Synergistic effects of olaparib combined with ERCCI on the sensitivity of cisplatin in non-small cell lung cancer

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Abstract. Non-small cell lung cancer (NSCLC) is a common malignant tumor. ERCC excision repair 1 endonuclease non-catalytic subunit (ERCCI) is a key mediator of nucleotide excision repair. The present study aimed to explore the synergistic effects of the poly(ADP-ribose) polymerase (PARP) inhibitor olaparib combined with ERCCI on the sensitivity of NSCLC cells to cisplatin. Preliminary experiments were performed to identify the optimal concentrations of cisplatin and olaparib for cellular treatment and subsequently NCI-H1299 and SK-MES-1 cells were treated with 20 µg/ml cisplatin combined with 50 µg/ml olaparib and 50 µg/ml cisplatin combined with 70 µg/ml olaparib, respectively. Subsequently, transfections were carried out to overexpress or knockdown the expression of ERCCI in NSCLC cell lines, including NCI-H1299 and SK-MES-1. The transfection efficiency was evaluated using reverse transcription-quantitative PCR and western blotting. The results demonstrated that cells with ERCCI overexpression and ERCCI knockdown were successfully constructed. Finally, the cell viability and apoptosis were determined using the Cell Counting Kit-8 and Annexin V-FITC cell apoptosis assays, respectively. In NCI-H1299 or SK-MES-1 cells treated with cisplatin combined with olaparib for 24 h, the cell viability significantly increased following ERCCI overexpression compared with the GV230 group (P<0.05), but significantly inhibited following ERCCI knockdown compared with the siRNA-NC group (P<0.05). However, ERCCI overexpression or knockdown had the opposite effect on apoptosis. In conclusion, olaparib combined with ERCCI expression may enhance the sensitivity of cisplatin in NSCLC. These findings may provide novel insight for the improvement of platinum drug sensitivity and treatment of NSCLC.

Introduction

Lung cancer is a leading cause of tumor-related mortality worldwide (1). Every year, 1.8 million people are diagnosed with lung cancer, and 1.6 million people die as a result of the disease, as well as 5-year survival rates vary from 4-17% depending on stage and regional differences (2). Non-small cell lung cancer (NSCLC) accounts for ~85% of lung cancer cases, and most deaths from lung cancer can be attributed to NSCLC (3). After radical surgery, >60% of patients with early-stage NSCLC can experience in situ recurrence of the tumor or distal metastasis (4). Recently, adjuvant chemotherapy based on platinum drugs has been widely applied to the clinical treatment of NSCLC after surgery (5). Platinum drugs can destroy the structure of DNA by forming platinum-DNA complexes, thereby contributing to the apoptosis of tumor cells (6). However, the long-term use of platinum drugs leads to drug resistance, which is one of the major obstacles of cancer treatment (7). Thus, an improved understanding of the mechanisms of platinum drug resistance, as well as the development of methods that can overcome resistance, is necessary to improve the prognosis of patients with cancer.

As the major target of platinum drugs is DNA, the sensitivity/resistance to these drugs may be affected by the ability of cells to recognize and repair DNA damage (8). Nucleotide excision repair (NER) is the major pathway for the removal of platinum-DNA adducts (9). ERCC excision repair 1, endonuclease non-catalytic subunit (ERCCI), a key component of NER, is involved in interstrand cross-linking repair, double-strand break repair, homologous recombination and telomere maintenance (10). Increasing evidence has indicated that the differential expression of ERCCI may be a cause of cell resistance to platinum drugs. Selvakumaran et al (11) found that downregulation of ERCCI altered the DNA repairing ability of cisplatin-resistant ovarian cancer cells and increased their sensitivity to cisplatin. In a previous meta-analysis, patients with lung cancer that had low/negative ERCCI expression had a higher response to platinum drugs and longer...
median survival time compared with those with high/positive ERCC1 expression (12).

In addition, poly(ADP-ribose) polymerase 1 (PARP1), is a sensor for DNA strand break that responds to platinum-induced DNA damage and participates in DNA repair (13). PARP inhibitors can improve the sensitivity of tumor cells to chemotherapy drugs or directly kill tumor cells through a homozygous lethal mechanism (14,15). A randomized clinical study demonstrated that the PARP inhibitor olaparib significantly increased the sensitivity to platinum drugs and prolonged the median progression-free survival time of breast cancer (16). However, the effects of PARP inhibitors combined with ERCC1 expression on the sensitivity of platinum drugs remain unclear in NSCLC.

Hence, the present study aimed to investigate whether the expression of ERCC1 enhanced the sensitivity of platinum drugs in combination with PARP inhibitors, thereby improving the prognosis of NSCLC. These results may provide novel insight for the improvement of platinum drug sensitivity and treatment of NSCLC.

Materials and methods

Cell culture. NSCLC cell lines, including the NCI-H1299 (adenocarcinoma) and SK-MES-1 (squamous carcinoma) cell lines were purchased from the Cell Resource Center, Shanghai Institute of Biotechnology, Chinese Academy of Sciences. NCI-H1299 and SK-MES-1 cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.) and minimum essential medium (Thermo Fisher Scientific, Inc.) with 10% FBS, respectively. Both cell lines were incubated in an incubator with 5% CO2 at 37°C.

Cell transfection. Small interfering (si)RNA-negative control (NC, non-targeting; forward, 5'-UUCUCCGAACGUGUACAGU-3' and reverse, 5'-ACGUGACACGUCCGAGAATT-3'), siRNA-ERCC1-1 (GCGCTTTATCTCCAGTCACA), siRNA-ERCC1-2 (CGACGTAATTCCGACTAT), and siRNA-ERCC1-3 (CCGTCGAGTCAGTCAACAA) were designed and synthesized by Guangzhou RiboBio Co., Ltd. Briefly, different concentrations of cisplatin (Sigma-Aldrich; Merck KGaA) and PARP inhibitor olaparib (Selleck Chemicals) were prepared in dimethyl sulfoxide (Beijing Solarbio Science & Technology Co., Ltd.). The control, ERCC1-overexpressing and ERCC1-knockdown cells were seeded into 6-well plates (5x104 cells/well), then treated with cisplatin alone or in combination with olaparib at 37KC, as shown in Fig. 1. Following treatment for 24 h, 10 µl of CCK-8 reagent (Beyotime Institute of Biotechnology) was added to the cells and incubated at 37°C for 2 h. Absorbance was detected at 450 nm using a microplate reader.

Cell viability assay. Cell viability of NCI-H1299 and SK-MES-1 cells was determined using the Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology). The schematic workflow of the cellular experimentation is presented in Fig. 1. Briefly, different concentrations of cisplatin (Sigma-Aldrich; Merck KGaA) and PARP inhibitor olaparib (Selleck Chemicals) were prepared in dimethyl sulfoxide (Beijing Solarbio Science & Technology Co., Ltd.). The control, ERCC1-overexpressing and ERCC1-knockdown cells were seeded into 6-well plates (5x104 cells/well), then treated with cisplatin alone or in combination with olaparib at 37KC, as shown in Fig. 1. Following treatment for 24 h, 10 µl of CCK-8 reagent (Beyotime Institute of Biotechnology) was added to the cells and incubated at 37°C for 2 h. Absorbance was detected at 450 nm using a microplate reader.

Cell apoptosis assay. Effects of ERCC1 expression on apoptosis in NCI-H1299 and SK-MES-1 cells were determined using an Annexin V-FITC cell apoptosis assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. The NCI-H1299 or SK-MES-1 cells with ERCC1 overexpression or interference (1x104 cells/well) were treated with cisplatin and olaparib at 37°C for 24 h as detailed in Fig. 1. Subsequently, the cells were harvested and resuspended in

RT-qPCR. Total RNA was extracted from the transfected cells (5x104 cells/well) using TRIzol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using the PrimeScript™ II 1st Strand cDNA Synthesis kit (Takara Bio, Inc.). The temperature protocol used for reverse transcription was 37°C for 60 min and 85°C for 5 sec. Subsequently, qPCR was performed using SYBR Premix EX Taq (2X, Thermo Fisher Scientific Inc.), and the primer sequence of ERCC1 was as follows: Forward, 5'-TTGTCCAGGTGAGTGGAA-3' and reverse, 5'-GCTGTTCTGCTATAGGGC-3'. The qPCR thermocycling conditions were as follows: 95°C for 3 min; 95°C for 10 sec; followed by 40 cycles at 60°C for 30 sec and 60°C for 30 sec. The mRNA expression of ERCC1 was quantified using the 2-ΔΔCq method (18) and normalized to the reference gene GAPDH forward, 5'-AGACAGCCGCACTTCTTGTG-3' and reverse, 5'-CTTGCAGTGGGTAGATCAT-3'.

Western blotting. Total protein was isolated from transfected cells (5x105 cells/well) using radioimmunoprecipitation assay protein lysis buffer (Beyotime Institute of Biotechnology). Protein concentrations were measured using a Bicinchoninic Acid Protein Assay kit (Wuhan Boster Biological Technology, Ltd.) following the manufacturer's protocol. Protein samples (20 µg) were separated by 10% SDS-PAGE and transferred to PVDF membranes. After blocking with 5% skimmed milk for 2 h at 37°C, the membranes were incubated with anti-ERCC1 antibody (1:2,000; cat. no. 14586-1-AP; ProteinTech Group, Inc.) and anti-β-actin antibody (1:10,000; cat. no. 60609-1-Ig; ProteinTech Group, Inc.) overnight at 4°C. After washing 3 times with PBS (0.05% Tween-20 in PBS), the membranes were incubated with goat anti-rabbit mouse IgG (1:10,000; cat. no. 115-035-003; Jackson ImmunoResearch Laboratories, Inc.) at 37°C for 2 h. After 3 washes, protein bands were visualized using the ECL assay kit (Beyotime Institute of Biotechnology) and analyzed using Image-Pro Plus software v.6.0, (Media Cybernetics Inc.).
pre-cooled phosphate-buffered saline. After centrifugation at 1,000 x g for 5 min at room temperature, 195 µl binding buffer and 5 µl Annexin V-FITC (20 µg/ml) were added to the cells. After incubation at room temperature for 30 min in the dark, the cells were stained with 5 µl of propidium iodide (PI; 50 µg/ml) and incubated at room temperature in the dark for 10 min. Finally, flow cytometry (FACSCalibur; Becton-Dickinson and Company) was used to observe cell apoptosis, and the apoptosis rate (early plus late apoptosis) was calculated using the CellQuest software v.4.0 (Becton-Dickinson and Company).

Statistical analysis. Each experiment was performed in triplicate. All data are expressed as the mean ± standard deviation. GraphPad Prism 5.0 (GraphPad Software, Inc.) was used for statistical analysis. For multiple comparisons, one-way analysis of variance followed by Bonferroni correction was performed. Student's t-test with unpaired test was applied for comparisons between two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Selecting optimal concentrations of cisplatin and olaparib for cellular treatment. To select the optimum concentration of cisplatin, different concentrations were used to treat NSCLC cell lines for 24 h. In the NCI-H1299 cell line, when the cisplatin concentration was 10 µg/ml, the cell viability was significantly inhibited compared with the control group (P<0.05) and gradually decreased with an increase in cisplatin concentration (Fig. 2A). In the SK-MES-1 cell line, when the cisplatin concentration was 30 µg/ml, the cell viability began to be significantly inhibited compared with the control group (P<0.05), and when the concentration of cisplatin was 50 µg/ml, cell viability was further suppressed compared with the control group (P<0.05; Fig. 2B). Thus, in subsequent experiments, 20 and 50 µg/ml cisplatin were applied to the NCI-H1299 and SK-MES-1 cell lines, respectively.

To confirm the concentrations required for the PARP inhibitor olaparib, the NSCLC cell lines were treated with cisplatin combined with different concentrations of olaparib. When the NCI-H1299 and SK-MES-1 cell lines were treated with 20 and 50 µg/ml cisplatin, respectively, cell viability was significantly decreased compared with that of the control group (P<0.05; Figs. 2C and D). However, in the NCI-H1299 cell line, the cell viability was significantly lower after 50 µg/ml olaparib combined with cisplatin administration compared with after cisplatin treatment alone (P<0.05; Fig. 2C). Similarly, in the SK-MES-1 cell line, when the concentration of olaparib was 70 µg/ml, the cell viability was further significantly reduced compared with that of the cells treated without olaparib (P<0.05; Fig. 2D). These results indicated that in subsequent experiments, the NCI-H1299 and SK-MES-1 cells should be treated with 20 µg/ml cisplatin combined with 50 µg/ml olaparib and 50 µg/ml cisplatin combined with 70 µg/ml olaparib, respectively.

Cell transfection efficiency analyses by RT-qPCR and western blotting. To evaluate the transfection efficiency, the expression level of ERCC1 in NSCLC cell lines was determined by RT-qPCR and western blotting. In the NCI-H1299 cell line, western blot analysis demonstrated that the expression of ERCC1 significantly decreased following transfection with siRNA-ERCC1-1/2/3 compared with the siRNA-NC group (P<0.05) and that the siRNA-ERCC1-1 group had the
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best transfection efficiency as the ERCC1 mRNA expression was the lowest in the siRNA-ERCC1-1 group compared with the siRNA-NC, si-RNA-ERCC1-2 and siRNA-ERCC1-3 groups (Fig. 3A and B). In addition, RT-qPCR was performed to determine the mRNA expression levels of ERCC1 in the GV230, GV230-ERCC1+, siRNA-NC, and siRNA-ERCC1-1 groups. The expression level of ERCC1 in the GV230-ERCC1+ group was significantly higher compared with that in the GV230 group, while the expression level was significantly downregulated in the siRNA-ERCC1-1 group compared with that in the siRNA-NC group (both P<0.05; Fig. 3C). The trend of ERCC1 mRNA expression in the SK-MES-1 cell line was similar to that in the NCI-H1299 cell line (Fig. 3D). These results suggested that the NCI-H1299 or SK-MES-1 cells with ERCC1 overexpression and ERCC1 knockdown were successfully generated.

Effects of ERCC1 combined with olaparib on the cell viability of cisplatin-treated cells. In the NCI-H1299 cell line, after the cells were treated with 20 µg/ml cisplatin combined with 50 µg/ml olaparib for 24 h, cell viability was significantly increased in the ERCC1-overexpressing cells compared with the GV230 group, and markedly decreased in the ERCC1-knockdown cells compared with the si-NC group (P<0.05; Fig. 4B). These results suggested that ERCC1 combined with olaparib may enhance the sensitivity of NCI-H1299 and SK-MES-1 cells to cisplatin by affecting cell viability.

Effects of ERCC1 combined with olaparib on the cell apoptosis of cisplatin-treated cells. Effects of ERCC1 on the apoptosis of cells treated with cisplatin and olaparib were determined using flow cytometry. The flow cytometry dotplots evaluating the apoptosis of the NCI-H1299 and SK-MES-1 cell lines are shown in Figs. 5A and 6A, respectively. In the NCI-H1299 cell line, the cell apoptosis rates in the GV230, GV230-ERCC1+, siRNA-NC, and siRNA-ERCC1-1 groups were 67.88±2.1%, 50.14±1.53%, 6.3±0.29% and 11.7±0.98%, respectively (Fig. 5B). These results demonstrated that after NCI-H1299 cells were treated with 20 µg/ml cisplatin combined with 50 µg/ml olaparib for 24 h, the cell apoptosis rate significantly decreased in ERCC1-overexpressing cells compared with the GV230 group, and markedly increased in ERCC1-knockdown cells compared with the si-NC group (P<0.05; Fig. 5B). In addition, when the SK-MES-1 cells were treated with 50 µg/ml cisplatin in combination with 70 µg/ml olaparib, the trend of cell apoptosis rate in the SK-MES-1 cell line was similar to that in the NCI-H1299 cell line (Fig. 6B). In summary, the treatment of NSCLC cells with cisplatin and olaparib, cell apoptosis was inhibited in ERCC1-overexpressing cells, but enhanced ERCC1-knockdown cells.

Figure 2. Selecting optimal concentrations of cisplatin and olaparib for cellular treatment. Cell viability of (A) NCI-H1299 cells treated with different concentrations of cisplatin, (B) SK-MES-1 cells treated with different concentrations of cisplatin, (C) NCI-H1299 cells treated with 20 µg/ml cisplatin combined with different concentrations of PARP inhibitor olaparib. The control group was comprised of untreated cells; 0 µg/ml group consisted of cells treated with 20 µg/ml cisplatin combined with 0 µg/ml olaparib. (D) SK-MES-1 cells treated with 50 µg/ml cisplatin combined with different concentrations of PARP inhibitor Olaparib. The control group was comprised of untreated cells; 0 µg/ml group consisted of cells treated with 20 µg/ml cisplatin combined with 0 µg/ml olaparib. *P<0.05 vs. control group; **P<0.05 vs. 0 µg/ml olaparib group. OD, optical density; PARP, poly(ADP-ribose) polymerase.
Discussion

NSCLC is one of the most common malignant tumors and is harmful to human health and life (19). Platinum-based chemotherapy is usually used to assist in the treatment of NSCLC (20). However, clinical treatment is not always satisfactory due to the progression of drug resistance (21). Hence, there is an urgent need to develop new ways to combat drug resistance and improve the sensitivity of these drugs. The expression of ERCC1 regulates DNA damage induced by platinum drugs and is a candidate biomarker for predicting the sensitivity of platinum drugs (22,23). Additionally, olaparib can enhance the sensitivity of platinum drugs by inhibiting PARP-related pathways (24).
In the present study, 50 µg/ml olaparib combined with 20 µg/ml cisplatin significantly inhibited the viability of NCI-H1299 cells, while 70 µg/ml olaparib combined with 50 µg/ml cisplatin further inhibited the viability of SK-MES-1 cells. The findings of the present study indicated that olaparib can enhance the sensitivity of cisplatin in NSCLC. Ledermann and Pujade-Lauraine (25) demonstrated that olaparib increased the sensitivity of platinum-based drugs and prolonged the progression-free survival time of relapsed ovarian cancer, which is consistent with the results of the present study. The present study further demonstrated that olaparib combined with cisplatin can inhibit the viability of NSCLC cell lines.

In the present study, to further explore the synergistic effects of ERCC1 expression combined with olaparib on the sensitivity of cisplatin, NSCLC cell lines with ERCC1 overexpression and knockdown were successfully constructed. In the NCI-H1299 or SK-MES-1 cells with ERCC1 knockdown, olaparib combined with cisplatin inhibited cell viability and promoted cell apoptosis. In the present study, the trends of cell viability and apoptosis in the cells with ERCC1 overexpression were opposite to those in cells with ERCC1 knockdown. The low expression of some genes related to DNA damage repair, including ERCC1, cyclin dependent kinase 1 (CDK1), serine/threonine protein kinase CHK1 (CHK1), CHK2 DNA damage checkpoint kinase (CHK2), BRCA1 DNA repair associated (BRCA1), sperm hammerhead 2 (SH2), ATM serine/threonine kinase (ATM), RAD51 recombinase (RAD51), MRE11 homolog, double strand break repair nuclease (MRE11), phosphatase and tensin homolog (PTEN), X-ray repair cross complementing 1 (XRCC1), damage specific DNA binding protein 1 (DDB1), and XPA binding protein 2 (XAB2), can improve the sensitivity of PARP inhibitor therapy (26-28). A study by Xie et al (29) demonstrated that patients with NSCLC with low expression of both ERCC1 and PARP1 had the best prognosis in platinum-based chemotherapy compared to patients with NSCLC. Based on the findings of the aforementioned and present studies, it can be hypothesized that ERCC1 expression may have an effect on the sensitivity of olaparib to cisplatin and there may be a synergistic effect between PARP inhibitors and ERCC1 knockdown in NSCLC. Additionally, cisplatin-induced DNA damage triggers G2/M cell cycle arrest by activating checkpoint signaling (30). Hence, it can be hypothesized that olaparib combined with ERCC1 knockdown may mediate the proliferation and apoptosis of tumor cells by regulating DNA damage repair. However, this needs to be investigated in future studies.

Figure 5. Effects of ERCC1 on the apoptosis of NCI-H1299 cells treated with cisplatin and olaparib. (A) Representative flow cytometry images. (B) Cell apoptosis rate in different groups of treated cells. *P<0.05 vs. GV230 group; †P<0.05 vs. siRNA-ERCC1 group. ERCC1, ERCC excision repair 1 endonuclease non-catalytic subunit; si, small interfering; NC, negative control; PI, propidium iodide.

Figure 6. Effects of ERCC1 on the apoptosis of SK-MES-1 cells treated with cisplatin and Olaparib. (A) Representative flow cytometry images. (B) Cell apoptosis rate in different groups of treated cells. *P<0.05 vs. GV230 group. †P<0.05 vs. siRNA-ERCC1 group. ERCC1, ERCC excision repair 1 endonuclease non-catalytic subunit; si, small interfering; NC, negative control; PI, propidium iodide.
In addition, another study indicated that the predictive effect of ERCC1 expression on cisplatin treatment may be associated with the histological types of lung cancer (31). In the present study, olaparib combined with ERCC1 overexpression or ERCC1 knockdown enhanced the sensitivity of cisplatin in adenocarcinoma cells (NCI-H1299) and squamous carcinoma cells (SK-MES-1), thereby regulating cell viability and apoptosis. Pierceall et al (31) indicated that ERCCI expression in patients with squamous cell carcinoma displayed significant predictive value, while in patients with adenocarcinoma it was not significant. This was somewhat different from the findings of the present study, possibly due to the joint action of ERCCI and other genes related to DNA damage repair. BRCA1 expression is also considered a predictive biomarker for the sensitivity of platinum drugs and is associated with the expression of PARP in NSCLC (32,33). Further studies need to be performed to explore the combined effects of different genes related to DNA damage repair on the sensitivity/resistance of platinum drugs.

However, there are some limitations to our study. The relationship between Olaparib combined with ERCCI and DNA damage repair in NSCLC requires further investigation, and all underlying mechanisms need to be further explored. Additionally, the combination effects of Olaparib and ERCCI knockdown should be further verified in vivo; moreover, further studies associated with preclinical or clinical models are also required.

In conclusion, in the present study, olaparib combined with ERCCI knockdown enhanced the sensitivity of cisplatin, which inhibited cell viability and promoted cell apoptosis in NSCLC cell lines. The findings of the present study provide evidence for drugs that block ERCCI function during the treatment of NSCLC and may assist in the future development of new therapeutic strategies with olaparib combined with ERCCI for the treatment of NSCLC.

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Availability of data and material

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

Authors' contributions

HH conceived the study, KX performed the experiments, KX, XN and SL collected and analyzed the data. KX and HH drafted the manuscript and HH and GZ revised it for important intellectual content. HH supervised the study. HH and KX confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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