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# CLCA1 and TMEM16A: The link towards a potential cure for airway diseases

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## Abstract

The hallmark traits of chronic obstructive airway diseases are inflammation, airway constriction due to hyperreactivity and mucus overproduction. The current common treatments for asthma and COPD target the first two traits with none currently targeting mucus overproduction. The main source of obstructive mucus production is mucus cell metaplasia (MCM), the transdifferentiation of airway epithelial cells into mucus-producing goblet cells, in the small airways. Our current understanding of MCM is profusely incomplete. Few of the molecular players involved in driving MCM in humans have been identified and for many of those that have, their functions and mechanisms are unknown. This fact has limited the development of therapeutics that target mucus overproduction by inhibiting MCM. Current work in the field is aiming to change that.

### Keywords

mucus overproduction; CLCA1; TMEM16A; asthma; COPD; calcium activated chloride channel

The main source of obstructive mucus production is mucus cell metaplasia (MCM), the transdifferentiation of airway epithelial cells into mucus-producing goblet cells, in the small airways. MCM results in the upregulated expression of mucin genes that is driven by inflammatory mediators (1–3). MCM is triggered by interleukin-13 (IL-13) and IL-4, $T_H2$

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cytokines produced by inflammatory immune cells in response to allergens, pathogens, and other insults (4–6). Consequently, asthma therapeutics targeting IL-13, for example anti-IL-13 antibodies (Lebrikizumab and Tralokinumab) and anti-IL-13 receptor antibodies (Dupilumab), have been developed and have reached clinical trials. Some have been shown partially effective within some subgroups (7,8) or to have no effect on severe asthmatics (9). Additionally, initial studies also reported some adverse side effects associated with the use of these therapeutics, likely because of the fact that IL-13 also plays immunoregulatory roles. For example, IL-13 suppresses $T_H17$ cytokine production in an IL-10 dependent manner and thus may play a role in $T_H17$-associated autoimmune diseases. Accordingly, some patients who were administered IL-13-targeted biologics had significantly increased detrimental events involving musculoskeletal and autoimmune-related diseases (10,7). These observations give rise to three main points: 1) targeting the IL-13 pathway could be effective for treating asthma and COPD; 2) however, IL-13 may not be the optimal target since it is involved in numerous important processes; 3) thus identifying therapeutically manipulatable targets specific to the MCM pathway and understanding their mechanism of function is crucial.

Towards that end, recent work in this field has identified a number of new potential molecular targets in this pathway for the treatment of chronic inflammatory airways diseases (e.g., SPDEF (11), MARCKS (12), etc.). In this editorial, I will focus on three of them, each of which represent a different molecular class: a kinase (MAPK13/p38δ); a calcium-activated chloride channel regulator (CLCA1); and a calcium-activated chloride channel (TMEM16A), paying particular attention to the emerging importance of the last two.

Calcium-activated chloride channel regulator 1 (CLCA1, the human orthologue of mouse CLCA3/Gob-5) has had a long and highly undefined association with chronic inflammatory airway diseases. The expression of CLCA1 is highly upregulated in response to IL-13 in both mouse (13) and human models (14,15) of MCM. It is also highly expressed in COPD airways (15). A factor contributing to the confusion regarding CLCA1 function has been observations from *Clca3* (the mouse orthologue of CLCA1) knockout mouse, which suggest that it does not play a role in IL-13-induced mucus overproduction or $Cl^-$ secretion (13,16). This is likely due to functional redundancy due to the fact that mice encode at least 7 different CLCA proteins (13,17). The situation in humans appears to be much simpler, as they encode only 3 CLCA proteins. Overexpression of CLCA1 alone in human airway epithelial cells can drive mucus production, and siRNA knockdown of CLCA1 in primary airway cell models of IL-13-induced MCM prevents mucus production (15). This puts CLCA1 function in the middle of the IL-13 to mucus pathway. CLCA1 is a secreted protein which can be found in isolated mucin granules (18) and is proteolytically cleaved into two fragments during secretion (19). This secreted protein can be detected in bronchoalveolar lavage (BAL) fluid from asthmatic patients (20), and recently it was demonstrated that CLCA1 protein levels in sputum are an accurate biomarker of asthma endotypes (either $TH_2$-low or $TH_2$-high) (21).

Although the expression of CLCA1 has been associated with airway diseases, understanding the role that it plays in development of disease has lagged far behind. This is largely due to the fact that CLCA1 belongs to a unique family of proteins whose function and mechanism

of action were largely unknown until recently. Originally identified over 20 years ago, CLCA proteins were originally annotated as calcium-activated chloride channels since cells display increased calcium-dependent chloride currents when these proteins are expressed. However, subsequent *in vivo* and *in vitro* studies indicated that these proteins are largely secreted soluble proteins that do not contain the numerous transmembrane domains required to form a channel (20,17,16) and subsequent analysis indicated these were general properties of the CLCA family (17). Consequently, it was demonstrated that CLCA1 was able to mediate calcium-dependent chloride currents by activating an endogenous calcium-activated chloride channel in human cells (22,19).

How then does CLCA1 activate calcium-activated chloride channels (CaCCs)? We recently demonstrated that full-length CLCA1 is unable to activate CaCCs and that cleavage of the protein is required activate it (19). The protease responsible is a novel matrix metalloprotease (MMP)-like domain located within the N-terminus of CLCA1 itself. This self-cleavage then allows the N-terminal fragment of CLCA1 to interact with and activate its target CaCC. What is the identity of that target CaCC and how does CLCA1 activate it? We recently elucidated that CLCA1 activates the CaCC TMEM16A and that it can do so in a paracrine fashion (23). This last point is important, given the observation that secreted CLCA1 is highly abundant under inflammatory conditions in the airway. CLCA1 directly engages TMEM16A on the surface of cells, and this interaction appears to stabilize the surface expression of TMEM16A, increasing the number of channels on the surface, and consequently increasing the overall cellular currents through these channels.

These recent exciting results have uncovered many details regarding the function of CLCA1 and how it activates calcium-dependent chloride currents through TMEM16A. Several questions remain however, with the most prominent ones regarding whether or not the CLCA1-TMEM16A interaction contributes to the development of chronic inflammatory airways diseases, and if so, how? There are several lines of evidence that would suggest that these two molecules act synergistically. For example, the expression of both CLCA1 and TMEM16A is upregulated by the inflammatory cytokines IL-4 and IL-13, with this expression largely limited to goblet cells in the airway (24,15,25). In addition, both CLCA1 and TMEM16A have been tied to mucus production in some way. As mentioned above, overexpression of CLCA1 appears to be central to mucus overproduction. Likewise, TMEM16A activity has been linked to mucus secretion, as TMEM16A inhibitors block ATP-stimulated mucus secretion in IL-13-treated human bronchial epithelial cells (26). What is unknown at this time, however, is whether or not the CLCA1-TMEM16A interaction plays a central or an auxiliary role in MCM. We previously observed that IL-13-driven overexpression of CLCA1 in human airway epithelial cells activates a signaling cascade involving MAPK13 and that inhibitors to MAPK13 developed by structure-based drug design are able to block the IL-13 driven mucus production (15,27). It is unknown at this time whether or not the CLCA1-mediated activation of TMEM16A directly drives the activation of MAPK13 and the subsequent signaling cascade leading to the expression of MUC5AC, or whether this function of CLCA1 is just an important auxiliary function in the pathway. It is well known that anion channel activity is required for secreted mucins (MUC5AC and MUC5B) to function properly in a mucosal immunity and mucocilliary clearance capacity (28). Mucin proteins are secreted in dense, dehydrated granules and

require anion channel passage of chloride and bicarbonate ions to ensure proper hydration, salination, and pH control (29). This is exemplified by the disease cystic fibrosis, where the impaired function of the anion channel CFTR produces mucus that does not function properly and causes disease. In this regard, TMEM16A has been shown to carry both chloride and bicarbonate anions (30). Thus, regardless of whether or not the CLCA1-TMEM16A interaction is required within the signaling pathway driving MCM, it more than likely does play a role in ensuring that the mucus produced will function properly. This would be similar to role that has recently been attributed to the $Cl^-$ channel SLC26A9, whose activity is increased by IL-13 stimulation and is essential to prevent mucus plugging in the setting of MCM (31).

In conclusion, we have learned much recently regarding the function of CLCA1 and mechanistically how it activates chloride currents through TMEM16A. However, much more information will be required before one can develop asthma and COPD treatments that target this duo. First and foremost among those is defining the role of the CLCA1-TMEM16A interaction within the MCM pathway, i.e., does it drive mucus production and disease, or does it solely play a protective role in contributing to improved mucus hydration and clearance. In addition, more structural data for both proteins in required. Currently, there are no structural data for any portion of CLCA1, and although the groundbreaking crystal structure of a fungal orthologue of TMEM16A was recently reported and provided several enormous insights into the structure and function of this protein (32), the structure of a more highly related mammalian orthologue will be needed to effectively design specific TMEM16A-targeting drugs. Furthermore, the precise molecular determinants that govern the CLCA1-TMEM16A interaction remain to be determined. These future studies will determine if the CLCA1-TMEM16A interaction should be targeted therapeutically for asthma and COPD, and if so, how.

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