Original Article

KRT14 knockdown reduces cisplatin resistance by lowering LRP11 expression levels in cisplatin-resistant ovarian cancer cell lines

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Background: Platinum resistance is a major cause of mortality in patients with advanced ovarian cancer. Understanding the mechanisms underlying this resistance is essential for developing effective treatments to improve patient survival. Therefore, this study aimed to explore the role and mechanisms of keratin 14 (KRT14) in regulating cisplatin resistance in ovarian cancer.

Methods: We utilized quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and western blotting to measure messenger RNA (mRNA) and protein expression levels, respectively. Cisplatin-resistant cell lines (SK-OV-3/DDP and A2780/DDP) were transfected with small interfering RNA (siRNA) targeting KRT14 (si-KRT14) or a plasmid containing low-density lipoprotein receptor-related protein 11 (LRP11) to knock down KRT14 or overexpress LRP11, respectively. Differentially expressed mRNAs were identified using Illumina RNA sequencing. Cell viability and half-maximal inhibitory concentration (IC_{50}) values were determined via cell counting kit-8 (CCK-8) assays, while apoptosis was assessed using flow cytometry and Hoechst 33258 staining.

Results: KRT14 mRNA and protein levels were significantly higher in SK-OV-3/DDP and A2780/DDP cells compared with their parental counterparts. KRT14 knockdown reduced the IC_{50} values, increased apoptosis, and decreased the levels of the multidrug resistance (MDR)-related proteins P-glycoprotein (P-gp) and MDR-associated protein 1 (MRP1). KRT14 knockdown in SK-OV-3/DDP and A2780/DDP cells revealed 24 differentially expressed mRNAs. Further analysis revealed that KRT14 knockdown notably reduced LRP11 expression. LRP11 overexpression increased IC_{50} values, suppressed apoptosis, and enhanced MDR-related protein expression, thus counteracting the effects of KRT14 knockdown.

Conclusions: Cisplatin-resistant ovarian cancer cell lines revealed elevated KRT14 expression. KRT14 knockdown reduced cisplatin resistance by lowering LRP11 expression. Therefore, KRT14 may play a crucial role in mediating cisplatin resistance in ovarian cancer.

Keywords: Ovarian cancer; cisplatin resistance; keratin 14 (KRT14); lipoprotein receptor-related protein 11 (LRP11); RNA sequencing

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Introduction

Ovarian cancer, a highly malignant gynecological tumor, recorded 324,398 new cases and 206,839 related deaths from 185 countries in 2022, ranking third in both incidence and mortality rates among gynecological malignancies (1). This cancer typically develops deep in the pelvic cavity, which makes early detection challenging; thus, more than 75% of ovarian cancer patients are diagnosed at late stages with extensive metastasis, resulting in a 5-year survival rate less than 50% (2-4). Initial treatment involves debulking surgery and platinum-based chemotherapy (5). Over time, the interval between treatments for platinum-resistant recurrent ovarian cancer shortens, ultimately resulting in platinum resistance (6). Non-platinum chemotherapy reveals limited effectiveness (10–20%) against platinum-resistant recurrent ovarian cancer, significantly impacting survival in patients with late-stage disease (7). Therefore, understanding the mechanisms behind platinum resistance in recurrent ovarian cancer is crucial for developing effective treatments to improve patient survival.

Multidrug resistance (MDR) in tumors is a major reason for chemotherapy failure (8). MDR involves various molecules including transport proteins, enzymes, and apoptosis-related proteins (8). P-glycoprotein (P-gp), or MDR1, is an adenosine triphosphate (ATP)-binding cassette pump that expels drugs from tumor cell membranes, reducing their accumulation inside cells. The MDR-associated protein (MRP) family, including MRP1 and MRP2, also acts as ATP-binding cassette pumps, increasing drug efflux and affecting drug sensitivity in tumor cells. Glutathione S-transferases aid in cellular metabolism and detoxification, lowering drug concentrations inside cells and thereby reducing drug effectiveness. Apoptosis-related proteins like the Bcl-2 family regulate cell death pathways, influencing tumor cell sensitivity to chemotherapy drugs. Together, these regulatory proteins heighten tumor cell resistance to chemotherapy, a significant cause of treatment ineffectiveness in cancer therapy.

Keratin 14 (KRT14), a member of the keratin family, plays a significant role across various cancers. It is overexpressed in the urine of patients with bladder cancer, where it serves as a marker for bladder progenitors (9). Additionally, in triple-negative breast cancer, KRT14 upregulation is facilitated by enhancer of zeste homolog 2 (EZH2)-mediated tri-methylation of lysine 27 on histone H3 (H3K27me3), promoting peritoneal metastasis (10). Elevated KRT14 expression has also been observed in ovarian cancer tissues compared with normal ovarian tissues; this differential expression is associated with poorer progression-free survival in patients undergoing platinum and taxol-based chemotherapy (11). Furthermore, migratory ovarian cancer cells show increased KRT14 expression, which plays a crucial role at the invasive interface, facilitating ovarian cancer cell invasion (11).

The present study explored the role and underlying mechanisms of KRT14 in regulating cisplatin resistance in ovarian cancer. To achieve this, we assessed KRT14 expression levels and examined the effects of KRT14 knockdown on the half-maximal inhibitory concentration (IC_{50}), apoptosis, and the expression of MDR-related proteins in cisplatin-resistant ovarian cancer cell lines. Additionally, we utilized Illumina RNA sequencing to identify differentially expressed messenger RNA (mRNA) resulting from KRT14 knockdown to better understand the role of KRT14 in cisplatin resistance. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1795/rc).

Highlight box

Key findings

- Keratin 14 (KRT14) expression was significantly elevated in cisplatin-resistant ovarian cancer cell lines. KRT14 knockdown in SK-OV-3/DDP and A2780/DDP cells revealed 24 differentially expressed messenger RNAs. KRT14 knockdown reduced cisplatin resistance in these cell lines by lowering low-density lipoprotein receptor-related protein 11 expression levels.

What is known and what is new?

- Elevated KRT14 expression has been found in ovarian cancer tissues compared to normal ovarian tissues, which correlates with worse progression-free survival for patients receiving platinum and taxol-based chemotherapy. Additionally, migratory ovarian cancer cells exhibit higher levels of KRT14 expression.
- This research further investigates the role and mechanisms by which KRT14 regulates cisplatin resistance in ovarian cancer, with the goal of enhancing our understanding of how KRT14 plays a part in the treatment difficulties associated with this condition.

What is the implication, and what should change now?

- Present findings suggest that KRT14 could be a novel target for overcoming cisplatin resistance in ovarian cancer.

Methods

Cell lines

Human ovarian cancer cell lines SK-OV-3 (catalogue

Table 1 The primer sequences for the target genes

Primer name	Sequence (5'-3')
KRT14-F	CTCCCAGTTCTCCTCTGGAT
KRT14-R	TCAGTTCTTGGTGCGAAGG
LRP11-F	AGGAGGGAACCTACACCTTC
LRP11-R	GCAAGTGTGCAAACATCCTC
MTAP-F	GGCAAGAAGGACAGCAGTAA
MTAP-R	TGCATGCCGAGATTGTCTTT
NUDT16-F	GAAGAGGATGTGAGCACCAG
NUDT16-R	CCTCATTTCAGAGTCCCTGC
RBM15-F	AGAGGCCACTTAGGAACCTT
RBM15-R	ATCCGCTGTTCAACCAGTTT
GAPDH-F	GGGAAACTGTGGCGTGAT
GAPDH-R	GAGTGGGTGTCGCTGTTGA

F, forward primer; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; KRT14, keratin 14; LRP11, lipoprotein receptor-related protein 11; MTAP, methylthioadenosine phosphorylase; NUDT16, nudix hydrolase 16; R, reverse primer; RBM15, RNA binding motif protein 15.

number: MZ-0169) and A2780 (catalogue number: MZ-0614), and their cisplatin-resistant variants SK-OV-3/DDP (catalogue number: MZ-3291), and A2780/DDP (catalogue number: MZ-2815) were purchased from Ningbo Mingzhou Biotechnology Co., Ltd. (Ningbo, China). The cell lines were thawed, subcultured, and cryopreserved according to the instructions from supplier.

Cell transfection

Small interfering RNA (siRNA) targeting KRT14 (si-KRT14; target sequence: GCACCAAGGTCATGGATGT, catalogue number: stB0006898A) and negative control siRNA (si-NC; catalogue number: siN0000001-1-5) were purchased from Guangzhou Ribobio Co., Ltd. (Guangzhou, China). The full-length coding sequence of low-density lipoprotein receptor-related protein 11 (LRP11) isoform 1 precursor (NM_032832.6) was cloned into pcDNA3.1 to construct LRP11 overexpression plasmid (LRP11-OP). The transfection of si-NC, si-KRT14, empty plasmid pcDNA3.1 (EP), and LRP11-OP was performed using Lipofectamine™ 3000 transfection reagent (catalogue number: L3000150, Invitrogen, Carlsbad, CA, USA).

Fluorescent quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using Trizol reagent (catalogue number: 15596018CN, Invitrogen). After confirming that the purity and integrity of the extracted total RNA met experimental requirements, complementary DNA (cDNA) was synthesized using EasyScript First-Strand cDNA Synthesis SuperMix (catalogue number: AE301-02, TransGen Biotech, Beijing, China). The synthesized cDNA was served as the template for PCR reactions, and PCR reaction solution was prepared using PerfectStart® Green qPCR SuperMix (catalogue number: AQ601-01-V2, TransGen Biotech). Subsequently, PCR amplification was conducted on an ABI PRISM® 7500 Sequence Detection System. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was employed as an internal reference. The relative expression levels of the target genes were determined using the $2^{-\Delta\Delta Ct}$ method. The primer sequences for the target genes are listed in *Table 1*. Three independent experiments were performed with three technical replicates.

Western blotting

Total cellular protein was extracted using radioimmunoprecipitation assay buffer (catalogue number: P0013C, Beyotime, Shanghai, China) for lysis, followed by quantification using a bicinchoninic acid (BCA) protein assay kit (catalogue number: P0009, Beyotime). Thirty micrograms of the total protein were then loaded onto a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto a polyvinylidene difluoride (PVDF) membrane (catalogue number: 03010040001, Merck Millipore, Billerica, MA, USA). After washing the PVDF membrane with tris-buffered saline with Tween 20 (TBST), it was blocked with 5% non-fat milk at room temperature for 2 h. Subsequently, the membrane was incubated overnight at 4 ℃ with the primary antibodies against LRP11 (catalogue number: orb674844, Biorbyt, Cambridge, UK), KRT14 (orb1259684, Biorbyt), P-gp (catalogue number: orb11034, Biorbyt), and MRP1 (catalogue number: orb1150958, Biorbyt), following three washes with TBST for 8 min each. The goat anti-rabbit immunoglobulin G-horseradish peroxidase (HRP) secondary antibody (catalogue number: 4030-05, Southern Biotech, Birmingham, AL, USA) was then diluted and incubated at 37 ℃ for 50 min. After washing the membrane

with TBST three times for 8 min each, chemiluminescent detection was performed using immobilon western chemiluminescent HRP substrate (catalogue number: WBKLS0100, Merck Millipore, Billerica, MA, USA) in a darkroom, with the signal exposed onto X-ray film. Three independent experiments were performed with one technical replicate.

Measurement of cisplatin IC50

Cell suspensions for each group were dispensed into a 96-well plate and incubated with different concentrations of cisplatin (0, 2.5, 5, 10, 20, 40, and 80 μM) for 24 h in a humidified incubator at 37 ℃ with 5% CO_2. Cell counting kit-8 (CCK-8) solution (10 μL per well, catalogue number: CK04, Dojindo, Kumamoto, Japan) was then added to the plate. Next, the plate was incubated for 4 h in the incubator. Finally, the absorbance was measured at 450 nm using a microplate reader. The cell viability was calculated according to the absorbance values. The IC_{50} values were calculated using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Three independent experiments were performed with three technical replicates.

Detection of apoptotic cells by flow cytometry

Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (catalogue number: C1062S, Beyotime) was used to detect apoptotic cells by flow cytometry, Briefly, cells were centrifuged at 300 ×g for 5 min, the supernatant was discarded, and the cells were washed once with phosphate-buffered saline (PBS). The cells were then gently resuspended and counted. Next, 3×10^5 resuspended cells were centrifuged at 300 ×g for 5 min, the supernatant was discarded. The cells were washed with PBS and the supernatant was discarded again. The cell pellets were resuspended in 100 μL of diluted 1× Annexin V-binding buffer. Next, 2.5 μL of Annexin V- FITC reagent and 2.5 μL of propidium iodide (PI) reagent (50 μg/mL) were added to the cell suspension, and the mixture was gently vortexed. After incubating at room temperature in the dark for 15–20 min, 400 μL of diluted 1× Annexin V-binding buffer was added, and the sample was mixed. Finally, detection was immediately performed using flow cytometry analysis. Three independent experiments were performed with one technical replicate.

Detection of apoptotic cells using Hoechst 33258 staining

After washing twice with PBS, cells from each group were fixed with 4% paraformaldehyde for 20 min, followed by two washes with PBS to remove the fixative. Subsequently, the cells were incubated with 5 μg/mL Hoechst 33258 staining solution (catalogue number: BS116-25 mg, BioSharp, Hefei, China), ensuring complete coverage of the cells to be stained. After incubation at 37 ℃ in the dark for 20–30 min, the cells are washed twice with PBS and observed under a fluorescence microscope. Three independent experiments were performed with one technical replicate.

RNA sequencing

RNA was extracted using MagZol™ reagent (Axygen, Silicon Valley, CA, USA). Quality control included agarose gel electrophoresis, Nanodrop spectrophotometry, and Qubit fluorometry. Library preparation was simplified using a kit for double-strand synthesis, end repair, and dA-tailing. mRNA was enriched using VAHTS mRNA Capture Beads (Vazyme, Nanjing, China), fragmented, and used for double-stranded cDNA synthesis. The cDNA was purified and adapters were ligated. The libraries were evaluated using an Agilent Bioanalyzer and Qubit device. Sequencing on an Illumina Novaseq6000 (PE150 mode) provided high-quality data for bioinformatics analysis. Raw sequencing data underwent quality control analysis using fastp (v2.0) to remove adapters, trim low-quality reads (score <20), and exclude reads with >10% N content. Clean data were aligned to the reference genome using HISAT2 (v2.1.0) and assessed using RSeQC (v3.0.1). Expression was quantified using StringTie, and differential genes were analyzed using edgeR, using $|\log_2\text{fold change}| \geq 1$ and $P_{adj}<0.05$. The data were visualized using ggplot2, cluster, and heatmap2. All analyses were performed by Shanghai Origingene Bio-Pharm Technology Co., Ltd. (Shanghai, China) following academic protocols.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Data were presented as mean ± standard deviation. An unpaired *t*-test was used for comparisons between two groups, while one-way analysis of variance (ANOVA) was employed for comparisons among more than two groups.

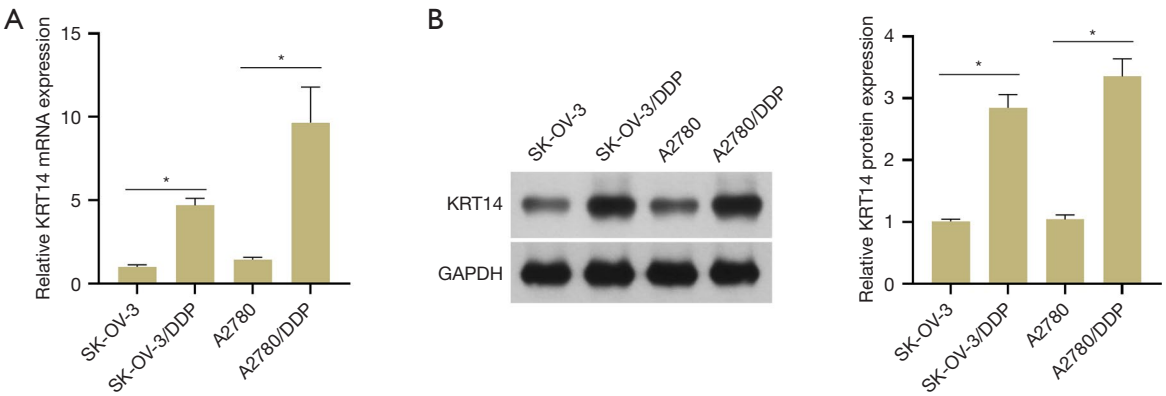

Figure 1 KRT14 expression levels in ovarian cancer cell lines SK-OV-3 and A2780 and their cisplatin-resistant variants SK-OV-3/DDP and A2780/DDP. (A) KRT14 mRNA expression levels detected using qRT-PCR. (B) KRT14 protein expression levels visualized using Western blot analysis. Right bar graph: KRT14 protein levels relative to GAPDH. *, statistical significance (P<0.05). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; KRT14, keratin 14; mRNA, messenger RNA; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

P<0.05 was considered statistically significant.

Results

Cisplatin-resistant cells reveal upregulated KRT14 expression

To investigate the role of KRT14 in the regulation of chemotherapy resistance in ovarian cancer, we first examined its expression levels in the SK-OV-3 and A2780 ovarian cancer cell lines and their cisplatin-resistant counterparts, SK-OV-3/DDP and A2780/DDP. Our results revealed significantly elevated KRT14 mRNA expression levels in the cisplatin-resistant SK-OV-3/DDP and A2780/DDP cells compared with their respective parental cell lines, SK-OV-3 and A2780, respectively (*Figure 1A*). Furthermore, KRT14 protein levels were also markedly increased in the cisplatin-resistant SK-OV-3/DDP and A2780/DDP cells (*Figure 1B*). These findings suggest a potential association between KRT14 upregulation and the acquisition of cisplatin resistance in ovarian cancer cells.

KRT14 knockdown reduces the IC50 value for cisplatin in cisplatin-resistant cells

To investigate the role of KRT14 in regulating cisplatin resistance, KRT14 levels in SK-OV-3/DDP and A2780/DDP cells were reduced through transfection with si-KRT14. The efficiency of KRT14 silencing was confirmed by qRT-PCR (*Figure 2A*) and western blot (*Figure 2B*) analyses. Subsequently, we assessed the viability of SK-OV-3/DDP and A2780/DDP cells, including those in the blank cells (untransfected cells), si-NC, and si-KRT14 groups, after treatment with varying concentrations of cisplatin (0, 2.5, 5, 10, 20, 40, and 80 μM). Cisplatin concentrations ranging from 2.5 to 80 μM effectively inhibited cell growth across all groups, with the si-KRT14 group exhibiting the lowest cell viability (*Figure 2C*). Based on these viability measurements, we calculated the IC_{50} values, with the fitting curves shown in *Figure 2D*. The IC_{50} values for SK-OV-3/DDP cells were 33.93 μM for blank cells, 32.55 μM for si-NC, and 13.22 μM for si-KRT14. For A2780/DDP cells, these values were 49.21, 50.75, and 24.04 μM, respectively. These results indicated that KRT14 knockdown decreased the cisplatin IC_{50} values in SK-OV-3/DDP and A2780/DDP cells.

KRT14 knockdown enhances apoptosis and down-regulates the expression of MDR-related proteins in cisplatin-resistant cells

The impact of KRT14 knockdown on apoptosis was assessed using flow cytometry and Hoechst 33258 staining. Both assays revealed significant increases in the apoptotic cell rates in cells treated with si-KRT14 compared with those treated with si-NC, suggesting that KRT14 knockdown enhanced apoptosis in SK-OV-3/DDP and A2780/DDP cells (*Figure 3A,3B*). Additionally, KRT14 knockdown reduced the expression levels of the MDR-related proteins P-gp and MRP1 (*Figure 3C*).

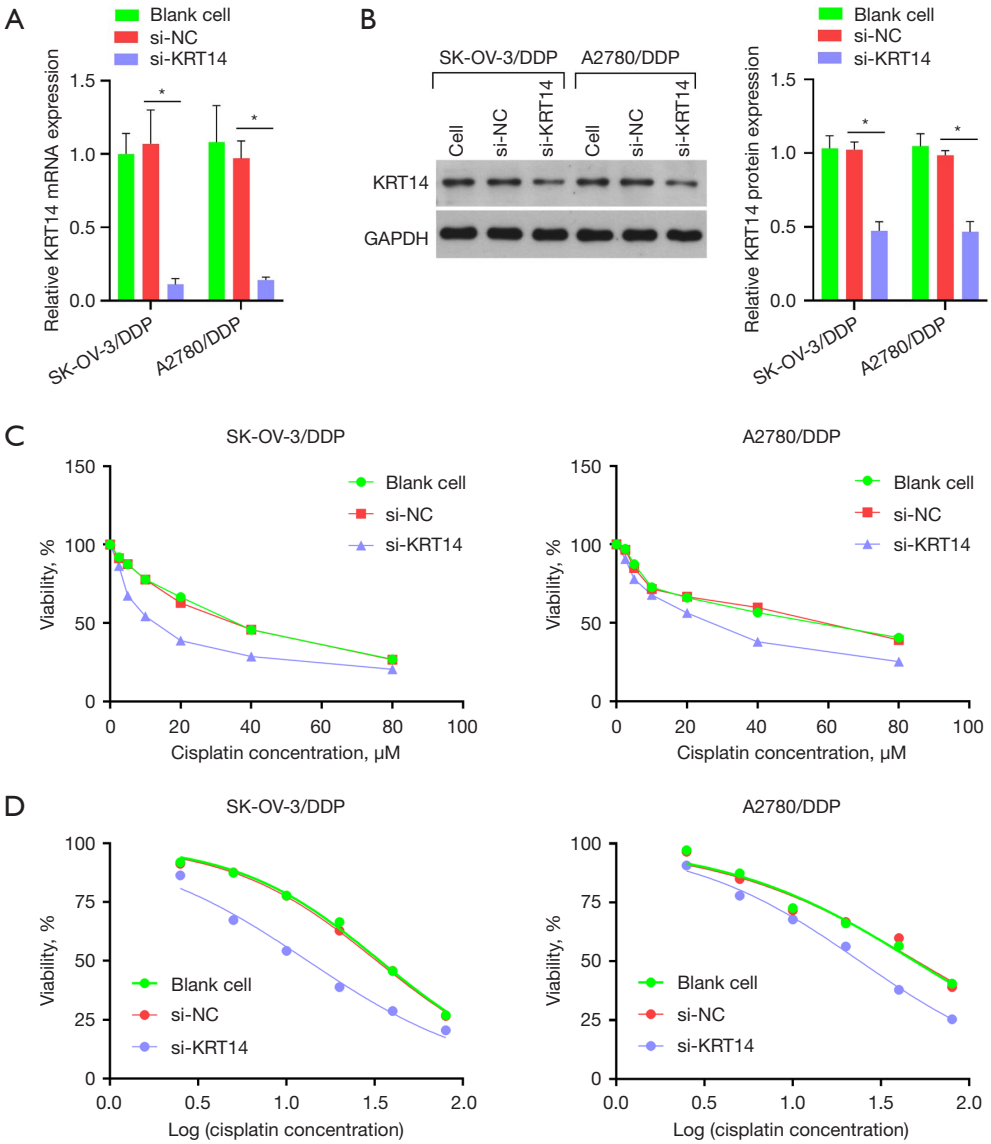

Figure 2 KRT14 knockdown reduces SK-OV-3/DDP and A2780/DDP cell viability. si-KRT14 was transfected into cells to achieve KRT14 knockdown, with cells transfected with si-NC and un-transfected cells (blank cells) serving as controls. (A) KRT14 mRNA levels. (B) KRT14 protein levels. Right bar graph: KRT14 protein levels relative to GAPDH. (C) Viability of SK-OV-3/DDP and A2780/DDP cells in the three groups after treatment with varying concentrations of cisplatin (0, 2.5, 5, 10, 20, 40, and 80 μM). (D) Fitting curves used to determine the IC_{50} values of SK-OV-3/DDP and A2780/DDP cells in response to cisplatin. *, statistical significance (P<0.05). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IC_{50}, half-maximal inhibitory concentration; KRT14, keratin 14; mRNA, messenger RNA; si-KRT14, siRNA targeting KRT14; si-NC, negative control siRNA; siRNA, small interfering RNA.

KRT14 knockdown reduces LRP11 expression in cisplatin-resistant cells

To explore how KRT14 regulates cisplatin resistance in ovarian cancer cells, we used Illumina RNA sequencing to identify differentially expressed mRNAs induced by KRT14 knockdown. The raw data have been deposited in the Gene Expression Omnibus (accession number GSE276216). In SK-OV-3/DDP cells, 200 mRNAs were up-regulated and 220 mRNAs were down-regulated after KRT14 knockdown compared to si-NC transfection. In A2780/DDP cells, 211 mRNAs were up-regulated and 229 mRNAs were down-

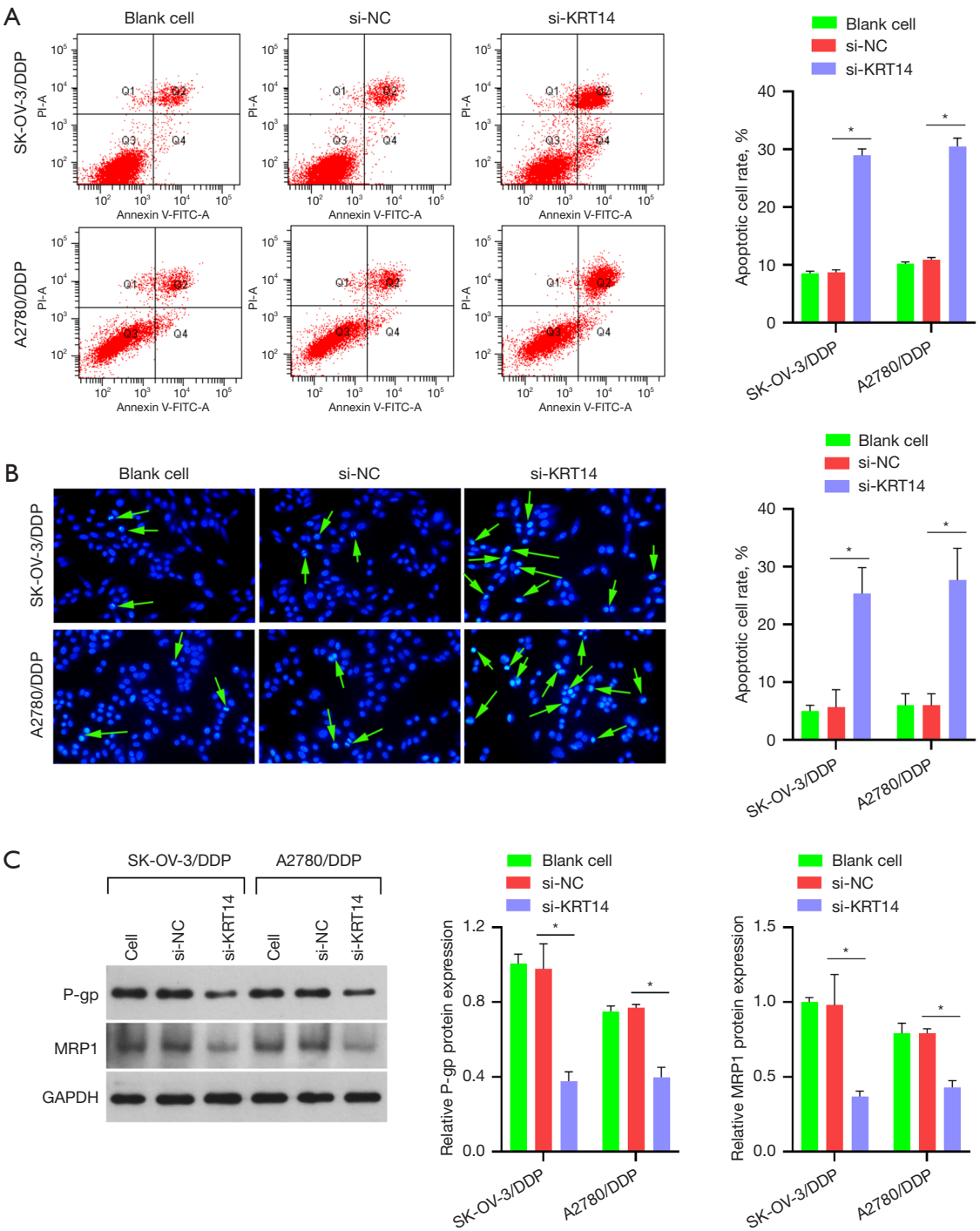

Figure 3 KRT14 knockdown increases apoptosis and decreases MDR-related protein expression in SK-OV-3/DDP and A2780/DDP cells. si-KRT14 was transfected into cells to achieve KRT14 knockdown, with cells transfected with si-NC and untransfected cells (blank cell) serving as controls. (A,B) Apoptotic cell rates assessed using flow cytometry and Hoechst 33258 staining, respectively. The magnification is 20× and the green arrows indicate the apoptotic cells. (C) P-gp and MRP1 protein levels. Right bar graph: P-gp and MRP1 protein levels relative to GAPDH. *, statistical significance (P<0.05). FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; KRT14, keratin 14; MDR, multidrug resistance; MRP1, multidrug resistance-associated protein 1; P-gp, P-glycoprotein; si-KRT14, siRNA targeting KRT14; si-NC, negative control siRNA; siRNA, small interfering RNA.

regulated under the same conditions (*Figure 4A*). Venn analysis revealed that 18 mRNAs were up-regulated and six mRNAs were down-regulated in both cell lines (*Figure 4B*). Among these 24 differentially expressed mRNAs, the top two up-regulated [nudix hydrolase 16 (NUDT16) and RNA binding motif protein 15 (RBM15)] and down-regulated mRNAs [methylthioadenosine phosphorylase (MTAP) and LRP11] were selected for further verification via qRT-PCR. Consistent with the RNA sequencing results, MTAP and LRP11 were down-regulated, while NUDT16 and RBM15 were up-regulated in both cell lines transfected with si-KRT14 compared to si-NC (*Figure 4C*), with LRP11 showing the most significant change. Western blot analysis further confirmed that KRT14 knockdown reduced LRP11 expression (*Figure 4D*).

LRP11 overexpression alleviates the effect of KRT14 knockdown on IC50 in cisplatin-resistant cells

To further investigate the role of LRP11 in KRT14-regulated cisplatin resistance in ovarian cancer cells, we examined the effects of LRP11 overexpression in SK-OV-3/DDP and A2780/DDP cells with and without KRT14 knockdown. LRP11 overexpression was achieved through transfection with LRP11-OP, which successfully elevated LRP11 expression levels (*Figure 5A*). Notably, LRP11 overexpression did not affect KRT14 expression (*Figure 5B*). LRP11 overexpression significantly increased cell viability after cisplatin treatment compared to cells transfected with an EP (*Figure 5C*). Specifically, cells in the si-KRT14 + LRP11-OP group exhibited higher viability than those in the si-KRT14 + EP group. Additionally, the IC_{50} values for each group were determined from the cell viability measurements, and the corresponding fitting curves are presented in *Figure 5D*. For SK-OV-3/DDP cells, the IC_{50} values were 34.35 μM for EP, 46.77 μM for LRP11-OP, 13.41 μM for si-KRT14 + EP, and 28.94 μM for si-KRT14 + LRP11-OP. For A2780/DDP cells, the IC_{50} values were 49.23 μM for EP, 66.17 μM for LRP11-OP, 24.54 μM for si-KRT14 + EP, and 44.90 μM for si-KRT14 + LRP11-OP. These results indicate that LRP11 overexpression increases the IC_{50} value, mitigating the effect of KRT14 knockdown on cisplatin resistance.

LRP11 overexpression alleviates the effects of KRT14 knockdown on apoptosis and MDR-related proteins in cisplatin-resistant cells

Both flow cytometry analysis and Hoechst 33258 staining demonstrated that LRP11 overexpression decreased the apoptotic cell rates in SK-OV-3/DDP and A2780/DDP cells (*Figure 6A,6B*). LRP11 overexpression also increased P-gp and MRP1 expression levels (*Figure 6C*). Additionally, the si-KRT14 + LRP11-OP group exhibited lower apoptotic cell rate and higher expression levels of P-gp and MRP1 than those in the si-KRT14 + EP group. These results indicated that LRP11 overexpression inhibited apoptosis and promoted the P-gp and MRP1 expression, thus mitigating the effect of KRT14 knockdown on apoptosis and MDR-related proteins.

Discussion

Cisplatin, a platinum-based chemotherapy drug, is a standard treatment for ovarian cancer (12,13). However, many patients eventually develop resistance to cisplatin, which can lead to cancer recurrence or metastasis, worsening the disease and diminishing patient quality of life. Addressing this issue requires a thorough understanding of the molecular mechanisms behind cisplatin resistance. Ongoing efforts are needed to identify new regulatory targets of cisplatin resistance to facilitate the development of drugs designed to increase cisplatin sensitivity. Therefore, the present study evaluated the potential role of KRT14 in regulating cisplatin resistance in ovarian cancer.

In this study, we observed increased KRT14 expression in cisplatin-resistant ovarian cancer cell lines. KRT14 knockdown results in a reduced cisplatin IC_{50} value, increased apoptosis, and decreased expression of MDR-related proteins in these resistant cells. Thus, elevated KRT14 levels may contribute to cisplatin resistance, and inhibiting KRT14 expression could help reduce cisplatin resistance in ovarian cancer. This conclusion is supported by the results of a study demonstrating a link between high KRT14 expression and decreased progression-free survival after platinum and taxol-based chemotherapy in patients with ovarian cancer (11). In addition, the oncogenic role of KRT14 has been reported in a variety of cancers, including triple-negative breast cancer and ovarian cancer (10,11). These studies also support our conclusion.

The results of the present study identified LRP11, also known as bA350J20.3 and MANSC3, as a downstream target of KRT14. Few studies have reported the functions of LRP11. The results of the Gene Ontology analysis in this study demonstrated its involvement in responses to heat, cold, water deprivation, mechanical stimulus, immobilization stress, and starvation. Recently, the roles of

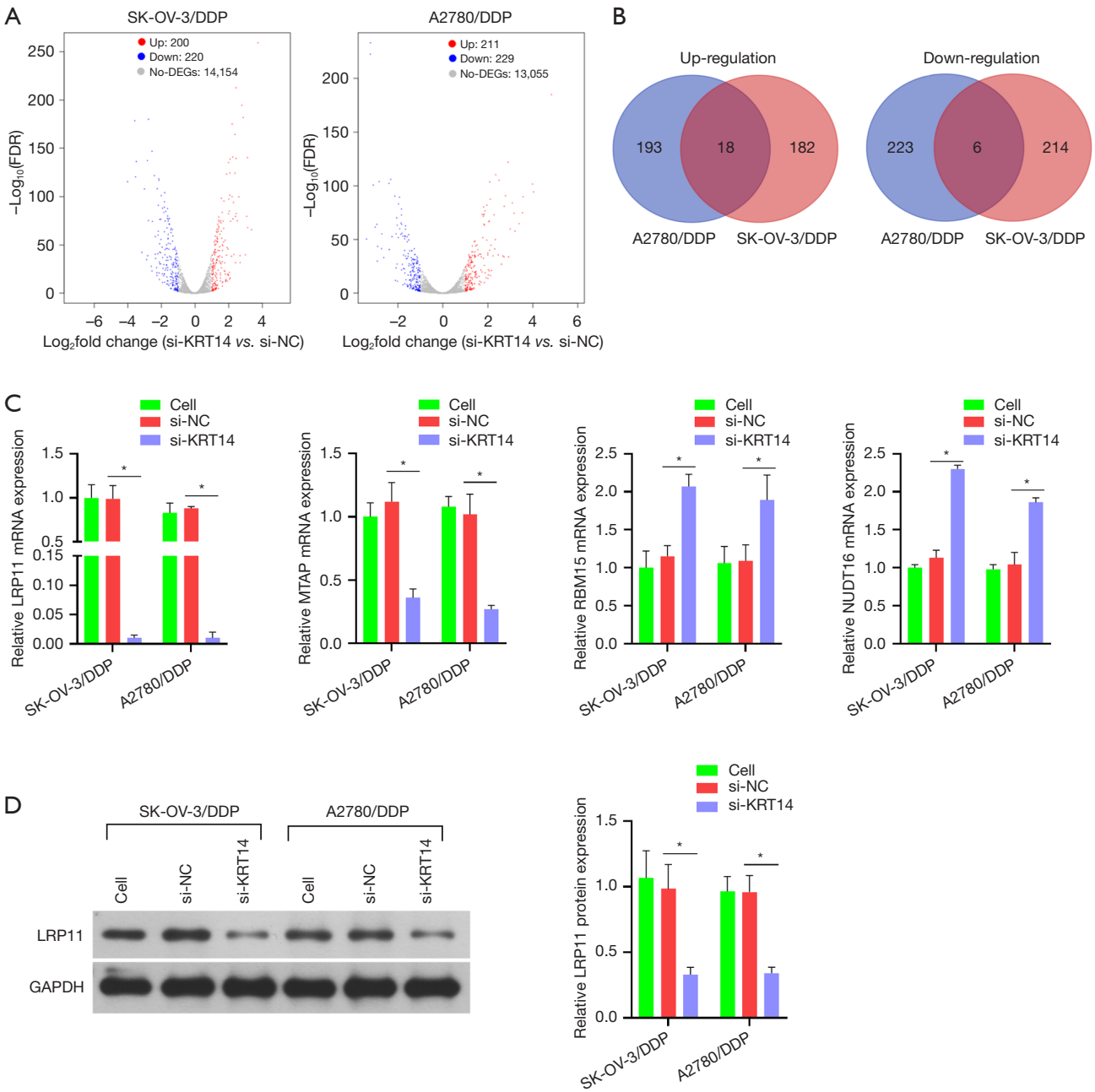

Figure 4 Effect of KRT14 knockdown on LRP11 expression. To achieve KRT14 knockdown, cells were transfected with si-KRT14, with cells transfected with si-NC used as a control. (A) Volcano plots of differentially expressed mRNAs identified by Illumina RNA sequencing following KRT14 knockdown. (B) Venn diagram of differentially expressed mRNAs in both SK-OV-3/DDP and A2780/DDP cells. (C) Expression levels of MTAP, LRP11, NUDT16, and RBM15 in KRT14 knockdown cells as measured by qRT-PCR. (D) Western blot results for LRP11 expression in KRT14 knockdown cells. Right bar graph: LRP11 protein levels relative to GAPDH. *, statistical significance ($P<0.05$). FDR, false discovery rate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; KRT14, keratin 14; LRP11, lipoprotein receptor-related protein 11; mRNA, messenger RNA; MTAP, methylthioadenosine phosphorylase; NUDT16, nudix hydrolase 16; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; RBM15, RNA binding motif protein 15; si-KRT14, siRNA targeting KRT14; si-NC, negative control siRNA; siRNA, small interfering RNA.

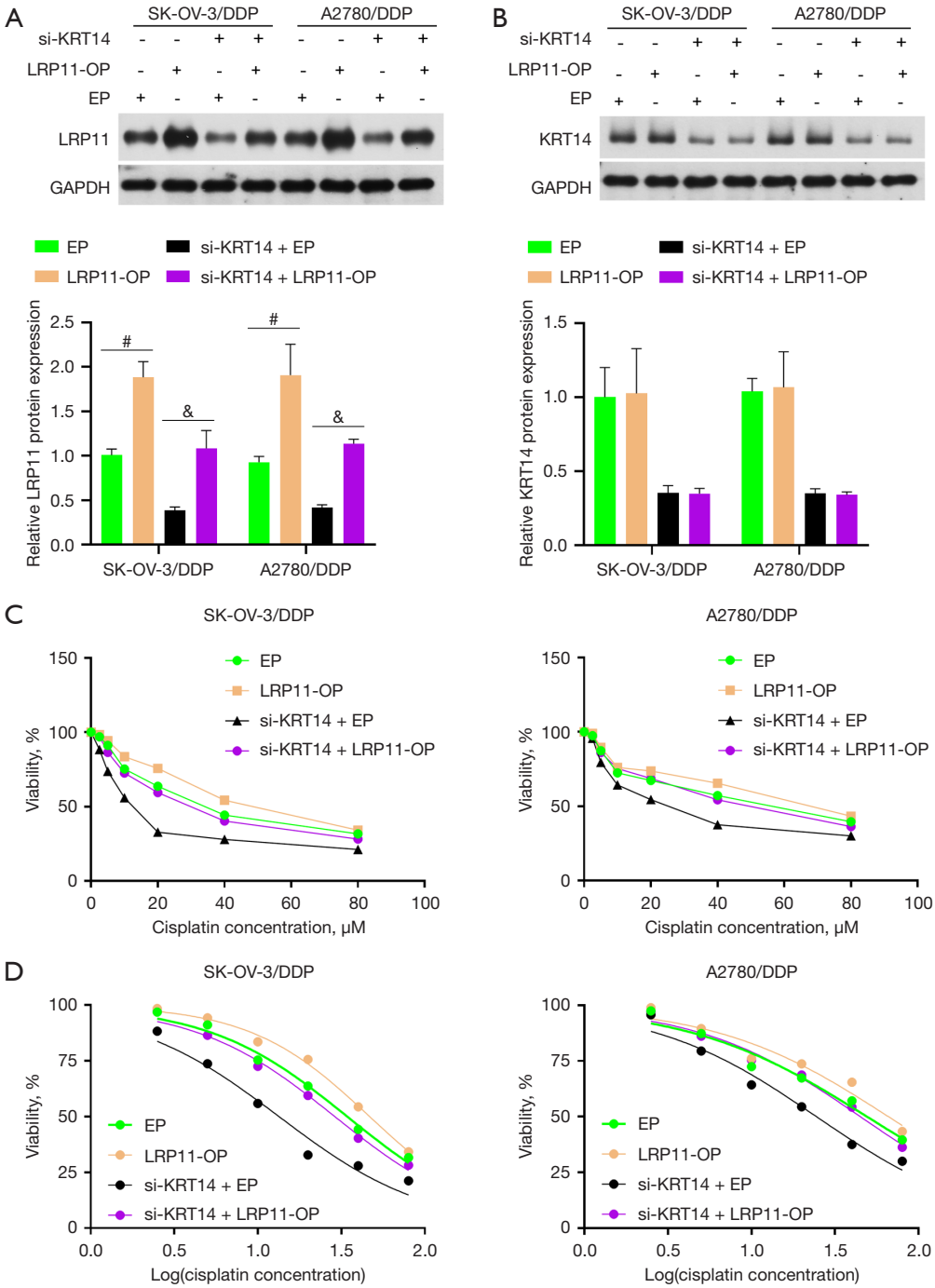

Figure 5 Effects of LRP11 overexpression on cell viability in SK-OV-3/DDP and A2780/DDP cells with and without KRT14 knockdown. Cells were transfected with si-KRT14 for KRT14 knockdown and LRP11-OP for LRP11 overexpression, while cells transfected with si-NC and EP served as controls. (A,B) LRP11 and KRT14 protein levels, with quantification relative to GAPDH provided below the graphs. (C) Viability of SK-OV-3/DDP and A2780/DDP cells in each group after treatment with various cisplatin concentrations (0, 2.5, 5, 10, 20, 40, and 80 μM). (D) Fitting curves used to determine the IC_{50} values for the cells in response to cisplatin. # and &, statistical significance ($P<0.05$). EP, empty plasmid pcDNA3.1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IC_{50}, half-maximal inhibitory concentration; KRT14, keratin 14; LRP11, lipoprotein receptor-related protein 11; LRP11-OP, LRP11 overexpression plasmid; si-KRT14, siRNA targeting KRT14; si-NC, negative control siRNA; siRNA, small interfering RNA.

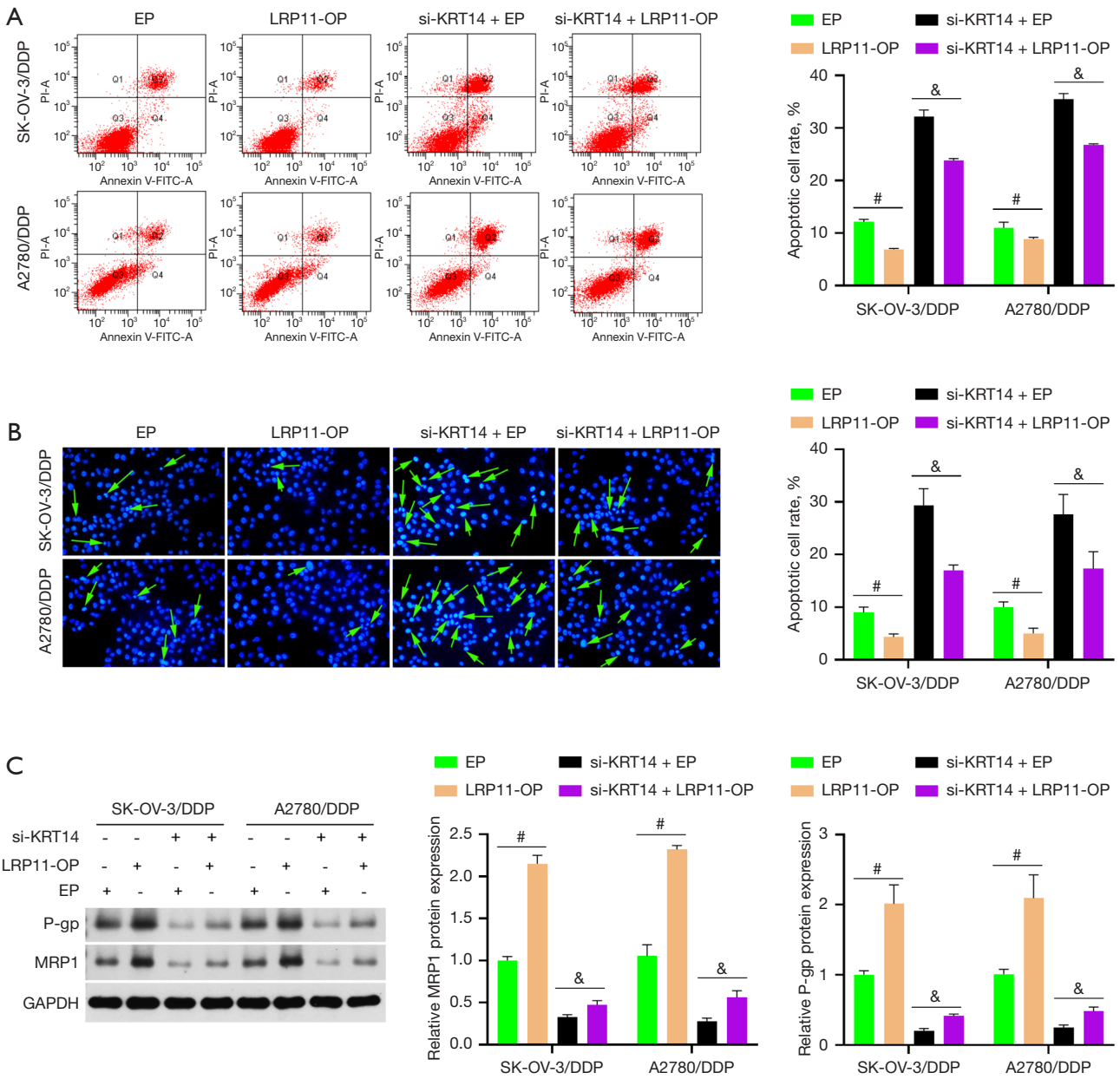

Figure 6 Effects of LRP11 overexpression on apoptosis and MDR-related protein expression in SK-OV-3/DDP and A2780/DDP cells with or without KRT14 knockdown. Cells were transfected with si-KRT14 for KRT14 knockdown and LRP11-OP for LRP11 overexpression, while cells transfected with si-NC and EP served as controls. (A) Apoptotic cell rates assessed using flow cytometry. (B) Apoptotic cell rates assessed using Hoechst 33258 staining. Magnification: 20×. The green arrows indicate the apoptotic cells. (C) P-gp and MRP1 protein levels. Right bar graph: P-gp and MRP1 protein levels relative to GAPDH. # and &, statistical significance ($P<0.05$). EP, empty plasmid pcDNA3.1; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; KRT14, keratin 14; LRP11, lipoprotein receptor-related protein 11; LRP11-OP, LRP11 overexpression plasmid; MDR, multidrug resistance; MRP1, multidrug resistance-associated protein 1; P-gp, P-glycoprotein; si-KRT14, siRNA targeting KRT14; si-NC, negative control siRNA; siRNA, small interfering RNA.

LRP11 in tumorigenesis have been reported. Bioinformatics analysis has revealed high LRP11 expression levels in a variety of tumors, which predicted worse survival in many cancer types (14-18). Furthermore, experimental findings have demonstrated its up-regulation and oncogenic role in prostate and breast cancers (19,20). In the present study, LRP11 overexpression increased the IC_{50} value for cisplatin, suppressed apoptosis, and enhanced the expression of MDR-related proteins in cisplatin-resistant cell lines. Thus, LRP11 may contribute to cisplatin resistance in ovarian cancer. Our findings were supported by the results of an analysis using the Genomics of Drug Sensitivity in Cancer database, which reported a positive correlation between LRP11 expression and the IC_{50} values of 159 anti-tumor drugs (15). This correlation suggests that patients with elevated LRP11 expression may exhibit resistance to a broad spectrum of anti-tumor drugs (15). Moreover, our results also demonstrated that KRT14 knockdown led to reduced LRP11 expression in cisplatin-resistant cells. Additionally, LRP11 overexpression counteracted the effects of KRT14 knockdown on IC_{50} values, apoptosis, and the expression of MDR-related proteins in these resistant cells. These results suggest that KRT14 might influence cisplatin resistance in ovarian cancer by modulating LRP11 levels. However, KRT14 knockdown in the cisplatin-resistant ovarian cancer cell lines revealed many differentially expressed genes. The relationship between KRT14 and these differentially expressed genes in cisplatin resistance requires additional study. Some studies have reported differences in metabolite levels between cisplatin-sensitive and resistant ovarian cancer cell lines (21,22). Kyoto Encyclopedia of Genes and Genomes pathway analysis revealed that MTAP, a gene that is significantly downregulated in KRT14-knockdown cells, is involved in cysteine and methionine metabolism, as well as metabolic pathways. This suggests that KRT14 may play a role in regulating metabolism, which could represent another molecular mechanism through which KRT14 contributes to cisplatin resistance.

Conclusions

We observed that KRT14 expression was significantly elevated in cisplatin-resistant ovarian cancer cell lines. Furthermore, KRT14 knockdown reduced cisplatin resistance in these cell lines by lowering LRP11 expression levels. These findings suggest that KRT14 could be a novel target for overcoming cisplatin resistance in ovarian cancer.

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None.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1795/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1795/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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