Int J Clin Exp Med 2016;9(9):17569-17578
www.ijcem.com /ISSN:1940-5901/IJCEM0033460

Original Article

RITA has growth inhibitory activity on colon cancer HCT116 cells expressing wild-type p53, but not SW480 cells harboring mutant p53, via repressing wild-type p53 ubiquitination

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Received June 7, 2016; Accepted August 14, 2016; Epub September 15, 2016; Published September 30, 2016

Abstract: Functional deficiency and disturbance of p53, primarily induced by post-translational modification and gene mutation, are extensively associated with hallmarks of colorectal cancer, including tumorigenesis, progression and therapeutic responses. RITA is a small molecule that can modulate both wild-type p53 (WT-p53) and mutant p53 (MT-p53) in various of cancers and presents anti-cancer activity. This study investigated the growth-inhibitory effects of RITA on two different human colon cancer cell lines, HCT116 and SW480, which mainly express WT-p53 and MT-p53 respectively. Western blotting was conduced to assess WT-p53 protein level in these two types of cells after treated with RITA. Mechanistic studies investigated impacts of RITA on WT-p53 gene expression, WT-p53 ubiquitination, and protein levels of MDM2 (an E3 ubiquitin ligase mediated WT-p53 proteasomal degradation) and WT-p53 downstream targets including p21 and Bax. RITA elevated WT-p53 protein levels in HCT116 cells and triggered the growth inhibition and apoptosis elevation, but all these effects were not observed in SW480 cells. Silencing WT-p53 in HCT116 cells via RNA interference abolished regulation of RITA on WT-p53, p21 and Bax proteins and the effects on cell growth and apoptosis. RITA had not effect on WT-p53 gene expression and MDM2 protein level, but blocked the linkage of ubiquitin on WT-p53, as reflected by Immunoprecipitation assay. These results collectively indicate that RITA has preferential growth inhibitory activity on colon cancer cells that express WT-p53, but not those cells harboring MT-p53, via repressing WT-p53 ubiquitination, therefore suggesting a limited utilization of RITA in the management of colon cancer.

Keywords: RITA, colon cancer, wild-type p53, mutant p53, ubiquitination

Introduction

The transcription factor p53 has been long recognized to act as a principal tumor-suppressor in various cells, which functions to block tumor formation and development by triggering cell cycle arrest, apoptosis, or senescence. Wild-type p53 (WT-p53) is described as "the guardian of the genome". When DNA is impaired, WT-p53 transactivates many regulators (e.g. p21) involved in cell-cycle control, resulting in cell-cycle arrest in both G1 and G2 phases [1]. The cell-cycle arrest provides time for DNA repair and prevents transmission of incorrect genetic information to daughter cells during following cell divisions, which has been demonstrated to be critically important in decreasing the risk of tumorigenesis [2]. In response to a variety of cellular stimuli, like irreparable DNA damage induced by DNA-damaging agents or radiation, oncogene activation, hypoxia and telomere erosion, WT-p53 becomes stabilized and activated, and then, initiates apoptotic progression via activating several substrates (e.g. Bax protein) participating in apoptotic pathway [3, 4]. Therefore, WT-p53 is also implicated in the blockage of cancer development and in the regulation of responsiveness to therapy.

Colorectal cancer (CRC), a common malignant tumor of the digestive tract, remains one of the leading causes of cancer-related deaths worldwide, even though several strategies have been applied to cure this fatal disease, including sur-

gical resection, chemotherapy and radiation therapy [5]. Loss of p53 function, either through mutation or post-translational modification, has been extensively reported in CRC, and closely associated with unchecked proliferation, tumor progression, and therapeutic resistance [6]. Mutation of the p53 gene is one of the most frequent genetic alterations in CRC [6]. Mutant p53 (MT-p53) is incapable of recognizing WT-p53 DNA binding sites in the promoter of p53 target genes, resulting the loss of p53 function against cancer, and even worse, some p53 mutations acquire new and distinct oncogenic properties and contribute to malignant process through the interaction with sequence-specific transcription factors, such as NF-Y, E2F1, NF-κB and Vitamin D receptor [7, 8]. Indeed, MT-p53 seem to be capable of activating promoters of genes that are usually not activated by the WT-p53 protein, such as c-myc and MDR1 [9]. Inducible knockdown of endogenous MT-p53 in CRC cell line (SW480) and pancreatic cancer cell line (MIA-PaCa-2) triggers the proliferative defect [9]. Knockin mice that carry one null allele and one mutant allele of the p53 gene (R172H or R270H) developed novel tumors compared to p53-null mice [10, 11]. In other CRC cases, p53 retains its wild-type form, but the p53 is maintained at a low level or becomes functionally inactive by the effect of the over-expressed murine double minute 2 (MDM2) [12]. MDM2 is an E3 ubiquitin ligase, which mediates p53 ubiquitination and proteasomal degradation. Recent studies have unveiled numerous additional actions of MDM2 which are implicated in p53 inactivation. MDM2 binds to the transactivation domain of p53, prevents p53 from interacting with the transcriptional machinery and inhibits p53-responsive gene expression [13]. Besides, MDM2 exports p53 from the nucleus abolishing its transcriptional activity [13]. Therefore, in the past few years, much effort has been made toward identification of small molecules capable of restoring normal p53 functions in cells harboring MT-p53 and of preventing the interaction of MDM2 with p53 to stabilize WT-p53 and maintain its function.

RITA (reactivation of p53 and induction of tumor cell apoptosis, NSC 652287) is a small molecule that has been reported to modify both WT-p53 and MT-p53 in some cancers and presents anti-cancer activity [14]. Mechanistic studies reveal that RITA can bind to the N-terminal p53 domain, which probably leads to conformational changes of p53, and then, impedes p53/MDM2 interaction, resulting in up-regulation of intracellular WT-p53 [14]. RITA is also reported to restore wild-type function of MT-p53, although underlying mechanism remain incompletely defined. Fiorini et al., [7] found that treatment with RITA re-establish the WT-p53 function of pancreatic adenocarcinoma cells bearing MT-p53, reducing the growth rate and inducing apoptosis. In addition, RITA activates WT-p53 response in MT-p53-carrying neuroblastoma cells, inducing the apoptotic cell death [14]. This study was conducted to investigate the growth-inhibitory effects of RITA on two different human colon cancer cell lines, HCT116 and SW480, which express WT-p53 and MT-p53 respectively. Mechanistic studies were carried out to elucidate the interaction of RITA with WT-p53.

Materials and methods

Cell lines and culture

Two different human CRC cell lines, HCT116 and SW480, which mainly express WT-p53 and MT-p53 respectively, were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). HCT116 and SW480 cells were cultured respectively in RPMI-1640 and DMEM mediums (Invitrogen, Life Technologies, Carlsbad, CA, USA) at 37°C in a humidified incubator containing 5% CO_2. These two types of mediums were supplemented with 5% fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin. The cells in the logarithmic growth phase were used to conduct the experiments described as follows.

Cell proliferation assay

The cells were seeded in 96-well plates in 100 μL complete medium and allowed to attach overnight. After the cells were subjected to diverse treatments, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (20 μL at 5 mg/mL; Sigma, St. Louis, MO, USA) was added to each well. The medium was removed 4 h later and 150 μL dimethylsulfoxide was added subsequently to terminate the reaction. The plates were read with a microplate reader at 570 nm.

Flow cytometry analysis of apoptotic cells

Apoptosis was detected using annexin V-FITC/propidium iodide (PI) staining followed by flow cytometry. After subjected to diverse treatments, the cells were washed twice with cold PBS and incubated with annexin V-FITC and PI at room temperature for 15 min in the dark. The cells were collected and analyzed by a FACSCanto II flow cytometer (Becton Dickinson Immunocytometry System).

Western blot analysis

The cells were lysed with RIPA buffer (Beyotime, Changsha, China). Equal amounts (20 μg) of total protein were loaded and separated on SDS-PAGE gels. Proteins were transferred to nitrocellulose membranes and subsequently immunoblotted with the primary antibodies, including anti-MDM2, anti-WT-p53, anti-Bax, anti-p21 and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies (Santa Cruz, CA, USA). Membranes were washed with TBS-T, and then incubated with secondary antibody. Proteins were detected using the Amersham enhanced chemiluminescence system (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. The blots were visualized by enhanced chemiluminescence using Fuji medical X-ray film (Fujiilm, Tokyo, Japan).

Construct of cell line with transient silencing of WT-p53

The cells were seeded at 60% confluence in the mediums without serum and antibiotics. WT-p53 in HCT116 cells was transiently silenced using three different small hairpin RNAs (shRNAs) targeting WT-p53 (shRNAs-WT-p53), which were synthesized by GenePharma Co., Ltd (Shanghai, China). shRNAs-WT-p53 and corresponding scrambled siRNA were transfected into HCT116 cells with using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. Validation of WT-p53 knockdown was assessed by qPCR and Western blotting.

Colony formation assay

Two hundred and fifty cells were plated in 6-well culture plates and cultured for two weeks. These cells were then fixed with methanol and stained with crystal violet solution. The numbers of colonies containing ≥ 30 cells were counted under the microscope. These experiments were repeated three times.

Quantitative real-time reverse transcriptase (qRT-PCR)

The total RNA of cells was extracted with TRIzol reagent (TaKaRa, Tokyo, Japan). The RNA was treated with DNA-free DNase and reverse-transcribed for cDNA synthesis. qRT-PCR analysis was performed using the SYBR Green Reaction Kit (TaKaRa, Tokyo, Japan) on a MJ Mini™ Gradient Thermal Cycler Real-Time PCR machine (Bio-Rad, California, USA). The amplification of WT-P53 and GAPDH genes (oligonucleotide primers: p53: 5'-AATTTGCGTGTGGAGTATTT-3' and 5'-GTGGAGTCTTCCAGTGTGAT-3'; GAPDH: 5'-TGGGCTACACTGAGCACCAG-3' and 5'-GGGTGTCGCTGTTGAAGTCA-3') was operated as follows: 5 min at 94°C (one cycle) and 30 sec at 94°C; 30 sec at the annealing temperature of 55°C; and 50 sec at 70°C (35 cycles) and 72°C for 10 min (one cycle). Expression data were uniformly normalized to GAPDH as an internal control, and the relative expression levels were evaluated using the $^{-\Delta\Delta}$Ct method as described previously [15].

Immunoprecipitation assay

Cells were lysed with immunoprecipitation assay lysis buffer (RIPA), in the presence of a protease inhibitor cocktail (Sigma). Equal amounts of cell lysates were then incubated with nickel beads conjugated to anti-WT-p53 antibody for 3 h, followed by wash IP buffer (50 mmol/L Tris, pH 7.5, 5 mmol/L EDTA, 150 mmol/L NaCl, 0.5% NP-40). Bound proteins were detected by Western blotting using anti-ubiquitin primary antibody and HRP-conjugated secondary antibody.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software package (version 16.0, Chicago, IL, United States). Data were expressed as mean ± S.D. For statistical analysis the Student's t test was applied. Every experiment was repeated at least three times. A P value < 0.05 was considered to be statistically significant.

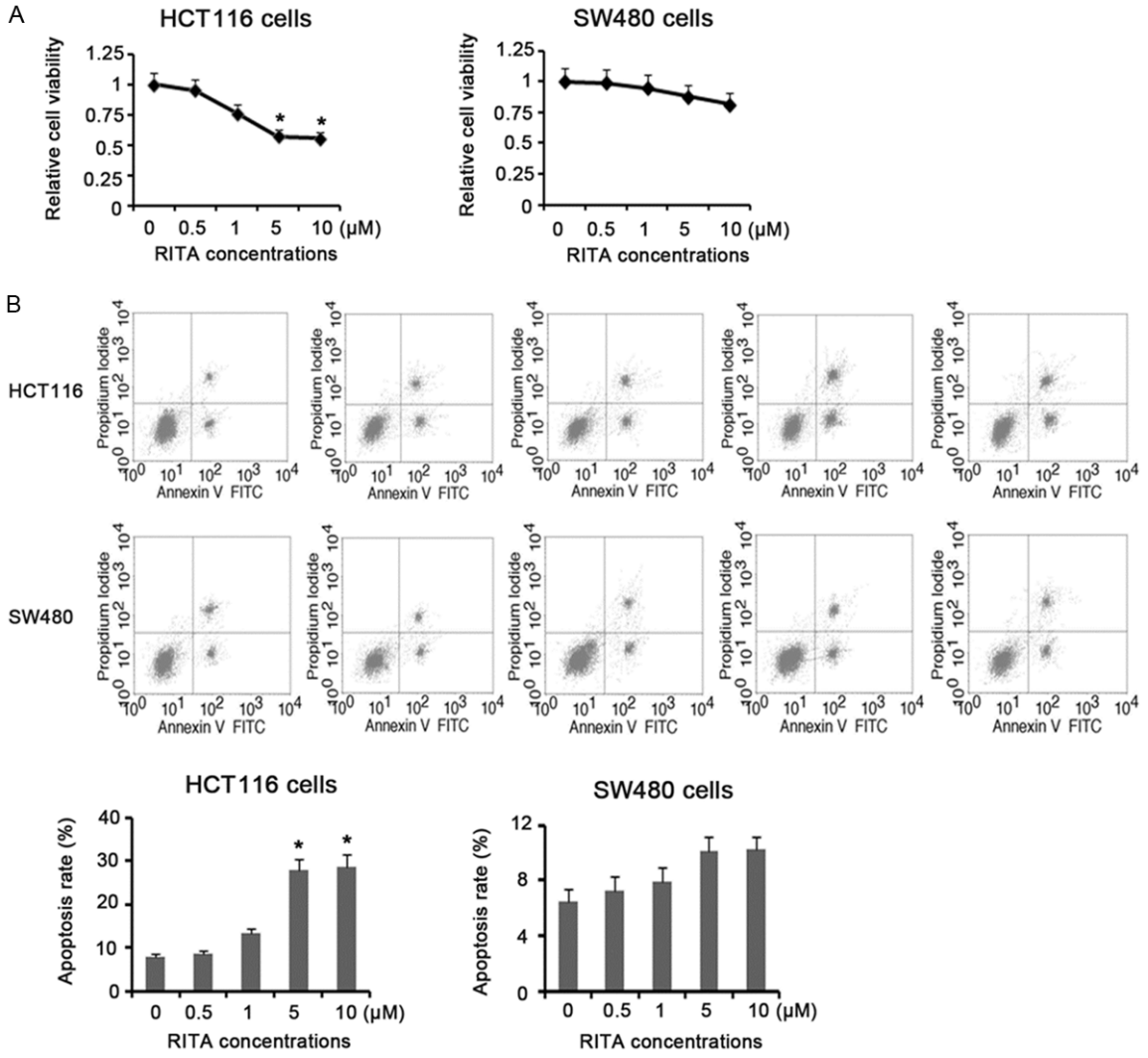

Figure 1. Anti-proliferative and apoptotic effects of RITA on human CRC cells. CRC HCT116 and SW480 cells were exposed to different doses of RITA (0, 0.5, 1, 5 and 10 μM) for 72 h. MTT assay (A) and flow cytometry detection (B) were performed to evaluate effects of RITA on the cell viability and apoptosis rate, respectively. Experiments were repeated at least three times. Each bar represents the mean of three independent experiments. *$P < 0.05$ *vs.* control group.

Results

Anti-proliferative and apoptotic effects of RITA on human CRC cells

We initially exposed HCT116 and SW480 cells to different doses of RITA (0, 0.5, 1, 5 and 10 μM) for 72 h, to determine anti-proliferative and apoptotic effects of RITA on these two types of cells. MTT detection showed viability of HCT116 cells was attenuated by RITA in a dose-dependent manner (**Figure 1A**). Treatment of HCT116 cells with 5 and 10 μM RITA caused significant decrease in the cell viability, compared with control group ($P < 0.05$). In contrast, viability of SW480 cells showed slight reduction after exposure to RITA at all tested dosages. After the exposure to RITA, apoptosis rate was detected by flow cytometry. As shown by **Figure 1B**, apoptosis rate of HCT116 cells was dose-dependently increased by RITA in the tested range of concentrations. Approximately 3-fold increase in apoptosis rate of HCT116 cells was observed with 5 and 10 μM RITA addition ($P < 0.05$). RITA also caused an increased trend in apoptosis rate of SW480 cells, but the increase did not reach to statistical significance, in despite of exposure to 10 μM RITA.

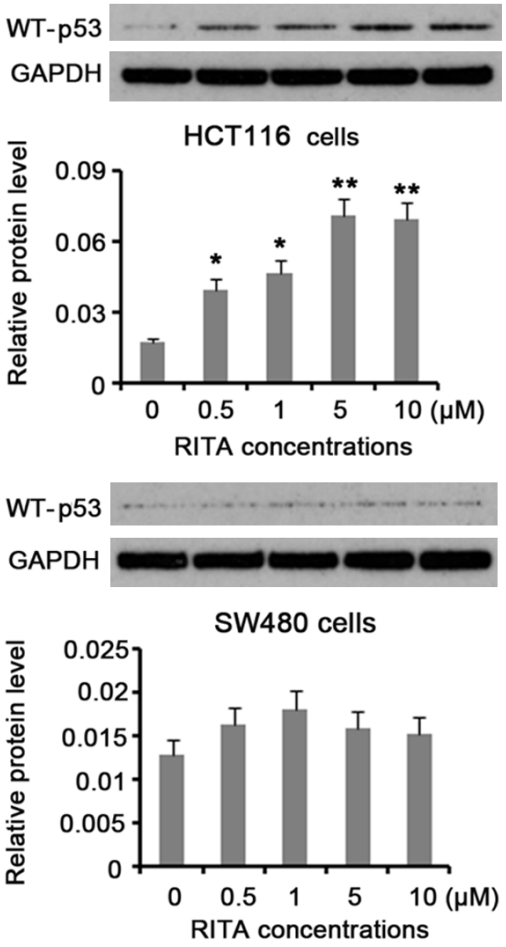

Figure 2. Effect of RITA on WT-p53 protein level in human CRC cells. Western blot assay was conducted following treatment of HCT116 and SW480 cells with different doses of RITA to detect effect of RITA on WT-p53 protein level. Experiments were repeated at least three times. Each bar represents the mean of three independent experiments. *$P < 0.05$ and **$P < 0.01$ *vs.* control group.

Effect of RITA on WT-p53 protein level in human CRC cells

To understand effect of RITA on protein level of WT-p53 in human colon cancer cells, Western blot assay was conducted following treatment of HCT116 and SW480 cells with different doses of RITA. The protein level of WT-p53 in HCT116 was increased with 0.5 and 1 μM RITA treatments compared to that in untreated cells ($P < 0.05$, **Figure 2**). Treatment with 5 and 10 μM RITA further elevated WT-p53 protein level ($P < 0.01$ *vs.* control). SW480 cells have been documented to mainly express mutant p53. The WT-p53 expression in SW480 was rather low compared with that in HCT116, as reflected by Western blotting. Treatment with RITA, regardless of the doses, had no notable effect on WT-p53 protein level in SW480 cells.

RITA inhibited the growth and proliferation of HCT116 cells via targeting WT-p53

To demonstrate that the inhibitory effects of RITA on growth and proliferation of HCT116 cells are involved in targeting WT-p53, we selectively silenced WT-p53 in HCT116 cells with three specific shRNAs (S1, S2, and S3), followed by validation with qPCR and Western blotting. qPCR detection showed that transfection of both S1 and S2 remarkably diminished WT-p53 gene expression ($P < 0.05$, **Figure 3A**). In western blot assay, WT-p53 protein level was also reduced after S2 ($P < 0.01$, **Figure 3B**) and S1 ($P < 0.05$) transfections compared to control. S2 was used in our study for WT-p53 knockdown in HCT116 cells. WT-p53 depletion with the RNA interference resulted in decreased cell viability ($P < 0.05$) and abrogated RITA-promoted effect on cell viability (**Figure 4A**). HCT116 cells with WT-p53 knockdown exhibited lower apoptosis rate than control cells ($P < 0.05$, **Figure 4B**). Remarkable increase in apoptosis rate induced by 10 μM RITA was abolished by silencing WT-p53. Colony formation assay was performed to evaluate clonogenic survival ability of treated HCT116 cells. As reflected by **Figure 4C**, WT-p53 knockdown promoted the colony formation efficiency of HCT116 cells ($P < 0.05$), even the cells treated with 10 μM RITA ($P < 0.05$).

RITA up-regulated WT-p53 in HCT116 cells through hindering its ubiquitination

qPCR analysis was conducted to determine whether RITA has the ability to modify gene expression of WT-p53. Treatment with RITA only triggered numeral increase in relative gene expression of WT-p53 ($P < 0.05$, **Figure 5A**). Down-regulated WT-p53 induced by RNA interference was not reversed following RITA treatment. Immunoprecipitation assay was undertaken to investigate the interaction between WT-p53 and ubiquitin, given previous documents have noted that MDM2 links ubiquitin to WT-p53 resulting in its degradation. After WT-p53 knockdown, WT-p53 protein that was linked to ubiquitin was almost undetectable (**Figure 5B**). RITA treatment significantly inhibit-

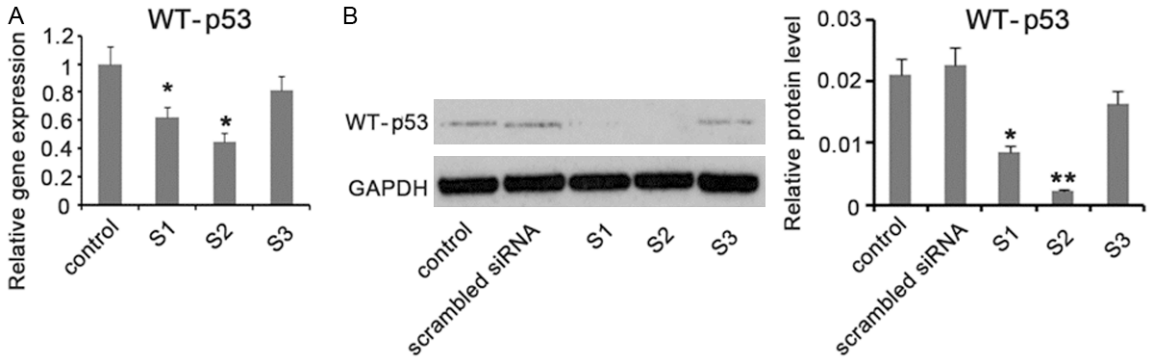

Figure 3. Knocking down WT-p53 in HCT116 via RNA interference. WT-p53 in HCT116 cells was selectively silencing via three specific shRNAs (S1, S2, and S3), followed by validation with qPCR (A) and Western blotting (B). Experiments were repeated at least three times. Each bar represents the mean of three independent experiments. *P < 0.05 and **P < 0.01 *vs.* control group.

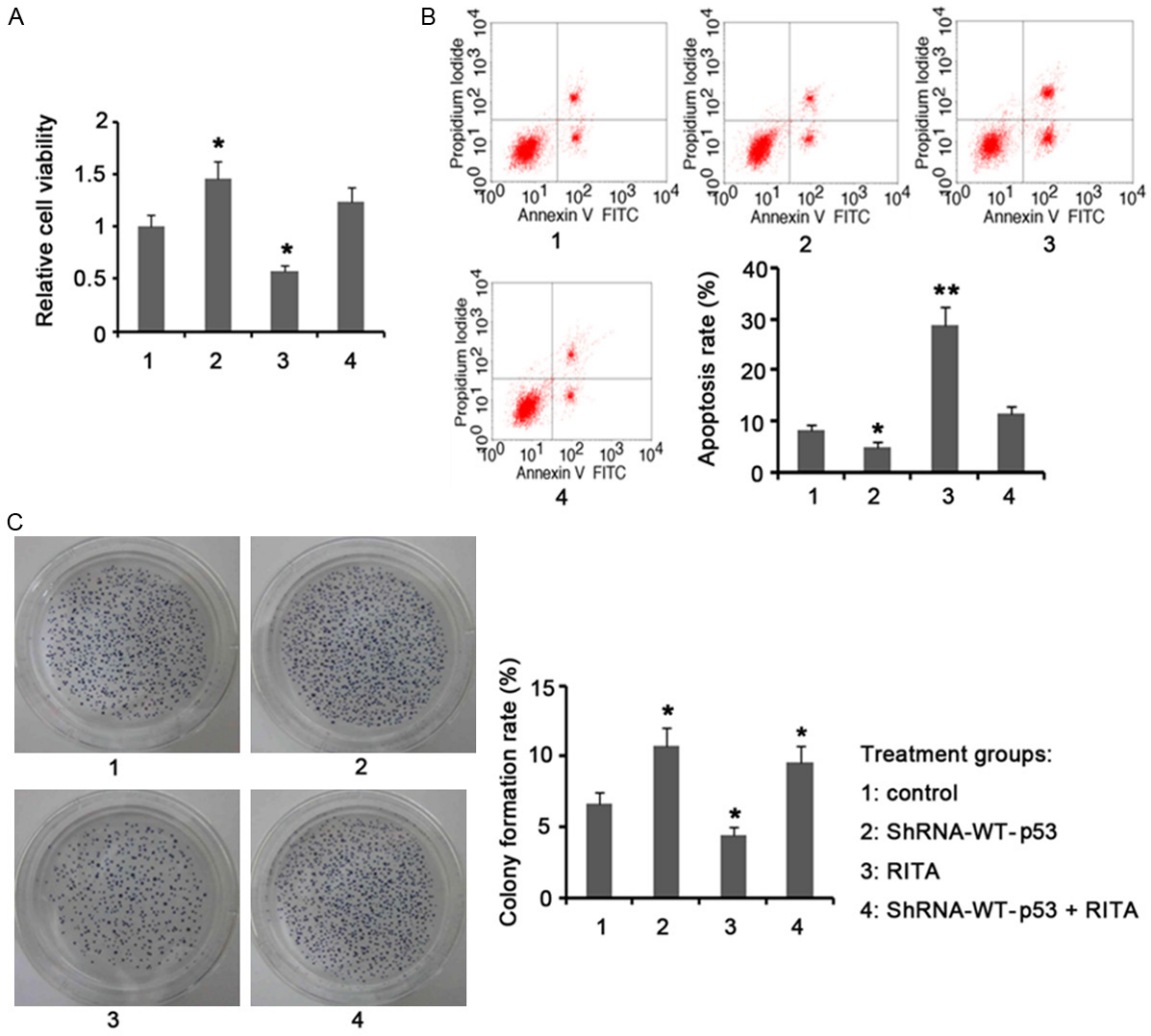

Figure 4. RITA inhibited the growth and proliferation of HCT116 cells via targeting WT-p53. HCT116 cells were subjected to following treatments: WT-p53 knockdown, exposure to 10 μM RITA for 72 h, or WT-p53 knockdown before the RITA treatment. MTT assay (A), flow cytometry detection (B) and colony formation assay (C) were performed after the treatments. Experiments were repeated at least three times. Each bar represents the mean of three independent experiments. *P < 0.05 and **P < 0.01 *vs.* control group. ShRNA-WT-p53: WT-p53 knockdown; RITA: exposure to 10 μM RITA for 72 h; ShRNA-WT-p53 + RITA: WT-p53 knockdown before the RITA treatment.

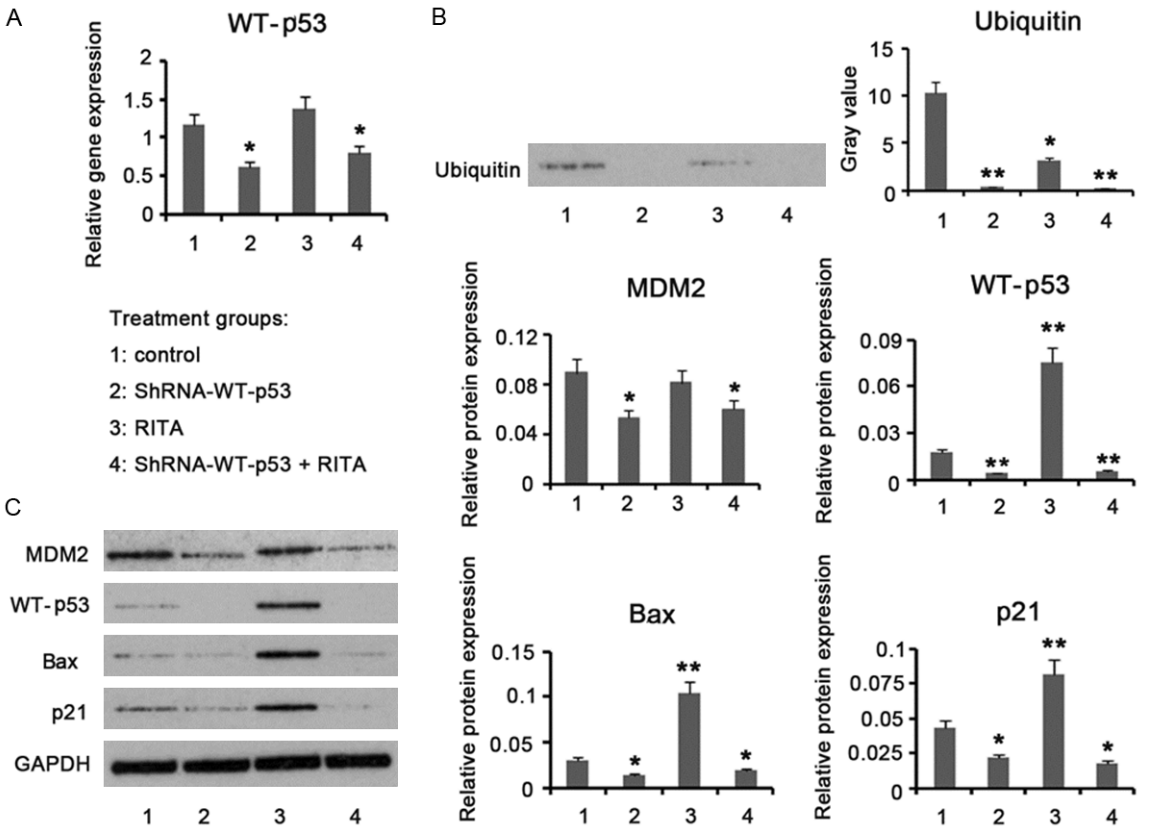

Figure 5. RITA up-regulated WT-p53 in HCT116 cells through hindering its ubiquitination. HCT116 cells were subjected to following treatments: WT-p53 knockdown, exposure to 10 μM RITA for 72 h, or WT-p53 knockdown before the RITA treatment. qPCR (A) Immunoprecipitation (B) and Western blot assays (C) were performed after the treatments. Experiments were repeated at least three times. Each bar represents the mean of three independent experiments. **P* < 0.05 and ***P* < 0.01 *vs.* control group. ShRNA-WT-p53: WT-p53 knockdown; RITA: exposure to 10 μM RITA for 72 h; ShRNA-WT-p53 + RITA: WT-p53 knockdown before the RITA treatment.

ed linkage of WT-p53 to ubiquitin ($P < 0.05$). To understand the effect of RITA on MDM2, we evaluated MDM2 protein level using Western blot assay. Results showed no significant alteration in MDM2 protein level following RITA treatment. Unexpectedly, MDM2 protein level was decreased with WT-p53 knockdown ($P < 0.05$, **Figure 5C**). Although RITA could up-regulate WT-p53 according to the Western blotting, RITA was unable to restore WT-p53 protein that was silenced by RNA interference.

Bax and p21 are the major downstream components of the TP53 tumor suppressor pathway, respectively controlling apoptosis and cell-cycle progresses. We detected the protein levels of Bax and p21 to assess the active status of WT-p53 in regulating apoptosis and cell-cycle arrest. Bax protein level was found to be decreased with WT-p53 knockdown ($P < 0.05$). HCT116 cells exposed to RITA displayed a notable increase in Bax protein expression compared to control group ($P < 0.05$). WT-p53 knockdown was in accompany with dramatic reduction in p21 protein level. Treatment with RITA promoted the p21 protein expression ($P < 0.05$). RITA was unable to up-regulated Bax and p21, when WT-p53 was knocked down.

Discussion

CRC remains one of the most frequent causes of cancer-related death worldwide, with less than 10% of 5-year overall survival rate in advanced stages of the disease [5]. Functional deficiency and disturbance of p53, primarily induced by post-translational modification and gene mutation, are commonly observed in CRC and closely associated with the more aggressive disease, worse overall survival and resistance to therapies. RITA is a drug (small molecule) that has been developed with the aim at

restoration and reactivation of WT-p53 functions in tumor with MT-p53 expression or low WT-p53 expression [14]. HCT116 is a human CRC cell line harboring WT-p53 gene, but the fact that it maintained at a low level probably results in attenuated functions in the regulation of apoptosis and other cellular processes, such as cell cycle arrest, proliferation, differentiation, and invasion. In present study, treatment with RITA successfully elevated WT-p53 protein level in HCT116 cells and caused notable decrease in the cell viability and increase in the apoptosis rate. Knocking down WT-p53 protein in HCT116 cells via small interfering RNA abolished the anti-proliferative and apoptotic effects of RITA. These data indicate that abilities of RITA to inhibit growth and induce apoptosis of HCT116 cells are associated to modulating expression and function of WT-p53.

In contrast, RITA had modest effect on the cell viability and apoptosis of SW480 cells which mainly express MT-p53 gene. Western blot assay reflected that RITA failed to elevate WT-p53 protein level in SW480 cells, implicating that RITA is unable to restore MT-p53 protein to that of WT-p53. However, inconsistent with our implication, previous data showed that RITA can re-establish the wild-type transcriptionally competent conformation of MT-p53 protein and restore transcriptional transactivation and transrepression function of several hot spot p53 mutants [7, 14, 16]. It is possible that some specific p53 mutants in SW480 are not completely restored to WT-p53 protein by RITA, therefore, RITA dose not, or only partly, lead to the reconstitution of WT-p53 function in SW480. Further studies are needed to identify RITA-induced modification at all mutants in p53 gene in SW480 cells.

We initially thought that RITA modulating WT-p53 gene expression might be an important way to up-regulate endogenous WT-p53 protein in HCT116 cells, but qPCR detection showed that RITA just exerted marginal effect on the elevation in WT-p53 mRNA, which suggests that other approach independent of genetic regulation is associated to WT-p53 up-regulation by RITA. Previous research has documented that WT-p53 generally has a short half-life, especially in the case of MDM2 amplification. MDM2 is an ubiquitin ligase for p53, which binds to the N-terminal transactivation domain of p53, and consequently, induces the rapid degradation by ubiquitination and proteolysis [13]. RITA is able to prevent the interaction between p53 and MDM2, which probably hinders MDM2 ubiquiltylating WT-p53 and facilitates WT-p53 accumulation in cells [14]. In present study, immunoprecipitation assay was performed to investigate the p53 ubiquitination in HCT116 cells following the RITA treatment. Our data showed that there is dramatic reduction in WT-p53 protein linked with ubiquitin. Western blot assay subsequently manifested that RITA did not significantly alter MDM2 protein level. These data suggest that RITA up-regulating endogenous WT-p53 protein is mainly associated with RITA repressing WT-p53 ubiquitination, but we cannot make a solid conclusion that RITA repressing WT-p53 ubiquitination is completely dependent on the blockage of MDM2 adding ubiquitin to WT-p53, although MDM2 is a well-established ubiquitin ligase responsible for p53 ubiquitination. On the one hand, p53 ubiquitination is under the regulation of several ubiquitin ligases; On the other hand, interaction of RITA with WT-p53 are rather complicated. An experimental study using nuclear magnetic resonance manifested that RITA fails to block p53-MDM2 binding [17]. Enge et al., [18] indeed observed that RITA induced the release of MDM2 from WT-p53, but remained unclear about how RITA does that. To further elucidate the mechanism underlying RITA repressing WT-p53 ubiquitination, a study designed to silence MDM2 and induce a forced MDM2 expression which is in the presence or absence of RITA is strongly recommended. An interesting finding in our study is that MDM2 protein level is dramatically decreased following WT-p53 knockdown. There is evidence that MDM2 is also one of the target genes of WT-p53 [19]. Thus, it is speculated that a negative feedback regulation is induced by WT-p53 to MDM2.

As a well-established p53-target genes, p21 is an essential mediator of p53-dependent cell-cycle arrest. p21-depleted mouse embryonic fibroblasts are unable to undergo p53-dependent G1 arrest after DNA damage. p21 serving as a cyclin-dependent kinase inhibitor causes a number of different cell lines to arrest in both G1 and G2 phases, which retards cell growth and proliferation. p53 is also a well-known positive regulator of Bax which participates in mitochondria-mediated apoptotic pathway [12, 20]. Thus, evaluating expression levels of p21 and

Bax is a suitable method to investigate active status of WT-p53 in the regulation of cell-cycle and apoptosis. Treatment of HCT116 cells with RITA induced the up-regulation of p21 and Bax protein levels. These effects were abolished by knockdown of WT-p53. It indicates that RITA facilitates WT-p53 activation and triggers up-regulation of p21 and Bax. Treatment with RITA, herein, is accompany with significant reduction in cell viability and elevation in apoptosis rate. An attenuated colony formation efficiency was observed with RITA addition. Silencing WT-p53 abrogated the adverse effects on HCT116 cells. Thus, our data strongly suggest that RITA exerted anit-proliferated and apoptotic actions on HCT116 cells via activation of WT-p53-p21 and WT-p53-Bax signals.

In conclusion, this study showed that RITA had preferential growth inhibitory activity on colon cancer HCT116 cells expressing WT-p53, but not SW480 cells harboring MT-p53, mainly because RITA can up-regulate WT-p53 in HCT116 cells but not in SW480 cells. The WT-p53 up-regulation in HCT116 cells is closely related to RITA blocking WT-p53 ubiquitination, rather that the impacts on WT-p53 gene expression and MDM2 protein level. The WT-p53 up-regulation facilitate the WT-p53 activating p21 and Bax, resulting decreased cell viability, elevated apoptosis rate, and attenuated colony formation efficiency.

Disclosure of conflict of interest

None.

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References

[1] Saha MN, Qiu L, Chang H. Targeting p53 by small molecules in hematological malignancies. J Hematol Oncol 2013; 6: 23.

[2] Li T, Liu X, Jiang L, Manfredi J, Zha S, Gu W. Loss of p53-mediated cell-cycle arrest, senescence and apoptosis promotes genomic instability and premature aging. Oncotarget 2016; 7: 11838-49.

[3] Li Y, Mao Y, Brandt-Rauf PW, Williams AC, Fine RL. Selective induction of apoptosis in mutant p53 premalignant and malignant cancer cells by PRIMA-1 through the c-Jun-NH2-kinase pathway. Mol Cancer Ther 2005; 4: 901-9.

[4] Pflaum J, Schlosser S, Müller M. p53 Family and Cellular Stress Responses in Cancer. Front Oncol 2014; 4: 285.

[5] Ginés A, Bystrup S, Ruiz de Porras V, Guardia C, Musulén E, Martínez-Cardús A, Manzano JL, Layos L, Abad A, Martínez-Balibrea E. PKM2 Subcellular Localization Is Involved in Oxaliplatin Resistance Acquisition in HT29 Human Colorectal Cancer Cell Lines. PLoS One 2015; 10: e0123830.

[6] Alam SK, Yadav VK, Bajaj S, Datta A, Dutta SK, Bhattacharyya M, Bhattacharya S, Debnath S, Roy S, Boardman LA, Smyrk TC, Molina JR, Chakrabarti S, Chowdhury S, Mukhopadhyay D, Roychoudhury S. DNA damage-induced ephrin-B2 reverse signaling promotes chemoresistance and drives EMT in colorectal carcinoma harboring mutant p53. Cell Death Differ 2016; 23: 707-22.

[7] Fiorini C, Cordani M, Padroni C, Blandino G, Di Agostino S, Donadelli M. Mutant p53 stimulates chemoresistance of pancreatic adenocarcinoma cells to gemcitabine. Biochim Biophys Acta 2015; 1853: 89-100.

[8] Carrero ZI, Kollareddy M, Chauhan KM, Ramakrishnan G, Martinez LA. Mutant p53 protects ETS2 from non-canonical COP1/DET1 dependent degradation. Oncotarget 2016; 7: 12554-67.

[9] Yan W, Liu G, Scoumanne A, Chen X. Suppression of inhibitor of differentiation 2, a target of mutant p53, is required for gain-of-function mutations. Cancer Res 2008; 68: 6789-96.

[10] Hanel W, Marchenko N, Xu S, Yu SX, Weng W, Moll U. Two hot spot mutant p53 mouse models display differential gain of function in tumorigenesis. Cell Death Differ 2013; 20: 898-909.

[11] Heinlein C, Krepulat F, Löhler J, Speidel D, Deppert W, Tolstonog GV. Mutant p53(R270H) gain of function phenotype in a mouse model for oncogene-induced mammary carcinogenesis. Int J Cancer 2008; 122: 1701-9.

[12] Melincovici CS, Mihu CM, Mărginean M, Boşca AB, Coneac A, Moldovan I, Crişan M. The prognostic significance of p53, Bax, Bcl-2 and cyclin E protein overexpression in colon cancer - an immunohistochemical study using the tissue microarray technique. Rom J Morphol Embryol 2016; 57: 81-9.

[13] Rezk MS, Abdel-Halim M, Keeton A, Franklin D, Bauer M, Boeckler FM, Engel M, Hartmann RW, Zhang Y, Piazza GA, Abadi AH. Synthesis and optimization of new 3,6-disubstitutedindole derivatives and their evaluation as anticancer agents targeting the MDM2/MDMx complex. Chem Pharm Bull (Tokyo) 2016; 64: 34-41.

[14] Burmakin M, Shi Y, Hedström E, Kogner P, Selivanova G. Dual targeting of wild-type and mu-

tant p53 by small molecule RITA results in the inhibition of N-Myc and key survival oncogenes and kills neuroblastoma cells in vivo and in vitro. Clin Cancer Res 2013; 19: 5092-103.

[15] Yang T, Li X, Zhu W, Chen C, Sun Z, Tan Z, Kang J. Alteration of antioxidant enzymes and associated genes induced by grape seed extracts in the primary muscle cells of goats in vitro. PLoS One 2014; 9: e107670.

[16] Zhao CY, Grinkevich VV, Nikulenkov F, Bao W, Selivanova G. Rescue of the apoptotic-inducing function of mutant p53 by small molecule RITA. Cell Cycle 2010; 9: 1847-55.

[17] Krajewski M, Ozdowy P, D'Silva L, Rothweiler U, Holak TA. NMR indicates that the small molecule RITA does not block p53-MDM2 binding in vitro. Nat Med 2005; 11: 1135-6.

[18] Enge M, Bao W, Hedström E, Jackson SP, Moumen A, Selivanova G. MDM2-dependent downregulation of p21 and hnRNP K provides a switch between apoptosis and growth arrest induced by pharmacologically activated p53. Cancer Cell 2009; 15: 171-83.

[19] Levav-Cohen Y, Goldberg Z, Tan KH, Alsheich-Bartok O, Zuckerman V, Haupt S, Haupt Y. The p53-MDM2 loop: A critical juncture of stress response. Subcell Biochem 2014; 85: 161-186.

[20] Sorrentino G, Mioni M, Giorgi C, Ruggeri N, Pinton P, Moll U, Mantovani F, Del Sal G. The prolyl-isomerase Pin1 activates the mitochondrial death program of p53. Cell Death Differ 2013; 20: 198-208.