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REVIEW

Therapeutic applications of CRISPR/Cas9 system in gene therapy

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Abstract Gene therapy is based on the principle of the genetic manipulation of DNA or RNA for treating and preventing human diseases. The clustered regularly interspaced short palindromic repeats/CRISPR associated nuclease9 (CRISPR/Cas9) system, derived from the acquired immune system in bacteria and archaea, has provided a new tool for accurate manipulation of genomic sequence to attain a therapeutic result. The advantage of CRISPR which made it an easy and flexible tool for diverse genome editing purposes is that a single protein (Cas9) complex with 2 short RNA sequences, function as a site-specific endonuclease. Recently, application of CRISPR/Cas9 system has become popular for therapeutic aims such as gene therapy. In this article, we review the fundamental mechanisms of CRISPR-Cas9 function and summarize preclinical CRISPR-mediated gene therapy reports on a wide variety of disorders.

Keywords CRISPR/cas9 · Gene therapy

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Introduction

Studies have shown that gene therapy is both much more challenging and technically complex than the simple idea of gene replacement. Many of these challenges are based on the ability to accurately control the introduction of genetic material into target cells. Techniques for adding exogenous genes have had remarkable improvements so far, however, there are still a few challenges including unforeseeable effects on gene expression and unpremeditated effects on adjacent genes, limitations in the delivery size of the transgene and finally the addition of exogenous genes cannot always directly address dominant mutations or eliminate undesirable genetic material such as viral genomes or receptors. Targeted gene editing with programmable nucleases, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats/CRISPR-associated nuclease 9 (CRISPR-Cas9) has emerged to make accurate, targeted corrections to genome sequences for resolving these fundamental limitations of conventional methods for gene addition. CRISPR-Cas9 is derived from the type II acquired immune system in bacteria and archaea and presents several advantages upon its counterparts and displays therapeutic potentials (Maeder and Gersbach 2016; Teimourian and Abdollahzadeh 2015). Unlike ZFNs and TALENs, CRISPR/Cas9 for targeting different regions of the genome do not require the engineering of novel

proteins also, able to do efficient multiplex genome editing and has high efficiency of introducing DSBs. In this article, we review the fundamental mechanisms of CRISPR-Cas9 in genome editing and summarize preclinical CRISPR-mediated therapy reports on a wide variety of diseases and disorders (Table 1).

Mechanism of CRISPR-Cas9 in genome editing

Generally, CRISPR/Cas9 works in a similar manner with other programmable nucleases, through RNA-directed endonuclease cleavage of the invading genomic sequence. Short sequences of invading DNA elements derived from phages or plasmids are incorporated into the host genome to form a CRISPR locus flanked by a repeat sequence. Upon subsequent infection, this locus is transcribed and processed into crRNA that harbors a short area of homology ($\approx$ 20 bp) and guides the complex to a special genetic locus. crRNA in cooperation with tracrRNA forms an RNA structure to recruit Cas9 to identify and cleave target DNA after Watson–Crick base pairing among crRNA and the target DNA. Generally four strategies utilize for double-strand break repair including gene knockout/disruption and gene deletion by NHEJ manner or gene correction and gene knock-in/insertion by HDR manner (Maeder and Gersbach 2016). The CRISPR system, as a powerful tool for targeted manipulation of the genome, has a very high potential for basic and clinical research. Recently, the use of this system has become popular for therapeutic purposes such as gene therapy.

Viral infection

CRISPR-Cas9 system as an adaptive immune defense system has shown therapeutic potential in manipulating and disabling viral genome to obstruct virus infection. There are two strategies to tackle latent HIV infection by CRISPR/Cas9 technology. In the first approach, host genes that needed for HIV-1 T cell infection is targeted. The most advanced genome editing-based antiviral therapy to date is the ablation of CCR5 gene by ex vivo adjustment of T cells and CD34+ hematopoietic stem and progenitor cells (HSPCs). In the second approach viral DNA is targeted for mutating vital sites or cleaving proviral DNA from infected cells. CRISPR/Cas9 strategies for targeting viral genome have also been applied to several other viral pathogens, including human papillomavirus, Hepatitis B virus, Herpesviruses, JC Virus and hepatitis C virus (Soppe and Lebbink 2017).

Cancer therapy

CRISPR-Cas9 technology for treating cancer has so far been used in three areas: cancer immunotherapy, manipulation of cancer genome and epigenome and elimination or inactivation of carcinogenic viral infections.

To confer higher affinity for tumor antigen, T cells have been genetically engineered to express altered αβ T-cell receptors (TCRs). Genome editing by CRISPR-Cas9 system for production of the chimeric antigen receptor (CAR) has been broadly recognized as one of the largest progress in personalized cancer immunotherapy. In CAR T cell therapy, autologous T cells are gathered, genetically engineered to attack cancer antigens ex vivo and then transferred back into the patient. Improving of CAR T cell function could be done by CRISPR-Cas9 system via interrupting the genes that coding T cell inhibitory receptors or signaling molecules (Zych et al. 2018).

Oncogenic virus infections are associated with tumorigenesis, for example, HBV and hepatitis C virus in liver cancer, human papillomavirus (HPV) in cervical cancer and Epstein-Barr virus (EBV) in nasopharyngeal carcinoma. CRISPR-mediated knock out of miRNAs can be used in cancer therapy, since miRNAs are involved in the regulation of cellular processes in both normal and pathological events (Chang et al. 2016). Suppression of the transcriptional activities of DNA elements can be done by binding of catalytically inactivated cas9 (dCas9) to them, a method called CRISPRi. Moreover, dCas9 that does not cleave DNA can fuse to histone modifiers and proteins involved in altering DNA methylation and under the steerage of sgRNAs, dCas9 and the modifiers are directed to the goal sites and perform epigenetic regulations (Fineran and Dy 2014).

Cardiovascular disease

Studies have been shown individuals with naturally occurring loss-of-function mutation in proprotein convertase subtilisin/kexin type 9 (PCSK9) have lower levels of LDLC thus disruption of the PCSK9 gene by CRISPR/Cas9 system is a hoping therapeutic

Table 1 Targeted gene therapy using CRISPR/Cas9

Target disease	Target site	Strategy	References
Viral infection			
	Host genome	Gene (HIV receptors) disruption	(Soppe and Lebbink 2017)
	Viral genome	Excising viral genome	(Soppe and Lebbink 2017)
Cancer			
	Cancer antigens	Production CAR-T cells	(Zych et al. 2018)
	Oncogenic virus genome	Elimination oncogenic virus	(Soppe and Lebbink 2017)
	miRNAs	Knock out	(Chang et al. 2016)
Cardiovascular disease			
Coronary heart disease	PCSK9	Gene disruption	(Wang et al. 2016)
WPW syndrome	PRKAG2	Disruption of mutant allele	(Xie et al. 2016)
HCM	MYBPC3	Gene correction	(Ma et al. 2017)
Eye disease			
Cataracts	Crygc	Correction of mutant allele	(Peddle and MacLaren 2017)
LCA10	CEP290	Disruption of aberrant splice site	(Peddle and MacLaren 2017)
AMD	VEGF-A	Gene disruption	(Peddle and MacLaren 2017)
RP	PDE6B, RPGR, Mertk	Gene correction	(Peddle and MacLaren 2017)
	Rho	Disruption of mutant allele	(Peddle and MacLaren 2017)
	Nrl, Nr2e3	Gene disruption	(Peddle and MacLaren 2017)
MECD	KRT12	Gene correction, disruption of mutant allele	(Peddle and MacLaren 2017)
Hematologic disease			
SCD	HBB	Correction of mutant allele	(Dever et al. 2016)
	BCL11A	Gene disruption	(Canver et al. 2015)
β-thalassemia	HBB	Gene correction	(Liu et al. 2017)
Haemophilia-B	F9	Gene knock-in	(Ohmori et al. 2017)
Haemophilia-A	F8	Gene correction	(Park et al. 2015)
Immunological disorders			
SCID	JAK3	Gene correction	(Chang et al. 2015)
CGD	CYBB	Gene correction	(De Ravin et al. 2017)
RA	miR-155	Knock-out	(Jing et al. 2015)
Inborn errors of metabolism			
HT-1	FAH	Gene correction	(Yin et al. 2016)
	Hpd	Gene disruption	(Pankowicz et al. 2016)
OTC deficiency	OTC	Gene correction	(Yang et al. 2016)
Arginase deficiency	Arg1	Gene knock-in	(Lee et al. 2016)
Muscular dystrophy			
DMD	Dys	Gene correction	(Zhu et al. 2017)
		KNOCK-in missing exon	(Li et al. 2015)
		Exon deletion	(El Refaey et al. 2017)
LGMD2B	dysferlin	Gene correction	(Turan et al. 2016)
LGMD2D	α-sarcoglycan	Gene correction	(Turan et al. 2016)
DM1	DMPK	Remove trinucleotides repeat	(van Agtmaal et al. 2017)
Neurological disease			
HD	HTT	Remove trinucleotides repeat	(Monteys et al. 2017)
		Deletion of open reading frame	(Talan 2015)
FA	Frataxin	Remove trinucleotide repeat	(Ouellet et al. 2017)

Table 1 continued

Target disease	Target site	Strategy	References
ALS	SOD1, FUS	Gene correction	(Wang et al. 2017)
Respiratory disease			
CF	CFTR	Gene correction	(Firth et al. 2015)
AATD	AAT	Gene correction, gene disruption	(Smith et al. 2015)
Skin disease			
RDEB	COL7A1	Gene correction	(Hainzl et al. 2017)
DDEB	COL7A1	Disruption of mutant allele	(Shinkuma et al. 2016)

target for the prevention of cardiovascular disease (Wang et al. 2016).

Selectively disrupting disease-causing mutation in WPW syndrome by use of SNP-derived PAM (a pathogenic mutation that leads to the occurrence of a novel PAM) promises an efficient approach in the treatment of WPW syndrome and other dominant inherited diseases (Xie et al. 2016).

Recently, CRISPR/Cas9 system has been used to correct a pathogenic mutation in the MYBPC3 gene in human embryos that causes hypertrophic cardiomyopathy (HCM) (Ma et al. 2017).

Eye disease

Cataracts were the first eye disorder that edited by CRISPR/Cas9 system for therapeutic aim. Wu et al. have corrected a dominant mutation in the Crygc gene via modification of mutant alleles in a CRISPR/Cas9-mediated HDR manner.

Leber Congenital Amaurosis type 10 (LCA10) is the most prevalent type of LCA result from mutations in the CEP290 gene. CRISPR/Cas9-mediated NHEJ approach has been used for deletion of an intronic region in the CEP290 gene comprising a mutation that disrupts the gene coding sequence by generating an aberrant splice site.

Age-related macular degeneration (AMD), Meesmann's epithelial corneal dystrophy (MECD), and Retinitis Pigmentosa (RP) are the other retinal diseases targeted by CRISPR/Cas9 system (Peddle and MacLaren 2017).

Hematologic disease

Two strategies have been used to treat sickle cell disease (SCD) using CRISPR/Cas9 system: correction of the sickle cell mutation and induction of fetal hemoglobin (HbF) expression. In gene correction approach haematopoietic stem cells derived from patients and corrected Ex vivo by CRISPR/Cas9 system, then transplanted to the patient (Dever et al. 2016). BCL11A is a transcriptional regulator that acts as a strong HbF silencer and suppresses the expression of γ-globin, thus suppression of BCL11A can be used to treat both SCD and β-thalassemia (Canver et al. 2015).

By the use of CRISPR/Cas9 system, β-thalassemia-causing mutations in patient-derived induced pluripotent stem cells (iPSCs) can be corrected and then transplanted the corrected iPSCs to the patient (Liu et al. 2017).

CRISPR/Cas9-mediated HDR is one of the strategies has been used to correct mutation in patients with haemophilia B and group C Fanconi Anemia (FA-C). In another approach, the insertion of an exon 2–8 cDNA into intron 1 of F9 has been used with regard to the subject that the majority of identifying F9 mutations located in the coding sequences of exons 2–8 (Ohmori et al. 2017). Near half of all severe haemophilia A are caused by two large (140 or 600 kb) chromosomal inversions in coagulation Factor VIII (F8) and correction of these inversions in patient-derived iPS cells have been made via transfer of an RNP complex of Cas9 and a pair of sgRNAs for targeting two different positions to induce reinversion of the mutations (Park et al. 2015).

Immunological disorders

Mutations in the JAK3 gene result in an autosomal recessive form of T-B+ SCID (Severe combined immunodeficiency). Recently, Chang et al. by use of CRISPR/Cas9-mediated HDR approach corrected point mutation in exon 14 of the JAK3 gene in patient-derived iPSC (Chang et al. 2015).

Patient-derived iPSC from X-linked chronic granulomatous disease (X-CGD) patients containing point mutations in the CYBB gene repaired by CRISPR/Cas9-mediated HDR approach and then transplant to the patients (De Ravin et al. 2017).

Knockout of miR-155, a key proinflammatory regulator in rheumatoid arthritis (RA), by CRISPR/CAS9 system showed inhibition of proinflammatory cytokine production in macrophage cell line and offered a hoping therapeutic strategy in RA treatment (Jing et al. 2015).

Inborn errors of metabolism (IEM)

Mutation in fumarylacetoacetate hydrolase (FAH) that catalyzes the ultimate step of tyrosine catabolism results in Hereditary Tyrosinemia type I (HT-I). By CRISPR/Cas9-mediated HDR homozygous G → A point mutation of the final nucleotide of exon 8 in FAH has been corrected (Yin et al. 2016). In a new gene therapy strategy, HPD gene deleted in liver via CRISPR/Cas9 system to reprogram the metabolic pathways showed higher therapeutic effect in the treatment of HT-I than directly correcting the pathogenic mutation in FAH (Pankowicz et al. 2016).

Ornithine transcarbamylase (OTC) deficiency, a disease resulting from an X-linked deficiency of the OTC enzyme, leading to hyperammonemia. Yang et al. corrected a G → A point mutation of the OTC gene by co-delivery of two AAV vectors, one carrying a sgRNA and the donor DNA and the other carrying Cas9 nuclease (Yang et al. 2016). Arginase deficiency corrected by addition of arginase 1 cDNA into exon 1 of hypoxanthine–guanine phosphoribosyltransferase (HPRT) locus in iPSCs (Lee et al. 2016).

Muscular dystrophy

The strategies used to treat Duchenne muscular dystrophy (DMD) include: correcting of point mutations, knock-in of the full missing exon, and restoring DMD's reading frame by deletion of the mutated exons. CRISPR/Cas9-mediated HDR approach has been used for correction of point mutation (Zhu et al. 2017) and the addition of missing exon 44 (Li et al. 2015). Deletion of one or more exons from the mutated transcript can restore the reading frame and generate a truncated but yet functional protein. This approach has been used to remove exon 23, exons 20–23, exons 50–54 and exons 45–55 by using multiple gRNAs that targeted regions surrounding desired exon(s) to generate more than one DSB simultaneously and removing large segments (El Refaey et al. 2017).

Limb-girdle muscular dystrophies types 2B (LGMD2B), types 2D (LGMD2D) and Myotonic dystrophy type 1 (DM1) are other muscular dystrophy that targeted by CRISPR/Cas9 (Turan et al. 2016; van Agtmaal et al. 2017).

Neurological disorders

So far, two strategies for gene therapy of progressive neurodegenerative diseases have been used, including deletion of pathogenic mutations and targeted gene correction. CRISPR/Cas9 system can be used for targeted deletion of the CAG repeats by sgRNA/Cas9 collections that flanked this domain and produced double strand breaks which subsequently induced non-homologous end joining (NHEJ) process (Monteys et al. 2017). Deletion of the open reading frame of HTT can also decrease mutant huntingtin mass (Talan 2015). Friedreich Ataxia and Amyotrophic lateral sclerosis (ALS) are other disease that targeted by CRISPR/Cas9 (Ouellet et al. 2017; Wang et al. 2017).

Respiratory disorders

The ΔF508 mutation is the first mutation identified in CFTR and present in 90% of cystic fibrosis (CF) patients and two studies have been done for the correction of ΔF508 mutation in iPCS via CRISPR/Cas9-mediated HDR approach (Firth et al. 2015). Alpha-1 Antitrypsin Deficiency (AATD) was another respiratory disease that edited by CRISPR/Cas9 system via both HDR based precise gene correction and NHEJ-mediated gene disruption (Smith et al. 2015).

Skin disease

Dystrophic Epidermolysis bullosa, a blistering skin disorder, caused by mutations in the COL7A1 gene and can be inherited in an autosomal recessive (RDEB) or dominant manner (DDEB). CRISPR/Cas9-mediated HDR approach can correct the causative mutation in patient-derived RDEB fibroblasts (Hainzl et al. 2017). Dominant negative mutations in the COL7A1 gene leading to DDEB and reading frame disruption of the mutant allele by CRISPR/Cas9-mediated NHEJ, while wild-type allele remains intact, has been applied for correction of these dominant negative disorder (Shinkuma, Guo, and Christiano 2016).

Commercializing the CRISPR technology

CRISPR/Cas9, as an incredibly powerful technology, will play a key role in how we diagnose and treat disease, thus a number of companies have invested in CRISPR technology. There are three big players in CRISPR-based gene-editing biotech companies including Editas Medicine (editas medicine. 2018), Intellia Therapeutics (Intellia Therapeutics. 2018), and CRISPR Therapeutics (CRISPR Therapeutics .2018). For example, CRISPR Therapeutics approaches classify in four main areas: (i) ex vivo approaches involving gene editing of hematopoietic cells for hemoglobinopathies, (ii) ex vivo approaches in immuno-oncology for cancer, (iii) in vivo approaches targeting the liver (haemophilia) and (iv) additional in vivo approaches targeting other organ systems, such as muscle (DMD) and lung (CF).So, if CRISPR is able to target all monogenic diseases diagnosed every year, this market worth billions dollar globally, also by addition of cancers, this market becomes more valuable.

Challenges and outlooks

CRISPR/Cas9 system showing new era in correcting gene defects to treating a numerous variety of human diseases by gene therapy, but several technical challenges still need to be solved regarding accuracy and efficiency issues. Various approaches have been explored to minimize Cas9-mediated off-target events that are mentioned in referenced papers (Chira et al. 2017; Ren and Zhao 2017). Several approaches for increasing HDR rate have been described, including inhibition of the NHEJ pathway, use of longer homologous donor DNA, cell cycle synchronization techniques for control the transfer time of Cas9 protein and sgRNA complexes (because HDR takes place mainly in S and G2 phases) and targeting non-target DNA strand that was free of protein interaction and located in the PAM – distal (Lin et al. 2014; Gibson and Yang 2017). Also, Homology-Independent Targeted Integration (HITI) approach may solve low HDR efficiency (Nygaard et al. 2016). After solving these current challenges and improvement of the delivery methods, the CRISPR-Cas9 system can apply for clinical applications in patients. Recently this system has been approved by an advisory committee at the US National Institute of Health as the first clinical trial to attack cancer cells (Reardon 2016).

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