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Original Article

Silence of URI in gastric cancer cells promotes cisplatin-induced DNA damage and apoptosis

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Abstract: URI, a prefoldin family member, has been implicated roles in cancer development. We have previously shown that URI can attenuate DNA damage in gastric cancer cells treated with potassium dichromate. The aim of this study was to investigate how URI involves cisplatin-induced DNA damage response (DDR) in gastric cancer cells and its possible mechanism relating to the ATM/CHK2 pathway. Here, MGC-803 and SGC-7901 gastric cancer cells were treated with different concentrations of cisplatin. Comet assay was used to detect DNA damage and the results confirmed the dose-effect of cisplatin-induced DNA damage in gastric cancer cells. *URI* knockdown cell lines were established with siRNA transfection. Cell viability and proliferation were detected by counting kit 8 (CCK-8) and 5-ethynyl-2'-deoxyuridine (EdU) assays respectively. Apoptosis and cell cycle were analyzed by flow cytometry. The results indicated that URI knockdown increased the sensitivity of cells to cisplatin by inhibiting proliferation and promoting apoptosis. The levels of P-ATM, P-CHK2 and γH2AX were detected by Western blot. Increased levels of P-ATM, P-CHK2, and γH2AX were observed in cisplatin treated cells, indicating that cisplatin induced a DNA damage response (DDR). *URI* knockdown in cisplatin-treated cells significantly decreased the levels of P-ATM and P-CHK2 at 12 hours, but not at 0 and 6 hours after drug withdrawal, while significantly increased γH2AX levels were detected at 6 hours, but not at 0 and 12 hours after drug withdrawal compared with the control cells. However, the levels of γH2AX were significantly increased in *URI* knockdown cells after cisplatin treatment for 12 hours. The cell cycle analysis showed that the number of cells entering S phase was significantly reduced and the cells were arrested in the G1 phase in *URI*-silenced cisplatin-exposed cells, indicating that cell cycle progression was inhibited. In conclusion, our results suggest that URI is involved in the cisplatin-induced DNA damage response via the ATM/CHK2 pathway, and silencing URI can increase cisplatin-induced DNA damage and enhance drug sensitivity in gastric cancer cells.

Keywords: URI, gastric cancer, cisplatin, DNA damage response, ATM/CHK2 pathway

Introduction

Gastric and gastroesophageal junction (G/GEJ) cancer is the third leading cause of cancer death worldwide, with an estimated one million new cases and 783,000 deaths in 2018 [1]. The highest incidence of gastric cancer is in China, accounting for approximately 50 percent of the world's cases [2]. Given its high rate of non-symptoms at early stage, gastric cancer is usually diagnosed at advanced stage with metastasis. Consequently, adjuvant chemotherapy, the cisplatin-based therapy in particular, becomes a main treatment option in addition to surgery for advanced gastric cancer, especially in Asia [3-5]. And that, the five-year survival rate of patients with advanced gastric cancer remains low due to drug resistance which limits the clinical applications and efficacy of the anti-cancer drugs [6].

The molecular mechanism causing the anticancer effect of cisplatin is complex, mainly involving DNA damage and mitochondrial apoptosis [7, 8]. Cisplatin may induce DNA lesions directly and cause cell cycle arrest and thus activation of DNA damage response (DDR) pathways. This will greatly affect the efficacy of the drug during

anti-cancer therapy [8]. The ATM-CHK2 and ATR-CHK1 pathways are two major kinase signaling cascades in cells activated in response to DNA damage in order to arrest the cell cycle and initiate the DNA repair process [9, 10]. It is noteworthy that ATM functions as the key transducer in DNA double-strand breaks (DSBs) which can be induced by most chemotherapeutic agents and transmits the message through protein phosphorylation [11, 12]. Targeting of ATM can significantly increase cisplatin sensitivity and apoptosis of cisplatin-resistant non-small cell lung cancer cells [13].

Unconventional prefoldin RPB5 interactor (URI), also known as the RNA polymerase II Subunit 5-mediating protein (RMP), participates in regulation of gene transcription by interacting with RPB5 [14, 15]. Previous studies have reported that URI is overexpressed in multiple cancers, including ovarian cancer, hepatocellular carcinoma, multiple myeloma, non-small cell lung cancer, and can promote cancer development both in vitro and in vivo [16-19]. Studies have also shown that URI can enhance the resistance of cancer cells to cisplatin, adriamycin, and oxaliplatin, but the molecular mechanisms remain incompletely understood [20-23]. Our previous studies have demonstrated that URI mitigates potassium dichromate-induced DNA damage and reduces the cell apoptosis and cell death [24]. This study aims to further investigate the effect and possible mechanism of URI silencing on cisplatin-induced ATM/CHK2 pathway activation and cisplatin resistance in gastric cancer cells.

Materials and methods

Chemicals and antibodies

Cisplatin was purchased from Selleck and dissolved in N'N-Dimethylformamide (DMF). Hiperfect transfection reagent was purchased from QIAGEN (301705). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (D120074) was purchased from Ruiyingbio (Suzhou, China). Primary antibody against RMP/URI (11277-1-AP) was obtained from Proteintech. Opti-MEM reagent was purchased from Invitrogen (Invitrogen, Carlsbad, CA). Primary antibodies against phospho-histone H2AX (Ser139) (9718), ATM (2873), phospho-ATM (Ser1981) (5883) and CHK2 (6334) were purchased from Cell Signaling Technology (Danvers, MA). Antibody for CHK2 (phospho Thr68) (YP0065) was purchased from ImmunoWay. Secondary antibodies, horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (AB10058) and anti-mouse IgG (D111050) were purchased from Sangon Biotech.

Cell culture

Human gastric cancer cell lines MGC-803 and SGC-7901 were gifts from Professor Wei Zhu of Jiangsu University. Both cell lines were maintained in Dulbecco's Modified Eagle Medium (HyClone, USA) with 10% fetal bovine serum (BI, Israel) and 1% penicillin-streptomycin mixture (Invitrogen). The cells were cultured at a constant temperature of 37°C in a humidified atmosphere of 5% CO_2.

Cell transfection

Human *URI* knockdown cells (MGC-803 and SGC-7901) were established by transfecting small interfering RNA-A (siRNA-A). The sequences of *URI* siRNA-A and scrambled control synthesized by Origene Technologies, were as follows: siRNA-A, AGAAGGUAGAUAAUGACUAUAAUGC. Scrambled control, CGUUAAUCGCGUAUAAUACGCGUAT. The cells were incubated in Dulbecco's Modified Eagle Medium without penicillin-streptomycin mixture and transfection was performed according to the manufacturer's protocol using Hiperfect Transfection Reagent (Qiagen, USA) and Opti-MEM (Invitrogen, Carlsbad, CA) when the cells were around 60-80% confluence. Untransfected cells served as blank controls. After 6 hours, the cells were incubated in fresh medium for another 24 hours or 48 hours. Expression of URI in all groups was detected by qRT-PCR and Western blot so as to verify the efficacy of *URI* knockdown.

Real-time PCR analysis

The total RNAs were extracted using ice-cold Trizol (Invitrogen, Carlsbad, CA) and reverse transcription was performed to synthesize cDNA with iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) according to the manufacturer's protocol. The gene was amplified and detected by RT-PCR using the Bio-Rad CFXTM96 Detection System and SYBR Green real-time PCR master mix (Bio-Rad, Hercules, CA) [20]. Endogenous GAPDH was used to normalize the

target gene and the result was calculated by $2^{-\Delta\Delta Ct}$ method. The primer sequences of URI were as the following: forward, TTTGCAGAAAATGAGCGATG, reverse, GCAATTCGGTGTTTTGCTTT. And the primer sequences of GAPDH were as follows: forward, TCTCTGCTCCTCCTGTTCGA, reverse, GCGCCCAATACGACCAAATC. Three independent experiments were performed.

Western blotting analysis

Western blotting was performed as described previously [21]. To extract total protein, the cells were lysed in RIPA buffer (Beyotime Biotechnology, CA, China) containing 10% phosphatase inhibitor and 10% protease inhibitor cocktail (KangChen, Shanghai, China) on ice and centrifuged at 14000 g for 15 minutes at 4°C. The supernatant was collected and boiled for denaturation. The protein was then fractionated with SDS-polyacrylamide gel electrophoresis and transferred onto the Immobilon-P membranes (Millipore, Billerica, USA). Specific primary antibodies were used to incubate the membranes overnight and HRP-conjugated secondary antibodies were used to incubate the immuno-complexes after washing the primary antibodies uncombined to the proteins. The results were then detected with enhanced chemiluminescence system (Minichemi, China). To ensure equal loading, expression of GAPDH was used as a protein loading control. The experiment was conducted independently in triplicate.

Comet assay

Comet assay was taken to measure the DNA damage in gastric cancer cells as previously described [25]. Briefly, about 10^4 cells treated with different concentrations of cisplatin were collected and mixed with 100 μl of low melting point agarose (LMPA) at 37°C. Then the mix was added to normal melting point agarose (NMPA) layered on slide immediately and moved to refrigerator flatly for solidification at 4°C. The cells were then lysed in ice-cold lysis buffer (pH10) for 90 min and electrophoresis was performed with alkaline running buffer at 25 V, 300 mA for 30 minutes after unwinding for 20 minutes. The slides were neutralized and stained with gel-Red. Fluorescent microscope was used to record the images and CASP (Comet Assay Software Project) was used to analyze the results. The degree of injury was described by OTM (Olive Tail Moment). The experiment was performed in triplicate.

Cell viability assay

The viability of gastric cancer cells treated with different concentrations of cisplatin was estimated via a cell counting kit-8 (CCK-8) assay according to the manufacturer's protocol (Solarbio Life Sciences, Beijing, China). Exponentially growing cells with transfection were seeded into 96-well plates with 5000 cells per well and treated with different concentrations of cisplatin. A mixture of CCK-8 and DMEM was added into the 96-well plates with 100 μl per well while the original medium was wiped out. The cells were incubated for 2 hours away from light at 37°C and the optical density (OD) was measured at 450 nm wavelength with a microplate reader (Bio-Rad Model 680, Richmond, CA, USA). Five replicates were used to calculate the mean value of each concentration. The half maximal inhibitory concentration (IC50) of cisplatin was calculated according to the vitality with SPSS 16.0. Three independent experiments were conducted.

Cell proliferation assay

The EDU detection kit (Ribo Bio, Guangzhou, China) was taken to detect the proliferation of gastric cancer cells treated with cisplatin. In brief, the cells were seeded into 96-well plates and treated with cisplatin as described above. The EDU solution was diluted 1000 times with DMEM. 100 μl of mixture was added into every well to label the DNA. The immobilization and staining of cells were performed according to the manufacturer's protocol. Images were captured by fluorescent microscope. All of the nuclei were stained with Hoechst (33342) showing blue fluorescence and the nuclei of the cells in proliferating phase were stained with Apollo showing green fluorescence. Independent experiment was repeated in triplicate.

Apoptosis assay

The apoptotic cells in URI knockdown groups and control groups with cisplatin treatment were detected via flow cytometry using the FITC Annexin V apoptosis detection kit (BD PharmingenTM, CA, USA) according to manufacturer suggested protocol. The cells treated

with cisplatin were collected and washed with cold PBS. About 10^6 cells were resuspended in 1 ml of 1× Binding Buffer and 100 μl of the mixture was transferred into a flow tube. The cells were kept in dark for 15 minutes with 5 μl of Annexin V-FITC and 5 μl of propidium iodide (PI) per tube. Flow cytometry analysis (BD AccuriTM C6 system) was taken after mixing with 400 μl of 1× Binding Buffer per tube within 1 h. The experiments were repeated three times.

Analysis of the cell cycle

The cell cycle was detected by flow cytometry using PI/RNase standard assay (BD PharmingenTM, CA, USA). The cells in 6-well plate were repaired for 12 hours after treating with cisplatin for 4 hours and the suspension containing 10^6 cells washed with ice-cold PBS was collected into a 5 ml tube. More than 5 ml ice-cold 75% ethanol was added into the tube dropwise and the mixture was stored at -20°C away from light for immobilization. The immobilized cells were washed with ice-cold PBS to wipe off ethanol completely the next day. All cells were stained with PI/RNase for 15 minutes away from light before being detected. The experiments were performed in triplicate.

Statistical analyses

All data are expressed as the mean ± SD. Statistical analyses were carried out using GraphPad Prism software (v6; GraphPad Software Inc., La Jolla, CA). Homogeneity of variance was analyzed using Brown-Forsythe test. A one-way analysis of variance followed by Bonferroni's post-hoc test was performed to test the significance of differences between groups. Statistical significance was set as follows: NS-not significant ($P>0.05$); $^{*}P\leq0.05$; $^{**}P\leq0.01$; $^{***}P\leq0.001$ means statistically significant.

Results

URI expression in two gastric cancer cell lines after transfection with siRNA-A

MGC-803 and SGC-7901 gastric cancer cell lines were derived from poorly differentiated tumors and lymph node metastatic tumors respectively, and both of them had high URI expression levels as demonstrated previously [21]. After transfection with URI siRNA-A, the expression of URI in two gastric cancer cell lines were obviously reduced compared with the control groups as examined by qRT-PCR (**Figure 1A**) and western blot (**Figure 1B**) analyses.

Cisplatin results in DNA damage in gastric cancer cells and the damage was significantly increased in URI knockdown cells

The OTM value of comet assay was used to evaluate the degree of DNA damage of cells treated with cisplatin. The dose range of cisplatin in MGC-803 (**Figure 2A**) and SGC-7901 (**Figure 2B**) was 0-20 μm and 0-10 μm respectively. The comet assay showed increased OTM values in a dose-response relationship in cisplatin treated cells compared with control cells. This indicated that the degree of DNA damage increased with the increase of cisplatin concentration.

URI gene silencing reduced the viability, proliferation and facilitated apoptosis of cells treated with cisplatin

We measured the cell viability of gastric cancer cells treated with different concentrations of cisplatin by CCK-8 cell viability assay. Compared with the scrambled and untransfected groups, the cell viability of the *URI* knockdown cells was reduced in a dose-dependent manner (**Figure 3A**). In addition, the mean IC50 of *URI* knockdown groups was lower than the control groups (**Figure 3B**), which indicated that *URI* gene silencing attenuates resistance to cisplatin in gastric cancer cells. The EDU assay (**Figure 3C**) showed that URI knockdown inhibited proliferation of cells treated with IC50 cisplatin compared with the control cells in two cell lines. Effect of *URI* knockdown on cisplatin-induced apoptosis was assessed by annexin V/propidium iodide apoptosis assay. The percentage of total apoptosis in two gastric cancer cell lines with *URI* knockdown were significantly higher than the control groups (**Figure 3D**), suggesting that URI can inhibit cell apoptosis induced by cisplatin.

URI gene silencing decreased activity of ATM/CHK2 pathway in cisplatin treated cells

After cisplatin treatment for 4 hours, P-ATM and P-CHK2 were measured at 0, 6, and 12 hours by western blot to evaluate the DNA damage

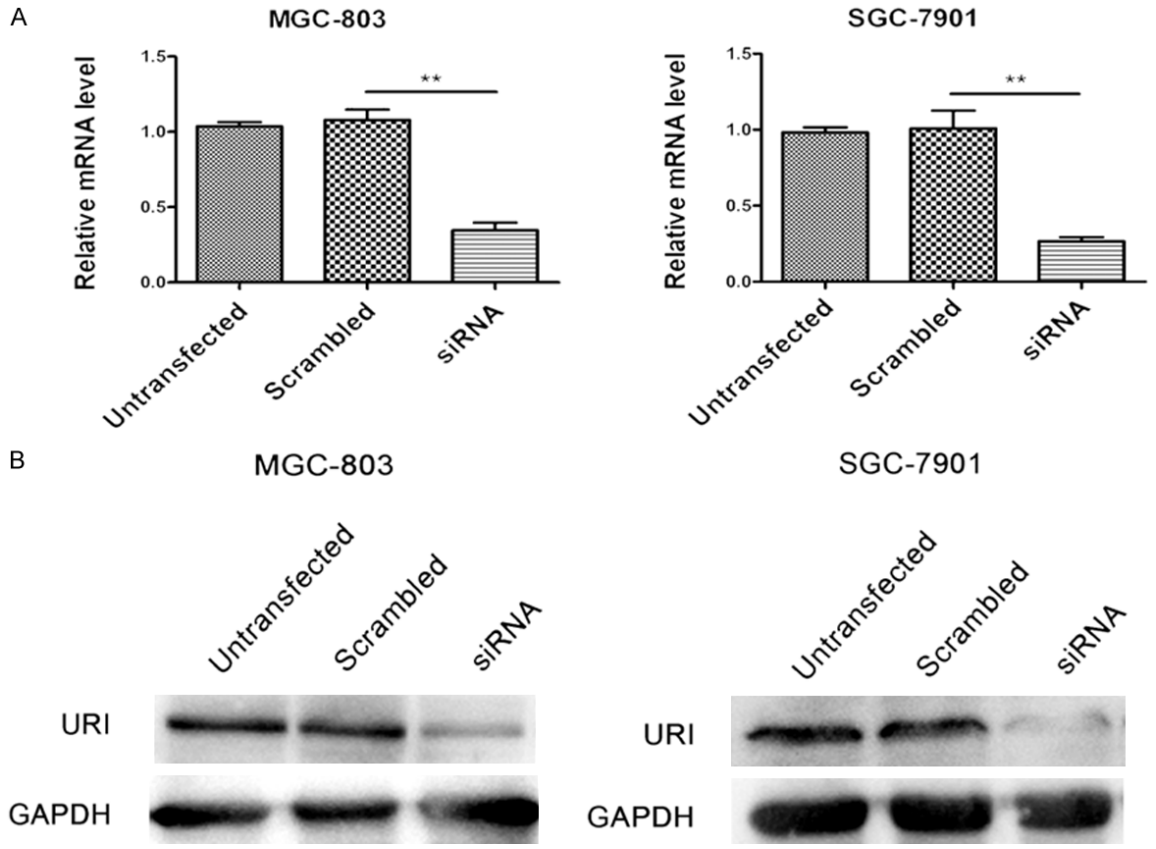


Figure 1. URI expression in two gastric cancer cell lines after transfection with siRNA-A. A. qRT-PCR analysis showed that the relative mRNA levels of URI were decreased in MGC-803 and SGC-7901 cells transfected with URI siRNA-A for 24 hours. B. URI knockdown was confirmed by western blot in MGC-803 and SGC-7901 cells transfected with URI siRNA-A. Untransfected group and scrambled group were used as controls, and GAPDH was used as internal control. Data was expressed as mean ± s.d. of three independent experiments, $^{**}P<0.01$.

response (DDR). The levels of P-ATM and P-CHK2 were significantly increased in cisplatin-treated cells compared with cells not treated with cisplatin. P-ATM and P-CHK2 levels in the *URI* knockout cells did not differ significantly from those in the control cells after 0 and 6 hours of repair (**Figure 4A**, **4B**), but were significantly lower in the *URI* knockout cells than in the control cells after 12 hours of repair (**Figure 4C**). These results suggest that *URI* gene silencing reduces the activity of the ATM/CHK2 repair pathway in cells that have been free of cisplatin for more than a few hours.

γH2AX levels were significantly increased in URI knockdown cells treated with cisplatin

Phospho-H2AX (γH2AX) is a marker of DNA damage and is often used to reflect the extent of DNA damage [26, 27]. MGC-803 and SGC-7901 cells were treated with 10 μM and 5 μM cisplatin, respectively. After 4 h of cisplatin treatment, MGC-803 and SGC-7901 cells were washed and released into fresh medium, and γH2AX was determined by Western blot at 0, 6 and 12 h (**Figure 5A-C**). γH2AX levels were significantly increased in cisplatin treated cells. After 6 h of cisplatin removal, the level of γH2AX in *URI* knockout cells was significantly higher than that in control cells. After 12 h of cisplatin removal, the γH2AX level of URI knockout cells did not change significantly compared with that of control cells. However, in both cell lines receiving continuous treatment with cisplatin for 12 h, γH2AX levels were significantly higher in *URI* knockout cells than in control cells (**Figure 5D**). These results suggested that *URI* silencing enhanced DNA damage of cells persistently treated with cisplatin and increased their drug sensitivity. The effect of *URI* knockdown depends on the presence of cisplatin.

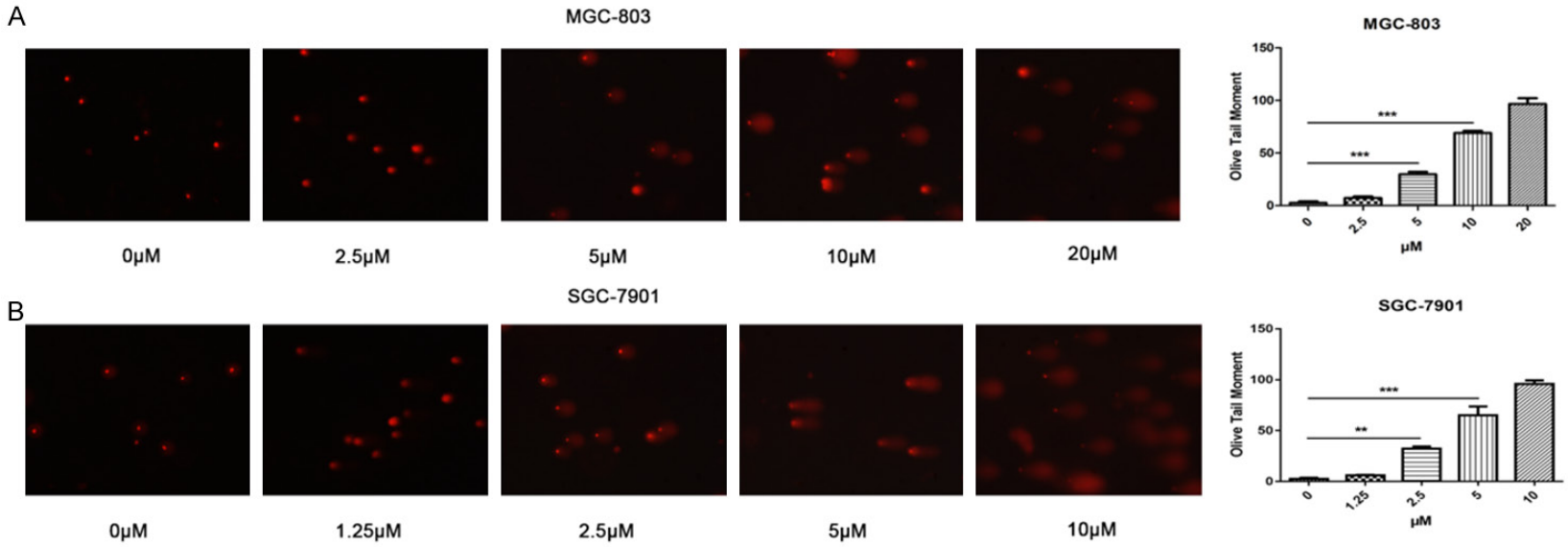
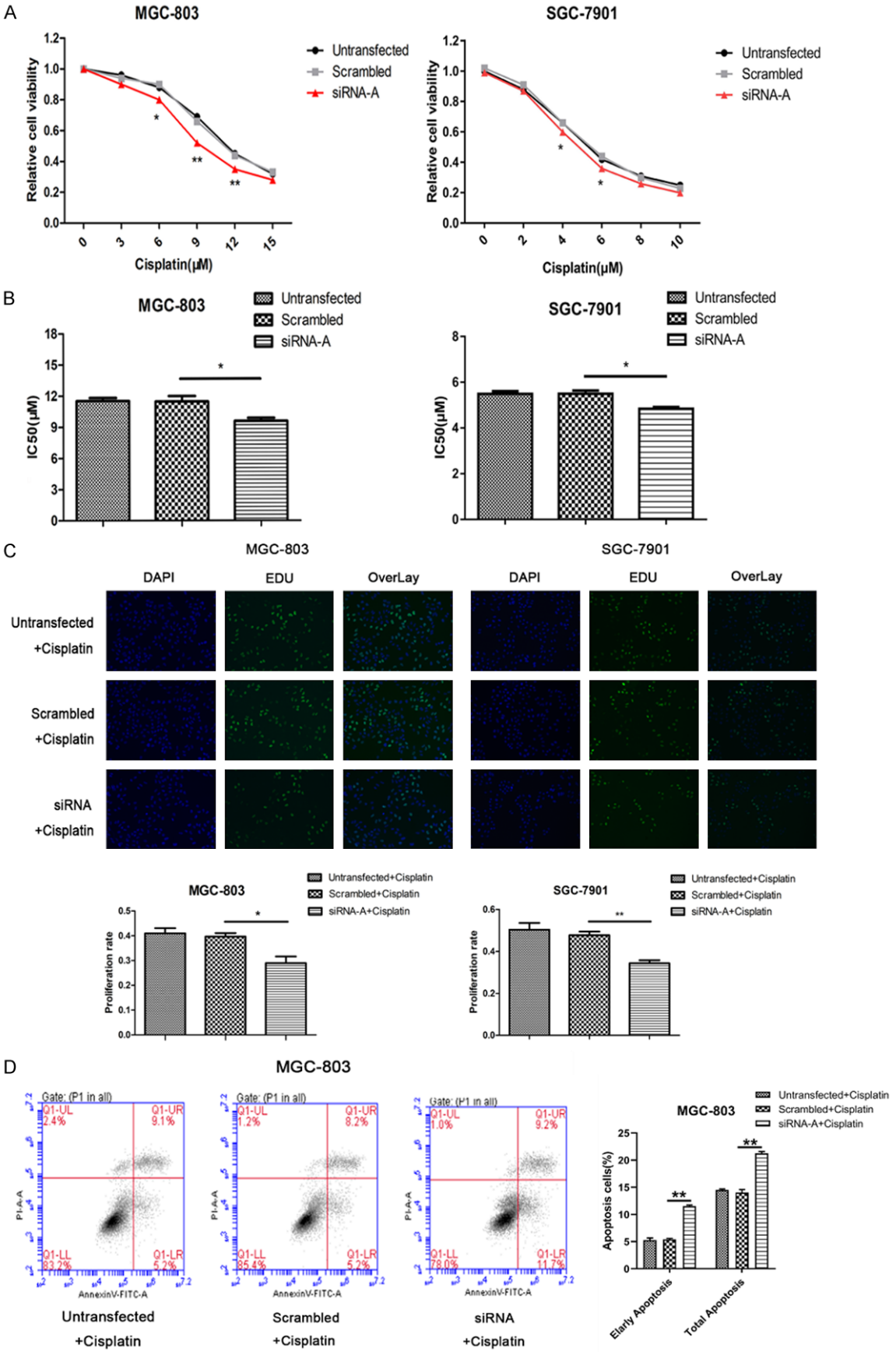


Figure 2. DNA damage caused by cisplatin in URI knockdown cells. DNA damage was measured by comet assay in MGC-803 (A) and SGC-7901 (B) cells treated with different concentrations of cisplatin for 12 h. Fluorescence images (left) and quantification bar plots of OTM (right) indicated that the extent of DNA damage was increased along with the increase of cisplatin concentration. Data was expressed as mean ± s.d. of three independent experiments, $^{**}P<0.01$, $^{***}P<0.001$.


A
MGC-803
SGC-7901
Relative cell viability
Cisplatin(μM)
Untransfected
Scrambled
siRNA-A
B
IC50(μM)
C
DAPI
EDU
OverLay
Untransfected +Cisplatin
Scrambled +Cisplatin
siRNA +Cisplatin
Proliferation rate
Untransfected+Cisplatin
Scrambled+Cisplatin
siRNA-A+Cisplatin
D
Gate: (P1 in all)
Q1-UL
Q1-UR
Q1-LL
Q1-LR
PI-A-A
AnnexinV-FITC-A
Apoptosis cells(%)
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Total Apoptosis

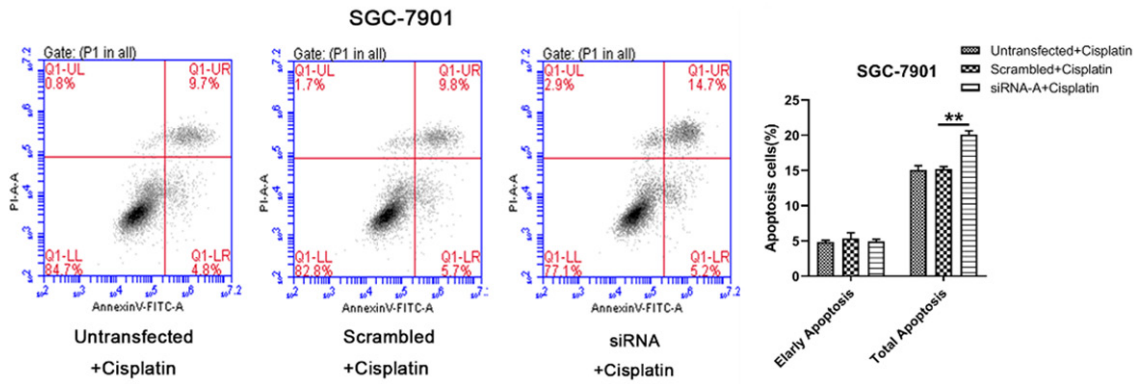

Figure 3. Effect of URI on the viability, proliferation and apoptosis of cells treated with cisplatin. A. CCK-8 assay indicated that the viability of the MGC-803 and SGC-7901 cells were reduced with the increase of cisplatin concentration. B. Histogram showed that the mean IC50 values of cisplatin in MGC-803 and SGC-7901 cells was decreased in URI knockdown group compared with the scrambled and untransfected groups. C. EDU assay (above) and the bar chart (below) showed that the proliferation of URI knockdown cells was lower than scrambled and untransfected cells in two cell lines. D. URI silencing can increase cisplatin-induced apoptosis in two cell lines. The Flow cytometry images (left) and the bar chart (right) were as shown. Data was expressed as mean ± s.d. of three independent experiments, $^{*}P<0.05$, $^{**}P<0.01$.

URI silencing attenuated cell cycle progression in cisplatin treated cells

We examined whether URI knockdown induced cell cycle arrest in MGC-803 and SGC-7901 cells exposed respectively to cisplatin with concentrations of 10 μM and 5 μM for 12 hours. Flow cytometry was used to analyze the cell cycle distribution. Cell cycle analysis showed that G2/M cells were reduced in cisplatin treated cells. However, in cisplatin treated *URI* knockdown cells, the number of cells entering S phase significantly decreased relative to control cells. At the same time, G1 cells were arrested and G2/M cells were relatively unchanged (**Figure 6A**, **6B**), suggesting an overall decrease in cell-cycle progression, thereby reducing the cell proliferation and promoting cell apoptosis.

Discussion

The cytotoxicity of platinum-based drugs leads to DNA crosslinks and the sustained development of DSBs (Double-strand breaks) in eukaryotic cells which in turn promote cell cycle arrest and apoptosis [28]. Studies have shown that cisplatin resistance depends on the activation of multiple non-overlapping mechanisms, so cisplatin resistance is often multifactorial and complex. Enhanced DNA damage repair is one mechanism of cisplatin resistance [29]. Comet assay is effective and sensitive in the monitoring of DNA damage in cancer patients [30]. γH2AX can also be used as a biomarker for DNA double stranded breaks (DSBs) caused by cytotoxic chemicals and environmental and physical damage [31]. In our study, DNA damage induced by cisplatin was confirmed by comet assay and increased γH2AX level in gastric cancer cells. The activation of DNA damage response (DDR) pathway in tumor cells plays an important role in platinum-based chemotherapy, but the enhanced DNA repair capacity of tumor cells promotes the development of cisplatin resistance [28, 32]. Three kinases, ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK), respond to DSBs, resulting in initial H2AX phosphorylation and recruiting DDR factors to damage sites [33-36]. This involves a series of processes of phosphorylation of relevant checkpoint kinase proteins, and eventually leads to cell cycle arrest, so as to allow cells time for DNA repair or initiate the apoptosis process when the repair processes failed [37, 38]. ATM is activated by DSBs through autophosphorylation at serine 1981. Active ATM regulates DNA repair, cell cycle arrest and apoptosis by phosphorylating many target proteins including CHK2, p53 and H2AX. ATM plays a critical role in the activation of G1/S cell cycle checkpoints [12]. We found that the phosphorylation levels of ATM (P-ATM) and CHK2 (P-CHK2) were significantly decreased in URI knockdown cells when the

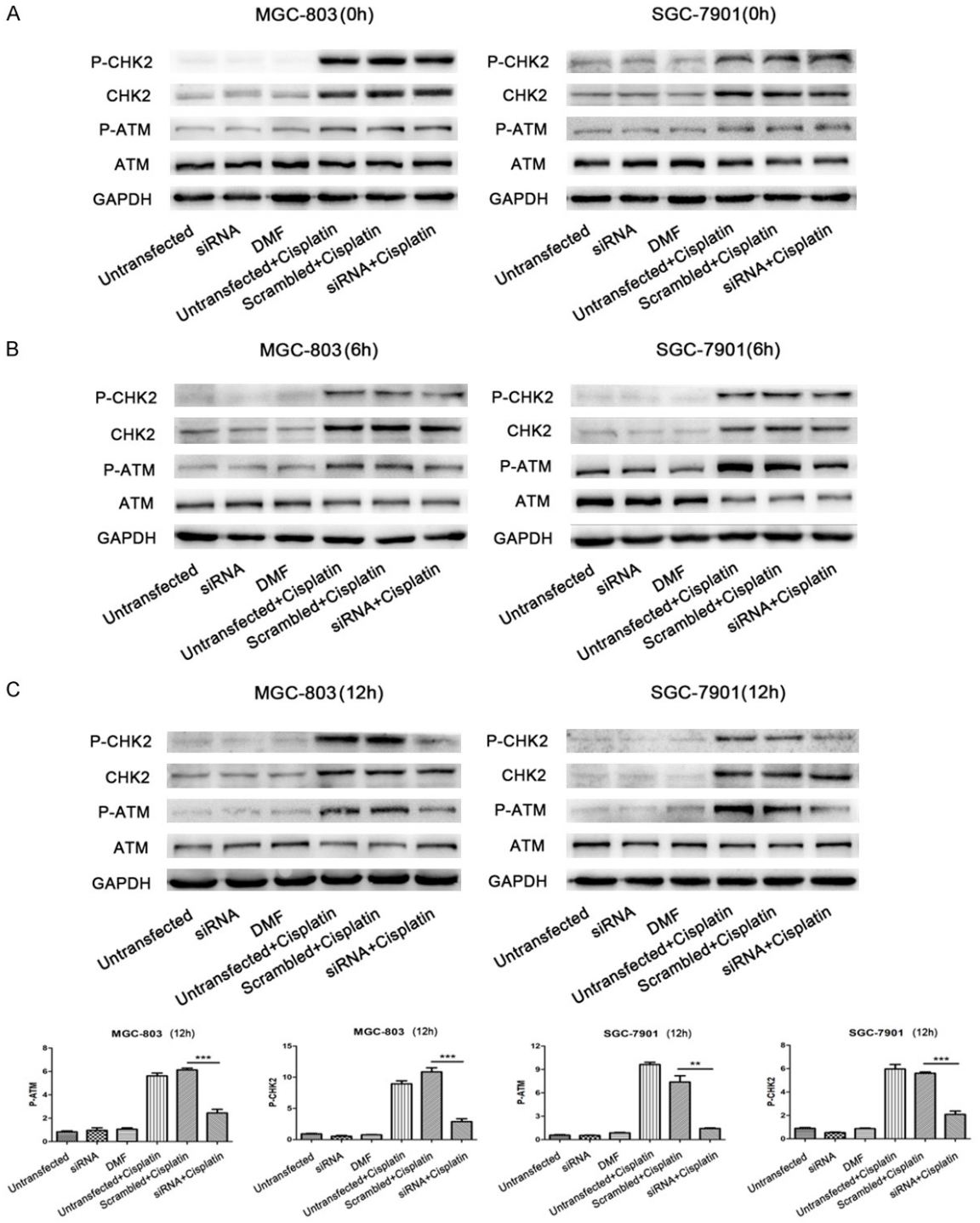

Figure 4. The levels of P-ATM and P-CHK2 in cisplatin-treated URI knockdown cells. A. After 4 h of cisplatin treatment and 0h of repair, there were no significant difference in P-ATM and P-CHK2 levels between URI knockdown cells and control cells as determined by Western blotting. B. After 4 h of cisplatin treatment and 6 h of repair, the levels of P-ATM and P-CHK2 were slightly different in the URI knockout group compared with the control groups. C. The western blot results (above) and histograms (below) showed that the levels of P-ATM and P-CHK2 significantly decreased in URI knockdown groups compared with control groups after cisplatin treatment for 4 h and repair for 12 h. DMF treatment group was used as a solvent control of cisplatin. GAPDH was used as internal control. Data was expressed as mean ± s.d. of at least three independent experiments, $^{**}P<0.01$, $^{***}P<0.001$.

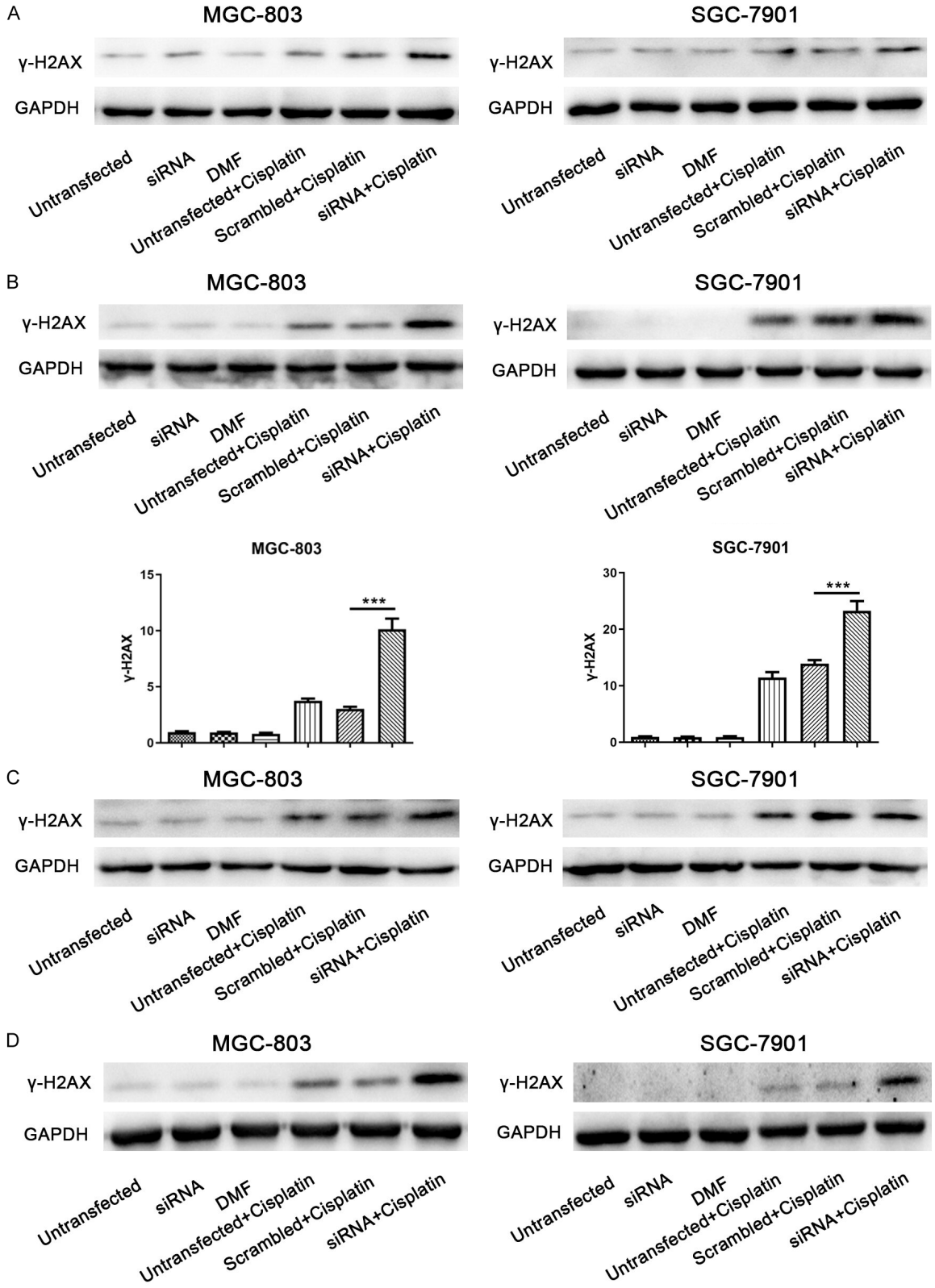

Figure 5. The γH2AX levels in cisplatin-treated URI knockdown cells. DNA damage was induced by cisplatin (MGC-803 for 10 μM, SGC-7901 for 5 μM) and Phospho-H2AX (γH2AX) levels were detected by Western blot. A. γH2AX level of cells after 0 h of cisplatin removal. B. γH2AX level of cells after 6 h of cisplatin removal. C. γH2AX level of cells after 12 h of cisplatin removal. D. γH2AX level of cells after continuous treatment with cisplatin for 12 h. DMF treatment group was used as a solvent control of cisplatin. GAPDH was used as internal control. Data was expressed as mean ± s.d. of three independent experiments, ***P<0.001.

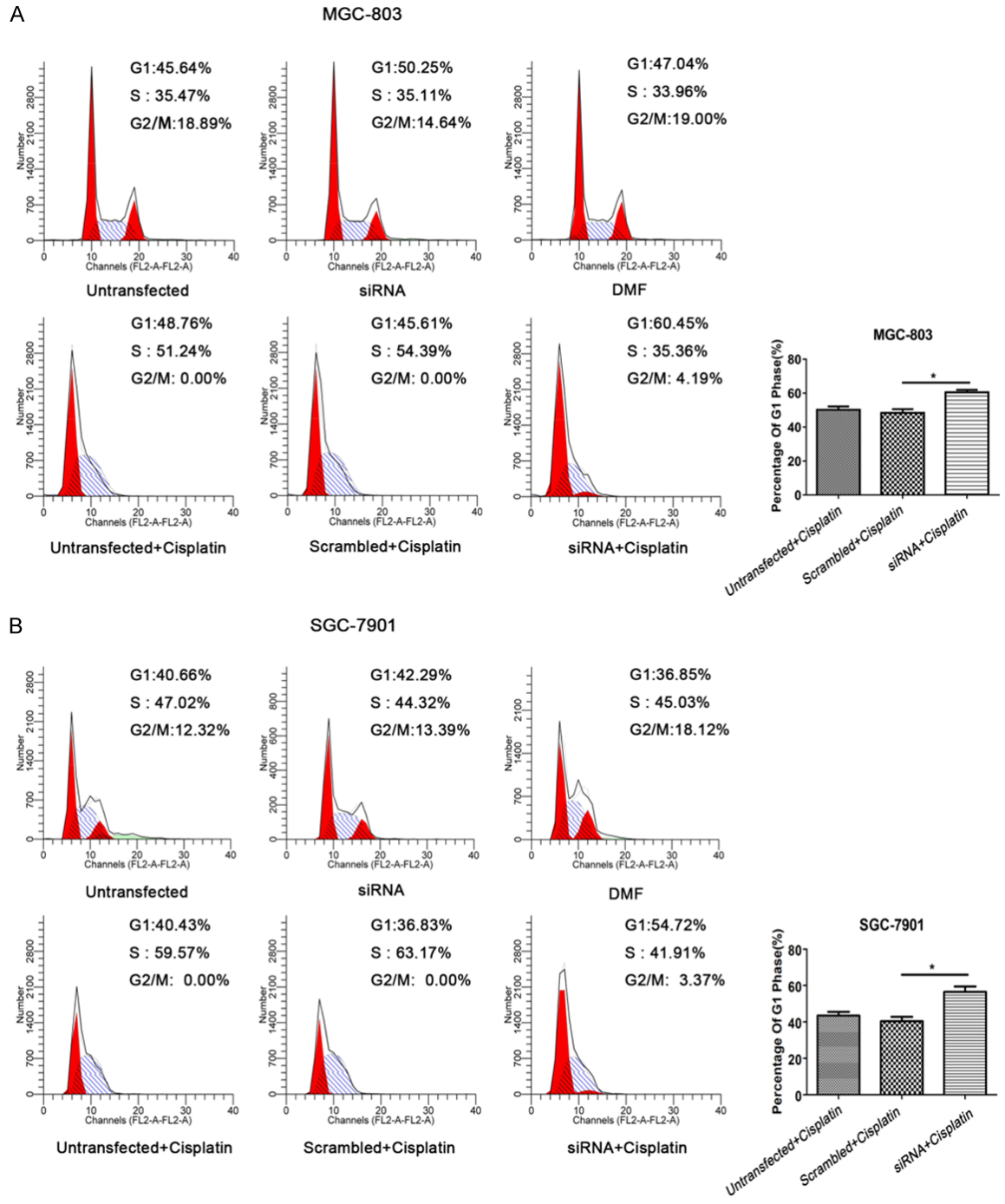

Figure 6. Cisplatin-induced cell cycle arrest in URI knockdown cells. Cell cycle distribution was analyzed by flow cytometry. A, B. In the absence of cisplatin treatment, there was no significant difference in cell cycle distribution in MGC-803 and SGC-7901 cells between URI knockdown group and control groups (top). The S phase was significantly decreased in URI knockdown group compared with control groups after cisplatin treatment for 12 h (bottom). DMF group was used as a solvent control of cisplatin. Data was expressed as mean ± s.d. of three independent experiments, $^{**}P<0.01$.

cisplatin-treated cells were given a certain period of time to repair. The decrease of IC50 values and increased apoptosis indicates that URI knockdown reduces resistance of gastric cancer cells to cisplatin. Our results suggested that silencing URI reduces cell resistance to cisplatin by inhibiting ATM/CHK2 pathway activation and reducing DNA damage repair.

We found that when URI expression was knocked down in gastric cancer cells, cisplatin-induced γH2AX level was significantly increased. This is consistent with a finding in uterine cancer cells that URI overexpression significantly reduced cisplatin-induced γH2AX phosphorylation and promoted cisplatin-induced DNA damage resistance [39]. These results indicate that URI attenuates the DNA damage induced by cisplatin, which supports our previous findings that URI attenuated the DNA damage caused by potassium dichromate in gastric cancer cells [24]. Sears et al. investigated the role of the DNA damage response (DDR) in cisplatin-mediated radiosensitization using two non-small cell lung cancer cell lines. The persistence of γ-H2AX foci is independent of ATM or DNA-PK activation. Unrepaired DSBs may present with persistent γ-H2AX foci. Cisplatin-mediated radiosensitization may be attributed to impaired DSB repair [40]. The function of γH2AX is very complex, and its function does not seem to restrict to DNA damage repair, it may also be involved in the regulation of apoptosis, possibly by induction of apoptosis. H2AX phosphorylation was critical for DNA degradation triggered by caspase-activated DNAse (CAD), and was required for DNA ladder formation in vitro [41]. Our data showed that γ-H2AX levels were significantly increased in URI-silenced cells under continuous exposure to cisplatin, suggesting that URI silencing inhibited DNA damage repair and induced apoptosis of gastric cancer cells.

Cisplatin can induce S phase arrest in cancer chemotherapy [42-45]. The effect of cisplatin on cell cycle progression has both dose and time effect. Significant S-phase accumulation was observed in ovarian cancer A2780 cells treated with 1.0 μM cisplatin for 12 h [46]. Kielbik et al. found that inhibition of ERK1/2 activation led to a shift of cell aggregation from S phase to G1 phase and increased the cytotoxicity of cisplatin to ovarian cancer cells [47]. In our study, we had similar observation that cisplatin exposure for 12 hours induced S-phase arrest in gastric cancer cells, but URI silencing significantly reduced the number of cells entering S-phase and the cells were arrested in G1 phase. The mechanism of URI regulating the cell cycle of cisplatin exposed cells remains to be explored. We hypothesized that URI inhibition may enhance cytotoxicity of cisplatin and reduce damage repair mainly in G1 phase.

In conclusion, this study reveals that cisplatin treatment causes induction of DNA damage which can activate the ATM/CHK2 signaling cascades. Inhibition of URI can enhance the sensitivity of gastric cancer cells to cisplatin by reducing DNA damage repair and inducing apoptosis. One of the limitation is that the in vitro effect of URI may not fully reflect the in vivo relevance which needs further investigation. URI may be a potential therapeutic target for gastric cancer patients with cisplatin resistance.

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Disclosure of conflict of interest

None.

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