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Review

The TMEM16A chloride channel as an alternative therapeutic target in cystic fibrosis☆

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ABSTRACT

Cystic fibrosis (CF), a multiorgan genetic disease, is caused by loss of function of CFTR, a cAMP-regulated anion channel. In CF airway epithelia, defective Cl^- and bicarbonate secretion impairs mucociliary clearance and other innate defense mechanisms, favoring the colonization of the lungs by highly virulent bacteria. The airway epithelium expresses TMEM16A, a second type of Cl^- channel that is activated by cytosolic Ca^{2+}. TMEM16A is particularly expressed in goblet cells. This specific localization could be important in the release and hydration of mucins. Activation of TMEM16A with pharmacological agents could circumvent the primary defect in CF. This strategy needs to be carefully designed and tested to avoid possible undesired effects due to the expression of TMEM16A in other cell types such as bronchial smooth muscle cells.

This article is part of a Directed Issue entitled: Cystic Fibrosis: From o-mics to cell biology, physiology, and therapeutic advances.

Contents

1. Introduction

Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene encoding the CFTR Cl^- channel (Riordan, 2008). CF affects several organs, but the most severe consequences are observed in the lung. The pathogenesis of lung disease in CF is still not perfectly understood. The loss of CFTR channel function in CF favors the colonization of the airway surface by highly virulent bacteria (Clunes and Boucher, 2007). However, the mechanism by which this happens is still a matter of debate. CFTR channel activity is certainly an important contributor to mucociliary clearance (Fig. 1). The fine balance between CFTR-dependent Cl^- secretion and Na^+ absorption through the ENaC channel controls the thickness of the periciliary fluid (PCF). In the absence of functional CFTR, fluid absorption prevails over secretion (Clunes and Boucher, 2007; Matsui et al., 1998). This imbalance dehydrates the airway surface thereby impairing ciliary beating. Immobilized mucus then becomes a niche for bacterial survival and proliferation. An alternative/complementary model postulates a more direct link between CFTR and bacteria. In CF pig airways, it has been shown that defective CFTR function impairs the innate anti-microbial activity of PCF (Pezzulo et al., 2012). This activity seems to require the secretion of bicarbonate through CFTR. Indeed, the direct delivery of a high bicarbonate concentration onto the airway surface restores the rapid bacterial

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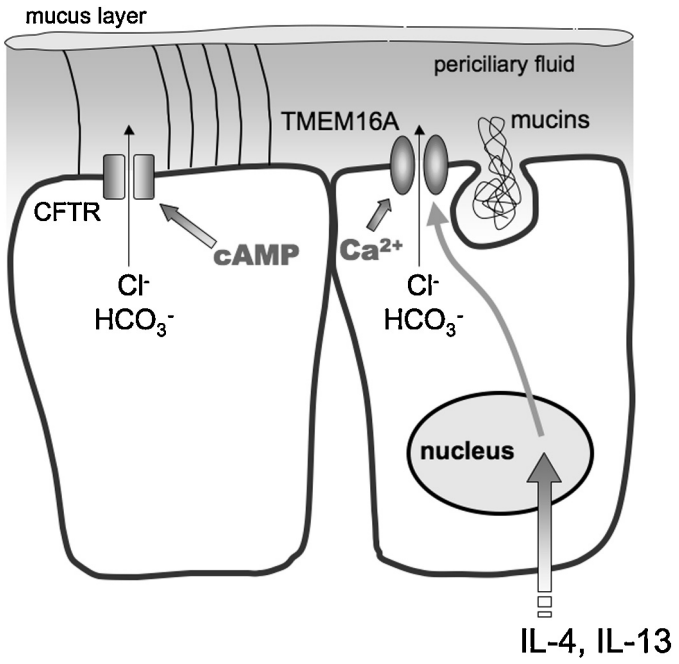

Fig. 1. Expression of CFTR and TMEM16A in the airway epithelium. Both types of channels contribute to the secretion of Cl^- and bicarbonate, a process that is important to maintain the correct thickness of periciliary fluid and fluidify mucus secretions. However, CFTR and TMEM16A are activated by different mechanisms (cAMP and Ca^{2+}) and expressed in separate cell types (ciliated and goblet cells). TMEM16A expression is also strongly stimulated by IL-4 and IL-13, cytokines that induce mucus cell metaplasia.

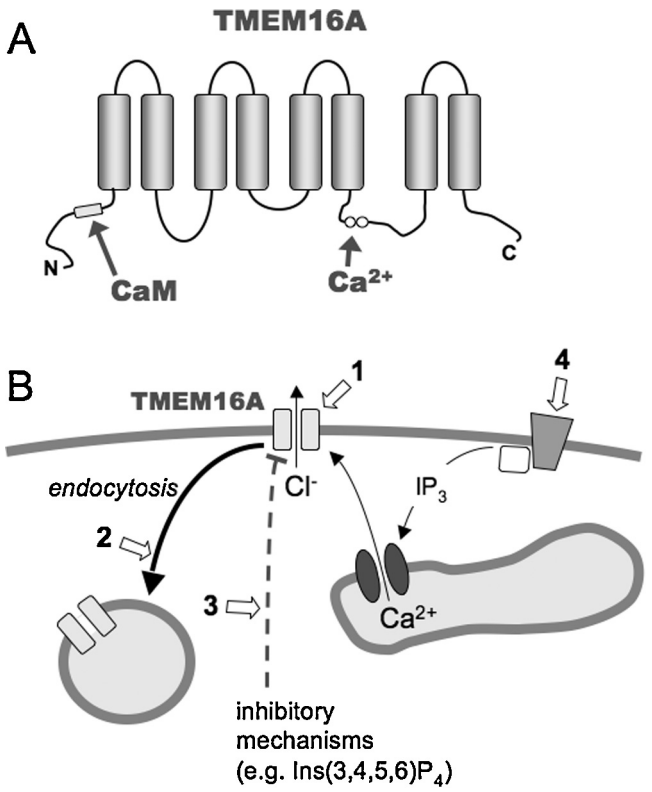

Fig. 2. Structure and regulation of TMEM16A. (A) TMEM16A consists of eight transmembrane segments. Two glutamic residues in the third intracellular loop appear to mediate direct activation by cytosolic Ca^{2+}. Channel activity may also be modulated by binding of calmodulin (CaM) to a region preceding the first transmembrane segment. However, regulation of TMEM16A gating by calmodulin is controversial. (B) Possible pharmacological strategies to upregulate TMEM16A activity. Pharmacological modulators could act as direct activators (1), inhibitors of endocytic internalization (2), blockers of inhibitory mechanisms (3), agonists of receptors linked to intracellular Ca^{2+} mobilization (4).

killing observed in non-CF airways (Pezzulo et al., 2012). Bicarbonate secretion may also be required to favor mucus expansion. In the absence of an adequate secretion of bicarbonate, the mucus released by goblet cells remains densely packed and tenaciously attached to the epithelial surface (Garcia et al., 2009; Gustafsson et al., 2012).

2. Identification of TMEM16A protein

More than 20 years ago, it was found that airway epithelial cells possess a second type of Cl^- secretory pathway (Mason et al., 1991). While CFTR is regulated by cAMP, the second pathway is activated by the increase in cytosolic Ca^{2+} concentration. Stimulation of airway epithelia with purinergic agonists such as ATP or UTP causes a strong, but transient, burst of transepithelial Cl^- transport (Mason et al., 1991). This effect is dependent on both release of Ca^{2+} from intracellular stores and influx of Ca^{2+} through the plasma membrane. Ca^{2+}-activated Cl^- secretion is independent of CFTR since it is not defective in CF patients. Interestingly, stimulation of Ca^{2+}-dependent Cl^- secretion has a positive effect on PCF thickness (Tarran et al., 2002). This effect suggests that stimulation of the Ca^{2+}-dependent pathway may compensate for defective CFTR.

The molecular identity of the Ca^{2+}-dependent Cl^- channel (CaCC) remained unknown for many years. Some membrane proteins, such as ClC-3 or bestrophins, were proposed to function as CaCCs. However, the biophysical and pharmacological properties of ion channels associated with ClC-3 or bestrophin expression did not resemble those of native CaCCs (Duran et al., 2010; Ferrera et al., 2010). In 2008, three independent teams identified the orphan protein TMEM16A as a component of CaCC (Caputo et al., 2008; Schroeder et al., 2008; Yang et al., 2008). Heterologous expression of TMEM16A in null systems elicited Cl^- currents very similar to those of native CaCCs. In one of the studies, TMEM16A was identified in the airway epithelium using interleukin-4 (IL-4) as a perturbing agent (Caputo et al., 2008). It was previously found that treatment with IL-4 or IL-13 causes a strong upregulation of CaCC activity in human bronchial epithelial cells (Danahay et al., 2002; Galietta et al., 2002). This effect appeared consistent with an increased expression of the gene encoding the CaCC. Analysis of global gene expression with microarrays identified TMEM16A as the membrane protein responsible for CaCC activity (Caputo et al., 2008).

TMEM16A, also known as anoctamin-1 (ANO1), belongs to a protein family composed of 10 members whose primary sequence and predicted structure (eight transmembrane domains) have no similarity with those of other anion channels (Fig. 2A). Activation by Ca^{2+} seems to involve direct interaction with TMEM16A protein at the level of the third intracellular loop (Yu et al., 2012). Indeed, mutagenesis of two highly conserved glutamic acid residues strongly decreased the sensitivity of TMEM16A to cytosolic Ca^{2+}. This conclusion is also supported by recent experiments in which TMEM16A protein was purified and reconstituted in artificial membranes (Terashima et al., 2013). Under this condition, TMEM16A was directly activated by Ca^{2+}, a finding that seems to exclude the requirement of other proteins, including calmodulin. Actually, there are conflicting results about the possibility that TMEM16A channel gating is regulated by calmodulin. In one study, calmodulin was found to be absolutely required for TMEM16A activation by Ca^{2+} (Vocke et al., 2013). In another study, calmodulin appeared to bind and modulate the activity of a specific TMEM16A isoform (Tian et al., 2011). However, in a very recent study no

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