Astragaloside IV: A multipotent phytochemical for treating fibrotic diseases (Review)

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Abstract. Fibrosis is a maladaptive response of tissues or organs to adverse stresses, such as chronic inflammation, infection and mechanical injury. It further promotes parenchymal cell loss, abnormal myofibroblast proliferation and excessive extracellular matrix buildup, eventually triggering scar tissue hyperplasia or organ injury. Although a moderate fibrotic response is beneficial for compensatory tissue repair induced by exogenous or endogenous injury, excessive fibrosis is the basis for the promotion of multiorgan pathologies, such as cardiac hypertrophy, idiopathic pulmonary fibrosis, or renal tubulointerstitial fibrosis. In industrialized countries alone, fibrotic diseases account for ~45% of all-cause mortality. Consequently, the development of medications that regulate the activation of growth factors, proliferation of fibrotic effector cells and deposition and degradation of the extracellular matrix is essential. Botanical compounds derived from Chinese medicine are generally considered natural tonics. Among these compounds, astragaloside IV (AS-IV) is a bioactive product isolated from the roots of *Astragalus membranaceus* Bunge. On the basis of the multitarget therapeutic mechanism of Chinese herbal medicine, AS-IV may have considerable benefits in improving multiorgan fibrosis and complex fibrotic diseases with multisignal cascades. It can effectively alleviate the fibrosis-induced dysfunction of major tissues or organs, including the heart, lungs, kidneys and liver, by regulating the signal transduction of reactive oxygen species/caspase-1/gasdermin D, transforming growth factor-β/Smads, Wnt/β-catenin and sirtuin 1-nuclear factor-κ B. The present review mainly focused on phytomedicine and highlights the potential of AS-IV as an antifibrotic medication. It aimed to provide a novel reference for the application of AS-IV in the nutritional intervention of fibrotic diseases.

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Abbreviations: AGEs, advanced glycation end-products; AKT, protein kinase B; AS-IV, astragaloside IV; BDL, bile duct ligation; CA, cycloastragenol; DKD, diabetic kidney disease; EMT, epithelial-mesenchymal transition; FNDC5, fibronectin type III domain-containing protein 5; GSDMD-N, gasdermin D N-terminal domain; HCC, hepatocellular carcinoma; IPF, idiopathic pulmonary fibrosis; LEF1, lymphoid enhancer binding factor-1; MAPK, mitogen-activated protein kinase; MCs, mesangial cells; MMPs, matrix metalloproteinases; Nrf2, nuclear factor erythroid 2-related factor 2; PF, pulmonary fibrosis; POAG, primary open-angle glaucoma; RTECs, renal tubular epithelial cells; T2DM, type 2 diabetes mellitus; TGF-β1, transforming growth factor-β1; TM, trabecular meshwork; UUO, unilateral ureteral obstruction; ZEB1, zinc finger E-box binding homeobox 1; α-SMA, α-smooth muscle actin

Key words: fibrotic disease, astragaloside IV, mechanism, signaling pathway, target

Contents

1. Introduction

Fibrosis is defined as the overabundant deposition of the extracellular matrix (ECM), which includes collagen and fibronectin (FN), leading to the accumulation of substantial fibrous connective tissue in areas of inflammatory infiltration or within and around damaged tissues (1). It is a pathological condition that eventually triggers severe scar hyperplasia; organ dysfunction, such as heart failure, idiopathic pulmonary fibrosis (IPF), end-stage renal disease (ESRD), end-stage liver disease and even death (2). If epithelial or endothelial regeneration capacity cannot be synchronized with recurrent damage during cell infection, exposure to toxic factors, or autoimmunity, or if other uncertain factors continuously induce

epithelial or endothelial damage, then the corresponding mesenchymal cells are activated and a considerable amount of ECM is deposited; this phenomenon is also the mechanism of fibrotic reaction in most organs (3). Organ fibrosis is a dynamic pathological process. In contrast to irreversible scar tissue formation, it is highly plastic and can reversely change the course of fibrosis by targeting key effector cells or signaling (4). Epidemiological data have shown that fibrosis-related health events pose a great threat to the health of a quarter of the population worldwide and that the annual incidence of major fibrosis-related diseases is close to 1 in 20 (5). Nintedanib and pirfenidone are prescription drugs approved by a number of developed and developing countries for intervening in fibrosis in specific organs and are available to patients with IPF. Thus far, however, drugs that specifically target the fibrosis of the liver, kidney, or heart have yet to be approved in the medical market. In addition, although nintedanib has been approved for the medical management of systemic sclerosis (SSc)-related interstitial lung disease and progressive fibrosing interstitial lung disorders, marked advances are needed regarding the development of fibrotic drugs, especially highly effective drugs that can specifically act on affected organs, thereby achieving reversible intervention in organ fibrosis diseases (6).

Multiple mechanisms determine the progression of fibrotic diseases and traditional Chinese medicine (TCM) can exert multicomponent, multitarget and multipathway therapeutic benefits. Medicinal resources, including Chinese herbal compounds, materials, extracts and formulas and their combinations may improve fibrotic diseases comprehensively with reduced pharmacological toxicity under the cumulative effects of the aforementioned therapeutic characteristics (7,8). Chinese herbal medicine has a protective effect against multiorgan fibrosis. This effect mainly manifests by improving myocardial perfusion and hemodynamic function, repairing ultrastructural damage in cardiac tissue, inhibiting cell apoptosis and cardiac remodeling and alleviating cardiac dysfunction (8). Active products derived from single herbs and TCM formulas effectively intervene in pulmonary fibrosis (PF) by targeting inflammation, oxidative stress and fibrotic effector molecules (7). Furthermore, a main mechanism through which Chinese herbal medicines alleviate renal fibrosis is the regulation of growth factors and fibrotic effector cytokines and the activation of related signaling pathways. These activities further improve the renal inflammatory microenvironment and inhibit reactive oxygen species (ROS) generation and renal cell proliferation (9). Given that multiple signaling molecules are involved in the development of liver fibrosis, treatment strategies for liver fibrosis are no longer limited to a single targeted inflammatory response and the multitarget therapeutic effects of Chinese herbal medicine may meet the needs of the multimodal alleviation of liver fibrosis (10). Intervention modes targeting metabolic mechanisms may become a key strategy to delay fibrosis. The widespread application of network pharmacology is conducive to constructing the affiliation between the main active ingredients in herbal medicines and metabolic targets, implying that the medical value of Chinese herbal medicine is receiving increasing attention (5,11). The present review is based on databases, such as PubMed, Web of Science, Elsevier and ScienceDirect and SpringerLink. 'Astragaloside IV (AS-IV)', 'fibrosis', 'pharmacokinetics', 'toxicity', 'bioavailability', and other keywords were used for retrieval, with relevant case reports, conference abstracts and duplicate studies being excluded. The present review mainly focuses on AS-IV, the natural saponin compound purified from *Astragalus membranaceus* Bunge (*Astragalus*), named *Huangqi* in Chinese and summarizes and discusses its metabolic regulatory mechanisms and key drug targets in alleviating fibrotic diseases from 63 basic experimental literatures (Fig. 1). It is hoped that the present review will provide additional theoretical support for the intervention of tissue fibroses by TCM or a combination of traditional Chinese and Western medicine and it calls for optimizing the bioavailability of natural products to improve the effectiveness of drug-targeted organ therapy.

2. Overview of AS-IV

Biological characteristics of AS-IV. Astragalus, a member of the leguminous family, is a perennial herb that is mostly produced in temperate and subtropical regions (12). It has been used as medicine for >2,000 years (13). More than 200 compounds, such as flavonoids, polysaccharides, triterpene saponins and certain trace elements, are found in *Astragalus* (14,15). Furthermore, in addition to being a type of TCM with tonic properties, *Astragalus* contains various antifibrotic pharmacological ingredients, including calycosin, AS-IV, polysaccharides and formononetin (16). *Astragalus* encompasses >2,000 species and different *Astragalus* species often have varying pharmacological components. This situation leads to variances in pharmacokinetics in mice administered *Astragalus* from different sources (16,17). Current pharmacokinetic research on *Astragalus* is limited because of seasonal diversity, growth location and planting years (16).

Astragalosides include various components, such as astragalosides I, II and IV. Among these natural compounds, high-purity AS-IV extracted from *Astragalus* has the strongest pharmacological activity (18). It can also be used as a representative marker to quantify the quality of *Astragalus* (19). Astragaloside undergoes mutual transformation during oral and intestinal digestion. This transformation is mainly reflected in the changes in AS-IV content. After the acetyl group is hydrolyzed, astragaloside II can undergo biotransformation, resulting in a corresponding increase in the amount of AS-IV during oral digestion. AS-IV content is stable during gastric digestion. During intestinal digestion, the contents of the three family members of astragaloside increase, indicating that other cycloartane-type triterpenoid saponins in *Astragalus* have been transformed into AS-IV. Moreover, this phenomenon reveals that transacetylation is involved in the quantitative change in astragaloside during digestion (20).

AS-IV is a highly polar lanolin alcohol-shaped tetracyclic triterpenoid saponin; it has poor hydrophilicity, the molecular formula of $C_{41}H_{68}O_{14}$ and molecular weight of 784.97 Da and is easily soluble in ethanol, methanol, or acetone (21,22) (Fig. 2). The extraction and separation of AS-IV through ultrafiltration, high-speed centrifugation and ultrasonic extraction can reduce the effect of the low water solubility of AS-IV and yield high contents of AS-IV with a shortened production cycle and reduced loss. This also indicates that the innovation of drug extraction and separation technology facilitates

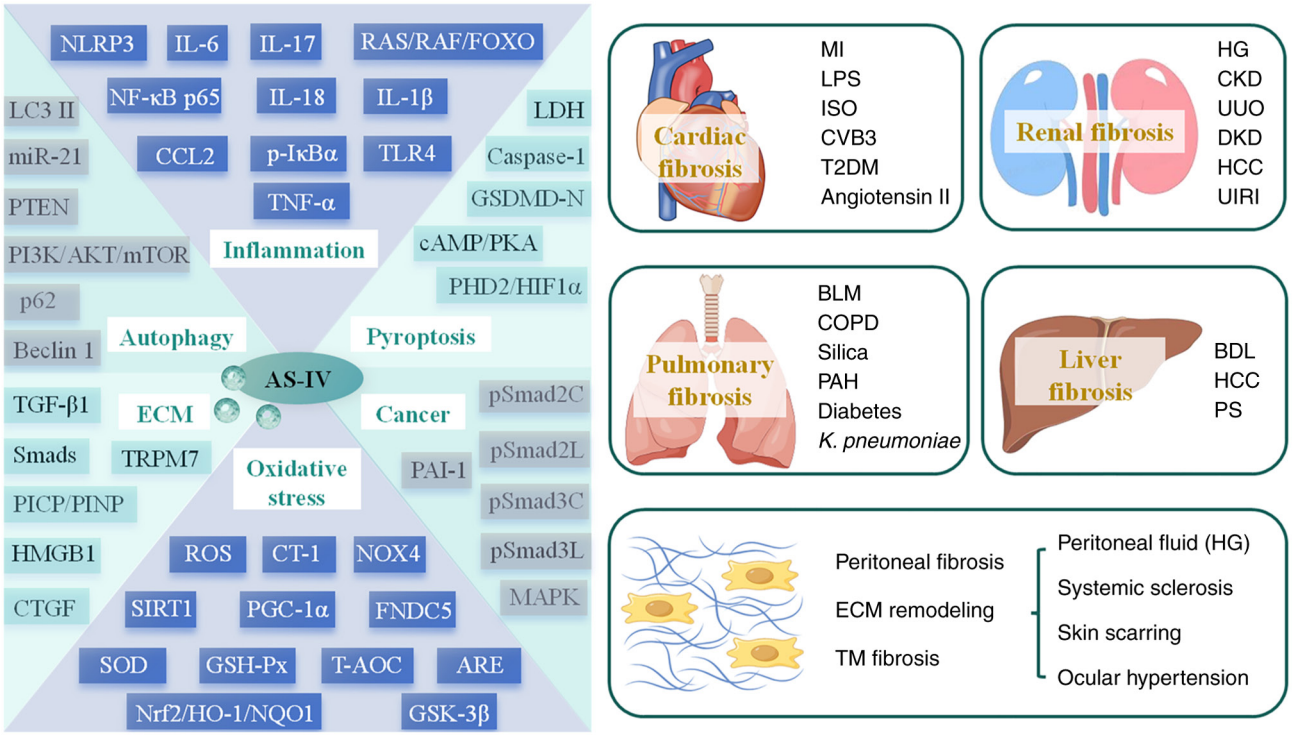

Figure 1. A general overview of AS-IV regulation of fibrotic diseases. AKT, protein kinase B; ARE, antioxidant response element; AS-IV, astragaloside IV; BDL, bile duct ligation; BLM, bleomycin; cAMP, cyclic adenosine monophosphate; CCL2, C-C motif chemokine ligand 2; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CT-1, cardiotrophin-1; CTGF, connective tissue growth factor; CVB3, coxsackievirus B3; DKD, diabetic kidney disease; ECM, extracellular matrix; FNDC5, fibronectin type III domain-containing protein 5; FOXO, forkhead box O; GSDMD-N, gasdermin D N-terminal domain; GSH-Px, glutathione peroxidase; GSK-3β, glycogen synthase kinase-3β; HCC, hepatocellular carcinoma; HG, high glucose; HIF1α, hypoxia-inducible factor 1α; HMGB1, high mobility group box-1; HO-1, heme oxygenase-1; IL-6, interleukin-6; ISO, isoproterenol; IκBα, inhibitor of NF-kappa B α; *K. pneumoniae*, *Klebsiella pneumoniae*; LC3 II, light chain 3 II; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MI, myocardial infarction; miR-21, microRNA-21; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NLRP3, nucleotide-binding oligomerization domain-like receptor thermal protein domain associated protein 3; NOX4, NADPH oxidase 4; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; PAH, pulmonary artery hypertension; PAI-1, plasminogen activator inhibitor-1; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1 α; PHD2, prolyl-4-hydroxylase 2; PI3K, phosphatidylinositol-3-kinase; PICP, procollagen type I carboxy-terminal propeptide; PINP, procollagen type I N-terminal propeptide; PKA, protein kinase A; PS, porcine-serum; pSmad3C, COOH-terminal phosphorylation of Smad3; pSmad3L, phosphorylation of the linker region of Smad3; PTEN, phosphatase and tensin homolog; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; ROS, reactive oxygen species; SIRT1, sirtuin 1; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus; T-AOC, total antioxidant capacity; TGF-β1, transforming growth factor-β1; TLR4, toll-like receptor 4; TM, trabecular meshwork; TNF-α, tumor necrosis factor-α; TRPM7, transient receptor potential cation channel, subfamily M, member 7; UIRI, unilateral ischemia-reperfusion injury; UUO, unilateral ureteral obstruction.

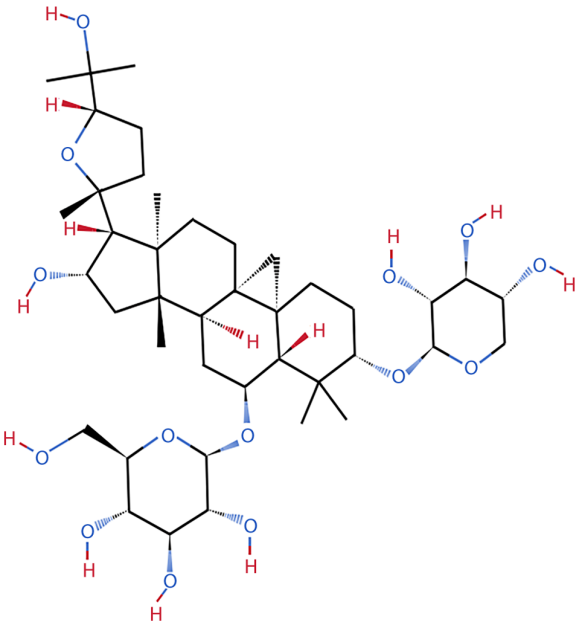

Figure 2. Molecular structure of astragaloside IV.

the gradual identification of various saponin components of *Astragalus* via gas chromatography-mass spectrometry and high-performance liquid chromatography. AS-IV, which has been approved as a medicine and food homologous bioactive product in China, has become increasingly popular in the clinical and food markets (23-25). It demonstrates considerable pharmacological benefits in improving hypertension (26), diabetes (27), infectious diseases (28), viral infections (29), ischemia-reperfusion injury (30) and anxiety (31), as well as anti-inflammation (32), antioxidative stress (33), antiperspirant and antidiarrheal (34) effects. Cycloastragenol (CA) is an aglycone and metabolite of AS-IV. The use of AS-IV and CA in the pharmaceutical market as telomerase activators indicates that AS-IV could also potentially be a viable antiaging medication (24).

Toxicity of AS-IV. The median lethal dose of *Astragalus* after acute oral administration to rats was found to exceed 250 g/kg body weight and no toxic reactions occurred after the oral administration of *Astragalus* for 90 days at a dose of 15 g/kg body weight (16). Furthermore, no adverse reactions were

observed after the intraperitoneal injection or intravenous administration of extracts containing *Astragalus* saponins and polysaccharides to rats and beagle dogs for three months. The safe dose ranges for rats and beagle dogs are 5.7-39.9 and 2.85-19.95 g/kg, respectively, which are 70 or 35 times that for humans (0.57 g/kg for an average weight of 70 kg) (35). AS-IV does not cause obvious symptoms or organ toxicity when used in clinical treatment and the oral administration of the conventional doses of AS-IV has no side effects on liver and kidney function (36). Notably, in Sprague-Dawley rats and New Zealand rabbits, the intravenous administration of 0.5-1.0 mg/kg AS-IV during pregnancy can produce certain toxic effects on the mother and fetus but does not cause fetal malformations (37). Further studies have shown that in rats, the administration of 1.0 mg/kg AS-IV for up to four weeks during gestation can induce fur formation, eye opening and neurodevelopmental lag in newborn rats but does not affect the cognitive function of newborn rats (38). Furthermore, the intravenous administration of 0.25-1.0 mg/kg AS-IV is detrimental to the reproductive ability of rats. Administering AS-IV at a dosage of 1.0 mg/kg/day to treat cardiovascular disease (CVD) during pregnancy may also induce side effects (37,38). Therefore, although AS-IV poses almost no toxicity risk to the general population, attention should be paid to the safe dose range of AS-IV during pregnancy and AS-IV should be taken with caution.

Compared with the oral administration of single drugs, the intravenous administration of AS-IV preparations often exhibits more remarkable bioavailability. In a test population, after the intravenous injection of 200-500 ml of astragaloside containing AS-IV for one week, only mild symptoms, such as transient episodes of elevated total bilirubin and rashes, appeared and subsided spontaneously (39). In addition, various organs or tissues of the body show good tolerance to astragaloside injection, with the highest tolerated dose reaching 600 ml. Even if astragaloside is intravenously infused at a rate of up to 4 ml/min, no remarkable adverse reactions in humans occurred (39).

3. Antifibrotic effects of AS-IV

Cardiac fibrosis. The occurrence of cardiac fibrosis is closely related to various CVDs, including chronic heart failure, atrial fibrillation, cardiac remodeling after acute myocardial infarction (MI), valvular heart disease, hypertension and ischemic injury (40). Between 60-70% of human heart cells are in the form of fibroblasts, which over-differentiate into myofibroblasts when the heart is stimulated by proinflammatory factors or ROS. These myofibroblasts are a major source of ECM deposited in the cardiac interstitium. They can further induce cardiac contractile and diastolic dysfunction and trigger pathological cardiac remodeling (41,42). ECM in the cardiac interstitium is mainly composed of type I collagen (collagen I). Myocardial fibrosis (MF) may lead to electrical heterogeneity and destroy cardiac ejection function. Severe cardiac fibrosis even predisposes to irreversible cardiovascular lesions, such as excessive arrhythmias and sudden cardiac death (43). Given that cardiovascular events have gradually become the main cause of global mortality and that fatal events related to CVDs once accounted for >40% of deaths in China, active intervention focusing on cardiac fibrosis has become a key strategy for promoting the development of human health (44). Anticoagulation, antiplatelet, thrombolysis and myocardial reperfusion strategies are widely used to reduce the area of MI and are the main medical means to relieve MI. However, they are often accompanied with side effects. For example, they indirectly lead to the aggravation of the extent of myocardial injury. Therefore, the application of high-efficiency drugs with low myocardial toxicity can help achieve ideal prognostic effects. Among these drugs, AS-IV is a derivative of Chinese herbal medicine that not only lacks organ toxicity when administered at regular doses but can also achieve considerable therapeutic benefits in anticardiac fibrosis.

Inflammation. The nucleotide-binding oligomerization domain-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome acts as a multiprotein complex that concurrently facilitates the onset of inflammation and cardiac fibrosis (45). The activated NLRP3 inflammasome not only maintains myocardial interstitial inflammatory infiltration, but its induced proinflammatory factors, such as interleukin (IL)-18 and IL-1β, also activate profibrotic growth factors and promote the deposition of collagen and development of a profibrotic population of fibroblasts (46). AS-IV administration markedly inhibits MF induced by isoproterenol (ISO) treatment. This effect may be associated with a decrease in the expression of caspase-1 and IL-18 in cardiac tissue caused by AS-IV through the targeted inhibition of NLRP3 inflammasome activation (41). In addition, ventricular remodeling phenomena, such as myocardial hypertrophy, fibrosis and cardiomyocyte apoptosis, are closely related to the development of end-stage CVDs. The C-C motif chemokine ligand 2 (CCL2) gene can regulate the expression of inflammatory factors, such as the proinflammatory factor IL-6, enriched in the nuclear factor-κ B (NF-κB) signaling pathway. Therefore, CCL2 is defined as a mechanistic target of AS-IV for antilipopolysaccharide-induced inflammation in H9C2 cardiomyoblasts. This relationship can be attributed to the ability of CCL2 inactivation to regulate negatively the lipopolysaccharide (LPS)-activated NF-κB signaling pathway and the high expression of its target genes IL-17 and tumor necrosis factor-α (TNF-α). Moreover, CCL2 may be an effector molecule for MF and the overexpression of fibrosis-related genes (proliferating cell nuclear antigen, collagen I and collagen III) induced by LPS is favorably connected with CCL2 activity. AS-IV can also serve as a potential CCL2 inhibitor to improve LPS-induced myocardial remodeling. Furthermore, AS-IV blocks NF-κB from entering the cytosol and hinders the inactivated inhibitor of NF-κB (IκB) to drive LPS-induced NF-κB p65 overexpression, thereby effectively inhibiting cardiac hypertrophy and the fibrosis of LPS-induced H9C2 cells (47).

Oxidative stress. Evidence indicate that cardiotrophin-1 (CT-1), as a key mediator of cardiac remodeling, is involved in cardiac fibroblast (CF) and vascular smooth muscle cell proliferation, collagen I deposition and ECM synthesis to a certain extent, with CT-1 being closely related to cardiomyocyte survival and cardiac hypertrophy (48,49). Notably, driving CT-1 overexpression is related to the release of ROS and treatment with AS-IV and the ROS inhibitor *N*-acetylcysteine targets the production of ROS in CFs, inhibits ISO-induced CF

proliferation and CT-1 overexpression and reduces collagen I deposition in cardiomyocytes. These effects indicate that inhibiting the driving effect of ROS on CT-1 expression is a potential mechanism of the anti-MF effect of AS-IV. However, whether mitochondrial NADPH oxidase 4, mitochondrial superoxide dismutase and mitochondrial catalase contribute to the negative regulation of oxidative stress in myocardial tissue by AS-IV remains inconclusive (50). ISO is a typical inducer of cardiac fibrosis and its profibrotic mechanism is intimately linked to the generation of ROS and activation of c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) (51,52). Notably, Dai *et al* (53) found that AS-IV can prevent profibrotic MAPK family members from undergoing phosphorylation and that the ROS inhibitor *N*-acetylcysteine mimics AS-IV's suppressive effect on MAPK activation. That is, their study finally demonstrated that AS-IV may exert an anticardiac fibrotic benefit by downregulating oxidative stress-mediated MAPK phosphorylation through the targeted inhibition of ISO-induced ROS production. Fibronectin type III domain-containing protein 5 (FNDC5) is highly active in the brain and heart and crosstalk has been found between the sirtuin 1 (SIRT1)/peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α)/FNDC5 signaling cascade and improvement in diabetic MF (54). Cong *et al* found that in mice, AS-IV activates the SIRT1/PGC-1α/FNDC5 signaling pathway, therefore effectively inhibiting atrial fibrosis, atrial fibrillation and oxidative stress induced by angiotensin II (55).

Lipid metabolism. Although the heart is not a typical organ for lipid storage, a pathological increase in serum lipid levels causes fatty acids to enter myocardial cells and some fatty acids are forced to be stored in myocardial cells in the form of triglycerides. When lipid deposition reaches a certain extent, the production of lipid-toxic intermediates, such as ceramides, diacylglycerols and ROS, become an important mechanism mediating myocardial inflammation and fibrosis in patients with diabetes and obesity (56). AS-IV not only helps alleviate cardiac lipid deposition by regulating plasma triglyceride, total cholesterol, high-density lipoprotein and myocardial free fatty acid (FFA) contents but also inhibits type 2 diabetes mellitus (T2DM)-related myocardial damage induced by increased serum creatine kinase isoenzyme and lactate dehydrogenase (LDH) levels and suppresses the driving effect of inflammatory infiltration on MF. AS-IV mainly exerts these effects by targeting the downregulation of TNF-α expression and alleviating the aggravating effect of TNF-α-activated inflammatory cytokines, including IL-1β and IL-6, on MF. It thereby simultaneously inhibits the proinflammatory and pro-MF effects of TNF-α because TNF-α not only originates from the heart but can also be targeted to change the pathophysiology of the heart (57). Therefore, AS-IV can inhibit inflammatory infiltration and fibrosis-induced myocardial damage. This inhibitory effect may be associated with mitigating the toxic damage of lipid deposition on the myocardium (57).

Pyroptosis. Pyroptosis is a form of programmed cell death that is intimately associated with the initiation of proinflammatory responses mediated by caspase-1 (58). However, the cleavage of pro-caspase-1 into active caspase-1 can be inhibited through the targeted inhibition of NLRP3 inflammasome activation, further blocking the transformation of the cytokine precursors pro-IL-1β and pro-IL-18 into bioactive IL-1β and IL-18, respectively. Therefore, this process is beneficial to relieve cardiac dysfunction caused by cardiomyocyte pyroptosis and inflammatory infiltration after MI. Zhang *et al* (59) clarified the upstream and downstream relationships between oxidative stress and cardiomyocyte pyroptosis. That is, AS-IV can inhibit MI-induced ROS release, thereby alleviating NLRP3 inflammasome activation and cardiomyocyte pyroptosis. This effect is achieved mainly through the suppression of the levels of NLRP3, cleaved caspase-1, cleaved IL-1β and cleaved IL-18 and the pyroptosis-related protein gasdermin D N-terminal domain (GSDMD-N) induced by MI. Furthermore, α-smooth muscle actin (α-SMA) and FN overexpression, as well as collagen I and III deposition, can be suppressed by AS-IV. AS-IV also decreases the number of apoptotic cardiomyocytes and compensatory hypertrophy of cardiomyocytes to improve cardiac function. AS-IV has been found to downregulate the ROS/caspase-1/GSDMD signaling pathway, thereby attenuating MF and cardiac remodeling induced by MI. This mechanism has also been demonstrated at the cellular level. That is, AS-IV may exert an anti-inflammatory effect by inhibiting macrophage pyroptosis *in vitro* (59).

Aging. The senescence-associated secretory phenotype (SASP) continuously drives inflammatory infiltration and ECM synthesis and progressively aggravates collagen deposition and fibrosis by inducing the release of transforming growth factor-β1 (TGF-β1), matrix metalloproteinases (MMPs) and the inflammatory cytokines TNF-α and IL-1β. Therefore, selectively regulating the release of SASP components effectively interferes with MF (60,61). Shi *et al* (62) detected a high correlation between cellular senescence and p53 signaling pathway-related genes and proteins through transcriptomics and proteomics analysis and found that the activity of the aging-related indicators p16, p21 and p53 of this pathway are markedly inhibited by AS-IV treatment in the ISO-induced MF group. Given that the senescence of CFs and release of SASP are triggered by oxidative stress and SASP secreted by senescent cells also promotes the oxidative stress effect again, these phenomena form a vicious cycle and induce a progressive exacerbation of the degree of MF. Therefore, the mechanism by which AS-IV inhibits MF may be attributed to regulatory effects on oxidative stress and the release of aging markers (62-64).

Collagen deposition. Coxsackievirus B3 (CVB3) not only serves as a human pathogen that induces acute and chronic viral myocarditis in adolescents but is also an important inducer of dilated cardiomyopathy (DCM) with MF (65). The synthesis and degradation of collagen I in cardiomyocytes depends on serum procollagen type I N-terminal propeptide (PINP) and procollagen type I carboxy-terminal propeptide (PICP) concentrations. AS-IV inhibits collagen deposition and TGF-β1 expression in the cardiomyocytes of a DCM model after CVB3 infection. Notably, although collagens I and III are substantially deposited in patients with DCM, only the content of collagen I in the myocardial tissue of DCM is markedly decreased by AS-IV. This effect is consistent with the downregulation of the PICP:PINP ratio and serum PICP concentration, both of which represent the excessive synthesis of collagen I in the myocardium. It also indirectly proves that AS-IV can exert considerable resistance against fibrosis

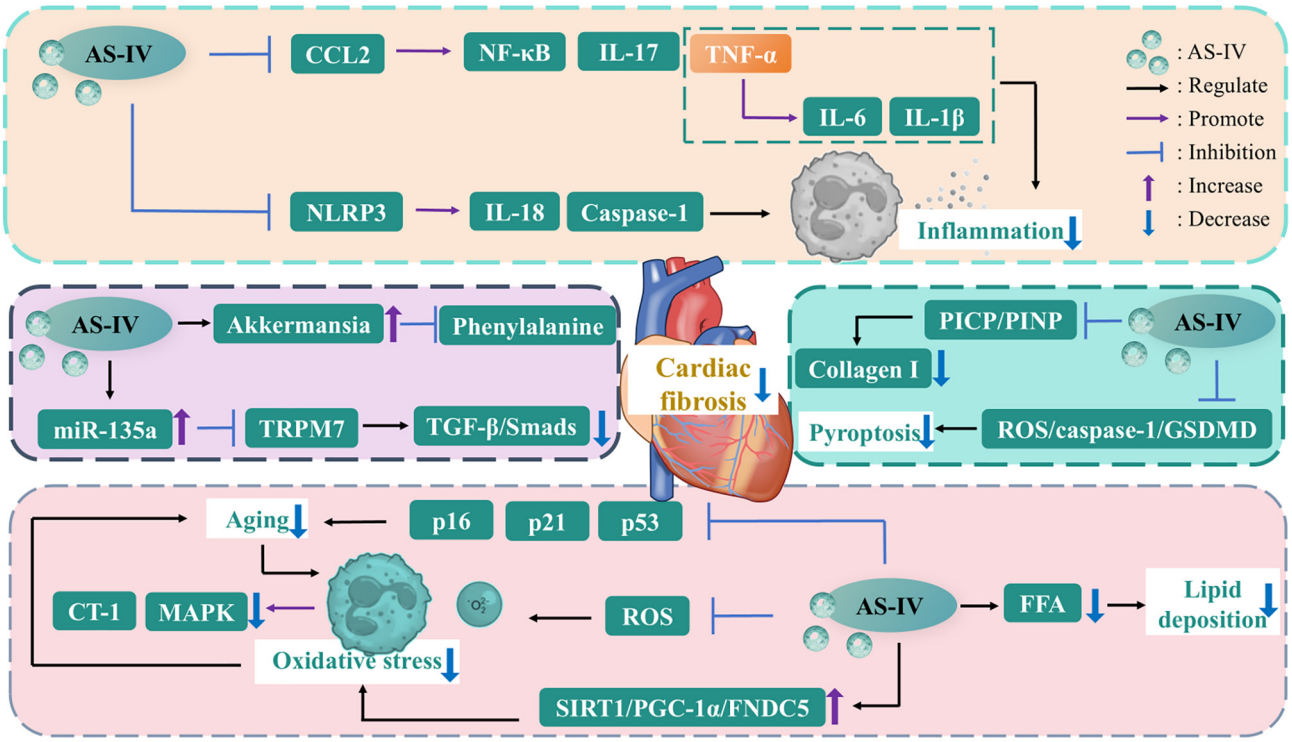

Figure 3. The mechanisms of AS-IV against cardiac fibrosis. AS-IV, astragaloside IV; CCL2, C-C motif chemokine ligand 2; collagen I, type I collagen; CT-1, cardiotrophin-1; FFA, free fatty acid; FNDC5, fibronectin type III domain-containing protein 5; GSDMD, gasdermin D; IL-17, interleukin-17; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; NLRP3, nucleotide-binding oligomerization domain-like receptor thermal protein domain associated protein 3; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1 α; PICP, procollagen type I carboxy-terminal propeptide; PINP, procollagen type I N-terminal propeptide; ROS, reactive oxygen species; SIRT1, sirtuin 1; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; TRPM7, transient receptor potential cation channel, subfamily M, member 7.

induced by ISO and CVB3 regardless of whether cardiac fibrosis is induced by the β-adrenergic receptor or not (65).

Gut microbiota. Various gut microbiota and fecal metabolites establish metabolic communication with the host or the intestinal microenvironment, thus forming metabolic crosstalk with cardiac fibrosis (66). In mammals, intestinal *Akkermansia* abundance plays a vital part in negatively regulating the development of metabolic syndrome and CVDs, such as atherosclerosis. In addition, intestinal *Akkermansia* can intervene in cardiovascular events by regulating the levels of fecal metabolites. Fecal metabolites, such as hydroxyprolyl-leucine, valyl-isoleucine and nepsilon-acetyl-L-lysine, are positively associated with the abundance of *Akkermansia*. *Akkermansia* negatively regulates phenylacetylglycine content. This phenomenon may be related to the increased risk of macrovascular disease because of the upregulation of phenylalanine, which is the hydrolysis product of phenylacetylglycine, in plasma (67,68). Studies have found that AS-IV can lessen the severity of ISO-induced cardiac fibrosis by increasing the abundance of the gut microorganism *Akkermansia*, reducing phenylalanine levels and increasing leucine and lysine levels (68).

TRPM7. Endogenous microRNAs (miRNAs/miRs) are a perennial research hotspot in the field of fibrotic disorders because of their involvement in regulating the Smad3-mediated TGF-β/Smads pathway. Transient receptor potential cation channel, subfamily M, member 7 (TRPM7) participates in inducing the proliferation and differentiation of fibroblasts and promoting ECM synthesis and collagen deposition. Among miRNAs, miR-135a is an upstream mediator that regulates the expression of the profibrotic factor TRPM7. AS-IV may alleviate cardiac fibrosis through the targeted intervention of miR-135a expression (42). Wei *et al* (42) revealed that AS-IV can inhibit the downregulation of miR-135a expression in ISO-treated rats and promote the negative regulation of TRPM7 by miR-135a and the inhibition of TRPM7 activity further promotes the inactivation of the TGF-β/Smads pathway. Furthermore, AS-IV treatment leads to a reduction in the expression levels of TGF-β1 and phosphorylated (p-)Smad3 while promoting the activation of Smad7 and intervention in rat neonatal CFs also shows a similar antifibrotic effect, indicating that the key mechanism for AS-IV to alleviate cardiac fibrosis may be the regulation of the miR-135a-TRPM7-TGF-β/Smads axis. Notably, TRPM7 channels can also regulate Ca^{2+} input in fibroblasts lacking voltage-gated calcium channels. Therefore, the mechanism through which AS-IV exerts its anti-MF effect may also involve the suppression of TRPM7 channel activity in CFs under hypoxic conditions, as well as downregulation in the ion-channel currents of fibroblasts (43) (Fig. 3).

Notably, most of the existing studies on the anticardiac fibrosis effect of AS-IV are based on *in vitro* and *in vivo* models with remarkable differences in pathophysiology from human cardiac fibrosis. Clinical research in this area is limited and the role of AS-IV in ischemic or nonischemic cardiac fibrosis remains unclear. Furthermore, although AS-IV is relatively safe, whether its coadministration with other drugs for CVD

treatment leads to drug interactions and whether the risks of such drug interactions increase the liver and kidney burden of patients with other comorbidities and trigger potential toxicity are unknown. Further studies, by incorporating the detection of indicators, such as ejection fraction and cardiac systolic or diastolic function, enrich the research value of AS-IV's anticardiac fibrosis effect. In addition, no research data on the targeting and accumulation of AS-IV in cardiac tissue exist. Therefore, evaluating the effective anticardiac fibrosis therapeutic dose, administration frequency and treatment course of AS-IV is difficult and the universality and clinical applicability of research results still need verification.

Pulmonary fibrosis. Diverse heterogeneous interstitial lung diseases can eventually develop into PF (69). PF can lead to the deposition of scar tissue in the lung parenchyma, impaired gas exchange function and even respiratory failure. IPF is also considered a chronic, fatal interstitial pneumonia with complex causes (70). Smoking, dust inhalation, drug use, gastroesophageal reflux, genetic factors, viral infection and autoimmunity are listed as clear PF-related risk factors (71). In the early stage of fibrosis, the lungs are dominated by chronic inflammation, tissue edema and congestion. This stage is followed by damage to alveolar epithelial cells; the massive migration and proliferation of mesenchymal cells; and the degradation of ECM, including collagen, laminin and tenascin-C. The continuously deposited ECM promotes scar tissue hyperplasia, eventually causing a progressive decline in lung function (72). The typical pathological features of PF include the persistent inflammatory responses and structural remodeling of the airways triggered by the gradual decline in lung function (73). Although glucocorticoids are mainly used to treat PF, they cannot achieve the expected clinical efficacy because of their certain side effects on the body (74). Pirfenidone and nintedanib, which target the inhibition of inflammasome and tyrosine kinase activities, respectively, have been approved for use in the global medical market and are mainly employed to improve symptoms of dyspnea and lung function decline related to IPF (75). Although the application of nanomaterials in the development of new antifibrotic drugs has begun to receive focus, paying attention to natural Chinese herbal resources against PF remains necessary (76).

Inflammation and oxidative stress. Exposing the body to silica dust particles for a long time can induce the diffuse nodular fibrosis of the lungs and chronic pulmonary inflammation involving alveolar macrophages, alveolar epithelial cells, fibroblasts, lymphocytes and other mediators. This condition, which is a typical pathological manifestation of silicosis, further promotes lung function decline, dyspnea and even death (77). In addition, the long-term stimulation of alveolar macrophages by silica particles drives the release of ROS and oxidative stress can further exacerbate inflammatory effects in the feed-forward cycle. AS-IV inhibits silicosis-related inflammation and oxidative stress by downregulating the expression of inflammatory factors, such as TNF-α, IL-1β and IL-6 and upregulating the activities of the antioxidant enzymes SOD and glutathione peroxidase (GSH-Px). This effect may stem from the ability of AS-IV to block the TGF-β1/Smads signaling pathway, thereby alleviating silicosis-related fibrosis (77). In summary, AS-IV negatively regulates fibroblast fibrosis, as primarily indicated by the remarkable promotion of the dephosphorylation of Smad2 and Smad3 in fibroblasts and restoration of Smad7 activity, indicating that AS-IV mediates positive feedback inhibition and the negative feedback enhancement of the TGF-β1/Smads signaling pathway (77,78). As aforementioned, NLRP3 is a multiprotein complex involved in fibrosis-related inflammatory responses. Hou *et al* (79) found that AS-IV administration could promote the inactivation of NLRP3 and the fibrosis-related effector proteins collagen I, collagen II and α-SMA in TGF-β-induced PF, thereby alleviating epithelial-mesenchymal transition (EMT). Furthermore, AS-IV dose-dependently inhibits pulmonary inflammatory infiltration and fibrosis induced by bleomycin (BLM) injection. This effect is associated with the downregulation of total cell, neutrophil, macrophage and lymphocyte counts in bronchoalveolar lavage fluid targeted by AS-IV (80). AS-IV can also inhibit increased levels of malondialdehyde (MDA) and ROS, as well as promotes the activity of the endogenous antioxidant indicator SOD and the total antioxidant capacity (T-AOC), thereby exerting considerable pharmacological benefits in alleviating BLM-induced PF-related oxidative stress (80).

Klebsiella pneumoniae (*K. pneumoniae*), a Gram-negative capsulated bacterium, is known to induce pneumonia (81). Li *et al* (82) found that AS-IV administration can target the downregulation of p-Smad2/Smad2, p-Smad3/Smad3 and p-IκBα/IκBα levels induced by *K. pneumoniae*. This finding suggests that the mechanism by which AS-IV downregulates the NF-κB inflammatory signaling pathway may be attributed to the negative regulation of the TGF-β1/Smad pathway. However, although the study demonstrated AS-IV's mediating function on the Smad and NF-κB pathways, it did not explore whether the quantity of lung colony-forming units during *K. pneumoniae* infection changed markedly with AS-IV intervention, suggesting that the effect of AS-IV on bacterial load in lung tissue is unclear (82). AS-IV and ligustrazine extracted from *Astragalus* and *Ligusticum chuanxiong*, respectively, are natural Chinese herbal components with antifibrotic activity (83,84). However, in view of their poor hydrophilicity, the introduction of nanoparticles is beneficial to improve their penetration into the mucosa. Therefore, AS-IV and LIG have been coloaded onto inhalable nanoparticles (AS_LIG@PPGC NPs) to achieve the maximum intervention for PF through noninvasive pulmonary inhalation therapy (85). NPs are not easily degraded after being inhaled by the body, can target lung tissue for more than 24 h and are heavily enriched in the right lower lobe and left lung. In addition, they are diffusely distributed in lung tissue, demonstrating that the use of polyethylene glycol-polylactic-co-glycolic acid NPs (PPGC NPs) can improve the ability of a single agent to target tissue (85). Zheng *et al* (85) found that the administration of AS_LIG@PPGC NPs inhibits the overexpression of the myofibroblast marker α-SMA and fibrotic effector factor TGF-β1. Moreover, it reduces the deposition of the ECM component collagen 1A1 and the release of the inflammatory cytokines TNF-α, IL-6 and IL-1β. In addition, the authors highlighted the therapeutic benefits of AS-IV in mitigating the levels of pulmonary inflammation and fibrosis from the perspective of integrated traditional Chinese and Western medicine.

These effects are mainly attributed to the ability of AS-IV to regulate the release of NOX4-derived ROS negatively and hinder ROS to drive the proinflammatory and profibrotic effects of the activated NLRP3 inflammasome (85). Targeting the reduction of ROS production can also prevent p38 MAPK from being activated and the excessive release of ROS further aggravates the upregulation of NOX4 activity. AS-IV's negative regulatory effect on the NOX4/NLRP3/p38 MAPK axis is complicated by the closed-loop chain formed among NOX4, ROS, the NLRP3 inflammasome and p38 MAPK. Applying AS_LIG@PPGC NPs to intervene in PF can further compensate for the limitations of treatment with a single TCM preparation (85).

Pyroptosis. The progressive pulmonary artery structural remodeling exhibited by pulmonary artery hypertension (PAH) induces a surge in right ventricular pressure. Hypoxia is a key inducer of pulmonary artery remodeling. The ability of mammalian cells to adapt to hypoxic signal stimulation relies on the promotion of oxygen transport and body metabolism by hypoxia-inducible factor 1α (HIF1α) (86,87). Under normoxic conditions, HIF1α hydroxylation and degradation depend on the catalytic effect of prolyl-4-hydroxylase 2 (PHD2) and the downregulation of PHD2 activity is positively associated with pulmonary vascular remodeling and PAH (88,89). Drugs, such as endothelin receptor blockers and prostacyclin, are insufficient to decrease the 20-30% mortality rate of patients with PAH. Xi *et al* (87) focused on natural herbal medicine to treat PAH-related fibrosis and found that AS-IV can inhibit the activity of the NLRP3 inflammasome. The authors also discovered that AS-IV can diminish the release of the proinflammatory cytokines IL-1β and IL-18 in the lung tissue of PAH rats; interfere with the upregulation of MMPs/tissue inhibitor of metalloprotease 4 and the deposition of FN and collagen I induced by PAH and negatively regulate the expression of the pyroptosis-related markers GSDMD-N and cleaved caspase-1 under hypoxic conditions and the activity of LDH in pulmonary artery smooth muscle cells (PASMCs). Furthermore, AS-IV promotes an increase in PHD2 expression and concurrently suppresses the expression of HIF1α. In short, NLRP3-mediated pyroptosis and fibrosis in PASMCs can be markedly inhibited by AS-IV targeting PHD2/HIF1α signaling.

Aging. In network pharmacology analysis, Yuan *et al* (90) selected PF-related signaling pathways through the Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis and used cytohubba to analyze the protein-protein interaction network associated with AS-IV and its PF targets. Given that the authors found that the top 10 key target proteins were markedly enriched in the cellular senescence pathways, they further used molecular docking to verify that the cellular senescence marker proteins p53, p21 and p16 are potential binding targets of AS-IV. By focusing on the mechanism of PF-related cell aging, they found that AS-IV further reduces oxidative stress-induced senescence by limiting ROS generation in the BLM-induced PF mouse model and alveolar epithelial (A549) cells. The metabolic imbalance in senescent cells induces pathological changes in protein expression and SASP release. However, AS-IV not only enhances the expression of the epithelial protein marker E-cadherin while blocking the synthesis of the mesenchymal protein marker vimentin but also suppresses the expression levels of the aging markers p53, p21 and p16. Therefore, alleviating EMT and cellular senescence is an anti-PF mechanism of AS-IV (90).

Collagen deposition. High mobility group box-1 (HMGB1), in addition to inducing inflammatory reactions, is an effector molecule related to PF. This function is related to its promotion of the transdifferentiation of fibroblasts and induction of EMT and ECM protein deposition (91). However, the extent of ECM protein deposition depends on the activity of cells that synthesize ECM. Such activity can be reflected by the expression of the myofibroblast marker α-SMA. ECM proteins contain a variety of collagen components. Given that hydroxyproline (HYP) is a primary ingredient of collagen, its content may be positively associated with the progression of PF (92). Li *et al* (92) revealed that AS-IV can target the downregulation of α-SMA, HMGB1, HYP and collagen III in lung tissue and further inhibit the synthesis of ECM components, including laminin and hyaluronic acid. This effect is mediated by promoting the inactivation of HMGB1, thereby effectively alleviating BLM-induced PF.

Non-coding RNAs (lncRNAs). Zinc finger E-box binding homeobox 1 (ZEB1) is involved in the regulation of the transcription factor networks linked to EMT and is a key inducer of PF (93). lncRNAs mediate the regulation of miRNAs, among which miR-200c, a member of the miR-200 family, is closely related to fibrotic effects, such as EMT, cell invasion and proliferation (94,95). Through dual-luciferase reporter gene experiments and bioinformatics, Guan *et al* (70) found that miR-200c has a consistent binding site with lncRNA-activated by transforming growth factor β (lncRNA-ATB) and that the downstream of miR-200c can regulate the expression of ZEB1. These findings are consistent with the fact that in patients with silicosis, overexpressed lncRNA-ATB can interact with miR-200c and target ZEB1 expression upregulation, thus driving EMT occurrence (70,96). However, in A549 cells used as an IPF-associated EMT model, AS-IV was found to inhibit lncRNA-ATB by acting as a competitive endogenous RNA to mediate the sponging of miR-200c in IPF to target the expression of ZEB1. That is, AS-IV negatively regulates the level of lncRNA-ATB, promotes the overexpression of miR-200c and further downregulates the nuclear level of ZEB1 in TGFβ1-induced EMT model cells, indicating that the lncRNA-ATB/miR-200c/ZEB1 regulatory axis is a target of AS-IV to alleviate IPF (70). Circular RNAs, a member of the endogenous non-coding RNA family, can combine with miRNA and serve as miRNA sponges to target changes in the biological functions of downstream genes (97). circ_0008898 overexpression is positively associated with PF progression. Zhu *et al* (98) discovered that TGF-β1-induced circ_0008898 overexpression in human fetal lung fibroblast 1 (HFL1) cells could be negatively regulated by AS-IV (98,99). TGF-β1 can also induce a reduction in miR-211-5p expression in HFL1 cells. That is, miR-211-5p may serve as a potential binding target of circ_0008898 given that miRNAs can target changes in mRNA expression and a rise in miR-211-5p expression is strongly connected to the inactivation of HMGB1 in HFL1 cells. HMGB1 has been further confirmed to be a downstream regulator of miR-211-5p through bioinformatics prediction and experimental validation (98). Notably, HMGB1 overexpression in TGF-β1-treated HFL1 cells has a crucial role

in driving the advancement of PF. In summary, AS-IV may rely on the circ_0008898/miR-211-5p/HMGB1 axis as a key signaling target to inhibit PF progression in TGF-β1-treated HFL1 cells (98).

The application of Danggui Buxue Decoction (DBT) as an antifibrotic supplement mainly relies on the pharmacological activities of AS-IV and ferulic acid (FA) (100). In addition, miRNA can pair with mRNA and mediate changes in protein expression through the transcription pathway. miR-29 can inhibit collagen fiber deposition by targeting various cytokines. For example, the 3'-UTR of the target gene TGF-β1 is one of its main sites of action. Tong *et al* (101) found that on the basis of the antifibrotic properties of miR-29, AS-IV + FA (*AF*) can use miR-29b as a drug target to induce Smad3 dephosphorylation further and alleviate oxidative stress and PF by triggering nuclear factor erythroid 2-related factor 2 (Nrf2) activation and interfering with TGF-β1/Smad3 signaling. This finding also indirectly shows that the antifibrotic effect of supplementation with combinations of Chinese herbal medicine preparations may be improved than that of supplementation with single drugs. Autophagy deficiency is a key cause of IPF. Its profibrotic mechanism mainly stems from fibrotic effects, such as inducing fibroblast transdifferentiation and ECM deposition (102). According to Li *et al* (103), AS-IV could prevent TGF-β1-treated A549 cells from showing a reduction in light chain 3B fluorescence signal intensity, indicating that AS-IV may function as an autophagy agonist. Although some studies have found that TGF-β1 treatment may motivate fibroblasts to overexpress miR-21 and overactivated miR-21 further exacerbates the induction effect of TGF-β1 on PF, AS-IV can inhibit the pathological upregulation of miR-21 expression. This effect is mainly attributed to the fact that the antifibrotic effect of AS-IV is reversibly mediated by miR-21 agonists (103,104). Bioinformatics analysis provides evidence that phosphatase and tensin homolog (PTEN) is one of the binding targets of miR-21 (103). Given that PTEN, a molecular inhibitor of phosphatidylinositol-3-kinase (PI3K), can negatively regulate PI3K/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling through downstream effects and mTOR is an effector molecule that drives autophagy blockage, in-depth research has confirmed that AS-IV can negatively regulate PI3K/AKT/mTOR signaling by targeting the upregulation of PTEN activity, thereby promoting the inhibitory effect of autophagy on IPF (103,105,106).

PM2.5 is a fine particulate matter involved in the formation of haze. PM2.5 suspended in the air can adsorb microorganisms and directly enter alveoli, markedly downregulating the activity of the anti-inflammatory factor miR-362-3p in alveolar epithelial cells and promoting the overexpression of the downstream target runt-related transcription factor 1 (RUNX1) of miR-362-3p, in which RUNX1 induces the transdifferentiation of fibroblasts into myofibroblasts, driving the occurrence of respiratory diseases, such as PF (107,108). AS-IV targets the overexpression of miR-362-3p and negatively regulates the activity of its downstream transcription factor RUNX1 in rat lung tissue and alveolar epithelial cells to alleviate the inhibitory effect of PM2.5 on the proliferation of rat alveolar epithelial cells L2 and its driving effect on LDH release. In addition, AS-IV suppresses PM2.5-induced alveolar wall thickening and collagen III deposition in lung tissue. Furthermore, it reverses the apoptosis rate of rat lung tissue and L2 cells; the secretion of the proinflammatory factors TNF-α, IL-1β and IL-6; and the overactivation of cleaved caspase-3, p-p65 and α-SMA, thereby inhibiting PM2.5-driven PF (108). Therefore, AS-IV may be a natural therapeutic agent for alleviating air pollution-related PF by regulating the miR-362-3p/RUNX1 signaling cascade.

Forkhead box O3a (FOXO3a). FOXO3a is extensively expressed in the majority of bodily tissues and cells. TGF-β1 is an upstream factor that activates the PI3K/AKT signaling pathway and promotes AKT phosphorylation, thereby markedly downregulating the activity of FOXO transcription factors. FOXO3a inactivation is an important factor for promoting fibroblast collagen deposition and α-SMA overexpression, which further induces the occurrence of IPF (109,110). However, AS-IV regulates the inactivation of the PI3K/AKT pathway by targeting TGF-β1, further blocking the hyperphosphorylation and inactivation of FOXO3a, downregulating EMT and the expression of α-SMA and increasing the levels of the epithelial cell marker E-cadherin, thus resulting in considerable resistance to BLM-induced IPF (84). Smoking has been confirmed to a key risk factor for inducing PF. Chronic obstructive pulmonary disease (COPD) is often triggered by excessive smoking and accompanied with persistent respiratory inflammatory infiltration, which further increases the risk of PF (111). Given that rat sarcoma (RAS) can regulate the downstream expression of the fibrotic effector molecule FOXO3a, RAS activity and EMT development have been demonstrated to be markedly positively associated (112). Through multiple protein and small-molecule interaction experiments and amino acid site mutation approaches, Zhang *et al* (113) found that RAS is a binding target of AS-IV. AS-IV can inhibit RAS expression to induce the dephosphorylation of c-Raf^{338} and FOXO3a. Therefore, by targeting the expression of the non-Smad signaling pathway RAS/rapidly accelerated fibrosarcoma (RAF)/FOXO signaling cascade and its downstream inflammatory factors TNF-α and IL-1β, AS-IV markedly inhibits the transdifferentiation of fibroblasts and alleviates LPS and cigarette smoke treatment-induced COPD-related EMT progression.

Advanced glycation end-product (AGE)-receptor for AGE (RAGE). Patients with T2DM can be complicated with diabetic PF (114). In view of the epidemiological data showing that T2DM and its complications have become the cause of death of ~6.7 million individuals, focusing on the application of Bu Yang Huan Wu Decoction, which has the effect of reducing blood sugar and blood lipids, in PF intervention (115) is essential. Elevated blood glucose concentration induces the nonenzymatic glycosylation of proteins, such as collagen and elastin, thereby promoting the production of AGEs. RAGE, which is found to be widely expressed in lung tissue, can act as an AGE inhibitor (115). Through network pharmacology analysis, Guo *et al* (115) discovered that regulating the AGE-RAGE signaling cascade and the gene expression of its downstream effector molecules is the key mechanism through which bioactive ingredients, such as AS-IV, in Bu Yang Huan Wu Decoction exert anti-PF effects. Hydrogen bonding is a key parameter indicating the binding degree of a protein to a ligand. Evidence from molecular docking and dynamics simulations supports that the complex system formed by the

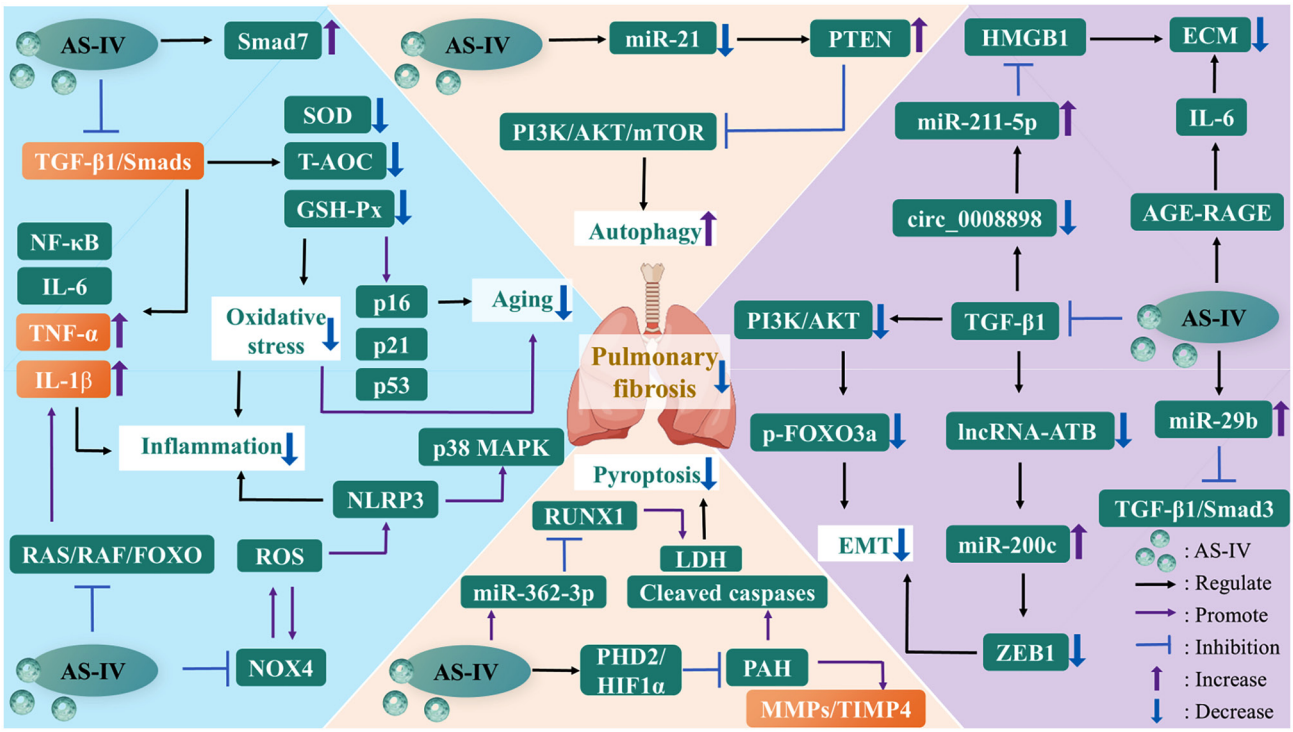

Figure 4. The mechanisms of AS-IV against pulmonary fibrosis. AGE, advanced glycation end-product; AKT, protein kinase B; AS-IV, astragaloside IV; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; FOXO, forkhead box O; GSH-Px, glutathione peroxidase; HIF1α, hypoxia-inducible factor 1α; HMGB1, high mobility group box-1; IL-1β, interleukin-1β; LDH, lactate dehydrogenase; lncRNA-ATB, long non-coding RNA activated by transforming growth factor β; MAPK, mitogen-activated protein kinase; miR-21, microRNA-21; MMP, matrix metalloproteinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NLRP3, nucleotide-binding oligomerization domain-like receptor thermal protein domain associated protein 3; NOX4, NADPH oxidase 4; PAH, pulmonary artery hypertension; PHD2, prolyl-4-hydroxylase 2; PI3K, phosphatidylinositol-3-kinase; PTEN, phosphatase and tensin homolog; RAF, rapidly accelerated fibrosarcoma; RAGE, receptor for AGE; RAS, rat sarcoma; ROS, reactive oxygen species; RUNX1, runt-related transcription factor 1; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TGF-β1, transforming growth factor-β1; TIMP4, tissue inhibitor of metalloprotease 4; TNF-α, tumor necrosis factor-α; ZEB1, zinc finger E-box binding homeobox 1.

downstream target gene IL-6 of the AGE-RAGE signaling pathway and AS-IV has certain stability likely because IL-6-AS-IV binds to an average of three hydrogen bonds. This phenomenon indicates that the binding affinity between the two is strong and also implies that AS-IV can target AGE-RAGE signaling and negatively regulate the induction of collagen deposition and ECM synthesis in lung tissue by IL-6, the downstream fibrotic effector molecule of the AGE-RAGE signaling pathway. That is, AS-IV can markedly inhibit high glucose (HG)-induced PF (Fig. 4).

PF is a heterogeneous disease and the therapeutic effect of AS-IV may vary across different subtypes of PF. In addition, animal models usually simulate acute or subacute PF, whereas PF in humans is a progressive condition. Therefore, the long-term intervention effect of AS-IV still needs to be verified in human clinical studies. The triggers of human PF, such as PF induced by drugs, connective tissue diseases, or inhaled dust, are difficult to replicate fully through animal experiments. Moreover, the evidence from tests encompassing pulmonary function indicators, including vital capacity and diffusion capacity, remains insufficient. Not only that, the comparative analysis of the therapeutic effects between AS-IV and anti-PF drugs, such as pirfenidone and nintedanib and the dose-response relationship between the dosage and anti-PF effect of AS-IV remain unclear. Finally, the oral bioavailability of AS-IV is limited. Additional data are required to confirm whether approaches, such as aerosol inhalation and dosage form optimization, can increase the concentration of AS-IV in lung tissues, ensure its accumulation in damaged lung tissues and enable its effective interaction with lung cells.

Renal fibrosis. The main characteristics of chronic kidney disease (CKD) include progressive renal tissue structural lesions and impaired renal excretory function, which may be due to functional nephron defects caused by diverse causative variables, including diabetes, interstitial nephritis, glomerulonephritis, obstructive nephropathy and polycystic kidney disease (116). CKD affects >10% of the overall population worldwide and in the United States alone, ~14% of adults are affected by pathological factors related to CKD (117,118). Among these factors, renal fibrosis is often a unified pathological manifestation of progressive CKD regardless of the progression of CKD. If the prognosis is inappropriate, then end-stage renal failure is often the final outcome (119). The transdifferentiation of intrinsic renal cells, such as mesangial cells (MCs), epithelial cells, endothelial cells, or fibroblasts, into myofibroblasts is a key mechanism for inducing renal fibrosis. Various cytokines that maintain the homeostasis of ECM deposition and degradation and proinflammatory mediators are also involved in the pathogenesis of renal fibrosis (120). In addition, the excessive deposition of fibrous connective tissue has adverse effects on the physiological function of renal tubules and glomerular filtration rate; this phenomenon also causes the tubulointerstitial space and glomerulus to become

key sites for frequent fibrosis (121,122). Although dialysis and kidney transplantation are currently commonly used to intervene in ESRD, renal replacement therapy may induce some uncertain risk factors, necessitating intervening in CKD as early as possible to avoid its progressive deterioration into ESRD (123). Given that whether fibrosis is a cause or a pathological manifestation of CKD is unclear and intervening in the progression of CKD by directly targeting the treatment of renal fibrosis requires further in-depth research, conservative antifibrotic treatments, such as the use of well-tolerated Chinese herbal medicines, may achieve the negative regulation of various kidney diseases in their initial phases (124).

Diabetic kidney disease-induced renal fibrosis

Lipid metabolism. Diabetic kidney disease (DKD) can cause glomerular ultrastructural alterations by inducing the deposition of ECM proteins, such as FN and DKD, lacking effective prognosis may cause the induction of ESRD. This situation can explain why ~1/3 of individuals with diabetes develop irreversible end-stage renal failure (125). Human glomerular mesangial cells (HMCs) can participate in the occurrence of DKD-related fibrosis via the uptake of FFAs flowing into the kidney. The uptake of medium- and long-chain FFAs mainly depends on the transport activity of cluster of differentiation 36 (CD36) protein, which is widely expressed in renal glomeruli and involved in the induction effect of FFAs on HMC fibrosis to a certain extent. AS-IV can negatively regulate the expression of FN and type IV collagen α 1 and the TGF-β1/Smad2/3 signaling pathway in the palmitic acid (PA)-treated HMC fibrosis model. It can also inhibit the activation of CD36 expression in a high-fat environment induced by PA. Given that NOX4-derived ROS play a vital role in regulating the progression of DKD and NOX4 can cross-talk with the TGF-β1/Smad2/3 pathway and become a fibrotic effector molecule, NOX4 may be the key target for the inhibition of DKD-related renal fibrosis (125). Nath *et al* (126) also verified the profibrotic effect of CD36. That is, the uptake of PA by CD36 induces the TGF-β1/Smad2/3 signaling cascade and mediates the occurrence of EMT in hepatocellular carcinoma (HCC). This situation indicates that NOX4 and TGF-β1 overexpression may be attributed to the regulatory effects of upstream CD36 activation. However, AS-IV markedly reduces the expression of CD36, NOX4 and TGF-β1 in PA-treated HMCs (125).

Inflammation and oxidative stress. In the diabetic phase, renal cells exhibit high glucose uptake. However, other cell populations, such as glomerular MCs, are unable to regulate the decrease in glucose transport rates. This inability induces an imbalance in intracellular glucose homeostasis and promotes cytosolic and mitochondrial ROS release (127). In addition, ROS-mediated oxidative stress induces the activation of the TGF-β/Smads signaling pathway, which in turn regulates the fibrosis of renal tubular epithelial cells (RTECs). Du *et al* (127) found that the combined administration of ginsenoside Rg1 (G-Rg1) and AS-IV is more effective than monotherapy in interfering with TGF-β1/Smads signaling mainly because G-Rg1 has strong pharmacological activity in the negative regulation of TGF-β1 expression, whereas Smad7 activity targeted by AS-IV is remarkably upregulated. In the treatment of DKD, AS-IV acts as an enhancer of the antioxidative stress effect of G-Rg1. The main manifestation of this effect is that after coadministration, the levels of catalase, GSH-Px and T-AOC maximally increase, whereas MDA shows reduced activity (127). In addition, abnormally elevated blood glucose concentrations can drive ROS release and oxidative stress. AGEs are protein derivatives that can mediate proinflammatory responses and oxidative stress. They all have an intimate connection with the occurrence of DKD. Zhang *et al* (128) found that in DKD rats, AS-IV administration can reduce the excessive synthesis of AGEs and the overexpression of inflammatory factors. Moreover, AS-IV treatment can reduce the excessive synthesis of collagen IV in the basement membrane and the excessive deposition of FN in the renal mesangium and interstitium. The authors also verified the activation effect of the excessive release of ROS on cytokines and transcription factors. This phenomenon is not only a reason for the induction of the massive deposition of ECM in the kidney area but also aggravates renal fibrosis complicated by DKD and the development of end-stage renal failure (129). Furthermore, transcriptomic analysis has shown that the inflammation-related NLR signaling pathway is a pharmacological target of AS-IV and can potentially participate in the way that AS-IV ameliorates renal fibrosis complicated by DKD in rats (128).

mTORC1/p70S6K. mTOR complex 1 (mTORC1) can regulate the phosphorylation of ribosomal protein S6 kinase β-1 (p70S6K) downstream, thus participating in cell proliferation or growth to some extent. However, the development of DKD is intimately associated with pathologically driven mTORC1/p70S6K signaling, which may further induce DKD complicated by RTEC transdifferentiation (130). Chen *et al* (131) found that in human proximal tubular epithelial (HK-2) cells treated with different concentrations of HG, AS-IV can target the upregulation of the epithelial cell marker E-cadherin and negatively regulate α-SMA. Moreover, AS-IV downregulates the phosphorylation of mTOR and its downstream molecule p70S6K. That is, it has an inhibitory effect on the signal transduction of the mTORC1/p70S6K pathway. In addition, a negative correlation exists between zinc finger transcription factors (Snail, Slug, Twist and ZEB1) and E-cadherin expression. Nevertheless, the mTORC1/p70S6K pathway is also highly associated with the activities of the transcription factors Snail and Twist (130). AS-IV can target the inactivation of HG-induced Snail and Twist proteins and that the ECM protein components FN and collagen IV are downregulated when AS-IV intervenes in DKD (131). In summary, AS-IV further negatively regulates renal tubular EMT induced by HG concentration by inhibiting the overactivation of the mTORC1/p70S6K signaling pathway and downregulates the expression of the transcription factors Snail and Twist in HK-2 cells, simulating DKD *in vitro* (131).

miRNA. Proteinuria is mainly used as a clinical diagnostic criterion of DKD, a microvascular complication of diabetes and is often accompanied with renal fibrosis. Highly differentiated podocytes participate in forming the outermost layer of defense for glomerular filtration. Adverse stress reactions may affect the differentiation structure of podocytes, further causing macromolecular plasma proteins to penetrate the damaged filtration barrier directly and flow into the urinary filtrate, thus aggravating the occurrence of renal fibrosis (132). MCs

are a type of glomerular cells. Their excessive activation also induces the enhanced expression of α-SMA and promotes the deposition of large amounts of ECM proteins, thereby participating in the formation of DKD-related renal fibrosis (133). The progression of mammalian kidney disease is known to be markedly associated with the expression of miR-21, which transcriptionally suppresses the expression of downstream target genes (134). However, AS-IV concentration-dependently decreases the levels of miR-21 and the mesenchymal marker α-SMA in HG-induced podocytes and MCs and the level of the epithelial marker nephrin in podocytes is positively associated with the concentration of AS-IV administration (135). In addition, podocyte damage and MC proliferation are inextricably linked to the involvement of the Wnt/β-catenin and TGF-β1/Smads signaling cascades. However, AS-IV markedly lowers the upregulated β-catenin, TGF-β1 and p-Smad3 levels in podocytes and MCs with miR-21 overexpression and promotes the recovery of Smad7 activity. In summary, AS-IV impedes the advancement of DKD-related renal fibrosis and maintains highly differentiated podocyte morphology and MC stability by negatively regulating the levels of highly activated miR-21 and inhibiting the driving effect of miR-21 on the downstream Wnt/β-catenin and TGF-β1/Smads signaling cascades. These functions provide new insights into the AS-IV targeted treatment of glomerular-related diseases (135).

Autophagy. As a deacetylase, SIRT1 plays a key role in mediating renal dysfunction, such as podocyte injury (136). In addition, during HG-induced podocyte apoptosis, activated NF-κB can serve as a signaling molecule that inhibits autophagy by targeting LC3 II downregulation. However, according to Wang *et al* (137), AS-IV further impedes podocyte EMT by reversing the negative effect of HG treatment on the activity of the autophagy markers Beclin1 and LC3 II. By contrast, SIRT1 activator helps enhance this phenomenon, indicating that AS-IV activates SIRT1 to deacetylate the NF-κB p65 subunit and may execute pharmacological activity as an autophagy agonist. At the same time, N-cadherin and α-SMA expression by AS-IV in podocytes treated with different HG concentrations are reversed under the mediation of the autophagy inhibitor 3-methyladenine, the activation of E-cadherin and nephrin levels and the inhibition of TGF-β. This effect further demonstrates that AS-IV can serve as a pharmacological autophagy inducer in DKD, thereby protecting glomerular structure and alleviating the HG environment that drives podocyte EMT (137). Moreover, Wang *et al* (138) found that AS-IV pretreatment concentration-dependently suppresses α-SMA, FN and collagen IV expression in MCs induced by HG and the prohibitive effect of AS-IV on the activation and proliferation of MCs induced by HG is still related to autophagy activation mediated by SIRT1-NF-κB signaling transduction.

RAF/MEK/ERK. The pathological upregulation of plasma C-X3-C motif ligand 1 (CX3CL1) concentrations in adult patients with CKD is often a key risk variable associated with the occurrence of CVD and diabetes (139). Furthermore, EMT is intricately linked to the activation of the RAF/mitogen-activated protein kinase (MEK)/ERK signaling cascade (140). Hu *et al* (141) revealed that AS-IV administration can negatively regulate the expression of the mesenchymal marker vimentin and myofibroblast marker α-SMA in db/db mice and HK-2 cells treated with HG; partially inhibit the overexpression of CX3CL1 and p-c-Raf/c-Raf, p-MEK/MEK and p-ERK/ERK; and upregulate the activity of the epithelial cell marker E-cadherin (141). In summary, AS-IV further inhibits the induction of EMT by the RAF/MEK/ERK signaling cascade by targeting the downregulation of CX3CL1 expression, thereby effectively alleviating the occurrence of EMT in DKD.

TGF-β1/UUO treatment-induced renal fibrosis

Inflammation. Renal function repair is facilitated by the synergistic action of fibroblasts, RTECs, endothelial cells, renal interstitial lymphocytes and macrophages that are involved in the inflammatory response and the preservation of ECM homeostasis when the kidney is under adverse stress (142). During repair, if the synthesis and degradation of ECM are out of balance and a large amount of collagen fibers are deposited, then the activation and proliferation of fibroblasts is induced and intrinsic renal cells are replaced. These processes constitute the occurrence of renal fibrosis. According to Zhou *et al* (143), AS-IV could inhibit the inflammatory infiltration induced by macrophages and lymphocytes in unilateral ureteral obstruction (UUO)-treated mice and upregulate IκBα expression. By contrast, in LPS-treated RTECs *in vitro*, AS-IV could negatively regulate Toll-like receptor 4/NF-κB signaling. This finding shows that AS-IV's anti-inflammatory action on RTECs and inflammatory cells may be one of its potential pharmacological mechanisms to impede the course of renal fibrosis and CKD.

Autophagy. EMT is associated with the G_2/M cycle and partial RTEC lesions induce G_2/M arrest and participate in the occurrence of EMT under the mediation of fibrotic effector cells. Furthermore, the mitochondrial physiological function is markedly positively associated with the activity of acetaldehyde dehydrogenase 2 (ALDH2). ALDH2 can target the inhibition of autophagic activity to mitigate the functional damage caused by LPS to the heart (144). Li *et al* (123) found that AS-IV can reverse the upregulation of the autophagy indicators ATG7, Beclin1 and LC3 II/LC3 I in an adenine-treated CKD rat model and a TGF-β1-induced *in vitro* HK-2 fibrosis model and drive p62 accumulation by inhibiting autophagy. The inhibitory effects of AS-IV on CKD-induced autophagy could be explained by the activation of the AKT/mTOR signaling cascade and upregulation of ALDH2 activity. In addition, following AS-IV administration, the activity of the mediators involved in G_2/M cell cycle arrest, including p21, p53, p-p53 and p-histone H3, markedly decreased, indicating that AS-IV can target CKD-related G_2/M cell cycle change. AS-IV induces the expression of the epithelial cell marker E-cadherin to be upregulated and that of renal mesenchymal cell markers to be downregulated; these effects further illustrate its inhibitory effect on EMT (123).

TGF-β/Smad. The TGF-β receptor activates the phosphorylation of the downstream fibrotic effector proteins Smad2 and Smad3, which form a complex with Smad4 and are transported into the nucleus. Smad family members, including Smad7, an inducer that promotes TGF-β1 receptor degradation and Smad2/3 inactivation, are mostly involved in the transcription of target genes. However, TGF-β1 has two sides: In addition to being an inducer driving fibrotic and anti-inflammatory responses, its inactivation may also mean exacerbating

proinflammatory effects, suggesting that antifibrotic intervention that directly targets TGF-β may trigger other unknown risks (145). Wang *et al* (146) reported that the expression of the myofibroblast marker a-SMA and the formation of fibrous connective tissue in a UUO-treated rat model were negatively regulated by AS-IV intervention. In addition, AS-IV has been shown *in vitro* to promote the inactivation of fibrotic effector connective tissue growth factor (CTGF) in rat renal fibroblasts (NRK-49F) treated with TGF-β1, thereby inhibiting its downstream induction of ECM synthesis and further resisting renal fibrosis. An *in vitro* experiment has also shown that AS-IV can activate the dephosphorylation of Smad2 or Smad3. Silencing the expression of the Smad7 gene in NRK-49F cells via the small interfering RNA technique further demonstrated that AS-IV alleviates renal fibrosis associated with obstructive nephropathy because of its targeted effect on the upregulation of Smad7 activity. In summary, AS-IV's antitubulointerstitial fibrosis effect and its targeted inactivation of the TGF-β/Smad signaling pathway are markedly positively associated (146).

PI3K/AKT/mTOR. Renal interstitial fibrosis often occurs when various CKDs progress to advanced stages (147). Gap junctions promote intercellular communication by mediating intercellular material transport and signal transmission. Connexin43 (Cx43) may crosstalk with the occurrence of fibrotic diseases (148). In addition, the inactivated PI3K/AKT signaling cascade can mitigate TGF-β1-induced EMT and highly activated mTOR is markedly positively associated with the occurrence of EMT (147,149). Lian *et al* (147) found that the inactivated AKT/mTOR pathway may be an integral mediating factor in the mechanism through which Cx43 negatively regulates EMT in TGF-β1-treated RTECs. That is, by inhibiting the TGF-β1-induced downregulation of Cx43 protein levels, AS-IV can interfere with PI3K/AKT/mTOR signaling, attenuate the expression of the mesenchymal marker α-SMA and vimentin and further prevent TGF-β1-induced RTEC EMT. This phenomenon suggests that AS-IV may be an effective drug for preventing EMT in RTECs and renal interstitial fibrosis.

PI3K/AKT/GSK-3β and Wnt/β-catenin. TGF-β1 not only targets the Smad transcriptional activator family but also regulates non-Smad-dependent signaling pathways, such as the PI3K/AKT signaling cascade, which contributes to fibroblast transdifferentiation and fibrosis. Network pharmacology analysis showed that AS-IV and renal fibrosis share multiple targets and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis found that the PI3K/AKT signaling pathway has intersectionality with these shared targets, among which AKT1 and glycogen synthase kinase-3β (GSK-3β) are markedly enriched in the PI3K/AKT signaling cascade (150). Further *in vitro* cell experiments revealed that AS-IV treatment inhibits the TGF-β1-induced upregulation of AKT phosphorylation in rat renal tubular epithelial (NRK-52E) and NRK-49F cells in a concentration-dependent manner, thereby promoting its induction of GSK-3β dephosphorylation (150). In addition, the dephosphorylation of GSK-3β can induce β-catenin degradation, thereby negatively regulating the progression of EMT. Consequently, the mechanism by which AS-IV can prevent TGF-β1 from activating β-catenin is linked to AS-IV targeting the inactivation of the AKT/GSK-3β signaling pathway, which alleviates EMT and further inhibits β-catenin to promote Wnt signaling pathway activation (150). Notably, disheveled (Dsh or Dvl) proteins often promote the dephosphorylation of β-catenin, thereby interfering with its degradation mainly because activated Dsh proteins drive the separation of GSK-3β from the degradation complex, thereby promoting Wnt/β-catenin signaling and targeting the elevation of downstream gene expression. According to Wang *et al* (151), AS-IV suppresses the overexpression of Wnt and Frizzled genes in a UUO-induced renal interstitial fibrosis rat model, with the activity of Dsh proteins in the cytoplasm and β-catenin in the nucleus also being downregulated after AS-IV intervention. In addition, stable RTEC structure depends on activated E-cadherin and E-cadherin is inversely associated with the nuclear level of β-catenin. Furthermore, the majority of the Wnt/β-catenin signaling pathway's downstream target genes, such as Snail, Twist, lymphoid enhancer binding factor-1 and Jagged1, are involved in the development of EMT. In the rat model of UUO-induced renal interstitial fibrosis, AS-IV can downregulate the nuclear levels of the aforementioned target proteins that are engaged in the Wnt/β-catenin signaling cascade downstream. To summarize, a key mechanism by which AS-IV decelerates renal interstitial fibrosis may be attributed to the partial inhibition or blockage of the Wnt/β-catenin signaling pathway (151).

MAPK and NF-κB. The fibrotic effector TGF-β regulates the occurrence of fibrosis during CKD and can participate in the induction of MAPK family members relying on noncanonical signaling pathways. Che *et al* (152) found that AS-IV substantially suppresses the overexpression of the cell proliferation marker proliferating cell nuclear antigen, the myofibroblast differentiation marker α-SMA, collagen I and FN in TGF-β1-treated renal fibroblasts. It also negatively regulates the activities of p-IκBα and the MAPK family members ERK1/2, p38 MAPK and JNK and the nuclear level of the NF-κB p65 subunit in TGF-β1-induced renal fibroblasts. Comparison revealed that the efficacy of MAPK and NF-κB inhibitors in regulating EMT is similar to that of AS-IV, further confirming that the suppression of the MAPK and NF-κB signaling pathways is a key mechanism through which AS-IV improves CKD and blocks fibroblast transdifferentiation (152). In addition, tubular cell apoptosis is involved in renal fibrosis (153). ERK, p38 MAPK and JNK are three major family members of the MAPK pathway that mediate apoptosis and inflammation. Targeting p38 MAPK inactivation is another key strategy for remitting renal structural atrophy and fibrosis induced by renal artery stenosis (154). Xu *et al* (155) found that the UUO-induced apoptosis of obstructed RTECs *in vivo* and TGF-β1-induced HK-2 cell apoptosis *in vitro* can be alleviated after AS-IV intervention and the level of activated caspase-3 is markedly inhibited. In addition, AS-IV promotes the dephosphorylation of the p38 MAPK and JNK pathways in obstructed kidneys and TGF-β1-treated HK-2 cells; negatively regulates serum creatinine and blood urea nitrogen levels, which are indicators of renal function injury, and the expression levels of FN and α-SMA, which are indicators of renal tubulointerstitial fibrosis. These effects provide pharmacological evidence for targeting AS-IV to improve renal tubular cell survival and alleviate renal fibrosis associated with obstructive nephropathy (155). Moreover, inhibiting the transdifferentiation of kidney resident cells, such as fibroblasts,

pericytes, tubular cells and endothelial cells, into myofibroblasts can negatively regulate tubulointerstitial fibrosis (156). Meng *et al* (156) focused on the negative regulatory mechanism of TCM compounds on fibrosis induced by UUO and, through mass spectrometry, identified AS-IV and FA as the main bioactive components in *Astragalus* and *Radix Angelica sinensis* extracts, respectively. The authors found that *AF* can downregulate the expression of serum creatinine, FN, α-SMA and TGF-β1 in UUO-induced obstructed kidneys; inhibit interstitial fibroblast activation; and upregulate the production of nitric oxide, which is a key mediator that negatively regulates renal injury (157). Furthermore, in TGF-β1-treated NRK-49F cells *in vitro*, *AF* mediates the downregulation of α-SMA and FN expression and in IL-1β-treated HK-2 cells *in vitro*, *AF* targets an increase in the dephosphorylation level of JNK. However, the phosphorylation levels of ERK and p38, two other members of the MAPK signaling pathway family, are not markedly affected by the interference effect of *AF*. These results suggest that *AF* administered on the basis of TCM compatibility can reduce tubulointerstitial fibrosis to a certain extent (156).

cAMP/PKA. The specific receptor G-protein-coupled receptor 14 of urotensin II (UII) is an upstream molecule that regulates cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) signaling. The activated cAMP/PKA signaling cascade further induces the downstream fibrotic effector molecule TGF-β to be overexpressed and promotes RTECs to acquire a mesenchymal phenotype, which triggers renal fibrosis (158). The phosphorylation of PKA is closely associated with inflammation and pyroptosis mediated by the NLRP3 inflammasome (159). However, according to Zhang *et al* (160), AS-IV administration could downregulate the UUO-induced renal fibrosis-related indicators cAMP and UII. Moreover, after *in vitro* UII intervention, the activity of PKA and expression of the pyroptosis-related indicators NLRP3, caspase-1 and GSDMD-N in NRK-52E cells were markedly inhibited by AS-IV. In addition, by suppressing the expression of α-SMA and FN and inducing the blockage of EMT, AS-IV can prevent UII-treated NRK-52E cells from losing epithelial cell morphology and acquiring the mesenchymal phenotype. In summary, AS-IV can target the downregulation of cAMP/PKA pathway activity and inhibit its induction of RTEC pyroptosis and EMT, thereby alleviating renal fibrosis (160).

TGF-β1/ZEB2. TGF-β treatment or HG induction is markedly associated with the upregulation of miR-192 activity (161). However, AS-IV administration can dose-dependently suppress the expression of miR-192 in HK-2 cells treated with TGF-β1 (162). In addition, ZEB2 is widely expressed in the kidney and miR-192 inactivates ZEB2 in HK-2 cells through downstream regulation. Notably, the negative regulatory effects of AS-IV on α-SMA, vimentin and collagen I are blocked under the mediation of miR-192 mimics and further enhanced by intervention with miR-192 inhibitors. This effect suggests that a potential mechanism of the antirenal fibrosis effect of AS-IV may be to target the inactivation of the TGF-β1/ZEB2 signaling pathway (162,163).

Liver cancer-induced renal fibrosis. The occurrence of liver cancer is often accompanied with an increase in the concentration of proinflammatory factors in the blood. This effect can easily lead to pathological communication, such as hepatorenal syndrome, between the liver and other organs, thereby further reducing the survival period (164,165). TGF-β receptor type 1 is a negative regulatory mediator of liver fibrosis primarily because of its capacity to drive the COOH-terminal phosphorylation of Smad3 (pSmad3C) and upregulation of p21 transcriptional activity (166). When JNK is activated in an inflammatory state, it further induces the phosphorylation of the linker region of Smad3 (pSmad3L), thereby targeting downstream plasminogen activator inhibitor-1 (PAI-1) activation and participating in fibrosis (167). This situation indicates that the phosphorylation of different sites of Smad3 is an important factor in inducing the completely opposite roles of pSmad3L/PAI-1 and pSmad3C/p21 signaling cascades in regulating fibrosis. However, Wang *et al* (165) revealed that AS-IV activates the pSmad3C/p21 pathway and simultaneously antagonizes pSmad3L/PAI-1 signaling, which in turn negatively regulates TGF-β1-induced α-SMA expression in HK-2 cells. In addition, after oxidative damage occurs in the kidney, AS-IV administration can regulate the Nrf2/heme oxygenase-1 (HO-1) pathway to strengthen the expression of pNrf2 and HO-1 further and maintain high Nrf2 activity. However, after Nrf2 is silenced, the regulation of the pSmad3L/PAI-1 and pSmad3C/p21 signaling pathways by AS-IV becomes imbalanced. Moreover, the kidneys of knockout mice exhibit collagen fiber deposition, implying that renal fibrosis also develops as liver fibrosis progresses to HCC. The regulatory effect of Nrf2 on pSmad3C/3L is a potential pharmacological mechanism of AS-IV in alleviating renal fibrosis complicated by liver cancer (165) (Fig. 5).

Notably, renal fibrosis induced in animal models cannot easily replicate the chronic progressive nature of human diseases and does not adequately reflect the progressive changes in clinical renal function indicators, such as estimated glomerular filtration rate and urinary protein. In addition, the recruitment and differentiation of endogenous renal stem cells and mesenchymal stem cells play a crucial role in regulating renal injury repair (168,169). Whether the antifibrotic mechanism of AS-IV involves targeting the proliferation and differentiation of renal stem cells remains to be investigated. Moreover, the low oral bioavailability of AS-IV may limit its enrichment in the renal cortex and medulla. Whether the end products of AS-IV after in vivo metabolism are also involved in the derivation of AS-IV's antirenal fibrosis activity requires a comprehensive mechanism integration analysis. Finally, human nephrons exhibit unpredictable regeneration and repair effects and the sole reliance on animal experiments cannot systematically define the dynamic effect of AS-IV on nephron remodeling during the multistage course of renal fibrosis (170).

Liver fibrosis. Viral hepatitis, cholestatic liver disease, alcoholic liver disease, nonalcoholic steatohepatitis, nonalcoholic fatty liver disease and other liver diseases can easily cause repeated damage to the liver, repair response imbalance and persistent inflammatory infiltration. Scar tissue and regenerative nodules may replace parts of liver tissue, resulting in liver fibrosis. As liver function gradually deteriorates and fibrosis develops, highly lethal cirrhosis may be induced and prognosis becomes increasingly challenging (171). A key contributing factor to liver fibrosis is chronic inflammation, which drives hepatic stellate cell (HSC) activation through

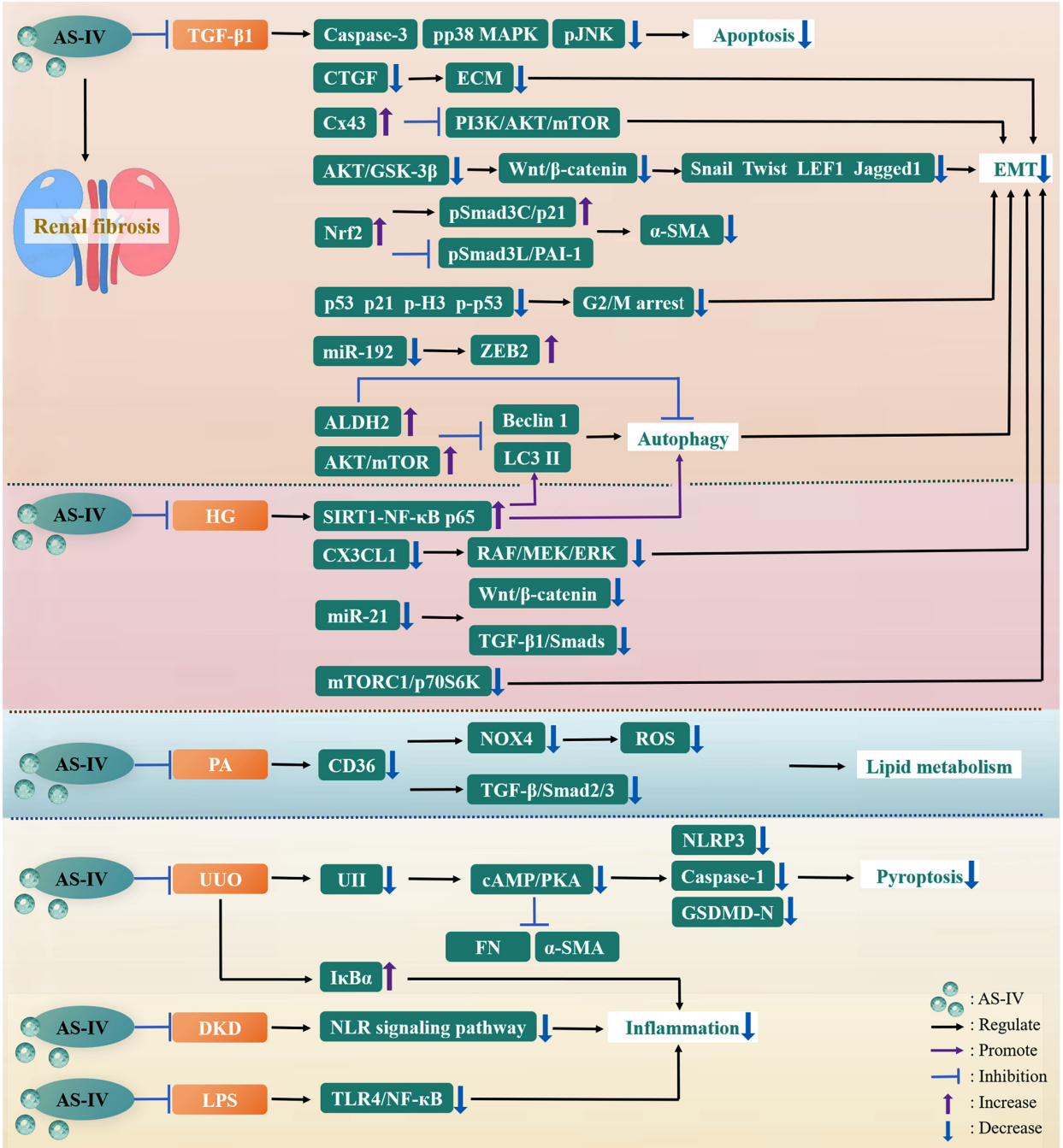

Figure 5. The mechanisms of AS-IV against renal fibrosis. AKT, protein kinase B; ALDH2, acetaldehyde dehydrogenase 2; AS-IV, astragaloside IV; cAMP, cyclic adenosine monophosphate; CD36, cluster of differentiation 36; CTGF, connective tissue growth factor; CX3CL1, C-X3-C motif ligand 1; Cx43, connexin43; DKD, diabetic kidney disease; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; FN, fibronectin; GSDMD-N, gasdermin D N-terminal domain; GSK-3β, glycogen synthase kinase-3β; HG, high glucose; IκBα, inhibitor of NF-kappa B α; JNK, c-Jun N-terminal kinase; LC3 II, light chain 3 II; LEF1, lymphoid enhancer binding factor-1; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; miR-192, microRNA-192; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; NF-κB, nuclear factor-kappa B; NLR, nucleotide-binding oligomerization domain-like receptor; NLRP3, nucleotide-binding oligomerization domain-like receptor thermal protein domain associated protein 3; NOX4, NADPH oxidase 4; Nrf2, nuclear factor erythroid 2-related factor 2; p70S6K, ribosomal protein S6 kinase β-1; PA, palmitic acid; PAI-1, plasminogen activator inhibitor-1; p-H3, phosphorylated histone H3; PI3K, phosphatidylinositol-3-kinase; PKA, protein kinase A; pSmad3C, COOH-terminal phosphorylation of Smad3; pSmad3L, phosphorylation of the linker region of Smad3; RAF, rapidly accelerated fibrosarcoma; ROS, reactive oxygen species; RTEC, renal tubular epithelial cell; SIRT1, sirtuin 1; TGF-β1, transforming growth factor-β1; TLR4, toll-like receptor 4; UII, Urotensin II; UUO, unilateral ureteral obstruction; ZEB2, zinc finger E-box binding homeobox 2; α-SMA, α-smooth muscle actin.

the recruitment of inflammatory cells, such as monocytes and macrophages. Among these cells, macrophages further regulate the transdifferentiation of stellate cells into myofibroblasts by inducing fibrogenic cytokines and TGF-β overexpression, and the increase in the number of myofibroblasts can promote the massive deposition of ECM (172-174). Therefore, the key mechanism targets for intervening in liver fibrosis mainly include inhibiting adverse stress, thus negatively regulating inflammatory responses, HSC activation, and ECM deposition (172). In addition to TGF-β, NLRP3-caspase-1, the

Wnt/β-catenin signaling cascade, and platelet-derived growth factor are important mediators involved in HSC activation and fibrosis progression (175-177).

Liver fibrosis is a pathological process that is dynamic and reversible. These characteristics suggest that the occurrence of liver fibrosis linked to chronic liver disease may be prevented through active interventions or targeting the reversal of fibrosis to basal levels (178). At present, no specific antihepatic fibrotic strategies exist, with antiviral therapy and the induction of the elimination of harmful factors being often used as conservative treatments to alleviate liver fibrosis. However, for patients with advanced fibrosis, especially those that progress to cirrhosis or are accompanied with some uncontrollable clinical symptoms, liver transplantation is ultimately the only option (10). Given that tissue or organ fibrosis tends to progress slowly and nonlinearly in the body and can last for years to decades, developing highly effective antifibrotic agents to prevent liver fibrosis from evolving into cirrhosis is crucial (179). Studies have confirmed that the use of TCM has considerable efficacy in fighting liver fibrosis (10). This section mainly focuses on the pharmacological mechanism and feasibility of AS-IV against liver fibrosis.

Liver cancer-related fibrosis. Primary liver cancer induced by tumor lesions related to hepatocytes or intrahepatic bile duct cells is highly related to the occurrence of liver fibrosis and preventing liver fibrosis from developing can decelerate the progression of liver cancer (167). The occurrence of liver fibrosis and cancer is intimately associated with the phosphorylation of different sites of Smad2/3, including pSmad3C, which transmits a tumor-suppressive TGF-β1 signal; pSmad2C; pSmad2L; and pSmad3L (166). Among these sites, pSmad3L can negatively regulate the C-terminal phosphorylation of Smad3C, thereby promoting the obstruction of pSmad3C signaling transduction and exacerbating liver cancer lesions (166). Zhang *et al* (167) found that in mice, fibrous connective tissue is present in the liver tissue at 12 weeks after diethylnitrosamine (DEN)/tetrachloromethane/ethanol-induced primary liver cancer. However, AS-IV markedly downregulates α-SMA expression, especially in areas where inflammatory cells are infiltrating and the extensive production of collagen fibers is reversed by AS-IV administration. In addition, AS-IV promotes the downregulation of the incidence and multiplicity of HCC nodules and induces the inactivation of the liver cancer marker α-fetoprotein. This effect indicates that AS-IV not only has antifibrotic activity but also may participate in the negative regulation of primary liver cancer. In primary liver cancer mouse models and HSC-T6 cells induced by TGF-β1 in vitro, AS-IV can promote the overexpression of pSmad3C, pNrf2, HO-1 and NAD(P)H:quinone oxidoreductase 1 (NQO1) and target the levels of TGF-β1, pSmad3L, pSmad2C and pSmad2L for downregulation (167). In primary liver cancer models *in vivo* and TGF-β1-induced human HCC (HepG2) cells *in vitro*, AS-IV can promote Nrf2/HO-1/NQO1 signaling transduction and upregulate the antioxidant activity of pNrf2 and its downstream proteins to alleviate the progression of liver fibrosis and primary liver cancer (167,180). Moreover, pSmad3C, pSmad3L and pNrf2 may have crosstalk *in vivo*. Given that TGF-β1 is upstream of pSmad3C/pSmad3L, the further stimulation of HSC-T6 and HepG2 with TGF-β1 can induce Nrf2 inactivation. This phenomenon may also imply that TGF-β1 is a negative regulator of Nrf2. However, additional research is still required to validate the upstream-downstream relationship of these signaling molecules (167). In summary, AS-IV, through its pharmacological activity against liver fibrosis, simultaneously inhibits the antagonism between pSmad3L and pSmad3C signaling, regulates the Nrf2/HO-1 signaling pathway and upregulates pNrf2 activity, thereby alleviating primary liver cancer (167).

Astragalus and *Salvia miltiorrhiza* Bunge (Lamiaceae) are often employed as TCM compounds. The main natural active ingredients derived from *Astragalus* and *S. miltiorrhiza* constitute Compound *Astragalus* and *S. miltiorrhiza* Extract (CASE), which includes AS-IV (181). The MAPK pathway can mediate TGF-β/Smad signaling transduction in tumors and participate in the induction of TGF-β-related oncogenic effects. The TGF-β signaling molecule can target downstream PAI-1 gene overexpression, thereby further promoting TGF-β signaling to drive tumor lesions. Furthermore, the development of liver fibrosis is markedly associated with the activation of the PAI-1 gene. Boye *et al* (181) found that TGF-β1 can stimulate the activation of ERK1, JNK1/2 and pp38 in HSCs and HepG2 cells *in vitro*. Although CASE pretreatment can inhibit the overexpression of pERK and pJNK, CASE may be a potentiator of TGF-β1-induced pp38 activation. Notably, in *in vivo* studies, CASE can markedly inhibit the activation of three family members of the MAPK pathway in a DEN-induced rat HCC model. Furthermore, CASE pretreatment can negatively regulate the stimulatory effect of TGF-β1 on the levels of p-Smad2C and p-Smad2L and the cancer-inducing factor p-Smad3L in HSCs and HepG2 cells. By contrast, under the interference of exogenous TGF-β1 stimulation, CASE can further promote the negative regulatory effect of p-Smad3C phosphorylation on tumorigenesis and inhibit Smad4 nuclear translocation. This regulatory effect is highly important in HepG2 cells. In addition, CASE pretreatment can inhibit PAI-1 overexpression in DEN-induced HCC models and TGF-β1-induced HSCs and HepG2 cells. In summary, CASE promotes cancer inhibitory signal output by activating the MAPK-dependent linker dephosphorylation of Smad2/3, further targeting the downregulation of PAI-1 expression, thereby inhibiting MAPK to regulate cancer-inducing signals related to TGF-β/Smad. This activity indicates that TCM compounds added with AS-IV can modulate the MAPK pathway and TGF-β/Smad signaling transduction in multiple targets, thereby achieving dual targeted benefits in the treatment of liver cancer and fibrosis (181).

Bile duct ligation-related fibrosis. Bile duct ligation (BDL) induces biliary epithelial cell damage and compensatory proliferation and serves as an inducer of cholestatic hepatic fibrosis by activating HSCs (182). Considering that multidrug combinations can simultaneously intervene in multiple targets, they can be applied in the treatment of liver fibrosis under the regulation of multiple mechanisms. Additionally, plants themselves can derive certain antioxidant substances, which help mitigate the damage to plant structures caused by the excessive release of ROS during photosynthesis. This situation is also applicable to TCM (183,184). Zhao *et al* (184) found that *AF* can markedly inhibit α-SMA overexpression and FN deposition in the livers of rats treated with BDL

and HSC activation and collagen I and III synthesis are also markedly inhibited by coadministration. In addition, the antifibrotic effect of *AF* is at least partially attributed to the improvement of oxidative stress by AS-IV. That is, by activating the endogenous antioxidant Nrf2, AS-IV upregulates the contents of glutathione (GSH) and SOD in the livers of rats treated with BDL and reduces MDA levels (184). Furthermore, GSK-3β is an inducer of Nrf2 degradation and AS-IV can activate the phosphorylation of hepatic GSK-3β at Ser9 (inactive form) in the BDL rat model. That is, AS-IV can inhibit excessive ROS release by targeting GSK-3β/Nrf2 signaling pathway activation (184). In conclusion, although the administration of AS-IV or FA alone can also undeniably improve BDL-induced liver fibrosis to a certain extent, the combined application of natural active products derived from Chinese herbal medicines is effective in inhibiting the activation of HSCs. This effect may be mediated by the synergistic mechanism of the upregulation of the antioxidant activity of Nrf2 targeted by AS-IV and the negative regulation of TGF-β signaling transduction by FA (184).

Fibrosis related to in vitro cultured activated HSCs. Compound TCM supplements often have greater pharmacological activity than single drugs. DBT, which is composed of *Astragalus* and *Radix Angelica sinensis*, is an effective TCM formula with a long history. Dong *et al* (185) found that the synergistic administration of *AF* extracted from DBT can markedly inhibit HSC activation and reduce ROS generation by activating Nrf2. These effects are mainly related to the regulation of the activation of the Nrf2/antioxidant response element pathway by AS-IV. Furthermore, the combined administration of *AF* negatively regulates the TGF-β1/Smad pathway. This action is mainly attributed to the suppression of TGF-β1 signaling transduction and the inactivation of Smad4 expression by FA. TGF-β can activate p38 MAPK downstream in HSCs by inducing ROS generation, thereby jointly mediating the regulatory effect of activated p38 MAPK on collagen fiber synthesis. AS-IV can promote p38 MAPK dephosphorylation and is further enhanced under the regulation of FA (185). Moreover, the simultaneous inactivation of Nrf2 and blocking of TGF-β signaling can completely reverse the inhibitory effect of *AF* on p38 MAPK phosphorylation. That is, the participation of p38 MAPK in the downstream of this signal transduction pathway has been demonstrated again. In summary, the aforementioned study further confirmed that the therapeutic effect of the synergistic application of antioxidants and TGF-β inhibitors on liver fibrosis is greater than that of single drug supplementation (185). Markedly, the nonenzymatic antioxidant GSH can serve as a second messenger in HSCs to mediate TGF-β1 signaling transduction, despite oxidative stress being considered as a third messenger (186,187). Oxidative stress is a key factor in inducing HSC activation. Li *et al* (187) revealed that AS-IV can markedly inhibit ROS release from activated HSCs and mediate the downregulation of lipid hydroperoxide content (188). In activated HSCs, AS-IV may promote activated Nrf2 to drive GSH synthesis by targeting Nrf2. However, after Nrf2 is blocked, the decline in ROS and collagen I content caused by AS-IV is reversed (187). In addition, activated p38 MAPK can mediate collagen synthesis in HSCs. When activated HSCs are treated with p38 MAPK kinase inhibitors, ROS, lipid hydroperoxide, GSH production and Nrf2 activity are unaffected. That is, in HSCs, Nrf2 may promote the GSH-mediated inactivation of p38 MAPK by targeting increased GSH synthesis, thereby further inhibiting the inducible effect of this kinase on collagen synthesis (187). Therefore, one possible explanation for AS-IV's suppression of HSC activation is its targeted effect on the upregulation of GSH content and Nrf2 activity.

Porcine-serum (PS)-related fibrosis. The strong immune rejection reaction of receptors to serum with an allogeneic origin can be complicated by liver fibrosis. This phenomenon is the main mechanism by which PS induces immune hepatic fibrosis in rats. This model differs from traditional postnecrotic liver fibrosis in that it can well simulate the clinical characteristics and pathological processes of human liver diseases and cause little damage to hepatocytes (189,190). Liu *et al* (190) revealed that in a rat model treated with PS, AS-IV may prevent TGF-β1 from being overexpressed in liver tissue and serum. Moreover, AS-IV can interfere with the influence of TGF-β1 stimulation on the incorporation of [^{3}H]proline in HSCs, as demonstrated by the decreased incorporation amount and inhibition of TGF-β1-induced proliferation and collagen synthesis in HSCs, thereby alleviating PS-induced immune hepatic fibrosis (Fig. 6).

Notably, in animal models, the induction pattern of liver fibrosis often relies on high-dose interventions, primarily causing acute or subacute liver injury. By contrast, the pathogenic process of liver fibrosis in humans follows a chronic low-dose pattern. Consequently, substantial differences may exist between humans and animal models in terms of the mechanisms by which AS-IV regulates cellular stress and acts on signaling pathways. Furthermore, the effect of AS-IV on regulating the activation of HSCs may be associated with the heterogeneity of HSCs. The subset differences of HSCs might lead to the diverse induction effects of AS-IV on the differentiation direction of HSCs, thereby influencing the progression of liver fibrosis. In basic experiments, fibrosis intervention is often conducted via the intraperitoneal injection of high concentrations of AS-IV. The dose used is considerably higher than the conventional oral dose, making realistically simulating clinical effects difficult. Therefore, the further introduction of liver organoids, liver chips and humanized models can enhance the reliability of AS-IV in the prevention and treatment of liver fibrosis.

Other tissue fibrosis

Peritoneal fibrosis

PGC-1α/ROS/apoptosis. Treatment with peritoneal dialysis (PD) is common for patients with end-stage renal failure. However, the long-term contact of acidic ions and high glucose concentrations in the dialysate with the peritoneum activates peritoneal intrinsic cells. Peritoneal mesothelial cells (PMCs) are induced to undergo hyperglycosylation and mesothelial-mesenchymal transition (MMT), leading to the decline in peritoneal barrier function and occurrence of peritoneal fibrosis and destroying the ideal PD efficacy (191-194). Mitochondrion-mediated redox metabolism imbalance promotes apoptosis that is directly related to peritoneal fibrosis. Xie *et al* (191) found that AS-IV can reduce the thickness of peritoneal tissue in rats and promote the overexpression of the mitochondrial synthesis-related protein

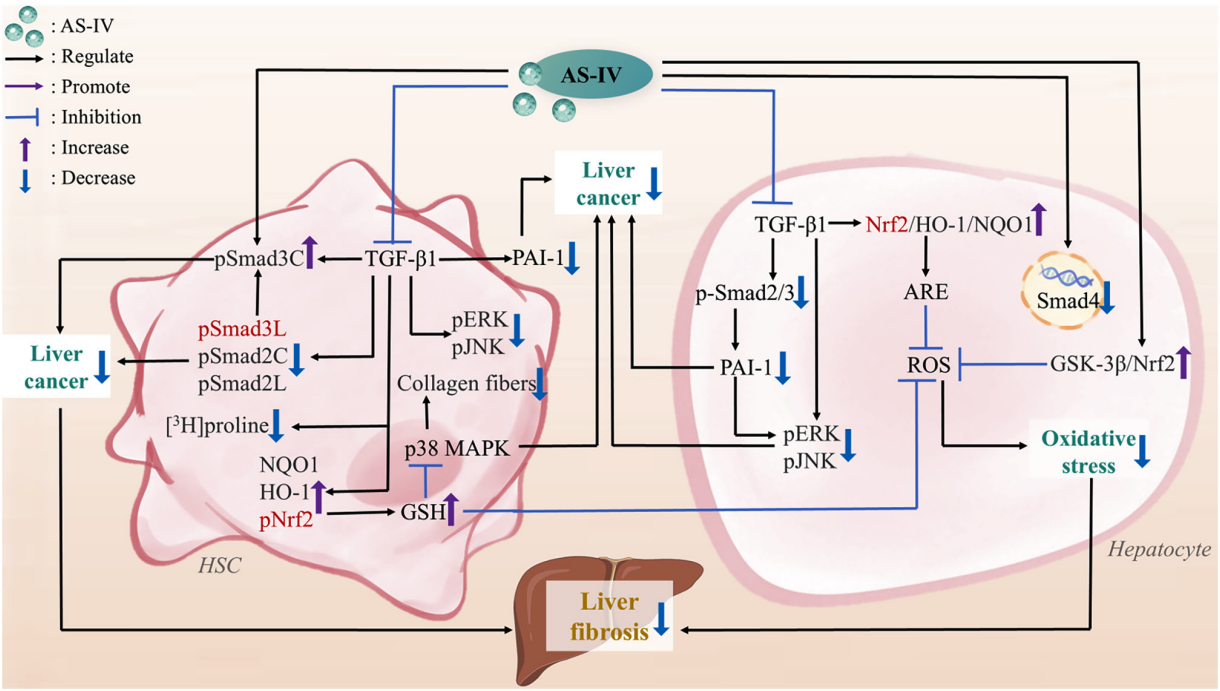

Figure 6. The mechanisms of AS-IV against liver fibrosis. ARE, antioxidant response element; AS-IV, astragaloside IV; GSH, glutathione; GSK-3β, glycogen synthase kinase-3β; HO-1, heme oxygenase-1; HSC, hepatic stellate cell; MAPK, mitogen-activated protein kinase; NQO1, NAD(P)H:quinone oxidoreductase 1; PAI-1, plasminogen activator inhibitor-1; pERK, phosphorylated extracellular signal-regulated kinase; pJNK, phosphorylated c-Jun N-terminal kinase; pNrf2, phosphorylated nuclear factor erythroid 2-related factor 2; pSmad3C, COOH-terminal phosphorylation of Smad3; pSmad3L, phosphorylation of the linker region of Smad3; ROS, reactive oxygen species; TGF-β1, transforming growth factor-β1.

PGC-1α and mitochondrial genes. Moreover, the authors discovered that AS-IV can downregulate the ubiquitination of PGC-1α; increase the half-life of PGC-1α; and, by negatively regulating the expression of cleaved-caspase-3, α-SMA and p-smad2/3, also execute considerable antiapoptotic and antifibrotic activities. In summary, under mediation by the PGC-1α/ROS/apoptosis signaling pathway, AS-IV promotes mitochondrial biosynthesis, as well as the upregulation of mitochondrial membrane potential, in the peritoneal tissue of peritoneal fibrosis rats by activating its pharmacological target PGC-1α. Furthermore, it reduces the excessive accumulation of ROS in the mitochondrial membrane, thereby inhibiting PMC apoptosis and alleviating peritoneal fibrosis progression (191).

miR-204-5p/Foxc1/β-catenin. miRNAs can mediate intercellular signaling transduction. Exosomes are responsible for transporting miRNAs. Exosomes carrying miRNAs circulate in the body and are taken up by different cells, thereby targeting functional changes in recipient cells (192,195,196). Shan *et al* (192) found that after LPS activates primary rat peritoneal macrophages and THP-1 monocyte cells, it induces the inflammatory infiltration of the abdominal cavity and promotes MMT. AS-IV pretreatment can alleviate the upregulation of vimentin and α-SMA and the downregulation of E-cadherin induced by inflammatory macrophages can also be reversed under AS-IV mediation. In addition, primary PMCs (RPMCs) uptake LPS-stimulated macrophage-derived exosomes. This phenomenon can further drive the occurrence of the MMT of RPMCs. However, AS-IV pretreatment can inhibit the promoting effect of exosomes on MMT. Furthermore, the identification of exosomal miRNA profiles revealed that AS-IV can activate miR-204-5p carried by inflammatory macrophage-derived exosomes. miR-204-5p has anti-inflammatory and antifibrotic activities and can negatively regulate the expression of forkhead box C1 (Foxc1). Foxc1 has been reported to drive MMT progression by activating β-catenin in fibroblasts (197). Therefore, the miR-204-5p/Foxc1/β-catenin axis may be a key signaling cascade in regulating the MMT of RPMCs. AS-IV can further inhibit the activating effect of downstream Foxc1 on β-catenin by targeting miR-204-5p overexpression, thereby alleviating peritoneal fibrosis and abdominal cavity inflammation associated with HG dialysis. Inflammatory macrophage-derived exosomes are also a key mediator for the regulation of signaling transduction between macrophages and RPMCs and alleviation of PD-induced peritoneal fibrosis by AS-IV (192).

SSc-related fibrosis. SSc, a heterogeneous, autoimmune connective tissue disease, is induced by the massive proliferation of fibroblasts and excessive ECM synthesis, which causes scar tissue to replace the physiological structure of blood vessels, skin and internal organs. Local skin tissue lesions and organ fibrosis eventually occur. Qi *et al* (198) demonstrated that AS-IV can reduce the deposition of collagen I and FN in SSc fibroblasts, as well as inhibiting highly activated p-Smad3 and the nuclear localization of p-Smad3 in SSc fibroblasts. Furthermore, AS-IV induces the blockage of TGF-β/Smad3 signaling transduction and further regulates the inactivation of the profibrotic mediator Smad3/friend leukemia virus integration 1 downstream of TGF-β, thereby alleviating SSc-related fibrosis.

Skin scarring and fibrosis. Skin wound treatment advocates rapid tissue repair while minimizing scarring. Notably, the activation of CTGF by TGF-β1 is a key factor in regulating

scar tissue hyperplasia. According to Chen *et al* (199), AS-IV could further enhance wound re-epithelialization during the proliferative phase of wound healing. This effect is mainly attributed to the remarkable induction of keratinocyte migration and proliferation. In addition, AS-IV exerts a scar-inhibiting effect similar to that of TGF-β antibodies by reducing TGF-β1 secretion. Most of the collagen in skin wounds is scarred and has lost its original highly organized structure. However, AS-IV can improve the tensile strength of healed skin (199). Moreover, scar tissue is often dominated by collagen I, whereas collagen III is lost. AS-IV can also maintain a low level of collagen I/III content, promote the content of collagen III related to epidermal repair and restore the elasticity of damaged skin. In short, AS-IV promotes skin wound repair and inhibits scar hyperplasia. These effects are related to inducing wound re-epithelialization, negatively regulating TGF-β1 secretion, regulating collagen I/III homeostasis and remodeling ECM (199).

Sodium alginate-gelatin (GT) hydrogel incorporated with AS-IV can improve skin wound repair and collagen synthesis and regulate serum TGF-β1 levels and skin tensile strength recovery. Intervening with a drug carrier that is compatible with AS-IV may be necessary in consideration of the frequency of medication. Solid lipid nanoparticles (SLNs) attracted the attention of Chen *et al* (200) because of their effectiveness in acting on epidermal tissue (201). By administering AS-IV-loaded SLN-enriched gel, the synthesis of collagen I can be induced, thereby promoting the physiological structure of the repaired epidermal wound to closely resemble that of the skin. In addition, AS-IV-based SLN-gel can regulate collagen fiber arrangement and collagen deposition and reduce scar formation. In summary, AS-IV-based SLN-gel, mediated by the caveolae endocytosis pathway, can induce keratinocyte proliferation and migration and promote the uptake of AS-IV by fibroblasts. The latter phenomenon is consistent with the ability of AS-IV-based SLN-gel to release drugs continuously and slowly and prolong the time of drug action on epidermal tissue repair (200).

About 2 million new cases of burns are reported every year worldwide. Covering with wound dressings often intervenes in the healing of local burn wounds, which are as severe skin traumas (202). Electrospun nanofibers can be used as structural mimics of ECM; although biocompatible silk fibroin (SF) scaffolds provide a supportive environment for cell adhesion and proliferation or tissue repair, their degradation is slow and may require further processing improvements (202). GT is not only beneficial for compensating for the disadvantages of SF scaffolds in degradation but is also a hydrolysis product of collagen. Therefore, Shan *et al* (202) found that the combined processing of GT-added SF scaffolds and nanofibrous wound dressings may be beneficial for the pharmacological activity of AS-IV in promoting healing and antiscarring. This benefit is mainly reflected in the ability of AS-IV-loaded SF/GT nanofibrous dressing (25/75) to release drugs quickly within 12 h, with their sustained release time reaching 12-24 h. AS-IV-loaded SF/GT nanofibrous dressing can promote postinjury vascular regeneration by upregulating the activity of vascular endothelial growth factors and regulate the relative regular arrangement of collagen fibers to inhibit the formation of scar tissue during burn healing. AS-IV-loaded SF/GT nanofibrous dressing (25/75) can increase the collagen I content of the healed skin and restore normal skin tissue structure. In addition, the swelling rate of AS-IV-loaded SF/GT nanofibrous dressing (25/75) is markedly increased. This effect is beneficial for absorbing excess exudate from local burned skin tissue and preventing bacterial infections. In summary, applying nanofibrous dressings with the optimal SF:GT ratio of 25:75 to heal burn wounds and successfully loading the insoluble drug AS-IV can improve the biological properties of functional burn wound dressings and promote cell proliferation and angiogenesis, thus inhibiting scar hyperplasia (202).

Trabecular meshwork fibrosis. Of patients with glaucoma, ~74% present with primary open-angle glaucoma (POAG) (203). The pathogenesis of POAG can be summarized as follows: endoplasmic reticulum (ER) stress promotes the excessive deposition of ECM in the trabecular meshwork (TM) by interfering with ECM protein processing, thereby inducing TM fibrosis. TM fibrosis further regulates the increase in aqueous humor (AH) outflow resistance. The latter becomes a key cause of increased intraocular pressure (IOP), which becomes a risk factor that ultimately leads to the occurrence of POAG. The overexpression of TGF-β2 in AH and TM is also an inducing factor for POAG (204). Kasetti *et al* (203) showed that AS-IV can markedly suppress TGF-β2-induced EMT and ER stress in TM cells. In addition, MMPs are involved in regulating AH outflow, IOP and ECM remodeling in the TM. AS-IV can negatively regulate TGF-β2-induced MMP9 inactivation and further enhance the compensatory upregulation of MMP2 expression by TGF-β2 (203). Moreover, ocular AS-IV treatment reverses TGF-β2-induced ocular hypertension and NF-κB overexpression in mice. The downregulation of pNF-κB may activate the endogenous TGF-β inhibitor, thereby blocking TGF-β2 signaling transduction and reducing TM fibrosis. In summary, AS-IV markedly alleviates TGF-β2-induced POAG-related ocular hypertension by inhibiting TM cell fibrosis and TM tissue ER stress and upregulating TM cell MMP activity (Table I) (203).

4. Clinical translation of AS-IV

Although the antifibrotic effect of AS-IV has been confirmed in various animal or cell models, preclinical animal experiments generally have limitations, such as methodological problems, high bias risk and low reproducibility, as well as differences in pathophysiology across different tissues (205). This situation indicates that further cross-species studies, such as large-sample, double-blind and randomized human clinical trials, remain necessary. The signaling pathways and molecular targets regulating fibrotic diseases are intricate and given that humans are complex organisms, the further in-depth integration of network regulatory relationships will promote the clinical translation of AS-IV.

In addition, most current safety evaluations of AS-IV are based on systemic administration models, with no toxicity dose testing specifically targeting the liver (206). In model organisms, the administration dose of AS-IV for antifibrotic purposes show large variations. Therefore, clarifying the reference range of safe and effective doses will ensure the reproducibility of the antifibrotic therapeutic outcomes of AS-IV. Given its activating effect on the immune system,

Table I. Summary of antifibrotic effects and underlying mechanisms of AS-IV.

Authors, year	Fibrotic disease	Dosages	Models	*In vivo*/ *in vitro*	Effects and related mechanisms	(Refs.)
Wan *et al*, 2018	Cardiac fibrosis	100/200 mg/kg AS-IV and 31.25/62.5 mg/kg CA, i.g.; 31.25 μg/ml CA.	ISO-induced cardiac fibrosis in mice; primary rat CFs.	*In vivo* and *in vitro*	Demonstrating a protective effect against cardiac fibrosis by blocking the NLRP3 inflammasome pathway.	(41)
Wei *et al*, 2020		10 mg/kg, p.o.; 10 μM.	ISO-induced cardiac fibrosis in rats; CFs of neonatal rats.	*In vivo* and *in vitro*	Targeting the miR-135a-TRPM7-TGF-β/Smads pathway to prevent cardiac fibrosis.	(42)
Lu *et al*, 2017		5 mg/kg, 10 mg/kg, i.p.; 1, 10 μM.	ISO-induced cardiac fibrosis in rats; neonatal rat CFs, TRPM7 knockdown NIH-3T3 cells.	*In vivo* and *in vitro*	Suppressing TRPM7 expression to prevent hypoxia-induced MF.	(43)
Chai *et al*, 2024		5, 10, 50 μM.	LPS-induced cardiac hypertrophy and fibrosis in H9C2 cardiomyocytes.	*In vitro*	Suppressing CCL2 expression and downregulating NF-κB signaling pathway in a CCL2-dependent manner.	(47)
Jia *et al*, 2017		80 mg/kg, i.g.; 100 μM.	ISO-induced cardiac fibrosis in rats; primary CFs.	*In vivo* and *in vitro*	Inhibiting collagen synthesis and CF proliferation by blocking ROS-mediated CT-1 upregulation.	(50)
Dai *et al*, 2017		100 μM.	ISO-induced CF proliferation.	*In vitro*	Reducing ROS-mediated MAPK signaling activation, which in turn prevents ISO-induced CF proliferation and cardiac fibrosis.	(53)
Cong *et al*, 2024		80 mg/kg, i.p.	Ang II-induced atrial fibrosis and atrial fibrillation in mice.	*In vivo*	Atrial fibrillation and atrial fibrosis brought on by Ang II are prevented by upregulating the SIRT1/PGC-1α/FNDC5 pathway.	(55)
Wang *et al*, 2020		80 mg/kg, i.g.	STZ and HFD treatments-induced T2DM in rats.	*In vivo*	Enhancing myocardial lipid metabolism, which in turn reduces MF and inflammation in T2DM rats.	(57)
Zhang *et al*, 2022		40 mg/kg, i.g.; 100 μM.	Ligation of the LAD coronary artery-induced MI in mice; BMDMs.	*In vivo* and *in vitro*	MI-induced cardiac remodeling and MF are lessened by inhibiting the ROS/caspase-1/GSDMD signaling pathway.	(59)
Shi *et al*, 2024		100, 200 mg/kg, p.o.; 20, 40, 80 μM.	ISO-induced cardiac fibrosis in mice; H9C2 cells.	*In vivo* and *in vitro*	Suppressing oxidative stress and SASPs, modulating the p53 signaling pathway to reduce ISO-induced MF.	(62)
Chen *et al*, 2011		AS-IV-containing drinking water (300 mg/l); 20 μg/ml.	CVB3-induced DCM; primary cardiomyocytes and fibroblasts of neonatal rats.	*In vivo* and *in vitro*	Reducing MF in DCM brought on by CVB3 by inhibiting TGF-β1-Smad signaling.	(65)
Du *et al*, 2022		100 mg/kg, p.o.	ISO-induced cardiac fibrosis in mice.	*In vivo*	Elevating Akkermansia abundance, which influences phenylalanine metabolism to alleviate cardiac fibrosis.	(68)

Table I. Continued.

Authors, year	Fibrotic disease	Dosages	Models	*In vivo*/ *in vitro*	Effects and related mechanisms	(Refs.)
Guan *et al*, 2024	Pulmonary fibrosis	5 ml/kg, i.g.; 100 μg/ml.	BLM-induced PF in rats; A549 cells.	*In vivo* and *in vitro*	Regulating the lncRNA-ATB/miR-200c/ZEB1 signaling pathway to suppress the EMT process.	(70)
Li *et al*, 2021		20 mg/kg, i.p.	Silica-induced PF in rats.	*In vivo*	Inhibiting the TGF-β1/Smad signaling cascade, inflammation and oxidative stress to alleviate PF.	(77)
Li *et al*, 2019		20 mg/kg, i.p.; 7*et al*, 20, 60 μg/ml.	Silica-induced rat model; silicosis fibroblasts.	*In vivo* and *in vitro*	Demonstrating the effect of anti-silicosis fibrosis by preventing Smad3 from being continuously phosphorylated.	(78)
Hou *et al*, 2021		7, 20, 60 μg/ml.	TGF-β-treated A549 cells.	*In vitro*	Preventing EMT and fibrosis by inhibiting the expression of the NLRP3 protein.	(79)
Yu *et al*, 2016		10, 20, 50 mg/kg, i.p.	BLM-induced PF in rats.	*In vivo*	Alleviating PF by inhibiting BLM-induced inflammation and oxidative stress.	(80)
Li *et al*, 2023		5, 10, 20 mg/kg, i.g.	*K. pneumoniae*-treated rats.	*In vivo*	Alleviating the inflammation by inhibiting the TGF-β1/Smad pathway.	(82)
Qian *et al*, 2018		20 mg/kg, i.g.; 100 μg/ml.	BLM-induced PF in rats; A549 cells.	*In vivo* and *in vitro*	Preventing FOXO3a downregulation brought on by TGF-β1/PI3K/AKT to alleviate EMT in BLM-induced PF.	(84)
Zheng *et al*, 2024		AS_LIG@PPGC NPs (AS-IV: 25 mg/kg, LIG: 50 mg/kg), inhalation.	BLM-induced PF in mice; the murine fibroblast line L-929.	*In vivo* and *in vitro*	Demonstrating the antifibrotic properties by blocking the NOX4-ROS-p38 MAPK and NOX4-NLRP3 signaling pathways.	(85)
Xi *et al*, 2023		30 mg/kg, i.p.; 20 μM.	MCT-induced PAH in rats; PASMCs.	*In vivo* and *in vitro*	Modulating the PHD2/HIF1α signaling pathway to inhibit NLRP3-mediated pyroptosis and fibrosis in PASMCs.	(87)
Yuan *et al*, 2023		50, 100 mg/kg; 12.5, 25, 50 μM.	BLM-induced PF in mice; . A549 cells	*In vivo* and *in vitro*	Alleviating PF through the inhibition of cellular senescence and EMT.	(90)
Li *et al*, 2017		10 mg/kg, i.g.	BLM-induced PF in rats.	*In vivo*	Inhibiting the release of HMGB1 and the deposition of ECM to exert the antifibrotic property.	(92)
Zhu *et al*, 2024		20 mg/kg, i.p.; 25, 50, 75 μg/ml.	BLM-induced PF in rats; HFL1 cells.	*In vivo* and *in vitro*	Impeding PF process by regulating the circ_0008898/miR-211-5p/HMGB1 axis.	(98)
Tong *et al*, 2021		FA (24 mg/kg) + AS-IV (40.8 mg/kg).	BLM-induced PF in mice.	*In vivo*	Mitigating PF by lowering oxidative stress and blocking the TGF-β1/Smad3 signaling pathway through miR-29b regulation.	(101)
Li *et al*, 2024		40 mg/kg, i.g.; 0-50 μmol/l.	BLM-induced IPF in mice; A549 cells.	*In vivo* and *in vitro*	Suppressing IPF by triggering autophagy via the PTEN/PI3K/AKT/mTOR pathway mediated by miR-21.	(103)

Table I. Continued.

Authors, year	Fibrotic disease	Dosages	Models	*In vivo*/ *in vitro*	Effects and related mechanisms	(Refs.)
Tian *et al*, 2024		100 mg/kg, i.p.; 50 μM.	PM2.5-induced PF in rats; rat alveolar epithelial cells L2.	*In vivo* and *in vitro*	Inhibiting PM2.5-induced PF by regulating the miR-362-3p/RUNX1 pathway.	(108)
Zhang *et al*, 2023		2 mg/kg, i.p.; 10 μM.	LPS or/and CS to simulate PF in COPD of mice; BEAS-2B cells, N-HLF cells.	*In vivo* and *in vitro*	Demonstrating the effect of anti-PF in COPD by downregulating the RAS/RAF/FOXO signaling pathway.	(113)
Guo *et al*, 2024		Bu Yang Huan Wu Decoction: 0.99, 1.98, 3.97 mg/kg.	HFD, STZ and BLM treatments-induced diabetic PF model in rats.	*In vivo*	Targeting the AGE-RAGE signaling pathway to suppress IL-6-induced PF.	(115)
Li *et al*, 2023	Renal fibrosis	40, 80 mg/kg; 10, 50, 100, 150 μM.	Adenine-induced CKD model in rats; HK-2 cells.	*In vivo* and *in vitro*	Regulating AKT/mTOR-mediated autophagy by enhancing ALDH2 expression, which alleviates renal fibrosis.	(123)
Su *et al*, 2019		20, 40, 80 mg/kg/d, i.g.; 20, 40, 80 μM.	HFD and STZ treatments-induced DKD in rats; HMCs.	*In vivo* and *in vitro*	Inhibition of oxidative stress and fibrosis caused by PA via suppressing CD36 expression.	(125)
Du *et al*, 2018		G-Rg1: 50 mg/kg, i.g.; AS-IV: 16 mg/kg, i.g.	STZ-induced DKD in rats.	*In vivo*	Preventing DKD by lowering oxidative stress and blocking the TGF-β1/Smads signaling cascade.	(127)
Zhang *et al*, 2020		80 mg/kg, i.g.	HFD and STZ treatments-induced DKD in rats.	*In vivo*	The downregulation of NLR signaling by AS-IV may be linked to the inhibition of renal fibrosis and inflammation.	(128)
Chen *et al*, 2018		50, 100, 200 μg/ml.	HG-treated HK-2 cells.	*In vitro*	Mitigating renal tubular EMT by suppressing the mTORC1/p70S6K signaling pathway.	(131)
Wang *et al*, 2018		40 mg/kg, i.g.; 12.5, 25, 50 100 μM.	HFD-induced DKD model in KK-Ay mice; MCs, primary mouse podocytes.	*In vivo* and *in vitro*	Suppressing podocyte dedifferentiation and MC activation caused by miR-21 overexpression.	(135)
Wang *et al*, 2019		40 mg/kg, i.g.; 25, 50, 100 μmol/l.	HFD-induced DKD in KK-Ay mice; immortalized mouse podocyte cell line.	*In vivo* and *in vitro*	Attenuating the EMT of podocytes by modulating the SIRT1-NF-κB pathway and autophagy activation.	(137)
Wang *et al*, 2018		40 mg/kg, i.g.; 25, 50, 100 μM.	HFD-induced DKD in KK-Ay mice; mouse glomerular MC line.	*In vivo* and *in vitro*	Regulating the SIRT1-NF-κB pathway and promoting autophagy to inhibit renal fibrosis and MC activation.	(138)
Hu *et al*, 2022		10, 20, 40 mg/kg, i.g.; 40 μM.	T2DM model in mice; HK-2 cells.	*In vivo* and *in vitro*	Down-regulating the RAF/MEK/ERK pathway by suppressing CX3CL1 expression, which alleviates the EMT.	(141)
Zhou *et al*, 2017		20, 40 mg/kg, i.g.; 10, 20 μM.	UUO-induced renal fibrosis in mice; HK-2 cells.	*In vivo* and *in vitro*	Suppressing inflammation through the TLR4/NF-κB signaling pathway to alleviate renal fibrosis.	(143)

Table I. Continued.

Authors, year	Fibrotic disease	Dosages	Models	*In vivo*/ *in vitro*	Effects and related mechanisms	(Refs.)
Wang *et al*, 2014		3.3, 10, 33 mg/kg; 2, 5, 10, 20, 40 μmol/l.	UUO-induced renal fibrosis in rats; NRK-49F cells.	*In vivo* and *in vitro*	The suppression of fibrosis is linked to the activation of Smad7, which in turn inhibits TGF-β/Smad signaling.	(146)
Lian *et al*, 2021		10, 40, 80 μg/ml.	EMT model of NRK-52E cells induced by TGF-β1.	*In vitro*	Decreasing the phosphorylation of AKT and mTOR and increasing Cx43 expression in RTECs to inhibit EMT.	(147)
Yu *et al*, 2022		80 mg/kg, i.g.; 10, 20, 40 μM.	UIRI-induced renal fibrosis in rats; NRK-52E and NRK-49F cells.	*In vivo* and *in vitro*	Mitigating renal fibrosis through the regulation of the AKT1/GSK-3β pathway.	(150)
Wang *et al*, 2014		3.3, 10, 33 mg/kg, i.g.	UUO-induced renal interstitial fibrosis in rats.	*In vivo*	Lowering the expression of proteins involved in the Wnt/β-catenin signaling pathway to alleviate renal interstitial fibrosis.	(151)
Che *et al*, 2015		10, 50, 100 μg/ml.	TGF-β1-induced mouse renal fibroblasts.	*In vitro*	Regulating the MAPK and NF-κB signaling pathways to alleviate renal interstitial fibrosis.	(152)
Xu *et al*, 2014		20 mg/kg, i.p.; 50, 100, 200 μg/ml.	UUO-induced renal tubulointerstitial fibrosis in mice; HK-2 cells.	*In vivo* and *in vitro*	Alleviating renal fibrosis via blocking the phosphorylation of p38 and JNK MAPKs and apoptosis.	(155)
Meng *et al*, 2011		*AF*-L/H (10.8 + 4.8/20.4 + 12 mg/kg), i.g.; *AF* (24 + 43 μmol·l^{-1}).	UUO-induced renal tubulointerstitial fibrosis in rats; NRK-49F and HK-2 cells.	*In vivo* and *in vitro*	Suppressing the tubular EMT and fibroblast activation and promoting NO generation to mitigate fibrosis.	(156)
Zhang *et al*, 2024		40 mg/kg, i.g.; 15 μg/ml.	UUO-induced renal fibrosis in rats; NRK-52E cells.	*In vivo* and *in vitro*	Preventing pyroptosis and EMT by blocking the cAMP/PKA signaling pathway.	(160)
Cao *et al*, 2019		20 mg/kg, i.g.; 7.5, 15, 30 mg/ml.	UUO-induced renal fibrosis model in mice; HK-2 cells.	*In vivo* and *in vitro*	Suppressing renal fibrosis, at least partly through the inhibition of the TGF-β1/ZEB2 signaling.	(162)
Wang *et al*, 2024		40 mg/kg, i.g.; 10, 20, 40 μM.	DCC treatment-induced model of liver fibrosis to HCC in Nrf2$^{-/-}$ mice; HK-2 cells.	*In vivo* and *in vitro*	Inhibiting renal fibrosis from hepatocarcinogenesis via the regulation of the Nrf2/HO-1 and pSmad3C/3L signaling pathways.	(165)
Zhang *et al*, 2021	Liver fibrosis	20, 40, 80 mg/kg, i.g.; 5, 10, 20 μM.	DCC-induced liver fibrosis and liver cancer in mice; HSC-T6 and HepG2 cells.	*In vivo* and *in vitro*	Preventing primary liver cancer by inhibiting the development of fibrosis and modulating pSmad3C/3L and Nrf2/HO-1 pathways.	(167)
Boye *et al*, 2015		CASE (60, 120, 240 mg/kg), i.g.; 20, 40, 80 μg/ml.	DEN-induced HCC in rats; HSCs, HepG2 cells.	*In vivo* and *in vitro*	HCC and liver fibrosis are alleviated by suppressing PAI-1 expression and modulating the MAPK-regulated TGF-β/Smad signaling.	(181)

Table I. Continued.

Authors, year	Fibrotic disease	Dosages	Models	*In vivo*/ *in vitro*	Effects and related mechanisms	(Refs.)
Zhao *et al*, 2020		FA (10.8 mg/kg) and AS-IV (4.8 mg/kg); i.g.	BDL-induced liver fibrosis in rats.	*In vivo*	HSCs are deactivated by promoting the Nrf2 pathway and inhibiting the TGF-β pathway.	(184)
Dong *et al*, 2017		4 μM FA and/or 2 μM AS-IV.	HSC-T6 cells.	*In vitro*	Suppressing HSC activation by inhibiting p38 MAPK phosphorylation through Nrf2/ARE pathway activation and TGF-β1/Smad pathway blocking.	(185)
Li *et al*, 2013		3, 10, 30, 100 μM.	HSCs.	*In vitro*	Suppressing HSC activation by activating Nrf2 and downregulating p38 MAPK activity.	(187)
Liu *et al*, 2009		2.0, 4.0 mg/kg, i.g.; 0, 1.5, 3, 6, 12, 24 mg/l.	PS-induced liver fibrosis in rats; primary cultured HSCs.	*In vivo* and *in vitro*	Down-regulating TGF-β1 and suppressing the activation of HSCs to alleviate liver fibrosis.	(190)
Xie *et al*, 2023	Peritoneal fibrosis	20, 40 mg/kg, i.p.; 10, 20, 30 μg.	HG peritoneal fluid-induced peritoneal fibrosis in rats; PMCs of rats.	*In vivo* and *in vitro*	Regulating the signaling cascade linked to mitochondrial biosynthesis, ROS and apoptosis to suppress peritoneal fibrosis.	(191)
Shan *et al*, 2024		5 or 10 mg/kg, i.p.; 150 μM.	HG peritoneal fluid-induced peritoneal fibrosis in rats; RMacs, RPMCs.	*In vivo* and *in vitro*	Mitigating the fibrosis caused by macrophage-derived exosomes in PD via the miR-204-5p/Foxc1 pathway.	(192)
Qi *et al*, 2014	Systemic sclerosis-related fibrosis	2, 4 mg/kg, i.g.; 1, 3, 10, 30 μM.	BLM-induced skin fibrosis mice model; dermal fibroblasts from normal and SSc samples.	*In vivo* and *in vitro*	Mitigating fibrosis by suppressing the TGF-β-Smad3 axis in SSc.	(198)
Chen *et al*, 2012	Skin scarring and fibrosis	The concentration of 0.5%; 12.5-200 μmol/l.	The full-thickness skin excision wounds are made in rats; HSFs and HaCaT keratinocyte cell line.	*In vivo* and *in vitro*	Lowering the levels of collagen I/III and TGF-β1 secretion to inhibit the scar complications of wound healing.	(199)
Chen *et al*, 2013		0.5 mg/2 d; 50, 100 μmol/l.	The rat full-skin excision model, skin irritation test in New Zealand rabbits; HSFs, keratinocytes.	*In vivo* and *in vitro*	Promoting the proliferation and migration of keratinocytes and the drug uptake on fibroblasts.	(200)
Peng *et al*, 2012		0.5 mg AS-IV-containing sodium alginate-GT hydrogel.	The rat skin excision model.	*In vivo*	Regulating skin regeneration and serum TGF-β1 levels, improving collagen synthesis and skin tensile strength.	(201)
Shan *et al*, 2015		0.5 mg/2 d; the AS-IV-loaded SF/GT nanofibrous dressing (25/75).	The partial-thickness burn wound model in rats; keratinocytes, human fibroblast cells.	*In vivo* and *in vitro*	Inducing pro-angiogenesis and cell proliferation and preventing scar complications in wounds by improving collagen organization.	(202)

Table I. Continued.

Authors, year	Fibrotic disease	Dosages	Models	*In vivo*/ *in vitro*	Effects and related mechanisms	(Refs.)
Kasetti *et al*, 2021	Trabecular meshwork fibrosis	AS-IV topical ocular eye drops, 1 mM, twice a day; 50, 100 μM.	TGF-β2-induced ocular hypertension in mice; human primary TM cells.	*In vivo* and *in vitro*	Lowering IOP via suppressing the TGF-β2-induced fibrotic lesions and ER stress in TM cells.	(203)

A549, alveolar epithelial cell; *AF*, AS-IV + FA; AGE, advanced glycation end-product; AKT, protein kinase B; ALDH2, acetaldehyde dehydrogenase 2; Ang II, angiotensin II; ARE, antioxidant response element; AS-IV, astragaloside IV; BDL, bile duct ligation; BEAS-2B, human bronchial epithelial cell; BLM, bleomycin; BMDMs, bone marrow-derived macrophages; CA, cycloastragenol; cAMP, cyclic adenosine monophosphate; CASE, Compound *Astragalus* and *Salvia miltiorrhiza* extract; CCL2, C-C motif chemokine ligand 2; CD36, cluster of differentiation 36; CFs, cardiac fibroblasts; CKD, chronic kidney disease; Collagen I, type I collagen; COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; CT-1, cardiotrophin-1; CVB3, coxsackievirus B3; CX3CL1, C-X3-C motif ligand 1; Cx43, connexin43; DCC, diethylnitrosamine (DEN)/tetrachloromethane (CCl_4)/ethanol (C_2H_5OH); DCM, dilated cardiomyopathy; DKD, diabetic kidney disease; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FA, ferulic acid; FNDC5, fibronectin type III domain-containing protein 5; Foxc1, forkhead box C1; FOXO3a, forkhead box O3a; G-Rg1, ginsenoside Rg1; GSDMD, gasdermin D; GSK-3β, glycogen synthase kinase-3β; GT, gelatin; HCC, hepatocellular carcinoma; HepG2, human hepatocellular carcinoma cell; HFD, high-fat diet; HFL1, human fetal lung fibroblast 1; HG, high glucose; HIF1α, hypoxia-inducible factor 1α; HK-2, human proximal tubular epithelial cell; HMCs, human glomerular mesangial cells; HMGB1, high mobility group box-1; HO-1, heme oxygenase-1; HSC, hepatic stellate cell; HSFs, human skin fibroblasts; IL-6, interleukin-6; IOP, intraocular pressure; IPF, idiopathic pulmonary fibrosis; ISO, isoproterenol; JNK, isoproterenol; KK-Ay, KK.Cg-A^y/TaJcl; *K. pneumoniae*, *Klebsiella pneumoniae*; LAD, left anterior descending; LIG, ligustrazine; lncRNA-ATB, long non-coding RNA-activated by transforming growth factor β; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCs, mesangial cells; MCT, monocrotaline; MEK, mitogen-activated protein kinase kinase; MF, myocardial fibrosis; MI, myocardial infarction; miR, microRNA; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; NF-κB, nuclear factor-kappa B; N-HLF, normal human lung fibroblast cell; NIH-3T3, mouse embryonic fibroblast cell line; NLRP3, nucleotide-binding oligomerization domain-like (NOD-like) receptor (NLR) thermal protein domain associated protein 3; NO, nitric oxide; NOX4, NADPH oxidase 4; Nrf2, nuclear factor erythroid 2-related factor 2; NRK-49F, rat renal fibroblasts; NRK-52E, rat renal tubular epithelial cells; p70S6K, ribosomal protein S6 kinase β-1; PA, palmitic acid; PAH, pulmonary artery hypertension; PAI-1, plasminogen activator inhibitor-1; PASMCs, pulmonary artery smooth muscle cells; PD, peritoneal dialysis; PF, pulmonary fibrosis; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1 α; PHD2, prolyl-4-hydroxylase 2; PI3K, phosphatidylinositol-3-kinase; PKA, protein kinase A; PMCs, peritoneal mesothelial cells; PPGC NPs, polyethylene glycol-polylactic-co-glycolic acid nanoparticles; PS, porcine-serum; pSmad3C, COOH-terminal phosphorylation of Smad3; pSmad3L, phosphorylation of the linker region of Smad3; PTEN, phosphatase and tensin homolog; RAF, rapidly accelerated fibrosarcoma; RAGE, receptor for AGE; RAS, rat sarcoma; RMacs, primary rat peritoneal macrophages; ROS, reactive oxygen species; RPMCs, primary PMCs; RTECs, renal tubular epithelial cells; RUNX1, runt-related transcription factor 1; SASP, senescence-associated secretory phenotype; SF, silk fibroin; SIRT1, sirtuin 1; SSc, systemic sclerosis; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TGF-β, transforming growth factor-β; TLR4, toll-like receptor 4; TM, trabecular meshwork; TRPM7, transient receptor potential cation channel, subfamily M, member 7; UIRI, unilateral ischemia-reperfusion injury; UUO, unilateral ureteral obstruction; ZEB1, zinc finger E-box binding homeobox 1.

AS-IV has been confirmed to potentially induce the development of autoimmune diseases, virtually increasing the challenge of quantifying its toxicity (207). Furthermore, in rats, AS-IV may trigger an increase in nerve conduction velocity and mechanical withdrawal threshold (207). This concurrent neurotoxicity implies that the adverse reactions of AS-IV administration in human clinical practice may need to be further and extensively defined through numerous toxicological experiments. However, AS-IV administered at conventional biological doses is generally considered safe and has been widely developed as a bioactive component in various TCM preparations, such as Shen-Qi-Jiang-Tang and Zhen-Qi-Fu-Zheng granules and Qi-Shen-Yi-Qi pills (27).

Although AS-IV has strong lipophilicity (theoretically calculated lipid/water partition coefficient of 2.0), it has a high molecular weight, low hydrophilicity, low permeability on the intestinal mucosa and undergoes paracellular transport in the form of passive transport, resulting in low absorption fraction of AS-IV in various target organs or tissues of the body (208,209). Moreover, AS-IV has poor *in vivo* target specificity that limits its bioavailability. Nevertheless, evidence has shown that copper silicate nanoparticles modified with polyethylene glycol and loaded with AS-IV exhibit considerable clinical efficacy in improving osteoarthritis (210). Conductive bioadhesive hydrogels that enable the sustained release of AS-IV have advantages in clinical translation for mediating cardiac repair (211). Moreover, biomimetic nanodelivery systems loaded with AS-IV have application value in sepsis treatment (212). Meanwhile liposomes loaded with AS-IV are valuable in breast cancer immunotherapy (213). All these drug delivery systems provide sufficient reference evidence for optimizing the organ-targeting properties of AS-IV in antifibrotic therapy. Not only that, chitosan and sodium deoxycholate have the biological activity of promoting the opening of tight junctions between cells and are considered enhancers of the paracellular transport pathway. Therefore, the synergistic administration of chitosan and sodium deoxycholate can enhance the permeability coefficient of AS-IV through intestinal mucosa and enhance its pharmacodynamic absorption index (209). Notably, the lipid solubility and cell membrane permeability of CA are superior to those of AS-IV. Therefore, the application of biotransformation technology and *Bacillus* LG-502 to promote the conversion of AS-IV into CA, as well as the synergistic hydrolysis of AS-IV using heat- and sugar-resistant enzymes purified from *Dictyoglomus thermophiles* to improve the conversion rate of AS-IV, enable the convenient enhancement of AS-IV's antifibrotic therapeutic efficacy without relying on structural modification or the introduction of novel formulations (214).

Due to the diversity of modification sites and the uniqueness of the backbone structure, natural triterpenoids have become a research hotspot in numerous fields (21). Among them, AS-IV is also a tetracyclic triterpene saponin derived from natural herbs and the side chain of the A-ring at the C-2 and C-3 positions and the D-ring are the main modification sites. AS-IV mainly depends on the introduction of hydroxyl, ester and nitrogen-containing groups for modification (21). However, the high biological activity of AS-IV dictates that it has a diverse set of active binding sites, but this also indirectly leads to a severe test of how to invent the highly soluble derivatives of AS-IV by introducing hydrophilic groups or polar hydroxyl groups (215). Astragalosidic acid (LS-102) is a water-soluble derivative synthesized through AS-IV. Its relative bioavailability is twice that of the parent compound. Compared with AS-IV, the maximum plasma concentration of the derivative and the area under the plasma concentration-time curve are markedly increased and the permeability through the intestinal mucosa and intestinal absorption index are improved. In addition, the half-life of LS-102 is markedly shorter than that of AS-IV, which means that LS-102 is not prone to excessive accumulation of drug toxicity in the body and can be rapidly metabolized. This can be confirmed in the toxicity experiments of acute administration of high doses of LS-102, even when LS-102 is administered at a single-dose of up to 5,000 mg/kg body weight, no adverse reactions or even death are observed in mice (215). Therefore, given that the solubility of LS-102 has been markedly improved compared to the precursor compound AS-IV, it can be employed as a novel synthetic drug containing carboxylic acid to treat metabolic diseases such as obesity through oral administration (21,215). Besides the development of LS-102 to improve the oral bioavailability of AS-IV, AS-IV-loaded nanomicelles, as a product of novel formulation technology, act as a crucial reference in advancing the clinical translation of AS-IV (27). In short, chemical group or site modification, development of derivative compounds, combined drug administration and application of green nanomaterials are beneficial to improving the low hydrophilicity of AS-IV. In the future, attention should be paid to the improvement effect of these new improved strategies on the bioavailability of AS-IV (16). Finally, differences in the sources of *Astragalus* (in terms of species, purity and quality) and extraction technologies can lead to variations in the antifibrotic effect of AS-IV. This situation indicates that the selection of high-quality *Astragalus* is an important prerequisite for ensuring the clinical antifibrotic efficacy of AS-IV.

5. Conclusions and perspectives

The antifibrotic activity of AS-IV, a natural compound extracted from *Astragalus*, is affirmed. AS-IV can regulate the abundance of gut microbiota, senescence of cells and ion-channel currents of fibroblasts and inhibit oxidative stress, inflammation, pyroptosis and lipotoxicity, thereby alleviating collagen deposition in myocardial tissue. Moreover, AS-IV regulates the TGF-β1/Smads, PHD2/HIF1α and AGE-RAGE signaling axes and diverse noncoding RNAs and aging markers. It also maintains FOXO3a activity, inhibits autophagy impairment and ECM synthesis. It can be combined with nanotechnology for pulmonary inhalation therapy, thereby demonstrating good bioactivity in alleviating PF. AS-IV targets the expression of CD36, NOX4, TGF-β1 and MAPK and regulates the Wnt/β-catenin, mTORC1/p70S6K, pSmad3C/p21, pSmad3L/PAI-1 and PI3K/AKT/mTOR signaling pathways, thereby further inhibiting HMC fibrosis, renal fibrosis complicated by DKD or liver cancer, obstructive nephropathy-related fibrosis and the EMT of RTECs. AS-IV relies on its anticancer, antioxidative and immune-regulating activities to exert multitarget benefits in alleviating liver fibrosis, which is closely related to the activation of Nrf2,

inhibition of MAPK phosphorylation and TGF-β1 overexpression and regulation of pSmad3C/pSmad3L toward outputting tumor-suppressive signals. Furthermore, AS-IV can inhibit EMT and PMC apoptosis by targeting mitochondrial biosynthesis. It can also mitigate peritoneal fibrosis mediated by macrophage-derived exosomes carrying miRNAs. Finally, AS-IV has the effects of antiscarring, promoting skin wound repair and alleviating SSc and glaucoma. These effects are related to its ability to regulate collagen homeostasis and wound re-epithelialization and inhibit TGF-β signaling transduction and TM fibrosis.

Studies have mainly explored the regulatory effects of AS-IV on fibrosis from the perspectives of experimental animal models at the cellular, molecular and genetic levels, with most only focusing on single drug components and large-sample placebo-controlled, randomized and standardized double-blind experimental data regarding TCM treatments awaiting further investigation (10,14). Therefore, further combining numerous clinical trials to confirm through convincing population studies that AS-IV is applicable not only to antifibrotic treatment in basic experimental research is imperative. Moreover, prospective cohort studies involving the antifibrotic effects of AS-IV are lacking. This situation indicates that further long-term follow-up experiments are still needed to define the effective treatment period of AS-IV. Therefore, introducing drug development technologies, such as computer-aided drug design, high-throughput screening, systems modeling and simulation to obtain derivatives of AS-IV with increased bioavailability and conducting targeted validation or further adopting biocompatible drug carriers, such as nanoparticles, liposomes and micelles encapsulating AS-IV, may be required to achieve its efficient delivery. Although some studies have already employed high-sensitivity bioinformatics analysis techniques to verify the potential binding targets of AS-IV for antifibrosis effects, relying solely on acute or subacute toxicity tests on animal models cannot directly reflect the dose-time-pharmacological/toxicological effects of AS-IV in humans. This limitation may become a main obstacle to the conversion of AS-IV into a novel clinical drug. The application of reverse pharmacokinetics may be a key strategy to improve the conversion potential of AS-IV and research focusing on the fibrotic microtissue model also strives to simulate the fibrotic microenvironment in the human body. Such an approach weakens doubts about the antifibrotic feasibility of AS-IV because of the metabolic differences between humans and experimental animals. Notably, although natural products are mostly bioactive substances produced by plants to resist microbial and other exogenous interference, their extractable medicinal content is low. In addition, compared with chemical compounds, natural products have more diverse structures and complex biological activities. These characteristics increase the difficulty of their subsequent total or semisynthesis. Therefore, optimizing lead compounds on the basis of the structure-activity relationship analysis of AS-IV and the innovation of plant tissue culture techniques may help further screen small-molecule derivatives with enhanced pharmacological activity. Finally, multiple studies have shown that AS-IV has potential protective advantages against diverse fibrotic diseases and its targeted pathways involved have overlaps. These overlaps include regulating the phosphorylation of MAPK and signal transduction of p53, TGF-β/Smads, PI3K/AKT/mTOR and other pathways. However, current research has not verified whether AS-IV can simultaneously inhibit multiorgan fibrosis by acting on a specific signaling molecule or cascade. Therefore, this topic may be worth further in-depth research in the future to enhance the multitarget, multibenefit antifibrosis activity of AS-IV and its TCM compounds.

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Availability of data and materials

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Authors' contributions

MW was responsible for conceptualization, data curation, writing and preparation of original draft. KL was responsible for data curation, writing, reviewing, editing and validation. JW was responsible for writing, reviewing, editing and validation. QZ writing, reviewing, editing and data curation. XM was responsible for writing, reviewing, editing and investigation. WD was responsible for writing, reviewing, editing and validation. HG was responsible for writing, reviewing, editing and investigation. XD was responsible for writing, reviewing, editing and validation. WW: was responsible for supervision, project administration, writing, reviewing and editing. WX was responsible for funding acquisition, project administration, supervision, writing, reviewing and editing. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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