

Research on the A549 cell apoptosis mechanism of the nude mouse model using MenSC-sTRAIL

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Abstract. – OBJECTIVE: To investigate the inhibitory effect of MenSC-sTRAIL on the apoptosis of A549 cells of lung cancer in a nude mouse model and the relevant molecular mechanism.

MATERIALS AND METHODS: The tumorigenesis curve of A549 cells in the nude mouse model was assayed using the growth curve plotted by the growth rate of tumor of the A549 cell nude mouse model. Cellular apoptosis was assayed using flow cytometer and Annexin V-FITC, and the effect of MenSC-sTRAIL on the expression of Bax was measured using Western blot.

RESULTS: MenSC-sTRAIL promoted the apoptosis of A549 cells. Results of the quantitative assay using Annexin V-FITC further confirmed the effect of MenSC-sTRAIL on inducing the cellular apoptosis. Western-blot results showed that MenSC-sTRAIL increased the expression of Bax.

CONCLUSIONS: MenSC-sTRAIL may vary the balance of regulation of heterodimer of Bcl-2 and Bax, which can induce the apoptosis of A549 cells.

Key Words:

MenSC-sTRAIL, Lung cancer, A549 cells, Apoptosis.

level, which was considered to be associated with a higher incidence of recurrence as well as metastasis observed in the advanced phase of lung cancer^{1,2}. Although chemotherapy may delay the pathological process, the risk its failure is high.

Chemotherapy usually has to be ceased due to multiple drug-tolerance or intolerance to the toxic side effects. Search for effective chemotherapy drugs with definite, safe and reliable efficacy has become an important area of interest^{3,4}. During the screening process for new anti-tumor drugs, the reaction of the animal model can hardly be substituted by *in-vitro* cell culture experiments^{5,6}. Additionally, the success rate of heterograft of some tumors is relatively low due to the characteristics of the tumor cells as well as the influence of internal environment of a nude mouse. It is necessary to establish an animal transplantation model with a high compatibility for accurately evaluating the target efficacy of anti-tumor drugs. To resolve this technological problem and increase the success rate of transplantation model, we used human lung cancer A549 cells, which may be served as a reliable reference for the establishment of relevant animal models in the future.

Introduction

Lung cancer, with the highest clinical prevalence rate among malignant tumors, shows no specific clinical manifestations in early stages. Most patients are usually diagnosed accidentally with the advanced lung cancer during a regular physical examination or when they are examined for other diseases. This way patients miss the best time-window for surgical treatment. Although the cure rate of lung cancer has been increased in recent years, the long-term survival rate remains poor. Various studies revealed that the 5-year survival rate remained lower than the expected

Materials and Methods

Reagent

We used the following reagents in this study: MTT (methyl thiazolyl tetrazolium, Sigma-Aldrich, St. Louis, MO, USA), cis-platinum (Sigma-Aldrich, St. Louis, MO, USA), streptomycin (Sigma-Aldrich, St. Louis, MO, USA), penicillin (Sigma, USA), DMSO (Guangdong Guanghua Sci-Tech Co., Ltd, Guangdong, China), DMEM and fetal calf serum (Gibco, Grand Island, NY, USA).

Cells and Experimental Animals

10% fetal calf serum was added into the DMEM containing 100 IU/mL of streptomycin and penicillin for the culture of human lung cancer A549 cells. BALB/c-nu nude mice with an age of 3.5 weeks and weight of 20 g were provided by Nanjing JLC (Biopharma Co., Ltd. Nanjing, China). This study was approved by the Animal Ethics Committee of Jiangsu University Animal Center.

Transplanted Tumor Model of A549 Cell on the Nude Mouse

A549 cells ($1 \times 10^6/0.2$ mL) were transplanted to the axilla of the mouse. Then, the variation in the volume of transplanted tumor was continuously monitored. Significant enlargement of the tumor was observed between the first and second weeks after transplantation. The volume of tumor was calculated using the max and min diameters according to the following formula: $\pi/6 \times (\text{the length of max diameter}/2 \text{ the length of min diameter}/2)^3$ and the growth curve was accordingly drawn. The mouse was sacrificed when the length of max diameter reached 1 cm, and the tumor mass was resected in a sterile manner for the preparation of single cell suspension (filtrated by mesh). We used this to conduct the experiment of cellular apoptosis.

Cellular Apoptosis Experiment

The cellular apoptosis was quantitatively assayed using the Annexin V-FITC. Cells were digested using 0.25% trypsin and dissociated using a pipette. After cells had been rinsed using PBS, they were centrifuged at 1000 rpm for 10 min at 4°C and the supernatant was discarded. The binding buffer was added and cells were re-suspended with the cell count being adjusted to 5×10^5 /ml. 195 μ l of cell re-suspension was mixed with 5 μ l AnnexinV-FITC at room temperature for 10 min. Binding buffer was added next (200 μ l) and cells were centrifuged at 1000 rpm for 10 min at 4°C. The supernatant was discarded and 190 μ l of binding buffer was added to re-suspend cells. Thereafter, 10 μ l of PI (20 mg/L) was added into cells for assay using the flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

Western Blot

After cells had been treated using lysate, total protein was extracted followed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and proteins were transferred to a membrane. The primary antibody (1:250) was added to the membrane and after incubation

membrane was washed with PBS and TBS for 10 minutes for a total of 3 times and twice, respectively. The secondary antibody (1:2500) was added onto the membrane followed by washing using PBS and TBS for 10 min for twice, respectively. Thereafter, the membrane was preserved in the ECL (liquid A: liquid B = 1:1) for 1 min followed by drying at the room temperature. Membrane was used for imaging.

Statistical Analysis

Software SPSS21.0 (Version X; IBM, Armonk, NY, USA) was used for statistical analysis. Comparison between groups was done using One-way ANOVA test followed by Post-hoc test (Least Significant Difference). Percentage (%) was used to express the enumeration data and chi-square test was used for data analysis. $p < 0.05$ indicated that the difference was statistically significant.

Results

Growth of Transplanted Tumor of A549 Cells

The growth of transplanted tumor was assessed using the growth curve of transplanted tumor. The growth rate of transplanted tumor of A549 cells is shown in Figure 1.

Apoptosis of A549 Cells

We used Annexin V-FITC for the quantitative assay. According to the results obtained from the flow cytometer and Annexin V-FITC, we discovered that the apoptotic rate of A549 cells that were treated by cis-platinum for 48 h was rela-

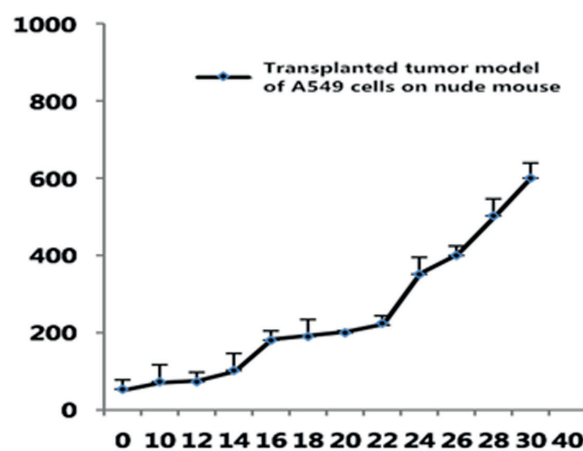


Figure 1. Growth curve of tumor model of A549 cells on nude model.

tively low. However, when treated with cis-platinum plus sTRAIL, the apoptotic rate increased to 47% (Figure 2), indicating the strong effect of sTRAIL on A549 cells apoptosis.

Apoptosis Mechanism of A549 Cells

A549 cells treated with cis-platinum and cells in the control group were collected and used for protein extraction to evaluate Bax expression level. The results revealed that Bax expression was gradually increased in cells treated with MenSC-sTRAIL. MenSC-sTRAIL protein expression level was also amplified (Figure 3). This suggested that MenSC-sTRAIL can promote cell apoptosis and showed the positive synergistic effect with the pro-apoptosis protein Bax.

Discussion

Cell apoptosis is affected by various factors, including the genetic regulation and exogenous

factors. In recent years, with the continuous progress being made in the research of molecular biology of tumor⁷⁻¹⁰, researchers have found that the occurrence and development of tumor are not only associated with the cell proliferation or differentiation, but also affected by the regulation of cell apoptosis which plays a crucial role in a series of pathological progression of tumor cells. Results of study through the establishment of the animal model by transplantation of tumors and inducing the cell apoptosis may provide the reference for research of molecular biological characteristics of tumor progression as well as the development of target drugs¹¹⁻¹³. In this study, we investigated the apoptotic effect and relevant mechanism of MenSC-sTRAIL on A549 cells of nude mouse model, and found that MenSC-sTRAIL promoted cell apoptosis and showed the positive synergistic effect with the pro-apoptosis protein Bax (Figure 4). Moreover, the quantitative assay of cell apoptosis using

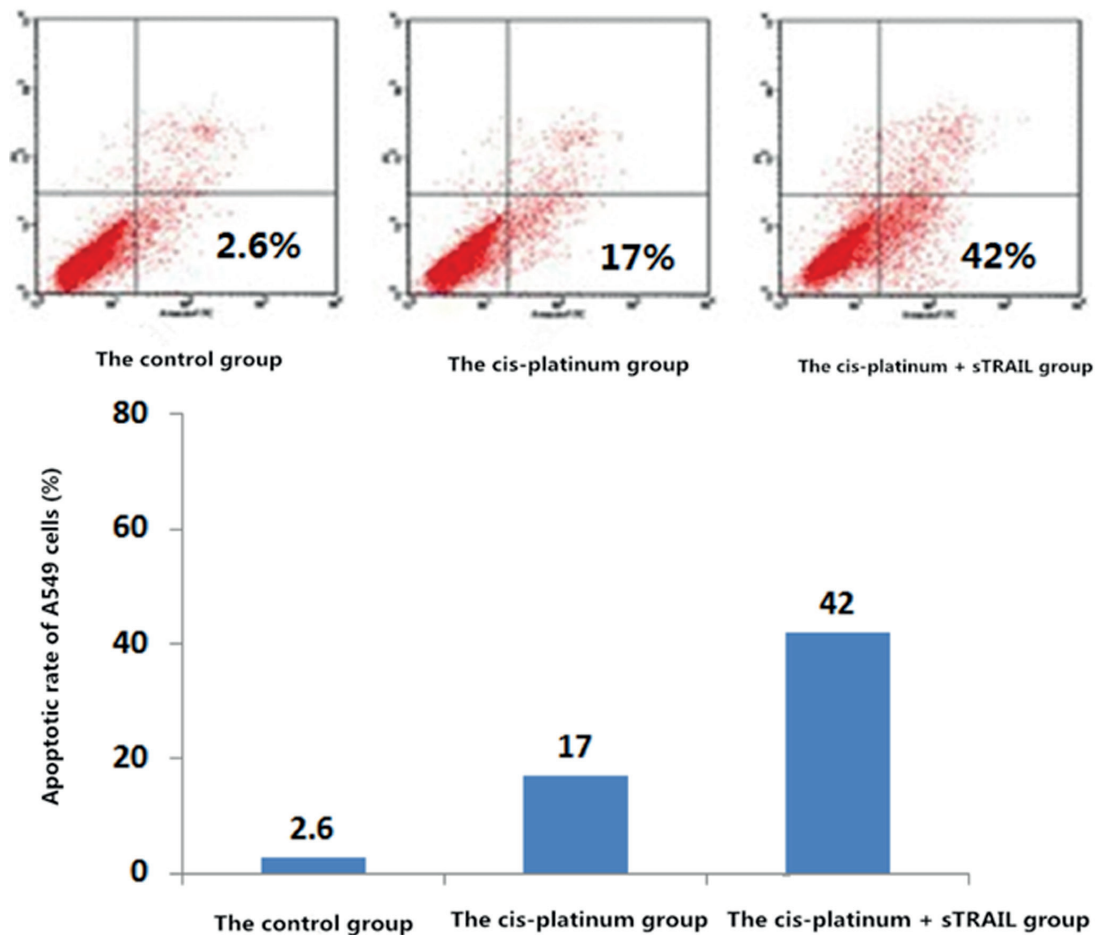


Figure 2. Apoptotic rate of A549 cells.

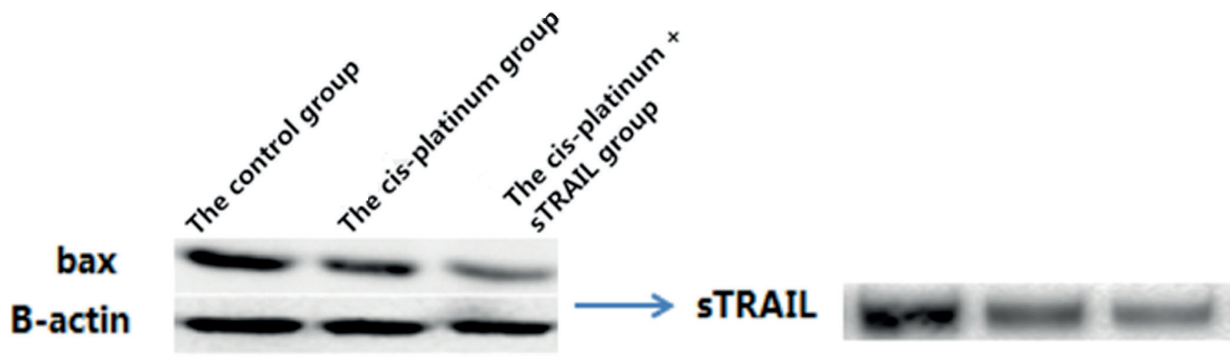


Figure 3. The expression of apoptotic protein as well as the effect of sTRAIL.

AnnexinV-FITC showed that MenSC-sTRAIL has a strong inducible effect of sTRAIL on the apoptosis of A549 cells.

Cell apoptosis is a complex phenomenon involving several genes¹⁴⁻¹⁶. Bcl-2 protein family plays an important role in apoptosis. Bcl-2 promotes cell apoptosis and participate in its depression progress. Bcl-2 and Bcl-XL are inhibitory factors in cell apoptosis, while Bax and Bcl-XS are pro-apoptosis factors. Bax is a 21 kD protein composed of 192 amino acids^{17,18}. The

combination of the Bax with Bcl-2 or Bcl-XL can form a heterodimer, inhibiting the generation of pro-apoptosis factors such as Bcl-2, thus producing the anti-apoptosis effect. The structural characteristics of the protein of Bax and Bcl-2 as well as their mutual action render them in a close relationship, i.e. regulating the generation of heterodimer composed by Bcl-2 or Bax, and participating in the regulation of cell apoptosis^{19,20}. Bcl-2 and Bax proteins levels are directly associated with the regulation of apoptosis²¹. An increase in Bcl-2 level can suppress the cell apoptosis, while an increase in Bax level promotes cell apoptosis.

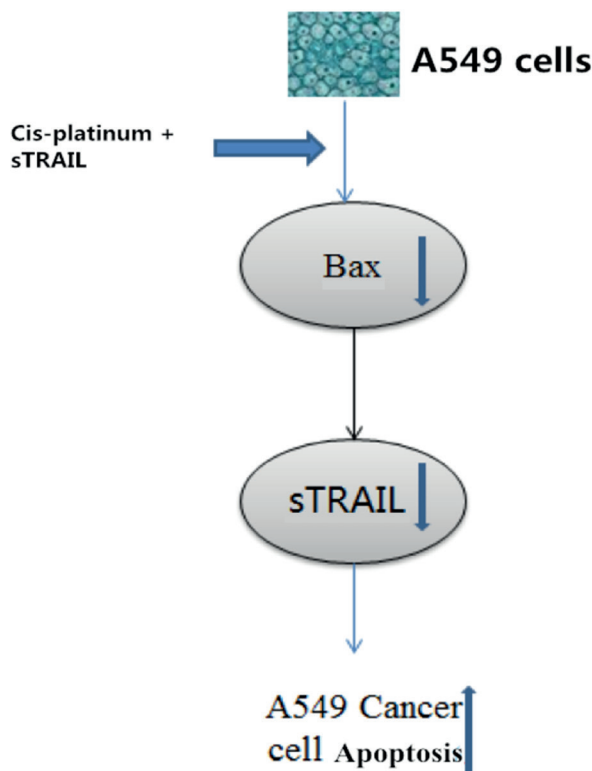


Figure 4. Investigation of relevant mechanisms.

Conclusions

In this study, we discovered that Bax protein expression level increased after the action of MenSC-sTRAIL on A549 cells. This suggested that MenSC-sTRAIL may vary the balance of regulation of heterodimer of Bcl-2 and Bax, which can induce the apoptosis of A549 cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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