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Original Article

Knockdown of FANCD2 alone or in combination with RAD51reverses the chemo-sensitivity of pancreatic cancer cells that acquire topotecan resistance

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Abstract: Acquired drug resistance is a challenging issue in clinical management of pancreatic cancer, resulting in the most of chemotherapy failure. Previous literature suggests that DNA repair mechanisms are employed by cancer cells to resist killing by genotoxic agents. Topotecan is such type of anti-cancer drug, leading formation of DNA double strand breaks (DBS). It is supposed that some mechanisms involved in the repair of DBS confer cancer topotecan resistance. In this study, we continuously cultured pancreatic cancer PANC-1 cells with topotecan at IC50 and finally obtained a modifiedpancreatic cancer cells, temporarily named as PANC-1(R) cells, which acquire strong topotecan resistance. With western blot and immuno-cytochemistry assays, it was observed that phospho (p)-ATM, γH2AX, p-BRCA1, p-BRCA2, FANCD2 and RAD51 were significantly up-regulated in PANC-1(R) cells compared to PANC-1 cells after cell exposure to topotecan. These proteins are involved in ATM-mediated DBS repair. FANCD2 knockdown in PANC-1(R) significantly decreased the cell viability and increased the caspase-3 expression and cell death rate after exposure to topotecan, but had modest effect on cell proliferation index. In contrast, the effectsinduced byRAD51 knockdown were marginal in all these respects. The knockdown of both FANCD2 and RAD51 showed more remarkable effects on the attenuation of chemo-resistance than FANCD2 knockdown alone. In conclusion, the acquired topotecanresistance of pancreatic cancer cells associates with ATM-mediated DBS repair; FANCD2 is identified as the key player in this process, therefore it is a promising target in clinical practice handling the topotecan resistance.

Keywords: ATM, FANCD2, RAD51, chemo-sensitivity, pancreatic cancer, topotecan

Introduction

Pancreatic cancer ranks as the fourth highest cause of death from cancer with an overall five-year survival rate at less than 5%. At initial diagnose, the majority of patients (approximately 80%) are at the late stage with a locally advanced or metastatic disease that portends their median survival only have 6-12 month in general [1, 2]. Although chemotherapy has been the main therapeutic option over the last decade, pancreatic cancer will relapse after the initial induction chemotherapy and nearly all patients will rapidly die from chemotherapy-resistant disease [3]. Experimental researchshows that the initial exposure to chemotherapeutic agents (e.g. cis-platinum and gemcitabine, which are commonly used in the first-line regimens) causes evident inhibition in the cell growth and proliferation *in vitro*, but many cells are not swept out and these cells transiently acquire resistance to the maximum tolerable dose of the chemotherapeutic agents in patients [2]. Based on the study, the rapid acquisition of drug resistance probably represents an important reason for the chemotherapy failure.

Topotecan, a semi-synthetic analogue of camptothecin, is used in the therapy of a vast array of cancers such as pancreatic cancer, melanoma, ovarian cancer, lung cancer colorectal carcinoma, and glioblastoma [3, 4]. Topotecan and other camptothecin derivates are the inhibitor of eukaryotic DNA topoisomerase I (Topo I). Topo I plays a critically important role in cell replication and transcription, through catalyzing a reversible single-stranded DNA break, leading

the unwinding of supercoiled DNA and relieving the torsional stresses [5]. In the present of topotecan, it forms a covalent complex between Topo I and DNA (also known as "Topo I-cleavable complex"), which prevents the religation of the DNA andinducesuncontrolled single and double strand breaks (DSB) in the DNA, thereby playingcytotoxic action [6-8]. Cancer resistance to topotecan, however, is increasingly observed in clinic practice, even though topotecan is commonly used as the second-line treatment option after the failure of first-line chemotherapy (Hagmann). Tomicic and Kaina discuss several potential mechanisms involved in the resistance to camptothecin-based drugs and highlightthat enhanced DNA repair actionis an important one [9].

DSB arising from normal cellular metabolism and extrinsic sources, such as ionizing radiation and xenobiotics, represents a major threat to cell survival. To combat with this DNA lesion, cells schematically operate an intricate network of pathways implicated in DNA damage repair, in whichthe kinase ATM (Ataxia telangiectasia mutated) is a key regulator [10, 11]. In response to DSB, activated ATM phosphorylates checkpoint kinase 2 to halt the progression of the cell cycle, preventing transmission of incorrect genetic information to daughter cells during following cell division. Further, activated ATM lead to the phosphorylation of several substrates participating in DNA damage repair. If the DNA lesion can be repaired timely and accurately, ATM-modulated signaling removes the cell cycle arrest and permits cell survival; otherwise, ATM is committed to initiateapoptotic pathways toeliminate the irreparable [12, 13]. It thus appears that ATM-mediated signaling has two sidesin the regulation of cell fate in response to DSBs. Selectively inhibiting the actions involved in ATM-mediated DNA repair would be a promising avenue to evoke apoptosis and repress chemo-resistance.

Previous literature documentsthat FANCD2 and Rad51 are important players in ATM-mediated DNA repair action. FANCD2 protein was first characterized in 2001. Both the knockdown andmutation of FANCD2 lead to decreased DNA repair efficiency. FANCD2 co-localizes with several proteins (e.g., BRCA1) in nucleus and plays a key role in DNA repair probably through rescuing stalled replication forks [14, 15]. RAD51, an evolutionarily conserved recombination, is believed to becrucial for cell survival, because knock-out of the Rad51 gene in the mouse led to an early embryonic death. Overexpression of RAD51 has been documented to promote chemotherapy and increases resistance of mammalian cells to ionizing radiation. RAD51 deficiency via siRNA transfection increases radio- or chemo-sensitivity of cancer cells. With the assistance of BRCA2 and other proteins, RAD51 is recruited to the DSB sites and polymerizes onto the resection-generated single strand-DNA ends, forming a nucleoprotein filament that promotes strand invasion and exchange between homologous DNA sequences. Strand invasion is followed by the initiation of DNA synthesis from the 3' end of the invading strand and eventual repair of the DSB16, 17. This study established a pancreatic cancer cell line that acquired strong topotecan resistance, followed by the identification of the potential mechanisms and molecules implicated in the acquired topotecan resistance.

Materials and methods

Establishing the pancreatic cancer cell line that acquires ability to resist topotecan

Pancreatic cancer PANC-1 cells purchased from American Type Culture Collection (ATCC) were incubated in F12 culture media containing 10% Fetal Bovine Serum under standard conditions (5% CO_2, 37°C). Topotecan hydrochloride powder (Meilunbio; Dalian, China) was dissolved in distilled water to produce appropriate loading solutions. The PANC-1 cells were exposed to different doses of topotecan for 72 h, before the cell viability measurement that was used to determine the IC50 (50% inhibitive concentration) of topotecan. In the following step, PANC-1 cells were cultured in the mediate supplemented with the topotecan at IC50 in company with the monitoring of cell viability. After one month continuous culture, we finally obtained a modifiedpancreatic cancer cells, temporarily named as PANC-1(R) cells, which showed strong resistance to the topotecan at IC50. These PANC-1(R) cells were used in further experiment.

Cell viability assay

CCK-8 Detection Kit (Beyotime Institute of Biotechnology, Shanghai, China) was used to measure the viability of PANC-1 and PANC-1(R)

cells. The cells seeded into 96-well plates were cultured with 100 μl of culture medium per well for 24 h and with 90 μl of culture medium plus 10 μl CCK-8 reagents per well for 1 h. The optical density at 490 nm (OD 490 nm) in each well was determined by an enzyme immunoassay analyzer (Bio-Tek ELX-800; Winooski, VT, USA).

Cell death rate assay

The cell death rate was assessed with an Annexin V-FITC/PI Apoptosis Detection Kit (Beyotime Institute of Biotechnology) according the manufacturer's instructions. The PANC-1 and PANC-1(R) cells were double-stained with Annexin V-FITC and propidium iodide in the dark, and then analyzed with a dual laser flow cytometer (Becton Dickinson, San Jose, CA, USA).

Cell proliferation index measurement

The PANC-1 and PANC-1(R) cells were fixed in 70% alcohol for 30 min on ice. The cells were treated with RNase A (Sigma, St. Louis, MO, USA) at 37°C and stained with propidium iodide in the dark for 30 min. DNA content was assayed by flow cytometry using a FACSCalibur (BD, Franklin Lakes, NJ, USA) and a cell cycle analysis was conducted using Cell Quest software. The cell proliferation index was obtained by calculating the ratio of cells in the S, G2, and M phases.

Immuno-cytochemical analysis of H2AX phosphorylation

H2AX is a variant of nucleosome core histone H2A. Phosphorylated H2AX, defined as γH2AX, can be detected immuno-cytochemically using γH2AX phospho-specific antibody (Millipore, Billerica, MA, USA). The PANC-1 and PANC-1(R) cells were fixed with 4% paraformaldehyde for 15 min and blocked with PBS containing 0.3% Triton X-100/5% BSA (w/v) for 1 h at room temperature, before the incubation with antibody specific for γH2AX (1:500). Incubation with the secondary fluorescent-labeled antibody (Alexa Fluor 488, Invitrogen) was performed in the dark prior to the microscopic analysis. Values given are the mean ± S.E. from three independent experiments.

Western blotting

Total cell extracts were prepared using ice-cold lysis buffer (20 mM Tris-HCl, 10 mM EDTA, 50 mM DTT, 100 mM NaCl, and 1% Triton X-100) containing protease inhibitor cocktail. Proteins in the PANC-1 and PANC-1(R) cell extract were separated by SDS-polyacrylamide gel electrophoresis (10-15% gels) and transferred onto nitrocellulose membranes. Membranes were blocked in 5% non-fat milk in TBS/0.1% Tween 20 for 2 h prior to immunoblotting overnight with antibodies against phospho (p)-ATM (pS1981; 1:5000; Abcam, Cambridge, UK), Caspase-3 (1:500; Abcam), p-BRCA1 (pS1423; 1:1000; Abcam), p-BRCA2 (pS3291; 1:500; Millipore), FANCD2 (1:1000; Abcam), RAD51 (1:1000; Abcam) and β-actin (1:1000, Santa Cruz Biotechnologies, California, USA). Incubation with the secondary fluorescent-labeled antibody (Alexa Fluor 488) was performed for 2 h at room temperature in the dark. The proteins were visualized by enhanced chemiluminescence (Amersham Bio-sciences, NJ, USA).

The Knockdown of FANCD2 and RAD51 alone or in combination in PANC-1(R) cells

Small hairpin RNA (shRNA) respectively targeting FANCD2 (shRNA-FANCD2) and RAD51 (shRNA-RAD51) were synthesized by GenePharma Co., Ltd (Shanghai, China). shRNA-FANCD2 and shRNA-RAD51 were transfected into PANC-1 (R) cells alone or in combination by using Lipofectamine™ 2000 (Invitrogen Life Technologies), according to the manufacturer's instructions.

Statistical analysis

For statistical analysis the Student's t test was applied (SPSS13.0 software; Chicago, IL, USA). The results are presented as the mean ±S.E. of three independent experiments. *P* values of < 0.05 were considered as significant and marked with an *asterisk*.

Results

The determination of the IC50 of topotecan to pancreatic cancer PANC-1 cells

PANC-1 cells were exposed to different doses of topotecan (0-800 nM) for 72 h, before the cell viability measurement that was used to determine the IC50 of topotecan. As can be seen from the **Figure 1**, topotecan attenuated PANC-1 cell viability in a dose-dependent manner, and the treatment with 400 nM topotecan induced a 48% loss in viability, thus 400 nM is

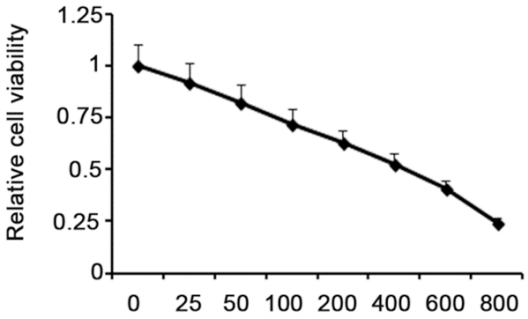

Figure 1. The determination of the IC50 of topotecan to pancreatic cancer PANC-1 cells. PANC-1 cells were exposed to different doses of topotecan (0-800 nM) for 72 h, before the cell viability measurement that was used to determine the IC50 of topotecan. Topotecan attenuated PANC-1 cell viability in a dose-dependent manner, and the treatment with 400 nM topotecan induced a 48% loss in viability, thus 400 nM is acted as the IC50 of topotecan to PANC-1 cells in our experiment.

acted as the IC50 of topotecan to PANC-1 cells in following experiment.

Inducing pancreatic cancer PANC-1 cell to acquire topotecan resistance

Continuous culture of PANC-1 cells in the mediate supplemented with 400 nM topotecan was performed with the monitoring of cell viability. After one month incubation, we established a modified pancreatic cancer cell line, temporarily named as PANC-1(R) cells, which only showed a modest decrease in the cell viability after exposure to 400 nM topotecan for 72 h (**Figure 2A**). We further investigated the cleaved caspase-3 protein level, cell death rate and cell proliferation index afterthese two types of cells were subjected to 400 nM topotecan for 72 h. Caspase-3 activation, which is dependent on the cleavage at its un-active form, is a key step for apoptosis execution and is widely regarded as the mark of early apoptosis. Incubation with 400 nM topotecan resulted in dramatic increase in cleaved caspase-3 protein level in PANC-1 cells ($P<0.05$, **Figure 2B**); The cleaved caspase-3 protein level was significantly lower in PANC-1(R) cells than in PANC-1 cells after exposure to the topotecan ($P<0.05$). Moreover, the rate of death cell (including apoptosis and necrosis) was considerably increased in the topotecan-treated PANC-1 cells relative to non-treated PANC-1 cells ($P<0.01$, **Figure 2C**); Topotecan-treated PANC-1(R) showed significantly lower cell death rate than topotecan-treated PANC-1 cells ($P<0.05$). The cell cycle is subdivided into the G1, S, G2, and M phases, which are controlled and coordinated by several "checkpoints." A major checkpoint, also known as a restriction point, has been identified before the G1-S transition, after which a cell is committed to division. Thus, the cell proliferation index was evaluated by estimating the percentage of cells in S, G2, and M phases. Our data showed 400 nM topotecan inhibited the proliferation index of PANC-1 cells ($P<0.05$, **Figure 2D**). PANC-1 and PANC-1(R) cells showed no significant difference in the cell proliferation index after exposure to 400 nM topotecan for 72 h. Collectively, PANC-1(R) cells were established and identified as a type of pancreatic cancer cells with strong resistance to topotecan.

ATM-mediated signaling responsible for DNA repair is activated in the PANC-1(R) cells

ATM kinase activation, relied on the phosphorylation on serine 1981, is the early event of ATM-mediated DNA repair in response to DNA damage, particularly DSB formation. The p-ATM expression level was significantly up-regulated in the PANC-1 cells after exposure to the topotecan ($P<0.05$, **Figure 3**). Topotecan-treated PANC-1(R) cells showed a much higher *P*-ATM protein level than topotecan-treated PANC-1 cells ($P<0.05$). BRCA1/2 are downstream signaling molecules of ATM and are involved in the regulation of FANCD2 and RAD51. Our data showed that treating PANC-1 cells with 400 nM topotecan for 72 h had no significant effect on the expression levels of BRCA1, BRCA2, FANCD2 and RAD51. Compared to the topotecan-treated PANC-1 cells, topotecan-treated PANC-1(R) cells showed dramatic increase in these protein levels ($P<0.05$).

Activated ATM is involved in thephosphorylation of H2AX on serine 139 (phosphorylated H2AX also known as γH2AX). This phosphorylation involves large chromatin domains forming nuclear foci that are easily detected by immuno-cytochemistry assay [13]. As can be seen from **Figure 4**, the fluorescence intensity of γH2AX in cell nucleus was slightly increased after PANC-1 cells were exposed to the topotecan. PANC-1(R) cells showed notable increase in the fluorescence intensity compared to PANC-1 cells ($P<0.05$).

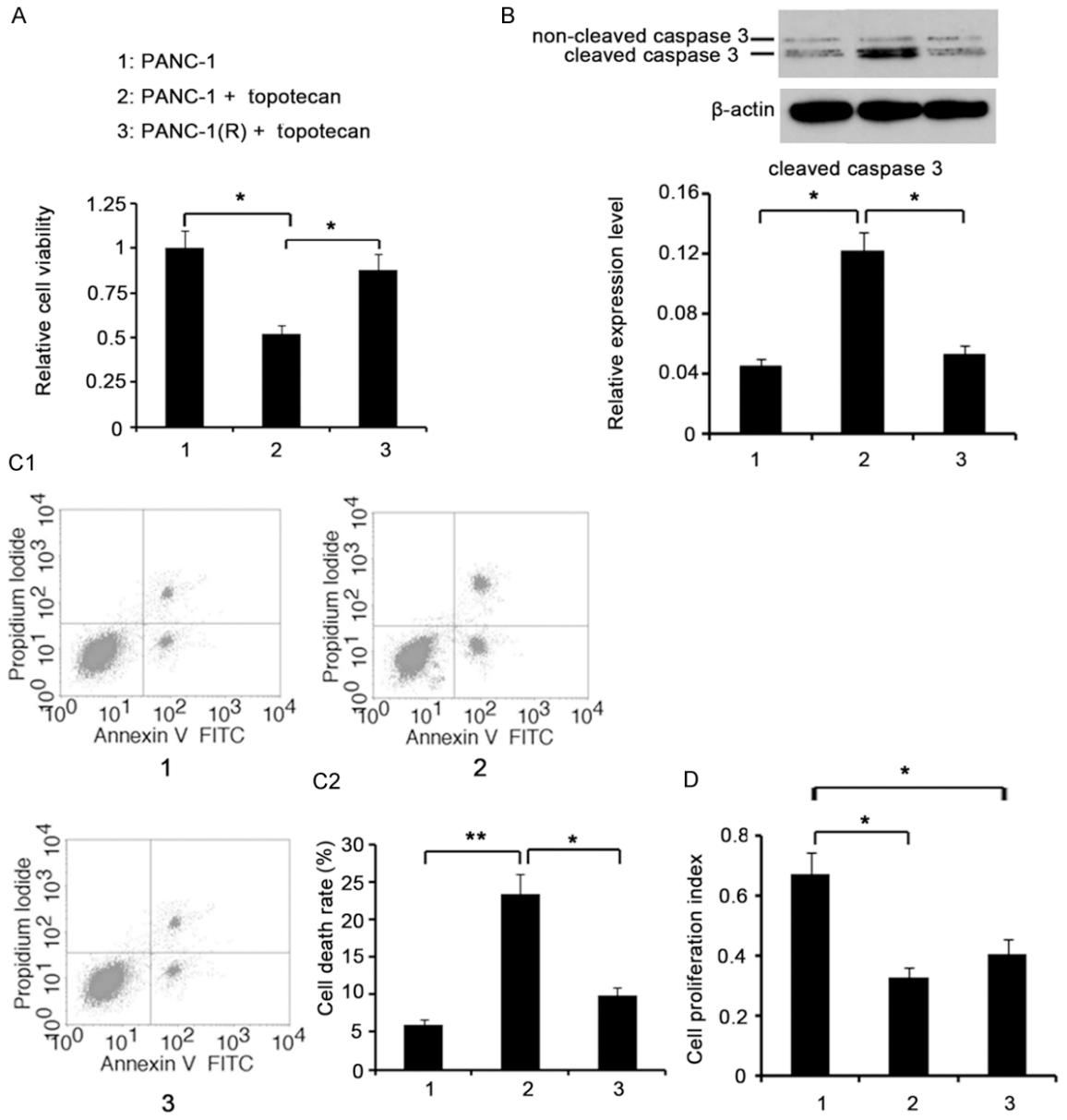

Figure 2. Inducing pancreatic cancer PANC-1 cell to acquire topotecan resistance. PANC-1 cells were continuously cultured in the mediate supplemented with 400 nM topotecan, and finally reached topotecan resistance after month--PANC-1(R) cells. A. Compared to PANC-1 cell, PANC-1(R) cells showed increased cell viability; B. Decreased cleaved caspase-3 protein level in PANC-1 cells; C. Decreased cell death rate in PANC-1 cell; D. Cell proliferation index. Bar graphs show mean ± S.E. of three independent experiments. (*) Indicates $P<0.05$.

The knockdown of FANCD2 and RAD51 alone or in combination in PANC-1(R) cells

Figure 5A shows that the transfection of PANC-1(R) cellswith shRNA-FANCD2 and shRNA-RAD51 respectively caused 89% decrease in FANCD2 expression ($P<0.01$) and 73% decrease in RAD51 expression ($P<0.05$). Further, FANCD2 and RAD51 were knocked down alone or in combination in PANC-1(R) cells prior to the cell exposure to 400 nM topotecan. FANCD2 expression level was decreased by 83% in the PANC-1(R) cells transfected with shRNA-FANCD2 compared to the non-transfected cells after the treatment with topotecan ($P<0.01$, **Figure 5B**). The transfection with shRNA-RAD51 had no effect on FANCD2 expression. The co-transfection with shRNA-FANCD2 and shRNA-RAD51 induced the same inhibition rate in FANCD2 expression with the transfection

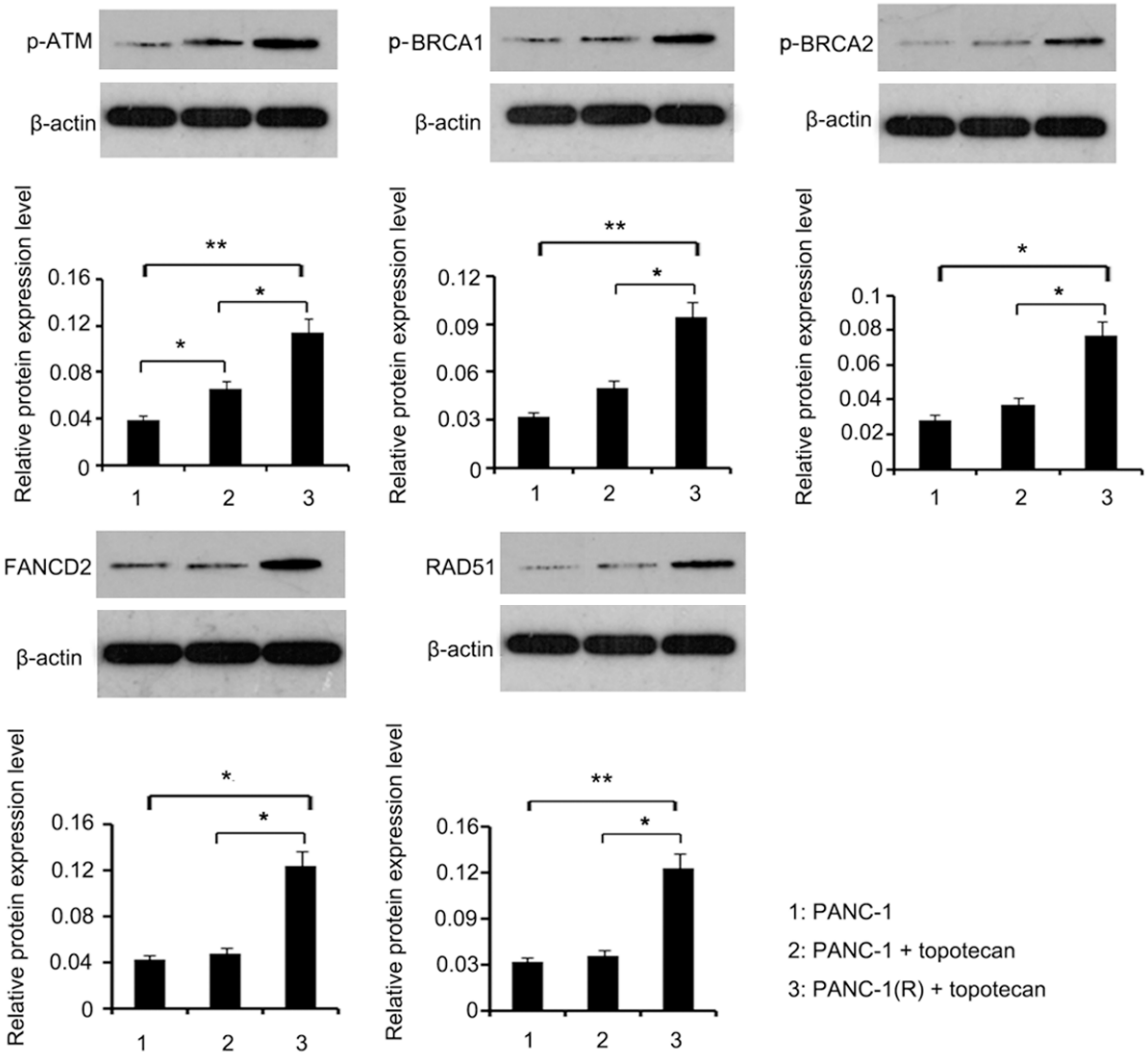

Figure 3. ATM-mediated signaling responsible for DNA repair is activated in the PANC-1(R) cells exposed to topotecan. Compared to PANC-1 cell, PANC-1(R) cells showed up-regulated phospho (p)-ATM, p-BRCA1, p-BRCA2, FANCD2 and RAD51 in protein levels after exposure to 400 nM topotecan for 72 h. Bar graphs show mean ± S.E. of three independent experiments. (*) Indicates $P<0.05$; (**) Indicates $P<0.01$.

with shRNA-FANCD2 alone. The transfection with shRNA-RAD51, alone or in combination with shRNA-FANCD2, induced 74% decrease in RAD51 expressioncompared to the non-transfected cells after topotecan treatment ($P<0.05$). The protein expression levels of p-ATM, p-BRCA1 and p-BRCA2 were not changed after the transfection with shRNA-FANCD2 and shRNA-RAD51 alone or in combination.

The knockdown of FANCD2 alone or in combination with RAD51 in PANC-1(R)cells attenuates the topotecan resistance

The PANC-1(R) cells transfected with shRNA-FANCD2 showed lower cell viability than the non-transfected cells after exposure to topotecan ($P<0.05$, **Figure 6A**). In contrast, the transfection ofPANC-1(R) cells with shRNA-RAD51 caused a marginal decrease in cell viability after exposure to topotecan. The Co-transfection with shRNA-FANCD2 and shRNA-RAD51 had more remarkable effect on cell viability reduction than transfection with shRNA-FANCD2 alone following the treatment with topotecan. Knocking down FANCD2 in PANC-1(R) cells increased cleaved caspase-3 protein level after cell exposure to topotecan ($P<0.05$, **Figure 6B**). In contrast, the effect of RAD51 knockdown was marginal on cleaved caspase-3 protein level. The cleaved caspase-3 protein level was

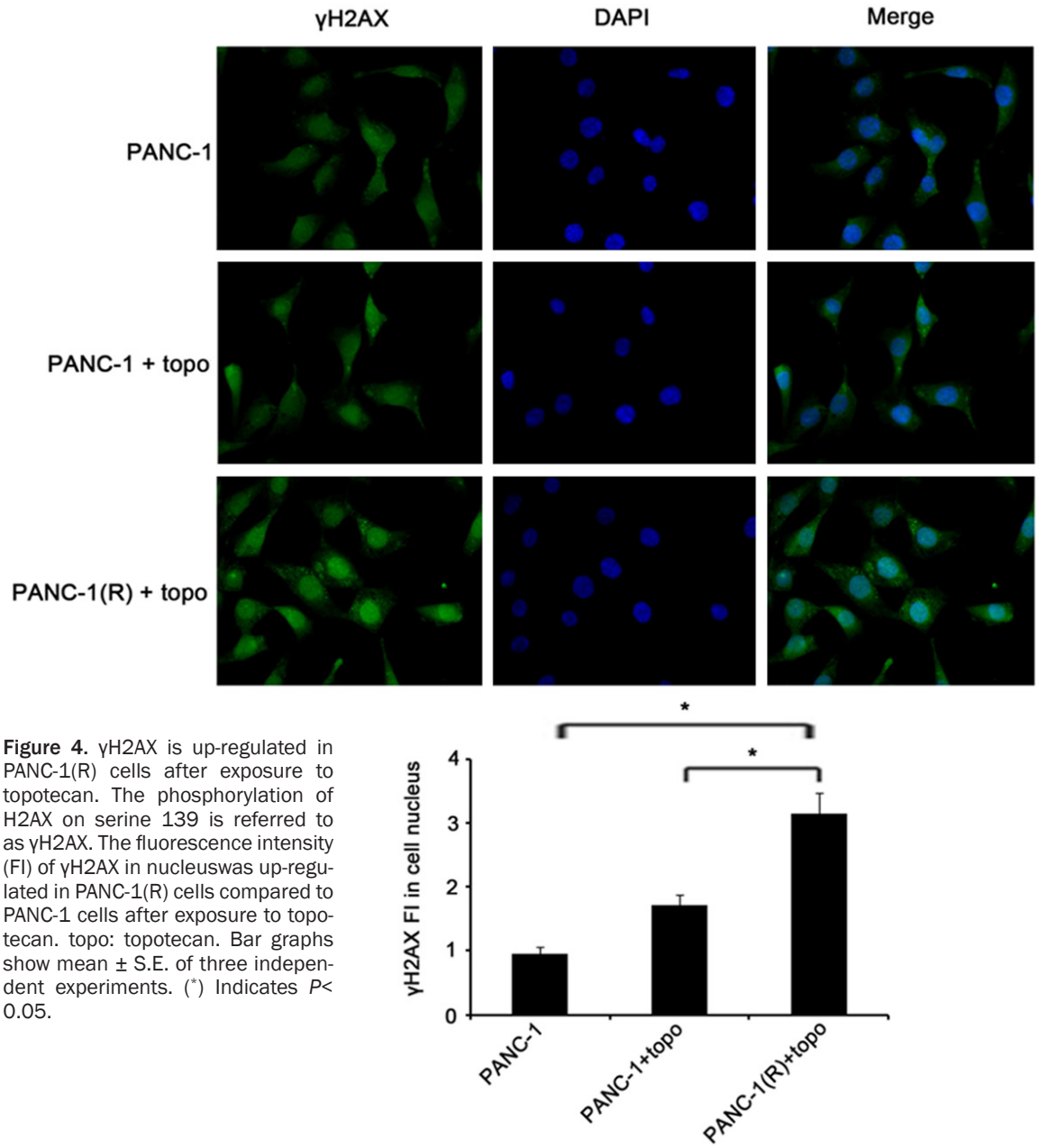

Figure 4. γH2AX is up-regulated in PANC-1(R) cells after exposure to topotecan. The phosphorylation of H2AX on serine 139 is referred to as γH2AX. The fluorescence intensity (FI) of γH2AX in nucleuswas up-regulated in PANC-1(R) cells compared to PANC-1 cells after exposure to topotecan. topo: topotecan. Bar graphs show mean ± S.E. of three independent experiments. (*) Indicates $P< 0.05$.

much higher in PANC-1(R) cells with both FANCD2 and RAD51 knockdown than in the cells with FANCD2 knockdown alone, after treatment with topotecan. Cell death rate showed similar changes with cleaved caspase-3 protein level. Cell death rate was significantly higher in PANC-1(R) cells with the knockdown of FANCD2 alone or in combination with RAD51, following the treatment with topotecan ($P<0.05$, **Figure 6C**). Knocking down FANCD2 and RAD51, alone or in combination, had modest effects on cell proliferation index (data not shown).

Discussion

Acquired drug resistance is a challenging issue in clinical management of pancreatic cancer, resulting in the most of chemotherapy failure [2]. Previous literature suggests that DNA repair mechanismsare employed by cancer cells to resist killing by the anti-cancer drugs that dedicate to leading DNA damage [9]. In mammal cells, multiple repair pathways exist that are specific to distinct DNA lesions. Topotecan is an anti-cancer drug able to induce DNA DBS for-

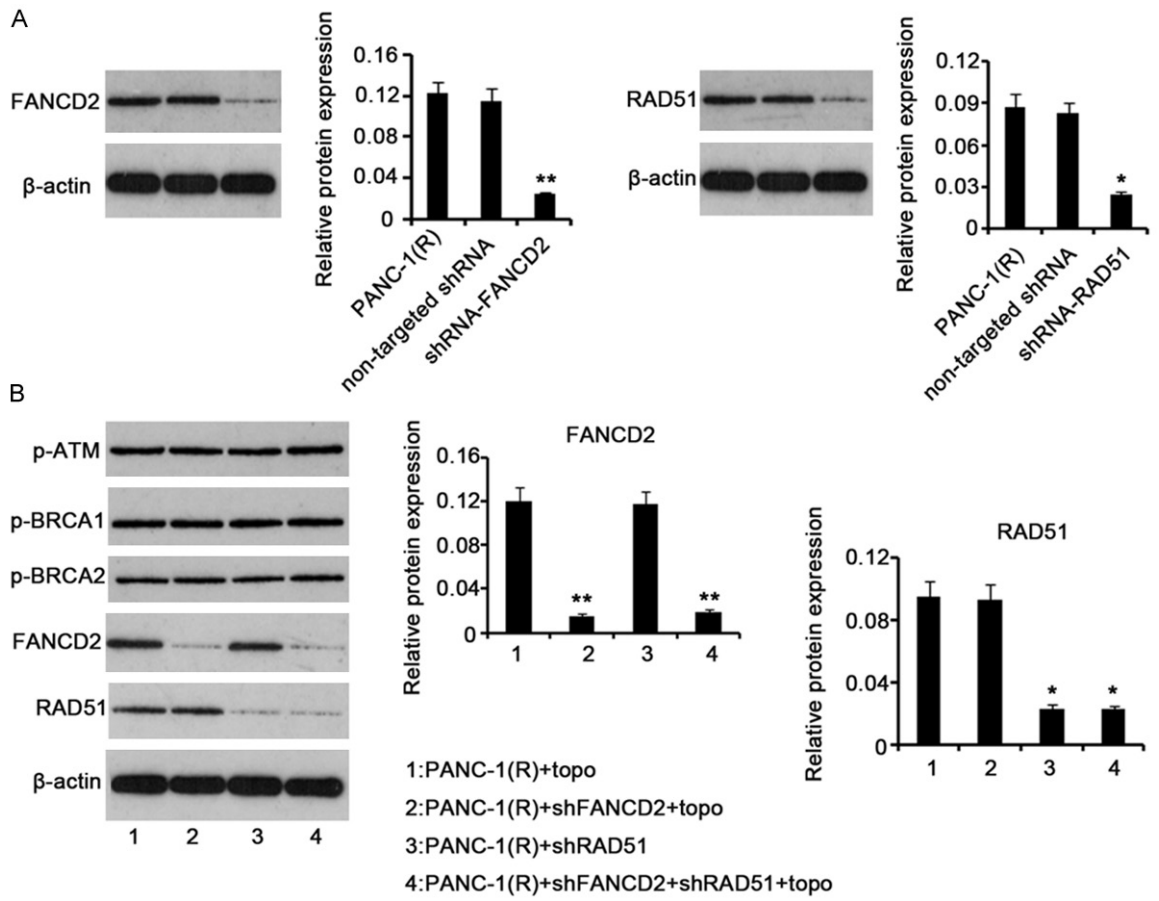

Figure 5. The knockdown of FANCD2 and RAD51 alone or in combination in PANC-1(R) cells. A. FANCD2 and RAD51 were knocked down in PANC-1(R) cells. B. The knockdown of FANCD2 and RAD51 alone or in combination in PANC-1(R) cells prior to the exposure to 400 nM topotecan for 72 h. shFANCD2: shRNA-FANCD2; shRAD51: shRNA-RAD51; topo: topotecan. Bar graphs show mean ± S.E. of three independent experiments. Bars marked with "*" indicate $P<0.05$ compared to the first bar; Bars marked with "**" indicate $P<0.01$ compared to the first bar.

mation. This prompted a speculation that DSB repairmechanism may be responsible for topotecan resistance in pancreatic cancer. As an important regulator in DNA DBS repair, ATM activates a complex network of pathways able to remove DNA lesion and regulate cell survival/apoptosis. In present study, p-ATM protein level was increased in pancreatic cancer PANC-1 cells after exposure to topotecan at IC50 for 72 h, indicating that ATM kinase activity is enhanced. However, enhanced ATM activity-failed to lead thesignificant elevation of its down-stream signaling molecules, including γH2AX, p-BRCA1, p-BRCA2, FANCD2 and RAD51, in protein levels as determined by western blot and immuno-cytochemistry assays. We speculated that ATM isnot activated to such a high extent or the time is too short for ATM activating its downstream molecules within 72 h. We continuously cultured PANC-1 cells with topotecan at IC50 in the following one month. At the end of the incubation, topotecan at IC50 only induced a modest decrease in cell viability and slightly increased cell apoptosis and death rate, suggesting thatthe PANC-1 cells have acquired the ability to resist the killing of topotecan at IC50. We defined these newly obtained PANC-1 cells as PANC-1(R) cells. Western blotting showed p-ATM protein level was significantly higher in PANC-1(R) cells than in PANC-1 cells after the exposure to topotecan. Moreover, the protein level of γH2AX, p-BRCA1, p-BRCA2, FANCD2 and RAD51 were dramatically increased in PANC-1(R) cells. These data indicate that DNA repair action regulated by ATM-mediated signaling is significantly strengthened in PANC-1(R) cells.

Previous researches showed that both mutations in ATM gene and treatment with selective

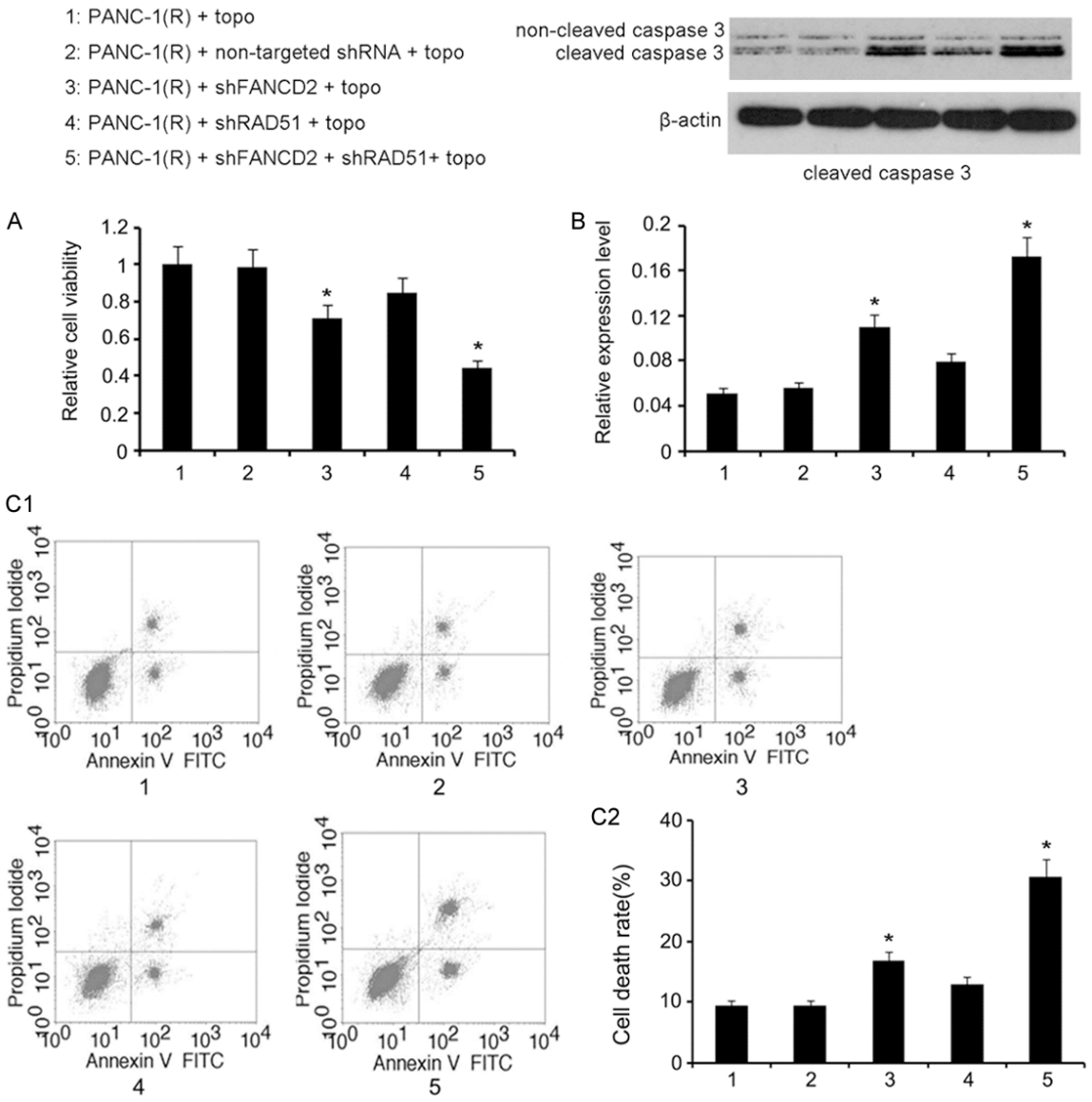

Figure 6. The knockdown of FANCD2 alone or in combination with RAD51 in PANC-1(R) cells attenuates the topotecan resistance. A. The knockdown of FANCD2 alone or in combination with RAD51 in PANC-1(R) cells attenuated cell viability; B. Increased cleaved caspase-3 protein level; C. Increased cell death rate after exposure to 400 nM topotecan for 72 h. shFANCD2: shRNA-FANCD2; shRAD51: shRNA-RAD51; topo: topotecan. Bar graphs show mean ± S.E. of three independent experiments. Bars marked with "*" indicate $P<0.05$ compared to the first bar.

ATM kinase inhibitors enhance cell chemosensitivity and radiosensitivity, thus suggesting that ATM is a promising clinical target [18, 19]. However, ATM is an apical signaling kinase involved in multiple cellular processes, including cell cycle checkpoint, DNA repair and cell survival/apoptosis, it may be a better choice to selectively inhibiting the ATM-downstream moleculessolely focusing on DNA repair function, which makes DNA unable to be repaired effectively after exposure togenotoxic agents, resulting in the activation of apoptosis-related factorsdownstream ATM (e.g. P53) and in ultimate apoptosis [6].

Once activated, ATM triggersthe phosphorylation of histone variant H2AX on Ser139, referred to as γH2AX, which is important for DSB repair because γH2AX anchors some DNA repair proteins (e.g., BRCA1 and RAD51) at the sites of DSB formation [20]. BRCA1and BRCA2 are tumor suppressor genes, as BRCA muta-

tions are the main known hereditary factors for breast and ovarian cancers [21, 22]. However, increasing evidence shows that cells with either BRCA1 or BRCA2 deficiencywere especially sensitive togenotoxic agents and ionizing radiation presumably due to the impaired ability to repair DSB [21, 22]. BRCA1/FANCD2 and BRCA2/RAD51 are ATM-cascade endpoints involved in DSB repair. In present study, we sought to knockdown FANCD2 and RAD51 alone or in combination in PANC-1(R) cells. Silencing FANCD2 via the transfection of shRNA-FANCD2 potentiates PANC-1(R) cell responses to topotecan as evidenced by the dramatic decreased cell viability and increased cell death rate after treatment with topotecan. RAD51 knockdown, herein, just induced a marginal change in cell viability and cell death rate after cell exposure to topotecan. It should be noted that the shRNA-RAD51 showed a lower efficiency than shRNA-FANCD2 in their gene-silencing function in our study. This might be an important reason for that RAD51 knockdown is not as effective as FANCD2 knockdown in enhancing the chemo-sensitivity of PANC-1(R) cells to topotecan. Silencing both FANCD2 and RAD51 induced more remarkable effect on the attenuation of chemo-resistance than silencing FANCD2 alone, implying that RAD51 isalso an important player correlated to the topotecan resistance of PANC-1(R) cells. In this study, knocking down FANCD2 and RAD51, alone or in combination, had modest effects on cell proliferation index, suggesting that FANCD2 and RAD51 have limited role in leading the G1/S checkpointthat is the early event following ATM activation.

In brief, this study initially induced pancreatic cancer PANC-1 cells toacquiretopotecan resistance through long-term incubation with topotecan at IC50. It was found the ATM-mediated signaling that is involved in DNA DSB repair is significantly activated in these newly obtained PANC-1(R) cells. Located at the downstream of this signaling, FANCD2 wasup-regulated in PANC-1(R) cells in company with the enhanced chemo-resistance, whereas FANCD2 knockdown strikingly attenuated the chemo-resistance. Our data collectively leads the conclusion: the acquired topotecan resistance of pancreatic cancer cells associates with ATM-mediated DNA DBS repair; FANCD2 is identified as the key player in this process, therefore it is a promising target in clinical practice handling the topotecan resistance.

Disclosure of conflict of interest

None.

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