PRECISION DELIVERY: USING AAV TO TARGET CRISPR TO SPECIFIC TISSUES

CRISPR gene editing is one of the most powerful tools in disease research with the potential to correct genetic defects that cause diseases. CRISPR-mediated gene correcting can either be used to develop curative therapies or prevent onset of disease by correcting the gene defect very early. CRISPR was first described in 2012 and there has been accelerated in the use of CRISPR gene editing for various applications including developing physiologically relevant preclinical animal models, investigating disease biology, confirming disease drivers and most importantly, correcting genetic mutations to cure disease[1]. While gene therapy using CRISPR has the potential to correct genetic disease drivers and reverse the course of a disease, it is dependent on accurate and safe targeting to the appropriate tissues or organs. The delivery system also has to have desirable ADME (absorption, distribution, metabolism, excretion) characteristics to ensure that the gene therapy is efficacious with minimal off target or toxic effects.

Gene therapy delivery methods can be broadly classified into two segments – viral and nonviral[2]. Viral delivery typically uses AAV (adeno-associated virus), adenovirus or lentivirus to delivery the genes of interest to specific tissues using the intrinsic ability of viruses to infect specific cells and introduce the transgene. Nonviral methods typically use either physical or chemical delivery systems and an example of physical delivery is electroporation where cell permeability is increased using electricity to facilitate transgene entry. Chemical delivery systems range from lipid particles that encapsulate the genetic material to RNA aptamers that bind to the cell surface and facilitate transgene uptake into the cell via endocytosis and other uptake pathways[2]. Both viral and nonviral delivery systems have benefits and challenges but viral approaches are typically more efficient and have been used for several studies to delivery CRISPR gene editing to specific tissues. AAV is the most favored viral vector as it has been used in several gene therapy trials with acceptable safety profiles. However, there are several issues to be considered when using AAV to deliver CRISPR and one of the major issues is the size limitation for the transgene. AAVs can typically hold less than 5kb of genetic material which is a challenge to deliver the guide RNAs, Cas9 enzyme and donor DNA that are components of CRISPR. One solution is to engineer 2 AAVs that contain the CRISPR components with the requirement that both AAVs will need to infect the same cells for the system to work. An alternative approach is to identify and engineer a smaller Cas9 that works as effectively as regular Cas9 enzymes, and this approach resulted in the identification of smaller potent Cas9 enzymes from staphylococcal bacteria[3].

Nevertheless, AAV mediated gene editing delivery has been successfully demonstrated in preclinical models. One of the earlier studies from George Church's group at Harvard showed that CRISPR-mediated excision of mutated exon 23 in the dystrophin gene allowed the expression of the correct protein in skeletal and cardiac muscle in the *mdx* mouse model of Duchenne muscular dystrophy[4]. In another study, James Wilson's group at the University of Pennsylvania delivered 2 AAVs with CRISPR components into mice to correct a urea cycle disorder that is a rare genetic liver disease[5]. While studies in preclinical mouse models are encouraging, the ultimate objective is to accurately deliver the CRISPR system in humans. The first *in vivo* clinical trial that uses viral delivery of CRISPR is being conducted by Editas Medicine to treat Leber congenital amaurosis (LCA), an inherited retinal disease that leads to blindness[6]. Luxturna, an AAV based gene therapy has been approved for LCA but is not curative and reports of retinal degeneration 1-3 years after treatment have been observed. A curative treatment is being evaluated in the clinic using CRISPR gene editing to remove a specific mutation from the *cep290* gene. The therapy is an AAV5 viral vector that contains Cas9 and two guide RNAs[6] that is delivered directly into the eye. In the past couple of years, clinical trials for CRISPR mediated gene therapies have been launched including one to cure chronic HIV[7] and it will be important to demonstrate the CRISPR can be delivered safely and effectively via systemic delivery. Despite the delivery challenges that still need to be addressed, the combination of improved CRISPR technology and engineered viral vectors will likely be used in several new clinical trials to cure difficult to treat diseases and fix "undruggable" disease drivers.

References:

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