

Tumor necrosis factor-related apoptosis-inducing ligand additive with Iodine-131 of inhibits non-small cell lung cancer cells through promoting apoptosis

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Abstract. Non-small-cell lung cancer (NSCLC) accounts for ~80% of human lung cancer cases and is the most common cause of cancer-associated mortality worldwide. Reports have indicated that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Iodine-131 (I-131) can induce tumor cell apoptosis. The purpose of the present study was to investigate the additive efficacy of TRAIL and I-131 on NSCLC cells. The present study demonstrated that additive treatment of TRAIL and I-131 (TRAIL-I-131) significantly inhibited the growth and aggressiveness of NSCLC cells compared with single TRAIL or I-131 treatment. Results demonstrated that TRAIL-I-131 treatment induced apoptosis of NSCLC cells, with western blot analysis confirming that TRAIL-I-131 treatment increased proapoptotic Bad and Bax expression levels, while antiapoptotic Bcl-2 and Bcl-w protein levels were decreased in NSCLC cells. The present study demonstrated that TRAIL-I-131 treatment inhibited vascular endothelial growth factor (VEGF) and activator protein-1 (AP-1) in NSCLC cells. Potential mechanism analyses identified that TRAIL-I-131 treatment induced apoptosis of NSCLC cells through caspase-9 activation. *In vivo* assays revealed that TRAIL-I-131 treatment significantly inhibited NSCLC tumor growth and increased apoptotic bodies in tumor tissues. Immunohistology demonstrated that caspase-9 was upregulated and VEGF was downregulated in tumor tissues in TRAIL-I-131-treated tumors. In conclusion, these results indicate that TRAIL combined with I-131 promoted apoptosis of NSCLC through caspase-9 activation, which may be a promising anticancer therapeutic schedule for the treatment of NSCLC.

Introduction

Lung cancer is a major public health problem and presents the leading cause of cancer-associated mortality worldwide since 2007 (1,2). Oncology studies have indicated that lung cancer is divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which have been demonstrated to comprise ~85 and ~15% of lung cancer cases respectively (3). A systematic review has indicated that radiotherapy with curative intent is a favorable treatment option in patients with early-stage NSCLC (4). At present, a number of clinical therapeutic methods have been explored and applied for the treatments of patients with NSCLC, including radiotherapy, chemotherapy, Chinese medicinal herb treatment, immunotherapy, genetic and targeted therapies (5,6). However, the overall 5-year survival has remained poor, at <15% in patients with NSCLC in developing countries since 2008 (7-9).

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a potential anticancer protein that strongly lyses various human tumors cells by inducing apoptosis (10). Analysis of mechanisms identified that TRAIL can induce apoptosis of tumor cells by binding with TRAIL receptors, which further induces caspase- or mitochondrial-dependent apoptosis (11). Studies have demonstrated that TRAIL exerts anticancer effects by inhibiting NSCLC cell aggressiveness and promoting NSCLC cell apoptosis (12,13). Further study has indicated that TRAIL can lead to augmentation of paclitaxel-induced apoptosis in human NSCLC cells by upregulation of Bcl-2-associated death protein (Bad) and Bcl-2-associated X protein (Bax) expression and downregulation of Bcl-2 and Bcl-w expression (14). In addition, TRAIL therapy enhances sensitization to death receptor-mediated apoptosis by the proteasome inhibitor bortezomib in NSCLC cells, and preclinical data has indicated that a combination therapy of TRAIL and bortezomib may be an effective strategy for treatment of NSCLC (15). Furthermore, combination of TRAIL and actinomycin D liposomes has been identified to significantly promote anti-tumor effects in NSCLC cells (16). Additionally, TRAIL downregulates fibronectin (FN), Vimentin (VN) and E-cadherin (EN) expression, which further leads to growth inhibition of tumor cells (17). These

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studies suggest that TRAIL may be a potential anti-cancer agent for the treatment of human NSCLC.

Iodine-131 (I-131) therapy has been accepted for the treatment of thyroid cancer and other carcinomas as the decay of I-131 emits β rays that further destroy tumor cells (18-20). The efficacy of I-131 has been widely investigated to establish the side effects for patients with cancer (21,22). A pivotal study demonstrated that I-131-labeled chimeric tumor necrosis radioimmunotherapy achieved an objective response rate (ORR) of 34.6% (complete response, 3.7%; partial response, 30.8%; no change, 55.1%; and progressive disease, 10.3%) in all patients and 33% in 97 NSCLC patients (23). The efficacies of Iodine-131 in theranostic action or ionizing effect on the cells mimicking the role of radiotherapy have been investigated in clinical studies (24,25). Notably, the *in vitro* and *in vivo* targeting properties of iodine-123- or I-131-labeled monoclonal antibody 14C5 provided a novel antibody-based agent for radioimmunodetection and radioimmunotherapy of patients bearing antigen 14C5-expressing NSCLC tumors (26). Additionally, studies have indicated that TRAIL induced apoptosis of tumor cells through inhibition of activator protein-1 (AP-1) and vascular endothelial growth factor (VEGF) expression (27,28). However, the sole use of I-131 is not sufficient for the treatment of NSCLC.

Based on the efficacy of TRAIL and I-131 for inhibition of NSCLC cells, we hypothesized that a combination of TRAIL and I-131 would generate an additive inhibition of NSCLC cells. In the present study, the efficacy of additive treatment of TRAIL and I-131 on NSCLC cells was investigated *in vitro* and *in vivo*, the inhibitory effects of additive treatment of TRAIL and I-131 on growth and aggressiveness in NSCLC cells were also analyzed. The numbers of apoptotic cells were examined and caspase-9 activation was also investigated in NSCLC tissue and cells. The present study presents evidence that the combination of TRAIL and I-131 significantly inhibits the growth and aggressiveness of NSCLC cells through upregulation of apoptosis, and the apoptosis of NSCLC cells may be markedly promoted by additive management of TRAIL and I-131.

Materials and methods

Ethics statement. The experiments were implemented according to the Guidelines for the Care and Use of Laboratory Animals of the Animal Protection Institute of China. This study was also approved by the Ethics Committee of the Central Hospital of Zibo (Zibo, Shandong, P.R. China).

Cells and reagents. The NSCLC cell lines A549 and H358 were purchased from American Type Culture Collection. All tumor cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.). All cells were cultured in a 37°C humidified atmosphere of 5% CO₂.

MTT assay. A549 and H358 cells (1x10³/well) were cultured for 12 h at 37°C and then incubated with I-131 (10 mg/ml) or/and TRAIL (10 mg/ml) in 96-well plates for 24, 48 and

72 h in triplicate at 37°C. Following incubation, 20 μ l of MTT (5 mg/ml) in PBS solution was added to each well and was further incubated for 4 h at 37°C. The medium was then removed and 100 μ l DMSO was added into the wells to solubilize the crystals. The optical density (OD) was measured by a microplate reader (BD Biosciences, Franklin Lakes, NJ, USA) at a wavelength of 570 nm.

Cells invasion and migration assays. A549 and H358 cells were incubated with I-131 (10 mg/ml) or/and TRAIL (10 mg/ml), with non-treated cells as control. Cultured A549 or H358 cells were suspended at a density of 1x10⁶ in 100 μ l in serum-free DMEM for 24 h using a Transwell insert (BD Biosciences, Franklin Lakes, NJ, USA) instead of a Matrigel invasion chamber to assess migration. DMEM medium with 5% PBS were placed in the lower chamber of the BD BioCoat Matrigel (BD Biosciences). Cells were then added to the upper chambers of BD BioCoat Matrigel Migration or invasion Chambers (BD Biosciences) according to the manufacturer's protocol. A549 and H358 cell migration and invasion were stained with 1% crystal violet (Beyotime, Shanghai, China) for 15 min at 37°C. At least three randomly selected fields from each sample were examined using light a microscope at x40, magnification. The extent of migration and invasion of A549 and H358 cells was mapped and quantified the results analysis.

Apoptosis assay. A549 and H358 cells were grown at 37°C with 5% CO₂ until they reached 90% confluence. The apoptosis rate of tumor cells was assessed by incubating the cells with I-131 (10 mg/ml) or/and TRAIL (10 mg/ml) for 48 h. After incubation, the tumor cells were trypsinized and collected. The cells were then washed in cold PBS, adjusted to 1x10⁶ cells/ml with PBS, labeled with Annexin V-FITC and propidium iodide (Annexin V-FITC Kit; BD Biosciences), and analyzed with a FACScan flow cytometer (BD Biosciences). The treatments were performed in triplicate, and the percentage of labeled cells undergoing apoptosis in each group was determined and calculated using Expo32-ADC software (version 1.2B; Beckman Coulter Inc., Brea, CA, USA).

Reverse-transcription quantitative polymerase chain reaction (RT-qPCR). Total RNA from A549 and H358 cells was extracted using RNAzol (Sigma-Aldrich; Merck KGaA), and DNase RNase-free was adopted to digest total RNA at 37°C for 15 min. An RNeasy kit was used to purify RNA and its concentration was adjusted to 1 μ g/ μ l. The RNA (2 μ g) was used as the template to synthesize cDNA by reacting with reverse transcriptase at 37°C for 120 min, at 99°C for 4 min, and at 4°C for 3 min using a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc.). Subsequently, qPCR was adopted to amplify the gene expression of FN, VN and EN (Table I) to determine the transcription level of mRNA, and β -actin was used as the internal control group. Agarose electrophoresis with 1% ethidium bromide was adopted to check the PCR amplified products. Relative mRNA expression changes were calculated by the 2^{- $\Delta\Delta$ C_q} method (29). The results are expressed as fold expression compared with β -actin.

Western blotting. A549 and H358 cells were then incubated with I-131 (10 mg/ml) or/and TRAIL (10 mg/ml) for 48 h. Cells

Table I. Sequences of primers used in the present study.

Gene name	Sequence
FN	Reverse: 5'-CGTTGCCCTATCTCCGTCTC-3' Forward: 5'-GGGAGCAATCGGGTAATTTTCC-3'
VN	Forward: 5'-ATAACATCAAGCCCAAATCTGC-3' Reverse: 5'-TTCCTTTTTTCTTTCCCAACA-3'
EN	Forward: 5'-GCCAATCCTGATGAAATTGGAA-3' Reverse: 5'-CAGAACCACTGCCCTCGTAATC-3'
β -actin	Reverse: 5'-CGGAGTCAACGGATTTGGTC-3' Forward: 5'-AGCCTTCTCCATGGTCGTGA-3'

FN, fibronectin; VN, vimentin; EN, E-cadherin.

were collected and lysed in RIPA buffer (M-PER reagent for the cells and T-PER reagent for the tissues; Thermo Fisher Scientific Inc.) followed homogenized at 4°C for 10 min. Protein concentration was measured using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.). A total of 20 μ g protein extracts was electrophoresed on 12.5% polyacrylamide gradient gels and then transferred to nitrocellulose membranes. The membranes were incubated in blocking buffer comprising 5% bovine serum albumin (BSA; Sigma-Aldrich; Merck KGaA) for 2 h at 37°C prior to incubation with primary antibodies at 4°C overnight. The primary rabbit anti-mouse antibodies used in the immunoblotting assays were: Bcl-2 (dilution 1:200, ab3214), Bcl-w (dilution 1:1,000, ab32370), Bax (dilution 1:500; ab32503), Bad (dilution 1:500; ab32445), FN (dilution 1:500; ab2413), VN (dilution 1:500; ab8978), EN (dilution 1:1,000; ab76055), VEGF (dilution 1:500; ab72278), AP-1 (dilution 1:500; ab207196) and β -actin (dilution 1:500; ab8226) (all from Abcam, Cambridge, UK). Horseradish peroxidase-conjugated anti-rabbit IgG (cat no. 5204-2504; Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used at a 1:5,000 dilution and detected using a western blotting Luminol Reagent (cat no. 32106; Thermo Fisher Scientific, Inc.). Experiments were repeated at least three times. Densitometric quantification of the immunoblot data was performed by using Quantity-One software (version 1.5; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Animal study. A total of 80 specific pathogen-free male Balb/c mice (8-weeks old; body weight, 32-38 g) were purchased from Slack Life Co., Ltd (Shanghai, China). All mice were housed in a temperature-controlled facility at 23 \pm 1°C and relative humidity of 50 \pm 5% with a 12 h light/dark cycle and free access to food and water. Experimental mice were injected with A549 or H358 tumor cells (1x10⁶ cells) into subcutaneous tissue and were divided into four groups: PBS, I-131, TRAIL and combination (I-131+TRAIL) (n=10 in each group). Mice were treated with of I-131 (10 mg/kg) or/and TRAIL (10 mg/kg) once a day, a total of 10 times. Treatments were initiated on day 3 after tumor implantation (diameter, 5-6 mm). The tumor volumes in each group were recorded from three randomly selected experimental mice. The tumor volumes were calculated

according to a previous study (30). Mice were sacrificed when tumor diameter reached 16 mm.

Immunohistochemistry. NSCLC tumors from xenograft mice were fixed in formaldehyde (10%) for 12 h at 4°C followed with embedding in paraffin. Tumor tissues were fabricated to 4 μ m-thick sections. Tissues were deparaffinized in 100% xylene for 30 min at 37°C and rehydrated in a graded alcohol series (70, 80, 90, 95 and 100%). Antigen retrieval was performed using Lab Vision™ Tris-HCl buffer for heat-induced epitope retrieval (cat no. AP-9005-050; Thermo Fisher Scientific, Inc.) and tumor sections were blocked with 5% BSA for 2 h at 37°C, then incubated with primary antibodies targeting caspase-9 (1: 1,200, ab32539; Abcam) for 12 h at 4°C. Subsequently, horseradish peroxidase-conjugated anti-rabbit IgG (1:2,000; cat. no., sc-362260; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were applied for specimens for 12 h at 4°C. A Ventana Benchmark automated staining system was used for observation of caspase-9 expression. The slides were examined with a Keyence Biozero BZ8100E fluorescence microscope (Keyence, Osaka, Japan) at a magnification of x40.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. Apoptotic cells in tumor specimens were analyzed using a TUNEL assay (DeadEnd™ Colorimetric TUNEL System; Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. Tumor sections were fixed with 4% PFA for 1 h at room temperature and then incubated with the reaction mixture (terminal deoxynucleotidyl transferase, equilibration buffer and biotinylated nucleotide mix) for 1 h at 37°C. Subsequently, Streptavidin and DAB-bound biotin were applied and the specimens were counterstained with hemalaun (Merck Millipore, Darmstadt, Germany) and mounted in aquatex (Merck Millipore) for 30 min at 37°C. DNA fragmentation was detected by selecting three random fields in the tumor sections (x40 magnification). The tissue sections were captured using a Keyence Biozero BZ8100E fluorescence microscope at a magnification of x40.

Statistical analysis. All data are expressed as the mean and standard deviation of triplicate dependent experiments and analyzed using one-way ANOVA (with Tukey HSD test). All data were analyzed using SPSS Statistics 19.0 (IBM Corp., Armonk, NY, USA) and Graphpad Prism version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Additive treatment of TRAIL and I-131 inhibits growth and aggressiveness of NSCLC cells. To evaluate the inhibitory effects of TRAIL-I-131 on NSCLC cell growth and aggressiveness, NSCLC cells were investigated following treatment with TRAIL or I-131. As shown in Fig. 1A, TRAIL-I-131 treatment significantly inhibited the growth of NSCLC cells compared with TRAIL or I-131 treatment. Fig. 1B and C revealed that TRAIL-I-131 treatment could achieve the maximum inhibitory effect against A549 and H358 cells after 48 h incubation. Migration and invasion assays demonstrated that TRAIL-I-131 treatment suppressed the migration and invasion of A549 and

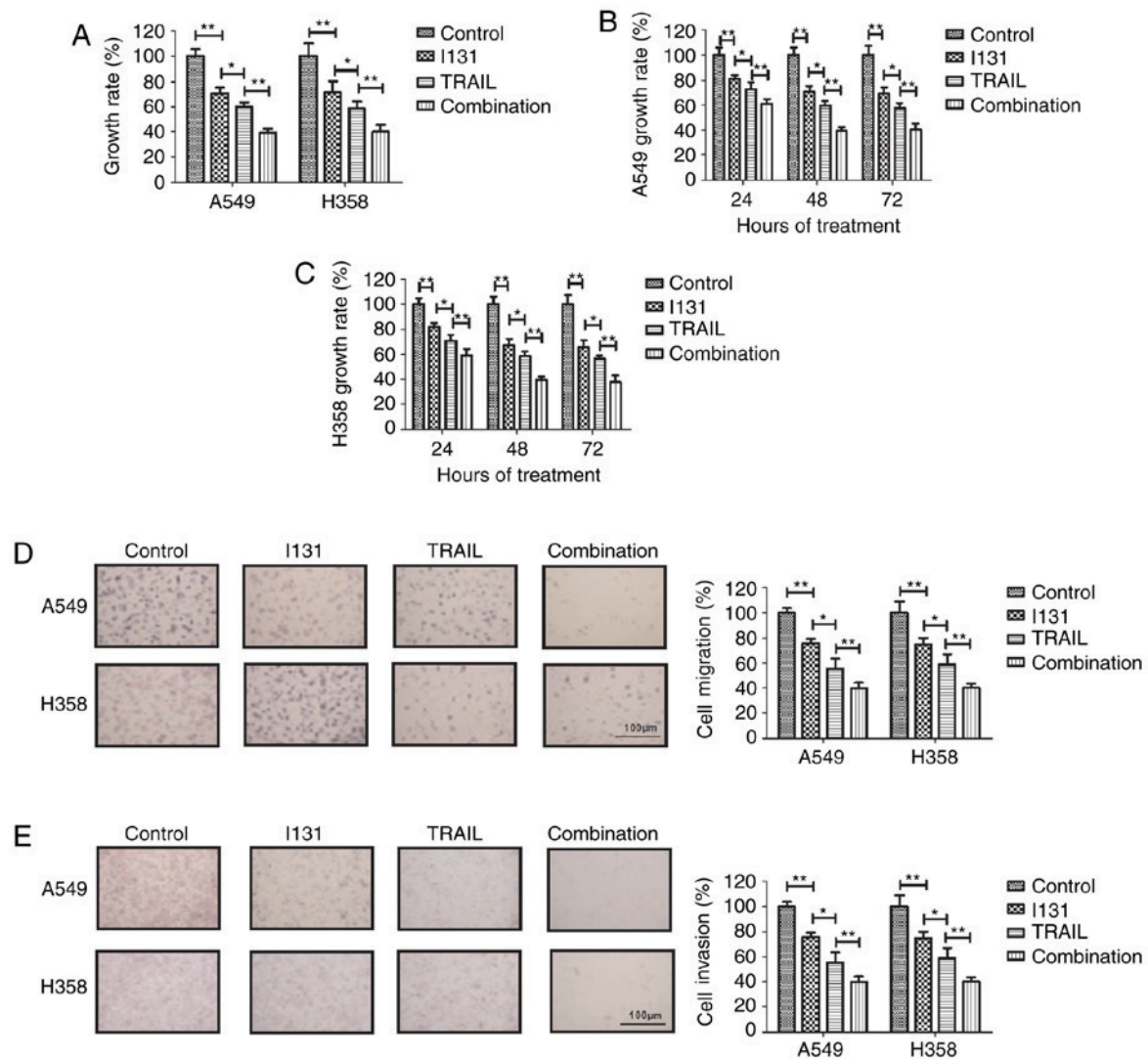


Figure 1. Additive treatment of TRAIL and I-131 inhibits the growth and aggressiveness of NSCLC cells. (A) Additive treatment of TRAIL and I-131 inhibited the growth of A549 and H358 cells. (B and C) Additive treatment of TRAIL and I-131 achieved the maximum inhibitory effect for (B) A549 and (C) H358 cells after 48 h incubation. (D and E) Additive treatment of TRAIL and I-131 inhibited (D) migration and (E) invasion of A549 and H358 cells. All data are expressed as the mean and standard deviation, and were analyzed using one-way analysis of variance with Tukey HSD test. *P<0.05; **P<0.01; TRAIL vs. I-131, I-131 vs. control, combination vs. TRAIL. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; I-131, Iodine-131.

H358 cells *in vitro* (Fig. 1D and E). These assays confirmed that the TRAIL was more efficient in inhibiting NSCLC cell growth and aggressiveness compared with I-131. Taken together, these results suggest that TRAIL-I-131 additive treatment can significantly inhibit the growth and aggressiveness of NSCLC cells.

Additive treatment of TRAIL and I-131 (TRAIL-I-131) promotes apoptosis of NSCLC cells. In order to investigate the role of TRAIL-I-131 in NSCLC cells, the efficacy of TRAIL-I-131 treatment in inducing apoptosis of NSCLC cells was analyzed. The results demonstrated that TRAIL-I-131 treatment markedly promoted apoptosis of A549 and H358 cells compared with either TRAIL or I-131 treatment (Fig. 2A). Western blot analysis demonstrated that TRAIL-I-131 treatment significantly decreased the levels of the anti-apoptotic proteins Bcl-2 and Bcl-w in A549 and H358 cells, while pro-apoptosis Bad and Bax protein levels were increased by TRAIL-I-131 treatment compared with either TRAIL or I-131 treatment

(Fig. 2B). TRAIL-I-131 treatment was also demonstrated to increase caspase-8 and caspase-9 activation in A549 and H358 cells compared with the TRAIL, I-131 and control groups (Fig. 2C and D). The results also demonstrated TRAIL-I-131 treatment inhibited VEGF and AP-1 expression in A549 and H358 cells (Fig. 2E). TRAIL-treated A549 cells exhibited lower Bcl-2 and Bcl-w expression than I-131-treated A549 cells, and TRAIL and I-131 had similar effects on Bad and Bax expression in A549 and H358 cells. These results suggest that TRAIL-I-131 additive treatment can markedly promote apoptosis of NSCLC cells by regulating apoptosis-associated gene expression.

Additive treatment of TRAIL and I-131 downregulates metastasis-associated gene expression levels. Metastasis-associated gene and protein expression levels were subsequently analyzed to understand the inhibitory effects of TRAIL-I-131 on NSCLC cells. RT-qPCR demonstrated that FN, VN and EN expression levels were downregulated by TRAIL-I-131 treatment in A549 and H358 cells (Fig. 3A and B). No statistically

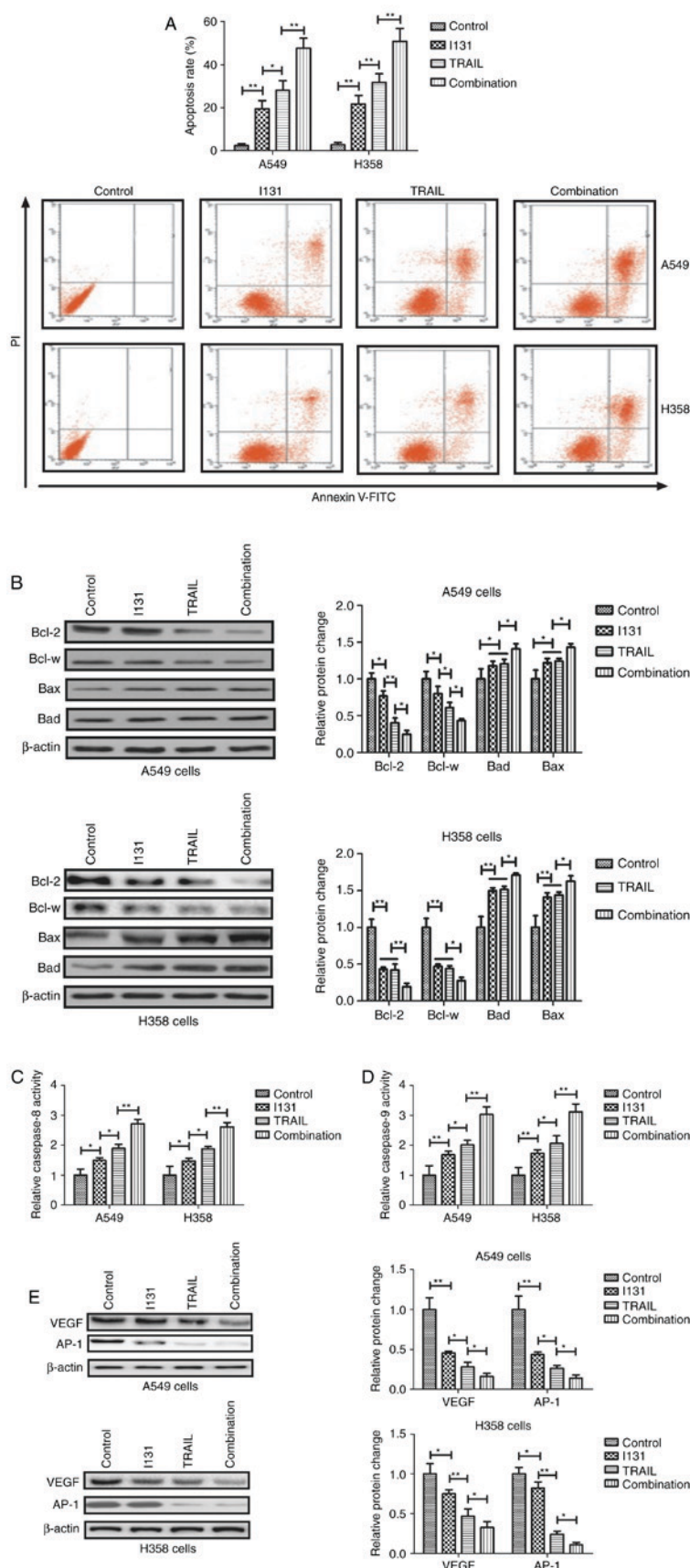


Figure 2. Additive treatment of TRAIL and I-131 promotes apoptosis of NSCLC cells. (A) TRAIL-I-131 treatment promoted the apoptosis of A549 and H358 cells compared with either TRAIL, I-131 or control treatment. (B) TRAIL-I-131 treatment increased proapoptotic Bad and Bax protein levels, and decreased antiapoptotic Bcl-2 and Bcl-w protein levels in A549 and H358 cells. (C and D) TRAIL-I-131 treatment increased (C) caspase-8 and (D) caspase-9 activation in A549 and H358 cells. (E) TRAIL-I-131 treatment downregulated VEGF and AP-1 expression levels in A549 and H358 cells. All data are expressed as the mean and standard deviation, and were analyzed using one-way analysis of variance with Tukey HSD test. * $P < 0.05$, TRAIL vs. I-131; ** $P < 0.01$, I-131 vs. control, combination vs. TRAIL. TRAIL, tumor necrosis factor related apoptosis-inducing ligand; I-131, Iodine-131; PI, propidium iodide; FITC, fluorescein isothiocyanate; VEGF, vascular endothelial growth factor; AP-1, activator protein-1.

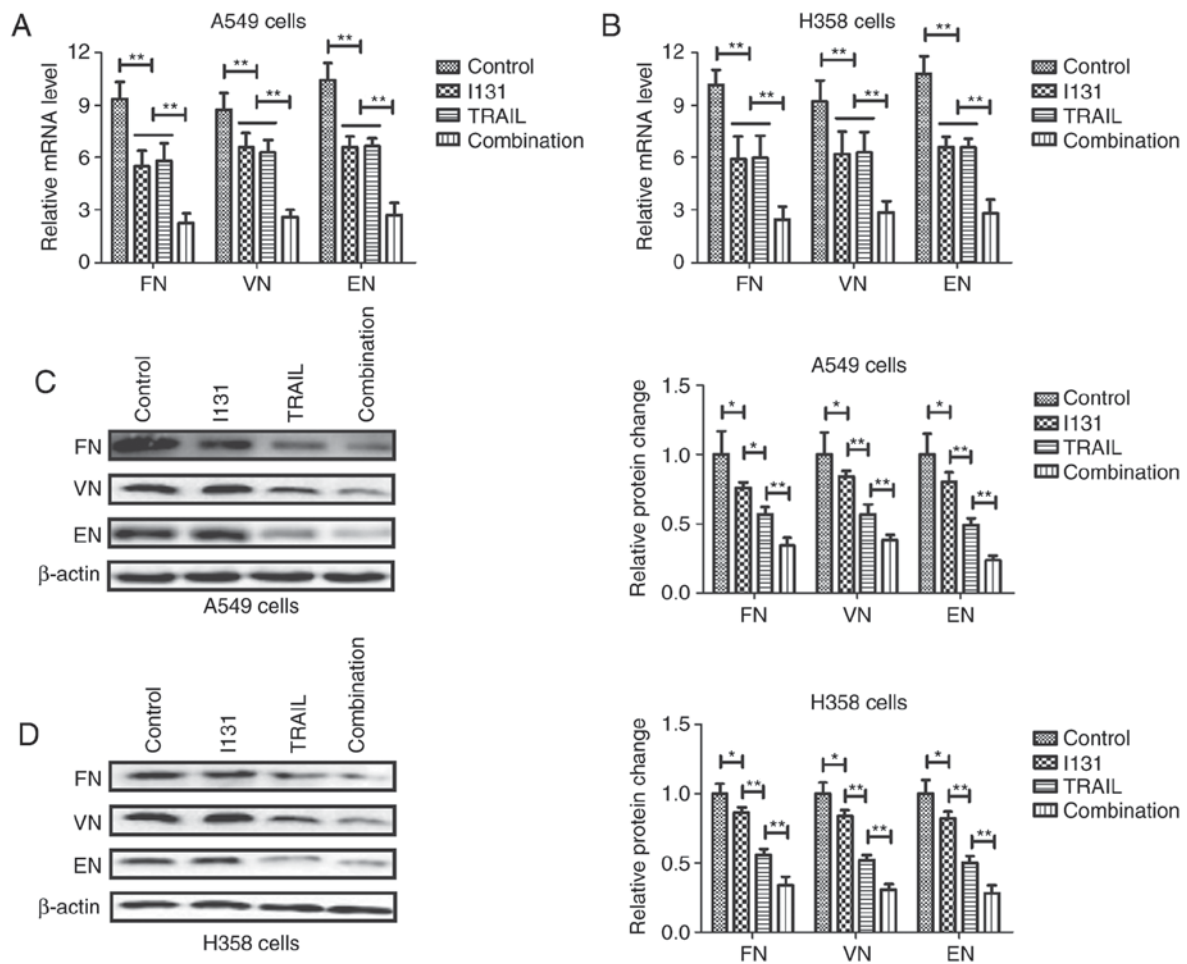


Figure 3. Additive treatment of TRAIL and I-131 downregulates metastasis-associated gene expression levels. (A and B) TRAIL-I-131 treatment downregulated FN, VN and EN mRNA expression levels in (A) A549 and (B) H358 cells. TRAIL-I-131 treatment downregulated FN, VN and EN protein expression levels in (C) A549 and (D) H358 cells. All data are expressed as the mean and standard deviation, and were analyzed using one-way analysis of variance with Tukey HSD test. *P<0.05; **P<0.01; I-131 vs. control, TRAIL vs. I-131, combination vs. I-131 or TRAIL. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; I-131, Iodine-131; FN, fibronectin; VN, vimentin; EN, E-cadherin.

significantly differences in miRNA levels were observed between the TRAIL and I-131 groups in A549 and H358 cells. Western blot analysis also demonstrated that TRAIL-I-131 treatment significantly decreased FN, VN and EN expression levels in A549 and H358 cells (Fig. 3C and D). TRAIL treatment significantly downregulated FN, VN and EN expression levels compared with the I-131 group in A549 and H358 cells, suggesting that TRAIL-I-131 additive treatment can decrease NSCLC metastasis-related FN, VN and EN expression levels in A549 and H358 cells.

Additive treatment of TRAIL and I-131 inhibits tumor growth and promotes apoptosis in tumor tissues. To further investigate the role of TRAIL-I-131, *in vivo* anti-cancer effects of TRAIL-I-131 were analyzed in NSCLC-bearing mice. As demonstrated in Fig. 4A and B, TRAIL-I-131 treatment was observed to inhibit tumor growth in A549- and H358-bearing mice compared with the TRAIL, I-131 and PBS groups. No significant difference was identified between the TRAIL and I-131 groups. A TUNEL assay demonstrated that TRAIL-I-131 treatment increased the number of apoptotic cells present in the tumor tissues examined (Fig. 4C). Immunohistochemistry demonstrated that caspase-9 expression was upregulated in

tumor tissue in TRAIL-I-131-treated tumors compared with the TRAIL, I-131 and PBS groups (Fig. 4D). These results demonstrate that TRAIL-treated tumors present a higher apoptosis rate and relative caspase-9 expression compared with the I-131 group in A549- and H358-bearing mice. Taken together, these results suggest that additive treatment of TRAIL and I-131 could inhibit tumor growth through increasing apoptosis of NSCLC cells.

Discussion

Systematic review and meta-analysis have indicated that drug-induced apoptosis contributes to the inhibition of tumor cell growth and aggressiveness (31). A previous study also identified the advantages of using TRAIL for human tumor cells by selectively inducing apoptosis by binding with death receptors in NSCLC cells (32). In addition, I131 radioimmunotherapy has presented more advantages in the treatment of human thyroid cancer due to its easy excretion and high accuracy (33). In the present study, the additive therapeutic effects of TRAIL and I-131 were investigated for NSCLC cells and an NSCLC-bearing mouse model, where TRAIL was demonstrated to possess increased efficiency compared

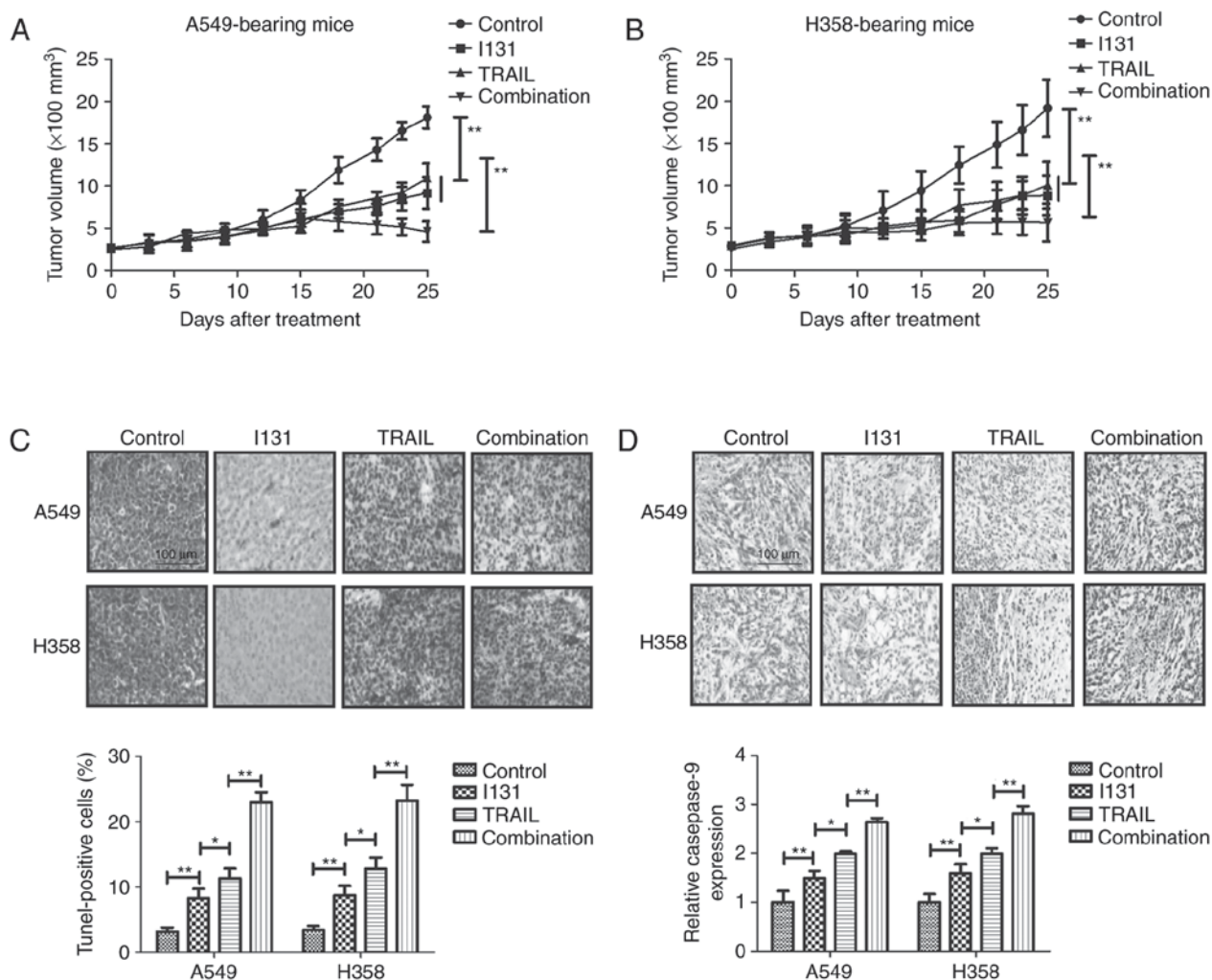


Figure 4. Additive treatment of TRAIL and I-131 inhibited tumor growth and promotes apoptosis of tumor cells. TRAIL-I-131 treatment inhibits tumor growth in (A) A549- and (B) H358-bearing mice compared with the TRAIL, I-131 and PBS groups. (C) TRAIL-I-131 treatment increased numbers of apoptotic cells in tumor tissues. (D) TRAIL-I-131 treatment upregulated caspase-9 expression in tumor tissues. All data are expressed as the mean and SD and analyzed using one-way ANOVA (with Tukey HSD test). * $P < 0.05$; ** $P < 0.01$; I-131 or TRAIL vs. control, combination vs. I-131 or TRAIL. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; I-131, Iodine-131.

with I-131 in the inhibition of NSCLC cell growth. However, the similar effects of TRAIL and I-131 on Bcl-2, Bcl-w, Bad, Bax, FN, VN and EN expression levels have been observed in NSCLC cells (14). These results may be associated with the different mechanisms of action of TRAIL and I-131 (34,35). The present study demonstrated that additive therapy of TRAIL and I-131 significantly inhibited NSCLC cell growth *in vitro* and *in vivo* through inducing apoptosis.

The therapeutic efficiency of I-131 in theranostic action has previously been identified in tumor therapy (36,37). In the present study, the inhibitory effects of I-131 on NSCLC cells *in vitro* and *in vivo* were analyzed, with results demonstrating that I-131 treatment inhibited the growth of NSCLC cells. The ionizing effects of I-131 target radiotherapy on cells, improving anticancer efficacy of vascular disruption treatment have been identified in rabbit VX2 tumor models (38). The results of the present study have indicated that I-131 decreased aggressiveness and induced apoptosis of NSCLC cells. However, the efficacy of single I-131 treatment was limited with unsatisfactory effects on tumor inhibition. In the present study, the results

obtained indicated that additive treatment of TRAIL and I-131 enhanced the inhibitory effects of TRAIL or I-131 for NSCLC cells. Kaewput and Pusuwan(39) previously reported that I-131 therapy may inhibit tumor growth and metastasis in a patient with papillary thyroid cancer and the results of the present study identified that I-131 promoted TRAIL-induced apoptosis and growth inhibition for NSCLC cells.

A previous study indicated that Bcl-2 mRNA expression may be regarded as a biomarker for patients with curatively resected NSCLC (40). The present study demonstrated that TRAIL-I-131 treatment decreased anti-apoptotic Bcl-2 and Bcl-w expression levels and increased pro-apoptotic Bad and Bax expression levels in A549 and H358 cells. The study of Checinska *et al* (41) suggested that caspase-9 may initiate a scaffold function and may serve as caspase substrate during NSCLC apoptosis. Additionally, therapy that targets VEGF has been demonstrated to be beneficial for the treatment of NSCLC (42,43). Furthermore, TRAIL-I-131 treatment markedly inhibited VEGF and AP-1 expression in A549 and H358 cells, which potentially contributed to the inhibitory efficacy of TRAIL-I-131 treatment for tumor

growth in the NSCLC-bearing mouse model. Furthermore, the sensitizing apoptotic effect of TRAIL could enhance the inhibitory effects for human lung cancer PC9 cells (44). Additionally, the study of Nazim *et al.* (45) ascertained that peroxisome proliferator-activated receptor- γ activation by troglitazone enhanced human lung cancer cell apoptosis induced by TRAIL. In the present study, an *in vivo* assay demonstrated that TRAIL-I-131 treatment increased the apoptotic rate and caspase-9 expression in tumor tissues compared with either TRAIL or I-131-treated tumors.

In conclusion, the results of the present study demonstrated that additive TRAIL and I-131 treatment effectively inhibited NSCLC cell growth and aggressiveness. These findings suggested that I-131 enhanced TRAIL-inhibited growth of NSCLC cells through the induction of apoptosis. Importantly, additive TRAIL and I-131 treatment demonstrated efficient tumor-suppressing effects on NSCLC tumor cells *in vitro* and *in vivo*, which suggests that additive TRAIL and I-131 treatment may be a promising anticancer therapeutic strategy for the treatment of NSCLC. However, this requires corroboration with further studies.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NY designed the present study; SY and DL performed all experiments and analyzed all data in the present study.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Central Hospital of Zibo (Zibo, Shandong, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Baker S, Dahele M, Lagerwaard FJ and Senan S: A critical review of recent developments in radiotherapy for non-small cell lung cancer. *Radiat Oncol* 11: 115, 2016.
- Jiang T, Zhai C, Su C, Ren S and Zhou C: The diagnostic value of circulating cell free DNA quantification in non-small cell lung cancer: A systematic review with meta-analysis. *Lung Cancer* 100: 63-70, 2016.
- Zhukovsky M, Varaksin A and Pakholkina O: Statistical analysis of observational study of the influence of radon and other risk factors on lung cancer incidence. *Radiat Prot Dosimetry* 160: 108-111, 2014.
- Falkson CB, Vella ET, Yu E, El-Mallah M, Mackenzie R, Ellis PM and Ung YC: Radiotherapy with curative intent in patients with Early-stage, medically inoperable, non-small-cell lung cancer: A systematic review. *Clin Lung Cancer* 18: 105-121.e5, 2017.
- Onesti CE, Iacono D, Angelini S, Lauro S, Mazzotta M, Occhipinti MA, Giusti R and Marchetti P: Unexpected long survival of brain oligometastatic non-small cell lung cancer (NSCLC) treated with multimodal treatment: A single-center experience and review of the literature. *Transl Lung Cancer Res* 5: 712-719, 2016.
- Cadranel J, Park K, Arrieta O, Pless M, Bendaly E, Patel D, Sasane M, Nosal A, Swallow E, Galebach P, *et al.*: Characteristics, treatment patterns, and survival among ALK+ non-small cell lung cancer (NSCLC) patients treated with crizotinib: A chart review study. *Lung Cancer* 98: 9-14, 2016.
- McKay C, Knight KA and Wright C: Beyond cancer treatment-a review of total lymphoid irradiation for heart and lung transplant recipients. *J Med Radiat Sci* 61: 202-209, 2014.
- Han RX, Liu X, Pan P, Jia YJ and Yu JC: Effectiveness and safety of chemotherapy combined with dendritic cells co-cultured with cytokine-induced killer cells in the treatment of advanced non-small-cell lung cancer: A systematic review and meta-analysis. *PLoS One* 9: e108958, 2014.
- Kuykendall A and Chiappori A: Advanced EGFR mutation-positive non-small-cell lung cancer: Case report, literature review, and treatment recommendations. *Cancer Control* 21: 67-73, 2014.
- Grosse-Wilde A, Voloshanenko O, Bailey SL, Longton GM, Schaefer U, Csernok AI, Schütz G, Greiner EF, Kemp CJ and Walczak H: TRAIL-R deficiency in mice enhances lymph node metastasis without affecting primary tumor development. *J Clin Invest* 118: 100-110, 2008.
- Dai X, Zhang J, Arfuso F, Chinnathambi A, Zayed ME, Alharbi SA, Kumar AP, Ahn KS and Sethi G: Targeting TNF-related apoptosis-inducing ligand (TRAIL) receptor by natural products as a potential therapeutic approach for cancer therapy. *Exp Biol Med* (Maywood) 240: 760-773, 2015.
- Yang F, Shi P, Xi X, Yi S, Li H, Sun Q and Sun M: Recombinant adenoviruses expressing TRAIL demonstrate antitumor effects on non-small cell lung cancer (NSCLC). *Med Oncol* 23: 191-204, 2006.
- Zhang X, Cheung RM, Komaki R, Fang B and Chang JY: Radiotherapy sensitization by tumor-specific TRAIL gene targeting improves survival of mice bearing human non-small cell lung cancer. *Clin Cancer Res* 11: 6657-6668, 2005.
- Odoux C, Albers A, Amoscato AA, Lotze MT and Wong MK: TRAIL, FasL and a blocking anti-DR5 antibody augment paclitaxel-induced apoptosis in human non-small-cell lung cancer. *Int J Cancer* 97: 458-465, 2002.
- Voortman J, Resende TP, Abou El Hassan MA, Giaccone G and Kruyt FA: TRAIL therapy in non-small cell lung cancer cells: Sensitization to death receptor-mediated apoptosis by proteasome inhibitor bortezomib. *Mol Cancer Ther* 6: 2103-2112, 2007.
- Guo L, Fan L, Ren J, Pang Z, Ren Y, Li J, Wen Z, Qian Y, Zhang L, Ma H and Jiang X: Combination of TRAIL and actinomycin D liposomes enhances antitumor effect in non-small cell lung cancer. *Int J Nanomedicine* 7: 1449-1460, 2012.
- Cho C, Horzempa C, Jones D and McKeown-Longo PJ: The fibronectin III-1 domain activates a PI3-Kinase/Akt signaling pathway leading to $\alpha v \beta 5$ integrin activation and TRAIL resistance in human lung cancer cells. *BMC Cancer* 16: 574, 2016.
- Kao PF, Chang HY, Tsai MF, Lin KJ, Tzen KY and Chang CN: Breast uptake of iodine-131 mimicking lung metastases in a thyroid cancer patient with a pituitary tumour. *Br J Radiol* 74: 378-381, 2001.
- Yu S, Feng F, Wang K, Men C, Lin C, Liu Q, Yang D and Gao Z: The therapeutic efficacy of I131-PSCA-mAb in orthotopic mouse models of prostate cancer. *Eur J Med Res* 18: 56, 2013.
- Schachter P, Shimonov M and Lorberboim M: Combined radio-iodine (I-131) treatment and radio-guided surgery for recurrent cervical well-differentiated thyroid cancer. *Harefuah* 144: 168-172, 232, 2005.
- van Ginkel RJ, Limburg PC, Piers DA, Koops HS and Hoekstra HJ: Value of continuous leakage monitoring with radioactive iodine-131-labeled human serum albumin during hyperthermic isolated limb perfusion with tumor necrosis factor-alpha and melphalan. *Ann Surg Oncol* 9: 355-363, 2002.

22. Dewaraja YK, Ljungberg M and Koral KF: Monte Carlo evaluation of object shape effects in iodine-131 SPET tumor activity quantification. *Eur J Nucl Med* 28: 900-906, 2001.
23. Chen S, Yu L, Jiang C, Zhao Y, Sun D, Li S, Liao G, Chen Y, Fu Q, Tao Q, *et al*: Pivotal study of iodine-131-labeled chimeric tumor necrosis treatment radioimmunotherapy in patients with advanced lung cancer. *J Clin Oncol* 23: 1538-1547, 2005.
24. Song JJ, Lin YS, Zhu L and Li F: Efficacy of iodine-131 in treating hyperthyroid heart disease. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 35: 166-170, 2013 (In Chinese).
25. Gultekin SS and Sahmaran T: The efficacy of patient-dependent practices on exposure rate in patients undergoing iodine-131 ablation. *Health Phys* 104: 454-458, 2013.
26. Burvenich I, Schoonooghe S, Cornelissen B, Blanckaert P, Coene E, Cuvelier C, Mertens N and Slegers G: In vitro and in vivo targeting properties of iodine-123- or iodine-131-labeled monoclonal antibody 14C5 in a non-small cell lung cancer and colon carcinoma model. *Clin Cancer Res* 11: 7288-7296, 2005.
27. Jin CY, Park C, Hong SH, Han MH, Jeong JW, Xu H, Liu H, Kim GY, Kim WJ, Yoo YH and Choi YH: Synergistic induction of TRAIL-mediated apoptosis by anisomycin in human hepatoma cells via the BH3-only protein Bid and c-Jun/AP-1 signaling pathway. *Biomed Pharmacother* 67: 321-328, 2013.
28. Na HJ, Hwang JY, Lee KS, Choi YK, Choe J, Kim JY, Moon HE, Kim KW, Koh GY, Lee H, *et al*: TRAIL negatively regulates VEGF-induced angiogenesis via caspase-8-mediated enzymatic and non-enzymatic functions. *Angiogenesis* 17: 179-194, 2014.
29. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
30. Bai FL, Yu YH, Tian H, Ren GP, Wang H, Zhou B, Han XH, Yu QZ and Li DS: Genetically engineered Newcastle disease virus expressing interleukin-2 and TNF-related apoptosis-inducing ligand for cancer therapy. *Cancer Biol Ther* 15: 1226-1238, 2014.
31. Shivapurkar N, Reddy J, Chaudhary PM and Gazdar AF: Apoptosis and lung cancer: A review. *J Cell Biochem* 88: 885-898, 2003.
32. Ouyang W, Yang C, Liu Y, Xiong J, Zhang J, Zhong Y, Zhang G, Zhou F, Zhou Y and Xie C: Redistribution of DR4 and DR5 in lipid rafts accounts for the sensitivity to TRAIL in NSCLC cells. *Int J Oncol* 39: 1577-1586, 2011.
33. Howard DM, Kearfott KJ, Wilderman SJ and Dewaraja YK: Comparison of I-131 radioimmunotherapy tumor dosimetry: Unit density sphere model versus patient-specific Monte Carlo calculations. *Cancer Biother Radiopharm* 26: 615-621, 2011.
34. De Miguel D, Gallego-Lleyda A, Ayuso JM, Erviti-Ardanaz S, Pazo-Cid R, del Agua C, Fernández LJ, Ochoa I, Anel A and Martínez-Lostao L: TRAIL-coated lipid-nanoparticles overcome resistance to soluble recombinant TRAIL in non-small cell lung cancer cells. *Nanotechnology* 27: 185101, 2016.
35. Zidan J, Hefer E, Iosilevski G, Drumea K, Stein ME, Kuten A and Israel O: Efficacy of I131 ablation therapy using different doses as determined by postoperative thyroid scan uptake in patients with differentiated thyroid cancer. *Int J Radiat Oncol Biol Phys* 59: 1330-1336, 2004.
36. Castellani MR, Aktolun C, Buzzoni R, Seregini E, Chiesa C, Maccauro M, Aliberti GL, Vellani C, Lorenzoni A and Bombardieri E: Iodine-131 metaiodobenzylguanidine (I-131 MIBG) diagnosis and therapy of pheochromocytoma and paraganglioma: Current problems, critical issues and presentation of a sample case. *Q J Nucl Med Mol Imaging* 57: 146-152, 2013.
37. Harisankar CN, Mittal BR, Bhattacharya A, Kashyap R and Bhansali A: Iodine-131 meta-iodobenzylguanidine single photon emission computed tomography/computerized tomography in diagnosis of neuro-endocrine tumors. *Indian J Nucl Med* 27: 55-58, 2012.
38. Shao H, Zhang J, Sun Z, Chen F, Dai X, Li Y, Ni Y and Xu K: Necrosis targeted radiotherapy with iodine-131-labeled hypericin to improve anticancer efficacy of vascular disrupting treatment in rabbit VX2 tumor models. *Oncotarget* 6: 14247-14259, 2015.
39. Kaewput C and Pusuwan P: Severe hyponatremia: A comorbidity with I131 therapy in a patient with papillary thyroid cancer. *J Med Assoc Thai* 97: 886-890, 2014.
40. Grimminger PP, Schneider PM, Metzger R, Vallböhrer D, Danenberg KD, Danenberg PV, Hölscher AH and Brabender J: The prognostic role of Bcl-2 mRNA expression in curatively resected non-small cell lung cancer (NSCLC). *Lung Cancer* 70: 82-87, 2010.
41. Checinska A, Giaccone G, Rodriguez JA, Kruyt FA and Jimenez CR: Comparative proteomics analysis of caspase-9-protein complexes in untreated and cytochrome c/dATP stimulated lysates of NSCLC cells. *J Proteomics* 72: 575-585, 2009.
42. Ballas MS and Chachoua A: Rationale for targeting VEGF, FGF, and PDGF for the treatment of NSCLC. *Onco Targets Ther* 4: 43-58, 2011.
43. Rades D, Setter C, Dunst J, Dahl O, Schild SE and Noack F: Prognostic impact of VEGF and VEGF receptor 1 (FLT1) expression in patients irradiated for stage II/III non-small cell lung cancer (NSCLC). *Strahlenther Onkol* 186: 307-314, 2010.
44. Li J, Wang Y, Liu L, Yuan Y and Bao Y: Thioridazine sensitizes apoptotic effect of TRAIL in human lung cancer PC9 cells through ER stress mediated upregulation of DR5. *Zhongguo Fei Ai Za Zhi* 20: 80-87, 2017 (In Chinese).
45. Nazim UM, Moon JH, Lee YJ, Seol JW and Park SY: PPAR γ activation by troglitazone enhances human lung cancer cells to TRAIL-induced apoptosis via autophagy flux. *Oncotarget* 8: 26819-26831, 2017.



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