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Cystic Fibrosis: Advancements in Gene-Therapy Approaches with Respect to Transfer Vectors

Muhammad Adnan Shah Bukhari* and Muhammad Imran Qadir

Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

***Corresponding author:** Muhammad Adnan Shah Bukhari, Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

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ABSTRACT

Cystic Fibrosis (CF) is a lethal genetic disorder caused by mutations of the CFTR gene on chromosome no. 7. This gene regulates the chloride ions, bicarbonate ions, and other body fluids and helps in maintaining homeostasis. Malfunction of this gene disturbs the salt and fluids level in the body which causes failure of multiple body organs. Lungs are mostly clogged with mucus in CF patients. Attempts to find a cure for CF fibrosis started early in 1989 when the CFTR gene was detected. Earlier studies attempted to fix CFTR mutations using Adenovirus vectors. Some human trial studies were successful in detecting expressed proteins in epithelial cells of air pathways. Adeno-associated virus-mediated trials and model-based studies also successfully expressed the CFTR gene. But their expression didn't last because epithelial cells of lungs and nasal pathways are frequently changed after two months. CRISPR/Cas9 is a new highly efficient DNA editing tool with huge potential. Some CRISPR/Cas9-based studies have been conducted to discover CF treatment. But this technology is in its very early phases. No complete cure for CF is available right now but the gathered data is very important. With recent advancements and ongoing research, successful CF treatment is expected in the near future.

Keywords: CFTR; CRISPR/Cas9; Adeno-Associated Virus; Adeno Virus

Abbreviations: CF: Cystic Fibrosis; CFTR: Cystic Fibrosis Transmembrane Regulator; AAV: Adeno-Associated Viruses

Introduction

Cystic Fibrosis is a genetic disorder that is caused by mutations in an autosomal recessive gene. This gene is named as CFTR (cystic fibrosis transmembrane regulator) gene and is located on the long arm of chromosome number 7 (7q31). It encodes for the protein that consists of 1480 amino acids. This protein is directly involved in the regulation of various body fluids. Chloride ions and bicarbonate ions transport at the cell membrane is directly regulated by CFTR protein. These ions are responsible for maintaining the pH of liquid layers on the surface of the pulmonic epithelium [1]. Disturbance in the regulation of these ions causes an imbalance between salt and fluids levels leading to the failure of multiple body organisms ultimately resulting in the death of the patients. It is mainly known for damaging the lungs and leading to their loss of function by sticky mucus clogging the airways making it harder to breathe for the patients [2]. Cystic fibrosis is one of the most common lethal genetic diseases. Many therapeutic discoveries have been made to improve the survival chances and life quality of cystic fibrosis patients since its early identification in 1989 [3]. Although scientists continuously have been trying to discover the ultimate cure for this deadly disease and we have gone through more than three decades of efforts in therapeutic research to find a proper cure for this horrible disease, no proper treatment is available for it. The reason behind the better life expectancy of cystic fibrosis patients is better disease management and symptomatic treatment. Even though we do not have any significant pharmacological cure for this disease, recent advances in the biotechnological field do have some promises for its treatment. Being a genetic disorder, it's more likely that a biotechnological treatment like gene therapy, mRNA, CRIPER,

etc. can be helpful in its treatment rather than pharmacological drugs. Some of the approaches in an attempt to treat cystic fibrosis are described here.

Gene therapy, as obvious by its name, is a genetic approach to the treatment of diseases. In gene therapy, usually mutated genes are targeted to restore their normal function and it eliminates the consequences caused by the lack of their function. Gene therapy utilizes different approaches to edit the DNA of required genes for the treatment of a disease. The first gene-therapy-based drug was approved in China only called 'Gendecine'. It was a p53 tumor suppressor gene carried by an adenovirus for patients having head and neck cancer [4]. Cystic fibrosis gene therapy has been on the tables of the researchers since early cloning of the CFTR in 1989. But still, no gene-therapy drug has got the approval to exist in the market to fix malfunctioning CFTR genes. The main cause of the failure in finding a successful gene-therapy-based drug is that the lungs proved more difficult to target than the initial anticipations [5]. However, the approval of Kalydeco gave some light of hope. Kalydeco is a drug that has been helpful for patients with certain types of CFTR mutation i.e. approximately 4% of the cystic fibrosis mutations. This has given hope for the treatment of CF patients independent of their type of mutations.

A vector is required for the gene transformation into cells. Most vectors used for CF gene therapy are adenoviruses, adeno-associated viruses (AAV), and lentiviruses. Non-viral vectors for CF gene therapy include peptide nanoparticles and lipoplexes.

Viral Vectors for Cystic Fibrosis Gene Therapy

Adenovirus Vectors

In the early 90s, adenovirus-mediated CFTR gene transfer was experimented with in a cotton-rat model which resulted in the successful detection of mRNA and protein [6]. In the following year, tests were conducted in humans for adenovirus-mediated CFTR gene transfer. But it was a failure as mRNA and proteins were not detected after a single nasal application [7]. For the first time in 1994, mRNA and protein were detected from nasal and pulmonary airways after administration of Ad-mediated transfer of the CFTR gene. This slightly improved the transfer of chloride ions channel but did not show some significant improvements [8]. The reason behind the significant expression of the CFTR gene transferred by Adenovirus in model-based studies but its lack of expression in human-based clinical trials was believed to be the absence of apical receptors from the epithelial cells of air pathways [9]. Therefore, having promising results from pre-clinical pulmonary and nasal model-based studies, Ad-mediated gene therapy of the CFTR gene did not prove to be successful in Cystic Fibrosis patients.

ADV Vectors

AAV (Adeno-associated Virus) vectors are one of the main highlighted viral vectors which have been found to be useful in gene therapy-based treatments. Some of the key features of AAV vectors are

(i) Broad tropism,

(ii) Minor immunogenicity,

(iii) Absence of relation with human pathology.

(iv) Capability in transferring the genes efficiently and

(v) Non-integrating nature [10].

These are the reasons why it has gained importance as a gene transfer vector for human therapeutic purposes. It has a carrying capacity of <5kb single-stranded DNA [11]. rAAV (recombinant adeno-associated virus) is a modified form of wild-type AAV with its two sets of genes removed that are involved in its replication and capsid formation. Some previously successful approvals of rAAV-mediated gene transfer for the treatment of diseases like congenital blindness, lipoprotein lipase deficiency, and hemophilia B promoted researchers to try CFTR gene transfer with rAAV vectors [12]. The very first rAAV-based gene therapy drug to get successful approval was Glybera which was approved by the European agency. Although, it did not take long for it to disappear from the drug market due to its costly downstream processing with some other reasons as well [13].

Preclinical Studies

Some preclinical studies demonstrated the successful transduction of rAAV2-CFTR and CFTR mRNA and protein expression for up to 6 months in primary cell cultures of nasal polyps of cystic fibrosis patients and the bronchi of rabbits [14,15].

Human Clinical Trails

In the first human clinical trial of AAV-CFTR transfer for the treatment of CF, tgAAV2-CF was administered into maxillary sinuses as it was considered the method with the least danger to testing the virus on human subjects [16]. The vector was detected for up to 10 weeks in the administered patients and no toxicity was seen. This study was promising because successful transduction and expression were detected persistently with no toxicity at all. Some other studies were also conducted on human lungs and trials were conducted on multiple phases, but the results did not meet the expectations, and no significant improvement was recorded in the lung functions [17-19]. This was mainly because of the limited carrying capacity of the AAV vector which leads to reduced expression than the anticipations. Although, so far, no groundbreaking cures for cystic fibrosis have been discovered in the conducted studies of AAV-mediated CFTR gene therapy, the data gained is encouraging. This data promises that with the significant improvements in the vector capabilities, it is possible to get desired result i.e. proper cure of CF by correcting CFTR functions.

Non-Viral Vectors for Cystic Fibrosis Gene Therapy

There are certain problems with using viruses as gene-transfer

vectors in humans, which can't be ignored. These include

(i) Undesired immune reactions,

(ii) The possibility of trans-gene miss-insertion,

(iii) Insertion of larger eukaryotic genes into viral vectors with low carrying capacity and

(iv) Mass production.

[19] These issues have increased the need for non-viral vectors to use in human subjects, more importantly for the treatment of diseases. Despite their benefits of not having issues like viral vectors, non-viral vectors are not very efficient for gene therapy. This might be due to their lack of specific cell entry mechanisms which is the normal feature of viral vectors. Several clinical trials of non-viral mediated CFTR transfer for CF treatment have been conducted with most being Phase I trials. Most of these studies demonstrated partial improvements in chloride ion channel regulation in nasal epithelium. One of these showed ~25% improvement in chloride channel regulation in the lungs [20]. Although the results of these studies look very promising, it will be too early to say that non-viral vectors are more of a success compared to viral vectors for CFTR gene transfer. Nor we can conclude that these vectors will help improve the lungs function in CF patients as none of these trials was sophisticated to analyze their efficacy in improving lungs function.

CRISPR/Cas9 Based Approach

CRISPR/Cas9, a biotechnological gift, is a marvelous gene-editing tool of high efficiency and accuracy with really high hopes and promises for the future. CRISPR stands for clustered Regularly Interspaced Short Palindromic Repeats. It is derived from the bacterial defense mechanism [21,22]. When a bacterial cell is invaded by a virus, some of its DNA is integrated into the bacterial genome. This is known as the CRISPR array. (Figure 1)Transcription of this array from multiple RNAs. In the next step, a nuclease protein like Cas9 and a tracrRNA (trans-activating small RNA) are associated with each of the transcribed crRNAs (CRISPR RNAs). This group, whenever detects a DNA that is similar to the sequence of crRNA, the Cas9 proteins, binds to that DNA sequence and makes a double-stranded cut in it. This mechanism can be used to make site-specific cuts in any DNA molecules by directing Cas9 protein by using customized crRNAs and it can serve as a highly precise genome editing tool for biotechnology [23]. In 2013, the very first CRISPR/Cas9-based study in an attempt of fixing the CFTR function was published [24]. A couple of other studies also demonstrated successful CRISPR-based CFTR mutation fixation. In 2017, Sanz et al. also tried to fix some CF mutations using a CRISPR-based method [25].

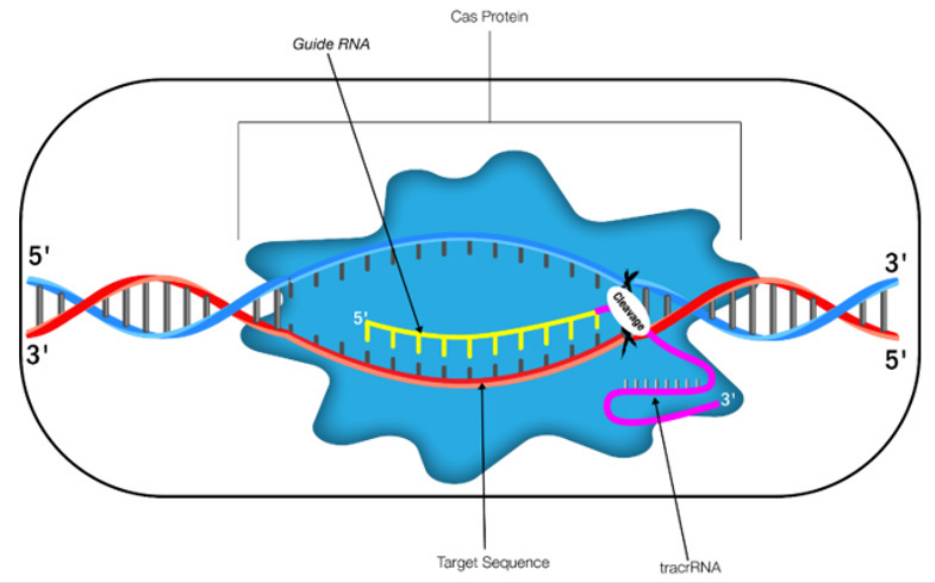

Figure 1: Mechanism of action for CRISPR Cas9.

Conclusion

Although a significant amount of work has been done on CF and researchers have been trying to overcome the obstacles for decades, we have not reached the point yet where we have available gene therapy-based drugs for CF. But it cannot be called a failure as we have come a long way and have got much more data than we knew initially. We have some high hopes for emerging more efficient technologies and tools like CRISPR/Cas9. With these technologies and past experiences and research, we can expect some gene-therapy-based treatments which will be independent of the type of mutations and will be a gift to humankind.

Conflict of Interest

None.

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Muhammad Adnan Shah Bukhari. Biomed J Sci & Tech Res

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