Original Paper

Downregulation of RIF1 Enhances Sensitivity to Platinum-Based Chemotherapy in Epithelial Ovarian Cancer (EOC) by Regulating Nucleotide Excision Repair (NER) Pathway

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Key Words

Ovarian Cancer • RIF1 (Rap1 interacting factor 1) • Cisplatin • NER (nucleotide excision repair) • Drug Resistance

Abstract

Background/Aims: Rap1 interacting factor 1 (RIF1) was deemed to be involved in replication timing regulation and DNA damage response. However, little is known about the role of RIF1 in malignancies. Thus, this study aimed to investigate whether the expression of RIF1 is relevant to the response of epithelial ovarian cancer (EOC) patients to cisplatin chemotherapy and its underlying mechanism. **Methods:** Immunohistochemistry was used for detecting the expression of RIF1 in 72 human ovarian cancer tissues followed by association analysis of RIF1 expression with patients' responses to platinum-based chemotherapy. The survival analysis of ovarian patients based on platinum chemotherapy was analyzed using online databases. RNA interference of RIF1 was carried out in OVCAR3 and A2780 cell lines, to determine the effect of lacking RIF1 expression on cellular responses to cisplatin by using MTS assay. The nucleotide excision repair (NER) capacity of these cells was assessed by using host-cell reactivation and UV sensitivity assay. Western Blot analysis was carried out to determine the effect of RIF1 on the proteins of NER and apoptosis signaling pathway by using RIF1 knockdown cells. BALB/c nude mice model was used for detection of response to cisplatin *in vivo*. **Results:** RIF1 expression was significantly associated with the response of ovarian patients to platinum-based chemotherapy ($P < 0.01$). In cohorts from online databases, high

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expression of RIF1 was associated with higher mortality of EOC patients based on platinum chemotherapy ($P < 0.01$). RIF1 knockdown increased sensitivity to cisplatin in EOC *in vitro* and *in vivo*. Deletion of RIF1 impaired the NER activity by inhibiting the NER proteins in ovarian cancer cells. Besides, knockdown of RIF1 enhanced cisplatin-induced apoptosis. **Conclusions:** RIF1 plays an important role in regulating the expression of NER proteins, which in turn contributes to cellular response to cisplatin and EOC patients' response to platinum-based chemotherapy. RIF1 knockdown also promotes cisplatin-induced apoptosis. RIF1 may serve as a novel biomarker for predicting platinum-based chemosensitivity and the prognosis of EOC patients.

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Introduction

Epithelial ovarian cancer (EOC) remains to be the most prevalent gynecologic malignancies as well as the fifth leading cause of death by cancer among women in the United States [1]. Most EOC patients are diagnosed at advanced phase of disease, and the overall five-year survival rate is relatively low [2]. The standard first-line treatment for EOC is primarily cytoreductive surgery (CRS) followed by platinum-based chemotherapy [3]. However, the efficacy of chemotherapy is limited, for approximately 25% of patients relapse within six months and the overall five-year survival rate is about 30% [4]. Therefore, it's imperative to find useful clinical markers to predict chemosensitivity of cisplatin in EOC and to decrease the failure of chemotherapy.

Platinum analogues induce DNA damage by binding to DNA in target cells, promoting apoptosis and leading to the anti-neoplastic activity [5-9]. One of the main mechanisms of platinum resistance is DNA repair [10]. Furthermore, nucleotide excision repair (NER) mechanism is the most primary way to repair DNA damages induced by platinum analogues among the several known DNA repair pathways [11-13]. Excision repair cross-complementation group 1 (ERCC1), replication protein A (RPA) and xeroderma pigmentosum complementation group A (XPA) protein all play important roles in NER pathway [11, 14-16]. Besides, increased NER proteins expression level is related to platinum-based chemoresistance in many cancers, including EOC [17-23] and suppressed NER proteins improves ovarian cancer patients' response to cisplatin-based chemotherapy [24]. Nevertheless, the underlying mechanism of increased NER proteins level in platinum-based chemoresistance cancers has not been disclosed yet.

Rap1 interacting factor 1 (RIF1) was firstly identified in *Saccharomyces cerevisiae* in 1992, which is crucial for the maintenance of telomere length [25]. In mammalian cells, RIF1 mainly participated in replication timing regulation and DNA damage response [26-29]. RIF1 was reported to participate in DNA double-strand break (DSB) repair by acting as the downstream of ATM/53BP1 in human cells [30, 31]. However, whether RIF1 is involved in NER pathway remains unexplored.

Interestingly, it's indicated that the expression level of RIF1 was obviously higher in human breast tumors than those in benign lesions. Besides, silencing of RIF1 sensitizes MCF7 cells to camptothecin (CPT) or staurosporine treatment [32]. Yet, little is known about the role of RIF1 in cancer chemosensitivity and the character of RIF1 in EOC patients.

In this study, we investigated the role of RIF1 in platinum-based chemotherapy in EOC treatments and explored the underlying mechanisms. We found that RIF1 expression correlated with the chemosensitivity of EOC patients to platinum-based chemotherapy. Knockdown of RIF1 expression increased the cellular sensitivity to cisplatin *in vitro* and *in vivo*. Further investigation revealed that RIF1 knockdown enhanced the activation of cisplatin-induced apoptosis signaling. Moreover, RIF1 mediated cellular NER activity by regulating the NER proteins expression, which in turn affected the cellular chemosensitivity to cisplatin.

Materials and Methods

Materials

Cisplatin was purchased from Sigma. Antibodies against RIF1 (sc-515573), β -actin (sc-58673) and GAPDH (sc-47724) were obtained from Santa Cruz Biotechnology. Antibodies against RPA32 (#2208), RPA70 (#2198) XPA (#14607), Cleaved-Caspase3 (#9964S), Caspase-3 (#9962S), Cleaved-PARP (#5625S), PARP (#9532S), Bcl2 (#15071S) and Bax (#2772S) were purchased from Cell Signaling Technology. Antibody against ERCC1 (D263621) was obtained from BBI life science. Cell culture media and reagents were obtained from Invitrogen. All other reagents were of molecular biology grade and were purchased from Sigma or Fisher.

Patients and samples

We obtained patient follow-up data from the Kaplan-Meier plotter database (<http://kmplot.com/analysis/>) for prognosis analysis [33]. We screened out the ovarian cancer patients with platinum-based treatment. The RIF1 (Affy ID: 214700_x_at) was selected as probe set.

The clinical cohort comprised of 72 ovarian cancer patients were enrolled at Xiangya Hospital of Central South University (Changsha, Hunan, China) and Hunan Cancer Hospital (Changsha, Hunan, China) from 2010 to 2015. All patients were given written informed consent and this study was authorized by the Ethics Committee of Xiangya School of Medicine, Central South University (Registration number: CTXY-140002-10). In this study, a total of 72 human ovarian cancer tissue samples were collected after surgery. After fixation in 10% formalin, the collected tissues were embedded in paraffin for histological diagnosis and IHC analysis. The qualified patients for the study had to meet the following criteria: (a) histologically confirmed ovarian cancer; (b) receiving no radiotherapy and biological therapy before chemotherapy; (c) having been treated with platinum-based chemotherapy as first-line treatment over 3 cycles; (d) with primary ovarian tumors. Platinum resistant was defined as patients who had substantiated disease progression on primary treatment or within 6 months from the end of primary treatment, and platinum sensitive was classified by no evidence of disease progression within 6 months since the end of primary treatment [34]. Exclusion criteria include: (a) active infection; (b) pregnancy or lactation; (c) symptomatic brain or leptomeningeal metastases; and (d) previous or other concomitant malignancies. All of the patients were staged on the basis of the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system [35]. All other demographic and clinical information were acquired from the 2 hospitals mentioned above.

Immunohistochemistry

All tissue specimens were collected via biopsy of paraffin-embedded samples for immunohistochemistry (IHC) analysis in the Pathology Department of Xiangya Hospital or Hunan Provincial Tumor Hospital. Tissue sections (4 μ m thick) were cut from paraffin embedded blocks. The tumor sections on slides were baked at 60°C for 30 minutes followed by incubation in xylene for 3 x 10 minutes and rehydration through graded ethanol to distilled water. Antigen retrieval was done by heating samples in 1 mmol/L EDTA for 20 minutes. Nonspecific staining was blocked by 10% goat serum in PBS buffer for 20 minutes at room temperature. The endogenous peroxidase activity was quenched by incubation in 3% H₂O₂ for 10 minutes. And then the slides were incubated with rabbit polyclonal monospecific RIF1 antibody or PBS control at 4°C overnight followed by incubation with biotinylated goat anti-rabbit antibody and peroxidase-conjugated streptavidin. The 3, 3'-diaminobenzidine tetrahydrochloride substrate kit (Zhongshan Goldenbridge) was used to visualize staining according to the manufacturer's instructions and the hematoxylin and eosin were used to counterstain all samples before viewing with a Leica DMI 4000B inverted microscope.

All ovarian cancer tissue sections were reviewed by two experienced pathologists and staining of RIF1 was independently scored by two pathologists blinded to the clinical data using the semiquantitative immunoreactive score (IRS) system. The score of the RIF1 staining intensity were performed as previously described [36]. The percentage of RIF1-positive cells was scored as follows: < 25% = 1, $\geq$ 25% to 50% = 2, $\geq$ 50% to 75% = 3, and $\geq$ 75% to 100% = 4. The staining intensity was scored as follows: negative = 0, weak = 1, moderate = 2, and strong = 3. A final IRS score was then calculated by multiplying these two scores. The cut-off score was set to 4.0 according to receiver operating characteristic curves (ROC) analysis. If the final score was < 4, the tumor was considered to have low RIF1 expression; whereas the score $\geq$ 4 indicated high RIF1 expression.

Cell culture and generation of stable cell lines

OVCAR3 and A2780 cell line was purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). These cells were cultured in RPMI-1640 media, supplemented with 10% fetal bovine serum (FBS) at 37°C under an atmosphere of 95% air and 5% CO₂.

Two siRNA sequences targeting human RIF1 cDNA were synthesized by GenePharma: RIF1 KD1: 5'-GCCUUUGAGUCCAUCCAUTT-3', KD2: 5'-GGUCAUACCUUUAGUGGUUTT-3'. The targeting sequence of scrambled control of siRNA was 5'-UUCUCCGAACGUGUCACGUTT-3'. Lentiviral vectors pGLV3/H1/GFP were acquired from GenePharma. The sequence of 5'-TTCTCCGAACGTGTCACGT-3' was used to generate lentivirus expressing shRNA targeting human RIF1, viral infection was performed according to the direction for use provided by GenePharma. RIF1 stable knockdown OVCAR3 cell lines were screened out over 15 days with 2 µg/ml puromycin. The western blot analysis and real-time reverse transcriptase PCR were performed after 30 generations of cell culture to verify the stable knockdown of RIF1 in OVCAR3 cell lines.

Survival assay

Cell viability was detected by MTS approach according to the protocol for Cell Titer 96 Aqueous-One-Solution Cell Proliferation Assay kit (MTS). RIF1 knockdown cells were seeded in 96-well plates at a density of 5×10^3 cells in 100 µL medium and incubated with cisplatin (Sigma) for 24 hours. The medium was then replaced by 100 µL MTS and RPMI-1640 media with a ratio of 1:9. Cell viability was then obtained by measuring the absorbance at a wavelength of 490 nm. All experiments were performed at least three times. The IC₅₀ was calculated from the dose-response curves using GraphPad Prism 5.0 program (GraphPad Software, Inc.).

Real-time reverse transcriptase PCR

Total RNA was isolated from cultured cells by TRIzol reagent (Invitrogen). Reverse transcription was performed using PrimeScript RT reagent Kit With gDNA Eraser (TaKaRa), and the quantitative RT-PCR was performed using SYBR Premix DimerEraser (Perfect Real Time) kit (TaKaRa Bio Inc) on the Roche LightCycler480 (Roche). The primers used are listed in Table 1. Data were analyzed using the $-2^{\Delta\Delta Ct}$ method and the expression of β -actin was used as normalization control.

Western blotting

Western blotting was carried out as described previously [37]. In brief, for Western blotting, proteins were extracted using RIPA buffer mixed with protease inhibitors (1:100), phenylmethylsulfonylfluoride (PMSF, 1:100) and dithiothreitol (DTT, 1:100) at 4°C for 30 minutes. The lysate was centrifuged at 13000 rpm at 4°C for 15 min. The supernatants were collected and protein concentrations were measured using the BCA method. For Western blot analysis, protein samples were separated by 8% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Bio-Rad). The membranes were then blocked with 5% nonfat milk and incubated with primary antibodies overnight followed by washing and reaction with horseradish peroxidase-conjugated secondary antibody for 1 hour at 37°C. The reaction was detected by using ECL reagents (GE Healthcare) and the signals were captured by X-ray films. The GAPDH or β -actin were used as normalization control.

Host-cell reactivation assay

The firefly luciferase assay-based host cell reactivation (HCR) assay was implemented as previously described [38]. In brief, the pCMVluc plasmid (178 ng/µL) in a 96-well plate was UV irradiated (254 nm) on ice by a Stratallinker UV Crosslinker (Stratagene). UV-induced damages were validated by using PCR [39] with a forward primer of 5'-CGGTATCTTATCATGTCTG-3' and a reverse primer of 5'-TGCTTCTGACACAACAGT-3'. The UV-damaged or unirradiated control plasmids (0.4 µg) were transfected to 5×10^4 cells in a 24-well plate by using Lipofectamine 2000 (Invitrogen). A pRL-TK (Promega) plasmid encoding renilla luciferase was taken as a control for transfection

Table 1. Sequences of primers for quantitative real-time PCR

Sequences of primers for quantitative real-time PCR		
Gene name	Forward primer sequence (5' → 3')	Reverse primer sequence (5' → 3')
β -actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
RIF1	TGGCAGATGACATTGATAGA	TAGATTGTGTAGTAGGAGAAGTT
XPA	CCAGGACCTGTTATGGAATTGA	GCTTCTTGACTACCCCAAACCTTC

efficiency. After transfection for 24 hours, cells were collected and both firefly and renilla luciferase activities were detected by using a Dual-Luciferase Reporter Assay System (Promega) on a luminometer (Berthold). The XPA-suppressed OVCAR3 cells was used as NER-deficient control. The siRNA sequences targeting human XPA cDNA was synthesized by GenePharma: XPA KD: 5'-UCAUCAACCAGGACCUGUUTT-3'.

UV sensitivity assay

The UV sensitivity assay was carried out as previous study with amendment [40]. On the one hand, the OVCAR3 cells were irradiated with UV (254 nm, 0, 5 or 10 J/m²) or not in 96-well plates and incubated in fresh media. After 24 h of recovery, the cell viability was detected by MTS approach as stated above. On the other hand, the OVCAR3 cells were UV irradiated (10 J/m²) or not in 96-well plates, incubated in fresh media, and then the cell viability was detected at various repair time points (0, 24 and 48 h) by MTS approach. All experiments were performed at least three times. The XPA-suppressed OVCAR3 cells was used as NER-deficient control.

Animal studies

All animal care and experimental procedures were carried out according to the National Institutes of Health's Guide for the Use and Care of Laboratory Animals, and were approved by the Institutional Review Board of Central South University (Changsha, China). Female BALB/c nude mice (4 weeks of age, 18–22 g) were obtained from Shanghai SIPPR - B&K Laboratory Animal Corp. Ltd., and fed under specific pathogen-free conditions at Department of Laboratory Animals, Central South University. In order to initiate tumors, 2×10⁶ scrambled control and RIF1 stable knockdown OVCAR3 cells were inoculated subcutaneously into each flanks of nude mice. The cisplatin was administered at a dose of 5 mg/kg every 4 days by intraperitoneal injection while the tumors were measurable. The tumors were examined every 4 days, maximum (L) and minimum (W) length of the tumor were measured with calipers, and the tumor volumes were calculated as 0.5LW². On day 24, the animals were euthanized and the tumors were excised and weighed.

Statistical analysis

All data were described as mean ± standard deviation (SD). For comparison of differences between groups, Pearson's χ^2 test or Fisher's exact test were used for qualitative variables, Student's t test or analysis of variance (ANOVA) were used for continuous variables. Survival curves were graphed using the Kaplan-Meier method, and the groups were compared using log-rank test. Statistical analysis was conducted using SPSS 18.0 (SPSS Inc.). P≤0.05 was considered statistically significant.

Results

Upregulation of RIF1 correlates with poor prognosis in EOC

To investigate the relationship between RIF1 expression and EOC prognosis, we screened out the data of ovarian cancer patients who received platinum-base treatment from the Kaplan-Meier plotter database (<http://kmplot.com/analysis/>). Kaplan-Meier survival curves and log-rank test survival analysis demonstrated that overexpression of RIF1 mRNA level was associated with worse overall survival (OS) of EOC patients (RIF1 low vs high expression patients: hazard ratio [HR] of survival = 1.24, 95% confidence interval [CI] = 1.08 to 1.43, P < 0.01) (Fig. 1A). Besides, overexpression of RIF1 mRNA level was also associated with worse progress free survival (PFS) of EOC patients (RIF1 low vs high expression

Table 2. Clinical features and RIF1 expression of ovarian cancer patients

Clinical features and RIF1 expression of ovarian cancer patients.					
Clinical features	n	RIF1 level		χ^2	P
		Low (%)	High (%)		
Age					
≥50	32	15(46.9)	17(53.1)	0.47	0.493
<50	40	22(55.0)	18(45.0)		
Histologic type					
Serous	37	15(40.5)	22(59.5)	3.586	0.058
No serous	35	22(62.9)	13(37.1)		
Histodifferentiation					
Low	31	14(45.2)	17(54.8)	0.845	0.358
Middle and High	41	23(56.1)	18(43.9)		
FIGO Stage					
I/II	28	21(75.0)	7(25.0)	10.225	0.001*
III/IV	44	16(36.4)	28(63.6)		
Chemosensitivity					
Sensitive	34	24(70.6)	10(29.4)	9.506	0.002*
Resistant	38	13(34.2)	25(65.8)		

patients: hazard ratio [HR] of survival = 1.53, 95% confidence interval [CI] = 1.33 to 1.76, $P < 0.001$) (Fig. 1B). These results indicated that high expression of RIF1 was associated with higher mortality of EOC patients with platinum-based chemotherapy.

Correlation of RIF1 expression with chemosensitivity

The RIF1 protein expression level in tissues of EOC patients was firstly evaluated by IHC (Fig. 1C). Then, we analyzed the association of RIF1 expression with chemosensitivity and the results were summarized in Table 2. RIF1 staining did not appear to have any statistically significant correlation with age, histologic type and histodifferentiation which were listed in the table. However, RIF1 staining had significant correlation with chemosensitivity of platinum-base treatment and FIGO Stage ($P = 0.002$ and $P = 0.001$, respectively). In general, chemotherapy resistant patients had higher RIF1 expression. Approximately 65.8% chemoresistant EOC patients of platinum-based treatment had high RIF1 level, these numbers went down to 34.2% for chemosensitive EOC patients of platinum-based treatment. Thus, the level of RIF1 expression correlated with chemosensitivity of EOC patients and the increased RIF1 expression level may increase chemoresistance.

Fig. 1. Clinical significance of RIF1 in human ovarian cancer patients. (A, B) Kaplan-Meier analysis of overall survival and progress free survival by low or high RIF1 expression in ovarian cancer patients who received platinum-based chemotherapy treatment were performed by using Cox proportional hazard models and follow-up data for 20 years. The data were obtained from Kaplan-Meier plotter database and all plots were analyzed by combing 15 data sets (GSE14764: $n = 80$, GSE15622: $n = 35$, GSE18520: $n = 63$, GSE19829: $n = 28$, GSE23544: $n = 28$, GSE26193: $n = 107$, GSE26712: $n = 195$, GSE27651: $n = 49$, GSE30161: $n = 58$, GSE3149: $n = 116$, GSE51373: $n = 28$, GSE63855: $n = 101$, GSE65986: $n = 55$, GSE9891: $n = 55$, and TCGA: $n = 565$). (C) Representative IHC staining of RIF1 expression in human ovarian tumor tissues. H&E: representative hematoxylin and eosin staining in ovarian cancer tissues. Sensitive: representative specimens of RIF1 staining of the patient sensitive to platinum. Resistant: representative specimens of RIF1 staining of the patient resistant to platinum. HR = hazard ratio.

Fig. 2. Silence of RIF1 in OVCAR3 and A2780 cells by RIF1-siRNAs and the validation of stable RIF1 knockdown (KD) OVCAR3 cells. (A, B) The silencing effect of the SiRNA1-RIF1 (Si-1) and SiRNA2-RIF1 (Si-2) were detected by RT-PCR and Western blot; (C) Stable RIF1-KD OVCAR3 cells were subjected to RT-PCR and western blot analyses of RIF1 expression. GAPDH was used as a loading control for Western blot. * $P < 0.05$. *** $P < 0.001$.

Fig. 3. Effect of RIF1 knockdown on cellular response to cisplatin in OVCAR3 and A2780 cells. (A, B) OVCAR3 and A2780 cells transiently transfected with siRNA1-RIF1 (Si-1) or scrambled control (Scr) siRNAs were subjected to treatment with cisplatin at various concentrations. Half maximal inhibitory concentration (IC₅₀) was calculated by Graphpad 5.0 software from 3 independent experiments. Relative resistance factor (RRF) of cisplatin was calculated by dividing the IC₅₀ of the control cells and by that of the cells with RIF1 knockdown. **P<0.01.

Silencing of RIF1 expression in EOC cells by siRNAs and established RIF1 stable knockdown cell lines

To investigate the functional role of RIF1 on EOC cells, the siRNA1-RIF1 (Si-1) and siRNA2-RIF1 (Si-2) were transfected into OVCAR3 and A2780 cells. After transfection for 48 hours, the expression of RIF1 was confirmed by RT-PCR and Western blot. As shown in Fig. 2A and 2B, RIF1-siRNAs could effectively decrease RIF1 expression in OVCAR3 and A2780 cells, respectively, as compared with scrambled control siRNA (Scr) groups. We also established RIF1 stable knockdown cell lines using OVCAR3 cells. Fig. 2C shown these stable clones had low expression of RIF1 at both mRNA and protein levels compared with the vehicle control. (Fig. 2 *** p < 0.01, * p < 0.05)

Effect of RIF1 knockdown on cellular response to cisplatin

After knockdown of RIF1 by siRNAs, the OVCAR3 and A2780 cells were subjected to MTS assay in the absence or presence of different concentrations of cisplatin. As shown in Fig. 3, these cells with reduced RIF1 expression were significantly more sensitive to cisplatin than the scrambled control cells with a nearly doubled relative resistance factor. (Fig. 3 ** p < 0.01)

Effect of RIF1 expression on cellular NER activity and UV sensitivity

Base on the observation that RIF1 expression contributed to cellular response to cisplatin, which was a DNA-damaging drug, we speculated that RIF1 might affect DNA repair activities. To test this hypothesis, we used HCR assay to analyze whether knockdown of RIF1 expression affected NER activity responsible for repair of DNA damages induced by cisplatin. For this purpose, a plasmid containing luciferase reporter was UV irradiated in order to generate DNA adducts which were confirmed by PCR analysis (Fig. 4C). The damaged reporter plasmids were then transfected into RIF1-silenced OVCAR3 or scrambled control cells followed by analysis of luciferase activity. We also used XPA-suppressed OVCAR3 cells as NER-deficient control. The luciferase activity decreased with the enhance DNA damages due to higher doses of UV irradiation, as shown in Fig. 4D. Furthermore, the luciferase activity in OVCAR3 cells with RIF1 or XPA knockdown had no significant difference, but they were both significantly lower than the control cells transfected with scrambled siRNA. These findings suggested that knockdown RIF1 expression probably suppressed cellular NER activity. We further

Fig. 4. Deletion of RIF1 expression on NER activities. (A, B) The OVCAR3 cells were subjected to RT-PCR and western blot analyses of XPA expression. (C) PCR analysis of UV damaged plasmids. Lane 1: Maker; Lane 2: unirradiated intact plasmid control; lanes 3–5: plasmids irradiated by 0.3, 0.6, 1.2 and 2.4 J/m². (D) OVCAR3 cells with RIF1 knockdown (Si-RIF1) and XPA knockdown (Si-XPA) or its scrambled control (Scr) were transiently transfected with a firefly luciferase reporter plasmids irradiated with different doses of UV followed by analysis of luciferase activity. A plasmid encoding renilla luciferase was cotransfected for normalization of transfection efficiency. Relative firefly luciferase activity was generated by first normalizing to renilla luciferase activity and then to that of transfection with the control cells transfected with intact firefly luciferase plasmids. (E) OVCAR3 cells were exposed to 0, 5 or 10 J/m² UV (254 nm), recovered for 24 h. Cell viability was calculated as percent of untreated (0 J/m²). (F) OVCAR3 cells were exposed to 10 J/m² UV (254 nm), incubated in fresh media, and then detected the cell viability after each recovery time point (0, 12, 24 and 48 h). Cell viability was normalized to the 0 h.

Fig. 5. Effect of knockdown of RIF1 expression on NER protein level. (A, B) OVCAR3 and A2780 cells transfected with SiRNA1-RIF1 (Si-1) and SiRNA2-RIF1 (Si-2) were subjected to Western blot of RPA70, RPA32, XPA and ERCC1. GAPDH was used as a loading control for Western blot. (C) Stable RIF1-KD OVCAR3 cells were subjected to Western blot analyses as aforesaid. (D-F) Quantification of A-C. * P<0.05, ** P<0.01, *** P<0.001.

analyze UV sensitivity of RIF1-suppressed OVCAR3 cells. As showed in Fig 4E, the cell viability of RIF1 or XPA knockdown OVCAR3 cells and scrambled control cells were reduced with the increased UV irradiation after 24 h of recovery. As shown in Fig 4F, the cell viability of RIF1 knockdown OVCAR3 cells was similar to that of XPA knockdown OVCAR3 cells, but they were both significantly lower than the scrambled control cells. Besides, the cell viability of scrambled control OVCAR3 cells with UV irradiated (10 J/m^2) increased over time (Fig 4F). But the cell viability of RIF1 or XPA knockdown OVCAR3 cells barely rose along the repair time compared with the scrambled control cell. These experiments indicated that silencing of RIF1 in OVCAR3 cells suppressed its repair ability under UV irradiation, which corroborated with the results of HCR assay.

Fig. 6. Silencing of RIF1 enhanced cisplatin-induced apoptosis signaling pathway. (A) RIF1-suppressed or scrambled control OVCAR3 cells were treated with cisplatin (0, 5 and 10 μM) for 48 h, cellular protein was harvested, and Western blot analysis was performed to investigate the levels of cleaved PARP, PARP, Bax, Bcl2 and cleaved caspase3 and caspase3. The β -Actin was detected as a loading control. (B-D) The ratio of Cleaved PARP/PARP, Cleaved Caspase3/Caspase3 and Bax/Bcl2 for each treatment (0, 5 and 10 μM) in RIF1-suppressed or scrambled control OVCAR3 cells. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Effect of RIF1 expression on NER proteins level

Because of the findings that knockdown RIF1 expression suppressed cellular NER activity, we hypothesized that RIF1 might regulate the expression of the proteins which were important for NER pathway. To test this hypothesis, we detected whether RIF1 knockdown affected the expression of NER proteins including RPA70, RPA32, XPA and ERCC1 using RIF1-silenced OVCAR3 and A2780 cells, as well as RIF1 stable knockdown OVCAR3 cells. As shown in Fig. 5A, 5B and 5C, the protein levels of RPA70, RPA32, XPA and ERCC1 were all decreased in these cells with RIF1 knockdown compared with the control cells. Thus, we concluded that RIF1 probably regulated NER proteins including RPA70, RPA32, XPA and ERCC1, which in turn, regulated the NER activity and cellular responses to cisplatin.

Silencing of RIF1 enhanced cisplatin-induced apoptosis signaling pathway

To further assess the effect of RIF1 on the sensitivity of ovarian cells to chemotherapy, we examined the proteins expression of apoptosis signaling pathway. RIF1-silenced and scrambled control OVCAR3 cells were treated with cisplatin (0, 5 and 10 μM) for 48 h, and Western blot analysis was implemented to investigate the expression levels of cleaved PARP, PARP, Bax, Bcl2 and cleaved caspase3 and caspase3 which are important for cell apoptosis. The β -actin was used as control. As showed in Fig.6 A, we observed upregulation of cleaved PARP, cleaved Caspase3 and Bax and downregulation of Bcl2. We further calculated the ratio of Cleaved PARP/PARP, Cleaved Caspase3/Caspase3 and Bax/Bcl2 for each treatment

(0, 5 and 10 μ M), and the ratio both rose with the increase of cisplatin concentration (Fig. 6B, 6C and 6D). Furthermore, the ratio of Cleaved PARP/PARP, Cleaved Caspase3/Caspase3 and Bax/Bcl2 for each treatment of RIF1-silenced OVCAR3 cells was higher than the scrambled control cells, indicating that silencing of RIF1 may enhance cisplatin-induced apoptosis signaling pathway in OVCAR3 cells.

Knockdown of RIF1 increases cisplatin sensitivity *in vivo*

To further investigate whether knockdown of RIF1 affected cisplatin sensitivity *in vivo*, RIF1 stable knockdown or control OVCAR3 cells were subcutaneously injected into the flanks of BALB/c nude mice. Mice were treated intraperitoneally with 5 mg/kg cisplatin every 4 days while the tumors were measurable. The tumor weight derived from the RIF1 stable knockdown cells was lighter than the vehicle cells (Fig. 7A and 7B). Moreover, RIF1 knockdown significantly inhibited the growth of xenografts (Fig. 7C). These results together indicated that knockdown of RIF1 increased cisplatin sensitivity *in vivo*.

Fig. 7. Knockdown of RIF1 increases cellular sensitivity to cisplatin *in vivo*. (A) 2×10^6 scrambled control and stable RIF1-KD OVCAR3 cells were inoculated subcutaneously into the left and right flanks of nude mice. When the tumors were palpable (~ 50 mm³), the mice were treated with cisplatin (5 mg/kg/4days). Representative images of xenografts are shown in A. Scale bar, 1 cm. (B, C) Tumor mass and volume of xenografts were evaluated. Data were presented as means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$.

Discussion

In the current study, for the first time, we found that RIF1 was highly expressed in chemoresistant EOC patients with platinum-based chemotherapy compared with the chemosensitive EOC patients, and overexpression of RIF1 mRNA level was associated with poor prognosis of EOC patients who received platinum-based chemotherapy. We discovered that RIF1 affected the chemosensitivity to cisplatin of EOC cells *in vitro* and *in vivo*. We further demonstrated that RIF1 regulated the protein expression of apoptosis signaling and NER pathway and then mediated the NER activities and cellular response to cisplatin.

Platinum analogues remain first-line therapy of various cancers including lung, bladder, cervical, testicular and ovarian cancers [41, 42]. However, resistance is the major barrier for successful chemotherapy. Thus, it's extremely essential to predict drug response before the initiation of chemotherapy, which may contribute to choosing the most appropriate treatment strategies for patients. In our study, we demonstrated that RIF1 expression was correlated with chemosensitivity and prognosis of EOC patients based on platinum therapy and RIF1 regulated the response to cisplatin *in vitro* and *in vivo*, which indicated that RIF1 might serve as a newly biomarker predicting patient drug response and prognosis. We speculate that assessing RIF1 level may be helpful for the individualized treatment for EOC patients. Because cisplatin is also a major anticancer drug for many other cancers, it is surmisable

that RIF1 may affect cisplatin chemosensitivity and serve as a prognostic marker in other cancer patients.

RIF1 has been reported to play a significant role in DNA double-strand break (DSB) repair [43, 44]. RIF1 acts downstream of 53BP1, suppressing the 5' end resection of broken DNA ends, which in turn, promoting the non-homologous end joining (NHEJ) repair and preventing homology directed repair [28, 31, 45-47]. And RIF1 can stabilize DNA ends and contribute to the repair of breaks through simple relegation [48, 49]. However, little is known about whether RIF1 participates in the DNA single-strand break (SSB). In our research, we found that RIF1 could regulate NER pathway, which is the major mechanism to repair SSB. Deletion of RIF1 could decrease the expression of NER proteins such as ERCC1, XPA, RPA70 and RPA32 and then inhibited the NER activity, leading to the defect in DNA repair and sensitivity to cisplatin. NER mechanism has been illustrated as the most primary way to repair DNA damages induced by platinum analogues among several known DNA repair pathways [11-13]. It has been found that the overexpression of ERCC1 and XPA are directly related to clinical resistance to platinum compounds therapy in human ovarian cancer [17] and the deletion of XPA or ERCC1 could sensitize the prostate cancer cells to cisplatin [50]. XPA level was also related to platinum resistance in non-small cell lung cancer [51]. The increased expression level of RPA70 and RPA32 reduced chemosensitivity of lung cancer cells to cisplatin, while decreased expression level of these proteins enhanced chemosensitivity [12]. Our results are consistent with the previous reported studies. However, the explicit mechanisms of how RIF1 interacts with the NER proteins need further exploration. It's the first time to discover that RIF1 also contributes to NER pathway, which orients a new direction for further investigation of the function of RIF1.

The NER process repairs the cisplatin DNA damage, eliminates the cytotoxic effect of cisplatin and decreases cisplatin-induced apoptosis signaling activation. Moreover, our previous study [52] found that silencing RIF1 increased cisplatin-induced apoptosis in human cervical cancer cells. Hence, it is possible that RIF1 knockdown potentiated ovarian cancer cells to platinum-based cancer treatment by disrupting the NER activity followed by increasing the apoptosis event. So we explored that whether RIF1 affects cisplatin-induced activation of apoptosis signaling in ovarian cancer. Consistently, we observed upregulation of cleaved PARP, cleaved Caspase3 and Bax and downregulation of Bcl2 in RIF1-suppressed OVCAR3 cells, indicating that knockdown of RIF1 promotes cisplatin-induced apoptosis signaling pathway. Our results are consistent with the results in the previous research performed by Wang et al [32]. Moreover, Accumulating evidence [30] demonstrates that RIF1 plays a critical role in DNA repair and the maintenance of genomic integrity which means that RIF1-silenced cells are more fragile to external stimuli. However, RIF1 has been reported to participate in NHEJ repair which is error-prone thus leading to mutation accumulation. In the initiation of cancer, although RIF1 could repair DNA damage, mutation may be accumulated gradually. After tumorigenesis, RIF1 may be an effective therapeutic target because suppressing RIF1 could inhibit the repair capacity of cancer cells to chemotherapeutic agents and promote apoptosis of cancer cells induced by chemotherapy.

In summary, we showed that RIF1 expression level is correlated with responses of EOC patients to platinum-based chemotherapy and their survival. We also demonstrated that silencing of RIF1 sensitized EOC cellular response to cisplatin both *in vitro* and *in vivo*. Besides, for the first time, we found RIF1 may regulate the expression of NER proteins, which in turn, influence cellular NER activity and the sensitivity of cisplatin. Furthermore, knockdown of RIF1 promoted the activation of cisplatin-induced apoptosis signaling. Our study indicated that RIF1 may serve as a novel biomarker for predicting platinum-based chemosensitivity and the survival of EOC patients.

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Disclosure Statement

The authors declare no competing financial interests. We confirmed that all patients were given written informed consent and this study was authorized by the Ethics Committee of Xiangya School of Medicine, Central South University. And all the protocols concerning animal experiments were approved by the Institutional Review Board of Central South University (Changsha, China).

References

- 1 Siegel RL, Miller KD, Jemal A: Cancer Statistics, 2017. *CA Cancer J Clin* 2017;67:7-30.
- 2 Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD: Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer. *Cancer Discov* 2015;5:1137-1154.
- 3 Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, Copeland LJ, Walker JL, Burger RA: Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N Engl J Med* 2006;354:34-43.
- 4 Jin L, Huo Y, Zheng Z, Jiang X, Deng H, Chen Y, Lian Q, Ge R, Deng H: Down-regulation of Ras-related protein Rab 5C-dependent endocytosis and glycolysis in cisplatin-resistant ovarian cancer cell lines. *Mol Cell Proteomics* 2014;13:3138-3151.
- 5 Wang QE, Milum K, Han C, Huang YW, Wani G, Thomale J, Wani AA: Differential contributory roles of nucleotide excision and homologous recombination repair for enhancing cisplatin sensitivity in human ovarian cancer cells. *Mol Cancer* 2011;10:24.
- 6 Rabik CA, Dolan ME: Molecular mechanisms of resistance and toxicity associated with platinating agents. *Cancer Treat Rev* 2007;33:9-23.
- 7 Zhu S, Pabla N, Tang C, He L, Dong Z: DNA damage response in cisplatin-induced nephrotoxicity. *Arch Toxicol* 2015;89:2197-2205.
- 8 Yu X, Li W, Xia Z, Xie L, Ma X, Liang Q, Liu L, Wang J, Zhou X, Yang Y, Liu H: Targeting MCL-1 sensitizes human esophageal squamous cell carcinoma cells to cisplatin-induced apoptosis. *BMC Cancer* 2017;17:449.
- 9 Wang G, Wang X, Xu X: Triptolide potentiates lung cancer cells to cisplatin-induced apoptosis by selectively inhibiting the NER activity. *Biomark Res* 2015;3:17.
- 10 Stewart DJ: Mechanisms of resistance to cisplatin and carboplatin. *Crit Rev Oncol Hematol* 2007;63:12-31.
- 11 Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S: Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* 2004;73:39-85.
- 12 Yin JY, Shen J, Dong ZZ, Huang Q, Zhong MZ, Feng DY, Zhou HH, Zhang JT, Liu ZQ: Effect of eIF3a on response of lung cancer patients to platinum-based chemotherapy by regulating DNA repair. *Clin Cancer Res* 2011;17:4600-4609.
- 13 Martin LP, Hamilton TC, Schilder RJ: Platinum resistance: the role of DNA repair pathways. *Clin Cancer Res* 2008;14:1291-1295.
- 14 Overmeer RM, Moser J, Volker M, Kool H, Tomkinson AE, van Zeeland AA, Mullenders LH, Foerster M: Replication protein A safeguards genome integrity by controlling NER incision events. *J Cell Biol* 2011;192:401-415.
- 15 Bessho T, Sancar A, Thompson LH, Thelen MP: Reconstitution of human excision nuclease with recombinant XPF-ERCC1 complex. *J Biol Chem* 1997;272:3833-3837.
- 16 Fisher LA, Bessho M, Wakasugi M, Matsunaga T, Bessho T: Role of interaction of XPF with RPA in nucleotide excision repair. *J Mol Biol* 2011;413:337-346.
- 17 Dabholkar M, Vionnet J, Bostick-Bruton F, Yu JJ, Reed E: Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J Clin Invest* 1994;94:703-708.
- 18 Steffensen KD, Waldstrom M, Jakobsen A: The relationship of platinum resistance and ERCC1 protein expression in epithelial ovarian cancer. *Int J Gynecol Cancer* 2009;19:820-825.
- 19 Du P, Wang Y, Chen L, Gan Y, Wu Q: High ERCC1 expression is associated with platinum-resistance, but not survival in patients with epithelial ovarian cancer. *Oncol Lett* 2016;12:857-862.

- 20 Park JS, Jeon EK, Chun SH, Won HS, Lee A, Hur SY, Lee KH, Bae SN, Yoon SC, Hong SH: ERCC1 (excision repair cross-complementation group 1) expression as a predictor for response of neoadjuvant chemotherapy for FIGO stage 2B uterine cervix cancer. *Gynecol Oncol* 2011;120:275-279.
- 21 Lord RV, Brabender J, Gandara D, Alberola V, Camps C, Domine M, Cardenal F, Sanchez JM, Gumerlock PH, Taron M, Sanchez JJ, Danenberg KD, Danenberg PV, Rosell R: Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. *Clin Cancer Res* 2002;8:2286-2291.
- 22 Lai KK, Chan KT, Choi MY, Wang HK, Fung EY, Lam HY, Tan W, Tung LN, Tong DK, Sun RW, Lee NP, Law S: 14-3-3sigma confers cisplatin resistance in esophageal squamous cell carcinoma cells via regulating DNA repair molecules. *Tumour Biol* 2016;37:2127-2136.
- 23 Liu Y, Bernauer AM, Yingling CM, Belinsky SA: HIF1alpha regulated expression of XPA contributes to cisplatin resistance in lung cancer. *Carcinogenesis* 2012;33:1187-1192.
- 24 Zhang Y, Yu JJ, Tian Y, Li ZZ, Zhang CY, Zhang SF, Cao LQ, Zhang Y, Qian CY, Zhang W, Zhou HH, Yin JY, Liu ZQ: eIF3a improve cisplatin sensitivity in ovarian cancer by regulating XPC and p27Kip1 translation. *Oncotarget* 2015;6:25441-25451.
- 25 Hardy CF, Sussel L, Shore D: A RAP1-interacting protein involved in transcriptional silencing and telomere length regulation. *Genes Dev* 1992;6:801-814.
- 26 Di Virgilio M, Callen E, Yamane A, Zhang W, Jankovic M, Gitlin AD, Feldhahn N, Resch W, Oliveira TY, Chait BT, Nussenzweig A, Casellas R, Robbiani DF, Nussenzweig MC: Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science* 2013;339:711-715.
- 27 Kumar S, Yoo HY, Kumagai A, Shevchenko A, Shevchenko A, Dunphy WG: Role for Rif1 in the checkpoint response to damaged DNA in *Xenopus* egg extracts. *Cell Cycle* 2012;11:1183-1194.
- 28 Silverman J, Takai H, Buonomo SB, Eisenhaber F, de Lange T: Human Rif1, ortholog of a yeast telomeric protein, is regulated by ATM and 53BP1 and functions in the S-phase checkpoint. *Genes Dev* 2004;18:2108-2119.
- 29 Kanoh Y, Matsumoto S, Fukatsu R, Kakusho N, Kono N, Renard-Guillet C, Masuda K, Iida K, Nagasawa K, Shirahige K, Masai H: Rif1 binds to G quadruplexes and suppresses replication over long distances. *Nat Struct Mol Biol* 2015;22:889-897.
- 30 Kumar R, Cheok CF: RIF1: a novel regulatory factor for DNA replication and DNA damage response signaling. *DNA Repair (Amst)* 2014;15:54-59.
- 31 Zimmermann M, Lottersberger F, Buonomo SB, Sfeir A, de Lange T: 53BP1 regulates DSB repair using Rif1 to control 5' end resection. *Science* 2013;339:700-704.
- 32 Wang H, Zhao A, Chen L, Zhong X, Liao J, Gao M, Cai M, Lee DH, Li J, Chowdhury D, Yang YG, Pfeifer GP, Yen Y, Xu X: Human RIF1 encodes an anti-apoptotic factor required for DNA repair. *Carcinogenesis* 2009;30:1314-1319.
- 33 Gyorffy B, Surowiak P, Budczies J, Lanczky A: Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. *PLoS One* 2013;8:e82241.
- 34 Patch AM, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, Nones K, Cowin P, Alsop K, Bailey PJ, Kassahn KS, Newell F, Quinn MC, Kazakoff S, Quek K, Wilhelm-Benartzi C, Curry E, Leong HS, Hamilton A, Mileskin L, Au-Yeung G, Kennedy C, Hung J, Chiew YE, Harnett P, Friedlander M, Quinn M, Pyman J, Cordner S, O'Brien P, Leditschke J, Young G, Strachan K, Waring P, Azar W, Mitchell C, Traficante N, Hendley J, Thorne H, Shackleton M, Miller DK, Arnau GM, Tothill RW, Holloway TP, Semple T, Harliwong I, Nourse C, Nourbakhsh E, Manning S, Idrisoglu S, Bruxner TJ, Christ AN, Poudel B, Holmes O, Anderson M, Leonard C, Lonie A, Hall N, Wood S, Taylor DF, Xu Q, Fink JL, Waddell N, Drapkin R, Stronach E, Gabra H, Brown R, Jewell A, Nagaraj SH, Markham E, Wilson PJ, Ellul J, McNally O, Doyle MA, Vedururu R, Stewart C, Lengyel E, Pearson JV, Waddell N, DeFazio A, Grimmond SM, Bowtell DD: Whole-genome characterization of chemoresistant ovarian cancer. *Nature* 2015;521:489-494.
- 35 Odicino F, Pecorelli S, Zigliani L, Creasman WT: History of the FIGO cancer staging system. *Int J Gynaecol Obstet* 2008;101:205-210.
- 36 Zeng ZL, Luo HY, Yang J, Wu WJ, Chen DL, Huang P, Xu RH: Overexpression of the circadian clock gene *Bmal1* increases sensitivity to oxaliplatin in colorectal cancer. *Clin Cancer Res* 2014;20:1042-1052.
- 37 Samaeekia R, Adorno-Cruz V, Bockhorn J, Chang YF, Huang S, Prat A, Ha N, Kibria G, Huo D, Zheng H, Dalton R, Wang Y, Moskalenko GY, Liu H: miR-206 Inhibits Stemness and Metastasis of Breast Cancer by Targeting MKL1/IL11 Pathway. *Clin Cancer Res* 2017;23:1091-1103.

- 38 Qiao Y, Spitz MR, Guo Z, Hadeyati M, Grossman L, Kraemer KH, Wei Q: Rapid assessment of repair of ultraviolet DNA damage with a modified host-cell reactivation assay using a luciferase reporter gene and correlation with polymorphisms of DNA repair genes in normal human lymphocytes. *Mutat Res* 2002;509:165-174.
- 39 Ahn B, Kang D, Kim H, Wei Q: Repair of mitomycin C cross-linked DNA in mammalian cells measured by a host cell reactivation assay. *Mol Cells* 2004;18:249-255.
- 40 Parikh D, Fouquerel E, Murphy CT, Wang H, Opreko PL: Telomeres are partly shielded from ultraviolet-induced damage and proficient for nucleotide excision repair of photoproducts. *Nat Commun* 2015;6:8214.
- 41 Kelland L: The resurgence of platinum-based cancer chemotherapy. *Nat Rev Cancer* 2007;7:573-584.
- 42 Basourakos SP, Li L, Aparicio AM, Corn PG, Kim J, Thompson TC: Combination Platinum-based and DNA Damage Response-targeting Cancer Therapy: Evolution and Future Directions. *Curr Med Chem* 2017;24:1586-1606.
- 43 Isobe SY, Nagao K, Nozaki N, Kimura H, Obuse C: Inhibition of RIF1 by SCAI Allows BRCA1-Mediated Repair. *Cell Rep* 2017;20:297-307.
- 44 Mattarocci S, Reinert JK, Bunker RD, Fontana GA, Shi T, Klein D, Cavadini S, Faty M, Shyian M, Hafner L, Shore D, Thoma NH, Rass U: Rif1 maintains telomeres and mediates DNA repair by encasing DNA ends. *Nat Struct Mol Biol* 2017;24:588-595.
- 45 45 Wilson MD, Benlekbir S, Fradet-Turcotte A, Sherker A, Julien JP, McEwan A, Noordermeer SM, Sicheri F, Rubinstein JL, Durocher D: The structural basis of modified nucleosome recognition by 53BP1. *Nature* 2016;536:100-103.
- 46 Fradet-Turcotte A, Canny MD, Escibano-Diaz C, Orthwein A, Leung CC, Huang H, Landry MC, Kitevski-LeBlanc J, Noordermeer SM, Sicheri F, Durocher D: 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. *Nature* 2013;499:50-54.
- 47 Buonomo SB, Wu Y, Ferguson D, de Lange T: Mammalian Rif1 contributes to replication stress survival and homology-directed repair. *J Cell Biol* 2009;187:385-398.
- 48 Chapman JR, Barral P, Vannier JB, Borel V, Steger M, Tomas-Loba A, Sartori AA, Adams IR, Batista FD, Boulton SJ: RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol Cell* 2013;49:858-871.
- 49 Escibano-Diaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, Tkac J, Cook MA, Rosebrock AP, Munro M, Canny MD, Xu D, Durocher D: A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol Cell* 2013;49:872-883.
- 50 Cummings M, Higginbottom K, McGurk CJ, Wong OG, Koberle B, Oliver RT, Masters JR: XPA versus ERCC1 as chemosensitising agents to cisplatin and mitomycin C in prostate cancer cells: role of ERCC1 in homologous recombination repair. *Biochem Pharmacol* 2006;72:166-175.
- 51 Weaver DA, Crawford EL, Warner KA, Elkhairi F, Khuder SA, Willey JC: ABCC5, ERCC2, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. *Mol Cancer* 2005;4:18.
- 52 Ying Mei, Chen Peng, Yong-Bin Liu, Jing Wang and Hong-Hao Zhou: Silencing RIF1 decreases cell growth, migration and increases cisplatin sensitivity of human cervical cancer cells. *Oncotarget* 2017;8:107044-107051.