Deficiency of 53BP1 inhibits the radiosensitivity of colorectal cancer

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Abstract. The present study aimed to observe the influence of p53 binding protein 1 (53BP1) silencing on the radiosensitivity of colorectal cancer (CRC) cells and investigated the potential underlying mechanisms. The differences in radiosensitivity among four CRC cells were detected by the clone formation assay, while the expression of their 53BP1 was detected by the western blot analysis. HCT116 cells with relatively high expression of 53BP1 were selected to silence the expression of 53BP1 by shRNA intervention. The influence on proliferation, apoptosis, and cell cycle distribution was detected by immunofluorescent staining of Ki-67 and flow cytometry. The expression of relevant proteins in the apoptotic pathway ATM-CHK2-p53 was further analyzed by western blot analysis. The expression of 53BP1 was found to be closely related to the radiosensitivity of the CRC cells. Decreased expression of 53BP1 led to the tolerance of HCT116 cells to radiation. The detection of tumor proliferation, apoptosis, and cell cycle showed that decreased expression of 53BP1 resulted in an increased S-phase percentage of HCT116 cells, an increased proliferating rate, and a decreased apoptotic rate after radiation. The analysis of the molecular pathway showed that the reduced expression of 53BP1 decreased the protein expression of ATM, CHK2, and the phosphorylated products associated with the p53 apoptotic pathway. In conclusion, decreased expression of 53BP1 leads to radiotolerance of CRC cells, and the underlying mechanism is probably related to the decreased expression of relevant proteins in the ATM-CHK2-p53 pathway, which affects cell cycle, apoptosis and proliferation.

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Introduction

Radiotherapy, following surgery and chemotherapy, is one of the most important therapies for colorectal cancer (CRC) in the advanced stage. Multiple clinical studies have verified that radiotherapy can, for example, lower preoperative staging and improve the operative resection rate and progression-free survival of patients (1). However, due to the indigenous biological properties of CRC, the tumor tissue itself shows low radiosensitivity. Moreover, radiotolerance is limited during the radiotherapy for CRC because of many normal risks to organs (such as small intestine and kidney) in its anatomical environment. Although radiotherapy instruments and technologies are constantly being upgraded, they exhibit restricted capacity of simultaneously increasing the dosage at the tumor region and decreasing the exposure to normal organs, thus limiting their application in gastrointestinal tumors (2). Therefore, enhancing the radiosensitivity of CRC cells based on the indigenous biological properties of tumors has become a critically urgent challenge to be addressed.

Cellular responses to external damage (due to radiotherapy and chemotherapy) depend on the self-repairing ability of cells, such as the ability to repair DNA double-strand breaks (DSBs) (3). Multiple signaling pathways are involved in the process of DSB repair, including homologous recombination repair, nonhomologous end joining, cell cycle regulation and apoptotic pathways (4,5). p53 binding protein 1 (53BP1) has been confirmed to contribute to apoptosis induction in DNA damage repair (DDR) and helps in determining the cellular response to different therapies (6,7). 53BP1 was originally believed to be the binding protein of tumor suppressor gene p53 and thought to be involved in p53-mediated apoptosis. 53BP1 has an important function as a significant reaction medium at the early stage in signal transduction pathways following DNA damage (6). Also, tumor tissues differ in their degree of 53BP1 deletion, which is closely related to the malignant staging of tumor cells and histological staging, and affects the sensitivity to drug, which is manifested as resistance to treatment (7,8). Moreover, 53BP1 can affect tumor (breast

cancer and glioma) responses to radiotherapy (9,10), although it has been rarely reported how abnormal expression of 53BP1 disturbs the response of CRC to radiotherapy. A previous study showed that epidermal growth factor receptor-tyrosine kinase inhibitor-icotinib hydrochloride enhanced the sensitivity of CRC to radiotherapy by increasing the expression of 53BP1 (11). In contrast, the radiosensitization effect of icotinib hydrochloride was notably eliminated when the expression of 53BP1 of CRC cells was decreased by shRNA, which implied that the abnormal expression of 53BP1 could influence the radiosensitivity of CRC. However, the exact effects and potential underlying mechanisms remain to be resolved.

Recent studies showed that on external damage, cells may activate multiple signaling pathways, which may contribute to cellular injury repair and induce apoptosis. The ataxia-telangiectasia mutated kinase (ATM)-checkpoint kinase-2 (CHK2)-p53 pathway plays a pivotal role in the DDR process. When activated, it may induce cell cycle arrest, leading to the activation of p53-related apoptotic pathways, which trigger apoptosis (12,13). 53BP1 is closely related to the ATM-CHK2-p53 pathway because of its special structure. It possesses two Tudor domains, which promote it to interact with ATM to involve in DDR (8,14,15), and a C-terminal BRTC domain, which activates CHK1 and CHK2 of the cell cycle to regulate the G1/S, S and G2/M checkpoints, phosphorylate p53, and then induce p53-dependent apoptosis (15,16). Thus, considering the mediator-featured structure of 53BP1, we speculated that the abnormal expression of 53BP1 could affect the tumor cell proliferation, apoptosis, and cell cycle distribution through interfering the ATM-CHK2-p53 pathway.

Previous studies have found that the expression of 53BP1 was deficient in CRC tissue, which was an early factor of poor prognosis. Therefore, we hypothesized that this deficiency may be an intrinsic factor that influences the response of colorectal tumors to radiotherapy. The present study aimed to verify the correlation between the expression of 53BP1 and radiosensitivity in various CRC cell lines and to investigate how 53BP1 affects the response to radiotherapy by disturbing the expression of 53BP1 in CRC cells *in vitro*. Moreover, the expression of proteins related to the ATM-CHK2-p53 pathway was also detected to explore the intrinsic factors of 53BP1 affecting the radiotherapy efficacy mechanically.

Materials and methods

Cell culture and animal care. HCT116, SW620, Caco2 and LoVo cells were cultured in an RPMI-1640 medium containing 10% fetal bovine serum (FBS) at 37˚C, 5% CO_2, and saturated humidity. Passages were performed to maintain monolayer growth. Cells were collected at the exponential growth phase for subsequent experiments. Female athymic nude mice (nu/nu; body weight, 20-25 g; 6-10 weeks of age) were purchased from Beijing HFK Bioscience Co., Ltd. (Beijing, China). The care and treatment of all experimental mice was in accordance with the institutional guidelines.

Cell transfection. This study used GCACAAGAACTTATGG AAAGT as the shRNA sequence of 53BP1 and TTCTCCGA ACGTGTCACGT as the shRNA sequence of the control group. The lentivirus plasmids containing the shRNA and green fluorescent protein lentivirus vector containing 53BP1 shRNA were purchased from Shanghai Genechem Co., Ltd. (Shanghai, China). The concentrated virus solution and HCT116 cells were co-cultured, and their fluorescence was observed by optical microscopy to confirm successful transfection. The transfection rates were verified by western blot analysis and reverse transcription-polymerase chain reaction (RT-PCR).

Clone formation assay. The cells at the exponential growth phase were digested into single-cell suspensions. After counting, the living cells were inoculated onto a 6-well plate at 100-5,000 cells/well. When cells grew in an adherent manner after a 24-h incubation, they were exposed to different radiation dosages (0, 2, 4, 6, 8 and 10 Gy) with a dosage rate of 2 Gy/min. Incubation was terminated after 10-14 days when >50 cells formed a clone in a visible cell mass. The cells were fixed with methanol and stained with Giemsa; colonies containing at least 50 cells were counted, and cell survival curves were plotted using the multitarget click model to compare the difference of radiosensitivity. Relative parameters, such as mean lethal dose (D_0), quasi-threshold dose (D_q), extrapolation number (N), surviving fraction at 2 Gy (SF_2), and a ratio of SF_2, termed as sensitization enhancing ratio (SER), were calculated. Three parallel tests were set.

Evaluation of tumor proliferation by immunofluorescent staining of Ki-67. The proliferation antigen Ki-67 was used to assess the proliferation rate of tumor. At the exponential growth phase, $2x10^5$ cells/ml were plated in chamber slides. After 24 h of irradiation (at 0, 2 and 4 Gy), the cells were fixed and permeabilized with 0.2% Triton X-100 (Wuhan Boster Biological Technology, Ltd., Wuhan, China). They were then incubated with anti-Ki-67 antibody (1:50, 19972-1-AP; Proteintech Group, Rosemont, IL, USA), and then with Alexa Fluor 488-conjugated goat anti-rabbit (Proteintech Group) at a dilution of 1:200. Finally, the sections were counterstained with 6-diamidino-2-phenylindole dihydrochloride (Vector Laboratories, Inc., Burlingame, CA, USA). The sections were examined on a confocal laser scanning microscope (Olympus, Tokyo, Japan) equipped with a camera. To determine the percentage of positive cells with Ki-67, at least 500-1,000 tumor cells per slide were counted, and the number of Ki-67-positive cells was scored and the positive rate was counted.

Cell cycle detection by flow cytometry. A total of $2x10^5$ cells/ml at the exponential growth phase were collected and inoculated onto a 6-well plate. When the cells attached to the wall after a 24-h incubation, they were exposed to different irradiation doses (at 0, 2 and 4 Gy). The cells were then digested with trypsin 24 h after irradiation. After overnight fixation with 70% ethanol, the cells were digested again with 1% RNase for 30 min at 37˚C, followed by 30-min staining with 20 mg/ml propidium iodide (PI). Finally, the cell cycle was detected using a flow cytometer (BD Biosciences, San Jose, CA, USA).

Apoptosis detection by flow cytometry. The cells were routinely digested, washed, suspended gently with 500 μl combining solution, gently blended with 5 μl Annexin V-FITC, and finally

blended with 5 µl PI using an Annexin V-FITC apoptosis detection kit, according to the manufacturer's instructions. Then, after 10-min incubation at room temperature (20-25˚C) in darkness, cells were analyzed using a flow cytometer (BD Biosciences). Ten thousand cells in each sample were analyzed, and the CellQuest software (BD Biosciences) was employed to analyze the data.

Western blot analysis. The cells were lysed in a radio immunoprecipitation assay lysis buffer for 30 min at 4˚C and centrifuged at 12,000 x g for 5 min. The supernatant was collected, combined with sodium dodecyl sulfate (SDS) buffer, and heated to 100˚C for 5 min. The proteins were separated by 10% SDS-PAGE and blotted to the polyvi-nylidene fluoride membranes, which were incubated with primary antibodies against 53BP1 (ab175933, 1:2,000; Abcam, Cambridge, MA, USA), ATM (#21147, 1:1,000; Signalway), ATMpS1981 (5883, 1:1,000; Cell Signaling Technology, Danvers, MA, USA), CHK2 (AP4999a, 1:1,000; Abgent, San Diego, CA, USA), CHK2pT68 (2197, 1:1,000; Cell Signaling Technology), p53 (AM2244B, 1:1,000; Abgent), p-P53 (9286, 1:1,000; Cell Signaling Technology), caspase-9 (10380-1-AP, 1:1,000; Proteintech Group), caspase-3 (BS1518, 1:1,000; Bioworl, Dublin, OH, USA), Bax (1063, 1:1,000; EpiGentek, Farmingdale, NY, USA), Bcl-2 (2870-P, 1:1,000; Cell Signaling Technology), or β-actin antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4˚C overnight. The membranes were washed with Tris-buffered saline and Tween-20 three times, incubated with secondary antibodies for 2 h at room temperature, and visualized by enhanced chemiluminescence. Band intensities were analyzed by the Gel-Pro analysis program.

Quantitative RT-PCR. The total RNA was extracted with a TRIzol reagent. Then, an RT-PCR kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to reversely transcribe RNA into cDNA. StepOne and StepOnePlus Real-Time PCR Systems (Applied Biosystems) were adopted for amplification analysis. More than three parallel tests were set for all trials.

Establishing the tumor-bearing nude mouse model. Cultured tumor cells were collected to prepare tumor cell suspensions with an RPMI-1640 (1:1) at a concentration of approximately $2x10^7$ cells/ml for the following animal experiments. Tumor cells (0.1 ml) (HCT116 and HCT116 with 53BP1 silencing) were subcutaneously injected into the posterior limbs of the mice and observed for 5-7 days. When tumors reached a diameter of 10 mm, all nude mice were randomly divided into different groups (n=3) to receive different treatments as observation by radiotherapy. For the radiotherapy group, irradiation was performed using a 6 MV linear accelerator (MDX, Siemens) with an exposure field of 5x5 cm^2, and the total dose was 10 Gy (2 Gy/day for 5 days). Since the beginning of the treatment, the caliper was used to measure the longest diameter L and the shortest diameter W of each tumor every other day, and the tumor volume was calculated based on the following formula: Tumor volume = (L x W^2)/2 mm^3. The growth time and volume of each tumor were recorded.
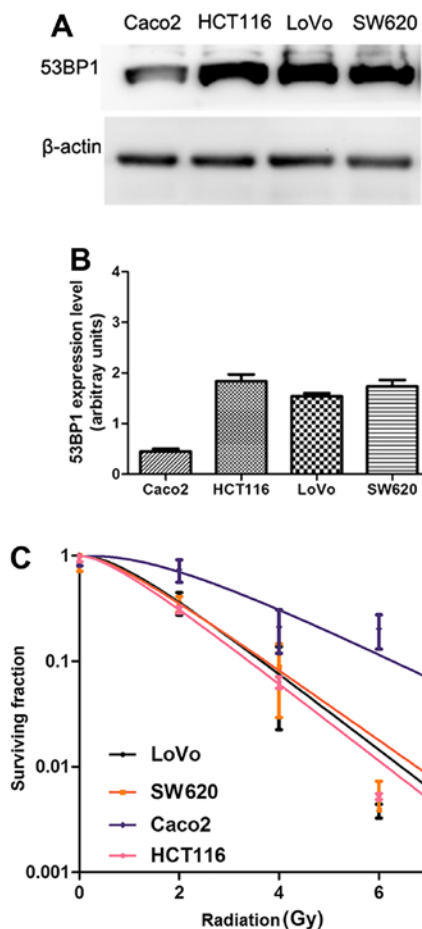

Figure 1. Expression of 53BP1 is related to radiation sensitivity in the four CRC lines. (A) 53BP1 protein level in CRC lines was detected using western blot analysis. (B) Histograms of quantification expression of 53BP1 protein based on the mean of three independent experiments; β-actin was used as the endogenous control. Error bar shows the standard error. (C) Survival curve of various CRC cell lines. The radiosensitivity of various CRC cell lines was detected by the clonogenic assay. The multitarget click model was used to fit the experimental data. Error bars show the standard error of the mean for three independent experiments.

Statistical analysis. SPSS 13.0 software was used for the statistical analysis. Quantitative data were expressed as mean 13.0. Mean value comparison among multiple groups was performed by one-way analysis of variance, and the comparison between the two groups was carried out by applying the Q-test. P≤0.05 indicated statistical significance.

Results

Expression of 53BP1 is closely related to the radiosensitivity of CRC cells. To investigate the correlation between the expression of 53BP1 and radiosensitivity of CRC cells, the expression of 53BP1 in the four CRC cell lines (HCT116, SW620, Caco2 and LoVo cells) was detected *in vitro* by the western blot analysis (Fig. 1A and B), and the radiosensitivity of these intestinal cancer cell lines was simultaneously detected by the clone formation assay (Fig. 1C). The results showed that the expression of 53BP1 was closely related to the radiosensitivity of CRC cells. The HCT116 cell line with the high expression of 53BP1 was relatively sensitive to irradiation with lower values of SF_2, D_0, D_q, and N in the cell survival curve analysis, while

Table I. The relative parameters of cell survival curves after irradiation.

Parameter/cell lines	Caco2	LoVo	SW620	HCT116
D_0	1.90	1.20	1.30	1.18
D_q	3.44	1.37	1.03	0.93
N	2.812	2.137	1.792	1.786
SF_2	0.74	0.36	0.35	0.31

D_0, lethal dose; D_q, quasi-threshold dose; N, extrapolation number; SF_2, surviving fraction at 2 Gy.

Table II. Effect of 53BP1 silencing on radiosensitivity in HCT116 cells.

Parameter/cell lines	Control	53BP1 silence
D_0	0.98	1.28
D_q	1.50	2.35
N	1.53	1.84
SF_2	0.30	0.49
SER		0.77

D_0, lethal dose; Dq, quasi-threshold dose; N, extrapolation number; SF_2, surviving fraction at 2 Gy; SER, sensitization enhancing ratio.
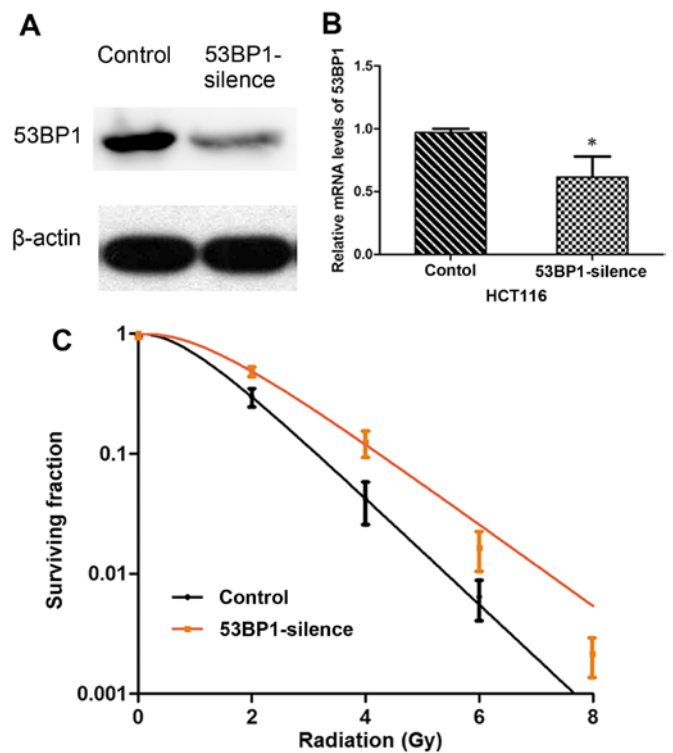

Figure 2. Decrease in the expression of 53BP1 inhibits the radiation sensitivity of HCT116 cells. (A and B) Western blot analysis and RT-PCR were used to confirm the transfection efficiency. (C) Survival curve of control and 53BP1 silencing cells of HCT116. The radiosensitivity of control and 53BP1 silencing cells of HCT116 was detected by the clonogenic assay.

the Caco2 cell line with the low expression of 53BP1 was relatively tolerant to irradiation with higher values of SF_2, D_0, D_q, and N in the cell survival curve analysis (Table I).

Decreased expression of 53BP1 inhibits the radiosensitivity of HCT116 cell line. The HCT116 cell line with relatively high expression of 53BP1 was selected, and stable HCT116 cell stains were constructed by RNA-intervened lentiviral vector transfection by decreasing the expression of 53BP1 via shRNA. The effective transfection rate was verified by western blot analysis and RT-PCR (Fig. 2A and B). Then, the effect of transfection on the cell radiosensitivity was detected, which showed that the decrease in the expression of 53BP1 increased SF_2, D_0, D_q, and N values, and decreased the value of SER. It indicated that the decreased expression of 53BP1 obviously inhibited the radiosensitivity of the HCT116 cells (Table II and Fig. 2C).

Decreased expression of 53BP1 increases the proliferation rate of HCT116 cells after irradiation. To determine the association between the radiosensitivity of HCT116 cells after 53BP1 silencing and tumor proliferation, the expression of Ki-67 was investigated by immunofluorescent staining. As shown in Fig. 3, the results revealed that the decrease in the expression of 53BP1 obviously increased the expression of Ki-67 compared with the control group. The proliferation rate was 31±4 vs. 18±4% before irradiation (P<0.05). After irradiation of 2 and 4 Gy, the 53BP1-silenced group still revealed stronger capacity to induce tumor proliferation compared with the control
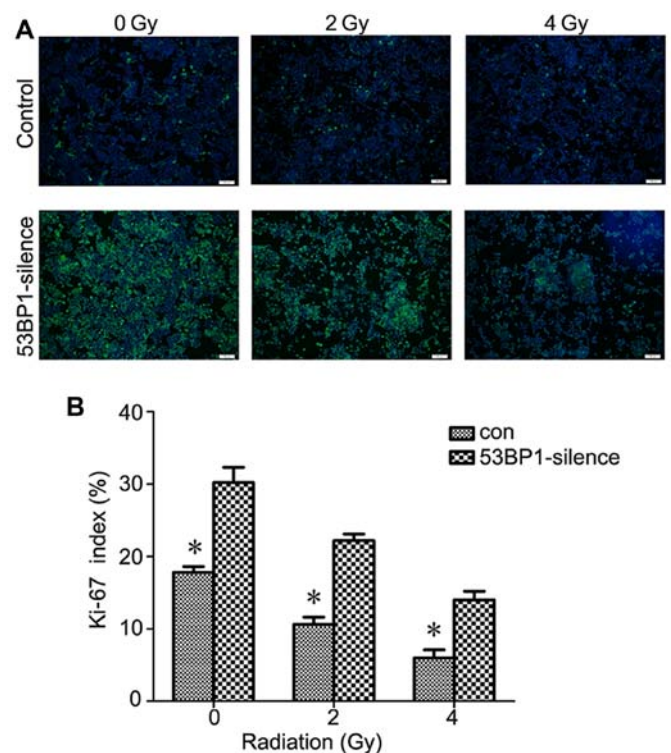

Figure 3. Decrease in the expression of 53BP1 increased Ki-67 proliferative marker in HCT116 cells. (A) Immunofluorescence staining of Ki-67 in HCT116 cells after the irradiation of 0, 2 and 4 Gy (magnification, x100). (B) Average proliferative index was determined by counting positive cells per microscopic field, and histograms of quantification of control and 53BP1 silencing cells of HCT116 was plotted. Columns, mean; error bars, standard error of mean from three independent experiments. *P<0.05.
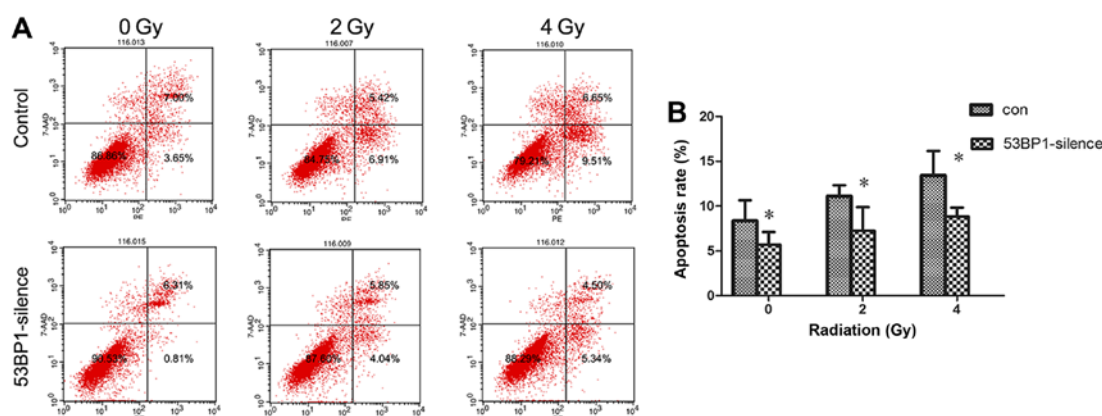
Figure 4. Decrease in the expression of 53BP1 decreased HCT116 cell apoptosis after radiation. (A) Apoptosis assays by flow cytometry in HCT116 after radiation. The doses are 0, 2 and 4 Gy. The apoptotic rate was calculated as the percentage of Annexin V positive cells. (B) Histograms of quantification of apoptosis rates of control and 53BP1 silencing cells of HCT116. Columns, mean; error bars, standard error of mean from three independent experiments. *P<0.05.
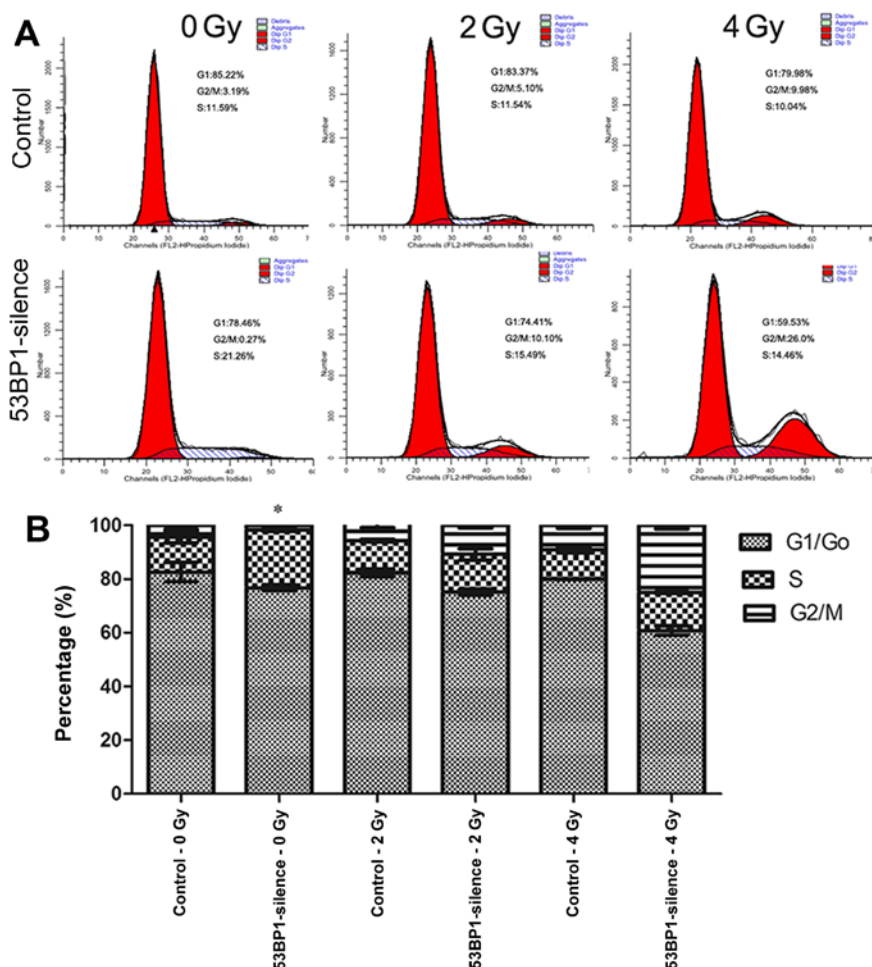

Figure 5. 53BP1 silencing increases S-phase accumulation in HCT116 and HT29 cells. (A) Cell cycle distribution in HCT116 control and 53BP1-silenced cells after radiation. The doses are 0, 2 and 4 Gy. (B) Histogram plots of HCT116 cell cycle distribution. Columns, mean; error bars, standard error of mean from three independent experiments. *P<0.05.

group. The proliferation rate was 22±3 vs. 11±4% for 2 Gy and 14±5 vs. 6±2% for 4 Gy. The difference between the two groups was obvious (P<0.05).

Decreased expression of 53BP1 reduces the apoptotic rate of HCT116 cells after radiotherapy. The effect of the decreased

expression of 53BP1 on tumor cell apoptosis was investigated after radiotherapy. The apoptotic rate of tumor cells before and after intervention at 24 h after exposure to irradiation doses of 0, 2 and 4 Gy was detected using flow cytometry (Fig. 4A). The results showed that the decrease in the expression of 53BP1 significantly reduced apoptosis of HCT116 cells (8.49±1.88 vs.
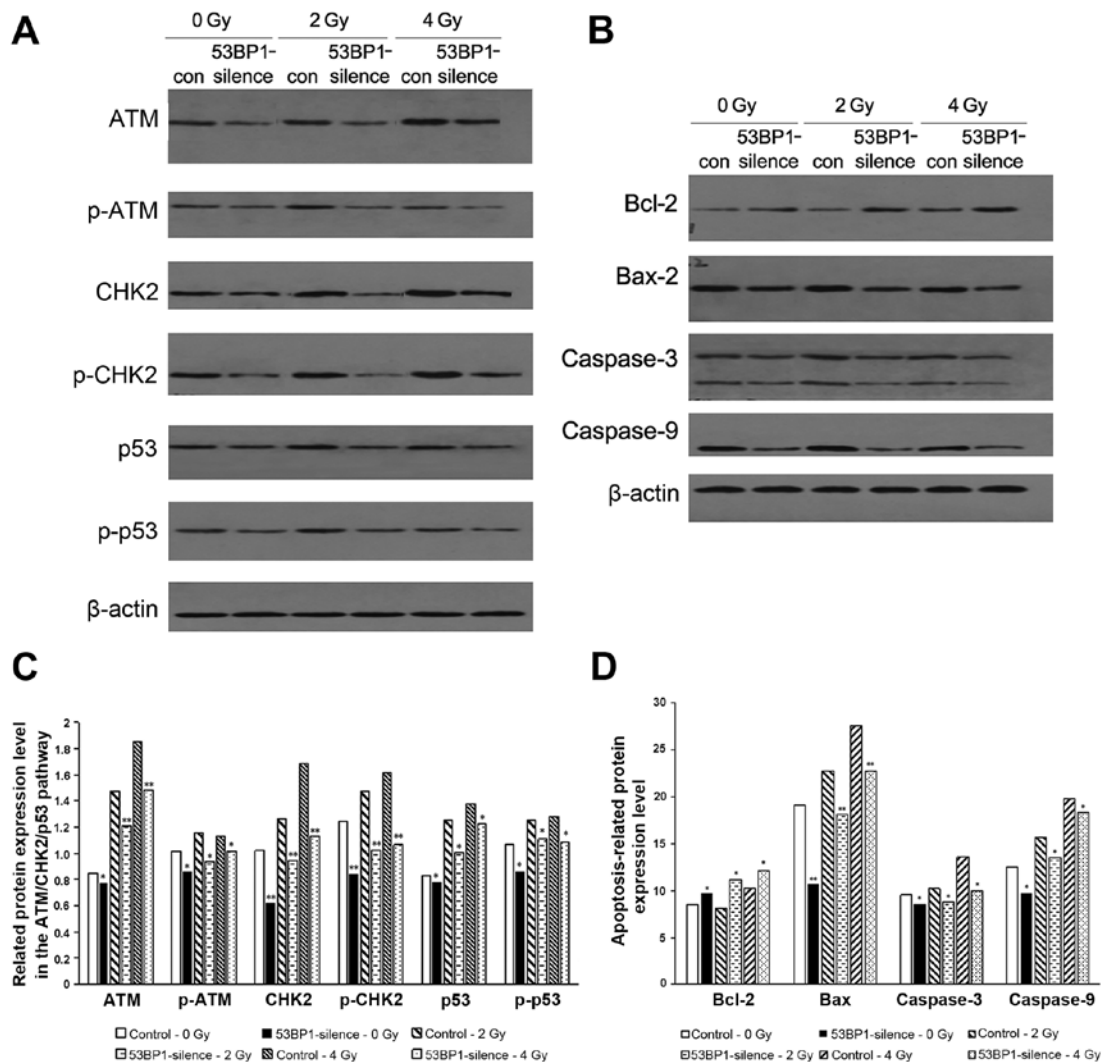
Figure 6. 53BP1 silencing inhibits related protein expression in the ATM-CHK2-p53 pathway. (A) Western blot detection of proteins and their phosphorylated variants in the ATM-CHK2-p53 pathway in HCT116 with 53BP1-silenced and control cells after radiation. The doses are 0, 2 and 4 Gy. (B) Western blot detection of apoptosis-related protein caspase-9, caspase-3, Bax and anti-apoptotic protein Bcl-2 in HCT116 with 53BP1-silenced and control cells after radiation. The doses are 0, 2 and 4 Gy. (C and D) Quantitative detection of ATM-CHK2-p53 and apoptosis-related protein bar chart. Columns, means; error bars, standard error of mean from three independent experiments. *P<0.05.

4.19±1.08%; P<0.05); the decrease in the expression of 53BP1 reduced apoptosis after irradiation (10.58±1.16 vs. 5.91±2.65% for 2 Gy, 13.43±3.15 vs. 8.82±1.17% for 4 Gy; P<0.05) (Fig. 4B).

Decreased expression of 53BP1 increases the percentage of S-phase of HCT116 cells after irradiation. The effect of the decreased expression of 53BP1 on the cell cycle of HCT116 cells after irradiation was investigated using the flow cytometer (Fig. 5A). The results showed that the decrease in the expression of 53BP1 remarkably increased the percentage of the S-phase in tumor cells (21.78±0.73 vs. 13.09±2.12%; P<0.05), indicating that the decreased expression of 53BP1 ushered tumor cells into the proliferation cycle with an accelerated proliferation rate. This result was consistent with the results of previous studies. After irradiation with an exposure to doses 2 and 4 Gy, the two 53BP1 silencing groups exhibited relatively higher percentages of S-phase compared with the control groups (14.92±0.71 vs. 11.91±0.53% for 2 Gy, 14.23±0.32 vs. 10.80±1.07% for 4 Gy) although the difference between

these two exposure groups was not significant (Fig. 5B). This indicates that the decrease in the expression of 53BP1 ushers tumor cells into a high-proliferation status, which inhibits the efficacy of regular radiotherapy.

Decreased expression of 53BP1 inhibits the expression of relevant proteins in the ATM-CHK2-p53 pathway. The ATM-CHK2-p53 pathway plays a pivotal role in the DNA injury repair process and that activation of this pathway may induce the cell cycle arrest, aggravate the DNA injury, and trigger the expression of p53-related apoptotic proteins. Therefore, western blot analysis was used to detect the expression of relevant proteins and their phosphorylated products in the ATM-CHK2-p53 pathway; apoptosis-related proteins caspase-9, caspase-3 and Bax; and the anti-apoptosis protein Bcl-2 before and after the decreased expression of 53BP1 and with or without radiation of 2 and 4 Gy. The results showed that the decrease in the expression of 53BP1 decreased the expression of apoptotic pathway-related proteins ATM, CHK2 and p53 as well as their phosphorylated products (Fig. 6A and C);
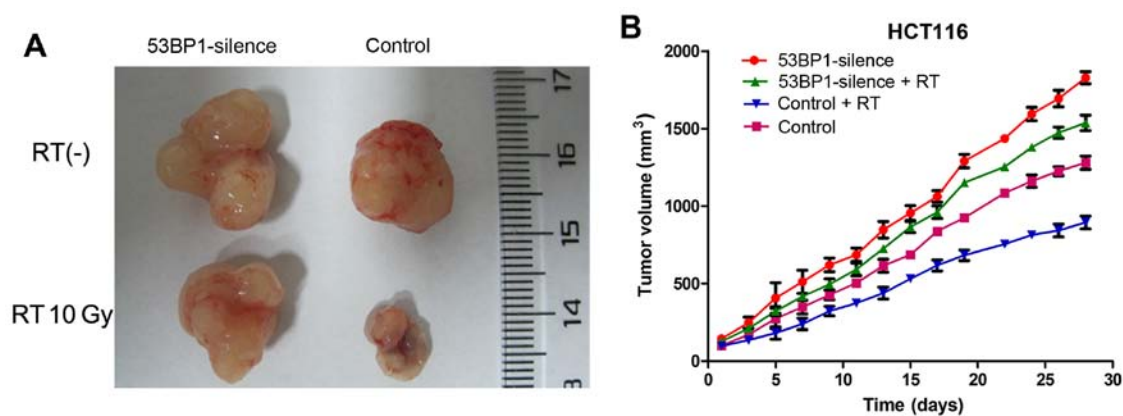
Figure 7. 53BP1 silencing decreases the radiation sensitivity in colorectal xenograft nude mice. The inhibition of *in vivo* tumor growth in nude mice treated with radiotherapy compared with the control group without radiation. (A) Representative tumor image of control and 53BP1-silenced HCT116 cells and with or without radiation. (B) Tumor growth curve of HCT116 with 53BP1-silenced and control cells with or without radiation. The dose is 10 Gy. Data points are the mean values of five animals in each group. Error bars are the standard error of mean of five experiments.

reduced the apoptosis-related proteins caspase-9, caspase-3 and Bax; and increased the expression of anti-apoptosis protein Bcl-2 (Fig. 6B and D), which may be the imminent cause of the decreased expression of 53BP1, resulting in an active proliferation of tumor cells. Similarly, the 53BP1-silenced group showed the reduced expression of the afore-mentioned apoptosis-related proteins and enhanced expression of the anti-apoptosis protein Bcl-2 compared with the control group after exposure to different radiation doses. This indicated that the decrease in the expression of 53BP1 led to the tolerance of tumor cells to irradiation via inhibiting the apoptosis-related ATM-CHK2-p53 pathway and the expression of relevant pro-apoptosis proteins and anti-apoptosis proteins.

Silencing 53BP1 in vivo induces the proliferation of tumor cells and inhibits reactivity to radiotherapy. To investigate the effect of 53BP1 on tumor proliferation and radiotherapy efficacy *in vivo*, the tumor-bearing nude mouse model was established. HCT116 and HCT116 with genetically silenced 53BP1 were subcutaneously injected into the right posterior limb of the mice. Tumor nodes were palpated after 7-10 days, and reached a diameter of 10 mm after 2 weeks. Subsequently, radiotherapy was performed (10 Gy/5F). The size of each tumor was recorded every other day starting from the beginning of treatment. When the tumors of a certain group reached a volume of 2 cm³, all nude mice were sacrificed to record the final tumor volume. The results showed that the tumors of the 53BP1-silenced group showed a relatively higher proliferation activity and reached a size of nearly 2 cm³ on the 28th day of the treatment. Then, all nude mice were sacrificed and the final volume of each tumor was recorded. The final tumor volumes of the 53BP1-silenced group without radiotherapy and the control group were 1.82±0.06 and 1.28±0.06 cm³, respectively, with a significant difference (P<0.01). The 53BP1-silenced group with radiotherapy had a markedly higher proliferation velocity compared with the control group (final tumor volume: 1.54±0.07 vs. 0.89±0.06 cm³; P=0.01), with a significant difference (Fig. 7). The results showed that the decrease in the expression of 53BP1 notably induced the growth of the transplanted colorectal tumor, which exhibited resistance to radiotherapy.

Discussion

Radiotherapy against CRC has now hit a bottleneck. Decades of radiosensitization studies have only realized a limited efficacy, which is primarily caused by the limited radiosensitivity of CRC cells. Therefore, the analysis of indigenous factors that affect tumor radiosensitivity is of crucial importance and has become an urgent problem that needs to be resolved. Multiple studies have shown that genetic stability is the necessary condition under which cells retain their normal function and maintain basic survival (8,17). However, DNA is quite vulnerable to various factors, and this injury may lead to gene mutations, genomic instability, chromosomal loss and reorganization, apoptosis, or even cancerization if timely and precise repair is unavailable (18,19). The DDR pathway plays an essential role in triggering DDR and maintaining the genomic stability of normal cells. Dysfunction of critical factors in the DDR pathway is closely related to the onset, development, and therapy resistance of multiple tumors (20-24). 53BP1 is a recently found cancer suppressor gene, which is also an important member of the DDR pathway family. It is involved in DDR, maintains genomic stability, and regulates apoptosis in coordination with p53 and ATM (25,26). The deficiency of 53BP1 may lead to failed anchoring of broken chromosome ends, which lays the foundation for chromosomal aberration (22). Studies have shown that different degrees of 53BP1 deletion exist during the onset and development of multiple tumors and that this deletion is closely related to tumor staging, malignancy grading, and even therapy resistance (8,17). Neboori *et al* (9) found that 53BP1 deletion resulted in an increased local recurrence rate of breast cancer after radiotherapy. Similarly, Han *et al* (27) found that the decrease in the expression of 53BP1 could enhance the response to DNA damaging agents. The deletion of the expression of 53BP1 in CRC tissue has been detected in previous studies, which was found to be related to the location of the tumors: the right half of the colon exhibited a higher 53BP1 deletion rate compared with the left half of the colon. Furthermore, 53BP1 deficiency was also an early risk factor of poor prognosis (28). Therefore, this study hypothesized that the deficiency of 53BP1 in CRC

tissue probably played a part in influencing the response to radiotherapy.

The present study performed *in vivo* and *in vitro* trials to verify this hypothesis. The expression of 53BP1 in various CRC cell lines was detected by western blot analysis together with differences in their radiosensitivity. The results showed that the expression of 53BP1 was closely related to the radiosensitivity of CRC cells. HCT116 cells with the high expression of 53BP1 were relatively sensitive to radioactivity, while Caco2 cells with low expression of 53BP1 were relatively tolerant to radioactive rays. In this study, the expression of 53BP1 was further silenced via gene interference to investigate the effects of 53BP1 on the radiosensitivity of CRC. The HCT116 cell line with the high expression of 53BP1 was selected, and its stable cell line was constructed with an RNA-intervened lentiviral vector to decrease the expression of 53BP1 by shRNA. The effects of 53BP1 on radiosensitivity were detected by the clone formation assay before and after 53BP1 silencing. The results showed that the decrease in the expression of 53BP1 inhibited the radiosensitivity of the HCT116 cell line. Furthermore, the effects of decreased expression of 53BP1 on proliferation and apoptosis were investigated, which revealed that the exposure to radiation doses of 0, 2 and 4 Gy remarkably induced tumor proliferation and inhibited tumor cell apoptosis. Then, the effect on the cell cycle was investigated using flow cytometry, which showed that the decreased expression of 53BP1 remarkably increased the percentage of the S-phase in HCT116 cells; the two exposure groups (irradiation of 2 and 4 Gy) had higher percentages of S-phase compared with the control groups, although the difference between these two groups was not significant. The previous studies also showed that the decreased expression of 53BP1 can increase the percentage of S-phase (28), which is correlated with cell proliferation (29,30). The results indicated that the decreased expression of 53BP1 prominently enhanced tumor proliferation, inhibited apoptosis, and ushered cells into the proliferation stage, which was associated with radioresistance.

However, the potential mechanisms of 53BP1 impacting the radiosensitivity of CRC is still uncertain. Han *et al* (27) found that the increased expression of 53BP1 can affect the expression of certain proteins in the DDR pathway to inhibit DDR, which was related to an enhanced sensitivity to therapy. Recent studies have recognized that ATM-CHK2-p53 as the primary pathway related to apoptosis is involved in the process of DDR repair, and the deletion of critical elements in this pathway results in the resistance to radiotherapy (17). 53BP1 has been found to be critically related to this pathway in the DDR process, thus, this study speculated that 53BP1 participates in regulating apoptosis and cell cycle distribution probably via affecting the ATM-CHK2-p53 pathway and then influencing the response of tumor tissue to radiotherapy. Therefore, after the expression of 53BP1 was decreased in HCT116 cells, mediated by shRNA interference, the proteins and their phosphorylated variants in the ATM-CHK2-p53 pathway were detected by western blot analysis, along with apoptosis-related protein caspase-9, caspase-3, Bax and Bcl-2. The results revealed that the decrease in the expression of 53BP1 decreased the expression of proteins related to the ATM-CHK2-p53 pathway (ATM and CHK2, as well as their phosphorylated products); decreased the expression

of apoptosis-related proteins caspase-9, caspase-3 and Bax; and increased the expression of the anti-apoptosis protein Bcl-2. This indicated that 53BP1 affected the response of CRC to radiotherapy through impacting the ATM-CHK2-p53 pathway and that 53BP1 played an important role in affecting the response of tumor cells to radiotherapy. The tumor-bearing nude mouse model was then established to investigate the effects of 53BP1 silencing on radiotherapy *in vivo*, which showed that 53BP1 silencing notably induced tumor proliferation and that the tumor had a relatively high-proliferation rate even after radiotherapy. Therefore, 53BP1 silencing was verified *in vivo* to inhibit the efficacy of radiotherapy.

Oncotherapy has now entered the era of precise treatment. Precise radiotherapy is required to maximize efficacy and minimize toxicity. However, no clear factors have been specified to predict the efficacy of radiotherapy. The *in vitro* and *in vivo* studies showed that 53BP1 deletion inhibited the ATM-CHK2-p53 pathway for inducing cell proliferation, inhibiting apoptosis, and then inhibiting the radiosensitivity of intestinal cancer. These results provide new perspectives for future studies in this field, but further clinical studies remain to be performed.

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