Anticancer Potential of Artemisinin and its Derivatives

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Abstract. Apart from the long-established anti-malarial efficacy, artemisinin and its derivatives have also been proved to have anti-tumor potentials. In recent studies, it has been discovered that artemisinin exhibits much less or nearly no cytotoxicity to healthy cells compared to traditional chemotherapy. Accordingly, the medical application of artemisinin has been a main focus in scientific fields. However, while its inhibiting effects on tumor cells has been confirmed, the specific mechanisms of anti-cancer artemisinin still need to be explored. This article mainly focuses on artemisinin-induced apoptosis and angiogenesis, which are two significant methods to inhibit tumor development. Artemisinin and its derivatives majorly induce apoptosis in cancer cells via mediating RECK, regulating Wildtype P53, inducing ROS-dependent apoptosis and enhancing lysosomal function and lysosomal degradation of ferritin, and affecting mitochondrial pathway and Bim/Bcl-2 balance. Besides, by targeting the NF-kappaB pathway, suppressing the Akt/mTOR pathway, and mediating the Wnt/CaMKII Signaling Axis, DHA and ART effectively inhibit angiogenesis. The essay provides a comprehensive perspective of two mechanisms of artemisinin's efficacy. Still, clinical research are required to be done to further investigate its effectiveness in medical field, which is likely to become the future research direction.

Keywords: Artemisinin; apoptosis; angiogenesis.

1. Introduction

Being one of the major causes of human death, cancer has long known for its difficulty to cure. It is a disease of body cells. Cells usually divide and grow in a controlled way, but sometimes they develop abnormally and continue to grow. These abnormal cells form masses termed tumors. The meaning of the word "cancer" is that these cell populations continue to expand and will perhaps eventually spread to the whole body. In 2020, cancer will be the primary cause of death for around 10 million people worldwide or roughly one in six fatalities [1]. The annual death rate is rising due to various cancers, such as stomach, liver, lung, breast, and colon cancer.

A traditional Chinese herbal (TCH), artemisinin (ART) contains numerous derivatives. It has a range of anticancer properties, including suppression of angiogenesis, prevention of cell invasion and metastasis, damage to DNA, and ferroptosis. In addition to its anti-malarial behavior, research has indicated that artemisinin possesses anti-inflammatory, anti-viral, anti-cancer, and anti-parasitosis qualities both in vivo and in vitro. The function of ART has been widely studied, and it has been found that the probability of adverse reactions and the side effects produced by the human body are small, so it has application prospects in cancer treatment.

In recent studies, the induction of apoptosis and the inhibition of angiogenesis have been greatly studied as two main approaches to fight against cancer. Researchers have gained a better perspective of the mechanisms of ART's anti-tumor characteristics and got down to clinical application step by step. Apoptosis, the process of programmed cell death, is used during early development to eliminate unwanted cells. ART-induced apoptosis also exhibits anti-tumor potentials through regulating protein expressions and mediating organelles [2-6].

Angiogenesis is the biological term of the formation of new blood cells. During this process, the endothelial cells lining blood vessels achieve migration, proliferation, and differentiation. The process of tumor proliferation is crucial because blood vessels supply nutrients and oxygen to the tumor cells. As a promising candidate for reducing tumor angiogenesis, ARTs inhibit tumor angiogenesis via down-regulating growth factors and up-regulating inhibitory factors. Through both in vivo and in vitro experiments, researchers have found that Dihydroartemisinin (DHA) has the

potential to inactivate angiogenesis by a process that is associated with nuclear factor (NF)-κB DNA-binding activity in pancreatic cancer cells [7]. Besides, DHA also finds its way to induce endothelial cell autophagy by suppressing of autophagy signaling pathway [8]. Through these two primary mechanisms, DHA has become one of the most promising therapies in artemisinin cancer treatment.

By observing ART-treated growth of Choroidal melanoma (CM), another important derivative of artemisinin, artesunate, is also considered to possess inhibitory effect on tumor cells [9]. Common therapies of CM, radiotherapy and surgical resection have exhibited undesired outcomes after clinical application. Both of them stimulate angiogenesis of CM that is associated with the vascular endothelial growth factor (VEGF). At the same time, ARTs have showed its inhibitory effect on Wnt signaling pathway, downstream of VEGF. Techniques like transfection and siRNA are implemented in in vitro experiments to validate its suppression.

In light of new research, this essay explores the mechanisms of ART and its derivatives' anti-tumor effects. Furthermore, it emphasizes the significance and prospect of ARTs' anti-cancer therapies from the scientific angle, which provides a summative reference for future research.

2. The Induction of Apoptosis

Recent studies have shown that DHA, a derivative of artemisinin, can upregulate RECK levels in glioma cells, thereby inhibiting cancer cells through apoptosis. RECK is a membrane protein that inhibits tumors by inhibiting metalloproteinases and cell invasion. Current research has found that down-regulated expression of RECK has been detected in some cancers, such as cervical cancer and prostate cancer. The study also found that DHA treatment can reduce the viability of ovarian cancer cells, increase the rate of cell apoptosis, and affect related protein and gene levels. In addition, DHA can also weaken the viability, migration and invasion of ovarian tumor cells, and enhance cell apoptosis. These findings indicate that DHA induces apoptosis by regulating the expression of RECK and has potential inhibitory effects on some known cancers. It is inferred that ARTs and their derivatives have considerable application prospects in the field of cancer treatment.

Compared with normal cells, tumor cells encapsulate higher iron content which allows proliferation and active metabolism, making tumor cells natural target of artemisinin. The formed radicals are associated with protein alkylation and reactive oxygen species (ROS) increase, causing DNA damage. This leads to the induction of cell apoptosis and, therefore, the reduction of proliferation. One effective mechanism against cancer cells in human body is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). It leads to the induction of apoptosis in cells that are transformed by cancer without affecting normal cells. This mechanism has turned TRAIL into a promising cancer therapy. TRAIL results in apoptosis through binding with either death receptor 4 (DR4) or death receptor 5 (DR5). Therefore, in a recent study, DR4-specific TRAIL variant 4C7 and DR5-specific TRAIL variant DHER were implemented to mediate and weaken the effect of decoy receptors [3]. However, due to its multiple phases of the TRAIL signaling pathway, cancers like colorectal tumor have gradually become resistant to apoptosis induced by TRAIL. The regulation of death receptor expression is highly responsible of the mutation in the p53 and K-Ras status. Besides, the unnatural down regulation or inactivation of caspase-8 reduces death receptor's signaling inside cells. Other targets like the inhibition of c-FLIP and other proteins that function against cell apoptosis down-regulate the same mechanism as well. In order to address the resistance of cell apoptosis caused by TRAIL, researchers treated the cell lines of the colon cancer with artemisinin and its derivatives, which may help human body revert to its sensitivity. In the experiment, researchers found out that artesunate and DHA were better anti-cancer therapies than artemisinin itself. ART/DHA send out oxidative stress through the production of ROS in tumors. This leads to DNA damage, p53 activation, apoptosis, or cell death that is not a result of apoptosis. The final result of the whole research indicated that combining ART/DHA and DHER together led to an increase in cell death in HCT116. Therefore, ART/DHA-induced expression of DR5 in HCT116 causes stimulation of cell lines, eventually resulting in apoptosis indused by DHER.

Meanwhile, artemisinin and its derivatives have proved to be great candidates in the induction of apoptosis in cancer cells through ROS and ferritin, traditional and significant mechanisms in anti-tumor therapies. Recent findings imply that artesunate causes endothelial cells to undergo apoptosis through a p38MAPK-mitochondrial pathway that is reliant on iron and ROS [4]. Mainly in vascular endothelial cells, artesunate enhanced the ratio of Bax to Bcl-2 and decrease the potentiality of the membrane of mitochondria. ART also triggered cytochrome C release and cleaved caspases 9 and 3, indicating that the mitochondrial apoptotic pathway was engaged in this specific mechanism. Artesunate induced activation of p38MAPK, while specific inhibitors of p38MAPK restrained apoptosis induced by artesunate in endothelial cells. The expressions of reactive oxygen species (ROS) were found to be increased by artesunate treatment in the research. Treatment with ferrous salt resulted in up-regulated ROS levels and enhanced cytotoxicity of ART on endothelial cells. At the same time, the agent deferoxamine which chelates iron down-regulated ROS levels and attenuated apoptosis induced by ART. In addition, another research has revealed the anti-tumor behavior of ART is associated with the preferred accumulations of it in the lysosomes [5]. It leads to activation of lysosomes by assembling certain enzymes. Lysosomes that function in human cells play a vital role in tumor apoptosis induced by ART. The study indicates that lysosomes treated with ART are observed a functional activation, which stimulates the degradation of ferritin. Then, the total amount of lysosomal iron present in tumor cells rises, allowing ART to use them so that it can achieve a cytotoxicity on transformed cells.

It is also found that the mitochondrial pathway, as well as the Bim/Bcl-2 balance, plays a significant role in DHA-induced apoptosis [6]. Through the suppression of tumor cells' growth, researchers have found that DHA exhibited cytotoxic effect on cells in the T-47D breast cancer. This result could also be achieved by inhibiting the progress of the cell cycle and initiating the apoptotic mitochondrial pathway. Besides, Bcl-2-interacting mediator of cell death (Bim) is probably engaged in the mediation of signals sent by the process of apoptosis. Inside human body, there are mainly two kinds of cell apoptosis. The first one is majorly driven by tumor necrosis factor receptors (TNF) and Fas. The other one of the two is strongly associated with mitochondria, as well as the Bcl-2 family. Researchers have found that treated with DHA, tBid is largely activated, which leads to the induction of damage to mitochondria. Other results of DHA treatment include releasing cytochrome c and activating caspase-9. The stimulation of caspase-9 subsequently initiates the downstream effector caspase cascade, which eventually causes tumor cell death. Being essential to the start of tumor cell death, Bim stands for a BH3-only member of the Bcl-2 family that positively regulates cancer apoptosis. In the experiment, the Bim/Bcl-2 relationship became unbalanced as a result of the DHA therapy, which increased pro-apoptotic Bim expression and decreased anti-apoptotic Bcl-2 expression. Plenty of Bim either directly triggered Bak and Bax to start the mitochondrial cell death process, or it attached to Bcl-2 proteins and formed oligomers to trigger cytochrome c release.

3. Angiogenesis

3.1. Inhibition of Angiogenesis by DHA

Studies have found that DHA has a significant effect on inhibiting angiogenesis (the process of new blood vessel formation). Angiogenesis plays a crucial role in various physiological and pathological conditions, including tumor growth and metastasis. DHA has demonstrated its ability to inhibit angiogenesis by targeting the binding activity of NF-κB, a transcription factor involved in the regulation of angiogenesis-related genes. Studies have shown that the inhibitory effect of DHA on angiogenesis is time- and dose-dependent. It was observed that DHA treatment reduced cell proliferation and the formation of tubular structures, particularly in human umbilical vein endothelial cells, which are known to be involved in angiogenesis. Additionally, DHA has shown the potential to prevent the survival of pancreatic cancer cells to some extent. In vitro studies have shown that DHA significantly reduces the expression of pro-angiogenic gene products targeted by NF-κB, such as VEGF, IL-8, COX-2, and MMP-9. These proteins play a crucial role in promoting angiogenesis

[7]. In vivo studies further confirmed the inhibitory effects of DHA on cancer by reducing tumor volume, microvessel density, and downregulating the expression of pro-angiogenic gene products associated with NF-κB. Other studies support DHA's ability to inhibit angiogenesis. Different assays, including cell counting, migration assays, and tube formation on collagen gels, have shown that DHA effectively inhibits angiogenesis in a dose-dependent manner. In contrast, DHA was found to be more potent than artesunate, another compound with anti-angiogenic properties [8]. Therefore, DHA has demonstrated its potential as a potent inhibitor of angiogenesis. Its ability to inhibit NF-κB binding activity and reduce expression of pro-angiogenic gene products has been shown to be effective in inhibiting cell proliferation, tube formation, and tumor growth. These findings highlight the potential therapeutic applications of DHA in treating angiogenesis-related diseases, including cancer.

Another way in which dihydroartemisinic inhibits angiogenesis is by inducing endothelial autophagy via inhibiting the Akt/mTOR pathway [9]. As a significant feature of autophagosome formation, autophagy associated protein LC3 is transferred, and the cytoplasmic form LC3-I in the cytoplasm binds to the membrane to form LC3-II. Researchers have observed significant increase in the protein levels of LC3-II treated with DHA with an increase of dose and time, indicating DHA's significance in autophagy. This mechanism is achieved by effect on PI3K/Akt/mTOR signaling pathway, which is a classical pathway to regulate autophagy (Fig.1). Akt activity is increased at the beginning and is significantly inhibited by DHA treatment later on. However, after incubation with DHA for a long time, the activity of Akt was no longer affected, which was specifically manifested as phosphorylation of Akt in this experiment. mTOR is a downstream protein of the PI3K/AKT pathway. The decreased protein activity level after DHA treatment was associated with the occurrence of autophagy later, which became a sign that mTOR might be involved in DHA-induced autophagy. Rapamycin, which is an mTOR inhibitor, can be used to verify whether mTOR plays a role in DHA-induced autophagy. Rapamycin alone largely increased LC3-II protein levels, while there was no significant increase in LC3-II protein levels after rapamycin pretreatment with DHA. These results are evidence that mTOR mediates DHA induced autophagy.

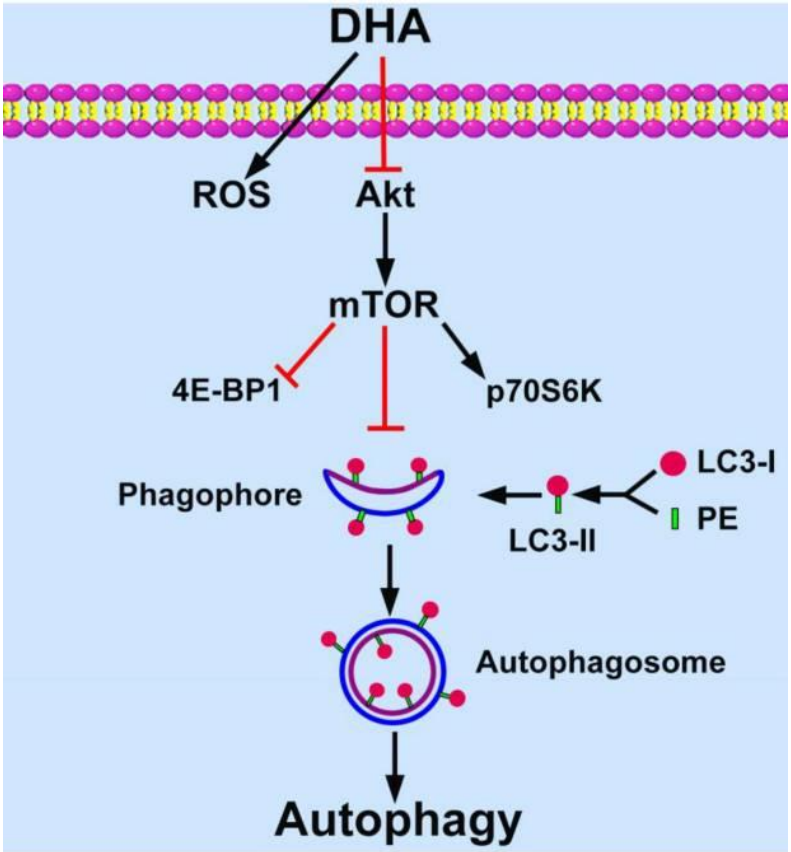

Fig 1. The mechanism of action of DHA in inducing autophagy [9].

3.2. Inhibition by ART

Although artesunate is inferior to DHA in a few cases, the significance of ART on cancer treatment cannot be ignored. Studies have shown that ART interferes with the Wnt5a/CaMKII signaling pathway to block VM formation in CM cells [10]. To analyze whether ART has inhibitory VM formation in CM through the Wnt5a/CaMKII signaling pathway, researchers used SiRNA technology to interfere with the expression of the Wn5a gene in CM cells, causing it to be specifically silenced. The results exhibited that transfection of Wnt5a siRNA was effective in reducing the level of Wnt5a protein. In addition, the expression of phosphorylated CaMKII transfected with Wnt5a siRNA was lower than in cells transfected with scrambled siRNA. These results showed that CM cells transfected with Wnt5a siRNA significantly reduced the number of newly formed tubes when compared with cells transfected with Scramble siRNA. (Figure 2).

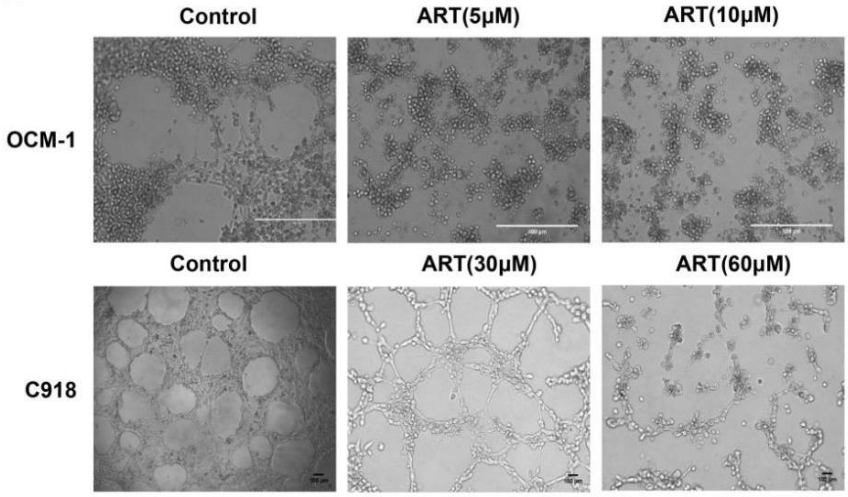

Fig 2. ART inhibited VM formation and angiogenesis [10].

4. Conclusion

Artemisnin has been widely studied not only as and effective anti-malarial therapy but also as a promising treatment for cancer. It possesses multiple efficacies like anti-viral and anti-parasitosis qualities. One of the significant ways to inhibit cancer development in human body is the induction of apoptosis by artemisinin and its derivatives. This process eliminates unwanted cells and therefore kills cancer cells to reduce uncontrollable proliferation. DHA plays a better role than artemisin itself and other derivatives in inducing apoptosis via mediating RECK. The combination of ART/DHA treatment induces expression of DR5 in HCT116, causing stimulation of cell lines. Among various pathways, this method effectively results in apoptosis induced by DHER. Two powerful derivatives, DHA and ART, induce ROS-dependent apoptosis and enhance lysosomal function and lysosomal degradation of ferritin. In addition, the mechanism of artemisinin's anti-tumor potentials is also involved with the mitochondrial pathway and Bim/Bcl-2 balance. Except for inducing apoptosis, artemisinin inhibits angiogenesis by targeting the NF-kB pathway and the Wnt/CaMKII signaling axis. Angiogenesis is also reduced by the suppression of the Akt/mTOR pathway. The investigations of mechanisms of artemisinin treatment serve as the foundation of medical application, while further research is required to explore other unknown mechanisms. This essay can be used as a comprehensive literature for medical application in terms of artemisinin-induced apoptosis and corresponding inhibition of angiogenesis. However, the mechanisms mentioned are mainly unique to certain types of cancers, which means more general patterns are expected to be explored. In conclusion, although there is a need for further investigations and clinical experiments before they are widely applied, there is no doubt that artemisinin and its derivatives have become promising therapy against cancer.

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