REV3L modulates cisplatin sensitivity of non-small cell lung cancer H1299 cells

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Abstract. Lung cancer remains the leading cause of cancer-related mortality worldwide and non-small cell lung cancer (NSCLC) accounts for approximately 80-85% of all cases of lung cancer. Cisplatin plays a significant role in the management of human lung cancer. Translesion DNA synthesis (TLS) is involved in DNA damage repair. DNA polymerase ζ (Pol ζ) is able to mediate the DNA replication bypass of DNA damage, which is suggested to be involved in chemoresistance. REV3L is the catalytic subunit of Pol ζ. Due to its critical role in translesion DNA synthesis, whether REV3L modulates cisplatin response in NSCLC cells remains unknown. In this study, REV3L overexpression and silencing H1299 cell lines were established. The reports showed that cisplatin induced the expression of REV3L by recruiting Sp1 to its promoter. Similar results were obtained when the ability of the cells to express luciferase from a platinated plasmid was measured. Co-transfection of the reporter with the REV3L overexpression vector or REV3L plus REV7L significantly enhanced the reporter activity. Nuclear condensation and fragmentation of shRNA-REV3L H1299 cells were more pronounced than shRNA-NC H1299 cells after cisplatin exposure, indicating that REV3L overexpression abolished cisplatin-induced DNA damage. Moreover, a forced expression of REV3L conferred the resistance of H1299 cells to cisplatin, whereas the knockdown of REV3L sensitized cisplatin efficacy in H1299 cells. Taken together, we demonstrated that inhibition of REV3L sensitized lung cancer H1299 cells to cisplatin treatment. Thus, REV3L may be a novel target for the chemotherapy of NSCLC.

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Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide and non-small cell lung cancer (NSCLC) accounts for approximately 80-85% of all cases of lung cancer (1). The overall prognosis and survival rate of patients with advanced NSCLC remain unsatisfactory, with a median survival time of 8-11 months and a one-year survival rate of 30% (2,3). Chemotherapy plays a significant role in the management of human lung cancer (4,5). Cisplatin (DDP) is widely used to treat many types of solid tumors and cisplatin-based adjuvant chemotherapy has significantly improved the progression-free survival of cancer patients, which has translated to a 5-10% improvement in the cure rate (6,7). However, few patients experience complete responses to chemotherapy, mainly because of the resistance of tumor cells and/or tolerance of the surrounding normal tissues (8). Thus, novel approaches to enhance the cisplatin efficacy and reverse chemoresistance are required, as well as chemosensitizers to reduce the dose of drugs administered and the length of time resulting in reduced side effects.

Cisplatin induces DNA crosslinks and can induce cell apoptosis, as is the case with many other chemotherapeutic drugs (9). Cancer cells elicits DNA repair mechanisms including nucleotide excision repair (NER), base excision repair (BER), double-strand break repair (DSBR) and mismatch repair (MMR) (10-12). In addition, eukaryotic cells have a DNA post-replication repair system that is composed of translesion DNA synthesis (TLS) and homologous DNA recombination (HR) pathways. The ubiquitous TLS consists

of a series of specialized polymerases, including polymerase (Pol) κ, ζ, η and ι (13). The catalytic REV3L subunit interacts with structural REV7L to form polymerase Pol ζ. Pol ζ cannot add nucleotides across DNA lesions, but it can extend from primers with terminal mismatches, which makes Pol ζ crucial in translesion DNA synthesis (TLS). Pol ζ is able to mediate DNA replication bypassing DNA damage, which may prevent chromosome instability in cells and be considered as a suppressor of spontaneous tumorigenesis. However, Pol ζ lacks 3' to 5' exonuclease activity and can insert a nucleotide into the lesion to complete the bypass of the lesion, which is important for cell survival when confronted with DNA damage (14-16). Loss of REV3L (also known as REV3 in vetebrates) has shown increased sensitivity to exogenous insults. For example, *REV3*-null mouse-derived embryonic fibroblasts are more sensitive to UV- and γ-irradiation with increased chromosomal abnormalities (17). The above mentioned results indicated that *REV3L* is involved in cell tolerance to various types of DNA damage.

The REV3(L) protein was partially conserved from yeast to vertebrates. It has been previously reported that human Pol ζ is expressed in human cancers including lung, stomach and colorectal cancers (18,19). Moreover, REV3L suppressed the focus formation suggesting a tumor-suppressor role. Due to its critical role in translesion DNA synthesis, whether REV3L is involved in chemoresistance remains unknown. In this study, we found that cisplatin induced the expression of REV3L by recruiting Sp1 to its promoter. Knockdown of REV3L sensitized cisplatin efficacy in H1299 cells.

Materials and methods

Reagents and plasmids. Benzo[a]pyrene (B[a]P), nicotine and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cisplatin was purchased from Qilu Pharmaceutical Co., Ltd. (Shandong, China). Geneticin (G418) was obtained from Life Technologies (Gaithersburg, MD, USA).

REV3L overexpression and RNAi vectors. The pcDNA3.1/neo-REV3L plasmid was kindly provided by Dr Yoshiki Murakumo, Nagoya University Graduate School of Medicine, Nagoya, Japan. Four shRNAs targeting REV3L were designed and constructed by GenePharma (Shanghai, China). The information of the four shRNAs is provided in Table I.

Cell culture and transfection. The human H1299 lung cancer cell line was maintained in DMEM supplemented with 10% FBS and antibiotics (Gibco, Grand Island, NY, USA). The cells were grown in a 37°C incubator with 5% CO_2. The cells were transfected by Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA, USA) with plasmids.

To generate stable *REV3L*-overexpressing or -silencing clones, the cells were grown in a 24-well culture plate to 70-80% confluence and then transfected with 1 μg of pcDNA3.1, pcDNA-REV3L, shRNA-NC or shRNA targeting *REV3L* vector using Lipofectamine 2000 (Invitrogen Life Technologies) according to the manufacturer's instructions. The medium was replaced with DMEM containing 600 μg/ml G418

Table I. Targeting sequences of shRNAs.

Name	Targeting sequence	Location (REV3L)
shRNA-NC	5'-GTCAATGGTCGTGTCGTGC-3'	
shRNA-1	5'-CGAAGATTGTGACCTGAATTA-3'	3611-3632
shRNA-2	5'-CTTCTGGTATGTCCTCAAAGA-3'	4447-4468
shRNA-3	5'-AGGAAAGCCAAATGCCTAATA-3'	4672-4693
shRNA-4	5'-CTCTAGTGATATCTCCAATTA-3'	6859-6880

48 h post-transfection. After 3-4 weeks, G418-resistant colonies were selected and screened for *REV3L* expression by reverse transcriptase-PCR (RT-PCR). The REV3L-overexpressing and -silencing clones were cultured in DMEM supplemented with 10% fetal bovine serum in the presence of 300 μg/ml G418 at 37°C in humidified air with 5% CO_2.

Reverse transcriptase-PCR analysis. Total RNA from lung tissues was extracted with TRIzol (Invitrogen Life Technologies) and reverse transcribed to cDNA using an oligo(dT)$_{12}$ primer and Superscript II (Invitrogen Life Technologies). The mRNA levels of target genes and the internal standard glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were measured by RT-PCR or quantitative PCR (qPCR) in triplicate on a Prism 7500 real-time PCR machine (Applied Biosystems, Foster City, CA, USA). The specific primers for the genes are listed in Table II.

Immunostaining. H1299 cells were fixed with 4% paraformaldehyde, washed with PBS, and permeabilized with 1% Triton X-100 in PBS. The cells were blocked with blocking buffer (PBS, 1% Triton X-100, and 5% BSA) and incubated at 4°C with the REV3L antibody (1:1000; Abnova, Taiwan) overnight. FITC-conjugated goat anti-mouse (1:100) was incubated for 30 min at room temperature. Nuclear counterstaining was performed using 4,6-diamidino-2-phenylindole (DAPI). Cells not treated with primary antibody served as the negative control.

Cell viability assay. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cells were plated in 96-well plates. The following day, the cells were transfected with plasmids or shRNAs according to the experimental design. The cells were then incubated with 20 μl MTT (5 mg/ml) for 4 h. After the medium was removed, 100 μl DMSO was added and the optical density (OD) at 490 nm was measured using a Microplate Reader (Bio-Rad, Hercules, CA, USA). The viability index was calculated as experimental OD value/control OD value. Three independent experiments were performed in quadruplicate.

Western blot analysis. The cells were lysed in lysis buffer (Promega, Madison, WI, USA) and centrifuged at 4°C for 10 min. The supernatant was collected and subjected to western blotting. Protein (50 μg) from each lysate was fractionated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). After

Table II. Primer sequences for RT-PCR and ChIP analysis.

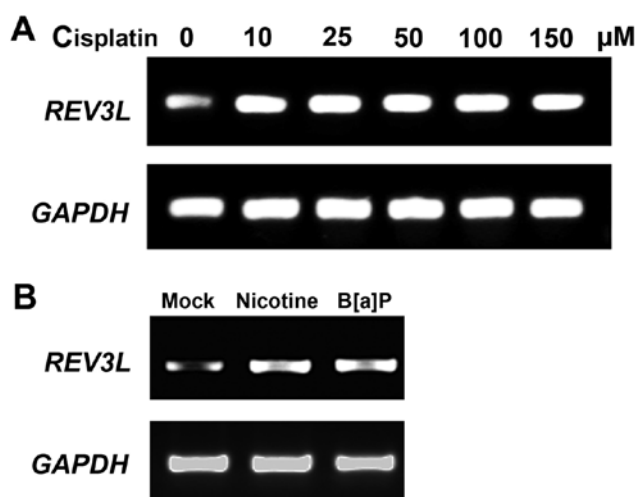|	RT-PCR	
Gene	Forward primer	Reverse primer
GAPDH	5'-GAAGGTGAAGGTCGGAGTC-3'	5'-GAAGATGGTGATGGGATTTC-3'
REV3L	5'-CGCGTCAGTTGGGACTTAAG-3'	5'-ACTATCGCCAACCTCAATGC-3'
	ChIP	
Gene	Forward primer	Reverse primer
Region 1	5'-GAAGGTGAAGGTCGGAGTC-3'	5'-GAAGATGGTGATGGGATTTC-3'
Region 2	5'-CGCGTCAGTTGGGACTTAAG-3'	5'-ACTATCGCCAACCTCAATGC-3'

Figure 1. Cisplatin induces the expression of *REV3L*. (A) H1299 cells were exposed to various concentrations of cisplatin for 24 h. (B) Cells were exposed to B[a]P or nicotine for 24 h. After treatment, RNA was extracted by TRIzol and the expression of *REV3L* and the internal control *GAPDH* was detected by RT-PCR.

blocking with 5% non-fat milk in PBS Tween-20 for 1 h at room temperature, the membranes were blotted with the appropriate Bax, Bcl2 or GAPDH primary antibody (All from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at a 1:1000 dilution. The membranes were then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody at a 1:2000 dilution for 1 h at room temperature. After TBST washes, the blot was incubated in the ECL detection kit (Amersham Bioscience, Freiburg, Germany).

Plasmid reactivation assay. A plasmid reactivation assay was performed as previously reported (20). The pGL3-promoter mammalian expression vector (Promega) containing the *Firefly luciferase* gene expressed high-level Firefly luciferase in mammalian cells. The plasmid DNA was dissolved in buffer containing 10 mM Tris and 1 mM EDTA (pH 7.4) and incubated with different concentrations of cisplatin for 1 h. The platinated DNA was then purified by ethanol precipitation and unbound free cisplatin was removed. This procedure resulted in plasmid DNA that was >90% supercoiled as verified by gel electrophoresis. Similar levels of platination have previously been shown not to affect the efficiency of transfection (21). For the luciferase assay, the pGL3-Promoter reporter vector plus pRL-TK (Promega) was co-transfected with pcDNA3.1-REV3L or shRNA-targeting REV3L. Luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega). Promoter activities were expressed as the ratio of *Firefly* luciferase to *Renilla* luciferase activities.

Quantitative chromatin immunoprecipitation (ChIP). H1299 cells were used for ChIP assays. The EZ ChIP kit (Upstate Biotechnology, Inc, Lake Placid, NY, USA) was used according to the manufacturer's instructions as previously reported (22). Briefly, 5×10^6 cells were cross-linked with 1% formaldehyde and sonicated to ~500 bp fragments. ChIP was conducted with antibodies against PAX2 and IgG. Input control DNA or immunoprecipitated DNA was amplified in a 20-μl reaction volume consisting of 4 μl eluted DNA template and primers specific for REV3L promoter (Table II). The immunoprecipitated fragments and the inputs were amplified by RT-PCR and qPCR. Results for the immunoprecipitated fragments were calculated compared to the Ct values obtained for the input samples in each case and were expressed as a percentage of the input.

Measurement of apoptosis. Cells were transfected with shRNA-NC or shRNA-REV3L for 24 h prior to treatment with cisplatin. Apoptosis was measured using propidium iodide (PI)/Annexin V double staining as per the manufacturer's instructions (Nanjing KeyGen Biotech. Co., Ltd., Nanjing, China). The cells were harvested 24 h after treatment with cisplatin and apoptotic fractions were measured using flow cytometry (Beckman Coulter, Miami, FL, USA). The Annexin V+/PI- cells indicated early apoptosis, while the Annexin V+/PI+ cells indicated late apoptosis. The percentage of the two types of cells was calculated.

Statistical analysis. Data were presented as the mean ± standard error of the mean (SEM) of at least three independent experiments. Standard error bars were included for all the data points. The data were then analyzed using the Student's t-test when only two groups were present or assessed by one-way analysis of variance (ANOVA) when more than two groups were compared. Statistical analysis was performed using
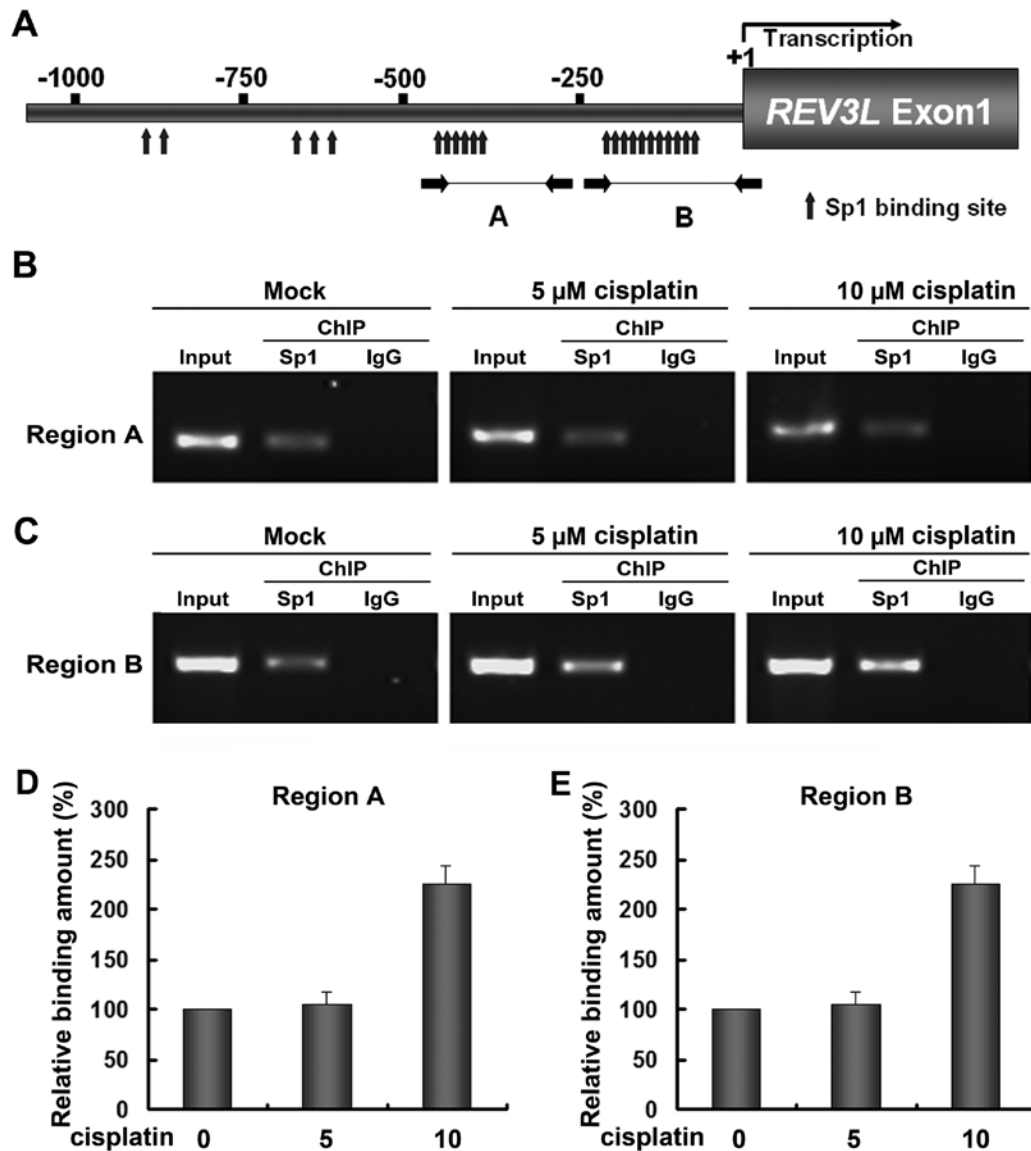
Figure 2. Sp1 is recruited for the cisplatin-induced REV3L expression. H1299 cells were mock-treated or treated with cisplatin for 24 h. (A) The H1299 cells were processed for ChIP using anti-Sp1 or IgG antibodies. (B) Relative binding amount of Sp1 to the *REV3L* promoter. The relative presence of Sp1 at the *REV3L* promoter was calculated relative to the corresponding input sample by real-time PCR.

SPSS software (Release 17.0, SPSS Inc.). Data were considered significant when $P < 0.05$.

Results

Cisplatin induces the expression of REV3L. It has been suggested that cisplatin can induce an increase in REV3L mRNA levels in both normal and cancer cells. Thus, we investigated whether cisplatin also increases REV3L mRNA levels in human H1299 NSLSC cells. As shown in Fig. 1A, 10 μM cisplatin increased the *REV3L* mRNA level while higher concentrations of cisplatin did not further increase REV3L expression. Moreover, DNA damage reagents B[a]P and nicotine upregulated REV3L expression in H1299 cells, indicating that REV3L was increased in response to DNA damage.

SP1 is recruited in the REV3L promoter with cisplatin. Bioinformatic tools predicted that the promoter of REV3L harbors multiple binding sites of transcription factor Sp1 (Fig. 2A). Two dense Sp1 binding regions were observed, the upstream one located between -450 and -360 bp (Region A) and the downstream one located between -200 and -75 bp (Region B, relative to the transcription start site). To investigate whether the interaction between Sp1 and the *REV3L* promoter conferred the cisplatin-induced increase of REV3L, quantitative ChIP was performed. In H1299 cells, the *REV3L* promoter was specifically precipitated with a Sp1 antibody but not with the control IgG in the two Sp1 dense regions (Fig. 2B and C), indicating the presence of Sp1 bound to the REV3L promoter *in vivo*. Results also showed that 5 μM cisplatin did not change the relative binding amount of Sp1 to the *REV3L* promoter, whereas 10 μM cisplatin significantly increased the amount of Sp1 bound to the REV3L promoter in the two regions (Fig. 3B-E). These results also indicated that changes in *REV3L* expression result in a corresponding change in Sp1 binding to the *REV3L* promoter.
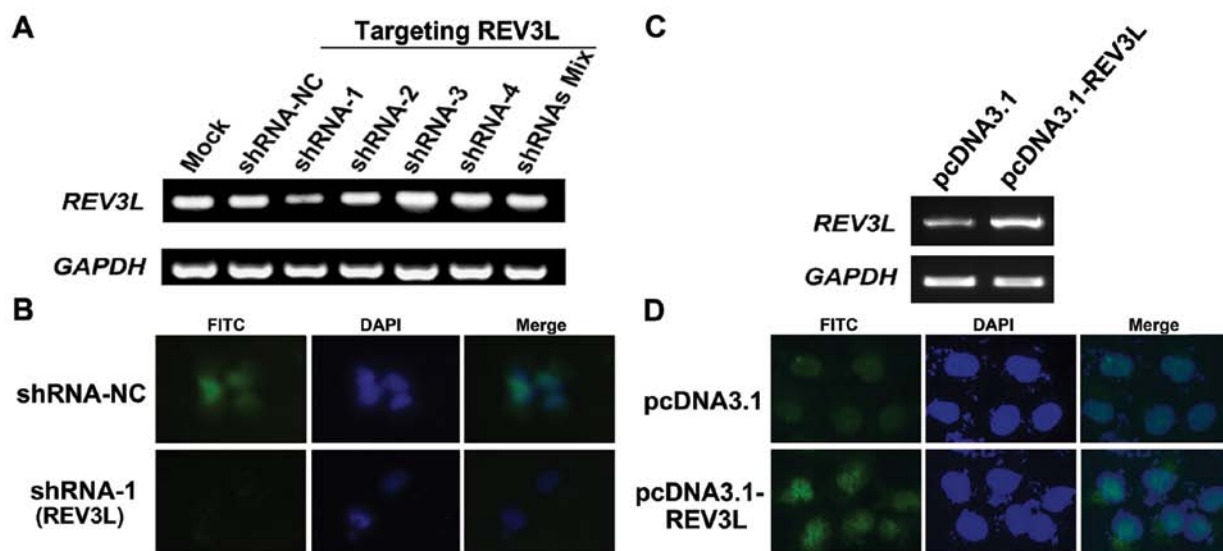
Figure 3. Evaluation of *REV3L* overexpression and silencing in H1299 cells. (A) H1299 cells were stably transfected with shRNA-NC or shRNA-targeting REV3L. *REV3L* mRNA expression was semi-quantified by RT-PCR. *GAPDH* was used as an internal control. (B) Stable H1299 cell lines were immunostained with an anti-REV3L and a FITC-conjugated secondary antibody. (C) H1299 cells were stably transfected with the REV3L overexpression vector. REV3L mRNA expression was semi-quantified by RT-PCR. *GAPDH* was used as an internal control. (D) Stable H1299 cell lines were immunostained with an anti-REV3L and a FITC-conjugated secondary antibody. Nuclei (blue) were stained with DAPI.
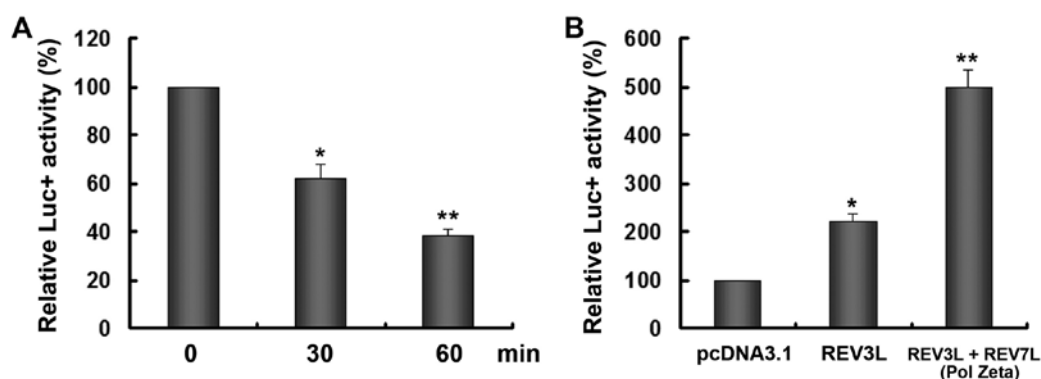

Figure 4. REV3L increases DNA repair following cisplatin treatment. (A) Luciferase assay of cells transfected with the cisplatin-treated pGL3-promoter reporter. $^*P<0.05$ and $^{**}P<0.01$, compared with the mock-treated plasmid. (B) REV3L modulates the expression of cisplatin-treated reporter. The cisplatin-treated luciferase reporter was co-transfected with indicated vectors into H1299 cells. After 24 h of incubation, luciferase activity was measured as described in Materials and methods. $^*P<0.05$; $^{**}P<0.01$, compared with pcDNA3.1-transfected cells.

Establishment of H1299 cell lines with overexpression and knockdown of REV3L. The function of *REV3L* in human lung cancer H1299 cells. We genetically manipulated *REV3L* expression in a cell line derived from H1299 was investigated. pcDNA3.1-REV3L was stably transfected into H1299 cells to generate REV3L-overexpressing cells. We also designed and constructed four shRNA vectors targeting different locations of the *REV3L* mRNA. REV3L cell lines stably transfected with the control, overexpression or shRNA vectors were screened by G418. As shown in Fig. 3A, compared with mock- or shRNA-NC-transfected H1299 cells, transfection of REV3L targeting shRNA-1 showed reduced *REV3L* transcripts (inhibition rate 71.4%, relative to shRNA-NC-transfected cells). Other shRNAs did not show obvious silencing ability of *REV3L*. To confirm this result, immunostaining was performed and the results demonstrated that in shRNA-1-transfected cells, the nuclear staining of REV3L was obviously reduced (Fig. 3B), validating the RT-PCR results. In addition, REV3L expression

was increased in *REV3L*-overexpressing stable cells, compared to the pcDNA3.1-transfected cells (Fig. 3C and D).

REV3L overexpression abolishes cisplatin-induced DNA damage. DNA is thought to be the primary biological target of cisplatin. The platinum atom of cisplatin forms covalent bonds with the N7 position of purine bases to form 1,2- or 1,3-intrastrand crosslinks and a lower percentage of interstrand crosslinks, both of which interfere with DNA transcription (23). To investigate whether REV3L affects cisplatin-treated DNA damage, the pGL3-promoter reporter was treated with cisplatin and then transfected into H1299 cells. The ability of cells in sweeping away cisplatin-induced adducts was assessed by determining the ability of the cell to successfully express the firefly luciferase from the extensively platinated reporter by treatment with cisplatin before transfection. As shown in Fig. 4A, the treatment of cisplatin decreased the reporter activity in a time-dependent manner possibly due
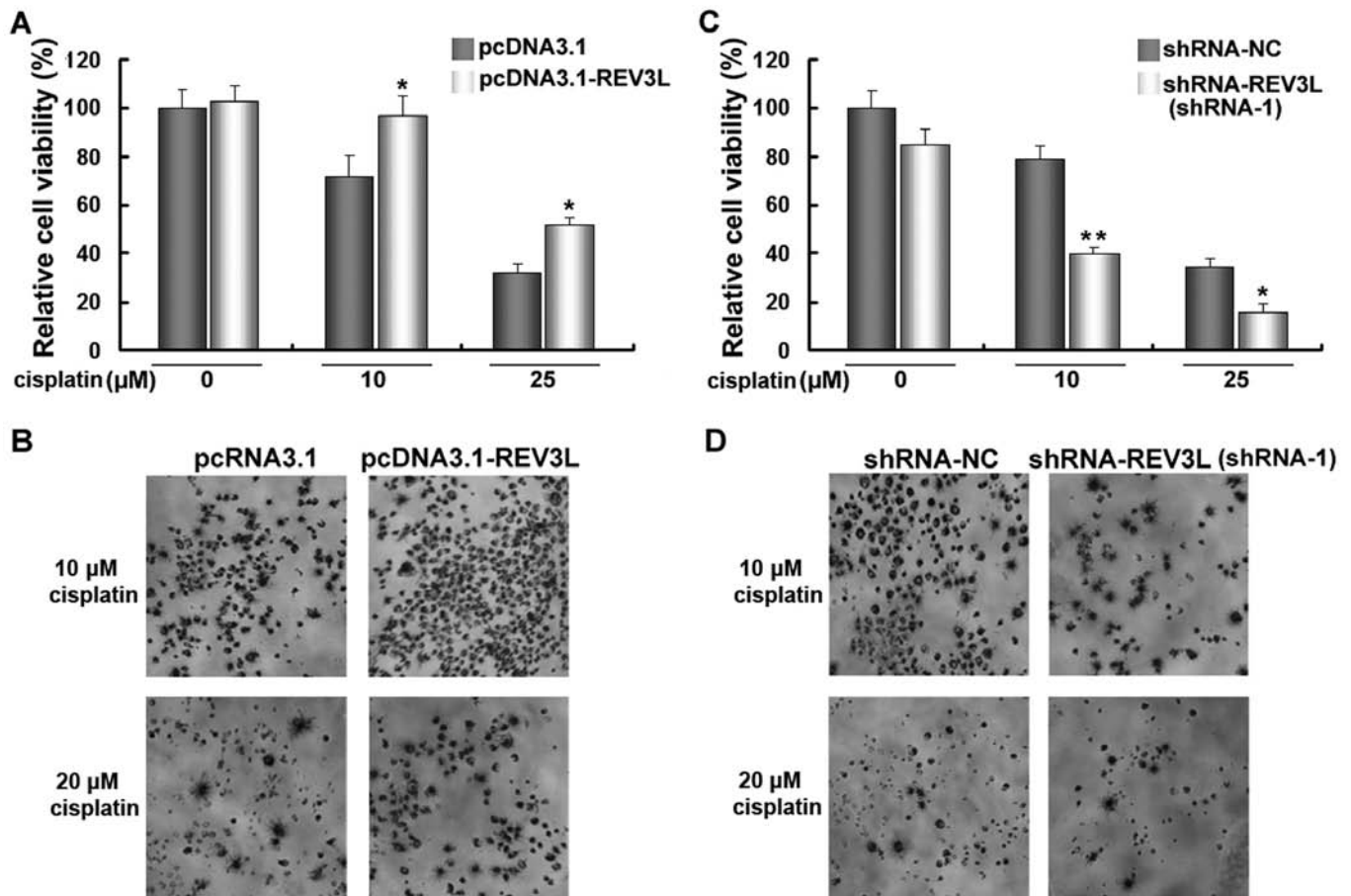
Figure 5. Knockdown of *REV3L* sensitizes cancer cells to cisplatin-induced cytotoxicity. (A) H1299 cells were stably transfected with pcDNA3.1 or pcDNA3.1-REV3L. The cells were treated with the indicated doses of cisplatin (DDP) for an additional 48 h. Cell viability was determined by MTT. Data are presented as a percentage of viable cells, where vehicle-treated cells were regarded as 100% viable. Points show the mean ± SEM of three independent experiments, *P<0.05 and **P<0.01. (B) Representative MTT image of pcDNA3.1- or pcDNA3.1-REV3L-transfected H1299 cells treated with 10 μM cisplatin for 48 h. (C) H1299 cells were stably transfected with shRNA-NC- or shRNA-targeting *REV3L*. The cells were treated with indicated doses of cisplatin for an additional 48 h. (D) Representative MTT image of shRNA-NC-transfected or *REV3L* silencing H1299 cells treated with 10 or 20 μM cisplatin for 48 h.

to the crosslink of the plasmid DNA. After 30 min treatment of cisplatin, the reporter was used for subsequent experiments. Co-transfection of the reporter with REV3L overexpression vector significantly enhanced the reporter activity by 2.23-fold. Moreover, the addition of REV3L and REV7L, which was able to form the complete Pol ζ, significantly increased the reporter expression by >5-fold.

REV3L knockdown sensitizes H1299 cells to cisplatin. We investigated the effect of REV3L expression on cell sensitivity to cisplatin treatment. As shown in Fig. 5A and C, transfection of pcDNA3.1-REV3L or shRNA-REV3L did not cause an obvious change of cell viability in H1299 cells. The cells were transfected with pcDNA3.1-REV3L, shRNA-REV3L or control vectors for 24 h, and then treated with 10 of 25 μM cisplatin for another 48 h. The results revealed that a forced expression of REV3L significantly increased cell viability (Fig. 5A and B), while the knockdown of REV3L significantly decreased the viability of H1299 cells treated with cisplatin (Fig. 5C and D), compared with cisplatin treatment alone. These results demonstrated that silencing of REV3L effectively enhanced the anticancer efficacy of cisplatin.

REV3L silencing promotes cisplatin-induced apoptosis. DAPI staining of nucleus was used to observe morphological changes such as nuclear condensation and fragmentation. The results showed that the nuclear condensation and fragmentation of shRNA-REV3L cells were more pronounced than shRNA-NC H1299 cells after 10 μM cisplatin exposure for 48 h (Fig. 6A).

We investigated whether silencing of REV3L affected cell apoptosis following treatment with cisplatin. As shown in Fig. 6B and C, knockdown of REV3L did not induce apoptosis (Annexin V+/PI- plus Annexin V+/PI+ cells). However, silencing of REV3L enhanced the response of H1299 cells to 10 or 25 μM cisplatin (shRNA-NC + 10 μM cisplatin 12.41% vs. shRNA-REV3L + 10 μM cisplatin 25.37%, P<0.05; (shRNA-NC + 25 μM cisplatin 31.84% vs. shRNA-REV3L + 25 μM cisplatin 46.68%, P<0.05). Taken together, these results demonstrated that REV3L inhibition enhances apoptotic cell death of H1299 cells in response to cisplatin.

Discussion

Repair of cisplatin-induced DNA damage has focused on the NER MMR and HR pathways (11). In a study conducted to explore the contribution of DNA damage response pathways
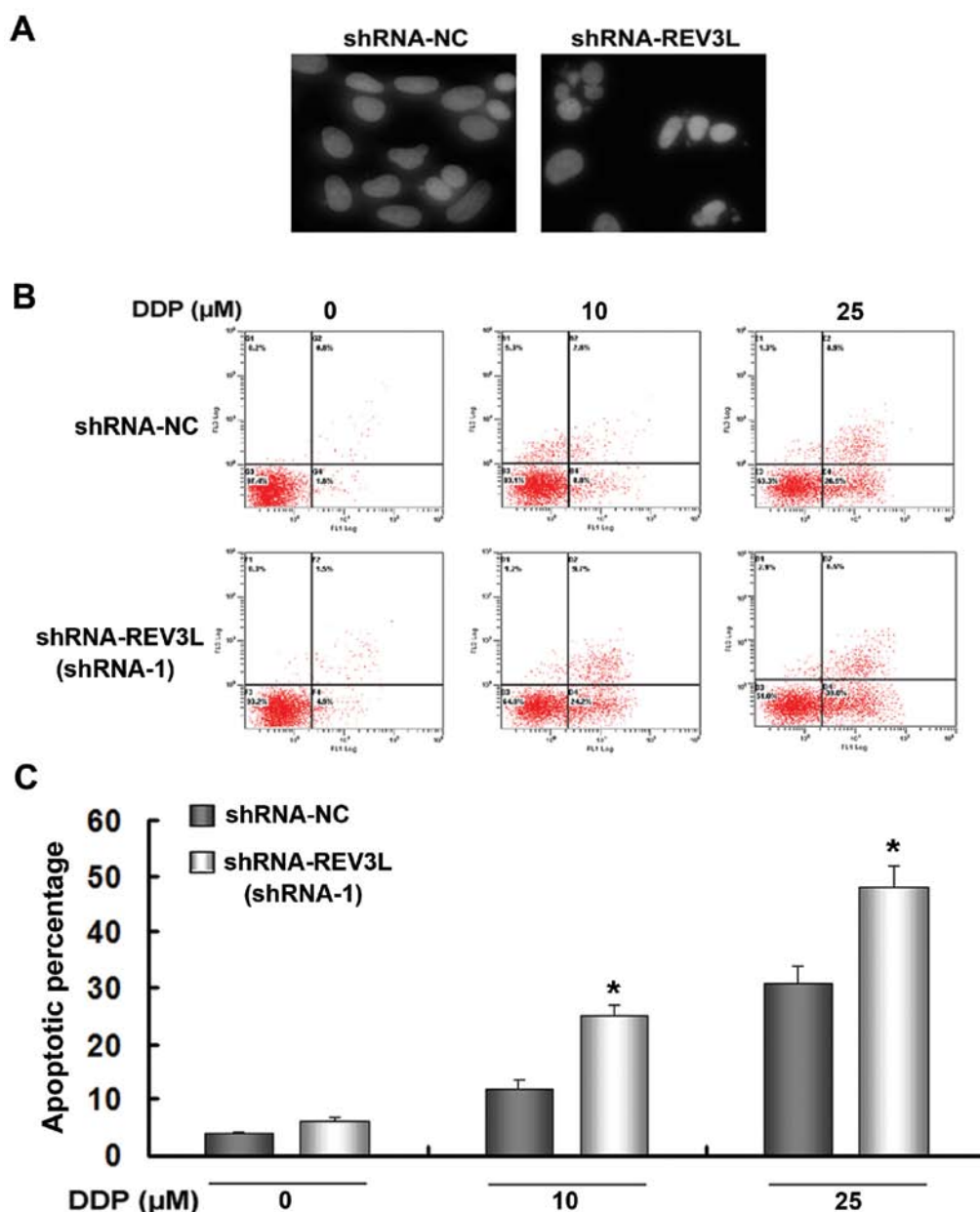
Figure 6. *REV3L* silencing promotes cisplatin-induced apoptosis. (A) Morphological examination to detect apoptosis by DAPI staining. Nuclear condensation and fragmentation of the REV3L knockdown of H1299 cells were more prominent than the vector control H1299 cells after treatment with 10 μM cisplatin (DDP) for 48 h. The cells were transfected with the indicated shRNA 24 h prior to 0, 10 or 25 μM cisplatin. Cell apoptosis was measured using propidium iodide (PI)/Annexin V double staining in H1299 cells. (B) Representative image of apoptotic analysis. (C) Calculated apoptotic percentage of the group of cells. The data are shown as mean ± SEM for three independent experiments. Statistical analysis between the groups was determined by ANOVA; *P<0.05.

in tolerance to cross-linking agents in vertebrates, a panel of gene-disrupted clones from chicken DT40 cells was used to measure the sensitivities of chicken DT40 cells to cross-linking agents, including cisplatin, mitomycin C, and melphalan. It was found that cells harboring defects in the TLS pathway, Fanconi anemia complementation groups (FANC) or homologous recombination pathway exhibited marked hypersensitivity to all the cross-linking agents, whereas NER played only a minor role (24). In particular, cells deficient in REV3 showed the highest sensitivity and markedly increased chrosomal abberrations to cisplatin (24).

The role of low-fidelity TLS polymerases in tumorigenesis remains controversial, because their existence is beneficial for the survival of human cells, while it accumulates mutations during DNA replication (25). Given the importance of TLS participants, studies have attempted to increase the efficacy of chemotherapy by inhibiting this pathway. Albertella *et al* found that Pol η is migrated to nucleus in response to cisplatin and its deficiency significantly decreased cell focus formation after cisplatin treatment (26). DNA polymerase η could replicate across intrastrand cross-link between cisplatin and two adjacent G residues (27). Wu *et al* found that cisplatin induced a concentration- and time-dependent increase in hREV3 mRNA and suppression of REV3L by transfection of a vector-expressing hREV3 antisense mRNA increased cisplatin sensitivity in human fibroblast (28).

hREV1, another important member in translesional replication and a Pol ζ interaction molecule, was also upregulated

in response to cisplatin in human ovarian carcinoma 2008 cells (29). Inactivation of REV1 causes it to become hypersensitive to a wide variety of DNA-damaging agents including cisplatin in human ovarian cancer cells (29,30). Similar results were obtained in colon cancer HCT-116 cells where the introduction of a shRNA against REV3L reduced the mutagenic bypass of cisplatin and cisplatin resistance. Comparatively, inhibition of the DNA MMP pathway did not have a significant effect on reporter expression, which emphasizes the importance of REV3L (17). Suppression of the structual unit of Pol ζ-REV7L (MAD2B) conferred hypersensitivity to a range of DNA-damaging agents, especially DNA cross-linkers such as cisplatin and γ-irradiation (31), suggesting that each of the TLS enzymes may have specificity to the chemotherapeutic drugs. We have previously reported that the downregulation of REV3L sensitized glioma cells to cisplatin via mitochondria-mediated apoptosis (32). Taken together, those results indicate that TLS enzymes are activated in response to chemotherapy as endogenous adaptive mechanisms and inhibition of these polymerases contributes to the efficacy of cisplatin.

In this study, we also found that transcription factor Sp1 is involved in cisplatin-induced REV3L overexpression. In addition, increased Sp1 binding was found in the proximal region of the *REV3L* promoter. To the best of our knowledge, this is the first study to show the transcriptional control of REV3L following cisplatin exposure. Sp1 is a ubiquitously expressed zinc finger-containing DNA-binding protein that can activate or repress gene transcription in response to various physiologic and pathological stimuli (33). Sp1 binds to GC-rich recognition elements (GC-boxes) through its C-terminal zinc finger motifs (34,35). Sp1 has been reported to be activated in response to oxidative stress and regulate neuronal survival in cortical neurons (36). DNA damage induces transient elevation in its DNA binding activity and phosphorylation of Ser-56 and Ser-101 residues on Sp1 in an ATM-dependent manner (35,37). In cancer cells, Sp1 is involved in the transcriptional control of pro-apoptotic NOXA following cisplatin exposure (38). Our results identified a new downstream target of Sp1 in response to cisplatin.

Taken together, we have demonstrated that cisplatin induced the expression of REV3L by recruiting Sp1 to its promoter. The knockdown of REV3L sensitized cisplatin efficacy in human lung cancer H1299 cells. Similar results were obtained when the ability of the cells to express luciferase from a platinated plasmid was measured.

Acknowledgements

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