# TCRP1 contributes to cisplatin resistance by preventing Pol β degradation in lung cancer cells

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**Abstract** Cisplatin (DDP) is the first-line chemotherapy drug widely used for the treatment of lung cancer patients, whereas the majority of cancer patients will eventually show resistance to DDP. The mechanisms responsible for DDP resistance are not fully understood. Tongue cancer resistance-associated protein 1 (TCRP1) gene was recently cloned and reported to specially mediate DDP resistance in human oral squamous cell carcinoma (OSCC) cells. However, the mechanisms of TCRP1-mediated DDP resistance are far from clear, and whether TCRP1 participates in DDP resistance in lung cancer cells remains unknown. Here, we show that TCRP1 contributes to DDP resistance in lung cancer cells. Knockdown of TCRP1 sensitizes the cells to DDP and increases the DDP-induced DNA damage. We have identified that Pol β is associated with DDP resistance, and Pol β knockdown delays the repair of DDP-induced DNA damage in A549/DDP cells. We find TCRP1 interacts with Pol β in lung cancer cells. Moreover, TCRP1 knockdown decreases the level of Pol β and increases the level of its ubiquitination. These results suggest that TCRP1 contributes to DDP resistance through the prevention of Pol β degradation in lung cancer cells. These findings provide new insights into chemoresistance

and may contribute to prevention and reversal of DDP resistance in treatment of lung cancer in the future.

## Introduction

Lung cancer is the most common and lethal malignant cancer in men and women throughout the world, and about 1.8 million new cases are diagnosed each year [1, 2]. Currently, platinum chemotherapeutic agent cisplatin (DDP) is the first-line drug for the treatment of patients with lung cancer [3, 4]. DDP forms a platinum complex inside of a cell, bind to DNA, and crosslink DNA in several different ways, including monoadducts, intrastrand adducts, and interstrand cross-links [5, 6]. The DNA adducts interfere with both RNA transcription and DNA replication, and the damage elicits multiple DNA repair mechanisms [7]. When the repair proves impossible, the apoptosis of those cells is triggered instead. The initial platinum responsiveness is effective, but the majority of cancer patients will eventually show resistance to DDP [8, 9]. The reasons responsible for the resistance involve in many complex processes, such as changes in cellular uptake and efflux of the drug, inhibition of apoptosis, increased detoxification, and increased DNA repair [10, 11]. However, the mechanisms responsible for DDP resistance are not fully understood, and exploring new mechanisms responsible for the resistance to DDP-based chemotherapy is still an urgent need.

Tongue cancer resistance-associated protein 1 (TCRP1) was cloned by our lab from the tongue cancer multi-drug resistance cell line Tca8113/Pingyangmycin (Tca8113/PYM) which showed resistance to DDP, PYM, pirarubicin,

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paclitaxel, and adriamycin [12]. Interestingly, we found that TCRP1 mediated a specific resistance to DDP in Tca8113 cells, and TCRP1 played important roles in DDP resistance through activation of the PI3K/Akt/NF-κB signaling pathway and decreased cells apoptosis [12–14]. A pilot study on clinical specimens of OSCC has shown that overexpression of TCRP1 is associated with resistance to DDP and poor clinical outcome in patients [15]. However, the mechanisms of TCRP1-mediated DDP resistance are far from clear, and whether TCRP1 participates in DDP resistance in lung cancer cells remains unknown.

In this study, the human lung cancer cell line A549 and DDP-resistant cell line A549/DDP were used as models to check the role of TCRP1 in lung cancer cells. We reported that TCRP1 contributed to DDP resistance in lung cancer cells. Knockdown of TCRP1 in A549/DDP cells sensitized the cells to DDP and increased the DDP-induced DNA damage. Pol β was downregulated following knockdown of TCRP1 in A549/DDP cells. We showed that Pol β was involved in DDP resistance, and Pol β knockdown delayed the repair of DDP-induced DNA damage in A549/DDP cells. In addition, we identified that TCRP1 interacted with Pol β and prevented the degradation of Pol β. Taken these together, our results suggested that TCRP1 might be a new target for prevention and reversal of DDP resistance in the treatment of lung cancer.

## Materials and methods

### Cell culture

The human lung cancer cell lines A549 and A549/DDP were obtained from China Center for Type Culture Collection (Wuhan, China). The cells were cultured in RPMI 1640 medium (Life Technologies) containing 10 % fetal calf serum (Life Technologies). To maintain drug resistance, A549/DDP cells were routinely cultured with an additional 2 μg/ml DDP; before the treatments, the cells were further cultured in drug-free RPMI 1640 medium for 1 week.

### Antibodies

Antibodies against TCRP1, Pol β, β-actin, and XRCC3 were purchased from Santa Cruz Biotechnology. Anti-ERCC3, anti-ATM, anti-XRCC1, and anti-DNA-PK antibodies were obtained from Cell Signaling Technology. Anti-γ-H2AX and anti-Ubiquitin antibodies were purchased from Millipore and Life Technologies, respectively. HRP-conjugated secondary antibodies were purchased from Cell Signaling Technology, and Alexa Fluor 488

donkey anti-mouse IgG was purchased from Invitrogen. DDP and MG132 were purchased from Sigma.

### Cytotoxicity assay

Cytotoxicity of DDP in different cells was determined by MTS assay as previously described [13]. The absorbance was recorded at 490 nm using a 96-well plate reader (Biotech). The values of 50 % inhibitory concentration (IC50) to DDP were determined by dose–response curve.

### Western blot

Whole-cell lysates were prepared with RIPA lysis buffer (50 mM Tris–Cl, pH 7.4, 150 mM NaCl, 1 % Triton X-100, 1 % sodium deoxycholate, 0.1 % SDS, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 1 % protease inhibitor cocktail). The proteins were separated by SDS-PAGE gel, and Western blot was performed as described previously [13].

### Real-time RT-PCR

Cellular RNA was extracted using Trizol reagent (Life Technologies), and cDNA was synthesized using first-strand cDNA synthesis kit (Thermo Scientific) according to the manufacturer's protocol. The expressions of β-actin, which was served as an internal control, and TCRP1 were verified by real-time RT-PCR using Applied Biosystems 7500 Real-Time PCR System with the SYBR Green detection method. The following primers were used in this study: TCRP1, 5′-GAACTC GTCTTCCTGTGGCA-3′ (forward) and 5′-GGGGTGG AGCAGTGTTACTC-3′ (reverse); Pol β, 5′-GAGAAGAA CGTGAGCCAAGC-3′ (forward) and 5′-AACTCGTAT-CATCCTGCCGA-3′ (reverse); β-actin, 5′-CTCACCATG-GATGATGATATCGC-3′ (forward) and 5′-AGGAAT CCTTCTGACCCATGC-3′ (reverse).

### Gene silencing and TCRP1 overexpression

Sequences of siRNA corresponding to human TCRP1, Pol β, and their scrambled controls were synthesized by Invitrogen (Table S1). The TCRP1 overexpression vector pcDNA3.1-TCRP1 was constructed as described in previous report [12]. Cells were transfected with siRNA or pcDNA3.1-TCRP1 plasmids using Lipofectamine 2000 (Life Technologies) according to the manufacturer's instructions. The expressions of TCRP1 and Pol β were determined by Western blot.

### Co-immunoprecipitation

Cells were lysed with RIPA lysis buffer, and cell lysates were immunoprecipitated overnight at 4 °C with indicated

antibody or control IgG which was served as negative control. Immune complexes were then captured with protein G magnetic beads (Cell signaling technology). The beads were washed extensively with lysis buffer, resuspended in 2× SDS sample buffer, and heated to boiling. The proteins were subsequently analyzed by SDS-PAGE and Western blot.

## Immunofluorescence microscopy

Cells were seeded on coverslips (VWR International). After indicated treatment, cells were washed with PBS and fixed in 4 % paraformaldehyde for 15 min. Cells were permeabilized using 0.25 % Triton X-100 solution for 5 min and then blocked in 5 % bovine serum albumin for 1 h at room temperature. Coverslips were incubated with anti-γ-H2AX (1:500) overnight at 4 °C. After washed with PBS, the cells were incubated with Alexa Fluor 488 donkey anti-mouse IgG (1:1000) for 1 h. The coverslips were washed with PBS and mounted onto slides using proLong gold antifade reagent with DAPI (Life Technologies). Slides were visualized on a microscopy system (Leica DM6000B).

## Alkaline single cell gel electrophoresis assay (comet assay)

The comet assay was performed as previously described [14]. Briefly, the roughened microscope slides were coated with 0.75 % normal melting agarose. After indicated treatment, the cells were washed with ice-cold PBS and suspended in low melting agarose (LMA) at 37 °C. The cells mixed with the LMA were spread on the slide using a coverslip. The cells were then lysed for 1 h at 4 °C in freshly prepared lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–Cl, pH 10; 1 % Triton X-100, and 10 % DMSO added just before use). The slides were placed in an electrophoresis unit containing freshly made electrophoretic buffer (300 mM NaOH and 1 mM EDTA, pH 13) for 30 min to unwind DNA. Subsequently, electrophoresis was conducted for 30 min at 300 mA and 25 V in an ice bath. The slides were then washed with Tris buffer (0.4 M Tris–Cl, pH 7.5), stained with ethidium bromide (20 μg/ml), and covered with cover slips. The pictures of 50 cells in each treatment were taken under a fluorescence microscope ((Leica DM6000B) at 200× magnification, and three independent experiments were performed for each treatment. The Olive tail moment [Tail DNA % × (Tail Mean−Head Mean)] was analyzed using Comet Assay Software Project (CASP).

## Ubiquitination assays

Cells were treated with 25 μM MG132 (Sigma) for 3 h and lysed with RIPA lysis buffer. Co-immunoprecipitation experiments were performed as described above. Ubiquitinated Pol β was affinity-purified using anti-ubiquitin or anti-Pol β antibody and captured with protein G magnetic beads. The bound proteins were eluted and subsequently analyzed by SDS-PAGE and Western blot.

## Statistical analysis

Quantitative values were expressed as mean ± standard deviation. The Student's $t$ test was used to compare the corresponding data. $P < 0.05$ was considered statistically significant.

# Results

## TCRP1 contributes to DDP resistance in lung cancer cells

We first carried out DDP sensitivity assays to validate the DDP resistance in A549 and A549/DDP cells. The cells were treated with different concentrations of DDP. The dose–response curves were plotted, and the values of IC50 to DDP were determined. As shown in Fig. 1a, dose-dependent anti-proliferative activities were observed in A549 and A549/DDP cells. The IC50 value of A549/DDP cells to DDP was significantly higher than that of A549 cells. To characterize the role of TCRP1 in DDP resistance in lung cancer cells, we compared the expression of TCRP1 in A549 and A549/DDP cells. The results of real-time RT-PCR and Western blot showed that A549/DDP expressed a significantly higher level of TCRP1 than the parental A549 cells in both mRNA and protein levels (Fig. 1a; Fig. S1a).

Next, we detected the effect of siRNA knockdown of TCRP1 in A549/DDP cells. The TCRP1 siRNA or control was transfected in A549/DDP cells. The siTCRP1-1 exhibited the best knockdown effect, and it was used in all the following experiments (Fig. S2a). The TCRP1-knockdown cells were treated with indicated concentrations of DDP, and the dose–response curves were presented. The IC50 value of A549/DDP-siTCRP1 cells to DDP was 2.5-fold lower than that of siRNA control (Fig. 1b). These data showed that knockdown of TCRP1 significantly increased sensitivity to DDP in A549/DDP cells (Fig. 1b). To further identify this role of TCRP1, we overexpressed TCRP1 protein in A549 cells and detected the IC50 values to DDP. As a result, we showed that overexpression of TCRP1 increased resistance to DDP in DDP-sensitive A549 cells (Fig. 1c). Therefore, all these findings suggest that TCRP1 plays a crucial role in mediating resistance to DDP in lung cancer cells.
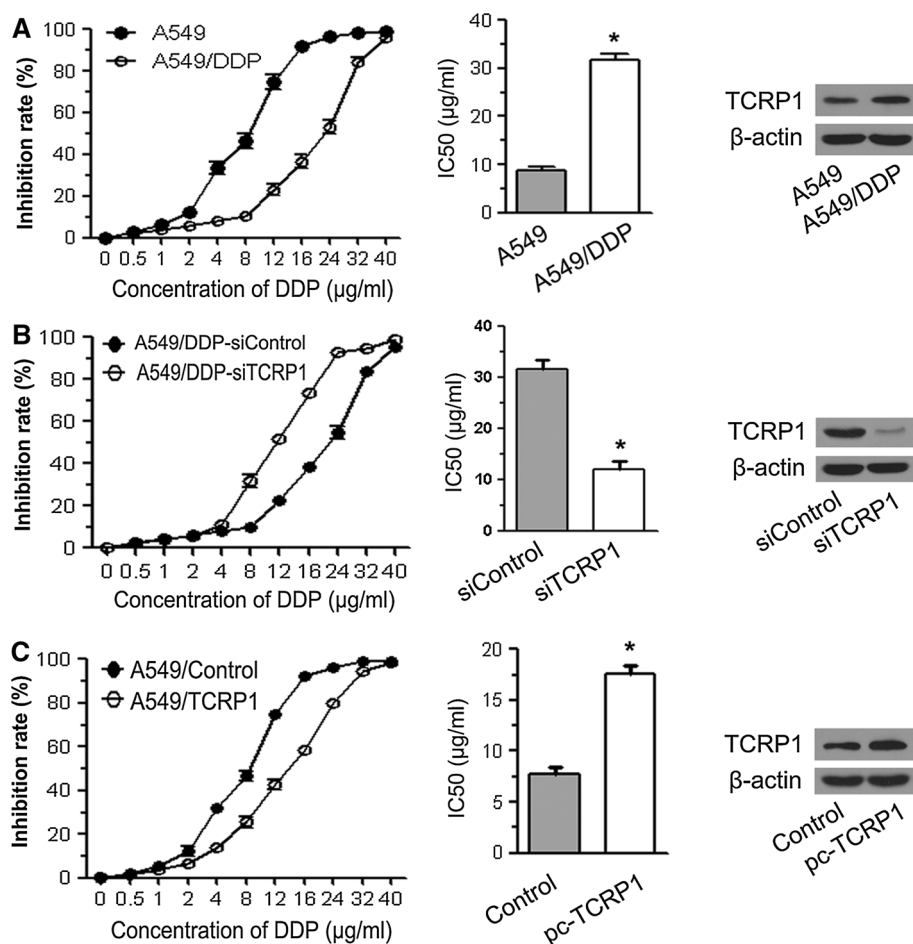
**Fig. 1** TCRP1 is associated with DDP resistance in lung cancer cells. **a** A549/DDP cells expressed a significantly higher level of TCRP1 than A549 cells. A549 and A549/DDP cells were treated with indicated concentrations of DDP and cultured for 72 h. MTS assay was performed, and the dose–response curves were presented (*left*). The values of IC50 to DDP were determined (*middle*). *$P < 0.05$ (*t* test). The protein expression levels of TCRP1 in A549 and A549/DDP cells were detected by Western blot (*right*). **b** Knockdown of TCRP1 increased sensitivity to DDP in A549/DDP cells. A549/DDP cells were transfected with siRNA for TCRP1 or scramble control. At 48 h after siRNA transfection, cells were treated with indicated concentrations of DDP for 72 h. The dose–response curves were plotted (*left*), and values of IC50 were determined by dose–response curve (*middle*). *$P < 0.05$ (*t* test). The effect of TCRP1 knockdown was confirmed by Western blot (*right*) and real-time PCR (Fig. S1b). **c** Overexpression of TCRP1 resulted in increased resistance to DDP in A549 cells. A549 cells were transfected with pcDNA3.1-TCRP1 or control plasmid and treated with different concentrations of DDP. Data in the *left pane* represented the dose–response curves, and *middle pane* showed the IC50 values. *$P < 0.05$ (*t* test). The Western blot result of TCRP1 overexpression was shown in the *right pane*, and real-time PCR result is presented in Fig. S1c

## Knockdown of TCRP1 exacerbates DDP-induced DNA damage in A549/DDP cells

An important mechanism of resistance to DDP is the increase of DNA repair [6]. To explore the functions of TCRP1 in repair of DDP-induced DNA damage, we evaluated the levels of H2AX phosphorylation on serine 139 (γ-H2AX) in TCRP1-knockdown cells. DDP can stimulate the foci formation of γ-H2AX, and γ-H2AX is the sensitive marker for DNA damage and DNA repair [16]. We first carried out immunofluorescence staining to examine γ-H2AX foci formation. As shown in Fig. 2a, upon DDP treatment, more γ-H2AX foci were observed in TCRP1-knockdown A549/DDP cells compared with control cells.

We classified the foci into three groups: foci = 0, foci = 1–20, and foci > 20. The percentage of cells in each group was calculated. We showed that higher percentage of DDP-treated A549/DDP cells with TCRP1 knockdown belonged to the foci > 20 group, as compared with those of control cells (Fig. 2b). Consistently, Western blot results showed that after exposure of DDP, the γ-H2AX level was higher in TCRP1-knockdown A549/DDP cells compared with the control group (Fig. 2c), suggesting that suppression of TCRP1 stimulated DDP-induced DNA damage. We also applied the alkaline single cell gel electrophoresis method, also known as the comet assay, to further identify the role of TCRP1 in repair of DNA damage induced by DDP. Comet assay is a standard
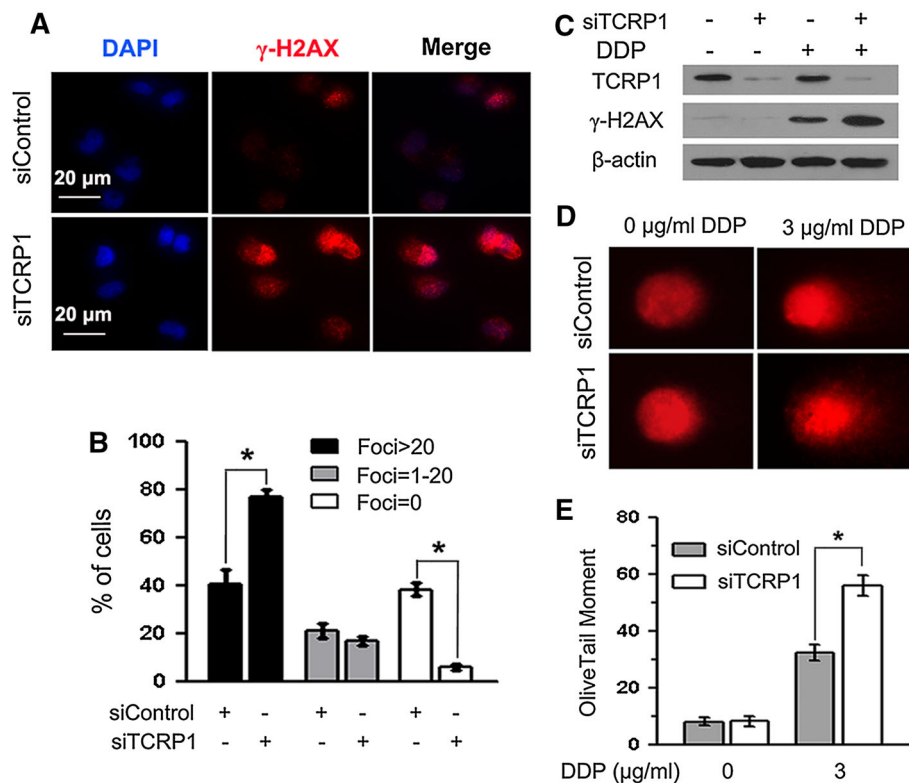
**Fig. 2** Knockdown of TCRP1 stimulates DDP-induced DNA damage in A549/DDP cells. **a** Immunofluorescence staining was performed to examine γ-H2AX foci formation. At 48 h after transfection of siRNA, TCRP1-knockdown or control A549/DDP cells were treated with 3 μg/ml of DDP for 24 h. γ-H2AX foci formation was detected by immunofluorescence staining. **b** From 600 single cells, γ-H2AX foci were quantified and categorized into the indicated groups. *$P < 0.05$ (*t* test). **c** Detection of γ-H2AX level by Western blot. A549/DDP cells transfected with TCRP1 or control siRNA were treated with 3 μg/ml of DDP for 24 h. The levels of γ-H2AX and TCRP1 were determined by Western blot. **d** Representative results of comet assay to identify DNA damage. A549/DDP cells were transfected with TCRP1 or control siRNA and treated with 3 μg/ml of DDP for 24 h. Comet assays were performed, and the pictures were taken individually under a fluorescence microscope at 200× magnification. **e** Olive tail moments were measured by the comet assay using comet assay software project. The *bars* represented the means ± standard deviations (SD) of three independent experiments. Fifty cells were analyzed in each experiment. *$P < 0.05$ (*t* test)

technique to measure DNA damage [17, 18]. Following DDP treatment, more extended migration of fragmented DNA was observed in the comet tails of TCRP1-knockdown A549/DDP cells compared with that of control (Fig. 2d), indicating that knockdown of TCRP1 sensitized A549/DDP cells to DDP-induced DNA damage. Olive tail moment was also used to assess the level of DNA damage [18]. Figure 2e showed that the mean value of olive tail moment in TCRP1-knockdown cells was significantly higher than that of siRNA control cells when the cells were treated with DDP. Collectively, all these results suggested that inhibition of TCRP1 contributed to the increase of DDP-induced DNA damage.

### Pol β knockdown increases DDP sensitivity and delays the repair of DDP-induced DNA damage in A549/DDP cells

As a variety of DNA repair proteins are involved in the DDP resistance, base excision repair proteins including Pol β and XRCC1, nuclear excision repair protein ERCC3, and double-strand break repair proteins including ATM, and PRKDC and XRCC3 were selected to check the protein levels in TCRP1-knockdown A549/DDP cells. We found that Pol β was downregulated following knockdown of TCRP1 in A549/DDP cells, while other tested DNA repair proteins did not show any changes in protein levels (Fig. 3a). Hence, we considered that Pol β might be involved in DDP resistance in A549/DDP cells, and a functional interaction might occur between TCRP1 and Pol β.

To investigate the effect of Pol β in DDP resistance in A549/DDP cells, siRNA knockdown of Pol β was performed. The siPol β-3 exhibited the best knockdown effect, and it was used in the following experiments (Fig. S2b). The dose–response curves to DDP were determined, and the IC50 values over 72 h treatment were measured (Fig. 3b–d). The data showed that the sensitivity to DDP was significantly increased following Pol β knockdown in A549/DDP cells, compared with control siRNA (Fig. 3c). This result implied that Pol β is associated with DDP
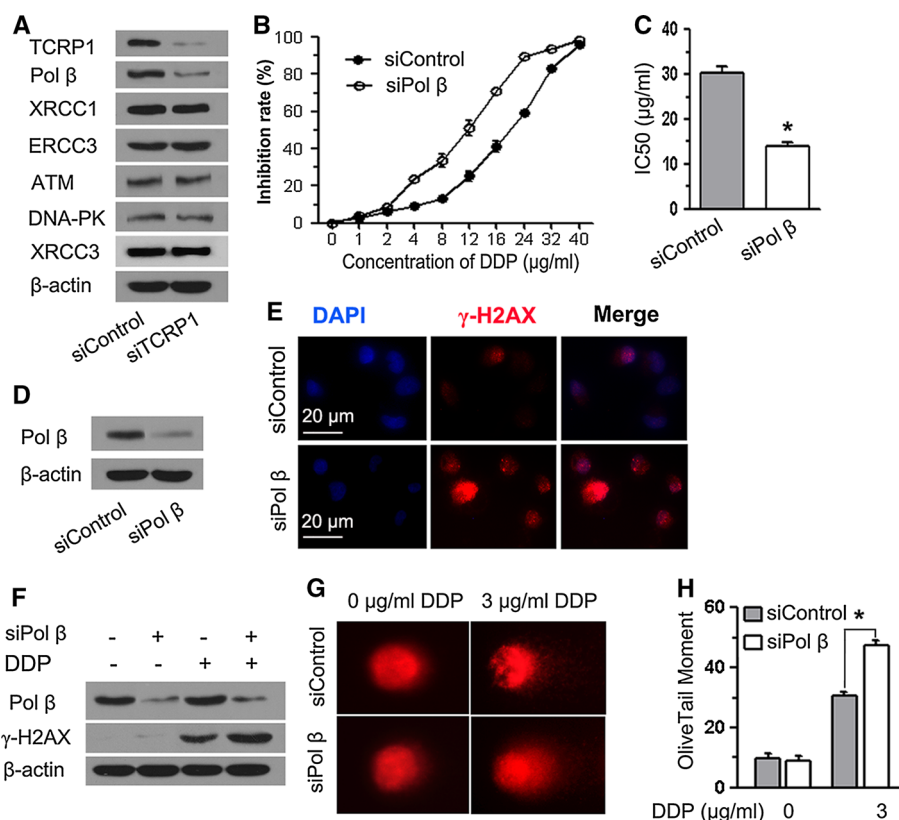
Fig. 3 Pol β contributes to DDP resistance. a Detection of important DNA repair proteins by Western blot. After siRNA knockdown of TCRP1 or control in A549/DDP cells, cells were lysed, and Western blot analyses were performed with the indicated antibodies. b–d Pol β knockdown in A549/DDP cells could sensitize cells to DDP. At 48 h after transfection of siRNA, Pol β-knockdown or control A549/DDP cells were treated with indicated concentrations of DDP for 72 h. The dose–response curves to DDP were represented (b), and IC50 values were determined (c). *$P < 0.05$ (t test). Western blot analysis was performed to confirm the effect of Pol β knockdown (d). e Immuno-fluorescence staining of γ-H2AX foci. Pol β-knockdown or control A549/DDP cells were treated with 3 μg/ml of DDP for 24 h, and γ-H2AX foci formation was detected by immunofluorescence staining.

f Western blot analysis of γ-H2AX induced by DDP. A549/DDP cells were transfected with Pol β or control siRNA for 48 h and treated with 3 μg/ml of DDP for 24 h. The levels of γ-H2AX and Pol β were detected by Western blot. g, h Comet assay in Pol β-knockdown or control A549/DDP cells treated with or without DDP as indicated. A549/DDP cells were transfected with siRNA for Pol β or scramble control. At 48 h after siRNA transfection, cells were treated with 3 μg/ml of DDP for 24 h. Comet assays were performed, and representative pictures were taken individually under a fluorescence microscope at 200× magnification (g). Olive tail moments were measured from 150 cells in three independent comet assays using comet assay software project (h). *$P < 0.05$ (t test)

resistance, and Pol β knockdown in A549/DDP cells could sensitize cells to DDP.

To assess the contribution of Pol β in the repair of DDP-induced DNA damage in lung cancer cells, we used immunofluorescence staining on γ-H2AX foci formation in Pol β-knockdown or control A549/DDP cells after the DDP treatment. We showed that more γ-H2AX foci were identified in Pol β-knockdown A549/DDP cells following DDP treatment, compared with control cells (Fig. 3e). Quantitative data of γ-H2AX foci showed that higher percentage of DDP-treated Pol β-knockdown cells belonged to the foci > 20 group, compared with the control cells (Fig. S3). In addition, Western blot showed that the γ-H2AX level was higher in Pol β-knockdown A549/DDP cells with the

exposure of DDP compared with the control cells (Fig. 3f), indicating that Pol β is involved in the DDP-induced DNA damage repair, and depletion of Pol β augments the damage in lung cancer cells. Next, we carried out comet assay in Pol β-knockdown or control A549/DDP cells. As shown in Fig. 3g, after DDP treatment, more extended tails were observed in Pol β-knockdown cells compared with control cells. The mean value of olive tail moment in Pol β-knockdown cells was significantly higher than that of siRNA control cells with the treatment of DDP in comet assay (Fig. 3h). Taken together, these results indicated that Pol β conferred DDP resistance, and Pol β silencing by siRNA decreased the repair of DDP-induced DNA damage in A549/DDP cells.
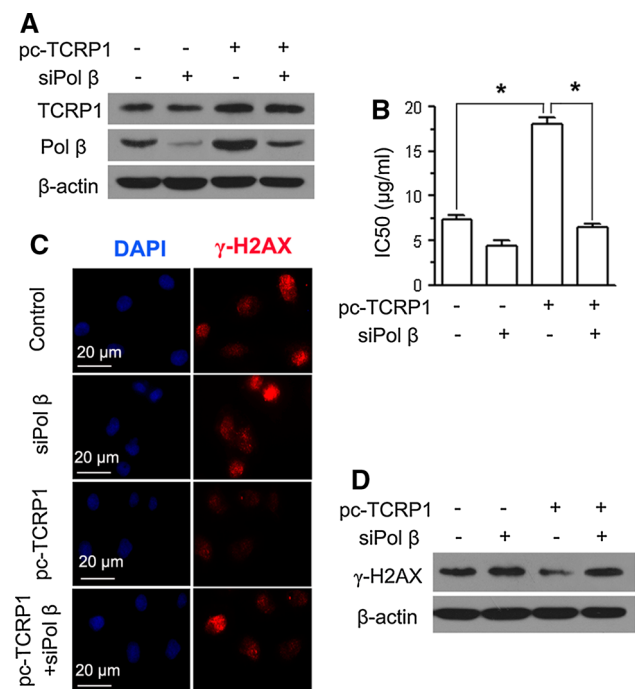
**Fig. 4** Pol β is involved in TCRP1-mediated DDP resistance. **a** A549 cells were co-transfected with expression vector pcDNA3.1-TCRP1 in combination with the Pol β siRNA. Whole-cell lysates of these cells were analyzed using Western blot. **b** Knockdown of Pol β could increase the sensitivity to DDP in TCRP1-overexpression cells. At 48 h after transfection of pcDNA3.1-TCRP1 and Pol β siRNA in A549 cells, the cells were treated with indicated concentrations of DDP, and IC50 values were determined. *$P < 0.05$ (*t* test). **c** Knockdown of Pol β markedly stimulated DDP-induced DNA damage in TCRP1-overexpression cells. A549 cells were transfected with different combinations of pcDNA3.1-TCRP1, Pol β siRNA, or controls. At 48 h after transfection, the cells were treated with 1 μg/ml of DDP for 24 h. The γ-H2AX foci formation was detected by immunofluorescence staining. **d** The cells treated as in (**c**) were harvested and subjected to Western blot for the detection of γ-H2AX foci formation



**Fig. 5** TCRP1 prevents the degradation of Pol β in lung cancer cells. **a** TCRP1 interacted with Pol β. Immunoprecipitation with anti-TCRP1 or anti-Pol β antibody was performed in A549/DDP cells, and the interaction proteins were identified by Western blot. **b** TCRP1 knockdown did not decrease the Pol β mRNA level. At 48 h after transfection of TCRP1 siRNA or control in A549/DDP cells, cells were subjected to RNA extraction, and Pol β mRNA levels were examined by real-time RT-PCR. **c** TCRP1 knockdown promoted the ubiquitination of Pol β. After knockdown of TCRP1, the A549/DDP cell lysates were incubated with anti-Pol β or anti-ubiquitin antibody. The immune complexes were immunoprecipitated by protein G magnetic beads and tested by Western blot with the indicated antibodies. **d** MG132 treatment inhibited Pol β degradation. A549/DDP cells were transfected with siRNA of TCRP1 or control, and 48 h later, the cells were treated with 25 μM of MG132 for 3 h. Western blot was performed with the indicated antibodies

## TCRP1 contributes to DDP resistance via the increased expression of Pol β in lung cancer cells

To test the possibility that TCRP1 mediated DDP resistance and repair of DDP-induced DNA damage via the expression of Pol β, we investigated whether knockdown of Pol β would increase the sensitivity to DDP in TCRP1-overexpression cells. A549 cells were co-transfected with expression vector pcDNA3.1-TCRP1 in combination with the Pol β siRNA. As shown in Fig. 4a, the expression of Pol β was up-regulated by transfection of TCRP1 expression vector, and the result was consistent with the earlier observation that decreased Pol β expression was detected in TCRP1-knockdown cells. After transfection of Pol β siRNA in TCRP1-overexpression cells, the Pol β gene decreased to the level similar to that of endogenous expression (Fig. 4a). These cells were treated with
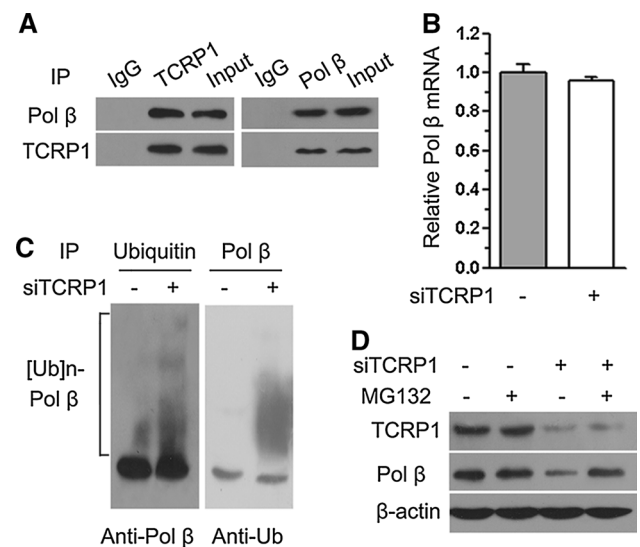
different concentrations of DDP, and values of IC50 were determined by dose–response curve. The TCRP1-overexpression cells exhibited increased resistance to DDP as identified previously, and knockdown of Pol β in TCRP1-overexpression cells leaded to decreased DDP resistance (Fig. 4b). Cells were then evaluated for their DNA damage repair using immunofluorescence staining of γ-H2AX foci. Overexpression of TCRP1 significantly decreased the γ-H2AX foci formation in DDP-treated A549 cells, and knockdown of Pol β markedly stimulated DDP-induced DNA damage (Fig. 4c). These results were further confirmed by Western blot (Fig. 4d). Overall, our results indicated that TCRP1 contributed to DDP resistance via the increased expression of Pol β and consequently enhanced repair of DDP-induced DNA damage in lung cancer cells.

## TCRP1 interacts with Pol β and prevents it degradation in lung cancer cells

To further investigate the functional effects of TCRP1 on Pol β, we focused on the interaction of these two

molecules. Physical interaction of endogenous TCRP1 and Pol β was examined by co-immunoprecipitation assays, and Fig. 5a verifies an interaction between the two proteins in A549/DDP cells. We previously found that TCRP1 knockdown decreased the Pol β in protein level (Fig. 3a). However, this downregulation of Pol β following TCRP1 knockdown was not observed at the mRNA level (Fig. 5b). Therefore, we considered that TCRP1 did not regulate the synthesis of Pol β. As shown in Fig. 5c, increased ubiquitination of Pol β was observed following siRNA knockdown of TCRP1 in A549/DDP cells. In addition, Pol β degradation that was induced by TCRP1 knockdown was inhibited by the treatment of proteasome inhibitor MG132 (Fig. 5d). Together, our results indicated that TCRP1 prevented the degradation of Pol β and consequently contributed to increased DNA repair and DDP resistance.

## Discussion

The purpose of this study was to characterize the mechanism that is involved in the TCRP1-mediated DDP resistance in lung cancer cells. Our results show that TCRP1 plays an important role in mediating DDP resistance in lung cancer cells, and knockdown of TCRP1 stimulates DDP-induced DNA damage. We have identified that Pol β is associated with DDP resistance, and Pol β knockdown delays the repair of DDP-induced DNA damage in A549/DDP cells. We also find that TCRP1 interacts with Pol β and regulates the ubiquitination of Pol β. Therefore, our results strongly indicate that TCRP1 contributes to DDP resistance through increasing the repair of DDP-induced DNA damage by preventing Pol β degradation in lung cancer cells.

Here, we showed that TCRP1 was associated with DDP resistance in A549 and A549/DDP cells. In previous study, the IC50 values to DDP and expression of TCRP1 were analyzed in nine different histopathological subtypes of lung cancer cell lines, and the result showed that the cell lines which had higher expression of TCRP1 in mRNA and protein levels showed increase in resistance to DDP (data not shown). These data suggested that TCRP1 might be a potential predictor of DDP resistance in lung cancer, and its expression might help to select cancer patients who would benefit from DDP chemotherapy. Further research will be needed to validate the results in large number of patients.

Although TCRP1 overexpression is found in many DDP-resistant cells (data not published), the mechanisms of TCRP1 in DDP resistance are still far from clear. Our previous work showed that the expression levels of Akt and NF-κB were reduced in TCRP1-knockdown Tca8113 cells, and PI3K/Akt/NF-κB signaling pathway was involved in the function of TCRP1 [13]. In this study, we show that TCRP1 contributes to DDP resistance in lung cancer cells, and Pol β-mediated DNA repair process is involved in the function of TCRP1. We hypothesize that there might be a crosstalk between PI3K/Akt/NF-κB signaling pathway and Pol β expression level in TCRP1-proficient cancer cells, and the interaction remains to be determined.

The DNA integrity and stability is essential for living cells. Platinum-based agents are one of the most potent anti-cancer drugs, and the main cytotoxic lesions are platinum–DNA intra- and inter-strand crosslinks, resulting in cell death [19]. Several cellular self-defense mechanisms including enhanced repair of DNA damage are activated and lead to platinum resistance in cancer cells [11, 20]. There are five major DNA repair pathways that are important for maintenance of genomic integrity: base excision repair (BER), double-strand break repair, nucleotide excision repair, mismatch repair, and direct repair [7]. The mechanism responsible for resistance to platinum is multifactorial, and some of these DNA repair systems are involved in platinum resistance [5, 7, 21]. In the present study, knockdown of TCRP1 increases the amount of phosphorylation on H2AX and olive tail moment values in comet assays after exposure of cancer cells to DDP, and these results confirm that TCRP1 is involved in the repair of DDP-induced DNA damage. Furthermore, our data show that DNA repair protein Pol β is involved in the TCRP1-mediated DDP resistance in lung cancer cells. Pol β is a primary DNA polymerase involved in gap filling during the repair process and thus plays a central role in the DNA BER [22, 23]. It is reported that the majority of endogenous and mutagen-induced DNA lesions are repaired by the BER pathway [24]. In this study, we report that knockdown of TCRP1 expression contributes to suppression of Pol β, and knockdown of Pol β leads to a decrease in DDP resistance. This result is consistent with previous studies which showed that downregulation of Pol β conferred sensitivity to DDP in human ovarian tumor cells and colorectal cancer cells [25, 26].

In this study, we report that TCPR1 could positively regulate the expression of Pol β. One possible mechanism could be TCRP1 binds to the promoter of Pol β and regulates the expression of Pol β directly. However, knockdown of TCRP1 in A549/DDP cells does not result in any changes to Pol β in mRNA level, and this result indicates that there must be some other mechanisms responsible for the TCRP1-mediated regulation of Pol β. Our data show that TCRP1 knockdown enhances the ubiquitination of Pol β, indicating that TCRP1 prevents the degradation of Pol β. The same as the majority proteins in mammalian cells, Pol β is degraded by the ubiquitin–proteasome pathway [27]. Up to date, some E3 ubiquitin ligases are reported to be involved in the degradation of Pol β [27, 28]. The E3 ubiquitin ligase Mule

(ARF-BP1) is reported to modulate the ubiquitination of Pol β, and knockdown of Mule leads to accumulation of Pol β and increased DNA repair [28]. CHIP, another E3 ubiquitin ligase, can regulate the cellular level of Pol β by ubiquitination and subsequent degradation [27]. In contrast, some deubiquitination enzymes are reported to inhibit the E3 ubiquitin ligase activities and counteract the Pol β degradation [29]. USP47 is identified as an important enzyme involved in deubiquitination of Pol β, and USP47 knockdown is shown to cause an increased level of ubiquitinated Pol β and a deficiency in BER [29]. In this study, we identify that TCRP1 regulates the level of Pol β by preventing its degradation, and our findings provide new insights into BER process and DNA repair. Currently, more details about this observation are being investigated.

In summary, we have shown that TCRP1 contributes to DDP resistance through preventing Pol β degradation in lung cancer cells. These findings provide new insights into chemoresistance and may contribute to prevention and reversal of platinum resistance in cancer treatment in the future.

## References

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