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REVIEW

Untapping the Potential of Astragaloside IV in the Battle Against Respiratory Diseases

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Abstract: Respiratory diseases are an emerging public health concern, that pose a risk to the global community. There, it is essential to establish effective treatments to reduce the global burden of respiratory diseases. Astragaloside IV (AS-IV) is a natural saponin isolated from *Radix astragali* (Huangqi in Chinese) used for thousands of years in Chinese medicine. This compound has become increasingly popular due to its potential anti-inflammatory, antioxidant, and anticancer properties. In the last decade, accumulated evidence has indicated the AS-IV protective effect against respiratory diseases. This article presents a current understanding of AS-IV roles and mechanisms in combatting respiratory diseases. The ability of the agent to suppress oxidative stress, cell proliferation, and epithelial-mesenchymal transition (EMT), to attenuate inflammatory responses, and modulate programmed cell death (PCD) will be discussed. This review highlights the current challenges in respiratory diseases and recommendations to improve disease management.

Keywords: astragaloside IV, *Radix astragali*, respiratory diseases, oxidative stress, inflammatory responses

Introduction

Respiratory diseases are one of the leading causes of death and disability globally, becoming a burden for patients and their caregivers.[1] Recently, the incidence of respiratory diseases worldwide has risen alarmingly, particularly in developed countries. A global investigation revealed an increase of 39.8% in 2017 from 1990, amounting to 544.9 million patients living with chronic respiratory disease.[2] Furthermore, chronic respiratory diseases were the third leading cause of death in 2017, behind cardiovascular diseases and neoplasms.[2] Despite recent advances in patient care and intervention, many respiratory diseases, such as pulmonary hypertension (PH) and idiopathic pulmonary fibrosis (IPF), still lack effective treatments and are thus impossible to cure. Therefore, researchers must discover new approaches to alleviate these debilitating diseases.

Natural products are an essential source for discovering and developing new drugs. Consequently, herbal medicine is gaining attention in the scientific community as a respiratory disease treatment.[3–13] One of the emerging natural compounds is astragaloside IV (AS-IV), a natural saponin (Figure 1) extracted from *Radix astragali* (Huangqi in Chinese) with multi-target therapeutic properties. Studies have reported the pharmacological effects of AS-IV, such as antioxidant,[14] anti-inflammatory,[15] anti-fibrotic,[16] and anticancer.[17] More importantly, AS-IV is potentially less toxic. For example, an earlier study revealed that oral administration of AS-IV at 10 mg/kg/day for 14 weeks had no adverse effects on rat liver and kidney functions.[18] Furthermore, AS-IV is the recommended treatment for various diseases in preclinical models, including cerebral ischemia,[19,20] atherosclerosis,[21] and cancer.[22] Various studies have also highlighted the potential therapeutic effects of AS-IV in various respiratory diseases, such as PH,[7] chronic obstructive pulmonary disease (COPD),[23] asthma,[24] lung cancer,[25] pulmonary fibrosis,[26] and lung injury.[27] Herein, this review discusses the role of AS-IV in respiratory diseases and the potential therapeutic efficacies from the existing literature.

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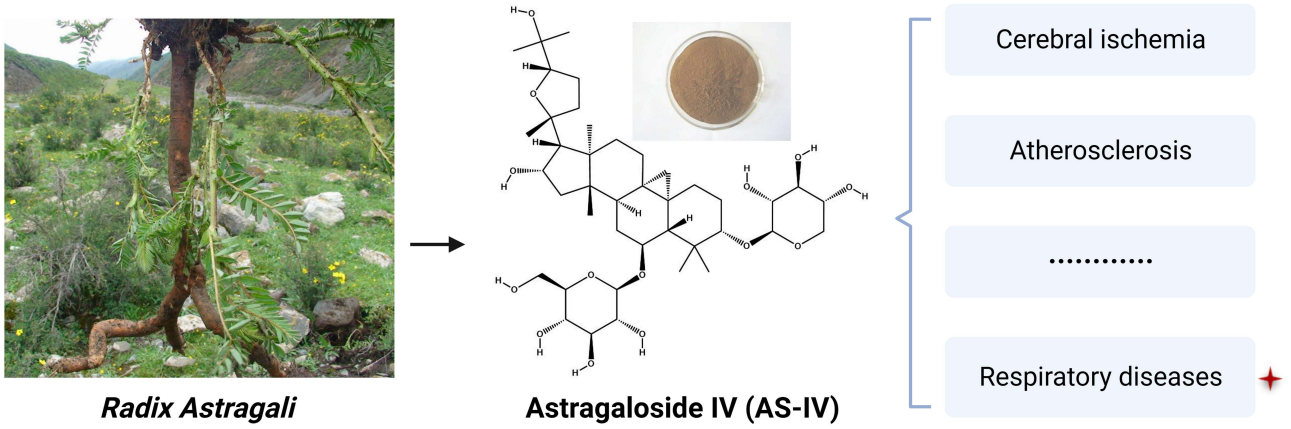

Figure 1 Brief introduction and profile of AS-IV. AS-IV is a natural saponin isolated extracted from *Radix astragali* (Huangqi in Chinese) and possesses a wide range of pharmacological activities including respiratory diseases treatment.

Roles of as-IV as a Treatment for Respiratory Diseases

In the past two decades, AS-IV reportedly improved the symptoms of respiratory diseases, especially PH and pulmonary fibrosis, in cellular and animal models. However, the key pharmacological mechanisms remain unclear and controversial. This section discusses in detail the effects and mechanisms of AS-IV in treating various respiratory diseases. The cellular signaling pathways of PH and pulmonary fibrosis affected by AS-IV are illustrated in Figures 2 and Figure 3, respectively.

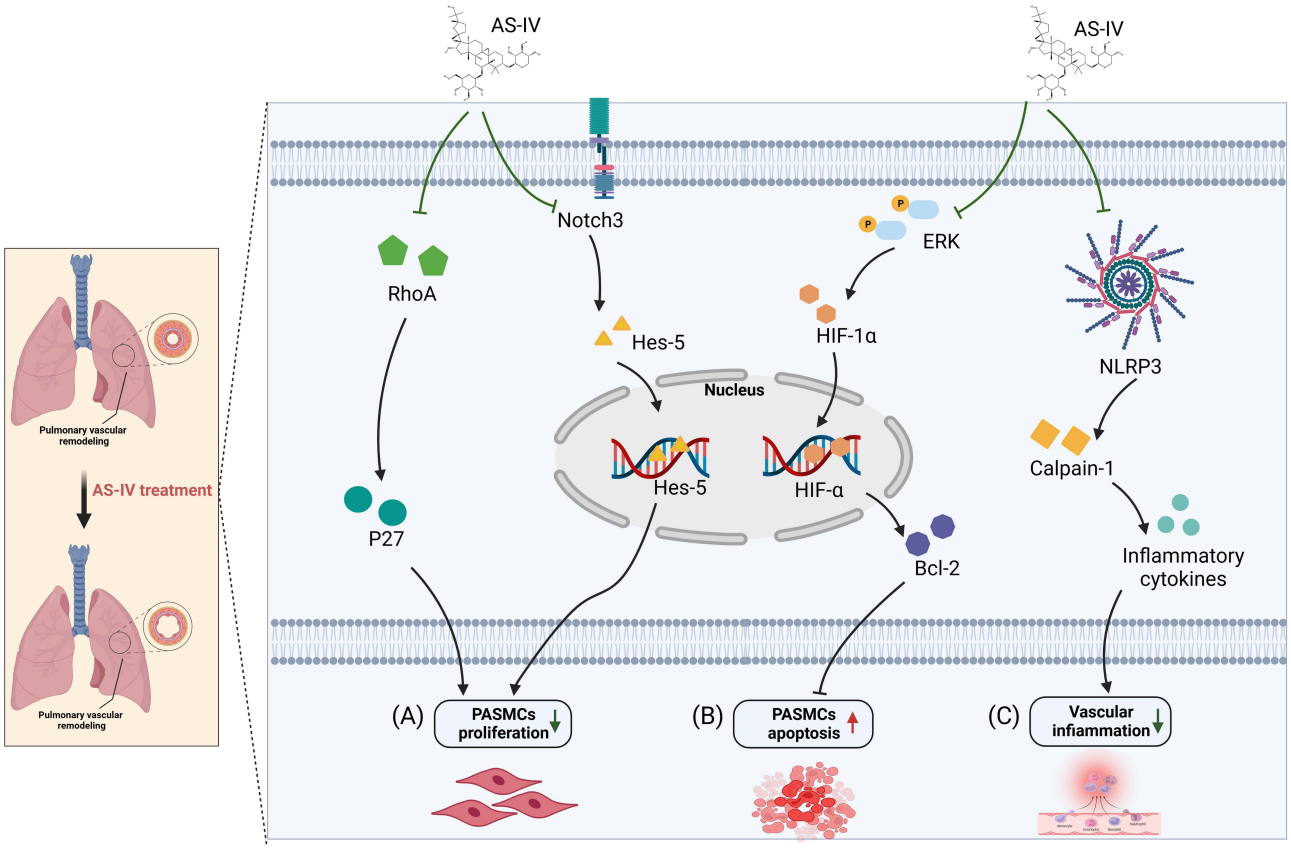

Figure 2 The targets and pathways of AS-IV in treating PH. (**A**). AS-IV inhibits PASMCs proliferation by suppressing Notch3 and RhoA pathway. (**B**). AS-IV ameliorated apoptosis resistance in PASMCs by downregulating Bcl-2, phospho-ERK, and HIF-1α expressions. (**C**). AS-IV attenuates inflammatory response mediated by NLRP-3/calpain-1. Created with BioRender.com.

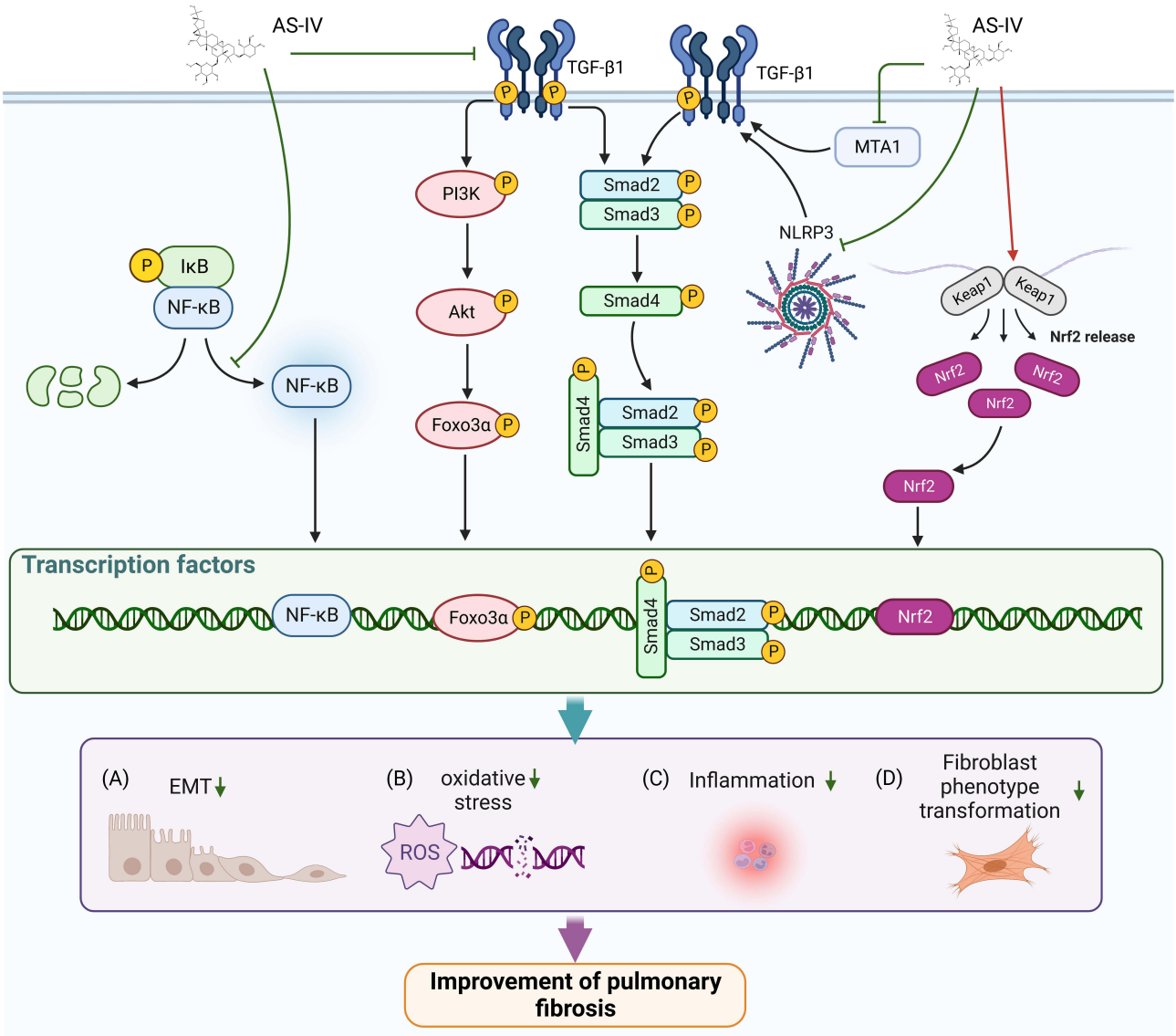

Figure 3 The targets and pathways of AS-IV in treating PF. (**A**). AS-IV inhibits the TGF-β1/Smads signaling pathway by suppressing MTA1 and NLRP3 expression, thereby inhibiting EMT; AS-IV also inhibits EMT by suppressing the TGF-β1/PI3K/AKT/Foxo3α signaling pathway. (**B**). AS-IV ameliorates oxidative stress via activation of Nrf2. (**C**). AS-IV alleviates inflammation by blocking NF-κB activation. (**D**). AS-IV blocks the differentiation of lung fibroblasts to myofibroblasts by inhibiting the TGF-β1/Smads signaling pathway. Created with BioRender.com.

AS-IV and PH

PH is a complex disorder characterized by pulmonary vascular remodeling and right ventricular hypertrophy, leading to right heart failure.[28] Currently, there are few FDA-approved treatments, and the clinical efficacy of these anti-PH drugs remains limited.[29] Plant-derived natural compounds with anti-PH properties offer new opportunities for developing low-toxicity and cost-effective drugs than synthetic alternatives.[30] Mean pulmonary artery pressure (mPAP) reduction and improved pulmonary vascular remodeling by AS-IV were first detailed by Zhang et al using a hypoxic PH rat model.[31] This finding became a starting point for further exploring of the anti-PH effects and mechanisms of AS-IV.

Inhibition of Pulmonary Arterial Smooth Muscle Cells (PASMCs) Proliferation in PH

Excessive proliferation of PASMCs is critical in the pathogenesis of pulmonary artery remodeling.[32] The Zhang group[31] reported that AS-IV is a potent PASMCs-proliferation inhibitor that could suppress pulmonary artery remodeling and lead to significant mPAP decline in rats with hypoxia-induced pulmonary hypertension. This study found that 50 μM AS-IV resulted in marked inhibition of hypoxia-induced PASMCs proliferation in vitro. Notch signaling, a highly

evolutionarily conserved signaling pathway, is vital in PASMCs proliferation. Notch-3 targets hes family bHLH transcription factor 5 (Hes-5), which is expressed exclusively in smooth muscle cells (SMCs) in adults and might be associated with SMC identity, maturation, and proliferation.[33,34] It has been proven in vitro and in vivo that AS-IV can reverse the hypoxia-induced PASMCs proliferation by suppressing Jagged-1, Notch-3, and Hes-5 expressions.[35] Furthermore, AS-IV inhibited the proliferation, migration, and adhesion of PASMCs under hypoxic conditions by downregulating RhoA and upregulating p27 at the protein level.[7]

Promoting PASMCs Apoptosis in PH

Resistance to apoptosis by PASMCs contributes to the pathophysiology of PH. Meanwhile, AS-IV ameliorated apoptosis resistance in PASMCs by downregulating Bcl-2, phospho-ERK, and hypoxia-inducible factor-1α (HIF-1α) expressions.[36]

Inflammation Regulation in PH

There is increasing evidence concerning the role of inflammation in pulmonary vascular remodeling. For instance, the Nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, comprising the NLRP3, the apoptosis speck-like protein containing a caspase-recruitment domain (ASC), and pro-caspase-1, mediate cytokine and inflammatory responses in PH.[37] Sun et al[38] reported that AS-IV treatments [(40 and 80 mg/kg/day, intraperitoneally (i.p.)] attenuated inflammatory response mediated by NLRP-3/calpain-1, thereby alleviating pulmonary vascular remodeling in monocrotaline (MCT)-induced PH in rats. In a recent study, AS-IV reportedly reduced the blood levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in MCT-induced PH rats.[36] Moreover, AS-IV reduced serum levels of endothelin-1 (ET-1), angiotensin II (AngII), TNF-α, and IL-6 in rats with hypoxia-induced PH.[31]

Summary Section

There are various causative factors of PH and AS-IV targets or pathways in treating the disease (Figure 2). Consequently, the preclinical mechanisms of AS-IV against PH have not been fully elucidated. Nonetheless, the literature indicated the potential of AS-IV as a treatment ingredient or prevention drug for PH, particularly in the absence of good clinical options for group 3 PH patients.

AS-IV and Pulmonary Fibrosis (PF)

PF, specifically IPF, is a highly confounding and fatal pathological process of unknown cause and is characterized by alveolar injury, fibroblast proliferation, and excessive deposition of extracellular matrix (ECM) proteins, progressively resulting in respiratory failure and death.[39] Current scientific evidence supports that AS-IV has a prominent anti-fibrotic and protective role against the progression of PF (Figure 3).

Inhibition of EMT in PF

Epithelial-mesenchymal transition (EMT) is a process in which fully differentiated epithelial cells are transformed into a mesenchymal phenotype. Numerous studies indicated EMT is a major driver of fibrosis and is involved in the pathological process of PF.[40] Transforming growth factor-β1 (TGF-β1) is an essential pro-fibrotic factor that induces EMT via Smad-dependent or Smad-independent pathways. In 2018, Qian et al[41] reported that AS-IV demonstrated protective effects against EMT in bleomycin (BLM)-induced pulmonary fibrosis in rats by suppressing TGF-β1/phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (Akt)-induced Forkhead box O3α (FOXO3α) hyperphosphorylation. Subsequently, they demonstrated that AS-IV blocked TGF-β1-induced EMT in alveolar type II epithelial (RLE-6TN) cells by inhibiting metastasis-associated gene 1 (MTA1) expression.[42]

In another study, NLRP3 inflammasome activation reportedly promoted EMT in alveolar epithelial cells with BLM treatment by upregulating TGF-β1/Smad2/3-mediated transcriptional activity.[43] Meanwhile, Hou et al[44] indicated that AS-IV could attenuate EMT in alveolar epithelial cells with BLM treatment by regulating NLRP3/TGF-β1 signaling pathway. Finally, AS-IV treatment could also retard pathological progression by inhibiting EMT in other disease models, such as diabetic nephropathy,[45] renal fibrosis,[46] and gastric cancer.[47]

Regulation of Inflammation and Oxidative Stress in PF

A recent study[48] illustrated that increased levels of malondialdehyde (MDA), total antioxidant capacity (T-AOC), reactive oxygen species (ROS), IL-1β, TNF-α and IL-6 in the bronchoalveolar lavage fluid (BALF) in BLM-induced PF rats were substantially down-regulated by AS-IV treatment (10, 20 and 50 mg/kg/day, i.p.). This outcome indicated the potential of AS-IV in reducing lung tissue injury by inhibiting inflammation and oxidative stress in BLM-induced PF rats. In addition, AS-IV (20 mg/kg/day, i.p.) treatment inhibited inflammation by impeding NF-κB pathway in silica-induced PF rats. Tong et al[49] also reported that AS-IV, combined with ferulic acid, protected lung tissue from oxidative stress by upregulating nuclear factor-E2-related factor (Nrf2) in BLM-induced PF mice.

Inhibition of Lung Fibroblast to Myofibroblast Differentiation

It is well-recognized that the uncontrolled proliferation of lung fibroblasts and excessive differentiation of fibroblasts into myofibroblasts produce ECM proteins that contribute to lung fibrosis. In 2019, Li et al[50] demonstrated that AS-IV inhibited fibroblast collagen production and myofibroblast transformation via TGF-β1/Smads signaling pathway. Furthermore, it was concluded that AS-IV (10 mg/kg/day, i.p.) reduced blood levels of type III collagen (collagen-III), laminin (LN), and hyaluronic acid (HA) and hydroxyproline (HYP) in lung tissues of BLM-induced PF rats.[51]

Summary Section

Excessive deposition of ECM proteins, inflammation, oxidative stress, and chemoresistance are the major factors of PF progression. Though pirfenidone and nintedanib are approved for IPF treatment, their clinical utility is limited by toxic side effects such as gastrointestinal problems, photosensitivity and skin reactions. Therefore, it is postulated that AS-IV alone or in combination with other drugs is a promising alternative for IPF treatment, and further research is needed to meet increasing clinical demands.

AS-IV and Lung Cancer

The anticancer properties of AS-IV are observed in the immunomodulatory action in cancer treatment and antagonizing the development of lung cancer through multiple pathways, such as inhibiting cell proliferation, migration, and invasion and enhancing the sensitivity of chemotherapy and targeted therapy.[52]

Improvement of Tumor Immunosuppressive Microenvironment in Lung Cancer

Cancer is characterized by immune escape and an immunosuppressive microenvironment. Consequently, tumor microenvironment (TME) remodeling has become an important research direction for current lung cancer treatment.[53–55] Indoleamine 2.3-dioxygenase (IDO) is a tryptophan catabolic enzyme that induces immune escape in lung cancer cells.[56,57] Zhang et al[58] reported that AS-IV reduced tumor growth in the Lewis lung cancer model by suppressing IDO expression to upregulate cytotoxic T lymphocytes (CTLs) and downregulate regulatory T cells (Tregs) activities. In addition, GBP1 binds to IDO1 and promotes the extracellular secretion of IDO.[59] In vitro and in vivo studies exhibited that AS-IV reduced the extracellular secretion of IDO1 by blocking the interaction between IDO1 and GBP1, reducing T-cell depletion and inhibiting lung cancer progression.[59]

Tumor-associated macrophages (TAMs) or M2-polarized macrophages are essential immunosuppressive cells in the TME,[60–62] and their increased infiltration in tumor tissue is often associated with poor prognosis. Thus, inhibiting M2-polarized macrophage activity becomes a promising therapeutic strategy for lung cancer. Xu et al[63] found that AS-IV significantly decreased IL-13- and IL-4-induced M2 macrophage polarization and attenuated M2-CM-induced invasion, migration, and angiogenesis in A549 and H1299 cells by inhibiting adenosine monophosphate (AMP)-activated protein kinase α (AMPKα) activation. Likewise, an in vivo study demonstrated that AS-IV inhibited tumor growth in the Lewis lung cancer mice model and reduced M2 macrophage infiltration in tumor tissue.[63] Therefore, TME remodeling could be a new avenue to explore the AS-IV anti-tumor mechanism of action.

Inhibition of Cell Proliferation, Invasion, and Migration in Lung Cancer

A study reported that AS-IV could inhibit the invasion and migration of A549 cells by restraining the protein kinase C-alpha/extracellular signal-related kinases 1 and 2/nuclear factor-κB (PKC-α/ERK1/2/NF-κB) signaling pathway and

downregulating matrix level metalloproteinase-2 (MMP-2), MMP-9, integrin β1, transforming growth factor β1 (TGF-β1), TNF-α and IL-6 in A549 cells.[64] Moreover, AS-IV inhibited the growth and promoted apoptosis in three NSCLC cell lines (HCC827, H1299, and A549) by suppressing the protein kinase B/glycogen synthase kinase-3β (Akt/GSK-3β)/β-catenin signaling pathway.[65]

Enhanced the Sensitivity to Chemotherapy and Targeted Therapies in Lung Cancer

Cisplatin resistance is the most critical cause of chemotherapy failure in lung cancer patients.[66–69] An in vitro study showed that AS-IV enhanced the sensitivity of non-small cell lung cancer (NSCLC) cell lines to cisplatin by inhibiting autophagy and endoplasmic reticulum (ER) stress.[70] Furthermore, AS-IV elevated the sensitivity of A549, HCC827, and H1299 lung cancer cells to cisplatin by inhibiting B7-H3 protein expression.[71] Targeted therapy is also an essential treatment for NSCLC and faces a similar challenge to chemotherapy in treatment resistance.[72] A recent study stated that AS-IV enhanced A549 cells sensitivity to bevacizumab, potentially by suppressing autophagy and activating the Akt/mTOR signaling pathway.[25] Moreover, gefitinib combined with AS-IV treatment was more effective in restraining NSCLC cell proliferation than gefitinib alone.[73]

Summary Section

It is increasingly recognized that chemoresistance and immune escape pose major obstacles to the therapeutic management of lung cancer. Encouragingly, existing evidence indicated that AS-IV ameliorated chemoresistance, reshaped the tumor immune microenvironment, and may shed the new dawn on the treatment of lung cancer.

AS-IV and Lung Injury

Various pathological factors such as fine particulate matter (PM2.5) and paraquat (PQ) can induce lung injury, damaging critical lung functions. Multiple studies have demonstrated that AS-IV could be a protector against lung injury by decreasing inflammatory responses and regulating PCD.

Regulation of Programmed Cell Death in PM2.5-Induced Lung Injury

PM2.5 is currently the most critical factor in lung diseases caused by environmental pollution.[74,75] Previous studies indicated that PM2.5-induced pulmonary injury is associated with the activation of multiple PCD pathways, including ferroptosis,[76] pyroptosis,[77] and autophagy.[78]

Autophagy is essential for cellular homeostasis as an evolutionarily-conserved intracellular degradation pathway.[79] Multiple studies suggested that autophagy is pivotal in the pathogenesis of lung injury.[80] Pei et al[81] demonstrated that AS-IV exerted a protective role in PM2.5-induced lung injury in rats by inhibiting autophagy via PI3K/Akt/mammalian target of the rapamycin (mTOR) signaling pathway. Conversely, Wang et al[82] exhibited that AS-IV mitigated PM2.5-induced lung toxicity in rats by activating autophagy the AMP-activated protein kinase (AMPK)/mTOR signaling pathway. These results suggested that autophagy acts as a double-edged sword in PM-2.5-induced lung injury, and AS-IV exerted therapeutic benefits in lung injury via autophagy induction or autophagy flux inhibition.

Ferroptosis is characterized by the iron-dependent accumulation of lipid hydroperoxides and can induce various respiratory diseases.[83] This PCD mechanism is possibly involved in the pathological cell death associated with COPD,[84] PH,[85] and lung injury.[86] Glutathione peroxidase 4 (GPX4) is a known deterrent for ferroptosis. In a PM2.5-induced mouse model, AS-IV suppressed ferroptosis, inflammation, and oxidative stress through Nrf2/SLC7A11/GPX4 signaling pathway, resulting in a protective effect on lung tissue.[27]

Pyroptosis is a pro-inflammatory form of PCD resulting from the activation of caspase-1 within the inflammasome complex and caspase-11 (caspase-4/5 in humans) following intracellular lipopolysaccharide (LPS) recognition.[87] Huang et al[88] revealed the protective role of AS-IV against PM2.5-induced lung toxicity by suppressing NLRP3 inflammasome-mediated pyroptosis via NLRP3/caspase-1 axis inhibition. Consequently, PM2.5-induced lung inflammation and oxidative damage were prevented, leading to prolonged survival in mice. Furthermore, Wu et al[89] reported that AS-IV could prevent PM2.5-induced lung injury in rats by inhibiting the toll-like receptor 4 (TLR4)/MyD88/NF-κB signaling pathway and inflammation, besides delaying lung tissue injury.

Amelioration of Inflammation in Paraquat-Induced Lung Injury

PQ can cause multi-system injury, particularly severe lung tissue damage. Chen et al[90] revealed that AS-IV reduced Txnip/Trx expression and suppressed the Rho/ROCK/NF-κB signaling pathway in PQ-challenged mice, thus, alleviating pulmonary tissue injury.

Hypoxia/reoxygenation (HR) of pulmonary organization can also induce apoptosis in alveolar epithelial cells, resulting in lung impairment. Li et al[91] demonstrated that 1nM AS-IV inhibited TLR4/NF-κB pathway through the upregulation of miR-21-5p, thereby attenuating HR injury-induced type II alveolar epithelial cell apoptosis in vitro. Furthermore, pulmonary ischemia/reperfusion (I/R) lung injury can severely limit the postoperative lung function recovery and contribute to complications such as PH, pulmonary edema, and respiratory failure, eventually leading to patient mortality. Notably, AS-IV attenuated I/R lung injury in rats by reducing myeloperoxidase (MPO) levels in lung tissue and lung wet-to-dry (D/W) ratio.[92]

Summary Section

Impaired lung function due to lung and alveolar injury is a hallmark of many acute and chronic lung diseases. Because of its antioxidant and anti-inflammatory effects, AS-IV could mitigate lung injury triggered by various pathological factors. However, most related studies are based only on animal experiments and relevant clinical data is required in the future.

AS-IV and Asthma

Asthma is a heterogeneous disease with multiple underlying inflammatory pathways and structural airway abnormalities that influence the disease persistence and severity.[93] A study has revealed that AS-IV could inhibit airway inflammation and reduce airway hypersensitivity (AHR) by regulating various inflammatory cells and mediators, such as neutrophils, IL-4, and IL-10. Precisely, AS-IV treatment ameliorated airway inflammation and AHR in the ovalbumin (OVA)-sensitized allergic asthma mouse model by inhibiting the Janus kinase 2/signal transducer and activator of transcription 6 (JAK2/STAT6) signaling pathway.[24] In addition, AS-IV attenuated allergic inflammation by downregulating IL-4 and IL-10, upregulating interferon-γ (IFN-γ), and enhancing CD4(+) CD25(+) Foxp3 T cells in OVA-induced asthma mouse model.[94]

Multiple studies have portrayed the importance of inflamed bronchial epithelial cells in the pathological features of asthma exacerbation. An in vitro study indicated that AS-IV suppressed inflammation and oxidative stress in human bronchial epithelial cells by blocking the NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways.[95] In contrast, Jin et al[96] found that AS-IV improved AHR, attenuated lung inflammation, and reduced the production of inflammatory mediators, such as IL-4, IL-5, and IL-17, by inhibiting the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway in vivo.

Eosinophils are involved in developing asthmatic characteristics, including airway remodeling, hyper-responsiveness, and initiating allergic inflammation of the airways. Thus, an effective treatment for this disease would be to reduce eosinophil infiltration in the airways. Du et al[97] reported that AS-IV inhibited eosinophil infiltration in airways, hence, suppressing mucus hypersecretion, airway inflammation, and hyperreactivity in allergen-sensitized and challenged mice. Likewise, 50 μg/mL AS-IV significantly suppressed eosinophil activation induced by house dust mite allergen Dermatophagoides pteronyssinus (Der p) in vitro.[98] In summary, the anti-inflammatory and antioxidant feature of AS-IV offer potential treatment options for asthmatic patients.

AS-IV and COPD

COPD is a common chronic inflammatory disorder of the airways characterized by irreversible airflow limitation, ranking as the third highest cause of death worldwide.[99] Despite the incomplete knowledge regarding the pathological mechanism of COPD, airway inflammation and oxidative injury are involved in many aspects of the disease pathophysiology. Prolonged airflow restriction in COPD patients can lead to respiratory muscle fatigue, further aggravating the disease. For example, impaired diaphragm function often induces respiratory failure. Wang et al[100] found that AS-IV attenuated IL-8-induced apoptosis and inflammatory response in diaphragm cells by inhibiting AKT phosphorylation, caspase-3, and −9 protein expression, ROS, and inflammatory factor production in vitro.

Cigarette smoking (CS) remains the most critical risk factor for COPD.[101] Therefore, long-term exposure to cigarette smoke is currently the best method for establishing COPD rodent models. Studies on mice have depicted that AS-IV alleviated CS-induced pathological injury in lung tissue in a dose-dependent manner by suppressing JAK3/STAT3/NF-κB pathway.[23] Likewise, AS-IV significantly repressed the protein levels in JAK3/STAT3/NF-κB pathway in cigarette smoke extract (CSE)-induced human bronchial epithelial cells.[23] Furthermore, AS-IV has been proven to be a potent NF-κB pathway inhibitor and an antioxidant by exerting a protective effect against CS-induced airway inflammation in COPD rats.[102]

AS-IV and Coronavirus Disease 2019 (COVID-19)

COVID-19 remains a global pandemic to this day. Once contaminated with SARS-CoV-2, the patient's immune cells regularly secrete excessive pro-inflammatory cytokines, also termed "cytokine storms." The excessive inflammatory response caused by cytokine storms leads to multi-organ functional impairment in COVID-19 patients, often causing them to end up in the intensive care unit (ICU).[103] Early clinical research indicated that plasma levels of cytokines (IL-10, TNF-α, IL-2, and IL-10) and monocyte chemoattractant protein 1 (MCP1) were higher in ICU patients than in non-ICU patients.[104] Thus, hyperinflammation suppression may improve the prognosis of patients with severe COVID-19.

Network pharmacology and molecular docking have demonstrated the potential of AS-IV as a treatment option that may alleviate excessive inflammation in COVID-19 patients by inhibiting the NOD-like receptor signaling pathway.[105] Furthermore, a study has revealed that SARS-CoV-2 virus proliferated human cells via angiotensin-converting enzyme 2 (ACE2), thus, one of the key targets for COVID-19 inhibition. Ye et al[106] used molecular docking in combination with the surface plasmon resonance technique to investigate the potential of AS-IV in binding with ACE2, which may impede the invasion of SARS-CoV-2 into host cells. However, no preclinical trials have been performed to confirm the efficacy of AS-IV in treating COVID-19.

AS-IV and Obstructive Sleep Apnoea (OSA)

OSA is characterized by intermittent hypoxia and sleep disruption. Long-term chronic intermittent hypoxia can lead to oxidative stress and inflammatory responses in lung tissues and cells of OSA patients, resulting in a series of complications. In vitro illustrated that AS-IV significantly reduced IL-6, IL-1β, IL-8, MDA, ROS, and LDH levels in intermittent hypoxia-induced Beas-2B cells by inhibiting the TLR4/MAPK/NF-κB signaling pathway.[95]

Challenges and New Strategies for Application

Despite the promising therapeutic outcomes on lung diseases, several problems limit the clinical application of AS-IV. For instance, this natural saponin is a relatively large molecule (molecular weight = 784.97 g/mol) with poor solubility in water, leading to low bioavailability at targeted sites, particularly through oral administration. Gu et al[107] revealed that AS-IV exhibited a low absorption rate in a perfused rat intestinal model, and the oral bioavailability was only 2.2%. Similarly, Zhang et al[108] discovered that the absolute oral bioavailability of AS-IV in Beagle dogs is only 7.4%. Although various studies have indicated that the potential toxicity of AS-IV is low, others have reported the reproductive toxicity of this compound. For instance, Zhu et al[109] reported that AS-IV was maternally toxic in rats at 1 mg/kg and fetotoxic at > 0.5 mg/kg.

Liposomes are effective carriers for hydrophobic drugs, known for the efficacy enhancement and toxicity reduction. Rajesh et al[110] developed a novel multifunctional liposome to load AS-IV for drug delivery, which significantly improved the bioavailability and efficacy of the compound. Furthermore, utilizing nanomaterials in developing novel drugs is trending due to the ability to enhance the native drug efficacy substantially.[110] Interestingly, a recent study by Zhou et al[111] exhibited that Licorice-derived protein nanoparticles solubilized the insoluble AS-IV via encapsulation. Moreover, Sun et al[112] reported that dry age-related macular degeneration can be effectively managed with AS-IV-loaded lipid nanocapsules. Despite that, AS-IV nanoformulations are years away from clinical translation and require the joint efforts of pharmacologists, chemists, and material scientists to develop a stable, scalable, and effective products. Structural modification of a natural product improves drug efficacy and minimizes toxicity. Nonetheless, studies on the structural modification of AS-IV are lacking in existing literature.

Although multi-target modulation is a therapeutic feature of AS-IV in respiratory diseases, this method poses a significant obstacle to clinical translation. Multiple drug side effects may occur if drugs are not accurately targeted owing to the diverse physiological conditions and complex signaling pathways in vivo, thus, reducing drug efficacy. Therefore, identifying the specific therapeutic targets for AS-IV in lung disease is currently one of the crucial research directions. Cao et al[113] revealed that AS-IV attenuated renal fibrosis in diabetic nephropathy rats by blocking the NLR signaling through transcriptomic techniques. Furthermore, Fu et al have identified miR-26b-5p/ATF3/JUN as the major mediator of AS-IV's cardioprotective effect by transcriptome screening and experimental validation.[114] In addition, Xia et al[115] utilized quantitative proteomics to display that AS-IV inhibited cervical cancer cell invasion by targeting DCP1A and TMSB4X for autophagy induction. Therefore, modern biological techniques such as transcriptomics, proteomics, and metabolomics are reliable for exploring AS-IV-specific therapeutic targets.

Clinical trials have demonstrated that intravenous infusion of 200 mL astragalosides injection (contained 18 mg AS-IV) is safe and well-tolerated in healthy Chinese volunteers,[116] suggesting that AS-IV has excellent potential for clinical application. Still, most studies on applying AS-IV for lung disease have focused on preclinical models. Thus, it is essential to conduct clinical studies to confirm the efficacy and safety of AS-IV in preventing and treating lung diseases. Long-term studies have found that applying drug combinations is critical to achieving optimal effectiveness, providing a novel idea for the clinical translation of AS-IV. For example, AS-IV and ferulic acid combination demonstrated synergistic effects in various fibrotic disease models, thus, a promising anti-fibrotic treatment.[49,117] Moreover, the combination of AS-IV with atorvastatin,[118] bevacizumab,[25] tanshinone IIA[119] and ginsenoside Rg1[120] has been used in preclinical studies to treat various diseases. In summary, the application of AS-IV combination therapy in respiratory diseases is worth investigating in the future.

Conclusion and Perspectives

AS-IV, a natural saponin with several beneficial biological activities, has made major progress in the research on the role and mechanism of respiratory diseases prevention (Table 1 and Figure 4). This natural saponin inhibited respiratory

Table 1 The Summary of Mechanisms of as-IV in Respiratory Diseases

Respiratory Diseases	Animal Models	Cell Lines	Targets and Mechanisms	Ref.
Pulmonary hypertension	SD rats	PASMCs	Jagged-1↓ Notch-3↓ Hes-5↓ PCNA↓ α-SMA↓; PASMCs proliferation↓	[35]
	C57BL/6 mice	PASMCs	Ki-67↓ RhoA↓ p27↓; PASMCs proliferation↓	[7]
	SD rats	PASMCs	ERK/HIF-1α pathway↓ Bcl-2↓; PASMCs apoptosis↑	[36]
	SD rats	PAECs	NLRP3 inflammasome/calpain-1 pathway↓; pulmonary vascular inflammation↓	[38]
Pulmonary fibrosis	SD rats	A549 cells	Collagen-I↓ MDA↓ SOD↑ GSH-Px↑ TNF-α↓ IL-6↓ E-cad↑ α-SMA↓ TGF-β1↓ PI3K↓ AKT↓ FOXO3α↑; epithelial-mesenchymal transition↓	[41]
	SD rats	RLE-6TN cells	MTA1↓E-cad↑ α-SMA↓ TGF-β1↓; epithelial-mesenchymal transition↓	[42]
	NA	Fibroblasts	NLRP3↓ TGF-β1↓ α-SMA↓ collagen-I↓ collagen-III↓ E-cad↑ N-cad↓ Smad2↓ Smad3↓; epithelial-mesenchymal transition↓	[43]
	SD rats	NA	TNF-α↓ IL-6↓ IL-1β↓ NF-κB↓; inflammation↓	[48]
	C57BL/6J mice	NA	HYP↓ TGF-β1↓ MDA↓ SOD↑ ROS↓ Nrf-2↑ Smad3↑; oxidative stress↓	[49]
	SD rats	Fibroblasts	α-SMA↓ collagen-I↓ collagen-II↓ collagen-III↓ TGF-β1/Smad pathway↓; fibroblasts transformation to myofibroblasts↓	[50]

(*Continued*)

Table 1 (Continued).

Respiratory Diseases	Animal Models	Cell Lines	Targets and Mechanisms	Ref.
Lung cancer	C57BL/6 mice	NA	IDO1↓; cytotoxic T lymphocytes activities↑ regulatory T cells activities↓	[58]
	Nude mice	H460 cells	IDO1↓ GBP1↓; T-cell depletion↓	[59]
	C57BL/6 mice	A549, H1299, THP-1 cells	VEGFA↓ Arg-1↓ CD31↓ CD206↓ PPARγ↓ IL-10↓ TGF-β1↓ CCL-7↓ MMP-9↓ MMP-10↓ MMP-14↓ ICAM-1↓ IGF-1↓ CCL-2↓ AMPKα↓; M2-polarized macrophage activity↓	[63]
	C57BL/6 mice	A549 cells	PKC-α/ERK1/2/NF-κB pathway↓ integrin β1↓ MMP-2↓ MMP-9↓; cell invasion and migration↓	[64]
	NA	HCC827, H1299, A549 cells	Akt/GSK-3 pathway↓; cell proliferation↓	[65]
	NA	A549, H1299 cells	GRP78↓ Beclin1↓; ER stress and autophagy↓	[70]
	NA	HCC827, H1299, A549 cells	B7-H3↓; the sensitivity of lung cancer cells to cisplatin↑	[71]
	NA	A549 cells	P62↓ Beclin1↓ LC3I/LC3II↑ Bcl-2↓ Bax↑caspase-3↑ Akt↑ mTOR↑; autophagy↓ the sensitivity of lung cancer cells to bevacizumab↑	[25]
Lung injury	SD rats	NR8383 cells	p62↓ LC3BI/LC3BII↓ PI3K↓ AKT↓ mTOR↓ p65↓; autophagy↓	[81]
	SD rats	NA	AMPK↓ mTOR↓ Bcl-2↑ Bax↓ caspase-3↓ PARP1↓ p65↓; autophagy↓	[82]
	C57BL/6 mice	NA	MDA↓ MPO↓ SOD↑ IL-6↓ IL-1β↓ GSH↑ ROS↓ Nrf-2↑ SLC7A11↑ GPX4↑ FTH1↑ TFRC↓ COX2↓ HO-1↑; ferroptosis↓	[27]
	C57BL/6 mice	NA	NLRP3/caspase-1 pathway↓ IL-1β↓ IL-18↓; pyroptosis↓	[88]
	SD rats	NA	TNF-α↓ IL-6↓ CRP↓ MDA↓ CAT↑ SOD↑ TLR4↓ MyD88↓ NF-κB↓; inflammation↓	[89]
	BALB/C mice	NA	Rho/ROCK/NF-κB pathway↓ IL-6↓ IL-1β↓TNF-α↓; inflammation↓	[90]
	SD rats	RLE-6TN cells	Bcl-2↓ Bax↑ caspase-3↑ miR-21-5p↑ TLR4↓ NF-κB↓; inflammation↓	[91]
Asthma	BALB/c mice	NA	IL-4↓ IL-5↓ IL-13↓ JAK2↓ STAT6↓; inflammation↓	[24]
	BALB/c mice	NA	IL-4↓ IFN-γ↑ IL-10↓ Foxp3↑; inflammation↓	[94]
	BALB/c mice	NA	IL-4↓ IL-5↓I L-17↓ mTORC1 pathway↓; airway hypersensitivity and inflammation↓	[96]
	ICR mice	Human bronchial epithelial cells	IL-1β↓ TNF-α↓ IL-6↓ MDA ↓ SOD↑ JAK3↓ STAT3↓ NF-κB↓; inflammation and oxidative stress↓	[23]
COPD	Wistar rats	NA	IL-1β↓ TNF-α↓ NF-κB↓ GSH↑ NO↓; inflammation and oxidative stress↓	[102]
	BALB/c mice	NA	IL-4↓ IL-5↓I L-17↓ mTORC1 pathway↓; airway hypersensitivity and inflammation↓	[96]
	ICR mice	Human bronchial epithelial cells	IL-1β↓ TNF-α↓ IL-6↓ MDA ↓ SOD↑ JAK3↓ STAT3↓ NF-κB↓; inflammation and oxidative stress↓	[23]

(*Continued*)

Table 1 (Continued).

Respiratory Diseases	Animal Models	Cell Lines	Targets and Mechanisms	Ref.
COVID-19	NA	NA	Binding with ACE2 in silico	[105,106]
Obstructive sleep apnoea	NA	Beas-2B cells	MDA↓ ROS↓ IL-1β↓ IL-6↓ IL-8↓TLR4/MAPK/NF-κB pathway↓; inflammation and oxidative stress↓	[95]

Abbreviations: PASMCs, pulmonary artery smooth muscle cells; Hes-5, hes family bHLH transcription factor 5; PCNA, proliferating cell nuclear antigen; α-SMA, α-smooth muscle actin; HIF-1α, hypoxia-inducible factor-1α; NLRP3, nod-like receptor family pyrin domain-containing 3; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; TGF-β, transforming growth factor-β; PI3K, phosphatidylinositol 3-kinase; FOXO3α, forkhead Box O3; NF-κB, nuclear factor-κB; Nrf-2, nuclear factor E2-related factor; MTA1, metastasis-associated gene 1; MDA, malondialdehyde; T-AOC, total antioxidant capacity; ROS, reactive oxygen species; IDO1, indoleamine 2.3-dioxygenase 1; GBP1, guanylate binding protein 1; VEGFA, vascular endothelial growth factor A; PPARγ, proliferator-activated receptor γ; AMPKα, adenosine monophosphate (AMP)-activated protein kinase α; PKC-α, protein kinase C-alpha; ERK1/2, extracellular signal-related kinases 1 and 2; MMP-2, matrix metalloproteinase-2; Akt, protein kinase B; GSK-3β, glycogen synthase kinase-3β; mTOR, mammalian target of rapamycin; PARP1, poly(ADP-ribose) polymerase-1; GPX4, glutathione peroxidase 4; FTH1, ferritin heavy chain 1; HO-1, heme oxygenase-1; TLR4, Toll-like receptor 4; MyD88, myeloid differentiation factor 88; JAK2, Janus kinase 2; STAT6, signal transducer and activator of transcription 6; mTORC1, mechanistic target of rapamycin complex 1; ACE2 angiotensin-converting enzyme 2; MAPK, mitogen-activated protein kinase.

diseases progression through anti-inflammatory, antioxidant, cell proliferation, EMT inhibition, and PCD modulation. Precisely, AS-IV combined with ferulic acid, cisplatin, and bevacizumab demonstrated synergistic effects and significantly improved drug efficacy. More importantly, AS-IV showed low toxicity in vivo, thus, facilitating future clinical translation. Despite the promising therapeutic impacts of AS-IV in multiple preclinical models of respiratory diseases, the mechanism of action and direct targets have yet to be elucidated. Modern biological techniques such as transcriptomics, surface plasmon resonance, and protein microarray technologies offer new strategies to fill knowledge gaps in AS-IV studies. Nevertheless, a major hurdle in AS-IV application in respiratory diseases is the lack of clinical data. Therefore, future research should focus on clinical trials to confirm the efficacy and safety of AS-IV in treating respiratory diseases. In addition, the high molecular weight of AS-IV results in low bioavailability, further limiting the its clinical use. Novel

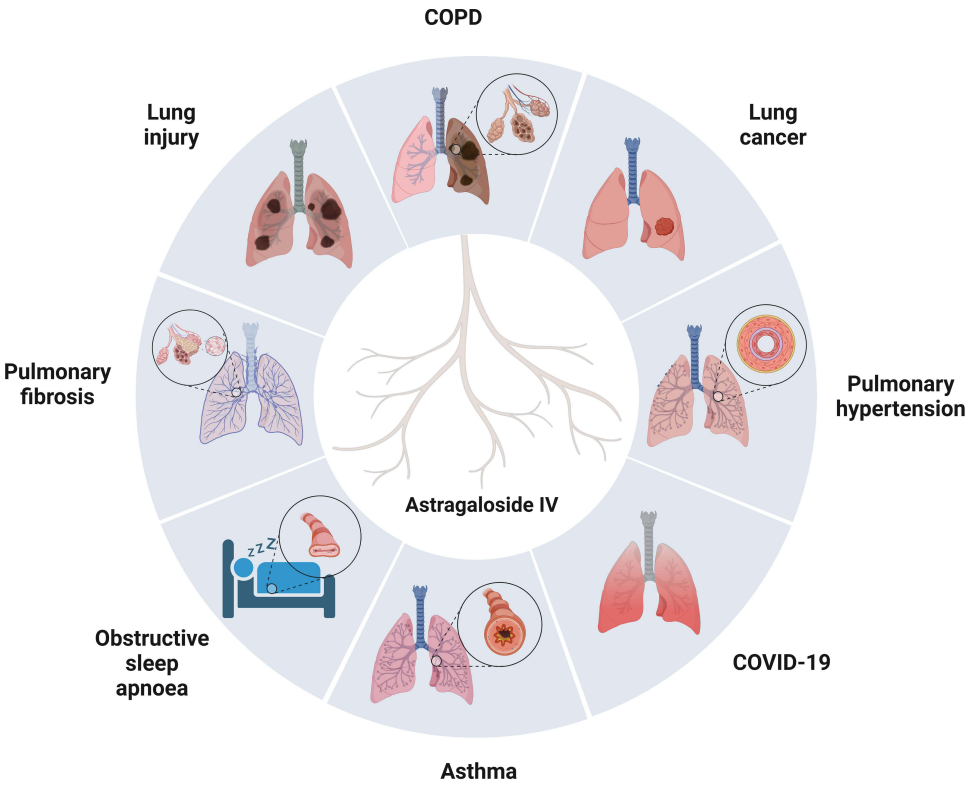

Figure 4 The general map of AS-IV in treating various respiratory diseases. Created with BioRender.com.

drug delivery systems, such as nanocapsules, have improved AS-IV bioavailability. In conclusion, AS-IV is a potential drug candidate for treating respiratory diseases and should be further explored in future research.

Data Sharing Statement

The data used to support the findings of this study are included in the article.

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Author Contributions

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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