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ORIGINAL ARTICLE

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Synergistic antitumor activity of TRAIL combined with chemotherapeutic agents in A549 cell lines in vitro and in vivo

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Abstract *Purpose*: To investigate the synergistic cytotoxicity of TRAIL in combination with chemotherapeutic agents in A549 cell lines, we systematically evaluated the cytotoxicity of TRAIL alone and TRAIL in combination with cisplatin, paclitaxel (Taxol) or actinomycin D in A549 cell lines in vitro and in vivo, and whether the sensitivity was correlated with the expression level of TRAIL receptors. *Methods*: We investigated the cytotoxicity of TRAIL alone and the synergistic antitumor effects of TRAIL in combination with chemotherapeutic agents in A549 cells by crystal violet staining and FACS in vitro. The expression levels of DR4, DR5, DcR1 and DcR2 were measured in TRAIL-treated and chemotherapeutic agent-treated A549 cells by Western blotting. The growth inhibition of tumors was evaluated in terms of incidence, volume and weight in a A549-implanted nude mice model. *Results*: Chemotherapeutic agents cisplatin (5.56 μg/ml), Taxol (10 and 30 μg/ml) or actinomycin D (9.26, 83.3 and 750 ng/ml) augmented the cytotoxicity of TRAIL in A549 cell lines within a range of concentrations of TRAIL (1.98–160 ng/ml) in vitro. The expression levels of DR4 and DR5 were not significantly different and the expression of DcR2 was slightly downregulated, but the expression of DcR1 was not detected in non-treated, TRAIL-treated and chemotherapeutic agent-treated A549 cells. The rates of tumor inhibition following treatment with TRAIL alone (15 mg/kg per day, daily for 10 days) and TRAIL/cisplatin (15 mg/kg per day TRAIL, daily for 10 days; 1.5 mg/kg per day cisplatin, daily for 10 days with 7-day intervals) were 28.3% and 76.8% by tumor weight ($P < 0.05$ for TRAIL alone versus control, $P < 0.05$ for TRAIL/cisplatin versus cisplatin alone and TRAIL alone) on day 65 in vivo. *Conclusion*: TRAIL in combination with chemotherapeutic agents cisplatin, Taxol or actinomycin D exerted synergistic antitumor effects in A549 cell lines in vitro and TRAIL/cisplatin demonstrated synergistic antitumor effects in vivo. The expression levels of TRAIL receptors suggested that the synergistic effects of TRAIL in combination with chemotherapeutic agents are not at the receptor level in A549 cell lines.

Keywords TRAIL · TRAIL receptor · Apoptosis · A549 · Chemotherapeutic agents

Abbreviations TNF: Tumor necrosis factor · TRAIL: TNF-related apoptosis-inducing ligand · NSCLC: Non-small cell lung cancer · DR: Death receptor · DcR: Decoy receptor · IB: Inclusion body · FACS: Fluorescence activated cell sorter · PI: Propidium iodide · IR: Rate of inhibition · PMSF: Phenyl methyl sulfonyl fluoride

Introduction

Lung cancer is the leading cause of cancer deaths in the world. Of all newly diagnosed lung cancers, 80% are non-small-cell lung cancer (NSCLC) [1]. Current protocols with present chemotherapeutic agents for therapy of NSCLC are not effective. Death receptors (DR), which mediate apoptosis, are increasingly being seen as an attractive anticancer target because they initiate apoptosis regardless of p53 phenotype and also have direct access to the caspase machinery [2]. A cloned member of the TNF family, Apo2L/TNF-related apoptosis-inducing ligand (TRAIL) [3, 4], may provide a new therapeutic option for NSCLC.

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TRAIL has been identified as a member of the TNF ligand family. It specifically kills various tumor cells and transformed cells, but is nontoxic to normal cells [5, 6]. TRAIL induces apoptosis in a number of cancer cell lines and has broad-spectrum activity against human malignancies. TRAIL can interact with four cell surface TRAIL receptors that belong to the TNF receptor family and are expressed constitutively in several normal human tissues. DR4 (TRAIL-R1) and DR5 (TRAIL-R2) can trigger the apoptotic signal to lead to a death cascade stimulated by TRAIL [7, 8]. However, normal cells are protected by decoy receptors DcR1 (TRAIL-R3) and DcR2 (TRAIL-R4), which compete for binding TRAIL but do not transduce death signals [9–11]. Although the precise mechanism of TRAIL-induced apoptosis is complex and unclear [12, 13], the apoptosis signal is initiated via extracellular TRAIL receptors DR4 and DR5 and intracellular downstream apoptotic modulators such as apoptosis-inducing proteins [14, 15].

Previous studies have shown that systemic administration of TRAIL is safe and effective in killing human breast and colon tumors in xenografted nude mice [7, 8, 16, 17]. However, there have been no systematic studies about the action of TRAIL in human lung carcinoma A549 cells (NSCLC) in vivo or in vitro so far. In this study, we sought to determine whether TRAIL mediates cytotoxicity in A549 cell lines in vivo and in vitro. We also investigated whether TRAIL-induced cytotoxicity could be augmented by chemotherapeutic agents in A549 cell lines in vitro and in vivo. Our results showed for the first time that TRAIL alone and in combination with cisplatin is effective as a cancer therapeutic agent in A549-implanted nude mice. The expression levels of TRAIL receptors in TRAIL-treated and chemotherapeutic agent-treated A549 cells were also determined for the first time.

Materials and methods

Reagents

Soluble TRAIL/Apo2L was produced in *E. coli* in our laboratory. The human NSCLC A549 cell line was purchased from the Cell Bank Center of the Chinese Academy of Science. Actinomycin D was purchased from Shanghai Songon Biological Engineering Technology Company. Taxol was obtained from Bristol-Myers Squibb Caribbean. Cisplatin was from QiLu Pharmaceutical Factory. A DNA-Prep Coulter reagents Kit was bought from Beckman Coulter. The following polyclonal antibodies were obtained: rabbit anti-DR4, anti-DR5, anti-DcR2 (1:500, Imgenex), anti-DcR1 (1:500, BD Biosciences) and antiactin (1:200, Santa Cruz Biotechnologies). Peroxidase-conjugated goat antirabbit IgG was purchased from Wuhan Boster Biological Technology Company. A SuperSignal West Femto kit was obtained from Pierce Technology. Flag-TRAIL and Anti-Flag M_2 antibody were a gift from Dr. Plascal Schneider, Institute of Biochemistry, University of Lausanne, Switzerland.

Expression and purification of TRAIL

Human TRAIL/Apo-2L cDNA (amino acids 114–281) was subcloned into pET-11a expression plasmid and expressed in *E. coli* strain BL21 in a 5-l fermenter. Cell pastes were sonicated and centrifuged to obtain the inclusion bodies (IBs). TRAIL contains an internal zinc atom bound by the cysteine residues at position 230, which is crucial for trimer stability and biological activity [18, 19]. Thus, the IBs were denatured in lysis buffer (50 m*M* Tris-HCl, 8 *M* urea, 14 m*M* β-mercaptoethanol, pH 8.5), and refolded by diluting into refolding buffer (20 m*M* Tris-HCl, 300 m*M* NaCl, 1 *M* urea, 100 μM $ZnCl_2$, 1 m*M* β-mercaptoethanol, pH 8.5) in our laboratory. The refolded soluble TRAIL was purified by chromatography with CM-Sepharose Fast Flow and Sephacryl S100. The purity was determined by silver staining using SDS-polyacrylamide gel electrophoresis and reverse-phase high-performance liquid chromatography. The purity of purified recombinant human TRAIL in phosphate-buffered saline (PBS) was not less than 95%.

Cytotoxicity assay of recombinant soluble human TRAIL

A549 cells were seeded in 96-well plate (5×10^4 per well) and incubated with TRAIL for 24 h in RPMI 1640 medium containing 10% bovine serum albumin (BSA) at 37°C under an atmosphere containing 5% CO_2. Cell viability was determined by crystal violet staining. Crystal violet granules were dissolved in 100 µl of 50% ethanol containing 0.1% HAc. The percentage cytotoxicity was calculated as follows: Cytotoxicity% $= (1 - OD_{570}$ of experimental well$)/OD_{570}$ of control well [7].

Propidium iodide staining for cytotoxicity analysis by FACS

The induction of apoptosis was assessed by fluorescence-activated cell sorter (FACS) analysis [20]. For FACS analysis, cells were cultured in RPMI 1640 medium containing 10% BSA at 37°C under an atmosphere containing 5% CO_2 overnight, then incubated with TRAIL at the designated concentrations and for the specified number of hours. Nonadherent cells and adherent cells trypsinized with 0.25% trypsin were collected together and centrifuged at 4000 *g*. The supernatant was aspirated, and the cells were washed with 2 ml PBS. The whole cells were resuspended in 50 µl fixing buffer at room temperature for 20 s, and 450 µl propidium iodide (PI) staining buffer was added in the dark at

room temperature for 30 min (procedure program of DNA-Prep Coulter reagents kit). A minimum of 1×10^4 treated cells for each experiment were analyzed using an EPICS XL-MCL model Coulter counter.

Synergistic cytotoxicity analysis

A549 cells were seeded in a 96-well plate (5×10^4 per well) and incubated with TRAIL combined with chemotherapeutic drugs for 24 h in RPMI 1640 medium containing 10% BSA at 37°C under an atmosphere containing 5% CO_2. Cell viability was determined by crystal violet staining. The synergy of the combinations of chemotherapeutic drugs and TRAIL in A549 cells was assessed using the following equations as described previously by Jin [21]: rate of inhibition (IR) = (OD_{570} of control well−OD_{570} of experimental well)/OD_{570} of control well−OD570 of blank well)×100%, and $q = E_{A+B}/[E_A + (1 - E_A)E_B]$, where E_{A+B} is the IR of the combination of drugs A and B, E_A is the IR of drug A, and E_B is the IR of drug B. Thus $q < 0.85$ indicates an antagonist effect, $q > 1.15$ indicates a synergistic effect, and $0.85 < q < 1.15$ indicates a combination effect.

Western blot

A549 cells were cultured in RPMI 1640 medium containing 10% BSA at 37°C under an atmosphere containing 5% CO_2 for 24 h, then incubated again in the presence or absence of actinomycin D (500 ng/ml), cisplatin (25 μg/ml), Taxol (30 μg/ml) or TRAIL (160 ng/ml) for 6 h. The cells detached in the buffer (1 m*M* EDTA, 0.9% NaCl) and nonadherent cells were harvested at 4000 rpm. The cells were washed with 1 m*M* EDTA, 0.9% NaCl, then lysed in lysis buffer (150 m*M* NaCl, 50 m*M* Tris-HCl, pH 7.6, 1 m*M* EDTA, 1% NP40, 0.25% sodium deoxycholate, 1 μg/ml aprotinin, 100 μg/ml PMSF). Protein concentrations were determined by the method of Lowry. An aliquot of total protein was diluted in an equal volume of 2× SDS sample buffer (50 m*M* Tris-HCl, pH 6.8, 100 m*M* dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol) and boiled for 5 min. The cell lysates (25 μg per well) were then electrophoresed on 12% SDS-PAGE and were transferred from the gel onto a nitrocellulose membrane. The nonspecific binding sites were blocked by incubation overnight at 4°C with freshly prepared 5% nonfat milk in TBST (20 m*M* Tris-HCl, 150 m*M* NaCl, 0.5% Tween 20) and incubated with antibody for 1 h at room temperature, then washed with TBST buffer three times, each for 10 min. After washing, the membrane was incubated for 1 h with horseradish peroxidase-conjugated goat-antirabbit IgG antibody. After washing with TBST three times, the membrane was developed with a SuperSignal West Femto kit (Pierce Technology).

In vivo study of antitumor activity

Female and male nude mice ($n = 36$, female or male, 20 ± 2 g, 8 weeks age) were purchased from Sino-British SIPPR/BK Laboratory Animals. Human lung cancer A549 cells in log phase were injected subcutaneously into the anterior flank of each mouse (nine mice per group). TRAIL and cisplatin were administered by intraperitoneal injection. Tumor volumes were calculated from the equation tumor volume (mm^3) = length × $width^2 \times 0.5$ [22], and tumor weights were determined after the mice had been killed.

Statistical analysis

For the determination of synergistic cytotoxicity, all assays were set up in triplicate, and Student's *t*-test was used for statistical analysis. The results from the animal experiments were analyzed using the rank sum test.

Results

Cytotoxicity of refolded recombinant soluble human TRAIL in A549 cells

To test the activity of TRAIL, A549 cells were incubated with threefold serial dilutions of purified TRAIL and cell viability was determined after 24 h. A representative standard histogram for the cytotoxicity of soluble recombinant TRAIL is shown in Fig. 1. The optimized preparation of soluble recombinant human TRAIL was cytotoxic to A549 cells and the IC_{50} was about 20 ng/ml. Purified recombinant soluble human TRAIL had the same antitumor activity as homologized Flag-TRAIL by Anti-Flag M_2 antibody (data not shown).

Time–response and dose–response relationships in A549 cells

To investigate further the biological activity of soluble TRAIL, the time- and dose-dependence of TRAIL-induced cytotoxicity was analyzed by FACS. The cytotoxicity of TRAIL in A549 cells was indicated by a subdiploid peak accompanied by an increase in the uptake of PI, and was time- and dose-dependent (Fig. 2). TRAIL-treated A549 cells and normal A549 cells also showed diploid peaks of different wavelengths (Fig. 3).

Synergistic cytotoxicity induced by TRAIL in combination with chemotherapeutic agents

To investigate the synergistic effects of TRAIL and chemotherapeutic agents, A549 cells were treated with various concentrations of TRAIL (1.98, 17.8 and 160 ng/ml) combined with various concentrations of

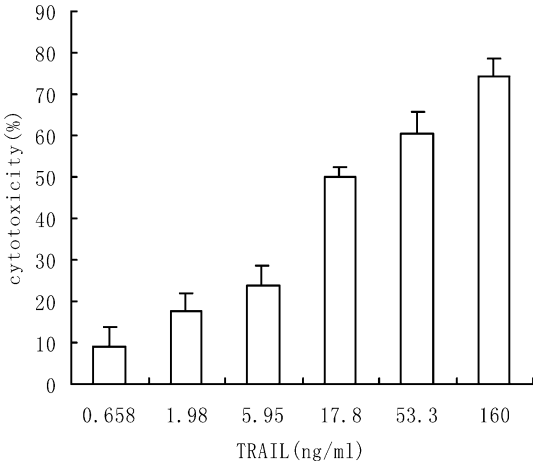


Fig. 1 Dose-dependent TRAIL-induced cytotoxicity in A549 cells as determined by crystal violet staining ($n=3$). A549 cells were incubated with soluble TRAIL (0.658, 1.98, 5.95, 17.8, 53.3, and 160 ng/ml) for 24 h, and cell viability was determined by crystal violet staining

cisplatin (0.62, 5.56 and 50 μg/ml), Taxol (0.37, 10 and 30 μg/ml) or actinomycin D (9.26, 83.3 and 750 ng/ml). The subcytotoxic doses of chemotherapeutic agents were determined in preliminary experiments so that one of the concentrations did not induce a high rate of apoptosis as a single treatment. As shown in Fig. 4, the cytoxicities of TRAIL at the various concentrations combined with cisplatin (5.56 μg/ml), Taxol (10 and 30 μg/ml) or actinomycin D (9.26, 83.3 and 750 ng/ml) were synergistic as compared with TRAIL alone in A549 cells ($P<0.01$).

Effect on the expression of death receptors and decoy receptors in A549 cells

The apoptosis of cancer cells is induced through TRAIL binding DR4 and DR5. However, DcR1 and DcR2 can protect against TRAIL-mediated apoptosis by acting as antagonists of the apoptosis-inducing TRAIL receptors (DR4, DR5). In the present study, we investigated the

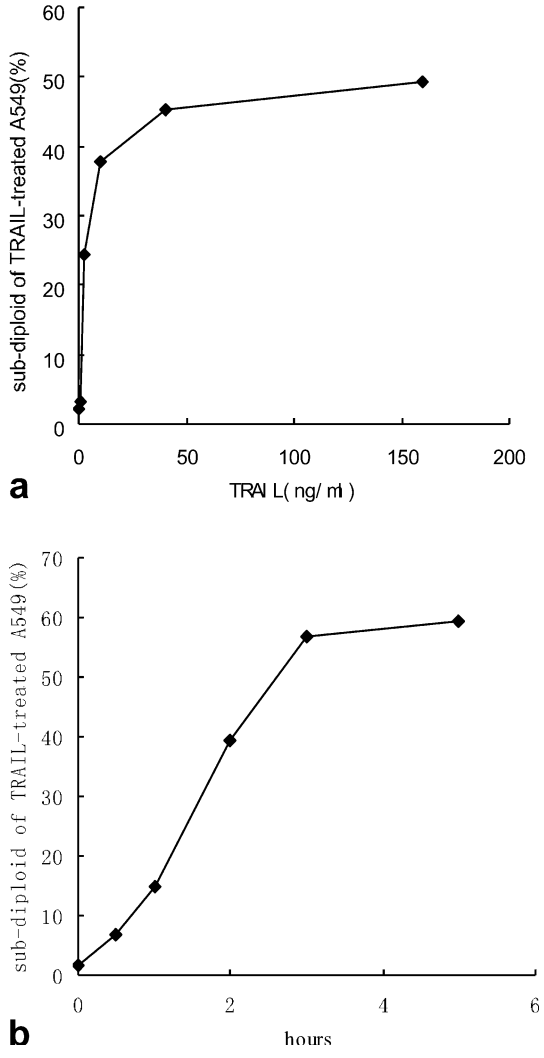


Fig. 2a, b Dose- and time-dependence of the subdiploid percentage of TRAIL-treated A549 cells as determined by FACS analysis. **a** A549 cells were cultured in RPMI 1640 medium overnight, and incubated with various concentrations of TRAIL (0.625, 2.5, 10, 40 and 160 ng/ml) for 24 h. **b** A549 cells were cultured in RPMI 1640 medium overnight, and incubated with soluble TRAIL (160 ng/ml) for 0.5, 1, 2, 3 and 5 h

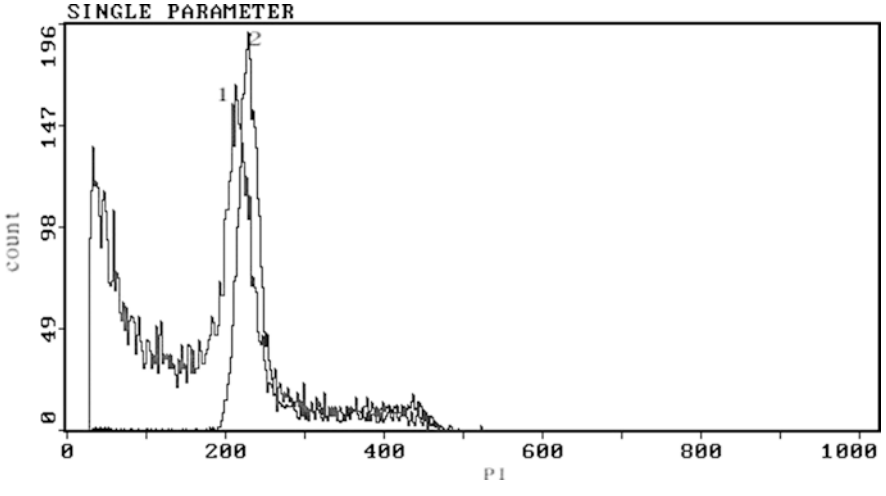


Fig. 3 PI uptake parameter of TRAIL-treated and normal nontreated A549 cells by FACS analysis. A549 cells were cultured in RPMI 1640 medium overnight, and incubated with soluble TRAIL (160 ng/ml) for 3 h. The subdiploid percentages of TRAIL-treated A549 cells were determined by FACS analysis. *1* indicates PI uptake by TRAIL-treated A549 cells; *2* indicates PI uptake by normal nontreated A549 cells

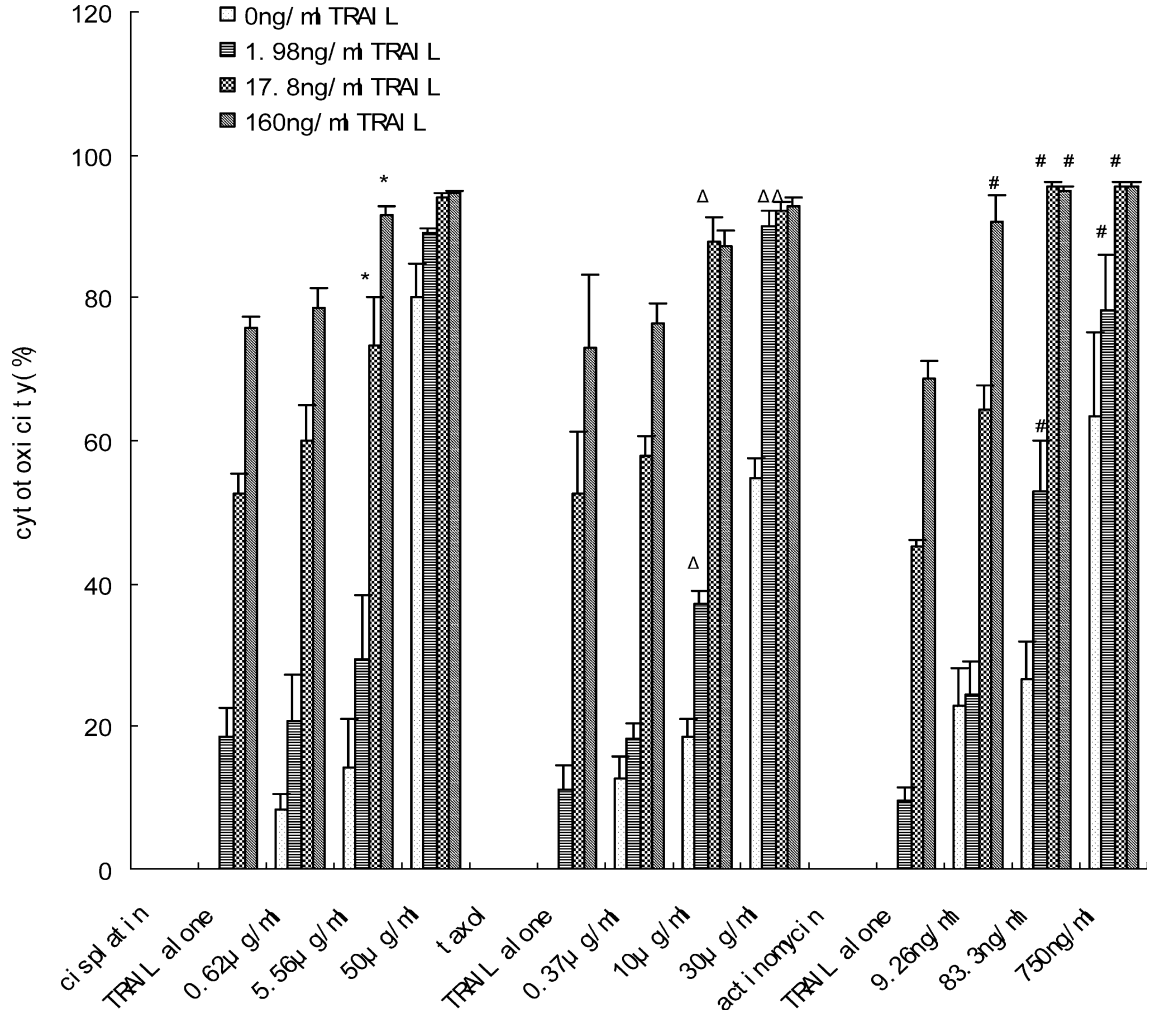


Fig. 4 Cytotoxicities of TRAIL in combination with cisplatin, Taxol or actinomycin D in A549 cells. A549 cells were incubated with various concentrations of TRAIL (1.98, 17.8 and 160 ng/ml) combined with cisplatin (0.62, 5.56 and 16.7 μg/ml), Taxol (0.37, 10 and 30 μg/ml) and actinomycin D (9.26, 83.3 and 750 ng/ml) for 24 h. Cytotoxicity was measured by crystal violet staining. Synergism is symbolized by *asterisks* (cisplatin), *open triangles* (Taxol) and *hash symbols* (actinomycin D). The data presented are means ± SD of triplicate cultures (*$P < 0.01$ versus TRAIL alone; $^{\triangle}P < 0.01$ versus TRAIL alone; $^{\#}P < 0.01$ versus TRAIL alone)

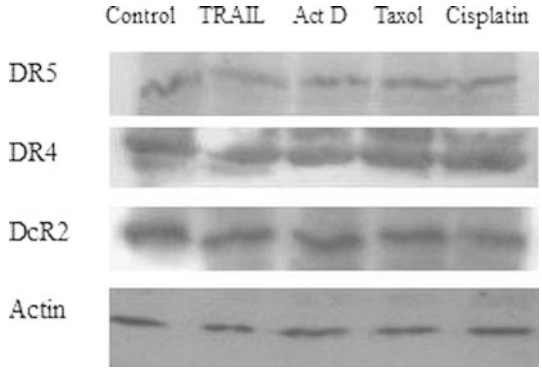
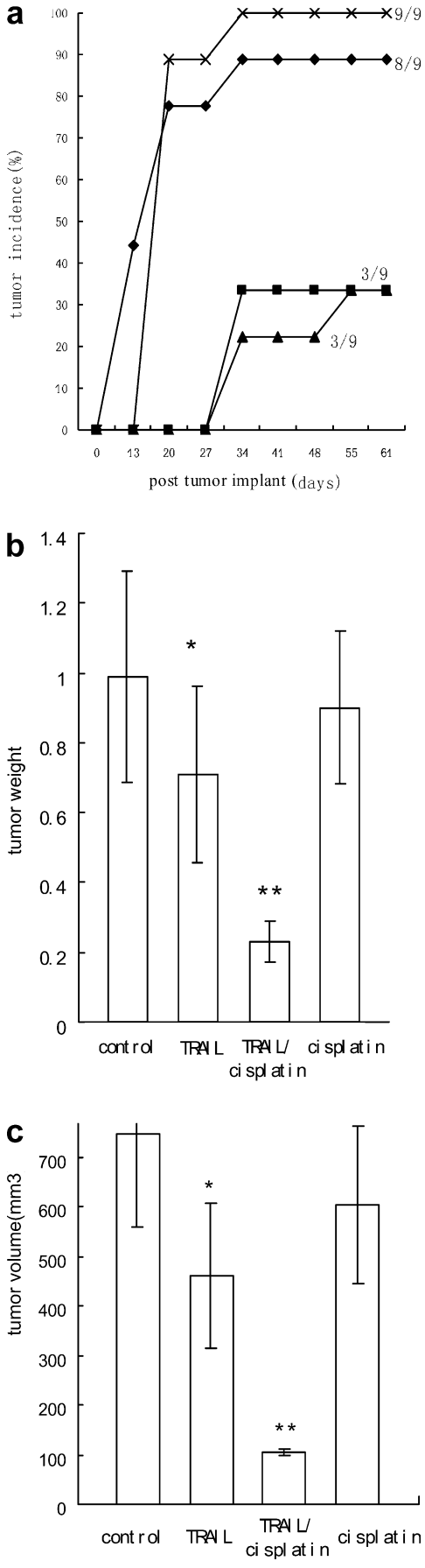


Fig. 5 Expression of death receptors (DR4, DR5) and decoy receptor DcR2 in A549 cells treated with TRAIL or chemotherapeutic agents. A549 cells were incubated with actinomycin D (500 ng/ml), cisplatin (25 μg/ml), Taxol (30 μg/ml) or TRAIL (160 ng/ml) for 6 h. The cell lysates (25 μg per well) were then electrophoresed on 12% SDS-PAGE and were transferred from the gel onto a nitrocellulose membrane. The expression of death receptors and decoy receptors was determined by Western blotting. Actin was used as an internal control for protein loading

possible alterations in the expression levels of DR4, DR5, DcR1 and DcR2 in A549 cells treated with TRAIL, actinomycin D, cisplatin or Taxol as compared with nontreated A549 cells. As shown in Fig. 5, the expression of DcR2 was slightly downregulated. However, there were no significant differences in the expression levels of DR4 or DR5. Expression of DcR1 was not detected in nontreated, TRAIL-treated or chemotherapeutic agent-treated A549 cells.

Antitumor activity of TRAIL in nude mice

Next, we tested the effects of TRAIL alone and TRAIL/cisplatin on the establishment of new tumors. We injected nude mice with A549 cells, and 24 h later, the mice were treated with vehicle control (PBS for 10 days), TRAIL (15 mg/kg per day for 10 days, on days 2–12), cisplatin



(1.5 mg/kg per day for 10 days, on days 2–7 and 15–19), and the combination of TRAIL (15 mg/kg per day for 10 days, on days 2–12) and cisplatin (1.5 mg/kg per day for 10 days, on days 2–7 and 15–19). The concentration and schedule of cisplatin were determined from previous reports [22, 23]. TRAIL significantly decreased the incidence of tumors. There were only three tumor-burdened mice out of nine mice in the TRAIL-treated group and the TRAIL/cisplatin-treated group. In contrast, there were eight tumor-burdened mice out of nine mice in the control group and nine tumor-burdened mice out of nine mice in the cisplatin-treated group. Tumor establishment in TRAIL-treated and TRAIL/cisplatin-treated mice was also postponed for 15 days in comparison with the control group (Fig. 6 a). Tumor inhibition on day 65 following treatment with TRAIL alone (15 mg/kg, daily for 10 days) and TRAIL/cisplatin (15 mg/kg TRAIL, daily for 10 days; 1.5 mg/kg cisplatin, daily for 10 days with intervals of 7 days) were 28.3% and 76.8% by tumor weight, 38.3% and 86% by tumor volume. The antitumor activities of the different treatments were significantly different by the rank sum test ($P < 0.05$ for TRAIL alone and TRAIL/cisplatin versus control; Fig. 6b,c). The synergistic effects on the inhibition of tumor growth for TRAIL/cisplatin versus TRAIL alone are also shown in Fig. 6b,c ($P < 0.05$).

Discussion

TRAIL is a member of the TNF family. Despite the ability of TNF and FasL to induce apoptosis in many cancer cells, the application of these death ligands to cancer therapy has been restricted by their severe toxicity to normal tissues [24, 25]. In contrast, the toxicity of TRAIL appears to be much less, while it is as efficient as CD95L and TNF in killing tumor cells. Therefore, TRAIL seems more suitable for therapeutic strategies directed against tumor cells than CD95L and TNF. The safety of TRAIL in nonhuman primates has been proved [5, 17]. TRAIL is an ideal potential antitumor

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Fig. 6a–c Effects of TRAIL and TRAIL/cisplatin on the establishment and inhibition of tumor in A549-implanted nude mice (nine mice per group). Nude mice were injected subcutaneously with A549 cells, and 24 h later they were injected intraperitoneally with vehicle control (PBS for 10 days), TRAIL (15 mg/kg per day for 10 days), cisplatin (1.5 mg/kg per day for 10 days with a 7-day interval) or TRAIL (15 mg/kg per day for 10 days) plus cisplatin (1.5 mg/kg per day for 10 days with a 7-day interval). **a** Tumor incidences over a period of 65 days: TRAIL alone (*filled squares*), TRAIL/cisplatin (*filled triangles*), cisplatin alone (*crosses*) and control (*filled diamonds*). **b** Tumor weights (means ± SD) on day 65 after inoculation (rank sum test): $*P < 0.05$ for TRAIL alone versus control; $**P < 0.05$ for TRAIL/cisplatin versus control, cisplatin alone and TRAIL alone. **c** Tumor volumes (means ± SD) on day 65 after inoculation (rank sum test): $*P < 0.05$ for TRAIL alone versus control; $**P < 0.05$ for TRAIL in combination with cisplatin versus control, cisplatin alone and TRAIL alone

therapy agent; it is cytotoxic to a broad spectrum of cancer cells but not to normal cells [7, 8, 16, 17]. To investigate the antitumor activity of TRAIL, we systematically evaluated the cytotoxicity of TRAIL alone and TRAIL combined with chemotherapy agents in A549 cell in vitro, and for the fist time report the antitumor activity of TRAIL alone and TRAIL/cisplatin in A549-implanted nude mice.

Treatment of NSCLC is not effective in the clinic. In this study, we explored the cytotoxicity of recombinant human TRAIL against A549 cells in vitro and in vivo. The present studies provide evidence that TRAIL combined with cisplatin, Taxol or actinomycin D augmented the cytotoxicity in A549 cells in vitro. Our results are not in agreement with those of an earlier study by Frese et al. in which no synergistic effects were observed in A549 cells treated with TRAIL (100 ng/ml) combined with paclitaxel (8.45 and 85.4 mg/ml) or cisplatin (3 and 30 μg/ml) [26]. We found that TRAIL in combination with cisplatin (5.56 μg/ml) or Taxol (10 and 30 μg/ml) acted synergistically in A549 cell lines (Fig. 4). The synergistic effects of TRAIL in combination with paclitaxel or cisplatin were obtained with subcytotoxic concentrations of TRAIL and chemotherapeutic agents. If the concentrations of chemotherapeutic agents are too high or too low, the synergistic cytotoxic effects cannot be achieved. The combinations of TRAIL with cisplatin, Taxol or actinomycin D increased cell apoptosis synergistically at subcytotoxic concentrations of chemotherapeutic agents [27–29]. The synergisms of TRAIL in combination with chemotherapeutic agents also provide the theoretical basis for decreasing the dose of chemotherapeutic agents and cytotoxicity in the clinic.

Previously, it has been shown that chemotherapeutic agents and ionizing radiation can enhance TRAIL-induced cytotoxicity [16, 28, 30, 31]. Such synergistic effects of combined treatment with TRAIL and chemotherapeutic agents or ionizing radiation primarily result from an increase in the expression of TRAIL death receptors. Different degrees of alteration in the expression of death receptors and decoy receptors induced by different chemotherapeutic agents have been reported in various cancer cell lines [31–33], but there are no reports about the expression levels of DR4, DR5, DcR1 and DcR2 in A549 cells treated with TRAIL, cisplatin, actinomycin D or Taxol. In this study, to demonstrate the mechanism of the synergistic effects of TRAIL with cisplatin, actinomycin D or Taxol, we investigated the expression levels of DR4, DR5, DcR1 and DcR2, and found no significant changes in the expression levels of DR4 and DR5. The expression levels of DcR2 were slightly downregulated, but the expression of DcR1 was not detected in A549 cells treated with TRAIL and cisplatin, actinomycin D or Taxol. These findings indicate that intracellular apoptosis-inducing factors might play an important role in the modulation of the apoptotic response, rather than the expression levels of the receptors in A549 cells. In further investigations, we will explore the expression of intracellular protein regulation elements and with the aim of demonstrating the mechanism of the synergistic apoptotic activity in response to TRAIL and chemotherapeutic agents.

Previous studies [16, 17, 34] have demonstrated that TRAIL can inhibit the growth of breast, colon and glioma cancers in nude mice. The antitumor activities of TRAIL plus paclitaxel, etoposide, camptothecin, doxorubicin, CDDP, 5-FU or CPT-11 have been evaluated in nude mice implanted with LNCaP-derived C4-2, U87MG, COLO 205, HCT116 and MCF-7 [16, 28, 31, 35–37]. However, there are no reports about TRAIL alone or in combination with cisplatin inhibiting the establishment and growth of lung cancer in A549-implanted nude mice so far. To assess the in vivo activities of TRAIL alone and in combination with chemotherapy drugs in lung cancer cell lines, we experimented for the first time on the A549-xenografted nude mice. Our studies showed that TRAIL alone inhibited the growth and establishment of tumor, and a synergistic antitumor effect was observed in TRAIL/cisplatin-treated mice. The tumor incidences in mice treated with TRAIL alone and TRAIL/cisplatin were low as compared with those in control-treated and cisplatin-treated mice. The establishment of tumors in mice treated with TRAIL alone and TRAIL/cisplatin were also postponed for 15 days as compared with the control group (Fig. 6 a). Although the tumor incidence in mice treated with TRAIL/cisplatin was the same as that in mice treated with TRAIL alone, the tumor volume of mice treated with TRAIL/cisplatin was smaller than in mice treated with TRAIL alone, and the tumor weight of mice treated with TRAIL/cisplatin was lighter than that in mice treated with TRAIL alone (Fig. 6 b,c). We chose the doses of TRAIL based on our previous in vivo data showing dose-dependent effects of TRAIL alone (data not shown). The doses of cisplatin in the experiments investigating the synergistic effects were those used by Jean Marie and Shyamal, and are low in comparison with clinical doses [23].

In conclusion, we have generated a potently active recombinant soluble human TRAIL molecule. In vitro, soluble TRAIL exerted cytotoxic effects and exhibited synergistic effects in combination with chemotherapeutic agents cisplatin, actinomycin D and Taxol in A549 cells. In vivo, TRAIL showed antitumor activity in human lung cancer cell A549 xenografts, and exhibited synergistic activity in combination with cisplatin. Thus, TRAIL may be a useful new tool to fight lung cancer cells in the clinic, leaving normal cells unharmed.

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