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Authors

Li, Linhui
Yang, David C
Chen, Ching-Hsien

Publication Date

2021-02-01

DOI

10.1016/j.freeradbiomed.2020.12.438

Peer reviewed

Review Article

Metabolic reprogramming: A driver of cigarette smoke-induced inflammatory lung diseases

Linhui Li [a,b], David C. Yang [a], Ching-Hsien Chen [a,b,*]

[a] *Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine and Center for Comparative Respiratory Biology and Medicine, University of California Davis, Davis, CA, USA*
[b] *Division of Nephrology, Department of Internal Medicine, University of California Davis, Davis, CA, USA*

ABSTRACT

Cigarette smoking is a well-known risk factor for pulmonary diseases, including chronic obstructive pulmonary disease (COPD), asthma and pulmonary fibrosis. Despite major progress in dissecting the mechanisms associated with disease development and progression, findings only represent one aspect of multifaceted disease. A crucial consequence of this approach is that many therapeutic treatments often fail to improve or reverse the disease state as other conditions and variables are insufficiently considered. To expand our understanding of pulmonary diseases, omics approaches, particularly metabolomics, has been emerging in the field. This strategy has been applied to identify putative biomarkers and novel mechanistic insights. In this review, we discuss metabolic profiles of patients with COPD, asthma, and idiopathic pulmonary fibrosis (IPF) with a focus on the direct effects of cigarette smoking in altering metabolic regulation. We next present cell- and animal-based experiments and point out the therapeutic potential of targeting metabolic reprogramming in inflammatory lung diseases. In addition, the obstacles in translating these findings into clinical practice, including potential adverse effects and limited pharmacological efficacy, are also addressed.

1. Introduction

Smoking including both cigarettes and electronic cigarettes (e-cigarettes) has been reported to induce chronic lung, vascular, and neoplastic-related diseases, and tobacco smoking in particular can shorten life expectancy by at least one decade as compared with non-smokers [1,2]. Smoking prevention campaigns in addition to decades of cigarette smoking research have led to a gradual decline of adult smoking prevalence from 42% in 1965 to currently 13.7% in the United States; however, most smokers develop nicotine addiction which prevents them from quitting smoking [3–6]. The toxicity of tobacco smoking on human health was not fully accepted until the last decade of the 20th century, and cigarette smoking has been acknowledged as a risk factor for lung cancer since then [7]. The inhaled smoke resulting from combusting a tobacco cigarette can be separated into a gas phase (toxic gases, organic compounds, free radicals, etc.) and a particulate phase consisting of 0.1–1.0 μm particles like nicotine and carcinogens which can reach the deep lung [8]. Because of the thousands of compounds present in tobacco smoke which can adversely affect human health, an electronic vaping device, termed e-cigarette, was invented to minimize the chemical contents presented to a smoker. The device applies heat to convert glycerol and propylene glycol, flavors, and nicotine into vapor for inhalation [9]. Studies of this new product have just begun, but several studies thus far have observed the same detrimental effects on lung functions as compared with tobacco cigarettes in healthy subjects or in animals [9,10]. Unfortunately, even though most people are aware that cigarette smoking is harmful, tobacco products and cigarette products are still consumed by people of different ages.

The deleterious consequences of cigarette smoking are not limited to lung cancer-related morbidity and mortality. Chronic exposure to cigarette smoke (CS) increases the chances of developing inflammatory respiratory conditions such as chronic obstructive pulmonary disease (COPD), asthma, and pulmonary fibrosis [11–13]. Prior studies have established that cigarette smoking contributes to pulmonary disease development and progression through altering gene expression and signaling pathways. However, the concept of metabolic dysregulation as a driver for pulmonary diseases is now emerging [14]. Accumulative studies have demonstrated metabolic dysregulation in both patients with pulmonary diseases as well as in disease models. These findings point to the critical role of metabolism in the genesis and pathology of diseases, providing insights into novel disease mechanisms and

Abbreviations

3PO	3-(3-Pyridinyl)-1-(4-pyridinyl)-2-propen-1-one	IRP2	iron-responsive element binding protein 2
AM	Alveolar macrophage	Mfn2	mitofusin 2
ARG	arginase	mTOR	mammalian target of rapamycin
ASL	argininosuccinate lyse	NOS	nitric oxide synthase
ASS1	argininosuccinate synthase	NOX	NADPH oxidase complex
CPT1A	carnitine palmitoyltransferase	NOX4	nicotinamide adenine dinucleotide phosphate reduced oxidase 4
CS	cigarette smoke	ODC	ornithine decarboxylase
CSE	cigarette smoke extract	PDK-1	pyruvate dehydrogenase kinase
DCA	dichloroacetate	PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3
Drp1	dynamin-related protein 1	PINK1	PTEN-induced kinase 1
ECVC	e-cigarette vapor condensate	SASP	senescence-associated secretory phenotype
FAM13A	family with sequence similarity 13 member A	ROS	reactive oxygen species
FAO	fatty acid oxidation	Th 1 lymphocytes	T helper 1 lymphocytes
FEV_1	forced exhalation volume in 1 second	Th 2 lymphocytes	T helper 2 lymphocytes
HBEC	human bronchial epithelial cell	TGF-β	transforming growth factor beta
HK2	hexokinase 2	TSC 1	tuberous sclerosis complex 1
iNOS	inducible nitric oxide synthase	TSC 2	tuberous sclerosis complex 2

therapeutic approaches. Therefore, we will review research studies within the scope of inflammatory pulmonary diseases and discuss current views of cigarette smoking-induced metabolic reprogramming in this work.

2. Cigarette smoke, metabolism and inflammation

Given that the lungs are constantly and directly exposed to environmental insults, mechanisms involving both innate and adaptive immune responses are employed to protect against any potential damage. Immune cells develop metabolic reconfiguration through changing metabolic enzymes, intermediates, and metabolites in different immune cell subsets to accommodate cellular functions and influence the inflammatory response [15]. Of note, the effect of cigarette smoke (CS) on immune systems in smokers or passively exposed individuals is complex and could vary depending on factors such as age, sex, and smoke pattern, thereby complicating our understanding of CS and immune-related responses [16]. Due to the highly variable nature, many studies have employed cell-based or animal models to minimize variation. These studies have demonstrated that CS, an exceptionally rich source of reactive oxygen/nitrogen species and aldehydes, disturbs the oxidant: antioxidant balance in cells to induce DNA damage, cell senescence and apoptosis [17]. Multiple exposures of human lung fibroblasts to cigarette smoke extract (CSE) inhibited cell proliferation and induced cellular senescence by upregulating pro-senescence markers p53, p21 and p16 activity [18]. Lung tissues from mice exposed to CS had elevated the expression of pro-senescence markers, including p16, p21 and DNA damage marker, phospho-γH2AX [18,19]. When cells undergo senescence, they are metabolically active but irreversibly lose replicative capacity [17]. These senescent cells secrete a broad range of proinflammatory cytokines, chemokines, matrix metalloproteases, and growth factors to generate a proinflammatory microenvironment and facilitate immune cell recruitment to the sites of senescent tissues, representing a hallmark phenomenon termed senescence-associated secretory phenotype (SASP) [19–21].

2.1. Inflammation response in lung

In addition to the CS-induced premature senescence, reactive oxygen species (ROS), a short-lived constituent of CS, promotes chronic inflammation in the lung [22]. A complex mixture of ROS is present in both the particulate and gas phases of CS, where the particulate phase is composed of relatively long-lived radical molecules like

quinone/hydroxyquinone radicals, and the gas phase is dominated by short-lived but more reactive ROS including nitric oxide (NO), nitrogen dioxide, and peroxynitrite ($ONOO^-$) [22,23]. Short-lived ROS damages the epithelial cells of upper airways through inducing peroxidation of lipids, DNA damage and activation of signaling pathways as well as transcriptional factors such as NF-κB to enhance genes expression of pro-inflammatory mediators [22,24]. The secretion of inflammatory mediators consequently leads to chronic immune cell recruitment and inflammation. CS-induced chronic inflammation contributes to the progression of pulmonary diseases by altering both innate and adaptive immune responses [17,25]. Cigarette smokers have elevated numbers of macrophages and neutrophils in their lungs due to the presence of oxidants in CS that stimulate alveolar macrophages (AMs) to release mediator and recruit inflammatory cells like neutrophils to the lungs [24]. These AMs also generate NO˙ and superoxide anion ($O_2^{\bullet-}$) via nitric oxide synthases (NOSs) and NADPH oxidase complexes (NOXs), respectively, further augmenting oxidative stress and lung damage [23,24].

Apart from generating ROS, AM polarization was found to be different in nonsmokers versus healthy smokers. Genes associated with the anti-inflammatory M2 macrophage phenotype were upregulated, whereas pro-inflammatory M1 macrophages-related genes were downregulated in smokers [25]. Since M2 macrophage polarization contributed to tissue remodeling and immunoregulation, this alteration of AM polarization could promote disease development. Indeed, the pattern of gene expression was preserved in COPD smokers with progressive downregulation of M1-related genes and gradual upregulation of some M2-related genes [25]. CS exposure also changed the proinflammatory cytokines production by macrophages. Nicotine and CS treatment enhanced interleukin 8 (IL-8) expression, which was the key chemokine for promoting CS-induced lung inflammation, observed in both human and mouse macrophages [26]. In addition to innate immunity, cigarette smoking increased $CD8^+$ but lowered $CD4^+$ T cells in both bronchoalveolar lavage (BAL) fluid and peripheral blood of smokers compared with nonsmokers, suggesting the involvement of T lymphocytes in lung inflammation [27]. It was also shown that the balance between subsets of $CD4^+$ T cells (Type 1 T helper (Th1) and Th2 lymphocytes) was altered in lungs after continuous CS exposure, contributing to COPD and asthma progression [16].

2.2. Metabolic alteration and associated effects

Since AMs together with airway epithelial cells are the first line of

defense acting as phagocytes and antigen-presenting cells in the lung, studies have investigated the implication of tobacco and e-cigarette smoke in these cells. A prior study on human AMs exposed to e-cigarette vapor condensate (ECVC) have found that the condensate induced ROS production along with inhibition of bacterial phagocytosis [16,28]. However, treating the macrophages with N-acetyl cysteine, a free radical scavenger, could restore the phagocytic function of those ECVC-exposed macrophages [28]. A recent report has observed the downregulation of phagocytosis of AMs is due to cigarette smoke extract (CSE)-induced glycolysis and ROS generation [29]. In addition to ROS production, CSE exposure inhibited oxidative phosphorylation with a compensatory increase of glycolysis, suggesting a shift of metabolism in AMs [29]. The CSE-induced phagocytosis defect could be replicated by hydrogen peroxide treatment, and N-acetyl cysteine both decreased CSE-induced glycolysis and reversed the effect of CSE and hydrogen peroxide on phagocytosis [29]. This interplay between CSE-induced metabolic alteration and ROS generation indicates the potential attribution of metabolic dysregulation during pulmonary pathogenesis. The Kuwano research group has demonstrated that CSE-induced cell senescence and ROS production in human bronchial epithelial cells (HBECs) were associated with mitophagic degradation, termed mitophagy. Upon CSE exposure, accumulated mitochondrial fragmentation was followed with insufficient mitophagy, resulting in exacerbation of ROS generation and cell senescence [30].

Moreover, the effect of CS in epithelial cells was evaluated in animal studies. Alveolar type II epithelial cells isolated from mice exposed to CS for 4 or 8 weeks, displayed an upregulation of CD36 and carnitine-palmitoyl transferase 1 expression, through promoting palmitate utilization for energy production [31]. Such alteration also decreased phosphatidylcholine level, potentially impairing surfactant production by alveolar type II cells [31]. Due to the role surfactant proteins plays in modulating activity of cells involved in immune response, the downregulation of surfactant production potentially contributes to CS-induced immunosuppression [32]. Further study has showed that autophagy, the catabolic process for cellular homeostasis, was suppressed in human bronchial epithelial cells upon nicotine exposure, resulting in subsequent cell apoptosis and senescence [33]. Lungs isolated from mice exposed to e-cigarette vapor were also demonstrated to have impaired autophagy and cellular senescence [33]. The determination of autophagy is governed by the activity of the mammalian target of rapamycin (mTOR) [34]. Chronic CS exposure lowered mTOR expression but increased the expression of its inhibitor, tuberous sclerosis complex 2 (TSC2), to induce autophagy and cell death in murine lungs [34]. Knocking down *Mtor* gene expression in bronchial or alveolar epithelial cells of murine lungs augmented airway inflammation and airspace enlargement post 6-month CS exposures [34]. The above animal studies show the impacts of chronic CS exposure in metabolism, suggesting that metabolic dysregulation could influence inflammation, tissue remodeling, and possible disease progression. However, further work is required to elucidate if metabolism in different immune cells is also altered upon CS exposure to strengthen the link between metabolism and inflammation in pulmonary inflammatory diseases.

3. Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is currently the 4th largest cause of death worldwide [35,36]. This pulmonary disease is characterized by poorly reversible and usually progressive airflow limitation, including pathological conditions (e.g., bronchitis, chronic obstructive bronchiolitis, and emphysema) and patients with COPD develop varying degrees of these features [37,38]. With cigarette smoking being the well-known dominant risk factor, COPD has been considered as a preventable disease [11]. In a 25-year follow-up study, lung function from men and women was collected over the period. Male and female smokers tended to have abnormal lung functions as compared to nonsmokers, and at least 25% of smokers developed COPD

in the end [11]. Although passive smoking, environmental or occupational exposure, atmospheric pollution, and viral infections were also reported as potential risk factors for COPD, cigarette smoking accounted for 95% of the cases in developed countries [38,39]. To recapitulate pulmonary anatomy of COPD patients, rodents chronically exposed to cigarette smoke (CS) developed emphysema, small airway, vascular remodeling, and pulmonary hypertension [40]. In addition, mice exposed to nicotine containing e-cigarette solution for 4 months was found to develop COPD-like lung tissue destruction in addition to cytokine (IL-6 and IL8) expression, implicating a potential role of nicotine inhalation in airway disease [41] (Fig. 1). Perhaps, applying e-cigarette in future studies can reveal novel mechanistic insights in COPD.

3.1. Metabolic reprogramming and emphysema

Our understanding of COPD pathogenesis has evolved over time. Previous studies have established that abnormal inflammatory response to cigarette smoke (CS) exposure in lungs is as a well-documented contributor to COPD pathogenesis [42]. Specifically, CS-induced excessive oxidative stress disrupts cell junctions between adjacent epithelial cells and induces apoptosis and necroptosis (cell death) in lung parenchyma, resulting in structural changes and COPD phenotypes [43–45]. Recent metabolomic studies identified metabolomic dysregulation in patient's samples with COPD, suggesting metabolic reprogramming as a part of COPD pathogenesis [46]. Short-term (4 and 8 weeks) CS exposure to A/J mice induced upregulation of genes encoding glycolysis, tricarboxylic acid cycle, mitochondrial fatty acid oxidation pathway, and redox regulation [47]. Although mice underwent short-term CS exposure did not fully recapitulate the clinical features in COPD, this study suggests a relationship between CS and mitochondrial dysregulation [47]. Mizumura et al. found that CS exposure induced PTEN-induced kinase 1 (PINK1)-mediated mitophagy. PINK1 is a key regulator of mitophagy, which is an autophagy-dependent elimination of mitochondria, and it is commonly associated with CS-induced mitochondrial dysfunction in pulmonary epithelial cells [48]. Cigarette smoke extract (CSE) increased PINK1 expression, leading to PINK1-dependent mitophagy and cell death in epithelial cells [48]. As demonstrated in animal studies, this PINK1-dependent mitophagy contributed to airway dysfunction and air space enlargement in CS-exposed mice [48]. Fatty acid is an important source of energy and can be utilized to fuel cells through the process of fatty acid oxidation (FAO) [49] (Fig. 2). Previous studies have demonstrated that fatty acid level and FAO were altered in COPD [31,47,50]. In light of the fact that short-term CS exposure impaired energy homeostasis, human bronchial epithelial cells exposed to CSE displayed upregulated fatty acid β-oxidation (FAO) as well as increased carnitine palmitoyltransferase (CPT1A) expression, which was the rate limiting enzyme of FAO process [31,47,51]. Additionally, FAM13A (family with sequence similarity 13 member A), a protein with undefined biological functions, was found to be associated with emphysema development in COPD [51]. This protein interacted with sirtuin 1 (SIRT1), an activator of mitochondrial biogenesis, and their interaction led to cell death through promoting CPT1A-regulated FAO and mitochondria-derived reactive oxygen species (ROS) accumulation [51]. Besides dysregulating mitochondrial function, CS exposure could also trigger cellular senescence in lungs. These senescent cells have limited tissue renewal and contribute to chronic inflammation [21]. Since mTOR signaling pathway is critical for cell growth and survival, activation of mTOR signaling was detected in lung species and cells derived from COPD patients [52]. In mice with conditional TSC1$^{-/-}$ (an inhibitor of mTOR) deletion, greater cellular senescence along with downregulated autophagy proteins were noted in airway endothelial cells or alveolar epithelial cells [52]. These mice were shown to progress with COPD-like lungs by rapidly developing emphysema, pulmonary hypertension, and vascular remodeling [52].
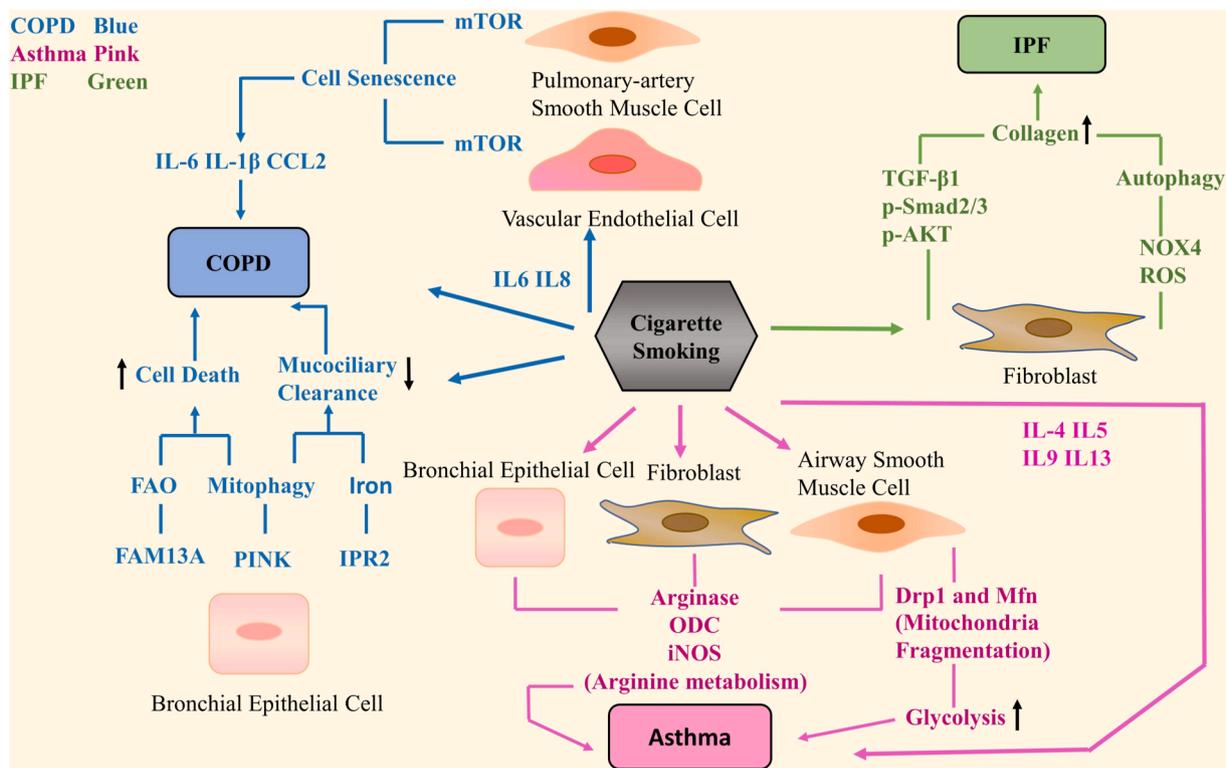
Fig. 1. A summary of cigarette smoking induced airway inflammatory diseases with indicated pathways contributing to disease development and progression. COPD, chronic obstructive pulmonary disease; Drp1, dynamin-related protein 1; FAM13A, family with sequence similarity 13 member A; iNOS, inducible nitric oxide synthase; IPF, idiopathic pulmonary fibrosis; IRP2, iron-responsive element binding protein 2; Mfn2, mitofusin 2; mTOR, mammalian target of rapamycin; NOX4, nicotinamide adenine dinucleotide phosphate reduced oxidase 4; ODC, ornithine decarboxylase; PINK1, PTEN-induced kinase 1; ROS, reactive oxygen species; TGF-β, transforming growth factor beta.

3.2. Targeting mitochondria

Since several metabolic pathways are related to mitochondrial function and in view of a close relationship between mitochondrial function and metabolic reprogramming, targeting the dysregulation of mitochondrial metabolism pathways could yield therapeutic opportunities for COPD [53]. For instance, lung tissue derived from COPD displayed an increase in the iron-responsive element binding protein 2 (IRP2), a major regulatory protein for cellular iron homeostasis [54]. In a comparison of iron load between wild-type mice with Irp2$^{-/-}$ mice, IRP2 was demonstrated to function in facilitating iron import into mitochondria after exposing mice to CS [54]. Moreover, low-iron diet or iron chelator (Deferiprone) treatments protected wild-type mice from the CS-induced impaired airway mucociliary clearance and pulmonary inflammation as well as lung injury, suggesting the use of iron chelator as a novel therapeutic approach for COPD [54]. Another strategy of improving mitochondrial function was through supplementing COPD patients with Qter (ubiquinone) and creatine [55]. Qter promotes mitochondrial oxidative phosphorylation to produce energy in the form of ATP, whereas creatine facilitates ATP recycling [55]. Following taking the supplement for 2 months, COPD patients had improved exercise capacity, body composition, and dyspnea accompanied with a change of the metabolic profile in mitochondria [55]. Given these observations, dietary supplement of Qter and creatine may provide beneficial effects to COPD patients; however, future studies are required to determine their effects in longer treatment period.

4. Asthma

Asthma is the most common chronic lung disease affecting millions of people worldwide [56]. The prevalence of asthma in developed western countries is rising on a year-by-year basis, while it is far lower in developing countries [56]. Asthma is a constellation of clinical symptoms encompassing wheezing, breathlessness, chest tightness and coughing in both children and adults [56,57]. The onset of these symptoms is influenced by individual immunological factors, age, and sex as well as extrinsic stimuli like exercise and inhalation of irritants, so as the disease progresses overtime, individuals experience different level of airway inflammation, airway hyper-responsiveness and airway remodeling [56,57]. Apart from the above-mentioned factors, cigarette smoking is another independent risk factor for asthma [58,59]. Plaschke et al. have found that smoking was a risk factor for onset of asthma, and there was a strong association of smoking and onset of asthma in non-atopics [12]. Particularly, persistent cigarette smoke (CS) exposure in allergic individuals augments T helper 2 (TH2) driven inflammation, releasing cytokines such as interleukin (IL)-4, IL-5, IL-9, IL-13 to trigger allergic response through facilitating IgE production, eosinophils and mast cell activation and airway hypersensitivity [60–62] (Fig. 1).

4.1. Metabolic reprogramming and airway remodeling

Although inflammation is an important component of allergic response, metabolic reprogramming contributes to the pathogenesis of asthma as well. The TH2 driven inflammation in asthma is related to arginine metabolism [63]. In arginine metabolism, L-arginine is metabolized by either arginase (ARG I and II) or nitric oxide synthase (NOS) to support distinct cellular functions [64,65]. Both ARG I/II hydrolyze L-arginine into urea and ornithine [64,66]. ARG I is predominantly expressed in liver, while mitochondrial ARG II is found in extrahepatic tissues [66,67]. The distinct expression patterns of ARGs leads to a hypothesis that ARG I predominantly carries out ureagenesis, and ARG II engages in other functions that it utilizes ornithine as the precursor for proline and polyamine biosynthesis to produce collagen and promote cell proliferation, respectively [66]. On the other hand,
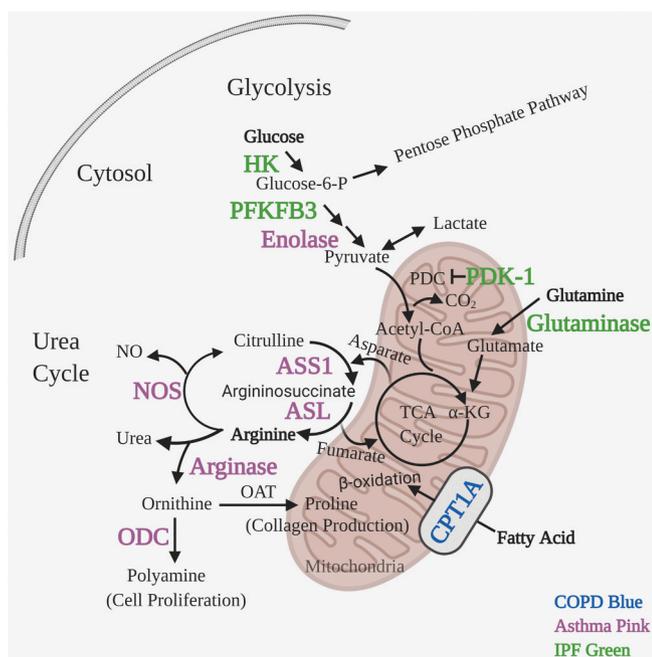
Fig. 2. Glycolysis, TCA cycle, fatty acid oxidation, and urea cycle are components of cellular metabolism. As metabolic reprogramming is identified in each airway inflammatory disease, metabolic enzymes are color-coded to match with their corresponding diseases. COPD, chronic obstructive pulmonary disease; ASL, argininosuccinate lyse; ASS1, argininosuccinate synthase; CPT1A, carnitine palmitoyltransferase; HK, hexokinase; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; PDK-1, pyruvate dehydrogenase kinase; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3.

L-arginine can be metabolized by inducible NOS (iNOS) and two other isoenzymes (neuronal NOS and endothelial NOS) to generate nitric oxide (NO) and citrulline [65]. NO is a pivotal gaseous signaling molecule in cells, whereas citrulline can return to urea cycle and supply for arginine biosynthesis [65]. Because both arginase and nitric oxide synthase contribute to L-arginine metabolism, some findings even suggested that an increased arginase expression reduces substrate (L-arginine) availability to NOS pathways, although more studies are required to support this notion [65,68]. In the case of asthma, the expression of argininosuccinate synthase (ASS1) and argininosuccinate lyase (ASL) in de novo arginine biosynthesis along with arginase II (ARG II) and iNOS in arginine metabolism were all upregulated in asthmatic airway epithelial cells [63] (Fig. 2). The overexpression of ARG II increased mitochondrial oxidative metabolism in addition to suppressing STAT6 phosphorylation [63]. In light of the fact that STAT6 was the central downstream signal of TH2 inflammation, ARG II overexpression was demonstrated to blunt TH2 immunity responses in bronchial epithelial cells [63]. The study indicated a higher ARG II and iNOS expression, but no change was associated with ARG I expression in asthmatic airway cells. However, another study detected an elevated ARG I expression in smoking asthmatic airway [69]. As compared to nonsmoking asthmatics, a higher immunostaining as well as mRNA expression of ARG I and ornithine decarboxylase (ODC) were observed in epithelium and smooth muscle bundle derived from smoking asthmatics, whereas no significant change of iNOS was observed in both smoking and nonsmoking asthmatics [69]. Nicotine exposure was shown to cause the upregulation of ARG I and ODC mRNA in airway epithelial cells [69], indicating a CS-induced arginine reprogramming in smoking asthmatics.

In addition to immune response, CS exposure was reported to affect airway smooth muscle (ASM) cells to induce airway hyperresponsiveness and remodeling [70,71]. Exposure of nonasthmatic ASM cells to cigarette smoke extract (CSE) triggered mitochondrial fragmentation by inducing dynamin-related protein 1 (Drp1) expression, a

master regulator of mitochondrial fission, while downregulating the expression of mitochondrial fusion regulator, mitofusin 2 (Mfn2) [72]. This alteration of fission and fusion proteins was also found in asthmatic ASM cells and could be further exacerbated upon CSE exposure [72]. In a follow up study, CSE exposure not only induced mitochondrial destabilization but also promoted ASM cell proliferation mediated by glucose reprogramming [73]. The expression of enolase, which converts 2-phosphoglycerate to phosphoenolpyruvate in glycolysis, was elevated in ASM cells upon CSE or Mfn siRNA treatments [73]. They also observed that the airways derived from mice exposed to CS were significantly thickened and fibrotic, implying that mitochondrial fragmentation and increased glycolysis influx consequently lead to airway remodeling [73]. Since the increased hypertrophy (cell size) and hyperplasia (cell number) of ASM cells augmented airway remodeling, the findings suggested CS-induced mitochondria fragmentation and glucose reprogramming contributed to ASM cells proliferation and airway remodeling in asthmatic smokers [73].

4.2. Targeting arginine metabolism

Active smokers with asthma may develop accelerated decline of lung function, limited response to short-term corticosteroid treatment and overall poor disease control [58,59]. This could be due to the potential additive or synergic effect of CS with inflammatory response and airway remodeling [58,59]. As CS exposure alters arginine metabolism in asthma, targeting arginine metabolism in treating severe asthma was a potential approach. Liao and his colleagues have examined clinical effects of L-arginine supplement in patients with severe asthma [74]. Because high fractional exhaled NO (FeNO) attributed to iNOS activity indicates an increase of arginine turnover and worse clinical outcomes, L-arginine supplement was given to asthmatic patients with low or high FeNO, and their accidents of exacerbation were evaluated after 3-month treatment [74]. In a subgroup of 28 subjects, 8 individuals responded to the treatment with at least 33% reduction in exacerbation [74]. Although no significant decrease in asthma exacerbation was observed, potential biomarkers including prostaglandin H2 and Nα-acetyl-L-arginine were found to distinguish responders versus non-responders [74]. Further work is required to validate these markers, and a larger sample size would allow for determination of the significance of L-arginine treatment in patients with severe asthma. In addition to the L-arginine supplement, an open-labeled study has revealed that 2-week L-citrulline supplement improved forced exhalation volume in 1 second (FEV$_1$) in obese female patients with late onset and poorly controlled asthma [75]. Their findings suggest the clinical relevance of targeting arginine metabolism in asthmatic patients. However, it remains unclear whether arginine reprogramming can crosstalk with other cellular signaling pathways to further influence asthma progression. If such crosstalk exists, this could limit the pharmacological efficacy of L-arginine or L-citrulline supplement in treating severe asthma.

5. Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and irreversible interstitial lung disease of unknown etiology [76]. Accumulative studies have suggested that cigarette smoking, occupational/environmental factors, and possibly viral infection are risk factors for IPF [76]. A case-control study has revealed that a history of smoking increased the risk of IPF development, but the mechanisms accounting for smoking-induced IPF progression remains elusive [13]. The initial hypothesis of IPF pathogenesis emphasized the role of inflammation in driving fibrosis development, but anti-inflammatory or immunosuppressant agents were indicated harmful to IPF patients during multiple clinical trials [77,78]. Therefore, a favored model of IPF pathogenesis has evolved since then. It holds that as insults repeatedly damage distal pulmonary epithelial cells, aberrant tissue repair triggers uncontrolled fibroblast activation and myofibroblast differentiation at lung

parenchyma, leading to the end-stage tissue scaring [76]. The aggregate of activate fibroblasts is a hallmark feature of IPF, termed fibroblastic foci, wherein myofibroblasts produce excessive extracellular matrix deposition that destroy alveolar space and ultimately respiratory failure [79,80]. Two antifibrotic drugs, nintedanib and pirfenidone, FDA-approved for IPF treatments were demonstrated to slow down decline of lung function. Unfortunately, fibrosis persists in IPF patients receiving the drugs, so most patients still die within 3–5 years after diagnosis [76,81].

5.1. Metabolic reprogramming and collagen production

To overcome the limitation of current IPF treatments, efforts have been made to identify novel cellular mechanisms and therapeutic targets. As a useful tool to provide mechanistic insights into a disease, global metabolomic profiling has been used to successfully predict biomarkers of cystic fibrosis exacerbation [82]. This finding, together with other metabolomics studies done on COPD and asthma, all indicate the promise of metabolomics approach in studying IPF. Characterization of the metabolic profiles in samples derived from IPF patients or healthy controls confirm that metabolites from glycolysis, TCA cycle, fatty acid, and arginine metabolisms were altered at different degrees [83,84]. For example, cellular level of arginine remains the same in both normal and IPF lung samples, but an increased level of polyamine, creatine and 4-hydroxyproline metabolites was observed in IPF lung, suggesting elevated cellular proliferation, ATP recycling and collagen biosynthesis, respectively [83]. Upon recognition of metabolic dysregulation in IPF, the transforming growth factor beta (TGF-β), a potent inducer of fibrosis, was found to influence metabolic pathways for promoting myofibroblast differentiation and their functions [85]. The expression of hexokinase isoform, hexokinase 2 (HK2), which phosphorylates glucose into glucose-6-phosphate, was higher in IPF lung fibroblasts to aid TGF-β mediated profibrotic events [86] (Fig. 2). Furthermore, glutaminolysis, the process of metabolizing glutamine into glutamate, was enhanced by TGF-β1 by inducing glutaminase 1 expression in lung myofibroblasts [87,88]. This process, as a result, facilitated fibroblast differentiation and collagen production [86–88].

Although how cigarette smoking mechanistically causes pulmonary fibrosis is not as well characterized as TGF-β1, the effect of cigarette smoke (CS) has been evaluated in rodent and cell-based experiments. Bleomycin-induced pulmonary fibrosis is a standard animal model for studying IPF, and co-administered CS and bleomycin to guinea pig increased α-smooth muscle positive cells and lung collagen content compared with bleomycin alone [89]. It was suggested that CS exposure created a profibrotic milieu to further potentiate the bleomycin-induced lung fibrosis [89]. *In vitro* cigarette smoke extract (CSE) treatment in lung fibroblasts induced p-Smad2/3 and p-AKT as well as collagen synthesis [90]. This treatment further enhanced TGF-β1 mediated p-Smad2/3 and p-AKT signaling pathways in the lungs of bleomycin-treated mice [90] (Fig. 1). In addition, human lung fibroblasts derived from IPF patients with smoking history had elevated expression of nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase 4 (NOX 4), generating intracellular reactive oxygen species (ROS) [91]. NOX4 expression interacted with TGF-β1-mediated p-Smad2/3 pathway to support myofibroblast differentiation [91]. The activity of NOX4 also contributed to defective autophagy when lung fibroblasts were exposed to CSE [92]. It is well-established that autophagy regulates cellular energetic balance by incorporating cellular contents in autophagosomes then degrading them in lysosomes, and the final products are recycled for cellular energy stores during nutrient deprivation or cellular damage [93]. In IPF, insufficient autophagy is detected, wherein it causes accelerated lung epithelial senescence and fibroblast differentiation [94]. Exposure of lung fibroblasts to CSE was found to cause insufficient autophagy, limiting the ability of collagen removal in cells [92]. These fibroblasts had elevated protein expression of α-collagen I and NOX4 with increased intracellular H_2O_2 production.

The impaired autophagy resulted from inhibition of lysosomal protease activity promoted collagen synthesis in lung fibroblasts. Despite the findings highly suggesting that CSE-induced defective autophagy enhances collagen production, no animal data were provided in this study. Therefore, it remains unclear whether the impaired autophagy can enhance fibrosis or collagen deposition in mouse lungs upon CSE and bleomycin challenge. Further work is needed to characterize CS-mediated metabolic dysregulation in lungs derived from IPF patients or the bleomycin model.

5.2. Targeting glycolytic flux

Currently, many pre-clinical studies have evaluated the effect of targeting glycolytic reprogramming in pulmonary fibrosis. A recent study has confirmed that inhibition of hexokinase with glucose analogue (2-deoxyglucose, 2-DG) or pyruvate analogue (3-bromopyruvate) suppressed lung myofibroblast differentiation [95]. Another key enzyme regulating glycolytic influx, termed 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), was also upregulated in myofibroblasts [95]. Treatment with its potent inhibitor, 3-(3-Pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) in lung myofibroblasts or the bleomycin/TGF-β1-induced mice was shown to attenuate myofibroblast-associated profibrotic functions as well as lung fibrosis. On the other hand, pyruvate dehydrogenase kinase 1 (PDK-1), a metabolic enzyme linking glycolysis, TCA cycle and fatty acid metabolism together, is also an attractive target [96,97]. Another preclinical study has reported that administration of dichloroacetate (DCA), the potent PDK inhibitor, effectively inhibited myofibroblast differentiation and pulmonary fibrosis in mice [96]. Table 1 summarizes a list of metabolic enzymes whose activities are implicated in the pathogenesis of COPD, asthma and IPF. These findings together suggest that targeting metabolic reprogramming is a potential therapeutic approach. However, it remains unknown whether combining the metabolic inhibitors with current treatments would provide any beneficial or adverse effects.

6. Conclusion

In addition to the significant advances in understanding pulmonary diseases, the application of metabolomics analysis provides information of putative biomarkers and novel mechanistic insights through indicating changes in metabolites of biofluids or tissue samples derived from patients. Indeed, the biological samples from COPD, asthma and IPF patients were demonstrated with metabolic dysregulation, suggesting the attribution of metabolism in pulmonary diseases. Although cigarette smoke (CS) exposure can lead to a change of metabolic enzymes expression in cells or animals, diseases share different patterns of metabolic dysregulation. In COPD studies, the expression of metabolic enzymes located at mitochondria was altered to induce cell death and subsequently COPD-like lungs in mice. *De novo* arginine biosynthesis in addition to arginine metabolism drive asthma progression, in which isoenzymes of arginases seemed to contribute significantly. The potent fibrotic stimulator, TGF-β, can broadly promote metabolic dysregulation in active fibroblasts to induce myofibroblast differentiation. Preclinical studies have demonstrated that targeting of metabolic enzymes is capable of preventing disease development and progression, and some clinical studies have also suggested the benefits of amino acid supplement in improving disease states. These exciting findings all together have demonstrated metabolic dysregulation as a driver of pulmonary diseases, and targeting metabolism represents a novel therapeutic approach.

However, there are challenges to be addressed in this approach. In light of the fact that metabolism delivers energy and metabolites for powering cellular functions, tissue-specific protein expression is crucial for governing proper functions. The isoenzymes, arginase I/II, predominantly express in different organs (liver versus extrahepatic organs) in healthy state, but arginase I expression is elevated in asthmatic

Table 1

The functional consequence of metabolic enzymes in airway inflammatory diseases.

Enzyme	Pathway	Disease/Functional consequence	Reference Number
PTEN-induced kinase 1 (PINK1)	Mitophagy	COPD: CS-induced PINK1-dependent mitophagy promotes cell death and mitochondrial dysfunction	[48]
Carnitine palmitoyltransferase (CPT1A)	Fatty acid oxidation (FAO)	COPD: CS elevated CPT1A expression to promote FAO-mediated ROS production and cell death	[51]
Mammalian target of rapamycin (mTOR)	Cell senescence	COPD: increases mTOR activation induces lung cell senescence	[52]
Iron regulatory proteins 2 (IRP2)	Iron homeostasis	COPD: CS-induced IRP2 overexpression increases mitochondrial iron loading and mitochondrial dysfunction	[54]
Argininosuccinate synthase (ASS1) Argininosuccinate lyase (ASL) Arginase II (ARGII) Ornithine decarboxylase (ODC) Inducible nitric oxide synthase (iNOS)	Urea cycle	Asthma: elevated ARGII expression suppresses pathological signaling pathways in asthma inflammation	[63]
Arginase I (ARG I) ODC iNOS	Arginine metabolism	Asthma: Upregulated expression of ARG I and ODC detected in smoking asthmatics	[69]
Dynamin-related protein 1 (Drp1) Mitofusin (Mfn) 2	Mitochondria	Asthma: CS increased Drp1 and decreased Mfn2 expression to induce mitochondrial fragmentation	[72]
Enolase	Glycolysis	Asthma: CS elevated enolase expression to promote glycolysis for cell proliferation	[73]
Hexokinase 2 (HK2)	Glycolysis	IPF: elevated expression of HK2 is required for profibrotic TGF-β-mediated cell migration and colony formation	[86]
Glutaminase 1 (GLS1)	Glutaminolysis	IPF: TGF-β1 induces GLS1 expression to increase glutaminolysis for myofibroblast differentiation and collagen production	[87,88]
Nicotinamide adenine dinucleotide phosphate reduced oxidase 4 (NOX4) α-Collagen I Synthesis	Autophagy	IPF: CS induces insufficient autophagy by upregulating NOX4 expression and α-Collagen I synthesis	[92]
Fructose-2,6-biphosphatase 3 (PFKFB3)	Glycolysis	IPF: upregulated PFKFB3 expression augments glycolysis to promote	[95]

Table 1 (*continued*)

Enzyme	Pathway	Disease/Functional consequence	Reference Number
Pyruvate dehydrogenase kinase 1 (PDK-1)	Glycolysis	myofibroblast differentiation and contractility IPF: overexpression of PDK-1 increases lactate accumulation to promote myofibroblast differentiation	[96]

CS: cigarette smoke.

airways from smoking asthmatics as compared with asthmatic nonsmokers. This observation suggested that activation of isoenzymes in tissues could promote disease progression. Likewise, different metabolic enzymes in glycolysis cycle were found overexpressed in IPF lung fibroblast, and targeting these enzymes were demonstrated as a potential therapeutic target for pulmonary fibrosis. However, cells from other tissues/organ also rely on the same metabolic pathways as a part of cellular homeostasis. It remains difficult to target the metabolic enzymes effectively and specifically without disturbing the metabolic pathways in healthy cells. Although preclinical studies have demonstrated that inhibiting the activity of a metabolic enzyme or metabolic pathways could alleviate disease development and progression, unknown adverse effects may diminish their clinical relevance. Since metabolism encompasses complexed pathways which form circuits with other signaling pathways, inhibiting an enzyme activity of a metabolic pathway could modulate other components within the pathway or different circuits to meet cellular demand in a compensatory manner. Furthermore, a comprehensive analysis of common metabolic landscape in cancer has been greatly investigated, but this piece of information is missing in pulmonary diseases [98,99]. It is unclear if specific metabolic alterations are common in inflammatory or fibrosis diseases, and whether the metabolic signature of pathological tissues is reminiscent of the normal. Further investigations are warranted to fill in the gap of our knowledge. By improving our understanding of metabolic dysregulation in lungs, we will be able to identify therapeutic targets that can be translated into clinical practice, improve pharmacological efficacy and minimize adverse effects of future treatments.

Author's contributions

Writing, review, and revision of the manuscript: LL; Review and revision of manuscript: DY; Review and study supervision: CHC.

Declaration of competing interest

The authors declare that they have no competing financial interests with the study.

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