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Abstract:

Since the discovery of the CFTR gene, researchers have been working to develop a gene therapy technique that helps to correct the causative gene in cystic fibrosis patients. Many vector delivery systems have been researched. However, even after years of clinical trials, there is still no FDA approved cystic fibrosis gene therapy technique. Researchers have found difficulty finding a vector system that has maintained high levels of expression over an extended period of time. This review investigates the early and current development of vector delivery systems, including viral and non-viral vectors, in a comparative study measuring efficiency. Currently, lentivirus vectors are a promising viral technique for CF patients, as well as nanoparticle delivery systems due to their enhanced gene expression and delivery efficiency. While the implementation of a cystic fibrosis gene therapy technique has been more challenging than previously expected, new research continues to bring the vector systems closer to reality.

Keywords: cystic fibrosis, gene therapy, viral vectors, non-viral vectors, CFTR gene

Introduction

Cystic fibrosis (CF) is a genetic disease caused by a mutation in the transmembrane conductance regulator gene, or CFTR. [1] The CFTR gene codes for the CFTR protein, which regulates the chloride channel and helps move chloride ions to the cell surface. [2] Without the chloride ion at the surface to attract water, mucus in various organs builds up and becomes very thick and sticky. [2] In the lungs of people who have CF, bacteria can get stuck in the build-up of mucus, which can cause inflammation, infection, and respiratory failure. [2] While current treatments help ease the symptoms, there is no cure for cystic fibrosis. Advancements in treatments have allowed patients to live a longer life if treatment is distributed early and managed consistently. [2]

Recent research, however, may give light to new ways of manipulating the CFTR gene through a technique known as gene therapy. Since the DNA sequencing of the causative gene, labs have been focusing on gene therapy techniques, specifically ones that transfer a functional CFTR gene into defective cells using vectors. [3] Vector carriers are genetically engineered to deliver the functional gene to the appropriate cell and, in this case, have the potential to correct the fault in CFTR function. [3] Researchers have had a hard time finding a vector that maintains high-level and long-term gene expression, large carrying capacity, low immunogenicity, and easy production levels. This review article will discuss the new experiments performed attempting to correct the CFTR channel defect using viral and non-viral vectors. In doing so, it will examine new research on some developing gene therapy techniques that, with further research and clinical trials, could eventually be implemented.

Viral Vectors

Most research has been focused on introducing a normal copy of the CFTR into lung epithelial cells, which are cells that line the outer surface of organs. Previous experiments have shown that expressing the CFTR gene in CF cells can help restore proper anion transport in these cells. [4] Since then, several viral vectors have been developed to deliver the correct DNA sequence in a CFTR gene. [4] Often, viruses are used as vectors because they have the ability to deliver the new gene by infecting the cell. [4] Depending on the type of viral vector, they can be inserted in an integrated technique or a non-integrated technique. Adenovirus vectors and adeno-associated viruses are non-integrated vector techniques, which means that DNA with the correct CFTR gene is inserted into an individual's cells, but the DNA does not become a part of the cell's genome. [4] The disadvantage of this technique is that it is not permanent. The lentivirus is an integrated vector technique, which means that the new copy of the CFTR gene would permanently become a part of the cell's genome. A disadvantage of this technique is that researchers have limited control over where this copy integrates into the genome. [4] With improved targeting and vector development, this problem could be resolved.

Adenovirus

Early gene therapy trials incorporated a normal CFTR gene into the DNA of an adenovirus vector, a vector which injects its DNA into the nucleus of the cell. This vector can successfully express the DNA of a CFTR gene in the cystic fibrosis epithelium using in vivo techniques. [5] These adenovirus vectors proved to be impractical, however, due to the low efficiency of these viral vectors inserting their DNA into epithelial cells, a

robust immune response from the cell, and a low genetic carrying capacity. [5] With a lack of DNA transfer, these cells therefore cannot sustain a long duration of gene expression. To maintain adequate CFTR expression, the vector would have to be re-administered to the cells over and over again.

Helper Dependent Adenoviral Vector (HDAds)

Helper dependent adenoviral vectors are constructed by removing all viral sequences from the vector except the packaging signals and small inverted terminal repeats needed for DNA transfer. [6] The absence of viral genes creates a system of a larger carrying capacity of DNA and allows the HDAd's to mediate long term expression without the immunogenetic effects.[6] After development of the adenovirus, researchers acknowledged the challenge of efficient DNA transfer in gene based therapies. In one study, they used an HDAd to deliver the functional CFTR gene to mouse and pig airway basal cells, cells located on the inner surface, in order to achieve long term therapeutic gene expression. [6] Because basal cells have the ability to renew themselves, targeting them for gene editing could give rise to permanent airway gene expression in cystic fibrosis patients, without the need for administering this therapy multiple times. [6] In addition, these vectors had a relatively good safety profile. Another study carefully investigated the behavior of the latest generation of helper dependent vectors after delivery.[7] Working with mice, these researchers developed a more advanced HD vector backbone, and found that almost no immune response was existent. [7] This study, like the previous one, observed prolonged gene expression with the HD vectors. Currently, HDAd's are being administered in different organs to achieve efficient DNA transfer in primates and rodents. [4] These studies suggest that human gene therapy by delivery of helper-dependent adenoviral vectors is feasible.

Adeno-Associated Virus (AAV)

Adeno-associated viral vectors are similar to the features of adenoviruses but are more advantageous in that they have the potential to prolong transgene expression and have a relatively good safety profile. [8] Efficient expression from recombinant AAV-associated cystic fibrosis transmembrane conductance regulator vectors (rAAV-CFTR) became a major finding in the 2000's. [3] Early studies show AAV serotype 2 vectors rapidly gained importance in CF gene therapy applications. In one study, a rhesus monkey airway epithelium model was successfully used to show persistence of the AAV2 vector up to 3 months after administration. [9] Another study that used these monkeys as a model found that average gene transfer increased with repeated administration. [10] Because of the small packaging capacity, researchers worked to develop a strategy to bypass this limit and achieve high levels of the CFTR gene expression from AAV vectors. More recently, rAAV5 vectors have gained attention from researchers. One study explained the advantage of using rAAV5: they contain larger promoters regions and an alternative capsid for enhancing gene expression in CF epithelial cells.[11] The AAV was designed with a DNA cassette that contained a special promoter, called a CB promoter, that was packaged into a rAAV5 capsid.[11] This study demonstrated that these features contributed to the correction of the CFTR chloride transport defect in CF mice models.[11] It was shown that gene expression is maximized by using rAAV5.[11] These studies provided the preclinical data necessary for the initiation of a clinical trial of rAAV in patients with CF.

Lentivirus

One of the most recent studies have been done on the lentivirus, another type of viral vector, and has become a promising therapeutic option due to a longer term of gene

expression, unlike other viral vectors previously mentioned. [3] Lentivirus is a type of retrovirus, a virus that contains genes that are encoded in RNA rather than DNA. [12] For the retrovirus to infect a cell, it first must be reverse-transcribed back into DNA before the material can be copied. One advantage of the lentivirus is the fact that it can persistently express the CFTR gene and possibly be readministered without the infected cell growing immunity to the virus, as shown by some studies. [12-13] To overcome this immunity, one study attached a compound, known as LPC to the lentivirus vector. [12] This allows the vector to open up areas called epithelial junctions, which often block access for pathogens to reach the cell receptor for target delivery. [12] While progress has been made by using mice as animal modules, further advancement has been hindered due to the fact that mice do not develop lung disease the same way people with cystic fibrosis do. [3] More recent studies have started using pigs as models, as they show more similarities in lung disease to humans. [3] In one study, a type of lentivirus, called the feline immunodeficiency virus, was delivered to the sinuses and respiratory tract of cystic fibrosis pigs. [14] Pigs that received the vector were seen to restore partial activity of the anion channel in cells.[14] Future studies with new animal models are needed to further investigate vector delivery systems. In 2017, a group of researchers proposed, through a series of experiments, that a lentivirus called rSIV.F/HN was set for a first-in-man clinical trial. [15] These studies as well as others have confirmed that that both primate and non-primate vectors are promising gene therapy candidates. Based on current research, more studies and clinical trials need to be completed in order to implement an effective viral vector therapy for cystic fibrosis patients.

Non-Viral Vectors

As an alternative to viral vectors, some researchers have focused on non-viral vectors as a new strategy for CFTR delivery. Non-viral vectors are made from synthetically produced biological molecules. [16] In addition, the rapid progress of nanotechnology has enabled the gene delivery of these nanosized particles. Typically, with nanoparticle delivery systems, the plasmid DNA which carries the gene expression cassette is bound to a synthetic chemical compound that is then released at the delivery site. [16] Early studies have shown that non-viral vectors can transfer the functional CFTR gene to the CF airway epithelia in vivo and partially correct the anion transport defect. [17] Carriers generally contain cationic lipids or cationic polymers because they bind with high affinity to the negatively charged DNA. [18] In terms of gene transduction, non-viral vectors are less efficient, but their availability, cost, and low immunogenicity make them a possible long-term solution.

These vectors are interesting because they have virtually no size restraints, but they are not delivered as efficiently as some of the viral vectors. [4] Because of this challenge, these non-viral carries are most often made from lipid-based plasmids, often a cationic lipid. [3] Most cationic lipids used for gene transfer are accompanied by a neutral co-lipid. [18] The cationic lipid is a vital component because the positive charge of the lipid interacts and binds to the negatively charged DNA. [18] One important study in the early stages of non-viral vector development was to understand the mechanisms by which these neutral co-lipids affect cationic lipid gene transfer. [18] This study found that these co-lipids increased DNA uptake and increased the ability for the DNA to dissociate from the lipid complex, two things that contribute to the overall amount of gene expression. [18]

An alternative class of non-viral vectors are cationic polymers, which are attractive for their chemical diversity and their great potential for function. [16] Two examples of these polymers are ploy(l-lysine) (PLL) and polyethylenimine (PEI). PLL has been known to be a rather toxic

polymer, so many researchers have created modified variants of it. [16] One example is when researchers covered the PLL with a PEG, a hydrophilic polymer which is designed to reduce any immune response and therefore increase duration of expression. [19] The clinical potential of PEGylated PLL was examined as a vector for CF, and in this study a clinical trial was carried out which supports the safety and efficiency of these DNA nanoparticles in nasal epithelial cells. [19] Another study focuses on the modification of a PEI polymer. [20] The aim was to develop a better strategy of cell uptake, efficiency and duration of gene expression, and safer delivery. They found that delivery was improved when a PLGA, a copolymer with good biocompatibility and a promising candidate for nanoparticulate drug delivery, was added but increased cell toxicity. [20] To combat this, researchers further modified the nanoparticles through PEGylation, which improved delivery efficiency while also mitigating toxicity. [20] If the low efficiency of gene transfer barrier could be overcome, non-viral vectors can be a safe and clinically satisfactory option for cystic fibrosis gene therapy.

Conclusion

While cystic fibrosis is an incurable inherited disease, researchers have come a long way to developing effective treatments that have increased the quality and lifetime of diagnosed individuals. The field of genetics is progressively learning from the extended research done on gene therapy including viral and non-viral vectors and new vectors that can efficiently deliver and correct the CFTR channel defect. [5-6, 8, 12-14] Major vector delivery systems are outlined in Table 1. The development of a more effective DNA delivery system for genetically altering more airway cells, however, is needed if treatment based on gene therapy is to be achieved. The lentivirus vectors are a promising viral technique for CF patients, as they are able to integrate DNA into the host genome and persistently express the new CFTR copy. As for non-viral vector systems, nanoparticle delivery systems are emerging due to their enhanced delivery efficiency.

Ideally, a vector should have high transduction efficiency, display a high safety profile which can be administrated without any genotoxic effects, and be relatively inexpensive. Therefore, future research should focus on improving vector designs and delivery systems, specifically the lentivirus vectors and nanoparticle systems, and the medical field will be on its way to creating new gene therapeutic techniques for patients with cystic fibrosis, as well as other diseases.

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