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Rab-like protein 1 A is upregulated by cisplatin treatment and partially inhibits chemoresistance by regulating p53 activity

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Abstract. Rab-like protein 1 A (RBEL1A), which is a predominant isoform of RBEL1, has been identified to serve an important function in breast tumorigenesis and may be upregulated in breast tumor cells. RBEL1A may block the transcriptional activity of p53, which is important in the induction of cisplatin sensitivity. Previous studies supported the association between the induction of chemoresistance and the inhibition of p53 by RBEL1A. However, the response of RBEL1A to chemotreatment and its interaction with p53 remains to be investigated. The present study revealed that the cisplatin treatment induced the expression of RBEL1A in MCF-7 cells. Consistent with previous studies, the present study demonstrated that cisplatin treatment and RBEL1A overexpression blocked the oligomerization of p53 in MCF-7 cells and led to a decrease of the transcriptional activity of p53 and its downstream target gene p21. Additionally, upregulation of RBEL1A decreased the protein level of p53 by promoting the ubiquitination of p53. A cytotoxicity assay demonstrated that upregulation of RBEL1A partially contributed to chemosensitivity via inhibiting p53 in MCF-7 cells. A pG13L (p53-responsive reporter plasmid) luciferase reporter and co-immunoprecipitation assay revealed that upregulation of RBEL1A led to an inhibition of the transcriptional activity of p53 or its target gene p21. Analysis of cellular proliferation, cell cycle and invasion also confirmed the regulatory activity of RBEL1A on the malignancy of breast cancer cells. Taken together, these results suggest that the induction of RBEL1A following cisplatin treatment may partially inhibit chemosensitivity in a p53-dependent manner.

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Introduction

RBEL1A represents one of the four isoforms of RBEL1 and harbors a N-terminus Ras/Rab-like GTPase domain followed by a GTP-binding regulatory domain, a protein-rich region and a C-terminus nuclear localization signal (1,2). RBEL1A functions as a GTPase by binding to GTP. Apart from its GTPase function, RBEL1A was identified to be upregulated in primary breast cancer tissues compared with its expression in adjacent normal tissues (3). Downregulation of RBEL1A led to a suppression of cell growth through cell cycle arrest and inhibition of cellular migratory and invasive abilities. Those results indicated the association of RBEL1A with poor prognosis in several types of cancer (4).

The p53 tumor suppressor is activated under stress conditions. Activated p53 functions as a positive transcriptional regulator for >60 genes, which in turn regulate cell cycle, DNA repair, apoptosis and senescence (5,6). In cancer, p53 is involved in the induction of chemosensitivity via its transcriptional activity to regulate cell cycle and apoptosis (7,8). The transcriptional activity of p53 depends on the process of oligomerization (9). In cancer cells, a number of strategies to prevent p53-mediated cellular control by inhibiting the transcriptional activity of p53 via dissociating tetramers have been revealed (10,11). S100B protein binds specifically to the tetramerization domain of p53 monomers but rarely with the p53 tetramers and leads to a shift of equilibrium favoring monomeric conformation (12,13). Apoptosis repressor with caspase recruitment domain (ARC) has been identified to interact with the C-terminus domain (amino acids, 301-393) of p53 and interferes with the tetramerization of p53 (14).

RBEL1A has been identified to serve an inhibitory function in the tetramerization of p53 (15). RBEL1A binds to residues 315-360 and decreases the oligomerization of the exogenously expressed C-terminus domain (residues, 301-393) of p53 *in vitro*. Depletion of RBEL1A increases the oligomerization of p53 and induces its transcriptional targets, including p21 and Puma in breast cancer cells (12). However, whether upregulation of RBEL1A serves any functions in regulating chemosensitivity via interaction with p53, remains unresolved.

In the present study, changes in the expression profile of RBEL1A in response to cisplatin treatment were assessed. Additionally, whether RBEL1A-p53 interaction is regulated

by chemotreatment was examined. Collectively, the results demonstrated that chemotreatment induced RBEL1A and negatively regulated the function of p53 by decreasing the protein level of p53 and blocking the oligomerization of p53 in MCF-7 cells. This may lead to the development of a promising therapeutic strategy for cancer through the targeting of p53.

Materials and methods

Cell lines. Human breast cancer cell line MCF-7 was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were incubated in minimum essential medium (MEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.). Cells were maintained at 37˚C in a humidified atmosphere containing 5% CO_2. To investigate the molecular mechanism underlying the effects of RBEL1A on p53 and p21, 10 μM MG132 (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was incubated with MCF-7 cells for 24 h at 37˚C in a 5% CO_2 incubator.

Plasmids and antibodies. Plasmids encoding HA-tagged p53 (HA-p53, Addgene, Inc., Cambridge, MA, USA), FLAG-tagged p53 (FLAG-p53, Addgene, Inc.) or RBEL1A (Addgene, Inc.) was cloned for mammalian expression from the cytomegalovirus immediate-early promoter in pcDNA3.1 vector (Thermo Fisher Scientific, Inc.). Antibodies used in chromatin-immunoprecipitation were as follows: Anti-HA tag antibody (1:500; cat. no. ab9110; Abcam, Cambridge, UK) and anti-FLAG tag antibody (1:500; cat. no. ab1162; Abcam). Polyclonal RBEL1A antibody was generated according to the protocol described by Montalbano *et al* (3). For western blot analysis, the primary antibodies used were as follows: Anti-p21 antibody (1:2,000; cat. no. ab109520; Abcam), anti-p53 antibody (1:2,000; cat. no. ab1101; Abcam), anti-MDM2 antibody (1:2,000; cat. no. ab16895; Abcam). The secondary antibody used were as follows: Goat anti-rabbit IgG H&L (HRP) antibody (1:5,000; cat. no. ab7090; Abcam), Rabbit anti-mouse IgG H&L (HRP) antibody (1:5,000; cat. no. ab6728; Abcam).

Transfection. MCF-7 cells were seeded at $3x10^5$ cells/well in a 6-well plate and attached overnight. Cells were transfected at 1.6 μg plasmid/well using Lipofectamine 2000® (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. After 4 h, medium was refreshed and cells were incubated in MEM supplemented with 10% FBS for 48 h.

Cytotoxicity assay. MCF-7 cells were plated at a density of $2x10^4$ cells/well in a 96-well plate and were left to attach overnight. Target cells were incubated with serial concentrations of cisplatin (1, 5, 10, 20, 40, 60, 80 and 100 μM; Sigma-Aldrich; Merck KGaA) for 24 h. The medium was then removed and 200 μl fresh medium supplemented with 20 μl MTT (5 mg/ml dissolved in PBS; Merck KGaA) was added to each well. Following 4 h incubation at 37˚C, supernatant was removed and 200 μl dimethyl sulfoxide (Merck KGaA) was added into each well. Absorbance at 570 nm was measured using a microplate reader (Synergy 2 Multi-Mode Microplate Reader; BioTek Instruments, Inc., Winooski, VT, USA).

RNA interference (RNAi). Knockdown of RBEL1A was performed by transfecting RBEL1A-specific small hairpin (sh)RNA construct in a pLKO.1 lentiviral vector. The structure of the primers for shRNA consisted of the following elements: Sense, loop (underlined), and antisense). The primers were as follows: shRBEL1A, 5'-CCGCCAGTGTTTCTCAGGGAT CTCGAGATCCCTGAGAAACACTGGCGG-3'; shScramble, 5'-AGGTTCCATGTGCGGTTCACCCTCGAGGGTGAA CCGCACATGGAACCT-3'; shp53, 5'-CCGACTCCAGTG GTAATCTACTTCAAGAGAGTAGATTACCACTGGAGT CTTTTT-3'; shScramble, 5'-CCAAGTCCTGGTTCAGCA CATTTCAAGAGAATGTGCTGAACCAGGACTTTTTT-3'. 293T cells were transfected with a target vector along with packaging plasmid psPAX2 and envelope plasmid pMD2.G (Addgene, Inc.) at the ratio of 1:1.5:1. At 4 h post-transfection, the medium was replaced with fresh medium supplemented with 10% FBS. Following 3 days of culture, the supernatants containing viral particles were collected and the titer was determined. Briefly, on day 1, $1x10^5$ MCF-7 cells were plated in a 12-well plate and were left to attach overnight. On day 2, cells were infected with 3-fold serial dilutions of the viruses in MEM containing 10 μg/ml polybrene (Sigma-Aldrich; Merck KGaA). GFP-positive cells were observed under microscopy and the optimal dilution was determined.

Dual-Luciferase® reporter assay. pG13-Luciferase (pG13-luc; a gift from Dr Jianjun Chen, Sichuan University, Chengdu, China) was stored in the laboratory. Cells were seeded into a 12-well plate for conducting the luciferase assays. Transfection of cells was performed using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. At 24 h post-transfection at 37˚C, cell lysates were subjected to the luciferase assay. To detect luciferase and β- galactosidase activity, a luciferase substrate (Promega Corporation, Madison, WI, USA) and the Galacto-Star™ β-galactosidase Reporter Gene Assay System for Mammalian cells (Cat. no.: T1012; Thermo Fisher Scientific, Inc.) were employed according to the manufacturer's protocol. Relative values of luciferase activity were calculated using β-galactosidase activity as an internal control for transfection.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA isolated from MCF-7 cells using Trizol (Thermo Fisher Scientific, Inc) was performed for first strand cDNA synthesis using Superscript III RT-qPCR kit (Thermo Fisher Scientific, Inc.). The following primers were used for the qPCR: RBEL1A, 5'-CCGATGTGACTGACG AGGATGAG-3' (forward) and 5'-GTGTTTGCTCTTCTT CTTGGCAGC-3' (reverse); β-actin, 5'-CATGTACGTTGC TATCCAGGC-3' (forward) and 5'-CTCCTTAATGTCACG CACGAT-3' (reverse); p53, 5'-CAGCACATGACGGAGGTT GT-3' (forward) and 5'-TCATCCAAATACTCCACACGC-3' (reverse); p21, 5'-TGTCCGTCAGAACCCATGC-3' (forward) and 5'-AAAGTCGAAGTTCCATCGCTC-3' (reverse). For chromatin immunoprecipitation analysis, the primers were as follows: p21 promoter region, 5'-CTGGACTGGGCACTC TTGTC-3' (forward) and 5'-CTCCTACCATCCCCTTCC TC-3' (reverse); and DHFR 5'UTR, 5'-TGTAAAACGACG GCCAGTC-3' (forward) and 5'-CCAGGAAACAGCTATGAC C-3' (reverse). The PCR program was as follows: 5 min 95˚C

hot start, 40 cycles of 10 sec 94˚C, 10 sec 60˚C and 1 min 72˚C; 10 min 72˚C incubation. Purified PCR products were cloned into a pCR2.1 vector (Thermo Fisher Scientific, Inc.) followed by DNA sequencing. In order to quantify gene expression, the $2^{-\Delta\Delta Cq}$ method was used (16). RT-qPCR was performed in triplicate.

Chromatin immunoprecipitation and western blot analysis. MCF-7 cells were plated in 100 mm tissue culture dishes and co-transfected with Flag-p53 and HA-p53 constructs for 24 h. Cells were trypsinized and placed in 6-well plates and left to attach overnight. Then, cells were treated with cisplatin [IC_{30} concentration, evaluated by the isobolographic method, as described previously (17)] for 24 h. Cells treated with equal volume of dimethyl sulfoxide were used as the control group. Whole cells were lysed using a lysis buffer [50 mM Tris/HCl (pH 8.0), 1 mM EDTA, 120 mM NaCl, 10% glycerol, 0.5% NP40, 1 mM dithiothreitol (DTT) and 0.5 mM phenylmethylsulfonyl fluoride (PMSF)], sonicated for 30 cycles (for each cycle, 30 sec on/30 sec off) using Diagenode Bioruptor Standard (Model UCD200), and centrifuged at 21,000 x g and 4˚C for 10 min. Supernatant was diluted 10 times with lysis buffer, and incubated with 20 μl protein A/G-agarose beads (Sigma-Aldrich; Merck KGaA) and anti-FLAG/anti-HA antibody. The beads were washed three times with buffer containing 20 mM HEPES (pH 7.9), 120 mM NaCl, 1 mM EDTA, 1 mM PMSF, and 1 mM DTT followed by centrifugation and boiled with 200 ul elution buffer (10 mM Tris-HCl, pH 8.0, 0.5 mM EDTA). Eluted sample was analyzed by RT-qPCR, with the aforementioned protocols. Then, western blot analysis was performed as aforementioned. Total proteins were isolated using radioimmunoprecipitation lysis and extraction buffer (Thermo Fisher Scientific, Inc.) and quantified using a bicinchoninic acid protein assay kit (Sigma-Aldrich; Merck KGaA) according to the manufacturer's instructions. Proteins (20 μg/lane) from total cell lysates were fractionated using SDS-PAGE and a 10-15% gel and transferred onto polyvinylidene fluoride membranes (Thermo Fisher Scientific, Inc.). Membranes were blocked with 5% skimmed milk in Tris-buffered saline with 0.2% Tween-20 (TBST) at room temperature for 1 h. Membranes were then incubated with anti-p21 antibody, anti-p53 antibody or anti-MDM2 antibody (dilution, 1:2,000) overnight at 4˚C, respectively. After three washes with TBST, secondary antibody (cat. no. ab7090; dilution, 1:5,000; Abcam) was incubated with the membrane at room temperature for 1 h. Imaging was performed using X-ray films (Kodak, Rochester, NY, USA) as described previously (3).

Cell cycle analysis. Cells were harvested by trypsinization and fixed for 4 h with 70% ice-cold ethanol at -20˚C. Fixed cells were washed with ice-cold PBS for three times and stained with 1 ml propidium iodide (50 μg/ml; Sigma-Aldrich; Merck KGaA) containing 0.1% Triton X-100 and 0.1 mg/ml RNase in darkness at room temperature for 30 min and analyzed by flow cytometry with ModFit LT software (Verify Software, Topsham, MN, USA).

Cell Counting kit-8 (CCK-8). MCF-7 cells were seeded at a density of $1x10^4$ cells/well in 96-well plates and incubated overnight. Following cisplatin treatment, 10 μl CCK-8 solution was added to each well and incubated for 4 h at 37˚C. Cell proliferation was determined by measuring the absorbance at a wavelength of 450 and 620 nm. Cell viability was calculated as (OD_{450}-OD_{620} in treatment group)/(OD_{450}-OD_{620} in control group) x100. Experiments were performed in triplicate. Two individual experiments were performed.

EdU incorporation assay. The apollo DNA labeling kit (Guangzhou RiboBio Co., Ltd., Guangzhou, China) was used to analyze cell proliferation. MCF-7 cells were seeded in a 12-well plate ($2x10^5$ cells/well), treated with 50 mM EdU for 2 h and fixed with 4% paraformaldehyde for 15 min at room temperature. The cells were incubated with 2 mg/ml glycine for 10 min to reverse fixation and washed with PBS three times. The cells were permeated with 100 μl/well permeabilization buffer containing 0.5% Triton X-100 and incubated with 100 μl of 1X apollo solution for 30 min in the dark. Following this, cells were observed under fluorescence microscope (magnification, x100; Olympus Corporation, Tokyo, Japan).

Invasion assay. Transwell membranes were precoated with 100 μl Matrigel (8%) in MEM and incubated at 37˚C for 4 h. A total of $5x10^3$ MCF-7 cells were plated in the upper chambers of Transwell plates in MEM (200 μl). MEM (600 μl) supplemented with 10% FBS was plated in the lower chambers. Following incubation for 24 h at 37˚C, the invasive cells were fixed with 4% paraformaldehyde at room temperature for 10 min and stained with 0.1% crystal violet stain (in PBS) at room temperature for 10 min. Stained cells were counted in five randomly-selected fields under a X71 fluorescence microscope (magnification, x100; Olympus Corporation).

Statistical analysis. Data were analyzed using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). The relevant data are expressed as the mean ± standard error of the mean. Statistical significance between treated and control groups was determined using one-way analysis of variance followed by Tukey's post hoc test and Student-Neuman-Keuls method. Statistical significance between two groups was determined using Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Regulation of mRNA and protein levels of RBELIA and p53 in response to IC_{30}-cisplatin treatment in MCF7 cells. It has been reported that RBEL1A is overexpressed in ~67% primary breast tumors (3), which indicates its potential function in regulating chemosensitivity. In order to explore whether RBEL1A is involved in the molecular mechanisms underlying chemosensitivity, IC_{30} cisplatin (22.4 μM) was employed to detect the expression of RBEL1A and p53 at 0, 4, 8, 12 and 24 h. As presented in Fig. 1A, mRNA levels of RBEL1A were significantly increased at 8, 12 and 24 h compared with the control. Consistently, the protein levels were also obviously increased. Additionally, mRNA and protein levels of p53 were decreased at 12 and 24 h after cisplatin treatment (Fig. 1A). In breast cancer cells, p53 is reported to be transcriptionally and post-transcriptionally regulated following overexpression of

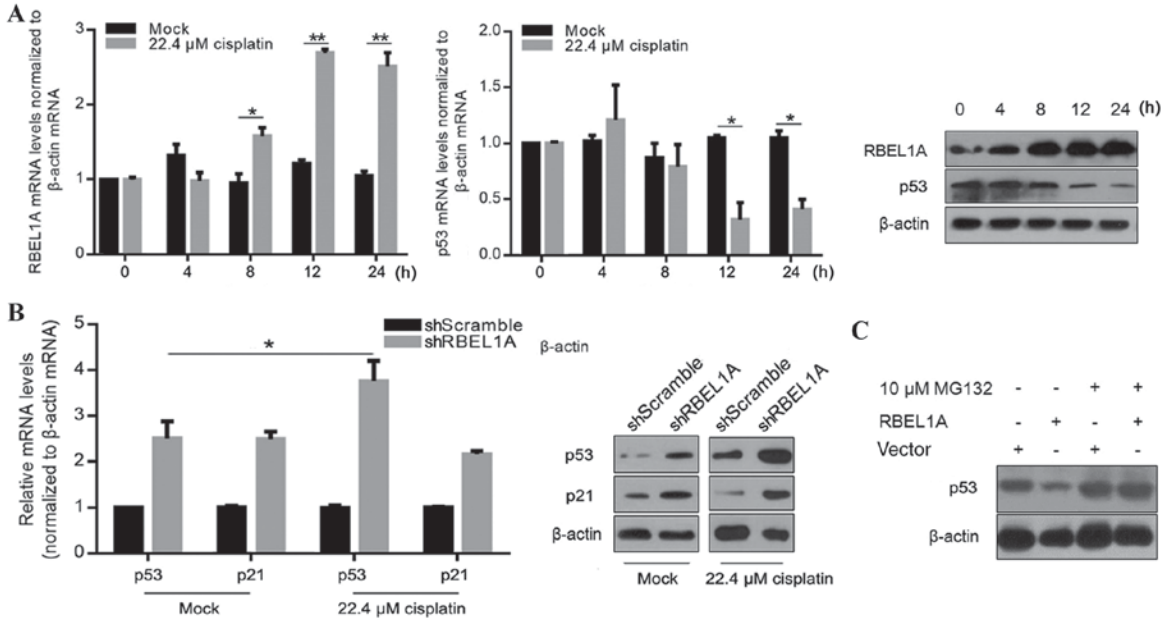

Figure 1. Cisplatin treatment transcriptionally regulates RBEL1A expression. (A) mRNA and protein levels of RBEL1A after cisplatin treatment at various timepoints as assessed using reverse-transcribed quantitative polymerase chain reaction and western blot analysis. *P<0.05, **P<0.01 vs. mock; Statistical analysis, one-way analysis of variance and Student-Neuman-Keuls test. (B) Expression levels of p53 and p21 following cisplatin treatment with or without shRBEL1A treatment, as assessed using RT-qPCR and western blot analysis. *P<0.05 vs. shRBEL1A/Mock; Statistical analysis, one-way analysis of variance and Tukey's post hoc test. (C) Expression of p53 in response to MG132 treatment using western blot analysis. RBEL1A, rab-like protein 1 A; sh, small hairpin.

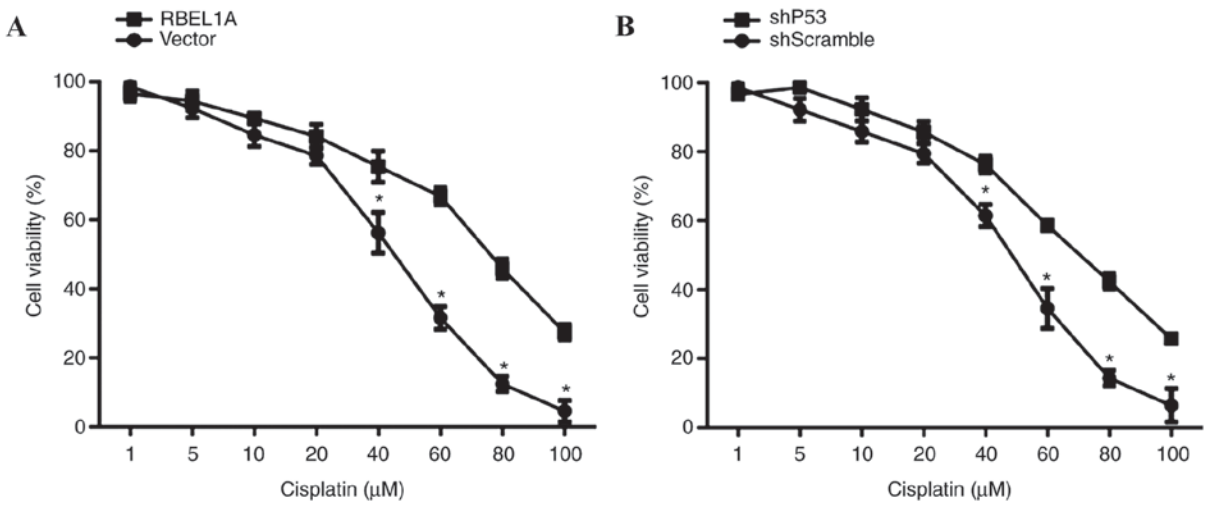

Figure 2. Cell sensitivity to cisplatin in the Cell Counting Kit-8 assay. (A) Cytotoxicity of cisplatin treatment to MCF-7 cells with RBEL1A. *P<0.05 vs. vector; Statistical analysis, one-way analysis of variance and Student-Neuman-Keuls test. (B) Cytotoxicity of cisplatin treatment to MCF-7 cells with p53-knockdown. *P<0.05 vs. shScramble; Statistical analysis, one-way analysis of variance and Student-Neuman-Keuls test. RBEL1A, rab-like protein 1 A; sh, small hairpin.

RBEL1A (2). Next, the effects of upregulation of RBEL1A on the target gene of p53, p21, were investigated in cisplatin-treated MCF-7 cells. The results demonstrated that downregulation of RBEL1A (using a shRNA target to RBEL1A, shRB3L1A) let to an upregulation of p53 and p21 in response to cisplatin treatment in MCF-7 cells (Fig. 1B). Knockdown of RBEL1A also resulted in an upregulation of p53 and p21 in untreated MCF-7 cells (Fig. 1B), indicating a regulatory effect of RBEL1A on p53 and p21 under normal conditions. Next, MCF-7 cells were treated with cisplatin and with 10 μM MG132, which mediates proteasome inhibition after ubiquitination, in order to investigate the molecular mechanism underlying the effects of RBEL1A on p53 and p21. The expression of p53 was examined using western blot analysis. Fig. 1C demonstrated that RBEL1A-mediated decrease in p53 protein levels was abrogated in cells treated with MG132. These results suggest that upregulation of RBEL1A following cisplatin treatment potentially decreased the expression levels of p53 by accelerating ubiquitination.

RBEL1A partially inhibits cisplatin sensitivity in MCF-7 cells. It has been demonstrated that p53 functions as a positive regulator of cisplatin-mediated chemotherapy in breast cancer (13). Variable responses to cytotoxicity were indicated

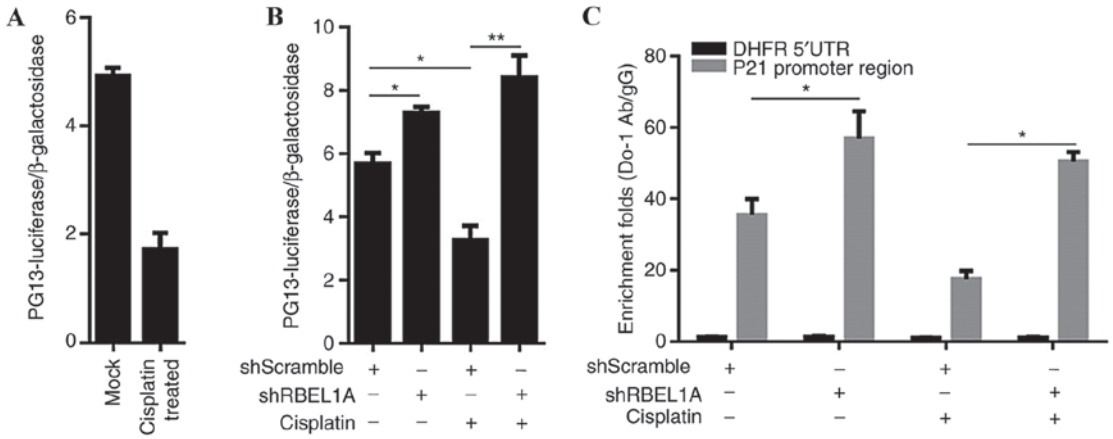

Figure 3. Cisplatin treatment induces RBEL1A-mediated transcriptional activity of p53. (A) The transcriptional activity of p53 was examined using a luciferase assay in mock- and cisplatin-treated MCF-7 cells. (B) The transcriptional activity of p53 was examined using a luciferase assay in response to shRBEL1A treatment. *P<0.05 vs. shScramble group; **P<0.01 vs. shScramble, cisplatin treated group; Statistical analysis, one-way analysis of variance and Student-Neuman-Keuls test. (C) Chromatin-imunoprecipitation was performed to detect the effects of RBEL1A on p53's transcriptional activity. *P<0.05 vs. shScramble; shScramble and cisplatin treated; Statistical analysis, one-way analysis of variance and Student-Neuman-Keuls test. RBEL1A, rab-like protein 1 A; sh, small hairpin.

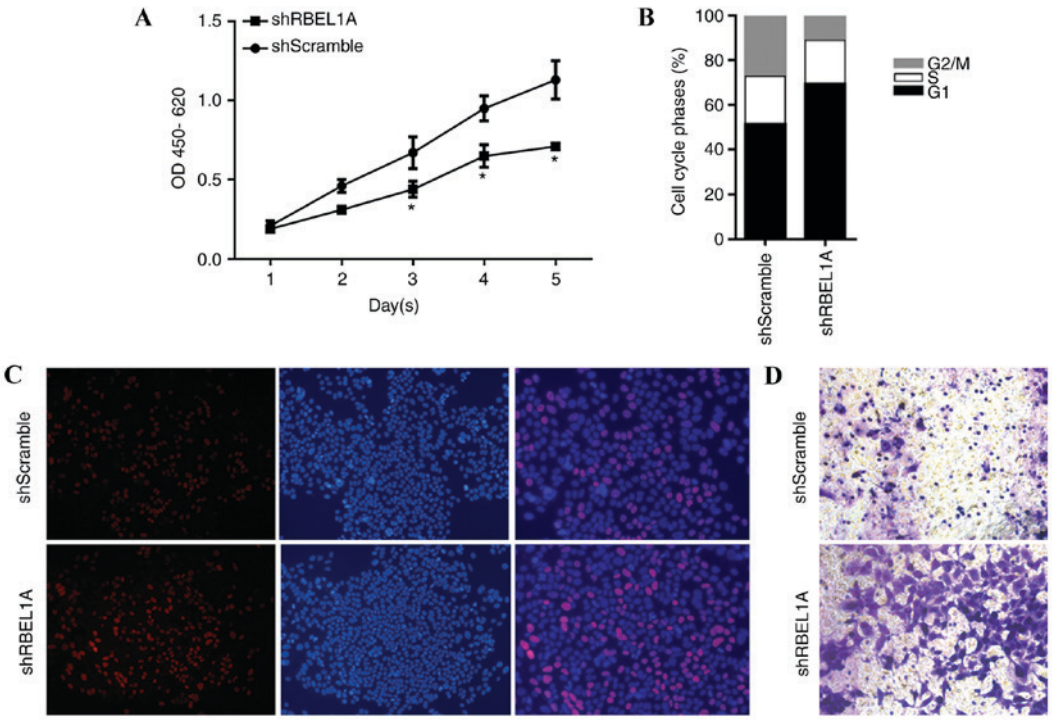

Figure 4. RBEL1A knockdown inhibits cellular proliferation by inducing cell cycle arrest and promoting invasion. (A) Cell CCK-8 in response RBEL1A knockdown in MCF-7 cells, as assessed using Cell Counting kit-8. *P<0.05 vs. shScramble; Statistical analysis, one-way analysis of variance and Student-Neuman-Keuls test. (B) Analysis of cell cycle in response to downregulation of RBEL1A. (C) Proliferation of MCF-7 cells in response to RBEL1A knockdown as assessed using EdU staining. Blue indicates nuclei and red indicates proliferating cells. Magnification, x100. (D) Analysis of invasive ability of MCF-7 cells in response to RBEL1A knockdown. Magnification, x100. RBEL1A, rab-like protein 1 A; sh, small hairpin; OD, optical density.

in response to cisplatin treatment in MCF-7-shRBEL1A and MCF-7-shp53 cells. The CCK-8 assay results illustrated that overexpression of RBEL1A increased cell viability compared with that of vector-transfected cells, indicating that RBEL1A led to a significant desensitization of MCF-7 cells to cisplatin (40, 60, 80 and 100 μM) (Fig. 2A). Additionally, knockdown of p53 (achieved using shp53) also led to a significant desensitization of MCF-7 cells to cisplatin (40, 60, 80 and 100 μM) as assessed using a MTT assay (Fig. 2B).

Cisplatin treatment stimulated the regulatory activity of p53 via upregulating RBEL1. The transcriptional activity of p53 is critical for inducing chemosensitivity (13), which may be tightly regulated by cisplatin-induced RBEL1A in MCF-7 cells. Therefore, whether RBEL1A-mediated p53 downregulation in cisplatin-treated MCF-7 cells may exhibit an effect on the interaction between p53 and its target DNA sequence was investigated. The transcriptional activity of p53 in cisplatin-treated MCF-7 cells was confirmed using a pG13L

luciferase reporter assay. Results demonstrated that cisplatin treatment reduced the transcriptional activity of p53 compared with mock-treated MCF-7 cells (Fig. 3A). In order to confirm the decrease of the transcriptional activity of p53, MCF-7 cells were treated with shRBEL1A prior to cisplatin treatment. According to the results, knockdown of RBEL1A failed to decrease the transcriptional activity of p53 in cisplatin- or mock-treated MCF-7 cells (Fig. 3B) and led to increased transcriptional activity of p53. Additionally, cisplatin treatment decreased the binding of p53 to p21l's promoter region, as assessed using chromatin-immunoprecipitation (Fig. 3C).

Knockdown of RBEL1A inhibited proliferation, blocked entry of cell cycle and invasive ability in cisplatin-treated MCF-7 cells. In order to identify the effects of cisplatin-induced RBEL1A expression on physiological processes, MCF-7-shScramble and MCF-7-shRBEL1A cells were treated with IC_{30} concentration of cisplatin. As presented in Fig. 4A and B, knockdown of RBEL1A significantly increased the proliferating rate (at day 3, 4 and 5) by accelerating cell cycle entry. By performing EdU staining (red-stained cell represents proliferating cells), it is demonstrated that treatment with shRBELIA regulated the proliferation of MCF-7 cells (Fig. 4C). Furthermore, invasive activity was also been promoted in MCF-7-shRBEL1 cells (Fig. 4D). Taken together, the results demonstrated that knockdown of RBEL1A may inhibit proliferation, and arrest cell cycle and invasion of MCF-7 cells in response to cisplatin treatment.

Discussion

Oligomerization is critical in p53-mediated regulation of apoptosis and chemosensitivity (14,15). The equilibrium of monomer and oligomer shifts under intracellular or extracellular stress. In normal conditions, p53 may predominantly exist as latent monomers, the monomers tend to oligomerize to form dimers, trimers, and dimers of dimer (tetramers) under stress conditions. Although monomers present slight DNA binding activity, tetramerized p53 binds tightly and specifically to transactivate promoters of various target genes that are involved in the regulation of cellular processes, including cell cycle arrest, apoptosis, cellular senescence and DNA repair (5). Despite mutations occurring in oligomerization domain of p53 (residues 301-363), proteins that block p53 oligomerization are expected to be a novel strategy for inhibiting p53' transcriptional activity (8). Several proteins have been reported to be involved in preventing p53's transcriptional activity via dissociating p53 oligomers by binding to p53 monomers. The predominant members of S100 protein family, S100A and S100B bind to the tetramerization domain of p53 specifically and lead to tetramer dissociation (10,11). These proteins were upregulated in various human malignancies, thus indicating their potential function in tumorigenesis and induction of chemoresistance (18,19). ARC has been reported to regulate p53's transcriptional activity via binding directly to p53's tetramerization domain (11). ARC was demonstrated to be upregulated in human colon cancer, and thus inhibited p53 tetramerization and nuclear translocation. Consequently, the transcriptional regulation of p53 to its target genes decreased (20). RBEL1A also interacts with p53 at p53's tetrameric domain and may lead to dissociation of p53 tetramers (12). However, whether RBEL1A is involved in the induction of chemoresistance via regulating p53's transcriptional activity remains unclear.

In the present study, it was demonstrated that mRNA and protein levels of RBEL1A were upregulated in response to cisplatin treatment. Expression of RBEL1B, which is one of the RBEL1 isoforms was unaffected in response to cisplatin treatment (data not shown), which is consistent with previous studies, indicating the positive association between RBEL1A but not RBEL1B with poor prognosis in breast cancer (3). It has been revealed that upregulation of RBEL1A inhibited p53 oligomerization in response to cisplatin treatment in 293 cells (15). Additionally, upregulation of RBEL1A decreased p53 protein level by transcriptional inhibition and accelerating protein degradation. Upregulation of RBEL1A regulated p53's transcriptional activity on reporter gene and downstream target gene as assessed using luciferase reporter assay and chromatin-immunoprecipitation. Although, the protein levels of RBEL1A increased following cisplatin treatment, its mRNA levels were unchanged, indicating that the effect of cisplatin on the expression of RBEL1A was on a post-transcriptional level. Several potential molecular mechanisms may be involved, including post-transcriptional regulation by microRNA targeting RBEL1A mRNA or accelerated ubiquitination. Future studies are required to unravel the molecular mechanisms underlying the regulation of RBEL1A by chemotreatment.

In breast tumors, nearly half of them contain mutant p53 and ~70% of mutations in p53 are missense mutations (21). Compared with p53 deficiency, p53 mutants demonstrate increased functional abnormality due to its multifunction. For example, mutant p53 was reported to positively regulate signaling pathways involved in cellular proliferation and metabolism (22). Mutant p53 has been revealed to promote the expressing level of 15-lypoxygenase, which is positively associated with tumor progression and survival rate of breast cancer cells (23). Mutant p53 was also reported to promote vascular endothelial growth factor expression in breast cancer (24). However, the interaction between RBEL1A and mutant p53 remains unclear.

To conclude, the results of the present study demonstrated that cisplatin treatment significantly induced the expression of RBEL1A, thus blocked the transcriptional activity of p53. This interaction may partially contribute to the induction of cisplatin-mediated chemosensitization.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

CC and ZZ designed the experiments. ST performed the gene expression analysis and cell-related experiments. CZ wrote the manuscript, provided funding support and performed data analysis.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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