

# CRISPR-Cas9 as a tool for treating cystic fibrosis through gene editing

**Jiabao Xu**

Chongqing Depu Foreign Language School, Chongqing, China, 400000

claytonhsu@outlook.com

**Abstract.** The most common cause of mortality is Cystic Fibrosis, a fatal genetic disease. However, in recent years, there have been a growing number of papers concentrating on CRISPR-Cas9, a gene-editing tool that is being used to permanently cure this genetic disease, named by a biopharmaceutical company EditasMedicine, invested in by Bill Gates. However, before the breadth of search and study of this technology continuously expands, challenges and remaining issues should be addressed. This paper reviews the mechanisms of cystic fibrosis and discusses its technical challenges, such as efficiency, safety and delivery of gene editing, potential side effects, and ethical issues, this paper also talks about the future applications of CRISPR-Cas9 in other diseases, so as to provide an alternative treatment method for the diseases with gene editing better results can be obtained.

**Keywords:** cystic fibrosis, CRISPR-Cas9, gene edit, CFTR.

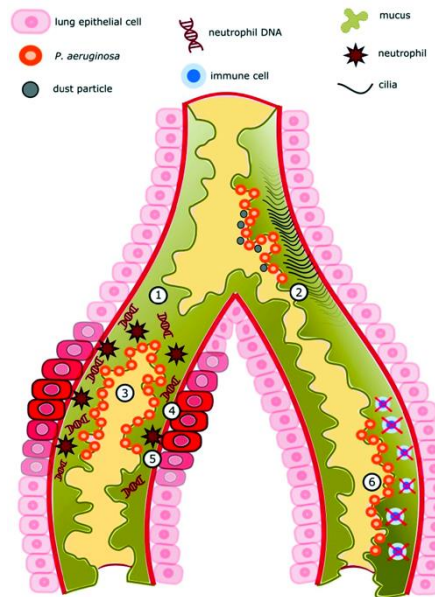
## 1. Introduction

Cystic fibrosis is an inherited disease that affects multiple organs of the body and causes a range of symptoms. Because so many exocrine organs are impacted by the autosomal recessive illness cystic fibrosis (CF), the condition is also known as "systemic exocrinopathy" [1], primarily affects the lungs, liver, pancreas, and reproductive system [2]. Steatorrhea, poor development, and pulmonary infections are some of the symptoms of this illness, which is defined by the malabsorption of proteins and lipids. Pancreatic injury and inadequate pancreatic enzyme secretion are the cause of malnutrition, which is thought to contribute to susceptibility to pulmonary infections, which are often the final event [3]. A novel genome editing technique called CRISPR-Cas9, which can modify the genome using a particular collection of tools, was presented in 2012 by Doudna et al [4]. Despite the great potential of CRISPR-Cas9 technology for gene editing, there are still some obstacles to be addressed, especially in terms of its efficiency, safety and delivery.

In this paper, the key aspect of analyzing the treatment of cystic fibrosis is the application of CRISPR-Cas9 to correct the gene mutations that cause cystic fibrosis, as measured by analyzing its effectiveness, safety, existing challenges and ethical issues. In addition to this, the future application of CRISPR-Cas9 in other similar diseases due to genetic variants will be discussed. This study may provide an alternative treatment for diseases for which better results can be obtained by gene editing.

## 2. Mechanism

CF is a result of defects in the CFTR gene, which encodes the CFTR protein responsible for forming an ion channel for chloride and bicarbonate ions at the tip of the airway epithelium. This passageway enables chloride ions to pass through the periciliary liquid layer and into the airway surface liquid (ASL), which is made up of a mucous layer (PCL). The ASL's constant water level and narrow PCL are both maintained by the osmotic pressure produced by ion movement. However, when CFTR is not functional, chloride ions are retained in the epithelial cells, and inhibition of the ENaC sodium channel is lost, leading to excessive sodium uptake. This results in water uptake in the ASL through transepithelial and paracellular means, causing ASL dehydration [5], as shown in Figure 1.



**Figure 1.** This image depicts a cross-section of a cystic fibrosis airway, showing the range of symptoms caused by dysregulation of CFTR protein [5].

Complications from lung disease are the top cause of death in individuals with cystic fibrosis (CF) [6]. The disease has multiple causes. First, mucous airway fluids, a mixture of mucus and sputum that prevents gas exchange, obstruct the airways [3]. Secondly, the movement of airway cilia is disrupted by the mucus, preventing the cilia from removing pathogens and debris from the lungs. Last but not least, impaired ion transfer causes the ASL pH to drop, which suppresses the lungs' antimicrobial peptide defence system [5].

In cases where the airway is obstructed by the spreading industry, removing secretions plays a crucial role in the treatment in the early stages of the disease [3]. Specifically, secretions are cleared with postural drainage and tapping ("ketchup bottle method") [7]. Bacteria are either killed or slowed down by antibiotics so that the body's immune system can better fight off infections [8]. There are several types of antibiotics used to treat CF. First, prophylactic antibiotics. People with CF can take antibiotics regularly to prevent infection from occurring in the first place, usually at low doses, and older patients infected with *Pseudomonas aeruginosa* have been shown to benefit from continued oral azithromycin therapy [9]. Second, long-term antibiotics. In 1993, a randomized trial of tobramycin in aerosols only showed that treatment every other month for 6 months improved lung function and reduced deterioration [10], which can be an effective way to deliver antibiotics directly to the infected site while minimizing side effects. It is worth noting that overuse of antibiotics can lead to antibiotic resistance, making the infection more difficult to treat, and because it is not clear whether the antibiotic has antibacterial activity or anti-inflammatory properties [3]. Long-term use may lead to side effects such as nephrotoxicity, vestibular toxicity and ototoxicity [8]. Therefore, antibiotic therapy for CF is carefully managed by

health care providers to ensure the most effective use of antibiotics while minimizing the risk of resistance.

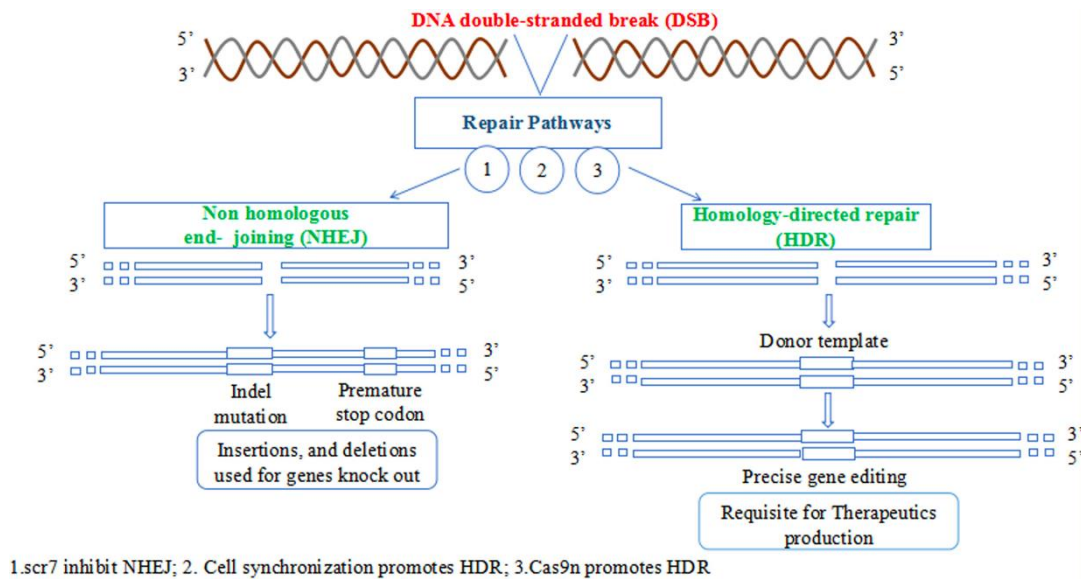
Chloride can be transported across epithelium cells' apical membranes in a number of organs thanks to CFTR, including the airway, intestine, pancreas, kidney, sweat glands, and male reproductive tract [11]. In addition to its main role, CFTR also controls the pH of airway surface fluids, secretes bicarbonate, and suppresses the activity of the epithelial sodium channel (ENaC), which is essential for hydrating secretions and mucus proteins [12].

There exists more than 2,000 CFTR genetic variation, while over 300 of them are able to cause CF [11, 13-14]. The mutations were categorized into six groups. The first group results in a complete halt in protein production, the second group causes CFTR to be improperly transported to the apical surface of epithelial cells, the third group causes impaired ion channel gating, the fourth group leads to decreased ion flow through the CFTR channel, the fifth group causes a reduction in protein production, and the sixth group leads to a reduction in surface retention of the protein on the cell [5]. The F508del mutation is the most prevalent CFTR mutation and is typically categorized as a II mutation. It involves the deletion of three base pairs, which leads to the elimination of a phenylalanine residue [15]. In general, CFTR malfunction results in a spectrum of diseases, which can affect different organs to varying degrees of severity [11].

### **3. Analysis of CRISPR-Cas9 to correct CFTR mutations**

In the 1990s, RNAi technology revealed gene activity, ushering in the age of reverse genetics. As a result, more effective instruments like ZFNs, TALENs, and CRISPRs were created [16, 17].

CRISPR-Cas9 technology has great potential to correct the gene mutation of CFTR which causes cystic fibrosis [18-20]. The CRISPR-Cas9 system works by using a molecule called guide RNA (gRNA) to target a specific location in the DNA sequence. Once the target sequence is bound by the gRNA, the Cas9 enzyme is activated, which creates a double-stranded break in the DNA [21]. Then, one of two mechanisms—homologous directed repair (HDR) or non-homologous end joining (NHEJ)—can be used to fix this split. NHEJ is an error-prone repair mechanism that can lead to DNA sequence insertions or deletions, which can lead to gene disruption or knockout, and therefore, It is not appropriate for the use of CRISPR as a medicinal tool. HDR, on the other hand, uses the same or similar repair template as the sequence being repaired, allowing precise correction of DNA sequences, but is not the most preferred correction pathway for double-strand breaks (DSBs) in nature [21], as shown in Figure 2. The NHEJ repair mechanism of DNA is triggered when CRISPR-Cas9-guided DSBs are introduced into the target cell. This mechanism generates insertions/deletions (INDELs) that may ideally result in the loss of specific genes [4]. In addition, if the HDR mechanism is activated, DNA breaks will be repaired using the homologous strand. Therefore, CRISPR-Cas9 technology can edit the chromosomal regions that regulate the expression of CFTR mutations, targeting and correcting them [4]. This technology can be used to target and correct chromosomal regions that regulate CFTR mutation expression, potentially leading to a cure for cystic fibrosis.



**Figure 2.** Schematic representation of non-homologous end joining (NHEJ) and homologous directed repair (HDR) mechanisms for DNA repair [21].

In one research, the CFTR locus was corrected in cultured intestinal stem cells derived from cystic fibrosis patients using the CRISPR-Cas9 genome editing method. Homogeneous mucous recombination was used to accomplish this correction. The corrected organoids were clonally expanded and analyzed, revealing a rapid expansion of their surface area. Additionally, the corrected alleles were expressed and found to be fully functional [22].

#### 4. Challenges of CRISPR-Cas9 genome editing technology

CRISPR-Cas9 technology has greatly facilitated precise genomic targeting manipulations. In complete clinical uses, numerous issues regarding efficacy and safety must still be resolved, including the adaptability of the edited cells, editing effectiveness, delivery strategies, and potential side effects [21].

A major challenge in genome editing is the adaptability of the edited cells, which may exhibit health defects and reduced proliferate and differentiate abilities, leading to suboptimal therapeutic results compared to unedited cells [23]. One study examined mouse models of cancer with mutations in the Pten and p53 genes, and transfected mice with vectors containing specific CRISPR through the tail vein. This resulted in 20% of hepatocytes being blood transformed and mutations in the  $\beta$ -catenin gene, which is often associated with cancer, were successfully corrected by CRISPR [24]. However, these potential problems and harms posed by the in vivo immune system triggered by CRISPR-Cas9 need to be demonstrated in large animal models and clinical researches [4].

Besides, potential off-target impacts are also important and should not be disregarded. Previous research has demonstrated that the CRISPR-Cas9 system commonly causes indels to be produced at undesirable genetic locations [25, 26]. Kinase-induced off-target events must be kept to a minimum in order to guarantee the secure implementation of CRISPR-Cas9 in vivo. Continued gene alteration can result in undesirable mutations, possible toxicity, and decreased precision of editing. It also raises the chance of off-target cleavage.

#### 5. Ethical issues of CRISPR-Cas9

Ethical issues should be carefully considered when using CRISPR-Cas9. It has the potential to provide treatments for a variety of genetic disorders, but it is also important to ensure that it is used in an ethical manner. The ethical issues surrounding the application of gene editing in humans can be divided into two broad categories, namely aiming at correcting faulty genes (gene therapy) and modifications, gene modifications focused on improving physiologically normal genes (gene enhancement) [27].

The greatest concern is that the off-target effect of that anticipated genomic change may produce uncontrollable genetic changes in individuals. If such mutations occur in the reproductive system, they will be passed on to offspring [28]. Second, while CRISPR can correct genetic mutations that cause disease, there is greater concern about the broader impact of gene editing on society. For example, there are concerns that gene editing may lead to a lack of diversity in the population.

## 6. Conclusion

As an effective modifying tool, CRISPR-Cas9 may revolutionize the treatment of genetic diseases such as cystic fibrosis by correcting pathogenic mutations in the CFTR gene. Clinical uses of gene therapy are also becoming more and more likely thanks to the ground-breaking creation of the gene-editing technique CRISPR-Cas9 [29].

In cancer therapy. For instance, the first CRISPR phase I clinical study in the United States, which aims to use CRISPR-Cas9 to modify autologous T cells for cancer immunotherapy, began in 2018 [30]. Since then, numerous CRISPR-mediated cancer immunotherapy trials have begun, and CRISPR gene therapy is now a very popular form of treatment [31]. Also, genetic modification of immune cells by CRISPR-Cas9 technology can be used to enhance the ability of cells in cystic fibrosis patients to resist infection, and the more effective immune system established by this technology can also improve patient outcomes.

The short journey of CRISPRs so far has been fascinating and its clinical applications have made promising progress [32], and offer significant promise for medical treatment of deadly diseases [21]. However, much work needs to be done before CRISPR-Cas9-mediated genome editing technology can be fully applied to target cancer-related genes in human patients.

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