Tumor necrosis factor-related apoptosis-inducing ligand inhibits the growth and aggressiveness of colon carcinoma via the exogenous apoptosis signaling pathway

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Abstract. Colon cancer is one of the most common types of gastrointestinal tumor. Previous studies have demonstrated that tumor necrosis factor-(TNF)-related apoptosis-inducing ligand (TRAIL) reduces the aggressiveness of colon cancer tumors and promotes the apoptosis of colon carcinoma cells. In the present study, the inhibitory effects of TRAIL were investigated and the potential mechanism of TRAIL-mediated apoptosis was explored in colon cancer cells. Reverse transcription-quantitative polymerase chain reaction, western blotting, immunofluorescence, immunohistochemistry, TUNEL and flow cytometry assays were used to analyze the effects of TRAIL on the growth, migration, invasion and apoptosis of colon tumor cells. *In vivo* experiments were performed in mice to analyze the therapeutic effects of TRAIL. The results demonstrated that TRAIL significantly suppressed the growth of colorectal tumor cells in a dose-dependent manner (0.5-2.5 mg/ml) and also promoted colon tumor cell death. The migration and invasion of colon tumor cells were inhibited by the downregulation of fibronectin, Vimentin and E-cadherin. The apoptotic rate revealed that TRAIL (2.0 mg/ml) significantly promoted the apoptosis of colon tumor cells by regulating apoptosis-related gene expression. TRAIL administration promoted the apoptosis of colon tumor cells via the exogenous apoptosis signaling pathway due to the upregulation of caspase-3, caspase-8 and nuclear factor-κB protein expression. *In vivo* assays revealed that TRAIL administration significantly inhibited tumor growth and promoted apoptotic body and lymphocyte infiltration, which led to increased survival in tumor-bearing mice compared with the control group. Immunohistochemistry revealed that P53 and B-cell lymphoma-2 were downregulated in TRAIL-treated tumors. In conclusion, TRAIL treatment significantly inhibited the growth and aggressiveness of colon tumors by inducing apoptosis via the exogenous apoptosis pathway, which suggests that TRAIL may be a potential anticancer agent for colon carcinoma therapy.

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Introduction

Colon cancer is the second most common cancer in women and the third most common in men worldwide (1). A recent systematic review by Gall *et al* (2) evaluated the treatments available for colon cancer and determined the accuracy of mini-probe endoscopic ultrasound in determining the clinical stage of colon cancer. The results suggested that screening, treatment options and prognoses for patients with colon cancer have improved over time (3). Although the systematic review included a number of targeted therapies for the treatment of advanced colorectal cancer and explored the potential of predictive biomarkers, at present there is no satisfactory therapy for colon cancer due to local disease migration and long distance metastasis (4,5). Metastasis and recurrence aggravates disease progression in patients with stage II and III colon cancer (6).

Colon cancer cell growth, metastasis and invasion are difficult to treat and increase the mortality of patients with colon cancer (7,8). Inhibiting apoptotic resistance and promoting apoptosis in colorectal cancer cells is an important part of cancer treatment, as well as the prevention of neoplasm metastasis (9,10). Previous researchers have developed targeted therapies, which suppress the underlying mechanisms of colorectal cancer cell metastasis and invasion (11-13). Advances in molecular bioinformatics have enabled scientists to screen for target molecules associated with diagnosis and therapy protocols, which suggests the potential of individual tailored treatments for patients with colorectal cancer and other chronic diseases (14,15).

Regulating apoptosis-associated protein expression is beneficial for the prevention and treatment of colon cancer as it may increase the apoptosis of tumor cells (16). Tumor necrosis factor-(TNF)-related apoptosis-inducing ligand (TRAIL) is a potential anticancer protein, which lyses various human tumor cells by inducing apoptosis (17). A previous study has demonstrated that TRAIL is safe for normal cells,

as it selectively induces apoptosis via binding with death receptors on tumor cells (18). Previous studies have demonstrated the inhibitory effects of TRAIL on tumor cells, which suggests that it is an effective oncolytic agent for the treatment of different types of human cancer (19-21). Similar results were demonstrated when binding with 'death-inducing' and 'decoy' receptors, while further activation of Fas-associated death domain or other proteins was observed in the caspase signaling pathway (22). In conclusion, these results suggest there is potential for the application of TRAIL in cancer therapy.

In the present study, the anticancer effects and mechanisms of TRAIL were examined in association with *in vitro* colon tumor cell growth, migration, invasion and apoptosis, as well as *in vivo* tumor growth inhibition. The immunoregulatory functions of TRAIL on colon tumors in a xenograft mouse model were analyzed following a 30-day treatment period. Exogenous apoptosis signaling pathways induced by TRAIL were also examined.

Materials and methods

Cells and reagents. Colon tumor cell lines LoVo and HT-29 were purchased from the American Type Culture Collection (Manassas, VA, USA). All tumor cells were cultured in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (Invitrogen; both Thermo Fisher Scientific, Inc., Waltham, MA, USA). All cells were cultured in a 37˚C humidified atmosphere containing 5% CO_2.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted from LoVo and HT-29 cells and tumors using an RNAeasy Mini kit (Qiagen Sciences, Inc., Gaithersburg, MD, USA). cDNA was synthesized with ReverTra Ace (Toyobo Life Science, Osaka, Japan) at 42˚C for 2 h. Fibronectin (FN), Vimentin and epithelial (E)-cadherin expression was analyzed using an iCycler thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc.). All forward and reverse primers (Table I) were synthesized by Invitrogen (Thermo Fisher Scientific, Inc.). The following thermocycling conditions were applied: 45 amplification cycles consisting of denaturation at 95˚C for 30 sec, primer annealing at 62.5˚C for 45 sec with touchdown at 56.5˚C for 50 sec and extension at 72˚C for 60 sec. The relative mRNA expression changes were calculated using the $2^{-\Delta\Delta Cq}$ method (23). Results are expressed as fold change compared with the control.

MTT cytotoxicity assays. LoVo and HT-29 cells were incubated with TRAIL (0-2.5 mg/ml; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in 96-well plates for 24, 48, 72 and 96 h in triplicate for each concentration. PBS was used as the control. Following the indicated incubation time, 20 μl MTT (5 mg/ml; Sigma-Aldrich; Merck KGaA) in PBS was added to each well and the plate was further incubated for 4 h. The majority of the medium was removed and 100 μl dimethyl sulfoxide was added into the wells to solubilize the formazan crystals. The optical density was measured using a microplate reader at a wavelength of 450 nm.

Cell invasion and migration assays. LoVo and HT-29 cells were incubated with TRAIL (0.20 mg/ml) for 12 h at 37˚C. For the invasion assay, LoVo and HT-29 cells were suspended at a density of $1x10^5$ in 500 μl serum-free DMEM. The cells were seeded in the upper chamber of a BioCoat Matrigel Invasion Chamber (BD Biosciences, Franklin Lakes, NJ, USA) in DMEM for 24 h at 37˚C. The lower chamber contained 10% FBS in DMEM. For the migration assay, cells were seeded on a control insert (BD Biosciences) instead of a Matrigel Invasion Chamber for 24 h at 37˚C. The migratory and invasive cells were fixed with 3% formaldehyde for 15 min at 37˚C and stained with 0.5% crystal violet for 10 min at 37˚C. The tumor cell migration and invasion were counted in a minimum of three randomly-selected stained fields using a light microscope at a magnification of x40.

ELISA. 96-well plates were incubated with rabbit anti-mouse TNF-α antibody (cat. no. T8300; 1:200; Sigma-Aldrich; Merck KGAa) for 24 h at 4˚C. Plates were washed with PBS three times at room temperature and then incubated with albumin (0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml) or TRAIL (0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml) for 1 h at 37˚C. Plates were washed with PBS three times at room temperature and then incubated with rabbit anti-mouse TRAIL antibody (1:500; cat. no. ab231063, Abcam) for 24 h at 4˚C. Plates were washed with PBS three times at room temperature and then incubated for 2 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (1:1,000; cat. no. ab6785; Abcam). The optical density value was detected at 450 nm using a microplate reader.

Cell cycle analysis. LoVo and HT-29 cells were incubated with TRAIL (0.20 mg/ml) for 48 h at 37˚C. Cells were harvested, stained with Annexin V-fluorescein isothiocyanate (FITC)/propidium iodine (PI; BD Biosciences) and analyzed as previously described (24). The cell cycle was analyzed using a flow cytometer and ModFit LT™ software (Version 4; Verity Software House Inc., Topsham, ME, USA).

Transfection of short interfering (si)RNA. All siRNAs were synthesized by Invitrogen (Thermo Fisher Scientific, Inc.) including the siRNA-Fas ligand (Si-FasL sense, 5'-CGC GGATCCGCGTTGCAGAA-3' and antisense, 5'-TCCCCG CGGGGAGCGACACTAA-3') and the Si-RNA-vector (sense, 5'-TCCCCGCGGGGAAGGTCTGTCTTATT-3' and antisense, 5'-CCATCGATGGTATACCGC-3'). LoVo or HT-29 cells ($1x10^6$) were transfected with 100 pmol of Si-FasL targeting FasL with si-RNA-vector as the control (both Thermo Fisher Scientific, Inc.) using a Cell Line Nucleofector kit L (Lonza Group, Ltd., Basel, Switzerland). Subsequent experiments were performed after a 72-h transfection. Si-Fas transfected cells were treated with TRAIL (0.20 mg/ml) for 48 h at 37˚C.

Animal studies. A total of 40 male Balb/c nude mice (age, 8 weeks; body weight, 22-28 g) were purchased from Shanghai SLAC laboratory Animal Co., Ltd. (Shanghai, China). All mice were housed at 23±1˚C, 50±5% humidity with a 12-h light/dark cycle and free access to food and water. Mice were inoculated with LoVo or HT-29 tumor cells ($1x10^6$) into the

Table I. Sequences of primers were used in this study.

Gene name	Sequence (5'-3')	
	Reverse	Forward
Fibronectin	TTCATTATAAATCTAGAGACTCCAGGA	CTTTGGGACTGGTGGAAGAATC
Vimentin	ACGTCTTGACCTTGAACGCA	TCTTGGCAGCCACACTTTCA
E-cadherin	GTGGCCCGGATGTGAGAAG	GGAGCCCTTGTCGGATGATG
β-actin	CGGAGTCAACGGATTTGGTC	AGCCTTCTCCATGGTCGTGA

subcutaneous tissue and divided into four groups: Control-LoVo, TRAIL-LoVo, Control-HT-29 and TRAIL-HT-29 (n=10 per group). Treatment was initiated on day 8 following inoculation, when tumor diameters reached 5-6 mm. Tumor-bearing mice were intravenously injected with TRAIL (0.20 mg/kg) or PBS (0.20 mg/kg) as the control. The treatment was performed once per day for 7 days. Tumor volumes were calculated every 3 days according to a previous study (25). The present study was approved by the Committee on the Ethics of Yantaishan Hospital (Yantai, China).

Immunohistochemistry. Mice (n=3 per group) were anesthetized with 60 mg/kg IP pentobarbital (Sigma-Aldrich; Merck KGaA) and sacrificed using cervical dislocation on day 31. Colon tumors from the xenograft mice were fixed using 10% formaldehyde for 2 h at room temperature and embedded in paraffin. Tumor tissues were fabricated to 5-μm-thick tumor sections. Antigen retrieval was performed on the tumor sections using Lab Vision™ Tris-HCl buffer for heat-induced epitope retrieval (cat no. AP-9005-050; Thermo Fisher Scientific, Inc.) for 15 min at 65˚C. The tumor sections were incubated with primary antibodies against P53 (cat. no. ab131442) and B-cell lymphoma-2 (Bcl-2; ab182858; 1:200; Abcam, Cambridge, UK) for 12 h at 4˚C. The membranes were then incubated with goat anti-rabbit horseradish peroxidase (HRP)-conjugated immunoglobulin G secondary antibodies (1:2,000; cat. no. PV-6001; OriGene Technologies Inc., Rockville, MD, USA) at 37˚C for 2 h. Blots were imaged using WesternBright ECL Chemiluminescent HRP Substrate (Advansta, Menlo Park, CA, USA).

Apoptosis assay. LoVo or HT-29 cells were grown at 37˚C in an atmosphere containing 5% CO_2 until 90% confluence was reached. Cells were incubated with TRAIL (0.20 mg/ml) for 48 h at 37˚C, following which the tumor cells were trypsinized and collected. Cells were washed in cold PBS, adjusted to a concentration of $1x10^6$ cells/ml with PBS and labeled with Annexin V-FITC and PI from the Annexin V-FITC kit (BD Biosciences) for 2 h at 4˚C. The results were analyzed using a FACScan flow cytometer and BD FACSDIVA™ software (version 1.2; BD Biosciences). The treatments were performed in triplicate and the percentage of apoptotic cells in each group was measured.

Western blotting. Colorectal tumors and cells were homogenized in a 10% RIPA buffer (Sigma-Aldrich; Merck KGaA) and centrifuged at 6,000 x g at 4˚C for 10 min to collect the supernatant. Transmembrane proteins were extracted using a Transmembrane Protein Extraction kit (Qiagen Sciences, Inc.) according to the manufacturer's protocol. The nuclear and cytoplasmic proteins were extracted using a Nuclear and Cytoplasmic Protein Extraction kit (Qiagen Sciences, Inc.). Protein concentration was measured using a BCA protein assay kit (Thermo Fisher Scientific Inc.). Protein samples (30 μg/lane) were separated on 12.5% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA) as previously described (26). Membranes were blocked with 5% skimmed milk for 1 h at 37˚C and then incubated with the following primary antibodies for 24 h at 4˚C: FN (cat. no. ab2413), Vimentin (cat. no. ab8978), E-cadherin (cat. no. ab1416), P53 (cat. no. ab131442), Bcl-2 (cat. no. ab182858; 1:1,000), caspase-3 (cat. no. ab13847), caspase-8 (cat. no. ab25901), NF-κB (cat. no. ab220803; all 1:1,000) and β-actin (cat. no. ab8226; 1:2,000; both Abcam). The membranes were then incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G monoclonal secondary antibodies (cat. no. PV-6001; 1:1,000; OriGene Technologies, Inc., Beijing, China) for 24 h at 4˚C. The results were visualized using WesternBright ECL Chemiluminescent HRP Substrate (Advansta, Menlo Park, CA, USA).

Statistical analysis. Data are presented as the mean ± standard deviation of three independent experiments. The results were analyzed using Student t-tests or one-way analysis of variance with Tukey's post hoc test. All the 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) with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

TRAIL inhibits colon cancer cell growth and induces cell cycle arrest. The affinity of TRAIL with TNF-α was analyzed by an ELISA assay. TRAIL could bind to TNF-α (Fig. 1A). An immunofluorescence assay revealed that TRAIL (0.20 mg/ml) could integrate with the surface of LoVo and HT-29 cells (Fig. 1B), while an *in vitro* assay demonstrated that TRAIL significantly inhibited colon cancer cell growth in a dose-dependent manner (Fig. 1C and D). Cell growth was most inhibited following treatment with 0.20 mg/ml TRAIL, and so this dosage was selected for use in further experiments. TRAIL inhibited LoVo (Fig. 1E) and HT-29 (Fig. 1F) cell growth in a time-dependent manner. The results also revealed

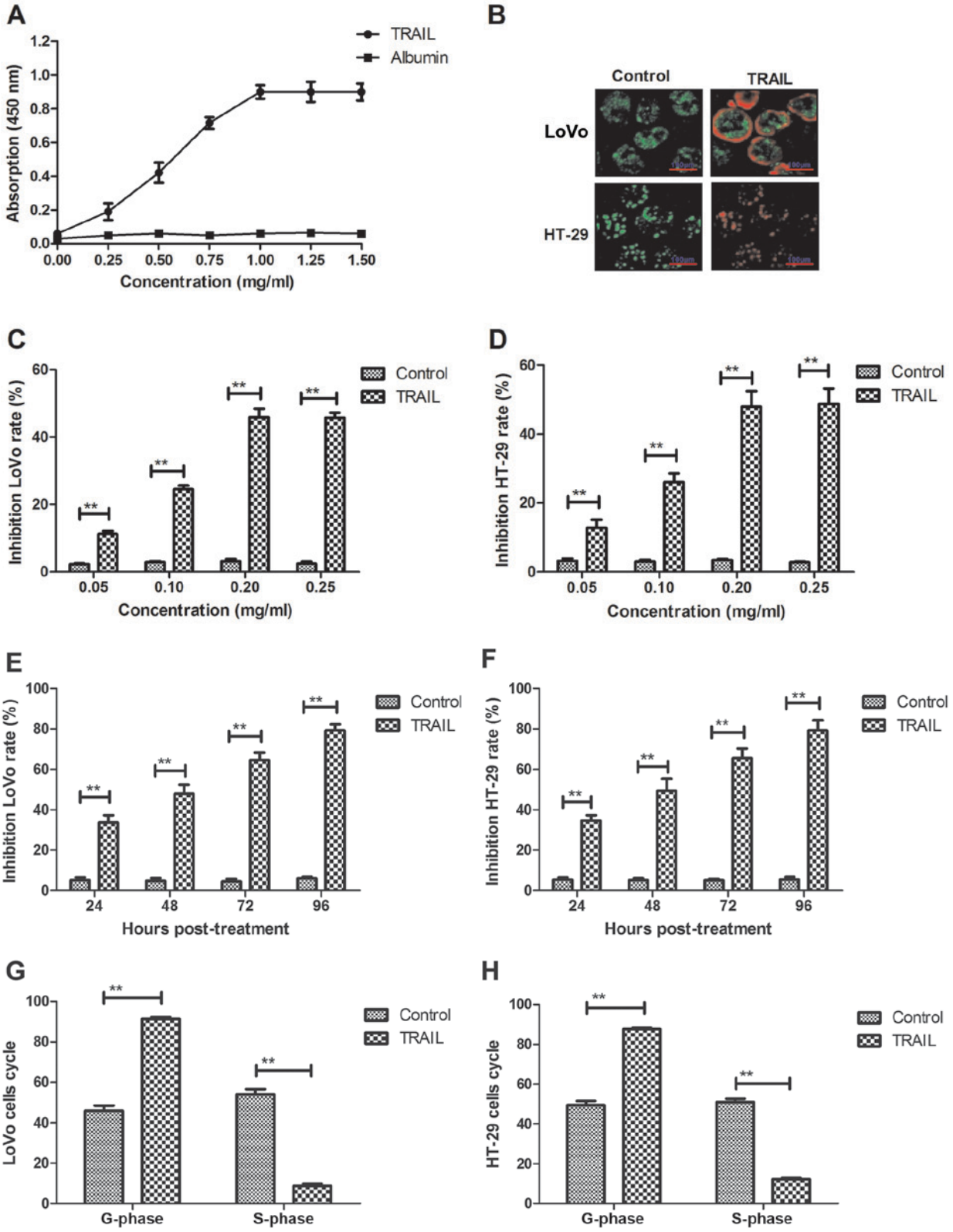

Figure 1. Effect of TRAIL on the growth and cells cycle progression of colon tumor cells. (A) Affinity of TRAIL with its receptor tumor necrosis factor-α in LoVo and HT-29 cells as determined by ELISA. (B) Immunofluorescence assay for the affinity of TRAIL with LoVo and HT-29 cells. TRAIL inhibits (C) LoVo and (D) HT-29 cell growth in a dose-dependent manner. TRAIL inhibits (E) LoVo and (F) HT-29 cell growth in time-dependent manner. TRAIL treatment induced cell cycle arrest in (G) LoVo and (H) HT-29 cells. **P<0.05 vs. control. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

that TRAIL treatment significantly arrested cell cycle progression in LoVo and HT-29 at the S phase (Fig. 1G and H). These results suggest that TRAIL may inhibit colon cancer cell growth by inducing cell cycle arrest.

TRAIL inhibits the migration and invasion of colon cancer cells by downregulating neoplasm metastasis-associated protein expression levels. Treatment with 0.02 mg/ml TRAIL for 48 h significantly inhibited the migration of LoVo (Fig. 2A)

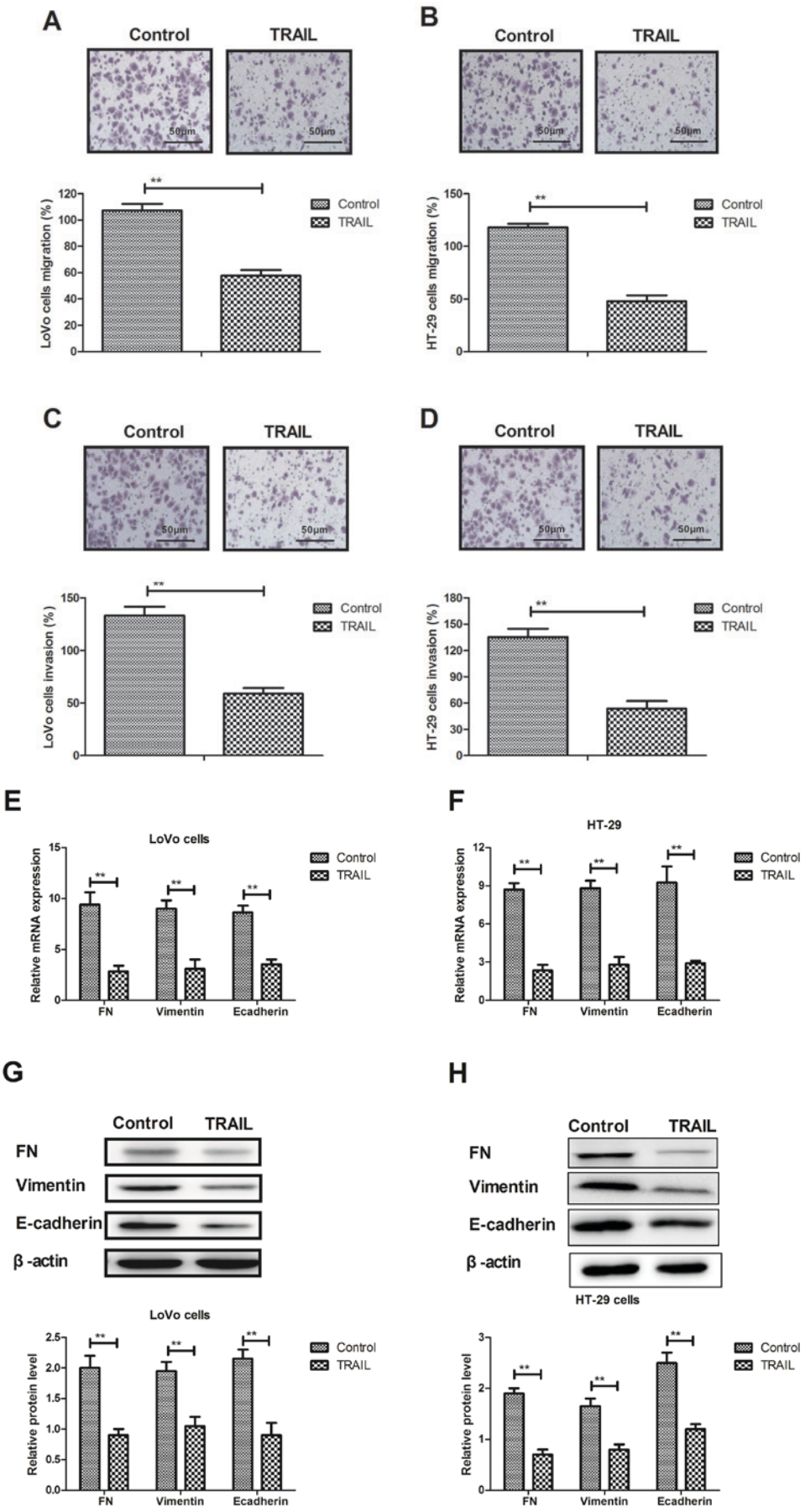

Figure 2. Effects of 48 h TRAIL treatment on the migration and invasion of colon cancer cells. TRAIL administration (0.20 mg/ml) inhibits the migration of (A) LoVo and (B) HT-29 cells. TRAIL administration (0.20 mg/ml) inhibits the invasion of (C) LoVo and (D) HT-29 cells. Reverse transcription-quantitative polymerase chain reaction analysis of FN, Vimentin and E-cadherin expression in (E) LoVo and (F) HT-29 cells. Western blotting of FN, Vimentin and E-cadherin protein expression in (G) LoVo and (H) HT-29 cells. **P<0.05 vs. control. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; FN, fibronectin.

and HT-29 (Fig. 2B) compared with the control. An invasion assay demonstrated that TRAIL administration (0.20 mg/ml) significantly inhibited the invasion of LoVo (Fig. 2C) and HT-29 (Fig. 2D) cells compared with the control, while RT-qPCR revealed that FN, Vimentin and E-cadherin expression was significantly downregulated following TRAIL administration in LoVo (Fig. 2E) and HT-29 (Fig. 2F) cells compared with the control. Western blotting results demonstrated that TRAIL administration significantly decreased FN, Vimentin and E-cadherin protein expression in LoVo (Fig. 2G) and HT-29 (Fig. 2H) cells compared with the control. These results suggest that TRAIL inhibits the migration and invasion of colon cancer cells by downregulating the expression of FN, Vimentin and E-cadherin.

TRAIL treatment promotes the apoptosis of colon cancer cells via the exogenous apoptosis pathway. The effects of TRAIL treatment on the apoptosis of colon cancer cells were analyzed *in vitro*. Flow cytometry revealed that TRAIL treatment (0.20 mg/ml) significantly promoted apoptosis in LoVo (Fig. 3A) and HT-29 (Fig. 3B) cells compared with the control. Western blotting also demonstrated that TRAIL treatment significantly increased the expression of caspase-8, caspase-3 and NF-κB proteins in LoVo (Fig. 3C) and HT-29 (Fig. 3D) cells compared with the control. However, the expression of P53 and Bcl-2 proteins was significantly downregulated by TRAIL treatment in LoVo (Fig. 3E) and HT-29 (Fig. 3F) cells compared with the control. In addition, TRAIL treatment significantly increased apoptosis in FasL-inhibited LoVo (Fig. 3G) and HT-29 (Fig. 3H) cells compared with FasL-inhibited cells. These results suggest that TRAIL treatment may promote the apoptosis of LoVo and HT-29 colon cancer cells via the exogenous apoptosis pathway.

TRAIL treatment inhibits tumors growth and prolongs the survival of LoVo and HT-29 tumor-bearing mice. TRAIL treatment significantly inhibited tumor growth in LoVo (Fig. 4A) and HT-29 tumor-bearing (Fig. 4B) mice compared with the PBS group. Immunohistochemistry revealed that TRAIL treatment notably increased the infiltration of apoptotic bodies and lymphocytes in tumors compared with the control group (Fig. 4C and D). P53 and Bcl-2 expression was markedly downregulated following TRAIL treatment in LoVo (Fig. 4E) and HT-29 tumor-bearing (Fig. 4F) mice compared with the PBS group. The survival rate of LoVo (Fig. 4G) and HT-29 tumor-bearing (Fig. 4H) mice was significantly increased following treatment with TRAIL, compared with the PBS group. These results indicate that TRAIL treatment inhibits tumor growth and prolongs survival in LoVo and HT-29 tumor-bearing mice by promoting apoptosis in colon tumor cells.

Discussion

Colon cancer is one of the most common types of gastrointestinal cancer worldwide, which can be highly invasive and is characterized by rapid local invasion of the lymphatic system (27). Death receptor 5 (DR5) and TRAIL have been reported to induce the apoptosis of human tumor cells and may represent a novel approach to cancer therapy by increasing the apoptotic sensitivity of cells (28). Numerous studies have demonstrated the effects of TRAIL by targeting the metabolic signaling pathway in human colon cancer (29-32). However, the exogenous apoptosis signaling pathway and TRAIL apoptotic death pathway are not well understood within colon cancer cells (33,34). In the present study, the inhibitory effects and underlying mechanisms of TRAIL in LoVo and HT-29 cells *in vitro* and *in vivo* were examined. The results revealed that TRAIL treatment significantly inhibited the growth and invasion of colon cancer cells. In addition, the results demonstrated that TRAIL treatment significantly promoted the apoptosis of colon cancer cells by increasing the expression of caspase-3, FasL and caspase-8 proteins, which are components of the exogenous apoptosis pathway.

TRAIL is a member of the TNF superfamily, which interacts with DRs on tumor cells and leads to the apoptosis of cancer cells (35). Gupta *et al* (32) demonstrated that TRAIL induces apoptosis through the extracellular signal regulated kinase-dependent upregulation of DRs p53 and Bcl-2-associated X protein. The increased expression of TRAIL receptors may enhance the apoptotic response induced by TRAIL and cause an increase in the apoptosis rate of tumor cells (36,37). The results of the present study revealed that TRAIL inhibited the growth of LoVo and HT-29 cancer cells and also promoted their apoptosis. Caspase-3 and caspase-8 expression was upregulated in TRAIL-treated colon tumor cells, which further increases apoptosis (38). The results also suggest that TRAIL-induced apoptosis was achieved via the exogenous apoptosis pathway via the downregulation of P53 and Bcl-2.

A previous study indicated that TRAIL may mediate apoptosis through the upregulation of DR5 by zerumbone and celecoxib (39). The results of the present study demonstrated that TRAIL treatment significantly increased the expression of caspase-8, caspase-3 and NF-κB proteins in LoVo and HT-29 cells. The current study indicated that the inhibition of FasL expression ameliorates the TRAIL-induced apoptosis of LoVo and HT-29 cells. In addition, a previous study has suggested that FasL-induced apoptosis serves a role in the elimination of tumor cells by natural killer cells (40). Contassot *et al* (41) have suggested that FasL is an important molecule in TRAIL-mediated apoptosis, which is associated with impaired DR and FLICE-inhibitory protein expression. The results of the present study demonstrated that TRAIL-induced colon tumor apoptosis occurred via the FasL-mediated exogenous apoptosis signaling pathway.

The results of the present study also revealed that TRAIL treatment markedly inhibits the invasion of colon tumor cells by downregulating FN, Vimentin and E-cadherin expression in LoVo and HT-29 cells. Kamoshida *et al* (42) reported that decreased matrix metalloproteinase production and FN expression may downregulate tumor cell invasion. Previous studies have also demonstrated that variable E-cadherin overexpression is a risk marker for the development of multiple tumors in animal models of colon tumors (43,44). Additionally, Vimentin mediates the regulation of cell motility through the modulation of integrin β4 protein expression in various tumor cells (45). The results of the present study suggest that TRAIL treatment may decrease FN, Vimentin and E-cadherin expression in LoVo and HT-29 cells, which inhibits the migration and invasion of colon tumor cells. It was also observed that TRAIL treatment inhibited tumor growth and prolonged the survival

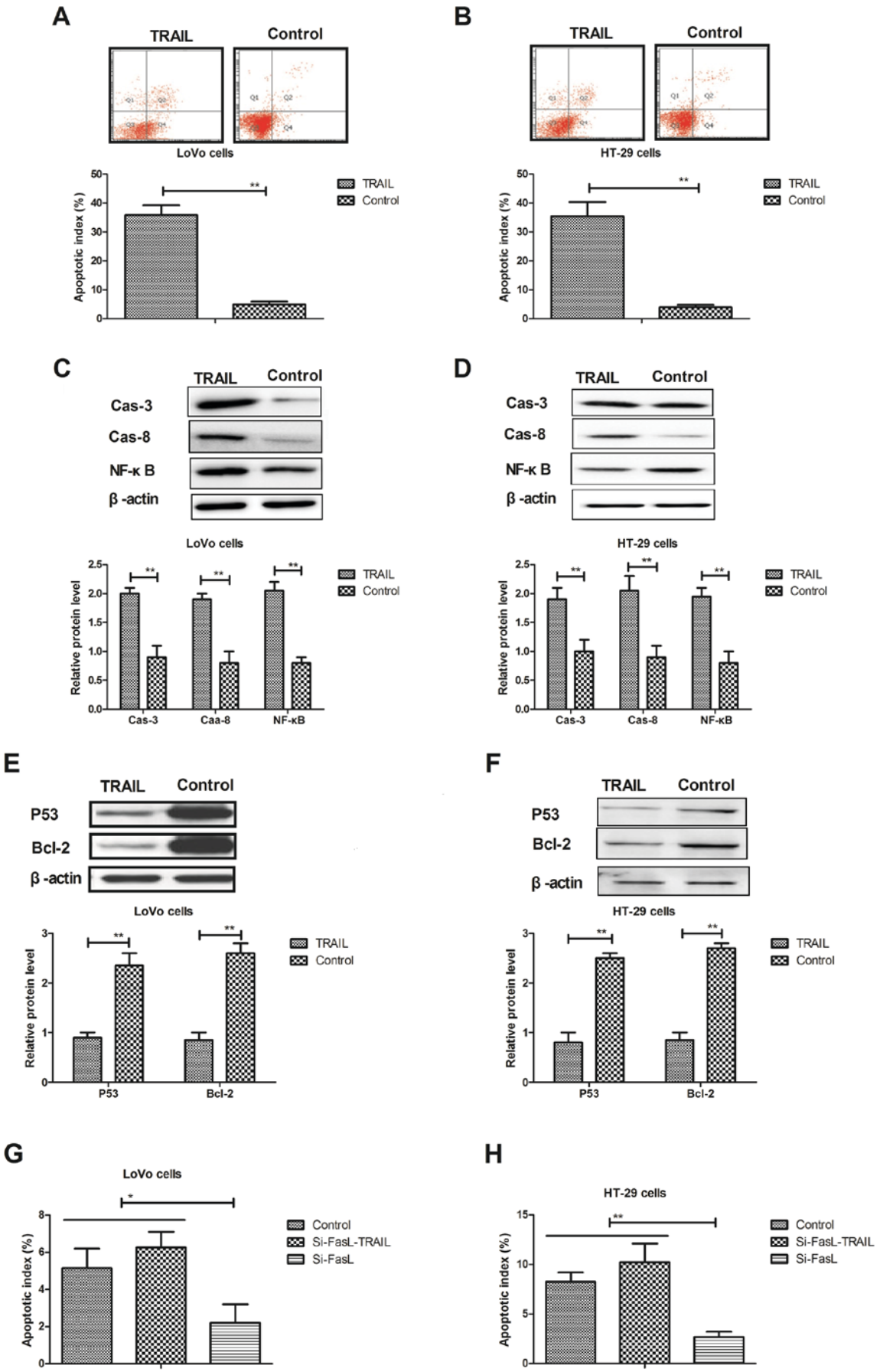

Figure 3. Effect of TRAIL on the apoptosis of colon cancer cells. TRAIL promotes the apoptosis of (A) LoVo and (B) HT-29 cells after 48 h incubation as determined by flow cytometry. Western blotting revealed that TRAIL promotes the expression of caspase-8, caspase-3 and NF-κB in (C) LoVo and (D) HT-29 cells, as well as P53 and Bcl-2 protein in (E) LoVo and (F) HT-29 cells. Si-FasL ameliorated TRAIL-mediated apoptosis in (G) LoVo and (H) HT-29 cells. *P<0.05 and **P<0.01 vs. control. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Bcl-2, B-cell lymphoma-2; NF, nuclear factor; Si-FasL, short interfering RNA-Fas ligand.

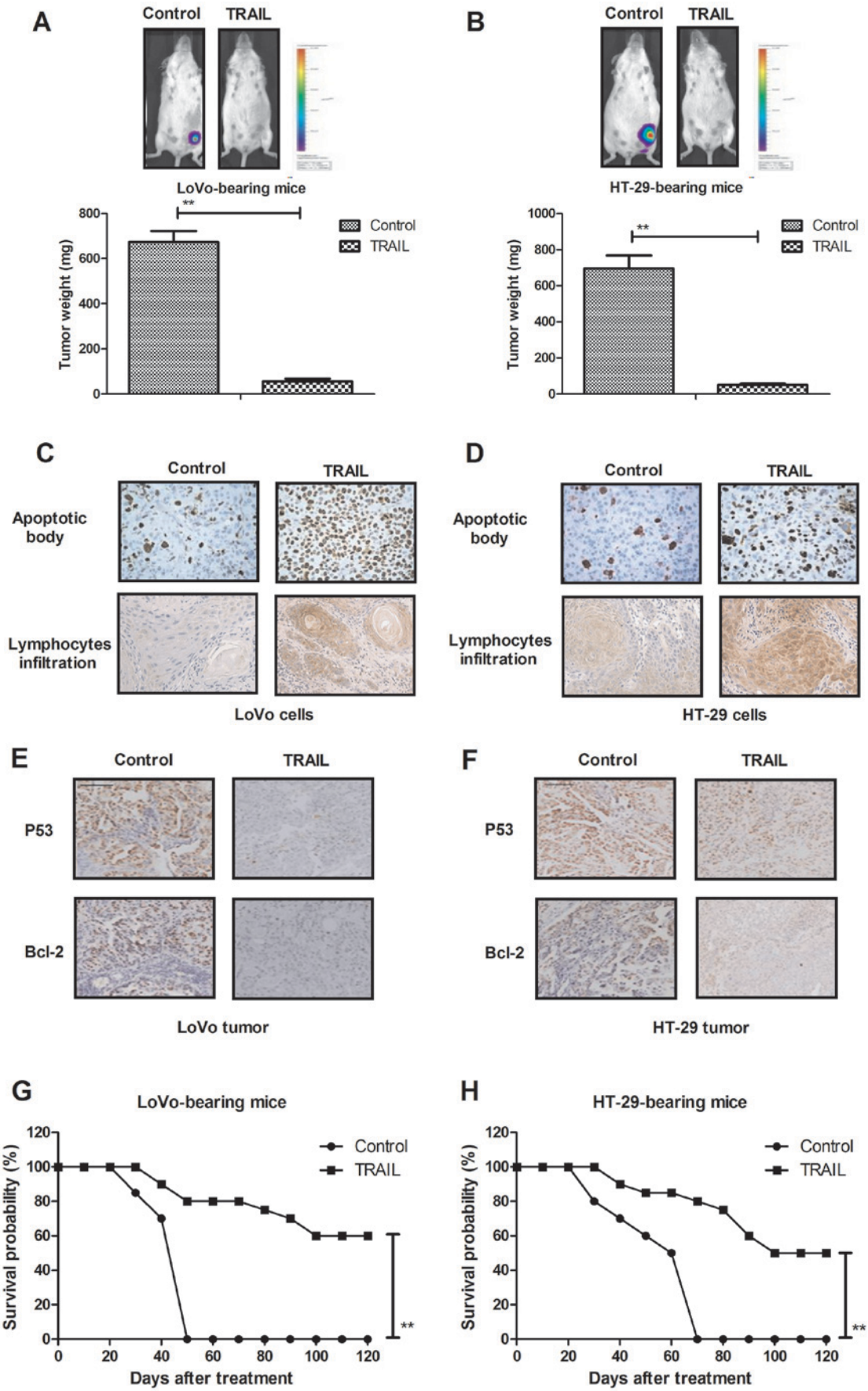

Figure 4. Effects of TRAIL on tumor growth and survival in LoVo- and HT-29-bearing mice. TRAIL suppresses tumor growth in (A) LoVo-bearing and (B) HT-29-bearing mice compared with the PBS group. Effects of TRAIL on apoptotic body and lymphocyte infiltration in (C) LoVo-bearing and (D) HT-29-bearing mice compared with the control group. TRAIL suppresses P53 and Bcl-2 expression levels in (E) LoVo-bearing and (F) HT-29-bearing mice compared with the control group. TRAIL prolongs the survival of (G) LoVo-bearing and (H) HT-29-bearing mice during a 120-day observation compared with the control group. **P<0.05 vs. control. Magnification, x100. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Bcl-2, B-cell lymphoma-2.

of tumor-bearing mice compared with the controls. However, further studies should be perform to explore the anticancer effects of TRAIL on other types of human cancer.

In conclusion, TRAIL treatment significantly promotes apoptosis and inhibits the growth and aggressiveness of colon tumor cells. In addition, TRAIL treatment had a tumor-suppressing effect on colon tumor cells *in vitro* and *in vivo*, which suggests that TRAIL may be a promising anticancer agent for the treatment of colon cancer.

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Funding

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Availability of data and materials

The analyzed datasets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

HG and WC designed the study. and performed the experiments. YW analyzed the data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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