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Precision treatment in neurology

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There is a growing interest in precision medicine treatment. Among the diseases likely to be targeted for precision medicine treatment is hereditary peripheral disease. In genetic diseases, gene mutation is the most important, causing disease; therefore, gene therapy can be used to treat it by precision medicine. However, in the case of complex diseases, it is not easy to control because there are not only genetic causes but also environmental epigenetic factors. Hereditary peripheral neuropathy (HPN) has more than 100 causative genes known. So far, several treatment options for HPN have been developed and clinically evaluated using small molecules, but gene therapy-based treatment strategies have not been actively attempted, because this may be mainly due to the inheritance manner of HPN. Given that gene therapy for genetic diseases began with the simple idea of replacing defective genes with functional replication, the clinical modality strategy is more difficult, as the majority of the causative mutations of HPN lead to gain-of-function rather than loss-of-function. Recent advances in genetic engineering techniques have brought new approaches to gene therapy to clinical applications in HPN therapy. In this review, we reviewed the precision medical treatment of hereditary peripheral neuropathy.

Key Words: precision, peripheral neuropathy, hereditary disease, gene, treatment

Introduction

Treatment options for hereditary peripheral neuropathy (HPN) are very limited. Although several attempts have been made to reduce or improve disease phenotypes through validation in animal studies, the clinical benefits are still uncertain. For example, the therapeutic efficacy of vitamin C has been shown to be successful in rodent models, but clinical trials have not demonstrated its efficacy.[1,2] Recently, a new combination of baclofen, naltrexone hydrochloride and D-sorbitol, PXT3003 is under clinical evaluation, but clinical benefits need to be submitted.[3,4] Unsatisfactory results in clinical practice may be attributed to inappropriate approaches to treating the disease. These approaches focus on the regulation of disease phenotypes by indirectly reducing the expression of toxic proteins or improving myelination. Therefore, direct manipulation of mutant gene expression should be considered to obtain acceptable therapeutic efficacy.

The disease was first described from the 19th century, but the first causative gene was isolated in 1991.[5-8] However, the advent of next-generation sequencing (NGS) technology has accelerated the identification of causative genes and more than 100 distinct genes have been raised as causative genes for HPN.[9]

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Among the numerous causative genes, the prevalence of several genes, such as peripheral myelin protein 22 (PMP22), myelin protein 0 (MPZ), gap junction protein beta 1 (GJB1), and mitofucin 2 (MFN2), are genetically isolated total more than 80%.[10-13] Therefore, most studies of the revealed pathophysiological mechanisms and development treatment options have been focused on these genes.

Applying gene therapy to genetic disorders is a basic and simple strategy to overcome genetic defects. Replacing the mutated gene with a functional replica through gene transfer may be the ultimate treatment strategy to reverse the pathogenesis of disease. Genetic therapy-based treatment options are expanding with new technological advances in genetic manipulation. Current strategies in gene therapy can be categorized into four types: gene replacement, gene addition, gene knockdown or regulation of gene expression and gene editing or correction.[14-17] The simple delivery of functional genes that manipulate the expression of mutated genes with toxic functions or ultimately correct the mutated genes with functional genes has become possible. Although the application of gene therapy has not been clinically attempted in HPN patients, breakthrough advances in gene therapy based therapies with the greatest therapeutic benefit for HPN are expected.

Precision medical treatment of peripheral neuropathy

Most genetic diseases are caused by a single genetic defect, so replacing a defective gene is a simple approach. Therefore, most gene therapy studies focus on gene replacement in recessive genetic diseases. Recently, several genetic replacement methods have been attempted in autosomal recessive and X-linked HPN cases by the European research group. For autosomal recessive cases of HPN, the efficacy of gene replacement therapy for SH3TC2 (SH3 domain and tetra tripeptide repeat 2) mutations in HPN was evaluated.[18] SH3TC2 protein is mainly expressed in myelinating Schwann cells and the loss of functional mutation of the SH3TC2 gene contributes to the development of CMT type 4C, a recessively inherited demyelinating neuropathy.[19] They generated lentiviral vectors expressing the SH3TC2 gene under the control of the Mpz promoter, a Schwann cell specific promoter.

Intrathecal injection of the lentivirus engineered to express the target gene was effectively delivered to Schwann cells in the mouse model and rescued the neuropathic phenotype. After 8 weeks, the mutant mice, exhibited improved myelination in the lumbar spinal roots and sciatic nerves and the motor behavior was also enhanced. Intriguingly, the same group also tried gene replacement therapy to the X-linked dominant type of HPN. GJB1 gene mutations cause loss of Connexin 32 (Cx32) in the gap junctions leading to a severe form of inherited demyelinating CMTX1 neuropathy.[20] The mutations in GJB1 cause dysfunction of Cx32 protein localizing in the paranodal loops of non-compact myelin and the Schmidt-Lanterman incisures in Schwann cells, which leads to demyelinating neuropathy.[21] Although the GJB1 mutation caused phenotype is considered as dominantly inherited, the clinical phenotypes are dramatically different according to gender. The affected female with the heterozygosity in GJB1 mutation exhibit later onset with mild phenotype compared to affected male with hemizygosity due to X-inactivation.[22] For the validation of therapeutic effect of gene replacement, they utilized GJB1-null/Cx32 knockout (KO) mice which exhibit severe demyelination as well as inflammation in the peripheral nerve.[23] Intraneural injection of lentivirus expressing GJB1 by MPZ promoter (LV. Mpz-GJB1) before the phenotype onset of GJB1-null mice significantly reduced the inflammation and ameliorated the peripheral neuropathic phenotype.[24] In the following study, they also validated the efficacy of intrathecal delivery of LV. Mpz-GJB1 in the same mouse model.[25] As intrathecal administration is less invasive than the intraneural delivery, the clinical feasibility of gene therapy is better improved for the demyelinating neuropathy. Recently, the same group also demonstrated that the

therapeutic benefit can be secured even the gene therapy is performed after the onset of peripheral neuropathic symptom.[26] These results increase the possibility of the successful clinical outcomes in future clinical trials for CMT1X patients.

Although demyelinating neuropathy occurs mainly due to dominant inheritance, one research group have consistently developed a phenotype modulating strategy using a neurotrophic factor. Nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3 (NT-3), and neurotrophin-4/5 are well-known nerve growth factors which bind to a protein tyrosine kinase receptor and activate the downstream signaling pathways in the neuronal cells.[27] When NT-3 was subcutaneously administered into Tr-J mouse model, a mouse model of demyelinating neuropathy with naturally occurred Leu16Pro mutation in PMP22 gene, the numbers of myelinated fiber forming regeneration units as well as axonal regeneration was elevated.[28] In the same report, the clinical efficacy of NT-3 in the CMT1A patients was also evaluated. The patients treated NT-3 exhibited enhanced nerve regeneration in the sural nerve. In the following study, they observed that administration of agonistic antibodies to TrkB and TrkC, NT-3 receptors, improved the neuropathic phenotype of a Tr-J mice.[29] Recently, as the long-term treatment of NT-3 is not clinically achievable due to its short half-life, they evaluated the gene delivery of NT-3 with recombinant adeno-associated virus (rAAV).[30] The intramuscular delivery of rAAV-NT-3 sustained the release of NT-3, which promote active myelination and nerve regeneration in Tr-J mice. The clinical benefit of neurotrophic factors in modulating the disease pathogenesis of the demyelinating neuropathy was also observed from other researchers. Administration of neuregulin-1 enhanced the myelination via stimulation of myelination pathways in the rodent models.[31,32]

As the mutation of a gene is translated into mutant proteins via mRNA intermediate, inhibiting the translation of mutant mRNA into mutant protein can be a potential therapeutic target for HPN. For this strategy, utilization of RNA interference (RNAi) is well studied. Small interfering RNA (siRNA) is a short double-stranded RNA with 19-22 nucleotides long[33] which can abrogate the gene expression by breaking down the mRNA transcripts with a sequence-specific manner. Recently, siRNA-based technique has been one of the powerful research tools for gene silencing in both basic and therapeutic research field.[34,35] By the introduction of siRNA or short hairpin RNA (shRNA), the gene expression level can be successfully modulated. In addition, the sequence-specific target mRNA breakdown enables easy discrimination of mutant allele from the wild type sequence in the genetic disorders. Since the dominantly inherited genetic disorders are caused by the toxic gain-of-function mutations rather than loss-of-function mutation in recessively inherited genetic disorders, mutant allele-specific targeting might be the primary therapeutic target rather than the addition of normal genes. Therefore, siRNAs are apt for specific targeting and silencing of the mutant allele in dominantly inherited disorders including neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Machado-Joseph disease, and amyotrophic lateral sclerosis.[36-40]

For HPN treatment, Lee et al. evaluated the efficacy of mutant allele-specific siRNA using Tr-J mice. They designed and isolated the mutant allele (c.47T>C, p.Leu16Pro in mouse Pmp22)-specific siRNA for Tr-J mice and evaluated the potency of allele specificity both *in vitro* and *in vivo*. They revealed that administration of allele-specific siRNA alleviates the neuropathic phenotype of Tr-J mice by improving the myelination and restoring the muscle volume. In the sciatic nerve of the treated mice, the expression level of mutant mRNA was reduced, whereas that of wild-type allele was increased. In this experiment, they validated the efficiency of the non-viral delivery method for HPN gene therapy for the first time, which would be a helpful information for future clinical applications. In addition, they also provided a couple of potent allele-specific siRNAs for human patients with same mu-

tation in PMP22 (CMT type 1E). These results implicate that the targeting mutant allele with specific siRNA might be a potential therapeutic option for dominantly inherited HPN.

CMT1A is the most frequent type of HPN with demyelination of Schwann cells due to a 1.5-fold overexpression of the PMP22, a myelinating protein. As the incidence of CMT1A is over 40% of total HPN, most of the research has been focused on the exploration of novel agents which can decrease the expression level of PMP22. By developing high-throughput screening method and by the aid of systems biology, a couple of research group isolated several repurposed drugs or their combination.[3,41] However, the mode-of-action as well as the potency of drugs in downregulating PMP22 expression is still unclear. Recently, two independent groups developed novel gene therapies which directly manipulate the gene dosage of PMP22. One group isolated novel microRNAs (miRNAs) which specifically target 3′-untranslated region (3′-UTR) of PMP22 mRNA and the other screened antisense oligonucleotides (ASO) which successfully downregulates PMP22 levels.

MicroRNAs (miRNA) are endogenous small noncoding RNAs of approximately 22 nucleotides in length.[37] miRNAs readily bind to the 3' untranslated region of the target mRNAs thereby inducing the degradation of their targets. In addition, miRNAs regulate gene expression by acting as modifiers in silencing the overexpressed genes. The significance of regulatory function of miRNAs in the development of the peripheral nervous system has been investigated. Ablation or reduction of Dicer from Schwann cells can impair normal myelination and axonal integrity.[42-45]

Regarding PMP22 gene expression, several miRNAs such as miR-9 and miR-29b are known to post-transcriptionally target 3′untranslated region (3′UTR) of PMP22.[46] Since miRNAs have great potential in regulating the expression level of target mRNAs, targeting PMP22 with its specific miRNA might be an excellent therapeutic option for controlling CMT1A caused by PMP22 overexpression. In this context, Lee et al. reported that the administration of miRNAs downregulated the Pmp22 expression levels in CMT1A mouse model.[47] They found that the expression level of several miRNAs are changed and miR-381 and miR-9 can modulate the expression level of PMP22. Using lentiviral system, LV-miR-381 as well as LV-miR-9 were administered into the sciatic nerve of the C22 mice which harbors 7 copies of human PMP22 gene and expression level of hPMP22 is 1.7 fold higher than mPMP22.[48-50] Expression of both miR-381 and miR-9 enhance the locomotor function, electrophysiological integrity (motor nerve conduction velocity and compound action potential), and myelination through the reduction of PMP22 level in the sciatic nerve of the C22 mice. This report opens a new way for developing potential HPN therapeutic strategies using miRNA-mediated regulation of gene expression.

RNA transcript can be modulated by ASOs which are synthetic nucleic acids with a single strand and readily binds to the target mRNA resulting in the degradation, interference with pre-mRNA processing or protein binding, and alteration of RNA structure.[51] Recently, the application of ASOs becomes emerging tool to manage various degenerative neuromuscular diseases. The clinical application of ASOs have exhibited successful outcomes in spinal muscular atrophy (SMA) and Duchenne`s muscular dystrophy (DMD) by modulating the splicing of the mRNA.[52-56]

The suppression of the PMP22 expression can be achieved by the hybridization of ASO which results in specific inhibition and degradation of PMP22 through the endogenous RNase H activity. Zhao et al. investigated the potency of PMP22 targeting ASOs in reducing the protein expression using two rodent models for CMT1A.[57] After ASOs treatment both mouse and rat models of CMT1A showed a 35% reduction in PMP22 mRNA, which result in slowing the disease progression as well as improvement of the CMT1 phenotypes. They also suggest that the skin biopsy samples are ideal for detecting the mRNA level of PMP22 and could serve as

a useful biomarker for future clinical trials on CMT1A based on the evidence of the decreased PMP22 mRNA levels in the skin.

Discussion

Through recent advances in genetic engineering techniques, novel gene therapies have been developed and evaluated for HPN using animal models. In order to effectively translate the valid preclinical results of gene therapy into clinical benefits for HPN patients, several aspects must be considered, such as ensuring efficacy and safety. We must carefully consider the selection of delivery vectors and route to improve the efficacy of gene therapy. Viral vectors have generally been used for the delivery of target genes. Virus delivery provides tissue-specific targeting, long-term effects, and large-scale feasibility for cargo genes, but there is still a risk of toxic-mediated immunotoxicity that can interfere with treatment outcomes. On the other hand, gene suppression mediated treatment strategies require relatively short nucleotides compared to the delivery of the whole gene, which allows in vitro synthesis of therapeutic agents and non-viral delivery. In gene therapy for HPN, all gene delivery strategies use Lentivirus or AAV, whereas most gene suppression methods use non-viral methods.

In addition, the determination of the delivery route is an important part of the efficacy and feasibility of gene therapy. Since the target tissue of HPN is the peripheral nerve, the best efficacy can be achieved by intranervous delivery. However, administration of therapeutic agents directly to the peripheral nerves can damage tissues that can worsen the disease phenotype. In this aspect, intrathecal or subcutaneous delivery may be an alternative option for HPN treatment. In the case of gene suppression strategies for target genes using siRNA, miRNA, ASO and CRISPR / Cas9 systems, securing safety is also an urgent task. Although most gene suppression strategies have demonstrated sequence-specificity in vitro and in vivo, the risk of unexpected results due to off-target effects still exists in human clinical trials. Therefore, further investigation is needed to verify safety.

Breakthrough advances in RNA interference or oligonucleotide-based therapies as well as genome editing technologies have developed new treatment options for HPN. In particular, the development of new treatment options for CMT1A can be beneficial to millions of patients with the same mutation in PMP22. The final interpretation of this intuitive concept into reality is a long way, but this meaningful innovation is expected to greatly expand the scope of gene therapy in the near future.

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