Technology in Cancer Research & Treatment
Volume 18: 1-7

DOI: 10.1177/1533033819853290
journals.sagepub.com/home/tct
SAGE

Synergistic Suppression Effect on Tumor Growth of Colorectal Cancer by Combining Radiotherapy With a TRAIL-Armed Oncolytic Adenovirus

Hangxiang Gao, BD[1], Xin Zhang, MD[2], Ying Ding, MD[3], Rong Qiu, BD[4], Yupeng Hong, PhD[5], and Wanyuan Chen, MD[2]

Abstract

The combination of gene therapy and radiation is a promising new treatment for cancer. This study aimed to clarify the synergistic effect of targeted oncolytic adenovirus (radiotherapy-tumor necrosis factor-related apoptosis-inducing ligand) and radiotherapy on colorectal cancer cells and elucidate the mechanisms of the underlying antitumor activity. Viability, cell cycle status, and apoptosis of treated colorectal cancer cells were determined via MTT and flow cytometric assays. The molecular mechanism underlying apoptotic pathway activation was elucidated through Western blot analysis of caspase-8, caspase-3, and PARP proteins. Combination treatment with radiotherapy-tumor necrosis factor-related apoptosis-inducing ligand and radiotherapy displayed significantly greater antitumor activity than either of the monotherapies. The primary mechanism behind the antitumor activity in the SW480 and Lovo colorectal cancer cell lines was apoptosis induction through the caspase pathway and G1 phase arrest. In an SW480 xenograft model of colorectal cancer, the combination therapy achieved a significantly greater reduction in tumor volume than the monotherapies. Overall, in this study, we demonstrate that the oncolytic radiotherapy-tumor necrosis factor-related apoptosis-inducing ligand construct can sensitize human colorectal cancer cells to radiation-induced apoptosis both *in vitro* and *in vivo*. Therefore, our findings point toward a novel synergistic approach to colorectal cancer treatment.

Keywords

oncolytic adenovirus, TRAIL, radiotherapy, colorectal cancer, cell cycle, apoptosis

Abbreviations

Ads, adenoviruses; CRC, colorectal cancer; FITC, fluorescein isothiocyanate; MOI, multiplicity of infection; PBS, phosphate-buffered saline; PI, propidium iodide; RT, radiotherapy; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

Received: January 25, 2019; Revised: March 7, 2019; Accepted: April 24, 2019.

[1] Department of Radiology, School of Medicine, Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou, Zhejiang, China
[2] Department of Pathology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang, China
[3] Department of Intensive Care Unit, School of Medicine, Sir Run Run Shaw Hospital Xiasha Campus, Zhejiang University, Hangzhou, Zhejiang, China
[4] Department of Pathology and Pathophysiology, Hangzhou Medical University, Hangzhou, Zhejiang, China
[5] Departments of Oncology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang, China

Corresponding Authors:
Yupeng Hong, PhD, Department of Oncology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang, 310004, China.
Email: hypbdn@zju.edu.cn;

Wanyuan Chen, MD, Department of Pathology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, 158# Shangtang Road, Hangzhou, Zhejiang, 310004, China.
Email: chenwanyuan@hmc.edu.cn

Introduction

Gene therapy has shown promising results in the treatment of various malignancies. Advanced vector systems, such as tumor-specific oncolytic adenovirus, which replicates selectively in tumor cells, have contributed to highly effective treatment strategies. Several preclinical and clinical studies have shown that oncolytic adenoviruses (Ads) are highly effective in colorectal cancer (CRC) therapy.[1]

Radiotherapy (RT), with or without a chemotherapeutic regimen, is used widely to eliminate solid tumors. In addition, it is also used as part of postsurgery adjuvant therapy. Several preclinical trials have shown promising results, both *in vitro* and *in vivo*, for combination therapies involving oncolytic viruses and radiation, with each therapy acting synergistically with the other to destroy tumor cells.[2-5] The encouraging results obtained in preclinical studies have prompted translational clinical trials that are currently in phases I and II.[6,7] Although the exact mechanism of this synergistic antitumor action is unknown, several hypotheses have been put forward: the oncolytic viruses may increase the radio-sensitivity of tumor cells, making them susceptible to radiation-induced death, and/or the radiation may increase viral uptake and replication in tumor cells and trigger cell death.[8] The exact effects of radiation, if any, on intracellular viral load and replication and on cytotoxicity need to be elucidated before introducing new oncolytic viruses for combination therapies. Therefore, preclinical mechanistic studies need to be performed before embarking on clinical trials.

Recently, targeted cancer therapy aimed at local tumors and not adjacent healthy tissues has gained much attention. Radiotherapy has also been adapted to such targeted treatment strategies with the help of genes that can specifically trigger apoptosis and inhibit oncogenesis and angiogenesis in tumor cells, thereby enhancing their radio-sensitivity. The apoptotic machinery of tumor cells is frequently targeted in such strategies because the apoptosis is typically dysregulated or inhibited during carcinogenesis.[9] Therefore, tumor cell-specific apoptosis induction can be a highly effective therapeutic approach in combination with radiation.[10] A key molecule in tumor gene-radiotherapy is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which selectively induces apoptosis in cancer cells but not in normal cells.[10-14] Tumor necrosis factor-related apoptosis-inducing ligand was first cloned from human lymphocytes and cardiac muscle cells by Wiley *et al*.[15] Tumor necrosis factor-related apoptosis-inducing ligand-based gene-radiotherapy has shown cumulative antitumor activity against breast cancer cells in preclinical and stage I and II clinical trials.[16] Other studies have shown that ionizing radiation can increase the levels of TRAIL and the death receptors DR4 and DR5 in tumor cells and accelerate apoptosis. The pro-apoptotic proteins DR4 and DR5, but not DcR1, DcR2 and osteoprotegerin receptors,[13] can activate caspase-8/10 pathways and trigger apoptosis.[17]

The cytotoxic action of gene-radiotherapy can be further maximized by including an oncolytic viral component. For example, the TRAIL gene can be combined with an oncolytic adenovirus to generate rAd-TRAIL virus. In this study, we report the antitumor effect of a combination of rAd-TRAIL infection and RT on *in vitro* and *in vivo* CRC models and explore the possible underlying mechanisms

Materials and Methods

Cell Lines and Viruses

The human CRC cell lines HCT116, HT29, Lovo, SW480, and SW620 and human renal epithelial cell line 293A were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (CBTCCAS, Shanghai, China) and cultured in Dulbecco modified Eagle's medium (GIBCO, Carlsbad, California) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO). Cells were incubated in a 5% CO_2 humidified incubator at 37°C. The recombinant oncolytic adenovirus rAd-TRAIL was constructed and produced as follows: The plasmid pXC1, which carries the adenovirus 5 E1A and E1B regions, was used to generate the adenovirus construction plasmid pZD55 by deleting the 55-kDa E1B gene and introducing a cloning site. The TRAIL gene (excised by EcoRI/XbaI) from pBlueScript-TRAIL was cloned into pCA13, which was excised by EcoRI/XbaI beforehand to construct pCA13-TRAIL. pZD55-TRAIL was constructed by inserting the entire foreign gene expression cassette cut from pCA13-TRAIL using BglII into the corresponding pZD55 site. All plasmid constructs were confirmed via restrictive enzyme digestion, polymerase chain reaction, and DNA sequencing. Generation of the recombinant adenovirus rAd-TRAIL was carried out according to the protocols of Microbix Biosystems. The recombinant rAd-TRAIL adenovirus was amplified by infecting 293A cells.

Cell Viability Assay

HCT116, HT29, Lovo, SW480, and SW620 cells were dispensed in 96-well culture plates at a density of 5×10^3 cells/well. After attachment, the cells were infected with RT, rAd-TRAIL, or RT plus rAd-TRAIL at the given concentration and time. Medium with phosphate-buffered saline (PBS) added was used as a blank control. The cell survival rate was evaluated using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma, St Louis, Missouri). Medium was removed, and fresh medium containing MTT (5 mg/mL) was added to each well. The cells were incubated at 37°C for 4 hours, after the supernatant was carefully drawn off each well, and then, 150 μL of dimethyl sulfoxide was added to each well and mixed thoroughly on a concentrating table for 10 minutes. The absorbance was read at 595 nm using a DNA Expert Microplate Reader Model GENios.

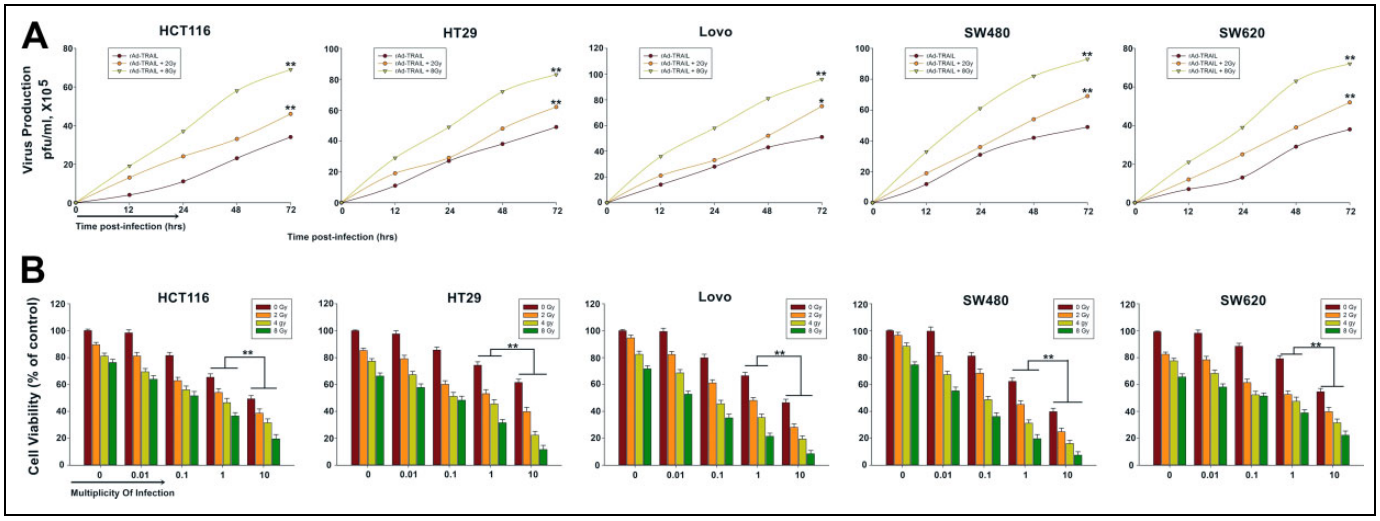

Figure 1. Combined rAd-TRAIL and RT shows synergistic cytotoxicity in a panel of colorectal cancer cell lines. A, Radiotherapy increases rAd-TRAIL viral replication in CRC cells. Cells were irradiated with either 2 or 8 Gy and 6 hours later infected with rAd-TRAIL (MOI 10). The cells were harvested and supernatant was collected at 48 hours after treatment. Viral titers were assessed using one-step growth curve assays. The data shown (mean [SD]) are representative of 3 experiments. $*P < .05$, $**P < .01$. B, Cells were treated with RT at either 2, 4, or 8 Gy and 6 hours later infected with rAd-TRAIL at a range of MOIs. Cell survival was measured 48 hours later using an MTT assay. The cytolytic effect of the oncolytic adenovirus rAd-TRAIL is enhanced with the increase in RT in the panel of CRC cell lines. The data shown (mean [SD]) are representative of 3 experiments. $*P < .05$ versus Control (RT = 0 Gy). $**P < .01$ versus Control (RT = 0 Gy). CRC indicates colorectal cancer; MOI, multiplicity of infection; RT, radiotherapy; rAd-TRAIL, radiotherapy-tumor necrosis factor-related apoptosis-inducing ligand; SD, standard deviation.

Western Blot Analysis

Cells were harvested in lysis buffer (Beyotime, Jiangsu, China) containing 1% Complete Mini-Protease Inhibitor Cocktail (Roche Diagnosis, Switzerland) and 5 mM NaF. Protein extractions were quantified using a BCA kit (Thermo Scientific, Massachusetts) and heated for 10 minutes at 100°C. Then, 30 μg of protein was resolved on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and transferred to a nitrocellulose membrane (Merck Millipore, Germany). After being blocked for 1 hour at 37°C, the membranes were immunoblotted with different antibodies (GAPDH [1:2000], caspase-8 [1:1000], caspase-3 [1:1000], PARP [1:500]) overnight at 4°C. The membranes were then washed with TBST and incubated with HRP-conjugated goat anti-rabbit or anti-mouse antibody (1:5000) for 1 hour at room temperature. Finally, blots were detected using a ChemiDoc MP Imaging System (Bio-Rad) with a SuperEnhanced chemiluminescence detection kit (Applygen, Beijing, China). To better compare changes in the caspase signaling pathway, gray values were calculated.

Flow Cytometric Analysis for Apoptosis

Cells infected with RT and/or rAd-TRAIL were trypsinized and washed once with complete medium. An aliquots of cells (5×10^5) was resuspended in 500 mL of binding buffer and stained with fluorescein isothiocyanate (FITC)-labeled annexin V and propidium iodide (PI, BioVision, Palo Alto, California) according to the manufacturer's instructions. Cell apoptosis and cell cycle were examined using FACS (FACStar cytofluorometer; BD Biosciences, San Jose, California).

Flow Cytometry Assay for Cell Cycle

After SW480 and Lovo cells were cultured in 6-well plates at 5×10^4 cells per well for 24 hours, they were treated with rAd-TRAIL or RT, respectively, or subjected to combination treatment. After 48 hours, when CPE was observed, the cells were trypsinized, washed once with phosphate-buffered saline, kept overnight at 4°C in 70% ethanol, and eventually treated with PI (50 mg/mL; Sigma) and RNase A (100 mg/mL). Cell cycle distribution was detected by flow cytometry (Beckman Coulter Epics XL, Ramsey, Minnesota).

Animal Experiments

All animal experiments were approved by the Institutional Animal Care and Use Committee and performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, following the guidelines of the Animal Welfare Act. Female BALB/c nude mice (4-5 weeks old) were purchased from Shanghai Experimental Animal Center (Shanghai, People's Republic of China). SW480 cells were injected subcutaneously into the lower right flank of the female nude mice, and the tumor xenograft model was established. Each group was composed of at least 8 animals, and tumor growth was monitored and measured every 3 days with a Vernier caliper. Tumor volume (V) was calculated according to the following formula: V (mm^3) = 1/2 × length (mm) × width

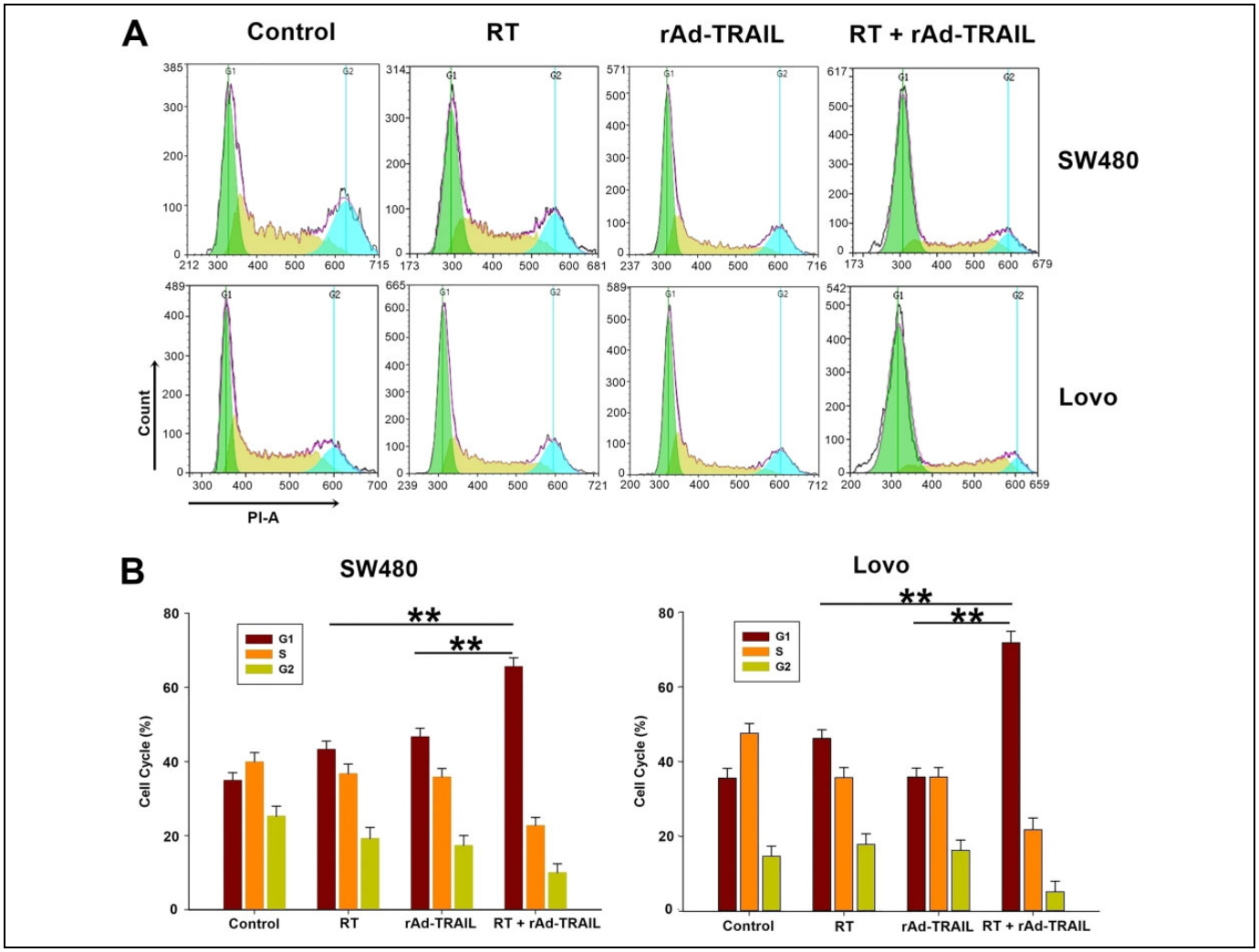

Figure 2. Colorectal cancer cells were arrested at G1 phase. A, The CRC cell lines SW480 and Lovo were infected with the oncolytic adenovirus rAd-TRAIL (0.1 MOI) at 6 hours post-RT (2 Gy), and cell cycle distribution was detected via flow cytometry 24 hours after treatment. B, The proportion of cells in the various cell cycle stages was determined. The data shown (mean [SD]) are representative of three experiments. $^{**}P < .01$ versus RT or rAd-TRAIL group. CRC indicates colorectal cancer; MOI, multiplicity of infection; RT, radiotherapy; rAd-TRAIL, radiotherapy-tumor necrosis factor-related apoptosis-inducing ligand; SD, standard deviation.

$(mm)^2$. Once the subcutaneous tumors reached $\sim 100\ mm^3$, the nude mice were randomly divided into 4 groups (8 mice in each group). Subsequently, mice were treated with RT, rAd-TRAIL, RT plus rAd-TRAIL, or PBS. Mice were either untreated, irradiated with 2 Gy fractions for 3 cycles, infected with rAd-TRAIL at 2×10^6 PFU/mL or administered combined treatment (2 Gy fraction for 3 cycles and 2×10^6 PFU/mL rAd-TRAIL).

Statistical Analysis

The experimental results are expressed as the mean (standard deviation). Analysis of variance was applied for comparison of 3 or more groups. Statistical analysis was performed with IBM SPSS Statistics software version 20 (SPSS Inc, Chicago, Illinois). Statistical significance was set at $P < .05$.

Results

The rAd-TRAIL and RT combination therapy shows synergistic cytotoxicity in CRC cells

To evaluate a possible effect of the selective replicative ability of rAd-TRAIL, a progeny assay was performed with 5 CRC cell lines—HCT116, HT29, Lovo, SW480 and SW620—that were treated with rAd-TRAIL or RT alone or in combination. Cells were irradiated with either 2 or 8 Gy and 6 hours later infected with rAd-TRAIL (multiplicity of infection [MOI] 10). The viral titer was determined using the TCID50 method. As shown in Figure 1A, the replication

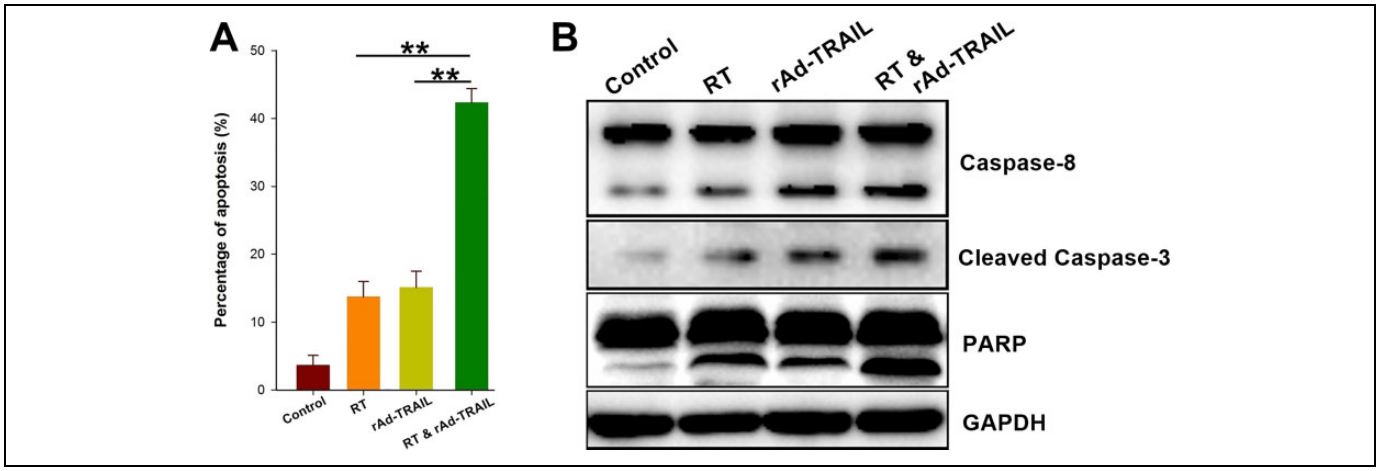

Figure 3. rAd-TRAIL enhances RT induced apoptosis in CRC cells. A, Radiotherapy (2 Gy), rAd-TRAIL (0.1 MOI), or RT (2 Gy) plus rAd-TRAIL (0.1 MOI) were used to treat SW480 cells; uninfected cells served as the control. After 48 hours, apoptosis was determined by flow cytometry. The data shown (mean [SD]) are representative of 3 experiments. $^{**}P < .01$ versus RT or rAd-TRAIL group. B, SW480 cells were infected with RT (2 Gy), rAd-TRAIL (0.1 MOI), or RT (2 Gy) plus rAd-TRAIL (0.1 MOI) for 48 hours. Whole-cell extracts were prepared and immunoblotted to detect activation of the caspase pathway. GAPDH was used as a loading control. To better compare changes in the caspase signaling pathway, gray values were calculated. $^{**}P < .01$ versus combination therapy group. CRC indicates colorectal cancer; MOI, multiplicity of infection; RT, radiotherapy; rAd-TRAIL, radiotherapy-tumor necrosis factor-related apoptosis-inducing ligand; SD, standard deviation.

efficiency of the oncolytic adenovirus rAd-TRAIL was enhanced with the increase in RT in the panel of CRC cell lines, which indicated that RT enhanced the replication efficiency of oncolytic adenovirus.

To assess the antiproliferative effect of each of these therapies, an MTT assay of the CRC cell lines was performed as described. The cells were either simply infected with rAd-TRAIL or first irradiated with 2, 4, or 8 Gy IR for 6 hours and then infected with rAd-TRAIL at a range of MOIs. The effects of both mono- and combination therapies were assessed 48 hours later with an MTT assay. As shown in Figure 1B, the cytotoxic effect of the oncolytic adenovirus rAd-TRAIL was enhanced with the increase in RT in the panel of CRC cell lines, which indicates that the combination of RT and rAd-TRAIL had an enhanced cytolytic effect on CRC cells.

Irradiated and rAd-TRAIL-Infected CRC Cells Are Arrested at G1 Phase

To elucidate the mechanistic basis of the effect of oncolytic adenovirus and irradiation on CRC cells, we monitored changes in cell cycle distribution using flow cytometry. The CRC cell lines SW480 and Lovo were infected with the oncolytic adenovirus rAd-TRAIL (0.1 MOI) at 6 hours post-RT (2 Gy). Compared to the control group, SW480 and Lovo cells infected with rAd-TRAIL and RT were arrested at G1 stage. In addition, the combination therapy group also showed an apoptotic peak in the cell cycle distribution. The RT treatment and rAd-TRAIL treatment did not significantly block the cell cycle (Figure 2A). Consistent with this, the cell cycle distribution analysis of the treated SW480 and Lovo cells revealed a significantly increased proportion of cells in G1 phase with a gradual decrease in the number of cells in G2 and S phases compared to the control cells (Figure 2B).

Combination Therapy with rAd-TRAIL and RT Shows Synergistic Pro-Apoptosis Activity in CRC Cells

To quantify the effects of rAd-TRAIL on RT-induced apoptosis, Annexin-V-FITC/PI double staining was performed (Figure 3A). The results showed that the apoptosis rate of SW480 cells treated with a combination of RT and rAd-TRAIL was 42.3%, which was nearly 3 times more than that seen with RT (13.7%) or rAd-TRAIL treatment alone (15.1%), which indicated that in cells treated with RT or rAd-TRAIL alone, apoptosis was significantly increased compared to Control cells. Cells treated with a combination of RT + rAd-TRAIL exhibited a significantly higher rate of apoptosis than those administered either treatment alone.

Consistent with the above findings, a Western blot assay was conducted to analyze the caspase-dependent apoptotic pathway, which involves caspase-8 and caspase-3 activation along with PARP cleavage. As shown in Figure 3B, in cells treated with RT or rAd-TRAIL alone, the caspase-dependent apoptotic pathway was significantly activated compared to Control cells, and caspase-dependent apoptotic pathway activation was further enhanced by co-treatment with rAd-TRAIL and RT.

Radiotherapy-Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Enhances RT-Mediated Colorectal Tumor Growth Suppression In Vivo

To assess the *in vivo* therapeutic effects of the RT and rAd-TRAIL combination, a CRC xenograft model was established

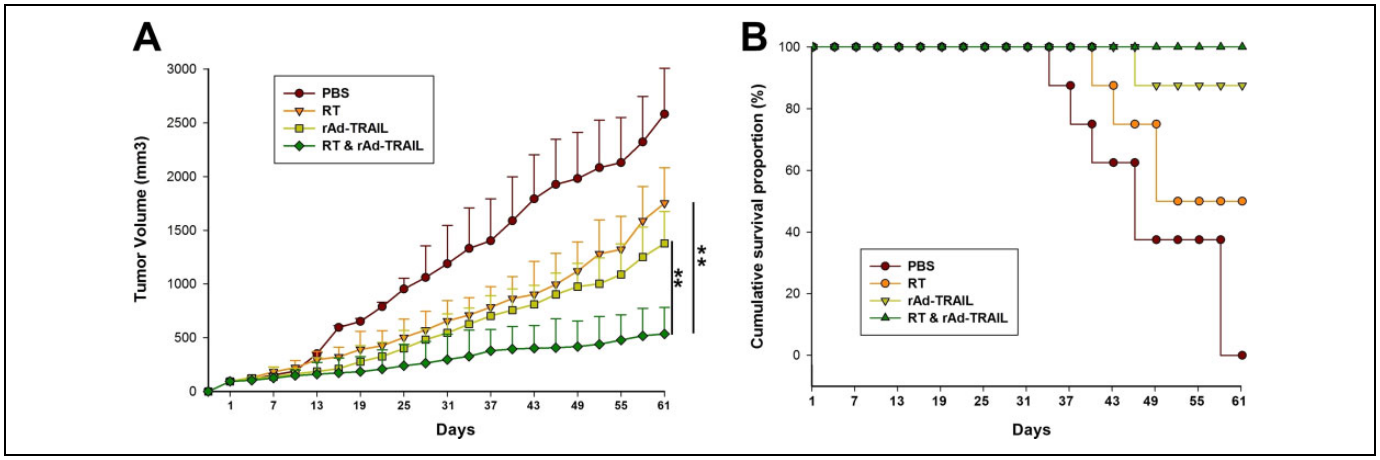

Figure 4. Synergistic effects of RT and rAd-TRAIL *in vivo*. A, Mice were either untreated, irradiated with 2 Gy fractions for 3 cycles, infected with rAd-TRAIL at 2×10^6 PFU/mL or administered combined treatment (2 Gy fraction for 3 cycles and 2×10^6 PFU/mL rAd-TRAIL). Tumor volume was measured at different times after treatment. The data are presented as the means (SD, n = 8). **, $P < .01$. B, Kaplan-Meier survival curves of animals. The percentage of surviving mice was calculated by monitoring the death of mice over a period of 61 days. A pairwise log-rank test was used to analyze survival rates in the various groups. NS, $P > .05$; *$P < .05$; **$P < .01$. PFU, plaque forming units; rAd-TRAIL, radiotherapy-tumor necrosis factor-related apoptosis-inducing ligand; RT, radiotherapy; SD, standard deviation.

using SW480 cells. Tumor growth curves were plotted to track changes in tumor volume in treatment and control groups for a 61-day period post treatment. As shown in Figure 4A, the mean tumor volume was significantly lower in animals receiving RT, rAd-TRAIL, or combination therapy than in control group animals that were injected with PBS. Furthermore, the combination treatment group had significantly smaller tumors than the monotherapy groups ($P < .01$ for both RT and rAd-TRAIL). Finally, the overall survival rate of the combination therapy group was improved compared to that of the control and monotherapy groups (Figure 4B).

Discussion

Mono-therapeutic approaches to cancer have had at best a mediocre success rate, primarily because tumors comprise genetically diverse clones that respond differentially to therapeutic agents and the tumor initiating cells often become recalcitrant to treatment. Recent strategies have therefore focused on combining two or more therapies, which have different individual antitumor mechanisms but act synergistically to increase tumor cell cytotoxicity without harming healthy tissues.[18,19] For example, several clinical studies have shown that a combination of oncolytic Ads with radiation or chemotherapy can synergistically enhance antitumor activity.[20,21] In parallel, mechanistic studies have focused on the basis of this cooperative interaction of oncolytic Ads and radiation with the aim of further optimizing targeted cancer therapy.[22,23]

Tumor cells are extremely radio-sensitive and unlike normal cells cannot survive sublethal radiation damage by activating DNA damage repair pathways. In other words, radiation treatment is effective in killing tumor cells by triggering massive damage to their DNA and plasma membranes. However, some tumors are radiation resistant, such as fibrosarcoma, osteosarcoma, liposarcoma, and rhabdomyosarcoma, and thus are not suitable for RT.[24,25] Furthermore, tumor cells that thrive in a hypoxic microenvironment are also insensitive to radiation. Finally, since radiation is effective on actively proliferating cells and approximately 20% to 50% of tumor cells are either dormant in G0 phase or stuck in S phase, these tumor cells are also radio-insensitive.[26]

Our study showed that CRC cells infected with the oncolytic rAd-TRAIL construct and radiation therapy underwent dose-dependent death *in vitro*. We found that the viability of SW480 cells treated with rAd-TRAIL and RT alone was 45% and 73%, respectively, and dropped drastically to 10% when cells were subjected to the combination treatment (Figure 1B), thereby demonstrating the synergistic antitumor effect of the monotherapies. Furthermore, the SW480 and Lovo cells were arrested at G1 stage after combination treatment, and the SW480 cells showed an increase in caspase-mediated apoptosis.

In summation, this study confirmed the synergistic therapeutic effect of oncolytic rAd-TRAIL and RT in CRC and provided some molecular insights into the underlying mechanisms. Our study provides a potential therapeutic strategy wherein the targeted oncolytic adenovirus rAd-TRAIL may act as a potential radiation sensitizer of cancer cells.

Acknowledgments

The authors would like to give our sincere gratitude to the reviewers for their constructive comments.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was part of the Program on the General Project Funds from the Health Department of Zhejiang Province, grant number 2018259783. The authors are grateful to all study participants.

ORCID iD

Wanyuan Chen, MD https://orcid.org/0000-0002-8216-9568

References

1. Zhou X, Xie G, Wang S, et al. Potent and specific antitumor effect for colorectal cancer by CEA and Rb double regulated oncolytic adenovirus harboring ST13 gene. *PLoS One*. 2012;7(10):e47566.
2. Kyula JN, Khan AA, Mansfield D, et al. Synergistic cytotoxicity of radiation and oncolytic Lister strain vaccinia in (V600D/E)BRAF mutant melanoma depends on JNK and TNF-alpha signaling. *Oncogene*. 2014;33(13):1700-1712.
3. Kyula JN, Khan AA, Wilkinson MJ, Mansfield D, Harrington KJ. Radiation lays down covering fire for viral shock troops. *Oncotarget*. 2013;4(9):1329-1330.
4. Mansfield D, Pencavel T, Kyula JN, et al. Oncolytic vaccinia virus and radiotherapy in head and neck cancer. *Oral Oncol*. 2013;49(2):108-118.
5. Twigger K, Vidal L, White CL, et al. Enhanced in vitro and in vivo cytotoxicity of combined reovirus and radiotherapy. *Clin Cancer Res*. 2008;14(3):912-923.
6. Harrington KJ, Karapanagiotou EM, Roulstone V, et al. Two-stage phase I dose-escalation study of intratumoral reovirus type 3 dearing and palliative radiotherapy in patients with advanced cancers. *Clin Cancer Res*. 2010;16(11):3067-3077.
7. Comins C, Spicer J, Protheroe A, et al. REO-10: a phase I study of intravenous reovirus and docetaxel in patients with advanced cancer. *Clin Cancer Res*. 2010;16(22):5564-5572.
8. Touchefeu Y, Vassaux G, Harrington KJ. Oncolytic viruses in radiation oncology. *Radiother Oncol*. 2011;99(3):262-270.
9. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med*. 2004;10(8):789-799.
10. Woynarowska BA, Roberts K, Woynarowski JM, Macdonald JR, Herman TS. Targeting apoptosis by hydroxymethylacylfulvene in combination with gamma radiation in prostate tumor cells. *Radiat Res*. 2000;154(4):429-438.
11. Perlstein B, Finniss SA, Miller C, et al. TRAIL conjugated to nanoparticles exhibits increased anti-tumor activities in glioma cells and glioma stem cells in vitro and in vivo. *Neuro Oncol*. 2013;15(1):29-40.
12. Tuckerkellogg L, Shi Y, White JK, Pervaiz S. Reactive oxygen species (ROS) and sensitization to TRAIL-induced apoptosis, in bayesian network modelling of HeLa cell response to LY303511. *Biochem Pharmacol*. 2012;84(10):1307-1317.
13. Niemoeller OM, Belka C. Radiotherapy and TRAIL for cancer therapy. *Cancer Lett*. 2013;332(2):184-193.
14. Li YB, Guo CX, Wang ZC, et al. Radiosensitization of breast cancer cells by TRAIL-endostatin-targeting gene therapy. *Neoplasma*. 2013;60(6):613-619.
15. Wiley SR, Schooley K, Smolak PJ, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity*. 1995;3(6):673.
16. Mahalingam D, Szegezdi E, Keane M, De JS, Samali A. TRAIL receptor signalling and modulation: are we on the right TRAIL? *Cancer Treat Rev*. 2009;35(3):280-288.
17. Sprick MR, Rieser E, Stahl H, Grossewilde A, Weigand MA, Walczak H. Caspase-10 is recruited to and activated at the native TRAIL and CD95 death-inducing signalling complexes in a FADD-dependent manner but can not functionally substitute caspase-8. *EMBO J*. 2014;21(17):4520-4530.
18. Wang SB, Tan Y, Lei W, et al. Complete eradication of xenograft hepatoma by oncolytic adenovirus ZD55 harboring TRAIL-IETD-Smac gene with broad antitumor effect. *Hum Gene Ther*. 2012;23(9):992-1002.
19. Wang S, Shu J, Chen L, et al. Synergistic suppression effect on tumor growth of ovarian cancer by combining cisplatin with a manganese superoxide dismutase-armed oncolytic adenovirus. *Onco Targets Ther*. 2016;9:6381-6388.
20. Ma B, Wang Y, Zhou X, et al. Synergistic suppression effect on tumor growth of hepatocellular carcinoma by combining oncolytic adenovirus carrying XAF1 with cisplatin. *J Cancer Res Clin Oncol*. 2015;141(3):419-429.
21. Ge Y, Lei W, Ma Y, et al. Synergistic antitumor effects of CDK inhibitor SNS-032 and an oncolytic adenovirus co-expressing TRAIL and Smac in pancreatic cancer. *Mol Med Rep*. 2017;15(6):3521-3528.
22. Zhang H, Wang F, Mao C, et al. Effect of combined treatment of radiation and tissue-specific recombinant oncolytic adenovirus on bladder cancer cells. *Int J Radiat Biol*. 2016;93(2):174-183.
23. Mcentee G, Kyula JN, Mansfield D, et al. Enhanced cytotoxicity of reovirus and radiotherapy in melanoma cells is mediated through increased viral replication and mitochondrial apoptotic signalling. *Oncotarget*. 2016;7(30):48517-48532.
24. Harada H. How can we overcome tumor hypoxia in radiation therapy? *J Radiat Res*. 2011;52(5):545-556.
25. Yoshimura M, Itasaka S, Harada H, Hiraoka M. Microenvironment and radiation therapy. *Biomed Res Int*. 2012;2013:685308.
26. Shim SH, Hah LC. Conditionally replicating adenovirus improves gene replication efficiency and anticancer effect of E1-deleted adenovirus carrying TRAIL in head and neck squamous cell carcinoma. *Cancer Sci*. 2010;101(2):482-487.