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TMEM16A Potentiators: Is There a Need for New Modulators in Cystic Fibrosis?

On October 21, 2019, the U.S. Food and Drug Administration approved a highly effective triple combination therapy (elexacaftor, tezacaftor, and ivacaftor) for patients with cystic fibrosis (CF) with at least one copy of the F508del mutation in CFTR (CF transmembrane conductance regulator). This combination therapy improved lung function, sweat chloride, weight, and quality of life in an unprecedented fashion for a folding mutation (1, 2). Thus, the currently available highly active modulators have the potential to reach ~90% of the population of patients with CF. This raises the question whether additional therapies for CF or other airway diseases related to CFTR dysfunction are still needed.

Given the remaining patients with CF without current highly effective treatment modalities, patients who cannot tolerate or who respond poorly to available modulators and the uncertainty of the treatment efficacy over time, the answer is a resounding yes. The question then is which mechanisms to target. Because lung disease is a major contributor to morbidity and mortality, a main aim of CFTR modulators is to hydrate mucus by restoring airway surface liquid volume to facilitate mucociliary clearance (MCC). Homeostasis of airway surface liquid is tightly regulated and maintained by the balance of ion channel activities in the airway epithelium, including chloride secretion by CFTR and calcium-activated chloride (CaCC) channels, potassium secretion by large-conductance calcium-activated and voltage-dependent potassium (BK) channels, and sodium absorption by the epithelial sodium (ENaC) channels (3, 4). Ion channels that compensate for defective CFTR therefore represent an important therapeutic target group.

Restoring airway surface hydration in CF by modulating ion channel function alternative to CFTR has shown promise as a therapeutic option *in vitro* and in animal studies, but strategies targeting these proteins had limited success in clinical trials. So far, no therapeutics have been developed for BK channel activation. Approaches to down-regulate the expression and/or function of ENaC have been proposed as a means to improve mucus hydration. Unfortunately, prototypical ENaC blockers such as amiloride showed no clinical efficacy, whereas amiloride analogs and other approaches provided thus far no measurable clinical benefit (5, 6), possibly because of low dosing to avoid toxicity.

Therapeutic strategies to enhance apical chloride secretion alternative to CFTR have been attempted even before the molecular identification of TMEM16A (transmembrane member 16A), also known as ANO1 (anoctamin 1), as one of the CaCCs (7–9). Early studies *in vitro* and in normal subjects provided evidence that stable uridine-5′-triphosphate analogs stimulate CaCC via increases in cytosolic calcium and improve MCC (10, 11). However, these selective P2Y2 purinergic receptor agonists also stimulated mucin secretion (12) and ultimately failed to demonstrate any clinical benefit in the large phase 3 clinical trial TIGER-2 (Transport of Ions to Generate Epithelial Rehydration).

TMEM16A is a highly conserved member of a larger family of proteins that comprise Ca^{2+}-dependent ion channels and phospholipid scramblases. Although TMEM16A is thought to be a significant contributor to CaCCs in the airways, the exact function of TMEM16A in airway disease remains controversial. This largely stems from findings that TMEM16A is expressed at low levels in the airway epithelium under normal conditions, and that its expression is highly induced by proinflammatory cytokines, including IL-4 and IL-13 that drive goblet cell hyperplasia (7, 13). Furthermore, the possible prosecretory effect of TMEM16A on mucin secretion (13) led to speculations that augmentation of TMEM16A activity may have adverse effects on MCC in CF.

In this issue of the *Journal*, Danahay and colleagues (pp. 946–954) describe the identification of a novel TMEM16A potentiator, ETX001, that enhances anion secretion and improves MCC both in primary CF bronchial epithelial cells *in vitro* (unstimulated and stimulated with IL-13) and in an *in vivo* sheep model of CF-like airway disease (14). ETX001 failed to stimulate TMEM16A activity in the absence of calcium, but rather

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enhanced uridine-5′-triphosphate-stimulated currents, consistent with ETX001 acting as a potentiator rather than an activator. ETX001 also significantly potentiated anion secretion without adversely affecting ENaC conductance or cAMP-dependent chloride secretion.

Importantly, ETX001 potentiated TMEM16A without affecting intracellular calcium, which can trigger excessive mucus secretion. Normal breathing produces shear stress, which causes the release of ATP. ATP-induced activation of purinergic receptors leads to the stimulation of CaCC, and likely other channels, which increases fluid secretion (3). In fact, the abundant presence of ATP in CF airways (15) may have contributed to the failure of uridine-5′-triphosphate analogs to enhance MCC via TMEM16A. However, treatment of CF bronchial epithelial cells with ETX001, independent of calcium and P2Y2 receptors, increased fluid secretion, as measured by airway surface liquid height, under shear stress conditions.

To test the benefit of ETX001 *in vivo*, the authors took advantage of an established CF-like sheep model (5, 16). As previously shown, inhalation of the CFTR inhibitor CFTRinh-172 depressed tracheal mucus velocity, a surrogate measure of MCC (16). However, tracheal mucus velocity was effectively restored by ETX001 for up to 12 hours after administration. Moreover, ETX001 improved whole-lung MCC even in the presence of functional CFTR. It is important to note that the pharmacological CF sheep model used in this study, although leading to an increase in mucus concentrations (16), does not mimic the hyperinflammatory state of CF airways, which increases goblet cell-related expression of TMEM16A. However, *in vitro* studies with IL-13 showed that ETX001 continues to enhance fluid secretion without any noticeable detrimental effect.

Thus, the authors provide compelling evidence that TMEM16A potentiators could have beneficial effects on MCC in CF in a mutation-agnostic manner. A sizeable population of individuals with CF who are either ineligible for triple combination therapy or respond poorly could benefit from ETX001 if its efficacy can be translated from the sheep to humans (the previous failures of other drugs were possibly linked to underdosing in human trials compared with the sheep). Furthermore, CFTR modulators and molecules targeting alternative ion channels are not mutually exclusive therapies and may have additive effects in maintaining airway homeostasis. Finally, the reach of ETX001 and other TMEM16A potentiators could also extend beyond CF and may hold promise as a therapeutic option for other mucoobstructive airway diseases. Indeed, these are unprecedented and exciting times in the development of novel therapies in CF and beyond. ■

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