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SPX-101 Is a Promising and Novel Nebulized ENaC Inhibitor

Optimal hydration of the airway lumen is required for effective mucociliary clearance of inhaled pathogens and particulate matter (1). Because the airway epithelium is relatively permeable to water, the volume and composition of the airway surface liquid (ASL) is determined by active ion transport. In cystic fibrosis (CF) airway disease, the ASL is depleted and excessively viscous because of the lack of cystic transmembrane conductance regulator-mediated chloride and bicarbonate secretion. In addition to this secretory deficit, the CF airway is hyperabsorptive as a result of dysregulated epithelial sodium channel (ENaC) activity (2–4). Because of these ion transport defects, the airway in CF is exceptionally vulnerable to dehydration, and the luminal contents are abnormally acidic and tenacious. Therefore, mucus clearance from the CF airway is reduced and causes chronic airway inflammation, obstruction, infection, and ultimately premature respiratory failure. One potential therapeutic strategy that has been proposed to mitigate the excessive ASL absorption in CF is to inhibit ENaC.

ENaC is the rate-limiting step of Na^+ absorption across the airway epithelium and is composed of three structurally related subunits, α , β , and γ . The ENaC subunits have large extracellular loops that play a key role in determining the channel's activity. Limited proteolysis of the extracellular loops on the α and γ subunits by a family of channel-activating proteases increases the channel's open probability and activity. The heightened ENaC activity observed in CF epithelia is believed to be partially a result of increased proteolytic activation of the channel (5–7). In an effort to isolate potential channel-activating protease inhibitors in the ASL, Garcia-Caballero and colleagues identified short palate, lung, and nasal epithelial clone 1 (SPLUNC1) as a potential ENaC inhibitor during proteomic analysis of the eluted proteins from trypsin affinity column (8). Tarran's laboratory has

subsequently confirmed that SPLUNC1 acts as an ENaC inhibitor, but not by acting as a protease inhibitor, as initially hypothesized. SPLUNC1 specifically binds to mature ENaC subunits and appears to promote channel internalization (9, 10). Interestingly, the SPLUNC1 interaction with ENaC is pH-sensitive, such that SPLUNC1 fails to inhibit Na^+ absorption in the abnormally acidic CF airway (11). Hobbs and colleagues then identified a limited region located near the unstructured amino terminus of SPLUNC1 that inhibits ENaC in a pH-independent manner. When applied to the apical surface of cultured CF airway epithelia, this 18-amino acid peptide (S18) not only reduced ENaC activity to the same extent as the full SPLUNC1 protein but also restored the ASL to a normal height (9).

In this issue of the *Journal*, Scott and colleagues (pp. 734–744) report on the development of SPX-101, a potential peptide therapeutic that has been derived from S18 to stabilize and optimize the peptide for nebulization (12). In primary cultures of airway epithelial from CF and non-CF donors, they demonstrate that SPX-101 causes a durable decrease in ENaC surface expression and reduces the amiloride-sensitive current. The effects of SPX-101 were then tested *in vivo* in $\beta\text{ENaC-Tg}$ mice that develop increased ASL absorption, excessive airway inflammation, and premature respiratory death similar to what is observed in human CF lung disease (13). Once-daily intranasal administration of SPX-101 improved survival and reduced airway inflammation in the $\beta\text{ENaC-Tg}$ mice. SPX-101 also increased the mucociliary velocity across explanted $\beta\text{ENaC-Tg}$ airways. In addition, tracheal mucus velocity was increased in an ovine model of CF after a single dose of SPX-101. The increased tracheal mucus velocity was dose-dependent, additive to hypertonic saline, and more durable than amiloride and hypertonic saline, with effects persisting for at least 8 hours. These findings, coupled with the unique mechanism of action, suggest SPX-101 is an optimal agent to reduce ENaC activity in the CF airway.

ENaC inhibition has long been a therapeutic target in CF, given the potential for ASL hydration and expected improvement in mucociliary clearance. However, the promising preclinical data have

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yet to translate into meaningful clinical effects. Amiloride, the prototypical ENaC inhibitor, increases the ASL height of cultured airway, but several clinical trials have failed to demonstrate appreciable benefit after nebulized administration (14, 15). A long-acting and potent amiloride analog, GS-9411, had initial promise as well, but unfortunately, systemic effects including hyperkalemia limited its potential in early clinical trials (16). An additional long-acting ENaC inhibitor, Parion-1037 (now VX-371), is currently in clinical trials, and the results remain pending. SPX-101 has several distinguishing features that may improve its efficacy compared with amiloride derivatives. First, SPX-101 has a prolonged duration of action that may provide durable ASL expansion, simplify dosing, and prolong the effects of hydration strategies such as nebulized hypertonic saline. Another compelling feature of SPX-101 is that systemic effects of ENaC inhibition, such as hyperkalemia or volume depletion, have not been observed in preclinical animal models. Although Tarran and colleagues have made impressive progress in identifying SPLUNC1 and developing SPX-101, it remains to be seen whether these efforts will translate into clinical benefit, particularly above that seen with hypertonic saline, for individuals with CF. Novel therapeutics such as SPX-101 and the next-generation cystic transmembrane conductance regulator correctors make the CF future bright, and we look forward to these upcoming studies. ■

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The Epigenetics of Intrauterine Smoke Exposure: Can Maternal Vitamin C Supplementation Prevent Neonatal Respiratory Disease?

Intrauterine smoke (IUS) exposure has been associated with a myriad of adverse health outcomes in the newborn, including decrements in lung function that persist into adolescence (1), early-onset wheeze (2), and asthma susceptibility (3). The

public health implications of these transgenerational effects of maternal smoking indicate an urgent need to better understand their biologic underpinnings and to identify early intervention strategies.

Developmental processes are highly regulated by epigenetic mechanisms, and the epigenome is most vulnerable to environmental perturbations during fetal development when the DNA synthesis

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