Hs6st2*	NM_001077202.1	NP_001070670.1	Q80UW0
		NM_015819.3	NP_056634.3	
Rattus norvegicus (rat)	*Hs6st2*	NM_001191726.1	NP_001178655.1	D3ZEK5
				D3ZBS5
Gallus gallus (chicken)	*HS6ST2*	NM_204490.1	NP_989821.1	Q76LW2
Danio rerio (zebrafish)	*hs6st2*	NM_194421.1	NP_919402.1	Q800H9
Q800H9				
Homo sapiens (human)	*HS6ST3*	NM_153456.3	NP_703157.2	Q8IZP7
Mus musculus (mouse)	*Hs6st3*	NM_015820.3	NP_056635.2	Q9QYK4

(*continued*)

Species	Gene symbol	Gene symbol	NCBI reference number (protein)	UniprotKB ID
Rattus norvegicus (rat)	*Hs6st3*	NM_001271404.1	NP_001258333.1	
Gallus gallus (chicken)	*HS6ST3*	XM_003640546.1	XP_003640594.1	F1NDE4
Danio rerio (zebrafish)	*hs6st3b*	NM_001080194.1	NP_001073663.1	A0MGZ7
HS6ST				
Drosophila melanogaster (fruit fly)	*Hs6st*	NM_079685.2	NP_524409.2	Q9VDR6
Caenorhabditis elegans (nematode)	*hst-6*	NM_076494.3	NP_508895.2	G5EFN7

Name and History

Name: Heparan sulfate 6-*O*-sulfotransferase

HS6STs transfer sulfate to position 6 of the *N*-sulfoglucosamine/*N*-acetylglucosamine residue in heparin/HS. Habuchi et al. found that heparan sulfate 6-*O*-sulfotransferase protein was secreted into the serum-free culture medium of CHO cells and purified the enzyme to an apparent homogeneity (Habuchi et al. 1995). The purified enzyme transferred sulfate to position 6 of the *N*-sulfoglucosamine residue when completely desulfated and *N*-resulfated heparin (CDSNS-heparin) was used as acceptor, but did not transfer sulfate to the amino group of glucosamine residue or to position 2 of the iduronic acid residue. HS also was served as an acceptor; however, chondroitin and chondroitin sulfate were not. The optimal pH of the enzyme was around 6.3. The enzyme was inhibited by dithiothreitol and stimulated strongly by protamine. From the partial amino acid sequence data of the purified enzyme, the cDNA was isolated by the reverse transcriptase-polymerase chain reaction followed by the screening of a cDNA library of CHO cells and the human fetal brain cDNA library by cross-hybridization (Habuchi et al. 1998). The isolated cDNA clones had a whole open reading frame that predicts a type II transmembrane protein composed of 401 amino acid residues. Then the new mouse and human isoforms of HS6ST, HS6ST2, which is a homologue to the original human HS6ST (HS6ST1) and subsequently the third HS6ST (HS6ST3), were cloned (Habuchi et al. 2000).

Structure

All three HS6STs are predicted to have type II transmembrane protein topology with short cytoplasmic domain at the N-terminus, followed by transmembrane and luminal domain which have PAPS binding site and sulfotransferase activity localized at the Golgi apparatus (Nagai et al. 2004; Kotani et al. 2005).

Enzyme Activity Assay and Substrate Specificity

HS6STs transfer sulfate to position 6 of the *N*-sulfoglucosamine residue in heparan sulfate but not to the *N*-acetylglucosamine residues.

$$\mathrm{nPAPS} + (\mathrm{IdoA}\alpha 1-4\mathrm{GlcNSO_3}\alpha 1-4)_n \rightarrow \mathrm{nPAP} + [\mathrm{IdoA}\alpha 1-4\mathrm{GlcNSO_3(6SO_4)}\alpha 1-4]_n$$

where PAPS is 3′-phosphoadenosine 5′-phosphosulfate, and PAP is 3′-phosphoadenosine 5′-phosphate.

The sulfotransferase activities were determined as follows: The standard reaction mixture (50 μL) contained 2.5 μmol of imidazole-HCl (pH 6.8), 3.75 μg of protamine chloride, 25 nmol (as hexosamine) of acceptor GAGs, 50 pmol of [^{35}S] PAPS (approx. 5×10^5 c.p.m) and enzyme. After incubation for 20 min at 37 °C, the reaction was stopped by heating at 100 °C for 1 min. Chondroitin sulfate A (0.1 μmol as GlcA) was added to the reaction mixture as a carrier, and the ^{35}S-labelled polysaccharides were precipitated with ethanol containing 1.3 % (w/v) potassium acetate and 0.5 mM EDTA, followed by gel chromatography on a fast desalting column to remove [^{35}S]PAPS and its degradation products. The radioactivity incorporated to high molecular weight fractions was counted by scintillation counter. The amounts of enzymes added to the reaction mixture were chosen so as to obtain a linear incorporation of [^{35}S] sulfate. The amount of enzyme activity required to transfer 1 pmol of sulfate/min to CDSNS-heparin was defined as one unit.

The HS6ST1 activity was also detected in mouse and human serum (Habuchi et al. 1998). All HS6STs transferred sulfate to CDSNS-heparin, *N*-sulfated heparosan, heparin, and heparan sulfate. Almost no activity was observed toward completely desulfated and *N*-acetylated (CDSNAc) heparin, suggesting that *N*-desulfated heparin is a poor acceptor. None of the isoforms showed significant activity toward chondroitin, any types of chondroitin sulfate, and keratan sulfate. Each isoform showed a different specificity toward the isomeric hexuronic acid adjacent to the targeted *N*-sulfated glucosamine; HS6ST1 appeared to prefer iduronic acid residues adjacent to the *N*-sulfoglucosamine, whereas HS6ST2 had a different preference, depending on the substrate concentrations (it preferred the glucuronic acid residue at high concentrations but the iduronic acid residue at low concentrations). The preference of HS6ST-3 was intermediate between those of HS6ST1 and HS6ST2. These three isoforms could also transfer sulfate to position 6 of *N*-sulfoglucosamine residue adjacent to the reducing side of iduronic acid 2-sulfate. Besides, there are two forms of HS6ST2, the original form and a short form with 40 amino acids deleted (HS6ST2-S) (Habuchi et al. 2003). HS6ST2-S also transferred the sulfate groups, but their preferences for sulfation sites in HS substrates were different. HS6ST-2 appeared to have a preference to transfer sulfate to position 6 of $GlcNSO_3$ residues in the IdoA($2SO_4$)-$GlcNSO_3$ unit. HS6ST-2S transferred sulfate groups to position 6 of $GlcNSO_3$ residues in the GlcA-$GlcNO_3$ in addition to IdoA($2SO_4$)-$GlcNSO_3$.

Preparation

Habuchi et al. purified HS6ST1 from the serum-free cultured medium of CHO cells as follows (Habuchi et al. 1995). All purification steps were performed at 4 °C. First, 0.1 volume (2 mL) of the medium fraction was applied to a column of heparin-sepharose CL-6B (20 × 65 mm, 20 mL) equilibrated with 0.15 M NaCl in buffer A (10 mM Tris-HCl, pH 7.2, 0.1 % (w/v) Triton X-100, 10 mM $MgCl_2$, 2 mM $CaCl_2$, 20 % (v/v) glycerol) at the flow rate of 70 mL/h. The column was washed with 10 column volumes (200 mL) of buffer A and then eluted with five column volumes (100 mL) of buffer A-1.0 M NaCl. The chromatography was repeated ten times. All eluates were combined, concentrated to 100 mL with polyethylene glycol 20,000 (molecular weight 15,000–25,000), and dialyzed against buffer A-0.05 M NaCl. Next, the 3′, 5′-ADP-agarose (14 × 90 mm, 1.9 μmol of 3′,5′-ADP/mL of gel) column was equilibrated with buffer A-0.05 M NaCl. Half of the fraction (50 mL) was applied at a flow rate of 13 mL/h, and the column was washed with 120 mL of buffer A-0.05 M NaCl. The sulfotransferase activity was eluted with a linear gradient of 0–0.2 mM 3′, 5′-ADP in buffer A-0.05 M NaCl of total volume (150 mL). The fractions containing sulfotransferase activity were pooled. The chromatography was performed twice, and the fractions containing the activity were combined. Finally, the heparin-sepharose CL-6B column (16 × 35 mm, 5 mL) was equilibrated with buffer A-0.15 M NaCl. The fraction was applied at the flow rate of 13 mL/h, and the column was washed with 50 mL of buffer A-0.25 M NaCl. The sulfotransferase activity was eluted with a linear gradient from 0.25 to 1.2 M NaCl in buffer A (total volume, 150 mL). The fractions containing sulfotransferase activity were pooled, dialyzed against buffer A-0.15 M NaCl, and stored at −20 °C.

Biological Aspects

Expression Pattern

From the EST profile database, mouse *Hs6st1* is expressed rather ubiquitously; *Hs6st2* primarily in connective tissue, heart, and spleen; and *Hs6st3* slightly in brain and eye. Human *HS6ST1* is also ubiquitously expressed with especially high expression observed in salivary gland. *HS6ST2* is expressed primarily in bladder, brain, testis, and thyroid and *HS6ST3* slightly in brain, connective tissue, and kidney. These results suggest that the expression of these isoforms may be regulated in tissue-specific manners and that each isoform may be involved in the synthesis of HS with tissue-specific structures and functions (Habuchi et al. 2000; Sedita et al. 2004; Cadwallader and Yost 2006; Buresh-Stiemke et al. 2012).

Signal Transduction

HS are important modulators for optimizing signal transduction of many pathways (Ebner et al. 2002; Kamimura et al. 2006; Lindahl and Li 2009; Kleinschmit et al. 2010; Qu et al. 2011; Kamimura et al. 2011; Song et al. 2011; Townley and Bulow 2011; Tran et al. 2012). The expression patterns of chick *HS6ST1* transcripts were preferentially localized at the anterior proximal region, while *HS6ST2* transcripts were localized mainly in the posterior proximal region and weakly in the anterior region (Nogami et al. 2004). Kobayashi et al. showed that the disruption of chick *HS6ST1* and *HS6ST2* in the prospective limb region by short hairpin RNA resulted in different decreasing patterns of 6-*O*-sulfation in HS in chick limb buds (Kobayashi et al. 2010). They performed an immunostaining with 10E4 antibody that specifically recognizes *N*-sulfated and 6-*O*-sulfated HS to show the level of 6-*O*-sulfation in the limb bud.10E4 staining was not observed in the anterior part of the mesenchyme in *HS6ST1* RNAi-treated limbs and decreased both in the anterior and posterior part of the limbs treated with *HS6ST2* RNAi. Staining with anti-HS antibody 3G10 that recognizes the HS stub regions generated by heparitinase digestion was not influenced by *HS6ST1* nor *HS6ST2* RNAi treatments, suggesting that HS polysaccharide synthesis was not affected. The expression levels of *Fgf-8* and *Shh* in *HS6ST1* RNAi limb buds were significantly decreased compared with control limb buds. The expression of *Fgf-8* decreased more in the anterior region where *HS6ST1* expression was preferentially observed. Significant decrease of *Fgf-10* expression in the mesoderm and concomitant upregulation of *Fgf-8* in the AER in the anterior region was observed in *HS6ST1* RNAi limb buds. In contrast, dramatic decrease of *Fgf-8*, *Shh*, and *Fgf-10* expression was observed in *HS6ST2* RNAi limb buds. When the *HS6ST1*-RNAi vectors were injected in the anterior or middle but not the posterior regions of the developing limb buds, the truncation was observed at a high frequency. Some truncation was also observed in limb buds at similar frequency when the *HS6ST2*-RNAi vector was injected in the anterior, middle, and posterior regions. The results suggest that *Fgf-8*, *Shh*, and *Fgf-10* expressions and signalings are regulated by 6-*O*-sulfation of HS regionally in chick limb buds, and thus, the 6-*O*-sulfation affects the limb pattern formation.

Chen et al. reported that *hs6st2* was expressed in the region around the caudal vein in zebrafish *Danio rerio* embryos, and the morpholino *hs6st2* antisense knockdown exhibited abnormalities in the branching morphogenesis of the caudal vein during embryonic development (Chen et al. 2005). In contrast, no vascular defects were observed in embryos injected with the *hs6st1* morpholino. Co-injection of morpholinos against *hs6st2* and *vegf-a* into embryos resulted in synergistic increase in the frequency of caudal vein branching defects, suggesting that Hs6st2 and Vegf-a interact in vivo during caudal vein formation. Bink et al. showed that *hs6st* morpholino-injected embryos displayed the phenotype of convergent-extension defects, which was reminiscent of *knypek* (*kny*)/*glypican* zebrafish mutants (Bink et al. 2003). Somite development was most affected in *hs6st* morphant embryos. In weakly affected embryos, muscle cells spanned across the somitic boundary. In more severe cases, breakdown of intersomitic boundaries was

observed. Expression of myoD, a bHLH transcription factor involved in muscle differentiation, was downregulated in wild-type embryos. However, in *hs6st* morphants, *myoD* expression was maintained at high level suggesting that 6-*O*-sulfate residues of HS may be involved in the muscle cell differentiation.

RNAi of Drosophila *Hs6st* gene produced defective branching in the tracheal system, a developmental system that shares many of the signaling components required for vertebrate angiogenesis (Kamimura et al. 2001). Highly specific expression of d*Hs6st* mRNA was detected in tracheal precursor cells, and its expression pattern was similar to that of *breathless* (*btl*), a Drosophila FGF receptor. 94 % of the d*Hs6st* dsRNA-injected embryos survived to stage 17, the final stage of embryogenesis, and died prior to the point immediately before hatching. They showed that tracheal branch formation was disordered in d*Hs6st* dsRNA-injected embryos without affecting tracheal cell differentiation. In d*Hs6st* dsRNA-injected embryos, downstream of FGF signaling MAPK was not activated, indicating that 6-*O*-sulfation of HS was required for FGF signaling in Drosophila during tracheal development.

Knockout Mice and Transgenic Mice

Disruption of the *Hs6st1* gene (Habuchi et al. 2007; Izvolsky et al. 2008; Habuchi and Kimata 2010) resulted in growth retardation and developmental abnormalities, with most embryos dying between the E15.5 and perinatal stages. Compared with their wild-type littermates, the *Hs6st1* knockout mice littermates showed an ~50 % reduction in the number of fetal microvessels in the labyrinthine zone of the placenta. VEGF-A mRNA and protein level was reduced to ~60 and 75 % in the placenta of *Hs6st1* knockout embryos. Disaccharide analysis showed a marked reduction of GlcNAc($6SO_4$) and HexA-GlcNSO_3($6SO_4$) levels in *Hs6st1* knockout embryos. Wnt-2 showed a decreased affinity to HS isolated from the knockout embryonic placenta. Alveolar enlargement was seen in the knockout mice, and when intra-alveolar distance was measured, mean linear intercept of the knockout mice was significantly increased, compared with the heterozygous mice (40 ± 5 versus 30 ± 4 μm, 7-day-old and 42 ± 6 versus 29 ± 4 μm, adult), indicating impaired alveolarization.

Recent studies indicate the important roles for HS in axon targeting (Irie et al. 2002; Conway et al. 2011). Different axon navigation phenotypes were observed in two HS sulfotransferase mutant mouse embryos, $Hs2st^{-/-}$ and $Hs6st1^{-/-}$. $Hs2st^{-/-}$ embryos displayed axon disorganization at the chiasm, with axons growing up the ventral midline (Pratt et al. 2006). Large number of retinal ganglion cell (RGC) axons in $Hs6st1^{-/-}$ embryos grew up the contralateral optic nerve and innervated the opposite eye, which resembled the phenotype of $Slit1^{-/-}$; $Slit2^{-/-}$ embryos (Plump et al. 2002). Quantitative RT-PCR analysis showed that both *Hs2st* and *Hs6st1* were expressed in the optic chiasm. High levels of *Hs6st1* expression were seen at the point at which RGC axons made errors. Retinal explants from wild-type and $Hs6st1^{-/-}$ knockout mice were cultured in collagen gels on a bed of a collagen

gel containing Slit2 expressing COS7 cells. RGC axons from wild-type explants showed a significant lower contact to COS7 cells expressing Slit2. In contrast, *Hs6st1*$^{-/-}$ RGC axons showed no difference in contact to COS7 cells whether they were expressing Slit2 or not. Thus, the ability of RGC growth cones to avoid Slit2 is HS6ST1 dependent.

Sugaya et al. examined the effects of HS 6-*O*-sulfation in growth factor signaling using fibroblasts derived from *Hs6st1*- and/or *Hs6st2*-deficient mice (6ST1-KO, 6ST2-KO, and double knockout (dKO)) (Sugaya et al. 2008). HS compositional analysis from mouse embryonic fibroblasts (MEF) prepared from E14.5 dKO mice showed that GlcNAc6SO_4 residue, HexA-GlcNSO_3(6SO_4) unit, and HexA(2SO_4)-GlcNSO_3(6SO_4) unit were hardly detectable. In contrast, *2-O*-sulfation in HS was increased 1.9-fold. HS6ST activity in the dKO-MEF was hardly detected, and HS2ST activity was 1.6-fold higher than that in wild-type MEFs. Neither the chain length nor the amount and composition of chondroitin sulfate (CS) were altered in dKO-MEFs. Although the expression levels of mRNAs encoding FGF receptors were similar between WT- and dKO-MEFs, FGF-4- and FGF-2-dependent signalings in dKO-MEFs were reduced to ~30 and 60 % of WT-MEFs, respectively, and FGF-1-dependent signaling was moderately reduced, compared with that of WT-MEFs. Surface plasmon resonance biosensor analysis demonstrated that the apparent affinity of dKO-HS for FGF-4 was markedly reduced to one-fifth. The Kd value of FGF-1 affinity to HS isolated from dKO-MEFs was 2.8-fold higher than that of HS isolated from WT-MEFs. In contrast, the FGF-2 affinity to HS from dKO-MEFs was 2.5-fold higher than that of HS from WT-MEFs, probably reflecting the higher content of *2-O*-sulfate groups in HS from dKO-MEFs. Thus, 6-*O*-sulfate in HS may regulate the signalings of multiple FGFs by affecting the interactions between ligands and their receptors.

Human Disease

As many signaling systems utilize HS for their efficient signal transduction, it is convincing that HS is involved in various disease processes (Li and Rossman 2001; Backen et al. 2007; Kato et al. 2007; Labbe et al. 2007; Waaijer et al. 2012). Papers reporting that HS6ST and 6-*O*-sulfation of HS participate in the pathogenesis of some diseases are summarized below.

Tornberg et al. reported that missense mutations in *HS6ST1* were identified in families with idiopathic hypogonadotropic hypogonadism (IHH) with variable degrees of olfactory dysfunction and gonadotropin-releasing hormone (GnRH) deficiency (Tornberg et al. 2011). IHH is a condition characterized by failure to undergo puberty in the setting of low sex steroids and low gonadotropins as a result of defects in gonadotropin-releasing hormone neuron development or function. In Kallmann syndrome, anosmia (loss of the sense of smell) is associated with IHH. *HS6ST1* mutations associated with IHH exhibited reduced sulfotransferase activity in vitro and in vivo, suggesting that HS6ST1 and the 6-*O*-sulfations of HS are critical for IHH pathogenesis. KAL1 gene encodes anosmin-1 protein, and the

mutation is associated in some individuals with Kallmann syndrome. Mutations of KAL1 affected the normal development of the olfactory system and migration of the neurons secreting GnRH. Transgenic expression of *C. elegans kal1* in AIY interneurons elicited an axon branching phenotype, which was suppressed to near-background levels by loss-of-function mutations in *hst6*, the single *HS6ST* in *C. elegans*. The suppression of the axonal branching phenotype was rescued by transgenic expression of a wild-type *HS6ST1* cDNA. Expression of the mutant *HS6ST1* cDNAs identified in IHH showed reduced capacity for transgenic rescue of the *hst6* loss-of-function phenotype. *HS6ST1* mutations were found in patients who had IHH with wide spectrum of severity. Two patients had microphallus, one of whom also had unilateral cryptorchidism. Three patients presented with absent puberty, whereas three male patients showed some spontaneous testicular development. Some carriers of *HS6ST1* mutations did not always manifest IHH. Though the same genetic mutation was observed, there were clinical variability in patients both within and across families, indicating that *HS6ST1* mutations display incomplete penetrance and variable expressivity of the IHH phenotype. Additional genetic factors may contribute to the clinical variability. In fact, mutations were found in other IHH-associated genes, *FGFR1*, and *NELF* in two different families. Thus, other genetic lesions are likely involved in this digenic or oligogenic disease in addition to *HS6ST1* mutations. *HS6ST2* or *HS6ST3* gene may be the possible candidate.

Osteoarthritis (OA) is a disease characterized by degeneration of cartilage that leads to pain and joint stiffness. The specific causes of osteoarthritis are unknown, but age, obesity, injury and physical activity, and other factors are believed to be risk factors for OA. Kashin–Beck disease (KBD) is a chronic, endemic osteochondropathy, which is mainly distributed in China and other countries of Asia. Using suppression subtractive hybridization technique, Wang et al. screened the genes that were differentially expressed in OA cartilage and found that HS6ST2 expression was downregulated in OA and KBD at both mRNA and protein levels (Wang et al. 2011). C28/I2 chondrocytes were transfected with three different *HS6ST2* siRNA oligonucleotides. They found that in *HS6ST2*-siRNA-transfected cells, cell viability was significantly decreased at 48 h after transfection. The mRNA expression of *SOX9* and *AGC1* (encoding the core protein of aggrecan) was remarkably decreased in *HS6ST2*-siRNA-treated cells at 24 and 48 h after transfection, respectively. At 72 h after transfection, *HS6ST2*-RNAi-treated cells were less stained by toluidine blue, suggesting that the aggrecan protein level was lower than in control RNAi-treated cells. When the mRNA expression levels of *MMP3*, *ADAMTS4*, and *ADAMTS5*, which are relevant to the degradation of aggrecan in cartilage, were examined, *MMP3* expression was found to increase significantly at 24 h after *HS6ST2*-siRNA transfection, *ADAMTS5* expression was to decrease significantly at 48 h after transfection, and *ADAMTS4* expression was to show no significant difference both at 24 and 48 h after transfection. These findings suggest that HS6ST2 may participate in the pathogenesis of OA and KBD by affecting aggrecan metabolism.

Premature ovarian failure (POF) is a condition characterized by amenorrhea, infertility, sex steroid deficiency, and elevated gonadotropin serum levels in women before the age of 40. POF is frequently associated with X chromosome

abnormalities ranging from various Xq deletions to complete loss of one of the X chromosomes. Two critical regions on the long arm of the X chromosome, at Xq13-q21 and Xq26-27, were identified (Davison et al. 2000). Bioinformatics analysis of those regions identified putative candidate genes for ovarian failure on the X chromosome or on autosomes that were involved in the translocation event (Baronchelli et al. 2012). Two isoforms of *HS6ST* gene were identified in two chromosomal breakpoints: *HS6ST1* gene at 2q21 (−216Kb) and *HS6ST2* gene at Xq26.2 (+720Kb). Although the definitive evidence for the involvement of HS6STs on POF is further needed, they are potent candidate genes, since *HS6ST2* was expressed preferentially in the ovary and the mutation might influence oocyte development by disturbing a proper interaction with follicular growth factors, and further, the *HS6ST1* gene mutations have recently been associated with IHH as described above, and these genes possibly play some roles in gonadal fertility.

Future Perspectives

In many diseases including infection and cancer, intracellular signaling systems are disturbed. Most studies tend to focus on the signaling molecule itself, but it should be considered that HS and its biosynthetic enzymes including 6-*O*-sulfotransferases might regulate the onset, the progression, and the severity of various diseases since HS serves as a modulator in the wide range of signaling systems and biological processes. It is becoming more important to gain a better insight into the role of HS in diseases, for example, by using mutant mice as model animals.

Cross-References

- ▶ Exostoses (Multiple)-Like 1-3 (EXTL1-3)
- ▶ Exostosin 1,2 (EXT1,2)
- ▶ Heparan Sulfate 2-*O*-Sulfotransferase (HS2ST)
- ▶ Heparan Sulfate (Glucosamine) 3-*O*-Sulfotransferase 1-6 (HS3ST1-6)
- ▶ Heparin-Heparansulfate Related GlcA C5-Epimerase
- ▶ *N*-Deacetylase/*N*-Sulfotransferase (Heparan Glucosaminyl) 1 (NDST1)
- ▶ *N*-Deacetylase/*N*-Sulfotransferase (Heparan Glucosaminyl) 2 (NDST2)
- ▶ *N*-Deacetylase/*N*-Sulfotransferase (Heparan Glucosaminyl) 3,4 (NDST3,4)

Further Reading

For review of the biological role of the zebrafish and the *Drosophila* Hs6st
Nakato and Kimata (2002)
Habuchi et al. (2004)
For review of the biosynthesis and biological roles of HS
Kusche-Gullberg and Kjellen (2003)

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