REVIEW

The Promising Effects of Astaxanthin on Lung Diseases

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ABSTRACT

Astaxanthin (ASX) is a naturally occurring xanthophyll carotenoid. Both in vitro and in vivo studies have shown that it is a potent antioxidant with anti-inflammatory properties. Lung cancer is the leading cause of cancer death worldwide, whereas other lung diseases such as chronic obstructive pulmonary disease, emphysema, and asthma are of high prevalence. In the past decade, mounting evidence has suggested a protective role for ASX against lung diseases. This article reviews the potential role of ASX in protecting against lung diseases, including lung cancer. It also summarizes the underlying molecular mechanisms by which ASX protects against pulmonary diseases, including regulating the nuclear factor erythroid 2–related factor/heme oxygenase-1 pathway, NF-κB signaling, mitogen-activated protein kinase signaling, Janus kinase–signal transducers and activators of transcription-3 signaling, the phosphoinositide 3-kinase/Akt pathway, and modulating immune response. Several future directions are proposed in this review. However, most in vitro and in vivo studies have used ASX at concentrations that are not achievable by humans. Also, no clinical trials have been conducted and/or reported. Thus, preclinical studies with ASX treatment within physiological concentrations as well as human studies are required to examine the health benefits of ASX with respect to lung diseases. *Adv Nutr* 2021;12:850–864.

Keywords: astaxanthin, lung diseases, oxidative stress, molecular mechanism, lung cancer

Introduction

Astaxanthin (ASX; 3,3′-dihydroxy-β,β′-carotene-4,4′-dione; **Figure 1**) is a naturally occurring red–orange oxycarotenoid pigment primarily found in marine organisms such as crustaceans, algae, shrimp, and salmon (1). In 1938, it was discovered for the first time in lobsters and was initially employed in aquaculture for the pigmentation. Later, in 1987, ASX was approved by the FDA as a food colorant in animal and fish feed. The European Commission considers natural ASX a food dye (2). In Japan, ASX is deposited in egg yolks by feeding laying hens *Phaffia rhodozyma*, a carotenoid-producing fermentative red yeast.

According to the European Food Safety Authority, the current acceptable daily intake of ASX is 0.034 mg·kg^{-1}·d^{-1}, which is equivalent to 2.38 mg·d^{-1} in a 70-kg human (3, 4). In 2011, the FDA allowed the usage of ASX at a concentration up to 12 mg per daily serving (5). However, it is important to note that these are suggested upper safe levels of use, not dietary guidelines. Also, these dietary guidelines are for a healthy population. There is a lack of information on the recommended daily intake dosages for individuals with certain conditions or people who have lower efficiencies in absorbing carotenoids. As a dietary supplement for humans and animals, ASX is obtained mainly from seafood or extracted from *Haematococcus pluvialis* (freshwater, unicellular microalga) (6). Due to technological developments in mass producing ASX by culturing *H. pluvialis*, a large amount of ASX is now available from natural sources (7).

Multiple in vivo studies have reported that ASX can protect the brain, eyes, salivary glands, skeletal muscle, liver, kidney, and lungs from oxidative stress (8–12). In rats fed *H. pluvialis*, ASX showed even better bioavailability than

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Abbreviations used: ALI, acute lung injury; AMPK, 5′-AMP–activated protein kinase; ARE, antioxidant response element; ASX, astaxanthin; BALF, bronchoalveolar lavage fluid; Bax, Bcl-2-associated X; BW, body weight; COPD, chronic obstructive pulmonary disease; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; ERK, extracellular signal-regulated kinase; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; JAK/STAT-3, Janus kinase–signal transducers and activators of transcription-3; JNK, Jun amino-terminal kinase; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MEK, meiotic chromosome axis–associated kinase; MKK, mitogen-activated protein kinase kinase; MMC, mitomycin C; NSCLC, non–small cell lung cancer; Nrf2, nuclear factor erythroid 2–related factor 2; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; ROF, roflumilast; SMA, α-smooth muscle actin; SOD, superoxide dismutase; Th1, T helper type 1; Th2, T helper type 2; TS, thymidylate synthase; XPC, xeroderma pigmentosum complementation group C.

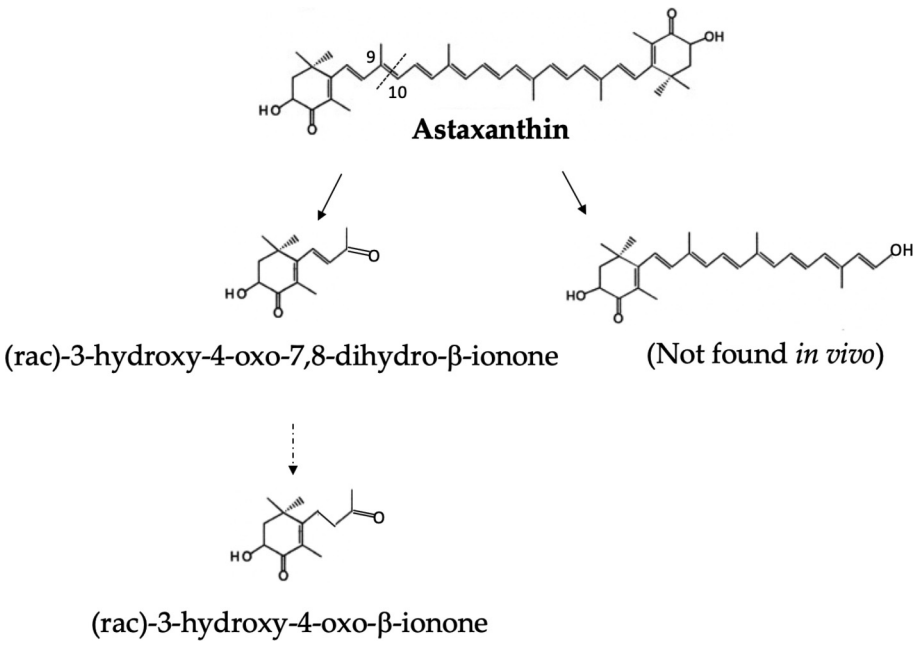

FIGURE 1 Metabolism of astaxanthin. Astaxanthin can be cleaved at C9 = C10 to (rac)-3-hydroxy-4-oxo-7,8-dihydro-β-ionone, which can be subsequently oxidized to (rac)-3-hydroxy-4-oxo-β-ionone (13).

β-carotene and lutein (10). Importantly, ASX is the only known ketocarotenoid that can be transported to the brain by transcytosis through the blood–brain barrier (14). Thus, this evidence further supports that ASX can be distributed throughout the body and may display systemic effects. ASX presents higher antioxidative properties based on its unique molecular structure: It contains hydroxyl group and keto moieties on each ionone ring (Figure 1), which contributes to donating the electrons and reacting with free radicals to convert them to more stable products, leading to the termination of free radical chain reactions (14, 15). In fact, in an in vitro setting, the antioxidant activity of ASX is 65 times more powerful than vitamin C; 55 times stronger than β-carotene; 10 times more potent than canthaxanthin, zeaxanthin, and lutein; and 100 times more effective than α-tocopherol (16). According to Davinelli et al. (17), the first comprehensive human study that investigated the efficacy of ASX in mitigating oxidative stress was performed by Park et al. in which participants who received 2 or 8 mg ASX for 8 wk had significantly lower 8-hydroxy-2′-deoxyguanosine (a DNA damage biomarker), higher total T and B cell subpopulations, and elevated natural killer cell cytotoxic activity compared with the control group (18). In a recent study, a continuous ASX supplementation (4 mg/d) for 4 wk substantially decreased plasma concentrations of malondialdehyde (a marker of oxidative stress), indicating that ASX is capable of decreasing systemic oxidative stress (19). Based on the previously mentioned reports, ASX has promising applications in nutrition and human health, and it may act as a potent compound in protecting against a wide range of diseases.

The bioavailability of ASX in human beings was reported previously (20, 21). In subjects without ASX supplementation, circulating ASX concentrations were nondetectable (20, 21). However, after consuming a single dose of 40 mg ASX, the plasma ASX concentration of 32 male subjects (average body weight: 81.5 kg) increased to ~190 μg/L (20). In another study, a single dose of ASX oral administration (100 mg, 168 μM) in 3 male volunteers (body weight: 90–100 kg) resulted in a substantial increase of plasma ASX concentration, reaching ~120 mg/L in 6.7 h (21). Nevertheless, it is important to note that the subjects received ASX as nutrient supplements in both studies.

Asthma is a common chronic respiratory disease due to chronic inflammation of the lower respiratory tract, affecting 300 million people worldwide. The prevalence of asthma in children and adults rises by 50% every decade globally, especially in developing regions (22). Researchers found that people with a medical history of asthma had a markedly higher risk of other chronic lung diseases later in life. For example, compared with healthy controls, people with asthma were 17 times more likely to acquire emphysema and had a 12.5 times higher risk of acquiring chronic obstructive pulmonary disease (COPD) (23). COPD is a lung disease characterized by obstruction of lung airflow that is usually associated with an enhanced chronic inflammatory response in airways (24). As one of the lung conditions included in COPD, emphysema is a progressive lung disease caused by the enlargement of airspaces distal to the terminal bronchioles accompanied by compressed lung parenchyma (25). Notably, COPD is the third leading cause of death globally; the current prevalence of COPD is ~10% (26).

COPD and emphysema can predispose patients to lung cancer (25, 27), the leading cause of global cancer according to a 2019 report from WHO (28). Lung fibrosis, a disease characterized by excess deposition of extracellular matrix by myofibroblasts, is another potent risk factor for lung cancer. Lung fibrosis patients have a 20% higher risk of developing lung cancer (29), and it takes only 2–4 y for this disease to proceed to end-stage respiratory insufficiency and death once symptoms occur (30). However, no current nutritional intervention has demonstrated efficacy in mitigating the aforementioned lung diseases.

In recent years, we have gained considerable knowledge of the biological effects of ASX, particularly its efficacy in alleviating chronic noncommunicable diseases. One example is its effect on multiple signaling pathways in cancer. Because the potent antioxidative efficacy of ASX has attracted growing interest and attention in recent years, much evidence has accumulated with regard to ASX treatment in alleviating lung diseases. Based on this background information, in this review we summarize the effects of ASX on lung diseases such as asthma, COPD and emphysema, acute lung injury (ALI), lung fibrosis, and cancer. We also highlight the potential underlying mechanisms of ASX in mediating its beneficial effects against these diseases. The details of the publications that reported the efficacy of ASX against lung diseases are summarized in **Table 1** (in vitro studies) and **Table 2** (in vivo studies).

ASX and Lung Diseases

Asthma

Asthma is a disease of the lungs in which the airways become blocked or narrowed, causing breathing difficulty, coughing, wheezing, and shortness of breath. Asthma progression can be affected by a combination of immunological, genetic, and environmental interactions. The onset of asthma is linked to increased pulmonary inflammation, which is characterized by infiltration of the airway wall with a variety of inflammatory cells mostly driven by activating T helper type 2 (Th2)-type lymphocytes, mast cells, and eosinophils (31).

Three in vivo studies investigated the efficacy of ASX supplementation in mitigating asthma. Two of these studies demonstrated that ochratoxin-induced lung damage and asthma were alleviated by the supplementation of ASX at a dosage of $\geq$5 mg/kg body weight (BW), in addition to observing decreased inflammatory cell infiltration in the lung (32, 33). In particular, Hwang et al. (32) demonstrated that the oral administration of ASX (5, 10, and 50 mg/kg BW) was capable of inhibiting ochratoxin-mediated respiratory system resistance, elastance, Newtonian resistance, tissue damping and tissue elastance in the lung, as well as reducing mucus production and lung fibrosis. These data provided strong evidence that ASX supplementation could alleviate the pathogenesis, progression, and symptoms of asthma. In addition to ASX alone, a combination of ASX (10 mg/kg), vitamin C (200 mg/kg), and ginkgo biloba leaf extract (10 mg/kg) significantly reduced asthma-associated inflammation in asthmatic guinea pigs (34). These researchers also found that compared with the control group, the ASX-fed animals had a significantly lower pulmonary content of cyclic nucleotides, which are second messengers in airway smooth muscle cells that decrease contractility and relax the airways, leading to decreased severity of asthma (35). The antiasthmatic effect of the combined remedies exceeded that of the single ASX compound and was even comparable with ibuprofen, a widely used nonsteroidal anti-inflammatory drug in asthma treatment (34). Previously, multiple studies reported that the combination of various nutrients exerted more potent anti-inflammatory and anti-antioxidant effects than a single nutrient (36, 37). The superior efficacy of the combined remedies against asthma might be due to the synergistic effects of the phytochemicals and vitamins.

COPD and emphysema

Two in vivo studies showed the efficacy of ASX supplementation against COPD and emphysema. The effect of a 12-wk ASX supplementation against cigarette smoke–induced COPD has been demonstrated in an animal model in which mice fed ASX developed less severe emphysema (8). These findings are in line with those of another study in which 9-d ASX supplementation at a dosage of 10 and 20 mg/kg BW inhibited cigarette smoke–induced COPD in mice (38). Furthermore, both studies reported decreased amounts of IL-6, TNF-α, and reactive oxygen species in the bronchoalveolar lavage fluid (BALF), suggesting an anti-inflammatory role of ASX (8, 38). Roflumilast (ROF) is a prescription medicine used in the treatment of COPD. Intriguingly, the effects against emphysema and inflammation were comparable between 20 mg/kg BW ASX and 10 mg/kg BW ROF (38), indicating that ASX may be used as a dietary strategy to mitigate emphysema and smoke-induced COPD.

ALI

ALI is a disorder of acute inflammation characterized by the disruption of lung endothelial and epithelial barriers (39). Multiple factors, including lung infection, aspiration, sepsis, multiple trauma, and shock, contribute to the onset of ALI, leading to alveolar–capillary membrane injury, lung inflammation, and increased pulmonary permeability edema (39).

Three in vivo studies reported the potential efficacy of ASX against the progression of ALI (33, 40, 41). One study showed that feeding mice 60 mg/kg BW ASX for 14 d significantly increased the overall survival rate in mice with cecal ligation and puncture (40). Also, ASX treatment significantly decreased pathological change in ALI, pulmonary apoptosis, and total cell and neutrophils in BALF (40). These results were in agreement with those of another study in which mice given 100 mg/kg BW ASX developed less severe ALI, less inflammatory cell infiltration, and decreased lung cell apoptosis compared with their counterparts fed a

TABLE 1 Characteristics of the in vitro studies examining the efficacy of ASX treatment against lung diseases[1]

Study	Year	Cell lines	Carcinogen	Dosage	Duration	Results
Wang et al. (42)	2013	MRC-5, A549	TGF-β1 (5 ng/mL)	8, 16, 32, 64, 128 μM	48 h	↓ Cell growth ↑ E-cadherin, ↓ vimentin ↑ Cell apoptosis, p53, ↓ Bcl-2
Song et al. (43)	2014	RLE-6TN	Hydrogen peroxide (65 μM)	8 μM	6, 12, 24 h	↑ Cell viability, ↓ cell apoptosis ↑ SOD, catalase activity ↑ Mitochondria morphology, ↑ MMP ↓ Bad and Bax, ↑ Bcl-xl and Bcl-2 ↓ Cytochrome c cytosolic translocation, ↓ caspase-9 and caspase-3 ↑ Nrf2 ↑ p53, ↓ PUMA
Zhang et al. (44)	2015	A549, MRC-5	TGF-β1 (5 ng/mL)	24 and 18 μg/mL for A549 and MRC-5 cells, respectively	48 h	↑ Cell apoptosis ↑ Bax ↑ Cytochrome c, Drp-1
Wu et al. (45)	2016	A549	NA	20, 40, 60, 80, 100 μM	NR	↓ Cell proliferation ↑ Cell apoptosis ↑ Bax ↓ Bcl-2, STAT3, JAK1
Ko et al. (46)	2016	A549, H1703	NA	2.5, 5, 10, 20 μM	24, 48, 72 h	↓ Cell viability ↓ *Rad51*, AKT phosphorylation ↓ *Rad51* stability
Liao et al. (47)	2016	H1650, H1703	NA	5, 10, 20 μM	3–24 h	↓ Thymidylate synthase ↓ Phospho-MEK1/2, phospho-ERK1/2 ERK1/2 inactivation decreased thymidylate synthase by ubiquitin-26S proteasome-mediated proteolysis
Chen et al. (48)	2018	A549, H1975	NA	20 μM	1–24 h	↓ Cell viability ↑ Erlotinib cytotoxicity ↓ XPC expression in a time- and dose-dependent manner in erlotinib-treated cells ↑ Phospho-MKK3/6 and phosphop38 MAPK protein concentrations
Cai et al. (41)	2019	Mouse primary peritoneal macrophage	LPS (dosage NR)	10, 25, 50, 100, 200 μM	1 h	Cell survival did not differ . 10 μM ASX ↓ apoptotic cells, TNF-α and IL-6 secretion ↓NF-κB p65 in the nuclear
Nian et al. (38)	2019	RAW264.7	LPS (1 μM)	5, 10, 20 μM	1 h	10 and 20 μM ASX ↓ NO, IL-6, TNF-α, IL-1β 20 μM ASX ↓ iNOS protein concentration

[1] ASX, astaxanthin; Bax, Bcl-2–associated X; bcl-xl, B cell lymphoma–extra large; Drp-1, dynamin-related protein-1; ERK, extracellular signal-regulated kinase; iNOS, inducible nitric oxide synthase; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MEK, meiotic chromosome axis–associated kinase; MKK, mitogen-activated protein kinase kinase; MMP, matrix metalloproteinase; NA, not available; NR, not reported; Nrf2, nuclear factor erythroid 2–related factor 2; PUMA, anti-p53-up-regulated modulator of apoptosis; RLE-6TN, rat lung epithelial-T-antigen negative; SOD, superoxide dismutase; STAT-3, signal transducers and activators of transcription-3; TGF-β1, transforming growth factor-β1; XPC, xeroderma pigmentosum complementation group C.

TABLE 2 Characteristics of the in vivo studies examining the efficacy of ASX supplementation against lung diseases[1]

Study	Year	Animal	Establish model	Treatment	Dosage	Duration	Results
Haines et al. (34)	2011	Guinea pig	0.35 mL 5% ovalbumin aerosol	Ginkgo biloba leaf extract (EGb761), ASX, and vitamin C respectively or in combination	ASX (10 mg/kg), vitamin C (200 mg/kg), and EGb761 (10 mg/kg)	26 d	ASX alone↓ Inflammatory cell infiltrate in BALF↓ Cyclic nucleotide content in lung The entire remedy↓ Inflammatory cell infiltrate in BALF and cyclic nucleotide content in lung to the same range as ibuprofen
Wang et al. (42)	2013	Rat	5 mg/kg bleomycin	ASX	0.5, 1, and 2 mg/kg body weight	25 d	↓ Fibrosis score (related to alveoli cavities, alveolar membrane thickness, and edema) ↓ Collagen and fiber contents in the interstitial lungs of the rats, ↓ hydroxyproline, α-SMA cells ↑ E-cadherin, ↓ vimentin, ↑ p53, ↓ Bcl-2
Song et al. (43)	2014	Rat	5 mg/kg bleomycin	ASX	1, 2 mg/kg body weight	7 d	↑ SOD, catalase activity ↑ Mitochondria morphology
Zhang et al. (44)	2015	Rat	5 mg/kg bleomycin	ASX	2 mg/kg body weight	7 or 14 d	↓ Lung parenchymal distortion, alveolar thickness, edema ↓ Collagen deposition, hydroxyproline, α-SMA cells ↑ Bax ↑ Drp-1 ↑ p53, PUMA
Bi et al. (40)	2017	Mouse	Cecal ligation and puncture	ASX	60 mg/kg	14 d	↑ Overall survival ↓ Pathological change in ALI ↓ Total cell and neutrophils in the BALF ↓ TNF-α, IL-6, IL-1β ↓ ROS, MDA, MPO, iNOS, nitrotyrosine, NF-κB P65 in the lung ↑ SOD ↓ Pulmonary apoptosis
Hwang et al. (32)	2017	Mouse	20 μg ochratoxin	ASX	5, 10, and 50 mg/kg body weight	5 d	↓ Respiratory system resistance, elastance, Newtonian resistance, tissue damping, tissue elastance ↓ Total cell number, IL-4, IL-5 ↑ IFN-γ in the BALF ↓ IgE, IgG1, OVA-specific IgG1 ↑ Ochratoxin-specific IgG2a ↓ Inflammatory cell infiltration in the lung, mucus production, lung fibrosis ↓ Caspase-1, caspase-3
Xu et al. (33)	2019	Mouse	5 mg/kg body weight ochratoxin	ASX	100 mg/kg body weight	27 d	↓ Inflammatory cells in lung ↓ Lung cell apoptosis ↓ MDA

(*Continued*)

TABLE 2 (*Continued*)

Study	Year	Animal	Establish model	Treatment	Dosage	Duration	Results
Kubo et al. (8)	2019	Mouse	18 cigarettes per day, 5 days per week	ASX	0.02% diet	12 wk	↑ SOD, GSH ↓ IL-1β, IL-6, TNF-α ↑ Nrf2, HO-1 ↓ Keap1 ↓ TLR4, MyD88, NF-κB p65 ↑ Nrf2 and HO-1 protein concentrations ↓ Macrophages and neutrophils in BALF Lymphocytes were not changed in BALF
Cai et al. (41)	2019	Mouse	20 mg/kg body weight LPS for inflammatory mortality	ASX	100 mg/kg	Pretreatment for 1 h before LPS injection; study lasted 7 d	↓ MLI and destructive index ↑ Survival rate ↓ IL-6 and TNF-α in the 4 h following LPS challenge ↓ Alveolar wall swelling, attenuated the decline in the number of pulmonary alveoli ↓ Degradation of IκB-α and phosphorylation of ERK1/2, p38, and JNK
Cai et al. (41)	2019	Mouse	5 mg/kg body weight LPS or ALI	ASX	5 mg/kg	ASX for 1 wk, then LPS	↓ ALI, lung edema ↓ Pulmonary congestion, inflammatory cell infiltration, alveolar wall thickening, interstitial edema ↓ CD68 expression ↓ IL-6, TNF-α, MPO activity ↓ IκB-α degradation; phosphorylation of ERK1/2, p38, and JNK
Nian et al. (38)	2019	Mouse	LPS + 8 cigarettes per day for 1 h, lasts for 10 d	ASX	10 and 20 mg/kg	9 d	↓ Neutrophils and macrophages in BALF ↓ IL-6, TNF-α, ROS in BALF ↑ Nrf2 phosphorylation and HO-1 protein expression in lung

[1] ALI, acute lung injury; ASX, astaxanthin; BALF, bronchoalveolar lavage fluid; Bax, Bcl-2–associated X; Drp-1, dynamin-related protein-1; ERK, extracellular signal-regulated kinase; GSH, reduced glutathione; HO-1, heme oxygenase-1; iNOS, inducible nitric oxide synthase; IκB, inhibitor of NF-IκB; JNK, Jun amino-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; MDA, malondialdehyde; MLI, mean linear intercept; MPO, myeloperoxidase; Nrf2, nuclear factor erythroid 2–related factor 2; OVA, ovalbumin; PUMA, anti-p53 upregulated modulator of apoptosis; ROS, reactive oxygen species; SMA, α-smooth muscle actin; SOD, superoxide dismutase.

regular chow diet (33). In another study, researchers fed mice 100 mg/kg BW ASX for 1 wk, followed by 5 mg/kg BW LPS to induce ALI; they found that ASX supplementation substantially inhibited the occurrence of ALI and lung edema and reduced pulmonary congestion, inflammatory cell infiltration, alveolar wall thickening, and interstitial edema (41). High plasma or pulmonary concentrations of proinflammatory cytokines TNF-α, IL-1β, IL-6, IL-8, and IL-18 are reliable biomarkers for predicting reduced morbidity and mortality in ALI patients (49). Interestingly, all 3 studies reported decreased IL-6, IL-1β, and TNF-α concentrations and inhibited myeloperoxidase activity in BALF or lung tissues of the ASX-treated mice compared with mice that did not receive ASX treatment. In summary, these preclinical studies revealed that ASX supplementation may protect against the onset of ALI.

Lung fibrosis

Two in vivo studies investigated the effect of ASX on lung fibrosis and found that ASX supplementation at 0.5–2 mg/kg BW significantly decreased lung parenchymal distortion, alveolar thickness, and pulmonary edema in rats with bleomycin administration (42, 44). They also reported that compared with no ASX supplementation, ASX feeding (0.5–2 mg/kg BW) resulted in pronounced attenuation in collagen deposition, decreased hydroxyproline, and reduced α-smooth muscle actin (SMA) in the lungs of rats (42, 44). α-SMA$^+$ is a specific marker for myofibroblasts, which contribute to producing collagen (44).

Further exploration of the effect of ASX on myofibroblasts showed that ASX treatment at 8–128 μM induced cell apoptosis in the lungs, which was concordant with the data that ASX (18 and 24 μM) promoted the apoptotic rate of transforming growth factor-β1–induced A549 and MRC-5 cells (42, 44). One of the driving forces behind fibrosis is the epithelial–mesenchymal transition (EMT), a process in which epithelial cells lose epithelial proteins (50). Among these proteins, E-cadherin is well studied due to its capability of strengthening tight junctions and maintaining cell–cell adhesion (50). During the progression of fibrosis, the epithelial cells may transfer to a more mesenchymal phenotype because the onset of fibrosis requires mesenchymal markers such as vimentin and fibronectin, which are proteins that contribute to the formation of extracellular matrix (50). Interestingly, in MRC-5 and A549 cells, treatment with ASX led to the upregulation of E-cadherin and downregulation of vimentin (42). The efficacy of ASX on promoting E-cadherin was also validated in rats with bleomycin-induced lung fibrosis (42). Thus, based on the previously discussed reports, ASX may be a potent compound in ameliorating lung fibrosis mainly by inducing the apoptosis of myofibroblasts and inhibiting EMT in lung tissues.

Lung cancer

Non–small cell lung cancer (NSCLC) accounts for the most lung cancer–related deaths (51). To our knowledge, no studies have shown the efficacy of ASX against NSCLC or other primary lung cancers in vivo.

In vitro, ASX treatment at 2.5–20 μM reduced the viability of NSCLC cells, including A549, H1650, H1703, and H1975, in a dose-dependent manner (47, 46, 45, 48). In A549 cells, ASX treatment at 20–100 μM enhanced cell apoptosis and decreased cell proliferation (45). In addition, ASX treatment at 20 μM inhibited the growth of A549 and H1975 cells by enhancing the cytotoxicity of the compounds that have demonstrated clinical activity (48). Erlotinib (Tarceva) is a selective epidermal growth factor receptor(EGFR) tyrosine kinase inhibitor prescribed for patients with NSCLC. ASX treatment at 2.5 μM synergistically enhanced cytotoxicity and cell growth inhibition of erlotinib (2.5 and 5 μM) in NSCLC cells, which were associated with the downregulation of xeroderma pigmentosum complementation group C (XPC) protein expression (48). Pemetrexed is one of the most frequently prescribed chemotherapeutic agents for advanced nonsquamous NSCLC treatment (52). In H1650 and H1703 cells, a combination of pemetrexed (5–20 μM) and ASX (10–20 μM) led to synergistic enhanced cytotoxicity and cell growth inhibition in these cells. Interestingly, ASX treatment alone (10–20 μM) or in combination with pemetrexed (5 μM ASX + 10–20 μM pemetrexed) significantly decreased the activation of thymidylate synthase (TS) (47), whose overexpression caused resistance to the antitumor effect of pemetrexed (53). Chemoresistant carcinomas exhibit high levels of *Rad51* expression, which plays a critical role in homologous recombination (54). In A549 and H1703 cells, ASX treatment (2.5–10 μM) significantly decreased *Rad51* expression (46). Moreover, the combination of ASX (20 μM) and mitomycin C (MMC) (2.5–10 μM), an antitumor antibiotic widely used in clinical NSCLC chemotherapy, substantially inhibited *Rad51* expression and further enhanced MMC-induced cytotoxicity (46). The findings of these in vitro studies suggest that ASX may improve the effectiveness of standard lung cancer treatments. However, there is a paucity of in vivo studies demonstrating the role of ASX in ameliorating lung cancer, with the exception of 1 study that reported the antimetastatic efficacy of ASX by suppressing metastasis of colon cancer cells into the lung and decreased matrix metallopeptidase 2 protein expression in the lung (55). Therefore, in vivo and human studies are warranted to verify the anti-NSCLC efficacy of ASX.

Molecular Mechanisms Underlying ASX Suppression of Lung Diseases

The nuclear factor erythroid 2–related factor/heme oxygenase-1 pathway

Nuclear factor erythroid 2–related factor 2 (Nrf2) is a basic region leucine zipper protein and a critical transcription factor in the regulation of cellular redox balance and phase II detoxification responses in mammals (56). Under endogenous and exogenous stresses, Nrf2 can be released from the inhibitor protein Kelch-like ECH-associated protein

1 (Keap1) and translocated into the nucleus to mediate activation of antioxidant genes (57).

The activation of the Nrf2 signaling pathway is known to be a primary mechanism in the defense against oxidative stress, especially in the lung (58, 59). Application of Nrf2-deficient mice identified an extensive range of protective roles for Nrf2 against the pathogenesis of pulmonary emphysema (58, 59). Heme oxygenase-1 (HO-1), one of the genes regulated through Nrf2, functions as a cytoprotective enzyme (56). Several studies have shown that HO-1 serves as a protective mediator in cigarette smoke–induced lung cell injury and COPD, whereas HO-1 deficiency leads to systemic inflammation (56, 60).

ASX exerts its antioxidative effects by activating the Nrf2–antioxidant response element (ARE) signaling pathway (11, 61–63). One in vitro study showed that when rat lung epithelial–T-antigen negative cells were exposed to hydrogen peroxide, Nrf2 was mildly activated as an adaptive response to the elevated oxidative stress, but the degree of Nrf2 activation was insufficient to restore the cells from apoptosis (43). However, ASX treatment at 8 μM significantly increased Nrf2 expression in the cells pretreated with hydrogen peroxide, and such increase was accompanied by elevated superoxide dismutase (SOD) and catalase activities, suggesting that ASX may ameliorate pulmonary oxidative stress partly by activating Nrf2 (43).

In addition, 3 in vivo studies showed that ASX inhibited the development of COPD or ALI through upregulating HO-1 expression, which was mediated by activating Nrf2 (8, 33, 38) and decreasing Keap1 protein expression (33). Furthermore, Nian et al. (38) showed elevated phosphorylation of Nrf2 with ASX treatment (10 and 20 mg/kg BW) in mice. Nrf2 can be phosphorylated at multiple sites. For example, 5′-AMP–activated protein kinase (AMPK) phosphorylates Nrf2 at the Ser558 residue in the canonical nuclear export signal, which, in conjunction with decreased glycogen synthase kinase 3β stability, leads to the intercellular translocation of Nrf2 from cytoplasm to nucleus for ARE-mediated gene expression (64). Protein kinase C (PKC) phosphorylates Nrf2 at Ser40, which promotes its intercellular translocation to the nucleus, further improving the antioxidant capability of Nrf2 (65). Finally, mitogen-activated protein kinases (MAPKs) phosphorylate Nrf2 at multiple sites, but direct phosphorylation of Nrf2 by MAPKs has limited contribution in modulating Nrf2 activity (66). Among these possible pathways, we highlight the AMPK–Nrf2 signaling pathway because evidence shows that ASX is capable of activating AMPK in vivo (67), whereas no studies have reported the effect of ASX in regulating PKC activity. We propose that ASX might ameliorate COPD and emphysema by activating the AMPK–Nrf2–HO-1 pathway (**Figure 2**).

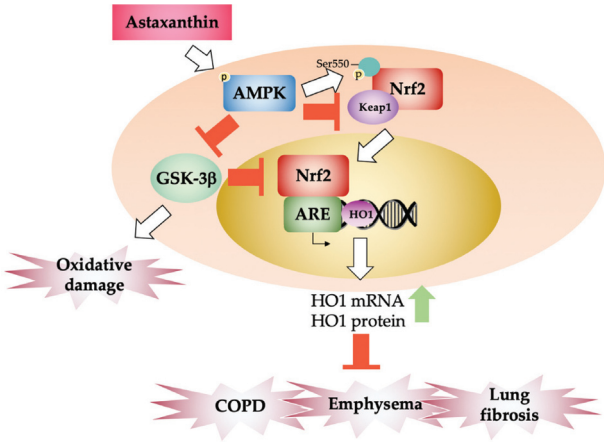

FIGURE 2 A model for the suggested pathways of astaxanthin inhibiting lung diseases by activating the Nrf2–HO1 pathway. Astaxanthin phosphorylates AMPK, which then phosphorylates Nrf2 at the Ser550 residue, accompanied by GSK3β inhibition, leading to nuclear accumulation of Nrf2 for the transactivation of ARE-driven genes, including HO-1. These changes result in inhibited oxidative damage and alleviated COPD, emphysema, and lung fibrosis. AMPK, 5′-AMP–activated protein kinase; ARE, antioxidant response element; COPD, chronic obstructive pulmonary disease; GSK3β, glycogen synthase kinase 3β; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor erythroid 2–related factor 2.

The NF-κB signaling pathway

NF-κB is a ubiquitous nuclear transcription factor that plays a crucial role in various physiological processes, such as mediating inflammatory and immune responses and governing the expression of genes related to cell survival, proliferation, and differentiation (68). Oxidative stress can induce the activation of the inhibitor of NF-κB (IκB) kinase β-dependent NF-κB pathway (69). Once activated, NF-κB promotes pulmonary inflammation and cancer mainly through mediating the secretion of inflammatory cytokines, including TNF-α, IL-1, and IL-6, and the CXC chemokine (69, 70). Indeed, overexpression of NF-κB was widely observed in tumor samples from patients with both NSCLC and small cell lung cancer (69), and it was associated with cancer progression, metastasis, and poor prognosis (71). Moreover, chemotherapy and cytotoxic treatments for cancer may enhance the expression and signaling of NF-κB, which in turn suppresses the anticancer and apoptotic potential of the chemotherapeutic reagents and causes drug resistance (72).

A large body of evidence indicates that ASX supplementation can inhibit NF-κB signaling as reported in both in vitro and in vivo studies (73–76). ASX treatment has shown efficacy in attenuating oral cancer (75) and colon cancer (77, 78) by blocking the NF-κB signaling pathway. In this review, we summarize 2 in vivo studies showing that ASX supplementation at 5 and 50 mg/kg BW, respectively, decreased the protein expression of NF-κB–p65, which is a subunit of the NF-κB transcription complex (33, 40). NF-κB inhibitor α (IκB-α) acts as a key negative feedback regulator of NF-κB through its ability to block the intercellular translocation of NF-κB from cytoplasm to nucleus (79). Interestingly, ASX supplementation (5 and 100 mg/kg BW)

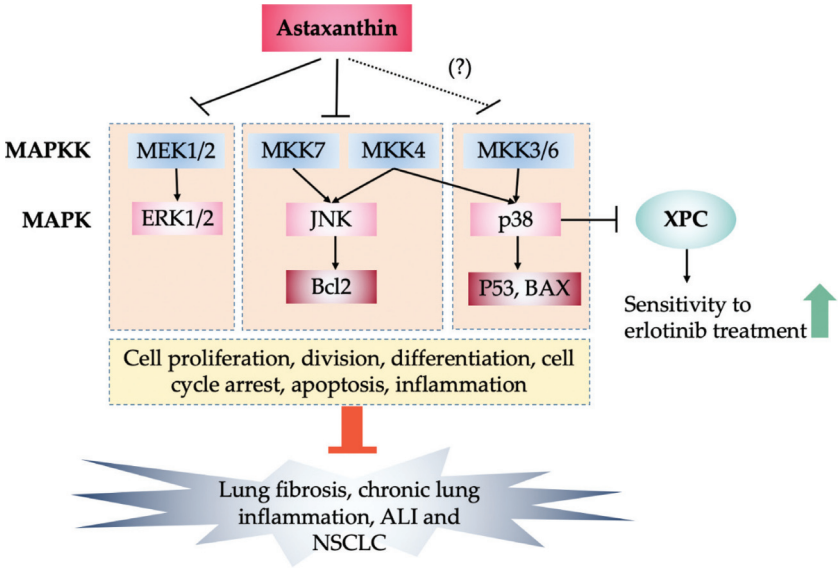

FIGURE 3 The suggested pathways of ASX blocking the MAPK signaling pathway. ASX suppresses the phosphorylation of ERK1/2, JNK, and p38 MAPK pathways. JNK dephosphorylation results in decreased Bcl2 expression. Dephosphorylated p38 MAPK leads to a decrease in p53 and BAX concentrations as well as XPC concentrations, which enhances sensitivity to erlotinib treatment for NSCLC. Altogether, ASX regulates cell proliferation, division, differentiation, cell cycle arrest, apoptosis, and inflammation via inhibition of the MAPK signaling pathway. Eventually, ASX can inhibit lung fibrosis, chronic lung inflammation, ALI, and NSCLC. ALI, acute lung injury; ASX, astaxanthin; BAX, Bcl-2–associated X; ERK, extracellular signal-regulated kinase; JNK, Jun amino-terminal kinase; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MEK, meiotic chromosome axis–associated kinase; MKK, mitogen-activated protein kinase kinase; NSCLC, non–small cell lung cancer; XPC, xeroderma pigmentosum complementation group C.

increased IκB-α protein expression in rodents by inhibiting its degradation (41), indicating that the inhibitory effect of ASX on NF-κB expression may be exerted via regulating IκB-α stability.

Consistently, in vitro, ASX treatment at a dosage of 10–200 μM significantly decreased LPS-induced NF-κB–p65 protein concentration in the nuclei of mouse primary peritoneal macrophages, which was associated with decreased apoptotic cells and decreased TNF-α and IL-6 secretion (41).

MAPK signaling pathway

MAPKs are serine and threonine kinases in crucial signal transduction pathways that regulate cell proliferation, cell differentiation, and cell death in humans (80). MAPKs can be classified into 3 major subgroups in mammals: extracellular signal-regulated kinases (ERKs), Jun amino-terminal kinases (JNKs), and p38 MAPK (80).

ERK1/2 signaling pathway.

ERK1 and ERK2 are the major ERK family members that consist mainly of a kinase domain. The ERK1/2 module responds preferentially to growth factors, differentiation stimuli, and mitogens to regulate cell growth and differentiation (81, 82). The activation of ERK1/2 is induced by a particular MAPK kinase (MAPKK). The MAPKKs of ERK1/2 are meiotic chromosome axis–associated kinase 1 (MEK1) and MEK2, which are dual-specificity protein kinases that mediate the phosphorylation of tyrosine and threonine in ERK1 and ERK2, leading to increased cell proliferation and migration (83, 84). It has been shown that the concentrations of ERK1/2 are elevated in NSCLC tissues compared with those in the corresponding normal surrounding lung tissues (85), and the amount of phospho-ERK1/2 is an independent prognostic factor for poor overall survival in NSCLC patients (86).

In mice, ASX showed substantial efficacy in inhibiting ERK1/2 activation in the chronic lung inflammation model (100 mg/kg BW ASX), as well as the ALI model (5 mg/kg BW ASX) (41).

In H1650 and H1703 cells, ASX treatment at 5–20 μM significantly decreased the phosphorylation of ERK1/2 and MEK1/2, which resulted in decreased protein expression of TS by inducing ubiquitin-26S proteasome-mediated proteolysis (47). A high concentration of TS is associated with poor prognosis in NSCLC patients after lung resection (87) and impaired response to the antifolate drug neoadjuvant pemetrexed chemotherapy (88). Thus, it is possible that ASX may enhance the cytotoxic effects of pemetrexed against NSCLC by suppressing ERK1/2 activation. One previous study also showed that TS protein expression was induced in a MEK1/2–ERK1/2–dependent manner in NSCLC cell lines (89). ASX is a promising natural compound against ALI, lung inflammation, and lung cancer via modulation of the MAPK signaling pathway (**Figure 3**).

JNK and p38 MAPK signaling pathway.

JNKs (with JNK1, JNK2, and JNK3 isoforms) belong to the MAPK family and play a central role in stress signaling pathways. JNK and p38 MAPKs are able to orchestrate cellular responses to various stresses such as oxidative stress, DNA damage, ionizing radiation, inflammation, and growth factors (90, 91). The phosphorylation of JNK and p38 MAPKs induces the transcription of multiple downstream molecules, such as B cell lymphoma/leukemia-2 (bcl-2), cyclin D1, Bcl-2-associated X (Bax), and p53, which are involved in regulating cell growth, differentiation, survival, and apoptosis (90). Notably, the expression of JNK and p38 MAPK pathway components is often altered in human tumors and cancer cell lines (91). Preclinical evidence suggests that JNK enzyme function is required for crucial steps in the process of lung remodeling and pulmonary fibrosis (92), ALI (93, 94), and lung cancer (95, 96). Interestingly, ASX treatment has been shown to significantly reduce JNK phosphorylation and activation in cells stimulated by UVB radiation, TNF-α, cobalt, dextran sulfate sodium, and insulin [reviewed by Kim and Kim (97)]. In addition, ASX can regulate autophagy through JNK and p38 MAPK pathways (97).

In vivo, ASX supplementation (20 mg/kg BW) for 7 d significantly decreased LPS-induced phosphorylation of p38 and JNK, resulting in alleviated chronic lung inflammation and ALI (41). In another study, ASX treatment significantly suppressed the pulmonary protein expression of Bcl-2 and enhanced p53 protein expression (42). This was consistent with the in vitro data that ASX treatment inhibited cell proliferation by enhancing cell apoptosis, mediated by inducing p53 and reducing Bcl-2 proteins (42, 45). In contrast, in A549 and H1975 cells, ASX treatment at a dosage of 20 μM enhanced phospho-p38 MAPK protein concentrations and the phosphorylation of mitogen-activated protein kinase kinase (MKK) 3/6, which is the MAPKK of p38 MAPK, leading to decreased NSCLC cell survival (48). Such discrepancies may be due to different experimental settings (in vivo compared with in vitro) or study designs (LPS induction compared with no stimulation). ASX (20 μM) enhanced erlotinib cytotoxicity toward NSCLC cells and inhibited the expression of XPC in a time- and dose-dependent manner, which improved the sensitivity of NSCLC cells to erlotinib (48). Taken together, ASX showed beneficial efficacy in alleviating lung fibrosis, chronic lung inflammation, ALI, and NSCLC in preclinical studies by modulating the MAPK signaling pathway (Figure 3).

Janus kinase–signal transducers and activators of transcription-3 signaling

The Janus kinase–signal transducers and activators of transcription-3 (JAK/STAT-3) signaling pathway is a pleiotropic cascade that can transduce a multitude of signals that regulate immunity, cell apoptosis, cell proliferation, and tumorigenesis in humans (98). In mammals, the JAK family comprises 4 members: JAK-1, JAK-2, JAK-3, and Tyk-2. The activated JAKs can phosphorylate additional targets, such as STATs (98, 99). STAT-3 is a latent transcription factor that is predominantly localized in cytoplasm (99). It functions through transmitting signals from the cell surface to the nucleus to promote oxidative phosphorylation and mitochondrial membrane permeability (99). Aberrant activation of JAK/STAT-3 signaling can lead to the development of multiple lung diseases, including lung cancer (100), lung fibrosis (101), ALI (102), and chronic lung inflammatory diseases (103).

Kowshik et al. (104) reported that in male Syrian hamsters, ASX supplementation at 15 mg/kg BW exerted anticancer actions through inhibiting the phosphorylation of STAT-3, subsequently decreasing its nuclear translocation. These events were associated with ameliorated oral cancer severity in rodents, indicating that ASX is capable of inhibiting tumor development and progression by modulating the JAK/STAT-3 signaling pathway.

In this review, we highlight reports that ASX showed anticancer activities in rat hepatocellular carcinoma cells (39 μM) and lung cancer cells (20, 40, 60, 80, and 100 μM) by inhibiting the phosphorylation of JAK1 and its downstream target, STAT-3, subsequently downregulating bcl-2, B cell lymphoma–extra large, the proto-oncogene protein c-myc, and Bax (45, 105). The effects of ASX on JAK/STAT-3, or other isoforms of JAK/STAT, such as JAK-1/3–STAT-6 and JAK-1/2–STAT-1/3/5 (106), warrant further investigation.

Phosphoinositide 3-kinase/Akt pathway

The phosphoinositide 3-kinase (PI3K)/Akt pathway is an intracellular signaling pathway linking oncogenes and various receptor classes to many aspects of cell growth and survival, both in physiological and in pathological conditions (107). PI3Ks are activated by receptor tyrosine kinases and convert phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate, followed by recruitment of the Ser–Thr kinase, Akt kinase, to the plasma membrane, where activated Akt elicits a wide range of downstream signaling events (108). The PI3K/Akt pathway is generally activated in NSCLC (109). It plays an essential role in promoting oncogenesis in lung cancer and mediating resistance to EGFR tyrosine kinase inhibitors (109). Therefore, therapy targeting this axis warrants further study. Indeed, a study reported that ASX supplementation at 15 mg/kg BW could prevent cancer hallmarks by suppressing the PI3K/Akt pathway, which was associated with inhibited NF-κB and STAT-3 signaling pathways in SCC131 and SCC4 oral cancer cells (in vitro), as well as in the hamster buccal pouch carcinogenesis model (in vivo) (104).

In NSCLC cells, ASX treatment at doses of 2.5–20 μM significantly decreased the phosphorylation of Akt at Ser473 in a dose-dependent manner, which was associated with a decreased transcription level of *Rad51*(46). *Rad51* is essential in the initiation and progression of carcinogenesis by promoting cancer cell survival (84), and it confers radiotherapy resistance, reduced survival, and poor prognosis in breast cancer, cervical cancer, and NSCLC (110, 111). *Rad51* was further decreased in cells with a combined treatment of ASX

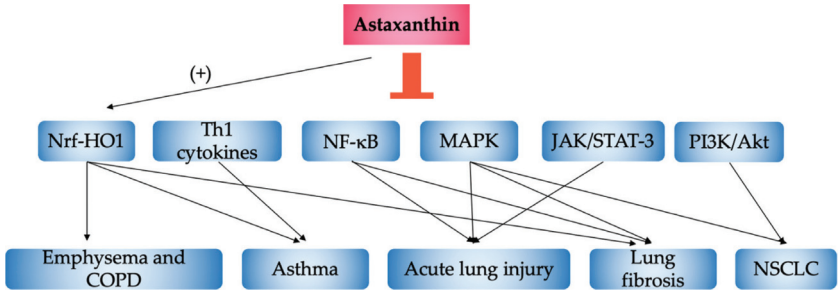

FIGURE 4 The suggested molecular mechanisms of action for ASX in the prevention of lung diseases. ASX activates the Nrf–HO-1 pathway to inhibit emphysema and COPD and asthma development. ASX suppresses the NF-κB, MAPK, JAK/STAT-3, PI3K/Akt pathways. ASX downregulates Th1-mediated immune responses to alleviate asthma. ASX inhibits acute lung injury through its effect on the NF-κB and JAK/STAT-3 pathways; alleviates lung fibrosis by suppressing the NF-κB and MAPK pathways and NSCLC by ASX supplementation via the inhibited MAPK and PI3K/Akt pathways. ASX, astaxanthin; COPD, chronic obstructive pulmonary disease; JAK/STAT-3, Janus kinase–signal transducers and activators of transcription-3; MAPK, mitogen-activated protein kinase; Nrf-HO1, nuclear respiratory factor-2/heme oxygenase 1; NSCLC, non–small cell lung cancer; PI3K, phosphoinositide 3-kinase; Th1, T helper type 1.

(20 μM) and PI3K inhibitors (46). MMC is an antitumor antibiotic that is widely used in clinical NSCLC chemotherapy. It was reported that a combination of ASX (2.5 or 5 μM) and MMC (2.5–10 μM) showed synergistic efficacy in decreasing cell growth in NSCLC cells, accompanied by reduced concentrations of AKT phosphorylation and *Rad51* protein expression (46), thus highlighting the possibility of applying ASX in the mitigation of lung cancer by targeting the PI3K/AST pathway.

Immune response

$CD4^+$ cells can be divided into T helper type 1 (Th1) and T helper type 2 (Th2) cell subsets. Compared with Th1 cells, a larger number of Th2 cells are found in the airways of patients with asthma (32). IL-4, IL-5, and IL-13 cytokines are the predominant cytokines secreted from Th2 cell subsets and play a key role in driving the disease pathology in asthma patients (112). Among these cytokines, IL-4 has long been recognized as a critical pro-inflammatory cytokine in the differentiation of $CD4^+$ cells to Th2 lymphocytes and is essential for allergic sensitization and IgE isotype switch (113). The biology of IL-13 resembles that of IL-4 and is crucial for eosinophil survival (112, 114, 115). Conversely, administration of the pro-Th1 cell cytokine IL-12 produces IFN-γ, which alleviated asthmatic conditions (116).

In vitro, direct administration of ASX at 300 nM induced cell proliferation and enhanced LPS-induced IFN-γ production in lymphocytes, although ASX treatment did not show an apparent effect on IFN-γ production in primary cultured lymphocytes (117).

Using an ovalbumin-induced asthma mouse model, Hwang et al. (32) found that oral administration of ASX (5, 10, and 50 mg/kg BW) significantly decreased IL-4 and IL-5 concentrations and promoted IFN-γ in BALF, compared with mice without ASX supplementation. In addition, they observed reduced total IgE, IgG1, ovalbumin-specific IgG1, IgG2a, and ovalbumin-specific IgG2a in the ASX-supplemented mice compared with their counterparts without ASX administration. Such effects led to suppressed lung inflammation, lung fibrosis, and caspase-1 and caspase-3 expression, suggesting that ASX may have therapeutic potential for alleviating asthma by inhibiting Th2-mediated cytokines while enhancing Th1-mediated cytokines. However, whether ASX is capable of regulating $CD4^+$ cell differentiation and which subset the $CD4^+$ cells differentiate into under stimulation by ASX remain enigmatic.

Conclusions

We reviewed experimental evidence showing the health benefits of ASX in alleviating asthma, COPD, and emphysema; ALI, lung fibrosis, and lung cancer. The potential beneficial effects of ASX were mediated by inhibiting the activation of the Nrf–HO-1 pathway, NF-κB signaling, MAPK signaling, JAK/STAT-3 signaling, and the PI3K/Akt pathway. ASX also exerts its efficacy against lung diseases by modulating the immune response, particularly reducing Th1 cytokines (**Figure 4**).

In the in vivo studies included in this review, animals were given ASX supplementation at a dosage between 0.5 and 50 mg/kg BW (8, 32, 33, 34, 38, 40, 41, 42, 44, 105). This is equivalent to a 60-kg man consuming ASX at a dosage between 0.065 and 6.5 mg/kg BW (118). Based on European consumption data of the proposed food categories, the mean and 95th percentile of daily intakes of ASX were 0.106 and 0.256 mg/kg BW, respectively (3). We are aware that the dosages of ASX used in high-dose groups exceed the daily intake of ASX by ~20-fold. However, even at low dosage that is within physiological relevance, the studies found that ASX significantly alleviated lung fibrosis (42, 44, 105) and improved the mitochondrial morphology as well as SOD and catalase activities in the animals (105). In addition, none of the studies reported genotoxicity or hepatotoxicity with ASX supplementation at high doses, indicating that ASX has the potential to be applied as a safe dietary

supplement or medical compound to alleviate pulmonary diseases.

One major limitation of this review is the usage of supraphysiologic concentrations of ASX in cell-based assays. As mentioned previously, an acute dose of 100 mg ASX supplementation in male volunteers (90–100 kg BW) resulted in circulating concentrations of ASX reaching a maximum of 120 μg/L (21). This is equivalent to 0.4 μ ASX treatment in the cells with 2 mL media. Notably, such concentration is far higher than allowed by the European Food Safety Authority or the FDA. However, in cell-based studies, the lung cells were treated with ASX at a dosage of 2.5–200 μM, which is $\geq$5-fold more than reported concentrations of ASX in humans supplemented with a high dose of ASX. Note that in subjects without ASX supplementation, circulating ASX concentrations were nondetectable. Also, like other dietary carotenoids, the absorption efficiency of ASX can be quite poor from whole foods in humans. Therefore, more studies need to be carried out with ASX treatment at a dosage within physiological relevance.

Although there is accumulating evidence that ASX is a potent antioxidant against numerous lung diseases, these studies have been performed exclusively in cells and animals, which lack generalizability to humans. However, preclinical studies have historically been implemented in discovering compounds or drugs that can be used to treat diseases. Therefore, despite lacking clinical evidence, the studies included in this review provide important information for the exploration of novel therapeutic approaches for ameliorating asthma, COPD and emphysema, ALI, lung fibrosis, and lung cancer.

This review poses several new research questions about the effects of ASX in lung disease studies. First, future studies should investigate the efficacy of ASX-enriched foods, such as algae and krill. It has been shown in vitro that krill oil has anticancer properties by inhibiting cancer cell growth and inducing cancer cell apoptosis (119). One study reviewed the anticancer properties of microalgal species and reported their capability of inducing the arrest of cell growth (120). It will be interesting to investigate whether consuming ASX-enriched foods may lead to amelioration in various lung diseases. Another topic of interest lies in exploring the effects of biological metabolites of ASX. As a non–provitamin A xanthophyll carotenoid, ASX cannot be cleaved at the central C15=C15′ double bond by β-carotene oxygenase 1 enzyme into retinol. However, it can be cleaved at the C9 position, yielding its polar metabolites, (rac)-3-hydroxy-4-oxo-7,8-dihydro-β-ionone and (rac)-3-hydroxy-4-oxo-β-ionone (13), as shown in rat hepatocytes (Figure 1). Several studies have shown that the metabolites of carotenoids may display more potent anticancer efficacy compared with their parent compounds (121, 122). Therefore, one can hypothesize that the cleavage products from ASX might impose equally strong, or even more potent, efficacy in lung diseases. Also, because the human body as an integrated whole is a highly dynamic system, another future direction is to investigate how ASX may affect systemic and lung diseases by regulating the cross-talk between the lung and other tissues. Finally, future in vitro and in vivo studies need to include an ASX dosage within physiological relevance. Also, clinical trials are warranted to investigate whether ASX could be given as a supplement to protect against pulmonary diseases.

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