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REVIEW

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Emerging Therapies For the Treatment of Mucopolysaccharidosis Type III

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Keywords

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ABSTRACT • Mucopolysaccharidosis type III (Sanfilippo) is a group of four autosomal recessive lysosomal storage diseases resulting from a failure to degrade glycosaminoglycans. The four biochemical subtypes of Mucopolysaccharidosis type III (MPS III A-D) are caused by the deficiency of one of the four enzymes required for heparan sulfate degradation. Unlike the other MPSs that present with extensive somatic involvement, patients with MPS III typically present with neurological signs and symptoms. Although enzyme replacement therapy is effective to some extent in management of somatic pathology of many lysosomal storage diseases, it seems to be of low efficacy in treatment of neurological symptoms because of the blood-brain barrier. In spite of some treatments for other MPS disorders, which mostly alleviate non-neurological symptoms, no therapy is currently available for MPS III. Treatment of the neurological symptoms of MPS III remains challanging due to blood-brain barrier that restricts the crossing of therapeutics to the central nervous system (CNS). Intraventricular enzyme replacement, gene therapy, hematopoietic stem cell transplantation, substrate reduction therapy, pharmacological chaperone therapy and stopcodon readthrough therapy are new experimental therapeutic approaches that circumvent this barrier. This review discusses some of the emerging treatment strategies to treat MPS III, and evaluates the outcomes of these treatments in animal models and human patients as well as those of in vitro.

INTRODUCTION

Mucopolysaccharidosis type III (MPS III) or Sanfilippo syndrome belongs to the group of approximately 50 inherited monogenic lysosomal storage disorders (LSDs) (1). Currently, there are four autosomal recessive subtypes of MPS III (A, B, C and D) recognized in humans (2); each is caused by the deficiency of one of four enzyme activities responsible for the degradation of a common glycosaminoglycan (GAG), heparan sulphate: heparan-N-sulfatase (MPS IIIA), N-acetyl-α-glucosaminidase (MPS IIIB), acetyl CoA: α-glucosaminide N-acetyltransferase (MPS IIIC), or N-acetylglucosamine 6-sulphatase (MPS IIID) (3). They result from mutations in SGSH (coding for heparan-N-sulfatase), NAGLU (coding for α-N-acetylglucosaminidase), HGSNAT (coding for acetyl-CoA:α-glucosaminide acetyltransferase), and GNS (coding for N-acetylglucosamine-6-sulfatase), respectively (4). MPS

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IIIA and IIIB are the most prevalent subtypes with incidences ranging between 0.2 and 1.89 per 100,000 live births while the incidence for MPS IIIC is reported to be 0.07-0.21 per 100,000 live births. MPS IIID is extremely rare with an incidence of 0.1 per 100,000 live births (3). Characterized by earlier onset, more rapid symptom progression, the clinical course in MPS IIIA is more severe than other subtypes (5).

Biochemically, MPS III is characterized by abnormal storage of heparan sulfate (HS) in lysosomes of all tissues and organs and its excretion in urine (6). Heparan sulfate is a negatively charged glycosaminoglycan (GAG) covalently bound to a number of proteins at the cell surface and in the extracellular matrix and catabolized within lysosome (7). Its degradation starts with endolytic cleavage by endoglycosidase and proceeds in a stepwise fashion by three exoglycosidases, at least three sulfatases and an acetyltransferase. The deficiency in three of them, α-L-iduronidase, iduronate sulfatase and β-glucuronidase, results in the lysosomal storage disorders MPS I, II and VII, respectively. The other four enzymes (SGSH, NAGLU, HGSNAT, GNS) are specific for HS and a deficiency leads to MPS III (for detailed review see (8). The abnormal storage of GAG affects different signaling pahways by interacting with molecules such as growth factors (9,10). The injury in neurons activates microglia and the constant release of inflammatory mediators. The accumulation in storage vesicles has been detected also in microglial cells in a mouse model of MPS IIIC (11). These cells play an important role in the brain defence and may release different toxic products. Thus, affection of the glial cells together with the inflammation may contribute to neuronal degeneration in MPS III (12). Lysosomal storage of heparan sulfate causes mitochondrial defects, altered autophagy, and neuronal death in the mouse model of mucopolysaccharidosis III type C (13). In addition to HS storage, the secondary accumulation of the gangliosides GM2 and GM3 is observed in lysosomes and other organelles such as mitochondria and Golgi bodies (14,15), either by direct GAG-mediated inhibition of lysosomal enzymes responsible for ganglioside degradation (16) or by deregulated trafficking or synthesis of gangliosides (15).

MOLECULAR GENETIC OF MPS III

MPS III is an autosomal recessive disease with four substypes according to the four enzymatic deficiencies caused by multiple mutations. MPS IIIA is caused by mutations in the SGSH gene resulting in sulfamidase or heparan N-sulfatase deficiency. A total of 137 mutations have been described to date (Human Genome Mutation Database, http://www.hgmd.cf.ac.uk/ac/index.php); most of these are missense mutations (77.3%); also, nonsense mutations, insertions and deletions have been reported. The mutation p.R245H is most common in Germany and the Netherlands, p.R74C in Poland, p.S66W in Sardinia and c.1091delC in Spain (17). The mutations in NAGLU gene encoding α-N-acetylglucosaminidase are responsible for MPS IIIB, where missense mutations outnumber nonsense and deletion mutations (17). Mapping the positions of known missense mutations onto the NAGLU protein revealed that they are scattered throughout the protein and only four missense mutations occur at the active site (18). These missense mutations reduce the stability of NAGLU thus resulting in less functional enzyme (19). MPS IIIC is caused by mutations in the HGSNAT gene localized in a pericentromeric region in chromosome 8p11.21 (20). Although the spectrum of mutations in MPS IIIC patients shows substantial heterogeneity, some of the missense mutations have a high frequency within the patient population such as p.R344C and p.S518F accounting for 22.0% and 29.3%, respectively, of the alleles in Dutch population (21). MPS IIID is caused by mutations in the GNS gene on chromosome 12q14, which encodes N-acetylglucosamine-6-sulfatase

(22). A homozygote c.1169delA (23) and a homozygote p.R355X (24) mutations were the first GNS mutations identified. The nonsense mutation p.Q272X and a large deletion (25), the nonsense mutation p.Q390X, a splice-site mutation (c.876-2A>G) and c.1138_1139insGTCCT are other GNS mutations identified (22).

CLINICAL ASPECTS

Generally, MPS III manifests at 2 to 3 years of age with developmental delays, initially appearing as language deficits followed by behavioral problems, sleep difficulties, progressive cognitive and motor function regression (26). Somatic symptoms in humans can include coarse facial features with broad eyebrows, dark eyelashes, dry and rough hair, and skeletal pathology that affects growth and causes degenerative joint disease, hepatosplenomegaly, macrocephaly, and hearing loss. Unlike other MPS types, major clinical characteristic of MPS III is however degeneration of the central nervous system (CNS), resulting in mental retardation and hyperactivity (7). Although four MPS III subtypes are assumed to be clinically indistinguishable, the clinical course in type A is more severe with earlier onset, rapid progression and shorter survival (27). It was reported that MPS IIIA patients lost their abilities to speak and walk earlier than the MPC IIIC patients. Median age at death is 15.22 ± 4.22 years in MPS IIIA patients, 18.91 ± 7. 33 years in MPS IIIB patients and 23.43 ± 9.47 years in MPS IIIC patients according to the data obtained from the Society of Mucopolysaccharide Diseases (UK) (2). Pneumonia was reported as the leading cause of death for both MPS IIIA and IIIB, accounting for more than 50% and 38%, respectively. Other causes of death include cardiorespiratory failure, gastrointestinal complications and central nervous systemcomplications according to the data obtained from the Society of Mucopolysaccharide Diseases (UK) (2).

PATHOLOGY OF MPS III

The storage of heparan sulfate, secondary accumulation of GM2 and GM3 gangliosides and neuroinflammation events were shown in the brains of MPS IIIA and IIIB mouse brains (12,28-31). In a study to compare neuropathology in mouse models of MPS I, IIIA and IIIB, quantitative immunohistochemistry showed significantly increased lysosomal compartment, GM2 ganglioside storage, neuroinflammation, decreased and mislocalised synaptic vesicle associated membrane protein, (VAMP2), and decreased post-synaptic protein Homer-1 in layers II/III-VI of the primary motor, somatosensory and parietal cortex. In addition, increased HS, abnormally N-, 6-O and 2-O sulphated compared to WT, neuroinflammation, dystrophic axons, axonal storage, and extensive lipid were observed (31). Substantial >30% reduction of neuronal density in somatosensory cortex and substantial loss of purkinje cells in cerebellar cortex have been demonstrated in homozygous Hgsnat-Geo MPS IIIC mice. Neurons of MPS IIIA, IIIB and IIIC mouse models contain SCMAS (subunit C of mitochondrial ATP synthase) aggregates, increased levels of ubiquitin and protein markers of Alzheimer disease and other tauopathies such as lysozyme, hyperphosphorylated tau (Ptau), Ptau kinase, Gsk3β, and β amyloid suggestive of mitophagy and a general impairment of proteolysis (32,33).

Post-mortem studies carried out on brain tissue from children with MPS IIIB revealed the accumulation of phosphorylated α-synuclein in spheroidal structures in the temporal cerebral cortex, hippocampus, periaqueductal gray, substantia nigra and anteroventral nucleus of the thalamus (34). In addition to post-mortem studies carried on brain tissues of patients and animal models, induced pluripotent stem cells (iPSCs) derived from fibroblasts of patients provide access to affected neurons and offer a good opportunity to model human neurodegenerative diseases. In a study to mod-

el MPS IIIB disease, patient iPSC and neuronal progeny of these cells expressed MPS IIIB disease that not apparent in parantel fibroblasts including storage vesicles and severe disorganization of Golgi ribbons associated with modified expression of the Golgi matrix protein GM130 (35).

THERAPY

Currently there is no treatment for MPS III. The cognitive and neurological problems are major clinical characteristics of MPS III. Management consists of supportive care and treatment of specific complications. The neurological nature of the disease makes treatment problematic due to the blood-brain barrier (BBB). There are numerous pre-clinical research projects examining various treatment strategies for MPS III. These recent treatment strategies are summarized and discussed in this review.

Enzyme replacement therapy

Although enzyme replacement therapy (ERT) has been shown to have a positive effect on systemic symptoms of the disease in many MPS types (MPS I, II, IVA, and VI), the main problem with this therapy is delivery of the enzyme to central nervous system (CNS) due to blood brain barrier (BBB) (36). This limits the utility of enzyme ERT for the treatment of neurological symptoms of MPSs. Recombinant caprine GNS enzyme was shown to correct liver pathology of a goat affected by MPS IIID, but it did not result in improvement in the encephalon due to fractional delievery of the enzyme to the CNS (37). A possible strategy to circumvent BBB is direct delivery of the enzyme in the cerebrospinal fluid (CSF) through either intracerebroventricular (ICV) injection into the lateral ventricle, or intrathecal injection into the lumbar spine or subarachnoid space at the cisterna manga (38). A phase 1/2 study of intrathecal heparan-N-sulfatase in patients with mucopolysaccharidosis IIIA appeared generally safe and well tolerated, and resulted in consistent declines in cerebrospinal fluid (CSF) heparan sulfate (39). However, immune responses of patients to recombinant enzyme, high cost of the enzyme, requirement of regular enzyme infusions in a hospital setting are other limitations of ERT. A recent ERT clinical trial for MPS II has shown the inconveniences about the implantation of such devices for periodic delivery of proteins to the CNS (40).

Hematopoietic stem cell transplantation

Although hematopoietic stem cell transplantation (HSCT) was shown to be effective for MPS I-Hurler with improvement of clinical parameters and increased life expectancy, it is not considered an effective method for MPS III because of concerns regarding neurological aspects (41). Patients can benefit HSCT if transplation is performed before somatic and intellectual development are severely affected (42). In this approach, HSCs repopulate the recipient and secrete enzyme which cross-corrects cells in the periphery but cannot cross BBB. However, monocytes traffic from the blood into the brain where they differentiate into microglial cells and mediate crosscorrection in the central nervous system (43). Allogeneic bone marrow transplantation was performed for children with MPS IIIA (44) and IIIB (45) but their neurological conditions were not prevented. Although lentiviral (LV)-transduced wild-type cells improved neuropathology in MPS IIIA mice, lentiviral-transduced autologous MPS IIIA cells were unable to mediate neurological correction, possibly due to insufficient enzyme production in brain (46). However, when transplanted into MPS IIIA mice, autologous HSCs expressing codon optimized SGSH under myeloid-specific promoters CD11b (CD11b-coSGSH vector) normalized MPS IIIA behavior, brain HS, GM2 ganglioside, and neuroinflammation to WT levels (47).

Gene therapy

Gene therapy attempts to introduce the coding se-

quence of the protein (cDNA) into the cells of patients via the use of a viral vector. Manipulated cells synthesize and secrete the enzyme of interest into circulation, which is taken up by unaltered cells (7). Intracerebral, intrathecal (IT), or intracerebroventricular (ICV) injection of adeno-associated viruses (AAV) and lentiviral vectors successfully treated brain disease in MPS I, IIIA, IIIB, and VII animal models, inducing stable expression of the vector and enzyme (48-53). Co-delivery of SGSH or sulfamidase and SUMF1 via intraventricular injection of a recombinant AAV vector resulted in increased sulfamidase activity in the mouse brain, decrease in lysosomal storage and microglial activation and enhancement of motor and cognitive capabilities (48). A clinical trial evaluating intracerebral injection of an AAVrh10hMPS3A vector, an AVV vector encoding both SGSH and the sulfatase modifying factor SUMF-1, in combination with immunosuppressive treatment showed moderate improvements in behavior, attention, and sleep (54). A similar gene therapy approach based on AAV-mediated NAGLU delivery for treating MPS IIIB mice resulted in a significantly prolonged lifespan and improved behavioral performences compared to untreated MPS III mice (55). An AAV-based vector designed to target liver, which included sulfamidase engineered to be fused to both the signal peptide of iduronate-2-sulfatase protein and the BBB binding domain of apolipoprotein B resulted in reduction of neuropathology and restoration of behavior in MPS IIIA mice, where BBB binding domain permitted rescue of sulfamidase in the brain (56).

Finally, a recent study showed that treatment of a new MPS IIID mouse model with adeno-associated viral (AAV) vectors of serotype 9 delivered to the cerebrospinal fluid completely corrected pathological storage, improved lysosomal functionality in the CNS and somatic tissues, resolved neuroinflammation, restored normal behaviour and extended lifespan of treated mice (57).

Substrate reduction therapy

Substrate reduction therapy (SRT) uses small molecules such as the isoflavone compound genistein to decrease the synthesis of HS and hence to improve the balance between the synthesis and degradation. Genistein is thought to impair GAG symthesis by inhibiting tyrosine autophosphorylation of the epidermal growth factor receptor (EGFR), which reduces the expression of factors responsible for GAG synthesis (58,59). Genistein treatment of cultured fibroblasts derived from MPS I, MPS II, MPS III, and MPS VII patients was shown to reduce GAG storage (58,60). GAG storage was also reduced in MPS II and MPS III mice after oral genistein administration (61,62). Although 8 weeks of daily genistein treatment reduced the total GAG content and the size of the lysosomal compartment significantly in the livers of male MPS IIIB mice, no change in total GAGs, lysosomal size or reactive astrogliosis in the brain cortex were observed despite evidence that genistein can cross BBB (61). However, genistein treatment over a 9 month period significantly reduced lysosomal storage, HS and neuroinflammation in the cerebral cortex and hippocampus in MPS IIIB mice, resulting in correction of the behavioural defects observed (63).

In clinical trials that administered genistein to MPS III patients orally in a soy isoflavone extract, mixed results were obtained. Patients treated with 5-10 mg/kg genistein for 12 months did not exhibit cognitive improvements (64,65); however, longer 36-month treatment improved cognitive function (66). In addition, in MPS IIIA mice treated with rhodamine B ((9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene)-diethylammonium chloride), GAG levels decreased both in somatic tissues and brain with an improvement in animal behavior (67,68). However, rhodamine was never tested at a clinical trial since its adverse effect on humans had already been reported (69). N-butyldeoxynojirimycin (miglustat), an inhibitor of ceramide glucosyl-

transferase and therefore of ganglioside synthesis, approved for the treatment for Niemann-Pick type C, has been shown to improve learning and restore the innate fear response in MPS IIIA mice by decreasing ceramide glucosyltransferase activity (70).

Stopcodon readthrough therapy

Premature termination codons (PTCs), also called as nonsense or stop mutations, represent a minor portion of the all mutations responsible for MPS III and cause neglible enzyme activity. In MPS III, they comprise about 10% in Type A, somewhere between 20-30% in Type B, somewhere between 10-20% in Type C and 8% in Type D, of all mutations. Since translation termination is not 100% efficient, a low level of translational read-through of termination codons occur, which results in the incorporation of an amino acid in place of a PTC (71). Some aminoglycosides combine with A site oligonucleotides of ribosome, thus reducing the fidelity of normal translation and promote stopcodon readthrough according to the results obtained from crystallographic and modelling studies (72). Gentamicin, amikacin, paromomycin, G418 (geneticin), lividomycin, tobramycin, and streptomycin were shown to suppress permature termination codons (PTCs) in mammalian cells and result in translation of full-lenght protein protein that is functional when the PTC is not at a crucial position (73). Glutamine (Gln) and tryptophan (Trp) are the most common amino acid insertions; UAG or UAA miscode Gln, whereas UGA miscodes Trp (74). In addition the identitiy of PTC itself and the sequence context around the PTC are crucial factors determining the efficiency of readthrough, with the highest readthrough efficiency observed for UGA codon, followed by UAG, and to a lesser extent, UAA (75).

The first demonstration that aminoglycosides could suppress PTC in a defective gene was carried out in cystic fibrosis (76,77). Since then PTC readthrough has been documented in vitro and in cell and animal models of different disorders including muscular dystrophy (78), methylmalonic-aciduria (79), Stüve-Wiedemann syndrome (80), propionic acidemia (81), phenylketonuria (82), xeroderma pigmentosum (83), mucopolysaccharidosis VI (84), Rett syndrome (85), mucopolysaccharidosis type I-Hurler (86). The toxicity of aminoglycosides in mammals has greatly restricted their potential for successful readthrough therapy and led to searching for better aminoglycoside derivatives with reduced toxicity and enhanced activity (87). A luciferase-based high-throughput screening by PTC Therapeutics identified a non-aminoglycoside readthrough drug, PTC124(88). PTC124 has not adverse effects in contrast to aminoglycosides and has a great potential for treating genetic diseases caused by PTCs. Clinical trials of this drug are underway for patients with cystic fibrosis (phase III), Duchenne muscular dystrophy (DMD) (phase II), and other diseases (89).

The first readthrough study on MPS III disease was carried out on NAGLU and HGSNAT mutations (90), where fibroblasts bearing the p.W168X (NAGLU), p.Q566X (NAGLU), and p.R384X (HGSNAT) mutations were treated with gentamicin, geneticin and five non-aminoglycoside (PTC124, RTC13, RTC14, BZ6 and BZ16) readthrough compounds. Neither of the tested drugs resulted in any recovery at the enzyme acitivity levels for all three mutations. However, a two-fold increase (75-90% of WT) in mRNA recovery for MPS IIIB fibroblasts treated with G418 and about 1.5 fold increase (45-50% of WT levels) in mRNA recovery for MPS IIIC fibroblasts treated with RTC14 and PTC124 was observed. Although no increase in enzyme activity was observed, G418 treatment resulted in high recovery of NAGLU mRNA for p.W168X/p.Q566X genotype, suggesting that the readthrough product was not active (90).

Pharmacological chaperone therapy

In the last last decade, protein misfolding due to missense mutations was demonstrated to be causative for increasing number of inborn errors of metabolism. Missense mutations tend to be more common although insertions, large deletions, premature stop codons and splicing mutations have been identified in many LSDs (91). They occur mostly outside the enzyme's active site and have negative effects on protein folding efficiency, thermodynamic stability, and lysosomal trafficking, although the mutant enzymes retain their catalytic properties (92). Misfolding of proteins due to mutations results in aggregations and hence a wide range of deleterious effects or a lack of catalytic activity. Misfolded proteins are recognized and retained in endoplasmic reticulum (ER) by a protein quality control system that relies on unfolded protein response (UPR) to recover from ER stres (93) and eventually routed for endoplasmic reticulum associated degradation (ERAD). Even in wild-type (WT) proteins, a significant fraction is misfolded or aggregated and degraded by the UPS within minutes of their synthesis despite chaperones (94). If prolonged ER stress continues and misfolded protein cannot be refolded or degraded, UPR causes the cells to undergo apoptosis (95). Another defense mechanism evolved by cells to cope with protein misfolding is chaperone machinery for proper protein folding and their trafficking to organelles. Both these machineries closely coordinate to maintain the proteome in soluble and functional state in different cellular compartments. Proteostasis regulators, chemical chaperones and pharmacological chaperones are small molecular weight compounds to rehabilitate misfolded proteins and therefore restore protein homeostasis in misfolding diseases (92). Chemical chaperones are low molecular weight and membrane-permeable molecules able to nonselectively stabilize mutant proteins, facilitate their folding, and rescue their physiological functionality. Various substances such as glycerol, polyols, dimethylsulfoxide (DMSO) or sodium 4-phenylbutyrate (4-PBA) represent chemical chaperones which also improve the folding of mutant proteins (96-100). From a functional point of view, chemical chaperones can be subgrouped into osmolytes and hydrophobic compounds. Osmolytes are uncharged or zwitterionic molecules that can change solvent properties, hence forcing thermodynamically unstable proteins to fold and stabilize (93). Polyols (glycerol, trehalose, sucrose), trimethylamine N-oxide (TMAO), taurine, β-alanin, glycin may act as osmolytes. Hydrophobic chaperones act as protectors by interacting with the exposed hydrophobic segments of unfolded proteins, thus preventing protein aggregation. 4-PBA is one of the most well-known chemical chaperones and it has been shown to reverse misfolding of various mutant proteins (101,102).

Pharmacological chaperones are small molecules that bind to proteins specifically via electrostatic forces, van der Waals forces, or hydrogen bonding, thus inducing thermodynamic stabilization and contributing to recover protein function. They are protein specific, and some are mutation specific (103). Pharmacological chaperones are competetive inhibitors of enzymes where weaker inhibitors shows minimum enhancement of mutant enzyme activity while more potent inhibitors act as more effective chaperones (104). Enzyme cofactors may act as another type pharmacological chaperones. An increase in the amount of the natural cofactor might stabilize misfolded proteins. A well known example is tetrahydrobiopterin (BH4), the natural cofactor of phenylalanine hydroxylase, the defective enzyme in phenylketonuria (PKU). BH4 treatment is effective in almost half of PKU patients (92). Many chaperone approaches have been assayed at different levels for LSDs such as Fabry (105), GM1-gangliosidosis (106), Pompe (107), Gaucher (108), Krabbe (109), and Niemann-Pick type C (110) diseases.

Iminosugars and azasugars represent a special class of small molecules for pharmacological chaperone therapy with high solubility and low toxicity (111,112). 1-deoxy-galactonojirimycin (DGJ) is an iminosugar used as a pharmacological chaperone for the treatment of Fabry disease and has been approved for use in the European Union under the brand name GalafoldTM (migalastat). Phase 3 studies conducted with patients whose mutations were responsive to migalastat monotherapy showed ≥50% reduction in the storage of globotriaosylceramide (GL-3) in the interstitial capillieries of the kidney following 6 months treatment (113). It was shown that even treatment of wild-type α-galactosidase with 1-deoxy-galactonojirimycin enhances its stabilization as shown by using scanning calorimetry (114), so that this effect of migalastat on α-galactosidase can be benefited for Fabry patients who do not have responsive mutation. By formulating with ERT with intravenous migalastat, the stability of the active form of the enzyme in circulation can be increased. Similarly, improved enzyme activity upon co-incubation of α-glucosidase and the chaperone N-butyldeoxynojirimycin (NB-DNJ) was shown both in vitro and in a mouse model of Pompe disease (107). In the case of type 1 Gaucher disease, pre-inbubation of glucocerebrosidase (GLA) with isofagomine significantluy increased stability of the enzyme to heat, neutral pH, and denaturing agents in vitro, thus resulted in increased intracellular enzyme activity (115).

Since the mutations that cause misfolding are relatively prevalent in MPS III disease, pharmacological chaperone therapy has the potential to be a suitable treatment strategy for the majority of affected patients. It is known that for these diseases, an enzyme activity above 10-20% is sufficient to preclude the development of clinical symptoms. The fact that pharmacological chaperones can be designed to cross the BBB make them candidates for the treatment of neurodegenerative damages of MPS III. A comprehensive evaluation of MPS IIIA mutations via a novel multiparametric algorithm demonstrated that the majority of the SGSH mutations impair proper folding of the three-dimensional conformation of the enzyme (116). This is especially relavant within the context of pharmacological chaperones, a highly promising therapy for the treatment of protein folding diseases. In addition, most of HGSNAT mutations results in misfolding of the enzyme, which is abnormally glycosylated and not targeted to the lysosome, but retained in the endoplasmic reticulum. Glucosamine, which is a competitive inhibitor of HGSNAT enzyme resulted in significant increases HGSNAT activity in eight out of nine patients' fibroblasts, indicating its therapeutic potential (117). Using CpGH89 from Clostridium perfringens, a close bacterial homolog of NAGLU, 2-acetamido-1,2-dideoxynojirimycin (2AcDNJ) and 6-acetamido-6-deoxycastanospermine (6AcCAS) were shown as potential inhibitors to act as pharmacologic chaperones by isothermal titration calorimetry (ITC) and kinetic methods (18).

CONCLUSION

MPS III are presented with serious neurodegeneration which does not have a cure. While other MPS diseases (MPS I, II, IVA and VI) can be treated by ERT and HSCT, there is no such an available therapy for MPS III. Although substrate reduction therapy was shown to be effective in MPS IIIA and IIIB mice, mix results were obtained in human clinical trials. There is not much research in the field of pharmacological chaperone therapy for MPS III except for few studies. Actually, the fact that the majority of disease causing mutations are missense variations that result in misfolding defects and the serious neurodegenerative nature of the disease hold great hopes for therapeutic application of pharmacological chaperones. Nonsense supression or stopcodon readthrough therapy is also an emerging therapy but it is feasible only for

the diseases mostly caused by PTCs such as MPS I-Hurler syndrome. In addition, the discovery of induced pluripotent stem cell (iPSC) technology is a revolution for the drug discovery and modelling of genetic diseases. While the existing animal models for MPS III and other LSDs are valuable, they suffer from partially mimicking the human phenotype. Furthermore, most in vitro studies focusing on pharmacological chaperone screening (and also screening of other small molecules for stopcodon readthrough and substrate reduction therapies) for LSDs have been performed on patient fibroblasts, a cell type not primarily affected in patients. Modelling of relevant neuronal defects using patient-specific iPSC obtained by re-programming of their fibroblasts provides access to human neurons and hence a drug screening platform for screeening of small molecules for therapy.

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