A Genetic Model of Substrate Reduction Therapy for Mucopolysaccharidosis*

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Inherited defects in the ability to catabolize glycosaminoglycans result in lysosomal storage disorders known as mucopolysaccharidoses (MPS), causing severe pathology, particularly in the brain. Enzyme replacement therapy has been used to treat mucopolysaccharidoses; however, neuropathology has remained refractory to this approach. To test directly whether substrate reduction might be feasible for treating MPS disease, we developed a genetic model for substrate reduction therapy by crossing MPS IIIa mice with animals partially deficient in heparan sulfate biosynthesis due to heterozygosity in Ext1 and Ext2, genes that encode the copolymerase required for heparan sulfate chain assembly. Reduction of heparan sulfate by 30–50% using this genetic strategy ameliorated the amount of disease-specific biomarker and pathology in multiple tissues, including the brain. In addition, we were able to demonstrate that substrate reduction therapy can improve the efficacy of enzyme replacement therapy in cell culture and in mice. These results provide proof of principle that targeted inhibition of heparan sulfate biosynthetic enzymes together with enzyme replacement might prove beneficial for treating mucopolysaccharidoses.

Glycosaminoglycans are catabolized in the lysosome by stepwise removal of monosaccharide residues and sulfate groups from the nonreducing end of the chain. Loss in any of the 12 enzymes involved in glycosaminoglycan catabolism results in the accumulation of their partially degraded substrates in the lysosome, causing lysosomal storage diseases known as mucopolysaccharidoses (1). The most severe MPS result from defects in heparan sulfate catabolism (MPS IIIa–e), causing extensive neuropathology with relatively minor somatic problems (1, 2). A major underlying cause of neurodysfunction in MPS is thought to be defective autophagosome-lysosome fusion, which can be visualized histologically by the appearance of cytosolic ubiquitin aggregates in sections of the brain (3, 4). Neuroinflammation, as assessed by an increase in microglial markers, and astrogliosis, as measured by increased glial fibrillary acidic protein (GFAP) immunoreactivity, are hallmarks of the disease (4, 5). Recent detection of hyperphosphorylated Tau (Ptau) and β-amyloid in the medial entorhinal cortex and dentate gyrus of MPS IIIa and IIIb mice (6, 7) suggests that neuropathology may also share some common mechanisms with age related dementias, such as Alzheimer disease.

The most common method of treatment for MPS remains enzyme replacement therapy (ERT), which restores lysosomal turnover by administration of exogenous enzyme (8). However, a major drawback of this approach is the inability of exogenous enzyme to traverse the blood-brain barrier and treat neurological symptoms (9). An alternative approach to ERT is substrate reduction therapy (SRT), a treatment strategy that employs small molecule inhibitors to reduce the biosynthesis of storage metabolites (10, 11). The most promising therapeutic of this class to date is N-butyldexoxynojirimycin, a glucosylceramide synthase inhibitor that has been shown to successfully reduce ganglioside accumulation in mouse models of Tay-Sachs and Sandhoff disease (12, 13), and is currently licensed for the treatment of type 1 Gaucher disease.

Silencing studies using siRNA directed against glycosaminoglycan biosynthetic enzymes suggested that SRT might reduce lysosomal storage of glycosaminoglycans in MPS fibroblasts (14, 15). However, progress in testing the efficacy of SRT for MPS in vivo has been stymied by a lack of specific inhibitors of
Genetic Substrate Reduction Therapy for MPS

glycosaminoglycan biosynthesis. Compounds thought to non-specifically affect glycosaminoglycan biosynthesis such as rhodamine B and genistein appear to reduce lysosomal storage in MPS mouse models (16–18). However, the non-specific nature of rhodamine B (a fluorescent dye (19)) and genistein (a soy isoflavone that modulates cell signaling and viability (20)) makes it impossible to discriminate the effects of these drugs on glycosaminoglycan biosynthesis from off-target effects that affect storage through alternative mechanisms. Thus, the ability of SRT to ameliorate storage and pathology in MPS remains poorly characterized.

To assess whether strategies that specifically mitigate glycosaminoglycan biosynthesis could be used to treat MPS, heparan sulfate biosynthesis was reduced in MPS IIIa mice by cross-breeding to mice deficient in Ext1 and Ext2, genes that encode the copolymerase required for chain polymerization. Our studies follow a rationale similar to genetic experiments carried out in the Sandhoff mouse model, wherein the impact of β1,4-N-acetylgalactosaminyltransferase ablation was used to characterize the efficacy of SRT in gangliosidoses (21). Here, we show that genetic substrate reduction therapy (gSRT) reduced the amount of disease-specific heparan sulfate nonreducing end biomarker for MPS IIIa. This biomarker, N-sulfoglucosamine, results from the absence of sulfamidase in the lysosome and correlates with the degree of lysosomal storage (22). In addition, pathological markers in key MPS IIIa mouse organs, including the brain, were reduced. Finally, gSRT significantly reduced the effective concentration of enzyme necessary for ERT in cell culture and showed an additive therapeutic effect in vivo, suggesting that a combined SRT/ERT therapeutic approach would prove effective for treating MPS III patients.

EXPERIMENTAL PROCEDURES

Mice Husbandry—Mice deficient in sulfamidase (B6.Cg-Sgshmps3a/PstI) were purchased from Jackson Laboratory. These mice carry a homozygous missense mutation in the sulfamidase gene Sgsh and exhibit <5% residual enzyme activity causing MPS type IIia (23–25). Mice heterozygous for the sulfamidase deficiency were bred with mice heterozygous for Ext1 and Ext2 (26–28) and then intercrossed to obtain MPS IIIa mice (Sgsh+/−) heterozygous for Ext1 or heterozygous for both Ext1 and Ext2.

Immunohistochemistry—Mice were perfused with PBS and 10% formalin (Fisher, SF93-4). Brains were embedded in paraffin and sectioned horizontally. Slices were stained with rabbit antiserum against ubiquitin (Dako, Z0458) at a 1:300 dilution or glial fibrillary acidic protein (Dako, N1506) at a 1:1000 dilution. Rabbit IgG (Dako, K5207) was used as a negative control. Sections stained for ubiquitin were incubated with biotinylated goat anti-rabbit antibodies (Jackson ImmunoResearch Laboratories (111-065-045) and horseradish peroxidase-conjugated streptavidin (016-030-084) at 1:500 dilutions. Sections stained for glial fibrillary acidic protein were reacted with horseradish peroxidase-conjugated anti-rabbit antibodies (111-035-003) at 1:500 dilution. Sections were counterstained with Mayer’s hematoxylin (Sigma-Aldrich). Sections were assessed for the number of Ptau AT270-positive inclusions in the dentate gyrus as described (6). The contrast of all images was adjusted equally, and the extent of staining was measured with ImageJ software (National Institutes of Health).

Glycosaminoglycan Purification—Mice (12 weeks old) were sacrificed, and organs were removed and placed in ice-cold buffer containing 50 mM sodium acetate (pH 6.0) and 0.2 M sodium chloride. After dissociation using a Polytron homogenizer, the resulting homogenates were digested on a shaker overnight at 37 °C with 0.1 mg/ml Pronase (type XIV protease from Streptomyces griseus) and 0.1% Triton X-100. Samples were filtered through a 0.45-μm membrane, and glycosaminoglycans were purified from homogenates by anion exchange chromatography as described previously (29). Enzymatic depolymerization of heparan sulfate was carried out using 1 milliunit each of heparin lyases I, II, and III (Seikagaku).

Glycosaminoglycan Analysis—The disaccharides resulting from enzymatic depolymerization of heparan sulfate were tagged by reductive amination with [12C6]aniline as described previously (30). Each sample was mixed with a known amount of [13C9]aniline-tagged internal unsaturated disaccharide standards as well as a monosaccharide standard for the saturated nonreducing end biomarker for MPS IIIa (N-sulfoglucosamine; Dextra) (22). Samples were analyzed by liquid chromatography-mass spectrometry (LC/MS) using an LTQ Orbitrap Discovery electrospray ionization mass spectrometer (Thermo Scientific) equipped with quaternary high performance liquid chromatography pump (Finnigan Surveyor MS pump) and a C-18 reverse-phase microbore column. Internal disaccharides and nonreducing end monosaccharides were identified based on their unique mass as described previously and quantified relative to the wet weight of tissue (22, 30).

Size and Turnover of [35S]Heparan Sulfate—Mouse embryonic fibroblasts (MEFs) were prepared from embryos harvested at embryonic day 14 and grown to confluence. Cell surface heparan sulfate was purified from cells following 48-h radiolabeling with [35S]O4 for 48 h and lysosomal heparan sulfate was purified from cells as the pool remaining after 48-h label/48-h chase with [35SO4] as described previously (31). To measure the size of heparan sulfate from these subcellular pools, the samples were subsequently treated with base to β-eliminate the chains and analyzed on a Sepharose CL-6B column (0.5 inner diameter × 75 cm, GE Healthcare) equilibrated with 0.1 M sodium chloride. The column was run at a rate of 0.1 ml/min, and 1-m1 fractions were collected. The elution position of the radiolabeled heparan sulfate was assessed by scintillation counting, and the peak $Kav$ values were calculated and compared with calibration curve described by Wasteson (32). Turnover experiments were carried out as described (31). Briefly, cells were labeled with 100 μCi/ml Na[35SO4] (PerkinElmer Life Sciences) for 48 h and then chased for 48 h in normal medium. Cells were treated with 0.05% trypsin/EDTA (Invitrogen) for 20 min and sedimented by centrifugation. The cell pellets were lysed using 0.1 M sodium hydroxide. [35S]Heparan sulfate was purified and quantified by scintillation counting, and the values were expressed relative to cell protein. In all experiments, the sensitivity of the samples to treatment with bacterial heparin lyases was used to validate that the measured material consisted predominantly of heparan sulfate and not other components.
Enzyme Production and Activity—Murine sulfamidase was cloned using RT-PCR, with primers designed to generate full-length constructs with a C-terminal histidine tag for rapid enzyme purification using Ni2+-affinity matrix chromatography. Recombinant sulfamidase was expressed by transient transfection of human embryonic kidney (HEK293) cells. The final preparation in 300 mM imidazole, 300 mM NaCl, and 2 mM Tris-HCl, pH 7.5, was desalted and concentrated from 20 ml to 1.3 ml in 150 mM NaCl and 25 mM Heps, pH 7.5, using an Amicon 10,000 MWCO filter. Protein was measured by BCA protein assay. Sulfamidase activity was determined using 4-methylumbelliferone-α-D-N-sulfoglucosaminidase as substrate according to the manufacturer’s instructions (Moscerdam), except sodium barbital/sodium acetate was replaced with Tris acetate buffer. The level of expression varied from 1 to 2 mg/litter, and enzyme activity ranged from 70 to 150 millunits/mg. SDS-polyacrylamide electrophoresis indicated a purity of >95%.

RESULTS

Genetic Substrate Reduction Therapy Improves Turnover and Reduces Lysosomal Storage in MPS IIIa MEFs—To generate a mouse model for gSRT, mice heterozygous for sulfamidase deficiency were crossed with mice heterozygous for the genes encoding the heparan sulfate copolymerase, Ext1 and Ext2. Interverbreeding these strains yielded mice with the genotypes Sgsh−/−, Sgsh−/−Ext1+/−, and Sgsh−/−Ext1+/−Ext2+/−. Complete loss of Ext1 or Ext2 results in embryonic lethality at embryonic day 7.5, but heterozygosity is well tolerated, causing only mild rib exostoses (26–28). The efficacy of gSRT was first assessed ex vivo using wild-type, Sgsh−/− (Illa), Sgsh−/−Ext1+/− (IllaE1), and Sgsh−/−Ext1+/−Ext2+/− (IllaE1E2) MEFs. The impact of Ext1 and Ext2 heterozygosity on heparan sulfate chain length was determined by radiolabeling cellular glycosaminoglycans with [35S]glucosamine followed by gel filtration chromatography of trypsin-releasable cell surface [35S]heparan sulfate, which is the source of material that is stored in lysosomes in MPS. As shown in Fig. 1A, the average size of cell surface heparan sulfate from wild-type and MPS IIIa fibroblasts had an apparent molecular mass of ~42 kDa. Heterozygous ablation of Ext1 alone (IIlaE1) reduced heparan sulfate chain length by ~20% to apparent mass of ~33 kDa, whereas the heterozygous ablation of both Ext1 and Ext2 (IIlaE1E2) reduced chain length by ~35% to an apparent molecular mass of ~26 kDa. Disaccharide analysis of cell surface heparan sulfate from these cells revealed no significant compositional change between wild-type and MPS IIIa fibroblasts.

The decrease in chain length of cell surface heparan sulfate corresponds to an overall decrease in the amount of heparan sulfate. Cell surface heparan sulfate is constitutively endocytosed and degraded in the lysosome. Thus, reducing the amount...
of material at the cell surface should diminish the amount of heparan sulfate in the lysosome that needs to be turned over. To assess the impact of reduced heparan sulfate chain length on lysosomal turnover of the chains, MEFs were incubated with $^{35}$S for 48 h to radiolabel the heparan sulfate proteoglycans to constant radiospecific activity. The cells were then chased for 48 h in the presence of nonradioactive sulfate. During this time, cell surface heparan sulfate proteoglycans either are shed from the cell surface or internalized by endocytosis and subsequently degraded in lysosomes. After 48 h, wild-type MEFs turned over almost all of the $^{35}$S heparan sulfate proteoglycans, whereas IIa MEFs accumulated large amounts of nontrypsin-releasable $^{35}$S heparan sulfate (Fig. 1B). IIaE1 MEFs exhibited a 35% reduction in $^{35}$S heparan sulfate accumulation compared with IIa MEFs, which increased to 50% reduction in IIaE1E2 MEFs (Fig. 1B). As shown in Fig. 1C, this lysosomal material had the characteristic size of short fragments as predicted from the action of lysosomal proteases on the proteoglycan core proteins and heparanase, an endogluconuronidase (33). This finding demonstrates that gSRT reduced the abundance and not the size of the accumulating material. The impact of gSRT on the diagnostic MPS IIa nonreducing end biomarker (N-sulfoglucosamine), whose amount correlates directly with the degree of heparan sulfate storage in the lysosome (22), is shown in Fig. 1D. Compared with IIa MEFs, IIaE1 and IIaE1E2 MEFs exhibited 30 and 50% reduced biomarker levels, respectively. Overall, these data demonstrate that strategies that shorten the length of heparan sulfate chains have the potential to improve lysosomal turnover significantly.

Genetic Substrate Reduction Therapy Improves Hepatomegaly in MPS IIa—Defects in heparan sulfate catabolism are associated with severe neuropathology but can also result in peripheral abnormalities such as hepatomegaly (23, 25, 34). As shown in Fig. 3, hepatomegaly in IIaE1E2 mice resulted in a ~20% increase in liver weight. A small reduction in liver weight was apparent in IIaE1 mouse livers, whereas IIaE1E2 mouse liver weights were almost completely normalized to that observed in wild-type mice.

Genetic Substrate Reduction Therapy Ameliorates Neuro-pathological Markers in MPS IIa Mice—Defects in heparan sulfate catabolism in MPS cause severe neurodegeneration (34). Blocked autophagy is thought to be a major underlying cause of brain dysfunction and can be visualized by the cytosolic accumulation of ubiquitinated aggregates (3, 35). At 12 weeks of age, IIa mice exhibited extensive ubiquitin aggregate staining.
in the midbrain (Fig. 4A). Ubiquitin staining appeared reduced in the IIIaE1 brain but failed to reach significance, whereas a ~65% decrease in ubiquitin was observed in IIIaE1E2 mice (Fig. 4B). Similar observations were made for GFAP staining (Fig. 4, C and D), a marker for astrocytosis (4, 5). Recent studies have identified enhanced Ptau immunostaining in the medial entorhinal cortex and dentate gyrus as markers of neurodegeneration in MPS IIIa and IIIb mice (6, 7). At 12 weeks of age, the number of Ptau AT270-positive inclusions was too faint in IIIa mouse brain to allow an accurate comparison. However, by 16 weeks of age the number of positive inclusions in the dentate gyrus of IIIa mice exhibited an intensity of 109 ± 8 (mean ± S.E.; n = 6), whereas IIIaE1E2 mice of the same age exhibited reduced staining (89 ± 4, mean ± S.E.; n = 4). Ptau inclusions in wild-type mice were negligible. These differences did not reach significance, but the trend in the data indicated that gSRT partially restored autophagy and reduced astrocytosis. Although these results show promising reductions in established pathological markers in the brain, we cannot be certain that these reductions are sufficient to reduce neuropathology in MPS disease, which is complex and may result from chronic secondary effects such as longstanding inflammation. Future behavioral analysis in gSRT mice should clarify whether SRT approaches may be sufficient to partially restore neurological function in MPS, at least during the early stages of disease, despite incomplete normalization of storage levels.

**Genetic Substrate Reduction Therapy Improves the Efficacy of Enzyme Replacement Therapy**—The ability of gSRT to systemically ameliorate storage and pathology in all tested tissues, including those typically resistant or refractory to ERT such as the brain, suggested that a combination of gSRT and ERT might elicit an additive therapeutic effect. To test whether gSRT can lower the effective dose of enzyme necessary to correct lysosomal turnover, we labeled and chased MEFs with radioactive sulfate in the presence and absence of recombinant sulfamidase. We then determined the effective dose of enzyme necessary to reduce the level of radiolabeled heparan sulfate to 30% of untreated disease cells (ED30). As shown in Fig. 5A, IIIa MEFs exhibited an ED30 of ~5.7 ng/ml sulfamidase. The ED30 for IIIaE1 and IIIaE1E2 MEFs was reduced 3–4-fold, i.e. to ~2.1 and 1.4 ng/ml, respectively. To test the impact of combined gSRT/ERT in vivo, we injected IIIa, IIIaE1, and IIIaE1E2 mice with 0.3 mg/kg sulfamidase and analyzed the amount of storage by biomarker analysis in different tissues 48 h after treatment. Treatment of IIIa mice with 0.3 mg/kg sulfamidase reduced storage in the spleen by ~40% compared with untreated IIIa (Fig. 5B). However, treatment of IIIaE1 and IIIaE1E2 mice was much more effective, reducing storage by ~60 and ~70%, respectively, compared with untreated IIIa mice. These findings suggest that gSRT can effectively reduce the level of enzyme needed for replacement therapy.

**DISCUSSION**

Defects in the catabolism of glycosaminoglycans have been shown to result in 11 distinct mucopolysaccharidoses in humans, each caused by the deficiency of a distinct lysosomal enzyme, resulting in severe and often lethal disease pathology in children (34). Although ERT is currently the most effective treatment for MPS, approved ERT therapies exist for only three disorders, MPS I, II, and VI (9). Unfortunately, intravenous injection of the recombinant enzyme missing in the patients does not reduce pathology in the brain due to exclusion of therapeutic enzyme by the blood-brain barrier (9). Intrathecal injection protocols are currently being tested as a method of delivering enzyme to the brain. However, this method is highly invasive (36). A further drawback of ERT is the weekly injection of enzyme to elicit any therapeutic benefit, which is quite disruptive to the lives of the patients and their families (9).

In light of these drawbacks, an urgent need exists to improve ERT or to develop new therapeutic strategies to address pathology associated with MPS. One potential approach might be
Genetic Substrate Reduction Therapy for MPS

SRT, which aims to ameliorate lysosomal storage by reducing the biosynthesis of the storage substrate. The primary advantage of SRT is the possibility to design low molecular mass biosynthetic inhibitors that are inexpensive, druggable, and that might traverse the blood-brain barrier to allow treatment within the central nervous system. Although SRT has been shown to successfully reduce storage and neuropathology in ganglioside storage disorders (12, 13, 37, 38), SRT approaches for MPS disease have remained largely unexplored due to a lack of biosynthetic inhibitors specific for glycosaminoglycans. To date, genistein is the only compound that has been tested in MPS mice and patients for its ability to elicit SRT. Although genistein is the only compound that has been tested in MPS mice and patients for its ability to elicit SRT, it is best characterized as an inhibitor of receptor tyrosine kinase activity with proapoptotic and immunosuppressive effects (20, 40, 41). These broad multifunctional activities make it difficult to distinguish whether the beneficial outcomes observed in MPS mice given ultrahigh doses of genistein result from SRT or other off-target effects (17, 18, 42). Further, recent open label studies using genistein in Sanfilippo patients showed no positive impact on pathology or well being (43), even when administered at 10 mg/kg per day (44), raising questions about the efficacy of SRT for MPS disease as a whole.

To address this issue, we have tested the ability of gSRT to treat MPS storage and pathology by using genetic strategies to reduce specifically and progressively the biosynthesis of heparan sulfate in MPS IIIa mice. Concerns might be raised regarding the use of SRT to treat MPS disease based on the observation that the complete loss of heparan sulfate biosynthetic enzymes can result in severe developmental defects (45). However, it is important to note that heterozygous enzyme deficiencies, such as those employed in this study, are often tolerated without profound physiological effects. This observation indicates that strategies used to reduce or alter heparan sulfate might be employed for the treatment of disease with minimal deleterious effects. Ext1 and Ext2 represent ideal targets for testing this therapeutic strategy in mice because heterozygous loss of either gene has been shown to reduce the length and number of heparan sulfate chains without causing severe developmental or physiological defects aside from exostoses (27, 28). It should be noted that a number of sulfotransferases are also involved in heparan sulfate biosynthesis (46). Thus, one might imagine customizing SRT to specific biosynthetic enzymes that correspond to lysosomal enzyme deficiencies, for example an inhibitor of the uronyl 2-O-sulfotransferase might prove beneficial for treatment of MPS II, which results from a deficiency in lysosomal 2-O-sulfatase.

In this study, we demonstrate the ability of gSRT to significantly reduce a disease-specific biomarker that correlates with the degree of lysosomal storage in multiple MPS IIIa mouse tissues, including the brain. Importantly, a partial reduction (~50%) in lysosomal storage in the brain, as assessed by biomarker analysis, was sufficient to dramatically reduce astroglialis and markers of aberrant autophagy. Small reductions in the neuropathological marker Ptau were also observed. Taken together, these results suggest that complete normalization of lysosomal storage may not be necessary to correct pathology in the brain and that small reductions in heparan sulfate biosynthesis might be sufficient to elicit these effects. Importantly, our findings suggest that a combined SRT/ERT therapeutic approach may be most effective for treating MPS disease. The ability of SRT to reduce the effective dose of exogenous enzyme needed to correct glycosaminoglycan turnover suggests that SRT approaches could dramatically lower the cost and perhaps the frequency of ERT. Taken together, these experiments establish the efficacy of SRT for treating MPS disease pathology and open the way for the development of specific glycosaminoglycan inhibitors for the treatment of this broad group of diseases.

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Genetic Substrate Reduction Therapy for MPS

B and related xanthene dyes. Histochemie 20, 244–249
20. Banerjee, S., Li, Y., Wang, Z., and Sarkar, F. H. (2008) Multi-targeted therapy of cancer by genistein. Cancer Lett. 269, 226–242
21. Liu, Y., Wada, R., Kawai, H., Sango, K., Deng, C., Tai, T., McDonald, M. P., Araujo, K., Crawley, J. N., Bierfreud, U., Sandhoff, K., Suzuki, K., and Proia, R. L. (1999) A genetic model of substrate deprivation therapy for a glycosphingolipid storage disorder. J. Clin. Invest. 103, 497–505
22. Lawrence, R., Brown, J. R., Al-Mafraj, K., Lamanna, W. C., Beitel, I. R., Boons, G. J., Esko, J. D., and Crawford, B. E. (2012) Disease-specific nonreducing end carbohydrate biomarkers for mucopolysaccharidoses. Nat. Chem. Biol. 8, 197–204
23. Bhaumik, M., Muller, V. J., Rozaklis, T., Johnson, L., Dobrenis, K., Bhattacharya, R., Wurzelmann, S., Finamore, P., Hopwood, J. J., Walkley, S. U., and Stanley, P. (1999) A mouse model for mucopolysaccharidosis type III A (Sanfilippo syndrome). Glycobiochem 9, 1389–1396
24. Bhattarcharya, R., Gliddon, B., Becci, T., Hopwood, J. J., and Stanley, P. (2001) A novel missense mutation in lysosomal sulfamidase is the basis of MPS III A in a spontaneous mouse mutant. Glycobiochem 11, 99–103
25. Crawley, A. C., Gliddon, B. L., Auclair, D., Brodie, S. L., Hirtz, C. King, B. M., Fuller, M., Hemsley, K. M., and Hopwood, J. J. (2006) Characterization of a C57BL/6 congenic mouse strain of mucopolysaccharidosis type IIIA. Brain Res. 1104, 1–17
26. Lin, X., Wei, G., Shi, Z., Dryer, L., Esko, J. D., Wells, D. E., and Matzuk, M. M. (2000) Disruption of gastrulation and heparan sulfate biosynthesis in EXT1-deficient mice. Dev. Biol. 242, 299–311
27. Stickens, D., Zak, B. M., Rougier, N., Esko, J. D., and Werb, Z. (2005) Mice deficient in Ext2 lack heparan sulfate and develop exostoses. Development 132, 5055–5068
28. Zak, B. M., Schuksz, M., Koyama, E., Mundy, C., Wells, D. E., Yamaguchi, Y., Pacifi, M., and Esko, J. D. (2011) Compound heterozygous loss of Ext1 and Ext2 is sufficient for formation of multiple exostoses in mouse ribs and long bones. Bone 48, 979–987
29. Esko, J. D. (1993) In Current Protocols in Molecular Biology (Ausubel, F., Brent, R., Kingston, B., Moore, D., Seidman, J., Smith, J., Struhl, K., Varki, A., and Coligan, J., eds) pp. 17.12.11–17.12.19, Greene Publishing and Wiley-Interscience, New York
30. Lawrence, R., Olson, S. K., Steele, R. E., Wang, L., Warrior, R., Cummings, R. D., and Esko, J. D. (2008) Evolutionary differences in glycosaminoglycan fine structure detected by quantitative glycan reductive isotope labeling. J. Biol. Chem. 283, 33674–33684
31. Lamanna, W. C., Lawrence, R., Sarrazin, S., and Esko, J. D. (2011) Secondary storage of dermatan sulfate in Sanfilippo disease. J. Biol. Chem. 286, 6955–6962
32. Wasteson, A. (1971) A method for the determination of the molecular weight and molecular weight distribution of chondroitin sulphate. J. Chrotnogr. 59, 87–97
33. Yanagishita, M., and Hascall, V. C. (1984) Metabolism of proteoglycans in rat ovarian granulosa cell culture: multiple intracellular degradative pathways and the effect of chloroquine. J. Biol. Chem. 259, 10270–10283
34. Neufeld, E. F., Lim, T. W., and Shapiro, L. J. (1975) Inherited disorders of lysosomal metabolism. Annu. Rev. Biochem. 44, 357–376
35. Settembre, C., Fraldi, A., Rubinstein, D. C., and Ballabio, A. (2008) Lysosomal storage diseases as disorders of autophagy. Autophagy 4, 113–114
36. Dickson, P. I., and Chen, A. H. (2011) Intrathecal enzyme replacement therapy for mucopolysaccharidoses I translating success in animal models to patients. Curr. Pharm. Biotechnol. 12, 946–955
37. Lachmann, R. H., and Platt, F. M. (2001) Substrate reduction therapy for glycosphingolipid storage disorders. Expert Opin. Investig. Drugs 10, 455–466
38. Cox, T., Lachmann, R., Hollar, C., Aerts, J., van Weely, S., Hrebícek, M., Platt, F., Butters, T., Dwek, R., Moyses, C., Gow, I., Elstein, D., and Zimran, A. (2000) Novel oral treatment of Gaucher’s disease with N-butyldesoxyojirimycin (OGT 918) to decrease substrate biosynthesis. Lancet 355, 1481–1485
39. Wegrzyn, A. (2012) Gene expression-targeted isoflavone therapy. IUBMB Life 64, 307–315
40. Polkowski, K., and Mazurek, A. P. (2000) Biological properties of genistein: a review of in vitro and in vivo data. Acta Pol. Pharm. 57, 135–155
Genetic Substrate Reduction Therapy for MPS

41. Jakóbkiewicz-Banecka, J., Piotrowska, E., Narajczyk, M., Barańska, S., and Wegrzyn, G. (2009) Genistein-mediated inhibition of glycosaminoglycan synthesis, which corrects storage in cells of patients suffering from mucopolysaccharidoses, acts by influencing an epidermal growth factor-dependent pathway. J. Biomed. Sci. 16, 26

42. Friso, A., Tomanin, R., Salvalaio, M., and Scarpa, M. (2010) Genistein reduces glycosaminoglycan levels in a mouse model of mucopolysaccharidosis type II. Br. J. Pharmacol. 159, 1082–1091

43. Delgadillo, V., O’Callaghan Mdel, M., Artuch, R., Montero, R., and Pineda, M. (2011) Genistein supplementation in patients affected by Sanfilippo disease. J. Inherit. Metab. Dis. 34, 1039–1044

44. de Ruijter, J., Valstar, M. J., Narajczyk, M., Wegrzyn, G., Kulik, W., Ijlst, L., Wagemans, T., van der Wal, W. M., and Wijburg, F. A. (2012) Genistein in Sanfilippo disease: a randomized controlled crossover trial. Ann. Neurol. 71, 110–120

45. Bishop, J. R., Schuksz, M., and Esko, J. D. (2007) Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature 446, 1030–1037

46. Esko, J. D., and Selleck, S. B. (2002) Order out of chaos: assembly of ligand binding sites in heparan sulfate. Annu. Rev. Biochem. 71, 435–471

47. Lawrence, R., Lu, H., Rosenberg, R. D., Esko, J. D., and Zhang, L. (2008) Disaccharide structure code for the easy representation of constituent oligosaccharides from glycosaminoglycans. Nat. Methods 5, 291–292