Mitochondrial mechanism of apoptosis induced by resveratrol in nasopharyngeal carcinoma cells CNE-2Z

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Abstract: AIM To study whether resveratrol-induced apoptosis in nasopharyngeal carcinoma cells CNE-2Z is *via* a mitochondrial pathway. **METHODS** CNE-2Z cells were treated with 100 $\mu mol \cdot L^{-1}$ resveratrol for 0 (control), 2, 4, 8, 12, and 24 h and treated with 0 (control), 25, 50, 100, and 200 $\mu mol \cdot L^{-1}$ resveratrol for 8 h. The Bcl-2, Bax and cytosolic cytochrome C protein levels were analyzed by Western blot. The mitochondrial membrane potential ($\triangle\psi_m$) was detected by flow cytometry following rhodamine 123 fluorescence staining. The caspase-9 activity was determined by colorimetric assay. **RESULTS** ① After CNE-2Z cells were treated with 100 $\mu mol \cdot L^{-1}$ resveratrol for 0(control), 2, 4, 8, 12, and 24 h, respectively, Bcl-2 protein expression and $\triangle\psi_m$ were decreased, while cytosolic cytochrome C level and caspase-9 activity were increased in a time-dependent manner ($n = 3$, $P < 0.01$). But Bax protein expression had no change. All except Bax protein expression had been altered since 2 h. The inhibition of Bcl-2 protein expression and the loss of $\triangle\psi_m$ were most obvious during 4 – 8 h (compared with control group $P < 0.01$), but no significance at 24 h. Cytosolic cytochrome C level and caspase-9 activity reached the peak at 8 h and were still much higher than those in control group at 24 h ($P < 0.01$). Cytosolic cytochrome C level and caspase-9 activity were about 3.0-fold and 5.4-fold of those at 0 h, respectively. ② After CNE-2Z cells were treated with 0 (control), 25, 50, 100, and 200 $\mu mol \cdot L^{-1}$ resveratrol for 8 h, respectively, the Bcl-2 protein expression inhibition, $\triangle\psi_m$ loss, cytochrome C release, and caspase-9 activity increase showed in a dose-dependent manner ($n = 3$, $P < 0.01$). However, Bax protein expression was not affected. **CONCLUSION** Resveratrol can induce apoptosis in CNE-2Z *via* a mitochondria/caspase-9-specific pathway, but this may not be related to Bax.

Key words: resveratrol; mitochondria; cytochrome C; protein Bcl-2; protein Bax; caspases

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Resveratrol (3, 4′, 5-trihydroxystibene), a natural phytoalexin present in grapes and a variety of medicinal plants, has been used to treat fever, hyperlipidemia, inflammation and atherosclerosis. Recently, resveratrol has been found to have antitumor activity which was *via* the induction of apoptosis in some tumor cells[1-4]. In our previous studies, we also found that resveratrol can induce apoptosis of human nasopharyngeal carcinoma epithelial cells in badly differentiated CNE-2Z[5], but the mechanisms of apoptosis are not completely clear.

In recent years, contradictory results were reported on the mechanisms of apoptosis induced by resveratrol[4,6-11]. In 1998, Clement, *et al*[6] reported resveratrol could trigger CD95 (Fas) signaling-dependent apoptosis in HL-60 leukemia cells and T47D breast carcinoma cells. However, in 2000, Tsan, *et al*[7] found resveratrol could induce Fas signaling-independent apoptosis in THP-1 human monocytic leukemia cells. In recent years, mitochondria have been reported to play an important role in the apoptosis induced by resveratrol in some tumor cells[4,8-11]. Furthermore, mitochondrial membrane potential($\triangle\psi_m$) loss,

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cytochrome C release from mitochondria and caspase-9 activation were found during the apoptosis by resveratrol[4,7-10]. Nevertheless, Zini, *et al*[12] found that resveratrol fully inhibited the release of cytochrome C in a concentration-dependent manner and the mitochondrial membranes were partly protected by resveratrol at 0.1 μmol·L^{-1} when they investigated resveratrol protection on the main functions of purified rat brain mitochondria submitted to anoxia-reoxygenation. Thus, the effects of resveratrol on mitochondria are not unanimous.

In addition, although the mitochondrial mechanisms of apoptosis induced by resveratrol in some tumor cells have been found, it is not reported whether resveratrol can induce apoptosis in CNE-2Z cells *via* a mitochondrial pathway. Therefore, this study is to investigate the mitochondrial mechanism of apoptosis induced by resveratrol in CNE-2Z cells.

1 MATERIALS AND METHODS

1.1 Drugs and reagents

Trans-resveratrol (purity > 98%) was purchased from Nanjing Xuezi Medical and Chemical Research Center. A stock solution of resveratrol was made in dimethylsulfoxide (DMSO) at 200 mmol·L^{-1} and was stored at −20℃ in the dark. Monoclonal mouse anti-human Bcl-2 (IgG_1), Bax (IgG_{2b}), and β-actin antibodies were purchased from Beijing Zhongshan Company. Monoclonal mouse anti-human cytochrome C antibody ($IgG_{2b/\kappa}$) was obtained from NeoMarkers. Horseradish peroxidase conjugated-goat anti-mouse IgG was purchased from Wuhan Boster Biological Technology Company. Enhanced chemiluminescence (ECL) detection kit was obtained from Santa Cruz Biotechnology (Santa Cruz, California). Rhodamine 123 was obtained from Sigma Company (USA). Caspase-9 inhibitor (Leu-Glu-His-Asp-fmk, LEHD-fmk) was obtained from Clontech Company (USA). Caspase-9 substrate (AC-Leu-Glu-His-Asp-pNA, AC-LEHD-pNA) was obtained from Alexls Company.

1.2 Cell line and cell culture

CNE-2Z cell line was from the Institute of Biochemistry and Molecular Biology of Guangdong Medical College. Cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% new-born bovine serum, penicillin (100 kU·L^{-1}) and streptomycin (100 mg·L^{-1}) at 37℃ in a humidified 5% CO_2 atmosphere. The cells used in these experiments were in exponential growth phase.

1.3 Drug treatment

After being seeded into culture dishes for 12 h, CNE-2Z cells were treated with 100 μmol·L^{-1} resveratrol for 0(control), 2, 4, 8, 12, and 24 h and treated with resveratrol 0(control), 25, 50, 100, and 200 μmol·L^{-1} for 8 h. All experiments were repeated three times ($n = 3$).

1.4 Western blot analysis

After cells (1×10^9 L^{-1} in 3 mL medium) were treated with resveratrol according to drug treatment, proteins were extracted. Proteins used for the analysis of Bcl-2, Bax, and β-actin (internal control) expression were extracted as described[9]. Cytosolic extracts used for the analysis of cytochrome C release were prepared as described[13]. Western blot analysis was performed as previously described[14]. Detection of specific proteins was carried out with an ECL Western blotting kit according to the manufacturer's instructions. Integral absorbance of each protein band was analyzed by software Leica Qwin. The ratios of integral absorbance of Bcl-2, Bax and cytochrome C to β-actin (internal control) represented Bcl-2, Bax, and cytochrome C protein level, respectively.

1.5 Detection of $\triangle\psi_m$

After treatment with resveratrol according to drug treatment, cells (0.5×10^9 L^{-1} in 2 mL medium) were harvested and incubated in D-Hanks fluid with rhodamine 123 (final concentration 10 mg·L^{-1}) for 30 min at 37℃ in the dark. Then cells were detected on a flow cytometry (Epics-XL, Coulter, USA). Excitation wavelength was 488 nm and emission wavelength was 530 nm.

1.6 Caspase-9 activity assay

Cells were divided into three groups: ①

control group: untreated cells. ② LEHD-fmk + resveratrol group: after pretreatment with 100 μmol·L^{-1} caspase-9 inhibitor (LEHD-fmk) for 2 h, cells were exposed to 100 μmol·L^{-1} resveratrol for 0, 2, 4, 8, 12, and 24 h, respectively. ③ resveratrol group: cells were treated with 100 μmol·L^{-1} resveratrol for 0, 2, 4, 8, 12, and 24 h and treated with 0, 25, 50, 100, and 200 μmol·L^{-1} resveratrol for 8 h. The cells (1×10^9 L^{-1} in 2 mL medium) in the above three groups were harvested and pretreated as described[15]. Then the cells were incubated with caspase-9 substrate AC-LEHD-pNA (final concentration 50 μmol·L^{-1}) for 1 h at 37℃. Thereafter, the absorbance at 405 nm ($A_{405\ nm}$) was measured with a microplate spectrophotometer (Bio-Tek, ELx 800, universal microplate reader). $A_{405\ nm}$ represented caspase-9 relative activity.

1.7 Statistical analysis

One-way ANOVA in SPSS 10.0 for Windows was used to compare the difference among multiple groups and Bonferroni was further used to compare the difference between two groups. A difference was considered to be significant at $P < 0.05$.

2 RESULTS

2.1 Changes in Bcl-2, Bax, and cytosolic cytochrome C protein levels of CNE-2Z cells

The changes in the Bcl-2 protein expression and cytosolic cytochrome C level showed in a time-dependent manner, but Bax protein expression had no change (Fig 1 and Tab 1). Bcl-2 protein expression had been decreased and cytosolic cytochrome C level had been increased since 2 h. The Bcl-2 protein expression was most obviously decreased during the 4 – 8 h compared with control group. Cytosolic cytochrome C level reached the peak at 8 h about 3.0-fold of that in control group. Bcl-2 protein expression was decreased and cytosolic cytochrome C level was increased in a dose-dependent manner, but Bax protein expression was not affected (Fig 2 and Tab 2).

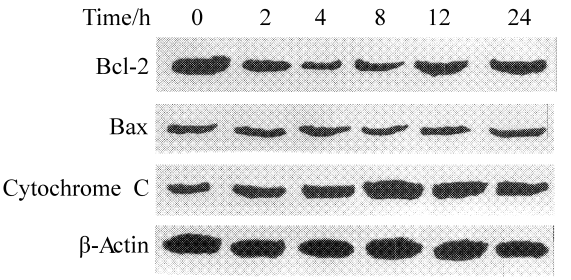

Fig 1. Changes in Bcl-2, Bax, and cytosolic cytochrome C protein levels of CNE-2Z cells by Western blot after treatment with 100 μmol·L^{-1} resveratrol for different time. Results are the representative of three separate experiments.

Tab 1. Ratio of integral absorbance of Bcl-2, Bax, and cytochrome C to β-actin in CNE-2Z cells after treatment with 100 μmol·L^{-1} resveratrol for different time

Time/h	Ratio of integral absorbance		
	Bcl-2: β-actin	Bax: β-actin	Cytochrome C: β-actin
0	0.838 ± 0.074	0.402 ± 0.048	0.282 ± 0.067
2	0.570 ± 0.057**	0.395 ± 0.062	0.536 ± 0.059*
4	0.341 ± 0.045**	0.395 ± 0.088	0.617 ± 0.035**
8	0.357 ± 0.070**	0.399 ± 0.057	0.856 ± 0.109**
12	0.627 ± 0.091*	0.409 ± 0.039	0.776 ± 0.074**
24	0.777 ± 0.056	0.400 ± 0.108	0.659 ± 0.051**

$\bar{x} \pm s$, $n = 3$. * $P < 0.05$, ** $P < 0.01$, compared with control group (0 h).

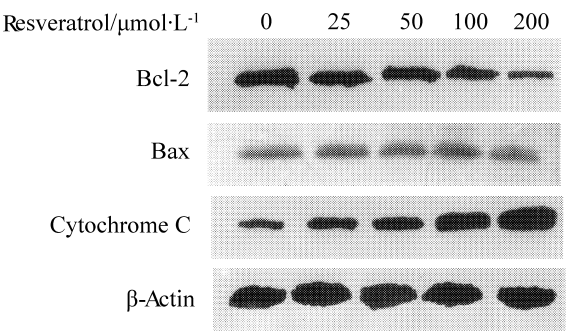

Fig 2. Changes in Bcl-2, Bax, and cytosolic cytochrome C protein levels of CNE-2Z cells by Western blot after treatment with different concentrations of resveratrol for 8 h. Results are the representative of three separate experiments.

Tab 2. Ratio of integral absorbance of Bcl-2, Bax, and cytochrome C to β-actin in CNE-2Z cells after treatment with different concentrations of resveratrol for 8 h

Resveratrol /μmol·L^{-1}	Ratio of integral absorbance		
	Bcl-2: β-actin	Bax: β-actin	Cytochrome C: β-actin
0	0.817 ± 0.098	0.400 ± 0.056	0.252 ± 0.035
25	0.566 ± 0.045**	0.397 ± 0.065	0.307 ± 0.063
50	0.475 ± 0.041**	0.387 ± 0.080	0.474 ± 0.047*
100	0.324 ± 0.040**	0.405 ± 0.041	0.808 ± 0.079**
200	0.186 ± 0.050**	0.382 ± 0.075	0.922 ± 0.101**

$\bar{x} \pm s$, $n = 3$. * $P < 0.05$, ** $P < 0.01$, compared with 0 μmol·L^{-1} group.

2.2 Changes in $\triangle\psi_m$

The number of the cells with low mitochondrial membrane potential ($\triangle\psi_{m\text{-}low}$) was increased in a time-dependent manner (Fig 3 and Tab 3), which demonstrated that loss of $\triangle\psi_m$ was in a time-dependent manner. $\triangle\psi_m$ had been decreased since 2 h and dropped to the lowest level during the 4 – 8 h compared with control group, but no difference at 24 h. The number of the cells with $\triangle\psi_{m\text{-}low}$ was increased in a dose-dependent manner (Fig 4 and Tab 4), which suggested that the loss of $\triangle\psi_m$ show in a dose-dependent manner.

Tab 3. Changes in $\triangle\psi_{m\text{-}low}$ after treatment with 100 μmol·L^{-1} resveratrol for different time

Time/h	$\triangle\psi_{m\text{-}low}$/%
0	2.2 ± 0.6
2	31.3 ± 5.0**
4	46.6 ± 8.4**
8	46.1 ± 5.0**
12	29.7 ± 2.8**
24	9.8 ± 3.1

$\bar{x} \pm s$, $n = 3$. ** $P < 0.01$, compared with control group (0 h).

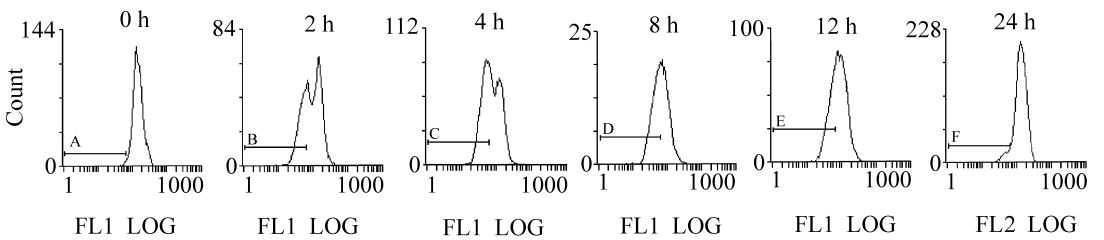

Fig 3. Results in $\triangle\psi_{m\text{-}low}$ after treatment with 100 μmol·L^{-1} resveratrol for different time by flow cytometry. Results are the representative of three separate experiments.

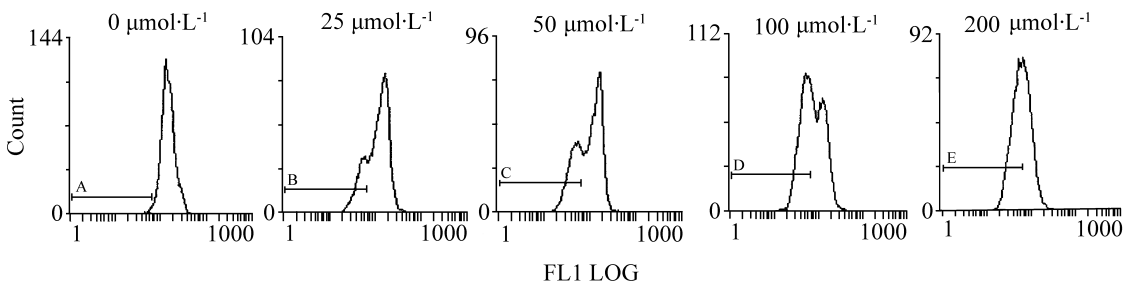

Fig 4. Results in $\triangle\psi_{m\text{-}low}$ after treatment with different concentrations of resveratrol for 8 h by flow cytometry. Results are the representative of three separate experiments.

Tab 4.　Changes in $\triangle\psi_{m\text{-}low}$ after treatment with different concentrations of resveratrol for 8 h

Resveratrol/μmol·L^{-1}	$\triangle\psi_{m\text{-}low}$/%
0	2.2 ± 0.6
25	22.1 ± 1.3**
50	31.3 ± 2.0**
100	44.4 ± 2.2**
200	54.1 ± 1.8**

$\bar{x} \pm s$, $n = 3$. ** $P < 0.01$, compared with 0 μmol·L^{-1} group.

2.3　Changes in caspase-9 activity

Caspase-9 activity was altered in a time-dependent manner (Tab 5). Caspase-9 activity in resveratrol group had been increased since 2 h compared with control group, reached the peak at 8 h about 5.4-fold of that in control group and was still higher than that in control group at 24 h. When the cells were exposed to 0, 25, 50, 100, and 200 μmol·L^{-1} resveratrol for 8 h, caspase-9 activity was 0.064 ± 0.008, 0.150 ± 0.018, 0.227 ± 0.036, 0.348 ± 0.020, and 0.416 ± 0.019, respectively. It was increased in a dose-dependent manner.

3　DISCUSSION

Recent evidences have demonstrated that there are at least 2 distinct pathways that mediate caspase activation and apoptosis. In the first pathway, the ligation of "death receptors" (such as Fas) result in activation of caspase-8 ("initiator" caspase), which activates "effector" caspases. In the second pathway, various forms of cellular stress trigger mitochondrial alterations: loss of $\triangle\psi_m$ and release of cytochrome C. Cytochrome C activates caspase-9 ("initiator" caspase). "Effector" caspases (such as caspase-3 and -6) were activated by caspase-9[16,17].

Resveratrol has been reported to induce apoptosis in some tumor cells, but the mechanisms of apoptosis are different in various tumor cells[4,6-11]. In our previous studies, we have found that "effector" caspase-3 and -6 could be activated during resveratrol-induced apoptosis in CNE-2Z cells[14,18], but capase-8 was not[19]. Thus, capase-3 and -6 may not be activated by caspase-8 and resveratrol-induced apoptosis in CNE-2Z cells may not be through the Fas/caspase-8-specific pathway. Is it *via* a mitochondria/caspase-9-specific pathway?

In this study, we found that Bcl-2 protein expression and $\triangle\psi_m$ were decreased, while cytosolic cytochrome C and caspase-9 activity were increased in a time- and dose-dependent manner, which is in agreement with the reports[8-10]. Our results showed that all except Bax protein expression had been changed since 2 h. Bcl-2 protein expression inhibition and $\triangle\psi_m$ loss were most obvious during 4 – 8 h, which were earlier than cytochrome C release and caspase-9 activation. The time course assays suggested that Bcl-2 protein expression inhibition and $\triangle\psi_m$ loss be prior to cytochrome C release and caspase-9 activation. Bcl-2 can prevent the loss of $\triangle\psi_m$ and the release of

Tab 5.　Detection of caspase-9 relative activity treated with 100 μmol·L^{-1} resveratrol for different time

Group	Caspase-9 relative activity ($A_{405\ nm}$)					
	0	2	4	8	12	24(h)
Control	0.056 ± 0.009	0.054 ± 0.008	0.047 ± 0.011	0.064 ± 0.008	0.063 ± 0.007	0.058 ± 0.005
LEHD-fmk + resveratrol	0.060 ± 0.007	0.053 ± 0.006	0.053 ± 0.007	0.059 ± 0.002	0.057 ± 0.006	0.062 ± 0.007
Resveratrol	0.047 ± 0.010	0.148 ± 0.022*	0.252 ± 0.019**	0.348 ± 0.020**	0.235 ± 0.019**	0.154 ± 0.026**

Control group: untreated cells. LEHD-fmk + resveratrol group: After pretreatment with 100 μmol·L^{-1} caspase-9 inhibitor (LEHD-fmk) for 2 h, cells were exposed to 100 μmol·L^{-1} resveratrol for 0, 2, 4, 8, 12, and 24 h, respectively. Resveratrol group: cells were treated only with 100 μmol·L^{-1} resveratrol for 0, 2, 4, 8, 12, and 24 h, respectively. $\bar{x} \pm s$, $n = 3$. * $P < 0.05$, ** $P < 0.01$, compared with the other two corresponding groups.

cytochrome C[20]. In the present study, the inhibition of Bcl-2 protein expression could cause the loss of $\triangle\psi_m$ and release of cytochrome C. The release of cytochrome C from mitochondria results in the formation complex containing Apaf1 and procaspase in presence of dATP, causing caspase-9 activation[21]. In addition, in our previous and present studies, we found caspase-9, -3, and -6 were sequentially activated during resveratrol-induced apoptosis of CNE-2Z cells[14,18], suggesting that caspase-9 trigger activation of caspase-3 and -6.

Bcl-2 family proteins can regulate the release of cytochrome C from mitochondria by affecting the activity of a novel ion channel[22]. In our study, we found Bcl-2 protein expression could be inhibited, but, worthy of note, Bax protein expression did not change with the increase of resveratrol-treated time and concentration. Thus, the loss of $\triangle\psi_m$ and the release of cytochrome C may be only related to Bcl-2 during resveratrol-induced apoptosis in CNE-2Z cells. Mahyar-Roemer, *et al*[11] also found that resveratrol at physiological doses can induce a Bax-mediated and a Bax-independent mitochondrial apoptosis. In the absence of Bax, membrane potential collapse was delayed, and apoptosis was reduced but not absent. These suggest that Bax be not essential during resveratrol-induced mitochondrial apoptosis.

According to the above, the mechanism of apoptosis induced by resveratrol in CNE-2Z cells may be as follows. Resveratrol can inhibit Bcl-2 expression, affecting the activity of ion channel. Then, the loss of $\triangle\psi_m$ and the release of cytochrome C from mitochondria are triggered. Cytosolic cytochrome C activates "initiator" caspase-9, resulting in activation of "effector" caspase-3 and -6. In conclusion, resveratrol induces apoptosis in CNE-2Z *via* the mitochondria/caspase-9-specific pathway, but this may not be related to Bax.

Surprisingly, we found that $\triangle\psi_m$ loss and cytochrome C release were decreased after they reached the peak at 8 h. Of interest, Zini, *et al*[12] reported that resveratrol could protect the mitochondrial membranes and inhibit cytochrome C release. Could resveratrol lead to $\triangle\psi_m$ loss and cytochrome C release in a fixed period of time? Could resveratrol protect the mitochondrial membranes and inhibit cytochrome C release when cells were treated with resveratrol for a long time because of other mechanisms? All these are to be explained.

4 REFERENCES:

[1] Niles RM, McFarland M, Weimer MB, Redkar A, Fu YM, Meadows GG. Resveratrol is a potent inducer of apoptosis in human melanoma cells[J]. *Cancer Lett*, 2003, **190**(2): 157 – 163.

[2] Zhou HB, Yan Y, Sun YN, Zhu JR. Resveratrol induces apoptosis in human esophageal carcinoma cells[J]. *World J Gastroenterol*, 2003, **9**(3):408 – 411.

[3] Joe AK, Liu H, Suzui M, Vural ME, Xiao D, Weinstein IB. Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines[J]. *Clin Cancer Res*, 2002, **8**(3):893 – 903.

[4] Roman V, Billard C, Kern C, Ferry-Dumazet H, Izard JC, Mohammad R, *et al*. Analysis of resveratrol-induced apoptosis in human B-cell chronic leukaemia[J]. *Br J Haematol*, 2002, **117**(4):842 – 851.

[5] Tang XD, Zhou KY, Zhao N. Effects of resveratrol on proliferation and apoptosis of nasopharyngeal carcinoma cell line (CNE-2Z)[J]. *Chin Tradit Herb Drugs*(中草药), 2004, **35**(3):287 – 290.

[6] Clement MV, Hirpara JL, Chawdhury SH, Pervaiz S. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells[J]. *Blood*, 1998, **92**(3): 996 – 1002.

[7] Tsan MF, White JE, Maheshwari JG, Bremner TA, Sacco J. Resveratrol induces Fas signaling-independent apoptosis in THP-1 human monocytic leukaemia cells[J]. *Br J Haematol*, 2000, **109**(2):405 – 412.

[8] Dorrie J, Geraucer H, Wachter Y, Zunino SJ. Resveratrol induces extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in acute lymphoblastic leukemia cells[J]. *Cancer Res*, 2001, **61**(12):4731 – 4739.

[9] Mouria M, Gukovskaya AS, Jung Y, Buechler P, Hines OJ, Reber HA, *et al*. Food-derived polyphenols inhibit pancreatic cancer growth through mitochondrial cytochrome C release and apoptosis[J]. *Int J Cancer*, 2002, **98**(5): 761 – 769.

[10] Tinhofer I, Bernhard D, Senfter M, Anether G, Loeffler M, Kroemer G, *et al*. Resveratrol, a tumor-suppressive compound from grapes, induces apoptosis *via* a novel mitochondrial pathway controlled by Bcl-2[J]. *FASEB J*,

2001，**15**(9)：1613－1615.
[11] Mahyar-Roemer M，Kohler H，Roemer K. Role of Bax in resveratrol-induced apoptosis of colorectal carcinoma cells [J]. *BMC Cancer*，2002，**2**(1)：27.
[12] Zini R，Morin C，Bertelli A，Bertelli AA，Tillement JP. Resveratrol-induced limitation of dysfunction of mitochondria isolated from rat brain in an anoxia-reoxygenation model [J]. *Life Sci*，2002，**71**(26)：3091－3108.
[13] Park JW，Choi YJ，Suh SI，Baek WK，Suh MH，Jin IN，*et al*. Bcl-2 overexpression attenuates resveratrol-induced apoptosis in U937 cells by inhibition of caspase-3 activity [J]. *Carcinogenesis*，2001，**22**(10)：1633－1639.
[14] Tang XD，Zhou KY，Ding H，Chen SZ. Activation of caspase-3 during resveratrol(Res)-induced apoptosis of CNE-2Z cells[J]. *J Immunol*(免疫学杂志)，2003，**19**(1)：23－29.
[15] Grub S，Perssohn E，Trommer WE，Wolf A. Mechanisms of cyclosporine A-induced apoptosis in rat hepatocyte primary cultures[J]. *Toxicol Appl Pharmacol*，2000，**163**(3)：209－220.
[16] Green DR，Reed JC. Mitochondria and apoptosis[J]. *Science*，1998，**281**(5381)：1309－1312.
[17] Yin XM，Wang K，Gross A，Zhao Y，Zinkel S，Klocke B，*et al*. Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis [J]. *Nature*，1999，**400**(6747)：886－891.
[18] Tang XD，Zhou KY，Chen XW，Chen SZ. Activation of caspase-6 during resveratrol(Res)-induced apoptosis of nasopharyneal carcinoma cells CNE-2Z cells[J]. *Chin J Immunol*(中国免疫学杂志)，2003，**19**(2)：100－103.
[19] Tang XD，Zhou KY，Ding H. Without participation of caspase-8 in resveratrol(Res)-induced apoptosis of CNE-2Z cells[J]. *Shanghai J Immunol*(上海免疫学杂志)，2003，**23**(6)：369，384.
[20] Yang J，Liu X，Bhalla K，Kim CN，Ibrado AM，Cai J，*et al*. Prevention of apoptosis by Bcl-2：release of cytochrome C from mitochondria blocked[J]. *Science*，1997，**275**(5303)：1129－1132.
[21] Li P，Nijhawan D，Budihardjo I，Srinivasula SM，Ahmad M，Alnemri ES，*et al*. Cytochrome C and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade[J]. *Cell*，1997，**91**(4)：479－489.
[22] Pavlov EV，Priault M，Pietkiewicz D，Cheng EH，Antonsson B，Manon S，*et al*. A novel，high conductance channel of mitochondria linked to apoptosis in mammalian cells and Bax expression in yeast[J]. *J Cell Biol*，2001，**155**(5)：725－731.

白藜芦醇诱导鼻咽癌细胞 CNE-2Z 凋亡的线粒体机制

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摘要：目的　探讨白藜芦醇诱导鼻咽癌细胞 CNE-2Z 凋亡的线粒体机制。**方法**　用 100 μmol·L^{-1}白藜芦醇分别处理细胞 0(对照)，2，4，8，12，24 h 和分别用 0，25，50，100，200 μmol·L^{-1}白藜芦醇处理 CNE-2Z 细胞 8 h，采用 Western 印迹分析 Bcl-2，Bax 和胞浆中细胞色素 C(Cyt C)的蛋白水平变化，罗丹明 123 荧光染色后经流式细胞术检测线粒体膜电位($\triangle\psi_m$)变化，比色法测定半胱天冬酶-9 的活性改变。**结果**　①100 μmol·L^{-1}白藜芦醇处理细胞不同时间，Bcl-2 蛋白表达和$\triangle\psi_m$减少、胞浆中的 Cyt C 和半胱天冬酶-9 活性增高均呈时间依赖性($P<0.01$)，但 Bax 蛋白表达无改变。除 Bax 以外的其他指标均自白藜芦醇处理 2 h 即有变化。Bcl-2 的蛋白表达受抑和$\triangle\psi_m$的丧失在 4～8 h 间最明显(与对照组比较 $P<0.01$)，在 24 h 时已无意义。胞浆中 Cyt C 水平和半胱天冬酶-9 活性在 8 h 达高峰(分别为 0 h 的 3.0，5.4 倍)，24 h 时仍明显高于对照组($P<0.01$)。②细胞经不同浓度的白藜芦醇处理 8 h 后，Bcl-2 的蛋白表达受抑、$\triangle\psi_m$的丧失、Cyt C 的释放和半胱天冬酶-9 活性的升高均具有剂量依赖性($P<0.01$)，但 Bax 的蛋白表达未受影响。**结论**　白藜芦醇可能经一个线粒体/半胱天冬酶-9 的特定途径诱导 CNE-2Z 细胞凋亡，但此凋亡过程可能与 Bax 无关。

关键词： 白藜芦醇；线粒体；细胞色素 C；蛋白 Bcl-2；蛋白 Bax；半胱天冬酶类

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