Progress on the study of the anticancer effects of artesunate (Review)

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Abstract. Artesunate (ART) is a derivative of artemisinin that is extracted from the wormwood plant *Artemisia annua*. ART is an antimalarial drug that has been shown to be safe and effective for clinical use. In addition to its antimalarial properties, ART has been attracting attention over recent years due to its reported inhibitory effects on cancer cell proliferation, invasion and migration. Therefore, ART has a wider range of potential clinical applications than first hypothesized. The aim of the present review was to summarize the latest research progress on the possible anticancer effects of ART, in order to lay a theoretical foundation for the further development of ART as a therapeutic option for cancer.

Contents

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Abbreviations: ART, artesunate; LDH, lactate dehydrogenase; ROS, reactive oxygen species; HCC, hepatocellular carcinoma; TCTP, translation-controlled tumor protein; NSCLC, non-small cell lung cancer; ATC, anaplastic thyroid carcinoma; HNC, head and neck cancer; MPNST, malignant peripheral nerve sheath tumor; EC, esophageal cancer; CRC, colorectal cancer; AR, androgen receptor

Key words: artesunate, artemisinin, antitumor, apoptosis, proliferation

1. Introduction

Cancer is a major health concern worldwide (1,2). According to GLOBOCAN 2020, which presented the latest estimates of cancer incidence and mortality (3), there were ~19.3 million new cases of cancer and 10 million cancer-related deaths worldwide in 2020. As such, the number of cancer cases worldwide is expected to reach 28.4 million by 2040, a 47% increase from 2020 (4). Asia, Latin America, the Caribbean and Africa are expected to experience particularly large increases in cancer morbidity and mortality rates (3). Therefore, it is crucial to develop novel anticancer agents.

Artesunate (ART) is a derivative of artemisinin that is characterized by high efficacy, rapid effects, low toxicity and reduced susceptibility to drug resistance (5,6). At present, ART is commonly used for the treatment of mild to severe malaria worldwide (7). However, accumulating evidence has shown that ART also displays anticancer properties, in addition to its antimalarial effect (8). For instance, ART has been reported to induce apoptosis and autophagy in human bladder cancer cells (9,10). Moreover, it can induce cell cycle arrest, reactive oxygen species (ROS) generation and ferroptosis in renal cell carcinoma (11). In the present review, the potential anticancer effects of ART and the underlying mechanism of action involved are summarized. The aim was to provide a theoretical basis for the further development of ART and its derivatives for the treatment of cancer.

2. Source and activity of ART

ART is a semi-synthetic, monomeric derivative of artemisinin isolated from *Artemisia annua* in the 1970s (12-14). The conversion from artemisinin to ART is a two-step process, starting with reduction of dihydroartemisinin with diisobutylaluminium hydride, followed by esterification with succinic anhydride (14). The chemical name of ART is dihydroartemisinin-1,2-α-succinate monoester, with the chemical formula of $C_{24}H_{39}O_8$ and a molecular weight of 455.56 g/mol (15).

ART has a hydrophilic group, and the 1,2,4-endoperoxide bridge is responsible for the antimalarial activity of the drug. ART acts on all stages of malaria parasite circulation. ART also may penetrate the cell membranes and generate ROS, and a small amount of ART reaches the mitochondria of the parasite, where ART and ROS react with each other, leading

to mitochondrial dysfunction (8). ART is the only artemisinin derivative with high water solubility, due to the addition of the hemisuccinate group. ART is metabolized to docosahexaenoic acid (DHA) as it enters the body (16-18). ART induces the generation of ROS, increasing malondialdehyde levels and decreasing the levels antioxidants such as superoxide dismutase and catalase, thereby causing alkylation of the proteins of the *Plasmodium* parasite (19). At present, ART is mainly used for the treatment of malaria of all types (20), for immune regulation (in type 1 diabetes in NOD mice) (21), as well as for liver (22), breast (23) and lung (24-26) cancer.

3. Anticarcinogenic mechanism of ART

There is considerable evidence that ART can exert anticancer effects on several types of cancer cells (6,15). ART has been reported to induce apoptosis, differentiation and autophagy in colorectal cancer cells by impairing angiogenesis (27), inhibiting cell invasion and migration (28), inducing cell cycle arrest (11), upregulating ROS levels, regulating signal transduction [for example, activating the AMPK-mTOR-Unc-51-like autophagy activating kinase (ULK1) pathway in human bladder cancer cells] (9) and blocking immune escape (29). In addition, ART has been shown to restore the sensitivity of a number of cancer types to chemotherapeutic drugs by modulating various signaling pathways; for example, ART can improve the apoptosis of HCC by inhibiting the PI3K/AKT/mTOR pathway (30), and can increase liver cancer cell sensitivity to sorafenib via suppression of the MEK/ERK pathway (31) (Fig. 1).

Apoptosis. Apoptosis is a type of programmed cell death that does not elicit inflammatory responses (32). A number of studies have shown that ART can induce apoptosis by activating the mitochondria-dependent pathway, specifically by mediating the activation of caspase-3 and -9 and the release of cytochrome c into the cytosol after permeabilization of the mitochondrial membrane (33). Additionally, ART can induce HL-60 human acute promyelocytic leukemia cell and KG1a acute myeloid leukemia cell death by regulating antiapoptotic proteins, such as Bcl-2, as well as proapoptotic proteins, such as Bid and Bak, through inhibition of the MEK/ERK and PI3K/Akt pathways (34). ART has also been demonstrated to induce T helper 1 cell differentiation and promote apoptosis in ovarian cancer cells via the microRNA (miR)-142/sirtuin 1 pathway (35).

Autophagy. Autophagy is a conserved, self-degrading system that is essential for maintaining cell homeostasis under stress conditions, and which has been demonstrated to serve an important role in cancer in association with a family of autophagy-related proteins (LC3B) (36). ART can induce autophagy and increase the levels of CD155 in uterine corpus endometrial carcinoma (UCEC) cells. Moreover, it also regulates the interaction between CD155 and its receptor on the NK92 natural killer cell line by upregulating the co-stimulator CD226 and downregulating the co-inhibitor TIGIT, thereby enhancing the cytotoxicity of these cells. Thus, ART has a dual anticancer effect on UCEC cells (37). ART also induces autophagy by upregulating ROS production and activating the AMP-activated protein kinase/mTOR/ULK1 pathway in human bladder cancer cells (9).

ROS. ROS have a dual role in cellular metabolism (38). Their production is impaired during normal cellular homeostasis, whilst excessive production can lead to oxidative stress (OS), a process that can lead to damage to cellular structure (39). A study has shown that higher levels of ROS are important for the initiation, progression, angiogenesis and metastasis of cancer (40). Dysregulation of ROS has been found to promote tumorigenesis through activation of various oncogenic, signaling pathways such as MAPK, PI3K/AKT/mTOR and NF-κB (18,40), DNA damage (41,42), immune escape, metastasis, angiogenesis and telomere elongation (40). ROS production has been demonstrated to play an important role in ART-induced apoptosis in various tumor cell lines, including glioblastoma (43), lymphoma (44), breast cancer cells (45). Yao *et al* (46) suggested that ART could increase ROS levels in the hepatocellular carcinoma (HCC) cell lines Huh7 and Hep3B. In addition, the combination of sorafenib and ART treatment was found to synergistically produce antiproliferative effects in HCC cells and induce apoptosis.

Inhibition of angiogenesis. Blood vessels provide oxygen and a nutrient supply for the growth of tumors, which also facilitate the proliferation, migration and subsequent invasion of malignant tumor cells in the long term (47). Angiogenesis is a dynamic and complex process that is regulated by a variety of mechanisms. Inhibition of angiogenesis has become a therapeutic strategy for pancreatic cancer (48), breast cancer (49) and ovarian cancer (50). Chen *et al* (51) demonstrated that ART could downregulate the expression of VEGF and angiopoietin-1 in RPMI8226 myeloma cells, decrease the activation of ERK1and inhibit angiogenesis. Their study indicated that ART possessed a potential anti-myeloma effect, which was mediated by the inhibition of angiogenesis.

Cell cycle arrest. Aberrant cell division is one of the characteristic features of cancer cells (52). ART inhibits the proliferation of bladder cancer cells (RT4, RT112, T24 and TCCSup), which is associated with G_0/G_1-phase cell cycle arrest and downregulation of cell cycle regulatory proteins [cyclin D1 and CDK4 (required for entry into the G_1 phase); CDK1 and cyclinA/B (essential during the late S phase and early M phase)] (10). ART can block cell cycle progression and lead to a significant reduction in the levels of the cell cycle activating proteins cyclin A, cyclin B, and CDK1, evoking G_0/G_1 phase arrest and inhibiting growth of the cells in renal cell carcinoma (11). In breast cancer cells (MCF-7 and MDA-MB-231), ART can block G_2/M progression by upregulating beclin-1 expression, which promotes autophagy (53). In glioblastoma cells (A172, U251 and U87), ART also increases the proportion of cells in the G_0/G_1 phase, reduces the proportion of cells in the S phase and inhibits proliferation by downregulating the expression levels of the cell cycle-related proteins CDK2, CDK4, cyclin D1 and cyclin B1 (54).

Ferroptosis. Ferroptosis is a recently identified form of regulated cell death, which is characterized by iron overload, lipid ROS accumulation and lipid peroxidation (55). Evidence suggests that ferroptosis is closely associated with the occurrence, development and inhibition of cancer (56). Zhang *et al* (26) demonstrated that ART could upregulate the mRNA levels of transferrin

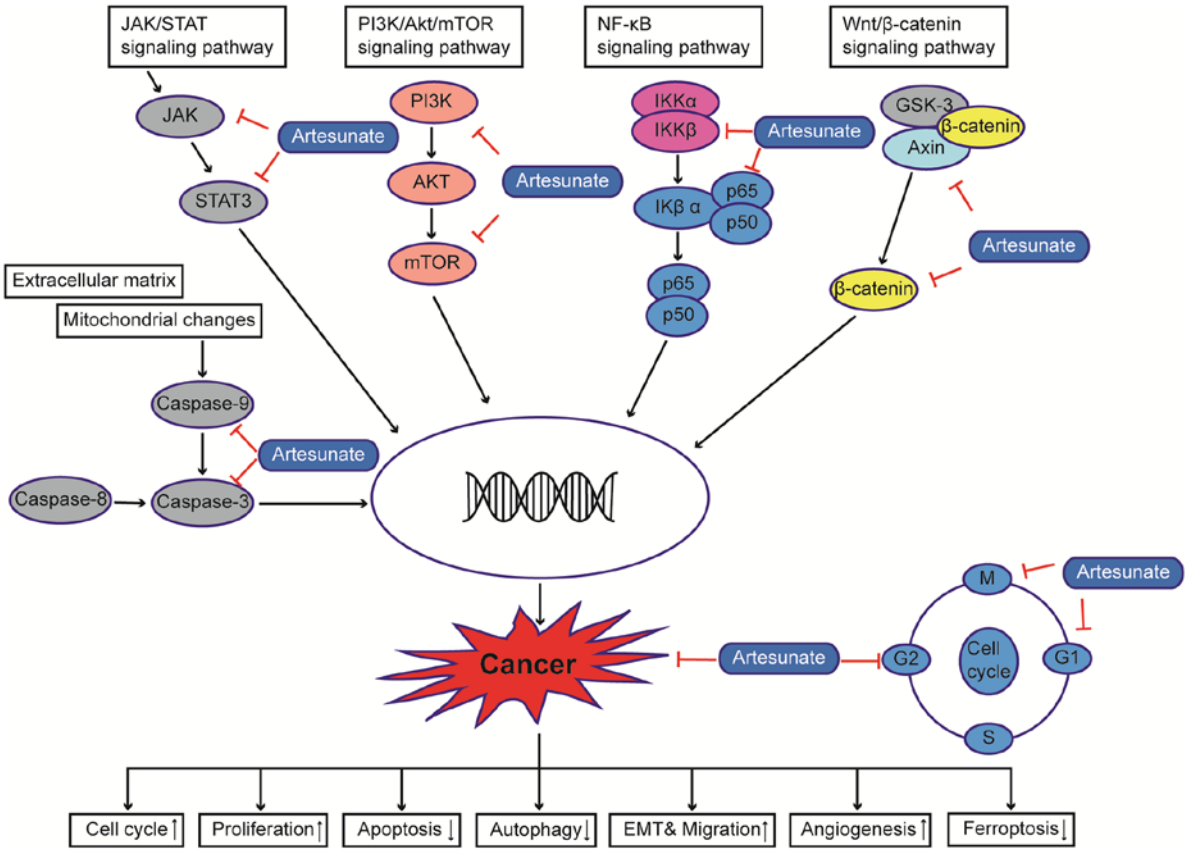

Figure 1. Anticarcinogenic mechanism of artesunate. Multiple molecular and signaling pathways regulate abnormal cell proliferation and migration, such as the NF-κB signaling pathway, the PI3K/Akt/mTOR signaling pathway and the JAK/STAT signaling pathway, among others, ultimately leading to tumorigenesis. Artesunate may affect the development of cancer by interfering with the cell cycle, proliferation, invasion, angiogenesis and apoptosis of cancer cells by acting on different sites. EMT, epithelial-mesenchymal transition.

receptor (a positive regulator of ferroptosis), thus inducing apoptosis and ferroptosis in A549 non-small cell lung cancer (NSCLC) cells. Li *et al* (57) showed that ART enhanced the anticancer effects of low-dose sorafenib (a novel multi-targeted oral drug for the treatment of gastroenteric tumors) against Huh7, SNU-449, and SNU-182 HCC cell lines *in vitro* and against a Huh7 cell xenograft model in BALB/c nude mice. In addition, ART-induced lysosome activation synergizes with the pro-oxidative effects of sorafenib to sequentially promote lysosomal cathepsin B/L activation, ferritin degradation, lipid peroxidation and ferroptosis (57).

4. Potential role of ART in human malignancies

Previous studies have reported that ART exerted minimal toxicity, was cost-effective and was effective for treating different types of cancer (Table I) (58-78). The potential anticancer properties of ART in different types of cancer are discussed below.

Leukemia. Leukemia is a clonal hematopoietic stem cell malignancy (79). ART can induce leukemic T cell apoptosis by promoting the generation of mitochondrial ROS (80). Previous studies have suggested that ART induces caspase-3/9-mediated apoptosis by targeting the outer mitochondrial membrane, leading to the activation and nuclear translocation of mitochondrial pro-apoptotic factors in human SKM-1 myelodysplastic syndrome cells (81). Nuclear translocation of apoptosis-inducing factors and endonuclease G were accompanied by low levels of ROS and increased mitochondrial production of superoxide, which occur prior to apoptosis and appear to be associated with the intracellular levels of divalent iron (59,60,82-84). Chen *et al* (34) found that ART may inhibit the levels of phosphorylated (p)-PI3K, p-AKT, p-MEK1 and p-ERK1/2 and promote the apoptosis of leukemia cells (HL-60 and KG1a cells) by inactivating PI3K/Akt and MEK/ERK signaling, ART also significantly reduced the expression of Ki67 and survivin, inhibited growth and stemness in KG1 xenograft models (34). In the MV4-11 cell line, ART combined with bortezomib (which is commonly used for the treatment of patients with multiple myeloma) resulted in significantly higher proliferation inhibition and reduced apoptotic rates compared with ART or bortezomib alone in the same concentration gradient. After the combination of the two drugs for 24 h, the expression of the pro-apoptotic proteins BIM and cleaved activated caspase-3 and the autophagy-related protein LC3B was upregulated in MV4-11 cells, whereas that of the anti-apoptotic protein Bcl-2 was downregulated (58).

Nervous system tumors. Central nervous system tumors comprise a group of malignancies that originate from tissues or structures of the central nervous system and exhibit an incidence of 5.6 per 100,000 person-years in children under the age of 19 (85-87). ART can selectively downregulate the expression

Table I. Antitumor activity of ART in different cancer types.

First author/s, year	Cancer type	Model/cell line	Mechanism/results	(Refs.)
Ishikawa *et al*, 2020	ATLL	HTLV-1	Cyclin-dependent kinase 1, 2, 4 and 6 ↑, cyclin B1, D2 and E ↓; p21 ↑; intracellular reactive oxygen species ↑; JunB↓; JunD↓	(5)
Chen *et al*, 2020	Leukemia	HL-60 and KG1a cells	Induced cell apoptosis and inhibited cell proliferation and stemness in a dose-dependent manner via the suppression of the MEK/ERK and PI3K/Akt pathways	(34)
Hu *et al*, 2019	Leukemia	MV4-11	Caspase-3 ↑; autophagy-related protein LC3B ↑; Bcl-2 ↓	(58)
Kim *et al*, 2015	CML	KBM-5	Antiproliferative and proapoptotic effects through suppression of multiple signaling cascades	(59)
Kumar *et al*, 2017	AML	AML MV4-11 and MOLM-13	Cellular and mitochondrial ROS accumulation, double-stranded DNA damage, loss of mitochondrial membrane potential and induction of the intrinsic mitochondrial apoptotic cascade	(60)
Wang *et al*, 2017	Pituitary adenoma	GH3 and MMQ	ART and BRC used in combination exert synergistic apoptotic and antitumor effects by suppressing miR-200c and stimulating PTEN expression	(61)
Karpel-Massler *et al*, 2014	Glioblastoma	U87MG and A172	A combination of ART and temozolomide resulted in increased cytotoxicity	(62)
Berte *et al*, 2016	Glioblastoma	LN229 and A172	Downregulation of RAD51 protein expression and HR activity. Inhibition of senescence induced by TMZ	(63)
Lian *et al*, 2016	Glioma	SHG44	Inhibition of cell proliferation, migration and invasion, and increase of cell apoptosis	(64)
Berdelle *et al*, 2011	Glioblastoma	LN-229	Oxidative DNA damage and DNA double-strand breaks, leading to tumor cell death	(65)
Button *et al*, 2014	Schwannoma	RT4	Combination with the autophagy inhibitor chloroquine potentiated cell death	(66)
Wei *et al*, 2020	Glioma	U251, U87, U138 and SK-N-SH	Impairing the nuclear localization of protein SREBP2 and the expression of target genes HMGCR through the mevalonate pathway, further affecting the metabolism of glioma cells	(67)
Greenshields *et al*, 2019	Breast cancer	MDA-MB-468 and SK-BR-3 cells	Inhibition of breast cancer cell proliferation via a ROS-dependent G_2/M arrest and ROS-independent G_1 arrest	(68)
Wen *et al*, 2018	Breast cancer	MCF7 cells	Inhibition of cell proliferation and increased G_2/M arrest through ATM activation and the 'ATM-Chk2-CDC25C' pathway	(69)
Greenshields *et al*, 2017	Ovarian cancer	Ovarian cancer cells	Induction of ROS; reduced proliferation; altered expression of cell cycle regulatory proteins, including cyclin D3, E2F-1 and p21; inhibition of mTOR signaling	(70)
Chen *et al*, 2019	Ovarian cancer	ID8	miR-142 expression in peripheral $CD4^+$ T cells ↑; Sirt1 levels ↓; Th1 differentiation from $CD4^+$ T cells ↑	(35)
Li *et al*, 2018	Ovarian cancer	SKOV3 and primary EOC	Induction of autophagy; cell cycle arrest; inhibition of EOC growth	(71)
Liu *et al*, 2015	Esophageal cancer	Eca109 and Ec9706	By downregulating mitochondrial membrane potential, Bcl-2 and CDC25A, upregulating Bax and caspase-3, induction of cell apoptosis and cell cycle arrest; concentration-dependent inhibitory activity *in vivo* and *in vitro*	(72)
Fei *et al*, 2018	Esophageal cancer	Irradiated TE-1 cells *in vitro* and *in vivo*	p21 ↑; cyclin D1, RAD51, RAD54, Ku70 and Ku86 protein ↓	(42)
Wang *et al*, 2018	Esophageal cancer	Eca109/ABCG2, xenograft tumor mouse model	Suppression of esophageal cancer drug resistance through the regulation of ABCG2 expression	(73)

Table I. Continued.

First author/s, year	Cancer type	Model/cell line	Mechanism/results	(Refs.)
Wang *et al*, 2017	Gastric cancer	SGC-7901	Inhibition of the cell growth; induction of apoptosis; may be related to the regulation of CDC25A, Bcl-2, Bax, caspase-3 and mitochondrial membrane potential	(74)
Zhang *et al*, 2015	Gastric cancer	HGC-27 cells	COX-2 ↓; Bax↑; Bcl-2 ↓; caspase-3 ↓; caspase-9 ↓	(75)
Jiang *et al*, 2018	Colon cancer	HCT116; *in vitro* and *in vivo*	Mitochondrial cleaved caspase 3, PARP, caspase-9 and Bcl-2-associated X protein ↑; Bcl-2 ↓	(76)
Kumar *et al*, 2019	Colorectal cancer	Rat model	Inhibition of cellular influx; decreased the levels of oxidative stress and inflammatory markers; cyclooxygenase-2, inducible nitric oxide synthase, NF-κB, and IL-1β ↓	(77)
Verma *et al*, 2017	Colon carcinogenesis	Rat model	β-catenin signaling ↓; angiogenic markers (VEGF, MMP-9) ↓; inhibition of cell proliferation	(27)
Li *et al*, 2019	Liver cancer	HepG2 and Huh7	Increased the expression levels of cleaved caspase-9 and cleaved poly ADP ribose polymerase, reduced VEGFR2 protein expression and reduced cell migration	(22)
Ilamathi *et al*, 2016	Hepatocellular carcinoma	HepG2	Suppression of STAT3; increased apoptosis	(78)
Wang *et al*, 2020	Lung cancer	A549	Reduced cell clone numbers; cell cycle arrest at the G_2/M phase; cell cycle and apoptosis-related proteins BAX, p21, p53 and caspase-3 ↑; Bcl-2 and cyclin B1 expression ↓	(24)
Zhao *et al*, 2020	Lung cancer	A549	Induction cell apoptosis and cell cycle arrest, Bcl-2 protein ↓; mitochondrial membrane potential ↓; Bax protein ↑	(25)

ART, artesunate; ATLL, adult T-cell leukemia/lymphoma; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; ROS, reactive oxygen species; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; SREBP2, sterol regulatory element-binding protein 2; Chk2, checkpoint kinase 2; CDC25, cell division cycle 25C; miR, microRNA; Sirt1, sirtuin 1; ABCG2, ATP binding cassette subfamily G member 2; COX-2, cyclo-oxygenase-2; PARP, poly(ADP-ribose) polymerase; BRC, bromocriptine; TMZ, temozolomide.

of survivin and induce the DNA damage response in glial cells to increase cell apoptosis and cell cycle arrest, resulting in increased sensitivity to radiotherapy (88). Previously, Wei *et al* (67) found that ART affected the nuclear localization of sterol regulatory element-binding protein 2 (SREBP2) by decreasing the expression of 3-hydroxy-3-methylglutaryl-CoA reductase and inhibited the mevalonate pathway, which in turn influenced the metabolism of glioma cells. In addition, ART disrupted the interaction between P53 and SREBP2 (which negatively regulates P53 and inhibits senescence), upregulated the expression of P21 and induced senescence in the U251, U87, U138 and SK-N-SH human glioma cell lines (67). The combination of ART and rapamycin (a specific inhibitor of mTOR) has been shown to synergistically decrease translation-controlled tumor protein (TCTP) expression and enhance the cytotoxicity of malignant peripheral nerve sheath tumor (MPNST) cells via mTOR-TCTP positive feedback loop, the results also suggested that TCTP may be a new target for the treatment of neurofibromatosis type 1-associated tumors and MPNSTs (89).

Thyroid carcinoma. Thyroid cancer is the most common cancer in the endocrine system, and its incidence is increasing worldwide (90). Anaplastic thyroid carcinoma (ATC) is an aggressive malignancy that is almost always fatal and lacks effective systemic treatment options. It is highly resistant to chemotherapy due to its undifferentiated and aggressive characteristics (91,92). Ma and Fei (91) showed that ART could inhibit growth and induce apoptosis in ATC cells (8505C, 8505C-r, KAT-4-r and KAT-4), by suppressing mitochondrial respiration and acting synergistically with chemotherapy drug doxorubicin without affecting glycolysis. Thus, ART led to oxidative stress and damage in ATC cells. Their work suggested that ART was a potential complement to the treatment of ATC, particularly cases with chemoresistance (91).

Breast cancer. Breast cancer is one of the most common malignancies among women (93). Despite decades of laboratory, epidemiological and clinical research, breast cancer rates continue to rise. Breast cancer remains the leading cancer-related cause of the burden of disease among women, affecting 1 in 20 women globally and 1 in 8 women in high-income countries (94,95). Systemic treatment (chemotherapy and endocrine therapy) of breast cancer is initially effective; however, after a period of time, drug resistance typically develops (96). ART has also been found to block the cell cycle progression of MCF-7 and MDA-MB-231 cells at the G_2/M phase and upregulate the expression of p21 and Beclin-1, thereby inhibiting the proliferation of breast cancer cells by inducing autophagy (53). ART treatment was also revealed to inhibit the proliferation of the triple-negative breast cancer cell line MDA-MB-468 and the human epidermal growth factor-2-enriched breast cancer cell line SK-BR-3 in a dose- and time-dependent manner. The proliferation of MDA-MB-468 and SK-BR-3 cells was inhibited by ROS-dependent G_2/M cell cycle arrest and ROS-independent G_1 cell cycle arrest (68). Furthermore, ART can inhibit breast cancer MCF-7 cell proliferation and promote G_2/M arrest by activating the ataxia-telangiectasia mutated/checkpoint kinase 2/cell division cycle 25 (CDC25) C pathway (69). By loading ART into the lipid core of a polymer-lipid hybrid carrier, the anticancer activity and physical stability of ART were found to be significantly increased and can be used for chemotherapy (97-99). Raza *et al* (100) found that ART generated the reactive oxygen species (ROS), resulted in DNA damage and enhanced the apoptosis of neighboring cells (Cx43-MCF7 cells) in breast cancer MCF-7 cells. In addition, the dose-dependent cytotoxicity of ART could be reduced by the gap junction (GJ) protein connexin-43 (Cx43). Li *et al* (101) found that ART could inhibit lysosomal function and clear dysfunctional mitochondria, and induce breast cancer cell apoptosis. In addition, ART was found to have a stronger inhibitory effect on drug-resistant breast cancer cells (A549/TAX and MCF-7/ADR) with higher lysosomal functional activity (101).

Ovarian cancer. Ovarian cancer is the seventh most common type of malignant neoplasm in women and the eighth cause of mortality (102-104). Most patients with ovarian cancer are typically diagnosed at an advanced stage of the disease (105). Ovarian cancer is treated with platinum chemotherapy following surgical resection (106). However, the recurrence rate is high (107,108) and the survival rates of ovarian cancer with International Federation of Gynecology and Obstetrics stage III and IV are only 10-30% (109). ART has been found to significantly reduce the expression of VEGF in the HO-8910 human ovarian cancer cell line, as well as that of KDR/flk-1 (VEGF receptor) in endothelial cells and HO-8910 cells, thereby significantly inhibiting angiogenesis in a dose-dependent form. Additionally, ART resulted in reduced xenograft tumor growth in nude mice, with no clear toxicity to the animal (110). ART could reduce the total amount of RAD51 and the formation of RAD51 foci in ovarian cancer cells sustaining DNA damage. Moreover, the downregulation of RAD51 conferred ovarian cancer cells an increased sensitivity to cisplatin (111). ART combined with cisplatin can synergistically induce DNA double-strand breaks and inhibit the proliferation of the HO8910 and SKOV-3 human ovarian cancer cell lines (111). ART induced the production of ROS and reduced proliferation in HEY1, HEY2 and SKOV-3 ovarian cancer cells, which were associated with downregulation in the expression levels of regulatory proteins of the cell cycle, including cyclin D3, CDKs (CDK4, CDK2, and CDK1), Rb, E2F-1 and CDC25C, while the tumor suppressor p21WAF1/CIP1, as well as phosphorylated Chk2 kinase which is important in the DNA damage response and an inhibitor of the CDC25 phosphatases were upregulated (70).

Esophageal cancer. Esophageal cancer (EC) is a common malignancy and has a high incidence rate in China (112). Although therapeutic approaches have improved, the 5-year survival of EC is <20% (113). ART can induce apoptosis and cell cycle arrest in the Eca109 and Ec9706 EC cell lines by upregulating Bax and caspase-3 and reducing mitochondrial membrane potential, as well as Bcl-2 and CDC25A expression in a concentration-dependent manner (72). In addition, an *in vivo* study showed that ART produced a dose-dependent Eca109-transplanted tumor regression in Balb/c nude mice, with little side effects. These results revealed that CDC25A was a molecular target of ART and that ART could inhibit the growth of EC cells by inducing apoptosis and G_0/G_1cell cycle

arrest (72). Fei *et al* (42) demonstrated that ART inhibited the proliferation of EC cells, enhanced radiosensitivity of TE-1 cells *in vitro* and enhanced the effect of apoptosis induced by irradiation in TE-1 cells by upregulating P21 and downregulating the expression of cyclin D1, RAD51, RAD54, Ku70 and Ku86 protein of irradiated TE-1 cells. Moreover, ART also could aggravate DNA damage of EC cells and prolong the formation of γ-H2AX foci induced by IR in TE-1 cells. The results indicated that ART may be a promising radiosensitizer for the treatment of EC. In another study, Wang *et al* (73) found that ART can reverse doxorubicin resistance in EC by downregulating the expression of ATP-binding cassette G2 in Eca109 cells. ART was reported to inhibit the proliferation, migration and invasion of KYSE-150 esophageal squamous cell carcinoma cells by suppressing cell elasticity and increasing adhesion; ART also may increase the apoptosis rate by altering the cytoskeleton of KYSE-150 cells (114).

Gastric cancer. Gastric cancer is the fourth leading cause of cancer-related mortality in the world, with a 5-year survival rate of <40% (115,116). ART can inhibit the proliferation of the gastric cancer cell lines SGC-7901, BGC-823 and AGS in a concentration-dependent manner, BGC-823 cells treated with ART exhibited calcium overload, downregulated expression levels of VEGF and upregulated expression levels of calpain-2 (117). ART treatment can also inhibit the proliferation of the SGC-7901 gastric adenocarcinoma cell line and induce apoptosis; the mechanism may be associated with Bax and caspase-3 upregulation and CDC25A and Bcl-2 downregulation (74). In addition, ART could prevent the growth of *Helicobacter pylori* and gastric cancer cells, inhibit the adhesion of *Helicobacter pylori* to these cells and reduce *Helicobacter pylori*-enhanced ROS production. Moreover, ART significantly reduces the number of tumor nodules and tumor size in a gastric cancer mouse model by inhibiting the NF-κB signaling pathway (118).

Colorectal cancer. Colorectal cancer (CRC) is one of the most common types of cancer worldwide and has incidence and mortality rates globally (119,120). ART was found to inhibit CRC proliferation and promote apoptosis in a dose-dependent manner to significantly suppress the growth of colorectal tumors, decrease the physiological activity of cancer and delay spontaneous liver metastasis in the CLY CRC cell line. These anticancer effects were associated with the membrane translocation of β-catenin and the inhibition of unrestricted Wnt/β-catenin signaling (121). In addition, ART can reverse the immunosuppression by downregulating the concentrations of TGF-β1 and IL-10 in Colon26 and RKO CRC cells (122). Jiang *et al* (76) found that ART induced apoptosis by increasing the protein levels of cleaved caspase-3, poly-ADP ribose polymerase (PARP), caspase-9 and Bax protein levels, while decreasing the levels of LC3 and beclin-1 in HCT116 colon cancer cells. ART can reduce the levels of oxidative stress and inflammatory markers, downregulate cyclo-oxygenase-2, induce nitric oxide (NO) synthase, NF-κB and IF-1β and reduce the risk of colon cancer (77).

Lung cancer. Lung cancer is the most common cancer in the world and the leading cause of cancer death (123), which has an overall 5-year survival rate of ~15% (124). Despite advances in treatment, progressive NSCLC still severely limits survival and requires new therapeutic compounds (125). ART can significantly inhibit the invasion and migration of NSCLC cells (H1395, A549, LXF289 and H460 cells) by downregulating the transcription of urokinase-type plasminogen activator, MMP-2 and MMP-7, whilst inhibiting AP-1 and NF-κB-transactivation (126). In addition, ART promotes radiosensitivity in A549 cells *in vitro* and *in vivo*, possibly by inducing cell cycle arrest at the G_2/M phase through the NO signaling pathway (127). Wang *et al* (128) found that ART could inhibit cell migration by upregulating the expression of the epithelial marker E-cadherin in A549 and H1975 NSCLC cell lines. In another study, ART could inhibit the invasion of A549 cells, and the mechanism may be associated with the reduced expression of intercellular adhesion molecule-1 and MMP-9 (129). Furthermore, ART inhibits the proliferation of A549 and H1299 cells by arresting the cell cycle at the G_1 phase and suppresses lung tumor progression by inhibiting the Wnt/β-catenin pathway (130). In A549 cells, ART combined with cisplatin blocks the cell cycle at the G_2/M phase and induces apoptosis by upregulating the expression of Bax, p53, p21, caspase-3, caspase-7 and caspase-9, whilst synergistically regulating the activity of the MAPK pathway by downregulating p-P38, p-JNK and p-ERK levels, which results in potentiated effects against cancer cell proliferation on A549 cells (131).

Liver cancer. Liver cancer is highly malignant and insensitive to cytotoxic chemotherapy, and is associated with a very poor patient prognosis (132,133). ART can activate caspase-3, increase the Bax/Bcl-2 ratio and PARP, whilst downregulating mouse double minute 2, which leads to induced apoptosis on human hepatocellular carcinoma (HCC) cells but had little effect on normal cells (134). The anticancer effects of ART nanoliposomes on human HepG2 cells was stronger than those mediated by ART active pharmaceutical ingredient at the same concentration (135). ART may function as a potential inhibitor of STAT3 in HCC cells to regulate STAT3 targets, including caspase-3, Bcl-xl and survivin, interfere with STAT3 dimerization and inhibition of both constitutive and IL-6-inducible STAT3, leading to cell apoptosis *in vitro* (78). Jing *et al* (30) also revealed that ART could inhibit phosphorylation of AKT and mTOR significantly, and induce apoptosis in HCC (SK-hep1 and SM-7721 cell lines) by inhibiting the PI3K/AKT/mTOR pathway. In addition, ART combined with sorafenib (which is a novel multi-targeted oral drug for the treatment of cancer) further increased the apoptosis of HCC cells by dual inhibition of both RAF/RAF/MAPK pathway and PI3K/AKT/mTOR pathway. Thus, the study identified a potential treatment strategy combining ART with sorafenib for the treatment of advanced HCC.

Other tumors. In a previous study, ART has been reported to induce lactate dehydrogenase release and cell death in necrosis-sensitive cholangiocarcinoma (136). Wang *et al* (137) found that ART could significantly inhibit proliferation in the Burkitt lymphoma Raji cell line, where it induced apoptosis and autophagy. The combination of ART and bromocriptine can synergistically promote apoptosis by inhibiting miR-200c expression and increasing that of PTEN in lactinomas (61).

Chauhan *et al* (138) found that ART induced ROS production and subsequent cell death in a receptor-interacting protein 1-dependent manner in human renal carcinoma. ART exerted a potent antiproliferative effect on polyomavirus-positive Merkel cell carcinoma (MCC) cells with good overall tolerance and induced ferroptosis (139). In addition, ART also significantly suppressed the growth of established MCC tumors in xenotransplanted mice, suggesting that ART may be used for the treatment of MCC (138). In another study, ART blocked the Wnt/catenin pathway to inhibit the proliferation, migration and invasion of uveal melanoma cells (primary 92.1 and metastatic Omm2.3 UM cells), mainly by suppressing the phosphorylation of GSK3β at Ser9 and decreasing the protein levels of β-catenin and its downstream targets (c-Myc and cyclin D1) (140). Wang *et al* (141) found that ART decreased androgen receptor (AR) expression, increased the expression and the catalytic activity of DNA methyltransferase3b (DNMT3b) in 22rv1 cells either in transplanted mice or *in vitro*. ART can suppress tumor growth of prostatic cancer cells through AR-DNMT3b pathway, suggesting it may be used for the treatment of prostate cancer in the future. Yang *et al* (142) found that ART induced mitochondrial dysfunction and cell apoptosis in the WERI-Rb1 and Y79 human retinoblastoma cell lines and in the ARPE-19 human retinal pigment epithelium cell line by upregulating Kruppel-like factor 6 expression, increasing the Bax/Bcl-2 ratio, promoting the release of cytochrome *c* and stimulating the cleavage of caspase-9 and -3. Roh *et al* (143) demonstrated that ART could induce ferroptosis in head and neck (HNC) cells via cellular glutathione depletion and ROS accumulation, and ART sensitivity decreased in some cisplatin-resistant HNCs as a result of Nrf2-ARE pathway activation. Berköz *et al* (144) suggested that ART treatment could decrease cell migration, invasion and colony formation in the A375 human melanoma cell line, possibly by inhibiting STAT3, Src activation and the protein expression of STAT3-associated molecules, including MMP-2, MMP-9, myeloid-cell leukemia 11, Bcl-xl, VEGF and Twist.

5. Summary and perspectives

Cancer is one of the most life-threatening diseases. With the increasing prevalence of cancer, the development of anticancer agents has become a key field of clinical and scientific research. Developments in medical science and technology have enabled the extraction of bioactive components from Traditional Chinese medicines for research due to their reported anticancer effects and lack of adverse reactions. ART has been demonstrated to be effective against leukemia, breast cancer, gastrointestinal tumors and other types of cancer (8,23,145). Importantly, since it is a drug that is already being used for the treatment of malaria, ART has a reliable safety record for clinical use. Although the amount of clinical data regarding the use of ART as an anticancer drug remains limited, preliminary results have been encouraging in terms of efficacy and tolerance (22). Combination therapy should be a key consideration in the future. In addition, development of modified derivatives of ART after structural modifications or modifying the treatment regimen to optimize the efficacy and toxicity profile are also possible directions for future research.

To conclude, existing information provides evidence supporting the use of ART as an anticancer agent. However, data from systematic *in vivo* animal and human studies are required to improve our understanding of the anticancer effects and mechanism of action of ART in the future.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the present study.

Authors' contributions

LL designed and supervised the study. JH, YZ and FW reviewed the references. XY wrote the manuscript. YZ, FW and JZ contributed to the table and figure. XY and LL revised the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing of interests

The authors declare that they have no competing interests.

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