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REVIEW

PI3K signalling in chronic obstructive pulmonary disease and opportunities for therapy

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Abstract

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease characterised by airway inflammation and progressive obstruction of the lung airflow. Current pharmacological treatments include bronchodilators, alone or in combination with steroids, or other anti-inflammatory agents, which have only partially contributed to the inhibition of disease progression and mortality. Therefore, further research unravelling the underlying mechanisms is necessary to develop new anti-COPD drugs with both lower toxicity and higher efficacy. Extrinsic signalling pathways play crucial roles in COPD development and exacerbations. In particular, phosphoinositide 3-kinase (PI3K) signalling has recently been shown to be a major driver of the COPD phenotype. Therefore, several small-molecule inhibitors have been identified to block the hyperactivation of this signalling pathway in COPD patients, many of them showing promising outcomes in both preclinical animal models of COPD and human clinical trials. In this review, we discuss the critically important roles played by hyperactivated PI3K signalling in the pathogenesis of COPD. We also critically review current therapeutics based on PI3K inhibition, and provide suggestions focusing on PI3K signalling for the further improvement of the COPD phenotype.

Keywords: PI3-kinase; leukocyte; cytokine; microRNA; immune response

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No conflicts of interest were declared.

Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive, multifactorial and irreversible disease that affects airways in afflicted people. In COPD, progressive airflow obstruction occurs as a result of exposure to cigarette smoke, air pollution and fossil fuels, leading to abnormal stimulation of mucosal secretion and narrowing of the small airways [1,2]. Chronic cough, chest pain, dyspnoea, intolerance for physical exercise, sputum overproduction and hypersecretion, depression and weight loss are among the most common complaints of COPD patients [3]. It seems that COPD is a consequence of the interaction of environmental factors with genetic factors [4]. Smoking is the primary cause of COPD, particularly in Western societies. Other factors, such as gender, chronic bronchitis, the use of fossil fuels for cooking and some microbial infections, are also considered as COPD risk factors. In fact, factors such as genetics, epigenetics, sex, immune system and microbiome are considered as background or predisposing factors [5], whereas cigarette smoke, air pollution, biomass smoke and microbial infections are risk factors [6]. The risk factors mainly induce oxidative stress [7,8], thereby influencing predisposing factors to develop the pathophysiology of the disease. These collective factors might lead not only to COPD but also to disorders such as atherosclerosis, cardiovascular disease and lung cancer (Figure 1). COPD exhibits an increasing trend in terms of both morbidity and mortality [9,10]. Although many diseases with high rates of mortality, such as coronary heart disease and stroke, show decreasing trends in mortality, COPD is the only primary cause of death showing an increasing trend [9]. In 2008, the World Health Organisation placed COPD as the fourth main cause of global mortality from non-communicable diseases and estimated that it would become the third ranked by 2020. However, COPD became so prevalent that it became the third main cause of worldwide mortality in 2016 [11], highlighting the rapid pace with which people get, and die of, COPD.

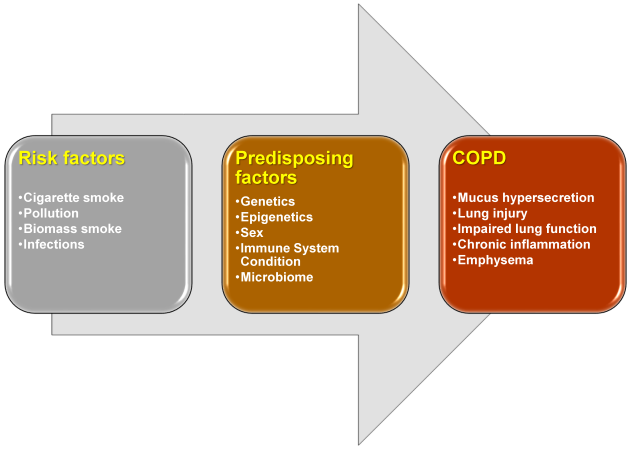

Figure 1. COPD development from risk factors and predisposing factors to the full-blown disease. Several risk factors, especially cigarette smoke, trigger the induction of oxidative stress in the lungs. The resulting oxidative stress will have different clinical consequences depending on genetic differences, sex, microbiome, etc. COPD development seems to result from interactions of risk factor with predisposing elements. The net consequence is the development of clinical manifestations that are typical of COPD.

The available treatments for COPD differ depending on disease severity and aetiology, and include smoking cessation, avoiding highly polluted urban areas, ameliorating medications, such as oral or inhaled bronchodilators, oral or inhaled corticosteroids, respiratory rehabilitation, antibiotics, oxygen therapy and, in specific cases, surgery [12,13]. However, these treatments are not sufficiently effective in most cases and it is therefore essential to develop new treatments that are both safe and highly effective. To identify new effective medicines, it is important to identify the molecular mechanisms underlying COPD pathophysiology, enabling the design of targeted and efficacious therapies for COPD.

In response to extrinsic factors stimulating the development of COPD, different molecular processes interact with each other to induce widespread changes in the gene regulatory network in epithelial and other cell types in the lung, which subsequently give rise to the damage seen in COPD [14]. In all cells, gene regulatory networks consist of tightly interconnected networks of chromatin regulators, transcription factors, metabolites, regulatory RNAs and signalling pathways [15–18]. These molecules engage in a dynamic interplay, whose net effect is the unique behaviour any cell elicits. Notably, extrinsic signalling pathways ripple through a cell's gene regulatory network, thereby shaping key cellular decisions and behaviours. The network in lung cells and COPD is composed of the same regulatory components mentioned above. However, as exogenous factors exert widespread effects on the development of this disease, the gene regulatory network in COPD is more profoundly affected by environmental cues and extrinsic signalling pathways [19–21].

Diverse signalling pathways, including transforming growth factor-β (Smad signalling pathway), mitogen-activated protein kinase (MAPK), nuclear factor kappa B (NF-κB) and phosphoinositide 3-kinase (PI3K), are reported to alter the gene regulatory network in COPD [22–25]. The interaction of these extrinsic pathways and endogenous regulatory networks determines how the diseased lung cells will behave and how COPD will proceed. As these pathways have major effects on COPD physiopathology, specific drugs have been developed to target the mediators of these pathways, including PI3K signalling [26].

PI3K signalling is among the key pathways in almost all cells. In fact, this pathway influences all cells in at least one stage during their lifetime [27]. PI3K signalling is activated by a series of extracellular factors, e.g. insulin and insulin-like growth factor (IGF), which bind specific cell-surface receptor tyrosine kinases (RTKs), stimulating several downstream mediators of this signalling [28]. These mediators in turn regulate many downstream substrates involved in critical processes, such as cell growth, cell cycle, protein synthesis and cell death [27]. Importantly, PI3K signalling is one of the most crucial pathways contributing to the pathophysiology of COPD.

This review aims to highlight the critical contribution of PI3K signalling to the physiopathology of COPD, the intracellular gene regulatory network involving PI3K signalling components, its roles in intercellular interactions between immune system cells and lung epithelial cells, and the regulatory processes involving PI3K signalling and microRNAs (miRNAs) in COPD. Moreover, we explore the current therapies and potential treatments modulating PI3K signalling in COPD, as well as clinical trials harnessing the potential of PI3K signalling targeting for COPD therapy. Finally, we provide key questions and suggestions utilising the PI3K pathway for improving therapeutic approaches for COPD.

The PI3K pathway

PI3K signalling is a critically important pathway for mediating various forms of cellular responses, from cell survival, growth, proliferation and differentiation to DNA repair and apoptosis in different developmental and tissue contexts [27,29,30]. In fact, embryonic development and organismal health, as well as many human diseases, including cancers, are regulated by PI3K signalling [29].

PI3K signalling is activated by extracellular ligands that trigger this signalling by binding to RTKs or G-protein-coupled receptors [27,29–31], whose activation leads to phosphorylation and activation of PI3Ks. PI3Ks are a family of lipid kinases that catalyse the phosphorylation of the 3'-hydroxyl group of the inositol ring of phosphatidylinositides of cell membranes and are historically divided into three classes (i.e. I, II and III) based on structure and function [32]. Class I PI3Ks are the most studied, and phosphorylate membrane-bound phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5) P2, also called PIP2) to phosphatidylinositol (3,4,5)-

trisphosphate (PtdIns(3,4,5)P3; PIP3). By acting as a secondary messenger, PIP3 serves as a docking site for protein kinases such as PI3K-dependent kinase-1 (PDK1). After recruitment to the membrane and subsequent activation, PDK1 phosphorylates and activates Ak strain transforming/protein kinase B (AKT/PKB), which then phosphorylates the mechanistic target of rapamycin (mTOR; usually found in two protein complexes – mTORC1 and mTORC2) [33] and many other downstream mediators, exerting numerous changes to cell behaviour (Figure 2).

Class I PI3Ks are heterodimers, consisting of a catalytic and a regulatory (or adaptive) subunit. Based on sequence and the type of adaptor bound to the catalytic subunit, they are further categorised into class IA PI3Ks and class IB PI3Ks. Class IA PI3Ks include PI3Kα, β and δ, whereas class IB PI3Ks are composed only of PI3Kγ [34]. Notably, class I PI3K isoforms play both isoform-specific and pan-PI3K roles [35], highlighting a high degree of dynamicity in the molecular function of different class I PI3K isoforms.

Class II and III PI3Ks are less studied monomers with no adaptive subunits, phosphorylating PI to PI(3)P (both classes) and PI(4)P to PI(3,4)P2 (class II only). As these phosphorylated lipid products are implicated only in protein and vesicular trafficking [32,34] with no documented roles in the pathogenesis of COPD, we will not further discuss class II and III PI3Ks in this review.

The enzymatic activity of PI3K is directly opposed by the dual-specificity protein phosphatase, phosphatase and tensin homologue (PTEN), which promotes the dephosphorylation of PIP3 into PIP2 (Figure 2). Therefore, PTEN functions as an inhibitor of PI3K activity and is considered a key therapeutic strategy in cancers and many other diseases in which PI3K signalling is a key driver [36].

The components of PI3K signalling have crosstalk with other regulatory pathways and cellular processes, such as cell survival, proliferation and differentiation, which explains how the deregulation of this pathway's mediators exerts profound effects on various (patho) physiological processes. In the next sections, we will highlight the critical functions of the PI3K pathway in the emergence and progression of COPD, as well as how we could exploit the available knowledge to target this pro-COPD signalling pathway more effectively.

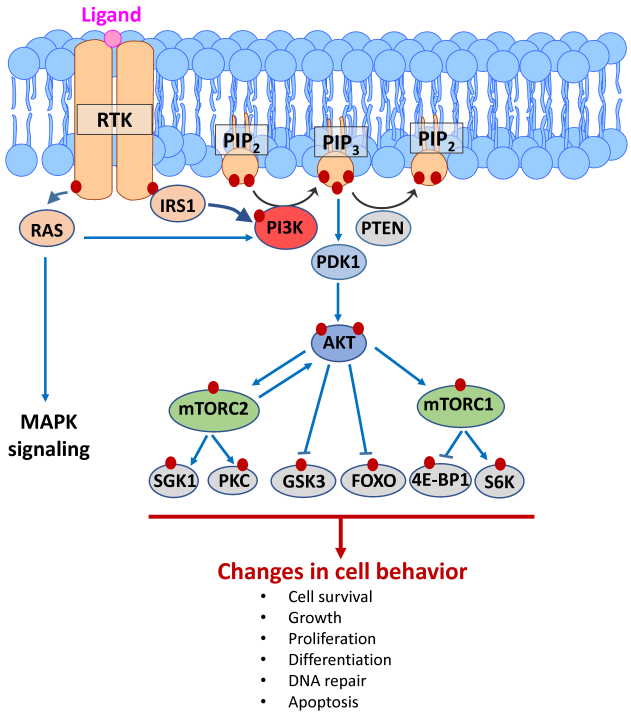

Figure 2. Schematic illustration of the PI3K signalling pathway. Extracellular ligands, such as IGF-1, bind to RTKs (which are transmembrane receptor proteins) and activate them. Activated RTKs induce the phosphorylation of PI3K, which subsequently phosphorylates the membrane phospholipid PIP2 to PIP3, providing a docking site for PDK1. Next, the protein kinase AKT/PKB, which is phosphorylated and activated by PDK1, induces mTOR activity by phosphorylation. AKT and mTOR have numerous downstream targets, which leads to enormous changes in cell behaviour, such as cell survival, growth, proliferation and differentiation, DNA repair and apoptosis. Red dots indicate phosphate groups, which are added to substrates by protein or lipid kinases.

Contribution of PI3K signalling to the development and exacerbation of COPD

PI3K and its downstream mediators are upregulated during lung and airway remodelling in COPD [23,37–41] (Table 1). The differential expression of PI3K signalling mediators during COPD progression implies their dynamic regulation under this pathological condition. Any dysregulation in PI3K signalling not only adversely affects the normal functioning of airway epithelial cells but also influences alveolar immune cells, leading to an exaggerated immune response [55,56]. This abnormally enhanced immune response gives rise to chronic inflammation, which is characteristic of COPD.

In addition to normal growth factors, environmental stressors can induce (hyper-) activation of PI3K signalling. In contrast to class I PI3Ks (i.e. PI3Kα/β), which are ubiquitously expressed and, hence, their knockouts are embryonic lethal, PI3Kγ and PI3Kδ are more highly expressed in leukocytes and their knockout is well tolerated [34]. Leukocytes lacking PI3Kγ and PI3Kδ suffer from impaired activation, migration and differentiation, as well as defective B and T cell antigen receptor signalling [28,57,58]. The impaired leukocyte migration and infiltration is due to regulation of cytoskeletal rearrangements by PI3K through the production of PIP3, asymmetric F-actin assembly and finetuning of Rac function and Ca^{2+} release [59,60] (Figure 3). PI3K has also been shown to be activated by an S-type lectin called galectin-3, similarly leading to dynamic polymerisation and reorganisation of F-actin through Rac activation [60]. These findings were confirmed by the application of pan-PI3K and/or PI3K-isoform-selective inhibitors, which reduced the chemotaxis of neutrophils and macrophages into the lung [61] (Table 2), resulting in

Table 1. Status of PI3K signalling mediators in COPD.

PI3K signalling mediator	Status in COPD	Cells	Reference
IGF-1R	Phosphorylated	Cigarette smoke-treated murine alveolar macrophages (*in vitro* study)	[42]
PI3K	Upregulated and phosphorylated	Primary human lung epithelial cells; lung epithelial cell lines; immune system cells in injured lung; mouse models of COPD; COPD patients	[23,40,43–46]
PTEN	Downregulated	Human lung epithelial cells; mouse models of COPD; COPD patients	[23,47]
AKT/PKB	Phosphorylated	Human lung epithelial cells; COPD patients	[23,48,49]
mTOR	Phosphorylated	Human airway epithelial cells and peripheral lung cells	[50,51]
PDE4	Upregulated	Peripheral blood neutrophils; alveolar macrophages; lung epithelial cells in COPD patients	[52–54]

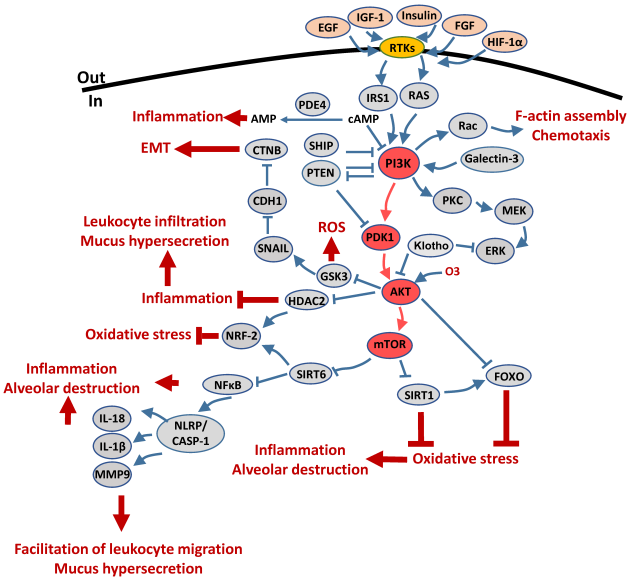

Figure 3. PI3K signalling in COPD. The PI3K/AKT/mTOR axis is upregulated in COPD. Hyperactivated PI3K signalling induces the activity of proteins that promote inflammation, such as NF-κB, cytokines and MMP-9, and suppresses proteins that exert antioxidant functions, such as FOXO, PTEN, SIRT1 and SIRT6. PI3K also induces Rac activity, leading to increased F-actin polymerisation and leukocyte chemotaxis.

dampening of innate and adaptive immune responses, as well as reduced inflammation.

PI3K indirectly activates AKT, a key protein kinase with a broad array of downstream targets in COPD. AKT activation correlates with PI3K activation and negatively correlates with PTEN activity. Importantly, PTEN is downregulated in the airway epithelial cells and peripheral lung tissue of COPD patients. Furthermore, cigarette smoke followed by reactive oxygen species (ROS) generation downregulates PTEN, enhancing AKT phosphorylation and vice versa. The resulting reduction in PTEN levels in bronchial epithelial cells also increases the production and secretion of CCL2 and CXCL8 chemokines, and interleukin-6 (IL-6) and other proinflammatory cytokines, promoting chronic inflammation via enhanced PI3K signalling [23,47]. This aberrant process in turn seemingly increases cell senescence in lung epithelial cells through ROS production and subsequently further PI3K activation [73,74]. As PTEN opposes the activity of PI3K signalling, activation of PTEN has been considered as a viable option against COPD exacerbation.

By phosphorylating MAPKs, S6 and other factors, AKT promotes significant changes in the gene regulatory network of the affected cells [48,75]. It also inhibits glycogen synthase kinase-3 (GSK3), thereby inducing ROS generation and epithelial-to-mesenchymal transition (EMT) [76]. By activating mTOR, AKT facilitates oxidative stress by inhibiting SIRT1 and SIRT6, which are involved in controlling the balance between free radicals and antioxidants [74,75,77]. AKT also inhibits another histone deacetylase, HDAC2, which subsequently leads to increased inflammation and enhanced oxidative stress [74,75]. In fact, the majority of the aberrantly activated mediators of PI3K signalling lead to further oxidative stress, which in turn gives rise to alveolar destruction, progressive airway inflammation and leukocyte infiltration (Figure 3). Ozone (O_3), which is produced under certain conditions, has been reported to promote leukocyte infiltration, inflammation, mucus hypersecretion and alveolar destruction through activating p38 MAPK and subsequent stimulation of IL-1B and matrix metalloproteinase-9 (MMP-9) release [78]. Notably, O_3 also enhances AKT and NF-κB protein levels only in females [79], highlighting the importance of sex difference in the pathogenesis of COPD. Finally, it has been found that the PI3K/AKT pathway is essential for uPAR-driven EMT in human small airway epithelial cells [80]. Taken together, each of these molecular interactions involving PI3K signalling promotes a vicious cycle of increased inflammation, EMT and oxidative stress, leading to COPD exacerbation.

Intercellular signalling involving the PI3K pathway in COPD

Oxidative stress, the main driver of COPD, is induced by various environmental stimuli as well as endogenous factors [62]. In response, lung epithelial cells upregulate PI3K signalling, inducing mucin overproduction and concomitant airway mucus hypersecretion, causing severe clinical consequences. The epithelial cells in COPD patients also secrete molecules that recruit leukocytes, particularly macrophages and neutrophils, to the stressed lung tissue [81]. It has been shown that the PI3K pathway plays a crucial role in COPD

Table 2. *In vitro* studies showing the effect of PI3K inhibition on COPD.

Inhibitor	Molecular target	Effects on COPD	Model	References
ZSTK474	Pan-PI3K	Decreases extracellular ROS production and MMP-9 release from neutrophils	Cultured blood and sputum neutrophils from COPD patients	[62]
Wortmannin	Pan-PI3K	Induces alveolar stem cell differentiation	Cultured human alveolar epithelial stem cells	[63]
LY294002	Pan-PI3K	Decreases neutrophil migratory speed but enhances their chemotactic accuracy; reduces MUC5AC, AKT and epidermal growth factor receptor expression	Migrating peripheral neutrophils from COPD patients; human bronchial epithelial cells	[64,65]
Roflumilast N-oxide (RNO)	PDE4	Inhibits prothrombotic functions of leukocytes and corticosteroid resistance in COPD neutrophils	Peripheral blood leukocytes from COPD patients	[66,67]
Erythromycin + dexamethasone	p-AKT	Increase steroid sensitivity induced by cigarette smoke extract	Peripheral blood mononuclear cells from COPD patients and U937 cells	[68]
Nortriptyline	PI3Kδ	Upregulates HDAC2 and inhibits steroid resistance	Cultured human monocytic cell line	[69]
IC87114	PI3Kδ	Enhances HDAC2 expression and reverses steroid insensitivity	Peripheral blood mononuclear cells from COPD patients	[44]
Formoterol	PI3Kδ	Decreases steroid insensitivity under oxidative stress conditions	Peripheral blood mononuclear cells from COPD patients	[70]
NVS-PI3-2 NVS-PI3-3 NVS-PI3-5	PI3Kα PI3Kδ PI3Kγ	Are well tolerated and do not impair phagocytosis	Cultured human macrophages isolated from COPD patients	[71]
Solithromycin (CEM-101)	Pan-PI3K	Inhibits corticosteroid resistance	Peripheral blood mononuclear cells from COPD patients	[72]
GSK045	PI3Kδ	Suppresses ROS and MMP-9 release from neutrophils during both the stable state and exacerbations	Cultured blood and sputum neutrophils from COPD patients	[62]

leukocytes, as its abrogation compromises leukocyte activation and infiltration into the lung [61,82,83].

PI3K signalling-mediated recruitment of leukocytes is followed by secretion of diverse proinflammatory cytokines and chemokines. Of note, cigarette smoke promotes IL-17A-induced production of IL-8 in bronchial epithelial cells [84]. These secreted proteins initiate a process leading to peri-bronchiolar fibrosis, alveolar destruction and mucus hypersecretion by airway epithelial cells [85,86] (Figure 4). Importantly, neutrophils secrete several proteases, including MMPs and elastase, that destroy the lung parenchyma and drive mucus hypersecretion [87]. Additionally, macrophages from COPD patients exhibit a defective response and polarisation towards the M2 phenotype, as well as high resistance to steroid therapy [88,89]. These abnormal characteristics are promoted at least partly by activation of PI3K signalling in COPD macrophages. Activation of PI3K signalling appears to compromise glucocorticoid responsiveness in cigarette smoke-exposed mice [90]. COPD macrophages also release proteases and ROS, contributing to further destruction of lung tissue and aggravating the inflammatory and oxidative microenvironment [81,86]. In addition, mast cells, which show increased numbers in inflammatory infiltrates of COPD patients, upregulate PI3K signalling, which facilitates their infiltration into the lung, their activation and degranulation followed by increased secretion of proinflammatory cytokines (e.g. IL-8 and tumour necrosis factor-α) and the enzyme hTryptase [91–93]. These mast cell-derived proteins are correlated with small airway remodelling and narrowing, increased airflow obstruction and decreased lung function (Figure 4). Therefore, PI3K signalling significantly contributes to the overactivation of the innate immune system in COPD patients.

The PI3K pathway also enhances adaptive immunity in COPD. Upon cigarette smoke exposure, B and T lymphocytes downregulate PTEN, leading to enhanced PI3K signalling in these cells, followed by increased inflammation in the lung parenchyma. The PI3K-powered infiltration of CD8+ T cells secretes enzymes, such as granzyme B and perforins, which promote alveolar destruction [94,95]. B lymphocytes display increased number, survival, activation and maturation in the lungs of COPD patients, due at least in part to PI3K pathway activation in these cells. The impaired processes in B cells of COPD patients are accompanied by an increase in the production of autoantibodies, which might play a role in increased lung injury and inflammation in COPD patients [94,96]. In addition, lung myeloid dendritic cells were observed to direct the induction of T-helper 1 (Th1) and T-helper 17 (Th17) responses in CD4 T cells in emphysema. Th17 cells secrete IL-17A, which increases CCL20 and MMP-12 secretion from pulmonary macrophages, thereby recruiting inflammatory cells and promoting lung destruction [97]. Furthermore, although IL-17A was upregulated in CD4 T cells from mouse lung upon exposure to cigarette smoke, its deficiency diminished cigarette smoke-induced emphysema, highlighting the important role of CD4 T cell-mediated autoimmunity [98]. Finally, human lung myeloid dendritic cells from smoke-induced emphysema lungs and mouse antigen-presenting cells exposed to cigarette smoke increased miR-22 expression, which subsequently enhanced autoreactive Th17 responses and lung damage via activation of HDAC4 and AP-1 [99]. Overall, the pathological events caused by complex interactions between immune cells, lung epithelial cells

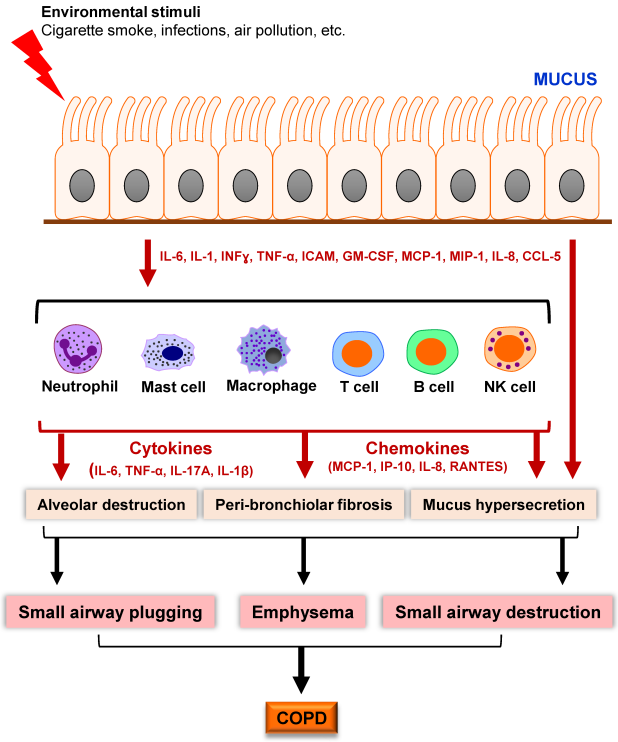

Figure 4. Interaction of lung epithelial cells and immune cells in COPD. Environmental stressors, particularly cigarette smoke, induce mucin overproduction and mucus hypersecretion in lung epithelial cells as the first and most important cell type in the lung to respond to exogenous stresses, gradually leading to increased airway obstruction. These cells also secrete cytokines and chemokines that trigger the induction, activation and infiltration of immune cells into the lung parenchyma. Various immune cells (in particular macrophages and neutrophils) start producing and secreting cytokines, chemokines and enzymes upon stimulation by epithelial cells, which together elicit an exaggerated immune response to the oxidative stress-induced lung inflammation, leading to further exacerbation of COPD. Overall, these aberrant interactions result in alveolar destruction, fibrosis and enhanced mucus hypersecretion, followed by emphysema and small airway obstruction, which are typical characteristics of COPD.

and oxidative agents ultimately lead to the plugging of small airways, trapping of air in the alveoli (emphysema) and small airway destruction (Figure 4), all of which are hallmarks of COPD pathogenesis.

Involvement of miRNAs targeting PI3K signalling in COPD pathogenesis

miRNAs constitute a unique class of short (~22-nt) non-coding RNAs that control their targets at the post-transcriptional level by cleaving the target mRNA or by suppressing its translation into protein. As miRNAs tolerate nucleotide mismatches when binding their target transcripts, they are thought to have 10s to 100s of targets [100]. It is believed that miRNAs regulate one-third of human transcripts, thereby modulating virtually all biological processes. They are crucially important for embryogenesis, tissue homeostasis, stem cell self-renewal [101–103], and cell fate programming and reprogramming

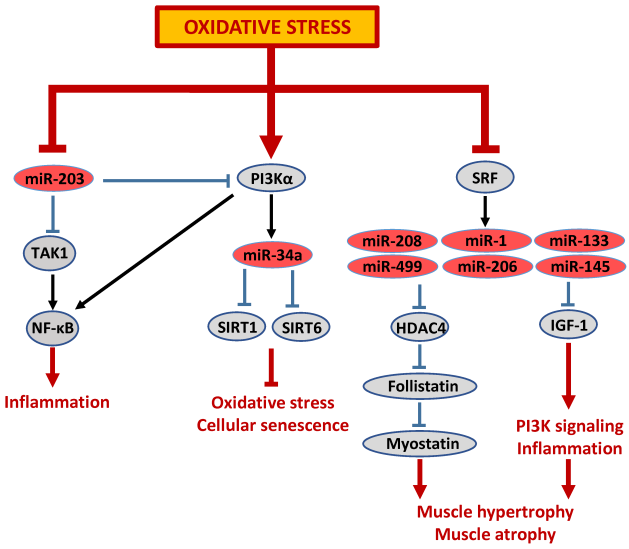

Figure 5. PI3K signalling–miRNA communications in COPD. Oxidative stress induces miR-203, thereby preventing it from suppressing TAK1 and NF-κB. PI3K itself induces miR-34a to block the translation of *SIRT1* and *SIRT6* transcripts, thereby promoting elevated oxidative stress. Finally, oxidative stress inhibits serum response factor, which acts as a driver of muscle-specific miRNA expression to repress IGF-1 and HDAC4. These two de-repressed proteins then promote further inflammation and enhanced muscle atrophy/hypertrophy that is observed in COPD patients.

[104–106]. Importantly, miRNAs are deregulated in many disorders, including cancer and lung diseases [107,108].

Chronic diseases of the lung induce major changes in miRNA expression. These changes are biologically significant, with certain miRNAs serving as inhibitors, whereas others are inducers of chronic lung pathologies [108]. Several miRNAs are influenced at the beginning or during the progression of COPD (Figure 5). For example, PI3K upregulates miR-34a, which blocked the translation of *SIRT1* and *SIRT6* and inhibited their antioxidative and antisenescence functions [109,110]. Importantly, suppression of miR-34a inhibited cell cycle arrest and features of senescence in airway epithelial cells of COPD patients [110].

miR-203, which is induced by oxidative stress, inhibits PI3K [111], thereby providing a therapeutic strategy for targeting PI3K to dampen inflammation and injuries triggered by its abnormal hyperactivation in COPD.

Oxidative stress also inhibits serum response factor, leading to the downregulation of miR-1 and other muscle-enriched miRNAs in the muscle tissue of COPD patients. This deregulation in muscle-specific miRNAs induces IGF-1 and HDAC4, which contribute to enhanced PI3K signalling, inflammation and muscle hypertrophy in COPD [112] (Figure 5). COPD is mostly associated with muscle dysfunction and wasting, which has been found to be accompanied by abnormal alterations in PI3K signalling in airway/lung smooth muscle cells [113–119]. Importantly, PI3K signalling blockade reversed the major characteristics of muscle wasting in COPD animal models [113–115].

Finally, miR-6724-5p, miR-424-5p and miR-195-5p, upregulated in COPD bronchial epithelial cells, potentially target fibroblast growth factor receptor-1 (an RTK involved in PI3K signalling), thereby inhibiting the apoptosis of bronchial epithelial cells [120]. It is therefore possible to exploit the potential of miRNAs to effectively target major PI3K mediators of COPD.

Impaired PI3K signalling makes COPD patients more susceptible to microbial infections

COPD patients show enhanced susceptibility to microbial infections, and infections contribute to the development of COPD [121]. PI3K signalling correlates with enhanced sensitivity of COPD patients to respiratory infections. For example, the mast cell-specific tryptase mMCP-6 promotes bacterial infections through suppressing immune responses [122]. Non-typeable *Haemophilus influenzae* exacerbates COPD by upregulating PI3K signalling [123–125]. It appears that bacterial lipopolysaccharide plays an important role by inducing ROS and a cascade of PI3K signalling in the lung [126–129]. In addition to bacteria, certain fungi exacerbate the COPD phenotype. For instance, COPD patients infected with *Aspergillus fumigatus* show increased mortality. Infection upregulates the PI3K pathway in alveolar macrophages, driving COPD exacerbation. In fact, COPD promotes alveolar macrophage dysfunction and upregulation of the TLR2/PI3K/Rac-1 signalling axis, facilitating *Aspergillus* invasion [130,131].

Notably, COPD patients are more susceptible to viral (in particular, influenza virus) infections. The upregulation of PI3K signalling enhances influenza virus infection and downregulates antiviral responses in COPD bronchial epithelial cells and in a cigarette smoke-induced mouse model of COPD [43]. Other viruses, such as rhinoviruses, also increase COPD-associated inflammation through PI3K signalling induction in the lung [132,133] and PI3K inhibitors aid immune responses against viruses, thereby increasing lung function [43,132,133]. These findings highlight the crucial importance of upregulated PI3K signalling in the enhanced susceptibility of COPD patients to microbial infections.

Targeting PI3K signalling in COPD: *in vitro*, preclinical and clinical studies

Smoking cessation is a major intervention to decrease COPD deterioration. However, inflammatory responses persist in the airways of COPD patients for several months and years, even after smoking cessation [134,135]. Smoking chronically increases the secretion of MMPs and elastase from lung neutrophils and epithelial cells, and alters the expression of RIG-I-like receptors, Toll-like receptors and receptors for advanced glycation end products (RAGE) on lung epithelial cells and leukocytes, increasing cell death [136–138]. Moreover, upregulation of RAGE promotes secretion of cytokines via Ras, NF-κB and PI3K signalling [139], maintaining the enhanced lung inflammation, even after stopping smoking. Importantly, the dynamics of PI3K pathway activation depend on the duration of exposure to cigarette smoke [23,140]. Short-term smoke exposure leads to Akt phosphorylation and PI3K signalling without PTEN suppression. However, PTEN is gradually downregulated upon longer exposures to smoke, promoting prolonged Akt phosphorylation and sustained PI3K signalling in the airways of COPD patients, even after smoking cessation [23]. This deregulation of PTEN can explain, at least partially, why inflammation persists even after the disappearance of cigarette smoke. In other words, chronic smoking-induced oxidative stress exerts lasting adverse effects on the cells' gene regulatory network that are usually not reversed by cessation [23,141]. As PTEN suppression enables persistent inflammation in ex-smokers, it would be worthwhile to develop strategies for activating PTEN in such COPD patients.

Current COPD treatments include inhaled β2-adrenergic receptor agonists, glucocorticosteroids and anticholinergics. Although these treatments have contributed to a decrease in COPD exacerbations, they are mostly ineffective in improving lung function and quality of life. For example, steroids are known to negatively affect the hypothalamic–pituitary–adrenal axis in COPD patients, thereby creating the need to taper consumption and seek alternative therapies.

PI3K signalling is known to serve as a major driver of COPD. Importantly, inhibition of key PI3K signalling mediators reduces inflammation and increases lung function. There is cumulative *in vitro* evidence that small-molecule PI3K pathway inhibitors inhibit the hyperactivated PI3K signalling in cultured lung cells and leukocytes (Table 2). For example, the pan-PI3K inhibitor ZSTK474 inhibits the release of MMP-9 and ROS from neutrophils isolated from COPD patients [62]. Another pan-PI3K inhibitor, Wortmannin, promotes the differentiation of alveolar stem cells into mature alveolar cells [63]. In animal models of COPD, Wortmannin significantly promoted the regeneration of alveoli [63] (Table 3) and therefore has potential to enhance lung regeneration in COPD patients. Notably, macrolides are a family of chemicals currently used as pan-PI3K inhibitors for effective suppression of inflammation, viral infections and sputum production in COPD [148,149] (Table 4).

On the other hand, isoform-specific PI3K inhibitors appear to be effective treatments for suppressing inflammation and other clinical manifestations of COPD. The PI3Kδ-specific inhibitor, nortriptyline, induces HDAC2 and inhibits glucocorticoid resistance in cultured monocytes [69]. Another PI3Kδ-selective inhibitor, theophylline, has not only reversed steroid insensitivity and inflammation in mice [44], but also in human phase IV clinical trials [151] (Tables 3, 4). Given that steroid

Table 3. Effects of PI3K signalling inhibitors in preclinical studies of COPD.

Inhibitor	Molecular target	Effects on COPD	Model	Reference(s)
Wortmannin	Pan-PI3K	Enhances pulmonary alveolar regeneration; decreases neutrophil activation	Murine COPD model; cigarette smoke-exposed mice	[63,142]
SHBM1009	PI3K/mTOR	Confers resistance to oedema, acute and chronic lung inflammation, injury, remodelling and emphysema	Rat model of lung inflammation	[143]
Theophylline	PI3Kδ	Reverses steroid resistance and inflammation	Cigarette smoke-exposed mice	[44]
IC87114	PI3Kδ	Reverses glucocorticoid insensitivity	COPD mouse model	[28,34]
TG100-115	PI3Kγ and PI3Kδ	Decreases neutrophils and downregulates tumour necrosis factor-α	Mouse COPD model	[144,145]
AS-506240	PI3Kγ	Inhibits neutrophil infiltration	βENaC-Tg mouse model	[83]
Genistein	PTK	Inhibits PI3K/AKT phosphorylation and activation	Rat model of chronic bronchitis	[146]
LY294002	Pan-PI3K	Inhibits both PI3K/AKT signalling and p38-MAPK and JNK activation	Rat model of chronic bronchitis	[146]
RV-1759	PI3Kγ and PI3Kδ	Suppresses allergen-induced inflammation	Mouse model of COPD	[147]

Table 4. Inhibitors of PI3K signalling in human clinical trials of COPD.

Inhibitor	Molecular target	Effects on COPD	Clinical phase	Reference(s)*
Macrolides	Pan-PI3K	Exert antimicrobial, anti-inflammatory and antiviral effects, and reduce sputum production	Phase IV clinical trial	[148]
Metformin	mTOR	Has useful effects on inflammation, antioxidant levels, the effectiveness of steroid treatment and recovery	Phase IV clinical trial	[150]
GSK2269557	PI3Kδ	Suppresses sputum levels of the proinflammatory cytokines IL-8 and IL-6	Phase II clinical trial	[45]; NCT02130635
Theophylline	PI3Kδ	Increases the responsiveness to corticosteroids, inhibiting inflammation	Phase IV clinical trial	[151]
Roflumilast	PDE4	Reduces COPD exacerbations and median time to rehospitalisation	Phase IV clinical trial	[152–154]
CHF6523	Pan-PI3K	Will investigate the safety, tolerability and pharmacokinetics as well as anti-inflammatory effect of inhaled CHF6523 in COPD subjects	Phase I clinical trial	NCT04032535
RV1729	PI3Kγ and PI3Kδ	Will analyse the safety, tolerability, pharmacokinetics and pharmacodynamics of RV1729 in COPD patients	Phase I clinical trial	NCT02140346

*References for published articles and/or the ClinicalTrials.gov Identifier for clinical trials.

resistance has greatly hindered the efficiency of COPD treatments, it is important to discover small molecules that sensitise lung cells and leukocytes to corticosteroid treatments. GSK045 is another selective PI3Kδ inhibitor that has been shown to diminish MMP-9 secretion by COPD neutrophils and decrease cytokine release from blood mononuclear cells from COPD patients [62,155]. It also inhibits ROS production by cultured COPD neutrophils [62], thereby potentially decreasing ROS-induced inflammation.

In a βENaC-Tg mouse model of COPD, in which the mice overexpress βENaC and exhibit enhanced airway inflammation and obstruction, the PI3Kγ-specific inhibitor, AS-5062, reduced the infiltration of neutrophils [83], potentially enabling a decrease in the exaggerated immune response of COPD patients. TG100-115 is an inhibitor of both PI3Kγ and PI3Kδ that blocks neutrophil migration [144,145]. Another dual-PI3K inhibitor, RV1729, not only reduces inflammation in COPD mice (Table 3), but is also being tested in human clinical trials (Table 3). In a phase II clinical trials, an inhaled PI3Kδ-specific inhibitor, GSK2269557 (Nemiralisib), could be safely used in COPD patients and effectively suppressed the secretion of proinflammatory cytokines into the sputum [45,156,157].

Finally, metformin, which can indirectly inhibit mTOR, reduced inflammation and oxidative stress and enhanced steroid responsiveness and patient recovery in a phase IV COPD clinical trial [150] (Table 4). These collective findings (summarised in Figure 6) highlight the potential of pan-isoform and isoform-selective inhibitors of PI3K signalling for more effective treatment of COPD.

Herbal medicine, a major component of traditional medicine, has been exploited for centuries to treat COPD signs and symptoms [158]. For example, a clinically used Chinese herbal formula known as Louqin Zhisou decoction has been reported to decrease sputum hypersecretion and enhance lung function in COPD patients. Its mechanism of action appears to involve AKT/PI3K signalling because it lowered the abundance of phospho-AKT and phospho-PI3K in a rat model [127]. Moreover, *Scutellaria baicalensis*, another medicinal herb used to treat several serious medical conditions, was reported to inhibit the AKT/PI3K/NF-κB signalling axis in COPD rat models [159]. Similar anti-COPD effects have been observed for crocin (a carotenoid chemical constituting a bioactive ingredient in *Crocus sativus*), ginsenoside Rg1 (a major bioactive compound in *Panax*

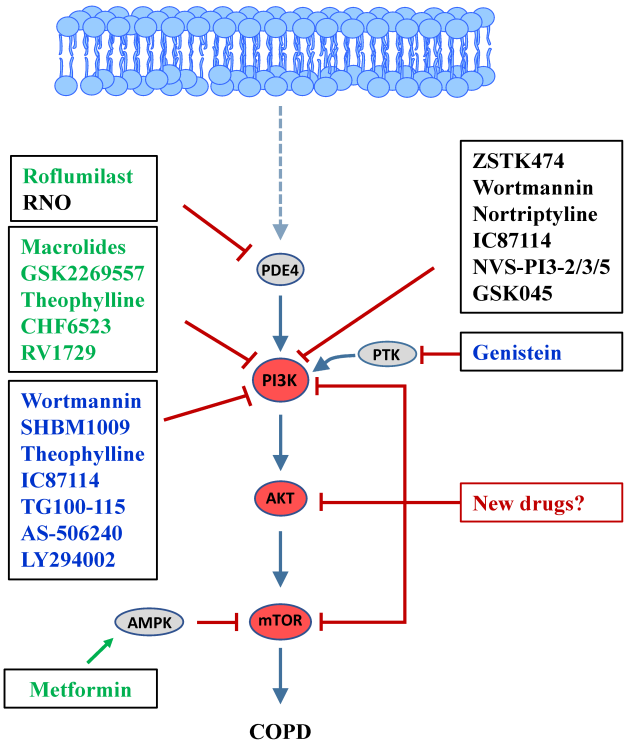

Figure 6. Pan-isoform and isoform-selective inhibitors of PI3K signalling in *in vitro*, preclinical and clinical studies of COPD. Various small-molecule inhibitors have been discovered to target PI3K signalling mediators in different model systems from *in vitro* analyses to human clinical trials of COPD. Inhibitors of PI3Kδ appear to exert the safest and most potent effects on COPD phenotype.

ginseng), 18β-glycyrrhetinic acid and glycyrrhizic acid (active ingredients in licorice) and isorhapontigenin (a dietary polyphenol), where various aspects of COPD were inhibited by these herbal medicines. Interestingly, all these herbs blocked PI3K signalling, thereby reducing inflammation and leukocyte infiltration [160–163].

Crocin has also been found to attenuate the COPD-associated depression via lowering PI3K/AKT-induced inflammation. It also inhibited cigarette smoke-induced NF-κB nuclear translocation. Notably, IGF-1 reversed the crocin-mediated suppression of NF-κB signalling, further confirming the important role of the enhanced PI3K pathway in increasing the pulmonary inflammation typical of COPD [164]. Importantly, certain herbal medicines, such as *Cordyceps sinensis*, were found to attenuate COPD-associated cellular senescence by reducing activation of the ROS/PI3K/AKT/mTOR axis [73]. Together, these findings indicate that traditional herbal medicine holds great promise in ameliorating COPD exacerbations through suppressing mTOR/AKT/PI3K signalling.

Concluding remarks

The current increasing trend of COPD morbidity and mortality highlights the need for more research into the underlying mechanisms of COPD development and exacerbations and for the development of highly efficacious therapeutics with less off-target or on-target adverse effects. Smoking is the main stressor leading to COPD development. Therefore, smoking cessation should be the first intervention in COPD therapy. However, even stopping smoking in combination with other treatments (e.g. corticosteroids) has not been successful enough in treating COPD and improving lung function. Therefore, new therapeutic strategies that are both safer and more efficacious should be developed. Cumulative evidence suggests that PI3K signalling mediators, particularly the various isoforms of the PI3K enzyme itself, hold great potential as effective targets in COPD treatment. So far, several small-molecule inhibitors of the PI3K signalling components have been investigated in different *in vitro* model organism and human clinical studies and the collective results demonstrate that inhibition of this pathway contributes to the considerable improvement of the various COPD phenotypes in patients.

As PI3K isoforms are expressed and play important roles in many cellular or tissue contexts and have several signalling crosstalks with other pathways, pan-isoform inhibitors of PI3K pathway are associated with higher side-effects. Therefore, it would be more suitable to use isoform-specific inhibitors (especially the small-molecule blockers of PI3Kδ) as well as develop new selective anti-PI3K inhibitors to minimise both off-target and on-target toxicities. One suggestion would be to reduce the dose to achieve comparable PI3K suppression but decreased toxicity. The development of inhaled therapeutics against PI3K signalling would be an important step towards safe and effective COPD therapy. Moreover, combination of PI3K inhibitors with other anti-COPD treatments might hold the key to treating this devastating disease. Furthermore, COPD patients might benefit from combinatorial treatment with anti-PI3K inhibitors and stem cell-based lung regeneration [165].

As COPD is associated with a high rate of microbial infections, it would be therapeutically more efficient to develop or discover therapies that inhibit both PI3K and microbial infections at the same time. It might also be needed to identify subpopulations of COPD patients who respond more effectively to some treatments than others, highlighting the potential of personalised medicine for tailoring patient-specific treatments. Importantly, some of the side-effects seen with highly selective PI3K inhibitors might stem from the fact that some small molecules are genotoxic. Alternatively, application of siRNAs against PI3K signalling might be a safer and/or more effective strategy. On the other hand, miRNAs might be even more powerful tools for effective targeting of PI3K signalling, as they can suppress 10s to 100s of targets. The multitarget rationale of miRNA regulation can be used to simultaneously repress several mediators of the PI3K pathway or both PI3K signalling and other signalling pathways. Further research on PI3K signalling is needed to develop safe and effective therapies for COPD patients.

Author contributions statement

SM, JS, MK, SAJ and MG designed the study. SM, EJ and SAJ reviewed the literature and wrote the manuscript. MK, MG and AZ reviewed and finalised the manuscript.

References

1. Soriano JB, Rodríguez-Roisin R. Chronic obstructive pulmonary disease overview: epidemiology, risk factors, and clinical presentation. *Proc Am Thorac Soc* 2011; **8:** 363–367.
2. Vestbo J, Hurd SS, Agustí AG, *et al*. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2013; **187:** 347–365.
3. Tan WC, Bourbeau J, Aaron SD, *et al*. Global initiative for chronic obstructive lung disease 2017 classification and lung function decline in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2018; **197:** 670–673.
4. Gnatiuc L, Caramori G. COPD in nonsmokers: the biomass hypothesis – to be or not to be? *Eur Respir J* 2014; **44:** 8–10.
5. Berry CE, Wise RA. Mortality in COPD: causes, risk factors, and prevention. *COPD* 2010; **7:** 375–382.
6. Müllerova H, Maselli DJ, Locantore N, *et al*. Hospitalized exacerbations of COPD: risk factors and outcomes in the ECLIPSE cohort. *Chest* 2015; **147:** 999–1007.
7. Kostikas K, Papatheodorou G, Psathakis K, *et al*. Oxidative stress in expired breath condensate of patients with COPD. *Chest* 2003; **124:** 1373–1380.
8. Kirkham PA, Barnes PJ. Oxidative stress in COPD. *Chest* 2013; **144:** 266–273.
9. Mannino DM. COPD: epidemiology, prevalence, morbidity and mortality, and disease heterogeneity. *Chest* 2002; **121:** 121S–126S.
10. GBD 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Respir Med* 2017; **5:** 691–706.
11. Cohen AJ, Brauer M, Burnett R, *et al*. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. *Lancet* 2017; **389:** 1907–1918.
12. Toy EL, Beaulieu NU, McHale JM, *et al*. Treatment of COPD: relationships between daily dosing frequency, adherence, resource use, and costs. *Respir Med* 2011; **105:** 435–441.
13. Niewoehner DE. Clinical practice. Outpatient management of severe COPD. *N Engl J Med* 2010; **362:** 1407–1416.
14. Barnes PJ. Cellular and molecular mechanisms of asthma and COPD. *Clin Sci (Lond)* 2017; **131:** 1541–1558.
15. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell* 2008; **132:** 567–582.
16. Hassani SN, Moradi S, Taleahmad S, *et al*. Transition of inner cell mass to embryonic stem cells: mechanisms, facts, and hypotheses. *Cell Mol Life Sci* 2019; **76:** 873–892.
17. Shahriari F, Satarian L, Moradi S, *et al*. MicroRNA profiling reveals important functions of miR-125b and let-7a during human retinal pigment epithelial cell differentiation. *Exp Eye Res* 2020; **190:** 107883.
18. Soleimani S, Valizadeh Arshad Z, Moradi S, *et al*. Small regulatory noncoding RNAs in *Drosophila melanogaster*: biogenesis and biological functions. *Brief Funct Genomics* 2020; **19:** 309–323.
19. Kusko RL, Brothers JF 2nd, Tedrow J, *et al*. Integrated genomics reveals convergent transcriptomic networks underlying chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2016; **194:** 948–960.
20. Chen W, Hong YQ, Meng ZL. Bioinformatics analysis of molecular mechanisms of chronic obstructive pulmonary disease. *Eur Rev Med Pharmacol Sci* 2014; **18:** 3557–3563.
21. Ezzie ME, Crawford M, Cho JH, *et al*. Gene expression networks in COPD: microRNA and mRNA regulation. *Thorax* 2012; **67:** 122–131.
22. Gomez-Cabrero D, Menche J, Cano I, *et al*. Systems medicine: from molecular features and models to the clinic in COPD. *J Transl Med* 2014; **12 (Suppl 2):** S4.
23. Yanagisawa S, Baker JR, Vuppusetty C, *et al*. Decreased phosphatase PTEN amplifies PI3K signaling and enhances proinflammatory cytokine release in COPD. *Am J Physiol Lung Cell Mol Physiol* 2017; **313:** L230–L239.
24. Edwards MR, Bartlett NW, Clarke D, *et al*. Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease. *Pharmacol Ther* 2009; **121:** 1–13.
25. Chung KF. p38 mitogen-activated protein kinase pathways in asthma and COPD. *Chest* 2011; **139:** 1470–1479.
26. Barnes PJ. Kinases as novel therapeutic targets in asthma and chronic obstructive pulmonary disease. *Pharmacol Rev* 2016; **68:** 788–815.
27. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149:** 274–293.
28. Hirsch E, Katanaev VL, Garlanda C, *et al*. Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* 2000; **287:** 1049–1053.
29. Follo MY, Manzoli L, Poli A, *et al*. PLC and PI3K/Akt/mTOR signalling in disease and cancer. *Adv Biol Regul* 2015; **57:** 10–16.
30. Yu JS, Cui W. Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. *Development* 2016; **143:** 3050–3060.
31. Zhang HX, Yang JJ, Zhang SA, *et al*. HIF-1alpha promotes inflammatory response of chronic obstructive pulmonary disease by activating EGFR/PI3K/AKT pathway. *Eur Rev Med Pharmacol Sci* 2018; **22:** 6077–6084.
32. Hemmings BA, Restuccia DF. PI3K-PKB/Akt pathway. *Cold Spring Harb Perspect Biol* 2012; **4:** a011189.
33. Laplante M, Sabatini DM. mTOR signaling at a glance. *J Cell Sci* 2009; **122:** 3589–3594.
34. Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, *et al*. The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol* 2010; **11:** 329–341.
35. Juss JK, Hayhoe RP, Owen CE, *et al*. Functional redundancy of class I phosphoinositide 3-kinase (PI3K) isoforms in signaling growth factor-mediated human neutrophil survival. *PLoS One* 2012; **7:** e45933.
36. Chalhoub N, Baker SJ. PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol* 2009; **4:** 127–150.
37. Sawa K, Koh Y, Kawaguchi T, *et al*. PIK3CA mutation as a distinctive genetic feature of non-small cell lung cancer with chronic obstructive pulmonary disease: a comprehensive mutational analysis from a multi-institutional cohort. *Lung Cancer* 2017; **112:** 96–101.
38. Wu Q, Jiang D, Chu HW. Cigarette smoke induces growth differentiation factor 15 production in human lung epithelial cells: implication in mucin over-expression. *Innate Immun* 2012; **18:** 617–626.
39. Yu H, Li Q, Kolosov VP, *et al*. Regulation of cigarette smoke-mediated mucin expression by hypoxia-inducible factor-1alpha via epidermal growth factor receptor-mediated signaling pathways. *J Appl Toxicol* 2012; **32:** 282–292.
40. Marwick JA, Caramori G, Casolari P, *et al*. A role for phosphoinositol 3-kinase delta in the impairment of glucocorticoid responsiveness in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2010; **125:** 1146–1153.

41. Marwick JA, Caramori G, Stevenson CS, *et al*. Inhibition of PI3K-delta restores glucocorticoid function in smoking-induced airway inflammation in mice. *Am J Respir Crit Care Med* 2009; **179:** 542–548.
42. Li L, Zhang M, Zhang L, *et al*. Klotho regulates cigarette smoke-induced autophagy: implication in pathogenesis of COPD. *Lung* 2017; **195:** 295–301.
43. Hsu AC, Starkey MR, Hanish I, *et al*. Targeting PI3K-p110alpha suppresses influenza virus infection in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2015; **191:** 1012–1023.
44. To Y, Ito K, Kizawa Y, *et al*. Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010; **182:** 897–904.
45. Cahn A, Hamblin JN, Begg M, *et al*. Safety, pharmacokinetics and dose-response characteristics of GSK2269557, an inhaled PI3Kdelta inhibitor under development for the treatment of COPD. *Pulm Pharmacol Ther* 2017; **46:** 69–77.
46. Lin G, Sun L, Wang R, *et al*. Overexpression of muscarinic receptor 3 promotes metastasis and predicts poor prognosis in non-small-cell lung cancer. *J Thorac Oncol* 2014; **9:** 170–178.
47. Hosgood HD 3rd, Menashe I, He X, *et al*. PTEN identified as important risk factor of chronic obstructive pulmonary disease. *Respir Med* 2009; **103:** 1866–1870.
48. Bozinovski S, Vlahos R, Hansen M, *et al*. Akt in the pathogenesis of COPD. *Int J Chron Obstruct Pulmon Dis* 2006; **1:** 31–38.
49. Kim MN, Lee KE, Hong JY, *et al*. Involvement of the MAPK and PI3K pathways in chitinase 3-like 1-regulated hyperoxia-induced airway epithelial cell death. *Biochem Biophys Res Commun* 2012; **421:** 790–796.
50. Wang Y, Liu J, Zhou JS, *et al*. MTOR suppresses cigarette smoke-induced epithelial cell death and airway inflammation in chronic obstructive pulmonary disease. *J Immunol* 2018; **200:** 2571–2580.
51. Houssaini A, Breau M, Kebe K, *et al*. mTOR pathway activation drives lung cell senescence and emphysema. *JCI Insight* 2018; **3:** e93203.
52. Wang D, Cui X. Evaluation of PDE4 inhibition for COPD. *Int J Chron Obstruct Pulmon Dis* 2006; **1:** 373–379.
53. Barber R, Baillie GS, Bergmann R, *et al*. Differential expression of PDE4 cAMP phosphodiesterase isoforms in inflammatory cells of smokers with COPD, smokers without COPD, and nonsmokers. *Am J Physiol Lung Cell Mol Physiol* 2004; **287:** L332–L343.
54. Lea S, Metryka A, Li J, *et al*. The modulatory effects of the PDE4 inhibitors CHF6001 and roflumilast in alveolar macrophages and lung tissue from COPD patients. *Cytokine* 2019; **123:** 154739.
55. Chung KF, Adcock IM. Multifaceted mechanisms in COPD: inflammation, immunity, and tissue repair and destruction. *Eur Respir J* 2008; **31:** 1334–1356.
56. Stark AK, Sriskantharajah S, Hessel EM, *et al*. PI3K inhibitors in inflammation, autoimmunity and cancer. *Curr Opin Pharmacol* 2015; **23:** 82–91.
57. Ghigo A, Damilano F, Braccini L, *et al*. PI3K inhibition in inflammation: toward tailored therapies for specific diseases. *Bioessays* 2010; **32:** 185–196.
58. Okkenhaug K, Bilancio A, Farjot G, *et al*. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science* 2002; **297:** 1031–1034.
59. Sadhu C, Masinovsky B, Dick K, *et al*. Essential role of phosphoinositide 3-kinase delta in neutrophil directional movement. *J Immunol* 2003; **170:** 2647–2654.
60. Mukaro VR, Bylund J, Hodge G, *et al*. Lectins offer new perspectives in the development of macrophage-targeted therapies for COPD/emphysema. *PLoS One* 2013; **8:** e56147.
61. Ferreira AM, Isaacs H, Hayflick JS, *et al*. The p110delta isoform of PI3K differentially regulates beta1 and beta2 integrin-mediated monocyte adhesion and spreading and modulates diapedesis. *Microcirculation* 2006; **13:** 439–456.
62. Gupta V, Khan A, Higham A, *et al*. The effect of phosphatidylinositol-3 kinase inhibition on matrix metalloproteinase-9 and reactive oxygen species release from chronic obstructive pulmonary disease neutrophils. *Int Immunopharmacol* 2016; **35:** 155–162.
63. Horiguchi M, Oiso Y, Sakai H, *et al*. Pulmonary administration of phosphoinositide 3-kinase inhibitor is a curative treatment for chronic obstructive pulmonary disease by alveolar regeneration. *J Control Release* 2015; **213:** 112–119.
64. Sapey E, Stockley JA, Greenwood H, *et al*. Behavioral and structural differences in migrating peripheral neutrophils from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2011; **183:** 1176–1186.
65. Yu Q, Chen X, Fang X, *et al*. Caveolin-1 aggravates cigarette smoke extract-induced MUC5AC secretion in human airway epithelial cells. *Int J Mol Med* 2015; **35:** 1435–1442.
66. Milara J, Lluch J, Almudever P, *et al*. Roflumilast N-oxide reverses corticosteroid resistance in neutrophils from patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2014; **134:** 314–322.
67. Totani L, Amore C, Di Santo A, *et al*. Roflumilast inhibits leukocyte-platelet interactions and prevents the prothrombotic functions of polymorphonuclear leukocytes and monocytes. *J Thromb Haemost* 2016; **14:** 191–204.
68. Sun XJ, Li ZH, Zhang Y, *et al*. Combination of erythromycin and dexamethasone improves corticosteroid sensitivity induced by CSE through inhibiting PI3K-delta/Akt pathway and increasing GR expression. *Am J Physiol Lung Cell Mol Physiol* 2015; **309:** L139–L146.
69. Mercado N, To Y, Ito K, *et al*. Nortriptyline reverses corticosteroid insensitivity by inhibition of phosphoinositide-3-kinase-delta. *J Pharmacol Exp Ther* 2011; **337:** 465–470.
70. Rossios C, To Y, Osoata G, *et al*. Corticosteroid insensitivity is reversed by formoterol via phosphoinositide-3-kinase inhibition. *Br J Pharmacol* 2012; **167:** 775–786.
71. Bewley MA, Belchamber KB, Chana KK, *et al*. Differential effects of p38, MAPK, PI3K or rho kinase inhibitors on bacterial phagocytosis and efferocytosis by macrophages in COPD. *PLoS One* 2016; **11:** e0163139.
72. Kobayashi Y, Wada H, Rossios C, *et al*. A novel macrolide/fluoroketolide, solithromycin (CEM-101), reverses corticosteroid insensitivity via phosphoinositide 3-kinase pathway inhibition. *Br J Pharmacol* 2013; **169:** 1024–1034.
73. Liu A, Wu J, Li A, *et al*. The inhibitory mechanism of *Cordyceps sinensis* on cigarette smoke extract-induced senescence in human bronchial epithelial cells. *Int J Chron Obstruct Pulmon Dis* 2016; **11:** 1721–1731.
74. Mercado N, Ito K, Barnes PJ. Accelerated ageing of the lung in COPD: new concepts. *Thorax* 2015; **70:** 482–489.
75. Karrasch S, Holz O, Jörres RA. Aging and induced senescence as factors in the pathogenesis of lung emphysema. *Respir Med* 2008; **102:** 1215–1230.
76. Ngkelo A, Hoffmann RF, Durham AL, *et al*. Glycogen synthase kinase-3beta modulation of glucocorticoid responsiveness in COPD. *Am J Physiol Lung Cell Mol Physiol* 2015; **309:** L1112–L1123.
77. Yanagisawa S, Baker JR, Vuppusetty C, *et al*. The dynamic shuttling of SIRT1 between cytoplasm and nuclei in bronchial epithelial cells by single and repeated cigarette smoke exposure. *PLoS One* 2018; **13:** e0193921.
78. Li F, Zhang P, Zhang M, *et al*. Hydrogen sulfide prevents and partially reverses ozone-induced features of lung inflammation and emphysema in mice. *Am J Respir Cell Mol Biol* 2016; **55:** 72–81.

79. Mishra V, DiAngelo SL, Silveyra P. Sex-specific IL-6-associated signaling activation in ozone-induced lung inflammation. *Biol Sex Differ* 2016; **7:** 16.
80. Wang Q, Wang Y, Zhang Y, *et al*. The role of uPAR in epithelial-mesenchymal transition in small airway epithelium of patients with chronic obstructive pulmonary disease. *Respir Res* 2013; **14:** 67.
81. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2016; **138:** 16–27.
82. Ali K, Camps M, Pearce WP, *et al*. Isoform-specific functions of phosphoinositide 3-kinases: p110 delta but not p110 gamma promotes optimal allergic responses in vivo. *J Immunol* 2008; **180:** 2538–2544.
83. Schmid MC, Avraamides CJ, Dippold HC, *et al*. Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3k-gamma, a single convergent point promoting tumor inflammation and progression. *Cancer Cell* 2011; **19:** 715–727.
84. Lee KH, Lee CH, Woo J, *et al*. Cigarette smoke extract enhances IL-17A-induced IL-8 production via up-regulation of IL-17R in human bronchial epithelial cells. *Mol Cells* 2018; **41:** 282–289.
85. Barnes PJ. Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 2018; **18:** 454–466.
86. Barnes PJ, Hansel TT. Prospects for new drugs for chronic obstructive pulmonary disease. *Lancet* 2004; **364:** 985–996.
87. Sng JJ, Prazakova S, Thomas PS, *et al*. MMP-8, MMP-9 and neutrophil elastase in peripheral blood and exhaled breath condensate in COPD. *COPD* 2017; **14:** 238–244.
88. Barnes PJ. Corticosteroid resistance in patients with asthma and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2013; **131:** 636–645.
89. Lu J, Xie L, Liu C, *et al*. PTEN/PI3k/AKT regulates macrophage polarization in emphysematous mice. *Scand J Immunol* 2017; **85:** 395–405.
90. Marwick JA, Stevenson CS, Chung KF, *et al*. Cigarette smoke exposure alters mSin3a and Mi-2alpha/beta expression; implications in the control of pro-inflammatory gene transcription and glucocorticoid function. *J Inflamm (Lond)* 2010; **7:** 33.
91. Kalenderian R, Raju L, Roth W, *et al*. Elevated histamine and tryptase levels in smokers' bronchoalveolar lavage fluid. Do lung mast cells contribute to smokers' emphysema? *Chest* 1988; **94:** 119–123.
92. Wei XM, Kim HS, Kumar RK, *et al*. Effects of cigarette smoke on degranulation and NO production by mast cells and epithelial cells. *Respir Res* 2005; **6:** 108.
93. Beckett EL, Stevens RL, Jarnicki AG, *et al*. A new short-term mouse model of chronic obstructive pulmonary disease identifies a role for mast cell tryptase in pathogenesis. *J Allergy Clin Immunol* 2013; **131:** 752–762.
94. van der Strate BW, Postma DS, Brandsma CA, *et al*. Cigarette smoke-induced emphysema: a role for the B cell? *Am J Respir Crit Care Med* 2006; **173:** 751–758.
95. Hogg JC, Chu F, Utokaparch S, *et al*. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004; **350:** 2645–2653.
96. Kirkham PA, Caramori G, Casolari P, *et al*. Oxidative stress-induced antibodies to carbonyl-modified protein correlate with severity of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2011; **184:** 796–802.
97. Shan M, Cheng HF, Song LZ, *et al*. Lung myeloid dendritic cells coordinately induce TH1 and TH17 responses in human emphysema. *Sci Transl Med* 2009; **1:** 4ra10.
98. Shan M, Yuan X, Song LZ, *et al*. Cigarette smoke induction of osteopontin (SPP1) mediates T(H)17 inflammation in human and experimental emphysema. *Sci Transl Med* 2012; **4:** 117ra119.
99. Lu W, You R, Yuan X, *et al*. The microRNA miR-22 inhibits the histone deacetylase HDAC4 to promote T(H)17 cell-dependent emphysema. *Nat Immunol* 2015; **16:** 1185–1194.
100. Bartel DP. Metazoan microRNAs. *Cell* 2018; **173:** 20–51.
101. Moradi S, Braun T, Baharvand H. miR-302b-3p promotes self-renewal properties in leukemia inhibitory factor-withdrawn embryonic stem cells. *Cell J* 2018; **20:** 61–72.
102. Moradi S, Sharifi-Zarchi A, Ahmadi A, *et al*. Small RNA sequencing reveals Dlk1-Dio3 locus-embedded MicroRNAs as major drivers of ground-state pluripotency. *Stem Cell Reports* 2017; **9:** 2081–2096.
103. Mathieu J, Ruohola-Baker H. Regulation of stem cell populations by microRNAs. *Adv Exp Med Biol* 2013; **786:** 329–351.
104. Lüningschrör P, Hauser S, Kaltschmidt B, *et al*. MicroRNAs in pluripotency, reprogramming and cell fate induction. *Biochim Biophys Acta* 2013; **1833:** 1894–1903.
105. Moradi S, Asgari S, Baharvand H. Concise review: harmonies played by microRNAs in cell fate reprogramming. *Stem Cells* 2014; **32:** 3–15.
106. Moradi S, Mahdizadeh H, Šarić T, *et al*. Research and therapy with induced pluripotent stem cells (iPSCs): social, legal, and ethical considerations. *Stem Cell Res Ther* 2019; **10:** 341.
107. Ha TY. MicroRNAs in human diseases: from cancer to cardiovascular disease. *Immune Netw* 2011; **11:** 135–154.
108. Stolzenburg LR, Harris A. The role of microRNAs in chronic respiratory disease: recent insights. *Biol Chem* 2018; **399:** 219–234.
109. Baker JR, Vuppusetty C, Colley T, *et al*. Oxidative stress dependent microRNA-34a activation via PI3Kalpha reduces the expression of sirtuin-1 and sirtuin-6 in epithelial cells. *Sci Rep* 2016; **6:** 35871.
110. Barnes PJ, Baker J, Donnelly LE. Cellular senescence as a mechanism and target in chronic lung diseases. *Am J Respir Crit Care Med* 2019; **200:** 556–564.
111. Shi L, Xin Q, Chai R, *et al*. Ectopic expressed miR-203 contributes to chronic obstructive pulmonary disease via targeting TAK1 and PIK3CA. *Int J Clin Exp Pathol* 2015; **8:** 10662–10670.
112. Lewis A, Riddoch-Contreras J, Natanek SA, *et al*. Downregulation of the serum response factor/miR-1 axis in the quadriceps of patients with COPD. *Thorax* 2012; **67:** 26–34.
113. Chuang CC, Zhou T, Olfert IM, *et al*. Hypoxic preconditioning attenuates reoxygenation-induced skeletal muscle dysfunction in aged pulmonary TNF-alpha overexpressing mice. *Front Physiol* 2018; **9:** 1720.
114. Wang Z, Li R, Zhong R. Extracellular matrix promotes proliferation, migration and adhesion of airway smooth muscle cells in a rat model of chronic obstructive pulmonary disease via upregulation of the PI3K/AKT signaling pathway. *Mol Med Rep* 2018; **18:** 3143–3152.
115. Ge Q, Moir LM, Trian T, *et al*. The phosphoinositide 3′-kinase p110delta modulates contractile protein production and IL-6 release in human airway smooth muscle. *J Cell Physiol* 2012; **227:** 3044–3052.
116. Zhang K, Shan L, Rahman MS, *et al*. Constitutive and inducible thymic stromal lymphopoietin expression in human airway smooth muscle cells: role in chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 2007; **293:** L375–L382.
117. Hong W, Peng G, Hao B, *et al*. Nicotine-induced airway smooth muscle cell proliferation involves TRPC6-dependent calcium influx via alpha7 nAChR. *Cell Physiol Biochem* 2017; **43:** 986–1002.
118. Xie S, Issa R, Sukkar MB, *et al*. Induction and regulation of matrix metalloproteinase-12 in human airway smooth muscle cells. *Respir Res* 2005; **6:** 148.
119. Barreiro E, Salazar-Degracia A, Sancho-Muñoz A, *et al*. Endoplasmic reticulum stress and unfolded protein response in diaphragm muscle dysfunction of patients with stable chronic obstructive pulmonary disease. *J Appl Physiol* 2019; **126:** 1572–1586.
120. Tsai MJ, Chang WA, Jian SF, *et al*. Possible mechanisms mediating apoptosis of bronchial epithelial cells in chronic obstructive pulmonary disease – a next-generation sequencing approach. *Pathol Res Pract* 2018; **214:** 1489–1496.
121. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. *Lancet* 2007; **370:** 765–773.

122. Thakurdas SM, Melicoff E, Sansores-Garcia L, *et al*. The mast cell-restricted tryptase mMCP-6 has a critical immunoprotective role in bacterial infections. *J Biol Chem* 2007; **282:** 20809–20815.
123. Wang B, Lim DJ, Han J, *et al*. Novel cytoplasmic proteins of non-typeable *Haemophilus influenzae* up-regulate human MUC5AC mucin transcription via a positive p38 mitogen-activated protein kinase pathway and a negative phosphoinositide 3-kinase-Akt pathway. *J Biol Chem* 2002; **277:** 949–957.
124. Morey P, Cano V, Martí-Lliteras P, *et al*. Evidence for a non-replicative intracellular stage of nontypable *Haemophilus influenzae* in epithelial cells. *Microbiology* 2011; **157:** 234–250.
125. Martí-Lliteras P, Regueiro V, Morey P, *et al*. Nontypeable *Haemophilus influenzae* clearance by alveolar macrophages is impaired by exposure to cigarette smoke. *Infect Immun* 2009; **77:** 4232–4242.
126. Weng JZ, Wang Y, Sun TY. Cathelicidin LL-37 restoring glucocorticoid function in smoking and lipopolysaccharide-induced airway inflammation in rats. *Chin Med J (Engl)* 2019; **132:** 569–576.
127. Feng F, Du J, Meng Y, *et al*. Louqin Zhisou decoction inhibits mucus hypersecretion for acute exacerbation of chronic obstructive pulmonary disease rats by suppressing EGFR-PI3K-AKT signaling pathway and restoring Th17/Treg balance. *Evid Based Complement Alternat Med* 2019; **2019:** 6471815.
128. Ngkelo A, Meja K, Yeadon M, *et al*. LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and Gialpha dependent PI-3kinase signalling. *J Inflamm* 2012; **9:** 1.
129. Binker MG, Binker-Cosen AA, Richards D, *et al*. LPS-stimulated MUC5AC production involves Rac1-dependent MMP-9 secretion and activation in NCI-H292 cells. *Biochem Biophys Res Commun* 2009; **386:** 124–129.
130. Zhang P, Xin X, Fang L, *et al*. HMGB1 mediates *Aspergillus fumigatus*-induced inflammatory response in alveolar macrophages of COPD mice via activating MyD88/NF-kappaB and syk/PI3K signalings. *Int Immunopharmacol* 2017; **53:** 125–132.
131. Wu Y, Xu H, Li L, *et al*. Susceptibility to *Aspergillus* infections in rats with chronic obstructive pulmonary disease via deficiency function of alveolar macrophages and impaired activation of TLR2. *Inflammation* 2016; **39:** 1310–1318.
132. Newcomb DC, Sajjan US, Nagarkar DR, *et al*. Human rhinovirus 1B exposure induces phosphatidylinositol 3-kinase-dependent airway inflammation in mice. *Am J Respir Crit Care Med* 2008; **177:** 1111–1121.
133. Liu K, Gualano RC, Hibbs ML, *et al*. Epidermal growth factor receptor signaling to Erk1/2 and STATs control the intensity of the epithelial inflammatory responses to rhinovirus infection. *J Biol Chem* 2008; **283:** 9977–9985.
134. Shapiro SD. End-stage chronic obstructive pulmonary disease: the cigarette is burned out but inflammation rages on. *Am J Respir Crit Care Med* 2001; **164:** 339–340.
135. Willemse BW, Postma DS, Timens W, *et al*. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. *Eur Respir J* 2004; **23:** 464–476.
136. Zhang Q, Wan H, Huang S, *et al*. Critical role of RIG-I-like receptors in inflammation in chronic obstructive pulmonary disease. *Clin Respir J* 2016; **10:** 22–31.
137. Todt JC, Freeman CM, Brown JP, *et al*. Smoking decreases the response of human lung macrophages to double-stranded RNA by reducing TLR3 expression. *Respir Res* 2013; **14:** 33.
138. Stogsdill MP, Stogsdill JA, Bodine BG, *et al*. Conditional overexpression of receptors for advanced glycation end-products in the adult murine lung causes airspace enlargement and induces inflammation. *Am J Respir Cell Mol Biol* 2013; **49:** 128–134.
139. Yonchuk JG, Silverman EK, Bowler RP, *et al*. Circulating soluble receptor for advanced glycation end products (sRAGE) as a biomarker of emphysema and the RAGE axis in the lung. *Am J Respir Crit Care Med* 2015; **192:** 785–792.
140. Pan Z, Yu H, Liao JL. Probing cellular and molecular mechanisms of cigarette smoke-induced immune response in the progression of chronic obstructive pulmonary disease using multiscale network modeling. *PLoS One* 2016; **11:** e0163192.
141. Willemse BW, ten Hacken NH, Rutgers B, *et al*. Effect of 1-year smoking cessation on airway inflammation in COPD and asymptomatic smokers. *Eur Respir J* 2005; **26:** 835–845.
142. Vlahos R, Wark PA, Anderson GP, *et al*. Glucocorticosteroids differentially regulate MMP-9 and neutrophil elastase in COPD. *PLoS One* 2012; **7:** e33277.
143. Fang X, Li K, Tao X, *et al*. Effects of phosphoinositide 3-kinase on protease-induced acute and chronic lung inflammation, remodeling, and emphysema in rats. *Chest* 2013; **143:** 1025–1035.
144. Doukas J, Eide L, Stebbins K, *et al*. Aerosolized phosphoinositide 3-kinase gamma/delta 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. *J Pharmacol Exp Ther* 2009; **328:** 758–765.
145. Ciraolo E, Iezzi M, Marone R, *et al*. Phosphoinositide 3-kinase p110beta activity: key role in metabolism and mammary gland cancer but not development. *Sci Signal* 2008; **1:** ra3.
146. Huang Y, Meng XM, Jiang GL, *et al*. Studies on mitogen-activated protein kinase signaling pathway in the alveolar macrophages of chronic bronchitis rats. *Mol Cell Biochem* 2015; **400:** 97–105.
147. Norman P. Evaluation of WO2013136076: two crystalline forms of the phosphatidylinositol 3-kinase-delta inhibitor RV-1729. *Expert Opin Ther Pat* 2014; **24:** 471–475.
148. Simoens S, Laekeman G, Decramer M. Preventing COPD exacerbations with macrolides: a review and budget impact analysis. *Respir Med* 2013; **107:** 637–648.
149. Sun X, Chen L, He Z. PI3K/Akt-Nrf2 and anti-inflammation effect of macrolides in chronic obstructive pulmonary disease. *Curr Drug Metab* 2019; **20:** 301–304.
150. Hitchings AW, Lai D, Jones PW, *et al*. Metformin in severe exacerbations of chronic obstructive pulmonary disease: a randomised controlled trial. *Thorax* 2016; **71:** 587–593.
151. Ford PA, Durham AL, Russell RE, *et al*. Treatment effects of low-dose theophylline combined with an inhaled corticosteroid in COPD. *Chest* 2010; **137:** 1338–1344.
152. Rabe KF, Calverley PMA, Martinez FJ, *et al*. Effect of roflumilast in patients with severe COPD and a history of hospitalisation. *Eur Respir J* 2017; **50:** 1700158.
153. Martinez FJ, Calverley PM, Goehring UM, *et al*. Effect of roflumilast on exacerbations in patients with severe chronic obstructive pulmonary disease uncontrolled by combination therapy (REACT): a multicentre randomised controlled trial. *Lancet* 2015; **385:** 857–866.
154. Facius A, Marostica E, Gardiner P, *et al*. Pharmacokinetic and pharmacodynamic modelling to characterize the tolerability of alternative up-titration regimens of roflumilast in patients with chronic obstructive pulmonary disease. *Clin Pharmacokinet* 2018; **57:** 1029–1038.
155. Khan A, Southworth T, Worsley S, *et al*. An investigation of the anti-inflammatory effects and a potential biomarker of PI3Kdelta inhibition in COPD T cells. *Clin Exp Pharmacol Physiol* 2017; **44:** 932–940.
156. Begg M, Wilson R, Hamblin JN, *et al*. Relationship between pharmacokinetics and pharmacodynamic responses in healthy smokers informs a once-daily dosing regimen for nemiralisib. *J Pharmacol Exp Ther* 2019; **369:** 337–344.
157. Wilson R, Jarvis E, Montembault M, *et al*. Safety, tolerability, and pharmacokinetics of single and repeat doses of nemiralisib administered via the ellipta dry powder inhaler to healthy subjects. *Clin Ther* 2018; **40:** 1410–1417.

158. Haifeng W, Hailong Z, Jiansheng L, *et al*. Effectiveness and safety of traditional Chinese medicine on stable chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Complement Ther Med* 2015; **23:** 603–611.
159. Xu F, Lin J, Cui W, *et al*. *Scutellaria baicalensis* attenuates airway remodeling via PI3K/Akt/NF-kappaB pathway in cigarette smoke mediated-COPD rats model. *Evid Based Complement Alternat Med* 2018; **2018:** 1281420.
160. Dianat M, Radan M, Badavi M, *et al*. Crocin attenuates cigarette smoke-induced lung injury and cardiac dysfunction by anti-oxidative effects: the role of Nrf2 antioxidant system in preventing oxidative stress. *Respir Res* 2018; **19:** 58.
161. Li F, Li X, Peng X, *et al*. Ginsenoside Rg1 prevents starvation-induced muscle protein degradation via regulation of AKT/mTOR/FoxO signaling in C2C12 myotubes. *Exp Ther Med* 2017; **14:** 1241–1247.
162. Kao TC, Wu CH, Yen GC. Glycyrrhizic acid and 18beta-glycyrrhetinic acid recover glucocorticoid resistance via PI3K-induced AP1, CRE and NFAT activation. *Phytomedicine* 2013; **20:** 295–302.
163. Yeo SCM, Fenwick PS, Barnes PJ, *et al*. Isorhapontigenin, a bioavailable dietary polyphenol, suppresses airway epithelial cell inflammation through a corticosteroid-independent mechanism. *Br J Pharmacol* 2017; **174:** 2043–2059.
164. Xie Y, He Q, Chen H, *et al*. Crocin ameliorates chronic obstructive pulmonary disease-induced depression via PI3K/Akt mediated suppression of inflammation. *Eur J Pharmacol* 2019; **862:** 172640.
165. Cheng Y, Gu W, Zhang G, *et al*. Activation of Notch1 signaling alleviates dysfunction of bone marrow-derived mesenchymal stem cells induced by cigarette smoke extract. *Int J Chron Obstruct Pulmon Dis* 2017; **12:** 3133–3147.