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CRISPR-CAS9 TECHNOLOGY AND ITS APPLICATION IN CONGENITAL DISORDERS

Medical Science

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ABSTRACT

Background: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated Cas9 (CRISPR/Cas9) system has been using from last few years in the field of biomedical research. Here, applications of CRISPR-Cas9 technology in congenital disorders, current and future directions are reviewed and discussed.

Methods: This non-systematic review was prepared using search engine: PubMed, Google Scholar and Medline for articles published from 2005 through 2017, using the following keywords: genome editing, CRISPR/Cas9, genetic disorders, DMD, Cystic fibrosa, hemophilia, Thalassemia etc.

Results: The defect in gene sequences can be corrected by deletion or insertion using CRISPR/Cas9 and this property has made this gene editing tool for wider applications.

Conclusion: CRISPR/Cas9 genome editing could be used in the future to correct inherited mutations and it is expected that in near future, genome editing technologies would help to accelerate the therapeutic directions towards understanding of molecular mechanism of disease at gene level.

KEYWORDS

CRISPR-Cas9; Congenital disorders; Genetic engineering; Gene therapy

Introduction

Gene targeting by homologous DNA repair has the potential to treat many different diseases, including clotting disorders such as hemophilia A and B, muscular dystrophy, cystic fibrosis, Fabry disease, Gaucher disease, Pompe disease, von Gierke disease, and Hurler and Hunter syndromes and so on. For gene manipulation at desired location, designer DNA endonucleases such as zinc finger nucleases (ZFN) based on eukaryotic transcription factors[1], transcription activator-like effectors (TALENs) from Xanthomonas bacteria has been used in several species[2-4]. Though, these technologies have some limitations that prevent their therapeutic use in congenital disorders. CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system, is an RNA-guided DNA nuclease are derived from an adaptive immune system that found in bacteria. In recent years, CRISPR-Cas9 system has been used to edit genome in many species, such as zebrafish[5], frog[6], mouse[7] and human[8]. These translational results showed that CRISPR/Cas9 have created new opportunities and would useful in near future for correcting congenital diseases. However, drawback of this tool is the recognition of off-target, which involves the deletion of nonspecific DNA sequences. The each programmable nucleases has own advantages and disadvantages, and can be executed diversely to treat particular disease.

Duchene muscular dystrophy (DMD) is an inherited X-linked fatal genetic muscle disease, produced by in-frame deletions affecting the dystrophin gene. Presently, there is no cure for DMD however, genome based editing approaches for exon removal has been used[9-10]. In general, 13% of DMD patient have mutation in exon 51. Restoring of exon 51 via correction of dystrophin gene expression would be great importance. Recently, functional dystrophin gene restoration has been demonstrated by genome editing in iPSCs derived from a patient lacking exon 44 and 45 using CRISPR-Cas9[10]. These results suggest that CRISPR/Cas9 tool possess the therapeutic potential to cure human congenital/fatal diseases including DMD. However, these approaches and strategies have some limitations and issues which need to be rectify before clinical application.

Hemophilia, one of the most common inherited blood diseases, is caused by mutations in the human hemoglobin beta (HBB) gene. To date there is only one curative treatment for b-thalassemia is allogeneic haematopoietic stem cell transplantation (HSCT). Gene therapy could be another option for treating hemophilia via correction of the defective gene. In recent years, CRISPR-Cas9 technology has been successfully applied to correct b-thalassaemia mutations in patient-derived induced pluripotent stem cells (iPSCs)[11-13]. These corrected iPSCs displayed normal function and could provide a source of cells for transplantation in patients, offering a new strategy to cure this disease suggests that CRISPR/Cas9 can be apply for treating hemophilia.

Cystic fibrosis (CF) is a chronic and progressive autosomal recessive genetic disorder, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on the long arm of chromosome 7. CF remains the most common and lethal genetic disease among the Caucasian population and need to develop better therapeutic intervention. It has been successfully demonstrated that CFTR mutation can be corrected using ZFN[14] and CRISPR[15-16]. In 2016, Vituret et al[17] demonstrated functional correction of the genetic defect in human CF cells by using microvesicles and exosomes as vectors. These studies suggest that mutation in CFTR gene can be corrected using gene editing tool.

Sickle cell anemia(SCI) and beta-thalassemia which was caused by single gene mutation of human b-globin gene (HBB) in hemoglobin chain. Till date, there is no available therapy to completely cure the sickle cell anemia except allogeneic hematopoietic stem cell transplantation (HSCT). It is reported that by using novel editing tools like ZFNs, TALENs and CRISPR/Cas9, these gene defect can be corrected. Recently, one of the studies has demonstrated correction of β-globin gene in $CD34^+$ cells using the optimized CRISPR/Cas9 system and donor template. Further, they showed that corrected cells gain the potential to develop into erythroid cells and started to produce β-globin protein[18]. Likewise, in a study, combination of CRISPR/Cas9 and the piggyBac transposon has been used to cleave the HBB gene and showed correction of two different β-thalassemia mutations in iPSCs from β-thalassemia patients[11]. This finding suggests that correction of faulty gene in monogenetic disorders is possible using CRISPR/Cas9 gene editing tool.

Huntington's disease (HD) is a severe neurodegenerative disorder caused by the autosomal dominant mutation in the first exon of the HTT gene encoding huntingtin protein. The symptoms of HD patient are movement disorders, cognitive impairment, and psychiatric disturbances due to the presence of excessive trinucleotide repeat (CAG repeats) in HTT as compared to normal person. Recent studies have demonstrated that use of CRISPR/Cas9 system useful to correct neurodegenerative disease in cell line as wells as in small animal models[19]. The multifactorial neurodegenerative disorders like Parkinson's disease (PD), Alzheimer's disease and amyotrophic lateral sclerosis are caused by abnormal protein folding. The gene editing tool

like CRISPR/Cas9 could be able to modify or correct abnormal protein production and prevent their accumulation in pathological condition. Therefore, CRISPR/Cas9 system can also be used to manipulate inherited diseases by targeting DNA regulators or enhancers of pathogenic genes.

Conclusion

Gene editing technologies like CRISPR/Cas9 could be used for desired genome manipulation. Further, studies have been suggested that CRISPR/Cas9 genome editing would become a novel tool to correct inherited mutations in near future. It is expected that in near future, genome editing technologies would also help to accelerate the therapeutic directions towards understanding of molecular mechanism of disease at gene level.

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Conflict of interest

The authors have no conflict to declare

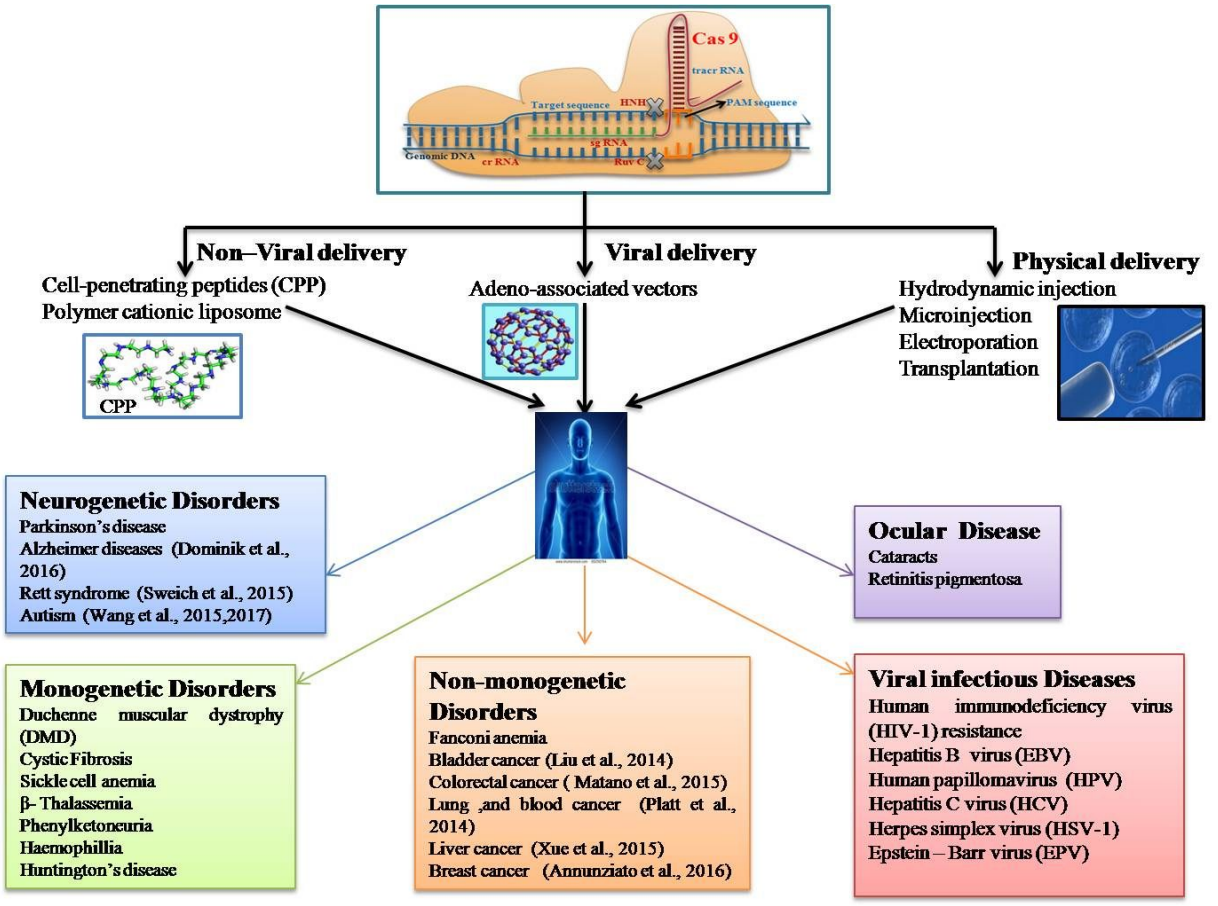

Figure 1. Applications of CRISPR/Cas9 in genetic disorders.

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