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Artesunate attenuates the growth of human colorectal carcinoma and inhibits hyperactive Wnt/β-catenin pathway

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Artesunate (ART), a remarkable antimalarial agent, also inhibited the growth of human colorectal carcinoma. As determined by MTT assay, flow cytometry analysis on apoptosis and indirect immunofluorescence analysis on the proliferation-associated marker Ki67, ART suppressed the proliferation and promoted the apoptosis of colorectal cancer cells in a dose-dependent manner. Furthermore, immunofluorescence analysis on β-catenin and RT-PCR analysis on Wnt/β-catenin target genes demonstrated ART translocated β-catenin from nucleus to adherent junctions of membrane and reduced transcription mediated by β-catenin. These results suggested the anticancer activity of ART correlated with the inhibition of hyperactive Wnt/β-catenin signaling pathway. *In vivo*, ART significantly slowed the growth of colorectal tumor xenografts. Bioluminescent imaging also revealed that ART decreased the physiological activity of tumor xenografts and delayed spontaneous liver metastasis. These antitumor effects were related to the membranous translocation of β-catenin and the inhibition of the unrestricted activation of Wnt/β-catenin pathway, which was confirmed by the immunohistochemical staining of tumor tissues. These results and the known low toxicity are clues that ART might be a promising candidate drug for the treatment of colorectal carcinoma.

Key words: artesunate (ART); anticancer; colorectal carcinoma; Wnt/β-catenin pathway

Artemisinin and its derivatives such as artesunate (ART) distinguish themselves as a new generation of antimalarial drugs with no obvious adverse reaction or noticeable side effects.[1–4] Recently, it is reported that the antimalarial artemisinin drugs also have antitumor activity.[5–8] The well-accepted antitumor mechanism is similar to the antimalarial mechanism: the artemisinin molecule contains an endoperoxide bridge that reacts with an iron atom to form free radicals,[9,10] which causes macromolecular damage and cell death.[11]

However, the cytotoxic theory is not sufficient to explain all the antitumor effects of artemisinin drugs, such as effects on angiogenesis and cellular differentiation.[7,8,12,13] Therefore, investigators continued to explore the antitumor mechanism and found that the anticancer activity was associated with the basal mRNA expression of various genes.[13–15] We hypothesize that the expression of these genes could be regulated by ART through some genetic pathways, such as Wnt/β-catenin signaling pathway. The Wnt/β-catenin pathway is a powerful signaling pathway that plays a crucial role in cell fate determination, survival, proliferation and movement in variety of tissues.[16] Misregulation of the Wnt/β-catenin pathway links to various human cancers.[16] The central player of Wnt/β-catenin pathway is β-catenin.[16,17] It is a multifunctional protein that is located in at least 3 distinct cellular compartments and performs at least 2 separate biochemical functions.[16,17] Normally, β-catenin serves as a component of the cytoskeleton, participating in cell–cell adhesion at the plasma membrane.[16,17] In normal cells, free cytoplasmic β-catenin is rapidly phosphorylated, ubiquitinated and degradated.[16,17] In the development of cancer, the mutations of Wnt/β-catenin components decrease the normal ubiquitination and degradation of β-catenin protein.[16,17] The protein subsequently accumulates in the cytoplasm and translocates to the nucleus, where it binds with transcription factors TCF/LEF to activate transcription of various target genes that have important roles in regulating cell proliferation, apoptosis, differentiation, metastasis, and so on.[16,17] Colorectal and many other cancers are related to the hyperactive Wnt/β-catenin pathway.[18] Accordingly, disruption of this signaling pathway holds promise for development of new anticancer drugs.[18] Therefore, we want to identify whether artemisinin drugs could interfere with tumor growth by blocking the unrestricted activation of Wnt/β-catenin pathway.

In this report, we investigated the anticancer effects of ART *in vitro* and *in vivo* and addressed the question whether the anticancer mechanism of ART is related to the inhibition of the hyperactive Wnt/β-catenin pathway.

Material and methods

Cells and cell culture

Colorectal cancer cell line CLY was established from liver metastasis of a 64-year-old Chinese man with colon adenocarcinoma.[19] It was characterized with β-catenin nuclear localization, hyperactive Wnt/β-catenin pathway and spontaneous liver metastasis.[19] The CLY cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO_2.

Chemicals and reagents

ART was purchased from Gulin Pharmaceutical (Guangxi, China). It was prepared as stock solution in DMSO and diluted with culture medium or sodium chloride injection for *in vitro* study or *in vivo* study, respectively. Control solution contained DMSO at an equivalent (v/v) dilution to that used for the highest concentration of ART. RPMI 1640 medium, FBS, penicillin, streptomycin, trypsin/EDTA, TRIzol, lipofectamine2000 and G418 were purchased from Invitrogen (Carlsbad, CA). DMSO, 3-(4,5-dimethyl thizol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), propidium iodine (PI), RNase A and Hoechst 33342 were purchased from Sigma-Aldrich (St. Louis, MO). Mouse anti-human β-catenin monoclonal antibody, mouse anti-human Ki67 monoclonal antibody and fluorescein isothiocyanate (FITC)-conjugated secondary antibody were obtained from Zymed (San Francisco, CA). Mouse anti-human β-actin monoclonal antibody, the horseradish peroxidase-conjugated secondary antibody and the enhanced chemiluminescence (ECL) reagent were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Polyvinyldenefluoride (PVDF) membranes and BSA protein assay kit were purchased from Amersham Biosciences (Arlington Heights, IL). Strept

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avidin biotin complex (SABC) kit was obtained from Wuhan Boster Bioengineering (China). D-Luciferin was obtained from Biosynth International (Naperville, IL). Cyclophosphamide (CYP) was purchased from Powerdone Pharmaceutical (Shanxi, China).

Animals

Six-week-old female athymic nude mice (Balb/c nu/nu) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and housed in specific-pathogen-free conditions in conformity with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health.

Growth inhibition assay

CLY cells were seeded in 96-well plates at a density of 3,000 cells/well. After 24-hr culture in the normal growth medium, cells were exposed to graded concentrations of ART for 24, 48 or 72 hr, respectively. After each time point, the number of viable cells was measured by thiazolyl blue (MTT) assay.[8] Data were expressed as percentage of viable cell number at a certain time-point compared to viable cell number at time when ART was added (time zero in Fig. 1*a*). IC_{50} value (50% inhibitory concentration) was determined from plots of percent control cell survival *vs.* log ART concentration (Microcal Origin 5.0 software).

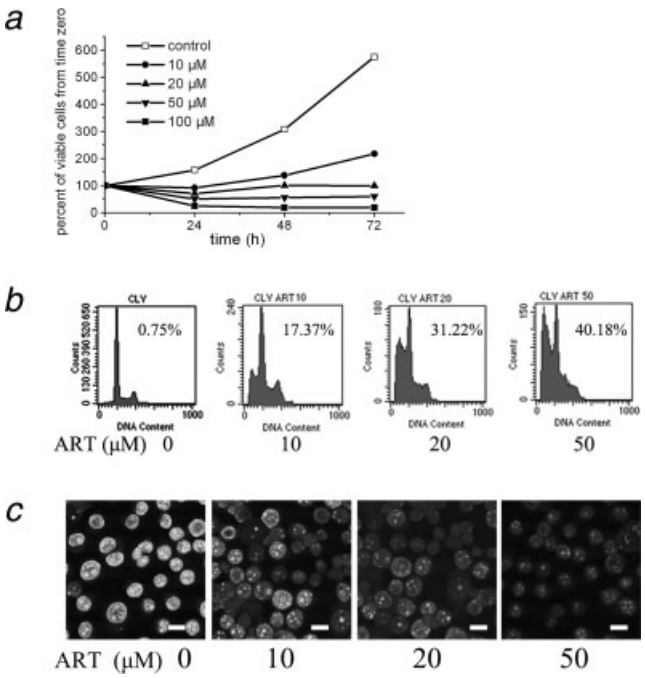

FIGURE 1 – Effects of ART on proliferation and apoptosis of CLY cells. (*a*) Different concentrations of ART were added to cell cultures at time zero, and the number of viable cells was measured by MTT assay at different times later. Data were expressed as viable cell number as percentage from that at time zero. (*b*) After 72-hr administration of ART, percent apoptosis was determined by flow cytometry analysis after staining with propidium iodide. A typical result out of 3 separate experiments is presented. The (subdiploid) peak to the left of the G1 peak represents apoptotic cells. Percentages of apoptosis are indicated. (*c*) Indirect immunofluorescence analysis of the proliferation-associated marker Ki67 was performed on CLY cells following 24-hr treatment with different concentrations of ART. The immunoreactivity of Ki67 decreased dramatically with increasing concentration of ART. Bar = 10 μm.

Analysis of cell apoptosis by flow cytometry

After incubation with different concentrations of ART for 72 hr, CLY cells were collected by trypsinization, washed with PBS and fixed in 70% ethanol at −20°C overnight. The cells were then centrifuged, washed with PBS and resuspended in PBS containing 50 μg/ml RNAase. After incubation, they were stained with 50 μg/ml of PI. The samples were analyzed using a FACSCalibur flow cytometer (Beckman Coulter; Miami, Florida) with an excitation wavelength of 488 nm. In the DNA histogram, the amplitude of sub-G1 DNA peak (the proportion of cells just before G1 phase) represents the number of apoptotic cells.

Indirect immunofluorescence analysis

CLY cells were grown on glass coverslips in 6-well plates. After ART treatment for 24 hr, the cells were washed with PBS, fixed with 4% formaldehyde, permeabilized in 0.3% Triton X-100 and blocked in 10% goat serum/PBS. The cells were then stained with anti-Ki67 or anti-β-catenin antibody. After a PBS wash, the cells were incubated with FITC-conjugated secondary antibody. The cells bound with anti-β-catenin were also counterstained with Hoechst 33342. Images were collected on a Bio-Rad Radiance 2100™ confocal system in conjunction with a Nikon TE300 microscope.

Western blot analysis

After CLY cells were incubated with different concentrations of ART for 24 hr, they were harvested in ice-cold PBS respectively. Total proteins were extracted and separated by SDS-PAGE and transferred onto PVDF membrane, as described previously.[20] The membranes were blocked with 5% nonfat milk and probed with primary antibodies against β-catenin and β-actin. After a PBS wash, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody, and the bands were visualized using the ECL system.

Reverse transcription-PCR analysis

C-myc and survivin mRNA expressions were evaluated semiquantitatively using RT-PCR. After CLY cells were incubated with different concentrations of ART for 24 hr, total RNA was extracted from cells using TRIzol™. RT-PCR analysis was conducted according to the method described previously[21] using the following primers: *c-myc* forward primer: 5′-CCCAGCGAGGACATCTGGAAGAA-3′, reverse primer: 5′-GAGAAGCCGCTCCACATGCAGTC-3′; *survivin* forward primer: 5′-AGCCCT TTCTCAAGGACCAC-3′, reverse primer: 5′-GCACTTTCTTCGCAGTTTCC-3′; β-*actin* forward primer: 5′-ACACTGTGTGCCCATCTACGAGG-3′, reverse primer: 5′-AGGGGCCGGACT CGTCGTCATACT-3′. To confirm the integrity of cDNA and to

TABLE I – ANTITUMOR ACTIVITY OF ART AGAINST CLY TUMOR XENOGRAFTS IN TRADITIONAL MURINE MODELS

Drug administration				Toxicity		Anticancer activity	
Drug	Dose (mg/kg)	Schedule	Route	Body weight loss (%)	Death	Tumor weight, mean ± SD (mg)	IR (%)
Control	–	Q3D × 7	i.v.	1.3	0/7	924 ± 177	–
ART	100	QD × 20	i.v.	7.5	0/7	597± 143*	35.4
ART	300	Q3D × 7	i.v.	4.6	0/7	457 ± 87*	50.5
CYP	100	Q7D × 2	i.v.	21.0	2/7	304± 87*	67.1

Q3D = every 3 days; QD = every day; Q7D = every 7 days. The significance of differences (*vs.* control) was determined by Student *t*-test. (*$p < 0.01$).

confirm equal loading on gels, the housekeeping gene β-*actin* was amplified concurrently.

Antitumor effect of ART in vivo

Experiment 1: traditional murine models. CLY cells (2×10^6) were injected subcutaneously into the right abdominal flanks of 28 nude mice. Tumor growth was measured with a slide caliper, and the volumes were estimated according to the following formula: Tumor volume (mm^3) = $L \times W^2/2$, where L is length and W is width. When the tumor volume reached about 100 mm^3, the mice were sorted into 4 groups ($n = 7$) with almost mean equal tumor sizes, and administration was started. Doses, schedules and routes of drug administration, including ART and the reference agent CYP, were shown in Table I. The experiment was stopped when the tumor volumes of control mice reached about 1,000 mm^3. At the end of the treatment, mice were killed for autopsy and the tumors were recovered and weighted. The tumor growth inhibitory rate (IR) was calculated as follows: IR (%) = [1 − (mean tumor weight of treated group)/(mean tumor weight of control group)] ×100. Toxicity was assessed by the lethality and body weight loss of nude mice. The significance of differences was determined by the Student *t*-test. In order to confirm the effects of ART on β-catenin localization on tissue levels, immunohistochemical staining of tumor tissues was carried out according to Wong *et al.*[22] SABC kit was used as a secondary reagent. Stainings were developed using DAB (brown precipitate). Slides were counterstained with hematoxylin.

Experiment 2: Bioluminescent imaging in vivo. CLY cells were cotransfected with plasmids expressing the firefly luciferase gene *luc* and antibiotic resistance gene *neo* (gift of Dr. Gang Liu) using lipofectamine2000, which were produced as previously described.[23] Stable transfectants were selected with G418 (800 μg/ml). The surviving colonies were screened for bioluminescence by IVIS™ camera system (Xenogen, Alameda, CA). CLY-*luc* cells characterized for stable luminescence expression were amplified and injected subcutaneously into the left abdominal flanks of 4 nude mice. These mice were sorted into 2 groups ($n = 2$): the control group and the ART treatment group (300 mg/kg, Q3D × 7). For *in vivo* bioluminescent imaging (BLI), animals were given the substrate D-luciferin, anesthetized and imaged by IVIS™ camera box as previously described.[23]

Results

Effects of ART on proliferation and apoptosis of CLY cells

ART inhibited the growth of CLY cells in concentration- and time-dependent manner by the MTT assay (Fig. 1*a*). The IC_{50} value for antiproliferative effect of ART at 72 hr was 20.34 ± 2.20 μM. Flow cytometry analysis also demonstrated the apoptosis rates of CLY cells increased with increasing ART concentration. In the DNA histograms, 17.37%, 31.22% and 40.18% of the cells treated with 10, 20 and 50 μM ART for 72hr, respectively, were in sub-G1 phase, which were higher than that of the control (0.75%) (Fig. 1*b*). According to the indirect immunofluorescence analysis, the immunoreactivity of Ki67, an important biological marker in cell proliferation, decreased dramatically with increasing concentration of ART (Fig. 1*c*).

Effect of ART on protein levels and subcellular localization of β-catenin

We were interested to determine whether the antitumor effects of ART described earlier were associated with changes in cellular content and localization of β-catenin. According to Western blot analysis, there were no significant changes in total β-catenin protein levels of CLY cells treated with ART (Fig. 2*a*). However, ART treatment was associated with a reduction in nuclear β-catenin immunofluroescence signal and a corresponding increase in membranous β-catenin localization. With the increase of ART concentration, nuclear β-catenin signal became weaker and mem-

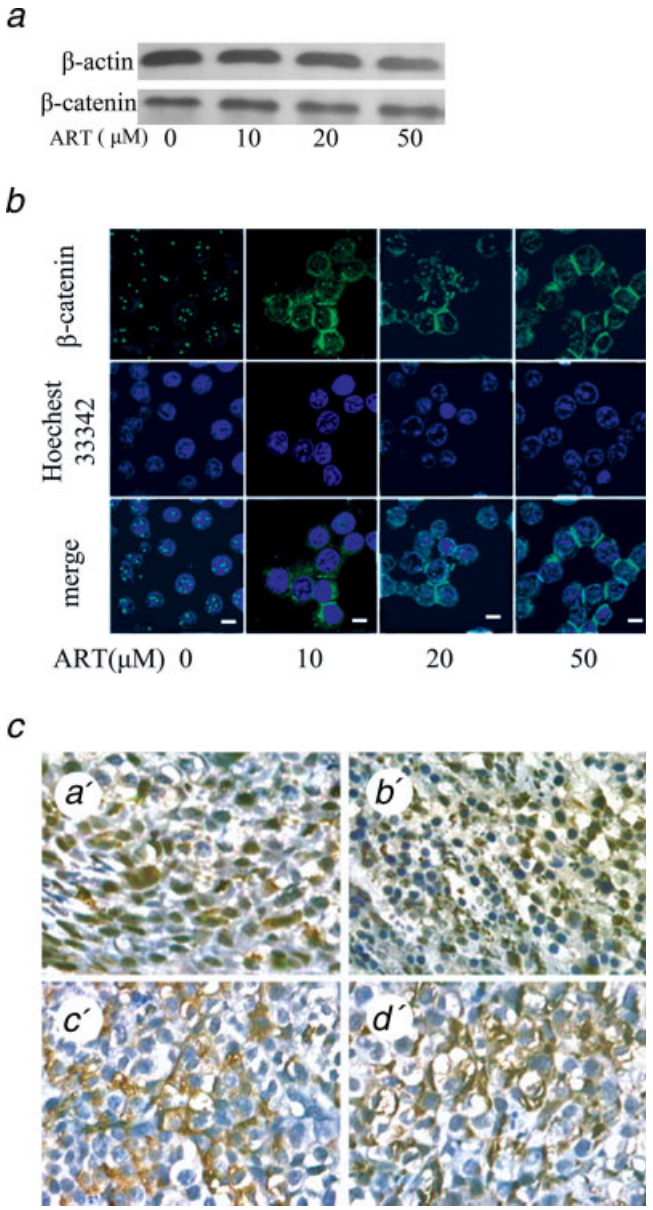

FIGURE 2 – Effects of ART on protein levels and subcellular localization of β-catenin. (*a*) Western blot analysis of β-catenin (92 kDa) and β-actin (42 kDa) protein expression in CLY cells was carried out after 24-hr incubation with different concentrations of ART. Expression of β-actin was used as an internal control. (*b*) Indirect immunofluorescence analysis of β-catenin in CLY cells was performed following 24-hr treatment with different concentrations of ART. β-Catenin labeling green and nucleus stained by Hoechst 33342 labeling blue. With the increase of ART concentration, nuclear β-catenin signal became weaker and membranous localization (especially at cell junctions) seemed to be more apparent. Bar = 10 μm. (*c*) Immunohistochemistry of tumor tissues from different treatment group showed different subcellular localization of β-catenin. Control group exhibited intense nuclear staining (*a′*); the reference agent CYP group also showed nuclear staining, although the pyknosis and karyorrhexis of nuclei were obvious (*b′*); persistent small dose treatment (100 mg/kg; QD × 20) of ART group demonstrated discrete membranous staining (*c′*); Intermittent large dose treatment (300 mg/kg; Q3D × 7) of ART group revealed visible cytoplasmic vacuolation and more prominent membranous staining (*d′*). (original magnification, ×220)

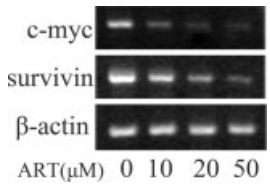

FIGURE 3 – Effects of ART on *c-myc* and *survivin* mRNA levels. RT-PCR analysis demonstrated that the decreases in *c-myc* and *survivin* mRNA were apparent in a concentration-dependent manner after 24-hr incubation with different concentrations of ART. β-actin was used as the loading control.

branous localization (especially at cell junctions) seemed to be more apparent (Fig. 2*b*).

Effects of ART on c-myc and survivin mRNA

We were then interested to determine whether the β-catenin membranous translocation induced by ART could inhibit transcription of the target genes of Wnt/β-catenin pathway, such as *c-myc* and *survivin*. RT-PCR analysis demonstrated the decrease in *c-myc* and *survivin* mRNA, which was apparent in a concentration-dependent manner after 24-hr incubation with different concentrations of ART (Fig. 3).

Antitumor effect of ART in vivo

Intermittent large dose treatment (300 mg/kg; Q3D × 7) and persistent small dose treatment (100 mg/kg; QD × 20) of ART exhibited significant antitumor effects with IR values of 50.5% and 35.4%, respectively. Although the antitumor activities of CYP (IR value of 67.1%) was superior to that of ART, the reference agent caused severe body weight loss and death of 2 nude mice on day 12 and day 14, respectively. It is worth mentioning that the ART-treated nude mice did not suffer from severe toxicity (Table I).

Furthermore, immunohistochemistry of tumor tissues confirm the effects of ART on β-catenin membranous translocation. Control group showed pronounced nuclear stain of β-catenin, while ART treatment group demonstrated weak nuclear staining and discrete membranous staining (Fig. 2*c*).

The bioluminescent murine models revealed more treatment information. The bioluminescent signals from the control mice continued to increase over time (Figs. 4*a* and 4*b*), which was consistent with the increasing mode of tumor volumes (Fig. 4*c*). However, the signals from ART treatment group (300 mg/kg; Q3D × 7) fluctuated in all the period of monitoring. Particularly in the later period, the bioluminescent signals declined significantly (Figs. 4*a* and 4*b*), while tumor volume continued to increase (Fig. 4*c*). We also found some bioluminescent signals from the liver of the control mice on day 23, which indicated that the control mice had developed liver metastasis, while there was no metastasis in ART-treated mice (Fig. 4*a*).

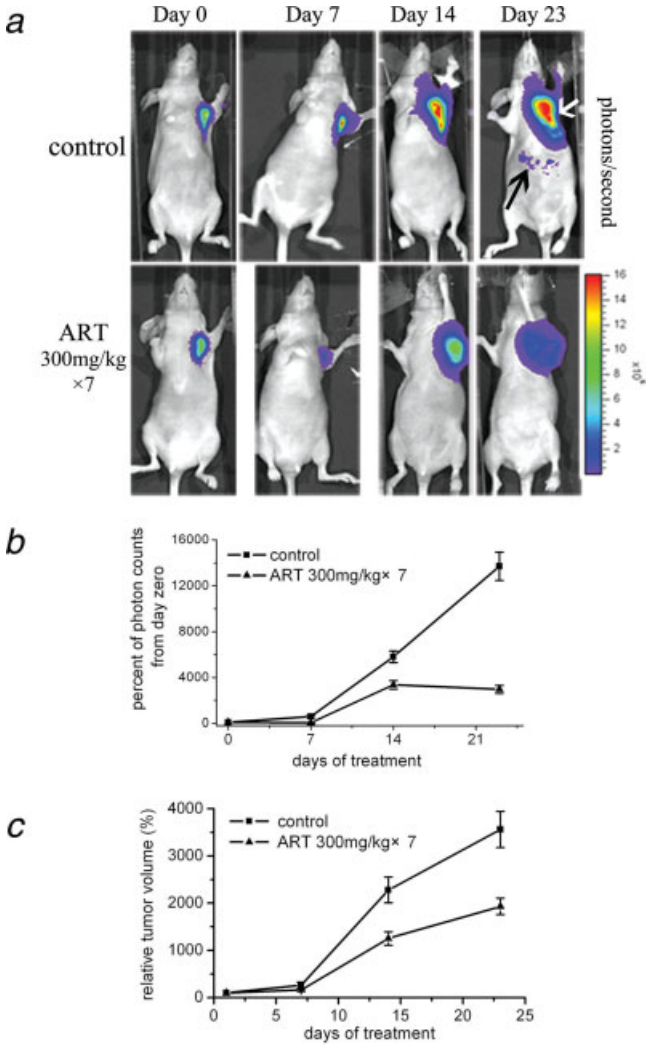

FIGURE 4 – Antitumor activity of ART against CLY xenografts in bioluminescent murine models. (*a*) Bioluminescent images of representative mice from control and ART-treated groups were shown from day 0 to day 23 after first administration. White arrow indicated primary tumor and black arrow represented liver metastasis. The images are representative of each group. (*b*) Bioluminescent data were expressed as photon counts as percentage from that on day 0. The results represent the mean value of each group. (*c*) The tumor growth was measured with a slide caliper and the relative tumor volumes were calculated by comparing the tumor volume on day *X* to the tumor volume on day 0. The results represent the mean value of each group.

Discussion

In the present investigation, we describe anticancer effects of ART on colorectal cancer cell line CLY. *In vitro*, ART suppressed the growth and promoted the apoptosis of CLY cells in a dose-dependent manner. Furthermore, the immunofluorescence analysis demonstrated that ART treatment made β-catenin translocate from nucleus to adherent junctions of membrane. Such translocation might promote apoptosis of CLY cells by inhibiting the hyperactive Wnt/β-catenin pathway. Up to date, we knew little about the effect of ART on β-catenin membranous translocation, but it suggested a novel explanation to the anticancer mechanism of ART. RT-PCR analysis further validated the inhibitory effect on the hyperactive Wnt/β-catenin pathway: ART treatment decreased mRNA levels of Wnt/β-catenin target genes, such as *c-myc* and *survivin*. The target genes *c-myc* and *survivin* are overexpressed in colorectal cancers.[24] Decreasing expression of the 2 genes could act together on the promotion of cell apoptosis and the inhibition of cell division.[25–28] In summary, ART treatment translocated β-catenin from nucleus to adherent junctions of membrane, which switched the function of β-catenin from prompting target genes expression to enhancing cells' adherence. Decreased transcription of crucial target genes might promote cell apoptosis and inhibit cell division, which produced obvious antitumor effects ultimately.

Moreover, we also demonstrate the antitumor effect of ART *in vivo*. ART slowed the growth of CLY xenografts. Although total dose of the 2 administration schedules was similar, the schedule of intermittent large dose treatment (300 mg/kg, Q3D × 7) was more efficient and safe than that of persistent small dose treat-

ment (100 mg/kg, QD × 20). It seemed that persistent administration could increase the potential side effect and drug resistance. In order to validate the anticancer effect of intermittent large dose treatment schedule, BLI was used to monitor *in vivo* chemotherapies of ART. BLI is a recently developed technique and used more and more in monitoring the physiological activity of tumor xenografts and the emerging of metastasis. There are several distinct advantages of BLI over traditional approaches (external tumor caliper measurement) to monitor tumors. First, BLI can reveal the physiological activity of tumor xenografts. BLI reflects the number of metabolically active tumor cells rather than a volumetric measurement of tumor mass, and so it may offer a closer assessment of treatment efficacy on tumor physiology than other detection methods.[23] Second, BLI is sensitive enough to detect micrometastases *in vivo*. Some researchers reported that spontaneous metastasis from a subcutaneous tumor can be detected by *in vivo* imaging as early as 2 or 3 weeks after primary tumor cells were implanted.[23] The BLI data demonstrated that photon counts of control mice increased notably and steadily with the development of tumor volumes. However, the fluctuation of photon counts of ART-treated mice was not consistent with the development of their tumor volumes. Particularly in the later period of the treatment, the tumor physiological activity of ART-treated mice declined, although the tumor volume continued to increase. Because of distinguishing physiological activity of subcutaneous tumor xenografts, spontaneous liver metastasis was not emerging in ART-treated mice but obvious in control mice. Another explanation to the antimetastasis effect is that ART treatment could induce the membranous translocation of β-catenin, which was confirmed by the immunohistochemical staining of tumor tissues. The membranous translocation of β-catenin enhanced cell adherence, and correspondingly decreased the cell migration.

Besides ART, some other established drugs also have important impacts on β-catenin signaling and strong antitumor activity against colorectal cancer cells.[18] These inhibitors of Wnt/β-catenin pathway includes indomethacin and aspirin (nonsteroidal antiinflammatory drugs); celecoxib and rofecoxib (selective cyclooxygenase-2 inhibitors); Glivec/Gleevec (protein tyrosine kinases inhibitor); endostatin (blood vessel formation inhibitor); and so on.[18] The molecular mechanisms of these Wnt/β-catenin signaling inhibitors range from relocalization of β-catenin to the plasma membrane, inhibition of β-catenin expression and induction of β-catenin degradation to disruption of β-catenin interactions with other proteins.[18] Therefore, the inhibition of hyperactive Wnt/β-catenin pathway is not a specific feature of these established drugs, including ART. However, all these Wnt/β-catenin signaling inhibitors could suppress the growth and promote the apoptosis of colorectal cancer cells.[18] The common features of these established drugs (inhibition of Wnt/β-catenin pathway and promotion of apoptosis of colorectal cancer cells) indicated that there may be some connection between the signaling pathway and regulation of apoptosis. Although several genes regulating apoptosis (including *survivin* and *c-myc*) were described to be activated by β-catenin, some investigators reported that initiation of the apoptotic cascade may not directly be attributable to the modulation of Wnt/β-catenin pathway.[29] Some researchers further observed that with an increasing percentage of apoptosis, β-catenin is processed by caspase-3.[30] Therefore, they presumed that the reduced β-catenin levels rather appeared to be a down stream effect of apoptosis.[30] However, anticancer effects of the aforementioned agents were restricted to cancer cells with hyperactive Wnt/β-catenin signaling,[18] which indicated that the inhibition of Wnt/β-catenin pathway was not simply a down stream effect or a bystander effect of apoptosis. Furthermore, the inhibitory effect on hyperactive Wnt/β-catenin pathway might be an accelerator of apoptosis, because the inhibition of the signaling can downregulate the expression of a series of proliferation and antiapoptosis genes, which may accelerate the apoptosis progress. Altogether, inhibition of hyperactive Wnt/β-catenin pathway might not initiate, but accelerate apoptotic cascade.

Apart from inhibition of Wnt/β-catenin signaling and promotion of apoptosis, the membranous translocation of β-catenin could enhance cell adhesion. It has been demonstrated that cell/cell or cell/matrix adhesion progresses are involved in chemoresistance.[31] Recent data indicate that inhibition of Wnt/β-catenin signaling pathway might affect cell adhesive function and chemosensitivity. Some investigators reported that, in epidermoid carcinoma cells, loss of catenins from cell surface after the cisplatin treatment destroyed the shape and functional integrity of cells, resulting in failure of entry mechanisms needed to bring agents into cells, which thereby resulted in resistance to the agents.[32] In addition, other researchers observed both inhibition of the Wnt/β-catenin pathway and enhancement of cell adhesion on leukemic cells triggered chemoresistance to anthracyclins through a GSK-3β/NF-κB-dependent pathway.[33] Therefore, β-catenin is not a simple marker for the response of cells to agents. Inhibition of Wnt/β-catenin pathway and enhancement of cell adhesion might act differently (triggering chemoresistance or enhancing chemosensitivity) to diverse agents in various cancer cells. As to the colorectal cancer cells with hyperactive Wnt/β-catenin signaling, we presume ART treatment might enhance the chemosensitivity of other chemotherapy (such as cisplatin treatment) by inducing membranous translocation of β-catenin, which remains to be determined.

In summary, ART had significant inhibitory effects on human colorectal carcinoma. *In vitro*, ART treatment strongly inhibited the hyperactive Wnt/β-catenin pathway and significantly promoted the apoptosis of CLY cells. *In vivo*, ART not only inhibited the volumetric development of tumor xenografts but also attenuated their physiological activity. These results and the known low toxicity are clues that ART might be a promising candidate drug for the treatment of colorectal carcinoma.

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