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Original Article

Resveratrol promotes melanoma cells autophagy and apoptosis by inhibiting Akt/mTOR signaling pathway

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Abstract: Objective: To study autophagy and mechanism of resveratrol (Res) effect on melanoma cells by inhibiting Akt/mTOR signaling path. Methods: Melanoma cells cultivated in vitro were divided into the control group (treated with culture medium RPMI-1640 only) and the Res group (treated with Res 5, 10, 20, 50, 100 umol/L). The proliferation and inhibition of MeWo cells in each group were detected by CCK for 12 hours and 24 hours after cells cultivation. The expression of Beclin-1 and LC3-II mRNA was detected by PCR. The protein expression of Beclin-1, LC3-II, Bax, Bcl-2, p-Akt, and mTOR was detected by WB. And the apoptosis rate was measured by flow cytometry for 48 hours in each group. Results: The control group was not inhibited at each process while the inhibition rate of the Res group increased with the rise of Res concentration, and the expression of the Res group was different from that of the control group ($P<0.05$). The apoptosis rate of the Res group increased with the rise of Res concentration, and differences were existed among the Res group ($P<0.05$). There was no difference between the Res group (except for the concentration of 5 and 10 micron oL/L) and the control group ($P>0.05$), while the apoptosis rate of other groups of Res was higher than that of the control group ($P<0.05$). The results showed that there was no difference in expression of Beclin-1 and LC3-II between the Res group (except for the concentration of 5 and 10 umol/L) and the control group. The expression of Beclin-1 and LC3-II mRNA in the rest of the Res groups was higher than that in the control group ($P<0.05$). The expression of Beclin-1 and LC3-II increased with the rise of Res concentration in the Res group, presenting a concentration-dependent trend. The protein expression of Beclin-1, LC3-II and Bax in the control group was lower than that of the Res group, while Bcl-2, p-Akt and mTOR was higher than that of the Res group ($P<0.05$). Among them, there was no difference in the protein expression between the Res group and the control group ($P>0.05$). The protein expression of Beclin-1, LC3-II and Bax increased with the rise of concentration in the Res group. The expression of Bcl-2, p-Akt and mTOR decreased with the rise of Res concentration. Conclusion: Res exerts an influence on proliferation and apoptosis of melanoma cells through promoting autophagy and apoptosis by inhibiting Akt/mTOR signaling pathway.

Keywords: Res, Akt, mTOR, melanoma, autophagy

Introduction

Melanoma, a common malignant tumor in dermatology, is mainly caused by excessive proliferation of melanocytes from the surface of skin. It may appear in any parts of the human body, especially arms, legs and rubbed parts, which is also characterized by invasiveness, metastasis and prognosis [1, 2]. In the past decade, the incidence of melanoma has increased sharply. Studies have shown that melanoma is one of the highest growth rates of malignant tumors, with an annual growth rate of 3% and 5% [3]. Survey in Chen [4] showed that there were 80,000 melanoma cases and 33,000 deaths in China in 2015. Although the incidence of melanoma was lower than that of other malignant tumors, it also causes a serious impact on the life of patients due to the lack of proper treatment.

Clinically, the treatment of early melanoma is surgical resection [4]. However, the manifestation of early melanoma is skin pigmented nevus or pigmentation, of which patients are difficult to notice. Patients who were admitted to the hospital are in the middle and late period of treatment, and radiotherapy and chemotherapy of melanoma cannot achieve the desired effect [5]. Now, doctors are desired to find a new treat-

Table 1. Primer sequence

Gene	Upstream primer	Downstream primer
Beclin-1	5'-GGUAACUGACAAAGUGAAATT-3'	5'-UUUCACUUUGUCAGUUACCAG-3'
LC3-II	5'-GCGAGAAUAUAGUGAAUUUTT-3'	5'-GCGAGAAUAUAGUGAAUUUTT-3'
GAPDH	5'-CATCACCATCTFCCAGGAGCG-3'	5'-TGACCTTGCCCACAGCCTTG-3'

Table 2. Cell growth inhibition rate at different time points in each group (%)

Group	24 h	48 h
Control group	0	0
5 μmoL/L	4.25±1.25***	10.24±2.12***
10 μmoL/L	6.84±1.44***	14.68±2.48***
20 μmoL/L	9.35±1.84***	18.94±3.44***
50 μmoL/L	16.33±2.35***	31.25±3.82***
100 μmoL/L	22.48±3.84***	43.28±5.22***

Note: ***compared with the control group (P<0.001).

ment to solve the problem. Autophagy, a process that maintains cell differentiation, growth and metabolism through lysosome degradation of intracellular substances, has been a hot research field in recent years, which plays an important role in maintaining intracellular environment [6]. In recent years, studies have shown that the pharmacological effects of Res are closely related to autophagy [7]. Res is a non-flavonoid polyphenol compound existing in natural plants, which has been proved that it plays an effective role in protecting cardiovascular, anti-inflammatory, regulating blood lipids and anti-tumor. Studies by Gurusamy [8] showed that Res could inhibit the mTOR signaling path directly and activate autophagy of MCF-7. Other studies showed that [9] Res could reduce the expression of Akt in hypoxia/reoxygenation H9c2 myocardial cells to control the activity of mTORC1, thereby activating autophagy. However, it remains unclear whether Res, or through what kind of ways, can mediate autophagy in melanoma.

Therefore, the intervention and effect of different dose of Res on melanoma cells MeWo are analyzed in this study, which provides a new perspective for clinic treatment.

Materials and methods

Source of cells

MeWo cells (ATCC cells bank, the United States, ATCC HTB-65).

Major materials and reagents

PCR kit TransScript II Two-Step RT-PCR SuperMix, total RNA extraction kit EasyPure PCR Purification Kit, Cell Proliferation Detection Kit TransDetect Cell Counting Kit (CCK) (TransGen Biotech, Beijing, China AH401-01, EP101-01, FC101-04), RPMI-1640 medium, bovine fetal serum, trypsin, penicillin streptomycin, ECL chemiluminescence, RIPA, BCA protein kit, Annexin V-FITC Kit (Thermo Fisher Scientific in Shanghai, China, 11875085, 12483020, A40007, 15070063, 32209, 89900, 23250, 331200), Res (Sigma Corporation of America, R5010), Beclin-1, LC3-II, Bax, Bcl-2, p-Akt, mTOR, β-Actin, Goat Anti-Mouse IgG (R&D Corporation, MAB5295, MAB85582, AF820, AF810, AF887, MAB4598, MAB8929, HAF008), PCR (American ABI, 7500), flow cytometry (American Beckmancoulter, CytoFLEX). The primer sequence was designed by Shanghai Sangon Biotech (**Table 1**).

Culture of cells

MeWo cell lines were cultured in RPMI-1640 medium (1% penicillin and streptomycin, 10% bovine fetal serum), and then transferred to 37°C and 5% CO_2 incubator for cultivating the next generation.

Cell proliferation detection

The second and third generation of cells were collected and inoculated into 96-well plates with 5×10^3/well. According to Res concentration, cells were divided into 5, 10, 20, 50, and 100 umol/L in the Res group (treated with RPMI-1640 medium culture). MeWo cells were used in the control group (treated without Res), and then transferred to 37°C and 5% CO_2 incubator for 48 hours. According to instructions of CCK kit, the cell proliferation in each group was detected and inhibition rate was calculated after 24 hours and 48 hours.

PCR detection

The second and third generation of cells were collected and inoculated into 96-well plates with 5×10^3/well. According to the Res concentration, cells were divided into 5, 10, 20, 50,

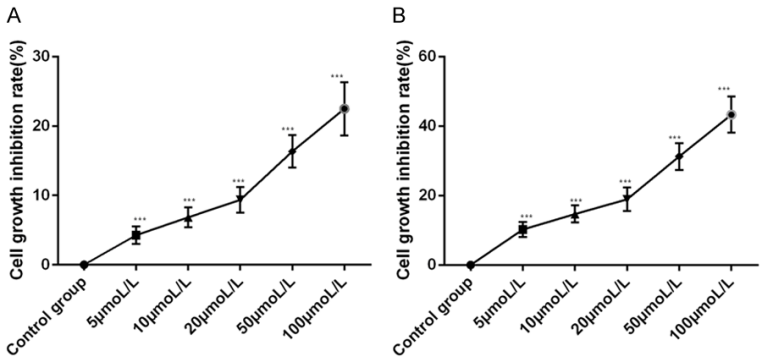

Figure 1. Cell proliferation inhibition rate at 24 h and 48 h in each group. A. Cell growth inhibition did not occur in the control group after 24 h, and the inhibition rate was lower than that in the Res group. B. There was no growth inhibition in the control group after 24 h, and the inhibition rate was lower than that in the Res group. ***indicates that compared with the control group (P<0.001).

and umol/L in the Res group (treated with RPMI-1640 medium culture). MeWo cells were used in the control group (treated without Res), and then transferred to 37°C and 5% CO_2 incubator for 48 hours. After that, the total RNA was extracted by using EasyPure PCR Purification Kit. The purity, concentration and integrity of the total RNA were detected by ultraviolet spectrophotometer and agarose gel electrophoresis. Reverse transcription of the total RNA was performed by using 5× TransScript® II All-in-One SuperMix for PCR. The operation was followed by the manufacturer's kit. And the PCR amplification experiment was carried out. The reaction system was as follows: cDNA 2 μL,1 μL of upstream and downstream primers, 2× TransScript® HIFI PCR SuperMix II 25 μL, Nuclease-free Water was used to supplement to 50 μL. The reaction conditions were as follows: 5 min for predenaturation at 94°C, 30 s for denaturation at 94°C, 30 s for annealing at 60°C, 45 s for extension at 72°C, with 40 cycles in total. Each sample has three repetition holes. The experiment was carried out for three times. In this study, GAPDH was used as an internal reference. The data was analyzed by using $2^{-\Delta\Delta ct}$.

Western blot detection

Total protein of cells was extracted by RIPA pyrolysis after 48 hours. BCA protein determination method was used to detect the concentration. The protein concentration was adjusted to 4 μg/μL and separated by 12% SDS-PAGE electrophoresis. The membrane was transferred to PVDF one, then dyed by ponceau and immersed in PBST for 5 min, with 5% skimmed milk powder sealed for 2 hours. And primary antibodies were added at 4°C overnight. The membrane was removed by getting out of primary antibodies. HRP (horseradish peroxidase) was added to label goat antirat IgG (H+L)-HRP (1: 5000), incubated at 37°C for 1 hour and immersed in PBST with 5 minutes for three times. Image was formed in dark room. The excess liquid in the membrane was absorbed by using filter paper. Light was given and developed by ECL. The protein bands were scanned, and the gray values were analyzed in the Quantity One, where the relative expression of the protein equals the gray value of the targeted protein band or the gray value of the β-Actin protein band.

Apoptosis detection

The cultured cells were transferred to 15 ml tapered tube and placed on the surface of ice after 48 hours. The cells in the plate were washed with 2 mL PBS. And then, PBS was removed while 0.5 ml 0.25% trypsin was added without EDTA and observe until the cells began to fall off from the culture plate under the microscope. The cells completely fell off from the culture plate wall through tapping. Resuspending the culture medium to adjust the cell density to 1×10^6 cells/ml and transfer 0.5 ml of the cell suspension to the clean centrifuge tube. And 1.25 μl Annexin V-FITC was added and the temperature was kept at 18~24°C in the room, away from light for 15 min. Centrifuging at 1000 rpm for 5 min at room temperature to get out of supernatant. The cells were resuspended and added with 10 μl Propidium Iodide, and kept on the ice. Flow cytometry was used for detection and analysis.

Statistical analysis

In this study, the collected data were analyzed by using the SPSS20.0 software, and the

Table 3. Comparison of apoptosis rate in 48 h in each group

Group	Apoptosis rate (%)
Control group	3.54±1.25
5 μmoL/L	4.22±1.72
10 μmoL/L	9.35±2.33***
20 μmoL/L	16.92±4.32***
50 μmoL/L	30.48±4.22***
100 μmoL/L	42.54±5.38***
F value	58.887
P value	<0.001

Note: ***indicates that compared with the control group P<0.001.

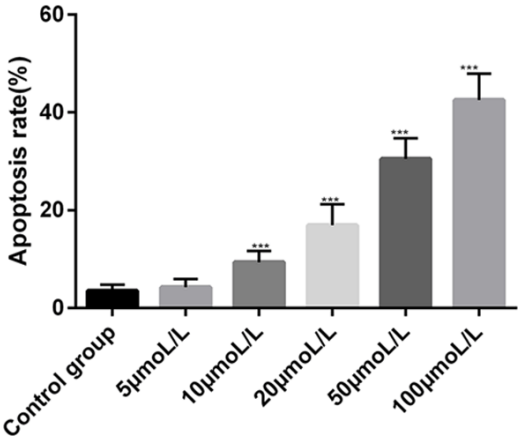

Figure 2. Apoptosis of cells in each group after 48 h. There was no significant difference in apoptosis rate between the Res group (except for concentration at 5 umol/L) and the control group. And the apoptosis rate in other groups of Res was higher than that in the control group. ***indicates compared with the control group (P<0.001).

images were plotted by using the GraphPad Prism 7. The measurement data were expressed using mean ± standard deviation (Mean ± SD). One-way analysis of variance (ANOVA) was used for comparison among groups and LSD-t was used for post hoc pairwise comparison. P<0.05 indicated that there was a statistically significant difference.

Results

Proliferation of cells

The inhibition rate of cells was measured in each group. In terms of 24 hours in the control group, the inhibition rate of concentration at 5, 10, 20, 50 and 100 μmoL/L was 0%, 4.25±1.25%, 6.84±1.44%, 9.35±1.84%, 16.33±2.35% and 22.48±3.84%. Under the same circumstance, the imbibition rate for 48 hours was 0%, 10.24±2.12%, 14.68±2.48%, 18.94±3.44%, 31.25±3.82% and 43.28±5.22%. The control group was not inhibited at each time point, while the inhibition rate of the Res group increased with the rise of Res concentration. The expression of each group was different from that of the control group (P<0.05) (**Table 2** and **Figure 1**).

Table 4. Expression of Beclin-1 and LC3-II mRNA

Group	Beclin-1	LC3-II
Control group	0.304±0.084	0.402±0.068
5 μmoL/L	0.389±0.088	0.439±0.071
10 μmoL/L	0.468±0.090	0.482±0.089
20 μmoL/L	0.535±0.132*	0.599±0.096*
50 μmoL/L	0.735±0.155*	0.842±0.166*
100 μmoL/L	0.894±0.179*	1.124±0.181*
F value	9.182	17.080
P value	0.001	<0.001

Note: *compared with the control group (P<0.05).

Apoptosis of cells

The apoptosis of cells in each group was detected by flow cytometry. The results showed that the apoptosis rate of cells in the Res group increased with the rise of Res concentration. And there was a difference in apoptosis among the groups in the Res (P<0.05). While there was no significant difference in apoptosis rate between the Res group (except for 5 μmoL/L) and the control group (P>0.05). The apoptosis rate of other groups was higher than that of the control group (P<0.05) (**Table 3** and **Figure 2**).

Expression of Beclin-1 and LC3-II mRNA

The expression of Beclin-1 and LC3-II in each group was detected by PCR. The results showed that there was no difference in the expression of Beclin-1 and LC3-II mRNA between the Res group (except for 5, 10 μmol/L) and the control group. The expression of Beclin-1, LC3-II mRNA in other groups of Res was higher than that in the control group (P<0.05). The expression of LC3-II and Beclin-1 increased with the rise of concentration in the Res group, presenting a concentration-dependent trend (**Table 4** and **Figure 3**).

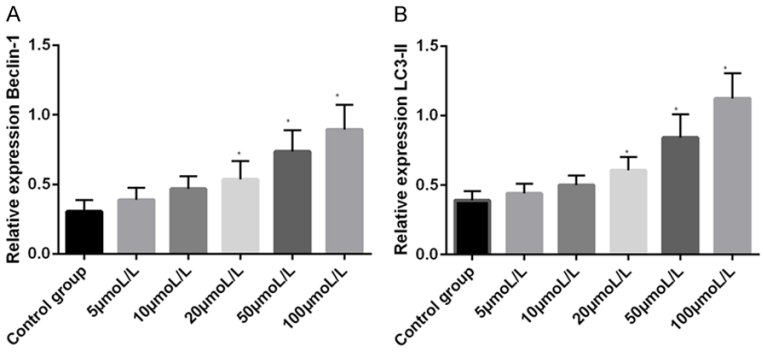

Figure 3. Expression of Beclin-1 and LC3-II mRNA in each group. A. PCR analysis showed that the expression of Beclin-1 mRNA has no difference between the control group and the Res group (except for 5, 10 μmol/L) after 48 h. The expression of concentration in other groups of Res was higher than that of the control group. B. PCR analysis showed that the expression of LC3-II mRNA was no difference between the control group and the Res group (except for 5, 10 μmol/L) after 48 h. The expression of concentration in other groups of Res was higher than that of the control group. *indicates that there is a difference compared with the control group ($P<0.05$).

Expression of autophagic protein and apoptotic protein

The expression of Beclin-1, LC3-II, Bax, Bcl-2, p-Akt and mTOR in each group was detected by WB. The results showed that the expression of Beclin-1, LC3-II and Bax in the control group was less than that in Res group, while the expression of Bcl-2, p-Akt and mTOR was higher than that in the Res group ($P<0.05$). Among them, the expression of protein in the 5 and 10 μmol/L concentration groups was not different from the control group ($P<0.05$). The protein expression of Beclin-1, LC3-II and Bax increased with the rise of concentration in the Res group. While the protein expression of Bcl-2, p-Akt and mTOR decreased with the rise of concentration of Res (**Table 5** and **Figure 4**).

Discussion

In this study, the proliferation and apoptosis of MeWo cells without intervention and MeWo cells with Res intervention have been examined. Clinically, apoptosis is the most effective way to treat tumors, which plays a key role in promoting the apoptosis of tumors and reducing the proliferation of tumors. Bax and Bcl-2 are the same kind of water-soluble proteins, during which Bax is a kind of gene that promotes while Bcl-2 is a kind of antagonist gene that inhibits apoptosis of cells. Many studies have shown that the expression imbalance and cell apoptosis of Bax and Bcl-2 was closely related [17]. Akt is a serine/threonine protein kinase that transmits extracellular stimuli signals such as growth factors, and is also an important signaling path pivotal enzyme for cell survival. Studies have shown that Akt phosphorylation is a kind of powerful BAD enzyme [18], which can regulate the expression of Bax and Bcl-2. In this study, the proliferation of MeWo cells decreased with the increase of Res concentration compared with the control group. Also, the expression of Bax protein increased with the increase of Res concentration, while the expression of Bcl-2 and p-Akt decreased with the increase of Res concentration. It indicated that Res may promote the expression of Bax protein by inhibiting p-Akt signaling path, thereby promoting the apoptosis of tumors.

Meanwhile, the expression of Beclin-1, LC3-II, mTOR protein and Beclin-1, LC3-II mRNA in cells was examined. As an important role of PIKK, mTOR is an atypical serine/threonine kinase that plays a key role in a variety of biological processes such as cell growth, proliferation, and autophagy. Studies have shown that autophagy can be promoted by inhibiting the mTOR signaling path [19]. LC3-II and Beclin-1, as important autophagic proteins, play a key role in the regulation of autophagy and cell death [20]. The relative expression of mTOR protein in each group showed that the protein expression of mTOR in the Res group was relatively decreased and there were differences at the concentration of 20 μmoL/L. It indicated that Res inhibition of the mTOR protein expression makes differences at 20 μmoL/L. Furthermore, the expression of LC3-II and Beclin-1 at the molecular and protein levels showed that the expression of LC3-II and Beclin-1 increased with the rise of concentration, indicating that Res can be activated by inhibiting the mTOR signaling path. Studies by Manzoni [21] showed that LRRK-2 inhibited mTOR signals to regulate LC3-II and Beclin-1 proteins, while studies by Wang [22] showed

Table 5. Protein expression

Group	Beclin-1	LC3-II	Bax	Bcl-2	p-Akt	mTOR
Control Group	0.584±0.065	0.422±0.048	0.284±0.064	0.842±0.082	0.958±0.068	0.866±0.077
5 μmoL/L	0.605±0.077	0.455±0.056	0.302±0.066	0.828±0.077	0.933±0.062	0.851±0.070
10 μmoL/L	0.658±0.069	0.522±0.058	0.415±0.060	0.721±0.065	0.867±0.060	0.739±0.062
20 μmoL/L	0.733±0.085*	0.705±0.078*	0.584±0.072*	0.604±0.070*	0.720±0.052*	0.621±0.060*
50 μmoL/L	0.924±0.088*	0.893±0.075*	0.765±0.077*	0.425±0.068*	0.543±0.049*	0.501±0.052*
100 μmoL/L	1.122±0.095*	1.013±0.089*	0.869±0.085*	0.301±0.055*	0.411±0.32*	0.433±0.047*
F value	20.665	37.757	35.364	29.702	26.674	25.317
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: *there is a difference compared with the control group (P<0.05).

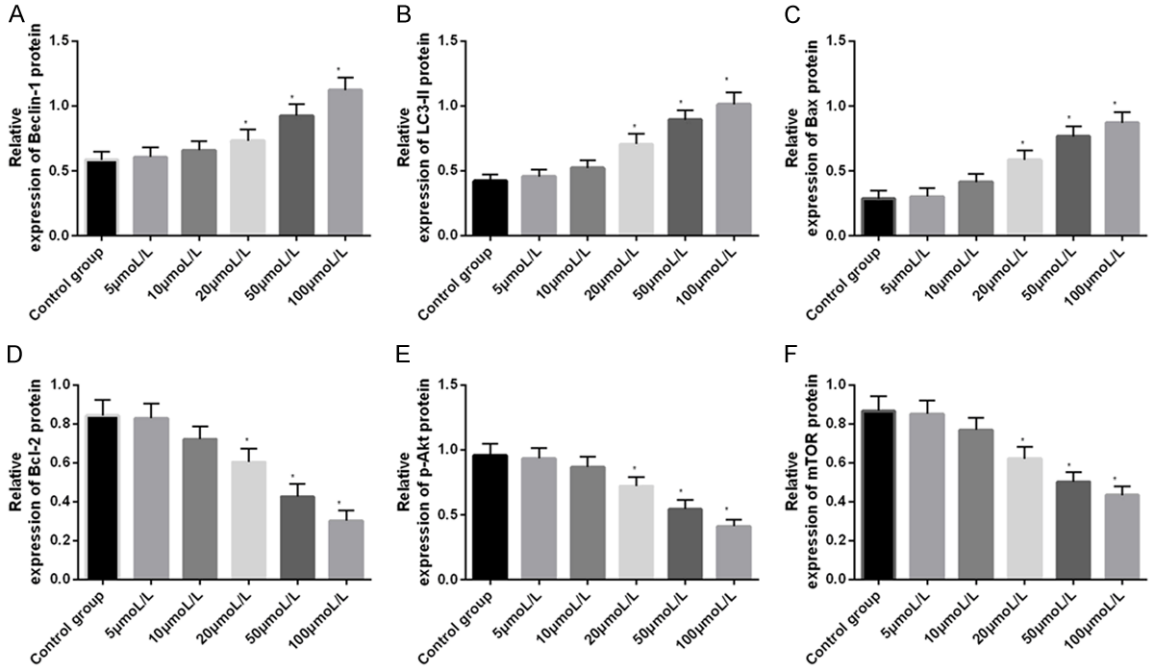

Figure 4. Protein expression of Beclin-1, LC3-II, Bax, Bcl-2, p-Akt and mTOR in each group. A. There was no difference in the protein expression of Beclin-1 in the Res group (except for 5, 10 μmoL/L) compare with the control group after 48 h, and the expression of other concentrations was higher than that of the control group. B. The protein expression of LC3-II in the Res group (except for 5, 10 μmoL/L) was higher than that in the control group. And there was a significance in the expression of other concentrations. C. The protein expression of Bax was no difference between the Res group (except for 5, 10 μmoL/L) and the control group. The expression of other concentrations was higher than that of the control group. D. The protein expression of Bcl-2 was no difference in the Res group (except for 5, 10 μmoL/L) and the control group. The expression of other concentrations was lower than that in the control group. E. The protein expression of p-Akt was no difference between the Res group (except for 5, 10 μmoL/L) and the control group. The expression of other concentrations was lower than that of the control group. F. The protein expression of mTOR was no difference between the Res group (except for 5, 10 μmoL/L) and the control group. The expression of other concentrations was lower than that in the control group. *indicates there is a difference compared with the control group P<0.05.

that the expression levels of autophagy-related proteins LC3 and Beclin-1 increased after SKOV3 cells were treated with Res at 25 umol/L for 24 hours. In this study, the protein expression of mTOR was inhibited when the dose of Res reached 20 umol/L. The LC3-II and Beclin-1 proteins in the Res group increased compared with the control group.

Some limitations still existed in this study. Firstly, the experiment was carried out in vitro, which has not been studied and proved in clini-

cal practice. Secondly, autophagy-related proteins such as LC3-II and Beclin-1 were detected. Other related proteins have not been detected through Akt/mTOR pathway. It remains unclear whether Res can induce autophagy through other autophagy-related proteins in the same way. Related experiments are hoped to be conducted to confirm the validity of the study in the future.

In conclusion, Res exerts an influence on the proliferation and apoptosis of melanoma through promoting autophagy and apoptosis by inhibiting Akt/mTOR signaling path.

Disclosure of conflict of interest

None.

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References

[1] Ascierto PA, Long GV, Robert C, Brady B, Dutriaux C, Di Giacomo AM, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbé C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Ny L, Arance A, Svane IM, Schadendorf D, Gogas H, Saci A, Jiang J, Rizzo J and Atkinson V. Survival outcomes in patients with previously untreated BRAF wild-type advanced melanoma treated with nivolumab therapy: three-year follow-up of a randomized phase 3 trial. JAMA Oncol 2019; 5: 187-194.

[2] Chen S, Zhao X, Ran L, Wan J, Wang X, Qin Y, Shu F, Gao Y, Yuan L, Zhang Q and Mi M. Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: a randomized controlled trial. Dig Liver Dis 2015; 47: 226-232.

[3] Singh CK, Ndiaye MA and Ahmad N. Resveratrol and cancer: challenges for clinical translation. Biochim Biophys Acta 2015; 1852: 1178-1185.

[4] Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, Hodi FS, Schachter J, Pavlick AC, Lewis KD, Cranmer LD, Blank CU, O'Day SJ, Ascierto PA, Salama AK, Margolin KA, Loquai C, Eigentler TK, Gangadhar TC, Carlino MS, Agarwala SS, Moschos SJ, Sosman JA, Goldinger SM, Shapira-Frommer R, Gonzalez R, Kirkwood JM, Wolchok JD, Eggermont A, Li XN, Zhou W, Zernhelt AM, Lis J, Ebbinghaus S, Kang SP and Daud A. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol 2015; 16: 908-918.

[5] Dummer R, Hauschild A, Guggenheim M, Keilholz U and Pentheroudakis G; ESMO Guidelines Working Group. Cutaneous melanoma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012; 23 Suppl 7: vii86-vii91.

[6] Zeng H, Zheng R, Zhang S, Zuo T, Xia C, Zou X and Chen W. Esophageal cancer statistics in China, 2011: estimates based on 177 cancer registries. Thorac Cancer 2016; 7: 232-237.

[7] Yin GF, Guo W, Chen XH, Liu ZY and Huang ZG. Clinical characteristic and prognostic analyses of 117 cases of head and neck mucosal melanoma. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2018; 53: 668-674.

[8] Gurusamy N, Lekli I, Mukherjee S, Ray D, Ahsan MK, Gherghiceanu M, Popescu LM and Das DK. Cardioprotection by resveratrol: a novel mechanism via autophagy involving the mTORC2 pathway. Cardiovasc Res 2010; 86: 103-112.

[9] Rozeman EA, Dekker TJA, Haanen JBAG and Blank CU. Advanced melanoma: current treatment options, biomarkers, and future perspectives. Am J Clin Dermatol 2018; 19: 303-317.

[10] Saranga-Perry V, Ambe C, Zager JS and Kudchadkar RR. Recent developments in the medical and surgical treatment of melanoma. CA Cancer J Clin 2014; 64: 171-185.

[11] Mariño G, Niso-Santano M, Baehrecke EH and Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 2014; 15: 81-94.

[12] Zhou W, Xu G, Wang Y, Xu Z, Liu X, Xu X, Ren G and Tian K. Oxidative stress induced autophagy in cancer associated fibroblast enhances proliferation and metabolism of colorectal cancer cells. Cell Cycle 2017; 16: 73-81.

[13] Sosinska P, Mikula-Pietrasik J and Ksiazek K. The double-edged sword of long non-coding RNA: the role of human brain-specific BC200 RNA in translational control, neurodegenerative diseases, and cancer. Mutat Res Rev Mutat Res 2015; 766: 58-67.

[14] Selvaraj S, Sun Y, Sukumaran P and Singh BB. Resveratrol activates autophagic cell death in prostate cancer cells via downregulation of STIM1 and the mTOR pathway. Mol Carcinog 2016; 55: 818-831.

[15] Niles RM, McFarland M, Weimer MB, Redkar A, Fu YM and Meadows GG. Resveratrol is a potent inducer of apoptosis in human melanoma cells. Cancer Lett 2003; 190: 157-163.

[16] Su Z, Yang Z, Xu Y, Chen Y and Yu Q. MicroRNAs in apoptosis, autophagy and necroptosis. Oncotarget 2015; 6: 8474-8490.
[17] Cillessen SA, Hijmering NJ, Moesbergen LM, Vos W, Verbrugge SE, Jansen G, Visser OJ, Oudejans JJ and Meijer CJ. ALK-negative anaplastic large cell lymphoma is sensitive to bortezomib through Noxa upregulation and release of Bax from Bcl-2. Haematologica 2015; 100: e365-e368.
[18] Vurusaner B, Gamba P, Testa G, Gargiulo S, Biasi F, Zerbinati C, Iuliano L, Leonarduzzi G, Basaga H and Poli G. Survival signaling elicited by 27-hydroxycholesterol through the combined modulation of cellular redox state and ERK/Akt phosphorylation. Free Radic Biol Med 2014; 77: 376-385.
[19] Saxton RA and Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017; 169: 361-371.
[20] Yu FS, Yu CS, Chen JC, Yang JL, Lu HF, Chang SJ, Lin MW and Chung JG. Tetrandrine induces apoptosis Via caspase-8, -9, and -3 and poly (ADP ribose) polymerase dependent pathways and autophagy through beclin-1/LC3-I, II signaling pathways in human oral cancer HSC-3 cells. Environ Toxicol 2016; 31: 395-406.
[21] Manzoni C, Mamais A, Roosen DA, Dihanich S, Soutar MP, Plun-Favreau H, Bandopadhyay R, Hardy J, Tooze SA, Cookson MR and Lewis PA. mTOR independent regulation of macroautophagy by leucine rich repeat kinase 2 via beclin-1. Sci Rep 2016; 6: 35106.
[22] Wang H, Peng Y, Wang J, Gu A, Li Q, Mao D and Guo L. Effect of autophagy on the resveratrol-induced apoptosis of ovarian cancer SKOV3 cells. J Cell Biochem 2018; [Epub ahead of print].