A Nod and a WNK (Kinase): Understanding Airway Surface Liquid pH

Cystic fibrosis (CF) is a progressive disease with high morbidity and mortality caused by mutations in the *CFTR* (CF Transmembrane Conductance Regulator) gene encoding for a cAMP-activated anionic channel, known to mainly secrete chloride (Cl^-) and bicarbonate (HCO_3^-) at the apical surface of epithelia. A key pathophysiological feature of cystic fibrosis is a reduction in HCO_3^- secretion, which in turn acidifies the airway surface liquid (ASL) acting as a thin fluid barrier on the luminal side of airway epithelia. Recent studies have revealed that there is reduced antimicrobial activity in CF ASL as a result of the decreased pH (1, 2), a defect that can be rescued with ASL alkalinization (2, 3). To date, the mechanisms regulating ASL pH remain poorly understood. Given the role pH homeostasis plays in CF disease pathogenesis, understanding the mechanisms that regulate HCO_3^- secretion in human airways may reveal new therapeutic targets in CF, including for those CFTR mutations where highly effective CFTR modulator therapy (HEMT) is not available.

WNK (with-no-lysine [K]) kinases are known as regulators of anion transport (4), with roles in HCO_3^- transport within the pancreatic ducts (5). In this issue of *AJRCMB*, Rehman and colleagues (pp. 491–502) report on studies that examine WNK kinase activity in airway epithelia, demonstrating that reduction of WNK kinase activity increases ASL pH and improves host defense mechanisms (6). Using human CF and non-CF primary cultures supported by single cell RNA sequencing analysis, the authors reveal the presence of two WNK kinase isoforms, WNK1 and WNK2, in secretory cells and ionocytes. They show that knockdown of *WNK1* and *WNK2* transcripts increases ASL pH, and pharmacologic inhibition of WNK kinases by WNK463 decreases ASL viscosity while increasing ciliary beat frequency and ASL-mediated *Staphylococcus aureus* killing in primary CF epithelia. Using a series of electrophysiological studies, they then suggest a mechanism for the restoration in CF host defense defects by experimentally confirming that WNK kinase inhibition decreases Cl^--mediated, but not HCO_3^--mediated current. Interestingly, they found that although WNK kinase inhibition reduced CFTR-mediated anion secretion, the channel's activity at the apical membrane remained unchanged, indicating that WNK kinase–related anion secretion was not dependent on apical anion channels.

The authors then interrogated basolateral Cl^- entry by inhibiting the loop-sensitive Na^+-K^+-$2Cl^-$ (NKCC) cotransporter using bumetanide. They found that WNK inhibition reduced bumetanide-sensitive short-circuit current in both non-CF and CF epithelia, suggesting WNK kinases regulate basolateral Cl^- uptake though NKCC activity. Indeed, the authors demonstrate that pharmacological NKCC inhibition or removal of intracellular Cl^- increases ASL pH, and WNK kinase inhibition–dependent alkalinization of CF ASL pH requires intracellular Cl^-. Further reinforcing the significance of the study, the authors provide evidence that TNFα and IL-17, critical drivers of CF neutrophilic inflammation (7, 8) and known to promote alkalization of ASL (9, 10), reduced WNK kinase expression. This effect of ASL pH alkalization by TNFα/IL-17 was increased by WNK inhibition with WNK463 or NKCC inhibition with bumetanide. The authors speculate that TNFα and IL-17 may work to promote apical HCO_3^- over Cl^- secretion, and NKCC or WNK kinase inhibition augmented this response by lowering basolateral Cl^- entry.

Like all noteworthy studies, this work raises many additional questions. For example, Rehman and colleagues demonstrate that either *WNK1* or *WNK2* knockdown increases ASL pH, leaving the question as to whether there is an additive and/or compensative effect between the two isoforms. Also, pendrin, a Cl^-/HCO_3^- exchanger, is expressed at low concentrations under basal conditions but is upregulated in the presence of proinflammatory cytokines (10) present in CF and other airway diseases, including asthma. Because TNFα/IL-17 reduces WNK kinase expression, might WNK kinases have a regulatory role in pendrin expression? Pendrin inhibition has been explored as a therapeutic target in asthma (11), and studies have suggested decreased pH in the airways of individuals with asthma as potentially pathogenic (12, 13). Interestingly, a recent study identified a single-nucleotide polymorphism in the WNK kinases substrate *OXSR1* (Oxidative Stress Responsive Kinase 1) to confer an increased risk of asthma exacerbations (14). Taken together, these works suggest a possible role for WNK kinase regulation of ASL pH homeostasis in diseases outside of CF. Although Rehman and colleagues did not elucidate the transporter responsible for apical HCO_3^- secretion, the authors' studies suggest an intracellular $[Cl^-]$-dependent regulation of HCO_3^- secretion. It will be important to determine the underlying mechanism of apical membrane HCO_3^- exit as well as the function of intracellular $[Cl^-]$ and whether the anion is simply increasing the driving force for Cl^-/HCO_3^- exchange or acting as a signaling molecule.

Finally, even though Rehman and colleagues performed their work in primary epithelia cultures, we believe their findings may have immediate clinical implications. For instance, introduction of HEMT has led to the goal of reducing inflammatory cytokine production (15). Might reductions in TNFα/IL-17 by HEMT lead to loss of the compensatory increase in ASL pH, and can this decrease in ASL pH be mitigated by WNK kinase inhibition? Likewise, the authors indicate that the addition of ivacaftor to WNK463-treated CuFi-4 (G551D/F508 del) epithelia further increased ASL pH, suggesting a complex relationship between the resolution of inflammatory response and epithelial-specific CFTR restoration.

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In addition, the authors limited testing of ASL antimicrobial activity to *S. aureus* killing. Although *S. aureus* is a major pathogen in CF, other emerging microbes are detected in CF airway fluids where host–pathogen interactions are complex and regulated by multiple bacterial and fungal phenotypes. More studies are needed to understand the role of the innate host defense, including cells of the immune system, host–pathogen interactions in CF lung disease, and their interplay with epithelia in the setting of CFTR functional restoration.

Altogether, research in the field of cystic fibrosis continues to suggest that ASL acidification acts as a key contributor to the pathogenesis of the disease and that drugs that target different aspects of epithelial-mediated ASL pH homeostasis, including WNK kinase activity as proposed by Rehman and colleagues, should be strongly considered for treatment of cystic fibrosis. ■

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