Resveratrol Suppresses Ovarian Cancer Cell Growth and Invasion Through Upregulation of microRNA-34a

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Abstract

Resveratrol (RES), a natural compound found in red wine, has previously reported to suppress ovarian cancer (OC) cell growth *in vitro* and *in vivo*; however, its potential molecular mechanisms are not fully elucidated. The aim of this study is to investigate the suppressive potential of RES in OC cell growth and invasion and reveal the underlying mechanisms. Herein, we found that RES treatment obviously suppressed the proliferative and invasive capacities of OC cells, and elevated cell apoptosis *in vitro*. Subsequent microarray and qRT-PCR analysis further showed that microRNA-34a (miR-34a) was significantly increased by RES treatment. Moreover, the inhibitory effects of RES on OC cells were enhanced by miR-34a overexpression, whereas weakened by miR-34a inhibition in OC cells. Of note, Bcl-2, an anti-apoptotic gene, was identified as a direct target of miR-34a. Then, we revealed that RES decreased the expression of Bcl-2 in OC cells in a dose dependent manner. Furthermore, the anti-tumor effects of RES were abolished by overexpression of Bcl-2 in OC cells. Overall, these results demonstrated that RES exerts the anti-cancer effects on OC cells through the miR-34a/Bcl-2 axis.

Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer-related death among women in the world, which has the highest mortality rate of all gynecologic neoplasms (Galdiero et al. 2015). Although remarkable advances in the surgery, chemotherapy and radiotherapy progress have been made, the overall OC survival rate has not improved in the past decades (Power et al. 2016; Zhou et al. 2017). Therefore, it's urgent to develop novel, evidence-based and safe approaches for its prevention and treatment.

Nowadays, a number of natural products have been considered as preventive and therapeutic agents in antitumor treatment due to little side effects (James et al. 2015; Tran et al. 2014). Resveratrol (RES, trans-3, 4, 5-trihydroxystilbene), a natural phytoalexin product discovered from the skin of grapes (Fremont 2000; Maxwell et al. 1994), has been shown to inhibit the initiation and progression in a wide range of malignancies, including lung, skin, liver, breast, prostate, pancreatic, ovarian, and colorectal cancer (Aggarwal et al. 2004). Clinical trials have assessed the use of RES as a cancer preventive and therapeutic agent. For example, Zhu et al. have shown the protective effects of RES on the breast cancer in women through altering mammary promoter hypermethylation (Zhu et al. 2012). Currently, several phase I and phase II clinical trials dealing with RES have been conducted for colon cancer and lymphoma patients (according to ClinicalTrials.gov). In addition, two previous studies have reported the profound anti-tumor efficacy of RES on human ovarian cancer (Liu et al. 2018; Wang et al. 2018). Although a large number of researches showed the antitumor effect of RES, the underlying responsible mechanisms need further study.

MicroRNAs (miRNAs) are small non-coding RNAs, which can bind to the 3' UTRs of target mRNAs, thereby preventing the translation of target mRNAs or inducing RNA degradation (Bartel 2009). Several studies have suggested that miRNAs are involved in the initiation and progression of OC by altering key signaling elements. For example, Zhang et al. showed that miR-338-3p suppressed OC cells growth and metastasis

through regulation of Wnt/catenin beta and MEK/ERK signaling pathways (Zhang et al. 2019). In another previous study, Xue et al. found that miR-139-3p regulated OC growth and metastasis by modulating the expression of ELAVL1 (Xue et al. 2019). Recently, a large body of evidence supports that miRNAs play important roles in the therapeutic and preventive effects of natural agents, including RES (Sheth et al. 2012). For example, miR-326, increased in response to RES treatment, participated in RES induced cancer cell apoptosis through reducing the expression of pyruvate kinase M2 (PKM2) (Wu et al. 2016). miR-520h-mediated forkhead box C2 (FOXC2) regulation was critical for inhibition of lung cancer progression by RES (Yu et al. 2013). RES also suppressed the growth and metastasis of human colorectal cancer cells by promoting miR-663 expression (Tili et al. 2010). These observations clearly suggest that miRNAs may be involved in the RES-mediated anti-tumor activities. However, whether miRNAs participate in the anti-tumor activities of RES in OC remains unclear.

In the present study, we investigated the effects of RES on OC cells and explored the underlying molecular mechanism. Our results highlight that miR-34a/Bcl-2 pathway mediated the anticancer effects of RES in OC. This study may provide new experimental evidence for the therapeutic effects of RES for the treatment of OC.

Materials And Methods

Cell culture and treatments

Ovarian cancer cell line SKOV-3, SNU-119 and OV90 were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China), and cultured in DMEM/F12 (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS) (Biowest, Nuaillé, France), 100 U/mL penicillin, and 100 mg/mL streptomycin (Hyclone). All cell lines were maintained at 37°C in a humidified atmosphere of 5% CO2/air.

Resveratrol (RES) was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in DMSO to make a 75 mM stock solution and was added directly to the media at different concentrations before use, the concentration of DMSO was ≤1% to ensure the lack of cytotoxicity.

Cell viability

The anti-proliferative effect of resveratrol against SKOV-3, SNU-119 and OV90 cells was measured by using CCK-8 assay. At the end of transfection, 10 μL CCK-8 solution (Beyotime, Jiangsu, China) was added to each well (1×10^5/well), and SKOV-3 and OV90 cells were cultured for 2 h. Then the OD absorbance of the samples at 450 nm was detected by iMark microplate reader (Bio-Rad, Hercules, CA, USA).

Cell apoptosis

After treatment for 24 h, cells were collected and cell apoptosis was analyzed using an Annexin V-FITC/PI apoptosis detection kit (Beyotime Institute of Biotechnology, China). After washing twice with PBS, the

cells were stained with Annexin V and propidium iodide, followed by incubation at room temperature in the dark for 15 minutes. Cell apoptosis was analyzed on a FACScan flow cytometer (FCM; Bechman Coulter, CA) and then analyzed by FlowJo 8.7.1 software (Ashland, OR).

Wound healing assay

SKOV-3, SNU-119 and OV90 cells (2 × 106/well) were seeded in 6-well plates overnight to allow cells to attach. Using a 200 μL pipette tip, monolayers were wounded vertically. The images were captured from each well at 0 h and 24 h.

Invasion assay

Transwell chambers (8-μm pore; BD Biosciences) coated with Matrigel (BD Biosciences) were used for invasion assay. Briefly, SKOV-3, SNU-119 and OV90 cell suspension containing 8×10^4 cells were added in the top chamber with DMEM/F12, while the lower chamber was added with DMEM/F12 containing 20% FBS. After incubation for 16 h by resveratrol treatment, we randomly selected and photographed five view fields from each sample using a CKX41 inverted microscope (Olympus Corp., Tokyo, Japan) to calculate the mean number of cells that passed through the Matrigel. The experiment was performed in triplicate wells and repeated three times.

miRNA microarray

Total RNA was isolated from SKOV-3 treated with or without resveratrol using TRIzol reagent (Invitrogen, USA) and purified using miRNeasy kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. The samples were assessed using the miRCURY LNA™ Array v. 18.0 (Agilent). The procedure and imaging processes were as described previously (Peng et al. 2018).

qRT-PCR

Total RNA was extracted from cells with the TRIzol reagent (Invitrogen, USA) according to the manufacturer’s protocol. For miRNA reverse transcription, cDNA was synthesized using the PrimeScript reverse transcription reagent kit (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's instructions. Real-time PCR were performed using a standard protocol from the SYBR Green PCR kit (Toyobo, Osaka, Japan) on an ABI 7300 system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Relative quantification was determined by normalization to U6. The primers for qRT-PCR analysis were as follows: miR-34a forward: 5’-CCCAGAACATAGACACGCTGGA-3’; miR-34a reverse: 5’-ATCAGCTGGGCACCTAGGACA-3’; U6 forward: 5’-TGCGGGTGCTCGCTTCGCAGC-3’; U6 reverse: 5’-CCAGTGCAGGGTCCGAGGT-3’. The PCR amplification protocol was as follows: an initial 95°C for 5min, followed by 40 cycles of 94°C for 10 s, 57°C for 31 s, and 70°C for 30 s. The qRT-PCR assays were performed in triplicate and the relative expression levels were calculated based on the $2^{-\Delta\Delta}$Ct method (Livak and Schmittgen 2001).

Cell transfection

The miR-34a mimics, mimics negative control (mimics NC), miR-34a inhibitor and inhibitor NC were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). In addition, the coding domain sequences of Bcl-2 mRNA were amplified by PCR, and inserted into pcDNA 3.0 vector to enhance its expression (Invitrogen, Grand Island, NY, USA), named as pcDNA-Bcl-2. , SNU-119pcDNA-Bcl-2 Subsequently, cells were cultured in DMEM/F12 supplemented with 10% FBS for 24 h. Then, the cells were treated with RES for 24 h and utilized in subsequent experiments.

Dual-luciferase reporter assays

pGL3-Bcl-2 wide type (Wt) or pGL3-Bcl-2 mutant type (mut) plasmids were co-transfected with 20 nM miR-34a mimics, 20 nM miR-34a inhibitor or 20 nM miR-NC into HEK 293T cells in 24-well plates (2×10^5/well) using Lipofectamine 2000 (Invitrogen). At 24 h post-transfection, the double luciferase activities were analyzed using the Dual-Luciferase Reporter Assay system (Promega Corporation).

Immunofluorescence assay

Immunofluorescence staining for Bcl-2 in cells was performed using anti-Bcl-2 antibody (cat no.#15071; Cell Signaling Technology, 1:2,000 dilution) in a humidified box at 4 °C overnight, and followed by incubation with an Alexa fluorescein-labeled secondary antibody for 1 h at 37 °C. The cell nuclei were stained with DAPI (5.0μg/mL). Immunostained samples were imaged by fluorescence microscopy (Olympus, Japan) at 200× magnification.

Western blot analysis

Total protein was extracted using radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The protein concentration was determined using a BCA kit (Beyotime Institute of Biotechnology, Haimen, China). Next, the proteins in the lysates were separated on SDS-PAGE gels and electro-transferred to PVDF membranes(GE Healthcare, Freiburg, DE), followed by blocking in a 5% skim milk solution for 1 h at room temperature. Primary antibodies against Bcl-2 (cat no. 15071) and β-actin (cat no.#3700, 1:2000) were incubation at 4°C overnight. All antibodies were bought from Cell Signaling Technology. Then, membranes were incubated with the corresponding horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (cat no.#8887, Cell Signaling Technology, 1:2,000) for 1 h at room temperature. The bands were detected by enhanced chemiluminescence (ECL) kit (GE Healthcare, Freiburg, DE). The intensity of the bands of interest was analyzed using Bio-Rad Laboratories Quantity One software 3.0 (Bio-Rad Laboratories, Inc.).

Statistical analysis

Data were presented as mean ± S.D. GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA) was used to perform all statistical analysis. When only two groups were compared, Student's t-test

was conducted. One-way analysis of variance followed by Tukey's post-hoc test was applied to compare differences between multiple groups. $P \leq 0.05$ was considered as statistically significant.

Results

Resveratrol exhibited an anti-tumor effect in both SKOV-3, SNU-119 and OV90 cells

To explore the anti-tumor effects of RES on the OC cells, we first examined the effect of RES on cell viability by using CCK8 assay after 48 h of drugs treatment. As shown in Figure 1A, resveratrol could suppress OC cell viability in a dose dependent manner. The half inhibitory concentration (IC50) values of RES in SKOV3, SNU-119 and OV90 cells were 163.31±11.09 μM, 114.42±10.05 μM and 132.26±15.34 μM, respectively. Based on these results, the low dose RES pretreatment partially inhibited cell proliferation, and therefore, 100 μM RES was used in the subsequent experiments. We next investigated whether the reduced cell viability was due to induction of apoptosis, and the results showed that resveratrol markedly promoted the cell apoptosis compared with the control group (Figure 1B,C). These data suggest that RES maybe inhibit the proliferation of OC cells by inducing cell apoptosis.

Next, we investigated the influences of RES on cell migration and invasion using transwell assay and wound healing assay, respectively. Transwell assay showed that RES markedly attenuated the invasiveness of SKOV3, SNU-119 and OV90 cells compared with that in the control group (Figure 1D). Wound healing assay indicated that RES significantly inhibited the migration of SKOV3, SNU-119 and OV90 cells compared with that in the control group (Figure 1E). These results implied that RES reduced the metastasis of SK-OV-3 cell *in vitro.*

Resveratrol increased the expression of miR-34a in ovarian cancer cells

Previous studies have indicated that RES exhibits its anti-tumor role in several types of cancers through modulation of miRNAs (Karimi Dermani et al. 2017; Wang et al. 2016; Wu et al. 2016). To determine the potential involvement of miRNAs in the inhibitory effect of RES on OC cells, we performed a microarray analysis to determine miRNA levels in OC cells after RES treatment. Our data revealed that compared with the control group, 30 miRNAs were up-regulated and 26 miRNAs were down-regulated in OC cells treated with RES (Figure 2A). Among them, miR-34a, a well-known tumor suppressor, is the most significantly up-regulated after RES treatment. Therefore, we focused on miR-34a in OC for further study.

To further verify the result of miRNA microarray, qRT-PCR was performed to measure the expression levels of miR-34a in OC cells treated with different concentrations of RES. As expected, RES could increase the expression of miR-34a in OC cells in a concentration dependent manner (Figure 2B). All data suggest that miR-34a may be involved in the anti-tumor role of RES in OC cells.

miR-34a overexpression enhanced the anti-tumor effects of resveratrol in ovarian cancer cells

To investigate the role of miR-34a in RES mediated the anti-tumor effects in OC cells, we overexpressed the miR-34a expression by transfection of miR-34a mimics into SKOV3, SNU-119 and OV90 cells. As

shown in Figure 3A, miR-34a mimics notably increased the miR-34a levels in SKOV3, SNU-119 and OV90 cells. It was observed that miR-34a overexpression enhanced the anti-proliferative and pro-apoptotic effects of RES in SKOV3, SNU-119 and OV90 cells (Figure 3B-D). Consistently, miR-34a overexpression enhanced the anti-invasive and anti-migratory effects of RES in SKOV3, SNU-119 and OV90 cells (Figure 3E-H). These results suggested that overexpression of miR-34a enhanced the anti-tumor effects of RES in OC cells.

miR-34a inhibition alleviates the inhibitory effects of resveratrol on the invasion and migration of ovarian cancer cells

Next, miR-34a inhibitor or inhibitor NC was transfected into SKOV3, SNU-119 and OV90 cells to further investigate the role of miR-34a on RES mediated the anti-tumor effects. As shown in Figure 4A, the levels of miR-34a in SKOV3, SNU-119 and OV90 cells were notably decreased after transfection with miR-34a inhibitor. The CCK-8 and flow cytometry assays showed that miR-34a inhibition attenuated the anti-proliferative and pro-apoptotic effects of RES in SKOV3, SNU-119 and OV90 cells (Figure 4B-D). Subsequently, the transwell and wound healing assays revealed that miR-34a inhibition alleviated the inhibitory effects of RES on the invasion and migration of SKOV3, SNU-119 and OV90 cells (Figure 4E-H). These results suggested that RES exerts its anti-tumor activity through suppressing miR-34a expression in OC cells.

Bcl-2 was a direct target of miR-34a in ovarian cancer cells

Through bioinformatics prediction using TargetScan 7.0 (targetscan.org/) and miRanda (microrna.org/) softwares, we found a putative target site of miR-34a in the 3´-UTR of Bcl-2 mRNA (Figure 5A). To experimentally conformed Bcl-2 as a target of miR-34a, we performed a dual luciferase reporter assay. As displayed in Figure 5B, overexpression of miR-34a significantly reduced luciferase activity of the 3'-UTR segment of Bcl-2, whereas knockdown of miR-34a increased the relative luciferase activity; however, there was no effect when the targeted sequence of Bcl-2 was mutated in the miR-34a-binding site. We confirm whether miR-34a could affect Bcl-2 expression in SKOV3 cells. Western Blot analyses indicated that the protein level of Bcl-2 was decreased by miR-34a mimics, while increased by miR-34a inhibitor in SKOV3 cells (Figure 5C). To investigate whether RES influenced Bcl-2 expression by regulating miR-34a, we measured the mRNA level of Bcl-2 in SKOV3 cells after different concentrations of RES treatment using qRT-PCR and indirect immunofluorescence (IFA) assays. The results showed that RES suppressed the expression of Bcl-2 in a dose dependent manner (Figure 5D, E). All these results suggest that RES inhibited Bcl-2 expression by upregulating miR-34a expression.

Overexpression of Bcl-2 reversed the anti-tumor effects of resveratrol in ovarian cancer cells

As mentioned above, Bcl-2 was regulated by RES in OC cells, therefore, the present study further investigated whether RES exerts the anti-tumor effects by regulating Bcl-2 expression. The Bcl-2 expression vector, pcDNA-Bcl-2 was transfected into SKOV3, SNU-119 and OV90 cells for 24 h. It was found that the expression level of Bcl-2 was significantly upregulated in these OC cells, compared with

that in the pcDNA-vector group (Figure 6A). Functionally, it was shown that the RES treatment inhibited the viability of SKOV3, SNU-119 and OV90 cells, whereas the overexpression of Bcl-2 partly abrogated the inhibitory effects of RES on the viability of these OC cells (Figure 6B). Subsequently, it was demonstrated that the overexpression of Bcl-2 reversed the apoptosis induced by RES in SKOV3, SNU-119 and OV90 cells (Figure 6C, D). Furthermore, the reduction in the invasion and migration induced by RES was markedly attenuated by Bcl-2 overexpression in these OC cells (Figure 6E-H). These data suggested that RES exerts the anti-tumor effects by downregulating Bcl-2.

Discussion

In the present study, we revealed the resveratrol (RES) inhibits OC cell proliferation, migration and invasion, and promotes cell apoptosis, thereby demonstrating that it is a highly potent anti-tumor agent. Moreover, we have identified that miR-34a/Bcl-2 axis mediated the anti-tumor effects of RES in OC cells. These findings provide a pharmacological basis on RES in the treatment of OC.

RES has always been thought to exert a role in antibacterial, anti-inflammatory, and immunoregulatory effects in various diseases (Bishayee 2009; Csiszar et al. 2006; Shankar et al. 2007). Previous researches have also found that RES have anti-tumor activity in connection with the lung, gastric, prostate and breast cancer (Ko et al. 2017; Yousef et al. 2017). Moreover, RES has been reported to reduce the cell proliferation and induce cell apoptosis in ovarian cancer cells (Liu et al. 2018; Vergara et al. 2017; Wang et al. 2018). Epithelial ovarian cancer is traditionally divided into four major histological subtypes: serous, endometrioid, clear cell and mucinous carcinoma (Coburn et al. 2017). The most aggressive sub-type, high-grade serous ovarian cancer (HGSOC), accounts for 90% of these serous carcinomas and two-thirds of all ovarian cancer deaths, making it by far the most extensively studied ovarian carcinoma (Torre et al. 2018). Currently, there are many cancer cell lines are frequently used as in vitro tumor models for HGSOC. However, the histopathological origins of most of these cells are unclear. Accordingly to a previous report, through comparison of genetic copy-number changes, mutations and mRNA expression profiles between commonly used ovarian cancer cell lines and HGSOC tumor samples, they found some popular cell line models (A2780, OVCAR-3, CAOV3 and IGROV1) do not closely resemble HGSOC tumors (Domcke et al. 2013). In this study, the three frequently used cell lines, SNU-119, SKOV-3 and OV-90 were selected for experiments. Our results showed that RES exhibits anti-tumor effect in OC cells, as evidenced by the reduction of cell viability, invasion and migration, and induction of apoptosis in SKOV3, SNU-119 and OV90 cells. These data suggested that RES may be a potential therapeutic agent in the treatment of ovarian cancer.

Increasing studies have reported that RES exerts its antitumor effects through modulating the expression of miRNAs. For example, Yan et al. have found that RES ameliorated the invasive and migratory abilities of pancreatic cancer cells through suppressing miR-21 expression (Yan et al. 2018). R Venkatadri et al. have shown that several miRNAs including miR-542-3p and miR-122-5p have a key role in RES-induced cell apoptosis in breast cancer cells (Venkatadri et al. 2016). In accordance with our microarray analysis, our study demonstrated that miR-34a was highly expressed in RES-treated OC cells. In several human

cancers, including cervical cancer, hepatocellular carcinoma, and breast cancer, miR-34a has been observed to have low expression with marked effect on the cell proliferation, apoptosis and invasion (Geng et al. 2015; Si et al. 2016; Sun et al. 2017). Also, miR-34a was documented to function as a tumor suppressor in OC (Dong et al. 2016; Li et al. 2015; Lv et al. 2018). Notably, one previous study has revealed that RES inhibited human colorectal cancer cell growth and induced apoptosis through up-regulating miR-34a expression (Kumazaki et al. 2013). We thus hypothesized that miR-34a may likewise be involved in the suppressive role of RES in OC. Our further study demonstrated that overexpression of miR-34a enhanced the anti-tumor effects of RES in OC cells, whereas knockdown of miR-34a has an opposite result. Overall, these data provide evidence that miR-34a is a viable target of RES for mediating its antitumor actions against OC.

One of the best ways to understand miRNA function is via the elucidation of their functional targets. Bcl-2 has been validated as a potential miR-34a target in a wide variety of human tumors. For example, miR-34a inhibited the proliferative and invasive of HCT116 cells by binding to Bcl2 (Li et al. 2017). In meningioma, miR-34a was indicated to modulate cell apoptosis by targeting Bcl2 (Werner et al. 2017). Of note, one previous study has reported that miR-34a induced apoptosis in OC cells by regulating Bcl-2 (Ding et al. 2017). Thus, we sought to determine whether miR-34a/Bcl-2 axis mediated the antitumor activity of RES in OC. Firstly, our data identified Bcl-2 as a direct target of miR-34a by binding site in its 3´-UTR, which reduced its expression. It was also found that RES treatment dose-dependently decreased the protein expression of Bcl-2 in OC cells, indicating RES may regulate the Bcl-2 expression through miR-34a. In addition, we found that Bcl-2 overexpression could abolish the anti-cancer effect of RES, which is similar with the role of miR-34a inhibition in OC cells. Taken together, our results suggest that RES exhibits its antitumor activity through regulation of miR-34a/Bcl-2 pathway in OC cells.

However, there are still some limitations in the present study. The main limitation of this study is the sole focus on the miR-34a/Bcl-2 pathway in the antitumor activity of RES. Moreover, we only investigate the role and molecular mechanism *in vitro*, not in animal models, thus we'll verify them later on. In addition, the underlying relationship between RES and other related pathways needs further investigations.

In conclusion, our results revealed that RES inhibited OC cell proliferation, invasion, migration and promoted cell apoptosis through miR-34a/Bcl-2 axis (Figure 7). It provides basic information to better understanding the molecular mechanism involved in the anti-cancer activity of RES in OC cells and suggested that RES could be a potent agent for OC.

Abbreviations

RES Resveratrol

OC ovarian cancer

PKM2 pyruvate kinase M2

FOXC2 forkhead box C2

HGSOC high-grade serous ovarian cancer

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions: Shangli Yao, Ming Gao, Zujun Wang, Wenyan Wang and Lei Zhan performed the experiments, contributed to data analysis and wrote the paper. Shangli Yao, Ming Gao, Zujun Wang, Wenyan Wang and Lei Zhan analyzed the data. Bing Wei conceptualized the study design, contributed to data analysis and experimental materials. All authors read and approved the final manuscript.

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Figures

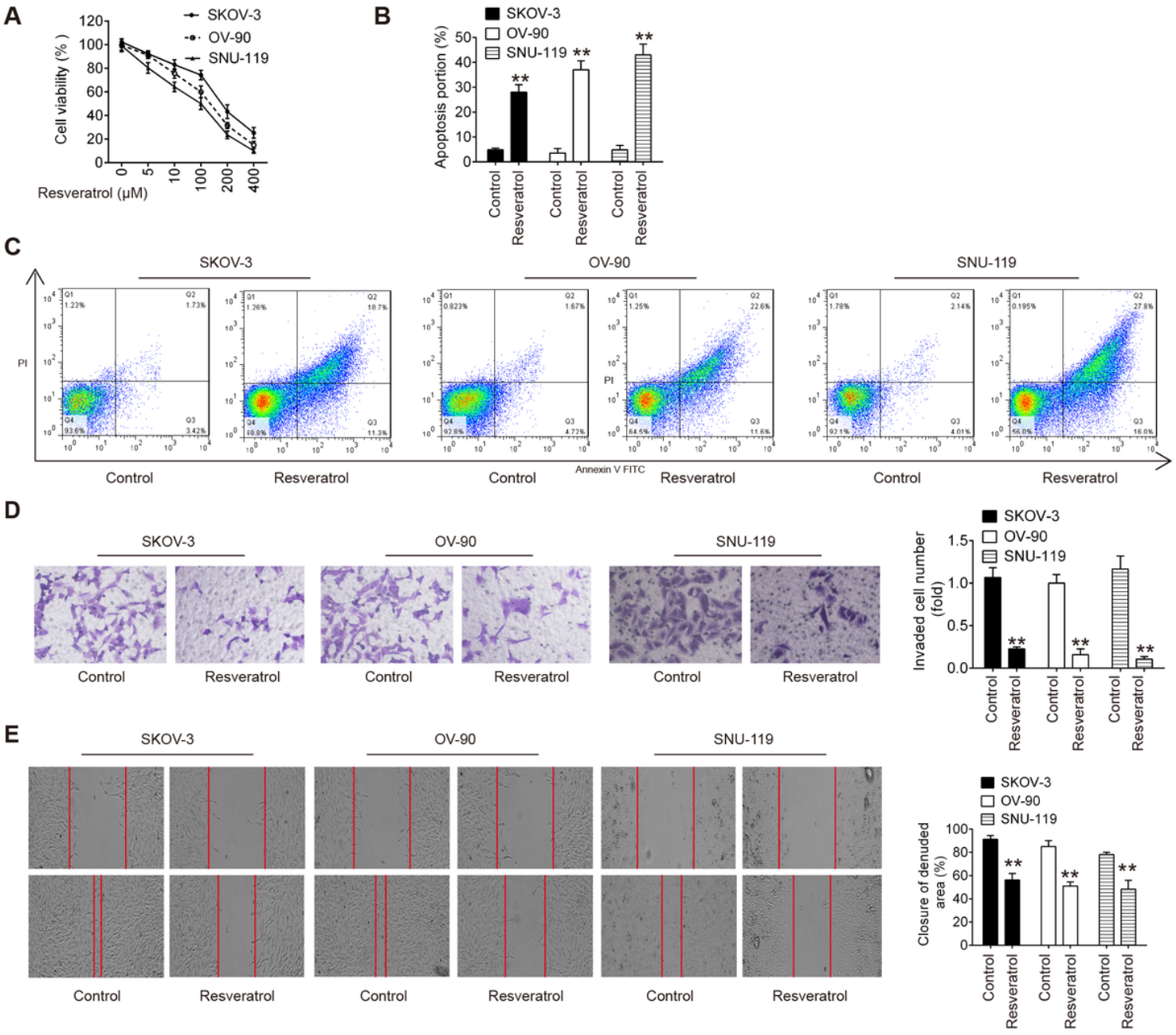

Figure 1

Resveratrol inhibits the cell viability, migration and invasion and induces cell apoptosis in ovarian cancer cells. (A) SKOV3, SNU-119 and OV90 cells were treated with the indicated doses of resveratrol for 24 h and then cell viability was measured by CCK-8 assay. (B, C) The flow cytometry analysis was performed to determine apoptotic cells in SKOV3, SNU-119 and OV90 cells. (D) Cell invasion was detected by Transwell assay in SKOV3, SNU-119 and OV90 cells. (E) Cell migration was assessed by Wound healing assay in SKOV3, SNU-119 and OV90 cells. Data were represented as the mean ± SD of three individual experiments. *p < 0.05, **p < 0.01 vs. control.

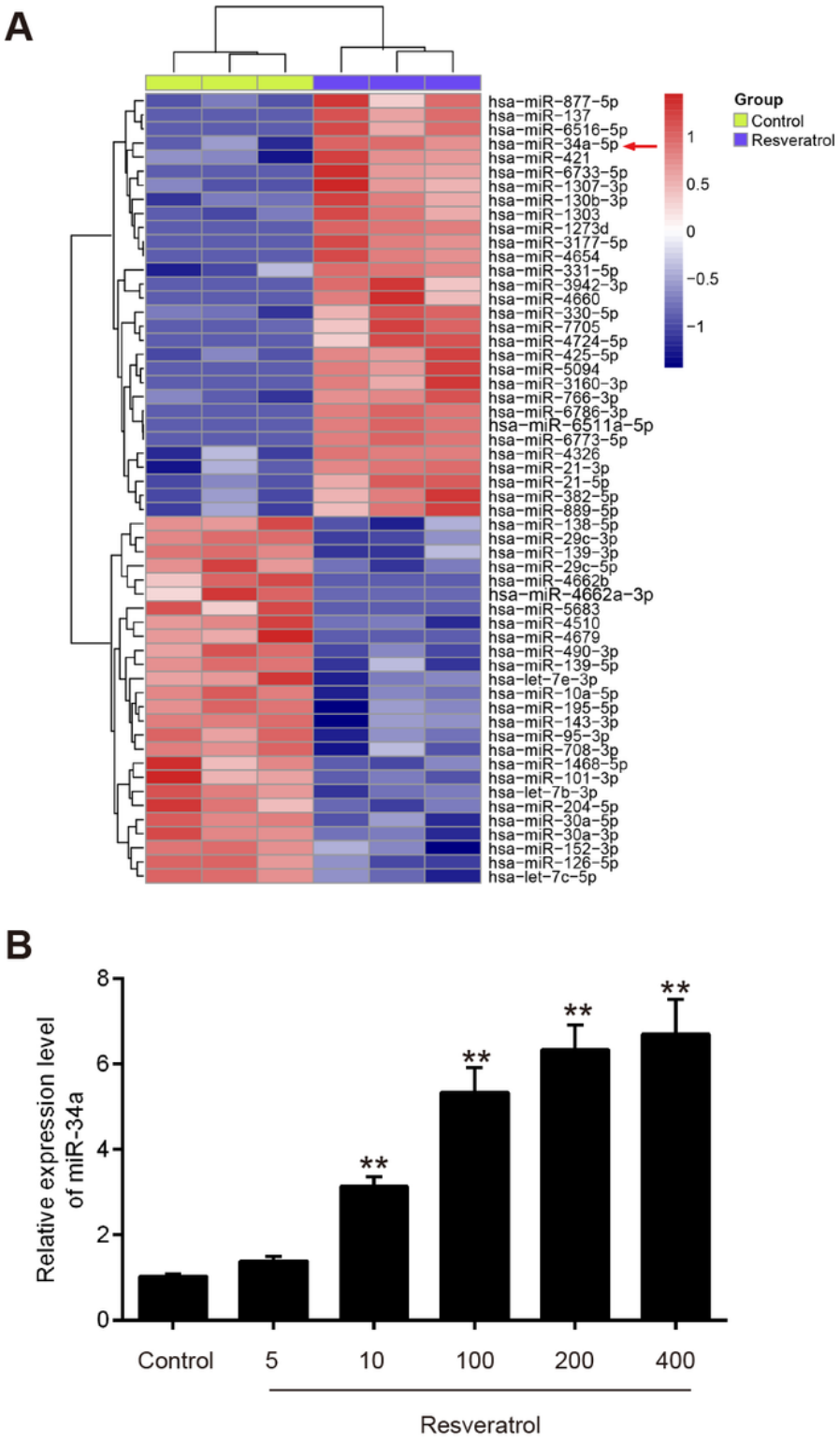

Figure 2

Resveratrol increased the expression of miR-34a in ovarian cancer cells. (A) The SKOV3 cells were treated with resveratrol (100 μM) for 24 h and the microarray analysis was used to determine miRNA levels. The heat map shows significant expressional changes of miRNAs in SKOV3 cell treated with resveratrol. The color code is linear within the heat map: green represents the lowest level of expression and red the highest. The miRNAs that were upregulated are shown from green to red, whereas the miRNAs that were

downregulated are shown from red to green. (B) The SKOV3 cells were treated with 5-400 μM of resveratrol for 24 h and qRT-PCR was conducted to determine miR-34a expression. Data were represented as the mean ± SD of three individual experiments. **p < 0.01 vs. control.

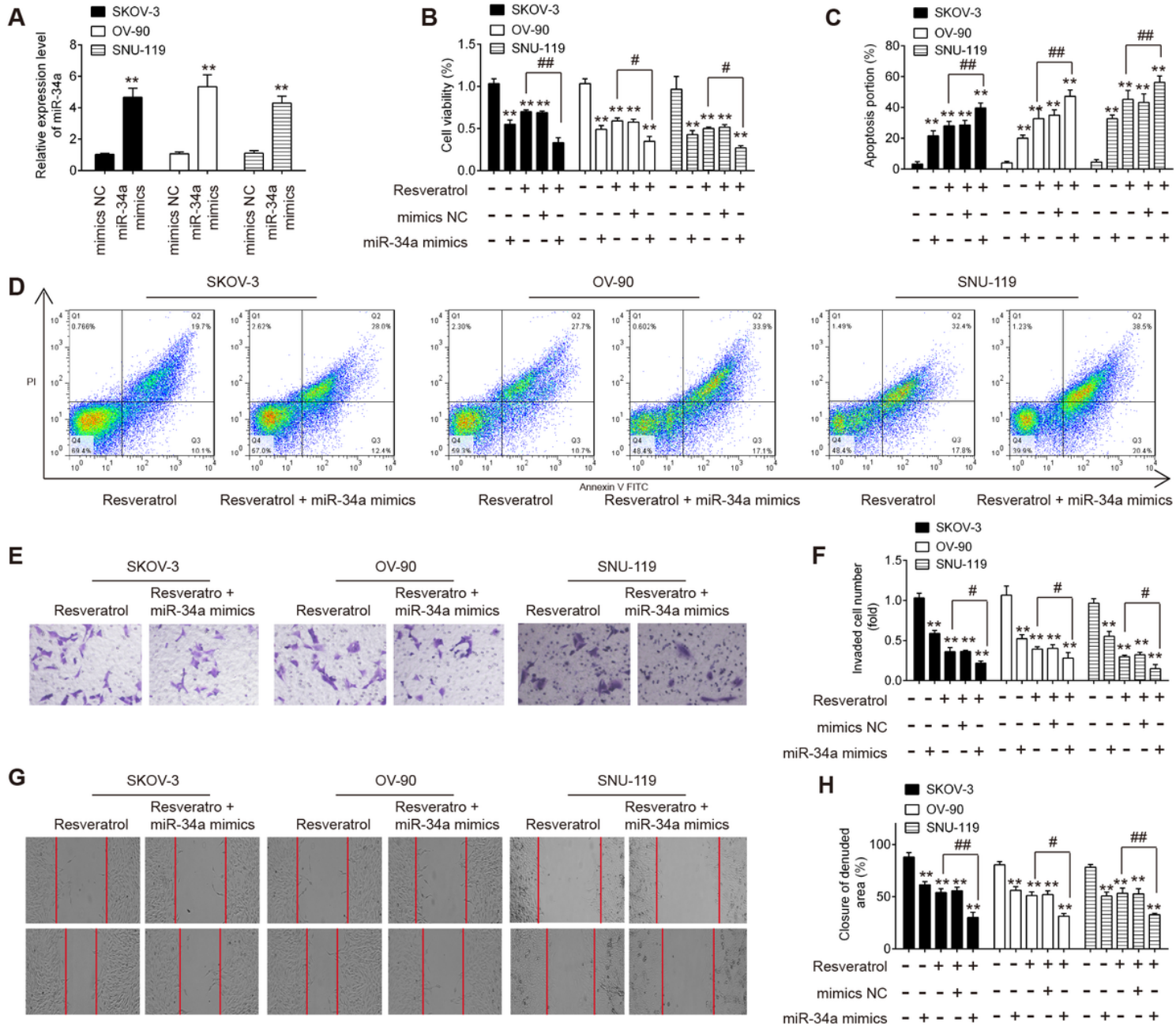

Figure 3

miR-34a overexpression enhanced the anti-tumor effects of resveratrol in ovarian cancer cells. The SKOV3, SNU-119 and OV90 cells were transfected with miR-34a mimics or mimic NC for 24 h, followed treatment with 100 μM of resveratrol for 24 h. (A) The expression of miR-34a was measured by qRT-PCR. (B) Cell viability was detected by CCK-8 assay. (C, D) The flow cytometric analysis was performed to determine apoptotic cells in SKOV3, SNU-119 and OV90 cells. (E, F) Cell invasion was detected by Transwell assay in SKOV3, SNU-119 and OV90 cells. (G, H) Cell migration was assessed by Wound

healing assay in SKOV3, SNU-119 and OV90 cells. Data were represented as the mean ± SD of three independent experiments. ** p < 0.01 vs. control. ##p < 0.01 vs. resveratrol group.

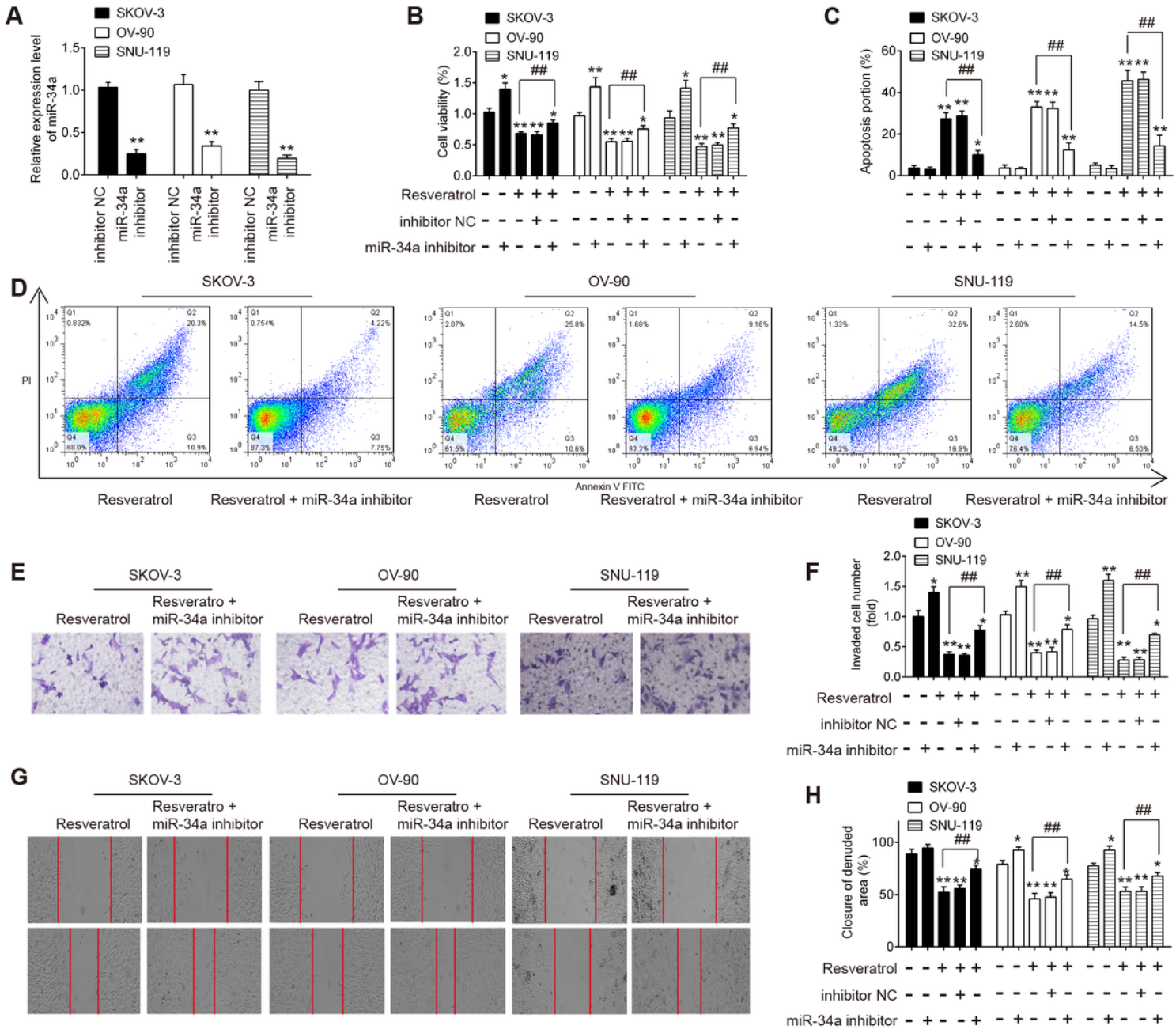

Figure 4

miR-34a inhibition attenuated the anti-tumor effects of resveratrol in ovarian cancer cells. The SKOV3, SNU-119 and OV90 cells were transfected with miR-34a inhibitor or inhibitor NC for 24 h, followed treatment with 100 μM of resveratrol for 24 h. (A) The expression of miR-34a was measured by qRT-PCR. (B) Cell viability was detected by CCK-8 assay. (C, D) The flow cytometric analysis was performed to determine apoptotic cells in SKOV3, SNU-119 and OV90 cells. (E, F) Cell invasion was detected by Transwell assay in SKOV3, SNU-119 and OV90 cells. (G, H) Cell migration was assessed by Wound healing assay in SKOV3, SNU-119 and OV90 cells. Data were represented as the mean ± SD of three independent experiments. ** p < 0.01 vs. control. ##p < 0.01 vs. resveratrol group.

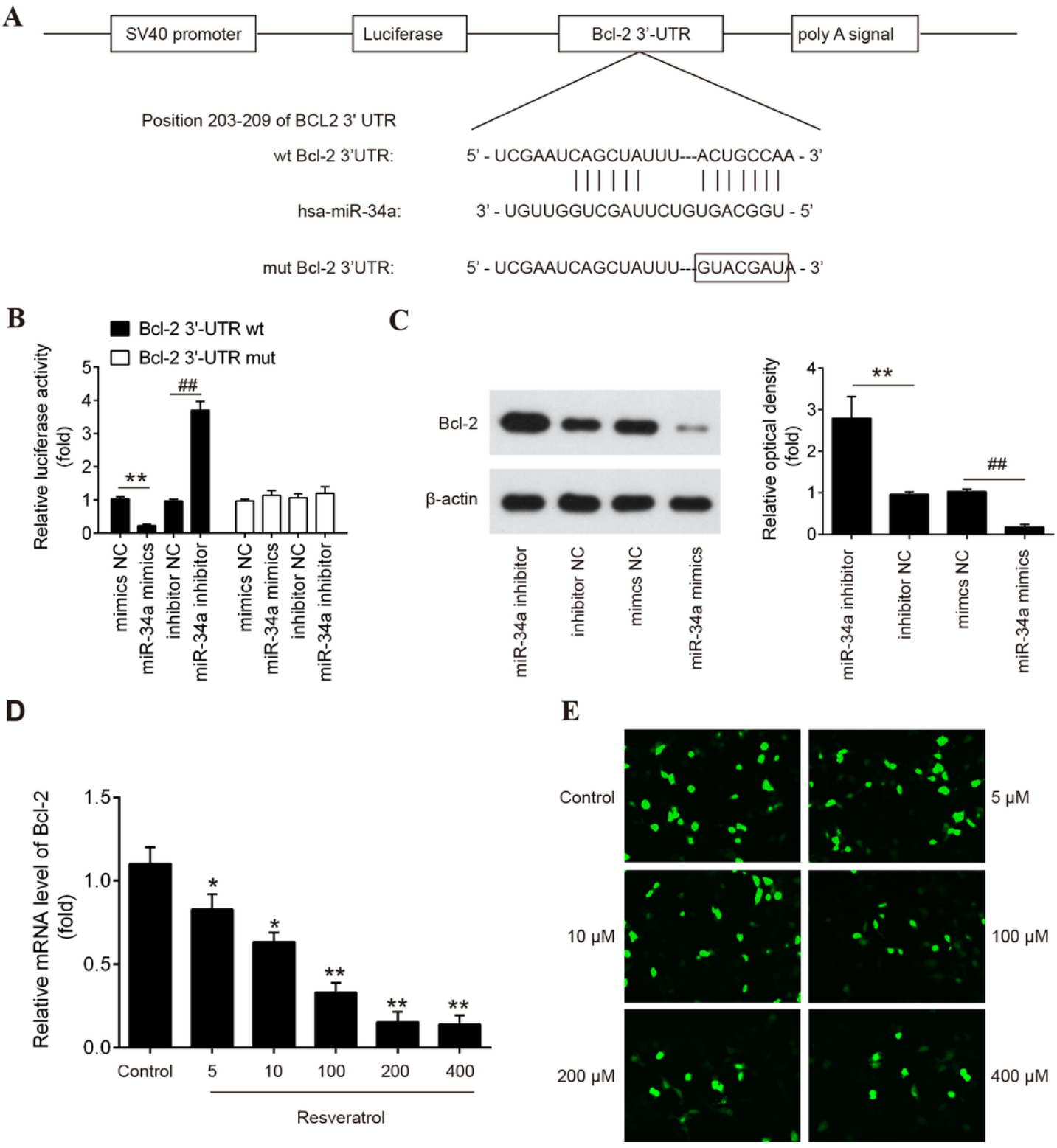

Figure 5

Bcl-2 was a direct target of miR-34a in ovarian cancer cells. (A) The predicted miR-34a binding sites on Bcl-2. (B) Luciferase reporter assay was conducted to detect luciferase activity of SKOV3 cells co-transfected with miR-34a mimics, miR-34a inhibitor or miR-NC and WT-BCL2-3′-UTR or MUT-BCL2-3′-UTR. (C) Western blot analysis was performed to determine the protein level of BCL2 in SKOV3 cells transfected with miR-34a mimics, miR-34a inhibitor or miR-NC. Data were represented as the mean ± SD

of three independent experiments. ** p < 0.01 vs. mimics NC. ##p < 0.01 vs. inhibitor NC. (D) The SKOV3 cells were treated with 5–400 μM of resveratrol for 24 h and qRT-PCR and indirect immunofluorescence assays were conducted to determine Bcl-2 expression. Data were represented as the mean ± SD of three individual experiments. **p < 0.01 vs. control.

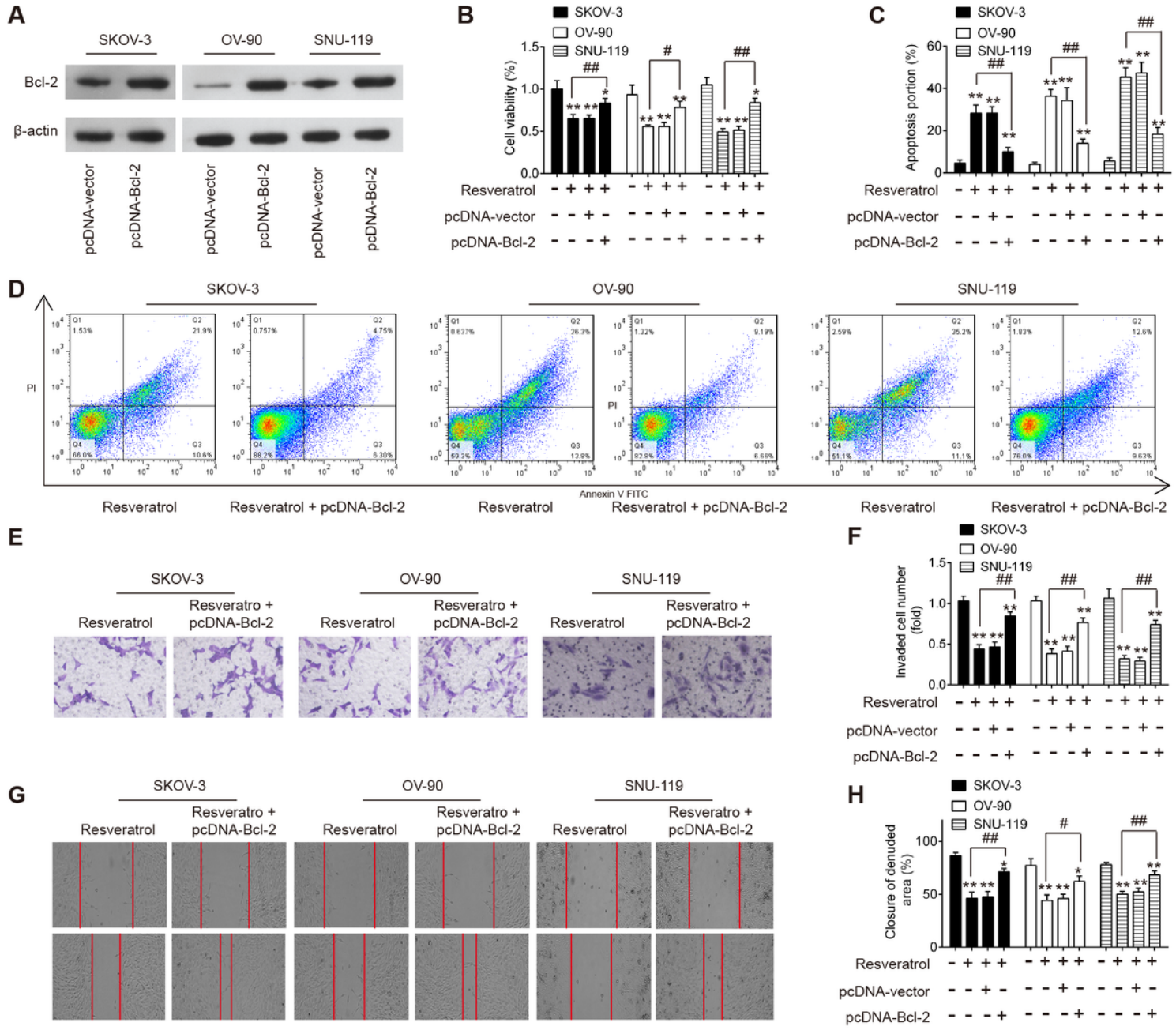

Figure 6

Bcl-2 overexpression reversed the anti-tumor effects of resveratrol in ovarian cancer cells. The SKOV3, SNU-119 and OV90 cells were transfected with pcDNA-Bcl-2 or pcDNA-vector for 24 h, followed treatment with 100 μM of resveratrol for 24 h. (A) The expression of Bcl-2 was measured by Western Blot. (B) Cell viability was detected by CCK-8 assay. (C, D) The flow cytometric analysis was performed to determine apoptotic cells in SKOV3, SNU-119 and OV90 cells. (E, F) Cell invasion was detected by Transwell assay in SKOV3, SNU-119 and OV90 cells. (G, H) Cell migration was assessed by Wound healing assay in

SKOV3, SNU-119 and OV90 cells. Data were represented as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01 vs. control. ##p < 0.01 vs. resveratrol group.

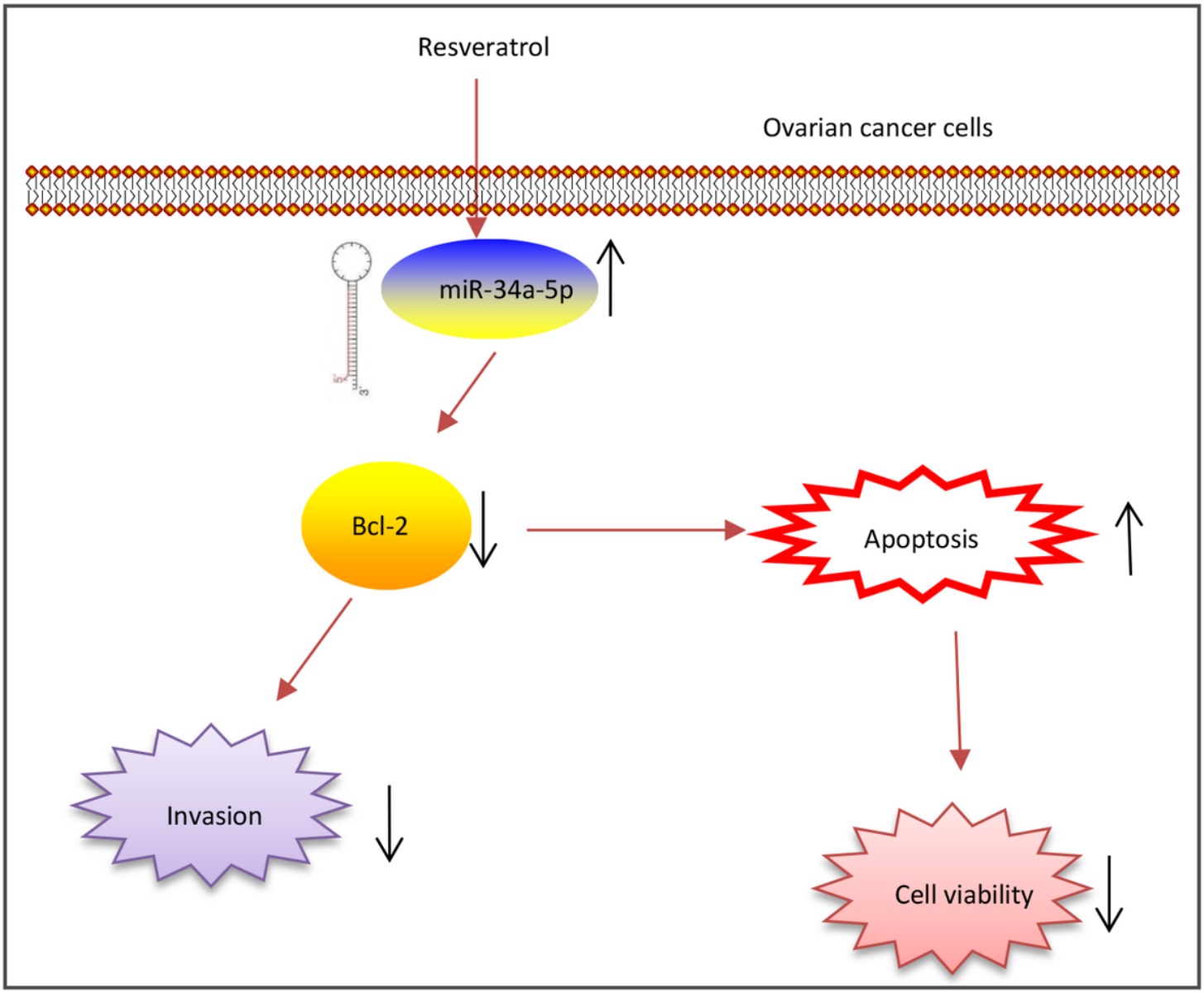

Figure 7

Schematic diagram of the molecular mechanism in which resveratrol exerts its anti-tumor effects in ovarian cancer cells. Resveratrol inhibited ovarian cancer cell proliferation, invasion and migration, and promoted cell apoptosis through miR-34a/Bcl-2 axis.