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Persistent induction of goblet cell differentiation in the airways: Therapeutic approaches[☆]

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ABSTRACT

Dysregulated induction of goblet cell differentiation results in excessive production and retention of mucus and is a common feature of several chronic airways diseases. To date, therapeutic strategies to reduce mucus accumulation have focused primarily on altering the properties of the mucus itself, or have aimed to limit the production of mucus-stimulating cytokines. Here we review the current knowledge of key molecular pathways that are dysregulated during persistent goblet cell differentiation and highlights both pre-existing and novel therapeutic strategies to combat this pathology.

1. Introduction

The human airway epithelium is the principal barrier protecting the lungs from the external environment. It is comprised of three major cell types, basal cells, ciliated cells and secretory cells (Goblet cells and Club cells) (Fig. 1). Goblet cells, together with submucosal glands are the principal secretors of MUC5AC and MUC5B mucin glycoproteins respectively (Hovenberg, Davies, & Carlstedt, 1996). These gel-forming mucins form the glycoprotein component of airway mucus.

Goblet cell differentiation (GCD) is believed to have evolved to combat parasitic infection and is an essential step for the clearance of inhaled pathogens from the airways (Evans, Kim, Tuvim, & Dickey, 2009). In healthy individuals, transient GCD results in an increase in goblet cell number at the affected areas of the epithelium, and temporarily upregulates mucus production. In contrast, chronic airways diseases, such as asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF), are characterized by a persistent goblet cell phenotype and continually high levels of secreted mucins

Abbreviations: cAMP, Cyclic Adenosine Monophosphate; COPD, Chronic Obstructive Pulmonary Disease; CCSP, Club Cell Secretory Protein; CF, Cystic Fibrosis; EGFR, Epidermal Growth Factor Receptor; EMT, Epithelial to Mesenchymal Transition; ERK, Extracellular Signal-Regulated Kinase; GCD, Goblet Cell Differentiation; HB-EGF, Heparin Binding-EGF-like Growth Factor; HDM, House Dust Mite; HNE, Human Neutrophil Elastase; mTOR, Mechanistic Target of Rapamycin; MAPK, Mitogen Activated Protein Kinase; mAb, Monoclonal Antibody; NICD, Notch Intracellular Domain; OVA, Ovalbumin; PI3K, Phosphatidylinositol-3 Kinase; AKT, Protein Kinase B; shRNA, Short-Hairpin RNA; STAT, Signal Transducer and Activator of Transcription; SOCS, Suppressor of Cytokine Signaling; TGF, Transforming Growth Factor; TNF, Tumor Necrosis Factor

[☆] Naming convention: uppercase lettering denotes protein (e.g. FOXA2). Italics denote genes and mRNA products (e.g. FOXA2 (human) or Foxa2 (murine)).

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Fig. 1. Normal vs diseased airway epithelium. In the normal airway epithelium (A) basal cells undergo regulated differentiation to form two major cell types, ciliated cells or secretory (Club and Goblet) cells. Goblet cells and submucosal glands are responsible for regulated mucus secretion into the airway which is normally present as a mucous bilayer. Club cells act to protect the airways through detoxification of harmful compounds and secretion of glycoproteins and lipids which physically protect surfactant and small airways. Mucociliary clearance which is dependent on ciliary function actively moves mucus to remove foreign particles. In chronic airways disease (B) persistent goblet cell differentiation (GCD) results in goblet cell hyperplasia. In addition to GCD, is the enlargement or hypertrophy of submucosal glands. These two events dramatically upregulate the production and secretion of mucin glycoproteins and result in a highly viscous and thickened mucus layer that is difficult to clear. This persistent GCD is directly linked to increased mucus accumulation in airways. MCVs: mucin containing vesicles.

(McCullagh, Jamieson, Blackwell, & Gupta, 1995). Over time this persistent mucin production is linked to intraluminal mucus accumulation, increased rates of infection and airway obstruction (Rose & Voynow, 2006).

Numerous signaling pathways have been implicated in the shift from 'normal', primarily ciliated epithelium to an overtly secretory phenotype. How these pathways become altered in diseases such as asthma, COPD/chronic bronchitis and CF remains an intense area of research and promises the development of new drugs targeting GCD and mucus overproduction. To date however, conventional therapies to treat intraluminal mucus accumulation have focused on targeting specific properties of mucus itself, such as altering mucus viscoelasticity, restoring 'normal' mucus structure as well as improving the rates of mucociliary clearance (Beeh, Beier, Esperester, & Paul, 2008). Expectant medicines, designed to promote the clearance of mucus from the airways, prove inadequate in curbing underlying mucus overproduction (Malerba & Ragnoli, 2008; Poole, Chong, & Cates, 2015). This is because they are largely interventions that aim to suppress the disease symptoms rather than pathogenic processes. Targeting the mechanisms that drive persistent GCD and downstream overproduction of mucus provides an alternative and potentially disease modifying solution. While not the focus of this review, submucosal gland morphology is also grossly altered in chronic airways disease and impacts

disease pathophysiology. During both asthma and COPD there is an enlargement of tracheobronchial submucosal glands (hypertrophy), which leads to the increased production of the MUC5B mucin (Hovenberg et al., 1996; Kirkham, Sheehan, Knight, Richardson, & Thornton, 2002). Dysregulation of gland function is also an early hallmark of CF and results in increased detection of MUC5B within submucosal glands (Ostedgaard et al., 2017). Increased MUC5B is associated with chronic airways disease, but is not sufficient for intraluminal mucus accumulation. It is only in the presence of high levels of MUC5AC that mucus accumulation occurs (Evans et al., 2009). Furthermore, experimentally reducing MUC5B has been shown to abolish mucociliary clearance and greatly increase infections at the airway in animal models; whereas reducing MUC5AC does not (Roy et al., 2014). As such, reducing the production of MUC5AC by therapeutically targeting goblet cells is a viable strategy for minimizing mucus accumulation during chronic airways disease.

In this article, we review the currently identified, as well as emerging molecular pathways that result in persistent GCD and how these overlap across a range of chronic airways diseases. Additionally, although the focus of this review is GCD in the human airway epithelium and emerging technologies that allow for more comprehensive studies *ex vivo*, reference is also made to critical experiments within the large airways of rodents. Although there is debate regarding how appropriate

rodent models are when focusing on the human airway epithelium, there are models that accurately reflect key disease features of asthma and COPD (Beckett et al., 2013; Kim et al., 2017; Thorburn et al., 2010), and it is clear that key mechanistic data remains applicable to both systems (Chen et al., 2009; Laoukili et al., 2001; Liu et al., 2016; Rajavelu et al., 2015; Starkey et al., 2013; Wills-Karp et al., 1998). Nonetheless a cautious approach must be taken when comparing the two systems and efforts be made to validate *in vivo* animal data in relevant human tissues. By providing an in-depth summary of the dys-regulated pathways currently associated with aberrant GCD, we highlight emerging novel targets that may influence excess mucus production from the airway epithelium. As such, we aim to emphasize the value of therapeutic strategies targeted towards persistent GCD and provide potential ways forward in the treatment of excess mucus production across a range of airways diseases.

2. Goblet cell pathology in chronic airways disease

Abnormal production of gel-forming mucin has been demonstrated in airways of asthmatic, COPD and CF patients (Caramori et al., 2009; Kirkham et al., 2002; Robinson & Bye, 2002). During normal gel-layer formation, linear polymers of MUC5B, released from submucosal glands, are coated and anchored by the MUC5AC released by goblet cells (Ermund et al., 2017; Thornton, Rousseau, & McGuckin, 2008). Healthy mucus consists of approximately 3% solids (including mucins, salts, lipids), however this proportion can reach almost 15% in chronic airways disease (Fahy & Dickey, 2010). The proportionate increase in solid mass leads to many more intermolecular interactions and results in a thicker, stickier gel-layer. Although there are some morphological similarities in airways when comparing; asthma, COPD and to some extent CF, these arise through very different processes (Kalinin et al., 1986) (Fig. 2; previously unpublished data).

2.1. Asthma

A range of overt structural anomalies are commonly exhibited by airways of asthmatics, such as smooth muscle cell hyperplasia/hypertrophy, goblet cell hyperplasia, subepithelial fibrosis and sub-mucosal gland hyperplasia/hypertrophy (Bai & Knight, 2005; Ordonez et al., 2001; Warner & Knight, 2008; Wilson & Li, 1997). This ‘airway remodeling’ is believed to occur in response to repeated epithelial injury through inhalation of allergens and numerous environmental stimuli, infection, as well as oversensitive and misperceived host inflammatory/immune responses (Fahy, Kim, Liu, & Boushey, 1995; Jackson & Johnston, 2010; Marini, Vittori, Hollemborg, & Mattoli, 1992; Woolnough et al., 2016). Indeed, the symptoms of asthma have been compared to infestation of the lungs by helminth worms, a downstream effect of which is the massive upregulation of goblet cells and immediate induction of mucin MUC5AC (Evans et al., 2009). Although asthma is understood to be a heterogeneous disorder of numerous distinct endotypes, a skewed T-helper class 2 (Th2) immune response is a prominent feature in most cases (Fahy, 2015; Hansbro, Kaiko, & Foster, 2011). During cases of both mild and severe asthma there is a significant increase in the number of goblet cells lining the upper airways compared to non-asthmatics (Jackson, 2001; Ordonez et al., 2001; Parker et al., 2013).

2.2. COPD and chronic bronchitis

The term COPD defines a complex range of airways/lung diseases that tentatively fall under two non-exclusive subtypes; chronic bronchitis and emphysema, and is characterized by fixed airflow obstruction. Airflow obstruction in individuals with primarily bronchitic COPD is caused by gross changes in airway morphology, including persistent GCD, while airflow obstruction in primarily emphysematous COPD is caused by the destruction of alveolar cells. There are similarities

Fig. 2. (A) Representative micrograph of a non-asthmatic human airway section. (B) Comparative section from an individual with fatal asthma, showing excessive intraluminal mucus accumulation (M), goblet cell hyperplasia (arrowhead) and basal lamina thickening (arrow). Tissue sections were obtained with consent from the UBC James Hogg Research Centre. Scale: 50 μ m.

between the airways of individuals suffering from COPD and those with asthma, such as chronic airway inflammation, airway wall thickening, increased airway smooth muscle deposition as well as persistent mucus plugging (Hartley et al., 2016; Jeffery, 1999; Pini et al., 2014). Unlike asthma, the epithelial pathophysiology of COPD is extremely heterogeneous and arises from exposure to toxic compounds, most commonly within cigarette smoke (Gao et al., 2015). Exposure to cigarette smoke has been shown to directly upregulate MUC5AC stores and increases goblet cell size and number (Innes et al., 2006). This exposure also compromises barrier integrity by disrupting cell-cell junctions, ciliary function and mucociliary clearance and increasing permeability of the epithelium, culminating in increased rates of infection (Ganesan, Comstock, & Sajjan, 2013). Additionally, the infiltration of neutrophils and CD8⁺ lymphocytes is associated with structural changes, including GCD in the airways of COPD subjects (Jeffery, 2001).

2.3. Cystic fibrosis

In the airways of patients with CF there is distinct, thickened mucus that results from a deficiency in the epithelium's ability to hydrate surface mucus. This dehydrated mucus manifests via a mutation in the CF transmembrane conductance regulator (CFTR) gene, resulting in epithelial cells that are deficient in a critical cAMP-dependent anion channel (Anderson et al., 1991). The majority of studies in CF airways have focused on the process of mucus hydration; however, there is also evidence, albeit controversial, of increased GCD. Burgel et al. (2007) found excessive goblet cell hyperplasia in the epithelium surrounding

Fig. 3. Key signaling events controlling the induction of goblet cell differentiation (GCD) at the airway epithelium. Critical regulators are highlighted (bold) that are known to be dysregulated in chronic airways disease. Inhibitors of selected signaling pathways are shown in red. *Unpublished data from our group suggests Notch based regulation of SPDEF.

mucus plugs in CF lung sections. Additionally, in mice with airway-specific overexpression of the epithelial Na^+ channel ($\beta\text{ENaC-Tg}$), GCD is stimulated by interactions with abnormal surface mucus (Mall et al., 2008). Conversely, Hays and Fahy (2006) showed increased volume of submucosal glands, but no change in GCD in bronchial biopsies taken from subjects with CF. Although mucus plugging has been shown in the CF airway, even in the absence of GCD (Gehrig et al., 2014), increased rates of infection within CF airways are known to trigger increases in GCD (Hao et al., 2012). The additive pathogenic effect of excess mucin secretion from increased goblet cell numbers would undoubtedly worsen the disease (Henderson et al., 2014).

3. Signaling pathways associated with GCD

Although very few studies have been conducted comparing the mechanisms of GCD between asthma, COPD, and CF it is likely that key signaling pathways, such as epidermal growth factor receptor (EGFR), interleukins (Th2 cytokines), Notch, signal transducer and activator of transcription 6 (STAT) 6, and WNT signaling are similar between these diseases (Fig. 3) (Takeyama et al., 1999; Takeyama, Fahy, & Nadel, 2001). Non-biological pathways, such as mechanical (shear and compressive) stress, may also contribute to GCD (Liu, Li, Zhou, Kolosov, & Perelman, 2013).

3.1. EGFR and GCD

EGFR is a multifunctional receptor protein required for the rapid transmission of growth responses. EGFR also regulates the stimulation of innate immune responses including GCD in the healthy airway. Dysregulation of EGFR-based signaling is linked to an enormous range of diseases/syndromes including cancers, as well as many pathophysiological features associated with asthma, COPD and CF (Acciani, Suzuki, Trapnell, & Le Cras, 2016; Allahverdian, Harada, Singhera, Knight, & Dorscheid, 2008; Chung, 2001; Kedzierski et al., 2017; Sharma, Bell, Settleman, & Haber, 2007; Stolarczyk et al., 2016; Voynow, Fischer, Roberts, & Proia, 2005). EGFR immunoreactivity is enriched in regions of increased GCD in human bronchi (Takeyama et al., 2001).

EGFR signaling involves four cell surface receptor proteins (EGFR/Erbb1, Erbb2, Erbb3 and Erbb4), and is directly activated by eight known ligand families; epidermal growth factor (EGF), transforming growth factor (TGF)- α , heparin binding-EGF like growth factor (HB-EGF), amphiregulin, betacellulin, epiregulin, epigen, and neuregulin (Cohen, 1964; Derynck, Roberts, Winkler, Chen, & Goeddel, 1984; Higashiyama, Abraham, Miller, Fiddes, & Klagsbrun, 1991; Jones, Akita, & Sliwkowski, 1999; Peles et al., 1992; Sasada et al., 1993; Shoyab, Plowman, McDonald, Bradley, & Todaro, 1989; Strachan et al., 2001). Upon ligand binding, ErbbB receptor dimers (both heterodimers

and homodimers) undergo a conformational change resulting in the activation of one or more downstream protein complexes including phosphatidylinositol-3 kinase/protein kinase B (PI3K/AKT), mitogen activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), STAT and mechanistic (or mammalian) target of rapamycin (mTOR), controlling airway homeostasis (Ikegami, Falcone, & Whitsett, 2008; Jiang, Zhu, Xu, Sun, & Li, 2010; Liu et al., 2013; Xu et al., 2015). Ligands such as TGF- α and amphiregulin are secreted by a range of inflammatory cells, including eosinophils and neutrophils as well as epithelial cells (Burgel et al., 2001; Calafat et al., 1997; Okumura, Sagara, Fukuda, Saito, & Okayama, 2005; Wong et al., 1990). The impact of these eosinophil and neutrophil-derived ligands on EGFR signaling in asthmatic, COPD and even CF airway epithelium remains an important area of investigation. Suppressor of cytokine signaling (SOCS)5 can downregulate EGFR signaling *in vitro* and as such elicits a protective role against influenza A infection in the mouse airway, a key inducer of MUC5AC production (Barbier et al., 2012; Kedzierski et al., 2017; Nicholson et al., 2005). Reduced SOCS5 expression has been observed in epithelial cells from COPD patients (Kedzierski et al., 2017). Tyner et al., implicated EGFR activation in the trans-differentiation of ciliated cells to goblet cells within mouse airways (Tyner et al., 2006). In this study EGFR signaling through PI3K was shown to prevent ciliated cells from undergoing apoptosis. These cells then were shown to transdifferentiate into goblet cells upon stimulation by interleukin-13 (IL-13).

Although EGFR has been clearly implicated in GCD, therapeutic approaches targeting EGFR receptors face considerable challenges. Progress is being made with regard to EGFR tyrosine kinase inhibitors but safety versus efficacy remains an issue (Ha & Rogers, 2016). Indeed, clinical trials using an inhaled EGFR inhibitor, BIBW 2948 failed to reduce epithelial mucin stores and were poorly tolerated by subjects with COPD (Woodruff et al., 2010). A number of proteins/molecules that act downstream of EGFR signaling, or act *via* crosstalk may therefore offer a more effective target for pharmacological therapy.

3.2. Role of type-2 interleukins in GCD

Acting in concert with EGFR signaling and essential in the control of healthy GCD in the airway epithelium are a number of well characterized interleukins; IL-13, IL-4, IL-5, and IL-9 (Grunig et al., 1998; Tanabe, Shimokawaji, Kanoh, & Rubin, 2014). IL-13 is arguably the most comprehensively studied molecule that can induce persistent GCD in airway epithelial cells across many species (Atherton, Jones, & Danahay, 2003; Grunig et al., 1998; Kanoh, Tanabe, & Rubin, 2011; Kondo et al., 2006; Kondo, Tamaoki, Takeyama, Nakata, & Nagai, 2002; Shim et al., 2001; Wills-Karp et al., 1998; Zhu et al., 1999).

Early studies suggested that IL-13 dependent GCD involved EGFR signaling since inhibition of a specific EGFR tyrosine kinase prior to IL-13 treatment ablated GCD in rat airway epithelium *in vivo* (Shim et al., 2001). However, a follow up study by Atherton et al., showed that the potent EGFR-tyrosine kinase inhibitor (AG-1478) did not prevent IL-13 driven GCD (Atherton et al., 2003). It is important to note that the study by Shim et al., was performed in rats *in vivo* and involved an intraperitoneal injection one-day prior to IL-13 exposure, whereas Atherton et al., treated primary cultures of human bronchial epithelium grown in air-liquid interface (ALI) culture with the inhibitor and cytokine simultaneously. This suggests species-based and/or temporal-based differences in IL-13/EGFR crosstalk. In the rat at least, the effect of IL-13 on GCD is leukocyte dependent as pre-treatment with an inhibitor of leukocyte cell production prevented IL-13 induced GCD (Shim et al., 2001). Although allergic inflammation increases IL-13 production, there is evidence in mouse models that Sendai-virus induces mucous cell metaplasia *via* macrophage-derived IL-13 secretion (Kim et al., 2008). Thus dysregulation of the innate immune response may also be a key stimulator of IL-13 signaling.

IL-4 and -5 have been well documented as mucus inducing agents,

particularly in asthmatics largely due to their well-documented roles in regulating allergic inflammatory responses (Hansbro et al., 2013; Lopez et al., 1988; Wang et al., 1989). Additionally, IL-4 exhibits a high structural similarity to IL-13, signals through the same receptor complex and both share several signaling pathways (Mueller, Zhang, Sebald, & Duschl, 2002). Although IL-4 and IL-5 overexpressing mice both exhibit significant GCD (Lee et al., 1997; Temann et al., 1997), mice deficient in these factors show similar GCD to controls following exogenous treatment with tumor necrosis factor (TNF)- α (Cohn et al., 1999; Cohn, Homer, Marinov, Rankin, & Bottomly, 1997). Thus IL-4 and IL-5 have been hypothesized to regulate GCD by recruiting TNF- α secreting cells to the epithelium (Cohn et al., 1997; Lee et al., 1997; Temann et al., 1997). Whether recruitment of TNF- α producing cells is a major function of IL-4 and IL-5 in human airways is unclear. IL-4 is becoming more recently acknowledged as a therapeutic target for chronic asthma, particularly in combination with IL-13 (Hansbro et al., 2013; Kau & Korenblat, 2014). Presumably this would include inhibition of mucus hypersecretion and GCD.

IL-9 appears to upregulate GCD in cells that are undergoing differentiation, such as would occur during injury repair or before a fully differentiated epithelium has developed (Vermeer, Harson, Einwalter, Moninger, & Zabner, 2003). The induction of IL-9 has been shown to occur in the presence of IL-4 and TGF β , which act in concert to differentiate Th2 cells into a Th9 subtype (Veldhoen et al., 2008). Th9 cells have been shown to elicit allergic airway inflammation and GCD when transferred to mice previously challenged with OVA to induce experimental asthma (Staudt et al., 2010). Furthermore, blocking IL-9 with monoclonal antibodies ameliorated the inflammation and GCD in treated mice (Staudt et al., 2010). Human airway epithelial cells grown in ALI and transdifferentiated into goblet cells *via* IL-13 treatment have recently been shown to release IL-4, -5, -9 and -13 at higher levels than ciliated cells (Tanabe & Rubin, 2016). Low level release of IL-4, -5 and -13 also occurs following epithelial damage (Allahveridian et al., 2008; Hodge et al., 2002; Wu et al., 2010). This release is hypothesized to enhance the inflammatory response in asthma to produce an increased level of GCD.

3.3. Critical proteins linked to persistent GCD

A number of proteins have been shown to act downstream of these initial receptor-activated signaling events in the control of GCD, including STAT6, forkhead box protein A2 (FOXA2), SAM Pointed Domain Containing ETS Transcription Factor (SPDEF) and γ -aminobutyric acid A receptor (GABA $_A$ R).

STAT6 is a key transcription factor selectively activated by IL-13 and IL-4 that influences the expression of multiple target genes, including those involved in GCD upon activation, and as such is a central mediator of cytokine signaling (Goenka & Kaplan, 2011). STAT6 deficient mice do not exhibit epithelial GCD following IL-13 exposure, but it is restored following epithelial reconstitution of STAT6, implicating a crucial role for the protein during IL-13 based signaling (Kuperman et al., 2002). STAT6 has also been shown to exhibit aberrant histone acetylation patterns at the transcriptional start site within the asthmatic airway epithelium (Stefanowicz et al., 2015). STAT6 upregulates GATA binding protein 3 (GATA3) expression, which is intimately linked to the production of Th2 cytokines and airway remodeling. Interestingly, although STAT6 deficient mice are insensitive to IL-13 stimulation, they are still able to exhibit GCD upon activation of the Notch signaling pathway (Guseh et al., 2009), suggesting that although IL-13-mediated induction of GCD is dependent on STAT6, IL-13/STAT6 independent pathways are also involved.

FOXA2 is a transcription factor required for maintaining surfactant homeostasis as well as host defense and has been shown to repress GCD in the healthy airway epithelium (Wan, Kaestner, et al., 2004; Wan, Xu, et al., 2004). FOXA2 deficient mice show increased goblet cell hyperplasia as well as increased numbers of neutrophils and macrophages in

bronchiolar lavage fluid (Wan, Kaestner, et al., 2004). Furthermore, deletion of *Foxa2* also leads to upregulation of IL-4 and IL-13 (Chen et al., 2010). A significant reduction in FOXA2 levels has been shown in airways from asthmatics, whilst aberrant methylation patterns have recently been observed in the *FOXA2* promoter in COPD epithelium (Park et al., 2009; Song et al., 2017). Downstream events following FOXA2 activation are currently unknown; however evidence suggests that FOXA2 is a negative regulator of myeloid dendritic cell recruitment and activation (Chen et al., 2010).

SPDEF is a transcription factor that controls GCD and a network of genes associated with airway epithelial mucin production. SPDEF plays a critical role during development of the airway epithelium as well as epithelial remodeling following exposure to allergen (Rajavelu et al., 2015). Chen et al. (2009) used transgenic mice with inducible *Spdef* expression combined with cell-lineage tracing analysis to demonstrate that increased SPDEF leads to goblet cell metaplasia (Chen et al., 2009). Microarray analysis has revealed upregulation of genes associated with allergen exposure following SPDEF induction, such as *FOXA3*, anterior gradient 2 (*AGR2*) and chloride channel accessory 1 (*CLCA1*) (Chen et al., 2014; Nakanishi et al., 2001; Schroeder et al., 2012; Zhen et al., 2007). The induction of SPDEF also resulted in downregulation of genes associated with differentiation of healthy airway epithelia, including *FOXA2* and thyroid transcription factor 1 (*TTF1*) (Chen et al., 2009; Maeda et al., 2011; Park et al., 2009). Additionally, forkhead box M1 (*FOXM1*) is required for induction of *Spdef* transcription and in mice conditional deletion of *FOXM1* from Club cells has been shown to inhibit expression of SPDEF and as a consequence, drastically reduces goblet cell number (Ren et al., 2013; Sun et al., 2017). Recently, Song et al. (2017), used a novel technique known as ‘targeted epigenetic editing’ to modify the *Spdef* promoter to increase binding of transcriptional repressors within human lung epithelial cells (Song et al., 2017). This downregulated SPDEF levels and resulted in a reduction of MUC5AC expression (Song et al., 2017). Dysregulated SPDEF and FOXA3 activity may be a hallmark of all persistent GCD associated diseases, as epithelial expression of each of these proteins is increased in asthma, COPD and CF (Chen et al., 2009). Targeting SPDEF and/or FOXA3 may therefore represent alternative strategies to alleviate mucus accumulation in chronic airways disease.

GABA_A receptors are pentameric Cl[−] channels that are upregulated in mice following sensitization and challenge with OVA. Treatment of cultured human small airway epithelial cells with IL-13 results in a dramatic increase in GABA_A β2 subunit protein concomitant with GCD and blocking of GABA signaling reduced GCD associated with IL-13 stimulation (Xiang et al., 2007). However this did not affect IL-13 production itself, suggesting a role for GABA_AR downstream of IL-13 signaling. As GABA_AR appears to regulate GCD in epithelial cells following IL-13 exposure it may prove an effective point of therapeutic manipulation to reverse GCD in a high IL-13 environment.

3.4. Role of Notch in GCD

The Notch family consists of four (NOTCH1–4) transmembrane receptor proteins and is most well-known for its roles in cell fate determination during development. Upon ligand binding, the Notch intracellular domain (NICD) is released via two proteolytic cleavage events and translocates to the nucleus to switch on/off key transcriptional profiles (Schroeter, Kisslinger, & Kopan, 1998). The key transcriptional effector and binding partner to NICD during Notch signaling is recombination signal binding protein for immunoglobulin kappa J region (RBPJ) (Castel et al., 2013; Tamura et al., 1995). Binding of the NICD to RBPJ is well documented to activate the transcriptional repressors hairy and enhancer-of-split (*HES*) and hairy and enhancer-of-split related with YRPW motif (*HEY*) (Fischer & Gessler, 2007), which repress airway ciliogenesis (Gerovac et al., 2014; Tsao et al., 2009). Notch signaling also represses expression of multicilin (*MCIDAS*) and forkhead box J1 (*FOXJ1*) in airway epithelium, which in turn inhibits

ciliated cell differentiation (Gerovac et al., 2014; Gerovac & Fregien, 2016). Additionally, recent cell lineage tracing studies have highlighted Notch as a key regulator of epithelial trans-differentiation in the adult lung (Lafkas et al., 2015). Ablation of Notch signaling using antibodies against the Notch-activating ligands Jagged-1 (*JAG1*) and Jagged-2 (*JAG2*) results in a direct conversion of Club cells into ciliated cells without increased cell division or cell death (Lafkas et al., 2015).

Although Notch is clearly implicated in the decisions of epithelial cell fate, one important caveat is that different Notch isoforms elicit different transcriptional responses. For example, Notch1 and to a lesser extent Notch2 activate the lymphoid enhancer binding factor 1 (*LEF-1*) transcription factor whilst Notch3 does not (Ross & Kadesch, 2001). Beatus, Lundkvist, Oberg, and Lendahl (1999), also showed that Notch1 upregulates transcription of *HES1* approximately 80-fold compared to a 3-fold induction by Notch3.

Recently, Notch2 has been identified as key for inflammatory cytokine-driven goblet cell metaplasia in murine lung *in vivo* and importantly, this effect was also seen in differentiated human bronchial epithelial cells *in vitro* (Danahay et al., 2015). In contrast to this finding, the overexpression of either Notch1 or Notch3 has been shown to drive goblet cell metaplasia in a system devoid of inflammatory cytokines such as IL-13 (Gomi, Arbelaez, Crystal, & Walters, 2015). This indicates that different Notch isoforms impact on GCD both within IL-13-high and IL-13-low environments (Gerovac & Fregien, 2016; Gomi et al., 2015; Guseh et al., 2009).

In addition to its role within the epithelium, Notch has also been shown to control cell fate during T-cell development and as such influences the production of Th2 associated cytokines, including IL-13 (Kang et al., 2009). Notch regulation of Th2 cytokine release by T-cells may then further regulate GCD at the airway epithelium. As such, Notch signaling is predicted to control mucus homeostasis through these epithelial and T-cell based mechanisms, and dysregulation of one or both will likely result in GCD (Zong, Ouyang, Li, Chen, & Chen, 2016). An improved understanding of the functional role and interactions of specific Notch isoforms in the control of ciliogenesis/GCD may identify therapeutically exploitable targets to reduce persistent GCD regardless of inflammatory cytokine profiles.

3.5. Wnt/β-catenin signaling and GCD

The WNT family of extracellular secreted ligands is crucial to lung morphogenesis and tissue maintenance in the adult lung. Wnt-signaling is generally characterized as either canonical (stabilization and translocation of β-catenin to the nucleus) or non-canonical (β-catenin independent) (Corda & Sala, 2017). In the canonical pathway, WNT ligands act to stabilize the cytosolic and nuclear expression of the transcriptional co-activator β-catenin, which can bind to and activate numerous downstream transcription factors, including the well-known *LEF-1* and *TCF-1/3/4* (Baarsma, Konigshoff, & Gosens, 2013). This is regulated by WNT ligand binding to Frizzled (*FZD*) receptors, which in the presence of the *LRP5/6* co-receptors inactivates the so-called destruction complex normally responsible for β-catenin degradation. This destruction complex, consisting of APC, Axin, casein kinase 1α and GSK-3β (Kimelman & Xu, 2006), targets β-catenin for phosphorylation and subsequent ubiquitination in the absence of WNT ligands, but is functionally repressed upon WNT activation. β-Catenin signaling in airway epithelial cells is key to differentiation and proliferation in response to WNT ligands and other extracellular ligands.

Okubo and Hogan initially discovered that surfactant protein C promoter (*SpC*)-driven expression of a constitutively active β-catenin/*LEF-1* fusion protein promotes a gene expression signature in the lung reminiscent of GCD (Okubo & Hogan, 2004). Transgenic expression of a constitutively active mutant form of β-catenin, lacking exon 3 (*Catnb^{Δ(ex3)}*), into club cell secretory protein (*CCSP*) rich airway epithelial cells yielded a similar phenotype with massive goblet cell hyperplasia (Mucenski et al., 2005). Furthermore, in a toluene

diisocyanate model of chemically-induced asthma, treatment with a tankyrase inhibitor (which stabilizes the β -catenin destruction complex) or with the β -catenin/CBP inhibitor ICG-001, sharply reduced GCD in both cases (Yao et al., 2017). In contrast though, ICG-001 treatment in OVA challenged mice had no such effect on GCD (Koopmans et al., 2016), indicating that the role of β -catenin in GCD may be stimulus-specific. A better understanding of this specificity and the signaling effectors controlling β -catenin mediated GCD may open therapeutic avenues for GCD treatment.

3.6. Other molecules implicated in GCD

In addition to the signaling pathways and proteins listed above, there are a number of emerging candidate proteins and miRNAs that also influence GCD within the airway epithelium. These molecules play roles downstream of selected signaling pathways listed above and may prove to be useful candidates when targeting persistent GCD.

3.6.1. Protein candidates suggested to regulate GCD

Kinesin family member 3A (*KIF3A*) is a known asthma susceptibility gene (Kim et al., 2011; Kovacic et al., 2011), regulating aeroallergen response through a Th2 mediated pathway in airway epithelial cells (Vummidi Giridhar et al., 2016). Mice with selective deletion of *Kif3a* from airway epithelial cells exhibited GCD and an increased level of eosinophilic inflammation following exposure to aeroallergen. These mice also showed increased expression of *Il-4*, *-13* and *17a* as well as *Ccl-11* (Vummidi Giridhar et al., 2016). Interestingly, *KIF3A* may also play a role in Notch signaling as primary epidermal keratinocytes extracted from *Kif3a* deficient mouse embryos exhibit a deficiency in NICD-3 translocation to the nucleus *in vitro* (Ezratty et al., 2011).

Neuregulin 1 β 1 (NRG1 β 1) treatment has recently been shown to increase MUC5AC and MUC5B as well as GCD via EGFR signaling in human bronchial epithelial cells grown under ALI conditions (Kettle et al., 2010). As inhibition of p38 MAPK, ERK and PI3K/AKT kinases blocked GCD *in vitro*, it appears that NRG1 β 1 operates upstream of EGFR signaling (Kettle et al., 2010). Whether NRG1 β 1 plays a primary role in GCD in chronic airways disease requires further investigation. If so it may provide a novel target for persistent GCD.

A transcription factor that has recently been implicated in the progression of GCD is Runt-related transcription factor 2 (Runx2). Following shRNA-induced knockdown of *RUNX2*, *SPDEF* expression was abolished in bronchial epithelial cell cultures (Shi, Zhang, & Chen, 2017). Mice administered with *Runx2* shRNA also exhibit significantly reduced airway mucus production and improved pulmonary function (Shi et al., 2017). Chromatin immunoprecipitation assays using antibodies against *RUNX2* showed enrichment of the protein at the human *SPDEF* promoter following IL-13 stimulation indicating a direct role for *RUNX2* in *SPDEF* mediated GCD (Shi et al., 2017). Additionally, increased expression of *RUNX2* is seen in airways from asthmatics proximal to areas enriched with goblet cells (Shi et al., 2017). Although further testing of the effects of *RUNX2* inhibition is required, it may also be a viable candidate in the treatment of persistent GCD.

TNF- α converting enzyme (TACE), also known as a disintegrin and metalloprotease 17 (ADAM17), is a membrane-tethered enzyme that cleaves numerous cell-surface proteins. A number of the proteins/signaling molecules that TACE cleaves have been shown to play a major role in GCD. These TACE substrates include EGFR ligands, Notch ligands and the NOTCH1 receptor (Brou et al., 2000). TACE has also been shown to be a mediator of human neutrophil elastase (HNE) induced GCD as mice deficient in TACE do not develop a goblet cell rich phenotype following exposure to HNE when compared to controls (Park et al., 2013). Due to its pivotal role in the regulation of neutrophilic inflammation TACE has been suggested as an ideal target to prevent GCD in severe asthma, COPD and CF (Park et al., 2013).

3.6.2. miRNA regulation of GCD

miRNAs are becoming increasingly well recognized in their ability to influence epithelial cell differentiation (Martinez-Anton et al., 2013; Moheimani et al., 2016). Understanding the contribution made by miRNAs in persistent GCD is necessary for the development of novel miRNA-based therapies. Here we provide a brief overview of miRNA species associated with GCD. miR125b is significantly reduced in the sputum of asthmatic children, especially those with eosinophilic asthma (Liu et al., 2016). Intranasal delivery of a miR-125b mimic in a house dust mite (HDM) induced mouse model of allergic airway inflammation significantly reduced GCD and mucus production (Liu et al., 2016). miR-125a and miR-125b have been shown to directly target TNF alpha induced protein 3 (TNFAIP3), which plays a role during inflammatory cytokine (e.g. TNF- α) signaling by negatively regulating nuclear factor kappa B (NF- κ B) activity (Hsu et al., 2017; Kim et al., 2012). Expression of miR-449a is significantly downregulated in the airway epithelium of asthmatics (Solberg et al., 2012). Mir-449a influences the NOTCH1 based repression of FOXJ1 and reduces GCD both within the airway and intestinal epithelium when overexpressed (Capuano et al., 2011; Marcet, Chevalier, Coraux, Kodjabachian, & Barbry, 2011). Furthermore, miR-449a has been shown to target *NOTCH1* mRNA, turning off the 'high-Notch' secretory cell differentiation pathway.

miR-106a has previously been found to inhibit expression of the anti-inflammatory cytokine IL-10 in human B-cells (Sharma et al., 2009). Knockdown of mouse miR106a in OVA-induced models significantly reduced features associated with allergic airway inflammation, including goblet cell metaplasia (Sharma et al., 2012). Finally, two other miRNAs, miR-124a2 and miR-205 have been shown to regulate GCD but not within airway epithelium directly. In mammary and kidney epithelium, miR-205 regulates the epithelial to mesenchymal transition (EMT) by targeting *ZEB1* and *ZEB2* and has recently been shown to upregulate GCD when induced in intestinal epithelial cells (Eyking et al., 2016; Suojalehto et al., 2013). Similarly, miR124a2 has been shown to regulate GCD within pancreatic B cells (Baroukh et al., 2007). Interestingly, a key target of miR-124a2 is *FOXA2* and as such is likely to play a role in pulmonary inflammation and GCD at the airway epithelium (Baroukh et al., 2007). It is clear that certain miRNAs regulate the induction of GCD in the epithelium. Supplementation of miR-125b reduces GCD and shows promise as an inhaled therapeutic component (Liu et al., 2016). Targeting miRNAs to regulate GCD in the future may produce novel, efficient and safe alternatives to small molecule therapies.

3.7. Mechanical stress and GCD

In addition to the dysregulation of protein/miRNA pathways listed above, mechanical stress has also been shown to contribute to GCD in chronic airways disease. Bronchoconstriction is the main physiological event in asthma and is also common in COPD and CF. Bronchoconstriction generates significant mechanical force within the airways during chronic airways disease (Tschumperlin & Drazen, 2001; Wiggs, Hrousis, Drazen, & Kamm, 1997). These forces include compression and shear stress which act perpendicular and parallel to the epithelial cell surface, respectively (Tarran et al., 2005; Tschumperlin, Shively, Kikuchi, & Drazen, 2003). It has been shown *in vivo* that repeated bronchoconstriction is associated with goblet cell hyperplasia at the human airway epithelium in the absence of inflammation and this can be mimicked by apical compression models *in vitro* (Grainge et al., 2011; Park & Tschumperlin, 2009).

Although compression and shear stress occur simultaneously during bronchoconstriction, the majority of work to date has focused on compressive forces in the airway (Ressler, Lee, & Randell, 2000; Swartz, Tschumperlin, Kamm, & Drazen, 2001; Tschumperlin & Drazen, 2001; Wiggs et al., 1997). These forces increase the concentration of secretory cell regulators into the lateral intercellular space, which impact the adjacent airway epithelial cells (Tschumperlin et al., 2004). These

regulators include TGF β_2 , endothelin (Tschumperlin et al., 2003), EGFR ligands such as HB-EGF, epiregulin and amphiregulin (Chu et al., 2005), plasminogen activator inhibitor-1 (PAI-1) (Chu et al., 2006), YKL-40 and chitinase-like protein (Park, Drazen, & Tschumperlin, 2010). Compression-induced increases in some of these mediators have been shown to increase goblet cell number and mucin production at the airway epithelium (Park & Tschumperlin, 2009; Zhu, Abdullah, Doyle, & Nguyen, 2015). Increased mucus production and goblet cell number has also been demonstrated *in vitro* via apical compression of human primary bronchial epithelial cells. This increase was significantly attenuated with an EGFR kinase inhibitor (AG1478) and abolished using anti-TGF- β_2 monoclonal antibody treatment (Park & Tschumperlin, 2009). To date, it is unknown if treatment with anti-TGF- β_2 would be effective in preventing compression induced GCD *in vivo*. Mechanical stress may therefore act to compound the problem of persistent GCD, particularly during episodes of bronchoconstriction. Whether compression and/or shear stress stimulate GCD-regulating pathways outside of EGFR is currently unknown. Future studies describing the role of these mechanical forces on GCD and mucus accumulation are clearly required.

3.8. Viral induced GCD

Virus infection, and particularly respiratory virus infection, has also been shown to upregulate GCD (Kim et al., 2008; Shibata et al., 2014; Villenave et al., 2012; Walter, Morton, Kajiwar, Agapov, & Holtzman, 2002). Respiratory syncytial virus (RSV) induces GCD in bronchial epithelial cells isolated from children (Villenave et al., 2012) and this correlates with excessive mucus production which is known to occur in RSV bronchiolitis (Hall, 2001). Infection with Sendai virus has been shown to cause chronic goblet cell metaplasia in the airway epithelium of mice. This persistence of goblet cells occurs in wild-type mice and even in ICAM-1 deficient mice, which are protected from infection-stimulated airway inflammation and hyperreactivity (Walter et al., 2002). Rhinovirus (RV), which is responsible for more than 50% of cases of the common cold (Leigh & Proud, 2015), is known induce GCD through various pathways. Neonatal mice, infected with RV1B demonstrate mucous cell metaplasia at the airway epithelium which persists for up to 60 days post infection (Schneider et al., 2012). Disruption of IL-13 signaling using either anti-IL-13 neutralizing antibody or IL-4R deficient mice prevents RV1B-induced mucous cell metaplasia, indicating a critical role for IL-13 signaling. RV infection has also been found to induce *Foxa3* expression in mice *in vivo* and human airway epithelial cells *in vitro* (Chen et al., 2014), leading to downstream increases in GCD.

4. Therapeutic strategies targeting GCD

To date, a number of different therapeutic approaches have been trailed for the treatment chronic airways disease including persistent GCD, albeit with some limitations (Table 1). As outlined above, numerous signaling pathways act in concert to regulate GCD within the airway epithelium. Most studies that have identified the pathways regulating GCD have been primarily conducted using *in vivo* animal models or *in vitro* culture of human bronchial epithelial cells. While no model can perfectly emulate what occurs in the human lung, the value of using such models cannot be overemphasized. *In vivo* animal models allow for variable mimicking of asthma, COPD and CF. This allows the assessment of GCD in the presence of a functional immune system, during development, following drug treatment or as a result of genetic modification. Conversely, *in vitro* studies in human tissues and cells from human donors enables the direct study of GCD in cells from asthmatic, COPD, CF and healthy cohorts. This has allowed for extremely high-throughput data collection and the ability to screen many potential therapeutic agents on human samples with minimal risk. Using such models to assess the efficacy of selected therapeutic

compounds not only helps us better understand the processes involved in dysregulated GCD, but is also critical in the identification of new targets for clinical application.

4.1. Drug treatments

For years, the symptoms of GCD (*i.e.* mucus hypersecretion), rather than GCD itself have constituted the major pharmacological target. This has led to a vast increase in the number of compounds with mucolytic/mucoregulatory activity being tested (Amaral et al., 2016). Ambroxol, N-acetylcysteine and carbocysteine are common expectorants used to treat diseases with a dysregulated mucus hypersecretion phenotype (Zhang & Zhou, 2014). Although such agents have been shown to improve mucociliary clearance with some effectiveness, they do not target the cause of mucus dysregulation and generally offer late term amelioration and symptom control only. A number of alternative therapeutic agents that instead target the upregulation of GCD may therefore offer a more effective preventative solution. Some progress has been made targeting key aspects of the pathways involved in GCD and has resulted in a number of therapeutic agents.

Corticosteroids are the most commonly prescribed medications for the treatment of asthma and are currently the most effective way to moderate airway inflammation in allergic asthmatics. They are often ineffective in patients with more severe asthma and COPD (Hansbro et al., 2017). As part of a larger clinical trial, budesonide has been shown to significantly reduce persistent GCD in patients exposed to both low and high dose HDM (De Kluiver et al., 2005). Additionally, corticosteroids such as dexamethasone have been shown to reduce MUC5AC production and GCD in animal models of asthma (Lundgren, Kaliner, Logun, & Shelhamer, 1988). However, separate studies have revealed that corticosteroid withdrawal can stimulate a rebound increase in GCD to levels above that of allergen induction alone (Southam, Ellis, Wattie, Glass, & Inman, 2008). In human bronchial epithelium grown at ALI, dexamethasone does not reduce GCD when cultures are co-stimulated with IL-13 (Kano et al., 2011). Recent studies by Leigh et al. (2016) present an exhaustive list of genes upregulated in human bronchial brushings and endobronchial biopsies following budesonide treatment. Numerous genes associated with GCD such as *ErbB4*, *RUNX2*, as well as JAK/STAT and PI3K/AKT signaling pathways, were upregulated in response to budesonide (Leigh et al., 2016). Thus, while it is clear that corticosteroids offer effective reduction in airway inflammation, the potential long-term induction of genes involved in GCD may increase the likelihood of persistent GCD outside of inflammation.

Anticholinergics, specifically tiotropium bromide, have shown promise in combatting COPD. Recently, clinical trials assessing tiotropium in patients with GOLD stage 1 or 2 COPD have shown significant improvements in lung function as measured by FEV1 (Zhou et al., 2017).

Furthermore, anticholinergics have been approved as add-on treatments for difficult-to-treat asthma (GINA guidelines, 2017). Although acetylcholine is best known for its role as neurotransmitter regulating bronchoconstriction and secretion of mucus from sub-mucosal glands, more recent findings also support a role for non-neuronal acetylcholine secreted by airway epithelial cells (Kistemaker & Gosens, 2015). GCD induced by IL-13 in human airway epithelial cells in ALI culture can be prevented and reversed by tiotropium treatment, suggesting a direct role for such non-neuronal acetylcholine (Kistemaker et al., 2015). Animal models confirm this contention and show reduced GCD after treatment with anticholinergics in both COPD and asthma models (Bos et al., 2007; Kistemaker et al., 2014; Pera et al., 2011).

Kaempferol is a common flavonoid that acts as an antioxidant and is known to block endoplasmic reticulum (ER) stress response. Kaempferol reduces eosinophil infiltration and inflammation as well as downstream mucous cell differentiation in OVA sensitized/challenged mice (Gong, Shin, Han, Kim, & Kang, 2012; Park et al., 2015). In

Table 1
Effectiveness and limitations of selected therapeutic compounds in alleviating goblet cell hyperplasia at the airway epithelium.

Target	Effector molecule (s)	Major treatment outcomes	Effect of treatment on GCD	Reference
IgE	Anti-IgE mAb	Significantly reduced; OVA/HDM-stimulated airway hyper-responsiveness, neutrophil/eosinophil infiltration, IgE and IgG1 production ^a	Significantly attenuated RSV and OVA/HDM-stimulated GCD during aerosol sensitization but not systemic sensitization ^a	Chan et al. (2001); Mizutani et al. (2015)
IgE	Omalizumab (anti-IgE mAb ^b)	Significantly reduced asthma exacerbations ^c	Goblet cell number not assessed ^c	Busse et al. (2001)
IL-13	Anti-IL13 mAb	Significantly reduced; transpulmonary resistance, airway hyper-responsiveness, eosinophil infiltration, proinflammatory cytokine production, serum IgE and airway remodeling during OVA-stimulation ^a Prevents subsequent allergic airways disease when exposed to <i>Chlamydia</i> infection during early life ^a	Significantly attenuated GCD ^a	Starkey et al. (2013); Yang et al. (2004)
IL-13	Tralokinumab (anti-IL13 mAb ^b)	Significantly reduced IL-13 induced airway hyper-responsiveness, airway eosinophilia and oesophageal eosinophilia ^a Potentially associated with increased lung function, increased peripheral blood eosinophils ^c	No significant effect on IL-13 induced GCD ^a	Blanchard et al. (2005)
IL-13	Lebrikizumab (anti-IL13 mAb ^b)	Significant increase in FEV ₁ in individuals with poorly controlled asthma ^c	Goblet cell number not assessed ^c	Piper et al. (2013)
IL-4Rα	Dupilumab (anti-IL4R mAb)	Significant reduction in asthma exacerbations. Significant increase in FEV ₁ when added to inhaled corticosteroids and LABAs	Goblet cell number not assessed ^c	Corren et al. (2011)
IL-5Rα	Benralizumab (anti-CD125 mAb)	Clinically significant improvement in ACOQ5 score ^c	Goblet cell number not assessed ^c	Wenzel et al. (2013)
mTOR	Rapamycin	Significantly reduced annual asthma exacerbation rate. Improved prebronchodilator FEV ₁ Significantly reduced airway hyperactivity, inflammatory cell number and IgE when administered together with HDM sensitization/challenge. Significantly reduced airway hyperactivity, IgE and T-cell activation but not inflammatory cell counts when administered between sensitization and challenge phases ^a	No current data on GCD ^c	Bleecker et al. (2016)
Bacterial 50S ribosome (primary)	Clarithromycin	Significantly reduced IL-13 stimulated increases in MUC5AC, CLCA1, pSTAT6 and pERK1/2 protein as well as <i>Muc5ac</i> , <i>Clca1</i> and <i>Spdef</i> mRNA in human bronchial epithelial cells grown in ALI ^d	Significantly attenuated IL-13 stimulated GCD ^d	Nagashima et al. (2016); Tanabe et al. (2011)
Various immunomodulatory targets (secondary)	Ciclesonide	Significantly prevented and reversed OVA-induced airway eosinophilia, T cell activation and bronchial hyper-responsiveness ^a	Significantly attenuated OVA-stimulated GCD ^a	Leung et al. (2005)
GCR	Fluticasone propionate	Significantly prevented and reversed OVA-induced airway eosinophilia and T cell activation, but was only effective at reversing bronchial hyper-responsiveness	Significantly attenuated OVA-stimulated GCD ^a	Leung et al. (2005)
GCR	Budesonide	Significantly reduced mucosal eosinophils, neutrophils and T lymphocytes ^c	Significantly reduced goblet cell number in asthmatics compared to placebo ^c	De Kluiver et al. (2005)
TGF-β ₂	Anti-TGF-β ₂ mAb	Completely abolished compression based increases in MUC5AC production ^b	Abolished GCD ^d	Park and Tschumperlin (2009)
EGFR kinase	Gefitinib	Significant reduction in airway inflammation, goblet cell and airway smooth muscle cell hyperplasia ^a . Chronic exposure significantly reduced MUC5AC and IL-13 in BALF	Significantly reduced OVA-stimulated GCD ^a	Song et al. (2016)
PDE4	Rolipram	Significantly prevented OVA-induced increases in airway resistance, eosinophil, lymphocyte, and neutrophil accumulation in the BALF ^a	Significantly attenuated OVA-stimulated goblet cell hyperplasia ^a	Kanehiro et al. (2001)
M ₃ muscarinic receptor	Tiotropium bromide	Increased FEV ₁ at 24 months in COPD patients of GOLD stage 1 or 2. Also ameliorated annual FEV ₁ decline after bronchodilator ^c Prevented allergen-induced mucous gland hypertrophy. Partially reduced the increase in MUC5AC-positive goblet cell numbers and eosinophil infiltration ^a Both attenuated and reversed IL-13 induced GCD. Restored aberrant FOXA2 and FOXA3 expression following IL-13 treatment ^d	Goblet cell number not assessed ^c Significantly reduced MUC5AC labelled goblet cell numbers in OVA-stimulated animals. Non-significantly (<i>p</i> = 0.06) reduced PAS labelled in OVA-stimulated animals ^a Significantly attenuated and reversed IL-13 stimulated GCD ^d .	Zhou et al. (2017) Bos et al. (2007) Kistemaker et al. (2015)

RSV respiratory syncytial virus; LABA long acting β-adrenoceptor agonist; ACOQ5 asthma control questionnaire (5-question variant); GCR glucocorticoid receptor; BALF bronchoalveolar lavage fluid; PDE4 phosphodiesterase 4.

^a Animal model.

^b mAb variants.

^c Clinical trial.

^d *In vitro* model.

addition to inhibiting lipopolysaccharide induced CCL11 expression, kaempferol dose-dependently reduces TNF- α stimulated epithelial expression of ICAM-1 and eosinophil expression of integrin β 2, which attenuates the eosinophil-airway interaction (Gong et al., 2012). Kaempferol is believed to exert these effects by moderating Janus activated kinase/signal transducers and activators of transcription (JAK/STAT) signaling, culminating in the disruption of NF- κ B activation (Gong et al., 2012).

Macrolide antibiotics including clarithromycin and rapamycin have been used on the basis of their immunosuppressive activity, to attenuate the symptoms of chronic airways disease (Essilfie et al., 2015; Gibson et al., 2017; Mushaben et al., 2011; Shinkai, Henke, & Rubin, 2008). In addition to reductions in airway hyperreactivity, cytokine secretion and airway inflammation, a decrease in goblet cell number is consistently observed following macrolide antibiotic treatment following allergen exposure (Mushaben et al., 2011; Nagashima et al., 2016). Clarithromycin treatment of human bronchial epithelial cells stimulated with IL-13 drastically reduces the levels of SPDEF, goblet cell specific CLCA1, ERK1/2 phosphorylation and overall goblet cell number (Nagashima et al., 2016; Tanabe et al., 2011). Likewise, rapamycin has been shown to suppress mucous cell metaplasia in mice challenged with HDM in an acute asthma model; however no changes in GCD were evident in a chronic/established asthma model (Fredriksson et al., 2012; Mushaben et al., 2011; Mushaben, Brandt, Hershey, & Le Cras, 2013). Similar to clarithromycin, rapamycin induces concomitant reductions in mouse CLCA3 (equivalent to human CLCA1) and SPDEF (Mushaben et al., 2013). This attenuation is likely due to the inhibition of mTOR (Mushaben et al., 2013). Indeed, although the widespread and consistent use of antibiotics such as rapamycin and clarithromycin may raise concerns in the treatment of persistent GCD, it is clear that understanding their mechanisms of action may provide us with a novel way forward in tackling this aspect of chronic airways disease.

Another drug that may prove beneficial is the EGFR tyrosine kinase inhibitor, gefitinib (IRESSA). To date, gefitinib has been identified as a potential inhibitor of goblet cell hyperplasia (Song et al., 2016). Pre-treatment of mice with gefitinib for 7 days alleviated OVA-induced allergic airway symptoms and pathologies including GCD. This alleviation appears to be time dependent as a 3 day intervention with gefitinib at the end of a 7 day OVA challenge failed to exert these effects (Song et al., 2016). Thus gefitinib may be better suited as a preventative drug to treat excessive GCD associated with allergic asthma. Gefitinib has also been shown to reverse the smoke-induced loss of ciliated cells in human bronchial epithelial cells grown under ALI culture (Valencia-Gattas, Conner, & Fregien, 2016). Furthermore, cells subjected to cigarette smoke recover a ciliated phenotype more quickly in the presence of gefitinib. This may offer an effective treatment for defective mucociliary clearance which is associated with the pathogenesis of COPD/chronic bronchitis (Sethi, 2000).

Preventative amiloride therapy has been shown disrupt GCD in mouse models of CF (Zhou et al., 2008). Amiloride directly blocks the epithelial sodium channel (ENaC), which improves mucus hydration, and when utilized as a preventative therapy in β ENaC-overexpressing mice, reduces airway inflammation along with IL-13 production, goblet cell metaplasia and mucus hypersecretion (Zhou et al., 2008). Together with a reduction in morphological characteristics associated with mucus obstruction, preventative amiloride treatment has also been shown to lower mortality and reduce the number of necrotic airway epithelial cells often seen in CF lungs (Zhou et al., 2008). Pilot studies have shown benefits from aerosolized amiloride treatment within the airways of CF patients slowing the loss of forced vital capacity (FVC) and reducing mucus viscoelasticity (Knowles et al., 1990). Nonetheless, follow up clinical trials revealed no significant benefit on forced expiratory volume in 1 s (FEV1) or FVC or sputum volume (Bowler et al., 1995; Graham et al., 1993; Pons et al., 2000). The effect of preventative amiloride treatment on GCD is not well documented in the human airway however and requires further investigation.

4.2. Monoclonal antibody therapy

A number of studies have utilized monoclonal antibody (mAb) treatment to attenuate signaling pathways involved in numerous airways diseases, including asthma and COPD (Arora, McDonald, Toews, & Huffnagle, 2006; Kondo et al., 2006; Kumar, Herbert, Webb, Li, & Foster, 2004; Syk et al., 2016). Monoclonal antibodies are thought to exhibit a number of advantages over small molecule based therapies; 1) mAbs are considered to exert greater specificity for the target molecule, 2) they do not form toxic metabolites following breakdown, and 3) they generally have longer half-lives (Chames, Van Regenmortel, Weiss, & Baty, 2009). The most well recognized of these is the chimeric monoclonal antibody that binds and neutralizes IgE, omalizumab. Omalizumab is the only mAb approved for treatment of early onset severe IgE dependent asthma and works by targeting the high-affinity domain of IgE, preventing its interaction with mast cells (Omalizumab, 2002). Omalizumab also significantly reduces circulating IL-13 and peripheral eosinophils in asthmatics underlying its significant impact on airway remodeling including GCD (Noga, Hanf, & Kunkel, 2003). Other emerging mAbs may also hold promise as effective pharmaceutical agents against persistent GCD and these include lebrikizumab (IL-13), tralokinumab (IL-13), benralizumab (IL-5R α) and dupilumab (IL-4R α) (Hansbro et al., 2011; Hansbro et al., 2013; Wenzel et al., 2013). To date however, in depth clinical analyses on how such mAb therapies may affect GCD are yet to be carried out (Table 1).

4.3. Therapies utilizing miRNA technology

A tantalizing approach to target the expression of specific proteins involved in persistent GCD is the administration of exogenous miRNAs and/or inhibitors of them, termed antagomirs. Antagomirs are small oligonucleotides that are perfectly complementary to a specific miRNA and have been shown to lead to the effective degradation of miRNA *in vivo* (Krutzfeldt et al., 2005). Indeed a number of antagomir based studies conducted in mice have yielded promising results. For example, inhibition of the miR-126 and the let-7 miRNA family in a mouse model of asthma reduces total number of inflammatory cells, cytokine production as well as levels of Muc5ac (Mattes, Collison, Plank, Phipps, & Foster, 2009; Polikepahad et al., 2010). Inhibition of miR-106a or miR-221 is shown to reduce inflammation in mouse models of asthma (Qin et al., 2012; Sharma et al., 2012). Likewise, deletion of miR-21 or miR-155 leads to significantly lower numbers of eosinophils in OVA-challenged mice (Lu et al., 2011; Malmhall et al., 2014). There is significant potential in the use of miRNA antagomirs and mimics to ameliorate not only persistent GCD, but other pathophysiological features of chronic airways disease.

One advantage in treating airway-based diseases in humans is the ability to deliver small molecule therapeutics *via* inhalation. Aerosolization, as well as dry powder inhalation, have been shown to be effective, low-risk methods of administering compounds to treat numerous airways diseases (Doukas et al., 2009; Guilleminault et al., 2014; Rubin & Williams, 2014). This is because aerosol-delivery increases compound bioavailability at the airway epithelium and reduces potential adverse effects typically associated with systemic delivery. Unfortunately, in chronic airways disease, the thicker and less hydrated mucus layer is a significant obstacle for aerosol-delivery. A potential solution to this problem is combining bioactive components, such as antagomir molecules, with a mucolytic aerosol performance enhancer, such as D-mannitol, to increase airway epithelial interactions and allow for transport into the cells (Li, Vogt, Hayes Jr., & Mansour, 2014; Muralidharan, Hayes Jr., Black, & Mansour, 2016). In addition to standard aerosol delivery has also been the advent of nanoparticle inhalation technology (Kuzmov & Minko, 2015). Nanoparticulate dry powder inhalation in combination with D-mannitol has been shown to effectively reach smaller airways in an *in vitro* predictive lung deposition model (Muralidharan et al., 2016).

For persistent GCD in the airway epithelium, antagomir therapy may prove to be an important novel approach to restore the expression of numerous proteins that are downregulated during chronic airways disease; one example being FOXA2 which is negatively regulated by miR-124a. Thioredoxin-interacting protein has been shown to down-regulate the expression of miR-124a, and increases expression of the goblet cell regulator FOXA2 (Jing et al., 2014; Wan, Kaestner, et al., 2004). Antagomir based therapies against miR-124a would further increase effectiveness in elevating FOXA2 expression. Furthermore, the addition of miRNA based therapies to inhaler technology would allow target delivery of antagomirs directly to the airway epithelium.

5. Conclusions

Accumulation of mucus in the airways is a key feature of chronic airways disease. Resolving persistent GCD during chronic airways disease is necessary to abolish the downstream pathogenic effects of intraluminal mucus accumulation. Currently, it is well recognized that dysregulation of several critical signaling pathways can lead to persistent GCD during chronic airways disease. These can include anomalies in EGFR signaling, interleukin production (IL-13, IL-4, IL-5, and IL-9), SPDEF, NOTCH, WNT/ β -Catenin, GABA_AR and KIF3A. A number of miRNAs also control the expression of several proteins within these pathways and have been shown to directly affect GCD. Gene-based approaches targeting the molecules regulating these pathways have been shown to limit GCD in mouse models of chronic airways disease. Recent advancements in laboratory based technologies, including novel cell culture/co-culture methods, such as bronchospheres and organ-on-chip microfluidics, will allow *ex vivo* studies to more accurately recapitulate *in vivo* events (Danahay et al., 2015; Esch, Bahinski, & Huh, 2015).

Therapeutic regulation of the above pathways at the human airway epithelium is the logical next step to combating persistent GCD. Although targeting GCD through inhibition of such master regulators as EGFR requires a cautious approach, one advantage of the airway epithelium is the ability to deliver drugs to the affected cells *via* aerosol. Monoclonal antibody therapy, such as the use of omalizumab, has been shown to significantly reduce Th2 cytokines, including IL-13, and is expected to have a dramatic effect on GCD. However, in depth studies of the response of the human airway epithelium to omalizumab are yet to be carried out. Dupilumab treatment, targeting IL-4 and IL-13 signaling also holds promise against persistent GCD during chronic airways disease and is currently undergoing clinical trials. With the pathways regulating persistent GCD becoming increasingly well understood and advancements in therapeutic design, *in vivo* mimicking technologies and drug delivery we are making clear strides toward resolving mucus associated pathogenesis during chronic airways disease.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Acciani, T. H., Suzuki, T., Trapnell, B. C., & Le Gras, T. D. (2016). Epidermal growth factor receptor signalling regulates granulocyte-macrophage colony-stimulating factor production by airway epithelial cells and established allergic airway disease. *Clinical and Experimental Allergy*, 46, 317–328.

Allahverdian, S., Harada, N., Singhera, G. K., Knight, D. A., & Dorscheid, D. R. (2008). Secretion of IL-13 by airway epithelial cells enhances epithelial repair via HB-EGF. *American Journal of Respiratory Cell and Molecular Biology*, 38, 153–160.

Amaral, E. P., Conceição, E. L., Costa, D. L., Rocha, M. S., Marinho, J. M., Cordeiro-Santos,

M., ... Andrade, B. B. (2016). N-acetyl-cysteine exhibits potent anti-mycobacterial activity in addition to its known anti-oxidative functions. *BMC Microbiology*, 16, 251.

Anderson, M. P., Gregory, R. J., Thompson, S., Souza, D. W., Paul, S., Mulligan, R. C., ... Welsh, M. J. (1991). Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science*, 253, 202–205.

Arora, S., McDonald, R. A., Toews, G. B., & Huffnagle, G. B. (2006). Effect of a CD4-depleting antibody on the development of *Cryptococcus neoformans*-induced allergic bronchopulmonary mycosis in mice. *Infection and Immunity*, 74, 4339–4348.

Atherton, H. C., Jones, G., & Danahay, H. (2003). IL-13-induced changes in the goblet cell density of human bronchial epithelial cell cultures: MAP kinase and phosphatidylinositol 3-kinase regulation. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 285, L730–L739.

Baarsma, H. A., Konigshoff, M., & Gosens, R. (2013). The WNT signaling pathway from ligand secretion to gene transcription: Molecular mechanisms and pharmacological targets. *Pharmacology and Therapeutics*, 138, 66–83.

Bai, T. R., & Knight, D. A. (2005). Structural changes in the airways in asthma: Observations and consequences. *Clinical Science*, 108, 463–477.

Barbier, D., Garcia-Verdugo, I., Pothlichet, J., Khazen, R., Descamps, D., Rousseau, K., ... Sallenave, J.-M. (2012). Influenza A induces the major secreted airway mucin MUC5AC in a protease-EGFR-extracellular regulated kinase-Sp1-dependent pathway. *American Journal of Respiratory Cell and Molecular Biology*, 47, 149–157.

Baroukh, N., Ravier, M. A., Loder, M. K., Hill, E. V., Bounacer, A., Scharfmann, R., ... Van Obberghen, E. (2007). MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic β -cell lines. *Journal of Biological Chemistry*, 282, 19575–19588.

Beatus, P., Lundkvist, J., Oberg, C., & Lendahl, U. (1999). The Notch 3 intracellular domain represses Notch 1-mediated activation through hairy/enhancer of split (HES) promoters. *Development*, 126, 3925–3935.

Beckett, E. L., Stevens, R. L., Jarnicki, A. G., Kim, R. Y., Hanish, I., Hansbro, N. G., ... Hansbro, P. M. (2013). A new short-term mouse model of chronic obstructive pulmonary disease identifies a role for mast cell tryptase in pathogenesis. *Journal of Allergy and Clinical Immunology*, 131, 752–762.

Beeh, K. M., Beier, J., Esperester, A., & Paul, L. D. (2008). Antiinflammatory properties of ambroxol. *European Journal of Medical Research*, 13, 557–562.

Blanchard, C., Mishra, A., Saito-Akei, H., Monk, P., Anderson, I., & Rothenberg, M. E. (2005). Inhibition of human interleukin-13-induced respiratory and oesophageal inflammation by anti-human-interleukin-13 antibody (CAT-354). *Clinical and Experimental Allergy*, 35, 1096–1103.

Bleecker, E. R., FitzGerald, J. M., Chaney, P., Papi, A., Weinstein, S. F., Barker, P., ... Goldman, M. (2016). Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta₂-agonists (SIRIOCCO): A randomised, multicentre, placebo-controlled phase 3 trial. *Lancet*, 388, 2115–2127.

Bos, I. S., Gosens, R., Zuidhof, A. B., Schaafsma, D., Halayko, A. J., Meurs, H., & Zaagsma, J. (2007). Inhibition of allergen-induced airway remodelling by tiotropium and budesonide: A comparison. *European Respiratory Journal*, 30, 653–661.

Bowler, I. M., Kelman, B., Worthington, D., Littlewood, J. M., Watson, A., Conway, S. P., ... Sheldon, T. A. (1995). Nebulised amiloride in respiratory exacerbations of cystic fibrosis: A randomised controlled trial. *Archives of Disease in Childhood*, 73, 427–430.

Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., ... Israel, A. (2000). A novel proteolytic cleavage involved in Notch signaling: The role of the disintegrin-metalloprotease TACE. *Molecular Cell*, 5, 207–216.

Burgel, P.-R., Lazarus, S. C., Tam, D. C.-W., Ueki, I. F., Atabai, K., Birch, M., & Nadel, J. A. (2007). Human eosinophils induce mucin production in airway epithelial cells via epidermal growth factor receptor activation. *The Journal of Immunology*, 167, 5948–5954.

Burgel, P. R., Montani, D., Danel, C., Dusser, D. J., & Nadel, J. A. (2007). A morphometric study of mucins and small airway plugging in cystic fibrosis. *Thorax*, 62, 153–161.

Busse, W., Corren, J., Lanier, B. Q., McAlary, M., Fowler-Taylor, A., Cioppa, G. D., ... Gupta, N. (2001). Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *Journal of Allergy and Clinical Immunology*, 108, 184–190.

Calafat, J., Janssen, H., hle-Bäckdahl, M. S., Zuurbier, A. E. M., Knol, E. F., & Egesten, A. (1997). Human monocytes and neutrophils store transforming growth factor- α in a subpopulation of cytoplasmic granules. *Blood*, 90, 1255–1266.

Capuano, M., Iaffaldano, L., Tinto, N., Montanaro, D., Capobianco, V., Izzo, V., ... Sacchetti, L. (2011). MicroRNA-449a overexpression, reduced NOTCH1 signals and scarce goblet cells characterize the small intestine of celiac patients. *PLoS One*, 6, e29094.

Caramori, G., Casolari, P., Di Gregorio, C., Saetta, M., Baraldo, S., Boschetto, P., ... Papi, A. (2009). MUC5AC expression is increased in bronchial submucosal glands of stable COPD patients. *Histopathology*, 55, 321–331.

Castel, D., Mourikis, P., Bartels, S. J. J., Brinkman, A. B., Tajbakhsh, S., & Stunnenberg, H. G. (2013). Dynamic binding of RBPJ is determined by Notch signaling status. *Genes and Development*, 27, 1059–1071.

Chames, P., Van Regenmortel, M., Weiss, E., & Baty, D. (2009). Therapeutic antibodies: Successes, limitations and hopes for the future. *British Journal of Pharmacology*, 157, 220–233.

Chen, G., Korfhagen, T. R., Karp, C. L., Impey, S., Xu, Y., Randell, S. H., ... Whitsett, J. A. (2014). Foxa3 induces goblet cell metaplasia and inhibits innate antiviral immunity. *American Journal of Respiratory and Critical Care Medicine*, 189, 301–313.

Chen, G., Korfhagen, T. R., Xu, Y., Kitzmiller, J., Wert, S. E., Maeda, Y., ... Whitsett, J. A. (2009). SPDEF is required for mouse pulmonary goblet cell differentiation and regulates a network of genes associated with mucus production. *The Journal of Clinical Investigation*, 119, 2914–2924.

Chen, G., Wan, H., Luo, F., Zhang, L., Xu, Y., Lewkowich, I., ... Whitsett, J. A. (2010). Foxa2 programs Th2 cell-mediated innate immunity in the developing lung. *The*

- Journal of Immunology*, 184, 6133–6141.
- Chu, E. K., Cheng, J., Foley, J. S., Mechem, B. H., Owen, C. A., Haley, K. J., ... Drazen, J. M. (2006). Induction of the plasminogen activator system by mechanical stimulation of human bronchial epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*, 35, 628–638.
- Chu, E. K., Foley, J. S., Cheng, J., Patel, A. S., Drazen, J. M., & Tschumperlin, D. J. (2005). Bronchial epithelial compression regulates epidermal growth factor receptor family ligand expression in an autocrine manner. *American Journal of Respiratory Cell and Molecular Biology*, 32, 373–380.
- Chung, K. F. (2001). Cytokines in chronic obstructive pulmonary disease. *European Respiratory Journal*, 18, 50s–59s.
- Cohen, S. (1964). Isolation and biological effects of an epidermal growth-stimulating protein. *National Cancer Institute Monograph*, 13, 13–37.
- Cohn, L., Homer, R. J., MacLeod, H., Mohrs, M., Brombacher, F., & Bottomly, K. (1999). Th2-induced airway mucus production is dependent on IL-4R α , but not on eosinophils. *The Journal of Immunology*, 162, 6178–6183.
- Cohn, L., Homer, R. J., Marinov, A., Rankin, J., & Bottomly, K. (1997). Induction of airway mucus production by T helper 2 (Th2) cells: A critical role for interleukin 4 in cell recruitment but not mucus production. *The Journal of Experimental Medicine*, 186, 1737–1747.
- Corde, G., & Sala, A. (2017). Non-canonical WNT/PCP signalling in cancer: Fzd6 takes centre stage. *Oncogenesis*, 6, e364.
- Corren, J., Lemanske, R. F., Hanania, N. A., Korenblat, P. E., Parsey, M. V., Arron, J. R., ... Matthews, J. G. (2011). Lebrikizumab treatment in adults with asthma. *The New England Journal of Medicine*, 365, 1088–1098.
- Danahay, H., Pessotti, A. D., Coote, J., Montgomery, B. E., Xia, D., Wilson, A., ... Jaffe, A. B. (2015). Notch2 is required for inflammatory cytokine-driven goblet cell metaplasia in the lung. *Cell Reports*, 10, 239–252.
- De Kluijver, J., Schrupf, J. A., Evertse, C. E., Sont, J. K., Roughley, P. J., Rabe, K. F., ... Sterk, P. J. (2005). Bronchial matrix and inflammation respond to inhaled steroids despite ongoing allergen exposure in asthma. *Clinical and Experimental Allergy*, 35, 1361–1369.
- Derynck, R., Roberts, A. B., Winkler, M. E., Chen, E. Y., & Goeddel, D. V. (1984). Human transforming growth factor- α : Precursor structure and expression in *E. coli*. *Cell*, 38, 287–297.
- Doukas, J., Eide, L., Stebbins, K., Racanelli-Layton, A., Dellamary, L., Martin, M., ... Cheresch, D. A. (2009). Aerosolized phosphoinositide 3-kinase γ /8 inhibitor TG100-115 [3-[2,4-diamino-6-(3-hydroxyphenyl)pteridin-7-yl]phenol] as a therapeutic candidate for asthma and chronic obstructive pulmonary disease. *Journal of Pharmacology and Experimental Therapeutics*, 328, 758–765.
- Ermund, A., Meiss, L. N., Rodriguez-Pineiro, A. M., Bähr, A., Nilsson, H. E., Trillo-Muyo, S., ... Hansson, G. C. (2017). The normal trachea is cleaned by MUC5B mucin bundles from the submucosal glands coated with the MUC5AC mucin. *Biochemical and Biophysical Research Communications*, 492, 331–337.
- Esch, E. W., Bahinski, A., & Huh, D. (2015). Organs-on-chips at the frontiers of drug discovery. *Nature Reviews Drug Discovery*, 14, 248–260.
- Essilfie, A. T., Horvat, J. C., Kim, R. Y., Mayall, J. R., Pinkerton, J. W., Beckett, E. L., ... Hansbro, P. M. (2015). Macrolide therapy suppresses key features of experimental steroid-sensitive and steroid-insensitive asthma. *Thorax*, 70, 458–467.
- Evans, C. M., Kim, K., Tuvim, M. J., & Dickey, B. F. (2009). Mucus hypersecretion in asthma: Causes and effects. *Current Opinion in Pulmonary Medicine*, 15, 4–11.
- Eyking, A., Reis, H., Frank, M., Gerken, G., Schmid, K. W., & Cario, E. (2016). MiR-205 and miR-373 are associated with aggressive human mucinous colorectal cancer. *PLoS One*, 11, e0156871.
- Ezratty, E. J., Stokes, N., Chai, S., Shah, A. S., Williams, S. E., & Fuchs, E. (2011). A role for the primary cilium in Notch signaling and epidermal differentiation during skin development. *Cell*, 145, 1129–1141.
- Fahy, J. V. (2015). Type 2 inflammation in asthma — Present in most, absent in many. *Nature Reviews Immunology*, 15, 57–65.
- Fahy, J. V., & Dickey, B. F. (2010). Airway mucus function and dysfunction. *New England Journal of Medicine*, 363, 2233–2247.
- Fahy, J. V., Kim, K. W., Liu, J., & Boushey, H. A. (1995). Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *Journal of Allergy and Clinical Immunology*, 95, 843–852.
- Fischer, A., & Gessler, M. (2007). Delta-Notch—And then? Protein interactions and proposed modes of repression by Hes and Hey bHLH factors. *Nucleic Acids Research*, 35, 4583–4596.
- Fredriksson, K., Fielhaber, J. A., Lam, J. K., Yao, X., Meyer, K. S., Keeran, K. J., ... Levine, S. J. (2012). Paradoxical effects of rapamycin on experimental house dust mite-induced asthma. *PLoS One*, 7, e33984.
- Ganesan, S., Comstock, A. T., & Sajjan, U. S. (2013). Barrier function of airway tract epithelium. *Tissue Barriers*, 1, e24997.
- Gao, W., Li, L., Wang, Y., Zhang, S., Adcock, I. M., Barnes, P. J., ... Yao, X. (2015). Bronchial epithelial cells: The key effector cells in the pathogenesis of chronic obstructive pulmonary disease? *Respirology*, 20, 722–729.
- Gehrig, S., Duerr, J., Weitnauer, M., Wagner, C. J., Graeber, S. Y., Schatterny, J., ... Mall, M. A. (2014). Lack of neutrophil elastase reduces inflammation, mucus hypersecretion, and emphysema, but not mucus obstruction, in mice with cystic fibrosis-like lung disease. *American Journal of Respiratory and Critical Care Medicine*, 189, 1082–1092.
- Gerovac, B. J., & Fregien, N. L. (2016). IL-13 inhibits multicilin expression and cilogenesis via janus kinase/signal transducer and activator of transcription independently of Notch cleavage. *American Journal of Respiratory Cell and Molecular Biology*, 54, 554–561.
- Gerovac, B. J., Valencia, M., Baumlin, N., Salathe, M., Conner, G. E., & Fregien, N. L. (2014). Submersion and hypoxia inhibit ciliated cell differentiation in a notch-dependent manner. *American Journal of Respiratory Cell and Molecular Biology*, 51, 516–525.
- Gibson, P. G., Yang, I. A., Upham, J. W., Reynolds, P. N., Hodge, S., James, A. L., ... Simpson, J. L. (2017). Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): A randomised, double-blind, placebo-controlled trial. *Lancet*, S0140-6736(17), 31281–31283.
- Global Initiative for Asthma (2017). Global Strategy for Asthma Management and Prevention. Available from www.ginasthma.org.
- Goenka, S., & Kaplan, M. H. (2011). Transcriptional regulation by STAT6. *Immunologic Research*, 50, 87–96.
- Gomi, K., Arbelaez, V., Crystal, R. G., & Walters, M. S. (2015). Activation of NOTCH1 or NOTCH3 signaling skews human airway basal cell differentiation toward a secretory pathway. *PLoS One*, 10, e0116507.
- Gong, J. H., Shin, D., Han, S. Y., Kim, J. L., & Kang, Y. H. (2012). Kaempferol suppresses eosinophil infiltration and airway inflammation in airway epithelial cells and in mice with allergic asthma. *Journal of Nutrition*, 142, 47–56.
- Graham, A., Hasani, A., Alton, E. W., Martin, G. P., Marriott, C., Hodson, M. E., ... Geddes, D. M. (1993). No added benefit from nebulized amiloride in patients with cystic fibrosis. *European Respiratory Journal*, 6, 1243–1248.
- Grainge, C. L., Lau, L. C. K., Ward, J. A., DULAY, V., Lahiff, G., Wilson, S., ... Howarth, P. H. (2011). Effect of bronchoconstriction on airway remodeling in asthma. *New England Journal of Medicine*, 364, 2006.
- Grunig, G., Warnock, M., Wakil, A. E., Venkayya, R., Brombacher, F., Rennick, D. M., ... Corry, D. B. (1998). Requirement for IL-13 independently of IL-4 in experimental asthma. *Science*, 282, 2261–2263.
- Guilleminault, L., Azzopardi, N., Arnould, C., Sobilo, J., Herve, V., Montharu, J., ... Heuze-Vourc'h, N. (2014). Fate of inhaled monoclonal antibodies after the deposition of aerosolized particles in the respiratory system. *Journal of Controlled Release*, 196, 344–354.
- Guseh, J. S., Bores, S. A., Stanger, B. Z., Zhou, Q., Anderson, W. J., Melton, D. A., & Rajagopal, J. (2009). Notch signaling promotes airway mucous metaplasia and inhibits alveolar development. *Development*, 136, 1751–1759.
- Ha, E. V. S., & Rogers, D. F. (2016). Novel therapies to inhibit mucus synthesis and secretion in airway hypersecretory diseases. *Pharmacology*, 97, 84–100.
- Hall, C. B. (2001). Respiratory syncytial virus and parainfluenza virus. *New England Journal of Medicine*, 344, 1917–1928.
- Hansbro, P. M., Kaiko, G. E., & Foster, P. S. (2011). Cytokine/anti-cytokine therapy — Novel treatments for asthma? *British Journal of Pharmacology*, 163, 81–95.
- Hansbro, P. M., Kim, R. Y., Starkey, M. R., Donovan, C., Dua, K., Mayall, J. R., ... Horvat, J. C. (2017). Mechanisms and treatments for severe, steroid-resistant allergic airway disease and asthma. *Immunological Reviews*, 278, 41–62.
- Hansbro, P. M., Scott, G. V., Essilfie, A.-T., Kim, R. Y., Starkey, M. R., Nguyen, D. H., ... Foster, P. S. (2013). Th2 cytokine antagonists: Potential treatments for severe asthma. *Expert Opinion on Investigational Drugs*, 22, 49–69.
- Hao, Y., Kuang, Z., Walling, B. E., Bhatia, S., Sivaguru, M., Chen, Y., Gaskins, H. R., & Lau, G. W. (2012). Pseudomonas aeruginosa pyocyanin causes airway goblet cell hyperplasia and metaplasia and mucus hypersecretion by inactivating the transcriptional factor FoxA2. *Cellular Microbiology*, 14, 401–415.
- Hartley, R. A., Barker, B. L., Newby, C., Pakkal, M., Baldi, S., Kajekar, R., ... Brightling, C. E. (2016). Relationship between lung function and quantitative computed tomographic parameters of airway remodeling, air trapping, and emphysema in patients with asthma and chronic obstructive pulmonary disease: A single-center study. *Journal of Allergy and Clinical Immunology*, 137(1413–1422), e1412.
- Hays, S. R., & Fahy, J. V. (2006). Characterizing mucous cell remodeling in cystic fibrosis: relationship to neutrophils. *American Journal of Respiratory and Critical Care Medicine*, 174, 1018–1024.
- Henderson, A. G., Ehre, C., Button, B., Abdullah, L. H., Cai, L.-H., Leigh, M. W., ... Kesimer, M. (2014). Cystic fibrosis airway secretions exhibit mucin hyperconcentration and increased osmotic pressure. *The Journal of Clinical Investigation*, 124, 3047–3060.
- Higashiyama, S., Abraham, J. A., Miller, J., Fiddes, J. C., & Klagsbrun, M. (1991). A heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science*, 251, 936–939.
- Hodge, S., Hodge, G., Flower, R., Reynolds, P. N., Scicchitano, R., & Holmes, M. (2002). Up-regulation of production of TGF-beta and IL-4 and down-regulation of IL-6 by apoptotic human bronchial epithelial cells. *Immunology and Cell Biology*, 80, 537–543.
- Hovenberg, H. W., Davies, J. R., & Carlstedt, I. (1996). Different mucins are produced by the surface epithelium and the submucosa in human trachea: Identification of MUC5AC as a major mucin from the goblet cells. *Biochemical Journal*, 318(Pt 1), 319–324.
- Hsu, A. C. Y., Dua, K., Starkey, M. R., Haw, T.-J., Nair, P. M., Nichol, K., ... Wark, P. A. (2017). MicroRNA-125a and -b inhibit A20 and MAVS to promote inflammation and impair antiviral response in COPD. *JCI Insight*, 2, e90443.
- Ikegami, M., Falcone, A., & Whitsett, J. A. (2008). STAT-3 regulates surfactant phospholipid homeostasis in normal lung and during endotoxin-mediated lung injury. *Journal of Applied Physiology*, 104, 1753–1760.
- Innes, A. L., Woodruff, P. G., Ferrando, R. E., Donnelly, S., Dolganov, G. M., Lazarus, S. C., & Fahy, J. V. (2006). Epithelial mucin stores are increased in the large airways of smokers with airflow obstruction. *Chest*, 130, 1102–1108.
- Jackson, A. D. (2001). Airway goblet-cell mucus secretion. *Trends in Pharmacological Sciences*, 22, 39–45.
- Jackson, D. J., & Johnston, S. L. (2010). The role of viruses in acute exacerbations of asthma. *Journal of Allergy and Clinical Immunology*, 125, 1178–1187.
- Jeffery, P. K. (1999). Differences and similarities between chronic obstructive pulmonary disease and asthma. *Clinical and Experimental Allergy*, 29, 14–26.
- Jeffery, P. K. (2001). Remodeling in asthma and chronic obstructive lung disease.

- American Journal of Respiratory and Critical Care Medicine, 164, S28–S38.
- Jiang, H., Zhu, Y. S., Xu, H., Sun, Y., & Li, Q. F. (2010). Inflammatory stimulation and hypoxia cooperatively activate HIF-1 α in bronchial epithelial cells: Involvement of PI3K and NF- κ B. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 298, L660–669.
- Jing, G., Westwell-Roper, C., Chen, J., Xu, G., Verchere, C. B., & Shalev, A. (2014). Thioredoxin-interacting protein promotes islet amyloid polypeptide expression through miR-124a and FoxA2. *Journal of Biological Chemistry*, 289, 11807–11815.
- Jones, J. T., Akita, R. W., & Sliwowski, M. X. (1999). Binding specificities and affinities of egf domains for ErbB receptors. *FEBS Letters*, 447, 227–231.
- Kaliner, M., Shelhamer, J. H., Borson, B., Nadel, J., Patow, C., & Marom, Z. (1986). Human respiratory mucus. *American Review of Respiratory Disease*, 134, 612–621.
- Kanehiro, A., Ikemura, T., Makela, M. J., Lahn, M., Joetham, A., Dakhama, A., & Gelfand, E. W. (2001). Inhibition of phosphodiesterase 4 attenuates airway hyperresponsiveness and airway inflammation in a model of secondary allergen challenge. *American Journal of Respiratory and Critical Care Medicine*, 163, 173–184.
- Kang, J. H., Kim, B. S., Uhm, T. G., Lee, S.-H., Lee, G. R., Park, C.-S., & Chung, I. Y. (2009). γ -Secretase inhibitor reduces allergic pulmonary inflammation by modulating Th1 and Th2 responses. *American Journal of Respiratory and Critical Care Medicine*, 179, 875–882.
- Kanoh, S., Tanabe, T., & Rubin, B. K. (2011). IL-13-induced MUC5AC production and goblet cell differentiation is steroid resistant in human airway cells. *Clinical and Experimental Allergy*, 41, 1747–1756.
- Kau, A. L., & Korenblat, P. E. (2014). Anti-interleukin 4 and 13 for asthma treatment in the era of endotypes. *Current Opinion in Allergy and Clinical Immunology*, 14, 570–575.
- Kedzierski, L., Tate, M. D., Hsu, A. C., Kolesnik, T. B., Linossi, E. M., Dagley, L., ... Nicholson, S. E. (2017). Suppressor of cytokine signaling (SOCS)5 ameliorates influenza infection via inhibition of EGFR signaling. *eLife*, 6, e20444.
- Kettle, R., Simmons, J., Schindler, F., Jones, P., Dicker, T., Dubois, G., ... Jones, C. E. (2010). Regulation of neuregulin 1 β -induced MUC5AC and MUC5B expression in human airway epithelium. *American Journal of Respiratory Cell and Molecular Biology*, 42, 472–481.
- Kim, E. Y., Battaile, J. T., Patel, A. C., You, Y., Agapov, E., Grayson, M. H., ... Holtzman, M. J. (2008). Persistent activation of an innate immune axis translates respiratory viral infection into chronic lung disease. *Nature Medicine*, 14, 633–640.
- Kim, J.-H., Cha, J.-Y., Cheong, H. S., Park, J. S., Jang, A. S., Uh, S.-T., ... Shin, H. D. (2011). KIF3A, a cilia structural gene on chromosome 5q31, and its polymorphisms show an association with aspirin hypersensitivity in asthma. *Journal of Clinical Immunology*, 31, 112–121.
- Kim, R. Y., Horvat, J. C., Pinkerton, J. W., Starkey, M. R., Essilfie, A. T., Mayall, J. R., ... Hansbro, P. M. (2017). MicroRNA-21 drives severe, steroid-insensitive experimental asthma by amplifying phosphoinositide 3-kinase-mediated suppression of histone deacetylase 2. *Journal of Allergy and Clinical Immunology*, 139, 519–532.
- Kim, S. W., Ramasamy, K., Bouamar, H., Lin, A. P., Jiang, D., & Aguiar, R. C. (2012). MicroRNAs miR-125a and miR-125b constitutively activate the NF- κ B pathway by targeting the tumor necrosis factor alpha-induced protein 3 (TNFAIP3, A20). *Proceedings of the National Academy of Sciences of the United States of America*, 109, 7865–7870.
- Kimelman, D., & Xu, W. (2006). Beta-catenin destruction complex: Insights and questions from a structural perspective. *Oncogene*, 25, 7482–7491.
- Kirkham, S., Sheehan, J. K., Knight, D., Richardson, P. S., & Thornton, D. J. (2002). Heterogeneity of airways mucus: Variations in the amounts and glycoforms of the major oligomeric mucins MUC5AC and MUC5B. *Biochemical Journal*, 361, 537–546.
- Kistemaker, L. E., Bos, S. T., Mudde, W. M., Hylkema, M. N., Hiemstra, P. S., Wess, J., ... Gosens, R. (2014). Muscarinic M3 receptors contribute to allergen-induced airway remodeling in mice. *American Journal of Respiratory Cell and Molecular Biology*, 50, 690–698.
- Kistemaker, L. E., & Gosens, R. (2015). Acetylcholine beyond bronchoconstriction: Roles in inflammation and remodeling. *Trends in Pharmacological Sciences*, 36, 164–171.
- Kistemaker, L. E., Hiemstra, P. S., Bos, I. S., Bouwman, S., van den Berge, M., Hylkema, M. N., ... Gosens, R. (2015). Tiotropium attenuates IL-13-induced goblet cell metaplasia of human airway epithelial cells. *Thorax*, 70, 668–676.
- Knowles, M. R., Church, N. L., Waltner, W. E., Yankaskas, J. R., Gilligan, P., King, M., ... Boucher, R. C. (1990). A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *New England Journal of Medicine*, 322, 1189–1194.
- Kondo, M., Tamaoki, J., Takeyama, K., Isono, K., Kawatani, K., Izumo, T., & Nagai, A. (2006). Elimination of IL-13 reverses established goblet cell metaplasia into ciliated epithelia in airway epithelial cell culture. *Allergy International: Official Journal of the Japanese Society of Allergy*, 55, 329–336.
- Kondo, M., Tamaoki, J., Takeyama, K., Nakata, J., & Nagai, A. (2002). Interleukin-13 induces goblet cell differentiation in primary cell culture from Guinea pig tracheal epithelium. *American Journal of Respiratory Cell and Molecular Biology*, 17, 536–541.
- Koopmans, T., Crutzen, S., Menzen, M. H., Halayko, A. J., Hackett, T. L., Knight, D. A., & Gosens, R. (2016). Selective targeting of CREB-binding protein/beta-catenin inhibits growth of and extracellular matrix remodelling by airway smooth muscle. *British Journal of Pharmacology*, 173, 3327–3341.
- Kovacic, M. B., Myers, J. M., Wang, N., Martin, L. J., Lindsey, M., Ericksen, M. B., ... Hershey, G. K. (2011). Identification of KIF3A as a novel candidate gene for childhood asthma using RNA expression and population allelic frequencies differences. *PLoS One*, 6, e23714.
- Krutzfeldt, J., Rajewsky, N., Braich, R., Rajeev, K. G., Tuschl, T., Manoharan, M., & Stoffel, M. (2005). Silencing of microRNAs in vivo with 'antagomirs'. *Nature*, 438, 685–689.
- Kumar, R. K., Herbert, C., Webb, D. C., Li, L., & Foster, P. S. (2004). Effects of anticytokine therapy in a mouse model of chronic asthma. *American Journal of Respiratory and Critical Care Medicine*, 170, 1043–1048.
- Kuperman, D. A., Huang, X., Koth, L. L., Chang, G. H., Dolganov, G. M., Zhu, Z., ... Erle, D. J. (2002). Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nature Medicine*, 8, 885–889.
- Kuzmov, A., & Minko, T. (2015). Nanotechnology approaches for inhalation treatment of lung diseases. *Journal of Controlled Release*, 219, 500–518.
- Lafkas, D., Shelton, A., Chiu, C., de Leon Boenig, G., Chen, Y., Stawicki, S. S., ... Siebel, C. W. (2015). Therapeutic antibodies reveal Notch control of transdifferentiation in the adult lung. *Nature*, 528, 127–131.
- Laoukili, J., Perret, E., Willems, T., Minty, A., Parthoens, E., Houcine, O., ... Tournier, F. (2001). IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells. *The Journal of Clinical Investigation*, 108, 1817–1824.
- Lee, J. J., McGarry, M. P., Farmer, S. C., Denzler, K. L., Larson, K. A., Carrigan, P. E., ... Lee, N. A. (1997). Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *The Journal of Experimental Medicine*, 185, 2143–2156.
- Leigh, R., Mostafa, M. M., King, E. M., Rider, C. F., Shah, S., Dumonceaux, C., ... Newton, R. (2016). An inhaled dose of budesonide induces genes involved in transcription and signaling in the human airways: Enhancement of anti- and proinflammatory effector genes. *Pharmacology Research & Perspectives*, 4, e00243.
- Leigh, R., & Proud, D. (2015). Virus-induced modulation of lower airway diseases: Pathogenesis and pharmacologic approaches to treatment. *Pharmacology and Therapeutics*, 148, 185–198.
- Leung, S. Y., Eynott, P., Nath, P., & Chung, K. F. (2005). Effects of ciclesonide and fluticasone propionate on allergen-induced airway inflammation and remodeling features. *Journal of Allergy and Clinical Immunology*, 115, 989–996.
- Li, X., Vogt, F. G., Hayes, D., Jr., & Mansour, H. M. (2014). Design, characterization, and aerosol dispersion performance modeling of advanced spray-dried microparticle/nanoparticle mannitol powders for targeted pulmonary delivery as dry powder inhalers. *Journal of Aerosol Medicine and Pulmonary Drug Delivery*, 27, 81–93.
- Liu, Z., Chen, X., Wu, Q., Song, J., Wang, L., & Li, G. (2016). miR-125b inhibits goblet cell differentiation in allergic airway inflammation by targeting SPDEF. *European Journal of Pharmacology*, 782, 14–20.
- Liu, C., Li, Q., Zhou, X., Kolosov, V. P., & Perelman, J. M. (2013). Rhythmic pressure waves induce mucin5AC expression via an EGFR-mediated signaling pathway in human airway epithelial cells. *DNA and Cell Biology*, 32, 423–429.
- Lopez, A. F., Sanderson, C. J., Gamble, J. R., Campbell, H. D., Young, I. G., & Vadas, M. A. (1988). Recombinant human interleukin 5 is a selective activator of human eosinophil function. *The Journal of Experimental Medicine*, 167, 219–224.
- Lu, T. X., Hartner, J., Lim, E. J., Fabry, V., Mingler, M. K., Cole, E. T., ... Rothenberg, M. E. (2011). MicroRNA-21 limits in vivo immune response-mediated activation of the IL-12/IFN- γ pathway, Th1 polarization, and the severity of delayed-type hypersensitivity. *The Journal of Immunology*, 187, 3362–3373.
- Lundgren, J. D., Kaliner, M., Logun, C., & Shelhamer, J. H. (1988). Dexamethasone reduces rat tracheal goblet cell hyperplasia produced by human neutrophil products. *Experimental Lung Research*, 14, 853–863.
- Maeda, Y., Chen, G., Xu, Y., Haitchi, H. M., Du, L., Keiser, A. R., ... Whitsett, J. A. (2011). Airway epithelial transcription factor NK2 homeobox 1 inhibits mucous cell metaplasia and Th2 inflammation. *American Journal of Respiratory and Critical Care Medicine*, 184, 421–429.
- Malerba, M., & Ragnoli, B. (2008). Ambroxol in the 21st century: Pharmacological and clinical update. *Expert Opinion on Drug Metabolism & Toxicology*, 4, 1119–1129.
- Mall, M. A., Harkema, J. R., Trojanek, J. B., Treis, D., Livraghi, A., Schubert, S., ... Boucher, R. C. (2008). Development of chronic bronchitis and emphysema in β -epithelial Na⁺ channel-overexpressing mice. *American Journal of Respiratory and Critical Care Medicine*, 177, 730–742.
- Malmhall, C., Alawieh, S., Lu, Y., Sjostrand, M., Bossios, A., Eldh, M., & Radinger, M. (2014). MicroRNA-155 is essential for TH2-mediated allergen-induced eosinophilic inflammation in the lung. *Journal of Allergy and Clinical Immunology*, 133, 1429–1438.
- Marcel, B., Chevalier, B., Coraux, C., Kodjabachian, L., & Barbry, P. (2011). MicroRNA-based silencing of Delta/Notch signaling promotes multiple cilia formation. *Cell Cycle*, 10, 2858–2864.
- Marini, M., Vittori, E., Hollemborg, J., & Mattoli, S. (1992). Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *Journal of Allergy and Clinical Immunology*, 89, 1001–1009.
- Martinez-Anton, A., Sokolowska, M., Kern, S., Davis, A. S., Alsaaty, S., Taubenberger, J. K., ... Shelhamer, J. H. (2013). Changes in microRNA and mRNA expression with differentiation of human bronchial epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*, 49, 384–395.
- Mattes, J., Collison, A., Plank, M., Phipps, S., & Foster, P. S. (2009). Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 18704–18709.
- McCullagh, C. M., Jamieson, A. M., Blackwell, J., & Gupta, R. (1995). Viscoelastic properties of human tracheobronchial mucin in aqueous solution. *Biopolymers*, 35, 149–159.
- Mizutani, N., Nabe, T., & Yoshino, S. (2015). IgE/antigen-mediated enhancement of IgE production is a mechanism underlying the exacerbation of airway inflammation and remodelling in mice. *Immunology*, 144, 107–115.
- Moheimani, F., Hsu, A. C.-Y., Reid, A. T., Williams, T., Kicic, A., Stick, S. M., ... Knight, D. A. (2016). The genetic and epigenetic landscapes of the epithelium in asthma. *Respiratory Research*, 17, 119.
- Mucenski, M. L., Nation, J. M., Thitoff, A. R., Besnard, V., Xu, Y., Wert, S. E., ... Whitsett, J. A. (2005). Beta-catenin regulates differentiation of respiratory epithelial cells in vivo. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 289, L971–979.

- Mueller, T. D., Zhang, J.-L., Sebald, W., & Duschl, A. (2002). Structure, binding, and antagonists in the IL-4/IL-13 receptor system. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1592, 237–250.
- Muralidharan, P., Hayes, D., Jr., Black, S. M., & Mansour, H. M. (2016). Microparticulate/nanoparticulate powders of a novel Nrf2 activator and an aerosol performance enhancer for pulmonary delivery targeting the lung Nrf2/Keap-1 pathway. *Molecular Systems Design & Engineering*, 1, 48–65.
- Mushaben, E. M., Brandt, E. B., Hershey, G. K., & Le Cras, T. D. (2013). Differential effects of rapamycin and dexamethasone in mouse models of established allergic asthma. *PLoS One*, 8, e54426.
- Mushaben, E. M., Kramer, E. L., Brandt, E. B., Khurana Hershey, G. K., & Le Cras, T. D. (2011). Rapamycin attenuates airway hyperreactivity, goblet cells, and IgE in experimental allergic asthma. *The Journal of Immunology*, 187, 5756–5763.
- Nagashima, A., Shinkai, M., Shinoda, M., Shimokawaji, T., Kimura, Y., Mishina, K., ... Kaneko, T. (2016). Clarithromycin suppresses CLCA1 and inhibits IL-13-Induced goblet cell hyperplasia in human bronchial epithelial cells. *Antimicrobial Agents and Chemotherapy*, 60, 6585–6590.
- Nakanishi, A., Morita, S., Iwashita, H., Sagiya, Y., Ashida, Y., Shirafuji, H., ... Fujino, M. (2001). Role of gob-5 in mucus overproduction and airway hyperresponsiveness in asthma. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 5175–5180.
- Nicholson, S. E., Metcalf, D., Sprigg, N. S., Columbus, R., Walker, F., Silva, A., ... Nicola, N. A. (2005). Suppressor of cytokine signaling (SOCS)-5 is a potential negative regulator of epidermal growth factor signaling. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 2328–2333.
- Noga, O., Hanf, G., & Kunkel, G. (2003). Immunological and clinical changes in allergic asthmatics following treatment with omalizumab. *International Archives of Allergy and Immunology*, 131, 46–52.
- Okubo, T., & Hogan, B. L. (2004). Hyperactive Wnt signaling changes the developmental potential of embryonic lung endoderm. *Journal of Biology*, 3, 11.
- Okumura, S., Sagara, H., Fukuda, T., Saito, H., & Okayama, Y. (2005). FcεRI-mediated amphiregulin production by human mast cells increases mucin gene expression in epithelial cells. *Journal of Allergy and Clinical Immunology*, 115, 272–279.
- Omalizumab (2002). Anti-IgE monoclonal antibody E25, E25, humanised anti-IgE MAb, IGE 025, monoclonal antibody E25, Olizumab, Xolair, rhuMAb-E25. *BioDrugs*, 16, 380–386.
- Ordóñez, C. L., Khashayar, R., Wong, H. H., Ferrando, R., Wu, R., Hyde, D. M., ... Fahy, J. V. (2001). Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *American Journal of Respiratory and Critical Care Medicine*, 163, 517–523.
- Ostergaard, L. S., Moninger, T. O., McMenimen, J. D., Sawin, N. M., Parker, C. P., Thornell, I. M., ... Welsh, M. J. (2017). Gel-forming mucins form distinct morphologic structures in airways. *Proceedings of the National Academy of Sciences*, 114, 6842–6847.
- Park, J.-A., Drazen, J. M., & Tschumperlin, D. J. (2010). The chitinase-like protein YKL-40 is secreted by airway epithelial cells at base line and in response to compressive mechanical stress. *Journal of Biological Chemistry*, 285, 29817.
- Park, S. H., Gong, J. H., Choi, Y. J., Kang, M. K., Kim, Y. H., & Kang, Y. H. (2015). Kaempferol inhibits endoplasmic reticulum stress-associated mucus hypersecretion in airway epithelial cells and ovalbumin-sensitized mice. *PLoS One*, 10, e0143526.
- Park, J.-A., Sharif, A. S., Shiomi, T., Kobzik, L., Kasahara, D. I., Tschumperlin, D. J., ... Drazen, J. M. (2013). Human neutrophil elastase-mediated goblet cell metaplasia is attenuated in TACE-deficient mice. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 304, L701–L707.
- Park, J.-A., & Tschumperlin, D. J. (2009). Chronic intermittent mechanical stress increases MUC5AC protein expression. *American Journal of Respiratory Cell and Molecular Biology*, 41, 459.
- Park, S. W., Verhaeghe, C., Nguyen, L. T., Barbeau, R., Easley, C. J., Nakagami, Y., ... Erle, D. J. (2009). Distinct roles of FOXA2 and FOXA3 in allergic airway disease and asthma. *American Journal of Respiratory and Critical Care Medicine*, 180, 603–610.
- Parker, J. C., Thavagnanam, S., Skibinski, G., Lyons, J., Bell, J., Heaney, L. G., & Shields, M. D. (2013). Chronic IL9 and IL-13 exposure leads to an altered differentiation of ciliated cells in a well-differentiated paediatric bronchial epithelial cell model. *PLoS One*, 8, e61023.
- Peles, E., Bacus, S. S., Koski, R. A., Lu, H. S., Wen, D., Ogden, S. G., ... Yarden, Y. (1992). Isolation of the neu/HER-2 stimulatory ligand: A 44 kd glycoprotein that induces differentiation of mammary tumor cells. *Cell*, 69, 205–216.
- Pera, T., Zuidhof, A., Valadas, J., Smit, M., Schoemaker, R. G., Gossens, R., ... Meurs, H. (2011). Tiotropium inhibits pulmonary inflammation and remodelling in a guinea pig model of COPD. *European Respiratory Journal*, 38, 789–796.
- Pini, L., Pinelli, V., Modina, D., Bezzl, M., Tiberio, L., & Tantucci, C. (2014). Central airways remodeling in COPD patients. *International Journal of Chronic Obstructive Pulmonary Disease*, 9, 927–932.
- Piper, E., Brightling, C., Niven, R., Oh, C., Faggioni, R., Poon, K., ... Molino, N. A. (2013). A phase II placebo-controlled study of talolimumab in moderate-to-severe asthma. *The European Respiratory Journal*, 41, 330–338.
- Polikepahad, S., Knight, J. M., Naghavi, A. O., Opl, T., Creighton, C. J., Shaw, C., ... Corry, D. B. (2010). Proinflammatory role for let-7 microRNAs in experimental asthma. *Journal of Biological Chemistry*, 285, 30139–30149.
- Pons, G., Marchand, M. C., d'Athis, P., Sauvage, E., Foucard, C., Chaumet-Riffaud, P., ... Lenoir, G. (2000). French multicenter randomized double-blind placebo-controlled trial on nebulized amiloride in cystic fibrosis patients. The Amiloride-AFLM Collaborative Study Group. *Pediatric Pulmonology*, 30, 25–31.
- Poole, P., Chong, J., & Cates, C. J. (2015). Mucolytic agents versus placebo for chronic bronchitis or chronic obstructive pulmonary disease. *The Cochrane Database of Systematic Reviews*, 7, CD001287 (electronic resource).
- Qin, H. B., Xu, B., Mei, J. J., Li, D., Liu, J. J., Zhao, D. Y., & Liu, F. (2012). Inhibition of miRNA-221 suppresses the airway inflammation in asthma. *Inflammation*, 35, 1595–1599.
- Rajavelu, P., Chen, G., Xu, Y., Kitzmiller, J. A., Korfhagen, T. R., & Whitsett, J. A. (2015). Airway epithelial SPDEF integrates goblet cell differentiation and pulmonary Th2 inflammation. *The Journal of Clinical Investigation*, 125, 2021–2031.
- Ren, X., Shah, T. A., Ustiyani, V., Zhang, Y., Shinn, J., Chen, G., ... Kalinichenko, V. V. (2013). FOXM1 promotes allergen-induced goblet cell metaplasia and pulmonary inflammation. *Molecular and Cellular Biology*, 33, 371–386.
- Ressler, B., Lee, R. T., Lee, R. T., Randell, S. H., Drazen, J. M., & Kamm, R. D. (2000). Molecular responses of rat tracheal epithelial cells to transmembrane pressure. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 278, 1264–1272.
- Robinson, M., & Bye, P. T. (2002). Mucociliary clearance in cystic fibrosis. *Pediatric Pulmonology*, 33, 293–306.
- Rose, M. C., & Voynow, J. A. (2006). Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiological Reviews*, 86, 245–278.
- Ross, D. A., & Kadesch, T. (2001). The Notch intracellular domain can function as a coactivator for LEF-1. *Molecular and Cellular Biology*, 21, 7537–7544.
- Roy, M. G., Livraghi-Butrico, A., Fletcher, A. A., McElwee, M. M., Evans, S. E., Boerner, R. M., ... Evans, C. M. (2014). Muc5b is required for airway defence. *Nature*, 505, 412–416.
- Rubin, B. K., & Williams, R. W. (2014). Aerosolized antibiotics for non-cystic fibrosis bronchiectasis. *Respiration*, 88, 177–184.
- Sasada, R., Ono, Y., Taniyama, Y., Shing, Y., Folkman, J., & Igarashi, K. (1993). Cloning and expression of cDNA encoding human betacellulin, a new member of the EGF family. *Biochemical and Biophysical Research Communications*, 190, 1173–1179.
- Schneider, D., Hong, J. Y., Popova, A. P., Bowman, E. R., Linn, M. J., McLean, A. M., ... Hershenson, M. B. (2012). Neonatal rhinovirus infection induces mucous metaplasia and airways hyperresponsiveness. *Journal of Immunology (Baltimore, Md. : 1950)*, 188, 2894–2904.
- Schroeder, B. W., Verhaeghe, C., Park, S.-W., Nguyen, L. T., Huang, X., Zhen, G., & Erle, D. J. (2012). AGR2 is induced in asthma and promotes allergen-induced mucin overproduction. *American Journal of Respiratory Cell and Molecular Biology*, 47, 178–185.
- Schroeter, E. H., Kisslinger, J. A., & Kopan, R. (1998). Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature*, 393, 382–386.
- Sethi, S. (2000). Bacterial infection and the pathogenesis of COPD. *Chest*, 117, 286S–291S.
- Sharma, S. V., Bell, D. W., Settleman, J., & Haber, D. A. (2007). Epidermal growth factor receptor mutations in lung cancer. *Nature Reviews: Cancer*, 7, 169–181.
- Sharma, A., Kumar, M., Ahmad, T., Mabalirajan, U., Aich, J., Agrawal, A., & Ghosh, B. (2012). Antagonism of mmu-mir-106a attenuates asthma features in allergic murine model. *Journal of Applied Physiology*, 113, 459–464.
- Sharma, A., Kumar, M., Aich, J., Hariharan, M., Brahmachari, S. K., Agrawal, A., & Ghosh, B. (2009). Posttranscriptional regulation of interleukin-10 expression by hsa-miR-106a. *Proceedings of the National Academy of Sciences*, 106, 5761–5766.
- Shi, N., Zhang, J., & Chen, S.-Y. (2017). Runx2 a novel regulator for goblet-cell differentiation and asthma development. *The FASEB Journal*, 31, 412–420.
- Shibata, T., Habel, D. M., Coelho, A. L., Kunkel, S. L., Lukacs, N. W., & Hogaboam, C. M. (2014). Axl receptor blockade ameliorates pulmonary pathology resulting from primary viral infection and viral exacerbation of asthma. *The Journal of Immunology*, 192, 3569–3581.
- Shim, J. J., Dabbagh, K., Ueki, I. F., Dao-Pick, T., Burgel, P. R., Takeyama, K., ... Nadel, J. A. (2001). IL-13 induces mucin production by stimulating epidermal growth factor receptors and by activating neutrophils. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 280, L134–L140.
- Shinkai, M., Henke, M. O., & Rubin, B. K. (2008). Macrolide antibiotics as immunomodulatory medications: Proposed mechanisms of action. *Pharmacology and Therapeutics*, 117, 393–405.
- Shoyab, M., Plowman, G. D., McDonald, V. L., Bradley, J. G., & Todaro, G. J. (1989). Structure and function of human amphiregulin: A member of the epidermal growth factor family. *Science*, 243, 1074–1076.
- Solberg, O. D., Ostrin, E. J., Love, M. I., Peng, J. C., Bhakta, N. R., Hou, L., ... Woodruff, P. G. (2012). Airway epithelial miRNA expression is altered in asthma. *American Journal of Respiratory and Critical Care Medicine*, 186, 965–974.
- Song, J., Cano Rodriguez, D., Winkle, M., Gjaltema, R. A. F., Goubert, D., Jurkowski, T. P., ... Hylkema, M. N. (2017). Targeted epigenetic editing of SPDEF reduces mucus production in lung epithelial cells. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 312, L334–L347.
- Song, L., Tang, H., Liu, D., Song, J., Wu, Y., Qu, S., & Li, Y. (2016). The chronic and short-term effects of gefinitib on airway remodeling and inflammation in a mouse model of asthma. *Cellular Physiology and Biochemistry*, 38, 194–206.
- Southam, D. S., Ellis, R., Wattie, J., Glass, W., & Inman, M. D. (2008). Goblet cell rebound and airway dysfunction with corticosteroid withdrawal in a mouse model of asthma. *American Journal of Respiratory and Critical Care Medicine*, 178, 1115–1122.
- Starkey, M. R., Essilfie, A. T., Horvat, J. C., Kim, R. Y., Nguyen, D. H., Beagley, K. W., ... Hansbro, P. M. (2013). Constitutive production of IL-13 promotes early-life Chlamydia respiratory infection and allergic airway disease. *Mucosal Immunology*, 6, 569–579.
- Staudt, V., Bothur, E., Klein, M., Lingnau, K., Reuter, S., Grebe, N., ... Bopp, T. (2010). Interferon-regulatory factor 4 is essential for the developmental program of T helper 9 cells. *Immunity*, 33, 192–202.
- Stefanowicz, D., Lee, J. Y., Lee, K., Shaheen, F., Koo, H.-K., Booth, S., ... Hackett, T.-L. (2015). Elevated H3K18 acetylation in airway epithelial cells of asthmatic subjects. *Respiratory Research*, 16, 95.

- Stolarczyk, M., Amatngalim, G. D., Yu, X., Veltman, M., Hiemstra, P. S., & Scholte, B. J. (2016). ADAM17 and EGFR regulate IL-6 receptor and amphiregulin mRNA expression and release in cigarette smoke-exposed primary bronchial epithelial cells from patients with chronic obstructive pulmonary disease (COPD). *Physiological Reports*, 4, e12878.
- Strachan, L., Murison, J. G., Prestidge, R. L., Sleeman, M. A., Watson, J. D., & Kumble, K. D. (2001). Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily. *Journal of Biological Chemistry*, 276, 18265–18271.
- Sun, L., Ren, X., Wang, L. C., Pradhan, A., Zhang, Y., Flood, H. M., ... Kalinichenko, V. V. (2017). The FOXM1 inhibitor RCM-1 suppresses goblet cell metaplasia and prevents IL-13 and STAT6 signaling in allergen-exposed mice. *Science Signaling*, 10, eaai8583.
- Suojalehto, H., Toskala, E., Kilpelainen, M., Majuri, M. L., Mitts, C., Lindstrom, I., ... Alenius, H. (2013). MicroRNA profiles in nasal mucosa of patients with allergic and nonallergic rhinitis and asthma. *International Forum of Allergy & Rhinology*, 3, 612–620.
- Swartz, M. A., Tschumperlin, D. J., Kamm, R. D., & Drazen, J. M. (2001). Mechanical stress is communicated between different cell types to elicit matrix remodeling. *Proceedings of the National Academy of Sciences*, 98, 6180.
- Syk, J., Malinowski, A., Borres, M. P., Unden, A. L., Andreasson, A., Lekander, M., & Alving, K. (2016). Parallel reductions of IgE and exhaled nitric oxide after optimized anti-inflammatory asthma treatment. *Immunity, Inflammation and Disease*, 4, 182–190.
- Takeyama, K., Dabbagh, K., Lee, H.-M., Agustí, C., Lausier, J. A., Ueki, I. F., ... Nadel, J. A. (1999). Epidermal growth factor system regulates mucin production in airways. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 3081–3086.
- Takeyama, K., Fahy, J. V., & Nadel, J. A. (2001). Relationship of epidermal growth factor receptors to goblet cell production in human bronchi. *American Journal of Respiratory and Critical Care Medicine*, 163, 511–516.
- Tamura, K., Taniguchi, Y., Minoguchi, S., Sakai, T., Tun, T., Furukawa, T., & Honjo, T. (1995). Physical interaction between a novel domain of the receptor Notch and the transcription factor RBP-J kappa/Su(H). *Current Biology*, 5, 1416–1423.
- Tanabe, T., Kanoh, S., Tsushima, K., Yamazaki, Y., Kubo, K., & Rubin, B. K. (2011). Clarithromycin inhibits interleukin-13-induced goblet cell hyperplasia in human airway cells. *American Journal of Respiratory Cell and Molecular Biology*, 45, 1075–1083.
- Tanabe, T., & Rubin, B. K. (2016). Airway Goblet Cells Secrete Pro-Inflammatory Cytokines, Chemokines, and Growth Factors. *Chest*, 149, 714–720.
- Tanabe, T., Shimokawaji, T., Kanoh, S., & Rubin, B. K. (2014). IL-33 stimulates CXCL8/IL-8 secretion in goblet cells but not normally differentiated airway cells. *Clinical and Experimental Allergy*, 44, 540–552.
- Tarran, R., Button, B., Picher, M., Paradiso, A. M., Ribeiro, C. M., Lazarowski, E. R., ... Boucher, R. C. (2005). Normal and cystic fibrosis airway surface liquid homeostasis. *Journal of Biological Chemistry*, 280, 35751.
- Temann, U. A., Prasad, B., Gallup, M. W., Basbaum, C., Ho, S. B., Flavell, R. A., & Rankin, J. A. (1997). A novel role for murine IL-4 in vivo: Induction of MUC5AC gene expression and mucin hypersecretion. *American Journal of Respiratory Cell and Molecular Biology*, 16, 471–478.
- Thorburn, A. N., O'Sullivan, B. J., Thomas, R., Kumar, R. K., Foster, P. S., Gibson, P. G., & Hansbro, P. M. (2010). Pneumococcal conjugate vaccine-induced regulatory T cells suppress the development of allergic airways disease. *Thorax*, 65, 1053–1060.
- Thornton, D. J., Rousseau, K., & McGuckin, M. A. (2008). Structure and function of the polymeric mucins in airways mucus. *Annual Review of Physiology*, 70, 459–486.
- Tsao, P.-N., Vasconcelos, M., Izvolsky, K. I., Qian, J., Lu, J., & Cardoso, W. V. (2009). Notch signaling controls the balance of ciliated and secretory cell fates in developing airways. *Development*, 136, 2297–2307.
- Tschumperlin, D. J., Dai, G., Maly, I. V., Kikuchi, T., Laiho, L. H., McVittie, A. K., ... Drazen, J. M. (2004). Mechanotransduction through growth-factor shedding into the extracellular space. *Nature*, 429, 83.
- Tschumperlin, D. J., & Drazen, J. M. (2001). Mechanical stimuli to airway remodeling. *American Journal of Respiratory and Critical Care Medicine*, 164, S90.
- Tschumperlin, D. J., Shively, J. D., Kikuchi, T., & Drazen, J. M. (2003). Mechanical stress triggers selective release of fibrotic mediators from bronchial epithelium. *American Journal of Respiratory Cell and Molecular Biology*, 28, 142.
- Tumas, D. B., Chan, B., Werther, W., Wrin, T., Vennari, J., Desjardin, N., ... Jardieu, P. (2001). Anti-IgE efficacy in murine asthma models is dependent on the method of allergen sensitization. *Journal of Allergy and Clinical Immunology*, 107, 1025–1033.
- Tyner, J. W., Kim, E. Y., Ide, K., Pelletier, M. R., Roswit, W. T., Morton, J. D., ... Holtzman, M. J. (2006). Blocking airway mucous cell metaplasia by inhibiting EGFR anti-apoptosis and IL-13 transdifferentiation signals. *The Journal of Clinical Investigation*, 116, 309–321.
- Valencia-Gattas, M., Conner, G. E., & Fregien, N. L. (2016). Gefitinib, an EGFR tyrosine kinase inhibitor, prevents smoke-mediated ciliated airway epithelial cell loss and promotes their recovery. *PLoS One*, 11, e0160216.
- Veldhoen, M., Uytendaele, C., van Snick, J., Helmsby, H., Westendorp, A., Buer, J., ... Stockinger, B. (2008). Transforming growth factor- β 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nature Immunology*, 9, 1341–1346.
- Vermeer, P. D., Harson, R., Einwalter, L. A., Moninger, T., & Zabner, J. (2003). Interleukin-9 induces goblet cell hyperplasia during repair of human airway epithelia. *American Journal of Respiratory Cell and Molecular Biology*, 28, 286–295.
- Villanave, R., Thavagnanam, S., Sarlang, S., Parker, J., Douglas, I., Skibinski, G., ... Power, U. F. (2012). In vitro modeling of respiratory syncytial virus infection of pediatric bronchial epithelium, the primary target of infection in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 5040–5045.
- Voytsov, J. A., Fischer, B. M., Roberts, B. C., & Proia, A. D. (2005). Basal-like cells constitute the proliferating cell population in cystic fibrosis airways. *American Journal of Respiratory and Critical Care Medicine*, 172, 1013–1018.
- Vummidiridhar, P., Bell, S. M., Sridharan, A., Rajavelu, P., Kitzmiller, J. A., Na, C. L., ... Whitsett, J. A. (2016). Airway epithelial KIF3A regulates Th2 responses to aero-allergens. *Journal of Immunology*, 197, 4228–4239.
- Walter, M. J., Morton, J. D., Kajiwaru, N., Agapov, E., & Holtzman, M. J. (2002). Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *The Journal of Clinical Investigation*, 110, 165–175.
- Wan, H., Kaestner, K. H., Ang, S.-L., Ikegami, M., Finkelman, F. D., Stahlman, M. T., ... Whitsett, J. A. (2004). Foxa2 regulates alveolarization and goblet cell hyperplasia. *Development*, 131, 953–964.
- Wan, H., Xu, Y., Ikegami, M., Stahlman, M. T., Kaestner, K. H., Ang, S.-L., & Whitsett, J. A. (2004). Foxa2 is required for transition to air breathing at birth. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 14449–14454.
- Wang, J. M., Rambaldi, A., Biondi, A., Chen, Z. G., Sanderson, C. J., & Mantovani, A. (1989). Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *European Journal of Immunology*, 19, 701–705.
- Warner, S. M., & Knight, D. A. (2008). Airway modeling and remodeling in the pathogenesis of asthma. *Current Opinion in Allergy and Clinical Immunology*, 8, 44–48.
- Wenzel, S., Ford, L., Pearlman, D., Spector, S., Sher, L., Skobieranda, F., ... Pirozzi, G. (2013). Dupilumab in persistent asthma with elevated eosinophil levels. *New England Journal of Medicine*, 368, 2455–2466.
- Wiggs, B. R., Hrousis, C. A., Drazen, J. M., & Kamm, R. D. (1997). On the mechanism of mucosal folding in normal and asthmatic airways. *Journal of Applied Physiology*, 83, 1814–1821.
- Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T. Y., Karp, C. L., & Donaldson, D. D. (1998). Interleukin-13: Central mediator of allergic asthma. *Science*, 282, 2258–2261.
- Wilson, J. W., & Li, X. (1997). The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. *Clinical and Experimental Allergy*, 27, 363–371.
- Wong, D. T., Weller, P. F., Galli, S. J., Elovic, A., Rand, T. H., Gallagher, G. T., ... McBride, J. (1990). Human eosinophils express transforming growth factor alpha. *The Journal of Experimental Medicine*, 172, 673–681.
- Woodruff, P. G., Wolff, M., Hohlfield, J. M., Krug, N., Dransfield, M. T., Sutherland, E. R., Criner, G. J., Kim, V., Prasse, A., Nivens, M. C., Tetzlaff, K., Heilker, R., & Fahy, J. V. (2010). Safety and efficacy of an inhaled epidermal growth factor receptor inhibitor (BIBW 2998 BS) in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, 181, 438–445.
- Woolnough, K. F., Richardson, M., Newby, C., Craner, M., Bourne, M., Monteiro, W., ... Wardlaw, A. J. (2016). The relationship between biomarkers of fungal allergy and lung damage in asthma. *Clinical and Experimental Allergy*, 47, 48–56.
- Wu, C. A., Peluso, J. J., Zhu, L., Lingenheld, E. G., Walker, S. T., & Puddington, L. (2010). Bronchial epithelial cells produce IL-6: Implications for local immune responses in the airways. *Cellular Immunology*, 264, 32–41.
- Xiang, Y. Y., Wang, S., Liu, M., Hirota, J. A., Li, J., Ju, W., ... Lu, W. Y. (2007). A GABAergic system in airway epithelium is essential for mucus overproduction in asthma. *Nature Medicine*, 13, 862–867.
- Xu, X., Balsiger, R., Tyrrell, J., Boyaka, P. N., Tarran, R., & Cormet-Boyaka, E. (2015). Cigarette smoke exposure reveals a novel role for the MEK/ERK1/2 MAPK pathway in regulation of CFTR. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1850, 1224–1232.
- Yang, G., Volk, A., Pettley, T., Emmell, E., Giles-Komar, J., Shang, X., ... Li, L. (2004). Anti-IL-13 monoclonal antibody inhibits airway hyperresponsiveness, inflammation and airway remodeling. *Cytokine*, 28, 224–232.
- Yao, L., Zhao, H., Tang, H., Xiong, J., Zhao, W., Liu, L., ... Cai, S. (2017). Blockade of beta-catenin signaling attenuates toluene diisocyanate-induced experimental asthma. *Allergy*, 72, 579–589.
- Zhang, T., & Zhou, X. (2014). Clinical application of expectorant therapy in chronic inflammatory airway diseases (Review). *Experimental and Therapeutic Medicine*, 7, 763–767.
- Zhen, G., Park, S. W., Nguyen, L. T., Rodriguez, M. W., Barbeau, R., Paquet, A. C., & Erle, D. J. (2007). IL-13 and epidermal growth factor receptor have critical but distinct roles in epithelial cell mucin production. *American Journal of Respiratory Cell and Molecular Biology*, 36, 244–253.
- Zhou, Z., Treis, D., Schubert, S. C., Harm, M., Schattner, J., Hirtz, S., ... Mall, M. A. (2008). Preventive but not late amiloride therapy reduces morbidity and mortality of lung disease in β ENaC-overexpressing mice. *American Journal of Respiratory and Critical Care Medicine*, 178, 1245–1256.
- Zhou, Y., Zhong, N.-s., Li, X., Chen, S., Zheng, J., Zhao, D., ... Ran, P. (2017). Tiotropium in early-stage chronic obstructive pulmonary disease. *New England Journal of Medicine*, 377, 923–935.
- Zhu, Y., Abdullah, L. H., Doyle, S. P., & Nguyen, K. (2015). Baseline goblet cell mucin secretion in the airways exceeds stimulated secretion over extended time periods, and is sensitive to shear stress and intracellular mucin stores. *PLoS One*, 10, e0127267.
- Zhu, Z., Homer, R. J., Wang, Z., Chen, Q., Geba, G. P., Wang, J., ... Elias, J. A. (1999). Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eosinophil production. *The Journal of Clinical Investigation*, 103, 779–788.
- Zong, D., Ouyang, R., Li, J., Chen, Y., & Chen, P. (2016). Notch signaling in lung diseases: Focus on Notch1 and Notch3. *Therapeutic Advances in Respiratory Disease*, 10, 468–484.