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Salidroside inhibits the growth, migration and invasion of Wilms' tumor cells through down-regulation of miR-891b

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ABSTRACT

Aims: Salidroside is a major functional component of *Rhodiola rosea* L. with a lot of pharmacological effects, including anti-tumor. The present work aimed to explore whether Salidroside could also exhibit anti-tumor functions in Wilms' tumor.

Main methods: WIT49 and RM1 cells were treated by various doses of Salidroside. CCK-8 assay, flow cytometry detection, colony formation assay, Transwell assay, RT-qPCR and Western blot analysis were conducted to measure WIT49 and RM1 cells proliferation, apoptosis, migration and invasion. The expression changes of miR-891b in response to Salidroside treatment were tested by RT-qPCR. Rescue assays were performed to test whether miR-891b was a downstream effector of Salidroside. Finally, the involvement of PI3K/AKT/mTOR and NF-κB signaling pathways was studied.

Key findings: Salidroside with concentration of 80 μM significantly reduced WIT49 and RM1 cells viability, survival capacity, migration and invasion, and significantly induced apoptosis. Meanwhile, down-regulation of Cyclin D1, MMP-2 and Vimentin, up-regulations of p53 and p21, as well as cleavage of caspase-3 and -9 were observed in Salidroside-treated cell. miR-891b was down-regulated by Salidroside. And Salidroside did not suppress WIT49 and RM1 cells growth, migration and invasion when miR-891b was overexpressed. Also, the deactivation of PI3K/AKT/mTOR and NF-κB pathways induced by Salidroside was reversed by miR-891b overexpression.

Significance: Salidroside inhibits Wilms' tumor cells growth, migration and invasion via down-regulating miR-891b, which leads to the deactivation of PI3K/AKT/mTOR and NF-κB signaling pathways.

1. Introduction

Wilms' tumor, also known as nephroblastoma, is one of the most common malignant tumors in children and infants, accounting for 7% of all pediatric tumors [1]. In 1899, Dr. Max Wilms, a German surgeon, firstly described the pathological characteristics of this tumor in detail, Wilms' tumor therefore named. From then on, scientists worldwide focused on investigating this tumor, but the etiology of Wilms' tumor is still unclear. It is generally considered that Wilms' tumor can be classified into three types: blastemal, epithelial, and stromal cell types [2]. Recent decades, remarkable achievement has been made in the treatment of Wilms' tumor [3,4]. Nephrectomy followed by radiotherapy and chemotherapy is usually recommended to treat this disease. However, for patients with tumor metastasis and recurrence, the five-year survival rate is still unsatisfactory [5].

Rhodiola rosea L. is a Chinese herbal medicine that is widely distributed in northern Europe, Asia and North America. *Rhodiola rosea* L. has been used as a natural remedy for ages to treat heart diseases, depression, and anxiety [6]. With the development of pharmacology, Salidroside ($C_{14}H_{20}O_7$, molecular weight 300.3) has been identified as one of the major functional components of *Rhodiola rosea* L. Salidroside has a lot of pharmacological effects including anti-apoptosis, anti-autophagy [7], anti-inflammation [8], anti-oxidative stress [9], neuroprotective properties [10], and anti-senescence [11]. Besides, multiple investigations have provided that Salidroside has certain anti-tumor effects in breast cancer [12], gastric cancer [13], skin cancer [8], ovarian cancer [14], colorectal cancer [15] and renal cell carcinoma [16]. However, the effects of Salidroside on Wilms' tumor have not been studied yet.

microRNAs (miRNAs) have been defined as a class of non-coding

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RNAs with length approximately 22 nt. miRNAs can contribute to almost all cell biological processes via post-transcriptional regulation. Not surprisingly, miRNAs are reported to be involved in the pathogenesis and progression of cancers [17,18]. And also, the aberrant expression of miRNAs and their oncogenic or tumor-suppressive functions have been reported in Wilms' tumor [19–21], suggesting the potential use of miRNAs in diagnose and treatment of Wilms' tumor.

In this study, WIT49 cell line which appears to represent an example of a parent tumor with epithelioid-like, spindle shaped and stromal-like phenotypes was used in this study [22]. WIT49 cells were treated by various doses of Salidroside for testing its anti-tumor functions. Also, whether the effects of Salidroside on WIT49 cells can be reproduced in RM1 cells were checked. Besides this, the regulatory role of Salidroside in miR-891b (a previously identified tumor biomarker [23,24]) expression was studied, in order to decode one possible underlying mechanism of Salidroside's function.

2. Materials and methods

2.1. Cell culture and treatment

WIT49 cells were kindly provided by Hospital for Sick Children (Toronto, Canada). RM1 cells were purchased from ATCC (Manassas, VA). WIT49 cells were cultured in DMEM:F12 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA), and 0.8% L-glutamine (Sigma-Aldrich). RM1 cells were cultured in DMEM medium (ATCC) supplemented with 10% FBS (Gibco). Both cell lines were maintained at 37 °C in a humid atmosphere with 5% CO_2.

Salidroside with purity ≥98% was purchased from Solarbio (Beijing, China). Salidroside with concentration of 0–100 μM was used to treat cells for 24 h.

2.2. Transfection

miR-891b mimic (sense, 5′-UGCAACUUACCUGAGUCAUUGA-3′; anti-sense, 5′-AAUGACUCAGGUAAGUUGCAUU-3′) and the scrambled negative control (NC) were purchased from GenePharma (Shanghai, China). miR-891b mimic or NC was transfected into the cultured cells with the mediation of Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA). At 48 h of transfection, the medium was replaced by DMEM:F12 medium with 10% FBS to stop the transfection.

2.3. CCK-8 assay

The transfected or untransfected cells were seeded in 96-well plates with 5×10^3 cells/well. After 12 h of incubation at 37 °C, the culture medium was replaced with 200 μL per well fresh medium containing 0–100 μM Salidroside. The cells were further incubated at 37 °C for 24 h to assay cell viability. After the indicated incubation, 20 μL Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD) was added into each well, and the plates were incubated for another 4 h at 37 °C. Finally, the absorbance was measured by a microplate reader (Bio-Rad, Hercules, CA) at 450 nm.

2.4. Apoptosis assay

The transfected or untransfected cells were seeded in 6-well plates with 5×10^5 cells/well and were treated by Salidroside for 24 h. Then, the cells were collected by trypsin (Invitrogen), and apoptotic cell rate of each sample was tested by using Annexin V-FITC Apoptosis Detection Kit (Beyotime, Shanghai, China). In brief, the collected cells were resuspended in 200 μL Annexin V-FITC Binding Buffer. 5 μL Annexin V-FIT and 10 μL PI were added in turn, and the sample was incubated at room temperature in the dark for 20 min. The sample was then immediately analyzed under a FACS can (Beckman Coulter, Fullerton, CA, USA).

2.5. Colony formation assay

The transfected or untransfected cells (500 cells/well) were treated by Salidroside for 24 h in 6-well plates. Then, the culture medium was replaced by fresh medium without Salidroside, and the cells were cultured for another 10 days. The culture medium was replaced every three days. Methanol was used to fix cells for 20 min at room temperature, and then the cells were stained by 0.1% crystal violet (Beyotime) for 10 min. The stained colonies were counted microscopically.

2.6. Transwell assay

A HTS Transwell® 24-well Permeable Support purchased from Corning (New York, USA) was utilized in this study for testing cell migration and invasion. For migration detection, the transfected or untransfected cells were pre-treated with Salidroside for 24 h and were placed in the upper side of the chamber with serum-free medium. The lower side of the chamber was filled with the complete medium. After 48 h of incubation at 37 °C, the cells in the upper side was removed by cotton swab, and the cells in the lower side was stained by crystal violet and counted microscopically.

For invasion assay, the HTS Transwell® 24-well Permeable Support was pre-coated with 100 μL Matrigel (Millipore, Bedford, MA) at 4 °C, overnight. The Matrigel was diluted in cold non-serum culture medium to obtain a final concentration of 5 mg/mL.

2.7. RT-qPCR

After the indicated transfection and treatment of Salidroside for 24 h, the cells were split to extract total RNAs by using Trizol reagent (Invitrogen). For the test of mRNAs, PrimeScript RT Master Mix and TB Green Fast qPCR Mix purchased from (Takara, Dalian, China) were used for cDNA synthetize and qPCR process. For the test of miRNAs, Taqman MicroRNA Reverse Transcription Kit and TaqMan MicroRNA Assay (Applied Biosystems, Foster City, CA, USA) were used. The primer sequences used in this process was listed in Table 1. Levels of miRNAs were normalized to β-actin, and miRNAs were normalized to U6. Data were analyzed by the $2^{-\Delta\Delta Ct}$ method.

2.8. Western blot

After the indicated transfection and treatment of Salidroside for 24 h, cellular protein was extracted by M-Per Mammalian Protein Extraction Reagent (Thermo Scientific, Carlsbad, CA). Equal amount of protein extracts were subjected to SDS-PAGE, and the separated proteins were transferred onto PVDF membranes (Millipore). The members were blocked in Blocking Buffer (Beyotime) for 1 h at room temperature

Table 1
Primer sequences used in the RT-qPCR analysis.

Gene name	Sequences
CyclinD1	Forward: 5′-AACTACCTGGACCGCTTCCT-3′ Reverse: 5′-CCACTTGAGCTTGTTCACCA-3′
p53	Forward: 5′-ATTCTGGGACAGCCAAGTC-3′ Reverse: 5′-TAGTTGTAGTGGATGGTGGTA-3′
p21	Forward: 5′-CTTCGACTTTGTCACCGAGA-3′ Reverse: 5′-GGTCCACATGGTCTTCCTCT-3′
β-actin	Forward: 5′-AATGTCGCGGAGGACTTTGAT-3′; Reverse: 5′-AGGATGGCAAGGGACTTCCTG-3′
miR-891b	Forward: 5′-ACACTCCAGCTGGGTGCAACTTACCTGAGT-3′ Reverse: 5′-TGGTGTCGTGGAGTCG-3′
U6	Forward: 5′-CTCGCTTCGGCAGCACA-3′ Reverse: 5′-AACGCTTCACGAATTTGCGT-3′

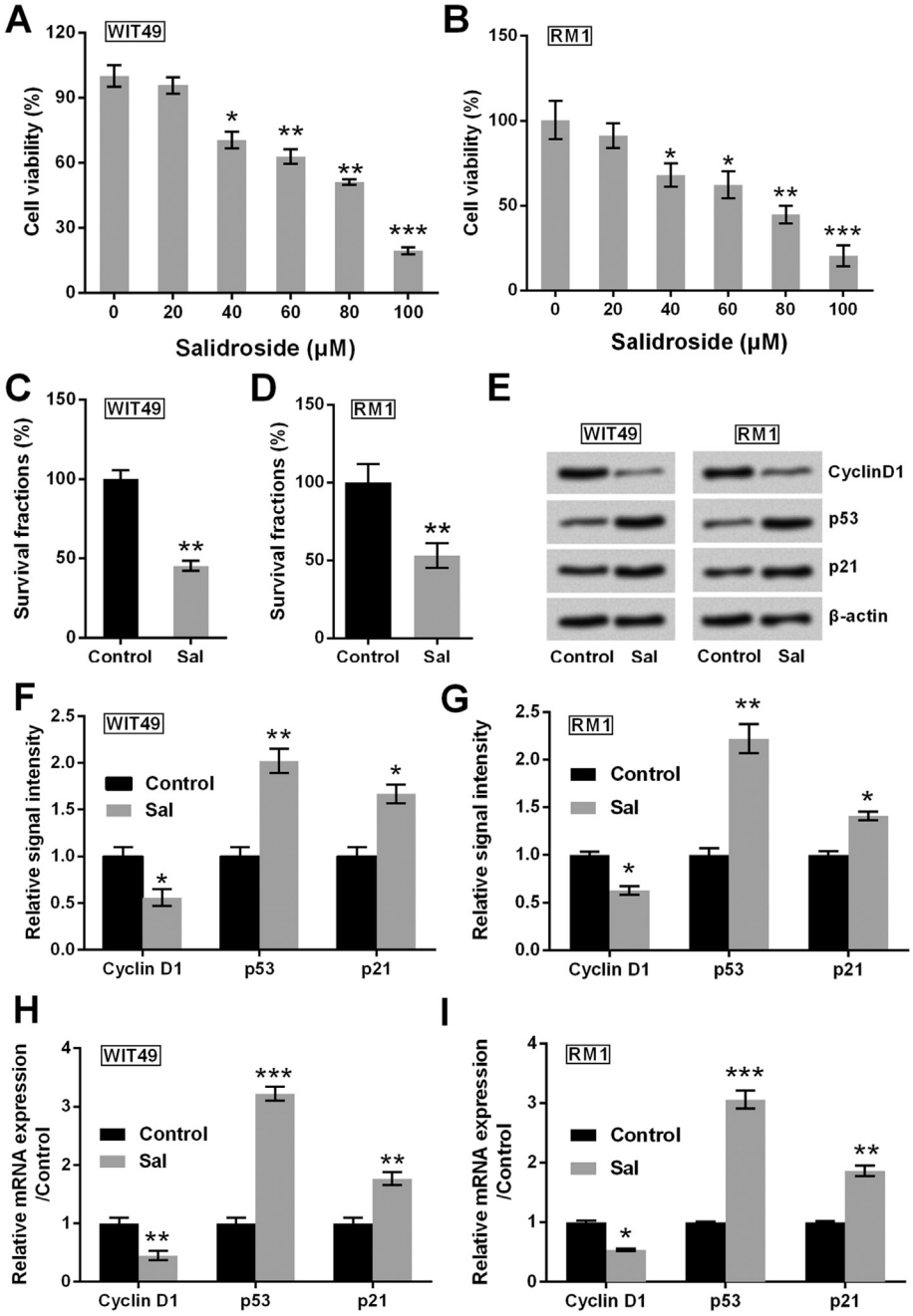

Fig. 1. Effects of Salidroside on the proliferation of cultured Wilms' tumor cells. The viability of (A) WIT49 and (B) RM1 cells was measured by CCK-8 assay, after treating with various doses of Salidroside for 24 h. (C–D) Survival fraction, and the expression changes of (E–G) protein and (H–I) mRNA levels of survival-related factors were determined by colony formation assay, Western blot and RT-qPCR, after treating with 80 μM Salidroside for 24 h. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

and incubated with primary antibodies at 4 °C overnight for the detection of Cyclin D1 (ab134175), p53 (ab131442), p21 (ab188224), pro capsase-3 (ab44976), cleaved caspase-3 (ab2302), pro caspase-9 (ab2013), cleaved caspase-9 (ab2324), MMP-2 (ab86607), Vimentin (ab8978), p65 (ab32536), p-p65 (ab86299), IκBα (ab32518), p-IκBα (ab133462), PI3K (ab191606), p-PI3K (ab182651), AKT (ab8805), p-AKT (ab38449), mTOR (ab2732), p-mTOR (ab109268), and β-actin (ab8227, Abcam, Cambridge, MA). After rinsing, the membranes were incubated in the secondary antibodies for 1 h at room temperature. The signal intensity was enhanced by EasyBlot ECL Kit (Sangon Biotech, Shanghai, China) and quantified by ImageJ 1.49 software (Bio-Rad).

2.9. Statistical analysis

Data represented as mean ± SD. Statistical analysis was performed by using SPSS 19.0 software (SPSS Inc., Chicago, IL). Student *t*-test and ANOVA were respectively conducted for the test of significant difference between two groups or more. A *p*-value of < 0.05 was considered as significant difference.

3. Results

3.1. Salidroside inhibited the proliferation of cultured Wilms' tumor cells

To start with, WIT49 and RM1 cells were treated by various doses of

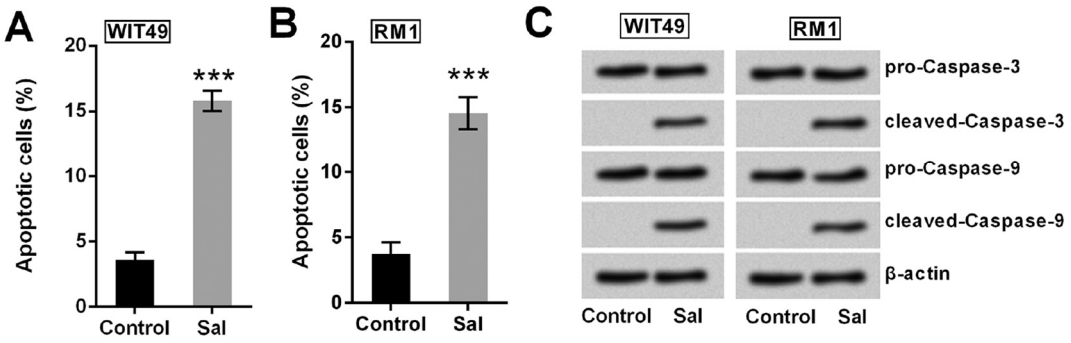

Fig. 2. Effects of Salidroside on the apoptosis of cultured Wilms' tumor cells. (A–B) Apoptotic cell rate and (C) expression changes of apoptosis-related proteins were determined by flow cytometer detection and Western blot, after treating with 80 μM Salidroside for 24 h. *** $p < 0.001$.

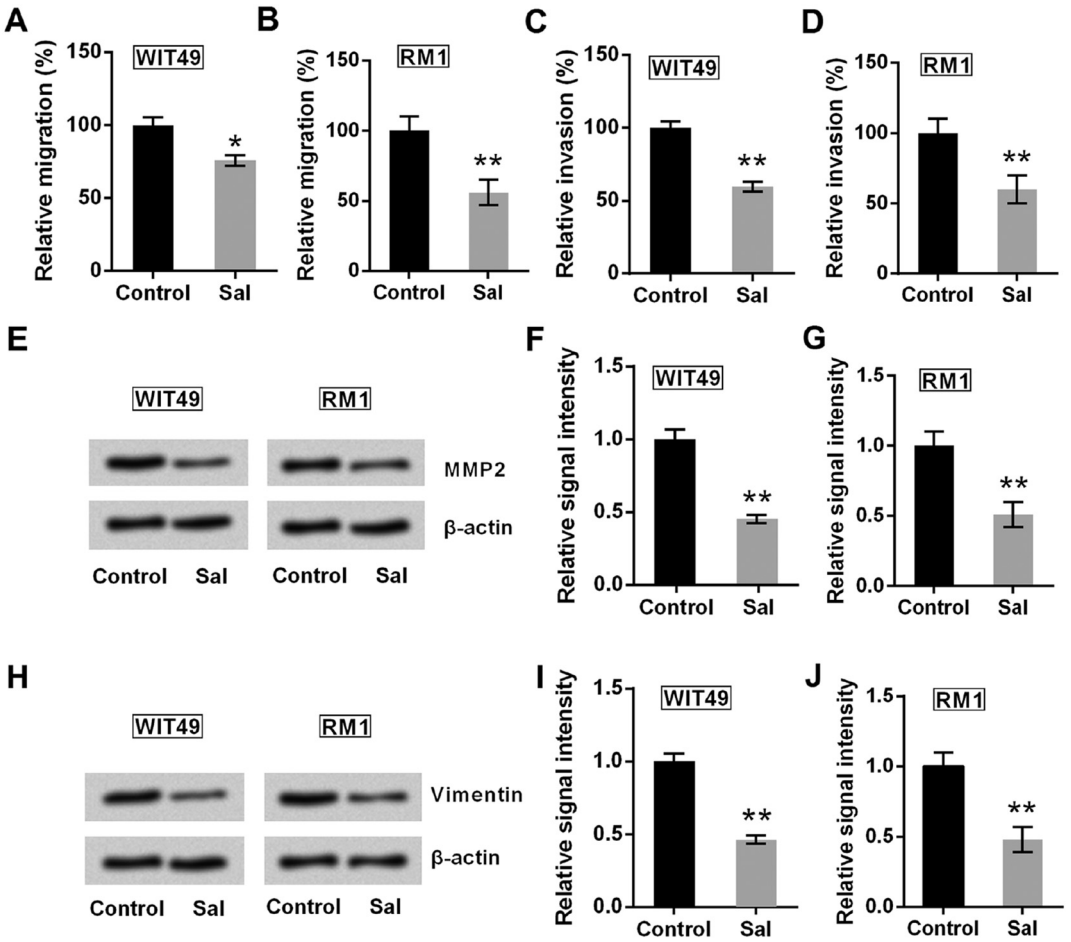

Fig. 3. Effects of Salidroside on the migration and invasion of cultured Wilms' tumor cells. (A–B) Relative migration, (C–D) invasion, and the expression changes of (E–G) MMP2 and (H–J) Vimentin proteins were determined by Transwell assay and Western blot, after treating with 80 μM Salidroside for 24 h. * $p < 0.05$, ** $p < 0.01$.

Salidroside for 24 h, and the changes of viability were monitored to see the cytotoxic effect of Salidroside on the cultured Wilms' tumor cells. As shown in Fig. 1A–B, the viability of WIT49 and RM1 cells was significantly reduced by Salidroside treatment with a dose-dependent manner. The IC50 value of Salidroside towards WIT49 and RM1 cells was 73.98 and 70.83 μM respectively; thus 80 μM was selected as a Salidroside-treating dose for use in the follow-up experiments. By performing colony formation assay, we found that the survival fraction was significantly reduced by Salidroside when compared to the control (untreated) group ($p < 0.01$, Fig. 1C–D). Besides this, Fig. 1E–I showed that the protein and mRNA levels of Cyclin D1 were down-regulated, while p53 and p21 levels were up-regulated in Salidroside group ($p < 0.05$, $p < 0.01$ or $p < 0.001$), as compared to the control group. These data suggested the anti-proliferating effects of Salidroside on cultured Wilms' tumor cells.

3.2. Salidroside promoted the apoptosis of cultured Wilms' tumor cells

The effects of Salidroside on the apoptosis of WIT49 and RM1 cells were tested by performing flow cytometry detection. Data in Fig. 2A–B showed that Apoptotic cell rate was significantly increased in Salidroside group, as compared to control group ($p < 0.001$). The induced apoptosis was further confirmed by Western blot analysis. As shown in Fig. 2C, caspase-3 and -9 were both remarkably cleaved in Salidroside

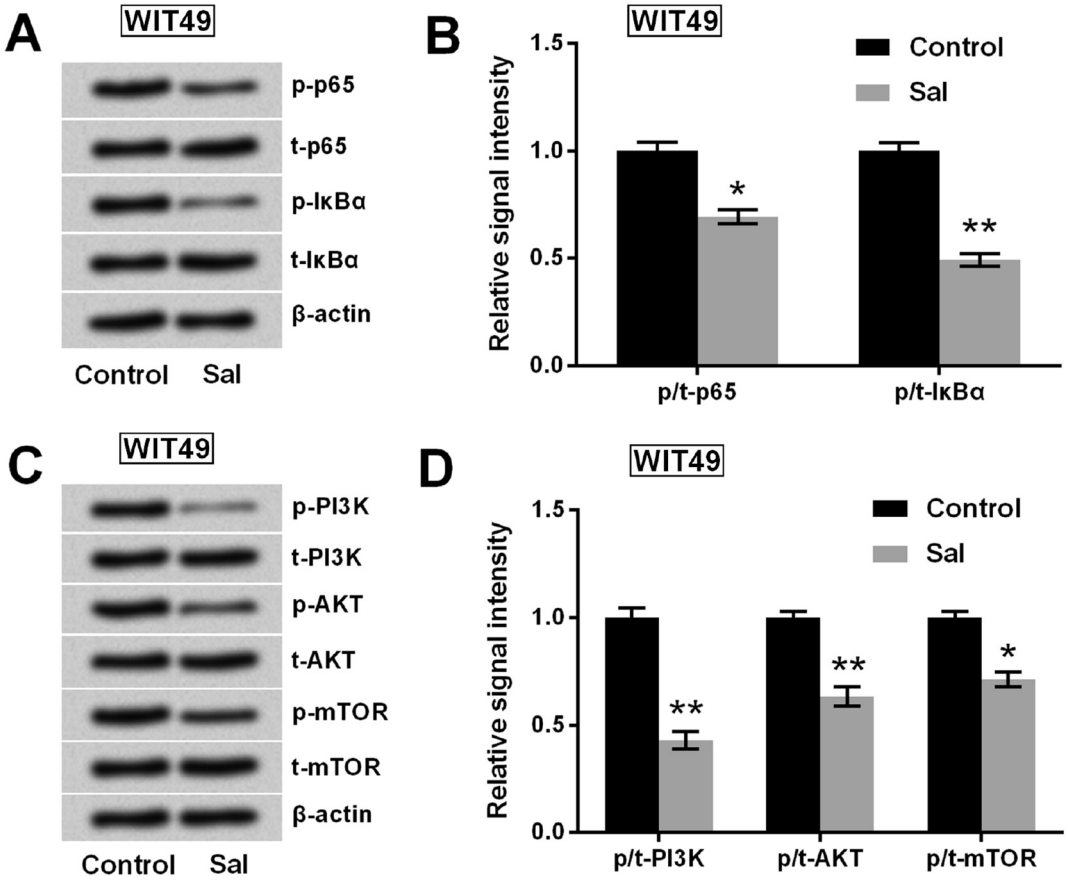

Fig. 4. Effects of Salidroside on NF-κB and PI3K/AKT/mTOR signaling pathways. Expression levels of core proteins in (A–B) NF-κB and (C–D) PI3K/AKT/mTOR signaling pathways were tested by Western blot, after treating with 80 μM Salidroside for 24 h. * $p < 0.05$, ** $p < 0.01$.

group compared to control group. All these suggested the pro-apoptotic effects of Salidroside on cultured Wilms' tumor cells.

3.3. Salidroside inhibited the migration and invasion of cultured Wilms' tumor cells

Next, whether Salidroside could impact the migratory and invasive capacities of WIT49 and RM1 cells were explored by using Transwell assay. Fig. 3A–D displayed that both the relative migration and invasion were significantly reduced by Salidroside when compared to control group ($p < 0.05$ or $p < 0.01$). By testing the expression changes of migration- and invasion-related proteins, we found that MMP-2 and Vimentin protein levels were significantly down-regulated by Salidroside ($p < 0.01$, Fig. 3E–J), further suggesting the inhibitory effects of Salidroside on the migration and invasion of cultured Wilms' tumor cells.

3.4. Salidroside repressed NF-κB and PI3K/AKT/mTOR signaling pathways

NF-κB and PI3K/AKT/mTOR are two known signaling pathways which are involved in regulating Wilms' tumor cells proliferation, apoptosis, migration and invasion [25–27]. Thus, we further investigated whether Salidroside impacted WIT49 cells via regulating these two signaling. Fig. 4A–D showed that, the phosphorylation levels of p65, IκBα, PI3K, AKT and mTOR were all significantly decreased in Salidroside group as compared to control group ($p < 0.05$ or $p < 0.01$). The total levels of these protein were unaffected by Salidroside. These data suggested that Salidroside treatment could repress the activation of NF-κB and PI3K/AKT/mTOR signaling pathways.

3.5. Salidroside down-regulated miR-891b to suppress Wilms' tumor cells growth, migration and invasion

Recently, miR-891b has been identified as a biomarker for several cancers, like gastrointestinal stromal tumor [23] and pancreatic cancer [24]. This work presented that miR-891b was significantly down-regulated by Salidroside ($p < 0.01$, Fig. 5A–B). In order to further reveal whether miR-891b was a downstream effector of Salidroside, the expression of miR-891b in WIT49 and RM1 cells was overexpressed by mimic transfection ($p < 0.001$, Fig. 1C–D). We found that transfection of cells with miR-891b mimic significantly attenuated Salidroside-induced viability loss ($p < 0.05$, Fig. 5E–F). Besides this, the expression of CyclinD1 was up-regulated, while p53 and p21 was down-regulated by transfection with miR-891b mimic (Fig. 5G).

Also, the changes in cell apoptosis, migration and invasion were checked following transfection. As data shown in Fig. 6A–C, transfection of cells with miR-891b mimic significantly attenuated Salidroside-induced apoptosis ($p < 0.05$), which accompanied by the repressed cleavage of caspase-3 and caspase-9. Not surprising, the inhibitory effects of Salidroside on the migration and invasion of WIT49 and RM1 cells were also attenuated by miR-891b mimic ($p < 0.05$, Fig. 6D–G). All together, these data suggested that Salidroside suppressed Wilms' tumor cells growth, migration and invasion possibly through down-regulation of miR-891b.

3.6. Salidroside down-regulated miR-891b to repress NF-κB and PI3K/AKT/mTOR signaling pathways

Finally, the involvement of miR-891b in Salidroside-modulated signaling pathways was studied. Fig. 7A–D showed that transfection of

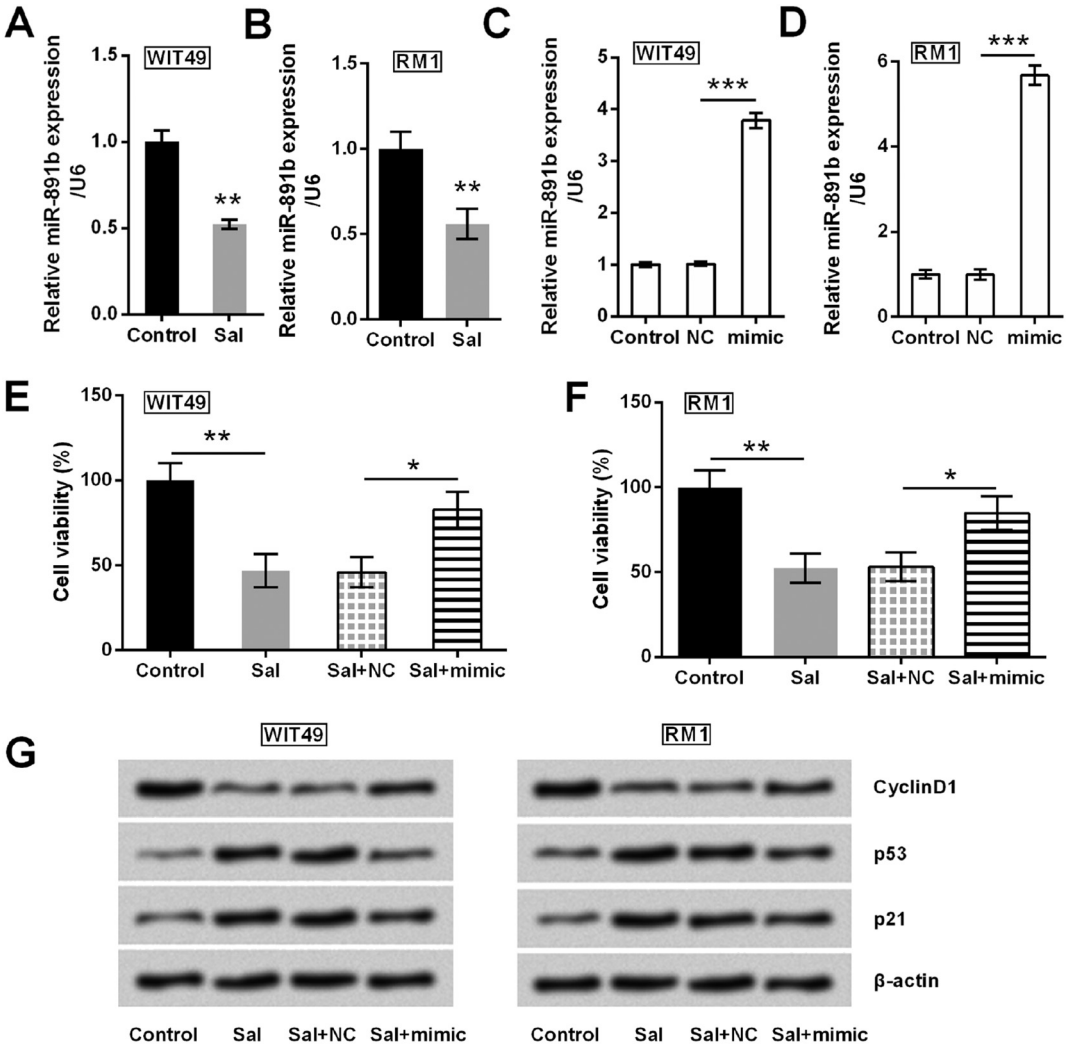

Fig. 5. Effects of Salidroside treatment together with miR-891b overexpression on Wilms' tumor cells proliferation. (A–B) Expression changes of miR-891b were measured by RT-qPCR, after treating with 80 μM Salidroside for 24 h. (C–D) Expression changes of miR-891b were measured by RT-qPCR, after transfection with mimic or NC specific for miR-891b. (E–F) Cell viability and (G) expression of survival-related proteins were respectively measured by CCK-8 assay and Western blot analysis, after transfection plus Salidroside treatment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

cells with miR-891b mimic significantly reversed the impacts of Salidroside on NF-κB and PI3K/AKT/mTOR signaling pathways. As compared to Salidroside + NC group, the phosphorylation levels of p65, IκBα, PI3K, AKT and mTOR were all significantly increased in Salidroside + mimic group ($p < 0.05$, $p < 0.01$ or $p < 0.001$). The total levels of these proteins were unaffected by either Salidroside or by transfection.

4. Discussion

Uncontrolled growth is one characteristic of cancer cells, including Wilms' tumor cells. Cyclin D1 and p21 are two main controllers of tumor proliferation, that Cyclin D1 is a critical target of proliferative signals in G1 [28], and p21 is a well-known inhibitor of cyclin-dependent kinase (CDK). Apart from Cyclin D1 and p21, p53 was also a key regulator of tumor growth, which has been identified as a tumor suppressive gene [29]. Its inactivation is an frequent event in many cancers, such as lung cancer [30] and pancreatic cancer [31]. More interestingly, p21 represents a major target of p53 activity [32]. In the current study, we found that Salidroside significantly suppressed WIT49 and RM1 cells viability and induced apoptosis, indicating the suppressive effects of Salidroside on Wilms' tumor cells growth. We additionally found that Salidroside treatment induced up-regulations of p53 and p21, down-regulation of Cyclin D1, and remarkable cleavage of caspase-3 and -9, suggesting Salidroside exerted anti-growth function via modulation of cell cycle progression and caspase-dependent apoptosis. Interestingly, the effects of Salidroside on cell apoptosis are dependent on different cell types. Based on the findings of previous studies, Salidroside is able to protect normal cells against various stimuli induced apoptosis, like amyloid β [33] and LPS [34]. While in tumor cells, Salidroside exerts potent pro-apoptotic effects [14], which is consistent with our findings.

Wilms' tumor carries a certain of metastatic potential, that it may spread to the lungs, liver, bone, brain, or nearby lymph nodes [35]. Inhibition of Wilms' tumor cells metastasis may be an efficient way to inhibit the progression of this cancer. Cancer cell metastasis requires the breakdown of extracellular matrix (ECM) [36]. MMPs are a class of enzymatic components which are responsible for ECM breakdown. Besides this, tumor cells achieved motility via a cellular process in which epithelial cells acquire a mesenchymal phenotype (so-called EMT), and Vimentin is a key marker of EMT [37]. The present work performed in Wilms' tumor cells showed that, Salidroside could significantly inhibit the migration and invasion of tumor cells. Besides, Salidroside treatment significantly down-regulated MMP-2 and Vimentin expression, further confirming the anti-migratory and anti-invasive functions of Salidroside in Wilms' tumor cells.

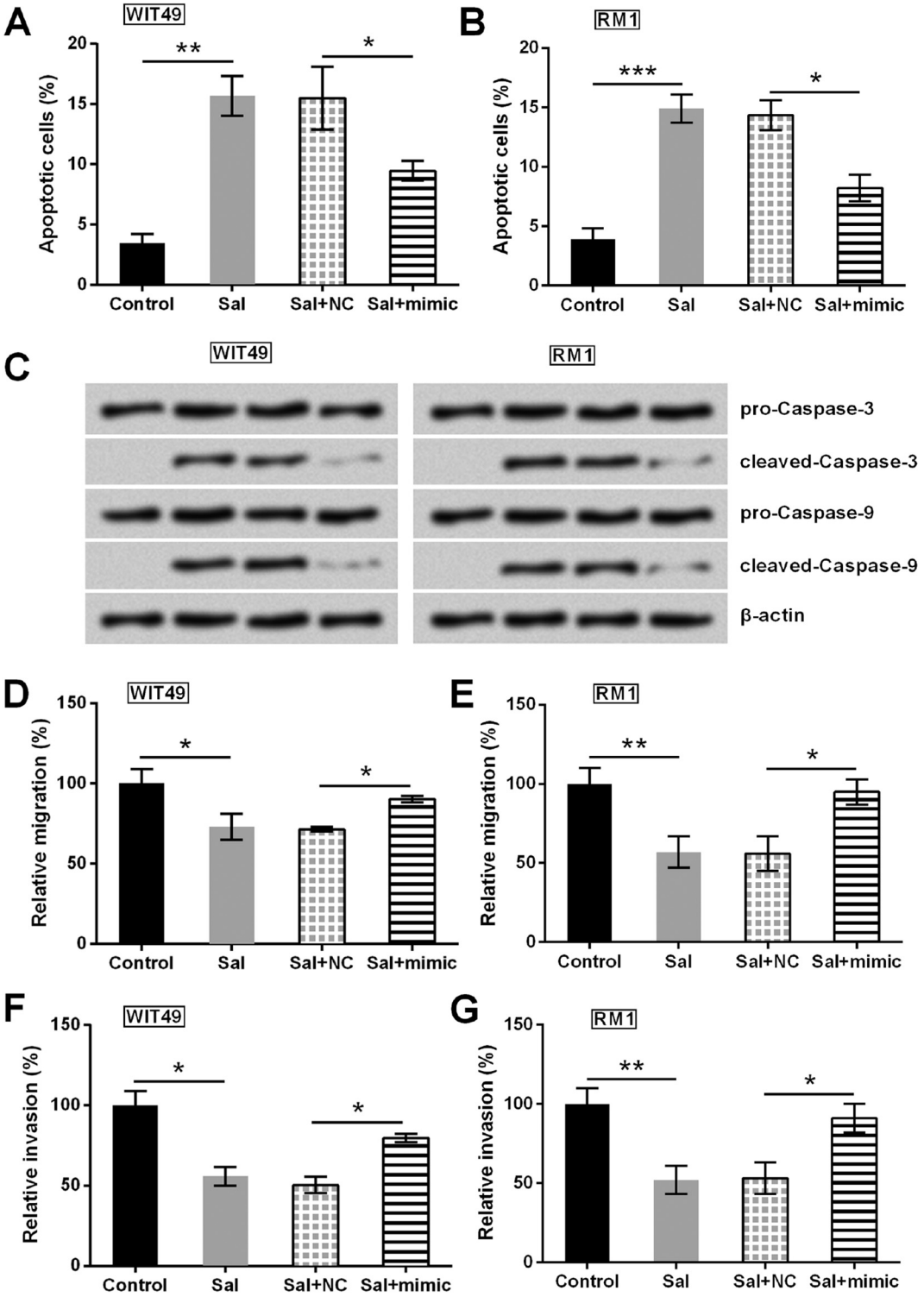

Fig. 6. Effects of Salidroside treatment together with miR-891b overexpression on Wilms' tumor cells apoptosis, migration and invasion. (A–B) Apoptotic cell rate, (C) expression of apoptosis-related proteins, (D–E) relative migration, and (F–G) relative invasion were measured by flow cytometer detection, Western blot and Transwell assay, after transfection plus Salidroside treatment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Actually, the suppressive functions of Salidroside on many cancers growth and metastasis have been previously reported. For the selected example, Kang and his colleagues have pointed out that Salidroside inhibited breast cancer cells viability, migration and invasion through modulation of MMP-2 [12]. Yu et al., demonstrated that Salidroside could induce a caspase-dependent apoptosis in ovarian cancer cells through up-regulation of p53 [14]. However, to our best of knowledge, our findings provided the first in vitro evidence that Salidroside also could suppress the growth and metastasis of Wilms' tumor cells.

Recently, some studies have tried to decode the underlying mechanisms of which Salidroside exhibited its pharmacological effects [9–16]. Some of them believed that Salidroside confers its function may be partially via miRNA regulation. For example, an in vivo study performed in Sprague-Dawley rats suggested that Salidroside protected

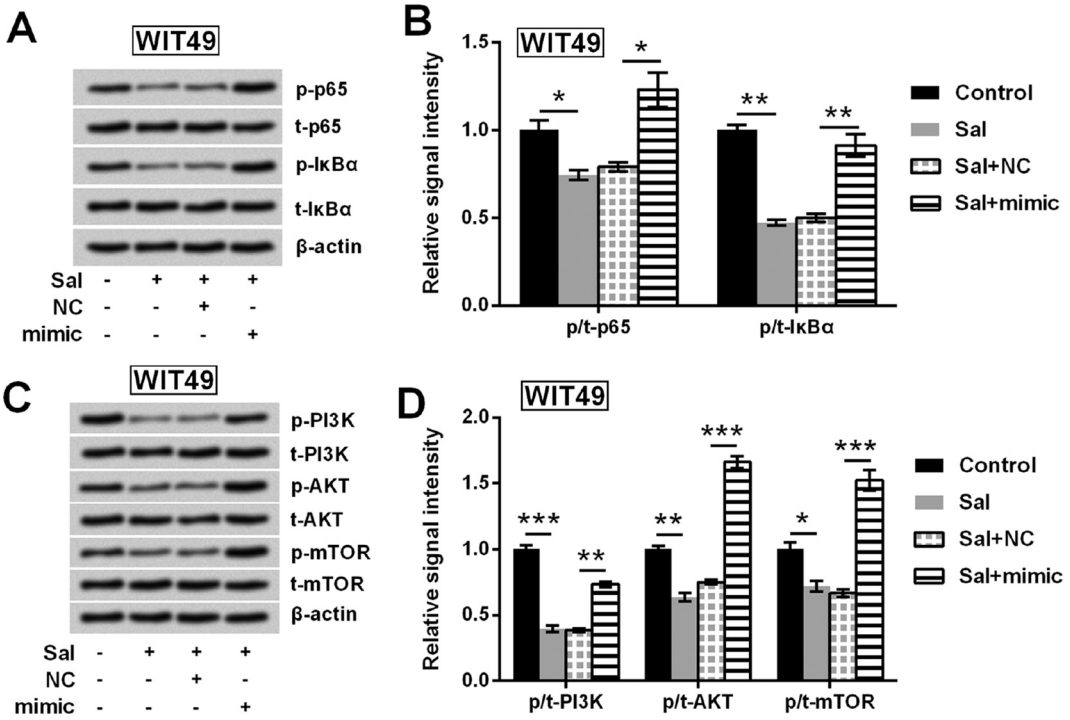

Fig. 7. Effects of Salidroside treatment together with miR-891b overexpression on the activation of NF-κB and PI3K/AKT/mTOR signaling pathways. (A) Expression levels of core proteins in (A–B) NF-κB and (C–D) PI3K/AKT/mTOR signaling pathways were tested by Western blot, after transfection plus Salidroside treatment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

neural stem cells against hypoxia injury via up-regulating miR-210 [38]. Another study showed that Salidroside regulated lipid metabolism of type 2 diabetic mice via down-regulating miR-370 [39]. It has been mentioned that targeting miRNAs by natural agent may open new avenues for the further elucidation of Salidroside's function [40]. This here, we explored the regulatory role of Salidroside in miR-891b expression, and found that miR-891b was down-regulated in response to Salidroside. miR-891b has been previously identified as a prognostic predictor of pancreatic cancer [24], and miR-891b could help chemotherapeutic agent *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) to kill breast cancer cells [41]. Rescue assay results in the current study showed that Salidroside did not suppress Wilms' tumor cells growth and metastasis when miR-891b was overexpressed. Thus, we inferred that Salidroside inhibited the growth and metastasis of Wilms' tumor cells possibly via down-regulating miR-891b.

At present, several pathways have been reported to be implicated in the development of Wilms' tumor, including Wnt/β-catenin, IGF2 [42], NF-κB [25], and PI3K/AKT/mTOR [27] signaling pathways. Besides, several studies have showed that Salidroside conferred its neuroprotective [38], anti-apoptotic, anti-autophagy [7], and anti-tumor [8] activities via modulation of PI3K/AKT/mTOR and NF-κB signaling. Therefore, in this study, we focused on these two signaling for further investigation, to explore whether they were involved in Salidroside's anti-tumor actions in Wilms' tumor cells. As a result, Salidroside treatment partially blocked the activation of these two signaling, but the impacts of Salidroside on signaling pathways were reversed when miR-891b was overexpressed. These data suggested that Salidroside deactivated PI3K/AKT/mTOR and NF-κB signaling pathways also via a miR-891b-dependent fashion.

To sum up, the present work provides in vitro evidences that Salidroside inhibits WIT49 and RM1 cells growth, migration and invasion, showing anti-tumor potentials in Wilms' tumor. Salidroside confers its anti-tumor activity possibly via down-regulating miR-891b, leading to the deactivation of PI3K/AKT/mTOR and NF-κB signaling pathways.

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Authorship

Conceives and designed the experiments: Shiying Hang, Hai Li and Delian Huang. Performed the experiments and analyzed the data: Hai Li and Delian Huang. Drafted the manuscript: Hai Li. Revised the manuscript and finally approved the article: Shiying Hang.

Conflict of interest

Authors declare that there is no conflict of interests.

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