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Heparan Sulphate Biosynthesis and Disease

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Proteoglycans carrying heparan sulphate (HS) chains are ubiquitously expressed at cell surfaces and in extra-cellular matrices, and HS chains interact with numerous proteins, including growth factors, morphogens and extra-cellular-matrix proteins. These interactions form the basis of HS-related biological phenomena. Thus, the biosynthesis of HS regulates key events in embryonic development and homeostasis, and deranged HS biosynthesis could cause diseases. *EXT1* and *EXT2* genes encoding the polymerase responsible for HS biosynthesis are known as causative genes of hereditary multiple exostoses, a dominantly inherited genetic disorder characterized by the formation of multiple cartilaginous tumours. In this review, we will summarize HS biosynthesis in several model animals, the effects on cellular functions by alteration of HS biosynthesis, and HS-associated diseases. This review suggests that HS biosynthetic enzymes would be potential candidates for drug targets in various diseases.

Key words: glycosyltransferase, heparan sulphate, hereditary multiple exostoses, proteoglycan, tumour-suppressor gene.

Abbreviations: Aβ, amyloid β-peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; EXT, exostosin; FGFs, fibroblast growth factors; GlcAT, glucuronyltransferase; GlcNAcT, *N*-acetylglucosaminyltransferase; α-GalNAcT, α-1,4-*N*-acetylgalactosaminyltransferase; Hh, Hedgehog; HME, hereditary multiple exostoses; HS, heparan sulphate; Ihh, Indian hedgehog; LDL, low-density lipoprotein; LDLR, LDL receptor; LRP, LDL-related protein; Ndst, GlcNAc *N*-deacetylase/*N*-sulphotransferase; PG, proteoglycan; PTH, parathyroid hormone; PTHrP, PTH-related peptide; TRL, triglyceride-rich lipoprotein; VEGF, vascular endothelial growth factor; Wg, Wingless.

Heparan sulphate (HS) exists ubiquitously as a proteoglycan (PG), and it has been implicated in cellular function by interacting with protein ligands, which include a wide variety of growth factors and morphogens (*1–3*) (Fig. 1A). For example, HS regulates not only fibroblast growth factor (FGF) signalling (*4*) but also the signalling and diffusion of Wingless (Wg) and Hedgehog (Hh) in *Drosophila melanogaster* (*5*) (Fig. 1A and B). Recent studies showed that HS proteoglycans (HS-PGs) function as a receptor for triglyceride-rich lipoproteins in the liver, and control lipid metabolism (*6*) (Fig. 1D). Therefore, disorder of the biosynthesis of HS causes impairment of cellular function and abnormal morphogenesis, which could lead to many diseases. Serendipitous discoveries have revealed that hereditary multiple exostoses (HME), which is a dominantly inherited genetic disorder characterized by the formation of multiple cartilaginous tumours, are mainly caused by mutations in genes encoding HS biosynthetic enzymes, EXT1 and EXT2. To date, all genes responsible for HS biosynthesis have been cloned and identified, which helps to investigate the possible association of these genes with several disease pathologies. Here, we will provide an outline of HME, the link between HS and the EXT family of proteins, and examples of other diseases associated with HS.

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HME AND *EXT* GENE FAMILY

HME, an autosomal dominant bone disorder, is the most common type of benign bone tumour. It is clinically characterized by cartilage-capped tumours located at the growth plate of long bones, known as osteochondromas or exostoses (*7*). As tumours grow with age, the compression of nerves and soft tissues causes paralysis, algia, interferes with joint movement and causes occlusion of blood vessels. Surgical intervention is often necessary, and many patients require multiple surgeries throughout the course of the disease. HME has an estimated occurrence of 1 in 50,000 among the general population, and usually presents early in life before the age of 10. Of greatest clinical concern, however, is malignant transformation of the benign tumour to a chondrosarcoma or an osteosarcoma, which occurs in 1∼2% of patients (*8*).

Extensive genetic linkage analysis has determined three different loci on chromosomes 8q24.1 (*EXT1*), 11p11–13 (*EXT2*) and 19p (*EXT3*) as causative agents of HME. *EXT1* and *EXT2* genes have been identified by positional cloning, while EXT3 has yet to be identified. In a variety of studies, mutations responsible for HME have been localized in either *EXT1* or *EXT2* genes in >80% of unrelated patients to date. Although the cellular functions of EXT1 and EXT2 were not clear at the time they were cloned, both were identified as tumour suppressor genes, since loss of heterozygosity at these loci occurred in HME patients, whose benign tumours were transformed into chondrosarcomas (*9*). In addition,

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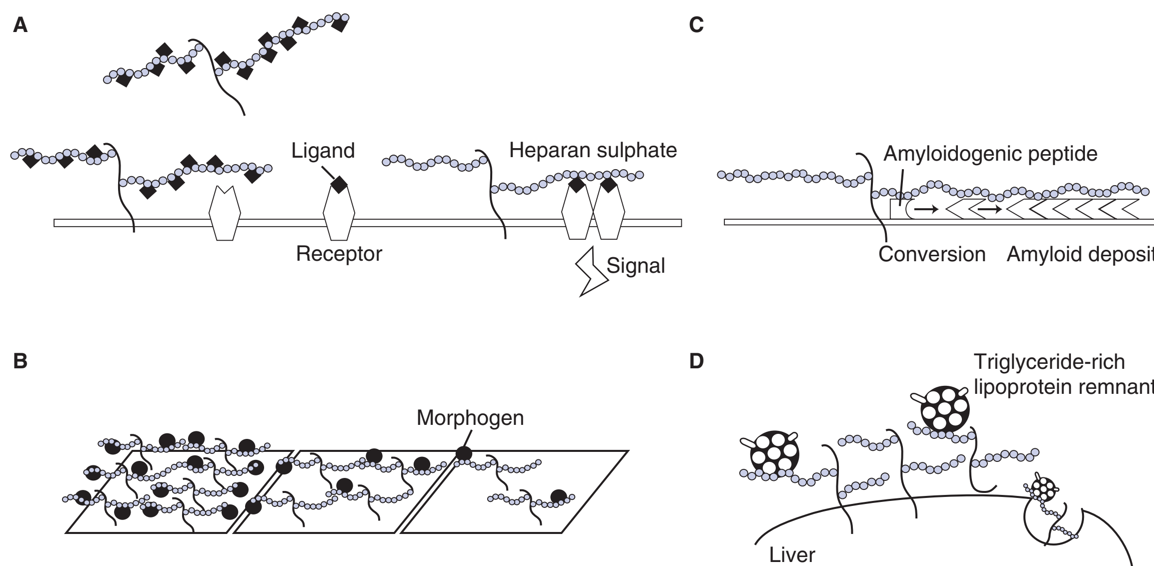

Fig. 1. **Various functions of HS-PGs.** (A) HS-PGs expressed on the cell surfaces or in the extra-cellular matrices act as reservoirs of ligands or co-receptors. (B) HS-PGs are involved in the gradient formation of morphogens. (C) HS-PGs accelerate the transition of monomeric amyloidgenic peptides to the β-sheet, leading to enhancement of fibril formation. (D) HS-PGs in the liver play a crucial role in the incorporation of lipoproteins.

three *EXT*-like genes, *EXTL1*, *EXTL2* and *EXTL3* (for *EXT*-like genes 1, 2 and 3) that share significant sequence homologies with *EXT1* and *EXT2* have been identified and cloned by database searches, revealing the *EXT* gene family, which consists of five members (Fig. 2A) (*10–13*). Although there is no evidence of an association of *EXT*-like genes with HME, genetic analysis of several cancers suggests that they may also belong to tumour suppression genes. All *EXT* genes encode type II transmembrane proteins with a short amino terminal cytoplasmic tail and, notably, the carboxyl terminal portion of EXTs is most highly conserved, suggesting a conserved function. However, their biological functions were not clarified until two different studies reported their involvement in HS biosynthesis, as described later.

ASSOCIATION WITH *EXT* GENE FAMILY AND DEFECTIVE HS BIOSYNTHESIS

As shown in Fig. 3, HS chains are covalently bound to Ser residues in core proteins through a common glycosaminoglycan-protein linkage tetrasaccharide structure, GlcUAβ1-3Galβ1-3Galβ1-4Xylβ1-*O*-Ser (*14*, *15*). Biosynthesis of HS is triggered by transferring the first GlcNAc residue to the tetrasaccharide linkage region, followed by synthesis of the repeating disaccharide region [(-4GlcUAβ1-4GlcNAcα1-)$_n$]. During the polymerization of HS chains, numerous modifications by corresponding sulphotransferases, an epimerase and sulphatases occur in the repeating disaccharide region. The specific sulphation patterns are recognized by a variety of proteins and their interactions are thought to be involved in the regulation of physiological functions.

A connection between HS-synthesizing glycosyltransferases and the HME genes was revealed by two independent studies. In one study, employing an HS-deficient mutant cell line, sog9 and the property of herpes simplex virus to infect cells with HS, cDNA that can rescue HS biosynthesis in that mutant cell line was isolated and identified as EXT1 (*16*). In the other study, EXT2 was identified as an HS co-polymerase by direct peptide sequencing of the HS polymerase purified from bovine serum (*17*). The expressed recombinant EXT2 as well as EXT1 displayed dual glycosyltransferase activities, of GlcNAc transferase (GlcNAcT) and GlcA transferase (GlcAT), representative of an HS polymerase (Fig. 2B) (*17*). These results suggest that EXT1 and EXT2 are HS polymerases responsible for the synthesis of the disaccharide-repeating region of growing HS chains. Interestingly, because mutations in either *EXT1* or *EXT2* result in the formation of clinically indistinguishable exostoses, EXT1 and EXT2 do not appear to be functionally redundant *in vivo*, even though the two proteins display similar substrate specificities *in vitro* (*18*). EXT1 and EXT2 are a functionally complementary pair because they form a stable complex *in vivo*. EXT1/EXT2 heterooligomeric complexes can reside in the Golgi and have considerably higher glycosyltransferase activity than either EXT1 or EXT2 alone (*19*). Furthermore, recombinant soluble enzymes expressed by the co-transfection of EXT1 and EXT2 exhibit polymerization activities and synthesized heparan polymer (*20*). Thus, EXT1 and EXT2 are engaged in the biosynthesis of HS as a polymerase complex with both GlcAT and GlcNAcT activities (Fig. 3).

Since, as described, three *EXT*-like gene products share amino acid sequence homology with EXT1 and EXT2, it was expected that these gene products were also involved in HS biosynthesis. In fact, biochemical analyses showed that EXTL1, EXTL2 and EXTL3 have GlcNAcT activities (Fig. 2) (*21*, *22*), and thus they were thought to be

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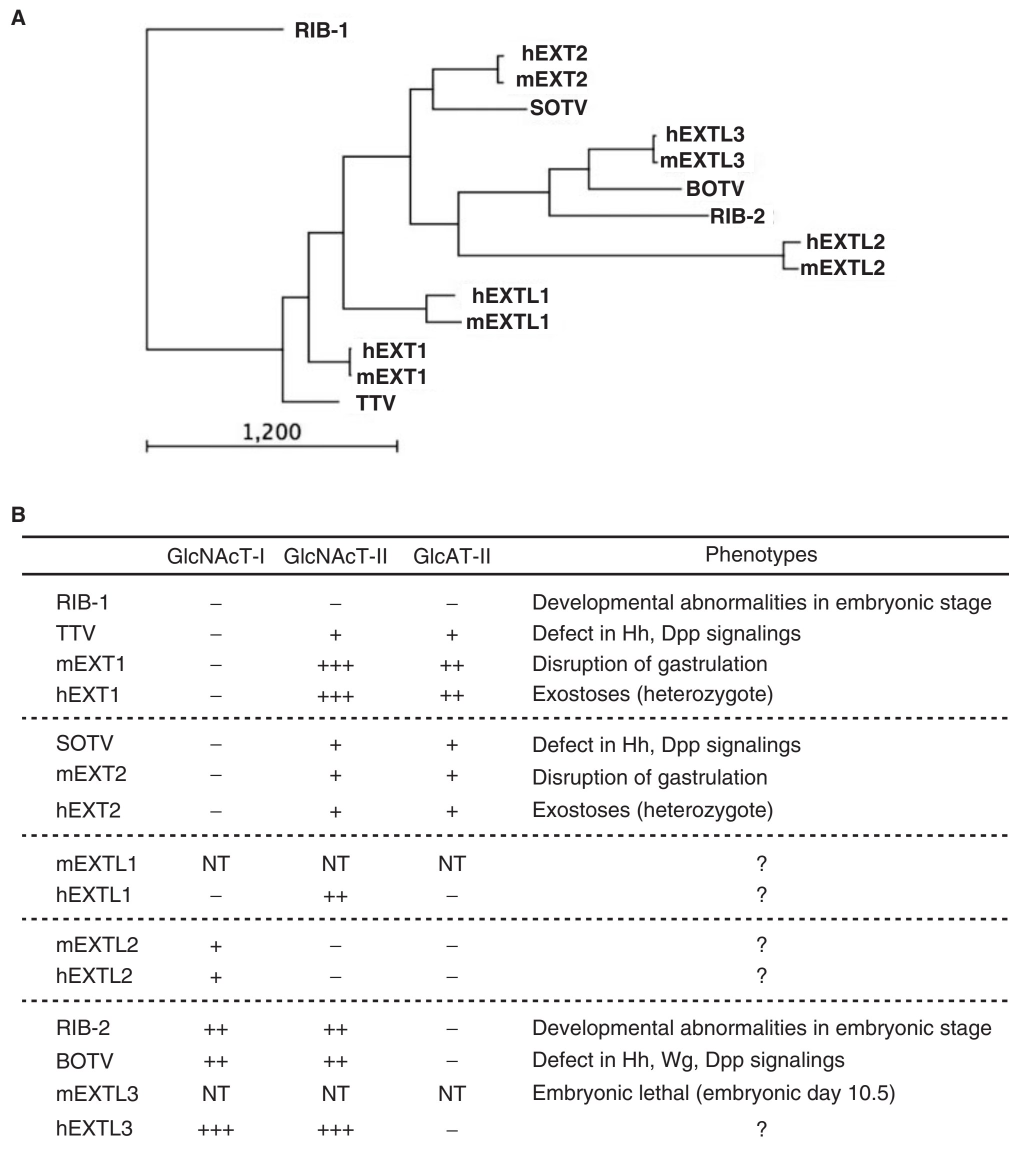

	GlcNAcT-I	GlcNAcT-II	GlcAT-II	Phenotypes
RIB-1	–	–	–	Developmental abnormalities in embryonic stage
TTV	–	+	+	Defect in Hh, Dpp signalings
mEXT1	–	+++	++	Disruption of gastrulation
hEXT1	–	+++	++	Exostoses (heterozygote)
SOTV	–	+	+	Defect in Hh, Dpp signalings
mEXT2	–	+	+	Disruption of gastrulation
hEXT2	–	+	+	Exostoses (heterozygote)
mEXTL1	NT	NT	NT	?
hEXTL1	–	++	–	?
mEXTL2	+	–	–	?
hEXTL2	+	–	–	?
RIB-2	++	++	–	Developmental abnormalities in embryonic stage
BOTV	++	++	–	Defect in Hh, Wg, Dpp signalings
mEXTL3	NT	NT	NT	Embryonic lethal (embryonic day 10.5)
hEXTL3	+++	+++	–	?

Fig. 2. **Phylogenic analysis and properties of EXT family members.** (A) Amino acid sequences of known EXT family members among humans, mice, *D. melenogaster* and *C. elegans* were aligned with CLC Free Workbench 4. (B) Glycosyltransferase activities of each EXT family member are listed. +, activity detected; –, no activity; NT, not tested. Phenotypes by defects in each EXT family member are shown to the right.

implicated in HS biosynthesis (Fig. 3). Because EXTL2 and EXTL3 possessed activity transferring the first GlcNAc residue to the tetrasaccharide-linkage region (so-called GlcNAcT-I activity), it was speculated that they were implicated in the initiation of HS biosynthesis as a GlcNAcT-I. Previous studies indicated that the Chinese hamster ovary cell mutant defective in HS polymerization (*23*) accumulated a pentasaccharide intermediate, GlcNAcα1-4GlUAβ1-3Galβ1-3Galβ1-4Xyl (*24*). These findings suggested the notion that GlcNAcT-I distinct from HS polymerase catalyses the initiation of HS. However, in view of the recent findings that *in vitro* polymerization of HS was induced on tetrasaccharide-linkage analogues as acceptor substrates by the enzyme complex of human EXT1/EXT2 without the aid of EXTL proteins, it can be believed that EXTL2 and EXTL3 might be dispensable for the initiation of HS in mammals (*20*). Hence, the biological roles of mammalian EXTL2 and EXTL3 in HS biosynthesis are less clearly defined.

We previously purified α-1,4-*N*-acetylgalactosaminyltransferase (α-GalNAcT) from the culture medium of a human sarcoma cell line, and found α-GalNAcT to be EXTL2 (*21*). Because EXTL2 possessed the ability to transfer GlcNAc and GalNAc to the tetrasaccharide representing the common glycosaminoglycan-protein linkage region, we considered EXTL2 to be the most likely critical enzyme that determines and initiates HS biosynthesis. To explore the physiological roles of EXTL2 in the biosynthesis of HS, we generated transgenic mice over-expressing the human *EXTL2* gene. Unexpectedly, the amounts of HS in *EXTL2*-transgenic mice decreased (unpublished data). The orthologue of mammalian

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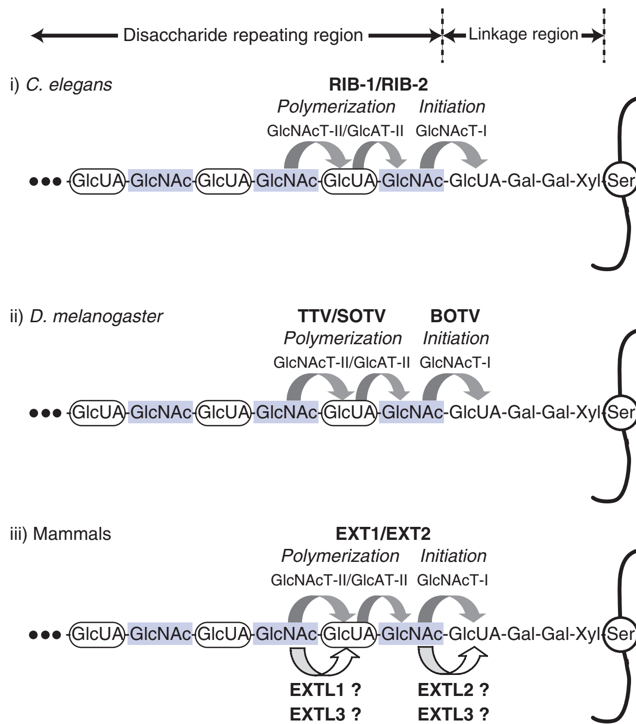

Fig. 3. **Comparison of HS biosynthesis among *C. elegans*, *D. melanogaster* and mammals.** The biosynthesis of HS is initiated by the addition of a Xyl residue to specific Ser residues in the core protein, followed by the sequential addition of two Gal residues and a GlcUA residue, forming the tetrasaccharide linkage structure GlcUAβ1-3Galβ1-3Galβ1-4Xylβ1-*O*-Ser. The transfer of a single GlcNAc residue to tetrasaccharide linkage regions initiates the assembly and polymerization of HS with GlcNAc and GlcUA residues added alternately. The mechanism of HS biosynthesis in these organisms is similar but distinct in that HS polymerase composed of TTV/SOTV complex in *D. melanogaster* exhibits no GlcNAcT-I activity for chain initiation. Thus, BOTV, which possesses GlcNAcT-I activity, is a trigger of HS chain initiation in *D. melanogaster*. On the other hand, mammalian HS is biosynthesized by EXT1/EXT2 polymerase complex even in the absence of EXTL2 and EXTL3. Therefore, the significance of EXTL2 and EXTL3 in the biosynthesis of HS remains unclear.

EXTL2 is absent in *Drosophila*, suggesting that it is not essential for HS biosynthesis (Fig. 2A). During the initial phases of our studies, EXTL2 was expected to act as a key enzyme for HS biosynthesis, but the recent study suggested that EXTL2 mainly functions *in vivo* as a regulatory molecule other than GlcNAcT-I implicated in the initiation of HS biosynthesis. Thus, further studies are needed to reveal the biological function of EXTL2.

It is also reported that EXTL3 may work as a biologically active molecule with a different function, a cell surface receptor for the pancreatic β-cell regeneration factor Reg (RegR) (*25*). Notably, it has been shown that $RegR^{-/-}$ ($EXTL3^{-/-}$) mice die by embryonic Day 10.5 (*26*), while *EXT1* or *EXT2* knockout mice die by embryonic Day 8.5 (*27*, *28*). These results also support that mammalian EXTL3 may not be involved in the initiation of HS biosynthesis. In addition, it was recently reported that gene silencing of *EXTL3* resulted in the synthesis of longer HS chains (*29*). Moreover, our recent study suggests that the chain length of HS is controlled by *EXTL3* gene expression (unpublished data). Taken together, these findings indicate that EXT1 and EXT2 are cooperatively responsible for the initiation and elongation of HS chains and that EXTL3 may regulate the length of HS chains.

The functional importance of *EXT* gene family members in HS biosynthesis has been demonstrated in several *in vivo* model animals. In particular, EXT orthologues have been identified and characterized in *Caenorhabditis elegans* and *D. melanogaster*. These orthologues can be organized by homology to their mammalian counterparts based on their sequences (Fig. 2A). In the *C. elegans* genome, only two genes, *rib-1* and *rib-2*, homologous to mammalian *EXT* genes have been identified (Fig. 2A). RIB-2 is most homologous to human EXTL3 and exerts both GlcNAcT-I and GlcNAcT-II activities involved in chain initiation and elongation of HS, and its acceptor specificity resembles that of human EXTL3, suggesting that RIB-2 is a *C. elegans* EXTL3 orthologue (Fig. 2B) (*30*). *rib-2* mutants exhibit developmental delay and egg-laying defects, which are most likely caused by a reduction in HS. In contrast, RIB-1 is unique among members of the EXT family identified to date in that the protein, composed of 382 amino acids, is about half the size of RIB-2 and shows significant homology only to the amino termini of EXT family members, especially to EXT1 (Fig. 2A). Although RIB-1 exhibits neither GlcNAcT-II nor GlcAT-II activities (Fig. 2B), the RIB-1/RIB-2 complex shows HS polymerization activities (Fig. 3) (*31*). In addition, *rib-1*-null mutants show reduced synthesis of HS and embryonic lethality as well as *rib-2*-null mutants (*31*). These findings show that the mechanism of HS biosynthesis in *C. elegans* is similar but distinct from that in mammals (Fig. 3) and that both RIB-1 and RIB-2 are indispensable for HS biosynthesis and embryonic development in the nematode (Fig. 3). In *Drosophila*, there are three orthologues of mammalian *EXT* genes, *EXT1* (*ttv*), *EXT2* (*sotv*) and *EXTL3* (*botv*) (Fig. 2). These invertebrate *EXT* genes are involved in Hh, Wg and Dpp signalling as well as the distribution of these morphogens, and their defects result in developmental abnormalities (Fig. 2). Biochemical and immunohistochemical studies on *Drosophila* have revealed that HS levels are dramatically reduced or are abolished in the absence of *ttv*, *sotv* or *botv* (*32–34*). Although TTV/SOTV complex can catalyse the HS polymerization reaction *in vitro*, the complex exhibits no GlcNAcT-I activity required for the initiation of HS in contrast to human EXT1/EXT2 complex (*35*), indicating that BOTV, corresponding to human EXTL3, which possesses GlcNAcT-I activity, is indispensable for HS biosynthesis in *Drosophila* (Fig. 3) (*36*). In this regard, Han *et al*. (*34*) demonstrated that *botv*-null embryos exhibited stronger segment polarity phenotypes than *ttv*- or *sotv*-null embryos and that Wg signalling is defective only in the *botv* mutant or *ttv*-*sotv* double mutant but not in the *ttv* or *sotv* mutant. These results altogether suggest that BOTV is essential for the initiation of HS and that all three EXT members, *ttv*, *sotv* and *botv*, are required for HS biosynthesis in *Drosophila*. Thus, the mechanism of HS biosynthesis in *Drosophila* is similar but distinct from that in *C. elegans* or mammals (Fig. 3). To gain more insight into the physiological significance of EXT members in mammals, genetically engineered mice were generated (Fig. 2B). Targeted deletion

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of *EXT1* in mice results in early embryonic lethality caused by failure to form a mesoderm and causes defects in egg cylinder elongation (*27*). Mice carrying a hypomorphic mutation in *EXT1* were also generated by gene-trap screening, although differences between these and *EXT1*-deficient mice were observed (*37*). Mice obtained by the targeted disruption of *EXT1* had undetectable levels of HS and died by embryonic Day 8.5, whereas mice generated by the gene-trap method died around embryonic Day 14 (*37*) and synthesized shorter HS chains, approximately one-third in length (*38*). The difference between these mice may be due to a small amount of wild-type *EXT1* transcript in animals generated with the gene trap. Furthermore, the embryonic mouse brain, where EXT1 alleles are conditionally disrupted, shows serious fallacy in midline axon guidance through Slit, Sonic hedgehog and FGF8 signalling (*39*). On the other hand, the lack of *EXT2*, similar to that of *EXT1*, also leads to a gastrulation defect and abnormalities in the formation of extra-embryonic structures. All *EXT2* heterozygous mice showed multiple abnormalities in cartilage differentiation, and about one-third of the animals formed one or more ectopic bone growths (exostoses) (*28*). In addition, combined *EXT1* and *EXT2* heterozygotes show a higher frequency of exostoses. These findings altogether highlight the importance of *EXT* genes in HS biosynthesis and the biological significance of HS.

HS AND BONE DISORDERS

In endochondoral ossification, a cartilaginous template is formed first, which is subsequently replaced by bone. Central to this process is the formation of a growth plate, a highly organized structure that generates all of the longitudinal growth. Growth-plate chondrocytes are arranged in three layers: resting chondrocytes, proliferating chondrocytes and hypertrophic chondrocytes. Different signalling pathways regulate the rate of proliferation and the conversion of proliferating chondrocytes into hypertrophic chondrocytes in a fine balance. The process is mainly regulated by the Indian hedgehog (Ihh)/parathyroid hormone (PTH)-related peptide (PTHrP) feedback loop and fibroblast growth factor (FGF) signalling pathway. Because HS-PGs required for diffusion and/or efficient signaling by Ihh in the growth plate regulate the Ihh/PTHrP feedback loop, they are important for normal bone formation. It was reported that HS biosynthetic enzymes, EXT1 and EXT2 mRNA expression merged with Ihh expression in the pre-hypertrophic chondrocytes (*40*). Also, a recent report showed that regulation of chondrocyte proliferation through Ihh is controlled by syndecan-3, which is a type of HS-PG expressed in proliferating chondrocytes (*41*). Mutations or targeted deletion of HS biosynthetic enzymes lead to a skeletal development disorder. Deletion of *EXT1* in mice results in failure to immobilize Ihh in the cell matrix (*27*). In addition, reduced amounts of HS potentiate the signalling of Ihh and PTHrP in mice carrying a hypomorphic mutation in *EXT1*, resulting in delayed hypertrophic differentiation and increased chondrocyte proliferation, and HME patients similarly have clusters of over-proliferating chondrocytes (*37*). The second signalling pathway is the FGF pathway, which plays a major role in the regulation of chondrocyte proliferation and differentiation. FGF receptor (FGFR) 1 and FGFR3 are both expressed in the growth plate. FGFR3 is expressed in proliferating chondrocytes, whereas FGFR1 is expressed in pre-hypertrophic and hypertrophic chondrocytes. Inactivation of FGFR3 in the mouse results in overgrowth of the long bones, whereas the expression of FGFR3 with an activating mutation results in dwarfism, suggesting that FGFR3 functions as a regulator of chondrocyte proliferation and/or hypertrophy. HS-PGs are required for functional binding of FGF to FGFR but also act as reservoirs of local ligand availability regulating diffusion, gradient formation and degradation of FGFs. Taken together, HS-PGs formed by HS biosynthetic enzymes, including EXTs, are critical mediators of skeletal development, and altered HS biosynthesis causes ectopic bone growth characteristic of HME.

HS AND TUMOURS

In addition to HME, *EXT1* expression is depressed in human cancer cells such as HL-60 by transcriptional silencing associated with CpG island promoter hypermethylation, and *EXT1* epigenetic inactivation causes inhibition of HS biosynthesis (*42*). In fact, hypermethylation of *EXT1* CpG island is reported to be common in leukaemia. Re-introduction of *EXT1* in HL-60 cells resumed HS biosynthesis, reduced colony formation density and tumour growth in nude mouse xenograft models, demonstrating that EXT1 exhibits tumour-suppressing activity (*42*).

Hypoxia is a tumour micro-environmental condition that plays pivotal roles in tumour progression. Recent studies show that hypoxia up-regulates enzymes involved in the synthesis of HS, such as HS-2-*O*-sulphoransferase and GlcNAc *N*-deacetylase/*N*-sulfotransferases-1 (Ndst1) and -2, resulting in an increase in FGF2-induced cell growth (*43*). Because the binding of FGF2 to its high-affinity receptors requires the presence of specific HS chains, it is likely that hypoxia increases responsiveness to FGF2 by regulating specific HS biosynthetic enzymes, leading to tumour-induced angiogenesis (*44*). Recently, Fuster *et al*. (*45*) generated mice bearing an endothelial-targeted deletion in *Ndst1*. A family of four Ndsts removes acetyl groups from subsets of GlcNAc residues of HS and adds sulphate to free amino groups. Binding of FGF2 and vascular endothelial growth factor (VEGF) to HS purified from *Ndst1*-deficient endothelial cells was dramatically reduced, and pathological angiogenesis in experimental tumours was altered (*45*). These results suggest that changes of HS biosynthesis in endothelial cells have profound effects on tumour angiogenesis.

HS AND NEURODEGENERATIVE DISORDERS

Alzheimer's disease (AD) is the most common neurodegenerative disease, and is characterized by cerebral neuritic plaques of amyloid β-peptide (Aβ). HS-PGs are considered to be associated with the pathology of AD because they play a role in Aβ plaque pathogenesis, Aβ binding, Aβ plaque and fibril formation and β-amyloid precursor protein (APP) processing (*46*). Aβ peptides are

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co-deposited with HS in tissue lesions *in vivo* (*47*). HS-PGs bind the HHQK (His[13]-His[14]-Gln[15]-Lys[16] within Aβ) site of APP (*46*), and Aβ plaque–microglia interactions through the HHQK domain of Aβ are mediated by membrane-bound HS-PGs (*46*, *48*). Glypican, known as a glycosylphosphatidylinositol-anchored type HS-PG, is reported to be a possible receptor for mediating Aβ neurotoxicity (*49*). The presence of HS accelerates the transition of monomeric Aβ peptides to the β-sheet, and affects fibril nucleation and growth (*50*). Furthermore, Alzheimer β-secretase (BACE1: β-site amyloid precursor protein-cleaving enzyme 1) is an aspartic protease that generates the amino terminus of β-amyloid protein from the APP. BACE1 is a key target for Alzheimer drug development, because it is implicated in the rate-limiting step in the formation of Aβ. HS has been identified as the first naturally occurring inhibitor of BACE1. HS interacts directly with BACE1 and inhibits *in vitro* processing of peptide and APP substrates. Although no genetic association of HS biosynthetic enzymes or HS-PGs with AD has been reported, it was recently suggested that an intronic single nucleotide polymorphism in *HSPG2* (*perlecan*) is associated with AD patients carrying *apolipoprotein epsilon 4* allele (*51*). Transgenic over-expression of heparanase rendered mice resistant to experimental amyloid protein A amyloidosis, presumably due to the sequestration of amyloid peptide by related HS oligosaccharides (*52*). Therefore, exogenous soluble HS oligosaccharides might be used for the treatment of AD. In addition, glucosamine analogues that inhibit formation of the HS precursor polysaccharide are anticipated for the treatment of various amyloid diseases (*53*).

Parkinson's disease is another common neurodegenerative disease, and is characterized by a loss of dopaminergic neurons (*54*). Analyses of patients with familial Parkinson's disease have revealed three genes responsible for the disease, encoding α-synuclein, Parkin and ubiquitin carboxy-terminal esterase L1. α-Synuclein is a critical molecular determinant in familial and sporadic Parkinson's disease, with the formation of α-synuclein aggregates called Lewy bodies, characteristics of Parkinson's disease. One HS-PG, agrin, binds to α-synuclein in an HS-dependent manner, induces conformational changes and enhances the insolubility of α-synuclein (*55*).

HS AND METABOLISM DISORDERS

Hyperlipoproteinaemia is reported to be linked to the defective clearance of both post-prandial- and hepatic-derived particles by the liver (*6*). Although hepatic clearance of triglyceride-rich lipoprotein (TRL) remnants is found to be mediated by low-density lipoprotein receptor (LDLR) and LDL-related protein (LRP) scavenger receptors, inactivation of LRP1 alone does not result in TRL remnant particle accumulation under normal physiological conditions (*56*), and combining LRP1 with LDLR mutations results in only a modest accumulation of TLRs (*56*). Therefore, it has been suggested that another receptor participates in the clearance of TRLs in addition to LRP and LDLR. Recent studies showed that liver HS-PGs mediate the clearance of TRLs independently of LDLR family members and represent long-sought-after receptors for TRL remnants *in vivo* (*6*). Inactivation of the HS biosynthetic enzyme gene *Ndst1* in hepatocytes could alter the liver HS fine structure, leading to the accumulation of TRL particles (*6*). These findings suggest that patients predisposed to hyperlipoproteinaemia should be screened for changes in HS structure and polymorphisms in the genes responsible for HS biosynthetic enzymes.

PERSPECTIVES

HS is involved in a variety of pathophysiological processes as described above, and disorder of HS–protein interactions by the alteration of HS biosynthesis is suggested to form the basis of several diseases. So far, all of the genes encoding the glycosyltransferases and sulphotransferases needed for the biosynthesis of HS have been cloned, and gene manipulation in various model animals has been carried out. The accumulating evidence obtained through genetically modified model animals provides insights into the molecular mechanism underlying HS-related diseases. A better understanding of the connection between HS biosynthesis and molecular pathogenesis of HS-associated diseases will enable a breakthrough in the development of marker genes for diagnosis and therapeutic agents.

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