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Chapter 24

Molecular Therapy for Lysosomal Storage Diseases

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Additional information is available at the end of the chapter

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1. Introduction

Lysosomes are organella involving the catabolism of biomolecules extracellularly and intracellularly incorporated, which contain more than 60 distinct acidic hydrolases (lysosomal enzymes) and their co-factors. Lysosomal storage diseases (LSDs) are caused by germ-line gene mutations encoding lysosomal enzymes, their activator proteins, integral membrane proteins, cholesterol transporters and proteins concerning intracellular trafficking of lysosomal enzymes [1,2]. The LSDs associate with excessive accumulation of natural substrates, including glycoconjugates (glycosphingolipids, oligosaccharides derived from glycoproteins, and glycosaminoglycans from proteoglycans) as well as heterogeneous manifestations in both visceral and nervous systems [1,2]. LSDs comprise greater than 40 diseases, of which incidence is about 1 per 100 thousand births, and recognized as so-called 'Orphan diseases'.

In the biosynthesis of lysosomal matrix enzymes, newly synthesized enzymes are N-glycosylated in the endoplasmic reticulum (ER) and then phosphorylated in the Golgi apparatus on the 6 position of the terminal mannose residues (M6P) via two step reactions catalyzed by Golgi-localized phosphotransferase and uncovering enzyme necessary to expose the terminal M6P residues [3,4]. The M6P-carrying enzymes then bind the cation-dependent mannose 6-phosphate receptor (CD-M6PR) at physiological pH in the Golgi. The enzyme–receptor complex is then transported to late-endosomes where the M6P-carrying enzymes dissociate from the receptor at acidic pH, while the CD-M6PR then traffics back to the Golgi as a shuttle. M6P-carrying enzymes are delivered to lysosomes via fusion with late-endosomes. A small percentage of lysosomal enzymes is known secreted from the cell. The secreted M6P-carrying enzymes or the dephosphorylated enzyme with terminal mannose residues can then bind either cation-independent M6P/IGFII receptor (CI-M6PR) or mannose receptor (MR) on the plasma membrane [4,5]. Thus, the extracellular lysosomal enzymes can be endocytosed via both glycan receptors to be delivered to the lysosomes where the captured enzymes can exhibit their normal catabolic functions.

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Many therapeutic approaches developed for LSDs, including bone marrow transplantation (BMT), stem cell-based therapy (SCT), enzyme replacement therapy (ERT) and *ex vivo* gene therapy, are based on this physiologic secretion/uptake system (cross-correction). In successful intravenous ERT for LSDs involving mainly visceral symptoms, including type 1 Gaucher disease [6,7] and mucopolysaccharidosis I (MPS I) [8], MPS VI [9], Fabry [10,11], and Pompe diseases [12,13], either MR or CI-M6PR have been utilized as delivery targets of the recombinant lysosomal enzyme drugs produced by mammalian cell lines including CHO cells and human fibrosarcoma cells. However, intravenous ERT has several disadvantages: i) long-life therapy, ii) requirement of large amounts of recombinant human enzymes, iii) high cost, iv) immune response to the exogenous enzymes [14], and v) ineffectiveness towards LSDs involving neurological signs because of the blood–brain barrier (BBB), although clinical trials are under-going of intrathecal ERT for MPS type I [15], II and IIIB patients. SCT using hematopoietic stem cell (HSC), hematopoietic precursor cell (HPC) and mesenchymal stem cell (MSC) derived from bone marrows has also been utilized as a treatment for LSDs animal models and patients [16-20]. BMT and SCT are based on that stem cells distribute widely *in vivo* as sources continuously producing the deficient enzymes. However, application of BMT is generally limited to LSDs that show a clear beneficial response and for which ERT is not available.

On the other hand, the gene replacement therapy (GT) [21-24] has advantages, including i) long-lasting therapy by a single transduction utilizing recombinant viral gene transfer vectors [25-29], ii) cross-correction effects, and iii) possible CNS-directed application to LSDs involving neurological symptoms [23,24,30-33], whereas GT has disadvantages, including i) low levels and persistence of expression in all tissues of patients, ii) incomplete response to therapy dependent on clinical phenotypes, and iii) insertional mutagenesis resulting in neoplasia. GT is one of the promising therapeutic approaches, especially toward LSDs involving CNS symptoms. In this review, we focus on the challenges to develop the CNS-directed GT for LSDs including GM2 gangliosidoses.

2. GM2 gangliosidoses

Lysosomal β-hexosaminidase (Hex, EC 3.2.1.52) is a glycosidase that catalyzes the hydrolysis of terminal N-acetylhexosamine residues at the non-reducing ends of oligosaccharides of glycoconjugates [34,35]. There are two major Hex isozymes in mammals including man, HexA ($\alpha\beta$, a heterodimer of α- and β-subunits) and HexB ($\beta\beta$, a homodimer of β-subunit), and a minor unstable isozyme, HexS ($\alpha\alpha$, a homodimer of α-subunit). All these Hex isozymes can degrade terminal β-1,4 linked N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) residues, while only HexA and HexS prefer negatively charged substrates and cleave off the terminal N-acetylglucosamine 6-sulfate residues in keratan sulfate. Hex A is essential for cleavage of the GalNAc residue from GM2 ganglioside (GM2) in co-operation with GM2 activator protein (GM2A) [34,35].

Tay-Sachs disease (TSD) (MIM 272800) and Sandhoff disease (SD) (MIM 268800) are autosomal recessive GM2 gangliosidoses caused by germ-line mutations of *HEXA* (locus 15q23-24)

encoding the Hex α-subunit, and *HEXB* (locus 5q13) encoding the Hex β-subunit, respectively [34,35]. The genes exhibit sequence homology, and the gene products exhibit 57% similarity in amino acid sequence. In TSD, the genetic defect of *HEXA* causes a deficiency of HexA (αβ with excessive accumulation of GM2 in the central nervous system (CNS), resulting in neurological disorders, including weakness, startle reaction, early blindness, progressive cerebellar ataxia, psychomotor retardation, and cherry red spots, and macrocephaly. In SD, the inherited defect of *HEXB* leads to simultaneous deficiencies of HexA and HexB with accumulation of GM2 in the CNS and of oligosaccharides carrying the terminal N-acetylhexosaminyl residues at their non-reducing ends, resulting in involvement of the visceral organs including cardiomegaly and minimal hepatosplenomegaly as well as the neurological symptoms. GM2 gangliosidosis AB variant (MIM 272750) is very rare autosomal recessive LSD caused by the gene mutation of GM2 activator protein (*GM2A* locus 5q31.3-q33.1) [34,36]. The gene product GM2A specifically binds GM2 to pull up from membranes in lysosomes, and present it to HexA for degradation of GM2. The deficiency of GM2A also cause the GM2 accumulation and neurological symptoms similar to those of TSD and SD [34,36]. The pathogenic mechanisms of these GM2 ganliosidoses has not been fully elucidated, although neurodegeneration and neuroinflammation have been reported to contribute to the pathogenesis [34,35,37,38].

GM2 gangliosidoses including TSD and SD exhibit a spectrum of clinical phenotypes, which vary from the severe infantile form (classical type), which is of early onset and fatal culminating in death before the age of 4 years, to the late-onset and less severe form (atypical type), which allows survival into childhood (subacute form) or adulthood (chronic form) [34,35,37,38]. Many mutations have been identified for each gene, including missense, deletion and insertion mutations [34,39-41]. Structural information on the basis of the crystal structures of human Hex B [42,43] and HexA [44] allowed us to predict the effects of missense mutaitons identified in TSD [34,39,40] and SD [34,39,41] on the protein structures of mutated gene products. According to these reports, the β-subunit of Hex comprises two domains (domain I and II). Domain I has an α/β topology, and domain II is folded into a $(\beta/\alpha)_8$-barrel with the active site pocket at the *C*-termini of the β-strands. An extrahelix that follows the eighth helix of the $(\beta/\alpha)_8$-barrel is located between domain I and the barrel structure. Only the α-subunit active site can hydrolyze GM2 due to a flexible loop structure that is removed post-translationally from β, and to the presence of αN423 and αR424. The loop structure is involved in binding the GM2A, while αR424 is critical for binding the carboxylate group of the N-acetylneuraminic acid residue of GM2. The β-subunit lacks these key residues and has βD452 and βL453 in their place. The β-subunit therefore cleaves only neutral substrates efficiently. The representative amino acid substitutions have been reported in the α-subunit, including R170W, R178H, W420C, C458Y, L484P, R499C/H, and R504C/H, as well as in the β-subunit, R505Q and C534Y. The dysfunctional and destabilizing defects in Hex α- and β-subunits well reflect biochemical and phenotypic abnormalities in TSD and SD, respectively. Such structural information should be useful to develop novel therapeutic approaches for these disorders [34,45].

3. General aspects of gene therapy for LSDs

Gene therapy (GT) utilizing various vectors for gene transfer has been preclinically and clinically applied for LSDs in recent years [21-33]. Recombinant viral vectors including retroviruses [25,32], adenovirus [26-28], herpes simplex virus (HSV) [33], adeno-associated virus (AAV) [29,46-48] and lentiviruses [24,49-51] are utilized currently as the effective means of gene transfer and enzyme expression. The retroviruses have been used primarily in *ex vivo* applications to transduce the dividing cells, such as HPC, HSC and other stem cells in culture, which are then transplanted into a recipient. However, the retroviral vectors are not suitable for *in vivo* GT due to lack of ability to transduce non-dividing cells. On the other hand, the adenoviruses can infect very efficiently non-dividing cells. However, the use of the early generation adenoviral vectors has been limited due to their strong antigenicity. In contrast, lentiviruses can infect both dividing and non-dividing cells, and are applicable to both *ex vivo* and *in vivo* GT. AAV vectors are able to transduce many cell types *in vivo* effectively, and it is often used as a safer tool for gene transfer because of the lower immunogenicity.

The application of recombinant viral vectors varies dependently on several factors, including ease of vector delivery, expression level in cell types and target tissues and organs mainly affected with LSD. At initial stage of development of GT for LSDs, the *ex vivo* transduction of HPC derived from type 1 Gaucher disease [25] and fibroblasts obtained from MPS VII model mice [32] using retroviral vectors was successful to secrete high levels of the enzymes and corrected the deficiencies. The *ex vivo* GT using retroviral vector and autologous HSC or HPC (human CD34+ cells) derived from bone marrow of the patient as donor cells for transplantation was clinically applied to type 1 Gaucher disease patients, and demonstrated the production of therapeutically effective levels of enzyme activity, resulting in persistent circulating enzyme available to tissues and organs [52]. The transduced cells also migrated into many tissues, expressed high levels of enzyme and reduced lysosomal storage in several critical tissues. However, several problems had been emerged, including less efficiency in transduction of human HSC or HPC using murine-based retroviral vector and difficulty in continuous production of sufficient amounts of recombinant enzymes to maintain the effectiveness.

The lentiviral vector based on human immunodeficiency virus had been expected to overcome the limitation of early generation of murine-based retroviral vectors in *ex vivo* GT [53]. Transduction efficiency of human HPC derived from Gaucher disease patient [54] was improved by using HIV-based lentiviral vector. β-Glucuronidase (GUSB)-deficient mobilized peripheral blood CD34(+) cells from a patient with MPSVII were transduced with a third-generation lentiviral vector encoding human GUSB, and then xenotransplantation to murine model with MPSVII. The corrected cells distributed widely throughout recipient tissues, resulting in significant therapeutic effects including improvements in biochemical parameters and reduction of the lysosomal distension of several host tissues [24].

Direct *in vivo* GT using adenoviral vector have been preclinically applied to murine models with Pompe, Fabry, and Wolman diseases, resulted in sufficient expression of circu-

lating enzymes and reduction of storage materials in the affected tissues [27,55, 56]. However, these therapeutic effects were transient because of the severe immune reactions directed against the adenoviral vector. Single intravenous administration of a modified adenoviral vector to Pompe disease mice was demonstrated to reduce glycogen storage with minimal immune response [57].

The AAV vector has been also developed as an alternative gene transfer tool for direct *in vivo* GT for LSDs. Intramuscular injection of AAV2 serotype vector [58,] in the murine models of Pompe disease, Fabry disease and MPS VII caused high level expression in the muscle tissues but lower levels of circulating enzyme activity [59-61], although the efficacy varied depending on the diseases. Intravenous injection of AAV2 vectors in young adult mice with MPS VII and Fabry disease reduced the lysosomal storage in many tissues [61,62] Significant improvement was observed in MPS VII and MPS I mice following intravenous delivery of AAV2 vector during the neonatal period [28,63]. These findings suggested the effectiveness of AAV vector delivery at early presymptomatic stage to prevent onset rather than delayed intervention for progressive LSDs.

As mentioned above, GT has therapeutic potency for LSDs involving neurological symptoms superior to that of clinically applied intravenous ERT, in which the enzyme cannot cross the BBB. Several CNS-directed *ex vivo* and *in vivo* GT have been performed for animal models of LSDs with brain involvement. Genetically modified bone marrow stromal cells using retroviral vector improved CNS pathology and cognitive function in MPS VII and GM1-gangliosidosis mice following intraventricular transplantation [64, 65]. Genetically modified human neuronal precursor cells (NPCs) differentiated into neurons and astrocytes and expressed β-glucuronidase for at least 6 months after injection into striata of adult MPS VII model mice. However, the cells did not migrate and correction was limited to regions adjacent to the transplantation site [66] *In vivo* GT of metachromatic leukodystrophy (MLD) by lentiviral vector corrected neuropathology and protected against learning impairments in the model mice [49]. CNS-directed in vivo GT using AAV vectors have been demonstrated to have therapeutic effects on the mouse model of LSDs involving neurological signs, including MPS IIIB [67], MPS VII [68], Globoid cell leukodystrophy (GLD) [69], Nieman-Pick A (NPA)[70] and α-mannosidosis [71] by intracranial administration of recombinant AAV vectors. Thus, AAV vectors exhibit a number of properties that have made this vector system an excellent choice for both CNS gene therapy and basic neurobiological investigations. In vivo, the preponderance of AAV vector transduction occurs in neurons where it is possible to obtain long-term, stable gene expression with very little accompanying toxicity. Promoter selection, however, significantly influences the pattern and longevity of neuronal transduction distinct from the tropism inherent to AAV vectors. AAV vectors have successfully manipulated CNS function using a wide variety of approaches including expression of foreign genes, expression of endogenous genes, expression of antisense RNA and expression of RNAi. With the discovery and characterization of different AAV serotypes, as well as the creation of novel chimeric serotypes, the potential patterns of in vivo vector transduction have been expanded substantially, offering alternatives to the more studied AAV 2 serotype. Furthermore, the development of specific AAV chimeras offers the potential to further re-

fine targeting strategies. These different AAV serotypes also provide a solution to the immune silencing that proves to be a realistic likelihood given broad exposure of the human population to the AAV 2 serotype. These advantageous CNS properties of AAV vectors have fostered a wide range of clinically relevant applications including Parkinson's disease, lysosomal storage diseases, Canavan's disease, epilepsy, Huntington's disease and ALS. In many cases the proposed therapies have progressed to phase I/II clinical trials. Each individual application, however, presents a unique set of challenges that must be solved in order to attain clinically effective gene therapies [72].

4. Gene therapy for GM2 gangliosidoses

4.1. Experimental and preclinical gene therapy using animal models

GM2 gangliosidoses, including Tay-Sachs disease (TSD), Sandhoff disease (SD) and the AB variant disorder, are characterized by excessive accumulation of GM2 and neurological symptoms due to progressive neurodegeneration and gliosis, as described above. However, there is no effective therapy for GM2 gangliosidoses at present, although we have reported and proposed the clinical potential of the intrathecal ERT using recombinant modified human HexA [73] and HexB [74,75] in recent years. It is crucial for treatment of GM2 gangliosidoses to develop the CNS-directed molecular therapy including such intrathecal ERT, *ex vivo* and *in vivo* GT or the combined methods including SRT [76]. In this chapter, we would focus on the CNS-directed GT and summarize the preclinical approaches using small and large animal models with GM2 gangliosidoses.

At early stage of development of GT for GM2 gangliosidoses, gene transduction of cultured cells was performed by utilizing recombinant vectors (virus or plasmids), and examined the effect of cross correction due to the secreted Hex isozymes. Guidotti, JE. *et al*. constructed a retroviral vector encoding for the α-subunit of human HexA (*HEXA* cDNA) and transduced the HexA-deficient fibroblasts derived from Tay-Sach disease model mice (*Hexa*$^{-/-}$ mice) [77]. Transduced cells overexpressed the human Hex α-subunit to produce the chimeric HexA composed of human α-subunit and murine β-subunit, which were taken up via CI-M6PR by non-transduced cells and exhibited the cross-correction effect.

On the other hand, Martino *et al*. also constructed a retroviral vector encoding for the α-subunit of human HexA (*HEXA* cDNA) and transduced NIH3T3 murine fibroblasts, resulting in production of large amount of human Hex activity. The secreted Hex was incorporated into the fibroblasts derived from TSD patient, but failed to correct intracellular GM2 storage, probably because of the absence of HexA isozyme sufficient for degrading the accumulated GM2 [78]. Akli S et al. produced a replication-deficient recombinant adenovirus (AdRSV) coding the human *HEXA* cDNA, and transduced the fibroblasts derived from TSD patients. Transdused cells restored the Hex activity ranging from 40 to 84% of the normal, and secreted the Hex a-subunit, which were delivered to lysosomes and degraded the GM2 accumulated in TSD fibroblasts [79].

We transfected an expression vector plasmid coding the human *HEXB* cDNA to fibroblasts derived from Sandhoff disease mice (*Hexb*$^{-/-}$ mice) and established a transformed murine cell line stably producing the human Hex β-subunit [80]. However, the GM2 accumulated in the transformed murine cell line was not reduced, while co-transfection of the human *HEXA* cDNA resulted in restoration of HexA activity and reduction of GM2 storage.

Yamaguchi *et al*. evaluated the systemic *in vivo* GT for *Hexb*$^{-/-}$ mice using cationic liposome-mediated plasmid using the *Hexb*$^{-/-}$ mice [81]. The mice received a single intravenous injection of two plasmids, encoding the human α and β subunits of hexosaminidase cDNAs. As a result, 10–35% of normal levels of Hex expression, theoretically therapeutic levels, were achieved in most visceral organs, but not in the brain, 3 days after injection with decreased levels by day 7. Histochemical staining confirmed widespread enzyme activity in visceral organs. Both GA2 and GM2 were reduced by almost 10% and 50%, respectively, on day 3, and by 60% and 70% on day 7 compared with untreated age-matched *Hexb*$^{-/-}$ mice.

These findings suggested that brain-directed *in vivo* GT based on direct transduction of the affected tissues by single gene transfer or *ex vivo* GT utilizing double genes (i.e. *HEXA* and *HEXB* cDNAs) for producing the homo-specific HexA should be required to achieve the therapeutic effects on TSD and SD. Since then, studies on the CNS-directed *in vivo* GT and *ex vivo* GT have been performed as two streams of development of molecular therapy for GM2 gangliosidoses.

4.2. CNS-directed *in vivo* gene therapy

Bourgoin *et al.* constructed the recombinant adenovirus coding the human *HEXB* cDNA, and transduced the fibroblasts derived from patient with SD resulting in high expression of HexA and HexB activities. They also administered the adenoviral vector intracerebrally to SD mice (*Hexb*-/- mice), and succeeded in expression of near-normal level of enzymatic activity in the entire brain. Co-injection of hyperosmotic concentrations of mannitol with low doses of the adenoviral vector enhanced the vector diffusion in the injected hemisphere without viral cytotoxicity. It was suggested that such combination will allow a high and diffuse transduction efficiency of adeno-viral vector in the brain with higher safety [82].

Martino *et al*. also constructed a non-replicating herpes simplex viral (HSV) vector encoding *Hexa* cDNA. They transplanted the encapsuled recombinant HSV into the brain of *Hexa*-/- mice. The diffusion of recombinant HSV and the secreted HexA derived from transduced neural cells corrected the GM2 storage in the brain during one month due to cross correction effects without adverse effects due to the viral vector [83].

Caillaud and co-workers reported that mono and bicistronic lentiviral vectors based on a simian immunodeficiency virus (SIV) containing the human *HEXA* or/and *HEXB* cDNAs were constructed and tested on the fibroblasts derived from the SD patient [84]. The bicistronic SIV.ASB vector encoding both *HEXA* and *HEXB* cDNAs enabled a massive restoration of Hex activity. A large reduction of GM2 accumulation in SIV.ASB transduced cells. Moreover, the Hex isozymes secreted by transduced SD fibroblasts were endocytosed in deficient cells via CI-M6PR, allowing GM2 metabolism restoration in cross-corrected cells.

Therefore, the bicistronic lentivector supplying both HexA and HexB isozymes may provide a potential therapeutic tool for the treatment of TSD and SD. A mechanistic link was demonstrated among GM2 accumulation, neuronal cell death, reduction of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity, and axonal outgrowth. Arfi *et al*. examined the ability of the SIV.ASB vector to reverse these pathophysiological events, hippocampal neurons derived from embryonic *Hexb*$^{-/-}$ mice, which were transduced with the lentival vector [85]. Normal axonal growth rates, the rate of Ca2+ uptake via the sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA) activity and the sensitivity of the neurons to thapsigargin-induced cell death were restored concomitantly with a decrease in GM2 and GA2 levels. Thus, the bicistronic SIV.ASB vector was revealed to reverse the biochemical defects and down-stream consequences in SD neurons, suggesting its potential of systemic and CNS-directed *in vivo* GT. Kyrkanides S. *et al.* performed the system in vivo GT utilizing the recombinant lentiviral vector FIV coding human *HEXB* cDNA to the neonatal *Hexb*$^{-/-}$ mice via intrapenitoneal administration. They also demonstrated the distribution of Hex isozymes into the CNS, including periventricular areas of the cerebrum as well as in the cerebellar cortex, and reduction of GM2 accumulated in these areas [86].

Cachon-Gonzalez *et al*. has reported that the *Hexb*$^{-/-}$ mice treated by stereotaxic intracranial inoculation of recombinant adeno-associated viral (rAAV) vectors encoding the human *HEXA* and *HEXB* cDNAs, including an HIV tat sequence as a protein transduction domain (PTD), to enhance protein expression and distribution [87]. *Hexb*$^{-/-}$ mice survived for >1 year with sustained, widespread and abundant enzyme delivery in the CNS. Onset of the disease was delayed with preservation of motor function; inflammation and GM2 storage in the brain and spinal cord was reduced. Gene delivery of the human HexA ($\alpha\beta$) by using AAV vectors has realistic potential for treating the TS and SD patients. Sargeant TJ. *et al.* demonstrated that intracranial co -injection of rAAV serotype 2/1 (rAAV2/1) vectors encoding the human *HEXA* and *HEXB* cDNAs prevents neuronal loss in the *Hexb*$^{-/-}$ mice brain tissues, including thalamus, brainstem and spinal cord, and correlated with increased lifespan [88]. Moreover, they performed intracranial co-injection of rAAV2/1 vectors into 1-month-old *Hexb*$^{-/-}$ mice [89]. As a result, the treated mice gave unprecedented survival to 2 years and prevented disease throughout the brain and spinal cord. Classical manifestations of disease, including spasticity were resolved by localized gene transfer to the striatum or cerebellum, respectively. Abundant biosynthesis of Hex isozymes and their global distribution via axonal, perivascular, and cerebrospinal fluid (CSF) spaces, as well as diffusion, account for the sustained phenotypic rescue—long-term protein expression by transduced brain parenchyma, choroid plexus epithelium, and dorsal root ganglia neurons supplies the corrective enzyme. Prolonged survival permitted expression of cryptic disease in organs not accessed by intracranial vector delivery.

4.3. CNS-directed *ex vivo* gene therapy

Ex vivo GT for GM2 gangliosidoses is based on the results of BMT previously reported [90,91]. BMT was demonstrated to prolong life span and ameliorate neurological symptoms in *Hexb*$^{-/-}$ mice [90], and the synergistic effects was also shown in combination with substrate

reduction therapy (SRT) utilizing deoxynojirimycin derivatives [91]. Transduction of neural cells derived from *Hexa*$^{-/-}$ and *Hexb*$^{-/-}$ mice by recombinant viral vectors was performed.

Lacorazza HD *et al.* constructed the ecotropic retrovirus encoding the human *HEXA* cDNA and transduced multipotent neural progenitor cell lines, which stably expressed and secreted high levels of active HexA and cross-corrected the metabolic defect including GM2 storage in TSD fibroblastic cell line. The genetically engineered CNS progenitors were transplanted into the brains of both normal fetal and neonatal mice, in which substantial amounts of human Hex α-subunit and activity were observed throughout the brain enough for therapeutic effect in TSD [92].

Tsuji D. *et al.* constructed a recombinant lentiviral vector encoding the murine *Hexb* cDNA, and transduced microglial cells established from the brains of *Hexb*$^{-/-}$ mice [50]. Transduced microglial cells produced and secreted Hex activity, in which the intracellularly accumulated GM2 and oligosaccharides with terminal N-acetylglucosamine residues were reduced. Transduced microglial cell line was expected as a donor for brain-directed *ex vivo* GT.

Mesenchymal stem cells (MSCs) derived from bone marrow stromal cells are one of the candidates for autologous donor cells for ex vivo GT, and have the multipotency to differentiate under specific culture conditions into other cell types such as osteoblasts, adipocytes, and chondrocytes [93,] as well as into neural lineages [94]. Recently, we established MSCs derived from bone marrow of adult *Hexb*$^{-/-}$ mice. The MSCs expressed cell-type specific markers, including CD29, CD90 and CD54, but not CD45, and exhibited the ability to differentiate into various cell types, including neuron-restricted precursor cells (NRPs) expressing N-CAM carrying polysialic acid (PSA-NCAM). We produced a bicistronic retroviral vector (MSV-*modB*) encoding for the modified human *HEXB* cDNAs (*modB*) causing six α-subunit type amino acid substitutions as well as *EGFP* gene [75]. The gene products, modified HexB (modB, a homodimer of the modified β-subunits) different from the wild-type HexB, can recognize negatively charged artificial substrates and bind to GM2A to exhibit GM2-degrading activity. We transduced the MSCs derived from *Hexb*$^{-/-}$ mice (SD MSCs) with the MSV-*modB*, resulting in restoration of HexA-like activity and reduction of the accumulated GM2 and GlcNAc-oligosaccharides. The modB was also secreted from the transduced SD MSCs. In addition, we performed intraventricular engraftment of the transduced SD MSCs expressing *modB* into the brain of *Hexb*$^{-/-}$ mice. As a result, the injected transduced SD MSCs expressing HexA-like activity and EGFP were observed in periventricular region of the brain (Figure 1). Reduction of the immunoreactivity towards natural substrates including GM2 and GlcNAc-oligosaccharides were also observed around the periventricular region of *Hexb*$^{-/-}$ mice brain (Figure 2). These results suggest that genetically modified MSCs can be utilized as a brain-directed donor cells for *ex vivo* GT for LSDs involving neurological manifestations, including Tay-Sachs and Sandhoff diseases.

Lee J-P. *et al.* demonstrated intracranial transplantation of neural stem cells (NSCs) delayed onset, improved motor function, reduced GM2 storage and prolonged life span in the *Hexb*$^{-/-}$ mice partly due to the cross correction effect of the Hex isozymes secreted from NSCs. Human NSCs derived directly from the CNS and secondarily induced from embryonic stem

(ES) cells also demonstrated a broad repertoire of potentially therapeutic actions, which are expected to be applied for the treatment of neurodegenerative diseases [95]

5. Conclusion

A number of preclinical and therapeutic approaches for GM2 gangliosidoses, including stem cell therapy, substrate deprivation therapy, gene therapy, and enzyme replacement therapy, are being examined and evaluated with disease model mice, although there is no effective therapy for treatment of the patients with GM2 gangliosidoses at present. However, according to the preclinical results obtained by using animal disease models, CNS-directed *in vivo* gene therapy utilizing recombinant viral vectors and *ex vivo* gene therapy based on the cross-correction by transduced autologous and heterologous stem cells are promising for development of novel therapies for LSDs associated with neurological abnormalities, including GM2 gangliosidoses. Improvement of these GTs and their combination with other clinical approaches will facilitate the development of efficient therapies for neurodegenerative disorders caused by neuroinflammation and gliosis.

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