RESEARCH ARTICLE

In vitro and *in vivo* Evaluation of the Antitumor Efficiency of Resveratrol Against Lung Cancer

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Abstract

Lung cancer remains a deadly disease with unsatisfactory overall survival. Resveratrol (Res) has the potential to inhibit growth of several types of cancer such as prostate and colorectal examples. In the current study, we evaluated *in vitro* and *in vivo* anticancer efficiency of Res in a xenograft model with A549 cells. Cell inhibition effects of Res were measured by MTT assay. Apoptotis of A549 cells was assessed with reference to caspase-3 activity and growth curves of tumor volume and bodyweight of the mice were measured every two days. *In vitro* cytotoxicity evaluation indicated Res to exert dose-dependent cell inhibition effects against A549 cells with activation of caspase-3. *In vivo* evaluation showed Res to effectively inhibit the growth of lung cancer in a dose-dependent manner in nude mice. Therefore, we believe that Res might be a promising phytomedicine for cancer therapy and further efforts are needed to explore this potential therapeutic strategy.

Keywords: Resveratrol - lung cancer - antitumor efficiency - therapeutic strategy

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Introduction

Lung cancer remains to be a deadly disease with unsatisfied overall survival (Jemal et al., 2009; Rocks et al., 2012). Though the treatment of lung cancer includes surgery, chemotherapy and radiotherapy, the overall survival remains poor. Moreover, due to their resistance to conventional therapy, the 5-year combined survival rates of patients bearing lung cancer of all stages is still only 16% (Yin et al., 2013). Therefore, it is important to identify potential drugs for the treatment of lung cancer. Resveratrol (Res), (trans-3,4,5-trihydroxystilbene), a natural polyphenolic extracted from red wine, has the potential to inhibit growth of several types of cancer such as prostate, and colorectal cancers (Miki et al., 2012; Sheth et al., 2012). Though the molecular mechanism is not fully understood, several studies have demonstrated that the antitumor effect of Res is via a ROS-dependent apoptosis pathway (Shao et al., 2009; Lu et al., 2013). Accordingly, the reported efficacy of Res makes it a novel and potential anticancer agent. These studies also support the concept of developing phytochemicals for anticancer applications.

In the current study, we evaluated the in vitro and in vivo anticancer efficiency of Res in a xenograft model of A549 cells. The cell inhibition effect of Res was measured by MTT assay. Apoptotis of A549 cells was measured by the activity of Caspase-3. The growth curve of tumor volume and bodyweight of the mice were measured every two days.

Materials and Methods

Materials

Resveratrol, was purchased from Sigma Chem. Co., (St. Louis, USA). All other chemicals were of analytical grade and used without further purification. Human lung cancer cell line A549 was obtained from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China).

Male and female nude mice (nu/nu; 6–8 weeks old and weighing 18–22 g) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). The mice were housed and maintained in the animal facility of the Animal Center of Nanjing Medical University. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

In vitro cytotoxicity

The half maximal inhibitory concentration (IC_{50}) of A549 cells were determined by the MTT assay. Briefly, cells were seeded in 96-well plates (1×10^4 cells per well) 24h prior to the assay. Then cells were exposed to a series of doses of Res. After 48 hrs of incubation, 20μL of 5 mg/

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mL MTT solution was added to each well and the plate was incubated for 4 h. Then, the media were removed and dimethylsulfoxide (DMSO) (150 μL) was added to each well. The optical density (OD) of each well was measured using a microplate reader at 560 nm (Bio-Rad, Hercules, USA).

Cell viability was determined by following formula: Cell viability (%) = OD (test well)/OD (reference well) × 100% (1)

All the results obtained from MTT assays were confirmed by repeating the experiment on at least three independent occasions and testing in triplicate each time.

Caspase-3 activity analysis

A549 cells were treated with a series of doses of Res for 48h. Determination of caspase-3 activity was performed by the caspase colorimetric protease assay kit (Keygen Biotech, Nanjing, China) by following the manufacturer's instruction. The optical density was measure at 405 nm. The obtained values were expressed as folds of controls.

In vivo antitumor efficacy

Nude mice implanted with A549 cell line were used to qualify the antitumor efficacy of Res through intravenous administration. The mice were raised under specific pathogen-free (SPF) circumstances and all of the animal experiments were performed in full compliance with guidelines approved by the Animal Care Committee of Nanjing Medical University. The mice were subcutaneously injected at the left axillary space with 0.1 ml of cell suspension containing 4–6*10^6 A549 cells. Treatments were started after 7-8 days of implantation. The mice whose tumor reached a tumor volume of 100 mm3 were selected and this day was designated as "Day 0".

On Day 0, the mice were randomly divided into four groups, with each group being composed of 6 mice. The mice were treated intravenously with saline and a series doses of Res, respectively. Res was administered at a equivalent dose of 15, 30, and 60 mg/kg. All mice were tagged, and tumors were measured every other day with calipers during the period of study. The tumor volume was calculated by the formula (W^2*L)/2, where W is the tumor measurement at the widest point, and L is the tumor dimension at the longest point.

Each animal was weighed at the time of treatment so that dosages could be adjusted to achieve the mg/kg amounts reported. Animals also were weighed every other day throughout the experiments. After 15 days of injections, the mice were sacrificed for the detection of peripheral blood parameters as well as liver and kidney functions.

Statistical analysis and research experience

Results were presented as Mean±SD. Statistical comparisons were made by t test or ANOVA analysis. The accepted level of significance was *P* value < 0.05. We have enough experience in conducting medical researches, and have published some results elsewhere (Huang et al., 2004; Zhou et al., 2009; Jiang et al., 2010; Yan et al., 2010; Gao et al., 2011; Huang et al., 2011; Li et al., 2011; Li et al., 2011; Li et al., 2011; Xu et al., 2011; Xu et al., 2011; Xu et al., 2011; Yan et al., 2011; Zhang et al., 2011; Gong et al., 2012; Li et al., 2012; Yu et al., 2012).

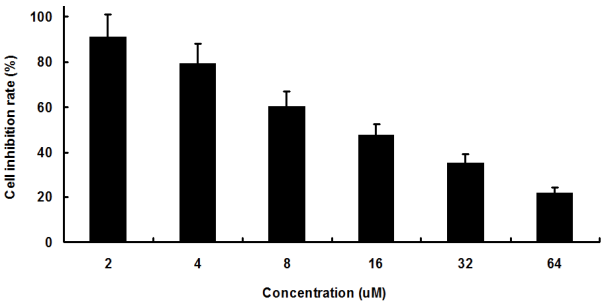

Figure 1. Effects of Res on A549 Cell Proliferation. (A) A5493 cells were treated with harmine at 2, 4, 8, 16, 32 and 64 μM for 48 hours

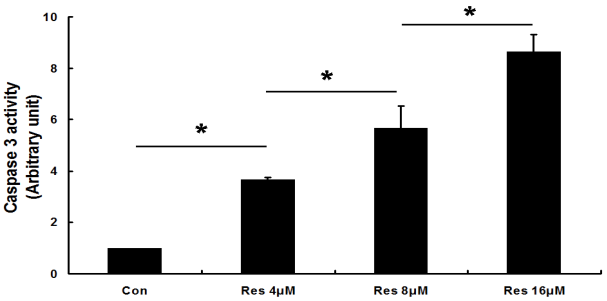

Figure 2. Analysis of Caspase-3 Activity. Values represents Mean ± SD. *represents $p < 0.05$

Results and Discussion

In vitro cytotoxicity of Res against A549 cells

Figure 1 shows the cytotoxicity of Res against A549 cells at different doses incubated for 48h. It is noted that Res showed similar dose- and time-dependent cytotoxicity against the cells at a dose from 4 to 64μM. As calculated from the cytotoxicity curve, the IC_{50} value of Res against A549 cells is 8.9±1.3μM.

Caspase-3 activation

Figure 2 indicates the acitivity of Caspase-3 in cells exposed to a series dose of Res. It is shown that Res could significantly activated Caspase-3 in a dose-dependent manner. Caspases are crucial mediators of programmed cell death (apoptosis) (Zhou et al., 2013). As reported, Caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins (Mcllwain et al., 2013). Thus, caspase-3 is essential for certain processes associated with the dismantling of the cell and the formation of apoptotic bodies, but it may also function before or at the stage when commitment to loss of cell viability is made. Obviously, Res shows the potential in effectively activating cellular apoptosis.

In vivo antitumor evaluation of Res against A549 xenograft

Antitumor efficacy of Res was investigated in A549 human lung cancer xenografts in nude mice. As shown in Figure 3A, Res exhibited a dose-dependent tumor growth inhibition effect. It is noted that the three doses of Res all significantly inhibited the growth of lung cancer since

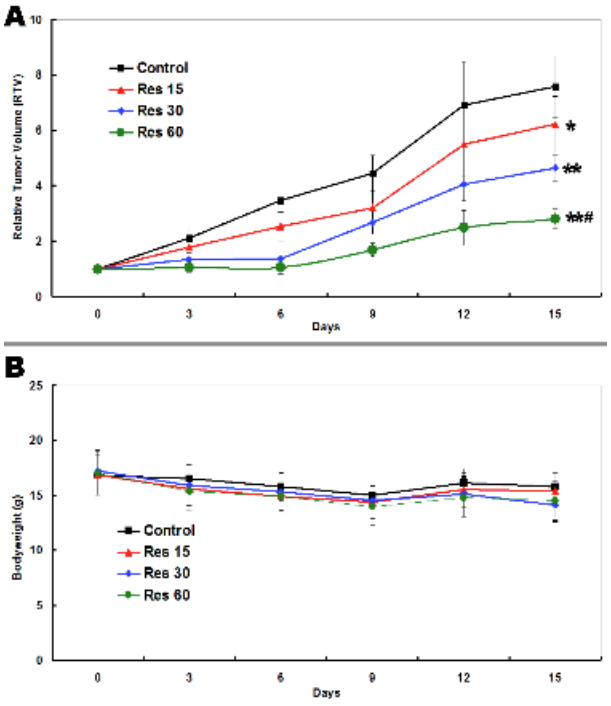

Figure 3. Antitumor Effect of Res in A549 Xenograft Models. (A) Tumor volume of established A549 xenografts in nude mice during therapy under different treatments. Mice were treated with different protocols on Day 0 as showed in the figure. Saline: vehicle; Res was administered at the doses of 15, 30 and 60 mg/kg. Different agents were delivered through intravenous pathway when tumor volume measured 100 mm3. Data are presented as mean±SD (n = 6). The difference between tumor volumes in the group of saline and Res is significant (* means $P < 0.05$, ** means $P < 0.01$). Significant difference (# means $P < 0.05$) also is observed between the group receiving 60 mg/kg Res and 30 mg/kg Res. (B) Bodyweight change of nude mice receiving different treatments during therapy. Data are presented as mean±SD (n = 6)

Day 6 ($P<0.05$ vs control). Moreover, the high dose of Res (60 mg/kg) showed the strongest antitumor effect. Among the four groups, the group that received 60 mg/kg was observed to maintain the greatest amount of antitumor activity (Figure 1A). In detail, The RTV of the group received low dose of Res (15 mg/kg) is nearly 6 at the end of treatment,. The RTV of the group received high dose of Res (60 mg/kg) is around 2, which is the lowest among all the groups indicating the strongest tumor inhibition. Statistical analysis reveals the significant differences between the group receiving Res and control group. It is also noted that the high dose of Res inhibited the growth of tumor more significantly than the other two doses of Res.

An analysis of body weight variations generally defined the adverse effects of the different therapy regiments (Figure 1B). No significance was observed among the four groups. The mice receiving even high dose of Res were in a good state in the aspects of movement and spirit.

In conclusion, the current study demonstrates the antitumor effect of Res in the treatment of lung cancer. In vitro cytotoxicity evaluation indicates that Res possesses a dose-dependent cell inhibition effect against A549 cells with the activation of Caspase-3. In vivo evaluation shows that Res effectively inhibits the growth of lung cancer in a dose-dependent manner in nude mice. Therefore, we believe that Res might be a promising phytomedicine in cancer therapy and further efforts are needed to explore this therapeutic strategy.

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