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Perspective

In-Vivo Gene Therapy Prospective of CRISPR-Cas19

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1. Introduction

Gene therapy (also known as human gene transfer) is a medical field that focuses on the therapeutic distribution of nucleic acid into a patient's cells as a drug to treat disease. The first therapeutic use of gene transfer as well as the first human DNA is inserted directly into the nuclear gene performed by Frenchman Anderson in the opening trial September 1990. It is believed that it can cure many people treat genetic disorders or those over time. The introduction of clustered regularly inter spaced short palindromic repeats CRISPR gene editing has opened new doors for its application and utilization in gene therapy, CRISPR associated nuclease 9 (CRISPR-Cas9) are genetic modification tools derived from a microbial adaptive immune system. Nucleus Cas9 can form Double Strand Breaks (DSBs) on target DNA sequences in a site specific way, guided by a single guide RNA (sg RNA) on the presence of a protoss Pacer Adjacent Motif (PAM) sequence. The resulting DSDs can be repaired by Non Homologous End Joining (NHEJ) or Homology Directed Repair (HDR), the former being prevalent. NHEJ may lead to index mutations, but HDR can provide precise genetic modification or addition. Since its introduction into mammalian cells and animals three years ago, CRISPR-Cas9 has revolutionized many areas of medical research and has been applied to the exploration of gene therapy for many human diseases.

2. Description

Recently, significant progress has been made in the gene therapy potential of CRISPR-Cas9. Three studies published simultaneously in science have demonstrated the potential of CRISPR-Cas9 *in vivo* gene therapy for local (intra muscular) or systemic (intra peritoneal or intravenous) Duchenne Muscular Dystrophy (DMD) in adult or neonatal mice. A pattern formed by a meaningless mutation in the exon 23 of the DMD gene. The CRISPR-Cas9 targeting intron 22 and intron 23 of the DMD gene can partially restore muscle function by removing the culprit mutation in muscle cells. In all three studies, components of CRISPR-Cas9 were distributed through Adeno Associated Virus Vectors (AAV8 or AAV9), which are preferred delivery tools in gene therapy for their extensive tissue tropism, low immunogenicity, and minimal invasive mutagenicity. To address the package size limit of AAV vectors, two studies used Cas9 from Staphylococcus Aureus (SaCas9) instead of the commonly used Cas9 from Streptococcus pyogenes (SpCas9) because the previous one was smaller, while the third study used spCas9 smaller promoter/ increase sequence to reduce package size.

Two other recent studies demonstrated the efficacy of CRISPR-Cas9 mediated HDR *in vivo* gene therapy by intravenous injection. In these two studies, the authors used a dual viral vector system or a combination of viral vector and lipid nanoparticles to provide the three therapeutic components of CRISPR-Cas9 (sg RNA, Cas9 and donor template) and obtained adequate HDR efficiencies. Protect disease pathogens in mouse models of hereditary liver diseases whose therapies require HDR mediated gene replacement. CRISPR-Cas9 is sufficient to correct the culprit genetic mutation of hereditary tyrosinemia in adult mice, initially to protect the disease phenotype by achieving genetic modifications in 0.25% of liver cells and 33.5% of liver cells

after 33 days. However, when genetic corrections provide growth negativity, genetically modified cells are overcome by their unmodified counterparts. In these cases, CRISPR-Cas9 mediated gene therapy may require much greater delivery and editing capabilities and may require repeated episodes of treatment.

As we all know the application potential of CRISPR-Cas9 in gene therapy is a fascinating area but efficiency is one of the major barriers to i*n vivo d*elivery. To date, most therapeutic discoveries using CRISPR-Cas9 have been performed on cells or animal germ line to correct, replace, or eliminate criminal genes. However, the strategy of *ex vivo* genetic correction by auto transplantation or allo transplantation is only applicable to some part of human diseases such as hematological malignancy and may require repeated episodes of treatment and germ line modification is not currently acceptable in humans. In mouse models of hereditary liver diseases, disease phenotypes are protected by HDR mediated gene replacement, which has a wider application spectrum for gene therapy because there are many diseases that need to be corrected for treatment rather than removing the culprit genes. Although these exciting advances suggest that we may not be far from the final applications of CRISPR-Cas9 to human gene therapy, there are still many challenges. First, there is a need to improve the efficacy of HDR for gene therapy of diseases requiring HDR treatment rather than a more effective NHEJ.

3. Conclusion

These recent advances represent important stages in the final application of CRISPR-Cas9 to human gene therapy. However, there are still many challenges such as delivery capacity, HDR capacity, off target effects and modified cell fitness. Future efforts to address these challenges are needed to pave the way for the clinical use of CRIPSR-Cas9 as a strategy for gene therapy.