rhTRAIL

Effects of recombinant human tumor-necrosis factor related to apoptosis inducing ligand protein on apoptosis of human lung adenocarcinoma cell line resistant to cisplatin  
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Abstract  Background and objective  Tumor-necrosis factor related to apoptosis inducing ligand protein (TRAIL), like tumor-necrosis factor (TNF) and Fas, is a member of TNF cytokine superfamily. Many researches have showed that TNF-α can reverse the resistance to some chemotherapeutic agents in cancer cell lines, and some anticancer drugs can result in up-regulations of death receptor (DR) and further lead to the enhancement of apoptosis induced by TRAIL. In order to clarify if TRAIL can reverse the resistance to cisplatin in cancer cells, the effects of recombinant human tumor-necrosis factor related to apoptosis inducing ligand protein (rhTRAIL) on apoptosis in human lung adenocarcinoma cell lines resistant to cisplatin (DDP) in vitro was explored. 

Methods  Human lung adenocarcinoma cell lines resistant to cisplatin, A549/DDP cells, were cultured in regular condition. At 24 hours after TRAIL and DDP, alone or combined, microculture tetrazolium (MTT) dye was used to evaluate the cytotoxic effects. And besides, to detect the apoptotic effects of rhTRAIL on A549/DDP cells, flow cytometry assay was used to test the apoptosis proportion, diphenylamine assay (DPA) was applied to detect the percent of DNA fragmentation and Caspase-3 fluorometric assay was performed to test the activity of Caspase-3 among these cells. 

Results  A549/DDP cells were not sensitive to low-dose rhTRAIL alone. The rate of growth inhibition and the apoptotic indexes such as the apoptosis proportion, the percent of DNA fragmentation and the activity of Caspase-3, had all no significant changes with rhTRAIL concentration less than 25 μg/L (P > 0.05). But treated with higher-dose rhTRAIL more than 50 μg/L, the four values changed obviously: 66.8% ± (27.13 ± 0.66)% (37.4 ± 0.2)% and 0.117 ± 0.011 (P < 0.05). With combination of different concentration of rhTRAIL and 3 mg/L DDP, the cyto-

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toxic and apoptotic effect was comparatively more apparent. The combination of rhTRAIL and 3 mg/L DDP presented synergistic effect on A549/DDP. 12.5 μg/L concentration of rhTRAIL together with 3 mg/L DDP could kill 30.4% of A549/DDP cells. Furthermore, the rate of cell apoptosis, percent of DNA fragmentation and activity of caspase-3 increased to (19.39 ± 0.54) %, (17.3 ± 4.1) % and 0.138 ± 0.009, which were significantly different from those of rhTRAIL alone (P<0.01). Conclusion High-dose rhTRAIL can also induce the cells resistant to cisplatin to apoptosis, but the cytotoxic and apoptotic effects of rhTRAIL alone were weaker than those of combination of rhTRAIL and low-dose cisplatin which can augment the apoptotic effect induced by rhTRAIL. rhTRAIL is expected to be an efficient biologic drug for treatment of lung cancer resistant to chemotherapy.

【Key words】 TRAIL  Lung neoplasms  Cell apoptosis  Multi-drug resistance

1.2 A549/DDP 10% 100 U/ml 100 U/ml RPMI1640 37 C .5%CO2 0 , 0.25% 5x10^4/ml.
1.3 100 µl(5x10^3) 96 200 µl 37 C .5%CO2 24 h 6 3 DMSO 50 ng/l.
1.4 4h 15 min 570 nm 50% (1-A0/A1)×100%.
1.4.1 TRAIL 24 h 2x10^6 (phosphate buffered saline, PBS)
40 C 4~72 h PBS RNase A 37 C 30 min PI 4 C 30 min.
1.4.2 DNA (1,000,000) (diphenylamine assay, DPA).
400 µl 13 000 r/min×10 min. 13 000 r/min×10 min. TCA 80 µl. 
90℃ 30 min, 2 000 r/min×10 min. 25 µl, 50℃ 4 h, 0-570 nm. DNA (% = A₀₀ / (A₀₀ + A₀₀) × 100%.

1. 4. 3 Caspase-3 2×10⁶, PBS 50 µl, 10 min, 4℃. Lowry 100 µg 37℃ 2 h, 510 nm. A. Caspase-3.

1. 5 x±s, t P<0.05.

2. 1 TRAIL A549/DDP (25 µg/L) A549/DDP 50 µg/L, A549/DDP 3 mg/L. TRAIL, 12.5 µg/L TRAIL (t = 4.678, P<0.05).

Tab 1 Effects of different concentrations of TRAIL on the cell viability of A549/DDP

<table>
<thead>
<tr>
<th>Group (µg/L)</th>
<th>TRAIL (0)</th>
<th>TRAIL (3 mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A growth inhibition</td>
<td>A growth inhibition</td>
</tr>
<tr>
<td></td>
<td>(x±s) rate(%)</td>
<td>(x±s) rate(%)</td>
</tr>
<tr>
<td>0</td>
<td>0,79±0,03</td>
<td>0,73±0,01</td>
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<tr>
<td>6,25</td>
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<td>0,66±0,06</td>
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<tr>
<td>12,5</td>
<td>0,72±0,12</td>
<td>0,55±0,10</td>
</tr>
<tr>
<td>25</td>
<td>0,68±0,07</td>
<td>0,42±0,03</td>
</tr>
<tr>
<td>50</td>
<td>0,25±0,03</td>
<td>0,17±0,07</td>
</tr>
<tr>
<td>100</td>
<td>0,20±0,05</td>
<td>0,14±0,02</td>
</tr>
</tbody>
</table>

Note: * vs negative control. P<0.01 Δ vs DDP control. P<0.01

2. 2 TRAIL A549/DDP (25 µg/L) A549/DDP 50 µg/L A549/DDP (27, 13±0,66) 0,117 ± 0,011(t = 11, 658, P<0.05).

2. 4 TRAIL A549/DDP Caspase-3 (t = 1, 148, P>0.05).

Fig 1 Effects of different concentrations of TRAIL on apoptotic index of A549/DDP cells

Fig 2 Effects of different concentrations of TRAIL on DNA fragmentation of A549/DDP cells
Fig 3 Effects of different concentrations of TRAIL on Caspase-3 activity of A549/DDP cells

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TRAIL  TNF, Fas L  5  TNF  5. (death receptor, DR) TRAIL-R1 (DR4)  TRAIL-R2 (DR5)  (Decoy receptor, DcR) TRAIL-R3 (DcR1)  TRAIL-R4 (DcR2)

DR4  DcR1  DcR2  DcR5  

DNA  . Caspase-3  


