Original Paper

Artesunate Decreases β -Catenin Expression, Cell Proliferation and Apoptosis Resistance in the MG-63 Human Osteosarcoma Cell Line

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Key Words

Artesunate • Human osteosarcoma MG-63 cells • β -catenin • Apoptosis • Proliferation

Abstract

Background: This study aims to determine the effects of artesunate on proliferation, apoptosis and β -catenin expression in the human osteosarcoma cell line MG-63. **Methods:** MG-63 cells in the logarithmic growth phase were collected and cultured with different concentrations of artesunate (12.5 μ g/mL, 25 μ g/mL and 50 μ g/mL) for 24 h, 48 h and 72 h. The total number of MG-63 cells and the morphological changes were observed under an inverted microscope. The MTT assay was adopted to test the inhibition rate (IR) of cell growth. The apoptosis rate was detected using annexin V-propidium iodide (PI) staining. Cell cycle distribution was identified by flow cytometry, and the expression levels of β -catenin, cyclins and cyclin dependent kinases (CDKs) were measured using Western blotting. **Results:** The results of the MTT assay indicated that artesunate could remarkably inhibit MG-63 cell proliferation compared with the rate of the untreated control group (0 μ g/mL artesunate), and the inhibitory effect was dose-dependent. The apoptosis rate of MG-63 cells was elevated as the concentration of artesunate increased, and all the rates were significantly higher than that in the control group. Additionally, as the artesunate concentration increased, the proportion of MG-63 cells in G0/G1 phase gradually declined whereas that of cells in the G2/M and S phases increased. Western blotting confirmed that a higher concentration of artesunate reduced the expression levels of β -catenin, cyclin A, cyclin D1 and CDK1 and increased the expression levels of cyclin B. However, artesunate had no impact on CDK2 expression in MG-63 cells. **Conclusion:** These results demonstrated that artesunate can inhibit β -catenin expression and cell proliferation as well as promote cell apoptosis in MG-63 cells, which indicates that artesunate may serve as a promising drug in the clinical treatment of osteosarcoma.

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Introduction

As one of the most common primary malignant bone tumors in the world, osteosarcoma is a highly malignant mesenchymal-based tumor that mainly presents in children and teenagers [1, 2]. The annual morbidity of osteosarcoma is approximately 5 million cases worldwide [3] with prevalence towards males at a ratio of 1.5 : 1. The knee joint periphery is a common pathogenic site [4], and the main clinical symptoms include pain, swelling, dysfunction, weight loss and anemia [5]. To date, the pathogenesis of osteosarcoma remains unclear, but Ottaviani et al. reported that this disease was related to both environmental and genetic factors [6]. The combined use of neoadjuvant chemotherapy (NACT) and surgical resection is the current first-line treatment strategy for osteosarcoma, which has greatly improved the 5-year survival rate of osteosarcoma patients to about 80% [7-9]. However, the effectiveness of this therapy has achieved little improvement in the past 20 years. Recent evidence has shown that pulmonary metastasis is the leading cause of death among osteosarcoma patients, and toxic side effects as well as multidrug resistance to conventional agents were too pronounced to provide any benefit to some patients [10]. Therefore, it is extremely urgent to develop new anti-osteosarcoma drugs.

Natural agents, in combination with conventional treatments, are proved to be novel options for the treatment of cancers and some of natural agents such as allixin, curcumin and formononetin play a crucial role in anticancer activities [11]. Artesunate is a major artemisinin derivative extracted from *Artemisia annua* and is often used to treat malaria [11]. A previous report indicated that artesunate could inhibit the growth of various tumors and induce cell apoptosis [12]. Moreover, it was reported that concomitant administration of conventional chemotherapeutic agents and artesunate could significantly extend the lifespan of patients with metastatic uveal melanoma [13]. Another study also showed that the Wnt/ β -catenin signaling pathway was closely related to the formation, translocation and apoptosis of osteosarcoma [14]. β -catenin, a central molecule in the Wnt/ β -catenin pathway, exhibited an abnormal expression pattern in osteosarcoma and was associated with osteosarcoma translocation [15]. Reducing β -catenin expression and inhibiting the Wnt/ β -catenin pathway could be targets for osteosarcoma treatment. Nevertheless, since many previous studies have specifically focused on the role of dihydroartemisinin in osteosarcoma, and studies regarding artesunate and β -catenin in osteosarcoma are rare, this study aims to investigate the impact of artesunate on the proliferation and apoptosis of MG-63 cells as well as on β -catenin protein expression in osteosarcoma in a systematic and complete manner, with the goal of providing basic *in vitro* data for the clinical treatment of tumors using natural agent-based drugs.

Materials and Methods

Cell culture and grouping

The human osteosarcoma cell line MG-63 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), and then diluted (1 : 10) with Roswell Park Memorial Institute (RPMI) 1640 culture medium (Beijing Thermo Fisher Scientific Company, Beijing, China). These cells were suspended and then centrifuged at a low speed (1000 rpm/ min) for 8 min (Heraeus Company, Germany). After repeating the above steps 3 times, the cell pellet was re-suspended and added into a culture flask (Beijing Thermo Fisher Scientific Company, Beijing, China) for adherent growth in Dulbecco's Modified Eagle's Medium (DMEM) sugar nutrient solution (HyClone Company, Logan, UT, USA; item No. 31600-034) containing 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin; these cells were cultured at 37°C in an incubator with saturated humidity and 5% CO₂ (SHEL LAB Company, USA). These cells were passaged every 2 to 3 days with cells in the logarithmic growth phase collected for experiments. Artesunate (60 mg/strip; Shanghai Fosun Pharmaceutical Limited Company, Shanghai, China; No: H10930195) was obtained. A total of 100 μ g of artesunate was collected and dissolved in 1 mL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich Company, USA) to prepare 100 μ g/mL stock solution that was then diluted using RPMI1640

into different concentrations of working solutions (12.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$), which were defined as the A1 group, A2 group and A3 group respectively. The cells were cultivated for 24 h, 48 h and 72 h as appropriate, sterilized using ultrafiltration and preserved at -20°C . In addition, a group cultured without RPMI 1640 medium was used as the control group.

Observation of the number and morphological changes of MG-63 cells

MG-63 cells in the logarithmic growth phase were collected and seeded into a 24-well plate for culturing. After the cells attached, different concentrations of artesunate were added to the plate, and cells were cultured for 48 h, after which the morphology of cells treated with different concentrations of artesunate was observed under an inverted microscope (Olympus Co., Ltd, China).

MTT assay

MG-63 cells in the logarithmic growth phase were collected, digested using 0.25% trypsin, and then diluted into single-cell suspensions using phosphate-buffered solution (PBS). Cells were seeded into 96-well plates at 2×10^4 cells per well (100 μL per well) and then cultured in a incubator containing 5% CO_2 . After complete cell attachment, 100 μL of different concentrations of artesunate (12.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$) was added to the cells as appropriate, while no artesunate was added to cells in the control group. A total of 100 μL of RPMI 1640 nutrient solution was then added into wells, with each condition performed in duplicate. In addition, 2 wells in each 96-well plate only received 200 μL of nutrient solution as a blank control. The plates were taken out after the cells were incubated with artesunate for 24 h, 48 h and 72 h, and 20 μL of MTT solution (5 mg/mL) (Wuhan Boster Biotechnology Limited Company, Wuhan, China; item No. AR1156) was added into all wells. After another time of incubation for 4 h at 37°C , the nutrient solution in each well was discarded, and 150 μL of DMSO was added to each well. The plates were shaken on a shaking table for 8 min. When the crystals were fully dissolved, the blank wells were used to zero the plate reader. The optical density (OD) of each well was detected at 490 nm using an enzyme-linked immunosorbent assay (ELISA) to analyze the inhibition and survival rates of the cells. The formulas for these rates are: inhibition rate (IR) = (OD value in the control group - OD value in the treated group) / OD value in the control group $\times 100\%$; survival rate (SR) = OD value in the treated group / OD value in the control group $\times 100\%$.

Annexin V/propidium iodide (PI) assay

The density of the osteosarcoma cells in the logarithmic growth phase was adjusted to 1×10^6 cells/mL, after which the cell suspension was seeded into a 12-well plate (Becton Dickinson Company, USA). A total of 2 mL of cell suspension and different concentrations of artesunate at different concentrations were added into each well with the final artesunate concentration in each group as 0 $\mu\text{g/mL}$, 12.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$. After cells were incubated for 48 h at 37°C , 1 mL of cell suspension per well was collected and centrifuged at 1000 r/min for 10 min at 4°C , after which the supernatant was removed. Then, 1 mL of cold PBS was added to the cell pellet, which was shaken lightly to resuspend the cells. The cells were centrifuged at 1000 r/min at 4°C for 5 min, and the supernatant was removed. These wash steps were repeated twice. The cells were resuspended in 200 μL of PBS, to which 10 μL of fluorescein isothiocyanate (FITC)-labeled annexin V (Annexin V-FITC) (Beijing Biosea Biotechnology Co., LTD) was added. The mixture was gently mixed and incubated in the dark at room temperature for 15 min. A total of 300 μL of PBS and 5 μL of PI were then added (Promega Corporation, USA), and flow cytometry (FCM) was conducted to test the levels of apoptotic cells (FACS Calibur Company, USA).

FCM detection

Osteosarcoma cells (1×10^5 cells/mL) in the logarithmic growth phase were seeded in 24-well plates (Becton Dickinson Company, USA) at a volume of 1 mL per well. A total of 3 groups were established with each condition performed in triplicate. In addition, 1 mL of artesunate solution was added to each group to final concentrations of 0 $\mu\text{g/mL}$, 12.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$. Cells were cultured for 48 h at 37°C under saturated humidity with 5% CO_2 . After that, the cells were harvested, washed once with PBS, and centrifuged to remove residual PBS. The cells were then fixed with 70% ethanol at 4°C for 1 h, centrifuged and washed by PBS another 3 times, after which the PBS was removed via centrifugation. The plates were added to a 37°C water bath for 30 min after adding RNA enzyme. Subsequently, 800 μL of PI was added,

mixed with cell solution and reacted with cells at 4°C in the dark for 30 min. The distribution of cells throughout the cell cycle was detected using FCM.

Western blotting

MG-63 cells treated with different concentrations of artesunate for 48 h were collected and established as their respective treatment groups. The cell density was 1×10^7 cells per flask (Becton Dickinson Company, USA), and the group without artesunate was used as the control group. A protein extraction reagent (Wuhan Boster Biotechnology Limited Company, Wuhan, China) was used to extract the proteins, and a BCA kit (Wuhan Boster Biotechnology Limited Company, Wuhan, China) was applied to measure the protein levels. Extracted proteins were separated using polyacrylamide electrophoresis on a 10% gel (Wuhan Boster Biotechnology Limited Company, Wuhan, China) and transferred to a polyvinylidene fluoride (PVDF) membrane. When the membrane transfer finished, the membranes were placed on a horizontal shaking table and blocked for 1 h with PBS containing either 5% bovine serum albumin (BSA) or dissolved skim milk powder. The sealed PVDF membrane was removed and incubated at 4°C overnight with the following primary antibodies ($100 \mu\text{L}/\text{cm}^2$): including β -catenin diluted by 5% BSA (Beyotime Biotechnology Company, Shanghai, China; item No. AC106), cyclin A (ab181591, Abcam Company, USA), cyclin B1 (ab32053, Abcam Company, USA), cyclin D1 (ab16663, Abcam Company, USA), CDK1 (ab133327, Abcam Company, USA) and p38 (ab64333, Abcam Company, USA). The secondary antibody was diluted in 5% BSA (Wuhan Boster Biotechnology Co., Shanghai, China; item No. A7007) at a density of $100 \mu\text{L}/\text{cm}^2$ and was added to the membranes, which were then sealed and incubated on a shaking table at room temperature for 1 h. Equal volumes of detection reagents A and B were mixed in a petri dish with no strong light throughout the procedure. The PVDF membrane (Bio-Rad Company, USA) immersed in PBS was placed in ECL working solution for color development, and was scanned using the Bio-Rad GelDoc 2000 gel imaging system. Image analysis and processing were conducted by Image Lab. Gray values of protein bands were quantitatively analyzed, with the gray value of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as reference.

Statistical analysis

Data were analyzed using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). Measurement data are presented as the mean $\pm$ standard deviation. Comparisons of normally distributed measurement data between two groups were conducted using the *t*-test, and comparisons among multiple groups were determined using one-way analysis of variance (ANOVA). Numeric data are presented as either ratios or percentages, and the chi-square test was used to compare between two groups. $P < 0.05$ was considered statistically significant.

Results

Artesunate-induced damage of the morphology of MG-63 cells

Forty-eight hours after MG-63 cells were treated with artesunate at final concentrations of 0, 12.5, 25, 50 $\mu\text{g}/\text{mL}$, visualization under a normal inverted microscope showed that in the treated groups with an increasing artesunate concentration, the cells exhibited a worse growth status, the number of cells gradually decreased, the cell size was smaller and the number of floating dead cells was increased. However, in the control group, there were many cells, the cell nuclei were relatively larger, the cytosol area was plentiful, and cells were healthy (Fig. 1).

Table 1. IR of MG-63 cells after treatment with different concentrations of artesunate for 24 h, 48 h and 72 h, IR, inhibition rate; *, $P < 0.01$ in comparison with the control group

Group	Concentration ($\mu\text{g}/\text{mL}$)	IR at 24 h (%)	IR at 48 h (%)	IR at 72 h (%)
Control group	0	0	0	0
A1 group	12.5	$28.02 \pm 1.22^*$	$42.05 \pm 2.29^*$	$56.88 \pm 1.87^*$
A2 group	25	$51.89 \pm 3.29^*$	$58.87 \pm 2.72^*$	$69.48 \pm 4.26^*$
A3 group	50	$59.95 \pm 1.31^*$	$66.45 \pm 1.54^*$	$73.55 \pm 3.02^*$

Fig. 1. The morphology changes of MG-63 cells after treatment with different concentrations of artesunate for 48 h. Notes: A, the morphology observations of MG-63 cells in the control group; B, the morphology observations of MG-63 cells in the group treated with 12.5 $\mu\text{g}/\text{mL}$ artesunate; C, the morphology observations of MG-63 cells in the group treated with 25 $\mu\text{g}/\text{mL}$ artesunate; D, the morphology observations of MG-63 cells in the group treated with 50 $\mu\text{g}/\text{mL}$ artesunate.

Artesunate inhibits the proliferation of MG-63 cells

The MTT method was adopted to detect the role of artesunate in the proliferation of MG-63 cells *in vitro*, and the results shown in Fig. 2 indicate that artesunate could significantly inhibit the growth of MG-63 cells. As the concentration of artesunate increased, its inhibitory effect on cell proliferation was enhanced, with a dose-dependent inhibition within a certain period. Compared with the control group (0 $\mu\text{g}/\text{mL}$ artesunate), MG-63 cells treated with 12.5 $\mu\text{g}/\text{mL}$, 25 $\mu\text{g}/\text{mL}$ or 50 $\mu\text{g}/\text{mL}$ artesunate showed a significant reduced IR at 24 h, 48 h and 72 h (all $P < 0.01$) (Table 1). Using the sigmoidal model, the half maximal inhibitory concentration (IC_{50}) of artesunate at 24 h, 48 h and 72 h was 39.19 $\mu\text{g}/\text{mL}$, 27.51 $\mu\text{g}/\text{mL}$ and 22.91 $\mu\text{g}/\text{mL}$, respectively. Thus, these data suggest that artesunate could inhibit the growth of MG-63 cells.

Fig. 2. The viability of MG-63 cells after treatment with artesunate for 24 h, 48 h and 72 h.

Artesunate promotes the apoptosis of MG-63 cells

The results of annexin V/PI double staining show that the natural apoptosis rate of MG-63 cells in the control group was relatively low. After MG-63 cells were treated with 12.5 $\mu\text{g}/\text{mL}$, 25 $\mu\text{g}/\text{mL}$ or 50 $\mu\text{g}/\text{mL}$ artesunate for 48 h, their apoptosis rates were 23.91%, 33.75% and 52.97%, respectively, which indicates that with increasing artesunate concentrations, the cell apoptosis rate gradually increased (all $P < 0.05$). The apoptosis rate of MG-63 cells in the three groups treated with artesunate was significantly higher than that in the control group (all $P < 0.05$) (Fig. 3, Table 2).

Effect of artesunate on the cell cycle distribution of MG-63 cells

FCM detection of the cell cycle distribution of MG-63 cells treated with 0 $\mu\text{g}/\text{mL}$, 12.5 $\mu\text{g}/\text{mL}$, 25 $\mu\text{g}/\text{mL}$ or 50 $\mu\text{g}/\text{mL}$ artesunate for 48 h is shown in Fig. 4. The results indicate that as the artesunate concentration increased, the number of cells in G0/G1 phase gradually decreased, whereas that of cells in the G2/M and S phases gradually increased. In addition, the differences between the control group and the treatment groups were all statistically significant (all $P < 0.05$) (Table 3).

Fig. 3. The apoptosis rate of MG-63 cells after treatment with different concentrations of artesunate for 48 h. Notes: A, the apoptosis rate of MG-63 cells in the control group; B, the apoptosis rate of MG-63 cells in the group treated with 12.5 µg/mL artesunate; C, the apoptosis rate of MG-63 cells in the group treated with 25 µg/mL artesunate; D, the apoptosis rate of MG-63 cells in the group treated with 50 µg/mL artesunate.

Table 2. Apoptosis rate of the MG-63 cells treated with artesunate with different concentrations for 48 h. A1, the group treated with 12.5 µg/mL artesunate; A2, the group treated with 25 µg/mL artesunate; A3, the group treated with 50 µg/mL artesunate; *, P < 0.05 when in comparison with the control group; #, P < 0.05 when in comparison with the A1 group

Groups	Concentrations (µg/ml)	Apoptosis ratio (%)
Control group	0	6.10 ± 0.25
A1 group	12.5	23.91 ± 2.61*
A2 group	25	33.75 ± 1.85*
A3 group	50	52.97 ± 6.32*

Table 3. Cell cycle distribution changes of the MG-63 cells treated with artesunate with different concentrations for 48 h. Notes: A1, the group treated with 12.5 µg/mL artesunate; A2, the group treated with 25 µg/mL artesunate; A3, the group treated with 50 µg/mL artesunate; *, P < 0.05 when in comparison with the control group; #, P < 0.05 when in comparison with the A3 group

Groups	Concentrations (µg/ml)	G0/G1	S	G2/M
Control group	0	62.74 ± 1.75#	20.34 ± 2.11#	16.92 ± 0.77#
A1 group	12.5	49.12 ± 1.57**	28.14 ± 2.35**	22.73 ± 1.03**
A2 group	25	39.62 ± 2.25**	33.78 ± 0.98**	26.60 ± 1.62**
A3 group	50	20.80 ± 0.73*	38.94 ± 1.52*	40.25 ± 1.89*

Expression of β-catenin and cell cycle-related proteins in MG-63 cells after treatment with artesunate

Western blotting analysis indicated that at 48 h after treatment with different concentrations of artesunate, all the treatment groups showed decreased protein expression of β-catenin, and the β-catenin protein expression gradually fell as the artesunate concentration increased (Fig. 5). In addition, concomitant with increases of the artesunate concentration, the protein expression levels of cyclin A, cyclin D1 and CDK1 gradually decreased, whereas those of cyclin B1 gradually increased. Differences between the control

Fig. 4. Cell cycle distribution of MG-63 cells after treatment with different concentrations of artesunate for 48 h. Notes: A, cycle distribution of MG-63 cells in the control group; B, cycle distribution of MG-63 cells in the group treated with 12.5 $\mu\text{g}/\text{mL}$ artesunate; C, cycle distribution of MG-63 cells in the group treated with 25 $\mu\text{g}/\text{mL}$ artesunate; D, cycle distribution of MG-63 cells in the group treated with 50 $\mu\text{g}/\text{mL}$ artesunate.

Fig. 5. Protein expression levels of β -catenin in MG-63 cells treated with different concentrations of artesunate for 48 h. Notes: A, protein bands of β -catenin and GAPDH; B, relative expression of β -catenin in all the groups, GAPDH, glyceraldehyde-3-phosphate dehydrogenase; *, $P < 0.05$ compared with the blank control group.

group and the treatment groups were all statistically significant (all $P < 0.05$). However, the protein expression of CDK2 showed no significant differences among all the groups ($P > 0.05$) (Fig. 6).

Discussion

Artemisinin, a widely used medical herb derived from the sweet woodworm *Artemisia annua* to treat fever, has long been proven to be of great therapeutic value in the treatment

Fig. 6. Expression of cell cycle-related proteins in MG-63 cells treated with different concentrations of artesunate for 48 h. Notes: A, proteins bands of cyclin A, B1, D1, CDK1, CDK2 and GAPDH in all the groups; B, relative expression of cyclin A, B1, D1, CDK1, CDK2 and GAPDH in all the groups; C, cyclins and cyclin-dependent kinases; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. * $p < 0.05$ compared with the blank control group.

of diabetes, malaria, ferroptosis, atherosclerosis and other diseases [16-19]. Recent studies indicated that artesunate can induce high anti-tumor activity *in vitro* and *in vivo* as evidenced by its inhibitory effects on breast carcinoma [20], pancreatic carcinoma [22], prostatic cancer [23] and skin cancer [24]. Therefore, this study aims to determine the impact of artesunate on the proliferation and apoptosis of MG-63 cells as well as on β -catenin expression thus further expanding Chinese research on artemisinin-based therapies in the field of pharmacology. In addition, the results in this study showed that artesunate could reduce β -catenin expression, inhibit proliferation and promote apoptosis of human osteosarcoma cells, which makes artesunate a new and promising drug in the clinical treatment of osteosarcoma.

An *in vitro* experiment showed that artesunate could remarkably inhibit the proliferation of MG-63 cells in a dose-dependent manner [25]. Recently, Xu et al. demonstrated that the antitumor mechanism of artesunate was achieved by inhibiting cell cycle progression and inducing apoptosis of human osteosarcoma cells *in vitro* and *in vivo* [26]. In this study, FCM was used to detect changes in cell cycle progression, and it was found that as the artesunate concentration increased, the proportion of MG-63 cells in the G2/M and S phases gradually increased while the proportion of cells at G0/G1 phase gradually decreased, which indicated that artesunate inhibited the progression of cell cycle thereby restraining cell proliferation. Dong et al. also observed similar findings in a study regarding the *in vitro* inhibition of artemisinin and artesunate on the human breast carcinoma cell line Michigan cancer cell line-7 (MCF-7), which supports the notion that artesunate exerted stronger inhibitory effect on MCF-7 cells than artemisinin [20]. Additionally, artesunate could induce DNA double-bond breakage in tumor cells and down-regulate the expression of homologous recombination protein [27]. Homologous recombination protein is a key factor in the repair of homologous recombination, and down-regulated expression of this protein could decrease the repair capacity of tumor cells. Therefore, artesunate was likely to block cell cycle progression by enhancing the level of DNA damage and thereby achieving the inhibitory effect on tumor cell proliferation.

Cell apoptosis is also known as programmed cell death, and its occurrence is accompanied by complex signal transduction pathways and control mechanisms [28]. To date, it has been acknowledged that cell apoptosis occurs via regulation of mitochondria, death receptors and endoplasmic reticulum signals. A previous study have verified that formononetin, an important natural anticancer agent extracted from red clover plants,

has inhibitory effect on the proliferation of osteosarcoma cells and induces cell apoptosis [29]. Increasing evidence has found that artesunate was likely to induce apoptosis of HL60 promyelocyte leukemia cells and A549 lung adenocarcinoma cells by activating caspase-3 (which is mitochondria-dependent) and inhibiting *Survivin* gene expression. Efferth et al. also confirmed that artesunate induced apoptosis of T cells in leukemia mainly through the mitochondria-dependent pathway [30]. Thus, it could be suggested that artesunate might promote MG-63 cell apoptosis in a mitochondria-dependent manner, which was consistent with our results.

It was also indicated in this study that in MG-63 cells treated with artesunate, higher concentrations of the drug resulted in lower β -catenin expression levels. β -catenin is a central component of the Wnt/ β -catenin pathway [31]. When the Wnt signal pathway is activated, β -catenin will accumulate in cytoplasm, translocate to the cell nucleus and then form complexes with TCF/LEFs, thus activating the expression of various genes that play an important role in the proliferation, differentiation, apoptosis and tumorigenesis of cells [32]. When the Wnt/ β -catenin pathway is inactivated, less β -catenin will translocate to the cell nucleus, which may interrupt the abovementioned pathway and disrupt normal cell cycle progression [33]. In addition, the relationship between β -catenin and cyclins is also of great significance. Inactivated cyclin D1, a transcription factor of β -catenin, was found to contribute to deficiencies in calvarial ossification [34]. Zhang et al. also reported that the expression of β -catenin and cell cycle-related proteins was associated with proliferation in HuLM cells [35]. Accumulating studies have emphasized the role of the Wnt/ β -catenin pathway by investigating the expression of cyclins, CDKs and β -catenin proteins [36-40]. It was reported that when WIF-1 was added to renal carcinoma cells (RCCs), β -catenin expression was weakened, which reduced the activation of the Wnt signal pathway and consequently inhibit the proliferation of RCCs [41]. Moreover, Xu et al. found that the mechanism by which artesunate acts as a natural therapeutic agent for the treatment of myelodysplastic syndromes was its capability to inhibit the Wnt/ β -catenin signal pathway [42]. Our research results showed that artesunate could inhibit the proliferation of MG-63 cells by blocking β -catenin expression.

Conclusion

Artesunate can inhibit the proliferation and promote the apoptosis of MG-63 cells as well as reduce the expression of β -catenin. Furthermore, compared with traditional anti-tumor drugs, artesunate, which was extracted from natural products, exhibited advantages such as high safety and low drug resistance, lending itself as a promising valid drug for treating tumors. Nevertheless, this study was limited to *in vitro* experiments; therefore, the regulatory mechanism of artesunate on molecules in the Wnt/ β -catenin pathway still requires further investigation.

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Disclosure Statement

None.

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