Defective Ion Channel in Cystic Fibrosis: Current Development in Treatment of Cystic Fibrosis

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Authors' contributions

This work was carried out in collaboration among all authors. Author NG designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors NC, MMG OGi and CAD managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Cystic fibrosis is an inherited disorder that causes severe damage to the lungs, digestive system and other organs in the body. Cystic fibrosis transmembrane conductance regulator (CFTR) is involved in the production of mucus, sweat and digestive juices. These secreted fluids are normally thin and slippery. But in people with cystic fibrosis, a defective gene in CFTR causes the secretions to become sticky and thick. Instead of acting as a lubricant, the secretions plug up tubes, ducts and passage ways, especially in the lungs and pancreas. This mucus leads to the formation of bacterial microenvironments known as biofilms (a niche that harbors bacteria; Staphylococcus aureus, Haemophilus influenzae, and Pseudomonas aeruginosa) that are difficult for immune cells and antibiotics to penetrate. Viscous secretions and persistent respiratory infections repeatedly damage the lung by gradually remodeling the airways, which makes infection even more difficult to eradicate. CFTR, a Cl⁻ selective ion channel, is a prototypic member of the ATP-binding cassette transporter super family that is expressed in several organs. Understanding how these complexes regulate the

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intracellular trafficking and activity of CFTR provides a unique insight into the aetiology of cystic fibrosis and other diseases associated to it. Cystic fibrosis patients exhibit lung disease consistent with a failure of innate airway defense mechanisms. The link between abnormal ion transport, disease initiation and progression is not fully understood, but airway mucus dehydration seems paramount in the initiation of CF lung disease. New therapies are currently in development that target the ion transport defects in CF with the intention of rehydrating airway surfaces.

**Keywords:** Cystic fibrosis; ion channel deficiency and treatment.

1. **INTRODUCTION**

The cystic fibrosis transmembrane conductance regulator (CFTR) is a member of the ATP-binding cassette (ABC) transporter super family. All ABC transporters bind to ATP and use its energy to drive the transport of several molecules across cell membranes. Mutations in ABC genes have been linked to many genetic diseases, including cystic fibrosis, Dubin–Johnson Syndrome (associated with mutations in ABCC2) etc. Cystic fibrosis is a pernicious disease that presents an exocrine pancreatic insufficiency, an increase in sweat NaCl concentration, male infertility and airway disease. Although the life expectancy of individuals with cystic fibrosis has increased dramatically in the past three decades, the average age of death, which is caused by respiratory insufficiency, is ~37 years [1]. Because mutations in the CFTR gene cause cystic fibrosis, considerable effort has been made to understand the function and regulation of CFTR.

CFTR is a plasma-membrane cyclic AMP-activated Cl⁻ channel that is expressed in several functionally diverse tissues, including the kidney, pancreas, intestine, heart, vas deferens, sweat duct and lung [2]. In epithelial cells, CFTR mediates the secretion of Cl⁻. In addition to its role as a secretory Cl⁻ channel, it also regulates several transport proteins, including the epithelial sodium channel (ENaC), K⁺ channels, ATP-release mechanisms, anion exchangers, sodium-bicarbonate transporters, and aquaporin water channels [3]. CFTR and transport proteins form large macromolecular signalling complexes, which are regulated by molecular switches. CFTR and molecular switches mediate trans-epithelial salt and water secretion into the lumen of kidney tubules, pancreatic ducts, and the intestine, but they also have important functions in pathophysiological conditions. CFTR might contribute to the enlargement of renal-cysts in individuals with poly-cystic kidney disease [4,5,6]. CFTR-mediated Cl⁻ secretion across many epithelial cells is regulated by modulating channel activity and by regulating the total number of CFTR channels in the membrane, which is achieved by the insertion and removal of CFTR channels from the plasma membrane. Mutations in CFTR affect the number of channels in the plasma membrane, channel activity and the intracellular trafficking of CFTR. Considerable effort is being made to examine the endoplasmic reticulum (ER) quality-control mechanisms that allow the export of wild-type CFTR but retain ΔF508-CFTR, the most common mutation in CFTR, and target it for degradation by the proteasome [7]. Because ΔF508-CFTR is partially functional as a Cl⁻ channel, the goal of many research laboratories and biotechnology companies is to identify drugs that allow ΔF508-CFTR to fold properly and thereby escape the ER quality-control mechanism. Other drugs in cystic fibrosis clinical trials are focused on correcting the other phenotypes of this complex disease [8]. There was no clear evidence that oscillation was a more or less effective intervention overall than other forms of physiotherapy. Furthermore, one study observed an increase in frequency of exacerbations requiring antibiotics whilst using an oscillating device compared to positive expiratory pressure [9].

2. **DESCRIPTION OF CYSTIC FIBROSIS**

CF is caused by a mutation in the gene cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation, ΔF508, is a deletion (Δ signifying deletion) of three nucleotides that results in a loss of the amino acid phenylalanine (F) at the 508th position on the protein. This mutation accounts for two-thirds (66– 70%) of CF cases worldwide and 90% of cases in the United States; however, over 1500 other mutations can produce CF [10]. Although most people have two working copies (alleles) of the CFTR gene, only one is needed to prevent cystic fibrosis. CF develops when neither allele can produce a functional CFTR protein. Thus, CF is considered an autosomal recessive disease [11].
The \textit{CFTR} gene, found at the q31.2 locus of chromosome 7, is 230,000 base pairs long, and creates a protein that is 1,480 amino acids long. More specifically, the location is between base pair 117,120,016 and 117,308,718 on the long arm of chromosome 7, region 3, band 1, sub-band 2, represented as 7q31.2. Structurally, \textit{CFTR} is a type of gene known as an ABC gene. The product of this gene (the CFTR protein) is a chloride ion channel important in creating sweat, digestive juices, and mucus. This protein possesses two ATP-hydrolyzing domains, which allows the protein to use energy in the form of ATP. It also contains two domains comprising six alpha helices apiece, which allow the protein to cross the cell membrane. A regulatory binding site on the protein allows activation by phosphorylation, mainly by cAMP-dependent protein kinase. The carboxyl terminal of the protein is anchored to the cytoskeleton by a PDZ domain interaction [12].

3. SIGNS AND SYMPTOMS

CF lung disease is characterized by persistent bacterial infection that is usually acquired in childhood and maintained throughout the patient’s life [13]. The infection is usually restricted to the airway lumen and persists despite intensive antimicrobial therapies. Clinical data suggests that the disease is typically initiated following viral infection or aspiration and that throughout adult life the disease progresses with discrete acute exacerbations, also potentially linked to viral infection [14]. The main signs and symptoms of cystic fibrosis are:

1. Salty-tasting skin,
2. Poor growth, and poor weight gain despite normal food intake
3. Accumulation of thick, sticky mucus,
4. Frequent chest infections, and coughing or shortness of breath.
5. Males can be infertile due to congenital bilateral absence of the vas deferens

Symptoms often appear in infancy and childhood, such as bowel obstruction due to meconium ileus in newborn babies. As the children grow, they exercise to release mucus in the alveoli. Ciliated epithelial cells in the person have a mutated protein that leads to abnormally viscous mucus production.

4. INHERITANCE PATTERN

This condition is inherited in an autosomal recessive pattern, which means both copies of the gene each cell have mutation. The parent of an individual with an autosomal recessive condition each carry one copy of muted gene, but they typically do not show signs and symptoms of the conditions.

5. STRUCTURE OF CFTR

The \textit{CFTR} consists of two repeated motifs made up of six membrane-spanning domains (MSDs) and a nucleotide-binding domain (NBD), separated by a regulatory domain (R domain). Site-directed mutagenesis targeting the MSDs

Fig. 1. Model analysis of CFTR by Philip Farrell [15]
appears to have two effects: (1) a permissive effect, perhaps releasing steric inhibition; and (2) a stimulatory effect, facilitating interaction between the NBDs and adenosine triphosphate (ATP). This phosphorylation is a requirement for pore opening and can be reversed by intracellular phosphatases. The ‘opening’ of the pore requires the hydrolysis of nucleoside triphosphates by the NBDs after the phosphorylation of the R domain [16].

6. TREATMENT AND MANAGEMENT OF CYSTIC FIBROSIS

There is no cure for cystic fibrosis, but treatment can ease symptoms and reduce complications. Close monitoring and early, aggressive intervention is recommended. Managing cystic fibrosis is complex, so consider obtaining treatment at a center staffed by doctors and other staff trained in cystic fibrosis. The goals of treatment include:

- Preventing and controlling infections that occur in the lungs
- Removing and loosening mucus from the lungs
- Treating and preventing intestinal blockage
- Providing adequate nutrition
- Medications
- The options may include: Antibiotics to treat and prevent lung infections.

7. NEW THERAPIES TO CORRECT AIRWAY HYDRATION

The main channels and receptors present in airway epithelium that represent potential therapeutic targets for the treatment of CF. Because CF is characterized by an absence of the CFTR protein at the apical membrane, ‘corrector’ drugs are currently in development which aim to promote the delivery of ΔF508 protein to the cell surface. Since ΔF508 CFTR is known to be active as a cAMP-regulated chloride channel [17], the aim is to direct mutated CFTR to the apical membrane to partially restore ion transport. Another class of drugs in development are termed ‘potentiators’, which are designed to increase the function of mutated CFTR in the apical membrane. As well as targeting ΔF508, these compounds have been demonstrated to increase the activity of at least one other common CFTR mutation, namely G551D [18].

8. DRUG TARGET

CFTR has been a drug target in efforts to find treatments for related conditions. Ivacaftor (trade name Kalydeco, developed as VX-770) is a drug approved by the FDA in 2012 for people with cystic fibrosis who have specific CFTR mutations [19,20].

![Structural model of C.F](image-url)
9. IVACAFTOR: A NOVEL GENE-BASED THERAPEUTIC APPROACH FOR CYSTIC FIBROSION

Ivacaftor (trade name Kalydeco) is a drug used to treat cystic fibrosis in people with certain mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (primarily the G551D mutation), who account for 4–5% cases of cystic fibrosis [21]. It is also included in a combination drug, lumacaftor/ivacaftor (trade name Orkambi), which is used to treat people with cystic fibrosis who have the F508del mutation in CFTR [20].

Invocator was developed by Vertex Pharmaceuticals in conjunction with the Cystic Fibrosis Foundation and is the first drug that treats the underlying cause rather than the symptoms of the disease. It was approved by the FDA in January 2012. It is one of the most expensive drugs, costing over US$300,000 per year, which has led to criticism of the high cost. The combination drug was approved by the FDA in July 2015.

10. MECHANISM OF ACTION OF IVACAFTOR

Ivacaftor, a CFTR potentiator, improves the transport of chloride through the ion channel by binding to the channels directly to induce a non-conventional mode of gating which in turn increases the probability that the channel is open [22,17]. Higgins et al. [23] concluded from their findings that Ivacaftor-treated patients had consistently favorable clinical outcomes relative to untreated comparators, and no new safety concerns were identified. While general limitations of observational research apply, these findings support disease modification by CF transmembrane conductance regulator (CFTR) modulator therapy with ivacaftor. Future, research of novel CFTR modulators will need to explore alternative methods for comparator selection for evaluation of clinical data given the evolving landscape of CF treatment.

![Fig. 3. Structure of Ivacaftor](Source: Gadsby et al., 2006)

![Fig 4. Mechanism of action of ivacaftor by michelle](24)

![Fig 5. Vest therapy]
11. CHEST PHYSICAL THERAPY

11.1 Mechanism of Action of Vest Therapy (High-Frequency Chest Wall Oscillation)

The machine mechanically performs chest physical therapy by vibrating at a high frequency. The vest vibrates the chest to loosen and thin mucus. Every five minutes, the person stops the machine and coughs or huffs [25]. The machine is made up of two pieces, an air-pulse generator and an inflatable vest that is connected to the generator by hoses. The generator sends air through the hoses, which causes the vest to inflate and deflate rapidly, as much as 20 times per second. This rapid inflation and deflation creates pressure on the chest similar to clapping. The vibrations not only separate mucus from the airway walls, they also help move it up into the large airways. Typically, a person uses the vest for five minutes and then coughs or huffs to clear the mucus. Sessions last about 20 to 30 minutes [26,11].

12. CONCLUSIONS

The treatment of CF has a long way to go as most of the existing therapeutics is for older children. Other limiting factors include mutation class, genetic profile, drug interactions, adverse effects, and cost. Novel approaches like gene transfer/gene editing, disease modeling and search for alternative targets are warranted.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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