

Review

Targeting ion channels in cystic fibrosis



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Received 20 April 2015; revised 28 May 2015; accepted 1 June 2015

Available online 23 June 2015

Abstract

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene cause a characteristic defect in epithelial ion transport that plays a central role in the pathogenesis of cystic fibrosis (CF). Hence, pharmacological correction of this ion transport defect by targeting of mutant CFTR, or alternative ion channels that may compensate for CFTR dysfunction, has long been considered as an attractive approach to a causal therapy of this life-limiting disease. The recent introduction of the CFTR potentiator ivacaftor into the therapy of a subgroup of patients with specific CFTR mutations was a major milestone and enormous stimulus for seeking effective ion transport modulators for all patients with CF. In this review, we discuss recent breakthroughs and setbacks with CFTR modulators designed to rescue mutant CFTR including the common mutation F508del. Further, we examine the alternative chloride channels TMEM16A and SLC26A9, as well as the epithelial sodium channel ENaC as alternative targets in CF lung disease, which remains the major cause of morbidity and mortality in patients with CF. Finally, we will focus on the hurdles that still need to be overcome to make effective ion transport modulation therapies available for all patients with CF irrespective of their CFTR genotype.

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Keywords: CFTR modulators; Alternative chloride channels; TMEM16A; SLC26A9; ENaC; Pharmacotherapy

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1. Introduction

CFTR, the protein that is defective in cystic fibrosis (CF), directly mediates the transport of chloride and other anions through the apical membrane of several types of epithelial cells [1,2]. Therefore, mutations impairing CFTR function cause a severe imbalance in ion and water transport that leads to a variety of negative effects in various organs. In the lungs, impaired secretion of chloride and bicarbonate appears to have multiple consequences. First, reduced secretion of electrolytes, and consequently of water, causes dehydration of airway surfaces with impairment of mucociliary clearance [3–5]. This alteration is further worsened by increased activity of the epithelial sodium channel (ENaC) in CF airways that has also been linked, at least in part, to CFTR malfunction [6–9]. Second, reduced bicarbonate secretion acidifies the apical surface fluid thus resulting in defective antibacterial mechanisms [10]. Third, defective bicarbonate secretion impairs the release and expansion of mucins from goblet cells and submucosal glands [11–13] (Fig. 1). It is not clear, if the alterations described above are all equally important, but the final result is that the airways of patients with CF are obstructed by dense mucus and are colonized by opportunistic bacteria such as *Pseudomonas aeruginosa* that trigger a severe inflammatory response [14]. This causes a progressive and irreversible structural lung damage and decline in lung function. CFTR malfunction also causes obstruction of pancreatic and biliary ducts (resulting in nutrient malabsorption and liver disease), infertility (particularly in men), meconium ileus at birth, and diabetes [15–18]. Another characteristic feature of most CF patients is the elevated concentration of sodium chloride in sweat [19]. Restoration of anion transport in defective cells is therefore considered as an important goal of therapeutic

strategies in CF. This goal may be achieved with small molecules that directly rescue CFTR function or stimulate the activity of other anion channels and transporters. In the lungs, improvement in mucociliary clearance may be also obtained by decreasing the activity of ENaC.

2. CFTR

CFTR belongs to the superfamily of ATP-binding cassette (ABC) transporters [1]. Its structure includes a membrane-spanning domain (MSD1) with six transmembrane helices, a nucleotide binding domain (NBD1), a regulatory (R) domain with multiple phosphorylation sites, a second membrane-spanning domain (MSD2), and a second nucleotide-binding domain (NBD2). Under resting conditions, the CFTR pore is closed. Concurrent phosphorylation of the R domain by cAMP-dependent protein kinase A and binding of two ATP molecules at the interface between NBD1 and NBD2 results in pore opening and anion transport [1]. CFTR is a notable exception among ABC transporters. While many members of this superfamily perform active transmembrane transport of solutes by consuming ATP, CFTR is an ion channel. ATP hydrolysis by NBDs is used to open the channel pore and the net flow of anions is solely dependent on the electrochemical potential, i.e. on the concentration gradient across the membrane and on electrical potential difference.

CF-causing mutations provoke CFTR impairment with a variety of mechanisms [2,16,20]. Class I mutations are represented by premature stop codons that lead to a truncated CFTR protein. Class II mutations, in particular F508del that is the most frequent among all CF patients, instead impair the stability and folding of the protein [21]. The consequence is the degradation of mutant

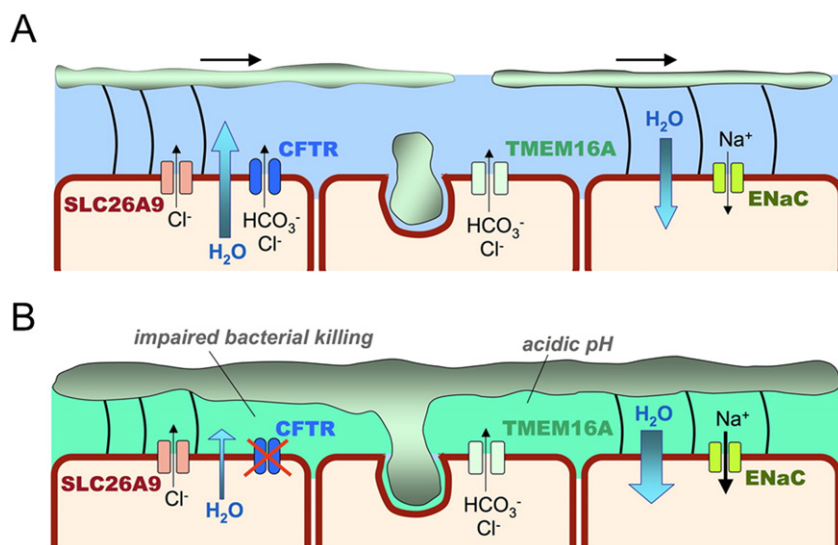


Fig. 1. Epithelial ion transport defects and impaired host defense in CF airways. (A) In healthy airways, coordinated secretion of salt and water driven by CFTR in conjunction with the alternative chloride channels TMEM16A and SLC26A9, and absorption by the epithelial sodium channel ENaC, result in proper hydration of the airway surface layer (ASL) that is essential for effective mucociliary clearance. Further, CFTR-mediated bicarbonate secretion contributes to pH regulation of airway surfaces. (B) In CF airways, CFTR malfunction impairs secretion of chloride/fluid and bicarbonate leading to dehydrated and acidic ASL and hyperconcentrated mucus. ASL dehydration is further aggravated by increased ENaC-mediated sodium/fluid absorption. As a result, mucociliary clearance and bacterial killing are impaired making CF airways vulnerable for infection and inflammation.

CFTR by the ubiquitin–proteasome system and the lysosome [22–24]. Class III includes all those missense mutations (e.g. G551D) that severely reduce the time spent by the CFTR channel in the open state (gating defect). Class IV and V mutations are less severe [16,25]. In Class IV, mutations partially decrease the ability of the channel pore to transport anions. In Class V, the mechanism is essentially a perturbation of the splicing mechanism that results in generation of aberrant mRNA and therefore decreased amount of normal CFTR protein. Hence, Class IV and V mutations retain some residual CFTR function that is associated with an overall milder disease phenotype including exocrine pancreatic sufficiency (PS) [26].

Importantly, it has been found along the years that mutant CFTR can be rescued by treatment with drug-like small molecules that increase the number (n) or open probability (P_o) of mutant CFTR chloride channels in the apical plasma membrane (Fig. 2A). This approach has been very successful for Class III mutations using compounds that are called potentiators [27,28]. These molecules strongly stimulate the activity of mutant CFTR, possibly by binding to the protein itself. Several potentiators have been identified, particularly by high-throughput screening of large chemical libraries [28–31]. When tested in clinical trials, the potentiator ivacaftor (also known as VX-770) showed a marked clinical benefit, with substantial improvement of lung function, reduction of pulmonary exacerbations, and increase in body weight in CF patients with G551D and 8 additional Class III

mutations (G178R, S549N, S549R, G551S, G1244E, S1251N, S1255P and G1349D) [32–35]. These positive results led to the approval of ivacaftor as the first causal therapy for patients with CF that carry one of these responsive mutations.

The pharmacological treatment of other CF mutation classes has revealed more difficulties. Class I mutations such as G542X can be targeted with so called read-through agents. In vitro studies have shown that these molecules, that include aminoglycoside antibiotics and PTC-124, reduce the energy barrier associated with the block represented by the premature termination codons (PTCs) allowing the synthesis of a full length CFTR [36–38]. However, results from clinical trials have been less positive. In a large phase 3 clinical trial, PTC-124 did not meet the primary end point, i.e. improvement in lung function [39]. It was suggested that this negative outcome could be the result of the concomitant use of tobramycin in a subgroup of patients participating in the study. Indeed, when these patients were not taken into account for statistical analysis, PTC-124 appeared to elicit a small but significant therapeutic effect [39]. It was therefore proposed that future trials with PTC-124 will need to remove tobramycin as a confounding factor since this aminoglycoside antibiotic may have intrinsic read-through activity that may compete with PTC-124. However, recent reports have questioned read-through activity of PTC-124, which may provide an alternative explanation for limited clinical efficacy [40]. Another factor that has to be considered for the pharmacological correction of class I mutations

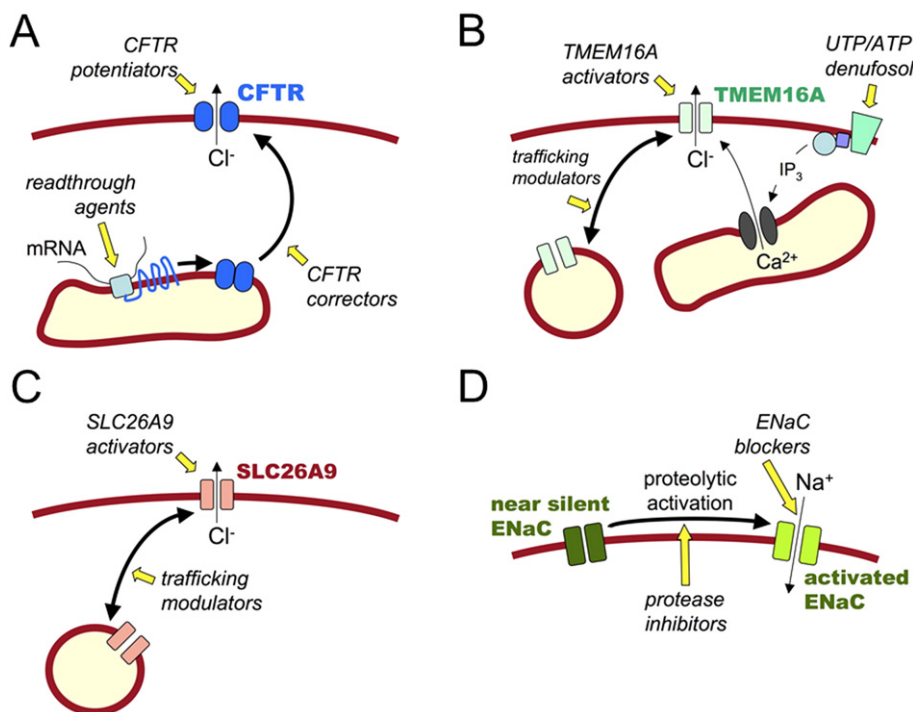


Fig. 2. Pharmacological strategies targeting ion channels in CF. (A) Depending on the underlying molecular mechanisms, pharmacological rescue of mutant CFTR function can be achieved by CFTR potentiators (gating mutations), CFTR correctors (folding mutations) and readthrough agents (premature termination codon mutations). (B, C) Strategies to compensate CFTR malfunction include activation of the alternative chloride channels TMEM16A (B) and SLC26A9 (C). In principle, these alternative targets could be activated by trafficking modulators that increase the number of chloride channels, and/or by activators that increase the open probability of TMEM16A (B) and SLC26A9 (C) channels in the apical plasma membrane. (D) In addition, inhibition of ENaC-mediated sodium/fluid absorption, either by preventing its proteolytic activation by protease inhibitors, or by direct inhibition of the channel by new generation ENaC blockers, can be used as a strategy to counteract airway surface dehydration in CF airways.

is the nonsense mediated RNA decay [41]. This phenomenon causes the degradation of mRNAs harboring PTCs and therefore limits the efficacy of readthrough agents.

The correction of F508del mutation is also a very difficult task. Deletion of phenylalanine 508 causes multiple defects to CFTR protein stability, folding, trafficking, and gating [21,42,43]. Treatment of cells with low temperature or with high concentrations of chemical chaperones (glycerol, DMSO) was found to improve the trafficking of F508del-CFTR to the plasma membrane with a significant increase in chloride transport [44,45]. This was a very important demonstration that the loss of function caused by F508del was reversible. Therefore, several academic laboratories and pharmaceutical companies have looked for small molecules, named generically correctors, which can rescue F508del-CFTR [27,28]. In theory, specific binding of a small molecule to mutant CFTR could favor stability and improve the folding. Another possible mechanism of action of a corrector is the modulation of one or more proteins that are involved in CFTR processing. However, several studies have shown that full rescue cannot be achieved with a single corrector alone [42,43,46]. Only a combination of molecules with different mechanisms of action is expected to induce a large degree of F508del-CFTR correction [46,47]. In agreement with this conclusion, clinical trials with the corrector lumacaftor (also known as VX-809) [48] have produced little effect on F508del homozygous patients [49]. Therefore, current clinical trials are based on the combination of lumacaftor or of another close analog, VX-661, with the potentiator ivacaftor. The rationale for this combination is that the potentiator, by targeting the gating defect caused by F508del, could maximize the rescue achieved with the corrector. However, two recent *in vitro* studies have revealed a possible problem with potentiators and F508del [50–52]. Cells were treated chronically with lumacaftor and ivacaftor or other potentiators. Under these conditions, potentiators appeared to destabilize F508del-CFTR and therefore to antagonize the effect of the corrector. Although limited to *in vitro* observations in human airway cultures, these studies may explain the moderate effects on sweat chloride and lung function observed in recent clinical trials testing combination therapy with lumacaftor and ivacaftor in CF patients homozygous for F508del [53,54]. In parallel to testing of these corrector–potentiator combinations in the clinical arena, the laboratory research continues to focus on the identification of novel more efficacious CFTR correctors and corrector combinations. Indeed, a treatment resulting in efficient correction of the F508del trafficking defect could be sufficient to translate into significant clinical benefits and even make the use of a potentiator unnecessary.

3. Alternative chloride channels

3.1. TMEM16A

More than 20 years ago, functional studies revealed that airway epithelial cells have a second mechanism of chloride secretion independent of CFTR [55,56] (Fig. 1). Stimulation of cells *in vitro*, or nasal epithelia *in vivo*, with calcium agonists (e.g. UTP or ATP) resulted in a large stimulation of chloride

transport. This response was presumably due to the calcium-dependent activation of a non-CFTR chloride channel since it also occurred in patients with CF [56]. The existence of an alternative chloride channel in airway epithelial cells paved the way for clinical studies in which stimulation of calcium-dependent chloride secretion was obtained by aerosol of denufosal [57]. This drug is a UTP analog that induces intracellular calcium elevation by binding to purinergic receptors (P2Y2) localized on the apical membrane of epithelial cells. Unfortunately, denufosal failed to improve lung function or reduce exacerbations in CF patients in recent phase 3 clinical trials [58]. The reasons for the failure are not clear but one possibility is the short half-life of the drug on the airway surface [59]. In addition, the discovery of compounds that activate alternative Ca^{2+} -dependent chloride channels (CaCC) directly was impeded by the fact that their molecular identity remained uncertain and that some of the early candidates, e.g. the CLCA family member hCLCA1/mCLCA3 could not be confirmed as CaCC in native airway tissues [60].

In 2008, three research groups identified TMEM16A as the protein that is responsible for calcium-activated chloride transport in many types of cells [61–63]. TMEM16A, also known as anoctamin-1 (ANO1), belongs to a family of proteins that includes ten members in total. Although other anoctamins, in particular TMEM16B [62], may also function as anion channels, TMEM16A is the one with a particular expression and function in epithelial cells. Silencing of TMEM16A expression in cultured cells or gene knockout in mice significantly reduced transepithelial calcium-dependent chloride secretion [61,63–65]. Interestingly, TMEM16A expression is controlled by pro-inflammatory stimuli. In particular, treatment of cultured airway epithelial cells with the Th2 cytokines IL-4 and IL-13 or induction of asthma-like conditions in mice strongly increased TMEM16A expression [66,67]. Such results were further supported by finding TMEM16A hyperexpression in the airway epithelium of asthmatic patients [67]. Importantly, TMEM16A was particularly found in the membrane of mucus-producing goblet cells [66] (Fig. 1). This localization seems to establish a link between TMEM16A and the mucus hypersecretion typical of Th2 immune response in the respiratory system. In theory, direct pharmacological stimulation of TMEM16A could result in enhanced anion transport with compensation of the basic defect in CF [68]. However, there are some issues that need to be clarified. In particular, the different localization of TMEM16A and CFTR (the former in goblet cells, the latter in ciliated cells) could indicate that the two proteins have different functions in fluid and mucin secretion, and thus mucus clearance by airway surfaces. In the near future, it would be particularly important to establish, with *in vitro* and animal models, the precise function of TMEM16A and its ability to affect the CF phenotype in the airways.

While waiting to better understand the pathophysiological role of TMEM16A in the respiratory system, it is already possible to look for pharmacological modulators (Fig. 2B). In a series of studies, TMEM16A activators and inhibitors were found by high-throughput screening using a fluorescence-based functional assay [69,70]. Activators of TMEM16A may stimulate anion

transport thus resulting in improved mucin release from goblet cells and improved mucociliary clearance. TMEM16A activators could also have other beneficial effects. In a recent study, loss of CFTR was found to induce a pro-inflammatory state characterized by IL-8 hypersecretion [71]. Intriguingly, stimulation of TMEM16A-dependent anion transport with activators corrected this defect. However, more work is needed to understand all consequences of TMEM16A activation in the airways. In particular, it is important to achieve selective modulation of TMEM16A in the airway epithelium to avoid the bronchoconstriction that may be theoretically induced by activating the channel in smooth muscle cells [67]. So far, the search for activators and inhibitors of TMEM16A has been performed by random screening of large collection of chemical compounds with maximally diverse structure. However, the recent publication of the 3D structure of a TMEM16 protein [72] may pave the way for the rational design of direct and specific TMEM16A openers.

3.2. SLC26A9

SLC26A9 belongs to the solute carrier 26 (SLC26) family of anion transporters that is expressed in epithelia of the lung and the stomach [73,74]. By contrast to other members of the SLC26 family that function as chloride and bicarbonate transporters participating in pH regulation, SLC26A9 functions as a chloride channel with minimal bicarbonate conductance [75,76]. The predicted topology of SLC26A9 includes 12 transmembrane domains that participate in the formation of the chloride channel pore and a STAS domain in the C terminus through which SLC26A9 can interact with CFTR, as well as WNK lysine deficient protein kinases, which were found to be implicated in the regulation of various ion transporters and channels participating in osmoregulation of epithelial cells [77–79]. Notably, it has been shown that SLC26A9 contributes to constitutive and cAMP-dependent chloride secretion in human bronchial epithelial (HBE) cells [76,80]. These results suggested that besides TMEM16A, SLC26A9 may also serve as an alternative chloride channel that may compensate for CFTR dysfunction in CF (Fig. 1).

Several lines of evidence from recent studies in mice support that SLC26A9 may indeed serve as an important modifier and potential therapeutic target in CF. First, recent studies in CFTR-deficient mice demonstrated that concomitant lack of SLC26A9 causes a significant increase in GI-related mortality suggesting that SLC26A9-mediated chloride secretion alleviates the severe meconium ileus-like intestinal obstruction in CF mice [81]. Second, studies in mice with experimental asthma demonstrated that similar to TMEM16A, SLC26A9 function was increased in Th2-mediated airway inflammation in parallel with goblet cell metaplasia and mucin hypersecretion [82,83]. These studies also demonstrated that upregulation of SLC26A9-mediated chloride secretion prevented airway mucus plugging in the presence of mucus hypersecretion. These results support a role of SLC26A9 as an alternative chloride channel that may contribute to the regulation of airway surface liquid (ASL) essential for mucus clearance under pathophysiological conditions. Third, data

emerging from genetic studies in humans provide independent evidence supporting the role of SLC26A9 as a disease modifier in CF and potentially other chronic obstructive lung diseases [83–87]. Specifically, consistent with a protective function of SLC26A9 in Th2-mediated airway inflammation, it was shown that a functional SNP (rs2282430) is associated with asthma in children [83]. This SNP resides in the 3' UTR of the SLC26A9 gene where it creates a binding site for a micro-RNA (has-miRNA-632). Binding of this micro-RNA to the 3' UTR results in reduced SLC26A9 protein expression, which may result in reduced chloride channel function *in vivo*. In the meantime, several additional polymorphisms were identified that alter SLC26A9 function by a variety of molecular mechanisms ranging from altered protein expression to decreased or increased chloride channel activity *in vitro* [84]. Interestingly, some of these variants were recently detected in patients with CF-like lung disease [85]. Further, SLC26A9 polymorphisms were also found to be associated with the risk to develop meconium ileus and early exocrine pancreatic disease in patients with CF [86,87].

Taken together, these studies support a role of SLC26A9 as a clinically relevant disease modifier and promising therapeutic target to counteract deficient chloride secretion and dehydration of mucosal surfaces of the airways and GI tract of patients with CF (Fig. 2C). However, current knowledge on the regulation and pharmacology of SLC26A9, as well as model systems and reagents required for the discovery process remain limited. Hence, considerable basic research efforts will be required before SLC26A9 can be explored as a novel therapeutic target in patients with CF and potentially other muco-obstructive lung diseases.

4. ENaC

The amiloride-sensitive epithelial sodium channel ENaC is expressed in the luminal membrane of absorptive epithelia including the conducting airways, alveolar airspaces, the distal colon, the sweat duct and the distal nephron of the kidney (Fig. 1). ENaC is a multimeric transmembrane protein composed of three homologous subunits (α , β and γ) that form the pore of a small conductance (~4 to 5 pS) sodium channel that is inhibited by amiloride with an IC₅₀ of ~0.1 μ mol/l [7,88]. ENaC is the limiting pathway for apical sodium uptake by absorptive epithelial cells. In tight epithelia with low water permeability, i.e. the sweat duct, coordinate activation of CFTR and ENaC in the same epithelial cell is a prerequisite for transcellular absorption of sodium and chloride that results in a hypotonic sweat and retention of salt in the body during sweating [89]. Accordingly, salt absorption in the sweat duct is impaired and the NaCl concentration of sweat is elevated in patients with CF, as well as patients with systemic pseudohypoaldosteronism, a rare genetic disorder caused by loss of function mutations in ENaC [90]. In less tight epithelia with paracellular permeability for water and ions, such as the respiratory epithelium lining the conducting airways, the transepithelial potential difference generated by active ENaC-mediated sodium absorption provides the driving force for absorption of chloride and water through the paracellular shunt pathway [91]. Therefore, in airway epithelia, CFTR is not necessarily required for absorption of salt and water,

and ENaC becomes the limiting pathway that plays an important role in volume regulation of the thin film of fluid that covers airway surfaces and is essential for effective mucociliary clearance [5,92].

In CF airway epithelial cells, CFTR is deficient, whereas ENaC function remains intact (Fig. 1). This defect leads to an imbalance between secretion and absorption of ions and fluid that makes CF airway surfaces vulnerable to dehydration. In addition, several lines of evidence suggest that ENaC is dysregulated in CF explaining the increased ENaC activity observed in airways from CF patients [93–95]. First, co-expression of ENaC with CFTR in heterologous cells showed that wild-type, but not F508del-CFTR inhibits ENaC activity [6,7,96]. These studies led to the hypothesis that CFTR has a dual function as anion channel and regulator of ENaC, and provided an explanation how normal airway cells can switch from ENaC-mediated absorption to CFTR-mediated secretion, and why ENaC is hyperactive in CF [9,97]. More recently, it was found that neutrophil elastase (NE), a major neutrophil product, other proteases released from inflammatory cells, as well as bacteria in CF airways, can activate ENaC directly by proteolytic cleavage independent of mutant CFTR [98–103].

Collectively, these results support that in the absence of CFTR-mediated anion secretion, continuing or even increased ENaC-mediated absorption of salt and water contributes critically to airway surface liquid (ASL) depletion and impaired mucociliary clearance in patients with CF. The importance of ENaC in the *in vivo* pathogenesis of CF lung disease was further buttressed by studies in mice that overexpress the β subunit of ENaC (β ENaC-Tg) in the airways [104,105]. These studies demonstrated that increased ENaC-mediated sodium absorption causes ASL depletion, hyperconcentrated mucus and impaired mucus clearance, and that these defects trigger CF-like lung disease with airway mucus plugging, spontaneous infection and inflammation, and structural lung damage *in vivo* [106–112].

Taken together, these studies suggest ENaC as an attractive alternative target to improve airway surface hydration and mucus clearance in patients with CF independent of their CFTR genotype (Fig. 2D). In fact, inhibition of ENaC by inhalation of the classical ENaC blocker amiloride was tested in clinical trials more than 20 years ago [113,114]. In these studies, inhaled amiloride did not improve lung function in CF patients with established lung disease, probably due to its limited potency and short half-life on airway surfaces [115,116]. Interestingly, when inhaled amiloride was revisited in preclinical studies in β ENaC-Tg mice, it was found that preventive treatment had significant effects on airway mucus obstruction and inflammation, and that the overall benefits were superior to preventive hypertonic saline in this model of CF lung disease [117–119]. This proof-of-concept suggests amiloride may also be beneficial in CF patients when applied in the preventive setting. With the widespread implementation of CF newborn screening, it could now be tested if preventive inhalation therapy started soon after birth has a potential to delay or even prevent irreversible lung damage in CF. However, the development of more potent and durable ENaC blockers remains critical for improving efficacy in patients with established CF lung disease.

Besides CFTR modulators, the preclinical development of novel more potent and long-acting ENaC inhibitors has been an active area of preclinical CF research [120–126]. In addition to direct pharmacological inhibition, several alternative strategies have been explored experimentally that may enable effective inhibition of ENaC *in vivo*. These strategies include inhibition of ENaC expression through silencing with specific short interfering RNAs (siRNAs) [127], inhibition of ENaC activity via inhibition of its activation proteases [99,101], and high throughput screening to identify novel ENaC modulators that may be druggable in CF lung disease [128]. The clinical development of inhalation therapy with some of these novel ENaC blockers has been hampered by their systemic absorption, leading to hyperkalemia resulting from inhibition of ENaC in the kidney [129]. However, several promising compounds have been identified with reduced potential to produce hyperkalemia that are expected to enter the clinical arena in early phase clinical trials in patients with CF in the near future.

Similar to therapeutic activation of alternative chloride channels, efficient inhibition of ENaC in the airways has the potential to provide clinical benefits for all patients with CF irrespective of their CFTR genotype. This approach may be of particular relevance in CF patients carrying mutations that are not responsive to CFTR modulator therapy. Further, inhibition of ENaC in the luminal membrane will hyperpolarize airway epithelial cells and thus increase the driving force for chloride secretion. Therefore, it may also be attractive to use ENaC blockers in combination with CFTR modulators to augment chloride secretion through pharmacologically rescued mutant CFTR.

5. Summary and outlook

During the last decade, tremendous progress has been made in therapeutic targeting of deficient ion transport in CF. The approval of ivacaftor as first causal therapy for a subgroup of patients with CF was a major milestone in the development of precision medicine for CF and an important proof-of-concept for other genetic lung diseases [32]. With CFTR corrector-potentiator combination therapies it may soon be possible to rescue CFTR function at least partially in a larger number of patients including those homozygous for the most common mutation F508del [53]. Importantly, systemic treatment with CFTR modulators may improve CFTR activity in all organs affected and may thus have therapeutic benefits beyond CF lung disease. However, the efficacy of pharmacological rescue of F508del, as well as other mutations that affect folding (Class II) and synthesis (Class I), remains limited and further improvement challenging [2,50,51,53]. Therefore, activation of alternative chloride channels and inhibition of ENaC remain attractive strategies to compensate for CFTR dysfunction in the airways, where these proteins are co-expressed. Because these alternative ion channels are not expressed in many of the other organs affected in patients with CF, potential benefits of therapeutic targeting are likely limited to the lungs. On the other hand, despite this limitation in the treatment of CF multiorgan disease outside the lungs, these alternative therapeutic strategies have a high potential to lead to an effective treatment of lung

disease in all patients with CF independent of their CFTR genotype. Significant progress in this area includes the molecular identification of TMEM16A and SLC26A9 as alternative chloride channels in CF airways [61,64] that will facilitate the development of specific activators of these alternative targets. Further, several new generations ENaC blockers have been developed with enhanced potency and duration of action and reduced potential to cause hyperkalemia in vivo [126]. In parallel, several animal models with CF-like lung disease have been generated [104,105,130–132] that can now be used for preclinical evaluation of emerging ion transport modulators to correct the CF ion transport defect and associated defects in mucus transport and anti-bacterial host defense in vivo. Finally, there has been a new trend in the pharmaceutical industry to invest into the discovery and clinical development of ion transport modulators for CF that will reduce the risk that promising compounds get lost in translation. Although still at the beginning, it seems realistic to expect that effective and safe ion transport modulators will become available in the near future, and that this new class of drugs will contribute to further improvement of life expectancy and quality of life of patients with CF. Importantly, although specifically developed for a rare genetic disease, these drugs may also be beneficial for patients with common chronic obstructive lung diseases including asthma and COPD [2,83,133,134].

Acknowledgments

This work was supported in part by grants from the German Research Foundation (MA2081/3-2 and MA2081/4-1 to MAM), the German Ministry for Education and Research (to MAM) and by grants from Ministero della Salute (Ricerca Finalizzata: GR-2009-1596824; Ricerca Corrente: Cinque per mille), Telethon Foundation (GGP10026), and Fondazione Italiana Fibrosi Cistica (FFC#2/2012) (to LJVG). The authors would like to thank their colleagues who contributed to the work cited in this review.

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