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Research Paper

Inhibition of PI3K/AKT Signaling Pathway Radiosensitizes Pancreatic Cancer Cells with ARID1A Deficiency *in Vitro*

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Abstract

Pancreatic cancer is among the most aggressive human cancers, and is resistant to regular chemotherapy and radiotherapy. The AT-rich interactive domain containing protein 1A (*ARID1A*) gene, a crucial chromatin remodeling gene, mutates frequently in a broad spectrum of cancers, including pancreatic cancer. Recent evidence suggests that ARID1A acts as tumor suppressor and plays an important role in DNA damage repair (DDR). However, the effect of ARID1A on the radiosensitivity of pancreatic cancer remains unclear. Herein, we investigated the involvement of ARID1A depletion in the radioresistance of pancreatic cancer cells, and explored the underlying mechanisms. The results reveal that knockdown of *ARID1A* enhances the radioresistance of pancreatic cancer cells through suppressing apoptosis, impairing G2-M checkpoint arrest, strengthening DDR, and accompanying activation of PI3K/AKT signaling pathway. Moreover, upon inhibition of PI3K/AKT pathway by PI3K-inhibitor LY294002 or AKT-inhibitor mk2206, the radiosensitivity of ARID1A-deficient pancreatic cancer cells is improved *in vitro* via increased apoptosis and weakened DDR. Taken together, these data suggest that loss of ARID1A expression enhances radioresistance of pancreatic cancer through activation of PI3K/AKT pathway, which maybe a promising target for radiosensitization of ARID1A-deficient pancreatic cancer.

Key words: ARID1A; pancreatic cancer; PI3K/AKT pathway; radioresistance

Introduction

Pancreatic cancer, one of the most hyper-invasive human cancers, is the fourth leading cause of cancer-related deaths in the United States [1]. Over 250,000 people develop pancreatic cancer every year worldwide [2]. Despite 50 years of research and therapeutic development, the 5-year relative survival is only 8% [1].

More than 80% of pancreatic cancers patients are inoperable when diagnosed [3]. For such patients, chemotherapy only affords a median survival of less than one year [4, 5], even in combination with erlotinib [6]. Recent progress in radiotherapy has raised extensive concerns, including the application of new radiation technologies [7], and new sensitizing drugs [8, 9], especially for targeted agents [10, 11]. Pancreatic cancer displays a variety of gene mutations, which may suggest new therapeutic targets.

AT-rich interactive domain containing protein 1A (*ARID1A*), a chromatin remodeling gene, has been recently found to be mutated in various cancers, including ovarian clear cell carcinoma [12], endometrioid carcinoma [13], gastric cancer [14], breast cancer [15], liver cancer [16], and pancreatic cancer [17, 18]. *ARID1A* encodes the protein BRG1-associated factor 250a (BAF250a), a crucial noncatalytic subunit of the human switch/sucrose non-fermentable (SWI/SNF) complex [19], which participates in several important cell functions, including transcription, DNA replication, and DNA repair [20-22]. SWI/SNF was reported as a central tumor suppressive complex in pancreatic cancer, and it affected at least one-third of all pancreatic cancer cases [17].

Mutations of *ARID1A* usually cause down-regulated protein expression [23]. Moreover, functional data suggest that ARID1A is a bona fide tumor suppressor [24]. In pancreatic cancer, the incidence of ARID1A mutations varies from 6% to 9% [25, 26]. A review of the COSMIC database reveals that ARID1A is among the five most frequent somatic mutations in pancreatic cancer [25]. Furthermore, Witkiewicz AK *et al.* found that ARID1A protein deficiency was significantly associated with poor outcome in pancreatic cancer [18], although controversy over this finding still exists [27, 28]. These conclusions all suggest the potential roles of ARID1A in pancreatic cancer, of which currently little is known.

As ARID1A has been reported to take essential part in DNA damage repair (DDR) [21], and DNA damage is known to be the prime cause of radiation-induced cell death, we hypothesize that ARID1A may play a key role in radioresistance of pancreatic cancer. We conducted the present study to investigate the effect of ARID1A on radiosensitivity of pancreatic cancer. Likewise, the possible underlying mechanisms were explored, which may help identify new targets for overcoming the radioresistance.

Materials and Methods

Cell culture and reagents

Human pancreatic cancer cell lines PANC-1 and SW1990 were purchased from the American Type Culture Collection (ATCC, US). Cells were cultured in RPM1640 medium (Hyclone Laboratories, US) supplemented with 10% fetal bovine serum (Gibco Waltham, US) and incubated in a humidified incubator with 5% carbon dioxide at 37 °C. LY294002 and mk2206 were purchased from Selleckchem (TX, US) and dissolved in DMSO.

Short hairpin RNAs (shRNAs)-mediated ARID1A knockdown

Lentivirus-*ARID1A*-RNAi vector and the corresponding empty vector were obtained from Shanghai GeneChem, Co., Ltd. (China). Two independent shRNA constructs (sequence: sh*ARID1A*#1, 5'-GCCTGATCTATCTGGTTCAAT-3'; sh*ARID1A*#2, 5'-CCTCTCTTATACACAGCAGAT-3') were designed and subcloned into the lentivirus vector. After confirmation of the constructed plasmid by DNA sequencing, lentiviral vector and packaging mix with lipofectamin2000 (Invitrogen, CA, US) were transfected into HEK293FT cells. 48 hours later, the supernatant containing lentivirus was collected, purified and supplemented with 8μg/ml polybrene (Santa Cruz biotech, CA, US). The target cells were infected using the virus solution. After culturing for another 72 h, the stably transfected cells were selected on 2 μg/ml puromycin (Sangon biotech, Shanghai, China) for 1 week. The protein expression level was examined by western blot.

Western blot

Cells were collected and lysed in urine buffer supplemented with 1% protease and 1% phosphorylation inhibitors (Roche Diagnostics, US). Equal amount of total protein (30μg) for each sample was loaded to gradient SDS-PAGE gel. After electrophoresis, protein was transferred onto polyvinylidene fluoride membranes (Millipore, US). The membranes were blocked in TBS with 0.1% Tween-20 and 5% non-fat dried milk for 1 hour at room temperature. After washed for three times, the membranes were probed with primary antibodies overnight at 4°C. The following antibodies were used in this study: ARID1A (1:500, Bethyl Laboratory); ATM, p-ATM, CHK1, p-CHK1 (Ser345) (1:500, Cell Signaling); PTEN, PI3K, AKT, p-AKT (Ser473) (1:500, Abcam, MA); H2AX, γH2AX antibodies (1:1000, Millipore) and β-actin antibody (1:2000, Sigma, US). On the next day, membranes were washed for three times, and incubated with HRP conjugated anti-mouse or anti-rabbit secondary antibodies (1:5000, Invitrogen, US) for 1 hour. Target proteins were visualized using an ECL detection kit (Thermo Fisher Scientific, US).

Clonogenic assay

Briefly, cells were seeded into 6-well culture plates. The next day, cells were treated with indicated conditions and cultured for 2 weeks. Clones were fixed with 4% polymerised formaldehyde, stained with 0.01% crystal violet (Sangon biotech, Shanghai, China) and counted.

Apoptosis Assay

Cells were planted in 6-well culture plates with about 40% fusion. 24 hours later, the cells were exposed to ionizing radiation. 72 hours later, cells were harvested and resuspended in binding buffer containing Annexin V-PE, then propidium iodide (PI) was added, using Annexin V/PI apoptosis kit (Invitrogen, US). Flow cytometry analysis (FACScan, Beckman Coulter, US) was conducted to detect the apoptosis. In each result figure (Fig. 2A and 5A), the percentage of cells in the upper right (including necrotic or late apoptotic cells) and lower right corners (including early apoptotic cells) was added as the apoptotic percentage.

Small interfering RNA (siRNAs)-mediated *ARID1A* knockdown

Cells were seeded to 6cm cell culture dish with 30% density and cultured overnight, then transfected with *ARID1A* or non-target siRNA (GeneChem, Shanghai, China) using oligofectamine transfection reagent (Invitrogen, CA, US) according to the protocol provided by the manufacture. The following siRNA sequences were used [29]:

ARID1A:
sense: 5'-GCCCUAACAUGGCCAAUAUTT-3'.
antisense: 5'-AUAUUGGCCAUGUUAGGGCTT-3'.

non-target control:
sense: 5'-UUCUCCGAACGUGUCACGUTT-3'.
antisense: 5'- ACGUGACACGUUCGGAGAATT-3'.

Cell cycle analysis

After indicated treatment, cells were collected and fixed with 70% ethanol at 4°C overnight. Then cells were washed, suspended and stained with propidium iodide (PI) staining solution (50μg/ml PI and 1mg/ml RNase in PBS). And cell cycle analysis was performed using Flow cytometer FACScan (Beckman Coulter, US).

Immunofluorescent staining

Cells grown on coverslips were fixed with 3% paraformaldehyde at room temperature for 20 min and then permeabilized with PBS containing 0.5% Triton X-100 for 5 min. The coverslips were blocked with PBS containing 5% goat serum for 30 min, and immunostained with anti-γH2AX (Millipore) antibody at 4°C overnight, then washed for at least three times and incubated with secondary antibody, Alexa Fluor 488 conjugated goat anti-rabbit IgG (1:50, Cell Signaling, US), for 1 hour at room temperature. DAPI (4, 6-diamidino-2-phenylindole, Sangon biotech, Shanghai, China) was used to counterstain the nuclei. Photographs were captured by Olympus Laser scanning confocal microscopy (Olympus optical Co., Tokyo, Honshu, Japan). At least 50 cells were scored for each treatment group, and the number of γH2AX foci was counted.

Immunohistochemistry (IHC)

Twenty pancreatic cancer primary tissue samples were collected between January 2016 and June 2017 with informed consent from the patients under institutional review board-approved protocols. This study was approved by the institutional research ethics committee of our university. Formalin-fixed and paraffin-embedded tissue sections (5μm thick) were deparaffinized and rehydrated. Subsequently, the sections were blocked of endogenous peroxidase and subjected to antigen retrieval. Then, 5% bovine serum albumin (BSA) was used to incubate the sections for 30 min at room temperature, followed by the primary antibody dilution (ARID1A, 1:2000; PI3K, 1:200; p-Akt, 1:100) overnight at 4°C. The slides were washed with Tris-buffered saline (TBS) and incubated with the secondary antibody dilution (anti-rabbit IgG and anti-mouse IgG, 1:200, Proteintech, Wuhan, China) for 2 hours at room temperature. 3,3'-diaminobenzidine (DAB) was used to develop visualization signal. Finally, the sections were counterstained with hematoxylin. The slides were viewed by two senior pathologists blinded to the clinical information. The staining scores were calculated based on the percentage of positive cells (0, <10% positive cells; 1, 10-25% positive cells; 2, 26-50% positive cells; and 3, >50% positive cells), and staining intensity (0, no staining; 1, weak staining; 2, moderate staining and 3 for strong staining). The total IHC score was calculated with the value of percent positive score $\times$staining intensity score and ranged from 0 to 9. The cut-off was defined as a score < 4 which represents low expression and a score ≥ 4 indicates high expression.

Statistical analysis

Each experiment was repeated at least three times, and the data were presented as mean $\pm$ standard deviation (SD) and were analyzed by SPSS 17.0 software. The student's t-test or ANOVA was used with a probability level of 0.05 indicating significant difference.

Results

Depletion of *ARID1A* enhances the radioresistance of pancreatic cancer cells

To explore whether ARID1A affects the radiosensitivity of pancreatic cancer cells, human pancreatic cancer cell lines PANC-1 and SW1990 were transfected with *ARID1A* shRNA. The efficacy of *ARID1A* knockdown was evaluated by western blot

analysis (Fig. 1A). The results indicated that both of the two *ARID1A* shRNA induced significant down-regulation of ARID1A protein in PANC-1 and SW1990 cells, while control shRNA (luciferase) did not show any effect on protein expression.

Subsequently, the above cells were exposed to the indicated doses of ionizing radiation (IR). A clonogenic assay was used to identify the radiosensitivity. The results (Fig. 1B) and statistical data (Fig. 1C) revealed that radiation induced significant less reduction of clone counts in ARID1A-depleted PANC-1 and SW1990 cells compared to that of the control, suggesting that ARID1A-deficient pancreatic cancer cells were more radioresistant.

Silencing *ARID1A* suppresses apoptosis and impairs G2-M checkpoint arrest after IR

To identify the possible underlying mechanism of radioresistance in pancreatic cancer cells with depletion of *ARID1A*, the apoptosis assay was conducted. The results revealed that the control cells presented a significant increase of apoptosis after IR (6Gy). The apoptosis of PANC-1 and SW1990 cells with ARID1A depletion (sh*ARID1A*) also increased after IR, but the magnitude of increase was significantly lower than that of the control (Fig. 2A and 2B).

Subsequently, we examined the distribution of cell cycle. First, the expression of *ARID1A* was knocked down in PANC-1 cells by siRNA, and the efficacy of silencing was evaluated with western blot (Fig. 2C). The cells were then exposed to IR of 6Gy, and the cell cycle distribution was assessed at the indicated time points. Results showed that ARID1A-depleted PANC-1 cells accumulated less significantly in the G2-M phase compared to that of control at both 4 and 8 hours after IR (Fig. 2D), which was verified by statistical analysis (Fig. 2E). These findings indicate that pancreatic cancer cells with ARID1A deficiency have impaired G2-M checkpoint arrest after IR-induced DNA damage, which leads to a quicker entrance into the mitosis phase and contributes to the radioresistance.

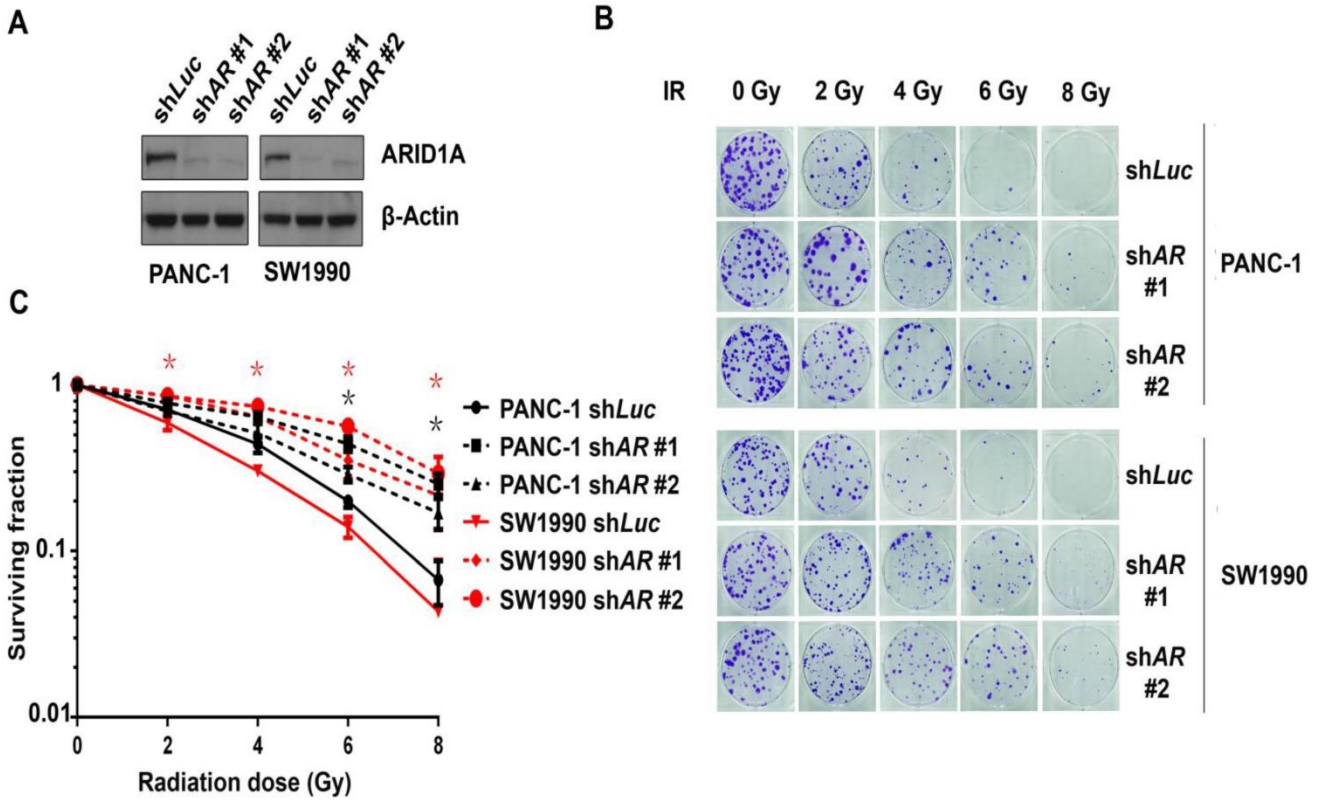

Figure 1. Depletion of *ARID1A* enhanced the radioresistance of pancreatic cancer cells. (A) Verification of knockdown efficacy. *ARID1A* was stably knocked down in PANC-1 and SW1990 cell lines by sh*ARID1A* #1 and sh*ARID1A* #2. Control cells were treated with sh*Luc*. (B) Clonogenic assays were conducted in *ARID1A* knocked down and control PANC-1 and SW1990 cells with IR of indicated dose. (C) Survival curves represented the radiosensitivity of control (sh*Luc*) and *ARID1A* depleted (sh*ARID1A* #1, #2) PANC-1 and SW1990 cells. Three independent experiments were performed with quantitative results representing the mean ± SD. The asterisk * represented $p < 0.05$. (AR, ARID1A)

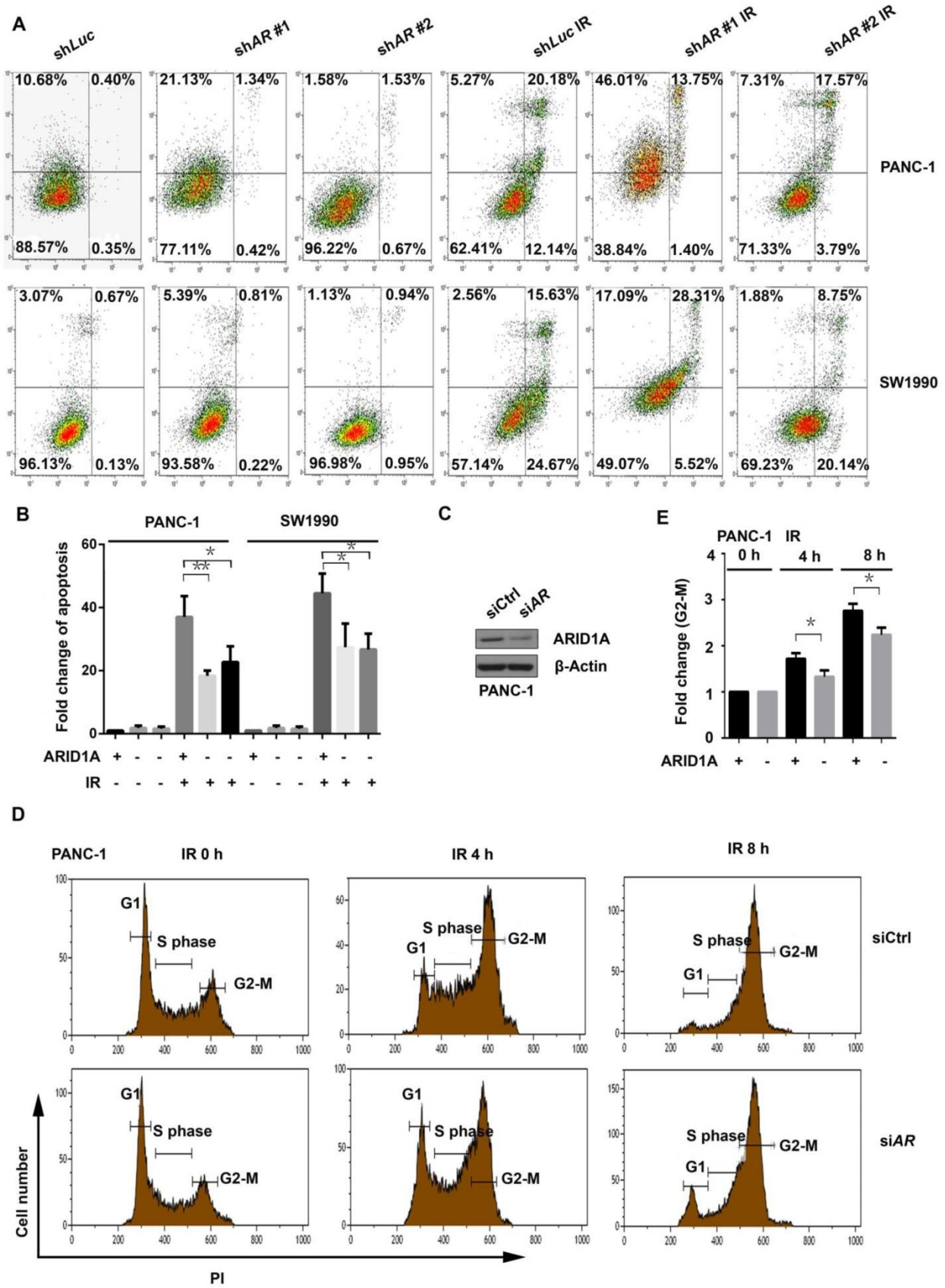

Figure 2. Silencing *ARID1A* suppressed IR-induced apoptosis and impaired G2-M checkpoint arrest after IR. (A) Apoptosis was detected with or without IR (6Gy) by Flow cytometry in *ARID1A* stably knockdown and control PANC-1 and SW1990 cells. (C) Western blot was used to detect ARID1A expression in transiently transfected PANC-1 cells with siCtrl or si*ARID1A*. (D) The cell cycle analysis was conducted in control (siCtrl) and *ARID1A* silencing (si*ARID1A*) PANC-1 cells after IR (6Gy) at indicated time points. (B) and (E) Quantitative results representing the mean ± SD of three independent experiments. (The asterisk * represented $p < 0.05$, ** represented $p < 0.01$; PI, propidium iodide; AR, ARID1A)

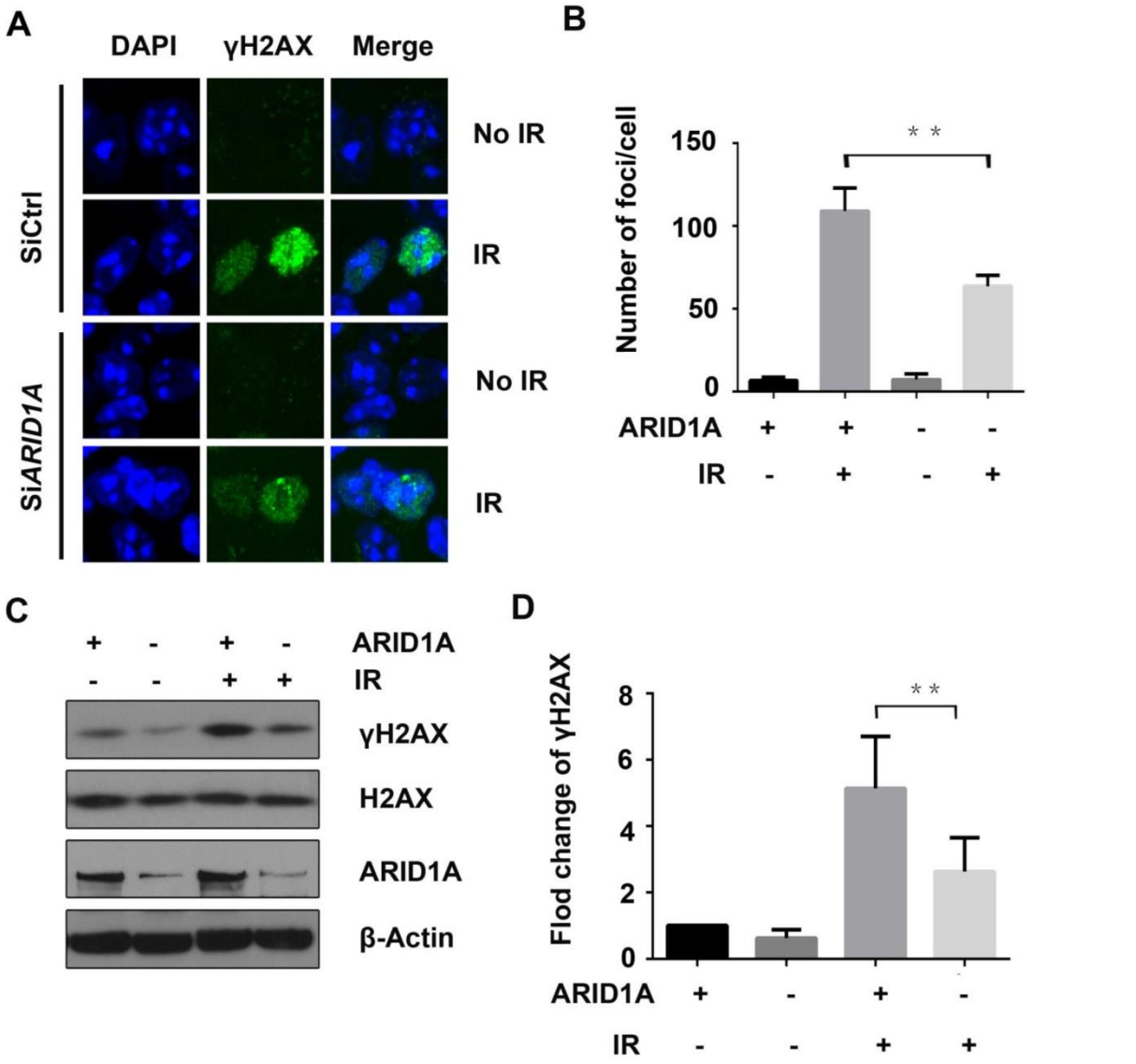

Figure 3. *ARID1A* knockdown strengthens DDR after IR. Control and *AIRD1A* depleted PANC-1 cells were exposed to IR (6Gy), and then (A) immunofluorescent staining for γH2AX foci and (C) western blot for γH2AX protein expression were conducted. Number of γH2AX foci and expression of γH2AX protein increased less significantly with *ARID1A* depletion compared to that of control after IR. (B) and (D) Quantitative results representing mean ± SD of three independent experiments. (The asterisk * represented $p < 0.05$, ** $p < 0.01$)

ARID1A knockdown strengthens DDR after IR

As ARID1A has been reported to play an essential role in DDR, which is important for radioresistance, we next evaluated the DNA damage marker, γH2AX, using immunofluorescence and western blot assays. PANC-1 cells transiently transfected with si*ARID1A* or siCtrl were exposed to IR of 6Gy. Two hours later, γH2AX was assessed. The results revealed that IR significantly increased the γH2AX foci (Fig. 3A) and the protein expression of γH2AX (Fig. 3C) in control cells. However, the foci and protein expression of γH2AX were significantly lower in *ARID1A*-silenced PANC-1 cells compared to that of the control (Fig. 3B and 3D), inferring that the DDR after IR was enhanced with ARID1A deficiency.

ARID1A depletion activates PI3K/AKT pathway, which participates in the radioresistance

DDR-related proteins were then evaluated by western blot assay, including ATM, p-ATM, CHK1, p-CHK1, PTEN, PI3K, AKT, and p-AKT (Ser^{473}), to identify the underlying target signaling proteins. The results showed that the expression of PI3K and p-AKT proteins significantly increased after IR in ARID1A-depleted PANC-1 cells compare to that of the control (Fig. 4A and 4B), whereas the expression level of other DDR-related proteins did not change notably (Fig. 4A).

Subsequently, the relation between the expression of ARID1A and PI3K or p-AKT in

pancreatic cancer patients were evaluated using IHC. Twenty sets of human pancreatic cancer tissue samples were collected. As shown in Fig. 4C, the expression of ARID1A is significantly negatively correlated with the expression of PI3K (R = -0.535, $p < 0.05$) or p-AKT (R = -0.462, $p < 0.05$). There were 75% (3/4) of the tumors with low expression of ARID1A showed high expression of PI3K or p-AKT, and 56.3% (9/16) of the tumors with high expression of ARID1A exhibited high expression of PI3K, or p-AKT (43.8%, 7/16).

To explore whether the activated PI3K/AKT signaling pathway was involved in the radioresistance, a clonogenic assay was addressed after IR of 6Gy with PI3K-inhibitor LY294002 or AKT-inhibitor mk2206. As demonstrated in Fig. 4D, in *ARID1A*-knocked down PANC-1 and SW1990 cells (sh*ARID1A*), PI3K-inhibitor LY294002 or AKT-inhibitor mk2206 could rescue the radiosensitivity, which was proved by significantly decreased clone counts after IR. However, in control cells (sh*Luc*), the above inhibitors did not change clone counts significantly (Fig. 4E). Such results indicate that the activated PI3K/AKT signaling pathway participates in the radioresistance induced by ARID1A depletion, and inhibition of PI3K/AKT signaling pathway sensitizes radiotherapy.

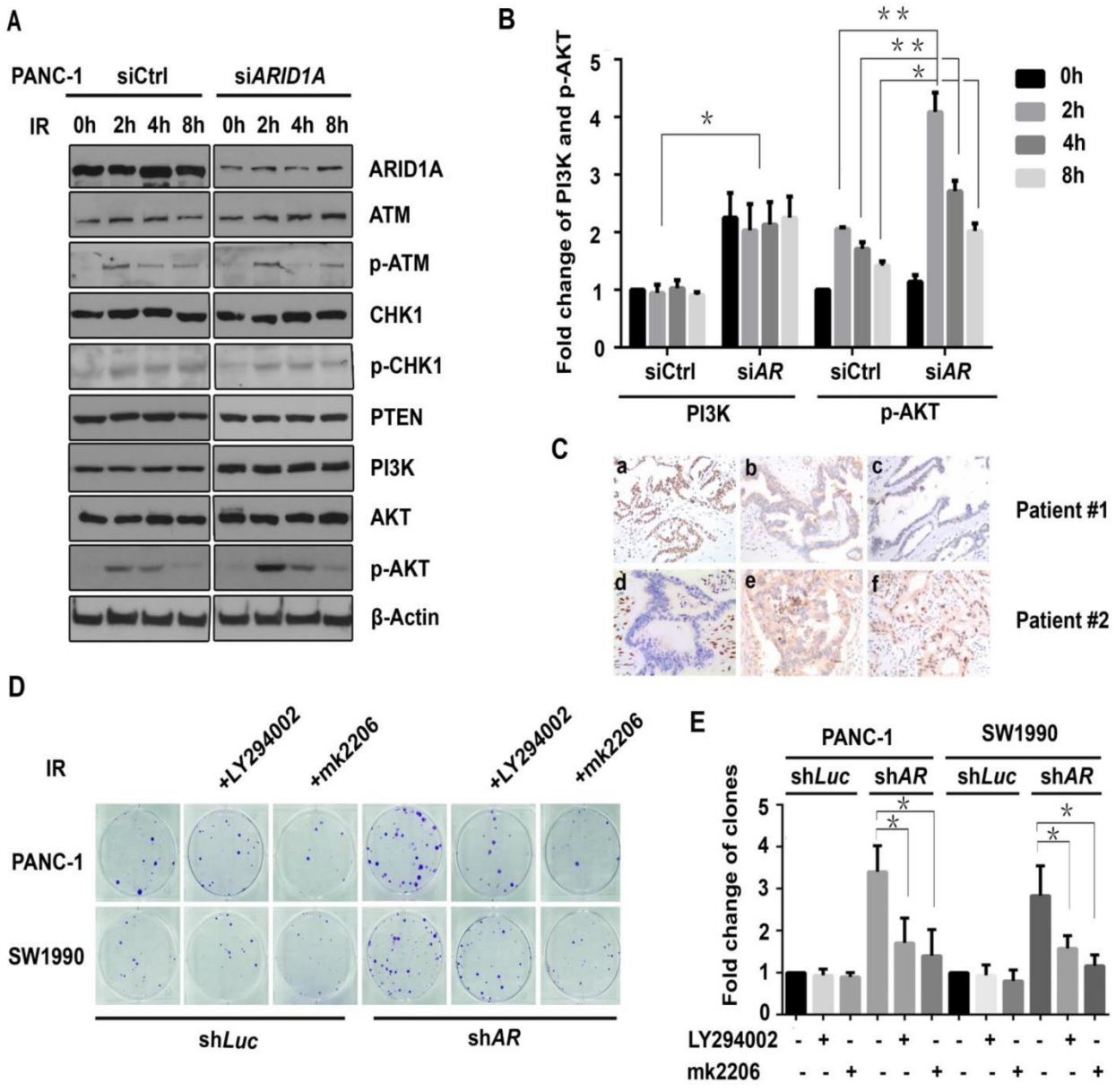

Figure 4. *ARID1A* depletion activates PI3K/AKT pathway, which participates in the radioresistance. (A) Western blot analysis for DDR-related proteins was performed in control (siCtrl) and *ARID1A* silencing (si*ARID1A*) PANC-1 cells after IR (6Gy) at indicated time points. (C) Immunohistochemical staining of ARID1A (a, d), PI3K (b, e) and p-AKT (c, f) in representative pancreatic cancer specimens (magnification, ×200). (D) Clonogenic assay was used in *ARID1A* depleted PANC-1 and SW1990 cells with or without inhibitors (LY294002 or mk2206) after IR. (B) and (E) Qantitative results representing the mean ± SD of three independent experiments. (The asterisk * represented $p < 0.05$, ** represented $p < 0.01$; AR, ARID1A).

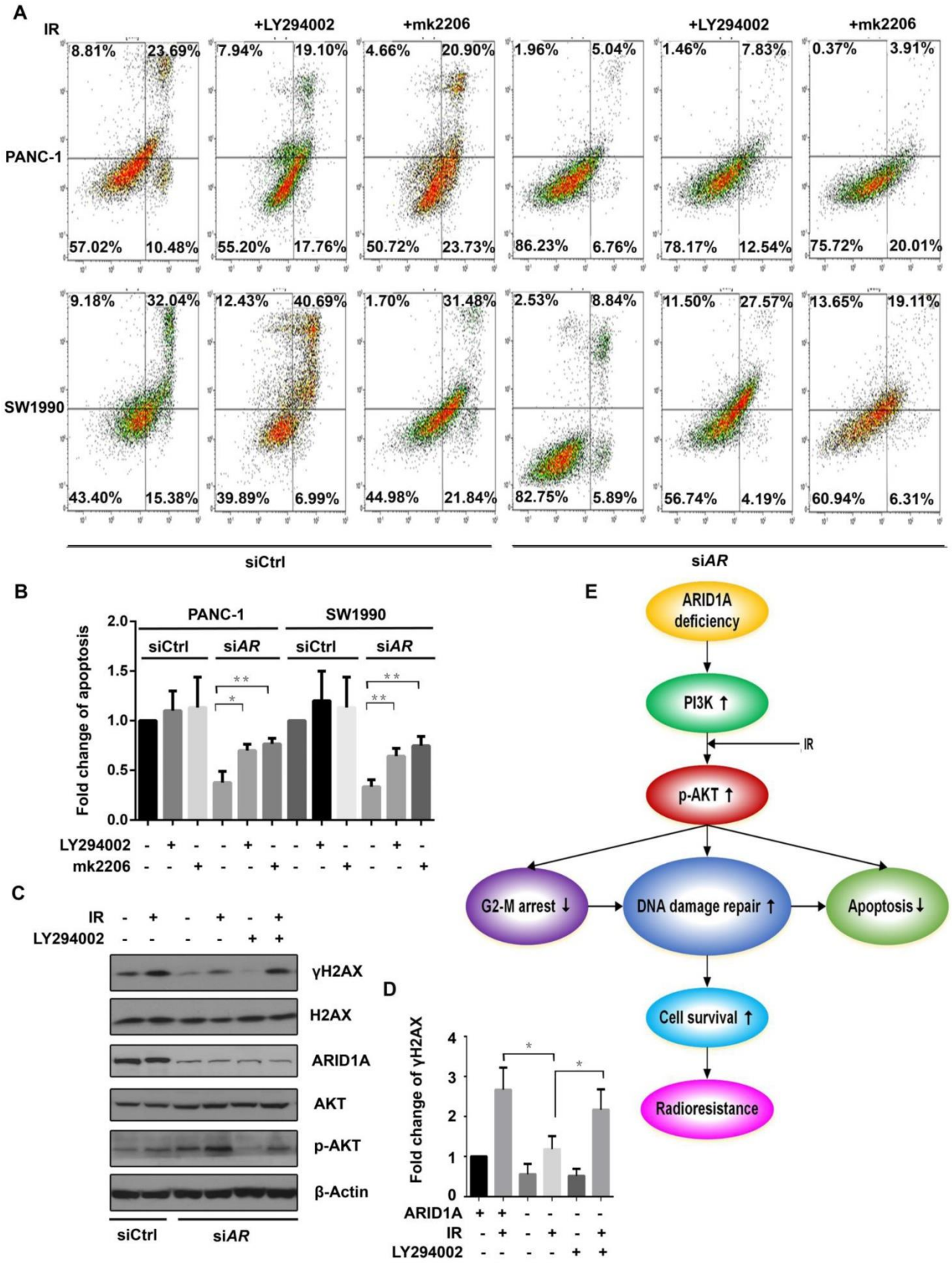

Figure 5. Inhibitors of PI3K/AKT pathway radiosensitize *ARID1A* deficient pancreatic cancer cells by increasing apoptosis and weakening DDR. (A) The apoptosis was checked after IR in *ARID1A* depleted PANC-1 and SW1990 cells (si*ARID1A*) and control, with or without inhibitors (LY294002 or mk2206). (C) Western blot was performed to detect the expression of γH2AX in PANC-1 cells with *ARID1A* deficiency and control at 2 hours after IR, with or without PI3K-inhibitor LY294002. (B) and (D) Quantitative results representing the mean ± SD of three independent experiments. (E) The mechanistic model of ARID1A regulation of the PI3K/AKT signaling pathway that involved in radioresistance of pancreatic cancer. (The asterisk * represented $p < 0.05$, ** represented $p < 0.01$; AR, ARID1A).

Inhibitors of PI3K/AKT pathway radiosensitize ARID1A-deficient pancreatic cancer cells by increasing apoptosis and weakening DDR

Apoptosis was evaluated in *ARID1A*-knocked down PANC-1 and SW1990 cells after IR of 6Gy. The results showed that LY294002 or mk2006 significantly increased the IR induced apoptosis in ARID1A depleted cells. However, in control cells, PI3K/AKT inhibitors did not increase the apoptosis significantly (Fig. 5A and 5B).

Consequently, γH2AX was assessed by western blot analysis. ARID1A knockdown significantly attenuated IR induced elevation of γH2AX expression in PANC-1 cells, which was abrogated upon treatment with LY294002 (Fig. 5C and 5D), suggesting the enhanced DDR by ARID1A deficiency could be reversed by inhibition of the PI3K/AKT pathway.

Discussion

ARID1A, a bona fide tumor suppressor, has been observed to regulate cell proliferation and migration in gynecologic [24] and gastric [29, 30] cancers. However, knockdown of ARID1A did not affect cell growth in pancreatic cancer [17]. Controversy still exists surrounding the prognostic role of ARID1A deficiency in pancreatic cancer [18, 27, 28]. Despite these findings, to the best of our knowledge, no further reports regarding the role of ARID1A on radioresistance of pancreatic cancer have been published.

Here, in the present study, our results reveal that knockdown of *ARID1A* enhances the radioresistance of pancreatic cancer cells by suppressing apoptosis, impairing G2-M checkpoint arrest, strengthening DDR and accompanying activation of PI3K/AKT signaling pathway. PI3K-inhibitor LY294002 or AKT-inhibitor MK-2206 could alleviate the radioresistance through increasing apoptosis and weakening DDR. Taken together, these data suggest that depletion of ARID1A enhances the radioresistance of pancreatic cancer cells by activation of the PI3K/AKT pathway, which affords a promising target to sensitize radiotherapy in pancreatic cancer.

It has been reported that ARID1A regulates the cell cycle by modulating related genes, such as transcription factor E2F1 [31], CCNE1 [32], and c-MYC [33]. Shen *et al* found that ARID1A deficiency led to impaired G2–M checkpoint initiation and maintenance [21]. These were in accordance with our findings. Pancreatic cancer cells with deficient ARID1A proceed faster from G2-M checkpoint arrest into the mitosis phase after IR, which contributes to the radioresistance.

ARID1A has been reported to participate in DDR in several studies [21, 34]. Watanabe *et al*. found that cancer cells lacking in the expression of certain SWI/SNF factors, including ARID1A, were deficient in DDR. They considered that such cancer cells were potentially vulnerable to DNA damage [34]. However, in several other studies, *ARID1A*-mutated ovarian clear cell carcinoma has been reported to be resistant to conventional platinum-based chemotherapy regimens [35-37]. Our findings also demonstrate that pancreatic cancer cells with depleted ARID1A are more radioresistant. These findings suggest that down-stream signaling pathways regulated by ARID1A deficiency may compensate for the impaired DDR and lead to resistance to chemotherapy and radiotherapy.

Interestingly, recent studies reveal inter-dependency between *ARID1A* mutations and PI3K/AKT pathway activation, which is one of the major survival pathways in cancer cells [38]. In several cancers, including endometrial cancer [39], ovarian clear cell carcinoma [40], colon cancer [41], and gastric cancer [30], it has been shown that loss of ARID1A expression up-regulates the phosphorylation of AKT. Zhang *et al* reported that ARID1A-involved SWI/SNF complex inhibited PIK3CA transcription by direct binding to its promoter [30]. Furthermore, *ARID1A* mutations were discovered to occur frequently in a synergistic fashion with mutations in PIK3CA [32, 39, 40, 42], which lead to an activation of the PI3K/AKT pathway. These findings indicate that cancer cells with ARID1A deficiency may depend more on the activation of PI3K/AKT pathway.

Thus, cancers with loss of ARID1A expression should be more vulnerable to PI3K/AKT pathway inhibition [43]. Currently, two studies have verified this viewpoint. First, Samartzis *et al* reported a synthetic lethal interaction between loss of ARID1A expression and inhibition of the PI3K/AKT pathway in breast cancer cells and ovarian clear cell carcinoma cell lines [44]. Subsequently, Zhang *et al* revealed that ARID1A depletion activated PI3K/AKT signaling pathway in gastric cancer, and inhibitors targeting AKT and PI3K could inhibit cellular growth *in vitro* and xenograft tumor growth *in vivo* efficiently [30]. However, in our pilot experiment, inhibition of PI3K/AKT pathway does not significantly affect the cell growth in pancreatic cancer with ARID1A depletion (Fig. S1). As AKT is directly involved in the control of DDR and radioresistance [45], we explored AKT-targeting inhibitors as effective strategies to overcome PI3K/AKT-dependent radioresistance. Our findings indicate that the radioresistance of ARID1A-deficient pancreatic cancer cells is PI3K/AKT dependent, and down-regulated

phosphorylation of AKT by LY294002 or mk2206 could sensitize them to radiotherapy.

Our current experiments reveal the role of PI3K/AKT signaling pathway on the radioresistance of pancreatic cancer cells induced by ARID1A deficiency *in vitro*. However, the results of *in vitro* studies have limit to extend to clinical significance, because microenvironment is important to the radiosensitivity. Although our IHC staining on patients' pancreatic cancer tissue showed the correlation between the expression of ARID1A and PI3K or p-AKT *in vivo*, more *in vivo* experiments are warranted in the future.

Collectively, our results demonstrate that depletion of ARID1A significantly enhances the radioresistance of pancreatic cancer cells and activates the PI3K/AKT signaling pathway. Moreover, PI3K- or AKT-inhibitors could radiosensitize pancreatic cancer cells with ARID1A deficiency via increasing apoptosis and weakening DDR. Our findings suggest that the PI3K/AKT-pathway is crucial for overcoming the radioresistance of ARID1A-deficient cancers, which is deserved to be exploited therapeutically in the future.

Abbreviations

ARID1A: AT-rich interactive domain containing protein 1A; DDR: DNA damage repair; IR: ionizing radiation; Luc: Luciferase.

Supplementary Material

Supplementary figure S1.
http://www.jcancer.org/v09p0890s1.pdf

Acknowledgments

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Competing Interests

The authors have declared that no competing interest exists.

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