RITA enhances irradiation-induced apoptosis in p53-defective cervical cancer cells via upregulation of IRE1α/XBP1 signaling

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Abstract. Radiation therapy is the most widely used treatment for patients with cervical cancer. Recent studies have shown that endoplasmic reticulum (ER) stress induces apoptosis and sensitizes tumor cells to radiotherapy, which reportedly induces ER stress in cells. Classical key tumor suppressor p53 is involved in the response to a variety of cellular stresses, including those incurred by ionizing irradiation. A recent study demonstrated that small-molecule RITA (reactivation of p53 and induction of tumor cell apoptosis) increased the radiosensitivity of tumor cells expressing mutant p53 (mtp53). In the present study, we explored the effects and the underlying mechanisms of RITA in regards to the radiosensitivity and ER stress in mtp53-expressing human cervix cancer cells. Treatment with 1 µM of RITA for 24 h before irradiation markedly decreased survival and increased apoptosis in C-33A and HT-3 cells; the effects were not significantly altered by knockdown of p53. In the irradiated C-33A and HT-3 cells, RITA significantly increased the expression of IRE1α, the spliced XBP1 mRNA level, as well as apoptosis; the effects were abolished by knockdown of IRE1α. Transcriptional pulse-chase assays revealed that RITA significantly increased the stability of IRE1α mRNA in the irradiated C-33A and HT-3 cells. In contrast, the same RITA treatment did not show any significant effect on sham-irradiated cells. In conclusion, the present study provides initial evidence that RITA upregulates the expression level of IRE1α by increasing the stability of IRE1α mRNA in irradiated mtp53-expressing cervical cancer cells; the effect leads to enhanced IRE1α/XBP1 ER stress signaling and increased apoptosis in the cells. The present study offers novel insight into the pharmacological potential of RITA in the radiotherapy for cervical cancer.

Introduction

Cervical cancer is the second most common type of cancer in females, with ~530,000 new cases each year and more than 274,000 mortalities worldwide (1). Since cervical cancer is considered a radiosensitive tumor, radiation therapy is the most widely used treatment modality in patients with cervical cancer, particularly for patients at an advanced stage or those who cannot be cured surgically (2). Therefore, cellular radiosensitivity has been a long-term research focus, for it is critical for therapeutic outcomes (3). However, despite progress in radiation technology, local recurrence still occurs in a large proportion of patients due to radioresistance (4). Thus, it is urgent to uncover new targets to enhance the cellular radiosensitivity in cervical cancer.

The endoplasmic reticulum (ER) is an essential subcellular compartment responsible for the synthesis and folding of proteins (5). Different physiological and pathological perturbations interfere with protein folding processes in the ER lumen, leading to accumulation of unfolded or misfolded proteins, a cellular condition termed ER stress (5). ER stress triggers the unfolded protein response (UPR), a transcriptional induction pathway aimed at restoring normal ER functioning (6). If UPR is insufficient to recover ER homeostasis, cells undergo apoptosis (5). The UPR is mediated by three ER stress receptors: protein kinase RNA-like ER kinase (PERK), inositol-requiring protein-1 (IRE1) and activating transcription factor-6 (ATF6), representing three branches of the UPR (7). In the IRE1 branch of UPR, activation of IRE1 leads to cleavage of a 26-nucleotide intron from the XBP1 mRNA. The spliced XBP1 mRNA encodes a stable, active transcription factor that binds to many UPR target genes (8,9). Recent studies have shown that ER stress induces apoptosis and sensitizes tumor cells to ionizing radiation and chemotherapy (10-12), suggesting that ER stress has the potential as a novel target to improve cancer radiotherapy and chemotherapy. In addition, radiotherapy reportedly induces ER stress in cells (13).

Classical key tumor suppressor p53 is involved in the response to a variety of cellular stresses (14). A number of stresses, including damage to chromosomal DNA incurred by ionizing irradiation and exposure to ultraviolet light may activate a p53-mediated growth-suppressive response. Approximately half of all human cancers harbor mutations in p53, which leads to loss of tumor suppressor function and/or
gain of new oncogenic activity (15). Loss of p53 function can contribute not only to aggressive tumor behavior, but also to therapeutic resistance (14).

Small-molecule RITA (reactivation of p53 and induction of tumor cell apoptosis) has been shown to bind p53 directly, inducing a conformational change that prevents its interaction with several inhibitory proteins including MDM2, Parc and iASPP (16). RITA reportedly induces p53-dependent apoptosis in tumor cells expressing wild-type p53 (wtp53), as well as in tumor cells expressing mutant or null p53 (15). A recent study demonstrated that RITA increases radiosensitivity in head and neck squamous cell carcinoma cells expressing mutant p53 (mtp53) (17).

Commonly used human cervical cancer cell lines, C-33A and HT-3, contain p53 codon mutations and are human papillomavirus (HPV)-negative. In contrast, five HPV-positive cervical cancer cell lines (HeLa S-3, Caski, SiHa, C-4I and ME-180) contain wild-type p53 (18,19). It has been reported that RITA may protect p53 from HPV-E6-mediated degradation of any cellular mRNA. Lentiviral transduction of rabbit anti-human polyclonal p53 (FL-393; sc-6243) antibody (Santa Cruz Biotechnology), rabbit anti-human polyclonal IRE1α (H-190; sc-20790) antibody (Santa Cruz Biotechnology) or mouse anti-human monoclonal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (6C5; sc-32233) antibody (Santa Cruz Biotechnology) and then washed and revealed using bovine anti-rabbit (sc-2370) or anti-mouse (sc-2371) secondary antibody (1:5,000, 1 h). Peroxidase was revealed with a GE Healthcare ECL kit (Shanghai, China). Three independent experiments were performed.

Materials and methods

Cell culture and radiosensitivity assay. Human cervix cancer cell lines C-33A (HTB-31) and HT-3 (HTB-32) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Dulbecco’s modified Eagle medium (DMEM; Life Technologies, Carlsbad, CA, USA) containing 10% heat-inactivated FBS (Life Technologies) and 100 U/ml penicillin-streptomycin (Sigma-Aldrich, Beijing, China) in an incubator with a humidified atmosphere of 95% air and 5% CO2 at 37˚C. Cells were plated at 4x105 cells/well into 96-well plates and cultured for 3 h for the cells to adhere to the plates. Then, the cells were cultured with or without 1 µM of RITA for 24 h before irradiation, which was performed at a dose rate of 200 cGy/min for the time required to generate dose curves of 2, 4, 6 and 8 Gy with linear accelerator Clinac 2100C (Varian Medical Systems, Palo Alto, CA, USA) operating at 6 MV. Corresponding controls were sham-irradiated. Colony-forming assays were performed immediately after irradiation by plating the cells into 6-well culture dishes. After 17 days, the colonies were fixed with 6.0% glutaraldehyde, stained with 0.5% crystal violet and counted. Survival curves were fitted with GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA). Each experiment was repeated three times in duplicates.

Stable lentiviral transduction. The p53 (sc-29435-V) and the IRE1α (sc-40705-V) shRNA lentiviral particles purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) contain expression constructs encoding target-specific shRNA designed to specifically knock down human p53 and IRE1α gene expression, respectively. The control shRNA lentiviral particles (sc-108080; Santa Cruz Biotechnology) contain a scrambled shRNA sequence that will not lead to specific degradation of any cellular mRNA. Lentiviral transduction was performed, and pools of stable transductants were generated via selection with puromycin (6 µg/ml; Sigma-Aldrich) according to the manufacturer’s instructions (Santa Cruz Biotechnology).

Western blot analysis. C-33A and HT-3 cells was lysed with a hypotonic buffer containing 2% Nonidet-P and a protease inhibitor cocktail (Sigma-Aldrich) by sonication three times for 3 sec on ice. The supernatant obtained after centrifugation at 2,000 x g for 15 min at 4˚C was used for protein concentration determination by the Coomassie blue method. Equal amounts of proteins for each sample were separated on 10% SDS-polyacrylamide gel and blotted onto a polyvinylidene difluoride microporous membrane (Millipore, Billerica, MA, USA). Membranes were incubated for 1 h with a 1:1,000 dilution of rabbit anti-human polyclonal p53 (FL-393; sc-6243) antibody (Santa Cruz Biotechnology), rabbit anti-human polyclonal IRE1α (H-190; sc-20790) antibody (Santa Cruz Biotechnology) or mouse anti-human monoclonal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (6C5; sc-32233) antibody (Santa Cruz Biotechnology) and then washed and revealed using bovine anti-rabbit (sc-2370) or anti-mouse (sc-2371) secondary antibody (1:5,000, 1 h). Peroxidase was revealed with a GE Healthcare ECL kit (Shanghai, China). Three independent experiments were performed.

Real-time quantitative reverse transcription PCR. RNA was prepared from the cells using TRIzol reagent. The cDNAs were synthesized using SuperScript II reverse transcriptase (Life Technologies). Real-time quantitative PCR was performed on an ABI-Prism 7700 Sequence Detection System, using the fluorescent dye SYBR-Green Master Mix (Applied Biosystems, Beijing, China) as described by the manufacturer. The primers used were as follows: for IRE1α, 5'-GCCACC CTGCAAGAGTAGTG-3' (forward) and 5'-ATGTTGAGG GAGTGGAGGTG-3' (reverse); for spliced XBP1 mRNA (XPB1spl), 5'-TGCTGAGTCCGCAGGCTTTG-3' (forward) and 5'-GCTGGCAGGCTCTGGGGAAG-3' (reverse); for GAPDH, 5'-GACTCATGACCACAGTCCATG C-3' (forward) and 5'-AGAGGCAGGGATGATGTTCTG-3' (reverse). Relative quantification of the level of IRE1α mRNA or spliced XPB1 mRNA was determined using the 2-ΔΔCt method and normalized against that of GAPDH in the same sample (19). Each experiment was repeated three times in duplicates.

Luciferase assay. Cells were transfected with a commercially available human IRE1α promoter/luciferase reporter (S720185; SwitchGear Genomics, Shanghai, China) using Lipofectamine 2000 transfection reagent (Life Technologies) and then cultured for 24 h. Luciferase assays were performed with the LightSwitch luciferase assay kit (LS010; SwitchGear Genomics) according to the manufacturer’s instructions. Each experiment was repeated three times in duplicates.

Transcriptional pulse-chase assay. A Click-iT Nascent RNA Capture kit (C-10365; Life Technologies) was used to determine the stability of IRE1α mRNA according to the manufacturer’s instructions. Briefly, C-33A and HT-3 cells were labeled with
To explore the effects of RITA on the radiosensitivity of mtp53-expressing cervical cancer cells, we treated human C-33A and HT-3 cervical cancer cells with 1 µM of RITA for 24 h before irradiation at 2, 4, 6 and 8 Gy. As shown in Fig. 1, RITA markedly decreased cell survival under irradiation compared with the controls, indicating that RITA may increase the radiosensitivity of C-33A and HT-3 cells. To determine the potential role of p53 in the radiosensitivity-enhancing effect of RITA, we knocked down p53 in both cell lines, even though the cells only express mtp53. As shown in Fig. 2, stable transduction of lentiviral p53-shRNA knocked down the endogenous p53 expression by over 85% in both the C-33A and HT-3 cells. Obviously, knockdown of p53 did not show any significant effect on RITA-induced radiosensitivity in the cells (Fig. 1).

To explore the effects of RITA on irradiation-induced apoptosis in mtp53-expressing cervical cancer cells, we next measured apoptosis in the C-33A and HT-3 cells with or without RITA treatment under sham 0 or 6 Gy of irradiation. As shown in Fig. 3, compared with the controls, RITA treatment at 1 µM for 24 h showed no significant effect on apoptosis in the C-33A and HT-3 cells under sham irradiation. The RITA IC50 value for C-33A and HT-3 cells under sham irradiation was calculated to be 15.6 and 18.3 µM, respectively. However, in the cells under irradiation, RITA treatment at 1 µM for 24 h increased the apoptosis rate by ~2-fold in both cell lines compared with the controls (Fig. 3). Knockdown of p53 did not significantly alter the apoptotic effect of RITA on the cells under irradiation (Fig. 3).

Collectively, the findings suggested that RITA induced radiosensitivity and irradiation-induced apoptosis in mtp53-expressing cervical cancer cells by a p53-independent mechanism.

**Effects of RITA/IRE1α signaling on survival and apoptosis of mtp53-expressing cervical cancer cells under irradiation.** As shown in Fig. 4, compared with the controls, RITA showed no significant effect on the protein level of IRE1α in the C-33A and HT-3 cells under sham irradiation. However, in cells under irradiation, RITA increased the protein level of IRE1α by 0.2 mM ethynyl uridine (EU) and incubated at 37°C for 4 h. The cells were then allowed to recover in EU-free medium for 0, 1, 2 or 4 h, respectively. Total RNA was extracted and 5 µg of total RNA was mixed with Click-IT reaction cocktail. Immediately, the reaction buffer additive 1 was added, followed by reaction buffer additive 2 exactly 3 min after addition of the first additive and the reaction was carried out for 30 min at room temperature. Following incubation, the ‘clicked’ RNA was re-purified with ammonium acetate precipitation and captured by streptavidin magnetic beads. The captured RNA was in-bead converted to cDNA using SuperScript III reverse transcriptase (Life Technologies). Then, the level of the IRE1α mRNA or the spliced XBP1 mRNA was determined with real-time quantitative reverse transcription PCR as described above.

**Statistical analysis.** Statistical analyses were performed with SPSS for Windows 10.0 (SPSS Inc., Chicago, IL, USA). All data values are expressed as means ± SD. Comparison of means between two groups was performed with Student’s t-tests. Comparisons of means among multiple groups were performed with one-way ANOVA followed by post hoc pairwise comparisons using Tukey’s tests. A two-tailed P<0.05 was considered statistically significant in the present study.

**Effects of RITA on survival and apoptosis of mtp53-expressing cervical cancer cells under irradiation.**

**Results**

**Effects of RITA on survival and apoptosis of mtp53-expressing cervical cancer cells under irradiation.** To explore the effects
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2.5-fold in the C-33A cells and by ~3.6-fold in the HT-3 cells, respectively (Fig. 4). Notably, compared with the controls under sham irradiation, the irradiation treatment increased the protein level of IRE1α by ~3-fold in the C-33A and by ~2-fold in the HT-3 cells, respectively (Fig. 4). Knockdown of p53 did not significantly alter the effect of RITA on the expression of IRE1α in the presence or absence of irradiation (Fig. 4).

To determine the potential role of IRE1α in the radiosensitivity-enhancing effect of RITA on mtp53-expressing cervical cancer cells, we knocked down IRE1α in the C-33A and HT-3 cells. As shown in Fig. 5, even under irradiation, stable transduction of lentiviral IRE1α-shRNA knocked down the endogenous IRE1α expression by ~80% in both the C-33A and HT-3 cells; the addition of RITA treatment (1 µM for 24 h) only partially restored the expression of IRE1α by 28% in the C-33A cells and by 37% in the HT-3 cells, respectively (Fig. 5). As shown in Fig. 6, RITA markedly decreased cell survival under irradiation compared with the controls, which was largely reversed by the knockdown of IRE1α. Notably, knockdown of IRE1α itself did not significantly improve cell survival compared with the controls (Fig. 6). Similarly, in the cell apoptosis assays, whereas knockdown of IRE1α reversed the apoptotic effect of RITA in the C-33A and HT-3 cells under irradiation, it did not significantly decrease...
cell apoptosis compared with the controls. Collectively, the findings suggested that RITA enhanced radiosensitivity and irradiation-induced apoptosis in the mtp53-expressing cervical cancer cells largely through inducing the expression of IRE1α.

In the IRE1 branch of ER stress-triggered UPR, activation of IRE1 leads to cleavage of a 26-nucleotide intron from the XBP1 mRNA. The spliced XBP1 mRNA is considered to be an important marker for ER stress, particularly for IRE1-mediated UPR (8,9). We next examined whether RITA enhanced ER stress in the C-33A and HT-3 cells under irradiation, using XBP1 as a marker. As shown in Fig. 8, compared with the controls, RITA showed no significant effect on the spliced mRNA level of XBP1 in the C-33A and HT-3 cells under sham irradiation. However, in cells under irradiation, RITA increased the spliced mRNA level of XBP1 by ~1.8-fold in the C-33A cells and by ~3-fold in the HT-3 cells, respectively, which was completely abolished by the knockdown of IRE1α (Fig. 8). The findings suggested that RITA enhances
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Effects of RITA on the stability of IRE1α mRNA in mtp53-expressing cervical cancer cells under irradiation. As shown in Fig. 9A, compared with the controls, RITA markedly elevated the mRNA level of IRE1α in the C-33A and HT-3 cells under irradiation, indicating that RITA induced the expression of IRE1α at the mRNA level in mtp53-expressing cervical cancer cells under irradiation. To test whether RITA exerted this effect through transactivation of the IRE1α gene promoter, we transfected the C-33A and HT-3 cells with a human IRE1α gene promoter/luciferase reporter. As shown in Fig. 9B, luciferase reporter assays revealed that RITA had no significant effect on the IRE1α promoter activity in the presence or absence of irradiation treatment, suggesting that RITA did not upregulate the IRE1α mRNA level in the irradiated C-33A and HT-3 cells at the gene promoter/transcription level. We next examined the effect of RITA on the stability of irradiation-induced ER stress through the IRE1α/XBP1 branch and promotes apoptosis in mtp53-expressing cervical cancer cells.

Effects of RITA on the stability of IRE1α mRNA in mtp53-expressing cervical cancer cells under irradiation. As shown in Fig. 9A, compared with the controls, RITA markedly elevated the mRNA level of IRE1α in the C-33A and HT-3 cells under irradiation, indicating that RITA induced the expression of IRE1α at the mRNA level in mtp53-expressing cervical cancer cells under irradiation. To test whether RITA exerted this effect through transactivation of the IRE1α gene promoter, we transfected the C-33A and HT-3 cells with a human IRE1α gene promoter/luciferase reporter. As shown in Fig. 9B, luciferase reporter assays revealed that RITA had no significant effect on the IRE1α promoter activity in the presence or absence of irradiation treatment, suggesting that RITA did not upregulate the IRE1α mRNA level in the irradiated C-33A and HT-3 cells at the gene promoter/transcription level. We next examined the effect of RITA on the stability of
IRE1α mRNA with transcriptional pulse-chase assays, using a Click-iT Nascent RNA Capture kit (Life Technologies). Briefly, immediately after irradiation treatment, the C-33A and HT-3 cells were labeled with EU and incubated at 37˚C for 4 h. Cells were then allowed to recover in EU-free medium for 0, 1, 2 or 4 h, respectively. Then, the labeled RNA was captured and subjected to real-time reverse transcription PCR assays to determine the IRE1α mRNA. As shown in Fig. 10, at 1 h, the IRE1α mRNA level in the control cells dropped to 62-65% of that at 0 h; at 4 h, the IRE1α mRNA level in the control cells dropped to ~10% of that at 0 h. In cells treated with RITA, however, the IRE1α mRNA level at 1 h remained above 85% of that at 0 h; at 4 h, the IRE1α mRNA level remained above 40% of that at 0 h. The findings suggested that in mtp53-expressing cervical cancer cells under irradiation, RITA induced the expression of IRE1α mainly by increasing its mRNA stability.

Discussion

In the present study, we demonstrated that RITA enhanced irradiation-induced apoptosis in mtp53-expressing cervical cancer cells mainly by upregulating the expression of IRE1α and IRE1α/XBP1 signaling.

RITA has been shown to reactivate wtP53 function as well as to rescue the function of p53 mutants in different types of human tumor cells and induce p53-dependent apoptosis (22). It reportedly may protect p53 from HPV-E6-mediated degradation in HPV-positive wtP53-expressing cervical cancer
Recent studies have explored the effect of RITA on mtp53-expressing cervical cancer cells. However, few studies have examined the role of RITA in enhancing radiosensitivity in mtp53-expressing cervical cancer cells. Thus, we employed C-33A and HT-3 cells, two human cervical cancer cell lines expressing mtp53, as cell models in the present study. At the concentration of 1 µM, RITA markedly enhanced apoptosis in the mtp53-expressing cervical cancer cells under irradiation, but not in those under sham irradiation. The radiosensitivity-enhancing effect of RITA at 1 µM was obviously p53-independent, since it was not altered by knockdown of p53. Much higher concentrations were needed for RITA to induce significant apoptosis in the mtp53-expressing cervical cancer cells under sham irradiation in the present study (15.6 for C-33A and 18.3 µM for HT-3 cells, respectively). Collectively, the findings suggest that RITA is an effective enhancer of radiosensitivity for mtp53-expressing cervical cancer cells and therefore, if used in conjunction with radiotherapy, may be potentially beneficial for patients with mtp53-expressing cervical cancer. This is important
since radiation therapy is the most broadly used treatment for patients with cervical cancer, particularly patients at an advanced stage or those who cannot be cured surgically (2).

ER stress induced by protein misfolding is an important mechanism in cellular stress (23). ER stress triggers the UPR to restore normal ER functioning (6). The UPR is mediated by three principal classes of stress sensors including PERK, ATF6 and IRE1 (8), which operate in parallel and use unique mechanisms of signal transduction to orchestrate adaptation to ER stress (5,8). However, if UPR is insufficient to reverse the ER stress or recover ER homeostasis, the cell fate switches to apoptosis (5). Recent studies have shown that ER stress induces apoptosis and sensitizes tumor cells to ionizing radiation (10-12), suggesting that ER stress has the potential as a novel target to improve cancer radiotherapy, which reportedly induces cellular ER stress (13). In the present study, irradiation led to irreversible ER stress in the C-33A and HT-3 cells, as indicated by the significantly increased IRE1α/XBP1 ER stress signaling and apoptosis in the irradiated cells compared with that in the sham-irradiated cells. RITA markedly enhanced IRE1α/XBP1 signaling and apoptosis in the irradiated C-33A and HT-3 cells, suggesting that RITA enhanced apoptosis in irradiated mtp53-expressing cervical cancer cells by enhancing irradiation-induced ER stress, mainly through the IRE1α/XBP1 signaling pathway. Knockdown of p53 did not alter this effect of RITA, confirming that it was through a p53-independent mechanism. Some previous studies have shown that ER stress sensitizes or stimulates wtp53-dependent apoptosis (24,25), while others have shown that ER stress prevents or inhibits wtp53-dependent apoptosis (26-29). The discrepancies can be attributed to different cell models or stimuli used in the studies. Nevertheless, since RITA is an established activator of p53, it may be intriguing to determine whether and how RITA may affect radiosensitivity and apoptosis in irradiated wtp53-expressing cervical cancer cells. We will focus on these issues in future studies.

We found that RITA increased the expression of IRE1α in the irradiated C-33A and HT-3 cells at both the protein and the mRNA levels. Transcriptional pulse-chase assays immediately after irradiation revealed that treatment with 1 µM of RITA for 24 h before irradiation significantly increased the stability of IRE1α mRNA. This effect was manifested at the IRE1α protein level, spliced XBP1 mRNA level and the cell apoptosis level 24 h after irradiation, confirming RITA as an effective and efficient enhancer of irradiation-induced ER stress/apoptosis in mtp53-expressing cervical cancer cells. The mechanism of how RITA increases IRE1α mRNA stability in irradiated mtp53-expressing cervical cancer cells is still unclear and will be explored in our future studies.

Our findings suggest that RITA may be useful as an enhancer of radiosensitivity for mtp53-expressing cancer cells. Since approximately half of all human cancers harbor mutations in p53 (15) and we only tested mtp53-expressing cervical cancer cell models in the present study, it would be beneficial to ascertain whether RITA can enhance irradiation-induced ER stress and apoptosis in other irradiation-sensitive cancers harboring mtp53. Moreover, as certain chemotherapeutic agents reportedly induce tumor cell apoptosis mainly through stimulation of ER stress, it may also be beneficial to discover whether RITA can enhance chemotherapeutic agent-induced ER stress/apoptosis in cancers harboring mtp53. Furthermore, PERK, ATF6 and IRE1 represent three branches of the UPR (5,8). We found in the present study that RITA is mainly affected through the IRE1 branch of UPR. Whether the PERK and the ATF6 branches of UPR could be critically involved in the potential effects of RITA on irradiation- or chemotherapeutic agent-induced ER stress/apoptosis in other types of mtp53-expressing cancers still needs to be elucidated in future studies.

In conclusion, the present study provides initial evidence that RITA upregulates the expression level of IRE1α by increasing the stability of IRE1α mRNA in irradiated mtp53-expressing cervical cancer cells; the effect leads to enhanced IRE1α/XBP1 ER stress signaling and increased apoptosis in the cells. The present study offers novel insight into the pharmacological potential of RITA in the radiotherapy for cervical cancer.

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