Paeonol exerts anti-tumor activity against colorectal cancer cells by inducing G₀/G₁ phase arrest and cell apoptosis via inhibiting the Wnt/β-catenin signaling pathway

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Received November 18, 2019; Accepted May 19, 2020

DOI: 10.3892/ijmm.2020.4629

Abstract. Paeonol is a simple phenolic compound isolated from herbal root bark, which has been reported to possess numerous biological and pharmacological characteristics, including a desirable anti-tumor effect. To date, the effect of paeonol against colorectal cancer (CRC) cells is yet to be fully elucidated. Therefore, the present study aimed to identify the underlying mechanism via which paeonol exerts its anti-tumor activity on HCT116 cells. After incubation with various concentrations of paeonol (7.8125, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml), the inhibitory effect of paeonol on cell viability was assessed using a Cell Counting Kit-8 assay. Cell apoptosis and cell cycle distribution were measured using flow cytometry. Moreover, caspase activity was measured using a colorimetric caspase assay. Luciferase assay was also used to determine the β-catenin-mediated transcriptional activity of T-cell specific transcription factor/lymphoid-enhancer binding factor (TCF/LEF), and western blotting analysis was performed to measure the related expression of proteins. The results indicated that paeonol exhibited a notable effect against HCT116 cells by inducing G₀/G₁-phase arrest, as demonstrated by downregulation of the cell cycle regulators cyclin D1 and cyclin D1 and upregulation of p21cip1 in a dose-dependent manner. Furthermore, paeonol dose-dependently induced cell apoptosis, accompanied by an increase in the Bax/Bcl-2 ratio, release of cytochrome c and further activation of caspases. Paeonol also dose-dependently blocked the activation of the Wnt/β-catenin signaling pathway by suppressing the expression of β-catenin, resulting in a decrease in β-catenin-mediated activity of TCF/LEF and downregulation of downstream target genes, including cyclin D1, survivin and c-Myc. Therefore, the present results suggested that paeonol exerted its anti-tumor effects on CRC cells, including the inhibition of cell proliferation, induction of cell cycle arrest and initiation of apoptosis, at least partly by suppressing the Wnt/β-catenin pathway, which may offer a promising therapeutic strategy for CRC.

Introduction

Colorectal cancer (CRC), a type of malignant gastrointestinal tumor, is the third leading cause of tumor-associated mortality worldwide (1). Moreover, in China, the CRC incidence in 2018 was 12.8% for men and 11.3% for women (2), and this rate is rapidly increases along with the development of the Chinese economy (3). Currently, the primary curative treatment for CRC is surgical resection; however, adjuvant chemotherapy has been incorporated to reduce high rates of adjacent tissue invasion and metastasis, thus decreasing the relapse rate (4). Due to the invariable incidence of drug resistance and serious side effects, including diarrhea, nausea, swelling, vomiting, abdominal pain, tiredness, low blood levels of albumin and other abnormalities, associated with standard anti-cancer drugs, the outcomes of chemotherapy and other effective measures are currently unsatisfactory for patients with CRC (5). Therefore, the investigation of novel treatment strategies with a safe profile that act via different signaling pathways is urgently required to develop improved targeted therapies.

Contrarily to traditional chemotherapeutic drugs, certain natural products, including flavonoids and jatrorrhizine (4), are considered to be potential candidates for neoplastic therapy on account of their substantial biological activities and relatively low adverse effects (6). Moreover, ongoing research for anti-cancer agents from medicinal plants has led to the examination of Traditional Chinese Medicine (7). As a simple phenolic compound extracted from the herbal root bark, paeonol (2'-hydroxy-4'-methoxyacetophenone) has substantial biological and pharmacological properties, including significant sedation, analgesic action, anti-inflammatory, anti-oxidation, anti-hypertension, neuroprotection and
immunomodulation (8, 9). In addition, paeonol has attracted increased attention in recent years due to its desirable anti-tumor effect against various types of cancer cell, both in vitro and in vivo, as revealed by its ability to inhibit cell proliferation and induce cell apoptosis (8, 10).

Previous studies have reported that several signaling pathways have important roles in the progression of cancer, including NF-κB, C-X-C motif chemokine ligand 4/C-X-C motif chemokine receptor 3B and the PI3K/Akt/NF-κB pathway (10-12). It has been shown that the role of the canonical Wnt signaling pathway is to regulate its downstream genes responsible for the cell cycle and cell survival (13). In addition, it is crucial to maintain homeostasis in multiple tissues throughout the body via the cell cycle and cell survival (13). In addition, it is crucial to maintain homeostasis in multiple tissues throughout the body via the cell cycle and cell survival (13).

Materials and methods

Major reagents. Paeonol (purity, >98%) was obtained from Sigma-Aldrich (Merek KGaA, cat. no. H35803) and the stock solution of paeonol in alcohol was diluted to obtain the required concentrations (7.8125, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml). RPMI-1640 medium and FBS were provided by Thermo Fisher Scientific, Inc. A Cell Counting Kit-8 (CCK-8) was obtained from Beyotime Institute of Biotechnology. The TRIzol® total extraction kit was from Invitrogen (Thermo Fisher Scientific, Inc.). Ribonuclease (RNase) and propidium iodide (PI) were purchased from Sigma-Aldrich (Merck KGaA). The Annexin-V-FITC/PI apoptosis detection kit was obtained from Beyotime Institute of Biotechnology. The Annexin-V-FITC/PI apoptosis detection kit was obtained from Beyotime Institute of Biotechnology. The Annexin-V-FITC/PI apoptosis detection kit was obtained from Beyotime Institute of Biotechnology.

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HCT116 cells/ml were incubated at 37°C with 0, 20, 40 and 80 µg/ml paeonol for a moderate period of time (48 h). After washing with ice-cold PBS, ice-cold cell lysis buffer was used to lyse cells for 15 min and then the supernatant was separated by centrifugation (12,000 g x 4°C for 10 min). The cell lysate was added to assay plates containing reaction buffer with 10 µl acetyl-Ile-Glu-Thr-Asp p-nitroanilide as a substrate for caspase-3, acetyl-Ile-Glu-Thr-Asp p-nitroanilide for caspase-8 or acetyl-Leu-Glu-His-Asp p-nitroanilide for caspase-9, followed by incubation at 37°C in the dark for 1.5 h. Finally, the A at 405 nm was measured with a microplate reader to quantify the formation of p-nitroanilide, and the relative increases of caspase-3, -8 and -9 activity were calculated by comparing the A of paeonol-treated HCT116 cells with the control group.

**TCF/LEF luciferase reporter assay.** The TCF/LEF dual-luciferase reporter assay was performed following the manufacturer's instructions with minor modifications. In brief, 1x10^4 HCT116 cells/well were seeded into 24-well microtiter plates and maintained in RPMI-1640 medium overnight at 37°C in an incubator with 5% CO₂ prior to transfection. After incubation at room temperature for 10 min, the mixture of 2 µg TCF/LEF reporter plasmid and 2 µl micropoly-transfecter was added to RPMI-1640 culture medium with HCT116 cells. Following the anti-biotic screening for 24 h, the transfection efficiency of HCT116 cells was performed by measuring the signals from TCF/LEF reporter (firefly luminescence). Then, the transfected cells were incubated at 37°C with either 0 (control), 20 or 80 µg/ml paeonol for 48 h. Finally, D-luciferin sodium at a final concentration of 15 mg/ml was added to each well to quantify the luciferase activity and the fluorescence images were acquired using the IVIS^®^ Spectrum system and Living Image® software (version 4.5; IVIS^®^ Spectrum; PerkinElmer, Inc.).

**Western blot analysis.** Following incubation with 20, 40 and 80 µg/ml paeonol at 37°C for 48 h, RIPA buffer was used to extract proteins from the harvested cells and then a BCA Protein Assay kit was used to determine the protein concentration in the supernatant after centrifugation at 12,000 x g and 4°C for 30 min. Aliquots containing 10 µg protein per lane were subjected to 10% SDS-PAGE and the separated proteins were transferred onto PVDF membranes (EMD Millipore). After blocking with a mixture of 5% skimmed milk/0.1% Tris-buffered saline containing 0.1% Tween-20 (TBST) at 25°C for ≥2 h, the membranes were probed with anti-CDK4, anti-p21^cip1^, anti-Bcl-2, anti-cytochrome c, anti-glycogen synthase kinase 3 β (GSK-3β), anti-c-Myc (all 1:1,000), anti-cyclin D1 (1:2,000), anti-survivin, anti-β-catenin (both 1:5,000) and mouse anti-β-actin (1:1,000) on a shaker table at 4°C for ≥12 h, followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (1:2,000) at room temperature for 2 h. After further rinsing with TBST three times, the membranes were visualized using an enhanced chemiluminescence substrate (Amersham; Cytiva). The intensity of each band relative to β-actin was determined semi-quantitatively using ImageQuant TL software (version 7.0; Cytiva) (21).

**Statistical analysis.** Data are presented as the mean ± standard deviation of ≥3 independent experiments performed in triplicate. Comparisons between two groups were analyzed with an unpaired Student's t-test, and one-way ANOVA followed by Tukey's post hoc test was performed to determine differences among ≥2 groups using SPSS 18.0 (SPSS, Inc.). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Paeonol reduces the number of viable HCT116 cells.** After incubation with paeonol for various intervals (12-72 h), it was demonstrated that paeonol significantly suppressed the proliferation of HCT116 cells, and the number of viable cells was inhibited with increasing concentrations of paeonol and the prolongation of incubation time (P<0.05; Fig. 1A). Moreover, the IC_{50} of paeonol was determined as 199.84 µg/ml at 24 h, 79.60 µg/ml at 48 h and 43.31 µg/ml at 72 h (Fig. 1B). Collectively, these results suggested that paeonol reduced the number of viable HCT116 cells in a dose- and time-dependent.

**Paeonol induces G0/G1-phase arrest in HCT116 cells.** As cell proliferation is closely regulated by the cell cycle (22), FCM was used to assess whether the effect of paeonol on inhibiting cell proliferation was due to induction of cell cycle arrest. Following incubation with 0, 20, 40 and 80 µg/ml paeonol for 48 h, the FCM results indicated that the proportion of HCT116 cells in G0/G1 phase was 53.42±2.14, 67.37±2.43 and 79.78±2.86%, respectively, which was significantly higher compared with the control group (38.68±1.96%; all P<0.05), demonstrating that paeonol dose-dependently induced a significant accumulation of HCT116 cells in G0/G1 phase. Furthermore, the cell cycle profile of HCT116 cells exposed...
to different doses of paeonol exhibited a distinctive broad sub-diploid DNA (sub-G1) peak at 48 h, which was significantly different compared with the control cells (Fig. 2A). The accumulation of HCT116 cells in G0/G1 phase was also accompanied by corresponding decreased percentages in the S and G2/M phases (Fig. 2A and B). In addition, the proportion of HCT116 cells in G0/G1 phase was time-dependently increased in the presence of paeonol (Fig. 2C), thus suggesting that paeonol dose- and time-dependently inhibited the proliferation of HCT116 cells by causing G0/G1-phase arrest.

Figure 2. Effect of paeonol causes cell cycle arrest in HCT116 cells. (A) After incubation with different doses of paeonol for 48 h, the cellular DNA content was analyzed using flow cytometry. (B) Histogram of cell cycle distribution in HCT116 cells exposed to different doses of paeonol for 48 h. (C) G0/G1 phase accumulation of HCT116 cells exposed to 40 µg/ml paeonol for various durations. *P<0.05, **P<0.01 and ***P<0.001 vs. control group (0 µg/ml); #P<0.05 vs. the groups at the time point of 12 and 24 h. PI, propidium iodide.
Paeonol induces apoptosis in HCT116 cells. To assess whether cell apoptosis was responsible for the reduction of viable cells following incubation with paeonol, an Annexin V-FITC/PI double staining assay with FCM analysis was used to monitor phosphatidylserine exposure. Following incubation with 20, 40 and 80 µg/ml paeonol for 24 h, the FCM results indicated that the apoptotic rate of HCT116 cells was 13.07±1.23, 17.13±1.65 and 30.97±2.01%, respectively, which was significantly higher compared with the control group 4.20±0.83% (all P<0.05; Fig. 3A and B), indicating that paeonol dose-dependently induced apoptosis of CRC cells. Moreover, the apoptotic rates increased with longer exposure time of paeonol (Fig. 3C).
Therefore, the results indicated that paonol dose- and time-dependently promoted apoptosis of CRC cells, which may be one of the underlying mechanisms of the anti-cancer activity of paonol.

**Paonol induces cell apoptosis via the caspase-dependent pathway.** Following incubation with increasing concentrations of paonol for 48 h, caspase-3 activity gradually increased in HCT116 cells and was higher compared with the control group (P<0.05). In addition, similar trends for caspase-8 and caspase-9 were observed in HCT116 cells exposed to paonol for 48 h (Fig. 4). Collectively, it was demonstrated that paonol dose-dependently increased the activity of caspase-3, -8 and -9.

**Paonol represses the β-catenin-mediated transcriptional activity of TCF/LEF.** To examine the effect of paonol on the activity of TCF/LEF mediated by β-catenin, a TCF/LEF luciferase reporter assay was performed. With increasing concentrations of paonol, the luciferase activity gradually decreased and there was a significant difference between the 20 µg/ml paonol-treated group and the control group (P<0.05). Furthermore, the luciferase activity following exposure to 80 µg/ml paonol was significantly weaker compared with the group treated with 20 µg/ml paonol (P<0.05; Fig. 5), suggesting that a high concentration of paonol significantly repressed the transcriptional activity of TCF/LEF.

**Paonol inhibits proliferation via the Wnt/β-catenin signaling pathway.** To elucidate the possible mechanism responsible for the inhibition of transition to the DNA synthesis phase, the cell cycle-associated proteins, including cyclin D1, CDK4 and p21cip1, which are able to promote cell cycle progression (23-24), were further investigated by western blot analysis. The protein expression levels of cyclin D1 and CDK4 were significantly downregulated, while p21cip1 expression was upregulated in HCT116 cells exposed to paonol for 48 h (Fig. 6). Thus, paonol may be able to cause G0/G1 phase arrest at least partly due to interference of the expression levels of the key G1-regulatory proteins CDK4, cyclin D1 and p21cip1.

To investigate the mechanisms underlying the anti-tumor effect of paonol against HCT116 cells, which were via inducing cell apoptosis, western blot analysis was used to detect the expression levels of apoptosis-associated proteins. Following incubation with 20, 40 and 80 µg/ml paonol for 48 h, the expression levels of Bax and cytochrome C were significantly upregulated, while those of Bcl-2 were significantly downregulated in HCT116 cells compared with the control group (P<0.05; Fig. 5). Moreover, the Bax/Bcl-2 ratio was elevated compared with the control group and was dose-dependently increased by paonol in HCT116 cells (P<0.05). Collectively, the results indicated that paonol induced cell apoptosis via increasing the Bax/Bcl-2 ratio in HCT116 cells.

To further elucidate whether paonol exerts its anti-tumor effect against HCT116 cells via the Wnt/β-catenin pathway, the protein expression levels of β-catenin, as well as its downstream signaling molecules cyclin D1, c-Myc and survivin proto-oncogene, were determined by western blot analysis. Following incubation with 20, 40 and 80 µg/ml paonol for 48 h, the expression levels of β-catenin, c-Myc and survivin were dose-dependently downregulated, while those of GSK-3β were dose-dependently upregulated in HCT116 cells compared with the control group (P<0.05; Fig. 6). In addition, paonol significantly inhibited the expression of cyclin D1 compared with the control group (P<0.05). Moreover, a possible mechanism responsible for the effects of paonol against human CRC cells is schematically presented in Fig. 7.

**Discussion**

Despite progress in systemic anti-cancer therapy, the effective treatment of CRC remains a major clinical challenge due to its high mortality and metastasis potential (25). Active components, including hydroxycarpin (15), aesculetin (16), baicalin (17), isobavachalcone (18), wogonin (20) and lyoncine (26), of Traditional Chinese Medicine formulations have attracted increased attention worldwide due to their unique advantages over western drugs in cancer treatment (26). For instance, experimental data have revealed that paonol has mild anti-tumor activities (10,27,28). In the present study, paonol time- and dose-dependently suppressed the viability of HCT116 cells, with an IC50 of 79.60 µg/ml at 48 h; these results are consistent with other CRC cell lines (LoVo and SW620) (29). Moreover, paonol has been shown to be a relatively safe medicine in mice with a median lethal dose of 3,430 mg/kg (30). Collectively, these experimental data suggest that paonol may be a novel candidate for anti-cancer therapy.

Cell proliferation is closely regulated by the cell cycle (22), and disruption of the cell cycle may inhibit cell proliferation and suppress tumor growth. The present results indicated that paonol induced an accumulation in the G0/G1 phase, accompanied by a concomitant decrease in S and G2/M phases in HCT116 cells, which is consistent with findings in other cancer cell lines (11,31). Cell cycle control is regulated by CDKs, cyclins and CDK inhibitors, such as p21cip1 and p27kip1 (23), and the activity of cyclin-CDK complexes may be affected by multiple signaling pathways (32). Furthermore, binding of cyclin D1 to CDK4 or CDK6 causes the formation of the cyclin D1-CDK4/6 complex, eventually driving cell transition from G1/S to S phase (33). In the present study, the expression levels of cyclin D1 and CDK4 were downregulated and that of p21cip1 was upregulated, subsequently blocking the cell cycle progression. Thus, it was hypothesized that
Figure 5. Effect of paeonol to interfere with T cell-specific transcription factor/lymphoid-enhanced factor activities. (A) Images of transfected HCT116 cells exposed to 0, 20 and 80 µg/ml paeonol in a 96-well dish were captured using the IVIS® Spectrum system (version 4.5 software). N1, N2 and N3 represent three experimental repeats. (B) Luciferase activities in the HCT116 cells exposed to various doses of paeonol for 48 h. **P<0.01 vs. control group (0 µg/ml); #P<0.05 vs. 20 µg/ml paeonol group. A.U., absorption units.

Figure 6. Effect of paeonol on the protein expression in HCT116 cells. (A) After incubation with different doses of paeonol for 48 h, the protein expression was detected by western blot analysis. (B) Quantification of cell cycle-coordinating proteins, apoptotic-associated proteins and GSK-3β, β-catenin, c-Myc and survivin proteins following exposure to different doses of paeonol for 48 h. (C) Ratio of Bax/Bcl-2. *P<0.05, **P<0.01 and ***P<0.001 vs. control group (0 µg/ml). GSK, glycogen synthase kinase; CDK, cyclin-dependent kinase.
and, ultimately, activation of caspase-3 (38). The present results release of cytochrome c, activation of the downstream caspase-9 fate of cancer cells (37). Bax causes mitochondrial disruption, receptor-mediated (extrinsic) apoptosis are the two principal metric caspase assay. Following incubation for 48 h, caspase-3, -8 and -9 activities of caspase-3, -8 and -9 were detected using a colori-
investigate which pathway was responsible for cell apoptosis, the
demonstrated an increase in the Bax/Bcl-2 ratio, via which the
reported that mitochondrial (intrinsic) and cell -surface death
Although the mechanisms of apoptosis are complex, it has been
displayed an increase in the Bax/Bcl-2 ratio, via which the
expression of Bcl-2 and increasing the expression levels of Bax,
showed that paeonol induces cell apoptosis via suppressing the
All of these results are consistent to a previous study, which
results suggested that the suppressive effect of paeonol on the
viability of CRC cells was associated with the induction of apoptosis via the caspase pathway.

Figure 7. Schematic illustration of possible mechanisms responsible for the effects of paeonol against human colorectal cancer HCT116 cells. Arrows represent promotion; red straight line represents inhibition; red dot line represents a decrease of inhibition. TCF/LEF, T cell-specific transcription factor/lymphoid-enhanced factor; GSK, glycogen synthase kinase; CDK, cyclin-dependent kinase; P, phosphate; AXIN1, axin 1.

paeanol exerted anti-proliferative effects by blocking cell cycle transition from G3 phase to S phase.

Induction of apoptosis is one of the most important and direct pathways that controls and eliminates cancer proliferation (11,34). The FCM results of the present study demonstrated that paeanol dose- and time-dependently induced cell apoptosis, which was in line with previous studies of CRC cells (29) and other cancer cells (11,31). In addition, HCT116 cells exposed to paeanol exhibited a distinctive broad sub-G1 peak at 48 h, the appearance of which is usually regarded as a result of the degradation of nuclear DNA in the early stages of cell apoptosis (35). Although the mechanisms of apoptosis are complex, it has been reported that mitochondrial (intrinsic) and cell-surface death receptor-mediated (extrinsic) apoptosis are the two principal pathways (36). Bcl-2 family proteins have a significant role in the intrinsic apoptotic pathway, during which the imbalance between pro- and anti-apoptotic proteins determines the ultimate fate of cancer cells (37). Bax causes mitochondrial disruption, release of cytochrome c, activation of the downstream caspase-9 and, ultimately, activation of caspase-3 (38). The present results demonstrated an increase in the Bax/Bcl-2 ratio, via which the apoptotic effect of paeanol on HCT116 cells was exerted. To investigate which pathway was responsible for cell apoptosis, the activities of caspase-3, -8 and -9 were detected using a colorimetric caspase assay. Following incubation for 48 h, caspase-3, -8 and -9 activities in HCT116 cells were enhanced with increasing doses of paeanol in comparison with those in the control group. All of these results are consistent to a previous study, which showed that paeanol induces cell apoptosis via suppressing the expression of Bcl-2 and increasing the expression levels of Bax, caspase-8 and caspase-3 (39). Furthermore, the aforementioned results suggested that the suppressive effect of paeanol on the viability of CRC cells was associated with the induction of apoptosis via the caspase pathway.

In most healthy cells, the Wnt pathway is commonly inactive and β-catenin is sequestered in the cytoplasm by a multi-protein complex containing axin 1 (AXIN1), APC regulator of Wnt signaling pathway, casein kinase 1 α and GSK-3β (40), resulting in a low level of β-catenin in the nucleus. Activated Wnt may cause the translocation of β-catenin from the cell cytoplasm to the nucleus, where it activates the β-catenin-mediated LEF/TCF transcriptional machinery, thus inducing the transcription of TCF/LEF-responsive genes, such as c-Myc (41) and cyclin D1 (42). Moreover, abnormal activation of the Wnt signaling pathway, a known hallmark of CRC, has been reported to be associated with cell proliferation, cell cycle and cell apoptosis, as a result of an activated canonical β-catenin and LEF/TCF pathway (43), in which suppressing the expression of β-catenin has a beneficial anti-tumor effect (44). In the present study, the expression level of β-catenin was significantly downregulated, while that of GSK-3β was upregulated by paeanol in a dose-dependent manner.

c-Myc, a downstream effector of the β-catenin pathway, has been revealed to be upregulated in ~30% of cancer cells and is associated with cancer progression (45). With the disruption of β-catenin/TCF activity, decreased c-Myc may cause the transcription of p21Cip1/Waf1, which in turn promotes cell cycle arrest at the G0/G1 phase and cell differentiation (46). Furthermore, cyclin D1, another downstream effector of the β-catenin pathway, exerts a vital role in regulating the cell cycle progression in different types of cells (47,48). The activated β-catenin signaling pathway may also stimulate the transcription of cyclin D1, which is an important marker for cells undergoing mitosis (49). The present findings identified the roles of c-Myc and cyclin D1 in cell cycle regulation, as demonstrated by the decreased TCF/LEF activity and concomitant downregulation of c-Myc and cyclin D1 protein. Apart from c-Myc and cyclin D1, survivin, an inhibitor of apoptosis, was identified as another target gene that is implicated in suppressing cell proliferation and regulating the cell life span (50). A previous study reported that downregulation of c-Myc, cyclin D1 and survivin may be an effective treatment strategy for CRC (51). Induction of TCF target gene transcription via activating the β-catenin pathway in CRC constitutes the primary transforming event, while TCF transcriptional activity may be reduced via suppressing the expression of β-catenin, subsequently followed by cell apoptosis via caspase-3 activation (52). The present results are in line with a previous study, reporting that blocking the interaction between β-catenin and TCF induced pancreatic cancer cell apoptosis via decreasing the expression levels of c-Myc and cyclin D1 (52). In addition, antagonism of Wnt/β-catenin signaling occurs at 20, 40 and 80 µg/ml paeanol to those required to suppress cell proliferation, block the cell cycle at G0/G1 phase and induce apoptosis in HCT116 cells. A schematic illustration of the possible mechanism underlying the anti-tumor activity of paeanol against CRC cells, including the induction of G0/G1 phase arrest and apoptosis via suppressing the Wnt/β-catenin signaling pathway is presented in Fig. 7. However, the lack of multiple cell lines to assess the present findings is a limitation of the current study. In addition, the suppressive effect of AXIN1 on Wnt signaling pathways and the intervention of Wnt/β-catenin signaling pathways on the cell cycle and apoptosis require further investigation in future studies.
In conclusion, the present results indicated that paeonol exerted an anti-tumor effect against CRC cells, which, at least partly, involved the blockage of the Wnt/β-catenin signaling pathway. Therefore, the current findings support the use of paeonol as a novel treatment for CRC, acting via distinct mechanisms. However, in subsequent studies, the results of the present study should be verified using multiple cell lines and the long-term effects of paeonol are required to be assessed in vivo.

Acknowledgements

Not applicable.

Funding

This study was financially supported by Jiangsu Provincial Administration of Traditional Chinese Medicine (grant no. YB2017099).

Availability of data and materials

All data generated or analyzed during the present study are included in this published article or are available from the corresponding author on reasonable request.

Authors’ contributions

LHL and RJS contributed to the conception and design of the study. LHL and ZCC performed the experiments and contributed to data analysis. LHL drafted the manuscript and RJS revised the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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