Opening up to cAMP Transport Mechanisms in Airway Smooth Muscle

The management of asthma and chronic obstructive pulmonary disease (COPD) is guided by the Global Initiative for Asthma and Global Obstructive Lung Disease guidelines, respectively (1, 2). In both the Global Initiative for Asthma and Global Obstructive Lung Disease guidelines, pharmacological interventions that increase levels of intracellular cAMP are used with the primary intention of relaxing airway smooth muscle and opening the airways. Indeed, the earliest asthma medications reported include adrenaline, which activates the β-2-adrenoceptor and downstream adenylyl cyclase production of cAMP, and caffeine, which functions as a PDE inhibitor to prevent cAMP degradation (3). Elevations in intracellular cAMP levels lead to smooth muscle relaxation by multiple mechanisms that may include cAMP-dependent PKA (protein kinase A) activation, PKA-independent activation of exchange proteins, and decrease of intracellular calcium concentrations and associated signaling (4). In this issue of the *Journal* (pp. 96–106), Cao and colleagues report that ABCC1 (MRP1), a member of the ATP-binding cassette transporter family, represents an additional candidate target potentially important in normalization of cAMP signaling and second messenger function in airway smooth muscle cells, with potential implications for patients with asthma and COPD (5).

Cao and colleagues used primary human airway smooth muscle cells from tracheas in observational and mechanistic experimental approaches to characterize the expression and function of ABCC1. Specifically, ABCC1 gene and protein expression were confirmed by real-time PCR, immunohistochemistry, and immunoblot of cultured primary human airway smooth muscle cells and corroborated with publicly available RNA sequencing data generated from a distinct set of human airway smooth muscle cells. These foundational observational steps were complemented by mechanistic approaches with siRNA knockdown of ABCC1 transcripts and pharmacological interventions. Using this multifaceted approach, the authors demonstrate prominent ABCC1 expression in primary human airway smooth muscle cells. Furthermore, functional cAMP transport assays with pharmacological interventions and siRNA knockdown were used to mechanistically implicate ABCC1 in cAMP efflux after activation of adenylyl cyclase. The cytoskeleton properties of human airway smooth muscle cells also were explored with magnetic twisting cytometry, another mechanistic approach. Collectively, their findings provide a compelling foundational demonstration of the expression and function of ABCC1 in human airway smooth muscle.

The major translational findings of ABCC1 function as a cAMP exporter in human airway smooth muscle present a new disease-modifying target that could be relevant in both asthma and COPD. Importantly, the principle behind ABCC1 as a new target is grounded in decades of clinical asthma and COPD management that focused on cAMP modulation. Building on that concept, the authors expand the two-dimensional approach of cAMP modulation (elevating and blocking breakdown) by adding the third dimension of extracellular transport. The novel concept that cAMP signaling is regulated by mechanisms that go beyond receptor activation and enzymatic degradation to include intracellular compartmentalization and transport requires deeper exploration (6), which will likely arise from the presented work.

A major strength of the study is the use of primary human airway smooth muscle cells and publicly available data sets for complementary confirmation of ABCC1 expression, which provide a solid foundation for the authors to perform their mechanistic studies. Their demonstration of the specificity of the antibodies used for detecting ABCC1 protein and the localization of ABCC1 at the cell membrane represent important validations. The use of both molecular (siRNA) and pharmacological (e.g., MK-571) interventions to both identify ABCC1 as the primary cAMP efflux transporter and target the protein's function are also strengths of the study. Their complementary approaches are valuable because neither approach alone is absolute—complete knockdown efficiency is challenging in primary cell culture systems and many pharmacological interventions have off-target effects.

Their study does have some limitations due to the exclusive focus on airway smooth muscle cells. The demonstration that ABCC1 functions as a cAMP transporter in human airway smooth muscle cells warrants the exploration of this molecule's expression and function in other lung and immune cells that may be important in asthma and COPD, including airway epithelial cells, where we have observed robust gene expression of this transporter (7). Importantly, ABCC1 has broad substrate specificity and can transport a variety of endogenous and exogenous molecules (8). The consequences of interventions targeting ABCC1 may therefore extend beyond airway smooth muscle relaxation and could impair other biological processes important in normal health. In addition, although the elevation of cAMP in airway smooth muscle via ABCC1 is grounded in decades of historical clinical practice with cAMP modulators (3), more recent evidence suggests that other cells, including airway epithelial cells, can respond to cAMP elevation with potentially deleterious proinflammatory consequences (9). Finally, of interest is the exploration of cAMP as a serum biomarker, although it remains to be determined if a mechanistic link exists between the modulation of cAMP efflux in the lung tissue and levels in circulation.

The use of cAMP elevating agents alone for the management of asthma has recently been revised in a change that strongly supports combinatorial therapies that include glucocorticoids (1). The interaction between cAMP elevating agents and glucocorticoids is additive and potentially synergistic, as suggested by studies exploring mechanisms of bronchodilation and antiinflammatory responses (10, 11).

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Correspondingly, the potential role for ABCC1 as an additional regulator of intracellular cAMP and modifier of glucocorticoid responses is interesting. Importantly, immediate translational studies may be possible as the authors use MK-571, a compound originally developed as a cysteinyl leukotriene receptor 1 antagonist, as an ABCC1 inhibitor and a compound that has been used safely in humans with asthma (12). The present manuscript provides an experimental rationale for exploring the repurposing of MK-571 or related chemistry, as a layered approach with glucocorticoids and cAMP elevating agents to both relax airway smooth muscle and attenuate inflammation. It is exciting to think that despite decades of clinical use of cAMP elevating agents for obstructive airway diseases, the authors have "opened up" the field by defining ABCC1 as an important regulator of airway smooth muscle contraction.

Their translational research comes at a time when we are reimagining how obstructive lung diseases are managed with complex biologics, personalized medicine, and guideline revisions. Despite these dynamic times, the fundamental strategy for managing obstructive lung diseases still involves modulating airway smooth muscle cell function. The discovery of ABCC1 expression and function as a cAMP transporter in airway smooth muscle cells lays the foundation for new treatment strategies that are grounded in historical practice. ■

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