Abstract. Radix Astragali (RA) is widely used in traditional Chinese medicine (TCM), and astragaloside IV (AS-IV) is the most critical component of RA. Previous studies have demonstrated that AS-IV exerts effects on the myocardium, nervous system and endocrine system, among others. In the present review article, data from studies conducted over the past 20 years were collated, which have evaluated the effects of AS-IV on tumors. The mechanisms of action of AS-IV on malignant cells both in vivo and in vitro were summarized and it was demonstrated that AS-IV plays a vital role, particularly in inhibiting tumor growth and metastasis, promoting the apoptosis of tumor cells, enhancing immune function and preventing drug resistance. Moreover, AS-IV controls several epithelial-mesenchymal transformation (EMT)-related and autophagy-related pathways, such as the phosphoinositide-3-kinase (PI3K)/protein kinase B (AKT), Wnt/β-catenin, mitogen-activated protein kinase (MAPK)/extracellular regulated protein kinase (ERK) and transforming growth factor-β (TGF-β)/SMAD signaling pathways, which are commonly affected in the majority of tumors. The present review provides new perspectives on the functions of AS-IV and its role as an adjuvant treatment in cancer chemotherapy.

1. Introduction
Cancer is the second leading cause of mortality worldwide and results in an increasing number of deaths annually. The World Health Organization postulates a 60% increase in cancer cases over the next 20 years globally (1). The medical treatment of the majority of cancers almost always involves several traditional approaches, such as surgery, chemotherapy and radiotherapy. Surgical resection is a suitable approach for tumor management in the early stages of primary tumors. However, surgery is still limited by post-operative recurrence and metastasis (2,3). Of late, chemoradiotherapy, molecular targeted therapy and immune checkpoint inhibitors have been considered the treatment approach for advanced stages of cancers; however, severe adverse events limit their use (4,5). Therefore, alternative therapeutic methods are required to address these existing shortcomings. Accordingly, traditional Chinese medicines (TCMs), such as ginseng, Radix Astragali (RA), Scutellaria barbata, Curcumae and turmeric, are used to enhance the efficacy and reduce the side-effects of chemoradiotherapy. TCMs are effective in suppressing tumor progression, relieving surgery-associated discomfort, improving immune function and preventing complications caused by the use of other treatment modalities (6).

RA is a dietary complement widely used in TCM and is known to modulate the immune system and attenuate the adverse effects of cytotoxic agents (7). Saponins are the primary constituents that are responsible for the suppression of tumor growth, which exert their effects via intrinsic and extrinsic apoptotic pathways, modulating intracellular signaling pathways, and inhibiting metastasis and angiogenesis. Astragaloside IV (AS-IV; chemical structure presented in Fig. 1) and astragaloside II are the 2 main components of RA (8).

AS-IV, chemically known as 3-O-β-D-xylopyranosyl-6-O-β-D-glucopyranosyl-cycloastragenol (C_{14}H_{38}O_{14}), is a lanolin-alcohol type of tetracyclic triterpenoid saponin. It is included in the Chinese and European Pharmacopoeia as a quality-control indicator of RA. It has long been used since ancient times in China without any evident hepatotoxic and nephrotoxic effects. Moreover, no side-effects have been reported in rats following 14 weeks of the continuous oral administration of AS-IV (10 mg/kg/day) (9,10). However, there is currently no data available regarding the safety of AS-IV in
humans, at least to the best of our knowledge. The methods used to extract AS-IV include ultrafiltration, high-speed centrifugation, ultrasonic extraction and alcohol precipitation. The present review article aimed to obtain and collate data from studies conducted over the past 20 years on the effects of AS-IV on tumors. In addition, the mechanisms of action of AS-IV on malignant cells both in vivo and in vitro are summarized in order to provide insight into the effects of AS-IV on cancer in humans.

2. Literature search

Search strategy. Studies in English and Chinese, as well as trials published before June 1, 2020, were searched on online databases. The databases in the English language that were used were PubMed, MEDLINE, Embase, ScienceDirect, Web of Science, BIOSIS Previews and the Cochrane Library and Cochrane Central Register of Controlled Trials (CENTRAL). The Chinese databases used for the searches included the China National Knowledge Infrastructure (CNKI) database and Wanfang Med Online.

In the present review, ‘astragaloside IV’, ‘Cancer’ and ‘mechanism’ were used as the key search concepts. Additionally, their synonyms were also included. Moreover, manual searches were also carried out using the aforementioned terms. The search methodology is described as follows as an example: i) astragaloside IV; ii) astraloside; iii) ASIV; iv) i OR ii OR iii; v) cancer[MeSH]; vi) tumor[MeSH]; vii) v OR vi; viii) pathway[MeSH]; ix) mechanism[MeSH]; x) viii OR ix; and xi) iv AND vii AND ix.

Inclusion criteria. The inclusion criteria were as follows: Studies exploring the molecular mechanisms of AS-IV in cancer; studies with comparable experimental and control groups, and those that successfully established animal models of cancer; studies in which animal experiments were approved by an ethics committee; and studies that investigated related pathways involving upstream and downstream molecular mechanisms and published experimental findings, which could be retrieved.

Exclusion criteria. The exclusion criteria were as follows: Studies that included only AS-IV or astragalus polysaccharide (APS) as the experimental group; studies that had an obvious risk of bias, including selection bias, performance bias, detection bias, reporting bias and attrition bias; case studies, cross-over studies and studies without a separate control group; studies combining AS-IV with other TCM interventions, in which data specific to the effect of AS-IV interventions on cancer could not be extracted separately.

3. Effects of AS-IV in cancer models

AS-IV has been widely used in the management of cardiovascular, digestive, endocrine, and nerve-related diseases (11-13). Furthermore, it exerts significant anticancer effects when used alone or as an adjuvant to other treatment modalities, as it sensitizes the host to other drugs (Table I).

To the best of our knowledge, there are no systematic reviews available that discuss the role of AS-IV in cancer; therefore, in the present review article, the efficacy and mechanisms of action of AS-IV in cancer therapy are presented and discussed.

Induction of apoptosis. Apoptosis, also known as programmed cell death, includes the initiation stage, effect stage and degradation stage. Apoptosis is characterized by surface blebbing, chromatin condensation, fragmentation of chromosomal DNA and the appearance of apoptotic bodies. As shown in Table I and Fig. 2, AS-IV leads to apoptosis mainly by the mitochondria-dependent intrinsic pathway and the death receptor-dependent extrinsic pathway. The intrinsic pathway leads to the release of cytochrome c (Cyt C) from the mitochondria, which activates caspase-9, -3 and -7 (14). However, Bcl-2 can inhibit the release of Cyt C and avoid the intrinsic apoptosis induced by Bax (15). Research has indicated that AS-IV can enhance the Bax/Bcl-2 ratio to induce intrinsic apoptosis in a number of types of cancer, including colorectal cancer (CRC), breast cancer, lung cancer, vulvar squamous cell carcinoma (VSCC) and hepatocellular carcinoma (HCC) (16-21).

In terms of extrinsic apoptosis, certain receptors, e.g., the Fas ligands and tumor necrosis factor (TNF)-α, can set off the caspase-8-dependent extrinsic apoptotic pathways and become activated following the caspase cascade, which finally triggers apoptosis (22). It has been reported that combined treatment with AS-IV and cisplatin (10 μM) markedly promotes the cleavage of caspase-8 and -3, and poly(ADP-ribose) polymerase (PARP) in MG-63 and 143B cells via the Fas/FasL signaling pathway, which considerably sensitizes the osteosarcoma cells to the effects of cisplatin (23). In CRC, AS-IV alone can increase the release of Cyt C into the cytoplasm and upregulate the Bax/Bcl-2 ratio, as well as activate PARP and the caspase cascade (16).

The IAP protein family may be the most important apoptotic regulator involved in both intrinsic and extrinsic apoptosis pathways, including the x-linked mammalian inhibitor of apoptosis (XIAP), survivin and cellular inhibitor of apoptosis protein 1 (cIAP1) (22,24).

HCC is associated with a high morbidity and mortality rate globally, and presents with increased levels of anti-apoptotic proteins, including myeloid cell leukemia 1 (MCL1), cellular FLICE-like inhibitory protein (c-FLIP) and XIAP. c-FLIP can suppress death receptor-mediated apoptosis, which inhibits caspase-8 (25-28). Additionally, studies have demonstrated that MCL1 can block apoptosis induced by various apoptotic stimuli, including chemoradiotherapy (29-31). Its high protein expression levels in cancer cells are associated with drug resistance (32). AS-IV has been shown to significantly decrease XIAP, MCL1, c-FLIP and survivin expression in HCC and C6 glioma cells (33,34).

Inhibition of proliferation. High levels of reactive oxygen species (ROS) are considered to be a driver of a number of diseases, such as cancer and neurodegeneration. ROS are capable of increasing the carcinogenic potential of cancer cells and activating hypoxia-inducing factor (HIF) in hypoxic tumor cells to maintain cell viability (32,35). On the other hand, cells are capable of eliminating surplus ROS via mechanisms involving superoxide dismutase (SOD) and glutathione
peroxidase (GSH-Px) (36). Yang proved that AS-IV interrupted the proliferation of Spc-A-1 cells and suggested that the mechanism was related to the activity of the antioxidant enzymes, SOD and GSH-Px, which modulate ROS levels in cancer (37).

In the B7/cluster of differentiation (CD)28 superfamily, the overexpression of B7-H3 is observed in various types of cancer. It can downregulate the T-cell-mediated immune responses, leading to immune escape (38–40). AS-IV can reduce B7-H3 by upregulating miR-29c, which inhibits cell growth and reduces the protein level of the cell-cycle regulators, cyclin D1 and CDK4, in CRC cells. Thus, the anticancer effects of AS-IV may be mediated via the B7-H3/nuclear factor (NF)-xB/cyclin D1 axis (41). It also increases the cytotoxicity of cisplatin in non-small cell lung cancer (NSCLC) by suppressing the expression of B7-H3 (42). Moreover, another study reported that AS-IV inhibited the proliferation of HCC HepG2 cells and promoted apoptosis by regulating oxidative stress and the NF-κB signaling pathway (43).

**Inhibition of metastasis.** Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes containing active Zn²⁺. Its functions include, but are not limited to, degrading the extracellular matrix (ECM). The interaction between MMPs and cell-surface ECM receptors affects the function of integrins and contributes to cell invasion (44). MMP-2 and MMP-9, in particular, have been considered to play a vital role in tumor progression (45).

The extracellular signal-regulated kinase pathway (ERK), an important upstream switch, has been known to regulate the secretion of MMPs in cells (46). Mitogen-activated extracellular signal-regulated kinase (MAPK) is a serine/threonine (Ser/Thr) kinase involved in cell proliferation, differentiation, growth and apoptosis. In general, the MAPK/ERK pathway, i.e., Ras-Raf-MEK-ERK pathway, is deregulated in various types of cancers (47). Recently, inhibitors against the MAPK/ERK pathway have been designed to combat glioma and have been shown to be effective in the U251, as well as the SGC7901 cell lines with the downregulation of the expression of MMP-2 and MMP-9 (48–52). Li et al and Cao reported that AS-IV inhibited the progression of glioma and gastric cancer by interfering with the MAPK/ERK signaling pathway (53,54). Moreover, ascites in H22-tumor-bearing mice have been shown to be decreased by AS-IV by inhibiting the angiogenesis- and metastasis-associated genes, as well as the expression of aquaporins (AQPs) (55).

Tissue inhibitors of metalloproteinases (TIMPs) comprise TIMP-1, TIMP-2, TIMP-3 and TIMP-4, all of which can form complexes with several MMPs via covalent bonds, thereby inhibiting MMPs (56,57). The NM23 gene is a widely studied metastasis suppressor gene. The protein encoded by NM23 has the function of inhibiting tumor metastasis (58). AS-IV can down-regulate the mRNA and protein expression of MMP-2, -7 and -9, can mediate multidrug resistance/P-glycoproteins (MDR1/P-gp) and multidrug resistance-associated protein 1(MRP-1), and upregulate TIMP-1 and NM23 to inhibit the proliferation of BGC823 (gastric cancer) cells and reverse drug resistance (59).

Epithelial-mesenchymal transformation (EMT) is the conversion of a polarized epithelial cell, which interacts with the basement membrane by means of its basal surface, to a mesenchymal cell. As regards the metastatic process, EMT can be detected based on specific molecular changes, such as diminished E-cadherin and cytoskeleton levels, and elevated levels of N-cadherin and vimentin (60). As the transforming growth factor β1 (TGF-β1) is a known factor in triggering the initiation and execution of EMT, the downregulation of TGF-β1 signaling can prevent EMT in tumor cells. As shown in Fig. 3, AS-IV can affect EMT via several pathways.

The Wnt/β-catenin signaling pathway regulates EMT. Using U251 cells, Han et al found that AS-IV treatment inhibited TGF-β1-guided EMT by interrupting the Wnt/β-catenin pathway (61). β-catenin can also modulate glycogen synthase kinase 3β (GSK3β). AKT is an upstream molecule that activates GSK3β phosphorylation, eventually leading to the accumulation of β-catenin in the cell nucleus (62). AS-IV has also been shown to attenuate EMT in HCC and NSCLC via the modulation of the Akt/GSK-3β/β-catenin pathway (18,63).

Phosphoinositide-3-kinase/protein kinase B/nuclear factor xB (PI3K/Akt/NF-xB) is another common pathway suppressing TGF-β1-induced EMT. It has been reported that AS-IV can inhibit TGF-β1-induced EMT by interfering with the PI3K/Akt/NF-xB signaling pathway in SiHa and MGC-803 cells (21,64). Moreover, it inhibits the phosphorylation of MAPK and mTOR to varying degrees, which is related to the proliferation of cancer cells.

Apart from the signaling pathways discussed, AS-IV may also interrupt the migration and invasion of A549 cells. This process is associated with the suppression of PKC-α-ERK1/2-NF-xB and can be detected based on specific proteins, e.g., E-cadherin, integrin β1 and MMPs (65). PKC-α expression can be affected by ROS, which can induce the downstream signaling of ERK1/2 and activate NF-κB to initiate the metastasis of carcinoma cells (66).

In parallel, several miRNAs take part in the inhibition of EMT signaling (67). For example, miR-134 from the miRNA gene family has been proven to inhibit EMT (68,69). CREB1 is an important transcription enhancer. A previous study reported that miR-134 activated by AS-IV markedly inhibiting EMT signaling and increasing the chemosensitivity of SW-480 cells to oxaliplatin by inhibiting CREB1 expression (70).
| Cancer type               | Observation | Cell type          | Effects                                                                 | Mechanism of action                                                                 | (Refs.) |
|--------------------------|-------------|--------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------|
| Colorectal cancer        | *In vitro* (10, 20, 40 µg/ml); *in vivo* BA/BI c mice (20 mg/kg) | HT29, SW480         | Inhibit proliferation, induce cell cycle G1 arrest, induce apoptosis    | p21↑, Bax/Be-2↑, cleavage of PARP↑, caspase-3/9↑                                  | (16)    |
| Breast cancer            | *In vitro* (10, 20, 40 µg/ml); *in vivo* BA/BI c nude mice (20 mg/kg) | MDA-MB-231          | Inhibit proliferation                                                  | pERK1/2↓, pJNK↓, MPP-2/9↓, Vav3↓, Rac1/MAPK pathway↓                                  | (75)    |
| Lung cancer              | *In vitro* (≥20 µg/ml) | A549               | Inhibit viability, invasion and migration                              | MMP-2↓, MMP-9↓, Integrin β1↓, E-cadherin↑, TGF-β1↑, TNF-α↓, IL-6↓, PKC-α-ERK1/2-2NF-κB↓ | (65)    |
| Lung cancer              | *In vitro* (40 M-100 µM) | A549, H1299        | Inhibit invasion, migration, angiogenesis                             | AMPKα↓, blocking the M2 polarization of macrophages through AMPK signaling pathway | (99)    |
| Cervical cancer          | *In vitro* (5, 10, 25 µM); *in vivo* BA/BI c nude mice (25 mg/kg/day) | HeLa, SiHa          | Inhibit tumor growth, inhibit invasion, induce autophagy               | LC3/I↑, DCP1A↑, TMSB4X↑, MGST3↓, ATRK12↓, ERL1N1↓, Atg7↑, Atg12↑, miR-214↑, miR-301a↓, SOX2↑, NANO↑, M-CSF↓, TIMP2↑ | (105)   |
| Gastric cancer           | *In vitro* (≥10 µmol/l) | BGC-823            | Inhibit cancer-associated fibroblasts, regulate tumor microenvironment, inhibit proliferation, migration and invasion-promoting capacities of GCAFs | JNK/c-Jun/AP-1↓, p-JNK↓, p-c-Jun↓ | (113)   |
| Non-small cell lung cancer | *In vitro* (12, 24 ng/ml) | HCC827, A549, NCI-H1299 | Inhibit migration and proliferation, induce apoptosis | Bax↑, Bcl-2↓, caspase-3↑, Akt/GSK3β/b-catenin↓ | (18)    |
| Gastric cancer           | *In vitro* (10 ng/ml, 20 ng/ml) | BGC-823, MKN-74     | Inhibit cell viability, invasion and migration                         | Inhibit TGF-β1-induced EMT through inhibition of PI3K/Akt/NF-κB pathway VEGF↑, NF-kBp65↑, MMP-2↑ | (66)    |
| Lung cancer              | *In vitro* (10, 20, 50 ng/ml) | A549               | Inhibit cell growth                                                   | JNK/c-Jun/AP-1↓, p-JNK↓, p-c-Jun↓ | (82)    |
| Liver cancer             | *In vitro* (0.1 mM) | 5-FU-resistant Bel-7402/FU human hepatic cancer cells | Reverse drug resistance of Bel-7402/FU cells | P-gp↓, MDR1↓ | (83)    |
| Liver cancer             | *In vitro* (0.08 mg/ml) | 5-FU-resistant Bel-7402/FU human hepatic cancer cells | Reverse drug resistance of Bel-7402/FU cells to 5-FU, enhance intracellular accumulation of 5-FU suppression of EMT by regulation of the Akt/GSK-3β/b-catenin pathway, E-cadherin↑, N-cadherin↓, Vimentin↑, α-SMA↓, Slug↓ | SMC7721, Huh-7 | (63)    |
| Liver cancer             | *In vitro* (100 µg/ml) | Huh7, MHCC97-H     | Suppress migration and invasion                                       | LncRNA-ATB↓, IL-11/STAT3 pathway↓ | (74)    |
| Liver cancer             | *In vitro* (10, 20, 40, 80, 160 µg/ml) | SMMC-7721, Huh-7   | Inhibit migration and cell viability, induce apoptosis               | p53↑, P21↑, Cyclin D1↑, Bax↑, cleaved caspase-3↑, Bcl-2↑, Bcl-xL↑, Beclin-1↑, LC3-B↑, P62↑, reverse dysregulation of TGF-β/Smad signaling by TGF-βRII↑ and Smad4↑ | (19)    |
| Vulvar squamous cell carcinoma | *In vitro* (100, 200, 400, 600 and 800 µg/ml) | SW962              | Inhibit cell proliferation, induce apoptosis and autophagy, induce cell-cycle arresting in G0/G1 phase | |
Table I. Continued.

| Cancer type               | Observation | Cell type                  | Effects                                                                 | Mechanism of action                                                                 | (Refs.) |
|---------------------------|-------------|----------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------|
| Osteosarcoma              | In vitro (20 mg/kg) In vivo (40 µM) BALB/c nude mice | MG-63, 143B | Inhibit cell survival, increase chemo sensitivity, enhance cisplatin-induced apoptosis | Cleaved caspase-8↑, cleaved caspase-3↑, cleaved PARP↑, GAPDH↑, regulate Fas/Fasl signaling       | (23)    |
| Glioma                    | In vitro (100 µg/ml); in vivo (20 mg/kg) athymic BALB/c mice | U251 | Inhibit proliferation in vitro and attenuate tumor growth in vivo, suppress migration and invasion | PCNA↓, ki67↓, MMP-2↓, MMP-9↓, VEGF↓, inactivation of MAPK/ERK signaling pathway        | (53)    |
| Liver cancer              | In vitro (150 µg/ml) | HepG2, T47D, MB-AMD-231, PC-3, 293T | Attenuate the clonogenic survival and anchorage-independent growth of cancer cells, inhibit the colony formation | Vav3.1↓, alteration of proteins like BIP/GRP78, HSP70-2, HSPA1A, HSPA8            | (76)    |
| Liver cancer              | In vitro (200, 400 µM) | SK-Hep1, Hep3B | Induced cytotoxicity, inhibit proliferation, suppress invasion, trigger G1 arrest | Caspase-3/8/9↑, XIAP↓, MCL1↓, CFLIP↓, Survivin↓                                       | (33)    |
| Cervical cancer           | In vitro (50, 200, 800 µg/ml); in vivo BABLc/nude mice (120 mg/kg) | SiHa | Inhibit invasion and migration in vitro and in vivo, inhibit EMT | p-p38↓, p-MAPK↓, p-PI3K↓, p-AKT↓, p-mTOR↓, TGF-β1↓, N-cadherin↓, Vimentin↓, E-cadherin↓ | (64)    |
| Colorectal cancer         | In vitro (≥50 ng/ml) | SW620, HCT | Reduce cell proliferation, arrest cell cycle in G0/G1 phase | B7-H3↓, miR-29c↑, cyclin D1↓, CDK4↓                                                | (41)    |
| Lung cancer               | In vitro (80, 160 µg/ml); in vivo C57Bl/6 mice with 3LL-luc-EGFP, 3LL-luc-IDO (40 mg/kg) | Lewis lung carcinoma cell | Inhibit tumor progression and prolong survival time | Enhance immune response by inhibiting the Treg frequency and induce the activity of CTLs, blocked IDO induction in vitro and in vivo. | (98)    |
| Glioma                    | In vitro (20, 40, 80 µg/ml) | U251 | Inhibit migration and invasion, promote apoptosis, inhibit proliferation | Interfered with the TGF-β1-induced Wnt/β-catenin signaling pathway to inhibit EMT | (61)    |
| Liver cancer              | In vitro (≥20 µg/ml) | SMMC-7721, Huh7 | Increase apoptosis | miR-150–5p↑, β-catenin↓, Bax↓, Bcl-2↑                                           | (20)    |
| Colorectal cancer         | In vitro (5, 10, 20 µg/ml) | SW-480 | Inhibit migration and invasion, increase chemo sensitivity | CREB1↓, miR-134↑, EMT↑                                                        | (70)    |
| Macrophages and Lewis lung carcinoma | In vitro (100 µg/ml) | RAW264.7 | Enhance immune function, induce G2/M phase arrest | NO↑, IL-4↑, IL-6↑, CD40↑, CD86↑, IL-1β↑, TNF-α↑, iNOS↑, cyclin D1↑, CDK4↑, CDK6↑, p50↑, p-p65↑, p50/β-actin↑, p-p65/p65↑, p-p38↑, p-ERK↑, p-JNK↑, p38↓, ERK↑, JNK↓, NF-κB/MAPK signaling pathway↑ | (102)   |
| Breast cancer             | In vivo (12 g/kg) 7, 12-dimethylbenzanthracene-induced SD rats | 7, 12-dimethylbenzanthracene-induced liver cancer | Inhibit tumor progression | IL-2↑, IFN-γ↑, CD3↑, CD4↑, CD4↑, CD8↑, IL-1↑, IL-6↑, TNF-α↑, CD8↓ | (101)   |
| Cancer type | Cell type | Observation | Mechanism of action | Refs. |
|-------------|-----------|-------------|---------------------|-------|
| Gastric cancer | C6 | In vitro | Induce apoptosis, trigger G1 arrest | AKT/NF-κB, BAX, BAX, Bel-2 | (120) |
| | BGC823 | In vitro | Increase chemosensitivity | MMP-2, MMP-7, MEF-9, TIMP-1, FAS, FAS-α | (149) |
| | SGC7901 | In vitro | Inhibit tumor growth and metastasis | COX-2, VEGF-1, PGE2 | (70) |
| | G875 | In vitro | Inhibit tumor growth | MMP-2, MMP-9, p-ERK-1 | (54) |
| | BN75 | In vitro | Inhibit tumor growth | CD44, CD88, IFN-γ, IL-4 | (100) |
| | SP-A1 | In vitro | Inhibit proliferation | SOD1, GSH-Px1, Bcl-2, Bax | (37) |
| | HepG2 cell | In vitro | Promote apoptosis, inhibit proliferation | ROS/NF-κB pathway, Ki67, Bel-2, NF-κB, IKK-α, IKK-β, ROS, Caspase-3, Bax | (87) |
| | | In vivo | Reverse MRP2 overexpression after Cis treatment | Bcl-2, BaX, Bcl-2/Bax | (114) |
| | | In vivo | Inhibit tumor growth | c45, c45-cd85 | (102) |
| | | | Inhibit invasion | MMP-2, MMP-9 | (13) |
| | | | Increase chemosensitivity | MRP2 overexpression after cis | (114) |
| | | | Inhibit tumor growth | ROS/NF-κB pathway | (43) |
| | | | Inhibit tumor growth | MMP-2, MMP-9, PGE2 | (54) |
| | | | Increase chemosensitivity | MMP-2, MMP-9, p-ERK-1 | (54) |
| | | | Inhibit tumor growth | CD44, CD88, IFN-γ, IL-4 | (100) |
| | | | Inhibit proliferation | SOD1, GSH-Px1, Bcl-2, Bax | (37) |
| | | | Promote apoptosis, inhibit proliferation | ROS/NF-κB pathway, Ki67, Bel-2, NF-κB, IKK-α, IKK-β, ROS, Caspase-3, Bax | (87) |
| | | | Reverse MRP2 overexpression after Cis treatment | Bcl-2, BaX, Bcl-2/Bax | (114) |
| | | | Increase chemosensitivity with high safety, induce G2/M cell cycle arrest | p-p38, activate eNOS/NO/3NT signaling by inhibiting CAV-1 | (17) |
| | | | Reverse MPP2 overexpression after Cis | B7-H1 | (13) |
| | | | Inhibit tumor growth | c45, c45-cd85 | (102) |
| | | | Inhibit invasion | MMP-2, MMP-9 | (13) |
| | | | Increase chemosensitivity | MRP2 overexpression after cis | (114) |
| | | | Promote apoptosis, inhibit proliferation | ROS/NF-κB pathway, Ki67, Bel-2, NF-κB, IKK-α, IKK-β, ROS, Caspase-3, Bax | (87) |
| | | | Reverse MRP2 overexpression after Cis treatment | Bcl-2, BaX, Bcl-2/Bax | (114) |
| | | | Increase chemosensitivity | MMP-2, MMP-9, p-ERK-1 | (54) |
| | | | Inhibit tumor growth | CD44, CD88, IFN-γ, IL-4 | (100) |
| | | | Inhibit proliferation | SOD1, GSH-Px1, Bcl-2, Bax | (37) |
| | | | Promote apoptosis, inhibit proliferation | ROS/NF-κB pathway, Ki67, Bel-2, NF-κB, IKK-α, IKK-β, ROS, Caspase-3, Bax | (87) |
| | | | Reverse MRP2 overexpression after Cis treatment | Bcl-2, BaX, Bcl-2/Bax | (114) |
| | | | Increase chemosensitivity with high safety, induce G2/M cell cycle arrest | p-p38, activate eNOS/NO/3NT signaling by inhibiting CAV-1 | (17) |
| | | | Reverse MPP2 overexpression after Cis | B7-H1 | (13) |
| | | | Inhibit tumor growth | c45, c45-cd85 | (102) |
| | | | Inhibit invasion | MMP-2, MMP-9 | (13) |
| | | | Increase chemosensitivity | MRP2 overexpression after cis | (114) |
| | | | Promote apoptosis, inhibit proliferation | ROS/NF-κB pathway, Ki67, Bel-2, NF-κB, IKK-α, IKK-β, ROS, Caspase-3, Bax | (87) |
| | | | Reverse MRP2 overexpression after Cis treatment | Bcl-2, BaX, Bcl-2/Bax | (114) |
| | | | Increase chemosensitivity with high safety, induce G2/M cell cycle arrest | p-p38, activate eNOS/NO/3NT signaling by inhibiting CAV-1 | (17) |
| | | | Reverse MPP2 overexpression after Cis | B7-H1 | (13) |
| | | | Inhibit tumor growth | c45, c45-cd85 | (102) |
| | | | Inhibit invasion | MMP-2, MMP-9 | (13) |
| | | | Increase chemosensitivity | MRP2 overexpression after cis | (114) |
| | | | Promote apoptosis, inhibit proliferation | ROS/NF-κB pathway, Ki67, Bel-2, NF-κB, IKK-α, IKK-β, ROS, Caspase-3, Bax | (87) |
| | | | Reverse MRP2 overexpression after Cis treatment | Bcl-2, BaX, Bcl-2/Bax | (114) |
The table below continues to discuss the effects of doxorubicin on cancer type, observation cell type, and effects on mechanisms of action:

| Cancer type | Cell type | Observation | Effects | Mechanism of action (Refs.) |
|-------------|-----------|-------------|---------|------------------------------|
| doxorubicin | In vivo | mg/kg) c57Bl/6 mice; Neonatal cardiomyocytes | Alleviate body weight loss, myocardial injury, apoptosis of cardiomyocytes, cardiac fibrosis and cardiac dysfunction | PARP, poly AdP‑ribose polymerase; ERK, extracellular regulated protein kinases; TGF‑β1, transforming growth factor‑β1; MMP, matrix metalloproteinases; AQP1, aquaporin 1; VEGF, vascular endothelial growth factor; AMPK, AMP‑activated protein kinase; EMT, epithelial‑mesenchymal transition; PI3K, phosphoinositide‑3‑kinase; JNK, c‑Jun N‑terminal kinase; NF‑κB, nuclear factor‑κB; PCNA, proliferating cell nuclear antigen; cOX‑2, cyclooxygenase‑2; HIF‑1α, hypoxia‑inducible factor‑1α; ROS, reactive oxygen species; α STAT3, signal transducer and activator of transcription 3; MNNG, N‑methyl‑N'‑nitro‑N‑nitrosoguanidine; TIGAR, TP53‑induced glycolysis and apoptosis regulator; PLGc, precancerous lesions |}

Inhibition of angiogenesis. Neovascularization relies on the secretion of vascular endothelial growth factor (VEGF) by tumor cells and the proliferation of endothelial cells (77). VEGF serves as a signal for cyclooxygenase‑2 (COX‑2)‑prostaglandin E2 (PGE2). PGE2 is involved in the major process of COX‑2 acting on malignant cells (78). AS‑IV inhibits the growth of SGC7901 cells with the downregulation of COX‑2, which leads to the suppression of its downstream product, PG2 expression, and the downregulation of VEGF, thereby decreasing tumor growth (79).<ref>

MDr and increase in chemosensitivity. MDr is the leading cause of the failure of chemotherapy and cancer renascence. The key to reversing tumor drug resistance is to prevent MDr pathways to reduce drug efflux, which can enhance the chemosensitivity of tumor cells (80). It has been found that MDr can be attributed to several factors, including P‑gpgs, lung resistance‑related proteins (LRPs), breast cancer resistance protein (BCRP) and multidrug resistance‑associated protein 2 (MRP2), all of which can pump drugs out from tumor cells and reduce the anticancer efficacy of drugs (81). Several studies have reported that AS‑IV can reverse MDr and increase the chemosensitivity or radiosensitivity of tumors (17,82‑87)

Caveolin‑1 (CAV‑1) is a constituent protein playing a role in signal transduction and other cellular activities. It has been confirmed that the expression of CAV‑1 is positively associated with cancer metastasis and has, therefore, been identified as a potential target to reverse MDr (88). Zheng et al reported that AS‑IV reduced CAV‑1 expression and reversed the Taxol‑induced increase in CAV‑1 expression; furthermore,
AS-IV administration resulted in initiating the endothelial nitric oxide synthase (eNOS)/nitric oxide (NO)/peroxynitrite (ONOO⁻) pathway and inhibiting CAV-1, which can induce severe oxidative stress and apoptosis (17).

Moreover, the MAPK pathway, which comprises the ERK, JNK and p38 pathways, controls several biological and cellular processes in cancer. Therefore, its activation is vital to MDR (89). Co-treatment with AS-IV and Taxol lowers ERK and JNK in malignant cells, which are associated with chemosensitizing effects (17).

Studies have found that inhibiting the JNK signaling pathway suppresses the expression of c-Jun and drug-resistant genes, e.g., MDR1 and P-gp, and increases drug-induced the apoptosis of tumor cells. Wang et al demonstrated that AS-IV
may reverse MDR by inhibiting the JNK/c-Jun/AP-1 pathway in Bel-7402/FU cells (82,83).

Silent information regulator 6 (SIRT6), an NAD+‑dependent deacetylase, plays a key regulatory role in genomic stability, metabolism, chromatin regulation, telomere integrity, gene transcription and glucose and lipid metabolism. Further exploration of these molecular mechanisms has indicated that the multiple roles of SIRT6 in tumorigenesis are realized by regulating the ERK, SMAD and Raf pathways (84).

SIRT6 also triggers lethal autophagy in human cancer cells (90). Recent studies have reported that the upregulation of SIRT6 enhances the sensitivity of NSCLC cells to other drugs and treatment modalities (91‑93). Accordingly, the study by Dai et al illustrated that AS‑IV acted on SIRT6 to heighten the tumor responses to gefitinib in the NCI‑H1299, HCC827 and A549 lung cancer cell lines (85).

Studies have indicated that NOTCH3 is highly expressed in tumor cells. It also has been shown that the depletion of NOTCH3 by sorafenib and adriamycin can increase the expression of p53, promote GSK3β phosphorylation and downregulate p21, thereby enhancing the efficacy of chemotherapy (94,95). In addition, NOTCH3 may be used as a biomarker for RC. In a previous in vitro study, AS‑IV was reported to enhance the chemosensitivity of CRC towards cisplatin by suppressing NOTCH3 (86).

The glucosylceramide synthase (GCS)‑mediated abolishment of ceramide‑induced apoptosis is one of the underlying mechanisms of acquired drug resistance in some resistant cells (96). AS‑IV can reverse drug resistance to doxorubicin in HepG2/GCS cells, suggesting that MDR can be prevented using AS‑IV as it reduces the expression of GCS (87).

Improvement of immunity. Owing to their high cytotoxicity and proliferation ability, cytotoxic T lymphocytes (CTLs) are useful in the monitoring and elimination of cancer cells. During tumor progression, the tumor microenvironment (TME) results in the suppression of immune function, which results in a loss of the functions of CTL, leading to immune escape.

Tumor‑associated macrophages (TAMs) constitute the most important inflammatory cell group in the TME. Recent studies have revealed that TAM may polarize to the M2‑type in terms of phenotypic characteristics. Macrophage colony‑stimulating factor‑1 (CSF‑1), interleukin (IL)‑4, IL‑10, TGF‑β and IL‑13 benefit M2 subgroup differentiation. Moreover, M2 and Tregs can reduce the levels of CTLs. Type 2 (M2) macrophages do not exert antitumor effects, but rather participate in the occurrence, development, invasion and metastasis of tumors; therefore, the phenotype M2 is a novel potential target for tumor therapy (97).

There are multiple mechanisms by virtue of which tumor cells escape recognition by CTLs. Indoleamine‑2,3‑dioxygenase (IDO) is a tryptophan‑degrading enzyme that participates in the immune‑escape program. In C57BL/6 mice bearing Lewis lung carcinoma cells, AS‑IV was shown to exert antineoplastic and immunity‑boosting effects to inhibit Tregs and augment CTL activity by suppressing IDO expression (98). AS‑IV has also been shown to partially block M2 differentiation via the AMPK signaling pathway, thereby inhibiting invasion, migration and angiogenesis (99).

In 7,12‑dimethylbenzanthracene‑induced liver and breast cancer in tumor‑bearing mice, the effect of co‑treatment of cisplatin and AS‑IV against breast cancer in vivo was more...
prominent than that of cisplatin alone. The mechanism of action may be related to the effective upregulation of the levels of immune factors IL-2, IFN-γ, CD3+, CD4+, CD4+/CD8+, and the downregulation of IL-1, IL-6, TNF-α and CD8+ in liver and breast cancer (100,101).

Moreover, in vivo experiments have demonstrated that AS-IV promotes host immunity by regulating the levels of cytokines, NO and cycle-related mRNA and/or protein expression, particularly IL-1β, IL-6 and TNF-α, under the influence of the NF-κB/MAPK pathway. As an inhibitor of proliferation, AS-IV also modulates the levels of cyclin D1, CDK4 and CDK6 in the host, promotes the secretion of CDS, such as CD40 and CD86, and arrests cells in the G2/M stage (102).

Promotion of autophagy. Autophagy is a process in which proteins or organelles are engulfed into vesicles and fused with lysosomes to form an autophagosome. Subsequently, the enclosed contents are degraded, thus achieving the metabolic needs of cells and the renewal of some organelles (103). Autophagy has a dual-directional effect on the progression and survival of malignant tumors. This progression could be measured based on the distribution of LC3-I and LC3-II, which are biomarkers indicating autophagy vesicle accumulation (104).

AS-IV elevates the level of autophagy-associated proteins, such as LC3III/I, Atg7 and Atg12 in cervical cancer cells. It also mediates differentially expressed proteins, including MGST3, AKR1C2, and ERL1N1, which are related to cancer proliferation and cytoskeleton composition. Two autophagy-related proteins, namely, DCP1A and TMSB4X, have been found to be increased in HeLa and SiHa cells following the administration of AS-IV (105).

The TGF-β/SMAD signaling pathway plays a crucial role in a number of types of cancer and the dysfunction of this pathway is an important pathogenic mechanism in cancers. SMAD and downstream TGF-β intracellular signaling transfer the ligand-receptor interaction signal from the cytoplasm to the nucleus. In a previous study, in VSCC cells, AS-IV was shown to improve the dysfunctions of the TGF-β/SMAD pathway, determined based on the elevated TGF-βRII and Smad4 levels; it was also found that AS-IV induced autophagy in SW962 cells, and markedly increased Beclin-1 and LC3-II levels, and decreased p62 protein levels (19).

Prevention of cancer. Aerobic glycolysis and oxidative phosphorylation are common energy sources in tumor cells. Owing to the rapid growth and high energy demand of tumor cells, there is a tendency for an increased glucose uptake and lactate production. Monocarboxylic acid transporters (MCT)1 and MCT4 can transport large amounts of lactic acid produced by tumor cells to the extracellular environment and play a key role in maintaining the acidic environment required for the glycolysis in tumor cells (106). CD147 is indispensable to the activity of MCT1 and MCT4 in gastric cancer. The study by Zhang et al suggested that AS-IV reduced the precancerous lesions of gastric carcinoma (PLGC), inhibited glycolysis by regulating the p53/miRNA-34a/LDHA and p53/TIGAR pathways, and restored the levels of MCT1/4, CD147 and HIF-1α (107).

AS-IV inhibits the activity of gastric cancer-associated fibroblasts (GCAFs) with an increased miR-214 and decreased miR-301a expression. AS-IV also inhibits GCAFs from increasing key factors, such as SRY-box2 (SOX2) and NANOG, in inducing pluripotency in somatic cells, decreasing M-CSF expression and increasing TIMP2 expression (108). All these studies demonstrate that AS-IV hinders the development of gastric cancer. This topic is worthy of further exploration in a clinical setting.

Remission of side-effects from chemotherapy. NADPH oxidase (NOX) is a plasma membrane-related enzyme protein family consisting of 7 members of DUOX1-2 and NOX1-5 families. Among the NOXs, NOX2 and NOX4 are expressed in the heart and are responsible for increasing intracellular ROS levels. Oxidative stress has been identified as a main cause of doxorubicin (DOX)-induced cardiomyopathy (109,110). DOX administration has been shown to increase the levels of NOX2 and NOX4 in animal hearts, thereby increasing ROS-induced cardiomyopathy. By contrast, AS-IV noticeably reduces the cardiomyopathy induced by DOX, decreases the oxidative stress caused by NOX2 and NOX4, attenuates the complications of doxorubicin, and, thus, appears suitable as an adjuvant to chemotherapy (111).

4. Conclusions and future perspectives

TCMs are commonly used in clinical treatment in several Asian countries. They significantly contribute towards enhancing the effects of other therapies and reducing toxicity. The in vitro and in vivo effects of AS-IV in inhibiting tumor proliferation and invasion and in promoting tumor cell apoptosis have been well-documented. Current findings highlight the role of AS-IV in suppressing EMT, as EMT plays a role in the majority of processes related to AS-IV in cancer. Furthermore, AS-IV has also been proven to exert significant preventive effects against MDR and in the regulation of immunity in antitumor therapy. In addition, the low-cost and ready availability of AS-IV further accentuates its potential in tumor therapy.

Despite these advantages, the use of AS-IV is still limited by several means: i) Its mechanisms of action have not been adequately elucidated. A previous study demonstrated that AS-IV enhanced the efflux activity of P-gp and BCRP through the Nrfr2-ARE signaling pathway, exerting the opposite effect on P-gp protein in liver cancer and gastric cancer cells, which may lead to herb-drug interactions following treatment with AS-IV (112); ii) there are no clinical studies (to the best of our knowledge) available that explore the role and safety of AS-IV in human cancers. The human body is complex compared to model organisms (in vivo or in vitro) used in a laboratory setting; iii) finally, the dose of AS-IV used in studies varies greatly; therefore, the safety window and effective dose of AS-IV need to be accurately established. Thus, further studies are warranted to determine the effects of AS-IV and large cohort clinical studies are required to further validate its efficacy in a clinical setting.

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Availability of data and materials

Not applicable.

Authors’ contributions

All authors (TC, PY and YJ) were involved in the conception and design of the study. TC was involved in the drafting of the manuscript and in the processing of the figures. PY and YJ were involved in the critical revision of the manuscript for important intellectual content. YJ was responsible for obtaining funding. TC and PY provided administrative, technical, or material support. PY and YJ supervised and edited the study. All authors have 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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