Loss of ATRX sensitizes HeLa cells in response to ionizing irradiation in a manner dependent on the ATM/Chk2 pathway

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Research

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Abstract

Identifying novel targets for molecular radiosensitization is critical to improve the efficacy of cancer radiotherapy. Alpha-thalassemia/mental retardation X-linked (ATRX), a member of the SWI/SNF-like chromatin remodeling protein family, functions in the maintenance of genomic integrity, alternative lengthening of telomeres, and the regulation of apoptosis and senescence. However, whether ATRX is directly involved in the DNA damage response (DDR) remains unknown. Here, we show that silencing ATRX increased the sensitivity of cells to ionizing radiation (IR). IR-induced DNA damage was greater and G2/M arrest was less efficient in ATRX knockdown cells. ATRX downregulation caused defects in apoptosis and senescence, indicating that ATRX has a biological function in the DDR. ATRX loss led to failure to trigger ataxia telangiectasia-mutated (ATM) auto-phosphorylation, inhibiting the activation of ATM/Chk2 downstream effectors. These data identify ATRX as a novel target for radiosensitization, and its effect depends on inhibition of the ATM/Chk2 pathway in the DDR.

Background

DNA is an important target of ionizing radiation (IR), and IR-induced DNA damage is the main mechanism mediating the effect of cancer radiotherapy. DNA is impaired by double-strand breaks (DSBs) and single-strand breaks. Although DSBs are not lethal, failure to repair DSBs can lead to cell death [1]. Repair of DSBs is mediated by nonhomologous end joining (NHEJ) or homologous recombination (HR). Failed repair can lead to genetic alterations, which may increase the aggressiveness of tumor cells or trigger the development of malignant cells. After DNA damage is repaired, cells continue to proliferate or undergo apoptosis [2].

The ataxia telangiectasia-mutated (ATM) gene can mediate the DNA damage response (DDR) to resist cytotoxic DNA damage [3]. ATM is an apical kinase thought to regulate, through phosphorylation of hundreds of substrates[4] and is regulated by the global cellular responses initiated by DSBs, including the coordination of DSBs repair events, and the activation of the cell cycle[5, 6]. ATM is activated by autophosphorylation, and then activates checkpoint kinase 2 (Chk2), resulting in cell cycle checkpoint initiation and/or apoptosis [7, 8]. ATM is recruited to sites of DSBs by the MRN (Mre11/Rad50/Nbs1) complex and phosphorylates the histone H2AX to generate γH2AX [5, 9–11]. ATM can also activate the G2/M cell cycle checkpoint by mediating the phosphorylation of Cdc25C by Chk2 [6, 12]. Inhibition of the ATM/Chk2 pathway can cause cell death [13, 14]. Loss of ATM promotes IR-induced responses, suggesting a potential strategy to induce cell death by suppressing the ATM/Chk2 pathway to reduce DNA damage repair in cancer radiotherapy.

Alpha-thalassemia/mental retardation X-linked (ATRX) has recently attracted increasing attention, and studies indicate that ATRX binds to repeated sequences of DNA [15, 16]. ATRX interacts with DAXX to form a histone chaperone complex that loads the histone 3 variant H3.3 onto telomeres, imprinted genes, and endogenous retroviral elements to establish and maintain a heterochromatin environment [17–19]. ATRX plays a direct role in DNA repair, including the restart of stalled replication forks and DSB repair via

HR or NHEJ [20]. ATRX may also play an important role in the alternative lengthening telomere (ALT) pathway [21, 22]. Loss of ATRX function causes p53-dependent apoptosis in ATRX-null neuroprogenitor cells and leads to genomic instability *in vivo* [23]. It also attenuates senescence induced by multiple treatments (CDK4i and doxorubicin) [24]. Additionally, loss of ATRX function sensitizes glioma cells to temozolomide by suppressing ATM [25]. These findings suggest that ATRX regulates DNA damage by modulating the activity of ATM, thereby affecting cell killing effects from radiation or drugs.

In the current study, shRNA-mediated ATRX silencing in HeLa cells was performed to explore the relationship between radiosensitivity changes and cell death, DNA damage repair, cell cycle arrest, and ATM/Chk2 pathway status. The results showed that ATRX silencing increased the sensitivity of HeLa cells to IR, as indicated by increased apoptosis and decreased senescence, as well as increased DNA damage, aberrant G2/M checkpoint, and inactivation of the ATM/Chk2 pathway. Taken together, these findings suggest that ATRX is a potential target for cancer radiosensitization.

Methods

Generation of ATRX-depleted HeLa cell lines

HeLa and 293T cells were obtained from American Type Culture Collection and grown in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA). To obtain stable ATRX-depleted cell lines, 293T cells were transfected with shRNAs (sense sequences are shown in Table 1) using the lentiviral expression vector pGIZ (a gift of Dr. Shen from the Cancer Institute of New Jersey, Rutgers, USA). 293T cells were seeded in a 6-cm dish at a density of 1.2×10^6, and 24 h later were transfected with 120 μl of transfection reagents (Shanghai YESEN Biotechnology Co., Ltd.), 3 μg shRNA plasmids, 1.5 μg pSPAX2 packaging plasmids, and 1.5 μg pMD2G enveloping plasmids according to the manufacturer's instructions. Lentiviral supernatants were harvested at 48 and 72 h after transfection and filtered with a 0.45 μm filter (Merck Millipore, Billerica, MA, USA). HeLa cells were cultured in 6-well plates at a density of 2.0×10^5 and infected with lentiviral supernatants supplemented with 10 mg/ml Polybrene (Sigma-Aldrich; St. Louis, MO, USA). HeLa Cells were sub-cultured into 6-cm dishes and selected with 1 μg/ml Puromycin (Sigma-Aldrich; St. Louis, MO, USA). ATRX protein expression was measured using an anti-ATRX antibody (Santa Cruz, CA, USA) by immunofluorescence (IF) and western blotting (WB) to identify ATRX-depleted HeLa cell lines.

Colony Formation Assay

Cells were seeded into 6-cm dishes at densities of 8×10^1 (0 Gy), 2×10^2 (2 Gy), 8×10^2 (4 Gy), 4×10^3 (6 Gy), and 2×10^4 (8 Gy), and were irradiated by with an X-RAD 320iX machine (Precision X-ray, Inc, USA) at a dose rate of 1.0 Gy/min. After 10 days, cells were fixed with methanol and dyed with Giemsa, and the colonies were counted and calculated. The survival fraction (SF) was calculated as the ratio of treated group colony number to untreated group colony number(*26*).

Cck8 Assay

Cells were seeded in 96-well plates at a density of 6×10^4 or 10-cm dishes at 2×10^6 cells, and irradiated with X-rays at 0, 2, and 8 Gy. In the 96-well plates, CCK-8 reagents were added into each well at 0, 6, 10, 24, and 48 h and A_{490} values were detected. In the 10-cm dishes, the supernatants were collected at 48 h, filtered through a 0.45 μm filter, and added to the HeLa cells cultured in 96-well plates, and A_{490} was detected as described above. [b] There were six replicate wells per group. The experiment was performed in triplicate.

Flow Cytometry

Cell cycle distribution, polyploid cells, and apoptosis were detected by flow cytometry. For cell cycle and polyploid analysis, cells were collected after 24 h of irradiation with 2 and 8 Gy, centrifuged at 1000 rpm for 5 min, the supernatant was discarded, and cells were washed twice with PBS. Cells were re-suspended in 500 μl propidium iodide (BD, Franklin Lakes, NJ, USA) and stained for 30 min at room temperature (RT). For apoptosis detection, cells were stained with 10 μl Annexin V and 7-AAD (BD) for 15 min in the dark. Cells were collected and analyzed using FACSCanto II (BD).

Senescence Analyses

Cells were seeded in 6-well plates at a density of 1×10^5 and irradiated with 4 Gy. After 0.5, 1, 2, 5, and 7 days, cell senescence was detected using senescence-associated β-galactosidase (SA-β-gal) kits (Beyotime® Biotechnology, Hangzhou, China). Cell number was quantified by β-galactosidase staining as a proportion of total cells [27].

Quantitative real time PCR (qRT-PCR)

RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) after 48 h of irradiation at 0, 2, and 8 Gy, and the complementary DNA (cDNA) was synthesized from 1 μg of each RNA sample using a high-capacity reverse transcription kit (Takara Bio Inc. Japan). Primer sequences are shown in Table 2. The qRT-PCR reaction was performed using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) and with the SYBR® Premix Ex Taq ™ II kit (Takara Bio Inc., Japan) protocol. The qRT-PCR data were analyzed using the $2^{-\triangle\triangle Ct}$ method[28].

Immunofluorescence

The shCon-, shATRX1-, shATRX2-, and shATRX3-HeLa cells were grown on coverslips placed in 6-well plates for 12 h. Then, the coverslips were removed, fixed with 4% paraformaldehyde for 10 min at RT, permeabilized, and blocked with blocking buffer (0.3% Triton X-100 and 2% BSA in PBS) for 1 h at RT. The cells were incubated with anti-ATRX (1:500 in blocking buffer) overnight at 4 °C, followed by incubation with secondary antibodies (1:1000, red fluorescence) for 1 h at 37 °C. The coverslips were mounted onto microscope slides, fluorescence was observed, and images were processed.

γh2ax Foci

Cells were irradiated with 4 Gy, and after 0, 0.5, 1, 3, and 6 h, the coverslips were removed and stained as described above. Anti-γH2AX (Cell Signaling Technology, Danvers, MA, USA) (1:500 in blocking buffer) and secondary antibodies (1:1000, red fluorescence) were used for the immune reaction. DAPI was used for nuclear staining. The coverslips were mounted on microscope slides, red fluorescence was observed, and γH2AX foci were quantified as a proportion of total cells [29].

Western Blot

Total proteins were extracted and quantified, and 40 μg protein were separated by SDS-PAGE, followed by transfer to nitrocellulose membranes (200 mA, 1.5 h; Merck Millipore, Billerica, MA, USA). After blocking with 5% milk, the membranes were incubated with primary antibodies including anti-ATRX, anti-PARP1, anti-ATM, anti-p-ATM, and anti-GAPDH (Santa Cruz, CA, USA), anti-γH2AX, anti-Chk2, anti-p-Chk2, anti-Cyclin B1, anti-caspase-9 (cleaved), and anti-caspase-3 (cleaved) (Cell Signaling Technology), anti-Rad51 (Abcam, Cambridge, MA, USA), and anti-cdc2 (GeneTex, Alton Pkwy Irvine, CA, USA) overnight at 4 °C. After washing with TBST, the membranes were incubated with IgG-HRP conjugated secondary antibody (Immunoway, Plano, TX, USA) for 1.5 h at RT. Finally, the membranes were detected using an enhanced chemiluminescence detection system (ECL detection kit, Santa Cruz, CA, USA). The films were scanned for quantification of protein bands.

Statistical analysis

All statistical analyses were performed using SPSS 24.0 (SPSS Inc., Chicago, IL, USA) or Graphpad Prism 5.0 (La Jolla, CA, USA). The results are presented as the mean ± SD and analyzed using the Student's t test. All differences were considered statistically significant at $P < 0.05$.

Results

ATRX loss increases the inhibiting effect of IR on cell proliferation

In this study, the lentiviral vector pGIZ was used to deliver shRNA targeting ATRX (NM_000489.4), and GFP expression was used to identify infected cells. ATRX protein expression was detected by IF and WB (Fig. 1A and B), and the results showed that ATRX was not expressed in shATRX1-, shATRX2-, and shATRX3-HeLa cells. This confirmed the generation of stable ATRX knockdown cell lines. To examine the effect of ATRX knockdown on radiosensitivity, we performed colony formation assays. Increased radiation doses were associated with decreased numbers of surviving cells in the sh-control group; knockdown of ATRX in shATRX2-HeLa cells further decreased the survival capability compared with that of sh-control cells (Fig. 1C). Assessment of cell proliferation using the CCK8 assay showed that radiation inhibited cell viability to a greater extent in shATRX2-HeLa cells than in sh-control cells (Fig. 1D).

Atrx Loss Increases Ir-induced Cell Apoptosis

To explore the mechanisms underlying the inhibition of cell proliferation by IR, apoptosis was assessed by flow cytometry with Annexin V and 7-AAD staining. The results showed that the apoptosis rate was

significantly higher in shATRX2-HeLa cells than in shCon-HeLa cells (Fig. 2A and B). The expression of PARP1 and cleaved caspase-9 and − 3 was higher in irradiated-shATRX2-HeLa cells than in shCon cells (Fig. 2C and D). These results demonstrated that loss of ATRX increased IR-induced apoptosis.

ATRX loss decreases senescence and increases proliferation inhibition

Irradiation with 4 Gy induced the accumulation of perinuclear SA-β-gal (Fig. 3A and B) in shCon- and shATRX2-HeLa cells; however, the percentage of β-gal positive cells was lower in shATRX2-HeLa cells than in shCon-HeLa cells in the presence or absence of IR (Fig. 3A and 3B). This decline in senescence seems to inhibit cell death; we therefore examined whether senescence induced by ATRX loss could inhibit cell proliferation. For this purpose, the supernatants collected from shCon- and shATRX2-HeLa cells after IR were used for cell culture. As shown in Fig. 3C, cells stimulated with senescent supernatants from shATRX2-HeLa cells grew slowly. The mRNA expression of interleukin (IL)-8, IL-6, and CXCL1 was decreased in shATRX2-HeLa cells after IR (Fig. 3D). These results indicated that cell senescence decreases caused by ATRX loss were beneficial for cancer radiotherapy.

Atrx Is Involved In The Dna Damage Repair Process

We observed a significant increase of γH2AX foci at 0.5 and 1 h after 4 Gy IR, followed by a decrease, whereas the number of γH2AX foci was higher in shATRX2-HeLa cells (Fig. 4A and B). WB assessment showed that the γH2AX protein was expressed at a higher level in shATRX2-HeLa cells (Fig. 4C). Previous studies demonstrated that ATRX loss impairs NHEJ, and Rad51 are involved in this process [30–32]. In contrast to γH2AX expression, Rad51 were downregulated in shATRX2-HeLa cells, and ATRX protein expression in shCon-HeLa cells increased after IR (Fig. 4D). Taken together, these results indicate that ATRX plays an important role in DNA damage repair, and depletion of the ATRX protein reduced the NHEJ capability.

ATRX loss regulates cell cycle progression dependent on ATM/Chk2 pathway to after IR

To further investigate the mechanisms by which ATRX regulates DNA damage repair and the DDR, cell cycle progression was analyzed by PI staining. Flow cytometry results showed G2/M arrest in ATRX-depleted HeLa cells in the absence of IR, whereas after exposure to 8 Gy IR, G2/M phase arrest was more severe. The percentage of cells in G2/M phase was lower, whereas that of cells in of G0/G1 phase was higher in shATRX2-HeLa cells than in shCon cells (Fig. 5A and C). As shown in Fig. 5B and C, analysis of hexaploid and octoploid cells showed that they were significantly increased; however, they were lower in shATRX2-HeLa cells than in shCon cells. Taken together, these results indicated that ATRX depletion induced G2/M arrest and hexaploid and octoploid cells; cells had mitotic abnormality, and after IR, cells underwent apoptotic death, thus the percentages of cells in G2/M phase and that of hexaploid and octoploid cells were reduced.

Our previous results demonstrated that ATRX depletion increased radiosensitivity (Fig. 1B), and was involved in DNA damage (Fig. 4) and cell cycle regulation (Fig. 5A–C). Additionally, activation of the ATM

pathway activated checkpoint proteins and the repair of DNA damage [33]. To determine whether the increased radiosensitivity was regulated by deactivation of the ATM protein in ATRX-depleted HeLa cells, we measured phosphorylated ATM (p-ATM) expression, as well as the expression of downstream p-Chk2. The results showed that p-ATM and p-Chk2 were expressed at lower levels in ATRX-depleted HeLa cells irradiated with 2 Gy (Fig. 5D). Cdc25A, cyclin B1, and cdc2 were also decreased, indicating G2/M arrest. However, after exposure to 8 Gy IR, the phosphorylation levels of ATM and Chk2 were lower in ATRX-depleted cells than in shCon cells, and Cdc25A and Cyclin B1 were upregulated (Fig. 5D). These results indicated that ATRX loss induced the DNA damage repair-dependent ATM/Chk2 pathway to increase radiosensitivity.

Discussion

Radiotherapy is an effective treatment that is used in 50% of solid tumors [34]. IR is a potent genotoxic agent inducing DNA damage. However, the mechanisms of cell death induced by cancer radiotherapy remain unclear. ATRX was shown to play multiple roles in cancer. ATRX mutation and the simultaneous occurrence of ALT were demonstrated in several types of tumors [35, 36]. ATRX also regulates cell cycle progression and maintains genomic stability. In HeLa cells, ATRX depletion can induce abnormal chromosome aggregation, reduced sister chromatid cohesion and cause cell proliferation inhibition [37]. In this study, we showed that 8 Gy IR induced changes in the G2/M phase in ATRX-depleted HeLa cells, which also showed a lower percentage of hexaploid and octoploid cells, indicating abnormal mitosis in ATRX-depleted HeLa cells. Activation of ATM and/or the ATR pathway blocks mitosis [38]. The ATM pathway is primarily responsible for G2/M checkpoint initiation; after ATRX loss, the phosphorylation of ATM and Chk2 was increased, and 2 Gy IR promoted the phosphorylation of both proteins, whereas 8 Gy IR decreased phosphorylation. It is likely that the diminished G2/M checkpoint response allows cells to proliferate [39]. Indeed, we found that the proliferation of shATRX-HeLa cells was reduced following IR, although the underlying mechanisms need to be further investigated.

We propose that loss of ATRX function might affect the DDR in a manner dependent on the ATM/Chk2 pathway. As described above, inactivation of ATRX might activate ATM/Chk2 to regulate Cyclin B, Cdc25A, and cdc2 expression and G2/M arrest [40]. However, the percentage of cells in G2/M phase was reduced following exposure to 8 Gy IR; the ATM/Chk2 pathway was suppressed, and different activation states were generated because of the DNA damage induced by different doses of IR. When cells are exposed to IR, DSBs are generated, rapidly resulting in H2AX phosphorylation by ATM; phosphorylation is considered as a sensitive marker of DNA damage [41]. The data presented here demonstrate that IR induced the formation of γH2AX foci and promoted γH2AX protein expression, and these effects were more obvious in ATRX-depleted HeLa cells. Indeed, G2/M checkpoint inhibitors to enhance the efficacy of clastogenic therapies are currently being developed and evaluated in clinical trials [42]. In this study, we found that radiosensitivity was increased in ATRX-depleted HeLa cells, as demonstrated by colony formation assays.

If DNA damage is very serious, repair cannot be completed, and certain pathways are activated to clean up the damaged cells. Therefore, we investigated cell apoptosis and senescence. IR-induced apoptosis was increased in shATRX-HeLa cells, and PARP1 and cleaved caspase-9 and − 3 were upregulated. Additionally, during cancer treatment, senescence is a suppressive factor for cancer cells [43]. Studies show that senescence has a neutral or anti-proliferative effect on surrounding cells [44, 45]. On the other hand, senescent cells stimulate a cytokine release to lead to "sterile" inflammation [46]. Senescent cells secrete characteristic pro-inflammatory cytokines, such as IL-6 and IL-8 [47]. Although these cytokines can mediate senescence-related effects, the underlying mechanism is unclear. We showed that IR could induce HeLa cell senescence; however, ATRX depletion decreased the number of senescent HeLa cells, which is consistent with the results reported by Kovatcheva et al [48]. We used supernatants from IR-treated cells to stimulate HeLa cells, and the cell proliferation was inhibited; a possible mechanism underlying this effect could involve the downregulation of IL-6, IL-8, and CXCL1. Our data provided a different demonstration for IR-induced proliferation inhibition.

Conclusions

In this study, we demonstrated the potential of ATRX as a target for radiosensitization in cancer and elucidated the underlying mechanisms (Fig. 6). This study provides evidence that loss of ATRX reduces cell proliferation by suppressing DDR dependent on the ATM/Chk2 pathway. ATRX depletion enhanced the effect of IR on inducing apoptosis and decreased its effect on inducing G2/M arrest and senescence; in addition, IL-6, IL-8, and CXCL-1 were downregulated to inhibit cell proliferation. These results suggest that ATRX regulates IR-induced HeLa cell death, and propose a novel mechanism for radiosensitization in cancer.

Abbreviations

ATM: ataxia telangiectasia-mutated; ATRX: Alpha-thalassemia/mental retardation X-linked; Chk2 checkpoint kinase 2; DDR: DNA damage response; DSBs: double-strand breaks; HR: homologous recombination; IR: Ionizing radiation; NHEJ: nonhomologous end joining; qRT-PCR: Quantitative real time PCR;SA-β-gal: senescence-associated β-galactosidase

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

Experiments were conceived and designed by Z.W. Experiments were performed by G. T., Y. Y., Y. L., B. H. and W. X. Data analysis were performed by F. F. and L. Q. Overall discussions of the data and implications involved all authors; and the manuscript was written by G. T. and Z. W.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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Figures

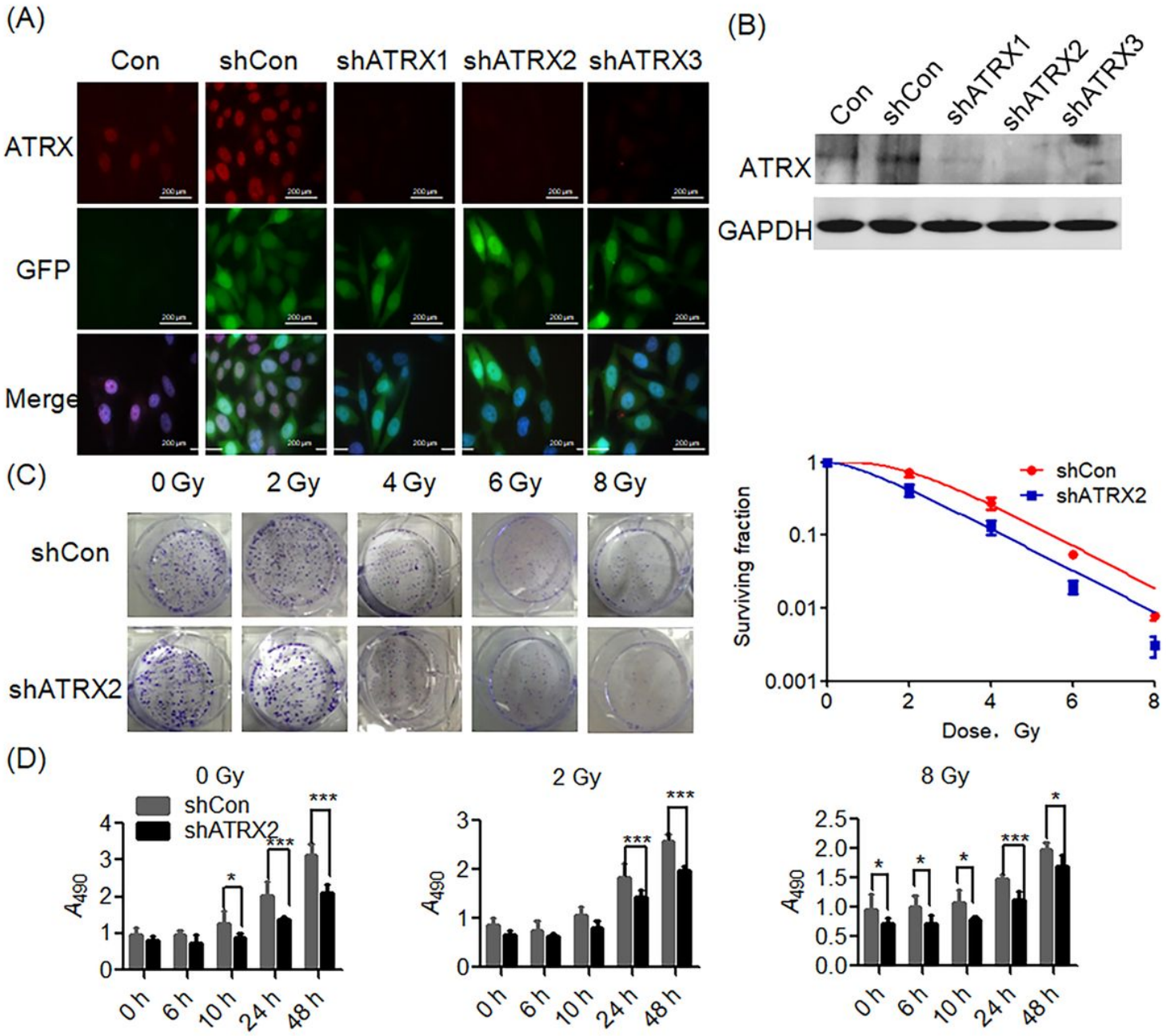

Figure 1

ATRX loss increases the proliferation inhibiting effect on HeLa cells after IR. (A) Immunofluorescence (IF) analysis of ATRX expression (red). HeLa cells were infected by pGIZ-lentivirus (green), and red fluorescence is present in HeLa and shCon-HeLa cells, but not in shATRX1-, shATRX2-, and shATRX3-HeLa cells, (magnification, 200×). (B) Western blot (WB) analysis showing that ATRX is downregulated in stable ATRX-depleted cell lines. (C) Colony formation assay of shCon- and shATRX2-HeLa cells following IR treatment as indicated, and cellular sensitivity to IR. (D) CCK8 assay demonstrates stronger effects of 2 and 8 Gy IR on ATRX-depleted HeLa cells in suppressing tumor proliferation. Bars represent the mean ± SD of triplicate measurements. *P < 0.05, **P < 0.01 and ***P < 0.001 versus shCon-HeLa cells.

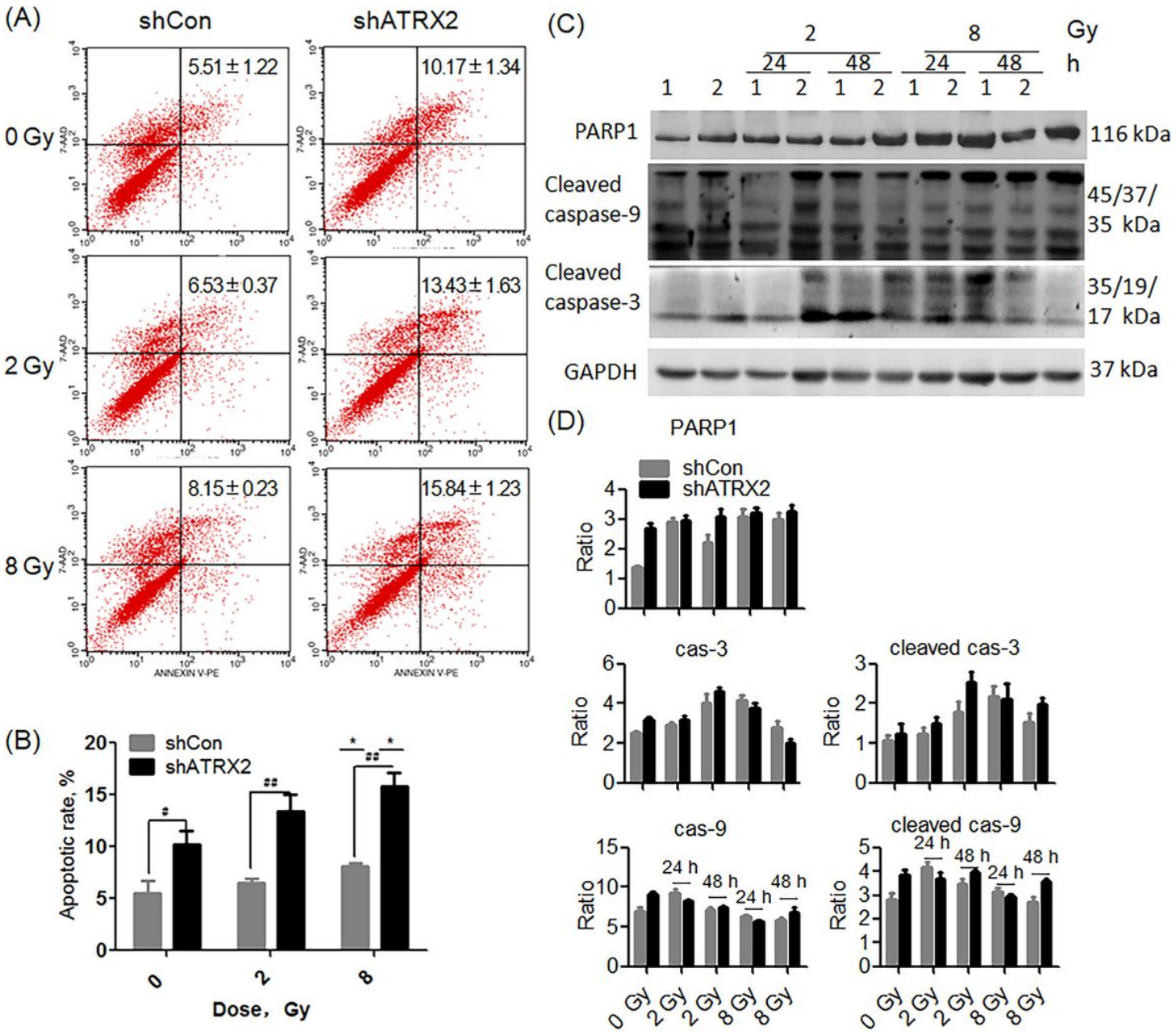

Figure 2

Apoptosis induced by IR and caspase-9 and -3 expression. (A) and (B) Representative flow cytometry (FCM) images of apoptosis and FCM analysis (%) in shCon- and shATRX2-HeLa cells stained by Annexin V and 7-AAD. (C) PARP1, cleaved caspase-9 and -3 were detected by WB, GAPDH was used as a loading control. Lane 1: shCon-HeLa cells and Lane 2: shATRX2-HeLa cells. Bars represent the mean ± SD of triplicate measurements. *P < 0.05 versus 0 Gy, #P < 0.05 and ##P < 0.01 versus shCon-HeLa cells.

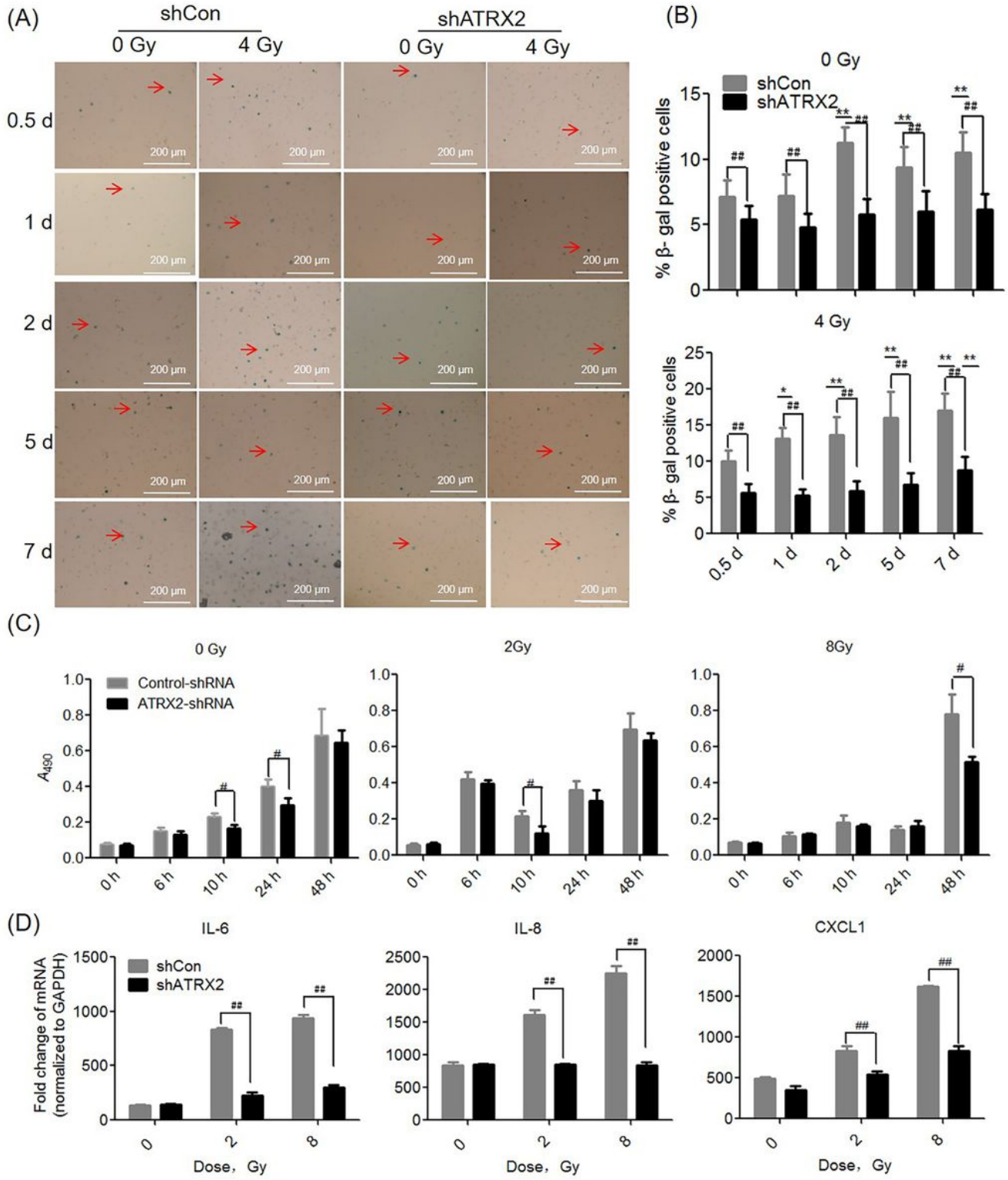

Figure 3

Reducing ATRX suppresses IR-induced senescence in HeLa cells. (A) Cells were stained with SA-β-gal kits to detect cellular senescence (blue, red arrow) (magnification, 100×). (B) The changes of β-gal positive cells. (C) The senescent supernatants in shCon- and shATRX2-HeLa cells were collected to stimulate HeLa cells, cell proliferation was measured by CCK8 assay. (D) The mRNA levels of IL-8, IL-6, and CXCL1

in shCon- and shATRX2-HeLa cells were measured by qRT-PCR. Bars represent the mean ± SD of triplicate measurements. *P < 0.05 and **P < 0.01 versus 0.5 d, #P < 0.05 and ##P < 0.01 versus shCon-HeLa cells.

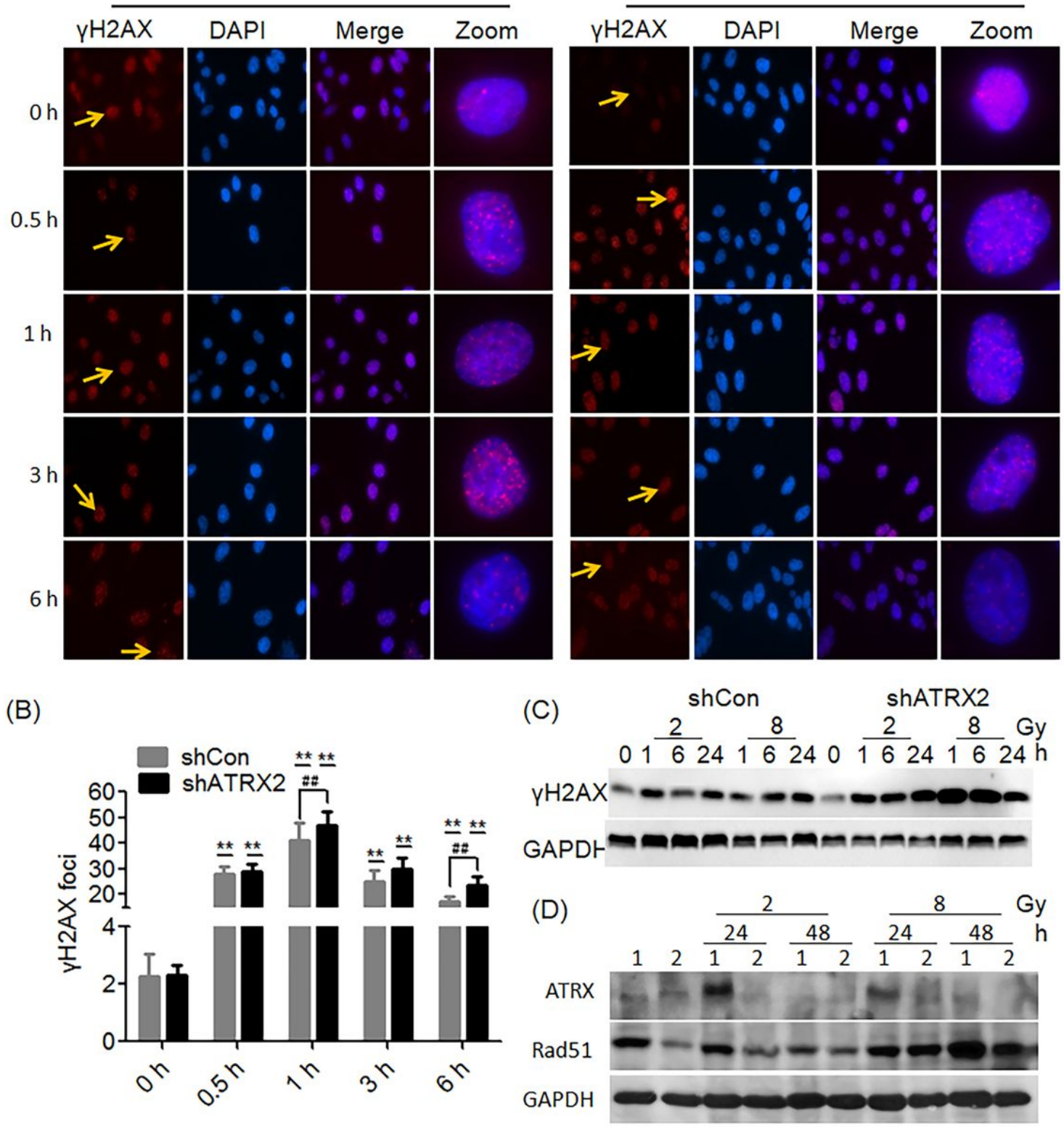

Figure 4

ATRX is involved in the DNA damage repair process. (A) Representative images of γH2AX foci, 200×. (B) IR induced more γH2AX foci in ATRX-deficient HeLa cells. (C) The expression of γH2AX was measured by WB, GAPDH was used as a loading control. (D) ATRX and Rad51 proteins were assessed by WB, GAPDH

was used as a loading control. Lane 1: shCon-HeLa cells and Lane 2: shATRX2-HeLa cells. Bars represent the mean ± SD of triplicate measurements. **P < 0.01 versus 0 h, ##P < 0.01 versus shCon-HeLa cells.

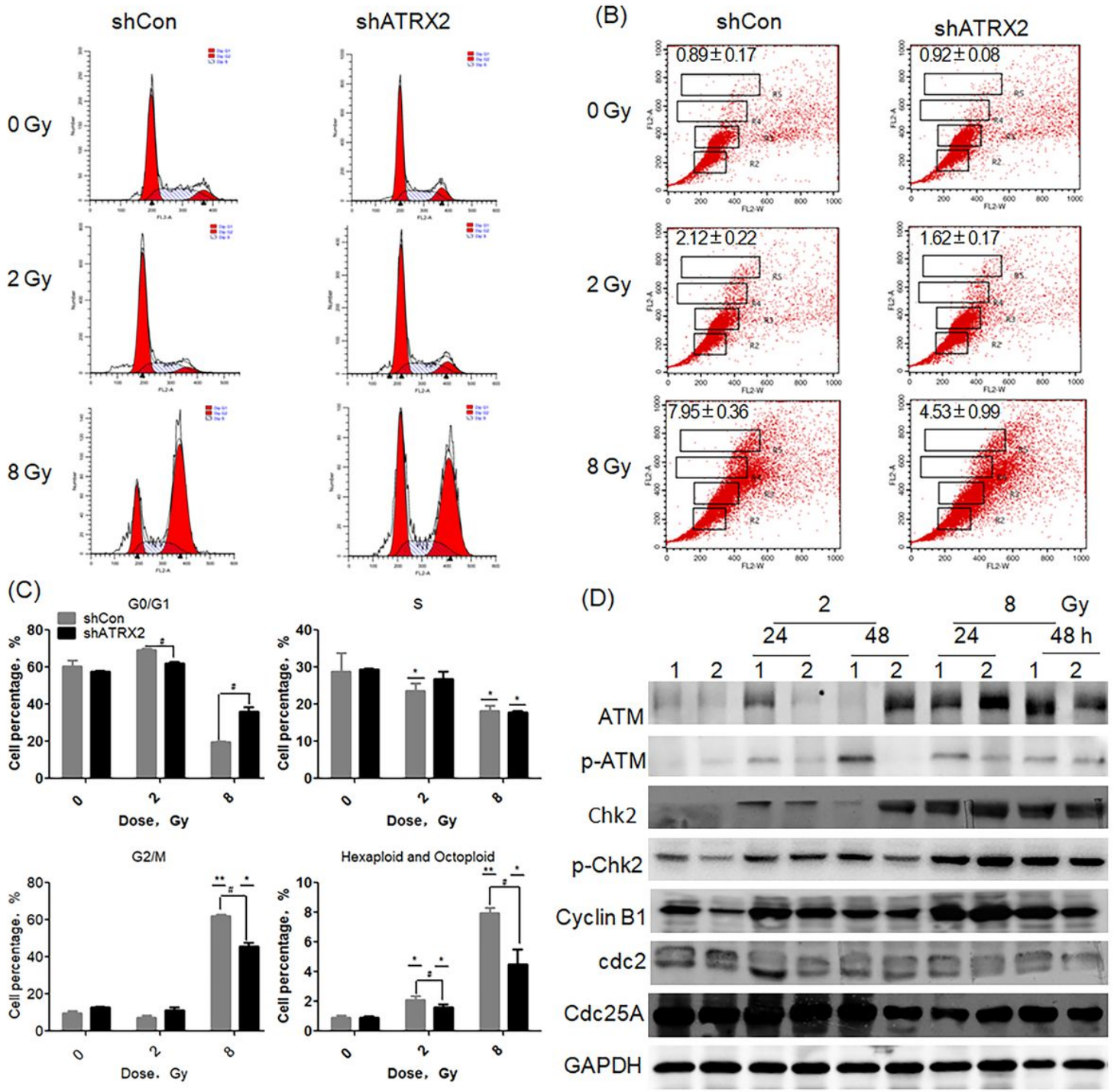

Figure 5

ATRX loss regulates G2/M arrest dependent on the ATM/Chk2 pathway after IR. (A) Representative images of cell cycle profile by FCM. (B) The hexaploid and octoploid (%) cells were detected by FCM. (C) The percentages of cells in different cell cycle phases detected by PI staining and FCM are shown. (D) WB analysis of ATM and its downstream proteins. Lane 1: shCon-HeLa cells and Lane 2: shATRX2-HeLa

cells. Bars represent the mean ± SD of triplicate measurements. *P < 0.05, **P < 0.01 versus 0 Gy, #P < 0.05 versus shCon-HeLa cells.

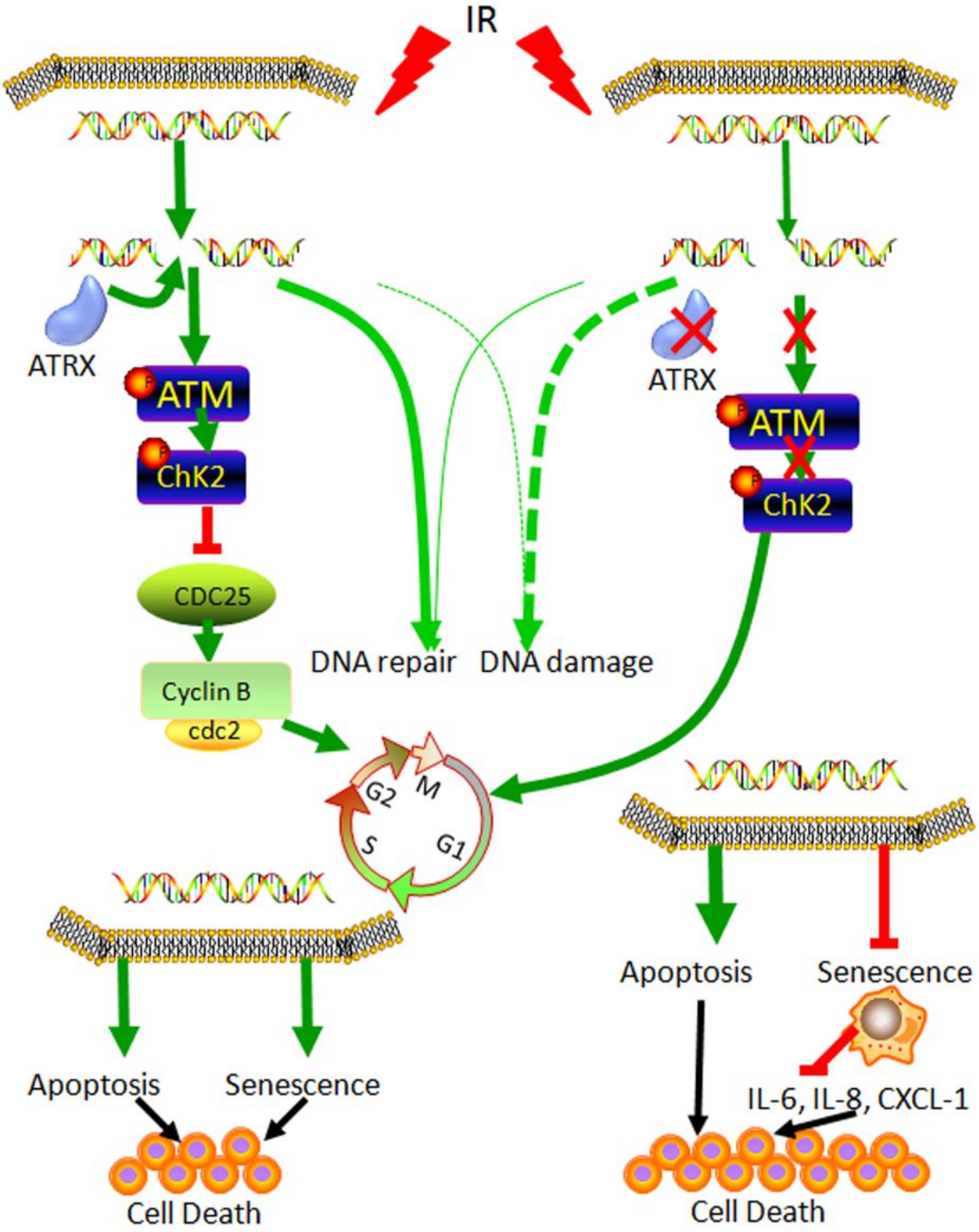

Figure 6

Schematic depiction of radiosensitization of ATRX-depleted HeLa cells. ATRX is involved in IR-induced DNA damage repair, G2/M arrest, apoptosis, and senescence in a manner dependent on the ATM/Chk2 pathway. When ATRX was depleted from HeLa cells, the phosphorylation of ATM and Chk2 was reduced,

and DNA damage repair was attenuated to induce more apoptosis and less G2/M arrest and senescence., IL-6, IL-8 and CXCL-1 were reduced to inhibit cell proliferation.