

# Resveratrol-mediated apoptosis in renal cell carcinoma via the p53/AMP-activated protein kinase/mammalian target of rapamycin autophagy signaling pathway

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**Abstract.** Resveratrol, known as phytoalexin, is a natural compound. Clinical studies have revealed that resveratrol has a variety of effects including anti-inflammatory, antiviral and tumor suppressor activities. It has been reported that it may serve an important role in renal cell carcinoma (RCC) however, the molecular mechanism underlying resveratrol-induced apoptosis in RCC is still unclear. The aim of the present study was to determine whether resveratrol could suppress RCC progression. Analysis of apoptosis demonstrated that resveratrol may act as a RCC suppressor in a dose- and time-dependent manner. In addition, the results of the MTT and cell migration experiments revealed that resveratrol significantly decreased cell viability and migration. In addition, the expression of the anti-apoptosis gene B-cell lymphoma 2 (Bcl-2) was downregulated by resveratrol, and the expression of pro-apoptosis gene Bcl-2-associated X was upregulated at the mRNA and protein levels. Resveratrol also promoted the expression of p53 and activated phospho-AMP-activated protein kinase (AMPK). The phosphorylation of mammalian target of rapamycin (mTOR) was inhibited and the autophagy-associated genes, light chain 3, autophagy related (ATG)5 and ATG7, were upregulated at the mRNA and protein levels. In conclusion, resveratrol suppressed RCC viability and migration, and promoted RCC apoptosis via the p53/AMPK/mTOR-induced autophagy signaling pathway.

## Introduction

Renal cell carcinoma (RCC), a complex metabolic disease that is associated with a number of different types of cancer,

occurs in the kidney. In recent years, RCC has become the most common urological malignancy (1), accounting for ~2-3% of adult malignancies and ~80-90% of adult kidney malignancies; thus, RCC has a serious impact on public health. The current treatments available for RCC include novel small molecular targeted drugs, immune targeted therapy, adjuvant and neoadjuvant therapies and biomarker research. The primary treatments applied for RCC include tyrosine kinase inhibitors such as sunitinib and pazopanib, the mTOR inhibitor temsirolimus, the monoclonal antibody inhibitor of vascular endothelial growth factor (VEGF) bevacizumab and cytokine treatments such as aldesleukin (2). The current principle of targeted RCC therapy is based on the known molecular mechanisms of renal cancer and aims to prevent the proliferation of tumor cells and inhibit angiogenesis; Von Hippel-Lindau tumor suppressor and mTOR (3) are the widely used targets in clinical practice. p53, a tumor suppressor protein, serves an important role in apoptosis and the inhibition of angiogenesis; it is essential for mouse double minute 2 proto-oncogene antagonist induced RCC cell apoptosis (4). Recently, a previous study demonstrated that co-treatment with curcumin and temsirolimus activated the expression of p53, which induced RCC cell apoptosis (5). In addition, p53 crosslinking with transglutaminase 2 led to p53 depletion and tumor survival (6); these findings suggested that p53 may be a key regulator in RCC.

mTOR is known as a serine-threonine kinase and the mTOR signaling pathway has been implicated in inflammatory, metabolic, degenerative and proliferative activities, and in cancer (7-10). It has been reported that mTOR is activated and hyperactivated in RCC (11). In addition, mTOR mutations have been observed in clear cell RCC (12). The results of these studies indicated that mTOR may have an important role in the regulation of RCC however, the molecular mechanism requires further investigation. Therefore, the present study will further evaluate the effect of mTOR in RCC.

Resveratrol (Trans-3,4',5-trihydroxystilbene) is a natural phytoalexin that is used to prevent human cardiovascular diseases, and induce anti-inflammatory (13,14) and anticancer (15) activities. A previous study revealed that resveratrol is a promising therapy for patients with chronic kidney disease (16). Liu *et al* (17) demonstrated that insulin- and leucine-stimulated

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mTOR signaling was inhibited by resveratrol. However, another previous study indicated that resveratrol may negatively regulate mTOR via AMP-activated protein kinase (AMPK) activation in sensory neurons (18). In RCC cells, resveratrol may inhibit RCC cell proliferation and apoptosis via the angiotensin II receptor type 1/VEGF signaling pathway (19). Whether resveratrol is able to regulate RCC through the AMPK/mTOR signaling pathway requires further study. In the present study, resveratrol-mediated cell apoptosis in RCC through the p53 AMPK/mTOR signaling pathway is investigated in order to provide a potential target for the treatment of RCC.

## Materials and methods

**Cell culture.** HK-2 and Ketr-3 cells were obtained from American Type Culture Collection (Manassas, VA, USA). The cells were maintained in Eagle's Minimum Essential Medium with 10% fetal bovine serum (FBS) (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd., Hangzhou, China), and were cultured at 37°C in a 5% humidified CO<sub>2</sub> atmosphere until they reached 90% confluence. Resveratrol was obtained from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany) and was dissolved in DMSO in order to create a stock solution at a concentration of 100 mM; this was subsequently diluted in the culture medium to the desired concentration (12.5, 25, 50 and 100  $\mu$ M) for experiments. DMSO was used as the blank control for all experiments.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis.** Total RNA was extracted from Ketr cells after 48 h using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) after the cells were treated with resveratrol (12.5, 25, 50 and 100  $\mu$ M) for 72 h. The concentration of extracted RNA was determined using a spectrophotometer at wavelength of 260 nm. Reverse transcription was performed using the PrimeScript™ RT reagent kit with gDNA Eraser (Perfect Real Time) according to the manufacturer's protocol (Takara Biotechnology Co., Ltd., Dalian, China). The reactions were as follows: 42°C for 60 min, followed by 95°C for 5 min. qPCR was performed using the SYBR Premix Ex Taq™ II reaction mixture (Takara Biotechnology Co., Ltd.). The thermocycling conditions were as follows: 95°C for 30 sec for initial denaturation, then 40 cycles of 95°C for 5 sec, 60°C for 30 sec and 72°C for 30 sec, followed by a final extension at 72°C for 5 min. PCR reactions were carried out using the ABI Step One Plus real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences used were as follows: B-cell lymphoma 2 (Bcl-2) sense, 5'-ACCAAGCTGAGC GAGTGTC-3' and antisense, 5'-ACAAAGATGGTCACG GTCTGCC-3'; Bcl-2-associated X (Bax) sense, 5'-ACCAAG CTGAGCGAGTGTC-3' and antisense, 5'-ACAAAGATG GTCACGGTCTGCC-3'; p53 sense, 5'-GTTTCCGTCTGG GCTTCTTG-3' and antisense, 5'-CCTGGGCATCCTTGAGT TCC-3'; light chain (LC)3 sense, 5'-TGTCCGACTTATTCG AAGCAGCA-3' and antisense, 5'-TTCACCAACAGGAAG AAGGCCTGA-3'; autophagy related (ATG)5 sense, 5'-TCG TACATATCCTGGCAGGTTCGC-3' and antisense, 5'-GAA GTCGCAGGGGCGTTCCAT-3' and ATG7 sense, 5'-CAT TCCGCTATAGGCACCAT-3' and antisense, 5'-CGGCAA

AGGAGAGAACAAAG-3'. Relative gene expression was calculated using the 2<sup>- $\Delta\Delta C_q$</sup>  method (20), and the results were normalized to those of  $\beta$ -actin (sense, 5'-AACCGCGAGAAG ATGACCCAGATCATGTTT-3' and antisense, 5'-AGCAGC CGTGGCCATCTCTTGCTCGAAGTC-3').

**Western blot analysis.** Ketr-3 cells were washed three times with cold PBS and proteins were extracted using ice-cold radioimmunoprecipitation buffer (containing 1% proteinase inhibitor PMSF; Beyotime Institute of Biotechnology, Shanghai, China). Cells were centrifuged at 14,500 x g for 10 min at 4°C. A Bicinchoninic Acid Protein Assay kit (Beyotime Institute of Biotechnology, Shanghai, China) was used to determine total protein concentrations. Total proteins (15  $\mu$ g) were separated by 4-12% SDS-PAGE gel and then transferred onto a nitrocellulose membrane (EMD Millipore, Billerica, MA, USA). Membranes were blocked with 5% non-fat dry milk for 2 h at room temperature. The membranes were then incubated with a diluted primary antibody at 4°C overnight; Bax (D2E11) rabbit (cat. no. 5023; 1:1,000), Bcl-2 (cat. no. 15071; 1:1,000), p53 (7F5) rabbit (cat. no. 2527; 1:1,000), phospho-AMPK $\alpha$  (Thr172) (40H9) rabbit mAb (cat. no. 2535S; 1:1,000), AMPK $\alpha$  (D63G4) rabbit mAb (cat. no. 5832S; 1:1,000), phospho-mTOR (Ser2448) (D9C2) rabbit (cat. no. 5536; 1:1,000) mTOR (7C10) rabbit mAb (cat. no. 2983; 1:1,000) LC3B (D11) XP® rabbit mAb (cat. no. 3868; 1:1,000) Atg5 (D5F5U) rabbit mAb (cat. no. 12994; 1:1,000) Atg7 (D12B11) rabbit mAb (cat. no. 8558; 1:1,000) GAPDH (D16H11) XP rabbit mAb (cat. no. 5174S; 1:5,000). All the primary antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Anti- $\beta$ -actin antibodies (cat. no. MAB-1501; 1:10,000) were obtained from EMD Millipore. Anti-rabbit horseradish peroxidase-conjugated IgG antibodies (cat. no. ADI-SAB-300; 1:2,000) were purchased from Enzo Life Sciences, Inc. (Farmingdale, NY, USA) and incubated with the membrane for 2 h at room temperature. The specific complexes were visualized using the SuperSignal West Pico chemiluminescent substrate (Shanghai Solarbio Science and Technology, Shanghai, China). Densitometric analysis was performed to quantify the signal intensity using ImageJ software (version 1.37; National Institutes of Health, Bethesda, MD, USA).

**MTT assay.** Ketr-3 cell viability was measured using an MTT assay. Briefly, 1x10<sup>6</sup> cells were seeded into 96-well plates, and following 24 h at 37°C to allow for cell adhesion, resveratrol was added at varying concentrations (12.5, 25, 50 and 100  $\mu$ M) for 12, 24, 48 and 72 h. Control cultures were treated with DMSO. A total of 20  $\mu$ l of 5 mg/ml MTT solution was added to each well and the plate was further incubated at 37°C for 4 h. The cells were then washed with PBS and 150  $\mu$ l DMSO was added to each well. Absorbance was read at 570 nm using a spectrophotometer (EnSpire 2300 Multilabel Reader; PerkinElmer, Inc., Waltham, MA, USA).

**Apoptosis assay.** Tumor cells were digested with trypsin at 37°C for 5 min and inoculated in a 50 ml culture flask at 5x10<sup>5</sup>/ml, then 1x10<sup>5</sup> Ketr-3 cells in each group were seeded in 6-well plates and treated with various concentrations of resveratrol (12.5, 25, 50 and 100  $\mu$ M) for 24 h; control cells treated with DMSO only. Cell apoptosis rate was detected

using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Assay kit (BD Pharmingen; BD Biosciences, San Jose, CA, USA), according to the manufacturer's protocol. A BD FACSCanto II flow cytometer (BD Biosciences) was used to collect data for analysis using FCSEXPRESS software (version 3.0; De Novo Software, Glendale, CA, USA).

**Cell migration assay.** Ketr-3 cells ( $1 \times 10^5$ ) after treatment with resveratrol (12.5, 25, 50 and 100  $\mu\text{M}$ ) were seeded in the top chamber of Transwell migration chambers (8-5 mm; BD Biosciences) in serum-free medium, and the lower chamber contained 10% FBS. Following culture for 24 h at 37°C, the cells in the bottom chamber were stained with 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) at 37°C for 20 min. The cells were then evaluated by a light microscopy (CK40; Olympus Corporation, Tokyo, Japan) at the magnification at  $\times 200$ . Images were captured, then the cells were counted randomly in five fields and the average was calculated.

**Statistical analysis.** The results were analyzed using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA). Data are presented as the mean  $\pm$  standard deviation of at least three independent experiments. An analysis of variance followed by a Tukey test was performed to compare the differences between the different groups.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Effect of resveratrol on Ketr-3 cell viability.** To investigate the effects of resveratrol on RCC cells, Ketr-3 cells were treated with various concentrations (0, 12.5, 25, 50 and 100  $\mu\text{M}$ ) of resveratrol for 12, 24, 48 and 72 h. Following treatment, cells were assayed by MTT and the results revealed that the Ketr-3 cell proliferation was significantly inhibited by resveratrol in a dose dependent manner (Fig. 1).

**Resveratrol treatment inhibits Ketr-3 cell migration.** To further evaluate the potential effect of resveratrol, its effect on Ketr-3 cell migration was determined by measuring the number of migratory Ketr-3 cells following resveratrol treatment. As shown in Fig. 2, the number of migrating Ketr-3 cells was significantly inhibited by resveratrol in a dose-dependent manner.

**Resveratrol induces apoptosis in Ketr-3 cells.** Previous studies have demonstrated that resveratrol induces apoptosis in cancer cells (21-24). The present study further confirmed the ability of resveratrol to induce apoptosis in Ketr-3 cells. Annexin V-FITC/PI staining was performed to detect the effect of resveratrol treatment on apoptosis in cells. The results shown in Fig. 3 revealed that resveratrol treatment significantly increased cell apoptosis. As Bcl-2 and Bax are markers of apoptosis in RCC (25), the present study detected their mRNA and protein expression. Resveratrol treatment increased and decreased the expression of Bax and Bcl-2, respectively, at both the mRNA and protein levels (Fig. 4A and B). The results indicated that resveratrol may serve as a tumor suppressor in RCC.

**Expression of p53 is downregulated in Ketr-3 cells and upregulated by treatment with resveratrol.** The resveratrol

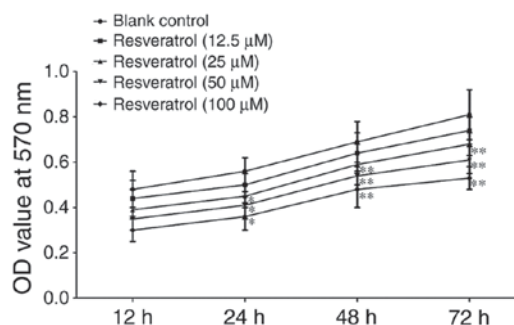


Figure 1. Resveratrol inhibits the Ketr-3 cell viability. Cells were seeded in a 96-well plate and treated with resveratrol at various concentrations (0, 12.5, 25, 50 and 100  $\mu\text{M}$ ) and detected by MTT assay at 12, 24, 48 and 72 h. DMSO was used as the blank control. Data are presented as the mean  $\pm$  standard deviation. \* $P < 0.05$  and \*\* $P < 0.01$  vs. blank control. OD, optical density.

treatment significantly increased the expression of p53 mRNA and protein in a dose-dependent manner (Fig. 4C). However, when compared with the normal kidney HK-2 cells, the expression of p53 was significantly decreased in Ketr-3 cells (Fig. 5). These results indicated that p53 may serve an important role in resveratrol-induced Ketr-3 cell apoptosis.

**Resveratrol induces Ketr-3 cell apoptosis through p53-mediated AMPK/mTOR signaling.** Recently previous findings have indicated that AMPK may serve a pivotal role in the control of the p53 signaling pathway (26-28), however, it is unknown whether AMPK is involved in resveratrol-mediated Ketr-3 cell apoptosis. The present study detected the expression of AMPK and demonstrated that resveratrol treatment upregulated phosphorylated (p)-AMPK (Fig. 6A), which indicated that resveratrol may activate p-AMPK and regulate downstream gene expression. As mTOR is key in the p53 signaling pathway, the present study detected the expression of mTOR and demonstrated that resveratrol treatment downregulated p-mTOR, however, not the total expression of mTOR (Fig. 6B). Thus, resveratrol may induce Ketr-3 cell apoptosis via the p53-mediated AMPK/mTOR signaling.

**Resveratrol mediated apoptosis in renal cell carcinoma cell potentially by promoting autophagy.** Previous studies have revealed that the AMPK/mTOR signaling pathway is important in autophagy, however, it is not clear whether resveratrol regulates RCC through autophagy (17,18,29). The present study detected the expression of the downstream genes of the AMPK/mTOR-induced autophagy pathway. The results revealed that the expression of LC3 (Fig. 7A), ATG5 (Fig. 7B) and ATG7 (Fig. 7C) were increased by resveratrol. These results suggested that p53 may regulate autophagy via AMPK/mTOR signaling, thereby promoting cell apoptosis.

## Discussion

Previous studies have demonstrated that resveratrol acts as an anticancer factor in leukemia, and breast, stomach, colon, prostate, ovarian and skin cancer (21,22,29-31). The results of these studies all indicated that resveratrol could effectively inhibit cancer cell activity, and that it could be used for chemoprevention and anti-inflammation (23). In addition, resveratrol



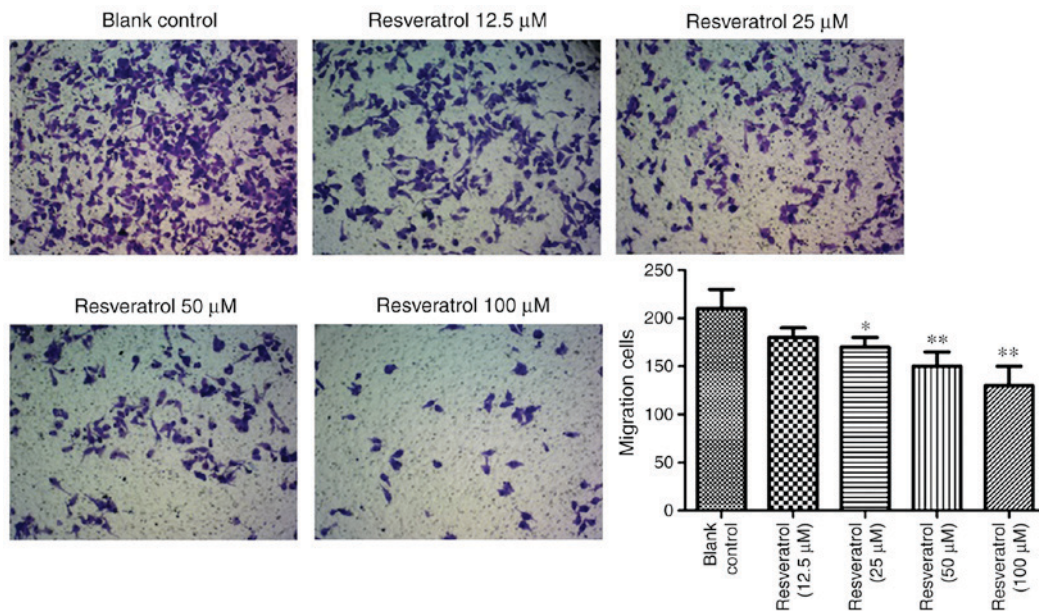


Figure 2. Migration of Ketr-3 cells is inhibited by resveratrol. Different concentrations of resveratrol (0, 12.5, 25, 50 and 100  $\mu$ M) were used to treat Ketr-3 cells and cell migration was detected by a cell migration assay. Magnification, x200. DMSO was used as the blank control. Data are presented as the mean  $\pm$  standard deviation. \* $P < 0.05$  and \*\* $P < 0.01$  vs. blank control.

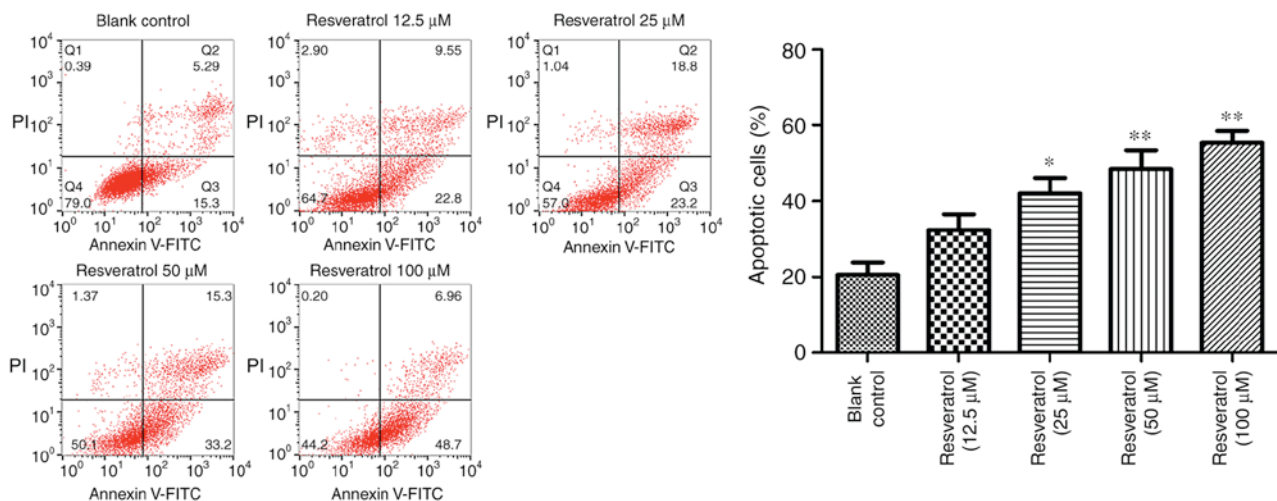


Figure 3. Different concentrations of resveratrol induce Ketr-3 cell apoptosis. Cells were treated with resveratrol at various concentrations (0, 12.5, 25, 50 and 100  $\mu$ M), then cell apoptosis was measured by flow cytometry. DMSO was used as the blank control. Data are presented as the mean  $\pm$  standard deviation. \* $P < 0.05$  and \*\* $P < 0.01$  vs. blank control. FITC, fluorescein isothiocyanate; PI, propidium iodide.

prevented malignant tumor invasion by preventing kinase function (31,32). In the present study, resveratrol induced RCC cell apoptosis, and inhibited cell viability and migration, which is consistent with the previous report (19). In addition, the present study also demonstrated that resveratrol may induce RCC apoptosis via the AMPK/mTOR autophagy pathway.

A previous study revealed that p53 was involved in resveratrol-induced cancer cell apoptosis (24). Resveratrol treatment in human breast cancer MCF-7 cells increased the expression of p53 and Bax/Bcl-2, and resveratrol can be used as a neo-adjuvant in human breast cancer (33). In addition, it has been reported that resveratrol can inhibit Glioblastoma multiforme growth by modulating p53 and protein kinase B (32). In RCC, p53 is a factor involved in cell-cycle arrest and apoptosis; although resveratrol has been reported to be associated with RCC, the specific role

of p53 in resveratrol anti-RCC remains unknown (34). In the present study, the expression of p53, as well as Bax, was significantly upregulated in resveratrol-induced RCC cell apoptosis. The results of the present study are consistent with those of a previous study, which observed transglutaminase 2-induced p53 mediated apoptosis in RCC (35).

The AMPK-targeting drug 5-Aminoimidazole-4-carboxamide riboside (26) and metformin may be used in clinical oncology (36). AMPK is a key regulator in cell growth and RCC tumorigenesis, and also when AMPK activation inhibits RCC growth and survival (26). In RCC, 8-chloro-adenosine activated AMPK and inhibited mTOR pathway, and Silibinin, as a cancer chemopreventive flavonoid, could induce an anti-metastatic effect on RCC by targeting AMPK (37). In the present study, the levels of p-AMPK were upregulated

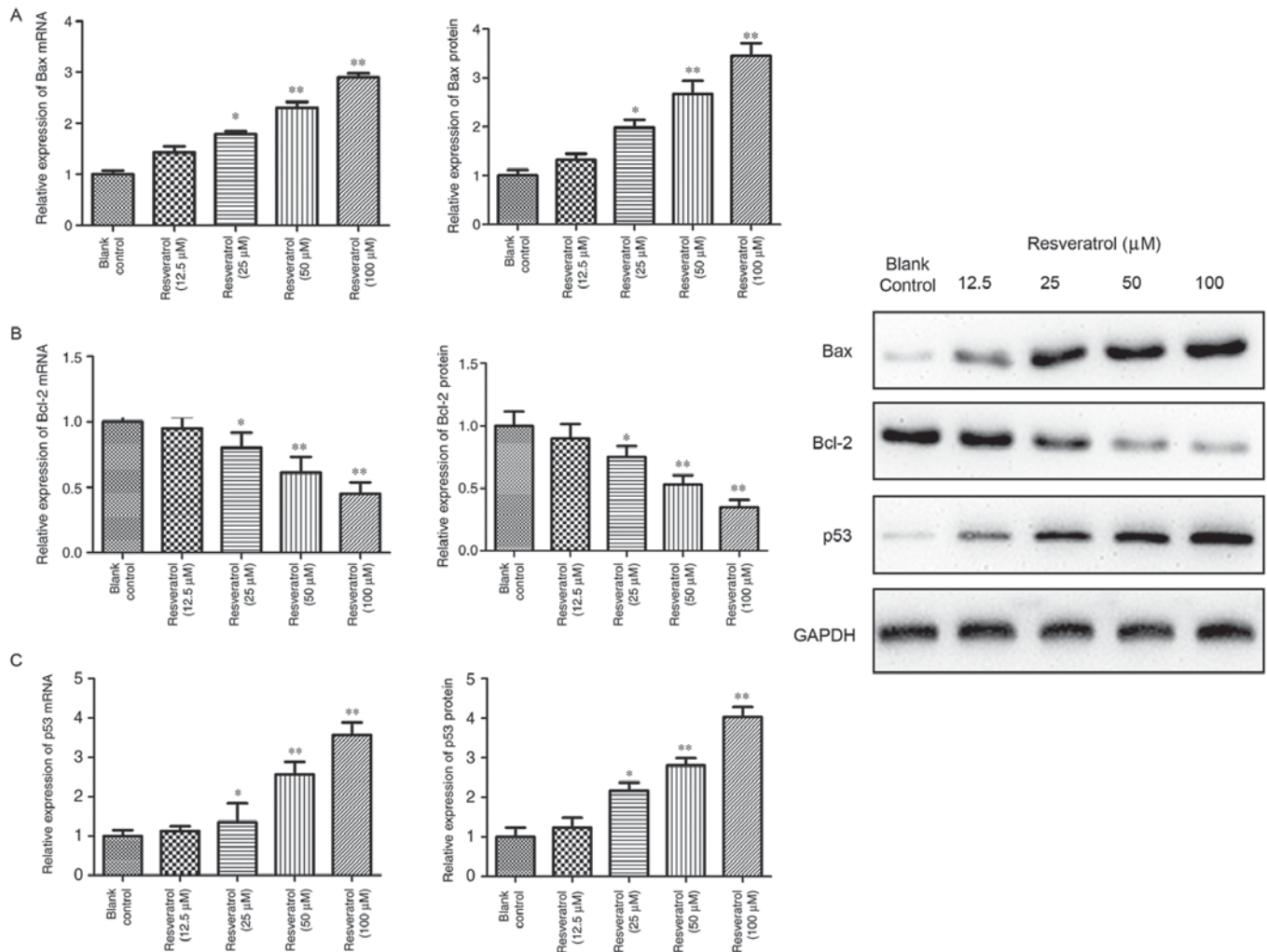


Figure 4. Expression of Bax, Bcl-2 and p53 in Ketr-3 cells treated with resveratrol. The relative mRNA and protein levels of (A) Bax, (B) Bcl-2 and (C) p53 were measured in Ketr-3 cells treated with 0, 12.5, 25, 50 and 100 μM resveratrol. mRNA was normalized to β-actin. DMSO was used as the blank control. Data are presented as the mean ± standard deviation. \*P<0.05 and \*\*P<0.01 vs. blank control. Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X.

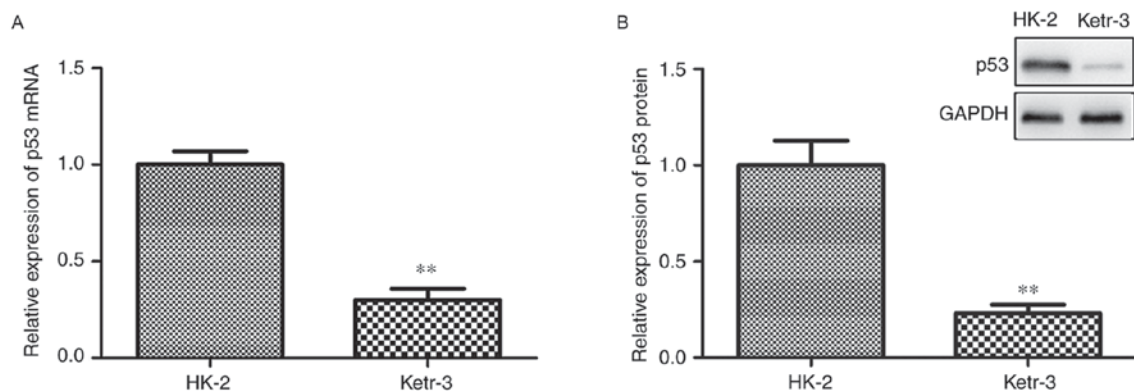


Figure 5. mRNA and protein expression of p53 in HK-2 and Ketr-3 cells. The relative (A) mRNA and (B) protein expression levels of p53 in normal HK-2 and Ketr-3 cells. β-actin and GAPDH were used as internal controls for mRNA and protein expression, respectively. DMSO was used as the blank control. Data are presented as the mean ± standard deviation. \*\*P<0.01 vs. blank control.

following treatment with resveratrol; thus, AMPK may participate in the apoptosis induced by resveratrol.

Autophagy regulation involves a variety of different signaling pathways, of which, AMPK and mTOR are key regulators. AMPK promotes and mTOR inhibits autophagy (38).

In Zucker diabetic fatty rats, liraglutide promotes autophagy by enhancing AMPK phosphorylation and inhibiting mTOR phosphorylation (27). In addition, in cancer cells induced by wild-type p53, docosahexaenoic acid attenuates autophagy by increasing AMPK activation and decreasing the activity

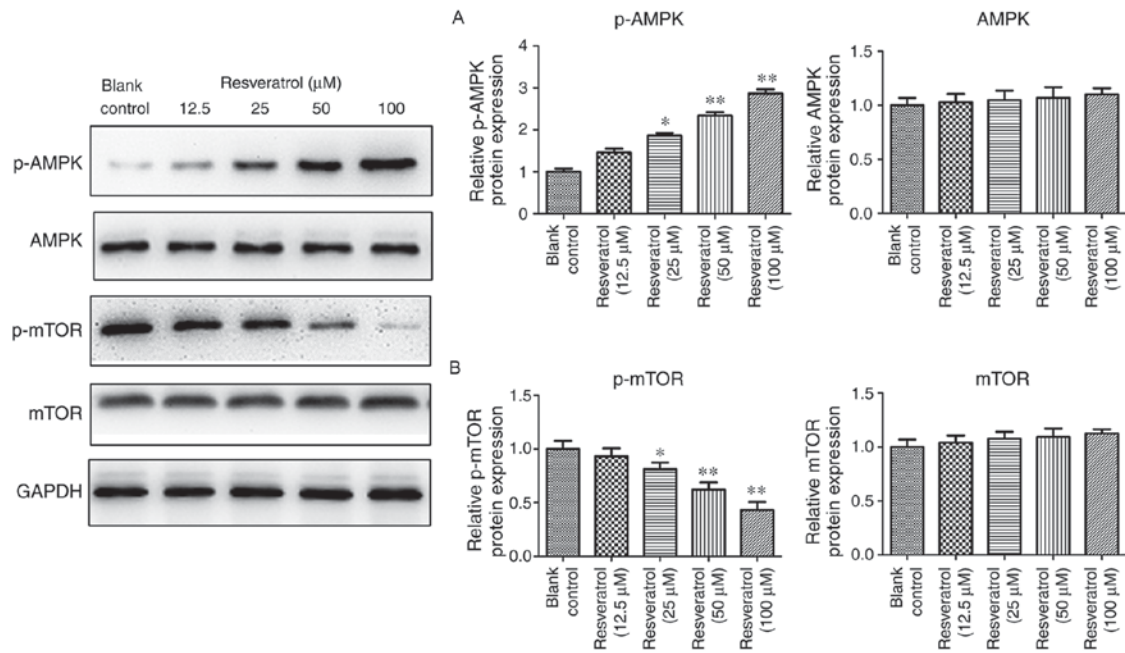


Figure 6. Effect of resveratrol on AMPK and mTOR protein expression. Cells were treated with 0, 12.5, 25, 50 and 100  $\mu$ M resveratrol and then the relative protein expression of (A) total and p-AMPK, and (B) total and p-mTOR was determined. Protein levels were normalized to those of GAPDH. DMSO was used as the blank control. Data are presented as the mean  $\pm$  standard deviation. \* $P$ <0.05 and \*\* $P$ <0.01 vs. blank control. AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; p-, phosphorylated.

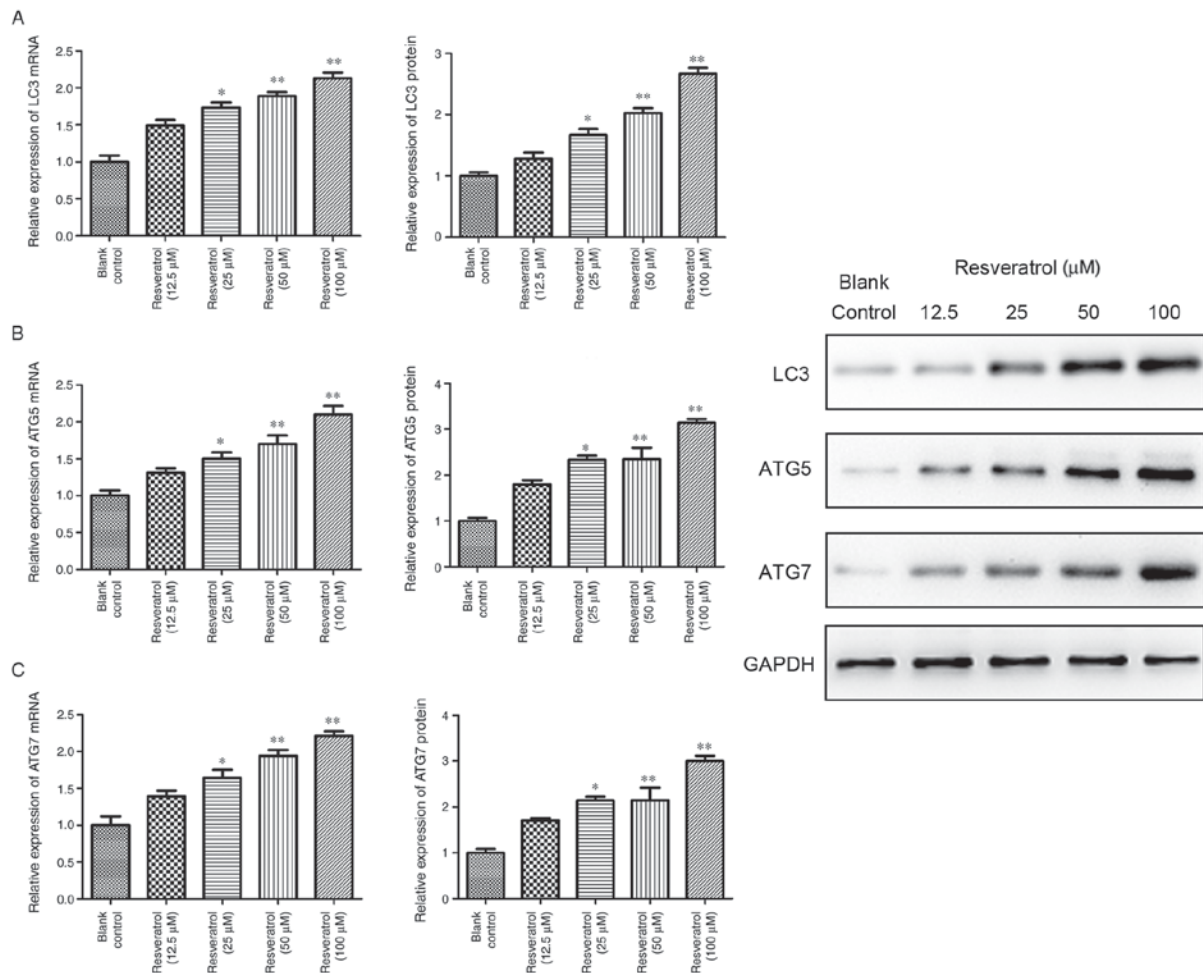


Figure 7. Autophagy is involved in resveratrol induced Kert-3 cell apoptosis. The relative mRNA and protein of (A) LC3, (B) ATG5 and (C) ATG7 were measured in cells treated with different concentrations of resveratrol (0, 12.5, 25, 50 and 100  $\mu$ M). DMSO was used as the blank control. Data are presented as the mean  $\pm$  standard deviation. \* $P$ <0.05 and \*\* $P$ <0.01 vs. blank control. LC3, light chain 3; ATG, autophagy related.



of mTOR (28). The present study detected the expression of the genes, LC3, ATG5 and ATG7, which are associated with autophagy. The results demonstrated that resveratrol upregulated their mRNA and protein levels, indicating that resveratrol may be involved in autophagy regulation in RCC. However, the role of resveratrol in the regulation of autophagy, in RCC or other human cancers, has yet to be elucidated.

In conclusion, the present study demonstrated that resveratrol treatment in RCC promoted apoptosis via p53-mediated AMPK/mTOR autophagy signaling. These results indicate that resveratrol may be a potential candidate for RCC treatment.

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## References

- Mitsunari K, Miyata Y, Watanabe SI, Asai A, Yasuda T, Kanda S and Sakai H: Stromal expression of Fer suppresses tumor progression in renal cell carcinoma and is a predictor of survival. *Oncol Lett* 13: 834-840, 2017.
- Merza H and Bilusic M: Current management strategy for metastatic renal cell carcinoma and future directions. *Curr Oncol Rep* 19: 27, 2017.
- Cho D, Signoretti S, Dabora S, Regan M, Seeley A, Mariotti M, Youmans A, Polivy A, Mandato L, McDermott D, *et al*: Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. *Clin Genitourin Cancer* 5: 379-385, 2007.
- Abolhasani M, Salarinejad S and Asgari M: P53 and MDM2 Over-expression and five-year survival of kidney cancer patients undergoing radical nephrectomy-iranian experience. *Asian Pac J Cancer Prev* 16: 5043-5047, 2015.
- Xu S, Yang Z, Fan Y, Guan B, Jia J, Gao Y, Wang K, Wu K, Wang X, Zheng P, *et al*: Curcumin enhances temsirolimus-induced apoptosis in human renal carcinoma cells through upregulation of YAP/p53. *Oncol Lett* 12: 4999-5006, 2016.
- Kang JH, Lee JS, Hong D, Lee SH, Kim N, Lee WK, Sung TW, Gong YD and Kim SY: Renal cell carcinoma escapes death by p53 depletion through transglutaminase 2-chaperoned autophagy. *Cell Death Dis* 7: e2163, 2016.
- Habib SL and Liang S: Hyperactivation of Akt/mTOR and deficiency in tuberlin increased the oxidative DNA damage in kidney cancer patients with diabetes. *Oncotarget* 5: 2542-2550, 2014.
- Lamming DW and Sabatini DM: A Central role for mTOR in lipid homeostasis. *Cell Metab* 18: 465-469, 2013.
- Kim YC and Guan KL: mTOR: A pharmacologic target for autophagy regulation. *J Clin Invest* 125: 25-32, 2015.
- Xu J, Pham CG, Albanese SK, Dong Y, Oyama T, Lee CH, Rodrik-Outmezguine V, Yao Z, Han S, Chen D, *et al*: Mechanistically distinct cancer-associated mTOR activation clusters predict sensitivity to rapamycin. *J Clin Invest* 126: 3526-3540, 2016.
- Pal SK and Quinn DI: Differentiating mTOR inhibitors in renal cell carcinoma. *Cancer Treat Rev* 39: 709-719, 2013.
- Garcia-Donas J, Rodriguez-Antona C and Jonasch E: Molecular markers to predict response to therapy. *Semin Oncol* 40: 444-458, 2013.
- Conte E, Fagone E, Fruciano M, Gili E, Iemmolo M and Vancheri C: Anti-inflammatory and antifibrotic effects of resveratrol in the lung. *Histol Histopathol* 30: 523-529, 2015.
- Bo S, Ciccone G, Castiglione A, Gambino R, De Micheli F, Villosio P, Durazzo M, Cavallo-Perin P and Cassader M: Anti-inflammatory and antioxidant effects of resveratrol in healthy smokers: a randomized, double-blind, placebo-controlled, cross-over trial. *Curr Med Chem* 20: 1323-1331, 2013.
- Wong JC and Fiscus RR: Resveratrol at anti-angiogenesis/anti-cancer concentrations suppresses protein kinase G signaling and decreases IAPs expression in HUVECs. *Anticancer Res* 35: 273-281, 2015.
- Saldanha JF, Leal Vde O, Stenvinkel P, Carraro-Eduardo JC and Mafra D: Resveratrol: Why is it a promising therapy for chronic kidney disease patients? *Oxid Med Cell Longev* 2013: 963217, 2013.
- Liu M and Liu F: Resveratrol inhibits mTOR signaling by targeting DEPTOR. *Commun Integr Biol* 4: 382-384, 2011.
- Tillu DV, Melemedjian OK, Asiedu MN, Qu N, De Felice M, Dussor G and Price TJ: Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain. *Mol Pain* 8: 5, 2012.
- Li J, Qiu M, Chen L, Liu L, Tan G and Liu J: Resveratrol promotes regression of renal carcinoma cells via a renin-angiotensin system suppression-dependent mechanism. *Oncol Lett* 13: 613-620, 2017.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) methods. *Methods* 25: 402-408, 2001.
- Kai L, Samuel SK and Levenson AS: Resveratrol enhances p53 acetylation and apoptosis in prostate cancer by inhibiting MTA1/NuRD complex. *Int J Cancer* 126: 1538-1548, 2010.
- Zhong LX, Zhang Y, Wu ML, Liu YN, Zhang P, Chen XY, Kong QY, Liu J and Li H: Resveratrol and STAT inhibitor enhance autophagy in ovarian cancer cells. *Cell Death Discov* 2: 15071, 2016.
- Singh SK, Banerjee S, Acosta EP, Lillard JW and Singh R: Resveratrol induces cell cycle arrest and apoptosis with docetaxel in prostate cancer cells via a p53/ p21WAF1/CIP1 and p27KIP1 pathway. *Oncotarget* 8: 17216-17228, 2017.
- Heiss EH, Schilder YD and Dirsch VM: Cvs. *J Biol Chem* 282: 26759-26766, 2007.
- Saker Z, Tsintsadze O, Jiqia I, Managadze L and Chkhotua A: Importance of apoptosis markers (MDM2, BCL-2 and BAX) in conventional renal cell carcinoma. *Georgian Med News*: 27-33, 2015.
- Woodard J, Joshi S, Viollet B, Hay N and Platanias LC: AMPK as a therapeutic target in renal cell carcinoma. *Cancer Biol Ther* 10: 1168-1177, 2010.
- Zhang Y, Ling Y, Yang L, Cheng Y, Yang P, Song X, Tang H, Zhong Y, Tang L, He S, *et al*: Liraglutide relieves myocardial damage by promoting autophagy via AMPK/mTOR signaling pathway in Zucker diabetic fatty rat. *Mol Cell Endocrinol* 448: 98-107, 2017.
- Jing K, Song KS, Shin S, Kim N, Jeong S, Oh HR, Park JH, Seo KS, Heo JY, Han J, *et al*: Docosahexaenoic acid induces autophagy through p53/AMPK/mTOR signaling and promotes apoptosis in human cancer cells harboring wild-type p53. *Autophagy* 7: 1348-1358, 2011.
- Park SY, Chae SY, Park JO, Lee KJ and Park G: Gold-conjugated resveratrol nanoparticles attenuate the invasion and MMP-9 and COX-2 expression in breast cancer cells. *Oncol Rep* 35: 3248-3256, 2016.
- Jing X, Cheng W, Wang S, Li P and He L: Resveratrol induces cell cycle arrest in human gastric cancer MGC803 cells via the PTEN-regulated PI3K/Akt signaling pathway. *Oncol Rep* 35: 472-478, 2016.
- Liu B, Zhou Z, Zhou W, Liu J, Zhang Q, Xia J, Liu J, Chen N, Li M and Zhu R: Resveratrol inhibits proliferation in human colorectal carcinoma cells by inducing G1/S-phase cell cycle arrest and apoptosis through caspase/cyclin-CDK pathways. *Mol Med Rep* 10: 1697-1702, 2014.
- Clark PA, Bhattacharya S, Elmayan A, Darjatmoko SR, Thuro BA, Yan MB, van Ginkel PR, Polans AS and Kuo JS: Resveratrol targeting of AKT and p53 in glioblastoma and glioblastoma stem-like cells to suppress growth and infiltration. *J Neurosurg* 126: 1448-1460, 2016.
- Ku BM, Kim DS, Kim KH, Yoo BC, Kim SH, Gong YD and Kim SY: Transglutaminase 2 inhibition found to induce p53 mediated apoptosis in renal cell carcinoma. *FASEB J* 27: 3487-3495, 2013.
- Shi T, Liou LS, Sadhukhan P, Duan ZH, Novick AC, Hissong JG, Almasan A and DiDonato JA: Effects of resveratrol on gene expression in renal cell carcinoma. *Cancer Biol Ther* 3: 882-888, 2004.
- Mondal A and Bennett LL: Resveratrol enhances the efficacy of sorafenib mediated apoptosis in human breast cancer MCF7 cells through ROS, cell cycle inhibition, caspase 3 and PARP cleavage. *Biomed Pharmacother* 84: 1906-1914, 2016.
- Ben Sahra I, Le Marchand-Brustel Y, Tanti JF and Bost F: Metformin in cancer therapy: A new perspective for an old anti-diabetic drug? *Mol Cancer Ther* 9: 1092-1099, 2010.
- Li F, Ma Z, Guan Z, Chen Y, Wu K, Guo P, Wang X, He D and Zeng J: Autophagy induction by silibinin positively contributes to its anti-metastatic capacity via AMPK/mTOR pathway in renal cell carcinoma. *Int J Mol Sci* 16: 8415-8429, 2015.
- Das G, Shrivage BV and Baehrecke EH: Regulation and function of autophagy during cell survival and cell death. *Cold Spring Harb Perspect Biol* 4: pii: a008813, 2012.