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Novel Approaches for the Treatment of Familial Hypercholesterolemia: Current Status and Future Challenges

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Familial hypercholesterolemia (FH) is an autosomal-dominant disorder that is characterized by high plasma low-density lipoprotein cholesterol (LDL-c) levels and an increased risk of cardiovascular disease. Despite the use of high-dose statins and the recent addition of proprotein convertase subtilisin/kexin type 9 inhibitors as a treatment option, many patients with homozygous FH fail to achieve optimal reductions of LDL-c levels. Gene therapy has become one of the most promising research directions for contemporary life sciences and is a potential treatment option for FH. Recent studies have confirmed the efficacy of a recombinant adeno-associated virus 8 vector expressing the human LDL-c receptor gene in a mouse model, and this vector is currently in phase 2 clinical trials. Much progress has also been achieved in the fields of antisense oligonucleotide- and small interfering RNA-based gene therapies, which are in phase 1−2 clinical trials. In addition, novel approaches, such as the use of minicircle DNA vectors, microRNAs, long non-coding RNAs, and the CRISPR/Cas9 gene-editing system, have shown great potential for FH therapy. However, the delivery system, immunogenicity, accuracy, and specificity of gene therapies limit their clinical applications. In this article, we discuss the current status of gene therapy and recent advances that will likely affect the clinical application of gene therapy for the treatment of FH.

Key words: **Familial hypercholesterolemia, MicroRNA, LncRNA, CRISPR/Cas9, Treatment**

Introduction

Familial hypercholesterolemia (FH, OMIM #143 890) affects more than 30 million people worldwide, with prevalence rates of 1/200−1/500 for heterozygous FH (HeFH) and 1/160,000−1/300,000 for homozygous FH (HoFH)[1]. FH is an autosomal-dominant monogenic disease that is mainly caused by mutations in the following three genes: low-density lipoprotein cholesterol receptor (LDLR), apolipoprotein B100 (apoB-100), and proprotein convertase subtilisin/kexin type 9 (PCSK9)[2]. Individuals with chronically high levels of low-density lipoprotein cholesterol (LDL-c) are more likely to develop early atherosclerosis and premature arteriosclerotic cardiovascular disease (ASCVD), particularly patients with HoFH[2]. Despite the recent global interest in FH, because of the associated high risk of ASCVD, the disease remains underdiagnosed and undertreated in most counties[2]. Therefore, many counties and regions around the world have recently launched FH registration studies and guidelines to gradually form a global, efficient, and reasonable FH management model[3, 4].

For most patients, the current lipid-lowering therapies are not able to reduce the LDL-c level to the target value, and this finding is particularly true for patients with HoFH[2]. Indeed, high-dose statins reduce LDL-c levels by only 10%−25%, and high-dose statins combined with ezetimibe result in only a 20%−30% decrease of LDL-c levels for patients with HoFH[2]. Although the combination of lomitapide and mipomersen with statin therapy decreases LCL-c levels by nearly 50%, this treatment strategy causes severe side effects[5, 6]. PCSK9 inhibitors were recently shown to decrease LDL-c levels by more than 50% when combined with statins[7]. However, because most drugs increase the LDLR level

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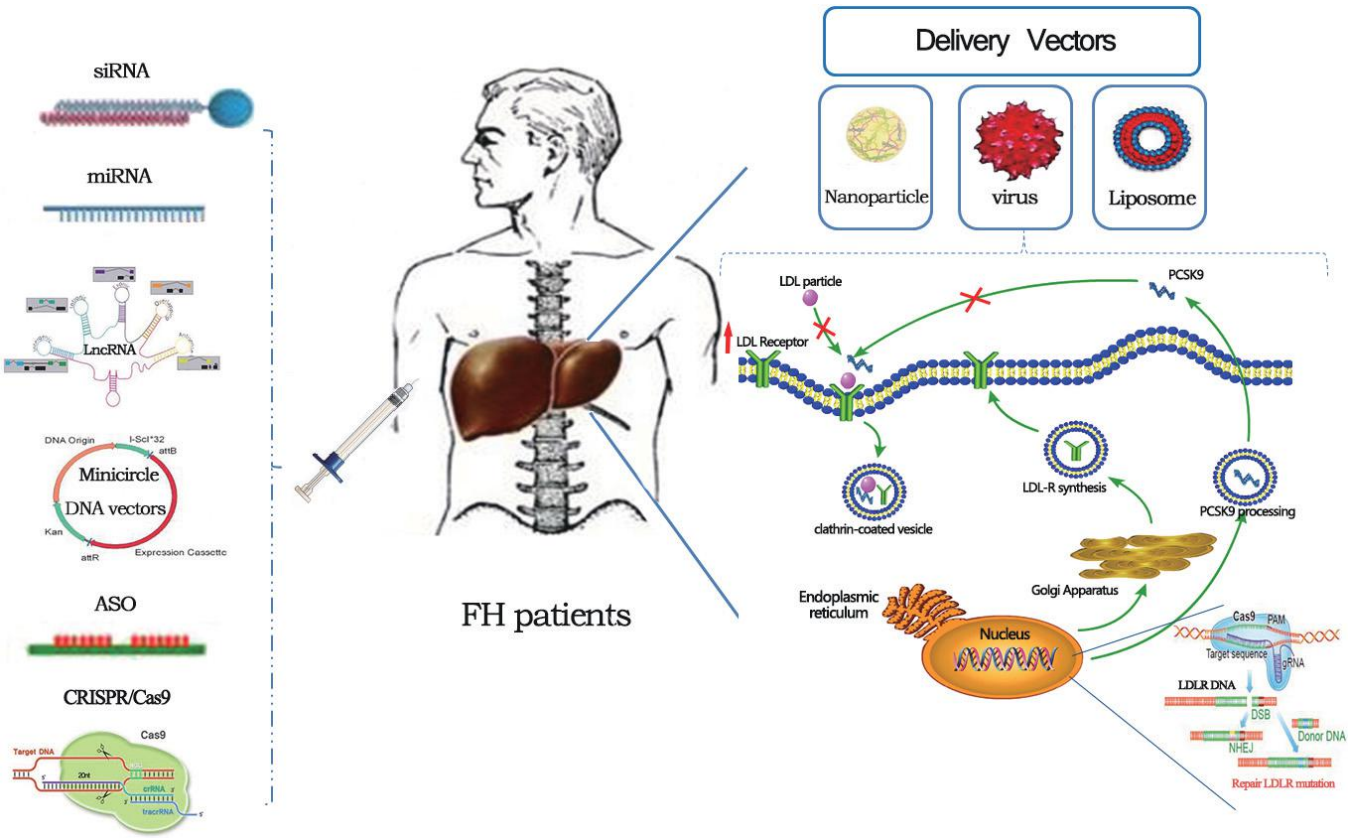

Fig. 1. The current and novel gene therapies for FH.

The siRNA, miRNA, lncRNA, minicircle DNA vector, antisense oligonucleotide (ASO), and CRISPR/Cas9-based methods can be used to treat patients with FH, by replacing the LDLR gene or targeting other lipid-relative genes; the nanoparticle-, virus-, and liposome-based delivery vectors can transport the above subjects into the liver.

by upregulating LDLR expression, structural defects of the LDLR limit the efficacy of those drugs, as they do not alter receptor function. Liver transplantation enhances LDL-c metabolism for patients with HoFH, but this method is difficult to employ because of the lack of donors and the risk of severe immune reactions. Therefore, scientists are seeking new strategies to restore the function of the LDLR *in vivo* and to develop a radical cure.

Gene therapy uses molecular biological methods to transport genes to a patient's body for the treatment or prevention of a disease. The first gene therapy for humans was launched in 1990, when it was used to treat a 4-year-old girl with severe combined immunodeficiency that was due to the lack of adenosine deaminase (ADA)[8]. Researchers performed retroviral-mediated transfer of the ADA gene into T cells, which were then returned to the patient's body, resulting in improved ADA function and continuous ADA expression at the 4-year follow-up, without any severe adverse reactions[8]. Since 2006, the emergence of gene-interference and gene-targeting technologies has highlighted the significance of applying gene interventions in biomedicine and in clinical research on human diseases. In fact, because of its great potential as a treatment for genetic diseases, gene therapy was included in Science magazine's top ten areas of scientific progress in 2009[9]. Currently, gene therapy has become one of the most promising research directions for contemporary life sciences. A review on this topic has been published, although many new technologies and drugs have since been developed and may potentially be applied to treat FH[10]. The present article is an update review that mainly focuses on discussing FH-associated gene therapy, the current confusion regarding treatment, the progress achieved, and the future challenges (**Fig. 1**).

Gene Therapy in the FH Field

1. The History of Gene Therapy for FH

The first use of gene therapy for FH treatment in an animal model was reported in 1991; the authors used recombinant retroviruses to correct autologous hepatocytes from the Watanabe heritable hyperlipid-

Table 1. Ongoing clinical trials for gene therapy for FH

Trial	Vector	Therapeutic Agent	Drug Name	Delivery	Study Design	N	Primary Endpoint	Trial Number
NA	AAV	hLDLR	AAV8.TBG.hLDLR	I.V. injection	Phase 1/2, Open Label	12	52 weeks safety; physical examinations; clinical laboratory parameters	NCT02651675
ORION-2	NA	siRNA-PCSK9	ALN-PCSsc	S.C. injection	Phase 2 Open Label	10	Changes in LDL-c levels at 90 or 180 days	NCT02963311
RADICHOL 1	NA	ASO-ApoB-100	Mipomersen	S.C. injection	Phase 3, RCT/Open Label	51	Changes in LDL-c levels up to week 28	NCT00607373, NCT00706849, NCT00794664, NCT00694109
NA	NA	ASO-ANGPTL3	IONIS ANGPTL3-LRx	S.C. injection	Phase 1/2, RCT	61	Safety and tolerability, pharmacokinetics, and pharmacodynamics up to day 127	NCT02709850

I.V.: intravenous injection; LDL-c: low-density lipoprotein cholesterol; S.C.: subcutaneous injection

emic (WHH) rabbit *ex vivo* and used a liver-directed method to transfer them back into the rabbits. The authors reported a 30%−50% decrease in total cholesterol (TC) levels after 122 days[11]. In 1995, the first clinical trial for FH treatment was launched, in which the authors employed recombinant retroviruses with a normalized LDLR gene to transfect hepatocytes from five patients with HoFH and then transferred the cells back into the patients. However, LDL-c levels decreased by 6%−25% in only three patients[12]. Different strategies were then applied to increase the therapeutic efficacy or reduce the immunogenicity of this approach, including the use of helper-dependent adenoviral vectors (HD-Ads) to deliver the very-low-density lipoprotein receptor (VLDLR) gene[13], transferrin-facilitated intravenous delivery of cationic liposome LDLR gene complexes[14], RNA interference (RNAi)-mediated knockdown of 3-hydroxy-3-methylgutaryl-coenzyme A (HMG CoA) reductase[15], and a balloon-catheter occlusion procedure[16]. However, most techniques are still in the preclinical phase, and many questions remain and must be addressed before these approaches are used in the clinic.

2. Current Gene Therapies in Clinical Trials for FH

(1) Virus Vector-Mediated Gene Therapy

The adeno-associated virus (AAV) is a member of the *Parvovirus* family. The AAV vector has been extensively utilized as a gene-delivery system, because of it offers several advantages such as safety, low immunogenicity, the ability to infect both dividing and non-dividing cells, and the ability to mediate stable gene expression[17]. The report of the first study to evaluate the efficacy of a recombinant AAV8 vector expressing the human LDLR gene in $Ldr^{-/-}$ mice was published in 2004[18]. Compared to AAV2, AAV8 exhibited more efficient hepatocyte transduction (4.2% vs. 81.2% of hepatocytes transduced), more efficient gene transfer (2.1 vs. 52 genome copies [gc]/cell), and an improved lipid profile (TC 227 mg/dL vs. 1,032 mg/dL, $p<0.001$)[18]. Subsequently, many related studies have confirmed that AAV-mediated LDLR gene-transfer therapy is effective. The first clinical trial of AAV8-directed hLDLR gene therapy began in 2016 and will end in 2019; the outcomes include physical examinations, clinical laboratory parameters, and adverse events observed for up to 52 weeks (NCT02651675). Recently, the findings of a phase 1 clinical trial of AAV8-mediated LDLR gene therapy for HoFH were published. No dose-limiting toxicities were observed for doses at or below 6.0×10^{13} gc/kg of AAV8.TBG.hLDLR, and the administration of 7.5×10^{12} gc/kg of the mouse LDLR vector resulted in a >80% reduction of serum cholesterol levels. Notably, an increase in the dose of either the mouse or the human LDLR vector to levels greater than 7.5×10^{12} gc/kg did not further decrease serum cholesterol levels[19]. The results of this clinical trial (NCT02651675) will be published in 2019 (**Table 1**).

(2) Antisense Oligonucleotides (ASOs)

ASOs are short single-stranded sequences of DNA that hybridize with complementary mRNAs in a sequence-specific manner via Watson−Crick base pairing[20]. The antisense drugs that are already available or in clinical development target apoB, lipoprotein(a) [Lp(a)], and angiopoietin-like 3 (ANGPTL3)[20] (**Table 1**).

Mipomersen is a synthetic single-strand antisense oligonucleotide analog that is designed to sequence-specifically bind to the mRNA encoding apoB-100, inhibiting its production and decreasing the secretion of apoB-containing particles from the liver. The first ran-

domized, double-blind, placebo-controlled, phase 3 study of patients with HoFH ended in 2010; this study included 51 patients with HoFH who were randomized in a 2:1 ratio. Mipomersen was administered at 200 mg weekly, and the mean LDL-c level was reduced by 24.7% at 26 weeks[21]. Another recent study performed a post hoc analysis of three randomized trials and an open-label extension study (NCT00607373, NCT00706849, NCT00794664, and NCT00694109) to evaluate major adverse cardiac events (MACEs) resulting from mipomersen treatment among patients with FH. One hundred four patients were included, and the mean LDL-c level was decreased by 28% and the Lp(a) level was decreased by 16.6% at the 1-year follow-up visit. Additionally, only 13 MACEs occurred after the initiation of mipomersen treatment, and a statistically significant difference in the proportion of patients who experienced a MACE before and after treatment with mipomersen was observed at the 2-year follow-up visit[22]. The most frequently reported adverse events were injection-site reactions (76% of patients), elevations of alanine aminotransaminase levels (12% of patients), and potential hepatic toxicity.

Patients with FH display higher Lp(a) levels, and the Lp(a) protein plays an important role in predicting the early onset and severity of coronary artery disease[23]. Therefore, drugs that reduce Lp(a) levels must be developed. In a phase 1 study of healthy subjects with Lp(a) concentrations of 25 nmol/L (100 mg/L) or greater, subcutaneous injections of 300 mg of the ISIS-APO(a)Rx ASO reduced Lp(a) levels and their associated oxidized phospholipid levels by up to 89% and 93%, respectively. Mild injection-site reactions were the most common adverse events[24]. ANGPTL3 is a secretory protein that regulates plasma lipid levels by inhibiting postprandial lipoprotein lipase activity[25]. Loss-of-function mutations in ANGPTL3 not only play key roles in the triglyceride (TG) metabolism of humans but also significantly reduce the incidence of cardiovascular disease[26, 27]. In a phase 1 study, ANGPTL3 ASO (IONIS ANGPTL3-LRx) reduced levels of the ANGPTL3 protein by up to 84.5%, TGs by up to 63.1%, LDL-c by up to 32.9%, very-low-density lipoprotein cholesterol by up to 60%, apoB by up to 25.7%, and apolipoprotein C-Ⅲ by up to 58.8% after 6 weeks of treatment; additionally, no serious adverse events were reported[28]. The phase 2 study of IONIS ANGPTL3-LRx for the treatment of patients with FH is underway [NCT02709850].

(3) Small Interfering RNAs (siRNAs) Targeting PCSK9 Synthesis

siRNAs are a type of short double-stranded RNA (dsRNA) of 21−23 nucleotides (nt) in length. One such siRNA is inclisiran (ALN-PCSsc), which inhibits PCSK9 synthesis and is administered as subcutaneous injections. In the ORION-1 randomized, double-blind, placebo-controlled, multicenter, phase 2 trial, 501 patients with elevated LDL-c levels who were at high risk of developing cardiovascular disease were randomly administered a single dose of placebo or 200, 300, or 500 mg of inclisiran on day 1 or two doses of placebo or 100, 200, or 300 mg of inclisiran on days 1 and 90. According to the results, least-square mean reductions of LDL-c levels at the 180-day follow-up visit were 27.9% to 41.9% after the administration of the single dose of inclisiran and 35.5% to 52.6% after the administration of two doses. More importantly, LDL-c and PCSK9 levels at the 240-day follow-up visit remained significantly lower than the corresponding baseline levels[29]. Based on the results of this trial, siRNAs targeting PCKS9 may be a useful therapy for patients at high risk of developing cardiovascular disease. Recently, an open-label, single-arm, multicenter pilot study was initiated to evaluate the safety, tolerability, and efficacy of ALN-PCSsc for subjects with HoFH; this study will end in 2018 [NCT02963311] (**Table 1**).

Novel Approaches in Pre-Clinical Development and Their Potential Prospects

1. Minicircle DNA Vectors

Minicircle DNA vectors are small (~4 kb), circular, nonviral plasmid derivatives that are generated by eliminating the backbone of DNA sequences from parental plasmids via DNA recombination. Minicircle DNA vectors exhibit several benefits, including longer periods of ectopic expression, higher transfection rates, and greater resistance to shearing forces, in comparison to the characteristics associated with plasmids[30, 31]. Researchers recently constructed special minicircle DNA vectors to mediate the therapeutic expression of the LDLR gene; the vectors include the LDLR promoter or the apolipoprotein E promoter, a hepatic control region, and a modified Kozak sequence[32]. The vector is only 5.23 kb in size, and it was able to physiologically control LDL-c levels *in vitro*. When the vectors were injected into C57BL/6 Ldlr$^{-/-}$ mice by using hydrodynamic methods, LDL-c levels decreased from 140−200 mg/dl to 90−150 mg/dl at 20 weeks, without any detectable toxicity[32]. However, the study did not evaluate the extent of atherosclerosis, and the authors also reported problems with the delivery of minicircle DNA vectors, limiting their application. More research is needed to confirm the potential clinical value of these molecules (**Table 2**).

Table 2. Gene therapies with future potential prospects for FH

Name	Description	Strategy	Potential Target	Animal experiment result	References
Minicircle DNA vectors	Small (~4 kb), circular, nonviral plasmid derivatives	Longer ectopic expression, higher transfection rate, and greater resistance to shearing forces	LDLR	LDL-c levels decreased from 140-200 mg/dl to 90-150 mg/dl at 20 weeks without any detectable toxicity in C57BL/6 LDLR$^{-/-}$mice	[32]
MicroRNAs	Endogenous non-coding single-stranded RNA containing 18-22 nucleotides	Small volume; target multiple genes	Genes involved in cholesterol metabolism	Hydrodynamic injection of pLDLR-LDLR-miR82 into Ldlr$^{-/-}$ mice reduced plasma levels of atherogenic lipids by ~32% and atherosclerosis by ~40% after 12 weeks	[34]
lncRNAs	RNA molecules that are greater than 200 nt in length	Easy association with homologous DNA sequences, homologous RNA sequences, and proteins	Genes involved in cholesterol metabolism	Ldlr$^{-/-}$ mice treated with AAV8.hTBG.LeXis exhibited reduced expression of genes involved in cholesterol biosynthesis, TC and TG levels and atherosclerotic burden	[36]
CRISPR/Cas9	A gene-editing system	Simple production, lower cost, high efficiency, can knock-in or knock-out multigene	Genes involved in cholesterol metabolism	Multiple studies have shown that PCSK9 targeting reduces lipid levels and atherosclerosis in Ldlr$^{-/-}$ mice	[38]

2. MicroRNAs

MicroRNAs (miRNAs) represent a new approach for regulating eukaryotic gene expression, and their use has greatly enriched our understanding of the mechanisms regulating gene expression. miRNAs are endogenous, non-coding, single-stranded RNAs of 18−22 nt; they bind to the 3′ untranslated region of a target gene via complementary base pairing, to inhibit transcription and translation. According to the results from *in vivo* animal studies, several miRNAs influence the LDL-c pathway and delay the progression of atherosclerosis[33]. A study, reported in 2016, included construction of a minigene vector (named pLDLR-LDLR-miR82 [LDLR-Hmgcr-RNAi]); it expressed a human LDLR cDNA and contained an efficient miRNA capable of targeting HMG CoA reductase[34]. Hydrodynamic injection of this episomal nonviral vector into Ldlr$^{-/-}$ mice reduced plasma levels of atherogenic lipids by ~32% and reduced atherosclerosis by ~40% after 12 weeks[34]. As reported in a recent review, many miRNAs regulate the post-transcriptional expression of genes encoding proteins such as LDLR, PCSK9, apoB, and LDLR adaptor protein 1 that are involved in FH pathogenesis[35]. The authors suggested that miRNAs might represent a potential novel therapy, because of the strong regulatory effects of miRNAs on the expression of FH-associated genes[35]. In summary, sufficient evidence is available to suggest that microRNAs may have therapeutic value for FH treatment (**Table 2**).

3. Long Non-Coding RNAs (lncRNAs)

LncRNAs, which are RNA molecules greater than 200 nt in length, do not encode proteins but regulate gene expression by affecting RNAs at various levels (such as epigenetic, transcriptional, and post-transcriptional levels). An AAV8 vector expressing a lncRNA targeting a liver-expressed liver X receptor-induced sequence (*LeXis*) under the control of the human liver-specific thyroxine-binding globulin (TBG) promoter (AAV8.hTBG.LeXis) was recently applied[36], and Ldlr$^{-/-}$ mice treated with AAV8.hTBG.LeXis exhibited reduced expression of genes involved in cholesterol biosynthesis, including Srebp2 and Hmgcr, and a significant decrease in TC and TG levels. In addition, AAV8.hTBG.LeXis significantly reduced the atherosclerotic burden[36]. Thus, lncRNA-based therapeutics have potential clinical implications for the treatment of FH and other diseases (**Table 2**).

4. The CRISPR/Cas9 System

Since 2013, the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system has been widely studied and has become a very hot research topic. A partially highly conserved CRISPR-associated gene (the Cas gene) encoding a functional domain that has nuclease activity that can specifically cleave DNA sequences is located within the vicinity of CRISPR[37]. Compared with traditional gene therapy, CRISPR technology has the advantages of simple production, lower cost, and high efficiency. In 2014, researchers first used CRISPR/*Streptococcus pyogenes* Cas9

(SpCas9) to perform adenovirus-mediated knockdown of PCSK9 expression in mouse liver. This method markedly inhibited PCSK9 expression (>50%) and decreased plasma cholesterol levels (by 35%−40%), without any detectable off-target mutagenesis[38]. A new system named CRISPR/Cpf1, which is small and can cleave DNA and RNA, has also been reported[39]; this system can edit up to four genes in mammalian cells and three genes in mouse brain[39], suggesting that CRISPR/Cpf1 can be applied to achieve multiplex gene editing *in vivo*.

In addition to knocking out genes, the CRISPR/Cas9 system can also knock in sequences, to repair mutations via homology-directed repair[40]. More importantly, CRISPR/Cas9 technology is precise, and the edited genes are heritable. Thus, CRISPR/Cas9-mediated gene editing has been widely studied for the treatment of monogenic inherited diseases[41, 42]. Arginase-1 deficiency is an autosomal recessive disease; diagnosis is delayed, as symptoms do not appear before the age of 3 years. A recent study reports the successful amelioration of an arginase-1 exon 7−8 deficiency of induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells by using the CRISPR/Cas9 system and piggyback technology[43], suggesting that CRISPR/Cas9 is useful for the early treatment of genetic diseases. Indeed, the CRISPR/Cas9 system is expected to be employed to repair LDLR mutations or to knock out multiple genes, such as PCSK9, apoB, and ANGPTL3, to treat FH in the future. A recent study used CRISPR/Cas9 genome-editing technology to permanently correct a three-base pair homozygous deletion in LDLR exon 4 of iPSCs derived from a patient with HoFH, providing an example of the successful use of CRISPR-mediated genetic modification to normalize HoFH-induced deficiencies of cholesterol metabolism at the cellular level[44]. With continuous development, the CRISPR/Cas9 technology will be a strong potential new therapeutic tool for FH treatment (**Table 2**).

Barriers to Gene Therapy

1. Gene Delivery

Delivery is a worldwide challenge for every gene-related therapy. The most widely used method in the contemporary era is virus-mediated delivery. However, this system has several drawbacks: (1) the dose-dependent immune response, which will be discussed further below; (2) neutralizing antibodies[45]; and (3) the limited capacity of AAV, preventing the delivery of the entire CRISPR/Cas9 system *in vivo*. Therefore, several nonviral delivery systems have been established, some of which have been applied in animal models of diseases. One recent study utilized lipid-like nanoparticles to effectively deliver the Cas9 mRNA and single-guide RNA (sgRNA) to the liver and achieved *in vivo* targeting of hepatitis B virus DNA and the Pcsk9 gene[46]. In addition to lipid-based systems, researchers have also employed polymer-based systems, to enable gene editing *in vivo*. For example, Ren *et al.* used pullulan-based ethanolamine-modified poly(glycidyl methacrylate) to deliver both lncRNAs and pDNAs, to treat hepatocellular carcinoma, and showed that the nanocomplexes effectively inhibited tumor growth and induced tumor necrosis, without affecting the health of mice[47]. Furthermore, the combination of AAVs encoding sgRNAs with lipid nanoparticle-mediated delivery of the Cas9 mRNA effectively enabled genome editing *in vivo*[48]. With the progression of this technology, researchers postulate that delivery will cease to be a problem for gene therapy in the near future.

2. Immunogenicity and Transgene Persistence

Immune responses also pose a challenge for gene therapy. Viral vectors, including AAV, have been shown to elicit a dose-dependent immune response against the viral capsid. Approximately 30% of patients from Western populations have preexisting anti-AAV8 neutralizing antibodies[49]; moreover, the anti-AAV8 seroprevalence is >80%[50] in non-Western populations, such as those of Chinese individuals, and many patients might be ineligible for AAV treatment. In addition, the presence of preexisting antibodies against other serotypes, such as AAV2, is even more prevalent[49]. Nonviral delivery systems, such as polymeric or lipidic nanoparticles, have also shown potential toxic and immunogenic effects, although vector-surface modifications may reduce the potential immunogenicity of the components of such systems[51]. Nonetheless, these issues mainly affect classical gene-replacement approaches. In addition, AAV-vector integration occurs at a rate of less than 10% and is associated with lower transgene persistence, because of host-mediated silencing of gene expression over time[52]. As newer methods, such as the application of the CRISPR/Cas9 system, complete genome editing in a few days and do not require sustained expression, immunogenicity is less of an issue.

3. Off-Target Effects

An additional concern is whether therapeutic molecules can exert their therapeutic effect by accurately reaching their target, without causing additional off-target effects. A tissue-specific vector is required to deliver siRNAs, miRNAs, ASOs, and the CRISPR/Cas9 system *in vivo*, and tissue-specific promoters and vector-surface modification can ameliorate these problems. For example, the addition of liver-specific promoters to the AAV system has been shown to achieve

the benefits of ubiquitous expression, while avoiding deleterious immune responses[53]. Interestingly, researchers have constructed a multifunctional nucleus-targeting "core-shell" artificial virus with natural hyaluronan that specially targets CD44 receptors, which are widely overexpressed on many types of tumor cells, thus improving cellular-uptake efficiency[54]. In fact, the artificial virus has been shown to accelerate endosomal escape and promote its penetration into the nucleus without the need for additional nuclear-localization signals. In addition, this system effectively targets ovarian cancer, accompanied by the minimal occurrence of side effects[54]. However, future studies should focus on this problem, to improve the accuracy of gene therapy.

Conclusions and Perspectives

FH has been widely studied, because it is associated with a high risk of coronary heart disease. Thirty-four registered clinical trials have been performed or are ongoing. Gene therapy is one of the most promising developments in this century, and it has led to many surprising discoveries in recent years. Gene therapy is becoming more precise and effective, as a result of the rapid improvements of gene-editing technology. In addition, the new CRISPR/Cas9 gene-editing system has shown great potential for application in gene therapy. In the future, gene therapy will undoubtedly be used to treat FH, without producing severe adverse reactions, thereby alleviating the enormous worldwide disease burden of FH.

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Conflict of Interest Statement

We have no conflicts of interest to declare.

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