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Neonatal Gene Therapy for Inherited Disorders

Koichi Miyake, Noriko Miyake and Takashi Shimada

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Abstract

In spite of developments of neonatal intensive care medicine, it is still difficult or impossible to treat several inherited genetic disorders using conventional pharmacological methods. Gene therapy is a promising alternate approach for treating a variety of genetic disorders. By the time the patient reaches adulthood, however, it is often too late for effective treatment. But in several of these cases, neonatal gene therapy appears potentially useful against inherited disorders that are not obviously treatable through any other methods. This chapter describes the strategy for neonatal gene therapy for inherited disorders and presents preclinical neonatal gene therapy data for two inherited disorders, metachromatic leukodystrophy and hypophosphatasia. We also discuss the utility, advantages, problems and potential of neonatal gene therapy for inherited disorders.

Keywords: neonatal gene therapy, AAV vectors, metachromatic leukodystrophy, hypophosphatasia

1. Introduction

Although there have been significant advances in neonatal intensive care medicine, several neonatal disorders remain major causes of mortality and morbidity. Consequently, there is an urgent need for development of new safe and effective therapies to improve the outcomes of these intractable and devastating neonatal disorders. Gene therapy is an exciting and promising approach to treat many diseases for which there are still no effective therapies. To date, more than 2400 clinical trials of gene therapy protocols have been attempted in effort to treat various genetic diseases as well as many types of cancers and infectious diseases (http://www.abedia.com/wiley/continents.php). The results of preclinical studies suggest that neonatal gene therapies represent potentially effective treatments for currently intractable neonatal

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disorders [1–6]. However, although neonatal gene therapies have several advantages over similar therapies used in adult patients, there is as yet no clinical protocol for use of gene therapy in newborn infants. This chapter describes a strategy for the use of neonatal gene therapy in the treatment of inherited disorders and presents preclinical neonatal gene therapy data for two inherited disorders, metachromatic leukodystrophy (MLD) and hypophosphatasia (HPP). We also discuss the utility, advantages, problems and the potential of neonatal gene therapeutic approaches for the treatment of inherited disorders.

2. Adeno-associated virus-mediated gene transfer to neonate

Among the numerous viral and nonviral vectors that have been developed to deliver genes of interest into target cells, adeno-associated virus (AAV) vector has emerged as a particularly promising tool for gene delivery, thanks to its safety (AAV is not pathogenic) and its ability to transduce nondividing cells [7–9]. We are now using several AAV vector serotypes (mainly 1–12), depending on the target [10–13]. **Figure 1** shows the results after intravenous injection into neonatal

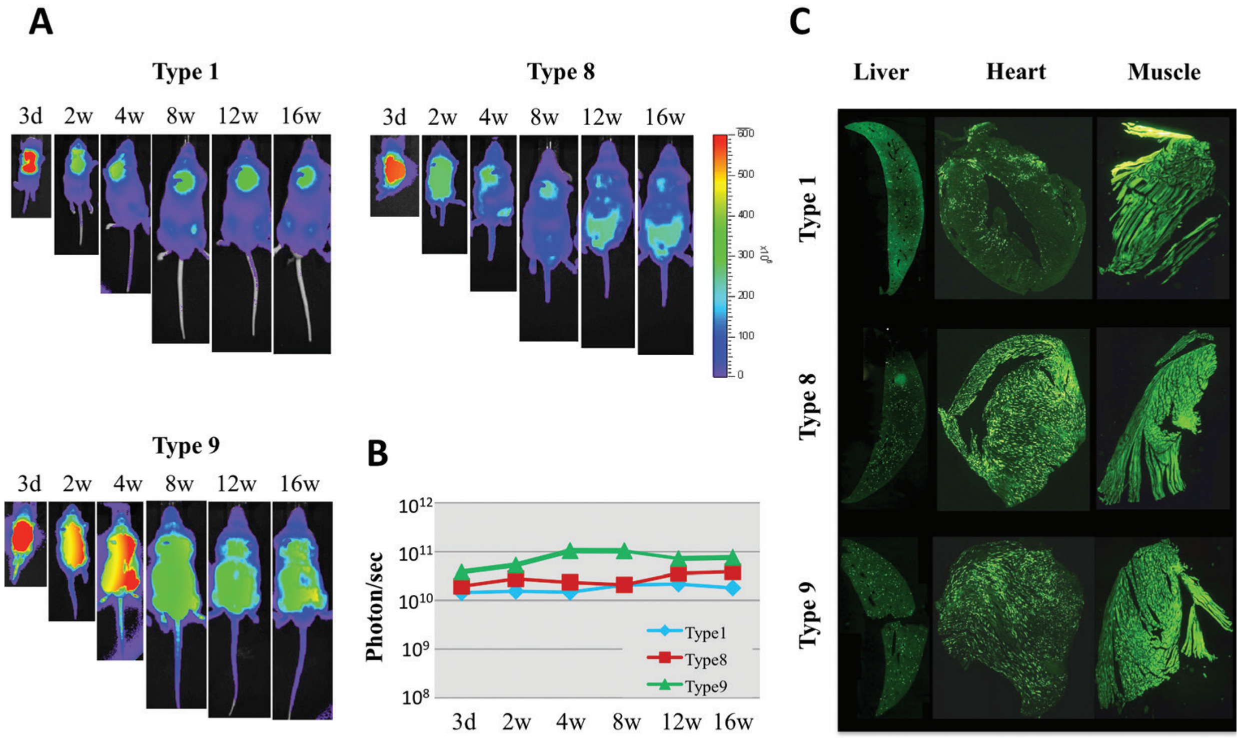

Figure 1. Systemic intravenous injection of AAV vectors into neonatal mice. (A) Approximately 5.0 × 10^{11} vector genomes (vg) of recombinant AAV vectors encoding the luciferase gene (AAV/Luc) (serotype 1, 8, 9) were injected into the external jugular vein of neonatal mice using a syringe with a 29-G needle. Bioluminescent images of mice were obtained using a Xenogen IVIS imaging system 3 days and 2, 4, 8, 12 and 16 weeks after administration. Color scale bar indicates radiant efficiency (photons s^{-1} cm^{-2} $steradian^{-1}$ per μW cm^{-2}). (B) Radiant efficiency of serotype 1 (blue), 8 (red), and 9 (green) AAV vectors injected mice was quantified. (C) Approximately 5.0 × 10^{11} vg of AAV vectors encoding green fluorescent protein (serotype 1, 8, 9) were injected into the external jugular vein of neonatal mice. Sixteen weeks after injection, liver, heart and muscle were stained with anti-GFP antibody.

mice of AAV vector serotypes 1, 8 and 9, harboring the luciferase gene. Expression of luciferase was detected within 3 days and continued for more than 16 weeks with no decrease in expression. Serotype 9 mediated the highest expression during the observation period (**Figure 1A, B**). In addition, using an AAV vector encoding green fluorescent protein (GFP), we determined that the organs most efficiently transduced are the liver, heart and muscle (**Figure 1C**). Moreover, although transduction efficiency was not as high, the central nervous system (CNS) was also transduced after intravenous injection of AAV vector, which apparently passes through the blood-brain barrier (BBB) [14] in neonatal mice [15]. Thus, a systemically administered AAV vector was able to transduce several important target organs in neonatal mice, including the CNS, and mediate expression of a gene of interest for a prolonged period of time.

3. Advantages of neonatal gene therapy

Systemic gene transfer to neonates has several advantages over treatment of the adults (**Table 1**). First, as mentioned above, neonatal gene therapy has the potential to overcome the limitation imposed by the BBB on treating genetic disorders of the CNS. Because the BBB is developmentally immature during the perinatal period, AAV-mediated neonatal gene therapy is a highly promising strategy for treating genetic neurological diseases. Second, because the immune system is immature, neonates are immunologically tolerant of the transgene and/or viral vector [16–18]. Immune rejection of the transgene product by neutralizing antibodies is a severe problem for gene therapy in adults. Third, treatment administered soon after birth may enable prevention of early-onset genetic disease. Finally, neonates can be effectively treated with a smaller amount of viral vector than adults. Using smaller amounts of viral vector is superior with respect to both safety and cost. Taken together, these advantages make systemic neonatal gene therapy a promising method for treating systemic genetic diseases.

- Penetrates the blood-brain barrier
- Induces immune tolerance
- Prevents early-onset genetic diseases
- Enables the use of smaller amounts of vector

Table 1. Advantages of neonatal gene therapy.

4. Application of neonatal gene therapy

4.1. Neonatal gene therapy for metachromatic leukodystrophy

Metachromatic leukodystrophy is an inherited, autosomal recessive lysosomal storage disease (LSD) caused by a deficiency in the lysosomal enzyme arylsulfatase A (ASA), which catalyzes the degradation of galactosyl-3-sulfate ceramide (sulfatide (Sulf)), a major myelin sphingolipid [19]. This disease is characterized by myelin degeneration, mainly in the CNS, and clinically by progressive motor and mental deterioration that is ultimately lethal. Therefore, the major target organ for treatment of this disease is the CNS, and the aim is to arrest or reverse the progression of the neurological symptoms. A major obstacle, however, is the BBB, which limits delivery of systemically administered therapeutic molecules to the brain [14]. It is therefore hoped that systemic administration of an AAV vector harboring ASA during the neonatal period would be useful for treating the CNS. We previously showed that a single systemic injection of AAV vector encoding human ASA (AAV/hASA) into neonatal ASA knockout (MLD) mice results in the wide distribution of ASA in the brain and correction of the biochemical and neurological phenotypes [20]. **Figure 2A** shows that a single systemic injection of AAV/hASA enables transduction of the CNS in neonates but not

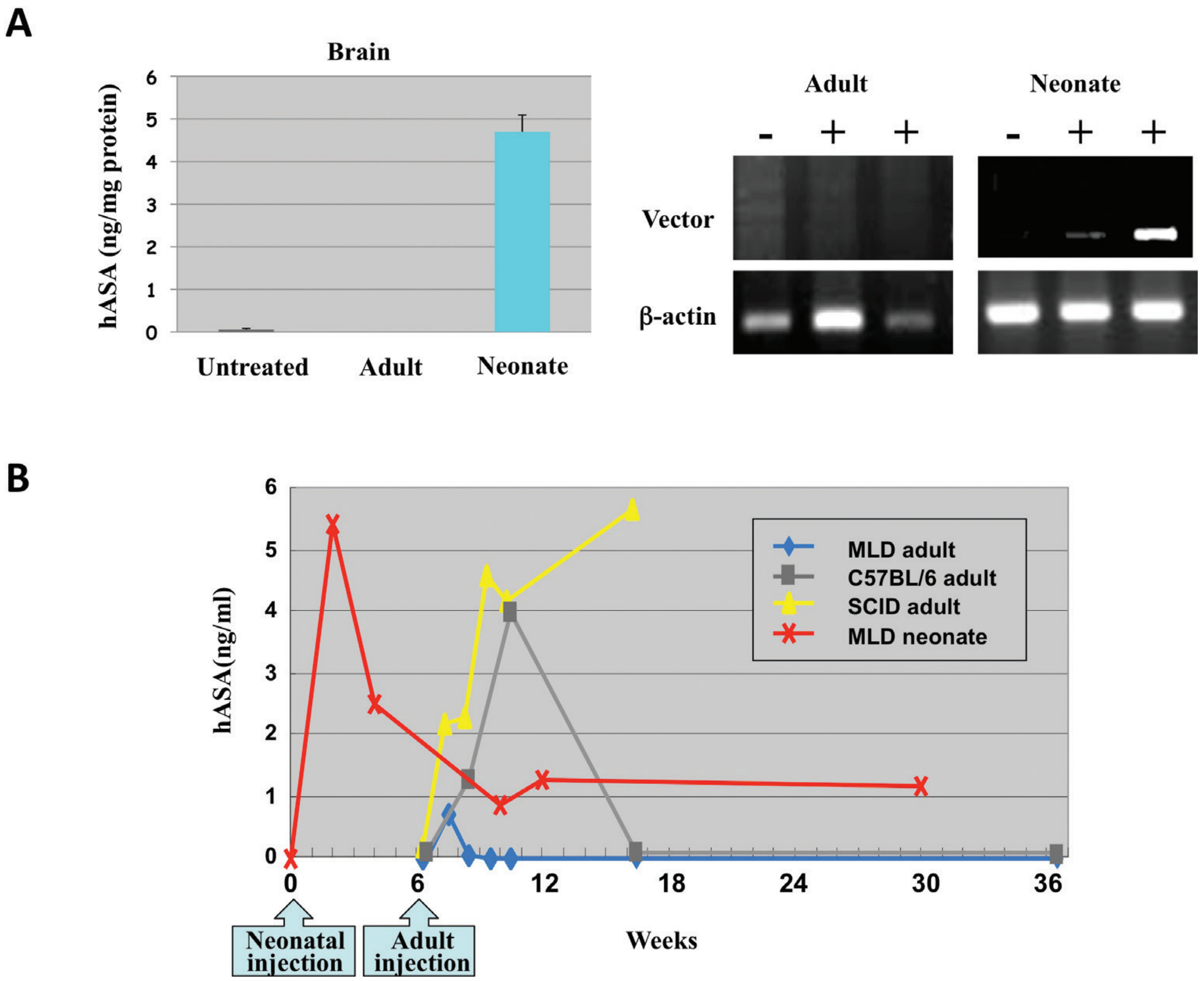

Figure 2. hASA expression of MLD mice following neonatal systemic administration of AAV/hASA vectors. (A) Fifty-two weeks after AAV/hASA injection, hASA concentration in the brain was determined by an indirect sandwich enzyme-linked immunosorbent assay (ELISA) (left panel). DNA from the brain was extracted and analyzed using PCR with hASA-specific primers (right panel). (B) hASA expression in plasma of AAV/hASA-injected mice. hASA concentration in plasma was determined by ELISA. Sustained expression was observed after neonatal injection of AAV/hASA.

in adults. Efficient hASA expression was detected in the brain of AAV/hASA treated at the neonatal period of MLD mice. PCR analysis confirmed that AAV vector genome was observed only in neonatal-treated MLD mice. Moreover, sustained expression of hASA in plasma was detected for at least 30 weeks after intravenous injection into neonatal MLD mice, while only transient increase in plasma hASA was obtained when injected into either adult MLD mice or wild-type C57Bl/6 mice (**Figure 2B**). Vector injection into adult NOD-SCID mice led to sustained secretion of hASA into the circulation, suggesting that immune responses to hASA are a major hurdle for successful gene therapy in immunocompetent adult MLD mice. It thus appears that the systemic injection of AAV vector during the neonatal period is a potentially useful means of treating neurological disorders.

4.2. Neonatal gene therapy for hypophosphatasia

Hypophosphatasia is an inherited disease caused by a deficiency of tissue-nonspecific alkaline phosphatase (TNALP) [21, 22]. The major symptom of human HPP is hypomineralization,

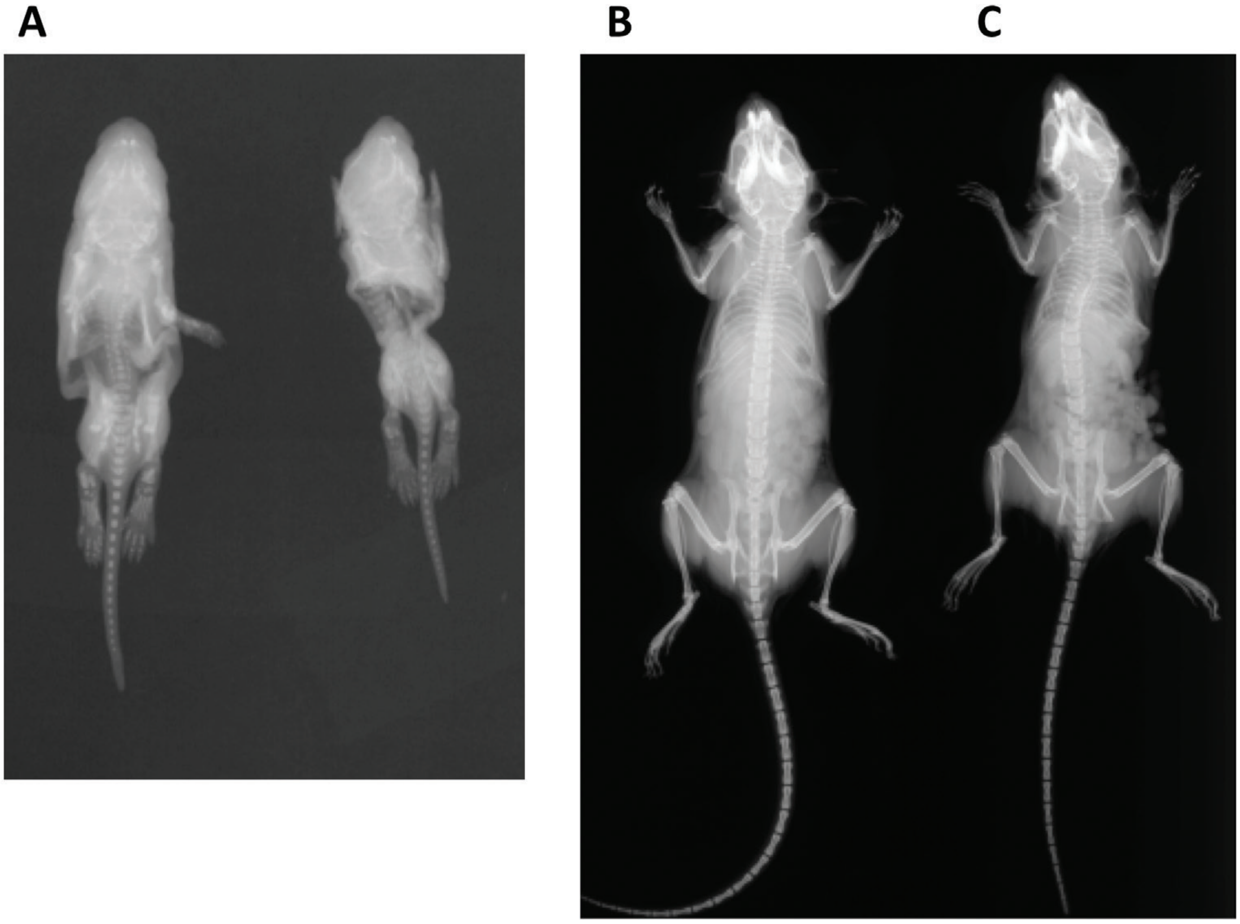

Figure 3. X-ray images of the whole bodies of TNALP knockout mice. Radiographic images were obtained on lFX1000 film (Fujifilm Corp., Tokyo, Japan) using a setup for analysis of small animals. The energy level was 25 kV, and the exposure time was 90 s for 10-day-old untreated TNALP knockout (A), normal wild-type (B) and AAV/TNALP-D10-treated TNALP knockout mice (C).

rickets or osteomalacia, although the clinical severity is highly variable. Patients with infantile HPP may appear normal at birth but gradually develop rickets before reaching 6 months of age. Neonatal gene therapy is a promising strategy for treating infantile HPP by preventing early onset. We have shown that the phenotype of TNALP knockout mice [23–25], which mimics the severe infantile form of HPP, can be prevented by a single neonatal injection of AAV vector encoding bone-targeted TNALP in which a deca-aspartate tail is linked to the C-terminus of soluble TNALP (AAV/TNALP-D10). Sustained expression of TNALP and phenotypic correction of TNALP knockout mice were observed following the neonatal gene therapy [26]. X-ray analysis showed that treated TNALP knockout mice grow as well as normal wild-type mice (**Figure 3**).

5. Problems of neonatal gene therapy

There are several problems that must be overcome before neonatal gene therapies can be used in humans. First, safety concern must be addressed, as there is the possibility of tumor development and of germ-line transmission. It was reported that liver and lung cancers appeared in some mice treated using AAV-mediated neonatal gene therapy [27, 28]. In addition, differences in developmental stages of organs in mice and humans may be another problem. The immune system in mice is less mature at birth than that in larger animals, and the human BBB is functionally mature before birth. It is therefore not clear whether the same beneficial effect of neonatal gene therapy seen in mice would be achieved in human infants. These problems must be overcome before there can be clinical trials of neonatal gene therapy.

6. Summary and future developments

We have shown that AAV-mediated gene transfer in neonatal mice has characteristics that could potentially overcome the problems encountered with current gene therapy protocols. However, before applying neonatal gene transfer to humans, several important issues must be addressed. In particular, the safety of neonatal gene transfer must be carefully evaluated using large animal models, including nonhuman primates. Nonetheless, because of its advantages over gene therapies used to treat genetic disorders in adults, safe and effective neonatal gene therapy has the potential to be an invaluable method for treating genetic diseases.

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