

Tat-SmacN7 induces radiosensitization in cancer cells through the activation of caspases and induction of apoptosis

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Abstract. A major concern in cancer therapy is resistance of tumors such as human non-small cell lung cancer and esophageal cancer to radiotherapy. Intrinsic radioresistance of these cancer cells limits therapeutic efficiency. Here, we determined in two cancer cell lines the potential radiosensitizing activity of Tat-SmacN7, a small molecule compound, which mimics the activity of Smac, a mitochondrial protein released during apoptosis. We found that Tat-SmacN7 can enter the cells and promote RNA expression and the activity of caspase-3, -8 and -9 and sensitized the cancer cells to radiation with a sensitization enhancement ratio (SER) of 1.5-1.6. Tat-SmacN7 radiosensitization was mediated by both extrinsic and intrinsic apoptosis pathways through activation of caspases. Consistently, blockage of caspase activation, through treatment with a caspase inhibitor, z-VAD-fmk, inhibited apoptosis and abrogated Tat-SmacN7 radiosensitization. Our study demonstrates that Tat-SmacN7 also has radiosensitization effects *in vivo*, so it could be further developed as a novel class of radiosensitizers for the treatment of radioresistant human non-small cell lung cancer and esophageal cancer.

Introduction

Cancer which performs progressive accumulation of genetic and epigenetic abnormalities, is a result of very complex multistep disorder. Six essential properties of cancer distinguish them from normal cells. Autocrine production of growth signals, resistance to apoptotic mechanisms, sustained angiogenesis, limitless replicative potential, tissue invasion and metastasis, and apoptosis avoidance (1). Resistance to apoptosis displays the very essential role among these various

properties of cancer cells in tumor development and progression. Evading apoptosis of cancer cells is related with their various biochemical properties. particularly, the upregulation of members of the inhibitor of apoptosis (IAP) family of proteins (2). Currently, there are two well-known apoptotic pathways, one initiated through the engagement of cell surface death receptors by their specific ligands (3) and the intrinsic pathway also called the mitochondrial pathway is triggered by changes in internal cellular integrity, which is induced by several different stimuli such as antineoplastic drugs, hypoxia, irradiation, growth factor withdrawal and heat shock (4). Both pathways eventually result in activation of caspases, cysteine proteases that comprise the effector arm of the apoptotic process (5). Radiation and other agents induce caspase activation mainly via the mitochondrial pathway, which involves mitochondrial integration of apoptotic signals and subsequent release of cytochrome c and Smac (the second mitochondria-derived activator of caspase)/DIABLO (direct IAP binding protein with low pI), Omi/HtrA2 and AIF into the cytosol (6). This release allows the assembly of the apoptosome (7). The apoptosome activates caspase-9, which in turn induces the activation of caspase-3, -6 and -7 (8). The effector caspases cleave their cellular specific substrates and generate the typical morphology of apoptosis.

Inhibitor of apoptosis proteins (IAPs) can inhibit apoptosis by interacting with and then regulating the ability of caspase-8 or caspase-9, -3 and -7 (9,10). c-IAP1 (cellular IAP-1), c-IAP2 (cellular IAP-2) and XIAP (X-linked IAP) are three important members of IAPs, especially XIAP, which has many domains interacting with different caspases such as caspase-3, -7 and -9 (11,12) and its BIR2 domain inhibits caspase-7 in a non-competitive manner (11). XIAP is highly expressed in many malignant tumors and has been associated with refractory disease and poor prognosis (13,14). Because XIAP blocks apoptosis at the effector phase, a point where multiple signaling pathways converge, treatments targeting XIAP may prove to be especially effective to overcome resistance. Smac was identified as a protein which can antagonize the inhibiting activity of IAPs to apoptosis after release from mitochondria in response to apoptotic stimulin (15-19). It has been proven that the domain of Smac interacts with IAPs is a particular NH2-terminal residue consisting of four amino acids, Ala-Val-Pro-Ile (18,20). It has been demonstrated that the AVPI

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sequence of Smac/DIABLO interacts with the BIR2 and BIR3 domains of XIAP, allowing the release of caspase-3 (18) and caspase-9 (17), respectively. Through this mechanism, SMAC/DIABLO prevents the sequestration of caspases by IAPs, thus facilitating the apoptotic pathway. Since the AVPI sequence is able to promote apoptosis, many Smac peptides that are able to mimic this tetrapeptide are synthesized and studied (21-26). We studied the Tat-SmacN7 protein to promote apoptosis of tumor cells.

Smac peptides can sensitize glioblastoma cells for TRAIL-induced apoptosis *in vitro* and *in vivo* by antagonizing XIAP (27). In addition, the genetic or pharmacologic inactivation of XIAP increases radiation-induced apoptosis in glioblastoma, neuroblastoma and pancreatic carcinoma cells (21-23). SM-164, a potent and well-characterized SMAC mimetic is an effective radiosensitizer both *in vitro* and *in vivo* of HNSCC cells by eliminating cIAP-1 (24-29). To translate the concept of improving the activity of caspases for radiosensitization into a clinically applicable approach to improve the efficiency of radiotherapy in esophageal carcinoma and the radioresistant human non-small cell lung cancer (NSCLC), we investigated in this study the therapeutic potential of Tat-SmacN7 for radiosensitization of EC109 cells and H460 cells.

Materials and methods

The human esophageal cell line (EC109) and non-small cell lung cancer (NSCLC) cell line NCI-H460 (H460). Human esophageal cell line (EC109) was generously provided by the General Hospital of Tianjin Medical University and human non-small cell lung cancer (NSCLC) NCI-H460 cells were purchased from Cell Culture Center of Chinese Academy of Medical Science and Peking Union Medical College. Cells were grown in 25-cm² flasks containing 3 ml of 1X RPMI-1640 (Hyclone, China) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and 1% streptomycin in a humidified incubator containing 5% CO₂ at 37°C. Cell lines were serially passaged following trypsinization using a trypsin/EDTA solution (Gibco).

Estimation of peptide internalization. To test the internalization efficiencies of the synthesized peptides, FITC-SmacN7 or FITC-Tat-SmacN7 at the concentration of 20 μ M, were added into the medium and continued to incubate for different time periods (2 and 24 h). Cells were washed three times with PBS and stained with DAPI. The distribution of fluorescein-labeled peptides was analyzed by a Leica DMI3000B fluorescence microscope.

WST-1 growth assay. Cells were seeded in 96-well plates and treated with Tat-SmacN7 peptides at various doses (10, 50, 100 and 500 nM, 1, 5, 10 and 100 μ M) for 24 h. WST-1 (Beyotime, China) was added to the microplates 4 h before measuring the cell viability using the Multi-Mode Microplate Reader (Synergy HT, BioTek).

Radiation exposure and clonogenic assay. The clonogenic colony formation assay was done on single-cell suspension. Briefly, cells were seeded in RPMI-1640 medium into 6-well plates (Corning, China), after adherence to the flask

the next day, cells were treated with Tat-SmacN7 peptides at the concentration of 20 μ M, 4 h later, cells were irradiated at room temperature in different doses of radiation (0, 2, 4 and 6 Gy) with Cr¹³⁷-ray (Atomic Energy of Canada) at a dose rate of 0.71 116 Gy/min. After 10-14 days, colonies were stained with gentian violet solution for 3 min and counted. Calculation of survival fractions (SF) was done using the equation SF = colonies counted/cells seeded \times (PE/100), taking into consideration the individual plating efficiency (PE). All experiments were repeated at least 2 times.

Annexin V-FITC/PI staining and flow cytometry for determination of apoptosis. Cells from single and combination treatments were harvested and processed with a FITC Annexin V Apoptosis Detection kit 1 (BD Biosciences) for flow cytometric analysis for quantitative determination of apoptosis.

Western blot analysis. Western blot analysis was done using the following antibodies: rabbit anti-XIAP from Abcam (AB21278), mouse anti- β -actin (CW0623A, Shanghai, China), goat anti-rabbit IgG and goat anti-mouse IgG conjugated to horseradish peroxidase (ZSGB, Biotechnology).

RNA extraction, cDNA synthesis and quantitative real-time PCR. Total RNA was purified and carried out as described previously (30). Equal amounts of total RNA was reverse-transcribed using PrimeScript RT reagent kit (Takara Co., Japan) according to the manufacturer's instructions. cDNA samples were mixed with primers and SYBR Master Mix (Life Technologies) in a total volume of 25 μ l. All samples were analyzed in triplicate using an ABI PRISM 7500 Sequence Detection system (Applied Biosystems-Life Technologies). The threshold cycle (C_T) values for each reaction were determined and averaged using the TaqMan SDS analysis software (Applied Biosystems-Life Technologies). The changes in the expression of a target gene were calculated by the comparative C_T method (fold changes = 2^[- $\Delta\Delta$ CT]). PCR primers for the caspase-3, -8 and -9 and the housekeeping gene GAPDH were obtained from Sangon Biotech (Shanghai, China). The specific primer pairs used were: CASP3, 5'-ATCACAGCAAAAG GAGCAGTTT-3' (forward) and 5'-ACACCACTGTCTGTC TCAATGC-3' (reverse); CAPS8, 5'-TAGGGACAGGAATGG AACACA-3' (forward) and 5'-TGGGAGAGGATACAGCAG ATG-3' (reverse); CASP9, 5'-TCTGGAGGATTTGGTGAT GTC-3' (forward) and 5'-CATTTTCTTGGCAGTCAGGTC-3' (reverse); GAPDH, 5'-ATGACATCAAGAAGGTGGTG-3' (forward) and 5'-CATACCAGGAAATGAGCTTG-3' (reverse).

Caspase activation assay. The activity of caspase-3, -8 and -9, was analyzed using a fluorogenic caspase assay with Ac-DEVD-AFC, Ac-IETD-AFC, Ac-LEHD-AFC (BD Pharmingen) as a substrate, respectively (31). The results are expressed as fold change compared to the control.

Human esophageal ectopic xenografts in athymic nude mice. Six-week-old athymic nude mice were purchased from Vital River (Beijing, China) and housed in the certified animal facility in the Institute of Radiation Medicine, Chinese Academy of Medical Sciences. All experimental procedures

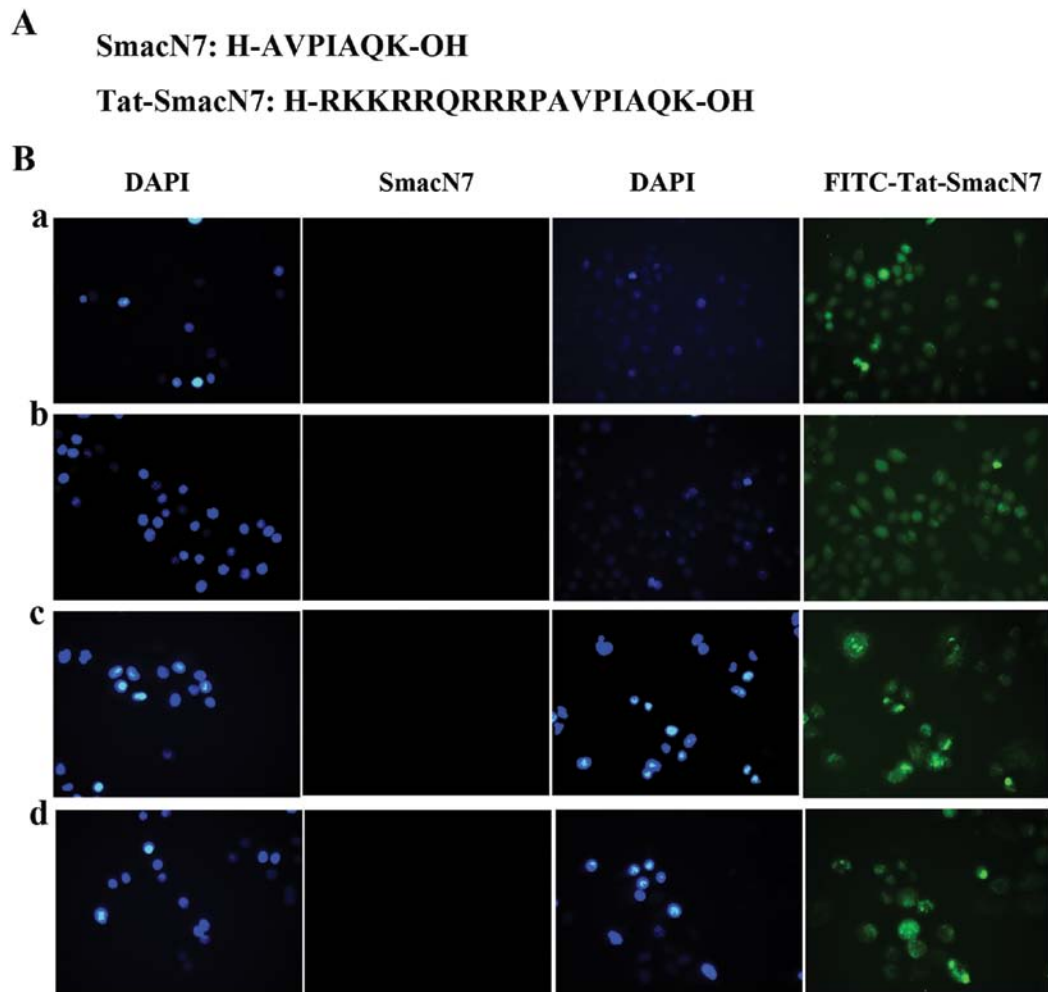


Figure 1. Tat-SmacN7 is cell permeable. (A) Structure of Tat-SmacN7 peptides used in this study. (B) Fluorescence microscopic observation of the cells treated with 20 μ M of SmacN7 or Tat-conjugated SmacN7 peptides (all labeled with fluorescein) respectively for 2 and 24 h, followed by three washes with PBS. (a) Ec109, 2 h; (b) Ec109, 24 h; (c) H460, 2 h; (d) H460, 24 h.

were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). For developing ectopic human esophageal cancer xenografts, Ec109 cells (5×10^6) in 100 μ l of PBS (1:1) were implanted by subcutaneous (s.c.) injection on the right leg of each mouse. Tumors were measured using an external caliper and volume was calculated by the formula: $\pi/6 \times \text{length} \times \text{width} \times \text{height}$. The mice were randomly divided into four groups: control (cont), Tat-SmacN7, radiation, Tat-SmacN7 combined radiation. The treatment started when the tumor size reached 100 mm³ at day 10 after tumor inoculation. Animals in control group did not receive any therapy. Tat-SmacN7 peptides (1 mg/kg) were administered locally twice at day 10 and 12 after tumor inoculation, radiation (2 Gy) were given once a day, 10 days in all. The same nude mice were anaesthetized using chloral hydrate solution (0.35 g/kg) intraperitoneally (i.p.) before radiation was delivered directly to the tumor with the rest of the animal shielded. For combination treatment, Tat-SmacN7 was given 30 min prior to radiation exposure. The tumor growth was measured once every other day, from at least 6 mice in each group.

Results

Tat domain-conjugated NH2 terminus of Smac acts as a cell-permeable compound. Before our investigation into whether synthetic Smac peptides containing the four N-terminal residues are essential for XIAP inactivation and would induce apoptosis in tumor cells in a manner similar to the cytosolic active form of Smac (25), we conducted the NH₂-terminal Smac peptide into cells. To address this question, we used the Tat-SmacN7 which linked the seven N-terminal residues of the Smac protein to the protein-transduction domain of the TAT protein to facilitate intracellular delivery. Cellular uptake of Smac peptides was estimated by fluorescence microscope (Leica DMI3000B). We saw that without the Tat residues, SmacN7 did not accumulate in cells, but Tat-SmacN7 showed sufficient amount of accumulation in cells after being added at 2 h and Tat-SmacN7 is accumulated in cells still after being added at 24 h (Fig. 1B).

Sensitivity of H460 and Ec109 cell lines to radiation or Tat-SmacN7 as a single agent or combination. We first determined radiosensitivity among the two cell lines by classic

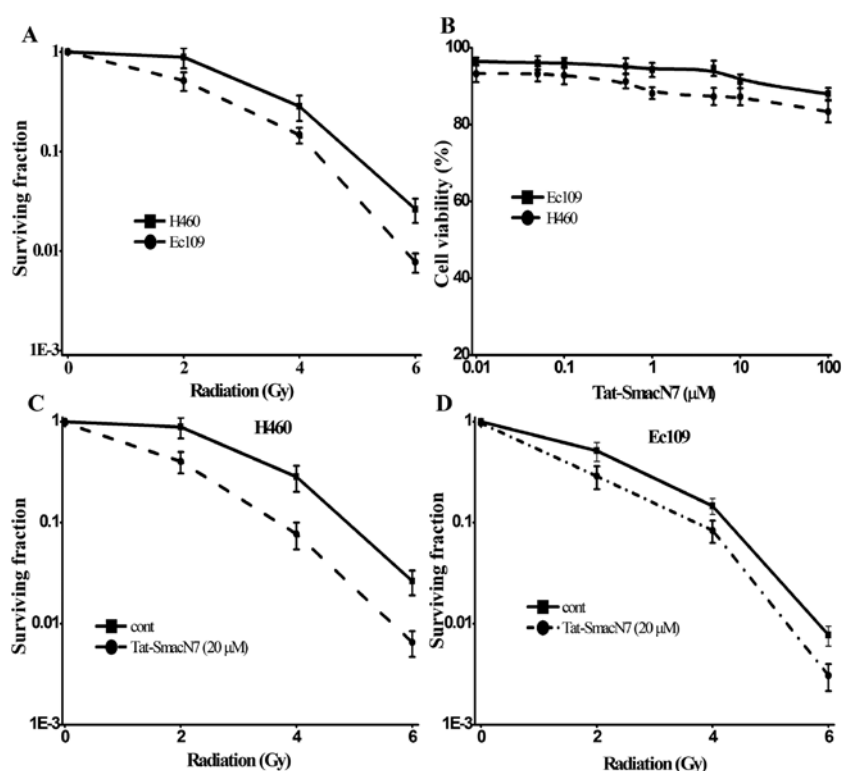


Figure 2. Tat-SmacN7 sensitizes H460 and Ec109 cell lines to radiation. (A) Sensitivity of two cell lines to radiation: the cells were seeded in 6-well plates in triplicate and irradiated. The colonies with >50 cells were counted after 10–14 days. Surviving fraction was calculated as the proportion of seeded cells following irradiation to form colonies relative to that of untreated cells (mean \pm standard deviation, $n=3$). (B) Sensitivity of the cell lines to Tat-SmacN7. Cells were seeded in 96-well plates in triplicate and treated the following day with various concentrations of Tat-SmacN7 for 24 h. Cells were then lysed for WST-1 assay (mean \pm standard deviation, $n=3$). (C and D) Radiosensitization by Tat-SmacN7. Cells were seeded in 6-well plates and irradiated 4 h before adding Tat-SmacN7 (20 μ M) to culture media. SER was calculated as the ratio of the mean inactivation dose under untreated control conditions divided by the mean inactivation dose after Tat-SmacN7 treatment (mean \pm standard deviation, $n=3$).

clonogenic assay after exposure to various doses of radiation (0, 2, 4 and 6 Gy). As shown in Fig. 2A, Ec109 cell lines were relatively sensitive, whereas the H460 were relatively resistant to radiation. We then determined the sensitivity of these lines to Tat-SmacN7 at various drug concentrations ≤ 100 μ M for 24-h treatment, using the WST-1 cell viability assay. Unlike human breast cancer MDA-MB-231 and ovarian cancer SK-OV3 cell lines, in which SM-164, another Smac mimetic compound was highly potent at low nanomolar concentrations (24,30,31), the two lines were highly resistant to Tat-SmacN7 as a single agent (21) as shown in Fig. 2B, two cell lines are resistant to Tat-SmacN7 as a single agent with only 15–20% growth inhibition at 100 μ M.

We next determined the potential radiosensitizing effect of Tat-SmacN7 using drug concentrations at 20 μ M in combination with different doses of radiation. Tat-SmacN7 dose-dependent radiosensitization was observed in H460 and Ec109 cells with a SER (sensitivity enhancement ratio) of 1.63 or 1.51, respectively, as shown in Fig. 2C and D.

Radiosensitization by Tat-SmacN7 is attributable to enhancement of induction of apoptosis. To determine the nature of Tat-SmacN7 radiosensitization, we performed FACS analysis of the two lines treated with Tat-SmacN7 or radiation, alone or in combination. Exposure to 20 μ M Tat-SmacN7 or radiation with 4 Gy induced a moderate level of apoptosis. The combination of radiation and Tat-SmacN7, significantly enhanced

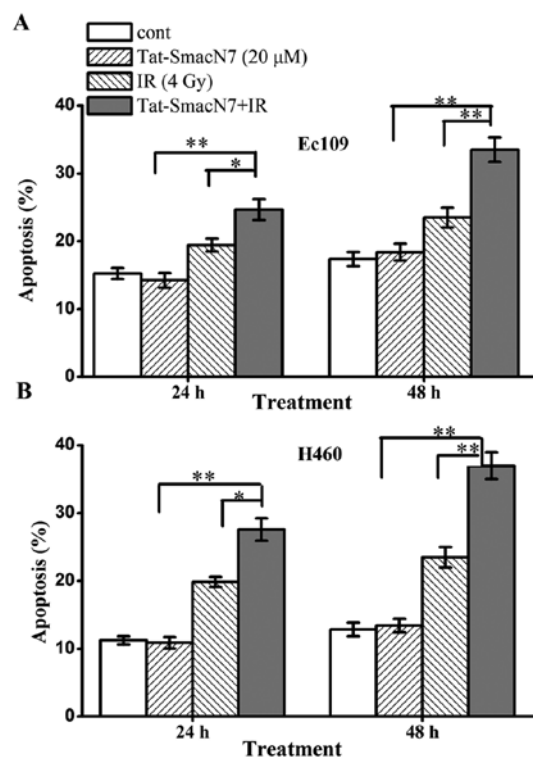


Figure 3. FACS profiling to determine the effect of Tat-SmacN7 on apoptosis. (A) Ec109; (B) H460, cells were treated with indicated agents, alone or in combination, followed by FACS analysis for apoptosis at 24 or 48 h later (* $p<0.05$; ** $p<0.01$) ($n=3$).

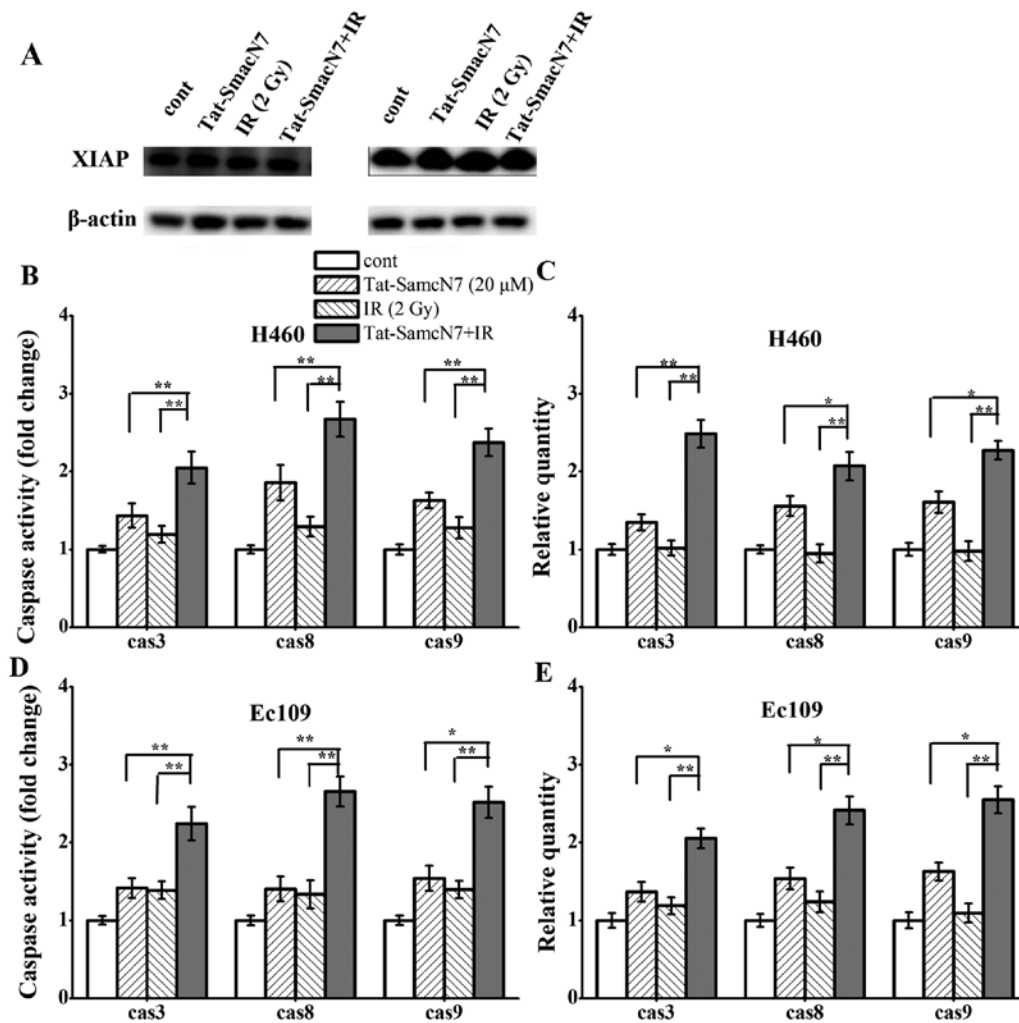


Figure 4. Tat-SmacN7 and radiation promotes caspase expression and activity. (A) Cells were treated with Tat-SmacN7 (20 μ M), radiation (2 Gy) or both and subjected to protein level assay for XIAP followed by immunoblotting. (B-E) RNA expression and activity of caspases. Cells were treated with Tat-SmacN7 (20 μ M), radiation (2 Gy) or both and subjected to RNA expression and activity assay for caspase-3, -8 and -9 (* p <0.05; ** p <0.01). (B and C) H460; (D and E) Ec109.

radiation-induced apoptosis (Fig. 3, p <0.05). Tat-SmacN7 alone caused a time-dependent induction of apoptosis, which was enhanced by radiation (from 24.6 to 33.5% at 48 h in Ec109 cells and from 27.6 to 37% in H460 cells), although radiation alone had a minimal effect on apoptosis induction. Taken together, these results suggest that Tat-SmacN7-mediated radiosensitization in the two cell lines is associated with enhanced apoptosis.

Tat-SmacN7 radiosensitization is dependent on caspase expression and activity. As shown in Fig. 4A, the expression of XIAP protein level varied slightly among the two cell lines with different treatment. Furthermore, in both lines, consistent with previous reports (17,20-22), Smac mimetic compound had no effect on XIAP levels (Fig. 4A). We investigated whether the two cell lines are sensitive to Tat-SmacN7 radiosensitization. Cells were treated with Tat-SmacN7, radiation alone, or the combination and the mRNA expression of caspase-3, -8 and -9 was measured. As shown in Fig. 4B and C, in H460 cells, Tat-SmacN7 alone treatment had a 1.34-, 1.55- and 1.60-fold RNA expression of caspase-3, -8 or -9 effect and had a 1.43-, 1.85- and 1.62-fold activity of the three caspases

respectively, whereas radiation alone had a minimal effect on caspase expression and activation. Combinational treatment caused a significant increase in the caspase RNA expression with a 2.48-, 2.07- and 2.27-fold and activity with a 2.04-, 2.67- and 2.37-fold for caspase-3, -8 and -9, respectively.

Similar results were observed in another sensitive line of Ec109 cells with combination treatment causing maximal activation of caspases (Fig. 4D and E). Tat-SmacN7 alone treatment had a 1.36-, 1.53- and 1.62-fold RNA expression of caspase-3, -8 or -9 effect and had a 1.41-, 1.40- and 1.53-fold activity of these three caspases respectively, the radiation alone had a 1.38-, 1.33- and 1.39-fold effect on caspases expression and activation. Combinational treatment caused a significant increase in caspases mRNA expression with a 2.05-, 2.41- and 2.54-fold and activity with a 2.24-, 2.65- and 2.51-fold increases for caspase-3, -8 and -9.

Blockage of caspase activation largely abrogated Tat-SmacN7 radiosensitization to enhance apoptosis. Finally, we tested if caspase activation, which leads to apoptosis induction, is the main cause for Tat-SmacN7 radiosensitization. We used the pan-caspase inhibitor -z-VAD-fmk to inactivate caspases. As

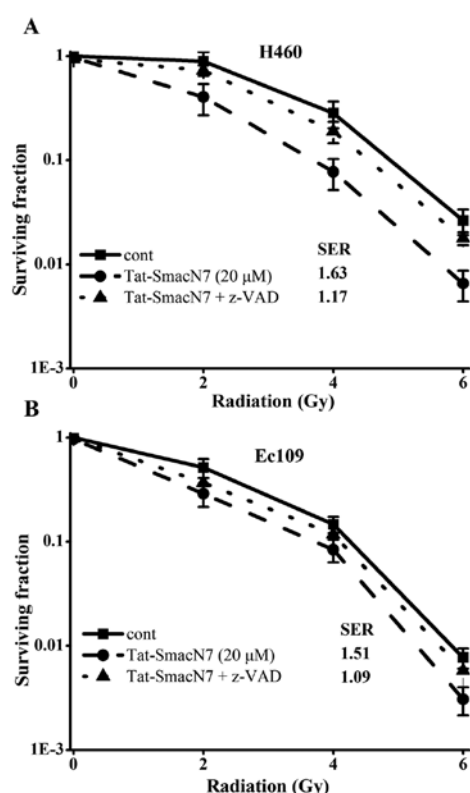


Figure 5. Partial abrogation of Tat-SmacN7 radiosensitization by a pan-caspase inhibitor. Cells were treated with Tat-SmacN7 alone (4 h prior to radiation) or in combination, respectively, with z-VAD-fmk (10 μ M, 6 h prior to radiation). Effect of z-VAD-fmk on Tat-SmacN7 radiosensitization was assessed by clonogenic survival assay (mean \pm standard deviation, $n=2$). (A) H460; (B) Ec109.

shown in Fig. 5, z-VAD-fmk treatment completely blocked apoptotic combination of Tat-SmacN7 and radiation in both Ec109 and H460 cells. Consequently, z-VAD-fmk largely abrogated Tat-SmacN7 radiosensitization with SER reduction from 1.63 to 1.17 in H460 cells and 1.51 to 1.09 in Ec109 cells, both are statistically significant ($p<0.05$).

Tat-SmacN7 sensitizes treatment with radiation in vivo. Finally, we investigated the effects of Tat-SmacN7 in malignant esophageal carcinoma xenograft model *in vivo*. Human Ec109 malignant cells were implanted into the right leg of athymic mice and Tat-SmacN7 peptides, radiation or combinations were locally administered from day 10 after tumor cell inoculation, Tat-smacN7 was given at day 10 and 12 after tumor inoculation, radiation was given every day for 10 days. Treatment with Tat-smacN7 peptides significantly sensitized esophageal carcinoma cells for apoptosis induced by radiation, we next determined the radiosensitizing activity of Tat-SmacN7 *in vivo*. As shown in Fig. 6, administration of Tat-SmacN7 alone at a dose of 1 mg/kg/day twice had no effect on tumor growth in nude mice. Radiation treatment at the clinically relevant dose of 2 Gy/day for 10 days had a moderate antitumor activity. In contrast, the combination of Tat-SmacN7 and radiation caused a remarkable suppression of tumor growth, which is statistically significantly greater than either treatment alone (Fig. 6C). The combination treatment was well-tolerated by the animals with a minimal loss of body weight (Fig. 6D). Radiation is the reason for the decreased body weight. Taken together, our results indicate that Tat-SmacN7 sensitizes Ec109 cells to radiation, as assayed in both *in vitro*

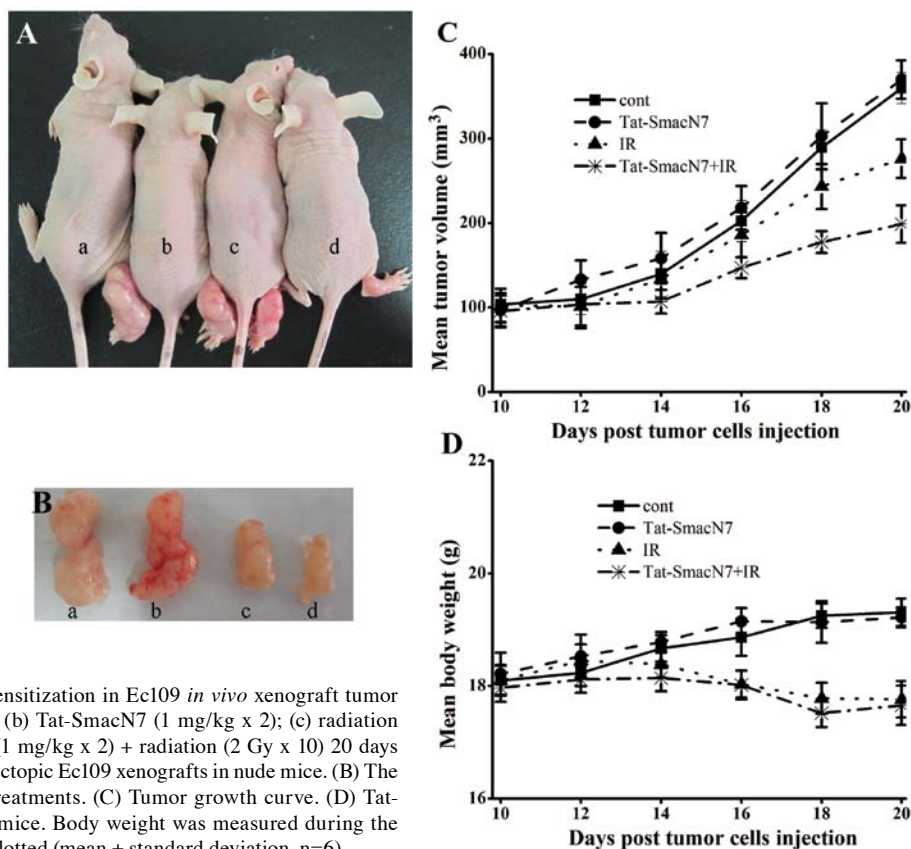


Figure 6. Tat-SmacN7 radiosensitization in Ec109 *in vivo* xenograft tumor model. (A and B) (a) control; (b) Tat-SmacN7 (1 mg/kg \times 2); (c) radiation (2 Gy \times 10); (d) Tat-SmacN7 (1 mg/kg \times 2) + radiation (2 Gy \times 10) 20 days post tumor cell injection. (A) Ectopic Ec109 xenografts in nude mice. (B) The representative tumors after treatments. (C) Tumor growth curve. (D) Tat-SmacN7 is well tolerated by mice. Body weight was measured during the treatment and thereafter and plotted (mean \pm standard deviation, $n=6$).

cell culture and *in vivo* tumor xenograft models and acts as a novel class of radiosensitizer.

Discussion

Because of intrinsic resistance of many tumors to established therapies, current attempts to improve the survival of cancer patients largely depend on methods to target tumor cell resistance and to identify novel anticancer agents (32). The key mechanism is to promote apoptosis of target cells for most antitumor therapies, including chemotherapy, γ -irradiation, immunotherapy and cytokines, but defects in apoptosis programs may cause resistance (30,31), whereas the mechanism of the defects have not been fully determined to find a way to increase the sensitivity of the tumor cells to the therapies. In this study, we determined the radiosensitizing activity of Tat-SmacN7, a small molecule protein that contain an apoptosis promoting AVPI sequence, which can disrupts inhibitory binding of XIAP to caspase-9 and -3 (24). We found that Tat-SmacN7, at non-toxic concentrations, significantly sensitized the tumor cells to radiation both *in vitro* cell culture and *in vivo* xenograft tumor models.

Our study revealed that the two cell lines are resistant to Tat-SmacN7 as a single agent, a potent inhibitor of IAPs (24) and H460 cell lines are more resistant to radiation than Ec109 cell lines. On the other hand, Tat-SmacN7 can act as a radiosensitizing agent equally effectively in both cell types, suggesting that Tat-SmacN7 can be studied as a potent radiosensitizer in some cancer cells, especially for the radioresistant cancer, which is consistent with recent studies showing that overexpression of SMAC protein itself enhanced radiation induced apoptosis in several cancer cell lines, including neuroblastoma, glioblastoma and pancreatic carcinoma and HNSCC cells (21,27). It is well proven that in vertebrate cells, apoptosis proceeds through either the signaling cascade known as the intrinsic mitochondrial or the extrinsic death receptor pathway, both of which converge on activating the executioner caspase-3 and -7 (33,34). Our study revealed mechanistically that cellular sensitivity to Tat-SmacN7 as a single agent is attributable to its intrinsic sensitivity to caspase-8 activation. On the other hand, Tat-SmacN7 radiosensitization in resistant cells is mainly mediated by activation of intrinsic apoptosis pathway because of disruption of XIAP-caspase-9 binding, leading to full activation of caspase-9. We elucidated that the underlying mechanism of Tat-SmacN7 radiosensitization is mainly through increase in the RNA expression of caspases and improve activation of caspases to induce apoptosis. This conclusion was strongly supported by the functional rescue experiments in which decrease of apoptosis through inactivation of caspases using pharmaceutical (z-VAD) approaches largely abrogates Tat-SmacN7 radiosensitization. Thus, induction of apoptosis through caspase activation plays a key role in radiosensitization by Tat-SmacN7 in the two cell types. Compared with the two cell lines, we found that the sensitivity to Tat-SmacN7 radiosensitization is not dependent on the expression of XIAP which can explain the radioresistance to but on the activity of caspases to Tat-SmacN7.

In conclusion, we report here that Tat-SmacN7 is a potent and novel class of radiosensitizer, regardless of the sensitivity to Tat-SmacN7 as a single agent. Tat-SmacN7 radiosensitization

is mainly mediated by caspase activation and the RNA expression through removal of negative blockers cIAP-1 (through degradation) and XIAP (through disrupting its inhibitory binding to active caspase-9), leading to induction of apoptosis. Our study suggests that cancer patients might benefit from Tat-SmacN7-radiation combinational therapy and provides the proof-of-concept for future development of Tat-SmacN7 or other SMAC mimetic compounds as a class of radiosensitizing drugs against cancer cells.

Acknowledgements

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