Ailanthone: A novel potential drug for treating human cancer (Review)

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Received September 12, 2019; Accepted May 5, 2020

DOI: 10.3892/ol.2020.11710

Abstract. Cancer is the second leading cause of death after cardiovascular disease. In 2015, >8.7 million people died worldwide due to cancer, and by 2030 this figure is expected to increase to ~13.1 million. Tumor chemotherapy drugs have specific toxicity and side effects, and patients can also develop secondary drug resistance. To prevent and treat cancer, scientists have developed novel drugs with improved antitumor effects and decreased toxicity. Ailanthone (AIL) is a quassinoid extract from the traditional Chinese medicine plant Ailanthus altissima, which is known to have anti-inflammatory and antimalarial effects. An increasing number of studies have focused on AIL due to its antitumor activity. AIL can inhibit cell proliferation and induce apoptosis by up- or downregulating cancer-associated molecules, which ultimately leads to cancer cell death. Antitumor effects of AIL have been observed in melanoma, acute myeloid leukemia, bladder, lung, breast, gastric and prostate cancer and vestibular neurilemmoma. To the best of our knowledge, the present study is the first review to describe the antitumor mechanisms of AIL.

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1. Introduction

Cancer, as a complex disease, is the result of long-term interaction of various exogenous and endogenous carcinogenic factors (1-3). Normal cell division has a certain maximum number of divisions, and it is precisely regulated by a series of internal factors such as genes, enzymes and proteins. Tumor cells often divide uncontrollably without following the principles of normal self-limited cell division, and can invade or spread to other healthy parts of the body, leading to the formation of tumors (4). Cancer is the second leading cause of death worldwide (5). With nearly 60% of the world’s population in Asia, 48.4% of new cancer cases and more than half of cancer-associated deaths (57.3%) occur in this region, where cancer mortality is higher compared with that in other regions, such as Europe, Africa and America (5). Lung cancer has the highest morbidity and mortality rates of all cancer types in men in China (6). In China, the most common cancer types among men and women are lung and breast cancer, respectively (6). Currently, the US Food and Drug Administration has approved ~150 anticancer drugs, which are classified into either cytotoxic or targeted drugs. Cytotoxic drugs can kill cancer cells by targeting mitotic and/or DNA replication pathways, whereas targeted drugs block the growth and spread of cancer by inhibiting molecular targets associated with cancer progression and migration (7). However, targeted drugs are often more expensive, and cytotoxic drugs often have various side effects and toxicity levels. Therefore, identification and isolation of natural compounds from medicinal plants has
received increasing attention for the development of novel anticancer drugs, with the aim overcome drug resistance and long-term survival.

2. Natural products as antitumor drugs

Medicinal plants have a long history of being used to treat various types of cancer. For example, numerous Asian countries, such as China, Japan and Thailand, have used traditional medicinal plants to treat cancer for thousands of years (8-10). Several of the antineoplastic drugs that have been used in a clinical setting originate from plants, some of which have significantly prolonged the survival time of patients. For example, vincristine is used to treat leukemia (11), lymphoma (12), breast cancer (13), lung cancer (14) and pediatric solid cancers (15); paclitaxel is used to treat ovarian, breast, lung, bladder and head and neck cancer (16); docetaxel is used to treat breast (17) and lung (18) cancer; and irinotecan is used to treat colorectal and lung cancer (19). In addition, a number of natural products (including evodiamine, peimine, isorhynchophylline, Coptis chinensis, ephedrine, oridonin and matrine) improve the drug resistance of cancer cells (breast cancer resistance to paclitaxel and epirubicin; gastric cancer resistance to fluorouracil; lung adenocarcinoma cell resistance to cisplatin and docetaxel; and hepatocellular carcinoma resistance to cisplatin), which is the primary cause of cancer chemotherapy failure (20). Recently, an increasing number of studies have focused on the anticancer mechanism of natural products. Wu et al (21) have reported that icariin induces apoptosis in human lung adenocarcinoma cells by activating the mitochondrial apoptotic pathway. Furthermore, lycorine has notable antitumor effects in various types of cancer, such as breast, esophageal, ovarian, prostate, melanoma and liver cancer (22). Acridone alkaloids are another class of natural products primarily obtained from Swinglea glutinosa, which has selective cytotoxicity against human prostate, lung, breast and liver carcinoma cell lines (23). In the Allium cepa assay and the yeast proliferation model, Kanchnar guggulu was evaluated for its cytotoxicity by inhibiting mitosis and anti-proliferation effects, confirming its potential in cancer treatment, and current studies have shown that it has anti-tumor effect (24,25). In addition, it has been shown that the use of a standardized Chinese herbal formula (including Radix and Rizoma Ginseng, Rhizoma Atractylodis, Poriae; Radix Glycyrrhiza Preparata, Rehmannia glutinosa, Radix Paeoniae alba, Radix Angelica sinensis, Rhizoma Chuang xiong, Ramulus Cinnamomum, Semen Armeniaca amara, Radix Platycodonos, Radix Saposhnikoviae, Fructus Jujubae, Massa Medica Fermentata, Cordyceps, RhizomaDioscoreae, Radix Ophiopogonis, Radix Bupleuri, Colla Corii asini, Semen Lablab album, Rhizoma Zingiberis, Ganoderma and Rhodiolae crenulatae) in patients with advanced lung cancer is acceptable and safe (26), therefore natural products have a broad application in the development and application of anti-tumor drugs.

3. Ailanthone (AIL) as an anti-tumor drug

Ailanthus altissima is a plant of the genus Ailanthus in the family Simaroubaceae (27). As a traditional Chinese medicine, it has a long history in China; for example, its bark, root bark and fruit have been used for the treatment of ascariasis, diarrhea, spermatorrhea, bleeding and gastrointestinal diseases (28). AIL, extracted from Ailanthus altissima, is a pentacyclic diterpene lactone compound (Fig. 1). It has notable clinical benefits in the treatment of inflammation (29), malaria (30), allergies (31), tuberculosis (32), ulcer s (33), amoeba-associated disease (34) and HIV (35) and has antitumor effects (36). Numerous in vitro studies have revealed that AIL has inhibitory effects on sever types of cancer cells, such as melanoma (37), acute myeloid leukemia (38,39), bladder (40), lung cancer (41,42), gastric (43), liver (44) and breast (27,45) cancer, vestibular schwannomas (VS) (46), osteosarcoma (47) and prostate cancer (48). The specific antitumor mechanism of AIL is summarized in Fig 2. In addition, AIL was found to improve the resistance of prostate cancer cells and leukemia cells to MDV3100 and doxorubicin (DOX), respectively (48,49). An overview of the antitumor mechanism of AIL in various types of cancer cells will be discussed in the present review.

Antitumor activity of AIL against melanoma. In 2011, a total of 9,128 melanoma deaths occurred in the United States. The overall age-adjusted melanoma death rate was 2.7 per 100,000 and the mortality rate of malignant melanin is even higher (50). It has become one of the most serious malignant tumors threatening human health (51). Liu et al (37) demonstrated that AIL inhibited cell proliferation and promoted apoptosis of B16 and A375 melanoma cells in a dose-dependent manner by downregulating the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway and inducing the activation of apoptotic initiating factors. The results of their study also revealed that the number of viable cells significantly decreased with increasing AIL concentrations 24-h following incubation (37). Subsequently, the potential mechanisms were explored; AIL induced G0/G1 phase arrest in B16 cells and G2/M phase arrest in A375 cells, significantly increasing the apoptotic rate in a dose-dependent manner (37).

Distinct apoptotic characteristics, such as nuclear condensation, irregular contraction of chromatin and apoptotic bodies were observed in the AIL-treated B16 cells, whereas the protein expression levels of p21 were increased and the levels of cyclins E and B were decreased. These results suggested that AIL inhibited cell proliferation by regulating cell cycle-associated protein expression to block the cell cycle of melanoma cells; the expression levels of PI3K, phosphorylated (p)-PI3K and p-AKT were also decreased, indicating that AIL inhibited the activity of the PI3K/AKT signaling pathway (37). In addition, the decrease of the mitochondrial membrane potential, increase of cytochrome c and Apaf-1 expression and activation of caspase-9 and -3 indicated that AIL mediated apoptosis through the mitochondrial pathway (37). Overall, these results suggested that AIL may be a potential antitumor agent to treat melanoma.

Antitumor activity of AIL against acute myeloid leukemia (AML). Acute myeloid leukemia (AML) is an aggressive, heterogeneous, myeloid malignancy. It is the most common adult acute leukemia and accounts for ~80% of cases (52). microRNAs (miRNAs/miRs) are short RNA
Antitumor activity of AIL against bladder cancer. Bladder cancer is one of the most common malignant tumors in the urinary system. Global cancer statistics in 2018 found that bladder cancer is more common in men, in whom it is the sixth most common cancer and ninth leading cause of cancer-associated death (5). Currently, the combination of surgery and cisplatin-based chemotherapy is the standard treatment (60); however, cisplatin-based chemotherapy is often accompanied by secondary drug resistance, which can reduce the long-term therapeutic effect and is particularly evident in invasive uterine cancer (61). Cisplatin resistance in patients with bladder cancer is associated with overexpression of NF-E2-related factor (Nrf2), and increased Nrf2 in resistant cells has been recognized as an important factor in maintaining drug resistance (62). Nrf2 also promotes the epithelial-mesenchymal transition by downregulating E-cadherin, and knockdown of Nrf2 impairs tumor cell migration and invasion (63). Yes-associated protein (YAP) is the primary effector of the Hippo pathway, which also participates in chemotherapy resistance of bladder cancer (64). When the Hippo pathway is inhibited, YAP is transported into the nucleus and binds to transcription factors, such as transcriptional enhanced factor (c-Myc, Cyp61 and survivin) that regulate cell proliferation, migration and survival (64). Conversely, knockdown of YAP and silencing of Nrf2 can enhance the sensitivity of bladder cancer cells to cisplatin and reduce the migration of tumor cells (62). Daga et al (40) demonstrated that AIL inhibited the proliferation and migration of bladder cancer cells by reducing the expression of Nrf2, YAP and c-Myc. Moreover, a similar effect was identified in cisplatin-resistant bladder cancer cells. The results of MTT and colony formation assays demonstrated that AIL was more effective compared with cisplatin in inhibiting the growth of 253J B-V and 253J bladder cancer cell lines (40). Of note, the growth inhibition rate of the cisplatin-resistant cell lines, 253J B-V-C-r and 253J C-r was the same as that of the sensitive cells, confirming the cytotoxicity and antiproliferative effect of AIL. Furthermore, AIL exhibited low cytotoxicity in normal adult HK-2 renal cortex cells, indicating that the toxicity of AIL to normal cells was lower compared with that of cancer cells (40). Further flow cytometry analysis demonstrated that AIL primarily arrested cells in the G0/G1 phase of the cell cycle and inhibited migration and invasion; however, this did not induce apoptosis. In addition, protein expression levels of Nrf2, YAP and c-Myc were decreased in the AIL-treatment group. Overall, Daga et al (40) demonstrated that AIL overcame cisplatin resistance in bladder cancer cells by downregulating the expression of Nrf2 and YAP, suggesting that AIL may be an effective drug for patients with bladder cancer that are resistant to chemotherapy.

Antitumor activity of AIL against lung cancer. Lung cancer is one of the most common malignancies in the world and is the leading cause of cancer-associated death, accounting for 18.4% of deaths among patients with cancer (5). Ni et al (42) screened 3,000 herbal monomers using an ATP luminescent high-throughput assay and demonstrated that AIL had the potential to inhibit the proliferation of non-small cell lung cancer (NSCLC) cells. AIL inhibited the proliferation and colony formation of NSCLC A549, H1299 and H1975 cells
in a dose- and time-dependent manner, and the growth inhibition ability of AIL was greater compared with that of cisplatin, which is a first-line chemotherapeutic drug for lung cancer (42). Orthotopic lung tumor models revealed that the volume and weight of tumors were smaller in the AIL-treated group. In addition, flow cytometry analysis demonstrated that AIL induced G1 or G2/M arrest of tumor cells in a dose-independent manner and induced apoptosis in H1975 cells, but not in A549 and H1299 cells (42). Western blotting showed that caspase-3 and poly-ADP-ribose polymerase (PARP) were activated in H1975 cells, but not in A549 and H1299 cells; similarly, following DAPI staining, AIL was observed to induce DNA damage in H1299 and H1975 cells, but not in A549 cells (42). This indicated that AIL-mediated growth inhibition was dependent on the induction of apoptosis and DNA damage. The authors further elucidated its mechanism of action using cDNA microarray analysis and reported that 1,222 genes were significantly differentially expressed in A549, H1299 and H1975 cells; among them, four genes, namely proliferating cell nuclear antigen (PCNA), replication protein A 1 (RPA1), acyl-CoA desaturase and DNA ligase 1, were involved in both nucleotide excision repair and DNA replication signaling pathways (42). Subsequent experiments revealed that the mRNA levels of PCNA and RPA1 were significantly decreased in all tested cell lines, while the protein expression levels of RPA1 were significantly decreased in a dose-dependent manner, and PCNA levels were not altered. Lastly, using animal experiments, it was confirmed that AIL inhibited subcutaneous xenograft and orthotopic lung tumor growth and prolonged the survival time of tumor-bearing mice (42). This indicated that AIL inhibited RPA1 expression in a dose-dependent manner, thus inhibiting DNA replication and tumor cell growth.

Hou et al. (41) demonstrated that AIL inhibited the PI3K/AKT and Janus kinase (JAK)/signal transducer and activator of transcription (STAT)3 signaling pathways by increasing the expression levels of miR-195, as well as by promoting apoptosis and autophagy in the A549 lung cancer cell line. Abnormal expression of miR-195 is associated with the development and progression of numerous types of tumors, such as breast (65), lung (66), liver (67) and prostate cancer (68). The viability of A549 cells treated with different concentrations of AIL was significantly decreased (P<0.01 or P<0.001) compared with that in the control group. Cell proliferation and cyclin D1 expression levels were also significantly decreased in cells treated with AIL (P<0.01), suggesting that AIL also inhibited the proliferation of lung cancer cells. Apoptotic analysis demonstrated that AIL significantly increased the rate of apoptosis of tumor cells, and the protein expression levels of cleaved-caspase-3 and -9 were increased, further indicating that AIL promoted apoptosis (41). However, Ni et al. (42) did not report that AIL induces apoptosis in A549 cells. Considering that a previous study identified that miR-195 was associated with lung cancer (66), Hou et al. (41) detected the expression levels of miR-195 and the protein expression levels of autophagy-related proteins Beclin-1 and p62 in AIL-treated A549 cells. It was concluded that AIL promoted apoptosis and autophagy by upregulating miR-195, which was verified by knockdown of miR-195. Also the upregulation of miR-195 inhibited the PI3K/AKT and JAK/STAT3 signaling pathways (41). Therefore, AIL was hypothesized to exert its anticancer effects by upregulating the expression of miR-195 in lung cancer.

Antitumor activity of AIL against gastric cancer. The incidence of gastric cancer in East Asia (including Mongolia, China, Japan and Korea) is notably higher compared with that in other regions, such as Northern America, Northern Europe, and Africa (5). Gastric cancer has become the fifth most frequently diagnosed cancer and the third leading cause of cancer-associated death in the world (5). Chen et al. (43)
explored the antitumor effect of AIL on human SGC-7901 gastric cancer cells. The results revealed that AIL inhibited the proliferation of SGC-7901 cells in a dose- and time-dependent manner. The IC₅₀ value of AIL in SGC-7901 cells at 24 h was significantly (P<0.05) lower than that of the taxol group, which was used as a positive control. The apoptotic rate was also significantly (P<0.001) increased with increasing concentrations of AIL. Furthermore, AIL significantly increased the percentage of cells in the G₀/G₁ phase in a dose-dependent manner. The protein expression levels of Bel-2 and Bax were down- and upregulated, respectively, in cells treated with AIL. Characteristic apoptotic morphology (nuclear shrinkage and chromatin condensation) were also observed in the AIL-treatment group following Hoechst 33258 staining, indicating that AIL induced apoptosis in SGC-7901 cells (43).

**Antitumor activity of AIL against liver cancer.** Liver cancer was the sixth most commonly diagnosed cancer and the fourth leading cause of cancer-associated death worldwide in 2018 (5). The incidence rate of liver cancer is often higher in countries with a lower Human Development Index (5). Primary liver cancer includes hepatocellular carcinoma (HCC), comprising 75-85% of total cases, intrahepatic cholangiocarcinoma, comprising 10-15% of cases, and other rare types (5). The 5 year survival rate of patients with liver cancer is still low (~18%), even following systemic treatment (69). Zhuo et al (44) investigated the anticancer effect of AIL in Huh7 human HCC cells. AIL reduced the viability of Huh7 cells in a dose- and time-dependent manner. Colony formation was also inhibited in a dose-dependent manner. Flow cytometry analysis showed that after 48 h of exposure to different concentrations of AIL (0, 0.2, 0.4, or 0.8 µM), the percentage of cells in the G₀/G₁ phase increased notably, confirming that AIL arrested the cell cycle. The expression levels of proteins regulating the cell cycle were investigated, and it was demonstrated that AIL decreased the expression of cyclins D and E, CDK2, CDK4 and CDK6, and increased the expression of p21 and p27 (44), even following systemic treatment (69). Zhuo et al (44) investigated the anticancer effect of AIL in MDA-MB-231 cells (45). The IC₅₀ of AIL on MDA-MB-231 cells was 9.8 µM at 48 h, which was lower compared with that of human non-tumorigenic breast epithelial MCF-12A cells; this indicated that AIL had lower cytotoxic effects on normal cells compared with that in cancer cells (45). In addition, AIL significantly decreased the percentage of BrdU-positive cells compared with the control group (P<0.05). An apoptosis assay revealed that AIL significantly increased the percentage of apoptotic cells, and AIL increased the protein levels of cleaved caspase-3 and -9 (P<0.001). Transwell assay results revealed that AIL significantly reduced cell migration and invasion; also the levels of migration- and invasion-associated proteins MMP-9 and vimentin were significantly (P<0.05, P<0.001) decreased in the AIL-treated group (45). The data from reverse transcription-quantitative PCR indicates that upregulation of miR-148a may mediate cell proliferation, apoptosis, migration and invasion affected by AIL in MDA-MB-231 cells. Furthermore, it was demonstrated that AIL inhibited AMP-activated protein kinase (AMPK) and Wnt/β-catenin signaling pathway by regulating miR-148a in MDA-MB-231 cells (45). Overall, these two studies suggested that AIL had potential in the treatment of breast cancer.

**Antitumor activity of AIL against breast cancer.** Breast cancer is the most commonly diagnosed cancer in the world, and was leading cause of cancer-associated death in women in 2018 (5). It has been reported that AIL significantly inhibits the proliferation of human MCF-7 breast cancer cells in a time- and dose-dependent manner (27). The inhibition rates of MCF-7 cells were 7.38-35.95, 22.73-47.6 and 35.64-56.76% at 24, 48 and 72 h, respectively, following treatment with different concentrations of AIL (0.5, 1.0, 2.0, 4.0 and 8.0 µg/ml) (27). AIL also promoted apoptosis in a dose-dependent manner, and the apoptosis rate in the 8.0 µg/ml group was 75.51%, which was significantly increased compared with that of the control group (P<0.01). In addition, the percentage of cells in G₀/G₁ phase were cells decreased, whereas the percentage of cells in S and G₂/M phase were notably decreased compared with that of the control group (27). In addition, the apoptosis of breast cancer MCF-7 cells in the AIL group was increased by upregulating the expression levels of Bax, caspase-3 and downregulating the expression of Bel-2 (27). This indicated that AIL arrested the cell cycle and promoted apoptosis in breast cancer cells.

Gao et al (45) reported that AIL significantly reduced the viability of breast cancer cells, suppressed cell proliferation and induced apoptosis. AIL downregulated cyclin D1 and upregulated p53 and p21 protein levels in MDA-MB-231 cells (45). The IC₅₀ of AIL on MDA-MB-231 cells was 9.8 µM at 48 h, which was lower compared with that of human non-tumorigenic breast epithelial MCF-12A cells; this indicated that AIL had lower cytotoxic effects on normal cells compared with that in cancer cells (45). In addition, AIL significantly decreased the percentage of BrdU-positive cells compared with the control group (P<0.05). An apoptosis assay revealed that AIL significantly increased the percentage of apoptotic cells, and AIL increased the protein levels of cleaved caspase-3 and -9 (P<0.001). Transwell assay results revealed that AIL significantly reduced cell migration and invasion; also the levels of migration- and invasion-associated proteins MMP-9 and vimentin were significantly (P<0.05, P<0.001) decreased in the AIL-treated group (45). The data from reverse transcription-quantitative PCR indicates that upregulation of miR-148a may mediate cell proliferation, apoptosis, migration and invasion affected by AIL in MDA-MB-231 cells. Furthermore, it was demonstrated that AIL inhibited AMP-activated protein kinase (AMPK) and Wnt/β-catenin signaling pathway by regulating miR-148a in MDA-MB-231 cells (45). Overall, these two studies suggested that AIL had potential in the treatment of breast cancer.
levels of cyclin D1, which is a critical target of proliferative signals in the G1 phase to promote cell cycle progression (46). AIL significantly increased the apoptotic rate (P<0.001) and the expression levels of cleaved caspase-9 and -3. In addition, increased expression levels of the autophagy-associated proteins Beclin-1 and LC3-II, and decreased levels of p62 in the AIL group compared with those in the control cells suggested that AIL can promote the autophagy of VS cells (46). Previous studies have demonstrated that high levels of miR-21 expression are associated with several types of cancer (72-74), thus it was explored whether miR-21 functioned in AIL-mediated growth inhibition of VS cells. miR-21 levels were increased in VS cells compared with those in healthy tissue using microarray analysis techniques (46). AIL significantly decreased the levels of miR-21 in tumor cells (P<0.01), indicating that miR-21 was negatively regulated by AIL; overexpression of miR-21 reversed the aforementioned results, suggesting that miR-21 participated in the antitumor mechanism of AIL (46). Furthermore, the possible mechanism by which AIL induced apoptosis and autophagy in VS cells was explored, reporting that AIL blocked the Ras/RAF proto-oncogene serine/threonine-protein kinase (Raf)/mitogen-activated protein kinase kinase (MEK)/ERK and mTOR pathways in a miR-21-dependent manner (46). Therefore, these results demonstrated that AIL may be a potential antitumor agent for treating VS.

**Antitumor activity of AIL against osteosarcoma.** Statistics from 1973 to 2004 show that osteosarcoma is the most common primary malignant tumor of bone in children and adolescents in the United States (75). At present, complete surgical resection combined with chemotherapy is the primary method of treatment for osteosarcoma (76). A number of studies have demonstrated that abnormal expression of miRNA (miR-27a, 95-3p, 195 and 133b) was associated with osteosarcoma growth, metastasis and prognosis (77-79). For example, Kong et al (47) revealed that different concentrations of AIL inhibited MG63 osteosarcoma cell viability (P<0.01 or P<0.001) and proliferation (P<0.01) compared with those in the control group by increasing the levels of miR-126. Furthermore, cell migration and invasion were also inhibited by AIL, whereas the rate of apoptosis was increased. Protein expression levels of cyclin D1 and Bcl-2 were decreased, while Bax, cleaved PARP and cleaved caspase-3 were increased following treatment of MG63 cells with AIL compared with untreated cells (47). In addition, PTEN protein expression levels were increased; however, PI3K and AKT phosphorylation levels were decreased (47). This indicated that the activation of the PI3K/AKT pathway was suppressed by AIL in MG63 cells. miR-126 was expressed at low levels in osteosarcoma cell lines MG63, U2OS, HOS and Saos-2 compared with those in normal osteoblast hFOB1 cells (47). These effects of AIL inducing MG63 cell proliferation, migration and invasion were all reversed when miR-126 was knocked down, indicating that AIL exerts its antitumorsarcoma effect by upregulating miR-126.

**Antitumor activity of AIL against prostate cancer.** In 2018, prostate cancer is the second most common type of cancer in the world and the fifth leading cause of cancer-associated death among men (5). Androgen deprivation therapy is the primary treatment for metastatic prostate cancer and includes three methods: Surgery, radiotherapy and castration drugs, such as Goserelin (80). Drug castration therapy primarily targets androgen receptors (ARs), which serve an important role in the development of prostate cancer. When ARs are phosphorylated following activation via endogenous androgen ligands (testosterone and dihydrotestosterone), the ligand-receptor complex, in association with coregulatory factors (for example, c-Fos, c-Jun, NFkB and sex-determining region Y gene translocates into the nucleus and binds to specific genomic DNA regions to regulate target gene expression (81). Androgen binding is the most important stimulator of androgen receptor activation (81); therefore, drug castration therapy is primarily aimed at eliminating this stimulus. A previous study has reported that >80% of patients respond to castration therapy in the early stages of treatment; however, almost all patients eventually progress to the terminal stage of castration-resistant prostate cancer (CRPC) (82). The drugs currently used in CRPC are docetaxel (83), cabazitaxel (84), abiraterone (85), radium-223 (86) and enzalutamide (87). In addition, the AR antagonist MDV3100 has also been reported to be effective against CRPC (88). Most of the AR antagonists used in clinic target the ligand-binding domain of the receptor. Therefore, AR shear variants (AR-Vs) that lack the ligand binding domain are resistant to antiandrogen therapy, including MDV3100 and abiraterone (89). He et al (48) demonstrated that AIL targeted p23 to overcome MDV3100 resistance in prostate cancer cell lines. The group used dihydrotestosterone to stimulate 22Rv1 prostate cancer cells to activate the ligand-dependent receptor full-length AR (AR-FL), and transfected with AR1-651 (this segment of AR lacks a ligand-binding domain (LBD), but can be continuously activated to introduce AR-Vs. After 12-h incubation with various natural compounds, a dual luciferase assay was used to detect AR transcriptional activity, and it was observed that AIL effectively reduced the transcriptional activities of AR-FL and AR-Vs. The same results were also demonstrated in LNCaP and c4-2b prostate cancer cell lines (48). A sulforhodamine B assay confirmed that AIL inhibited the proliferation of several AR-positive prostate cancer cell lines, including LNCaP, c4-2b, 22Rv1 and LAPC4. However, its proliferation inhibitory effect was weaker in AR-negative tumor cell lines and normal prostate cells (48). Similarly, AIL was more effective at inhibiting AR-positive cell migration compared with that of AR-negative prostate cancer cells. After combining AIL (0.1 µM) with the AR antagonists bicalutamide (BIC) and MDV3100, c4-2b androgen-insensitive and 22Rv1 castration-resistant cells proliferation was inhibited, indicating that AIL overcame drug resistance (48). AIL was also demonstrated to significantly inhibit the increase in tumor volume in 22Rv1 xenografts in animal experiments with BALB/c nude mice. In addition, the oral bioavailability of AIL was 25.7% and did not exhibit significant hepatotoxicity; however, there was some damage to the gastric mucosa (48). Of note, VCaP xenografts were more sensitive to AIL compared with MDV3100. CRPC 22Rv1 xenografts were resistant to BIC and MDV3100 treatment; however, AIL markedly inhibited tumor growth and reduced the tumor volume by 82% (95% confidence intervals, 70-95%) (48).

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**Statistics**

- Antitumor activity of AIL against osteosarcoma. Statistics from 1973 to 2004 show that osteosarcoma is the most common primary malignant tumor of bone in children and adolescents in the United States (75).
- Antitumor activity of AIL against prostate cancer. In 2018, prostate cancer is the second most common type of cancer in the world and the fifth leading cause of cancer-associated death among men (5). Androgen deprivation therapy is the primary treatment for metastatic prostate cancer and includes three methods: Surgery, radiotherapy and castration drugs, such as Goserelin (80). Drug castration therapy primarily targets androgen receptors (ARs), which serve an important role in the development of prostate cancer.

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**References**

- Kong et al (47)
- He et al (48)
- Li et al (48)
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AIL also significantly reduced the expression levels of AR proteins in LNCaP, 22RV1, LNCaP-MDV3100-R and VCaP prostate cancer cells in a dose-dependent manner, and these reductions were not associated with the presence or absence of androgen stimulation (48). An immunoprecipitation assay showed that AIL induced AR protein degradation and ubiquitination by preventing the interaction between AR and its molecular chaperones, heat shock protein HSP90 and HSP70; in addition, AIL downregulated AKT and CDK4 expression, which may be associated with inhibition of proliferation (48).

In the absence of a ligand, the AR resides in the cytosol bound to a complex of HS, chaperone and co-chaperone proteins (90). This protein complex is also termed foldosome and component proteins, including HSP90, HSP70 and p23, in which p23 serves an important role in maintaining the stability of the foldosome (90). Using the ProteOn XPR36 system, it was demonstrated that AIL could bind to p23 and inhibit its interaction with HSP90 in the foldosome, thus destabilizing the complex (90). In summary, targeting p23 is the main mechanism by which AIL induces the degradation of AR, and destabilizes the folding complex. Therefore, AIL is a potential candidate for the treatment of prostate cancer, which requires further investigation.

4. Preclinical safety evaluation of AIL

Unfortunately, most of the aforementioned studies did not evaluate the effect of AIL on normal cell lines (27,37-40,43,46,47). Only a few studies have evaluated the cytotoxicity of AIL in normal cells or the toxicity and the side effects in animal models (42,44,45,48). Gao et al (45) and He et al (48) have reported that cancer cells are more sensitive to AIL compared with normal cells. Traditional chemotherapeutic drugs have various side effects, such as myelosuppression, hepatotoxicity, nephrotoxicity, digestive tract reaction, neurotoxicity and pulmonary fibrosis, which markedly reduce the quality of life of patients with cancer and hinder the progress of treatment (91). Antitumor drugs extracted from traditional Chinese medicine exhibit less toxicity and fewer side effects compared with traditional chemotherapeutics. For example, Ni et al (42) demonstrated that AIL significantly inhibited NSCLC cell proliferation in vitro and tumor growth in vivo with low toxicity, and no damage was observed in the liver and kidney of SCID-Bg mice following AIL treatment. He et al (48) found that AIL does not induce significant hepatotoxicity in BALB/c nude mice; however, it can cause some damage to the stomach. To evaluate the in vivo cytotoxic effects of AIL, Zhuo et al (44) observed hematoyxin and eosin-stained sections from the heart, lung, liver, kidney and spleen and revealed no notable morphological changes were found in the AIL-treated animals.

In a recent study, Tang et al (92) used Kunming mice to evaluate the toxicity and safety of AIL. The acute toxicity experiments indicated that the main organs affected by AIL were the liver, spleen, intestine, colon and stomach. According to the toxicity of the classification standard, Globally Harmonized System of Classification and Labeling of Chemicals (93), the median lethal dose of AIL is 27.3 mg/kg, which is level 2 (severe). The primary causes of death from AIL included gastrointestinal hemorrhage and liver steatosis (92). In addition, the toxicity of AIL in the blood system was also investigated; AIL significantly reduced the numbers of red blood cells (RBC), hemoglobin, hematocrit and mean corpuscular hemoglobin concentration and increase the MCN, RDWCV and platelet counts in mice; however, this process was reversed following drug withdrawal, indicating that AIL may have hematologic toxicity, but it was not the primary cause of death in mice (92). In the autopsy report, in addition to gastrointestinal hemorrhage and liver steatosis in the high concentration group, hepatic steatosis, cholestasis, splenomegaly and chronic gastritis were also observed in the low concentration treatment group. In addition, the study showed that AIL had reproductive toxicity, as the testis and epididymis of male mice had marked atrophy and pathological damage, while ovarian follicle development was hindered, with corpus luteum necrosis in female mice (92).

5. Antitumor mechanism of AIL

Effects on apoptosis. One of the antitumor effects of AIL is the activation of the apoptosis pathway. Apoptosis is a form of programmed cell death regulated by genes, through which abnormal cells in the body can be removed and to maintain homeostasis. Defects within apoptosis are associated with numerous diseases, such as autoimmunity, degenerative diseases and cancer (94). Dysregulation of apoptosis may lead to the formation of tumor cells (95). Therefore, molecular pathways that promote apoptosis have become effective targets against tumor growth. AIL has been demonstrated to induce apoptosis in numerous types of cancer cells via the intrinsic and extrinsic pathways by regulating multiple molecular targets (37,43), including the caspase and Bcl-2 family proteins (43), transcription factors (such as β-catenin) (45), tumor suppressor genes (such as TP53) (45) and signaling pathways, such as PI3K/AKT (37) and JAK/STAT3 (41).

Members of the Bcl-2 family. The Bcl-2 family of proteins are key regulators of apoptosis via the mitochondrial apoptosis pathway and by promoting the caspase cascade activation (96,97). The balanced ratio of various Bcl-2 proteins determines whether the cell undergoes apoptosis or survives (98). AIL treatment downregulates Bcl-2 and upregulates Bax proteins in melanoma (37), gastric cancer (43), breast cancer (27) and osteosarcoma (47) cells. Mitochondrial membrane potential changes have been observed in melanoma (37) and hepatocellular carcinoma (44) cells treated with AIL compared with untreated cells, suggesting that the mitochondrial apoptosis pathway is involved.

TP53. The tumor suppressor protein p53, encoded by the TP53 gene in humans, serves an important role in preventing cancer development (99). TP53 is the most frequently mutated gene in human cancer (100). p53 can also promote apoptosis, relying on the induction of pro-apoptotic Bcl-2 proteins (99). In addition, p53 is an important mutual regulator of AMPK (101). AIL notably upregulated p53 protein levels in MDA-MB-231 breast cancer cells, which promoted apoptosis (45).

Effects on signaling pathways

PI3K/AKT/mTOR signaling pathway. The PI3K/AKT/mTOR signaling pathway is one of the most important intracellular
pathways that is frequently activated in a wide range of cancer types, including melanoma (102), breast cancer (103), lung and colorectal cancer (104). The PI3K/AKT/mTOR signaling pathway regulates cell proliferation, differentiation and metabolism, leading to anti-apoptosis and cancer cell survival. In addition, activation of the PI3K/AKT/mTOR pathway is also associated with tumor pathogenesis (including breast cancer, melanoma, gastric, lung, pancreatic and thyroid cancer and acute myeloid leukemia) and drug resistance, such as etoposide, DOX, cytarabine (105-107). PI3K, AKT or mTOR kinase inhibitors are already in clinical development (102,104). It has been demonstrated that AIL treatment suppresses the PI3K/AKT/mTOR pathway by decreasing the phosphorylation of PI3K and AKT, thus inducing apoptosis. In melanoma (37), acute myeloid leukemia (38), lung cancer (41), liver cancer (44), VS (46) and osteosarcoma (47), AIL exerts its antitumor activity mainly by inhibiting the PI3K/AKT pathway.

**JAK/STAT3 signaling pathway.** The JAK/STAT3 signaling pathway serves a key role in cell survival and apoptosis; it is activated and its components are abnormally expressed in a variety of tumors, including leukemia (108), prostate cancer (109), renal cell carcinoma (110), lung (111), colon (112) and pancreatic cancer (113). In recent years, the JAK/STAT3 signaling pathway has been considered as a potential target for antitumor therapy (114). The phosphorylation of STAT3 increased in various types of cancer (109); therefore, analysis of p-STAT3 protein expression levels can be used to determine whether AIL acts on the JAK/STAT3 signaling pathway. A previous study has demonstrated that AIL exerts its antitumor effects on lung cancer by upregulating miR-195, which inhibits the JAK/STAT3 signaling pathway (41).

**Ras/Raf/MEK/ERK signaling pathway.** The Ras/Raf/MEK/ERK signaling pathway also plays a pivotal role in tumor cell survival. Activation of the Ras protein is observed in ~30% of human cancer types, including pancreatic, lung, endometrium, ovary, prostate, stomach, liver and breast cancer (115). ERK promotes survival, metastasis and cell proliferation, primarily by activating the epidermal growth factor receptor and Ras small guanosine triphosphatases; p-ERK translocates into the nucleus and regulates various transcription factors, such as the Ets family of transcription factors (116). In VS, AIL acts on this signaling pathway to exert its antitumor effects (46).

**Wnt/β-catenin signaling pathway.** Dysregulation in the Wnt/β-catenin pathway has been observed in numerous types of human cancer, such as colon cancer, melanoma, pancreas cancer and adrenocortical carcinoma (117). Wnt/β-catenin is an important signal transduction pathway for the regulation of cell proliferation, apoptosis and metastasis (118). The expression levels of β-catenin are associated with poor prognosis in patients with breast cancer (119). Gao et al (45) reported that AIL inhibited the activation of the AMPK and Wnt/β-catenin signaling pathways by regulating miR-148a in MDA-MB-231 cells.

**Notch signaling pathway.** Notch can function as a proto-oncogene in tumors, including breast cancer and lymphoid malignancies (for example T cell acute lymphoblastic leukemia, B cell chronic lymphocytic leukemia and splenic marginal zone lymphoma), and can also serve as a tumor suppressor gene (120). Notch signaling pathway is regulated at the transcriptional or post-transcriptional levels. The ubiquitination pathway, miRNA (including miR-1, -34, -146, -199 and -200) and Cyclin/Cdk complex can all affect the Notch signaling pathway (121). Notch is particularly important in the hematopoietic system (122). Notch mediates the proliferation, self-renewal and differentiation of stem and progenitor cells to generate mature cells in the blood (122). The activation of Notch signaling is associated with poor prognosis of patients with AML (123), and targeting Notch1 has been considered as a novel strategy for AML treatment (124). AIL has been demonstrated to deactivate the Notch and PI3K/AKT signaling pathways by upregulating miR-449a expression (38).

**Effects on cell proliferation and cell cycle.** Cell proliferation is highly regulated in normal cells, and dysregulation of the cell cycle may lead to excessive or uncontrolled proliferation and promote metastasis (4). A number of studies have documented that AIL mediates its antiproliferative effect in cancer cells via modulation of various molecular targets, such as cyclin, CDKs, CDK inhibitors (CKIs) and the Rb gene (125). Cyclin binds to specific CDKs to form cyclin/CDK complexes that are important in regulating transcription, DNA repair, differentiation and apoptosis. The synthesis and destruction of cyclins is one of the primary means of regulating the cell cycle in vivo (125). CKI, as a negative regulator of the cell cycle, can be divided into two classes: The Ink4 family and the Cip/Kip family (126). AIL exerts its effects by regulating cyclin, CKD and CKI expression. Cyclins E and B were downregulated and p21 was upregulated in melanoma (37) and breast cancer (45), whereas cyclin D1 was downregulated in lung (41) and breast (45) cancer, VS (46) and osteosarcoma (47). In hepatocellular carcinoma, the expression levels of cyclins D and E were inhibited and CDK2, 4 and 6 were decreased, while the expression levels of p21 and p27 was increased (44). The expression of CDK4 was downregulated in prostate cancer (48). In addition, the Rb gene, one of the most important antioncogenes, can be phosphorylated by cyclin D/CDK4 or cyclin E/CDK2, releasing the transcription factor E2F. Transcription factor E2F regulates the expression of numerous genes, including cyclins E and A, cdk1, B-myb, dihydrofolate reductase, thymidine kinase and DNA polymerase (127). These genes serve an important role in the cell cycle and DNA synthesis (127,128). AIL can also act on the retinoblastoma (Rb) gene in HCC, reducing the expression of Rb protein, which is a positive regulator of the cell cycle (44).

Deregulation of cell metabolism and proliferation are major characteristics of tumor cells. AMPKs are activated when cells are metabolically stressed. AMPK activation regulates various cellular processes, such as cell proliferation, polarity, autophagy and apoptosis (129). Gao et al (45) demonstrated that AIL inhibited breast cancer cell proliferation by inhibiting AMPK.

**Effects on autophagy.** Autophagy serves a pivotal role in the cellular homeostasis of specific tissues, including liver tissue and skeletal muscle. Its functions include cell survival regulation (such as the response to metabolic alterations, recycling damaged macromolecules and organelles) and various forms of
Table I. Mechanism and biological effects of ailanthone in cancer cells.

### A. Melanoma

| Author, year | Mechanism | Biological effects | Model | Ref. |
|--------------|-----------|--------------------|-------|------|
| Liu et al, 2019 | G0/S arrest, G0/M arrest; Apoptotic body formation; ↑p21; ↓Cyclin E and B; ↓PI3K, p-PI3K, p-AKT; ↑Caspase-9, cleaved-caspase-3, -9; ↓Mitochondrial membrane potential; ↓Bel-2; ↑Bax; ↑Cytochrome c; ↑Apaf-1. | ↓Proliferation; ↑Apoptosis | Human melanoma cells B16, mouse melanoma cells A375 | (37) |

### B. AML

| Author, year | Mechanism | Biological effects | Model | Ref. |
|--------------|-----------|--------------------|-------|------|
| Zhang et al, 2019 | ↑miR-449a; ↓Notch1, Notch2, p-PI3K, p-AKT; ↓MMP-9, vimentin; ↑cleaved-caspase-7, -3, -9 | ↑Apoptosis; ↓Migration and invasion | Human AML cells (KG1, HL60, U-937, THP-1 and OCI-AML2) | (38) |
| Wei et al, 2018 | G0/G1 arrest; ↑Autophagy; Acidic vesicular organelles (AVOs); ↑Beclin-1, LC3-II; ↓p62, LC3-I | ↓Proliferation; ↑Apoptosis | Human AML cells HL60 | (39) |

### C. Bladder cancer

| Author, year | Mechanism | Biological effects | Model | Ref. |
|--------------|-----------|--------------------|-------|------|
| Daga et al, 2019 | G0/G1 arrest; ↓Nrf2, YAP, c-Myc | ↓Proliferation; ↓Migration and invasion | Human Bladder tumor cells (T24,253J B-V, 253J B-V and, 253J C-r) | (40) |

### D. Lung cancer

| Author, year | Mechanism | Biological effects | Model | Ref. |
|--------------|-----------|--------------------|-------|------|
| Ni et al, 2017 | DNA damage (H1299 and H1975 cells); G1 or G2/M arrest; ↓RPA1; ↑Cleaved- caspase-3(H1975); ↓DNA replication | ↑Apoptosis (H1975); ↓H1975 subcutaneously xenograft and orthotopic lung tumor growth; ↑Survival of tumor-bearing mice; ↓Viability | Human NSCLC cells (A549, H1299 and H1975) | (42) |
| Hou et al, 2019 | ↑miR-195; ↑Cleaved-caspase-3, -9; ↑Beclin-1; ↓p62; ↓p-PI3K, p-AKT, p-JAK, p-STAT3 | ↓Proliferation; ↑Apoptosis; ↑Autophagy | Human NSCLC cells A549 | (41) |
### Table I. Continued.

#### E, Gastric cancer

| Author, year  | Mechanism                                      | Biological effects       | Model                             | Ref. |
|---------------|------------------------------------------------|--------------------------|----------------------------------|------|
| Chen et al, 2017 | G₂/M arrest; ↓ Bel-2; ↑ Bax                    | ↓ Proliferation; ↑ Apoptosis; ↓ Viability | Human gastric cancer cells SGC-7901 |      |

#### F, Liver cancer

| Author, year  | Mechanism                                      | Biological effects       | Model                             | Ref. |
|---------------|------------------------------------------------|--------------------------|----------------------------------|------|
| Zhuo et al, 2015 | G₀/G₁ arrest; ↓ Cyclin D, cyclin E; ↓ CDK2, CDK4, CDK6; ↑ p21, p27; ↓ CDC25A; ↓ RB; ↑ H2AX; DNA damage; ↑ p-ATM, p-ATR, p-Chk1, p-Chk2; ↑ Cleaved-caspase-3, -9; ↑ Cleaved PARP; ↓ Mitochondrial membrane potential; ↓ p-PI3K, p-AKT | ↓ Proliferation; ↓ Viability; ↓ Huh7 subcutaneously xenograft | Human hepatic cell lines HepG2, Hep3B and Huh7 | (44) |

#### G, Breast cancer

| Author, year  | Mechanism                                      | Biological effects       | Model                             | Ref. |
|---------------|------------------------------------------------|--------------------------|----------------------------------|------|
| Wang et al, 2018 | G₀/G₁ arrest; ↓ Bcl-2; ↑ Bax; ↑ Caspase-3;       | ↓ Proliferation; ↓ Viability; ↑ Apoptosis | Breast cancer cells MCF-7 | (27) |
| Gao et al, 2019 | ↑ Cleaved-caspase-3, -9; ↓ MMP-9, vimentin; ↓ CyclinD1; ↑ p53, p21; ↑ miR-148a; ↓ p-AMPK, β-catenin | ↓ Migration and invasion; ↓ Viability; ↓ Proliferation; ↑ Apoptosis | Breast cancer cells MDA-MB-231, human non-tumorigenic breast epithelial cells MCF-12A | (45) |

#### H, Vestibular schwannoma

| Author, year  | Mechanism                                      | Biological effects       | Model                             | Ref. |
|---------------|------------------------------------------------|--------------------------|----------------------------------|------|
| Yang et al, 2018 | ↓ miR-21; ↑ Beclin-1; ↓ p62, LC3-1; ↑ Cleaved-caspase-3, -9; ↓ Viability; ↓ Cyclin D1; ↓ Ras, Raf, p-MEK, p-ERK, p-mTOR and p-p70S6K | ↓ Proliferation; ↓ Viability; ↑ Apoptosis; ↑ Autophagy | Human vestibular schwannoma cells | (46) |
| Author, year       | Mechanism                              | Biological effects                      | Model                                                                 | Ref. |
|-------------------|----------------------------------------|-----------------------------------------|----------------------------------------------------------------------|------|
| Kong et al, 2019  | ↑ Cleaved-caspase-3; ↑ Cleaved-PARP; ↓ CyclinD1; ↓ Bcl-2; ↑ Bax; ↑ PTEN; ↓ p-PI3K, p-AKT; ↑ miR-126 | ↓ Viability; ↓ Proliferation; ↑ Apoptosis; ↓ Migration and invasion | Osteosarcoma cell lines (MG63, U2OS, HOS and Saos-2), normal osteoblast hFOB1 cells | (47) |

**J, Prostate cancer**

| Author, year | Mechanism       | Biological effects                          | Model                               | Ref. |
|--------------|-----------------|---------------------------------------------|-------------------------------------|------|
| He et al, 2016 | ↓ AR protein; ↑ AR degradation; ↓ AKT; ↓ CDK4 | ↓ Proliferation; ↓ Migration; ↑ Drug(MDV3100) sensitivity; ↓ Tumor growth and metastasis (in vivo) | Prostate cancer cell lines (LNCaP, c4-2b, 22RV1 and LAPC4) | (48) |

AML, acute myeloid leukemia; NSCLC, non-small cell lung cancer; AR, androgen receptor; p, phosphorylated; Bcl-2, B cell lymphoma-2; Bax, Bcl-2-associated X; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; JAK, Janus kinase; STAT3, signal transducer and activator of transcription 3; Nrf2, NF-E2-related factor; YAP, Yes-associated protein; CDK, cyclin-dependent protein kinase; ATM, ataxia telangiectasia mutated proteins; ATR, ataxia telangiectasia and Rad3-related proteins; PARP, poly-ADP-ribose polymerase; AMPK, AMP-activated protein kinases; mTOR, mammalian target of rapamycin; PTEN, gene of phosphate and tension homology deleted on chromosome ten; RAF, RAF proto-oncogene serine/threonine-protein kinase; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal regulated kinases; MMP-9, matrix metalloproteinase-9.
programmed cell death (130). Autophagy can be considered as a tumor-suppressing process in specific tissues (131). The detection of autophagy-associated proteins Beclin-1, p62 and LC3-I/II can be used to analyze the role of AIL in promoting autophagy (130). Beclin-1 serves an important role in the formation of autophagosomes, which can initiate autophagy by binding to type III phosphatidylinositol and triphosphate kinase, and is considered to be a marker of autophagy initiation (132). p62 is an adaptor molecule involved in the activation of autophagy, targeting polyubiquitinated protein aggregates to autophagic lysosomes and degrading autophagic lysosomes (133). Therefore, p62 expression levels are a reliable indicator of autophagy (133). LC3 protein is sheared at the carboxyl end by cysteine protease ATG4 with endonuclease activity, which exposes glycine residues and produces LC3-I localized in the cytoplasm (134). After being modified and processed by a ubiquitin-like system, including Atg7 and Atg3, LC3 is covalently bound to phosphatidylethanolamine to form LC3-II, which is localized to the autophagosome membrane (135). The LC3-I/II ratio can be used to assess the rate of autophagy (134). By detecting the aforementioned proteins, it was demonstrated that AIL induced autophagy in promyelocytic leukemia (39), lung cancer (41) and VS (46) cells.

Effects on cell invasion and metastasis. Invasion and metastasis occur in the moderate and advanced stages of various types of cancer (136,137). As aforementioned, it has been shown that AIL inhibits metastasis in cancer cells by modulating molecular targets, including matrix metalloproteinases (MMPs). MMPs can degrade various protein components in the extracellular matrix, destroy the histological barrier of tumor cell invasion and serve a key role in tumor invasion and metastasis (138). Overexpression of MMPs, particularly MMP-2 (gelatinase A) and MMP-9 (gelatinase B) has been associated with tumor progression, metastasis and poor prognosis in breast, lung, colon, gastric, pancreatic, and prostate cancer (139). Zhang et al (38) and Gao et al (45) demonstrated that the inhibitory effect of AIL on invasion and migration of breast cancer cells and acute myeloid leukemia cells was associated with decreased MMP-9 expression.

Effects on drug resistance. Tumor chemotherapy is often accompanied by drug resistance difficulties. It has been demonstrated that traditional Chinese medicine plant extracts, such as curcumin (140), matrine (141) and resveratrol (142), can improve drug resistance and reduce the use of chemotherapy drugs. He et al (48) also confirmed that AIL improved resistance to MDV3100 in prostate cancer cell lines.

6. Conclusions

The antitumor effect of AIL involves numerous mechanisms (Table 1); however, the current research on the underlying mechanisms of AIL function is still relatively superficial. Based on existing studies, it has been observed in few studies in this review that the advantages of AIL as an antitumor agent are its relatively low toxicity and fewer side effects compared with existing chemotherapeutic drugs (42,44,48). However, it cannot be ignored that the median lethal dose in mice observed by Tang et al (92) was rated as level 2 (severe). Considering that the research investigating AIL is still in its infancy, there are few comparative studies on the efficacy of AIL and existing chemotherapy drugs. Therefore, this conclusion remains to be verified. Furthermore, the majority of studies lack in vivo experiments and clinical trials, and there are no further studies on the bioavailability and side effects of AIL. Therefore, in subsequent studies, researchers should focus on the efficacy of AIL compared with existing chemotherapy drugs, as well as in vivo and clinical trials. It is hypothesized that in the future, when its efficacy is demonstrated to be favorable compared with existing chemotherapy, AIL may be used as an effective novel anticancer treatment.

Acknowledgements

Not applicable.

Funding

This study was funded by the Traditional Chinese Medicine Science and Technology Project of Zhejiang Province (grant no. 2018ZJA109), Medical and Health Science and Technology Project of Zhejiang Province (grant no. 2018ZH026), Natural Science Foundation of Ningbo (grant nos. 2016A610157 and 2018A610371) and the Science and Technology Projects of Zhejiang Province (grant no. LGF19H030007).

Availability of data and materials

Not applicable.

Authors' contributions

HD and ZY conceived and designed the study and prepared the manuscript. XY, CH, KG and XL were responsible for the literature search, data visualization and analysis. XL and YJ searched for the relevant literature and revised the manuscript. 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.

References

1. Blattner WA: Human retroviruses: Their role in cancer. Proc Assoc Am Physicians 111: 563-572, 1999.
2. Tomlinson IP, Novelli MR and Bodmer WF: The mutation rate and cancer. Proc Natl Acad Sci USA 93: 14800-14803, 1996.
3. Wogan GN, Hecht SS, Felton JS, Conney AH and Loeb LA: Environmental and chemical carcinogenesis. Semin Cancer Biol 14: 473-486, 2004.
4. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.
Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Int J Cancer 68: 394-424, 2002.

6. Chen W, Zheng R, Zhang S, Zhang S, Zeng H, Xia C, Zuo T, Yang Z, Zhou X and Han J: The advantages of using traditional Chinese medicine as an adjunctive therapy in the whole course of cancer treatment instead of the whole term therapy. J Thorac Oncol 9: 16-34, 2014.

7. Nakamura K, Shinkozuka K and Yoshawba N: Anticancer and antimetastatic effects of cordycepin, an active component of cordyceps sinensis. J Pharmacol Sci 127: 53-56, 2015.

8. Lummelndik J, Taniwongse J, Booranasubkajorn S, Boonrak R, Akarsereenont P, Laohapand T and Heinrich M: Understanding cancer and its treatment in thai traditional medicine: An ethnopharmacological-anthropological investigation. J Ethnopharmacol 216: 259-273, 2018.

9. Bezwoda WR, MacDonald DF, Gear JS, Derman DP, Bothwel TH, Squ S, Hurwitz S and Lewis D: Combination chemotherapy including bleomycin in the treatment of advanced Hodgkin's disease. S Afr Med J 53: 369-373, 1978.

10. Duran J, Rams RA, Bartolucci AA and Dorfman RF: BCNU with and without cyclophosphamide and prednisone (COP) and cycle-active therapy in non-Hodgkin's lymphoma. Cancer Treat Rep 61: 1085-1096, 1977.

11. Wong MY and Chiu GN: Liposome formulation of co-encapsulation of vincristine and quercetin enhanced antitumor activity in a trastuzumab-insensitive breast tumor xenograft model. Nanomedicine 7: 834-840, 2011.

12. Munkner S, Vogelhuber M, Bornschein J, Stroszczynski C, Munker S, Vogelhuber M, Bornschein J, Stroszczynski C and Trotta F: The use of a standardized Chinese herbal formula in patients with advanced lung cancer: A feasibility study. J Int Med Res 16: 390-395, 2018.

13. Kasymjanova G, Tran AT, Cohen V, Pepe C, Sakr L, Small D, Akarasereenont P, Laohapand T and Heinrich M: Understanding cancer and its treatment in thai traditional medicine: An ethnopharmacological-anthropological investigation. J Ethnopharmacol 216: 259-273, 2018.

14. Akarasereenont P, Laohapand T and Heinrich M: Understanding cancer and its treatment in thai traditional medicine: An ethnopharmacological-anthropological investigation. J Ethnopharmacol 216: 259-273, 2018.

15. Wu X, Kong W, Qi X, Wang S, Chen Y, Zhao Z, Wang W, Liu W, Liu X, Pan Z, Wang D, Li M, Chen X, Zhou L, Xu M, Li D and Zheng Q: Ailanthone induces cell cycle arrest and apoptosis in human bladder cancer cells through down-regulation of Nrf2, YAP, and c-Myc expression. Phytomedicine 56: 156-164, 2019.

16. Yu D, Cheng Z, Wang W, Wu Y: Ailanthone exerts an antitumor function on the development of human lung cancer and c-Myc expression. Phytomedicine 56: 156-164, 2019.
Hallmarks of cancer. Cancer Cell 34: 21-43, 2018.

Barrera G: Crosstalk between Nrf2 and YAP contributes to main

Han LL: miR‑195 suppresses the metastasis and epithelial‑mesen

Pharmacother 80: 95-101, 2016.

Ciamporcero  E, Daga M, Pizzimenti  S, Roetto A, Dianzani  C,

Kocak I, Gravis G, Bodrogi I, Mackenzie MJ, Shen L, et al.: Prednisone plus cabazitaxel or mitoxantrone for metastatic castration‑resistant prostate cancer progressing after docetaxel treatment: A randomised open-label trial. Lancet 376: 1147-1154, 2015.

Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PFA, Sternberg CN,

Klosterges C, Zhao S, Zhang X, Zou C, Kung HF, Lin MC, Dress A, Wardle F,

and miR-342 in plasma are novel potential biomarkers for acute

microRNA‑144‑3p and its clinical value in non‑small cell lung cancer. J Pathol 248: 150-159, 2015.

Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roesser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL, et al.: AR-V7 and resistance to enzalutamide and abiraterone in pros
cancer. J Pain 36: 52-62, 2013.

Tao WW, Jiang H, Tao XM, Jiang P, Sha LY and Sun XC: Effects of acupuncture, tuina, tai chi, qigong, and traditional Chinese medicine five-element music therapy on symptom management and quality of life for palliative patients: A meta-analysis. J Pain Symptom Manage 51: 728-747, 2016.

Winder C, Azzi R and Wagner D: The development of the glob-
ally harmonized system (GHS) of classification and labelling of hazardous chemicals. J Hazard Mater 125: 29-44, 2005.

Hassan M, Watari H, AbuAlmayta A, Oiba Y and Sakuragi N: Apoptosis and molecular targeting therapy in cancer. BioMed Res Int 2014: 150845, 2014.
96. Tan SH and Barker N: Wnt signaling in adult epithelial stem cells and cancer. Prog Mol Biol Transl Sci 153: 21-79, 2018.
97. Taketo MM: Shutting down wnt signal-activated cancer. Nat Rev Cancer 26: 320-322, 2016.
98. Radiz-Fand Raj K: The role of Notch in tumorigenesis: Oncogene or tumour suppressor? Nat Rev Cancer 3: 756-767, 2003.
99. Huang T, Zhou Y, Cheng AS, Yu J, To KF and Kang W: NOTCH receptors in gastric and other gastrointestinal cancers: Oncogenes or tumour suppressors? Mol Cancer 15: 80, 2016.
100. Lobry C, Oh P, Mansour MR, Look AT and Aifantis I: Notch signaling: Switching an oncogene to a tumor suppressor. Blood 123: 2451-2459, 2014.
101. Xu X, Zhao Y, Xu M, Dai Q, Meng W, Yang J and Qin R: Activation of Notch signal pathway is associated with a poorer prognosis in gastric cancer. PLoS One 8: e58899, 2013.
102. Redza-Dutordoir M and Averill-Bates DA: Activation of apoptosis and roles in cancer development and treatment. Asian Pac J Cancer Prev 16: 2129‑2144, 2015.
103. Green DR and Reed JC: Mitochondria and apoptosis. Biochim Biophys Acta 1665: 2977‑2992, 2004.
104. Cory S, Roberts AW, Colman PM and Adams JM: Targeting BCL-2-like proteins to kill cancer cells. Trends Cancer 2: 443‑460, 2016.
105. Lowe SW, Schmitt EM, Smith SW, Osborne BA and Jacks T: P53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 362: 847‑849, 1993.
106. Kastenhuber ER and Lowe SW: Putting P53 in context. Cell 170: 1062‑1078, 2017.
107. Wang Z, Wang L, Liu P and Xie X: AMPK and cancer. Ex Suppl 107: 203-226, 2016.
108. Aziz SA, Jilaveau LB, Zito C, Camp RL, Rimm DL, Conrad P and Kluger HM: Vertical targeting of the phosphatidylinositol-3 kinase pathway as a strategy for treating melanoma. Clin Cancer Res 16: 6029‑6039, 2010.
109. Brachmann SM, Hofmann I, Schnell C, Fritschi C, Wee S, Lane H, Wang S, Echeverria CG and Maira SM: Specific apoptosis induction by the dual PI3K/mTOR inhibitor NVP-BEZ235 in non-small cell and PIK3CA mutant breast cancer cells. Proc Natl Acad Sci USA 106: 2299‑2304, 2009.
110. Martinelli E, Troiani T, D’Auito E, Morgillo F, Vitagliano D, Capasso A, Costantino S, Ciffreda LP, Merolla F, Vecchione L, et al.: Antitumor activity of pimasertib, a selective MEK1/2 inhibitor in combination with a PI3K inhibitor or with multi-targeted kinase inhibitors in pimmasertib-resistant human lung and colorectal cancer cells. Int J Cancer 133: 2089‑2101, 2013.
111. Luo Z, Zang M and Guo W: AMPK as a metabolic tumor suppressor: Control of metabolism and cell growth. Future Oncol 6: 457‑470, 2010.
112. Galluzzi L, Bravo-San Pedro JM, Levine B, Green DR and Kroemer G: Pharmacological modulation of autophagy: Therapeutic potential and existing obstacles. Nat Rev Drug Discov 16: 487‑511, 2017.
113. Chiou KS: Autophagy and cancer. Exp Mol Med 44: 109‑120, 2012.
114. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H and Levine B: Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 402: 672‑676, 1999.
115. Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, et al.: Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 131: 1149‑1163, 2007.
116. Tanida I, Minematsu-Kiyosue Y, Ueno T and Komai N: Lysosomal turnover, but not a cellular level, of endogenous LC3 is a marker for autophagy. Autophagy 1: 84‑91, 2005.
117. Winer A, Adams S and Mignatti P: Matrix metalloproteinase inhibitors in cancer therapy: Turning past failures into future successes. Mol Cancer Ther 17: 1147‑1155, 2018.
118. Hung WC, Tseng WL, Shiea J and Chang HC: Skp2 overexpression increases the expression of MMP-2 and MMP-9 and invasion of lung cancer cells. Cancer Lett 288: 156‑161, 2010.
119. Carthika C and Sureshkumar R: Can curcumin along with chemotherapeutic drug and lipid provide an effective treatment of metastatic colon cancer and alter multidrug resistance? Med Hypotheses 132: 109325, 2019.
120. An Q, Han C, Zhou Y, Li F, Li D, Zhang X, Yu Z, Duan Z and Kan Q: Matrine induces cell cycle arrest and apoptosis with recovery of the expression of miR-126 in the A549 non-small cell lung cancer cell line. Mol Med Rep 14: 4042‑4048, 2016.
121. Bjorklund M, Roos J, Gogvadze V and Shoshan M: Resveratrol induces SIRT1- and energy-stress-independent inhibition of tumor cell regrowth after low-dose platinum treatment. Cancer Chemother Pharmacol 68: 1459‑1467, 2011.