

LRPPRC contributes to the cisplatin resistance of lung cancer cells by regulating *MDR1* expression

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Abstract. The development of multidrug resistance is the major obstacle to successful lung cancer chemotherapy. Cancer cells gain resistance through increased levels of P-glycoprotein (P-gp), which is encoded by the multidrug resistance-associated protein 1 (*MDR1*) gene. Leucine-rich PPR motif-containing protein (LRPPRC), a member of the PPR family, has been verified to regulate the transcription of *MDR1*. This regulation is influenced by the methylation status of the GC -100 box in the *MDR1* promoter. The present study aimed to investigate the effect of LRPPRC on cisplatin (DDP) resistance in lung cancer cells and explore the underlying mechanism. DDP-resistant non-small cell lung cancer cell lines (A549/DDP, H1299/DDP) were generated. The expression levels of LRPPRC and P-gp/*MDR1*, investigated by qPCR and western blot analysis, were increased in the A549/DDP and H1299/DDP cells compared with that in the parental cells. LRPPRC silencing with shRNA increased DDP sensitivity *in vitro* and *in vivo*. LRPPRC silencing inhibited the level of

LRPPRC binding with the *MDR1* promoter, investigated by chromatin immunoprecipitation-qPCR, and the corresponding *MDR1* expression. Demethylation treatment rescued the decrease in the level of LRPPRC binding with *MDR1* and the corresponding expression of *MDR1* and the increase in DDP sensitivity due to LRPPRC silencing. Our study suggests that LRPPRC contributes to DDP resistance in lung cancer cells by regulating *MDR1* transcription. Thus, LRPPRC may serve as a potential molecular target for chemo-resistance reversal in lung cancer.

Introduction

Lung cancer ranks first among all emerging cancer cases and cancer-related deaths worldwide (1). Surgery is an important therapeutic method for early lung cancer. Surgery in combination with cisplatin (DDP)-based chemotherapy contributes to the long-term survival of patients. Chemotherapy is the main treatment for advanced and metastatic lung cancer. However, some patients still progress to relapse and metastasis. The development of multidrug resistance (MDR) is the major obstacle of successful cancer chemotherapy (2,3). Cancer cells can achieve MDR by transporting a broad range of cytotoxic drugs out of cells mediated through the overexpression of ATP-binding cassette (ABC) transporter proteins, thus reducing intracellular drug concentrations. Inhibition of the expression of ABC transporter proteins may reverse the MDR of cancer cells. Among the 48 known ABC transporter proteins, P-glycoprotein (P-gp) encoded by the *MDR1* gene, multidrug resistance-associated protein 1 (*MRP1*), breast cancer-resistant protein (*BCRP*) and lung resistance-related protein (*LRP*) are the four main efflux transporters that function in the MDR of cancers (4-6). Leucine-rich PPR motif-containing protein (LRPPRC), a member of the PPR family, is a multifunctional protein involved in mitochondrial gene transcription and tumorigenesis. LRPPRC mutation causes Leigh syndrome in French-Canadians. Many tumors highly express LRPPRC, which is associated with poor prognosis. Downregulation of LRPPRC inhibits the growth and induces the apoptosis of

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Abbreviations: LRPPRC, leucine-rich PPR motif-containing protein; DDP, cisplatin; NSCLC, non-small cell lung cancer; MDR, multidrug resistance; ABC, ATP-binding cassette; P-gp, P-glycoprotein; MRP1, multidrug resistance-associated protein 1; BCRP, breast cancer-resistant protein; LRP, lung resistance-related protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; qPCR, quantitative real-time polymerase chain reaction; ChIP, chromatin immunoprecipitation; IC₅₀, half-maximal inhibitory concentration

Key words: non-small cell lung cancer, multidrug resistance, cisplatin, *MDR1*, LRPPRC

tumor cells (7-10). More importantly, increasing evidence indicates that LRPPRC binds to the region of the *MDR1* gene promoter and regulates the transcription of *MDR1*. This regulation is influenced by the methylation status of the GC -100 box in the *MDR1* promoter (11,12). LRPPRC has been demonstrated to play functional roles in the tumorigenesis of lung cancer (9,13). In this study, it was demonstrated that downregulation of LRPPRC successfully reverses DDP resistance in lung cancer cells by regulating *MDR1* transcription.

Materials and methods

Cell culture. Human non-small cell lung cancer (NSCLC) cell lines A549 and H1299 were purchased from the Institute of Life Sciences Cell Resource Centre of the Chinese Academy of Sciences (Shanghai, China). DDP-resistant cell lines (A549/DDP and H1299/DDP) were generated by exposing these cell lines to increasing concentrations of DDP for several months. Cells were cultured in RPMI-1640 medium supplemented with 10% foetal bovine serum (FBS, Gibco; Thermo Fisher Scientific, Inc.), penicillin (100 U/ml) and streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO₂ at 37°C. The medium for DDP-resistant cells was further supplemented with DDP (1 µM).

Quantitative real-time polymerase chain reaction (qPCR). Total RNA was extracted from cells using Trizol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. One microgram of total RNA was converted to cDNA using TaKaRa PrimeScript RT reagent kit (Takara Bio, Inc.). The mRNA levels of *LRPPRC*, *MDR1*, *MRP1*, *BCRP* and *LRP* were analyzed through qPCR using an ABI 7500HT Fast Real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with 50 cycles of 20 sec at 95°C, 30 sec at 60°C and 30 sec at 72°C. GAPDH was used as the internal control. The 2^{-ΔΔC_q} method was used to analyze the data (14). Each sample was run in triplicate. The primers used are presented in Table S1.

Western blot analysis. Cellular total protein was extracted with radioimmunoprecipitation assay buffer containing protease inhibitors. Protein concentrations were investigated by bicinchoninic acid protein assay (Sigma-Aldrich; Merck KGaA). Equal amounts of protein lysates were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride membranes (EMD Millipore). The membranes were incubated with primary antibodies, including anti-LRPPRC (Abcam, ab97505, diluted 1:1,000), anti-P-gp (Abcam, ab262880, diluted 1:1,200) and anti-β-actin (Abcam, ab8227, diluted 1:1,000), overnight at 4°C and then with horseradish peroxidase-conjugated secondary antibodies (Boster, BA1054, diluted 1:5,000) for 1 h at room temperature. Visualization was performed using an enhanced chemiluminescence detection system (EMD Millipore). The expression of the target proteins relative to that of β-actin was determined via densitometric analysis using ImageJ software (National Institutes of Health, version 1.52n). Experiments were repeated in triplicate.

Cell transfection. A549/DDP and H1299/DDP cells were transfected with lentiviral vectors expressing the LRPPRC-specific short hairpin (shRNA) for LRPPRC silencing (Shanghai Genechem Co., Ltd.; forward, 5'-CCG GCCAUCUCGCUGCAGUCUAUUTTCTCGAGAUAGACUG CAGCGAGAUGGTTTTTTT-3' and reverse, 5'-AAT TCAAAAACCAUCUCGCUGCAGUCUAUUTTCTCGAGAU AGACUGCAGCGAGAUGGTT-3'). The shRNA lentiviral control vectors were used. Cells were transfected using X-tremeGENE HP DNA Transfection Reagent (Roche Diagnostics GmbH) as recommended by the manufacturer. After transfection, A549/DDP cells with stable endogenous LRPPRC silencing were obtained after treatment with 5 µg/ml of puromycin (Sigma-Aldrich; Merck KGaA).

Cell proliferation assay. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay was used to evaluate the proliferation capacity of the cells. Cells were incubated with 0.5 mg/ml MTT (Sigma-Aldrich; Merck KGaA) for 4 h at 37°C and mixed with 150 µl of dimethylsulphoxide (Sigma-Aldrich; Merck KGaA) to dissolve the generated formazan. Cell absorbance at 570 nm was measured using a microplate reader (Bio-Rad Laboratories, Inc.). Proliferation inhibition rate (%) = (1-Experimental optical density [OD]/Control OD) x100%. The half-maximal inhibitory concentration (IC₅₀) values of DDP for the inhibition of cell proliferation were calculated. Experiments were repeated in triplicate.

Flow cytometry. The effects of LRPPRC inhibition on apoptosis were determined through flow cytometry. A549/DDP and H1299/DDP cells were transfected with shLRPPRC or shControl for 24 h and then cultured with medium containing DDP (IC₂₀ concentration) for 48 h. Cells were stained with a combination of Annexin V-FITC and 7-AAD/PI for 15 min at 37°C in the dark to detect apoptosis, and the percentages of apoptotic cells were analyzed through flow cytometry using FACSCalibur (BD Biosciences; Becton, Dickinson and Company). Experiments were repeated in triplicate.

Chromatin immunoprecipitation-qPCR. Chromatin immunoprecipitation (ChIP) assay was evaluated through a Simple ChIP enzymatic ChIP kit (Cell Signalling Technology, Inc.) according to the manufacturer's protocol. Anti-LRPPRC antibody (Santa Cruz Biotechnology, Inc., sc-166178) was used to perform immunoprecipitation. Nonspecific mouse IgG was used as the control. DNA was extracted from bound fractions according to Abcam protocol. Then, the immunoprecipitated DNA was amplified using the sequence primers of the *MDR1* promoter (*MDR1*p (F) 5'-GCTGATGCGCGTTTCTCTACT-3' and *MDR1*p (R) 5'-CCGGGCCGGGAGCAGTCATC-3'). DNA amplification was quantified by qPCR analysis, and the percentage of DNA brought down by ChIP (percent input) was calculated. Experiments were repeated in triplicate.

Animal studies. Eighteen male BALB/c nude mice weighing 19-22 g, 6 weeks of age, were obtained from Silaike Experimental Animal Company, and housed in an air-conditioned room with a temperature of 23±1°C, relative humidity of 55±5% and a 12-h light/dark cycle. The bedding, food and

water were autoclaved. All the animals were acclimatized for 7 days. The mice were frequently assessed by animal care staff using daily inspections and health records. The Ethics Committee of the First Affiliated Hospital of Xi'an Medical University approved the protocol (no. 20180606). The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The mice were randomly divided into three groups ($n=6$ mice/group), including the A549/DDP, shControl-A549/DDP and shLRPPRC-A549/DDP groups. In each group, 5×10^6 cells were injected subcutaneously into the right back flank of each mouse. When xenografts had grown to a size of 100–200 mm³, the mice were treated with DDP (10 mg/kg) every week by intraperitoneal injection for 4 weeks. The volumes of the implanted tumors were measured twice a week. After 4 weeks of treatment with DDP, all of the mice were euthanized by intravenous injection of sodium pentobarbital (150 mg/kg), and tumor weights were measured.

Immunohistochemical examination. Immunohistochemistry (IHC) was performed to detect the expression of the indicated proteins in the xenograft tumors. Sections (4- μ m thick) were deparaffinized and dehydrated. The slides were incubated in 3% hydrogen peroxide solution for 10 min, washed with PBS buffer and then incubated for 15 min with normal goat serum. The sections were incubated with the primary antibodies, including anti-LRPPRC (Abcam, ab97505, diluted 1:500) and anti-P-gp antibody (Abcam, ab262880, diluted 1:500), overnight at 4°C. Thereafter, the sections were incubated for 40 min with an anti-rabbit secondary antibody (SA1050, Boster) and stained with diaminobenzidine (DAB; Boster). The negative control was processed identically but without the primary antibody.

Statistical analysis. Data are expressed as mean \pm standard deviation. Differences among the groups were analyzed by Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test using SPSS version 19.0 (SPSS, Inc.), and $P < 0.05$ was used to indicate a statistically significant difference.

Results

Overexpression of LRPPRC and MDR1 in A549/DDP and H1299/DDP cells. The DDP-resistant cell lines A549/DDP and H1299/DDP were established by long-term continuous exposure of the original DDP-sensitive cells to DDP. The proliferation inhibition of cells was investigated using MTT assay. Cells were cultured with medium containing different concentrations of DDP (1.7, 3.4, 6.8, 13.6, 27.2, 54.4, and 108.8 μ M) for 48 h. Then, MTT assay was performed, and IC₅₀ values were calculated. The cytotoxicity of DDP significantly differed between the DDP-resistant cells and their parental cells (Fig. 1). The IC₅₀ of DDP in the resistant cells was significantly higher when compared with that in the parental cells (Table I). The mRNA expression of LRPPRC and MDR-related efflux ABC transporter proteins, including MDR1, BCRP, MRP1 and LRP, in DDP-resistant and parental cells was investigated using qPCR. The mRNA

Table I. The IC₅₀ values of the lung cancer cells.

Cell line	IC ₅₀ (mM)		P-value
	Parental cells	Resistant cells	
A549	7.0 \pm 1.0	39.3 \pm 5.7	<0.01
H1299	11.7 \pm 2.7	54.0 \pm 11.0	<0.01

IC₅₀, half-maximal inhibitory concentration.

expression of LRPPRC (~3.2-fold change in A549/DDP and 2.4-fold change in H1299/DDP) and MDR1 (~2.8-fold change in A549/DDP and 2.3-fold change in H1299/DDP) was significantly increased in the resistant cells relative to that in the parental cells (Fig. 2A). The expression changes in LRPPRC and P-gp encoded by the MDR1 gene were confirmed through western blot analysis, and the results indicated significant increases in LRPPRC and P-gp expression in the A549/DDP and H1299/DDP cells compared with those in their parental cells (Fig. 2B and C).

LRPPRC silencing increases DDP sensitivity in vitro and in vivo. The effect of LRPPRC silencing on DDP sensitivity was investigated using MTT assay. The proliferation inhibition of tumor cells treated with different concentrations of DDP treatment for 48 h in combination with shLRPPRC was investigated, and IC₅₀ values were calculated. LRPPRC silencing significantly increased the DDP sensitivity of A549/DDP and H1299/DDP cells. The IC₅₀ values of DDP in the LRPPRC-silenced resistant cells were significantly decreased compared with those in LRPPRC-normal expression cells (9.3 \pm 1.7 vs. 41.1 \pm 3.9 μ M in A549/DDP cells; 17.0 \pm 3.2 vs. 54.5 \pm 4.0 μ M in H1299/DDP cells, $P < 0.01$, Fig. 3A). The percentage of apoptosis induced by DDP in LRPPRC-silenced A549/DDP and H1299/DDP cells was analyzed using flow cytometry. LRPPRC silencing synergized with DDP in increasing the percentage of apoptotic cells (Fig. 3B). We verified our results *in vitro* by examining the effect of LRPPRC silencing on DDP sensitivity in A549/DDP xenograft tumors. After DDP treatment for 4 weeks, the tumor volumes and weights of LRPPRC-silenced cells were significantly decreased compared with those of the shControl and mock groups (Fig. 3C). These findings indicate that downregulation of LRPPRC reverses DDP resistance in lung cancer cells.

LRPPRC silencing inhibits MDR1 expression. The mRNA expression of MDR1 was investigated in LRPPRC-silenced A549/DDP and H1299/DDP cells using qPCR. MDR1 mRNA levels were significantly decreased in LRPPRC-silenced resistant cells compared with that in LRPPRC-normal expression cells. Changes in P-gp expression were confirmed through western blot analysis (Fig. 4A and B). The *in vitro* results were verified through IHC in A549/DDP-derived xenograft tumors. P-gp expression was decreased in the tumors derived from LRPPRC-silenced cells compared with that in the shControl group (Fig. 4C).

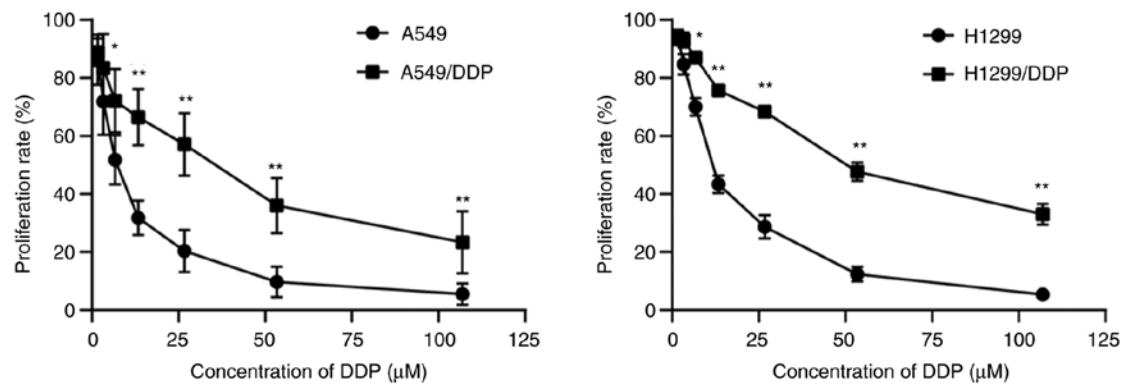


Figure 1. Analysis of the inhibition of proliferation of DDP-resistant lung cancer (A549/DDP and H1299/DDP) and parental (A549 and H1299) cells using MTT assay. Cells were treated with different concentrations of DDP (1.7, 3.4, 6.8, 13.6, 27.2, 54.4, and 108.8 μ M) for 48 h. Data are shown as mean \pm SD. * P <0.05; ** P <0.01 vs. the parental cells. DDP, cisplatin.

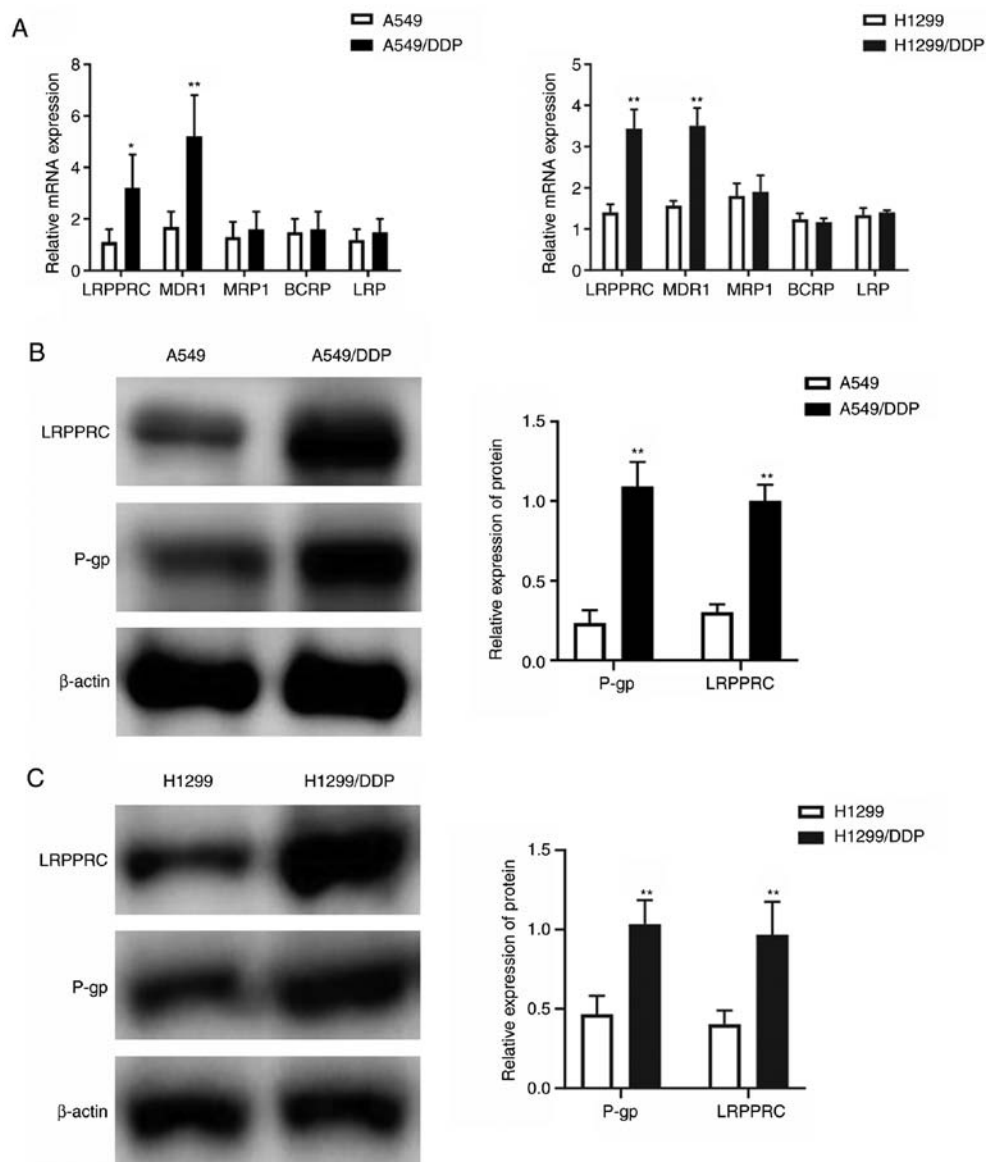


Figure 2. Expression of LRPPRC and MDR-related genes and proteins in DDP-resistant (A549/DDP and H1299/DDP) and parental (A549 and H1299) lung cancer cells. (A) The mRNA levels of LRPPRC and MDR-related genes were determined by qPCR. (B) Left panel: Expression levels of LRPPRC and P-gp proteins were investigated using western blot analysis in the A549 cell line. Right histogram: Data are the quantitative results of the left panel. (C) Left panel: Expression levels of LRPPRC and P-gp proteins were investigated using western blot analysis in the H1299 cell line. Right histogram: Data are the quantitative results of the left panel. Data are shown as mean \pm SD. * P <0.05; ** P <0.01 vs. parental cells. LRPPRC, leucine-rich PPR-motif-containing protein; MDR1, multidrug resistance 1; MRP1, multidrug resistance-associated protein 1; BCRP, human breast cancer resistance protein; DDP, cisplatin; MDR, multidrug resistance; P-gp, p-glycoprotein.

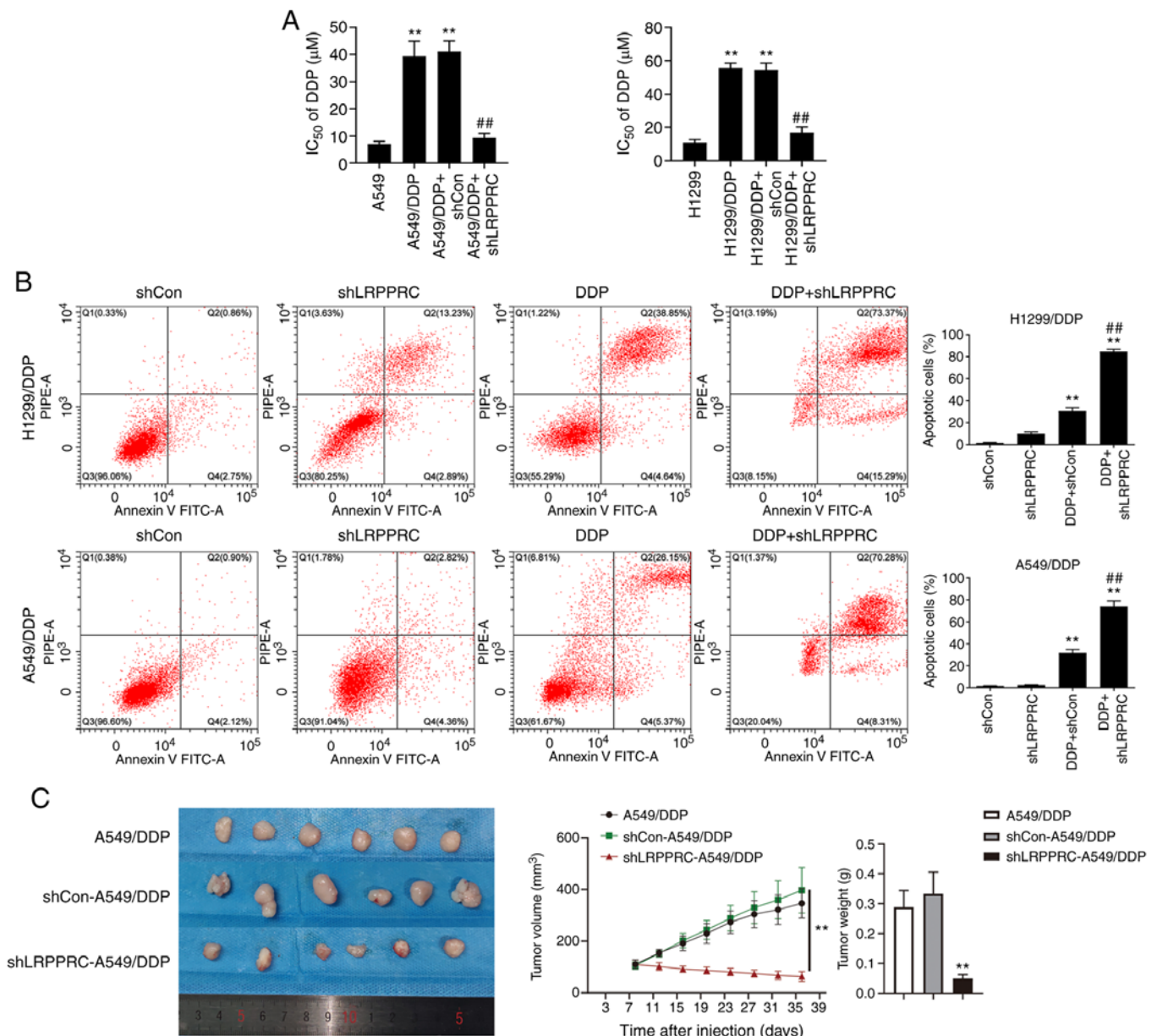


Figure 3. LRPPRC silencing increases the DDP sensitivity of DDP-resistant lung cancer cells *in vitro* and *in vivo*. (A) Effects of LRPPRC silencing on the IC₅₀ value of DDP using MTT assay. Data are shown as mean ± SD. **P<0.01 vs. the parental cells; ##P<0.01 vs. the resistant cells + shCon group. (B) Effects of LRPPRC silencing on apoptosis induced by DDP using flow cytometry. Left: Representative dot plots show the apoptotic status of cells with different treatments. Right: Percentages of apoptotic cells. Cells were treated with shLRPPRC, DDP or their combination. Data are shown as mean ± SD. **P<0.01 vs. the shCon or shLRPPRC group; ##P<0.01 vs. the DDP+shCon group. (C) Effects of LRPPRC silencing on DDP sensitivity in A549/DDP cell-derived tumors. Left panel: Images of the implanted tumors. Middle graph: Tumor growth curves. Right: Histogram representing mean tumor weights. BALB/c nude mice were divided into the A549/DDP, shCon-A549/DDP and shLRPPRC-A549/DDP groups and treated with DDP. Data are shown as mean ± SD (n=6/group). **P<0.01, shLRPPRC-A549/DDP vs. shCon-A549/DDP or A549/DDP group. LRPPRC, leucine-rich PPR-motif-containing protein; DDP, cisplatin; IC₅₀, half-maximal inhibitory concentration; DDP, cisplatin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide.

Demethylation treatment counteracts the effect of LRPPRC silencing. We investigated whether demethylation treatment could counteract the effect of LRPPRC silencing in lung cancer cells using the demethylation agent 5-Aza-dC (Sigma-Aldrich; Merck KGaA; cat. no. A3656). The viability of A549/DDP cells was decreased by approximately 10% after 1 μM 5-Aza-dC treatment (data not shown). Therefore, we used 0.5 μM 5-Aza-dC in the subsequent experiments. A549/DDP cells were treated with 5-Aza-dC combined with shLRPPRC for 48 h, and the resulting *MDR1* expression was investigated using

qPCR. 5-Aza-dC treatment rescued the decrease in *MDR1* mRNA level mediated by LRPPRC silencing (Fig. 5A). P-gp expression changes were confirmed by western blot analysis (Fig. 5B). The interaction between LRPPRC and the *MDR1* promoter was evaluated using ChIP-qPCR. The level of LRPPRC binding with the *MDR1* promoter showed a 4.0-fold increase in A549/DDP cells compared with that in the parental cells (Fig. 5C). 5-Aza-dC treatment rescued the decrease in level of LRPPRC binding with the *MDR1* promoter in A549/DDP cells brought about by LRPPRC silencing (Fig. 5D).

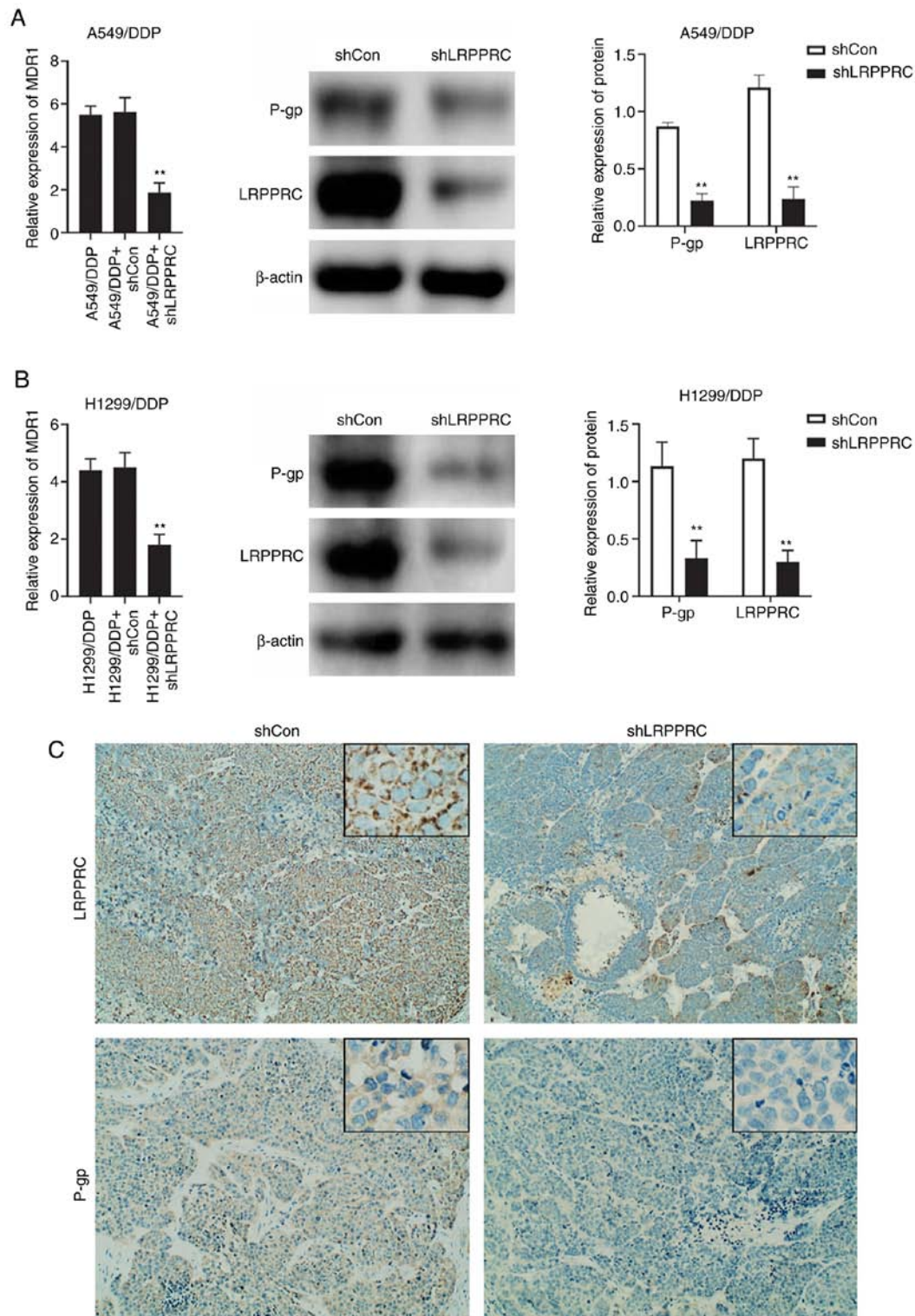


Figure 4. LRPPRC silencing decreases *MDR1*/P-gp levels in DDP-resistant lung cancer cells. (A) Left graph: mRNA levels of *MDR1* were determined using qPCR in the A549/DDP cell line. Middle panel: Expression level of P-gp protein was investigated using western blot analysis. Right graph: Data are the quantitative results of the middle panel. (B) Left graph: mRNA levels of *MDR1* were determined using qPCR in the H1299/DDP cell line. Middle panel: Expression of P-gp protein was investigated using western blot analysis. Right graph: Data are the quantitative results of the middle panel. (C) Representative LRPPRC and P-gp staining of A549/DDP-derived tumors from the LRPPRC-silenced (shLRPPRC) and control (shCon) groups. Magnification, $\times 100$ and $\times 400$. Data are shown as mean \pm SD. ** $P < 0.01$ vs. shControl. LRPPRC, leucine-rich PPR-motif-containing protein; DDP, cisplatin; *MDR1*, multidrug resistance-associated protein 1; P-gp, p-glycoprotein.

A549/DDP cells were pre-treated with $0.5 \mu\text{M}$ 5-Aza-dC for 3 weeks and then treated with different concentrations of DDP in combination with shLRPPRC for 48 h to investigate whether demethylation treatment could affect

DDP sensitivity regulated by LRPPRC. The inhibition of cell proliferation was evaluated using MTT assay, and IC_{50} values were calculated. The IC_{50} values of DDP of the LRPPRC-silenced cells treated with 5-Aza-dC increased

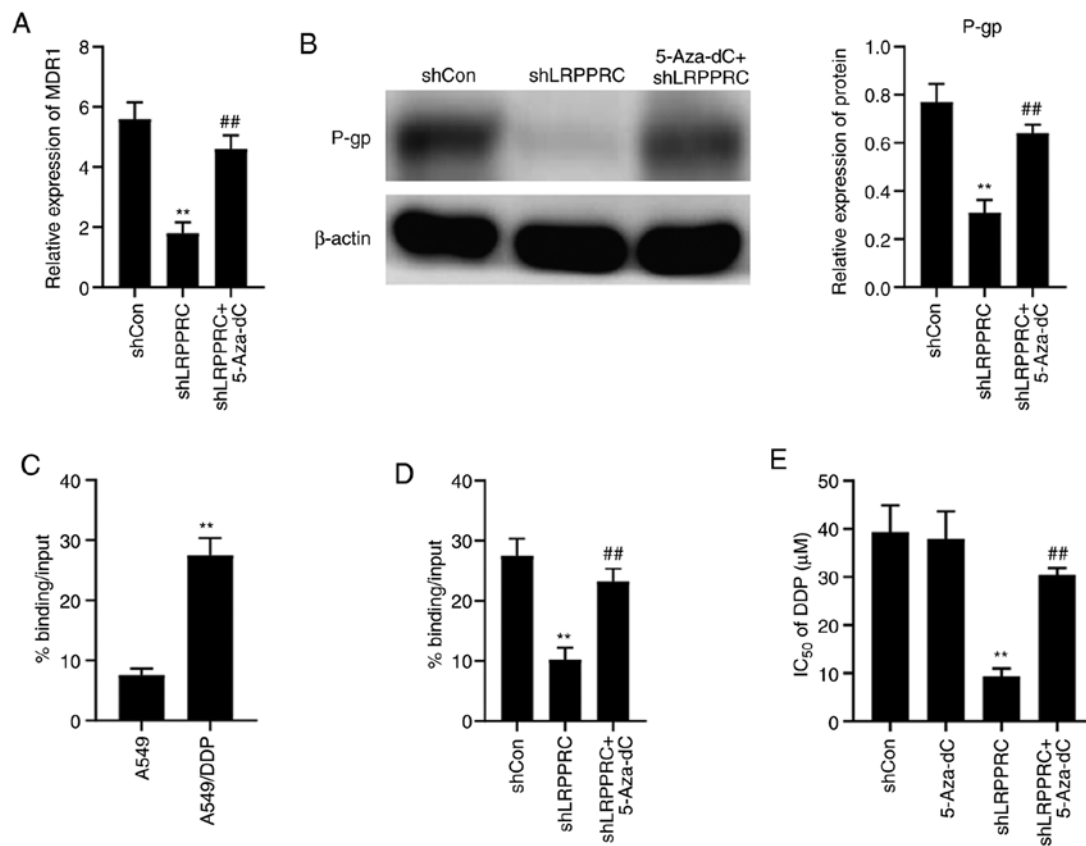


Figure 5. Demethylation treatment counteracts the effect of LRPPRC silencing. A549/DDP cells were treated with shLRPPRC or shLRPPRC+5-Aza-dC. (A) The mRNA levels of *MDR1* were determined using qPCR. ** $P < 0.01$ vs. the shCon group; ## $P < 0.01$ vs. the shLRPPRC group. (B) Left panel: Expression of P-gp protein was investigated using western blot analysis. Right graph: Data are the quantitative results of the left panel. ** $P < 0.01$ vs. the shCon group; ## $P < 0.01$ vs. the shLRPPRC group. (C) LRPPRC binding to *MDR1* was quantified in A549/DDP and parental cells using CHIP-qPCR. ** $P < 0.01$ vs. the parental cells. (D) Effects of 5-Aza-dC on LRPPRC binding to *MDR1* in A549/DDP cells using CHIP-qPCR. ** $P < 0.01$ vs. shCon; ## $P < 0.01$ vs. shLRPPRC. (E) Effects of 5-Aza-dC on the IC₅₀ value of DDP in A549/DDP cells using MTT assay. ** $P < 0.01$ vs. the shCon/5-Aza-dC; ## $P < 0.01$ vs. shLRPPRC. Data are shown as mean \pm SD. LRPPRC, leucine-rich PPR-motif-containing protein; MDR1, multidrug resistance-associated protein 1; P-gp, p-glycoprotein; DDP, cisplatin; IC₅₀, half-maximal inhibitory concentration; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide.

compared with the LRPPRC-silenced cells treated with PBS (Fig. 5E). These results indicate that demethylation treatment decreases LRPPRC silencing-induced increase in DDP sensitivity.

Discussion

Chemotherapy is an important therapeutic strategy for lung cancer. Although chemotherapy can improve patient survival, the development of resistance is generally inevitable, ultimately causing relapse and metastasis. Therefore, a better understanding of the relevant resistance mechanisms and identification of agents that reverse resistance are emergent issues concerning lung cancer.

Lung cancer highly expresses the leucine-rich PPR-motif-containing protein (LRPPRC). Downregulation of LRPPRC was found to decrease the anti-apoptotic, invasive and colony-forming abilities of lung adenocarcinoma cells (9). Overexpression of P-glycoprotein (P-gp) encoded by the multidrug resistance-associated protein 1 (*MDR1*) gene is one of the major mechanisms of cancer resistance. LRPPRC promotes *MDR1* transcription by binding with the *MDR1* promoter (11,12). Thus, we investigated whether LRPPRC plays a role in the chemo-resistance of lung cancer cells. We

successfully established two DDP-resistant lung cancer cell lines, including A549/DDP and H1299/DDP, via long-term DDP exposure of the cells. The obtained cells were more resistant to DDP than their parental cells. We compared the expression of LRPPRC and MDR-related proteins between DDP-resistant and parental cells using qPCR. *LRPPRC* and *MDR1* mRNA levels were significantly upregulated in A549/DDP and H1299/DDP cells. These results were confirmed by western blot analysis, thus revealing that long-term exposure of DDP increased *MDR1* expression and the LRPPRC overexpression may contribute to DDP resistance. We further determined whether downregulation of LRPPRC could increase DDP sensitivity. Downregulation of LRPPRC remarkably promoted the inhibition of proliferation and increased apoptosis of resistant cells induced by DDP. The results were verified by *in vivo* experiments, which showed that LRPPRC silencing improves DDP sensitivity in implanted A549/DDP tumors. Taken together, our findings indicate that LRPPRC contributes to DDP resistance in lung cancer cells.

LRPPRC has been demonstrated to be a transcription factor involved in the regulation of *MDR1* expression through *inMED1* binding sites in the promoter. This regulation is affected by the methylation status of *MDR1* promoter GC -100 box (11). We investigated changes in the mRNA levels

of MDR-related genes, including *MDR1*, *BCRP*, *MRP1* and *LRP*, in LRPPRC-silenced A549/DDP and H1299/DDP cells. LRPPRC suppression significantly decreased the mRNA levels of *MDR1* but had no effect on *BCRP*, *MRP1* and *LRP* expression. These results were confirmed by western blot analysis. We further investigated the effect of demethylation treatment on *MDR1* expression. Demethylation treatment using 5-Aza-dC rescued the decreased *MDR1* expression caused by LRPPRC silencing. Next, we compared the level of LRPPRC binding with the *MDR1* promoter between A549/DDP and parental cells and found that the level of LRPPRC binding with the *MDR1* promoter was significantly upregulated in resistant cells. Demethylation treatment rescued the decreased level of LRPPRC binding with the *MDR1* promoter in resistant cells due to LRPPRC silencing. We investigated whether demethylation treatment could affect DDP sensitivity regulated by LRPPRC and found that this treatment decreased the LRPPRC-silenced-mediated increase in DDP sensitivity. These results suggest that LRPPRC regulates *MDR1* transcription, which contributes to the chemo-resistance of lung cancer cells.

The effect of LRPPRC on multidrug resistance (MDR) has been studied in other tumor types. Overexpression of LRPPRC has been observed in chronic myeloid leukaemia MDR/IM cross-resistant cells (15,16). Li *et al* found that MDR gastric cancer cells highly express LRPPRC. Downregulation of LRPPRC was found to considerably increase cytotoxic drug sensitivity, reduce *MDR1* expression and the ability of P-gp protein to efflux adriamycin in gastric cancer cells (17,18). Our findings are consistent with previous reports but contrast those of Michaud *et al*, who found that the decreased expression of LRPPRC did not affect P-gp expression and the capacity of hepatocarcinoma cells to extrude cytotoxic drugs (19). Different cancer cells express different MDR-related genes for resistance (20,21), and the cytotoxic drug resistance of hepatocellular carcinoma cells may not depend on *MDR1* gene expression. This difference may contribute to the contradictory results obtained.

In conclusion, LRPPRC contributes to DDP resistance in lung cancer cells by regulating *MDR1* transcription. LRPPRC may serve as a potential molecular target for chemo-resistance reversal, which may benefit lung cancer patients.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

YH was concerned with the conceptualization, data curation, formal analysis, funding acquisition, software, investigation

and writing of the original draft. JC was responsible for the data curation, formal analysis and writing of the original draft. LJ was responsible for the software, formal analysis, validation, visualization of the findings and writing of the revision and editing. YS was responsible for the data curation, validation and visualization of the findings. XZ was concerning with the supervision of the research project, project administration, validation, methodology, writing, review and editing of the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The Ethics Committee of the First Affiliated Hospital of Xi'an Medical University approved the protocol (no. 20180606) for the animal study. The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Patient consent for publication

Not applicable.

Competing interests

The authors report no competing interests in this work.

References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 68: 394-424, 2018.
- Tian R, Zhang C, Xiong F and Chen H: PCAT1/miR-129/ABCB1 axis confers chemoresistance in non-small cell lung cancer. *Front Biosci (Landmark Ed)* 25: 948-960, 2020.
- Li Y, He LR, Gao Y, Zhou NN, Liu Y, Zhou XK, Liu JF, Guan XY, Ma NF and Xie D: CHD1L contributes to cisplatin resistance by upregulating the ABCB1-NF- κ B axis in human non-small-cell lung cancer. *Cell Death Dis* 10: 99, 2019.
- Vasilou V, Vasilou K and Nebert DW: Human ATP-binding cassette (ABC) transporter family. *Hum Genomics* 3: 281-290, 2009.
- Pérez-Tomás R: Multidrug resistance: Retrospect and prospects in anti-cancer drug treatment. *Curr Med Chem* 13: 1859-1876, 2006.
- Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, Hu T, Jiang L and Li J: Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade. *Cancer Lett* 370: 153-164, 2016.
- Manna S: An overview of pentatricopeptide repeat proteins and their applications. *Biochimie* 113: 93-99, 2015.
- Cui J, Wang L, Ren X, Zhang Y and Zhang H: LRPPRC: A multifunctional protein involved in energy metabolism and human disease. *Front Physiol* 10: 595, 2019.
- Tian T, Ikeda J, Wang Y, Mamat S, Luo W, Aozasa K and Morii E: Role of leucine-rich pentatricopeptide repeat motif-containing protein (lrpprc) for anti-apoptosis and tumorigenesis in cancers. *Eur J Cancer* 48: 2462-2473, 2012.
- Zhang HY, Ma YD, Zhang Y, Cui J and Wang ZM: Elevated Levels of autophagy-related marker ULK1 and mitochondrion-associated autophagy inhibitor LRPPRC are associated with biochemical progression and overall survival after androgen deprivation therapy in patients with metastatic prostate cancer. *J Clin Pathol* 70: 383-389, 2017.
- Corrêa S, Binato R, Du Rocher B, Ferreira G, Cappelletti P, Soares-Lima S, Pinto LF, Menciaha A and Abdelhay E: ABCB1 regulation through LRPPRC is influenced by the methylation status of the GC -100 box in its promoter. *Epigenetics* 9: 1172-1183, 2014.

12. Labialle S, Dayan G, Gayet L, Rigal D, Gambrelle J and Baggetto LG: New invMED1 element cis-activates human multi-drug-related ABCB1 and MVP genes, involving the LRP130 protein. *Nucleic Acids Res* 32: 3864-3876, 2004.
13. Fahrman JF, Grapov D, Phinney BS, Stroble C, DeFelice BC, Rom W, Gandara DR, Zhang Y, Fiehn O, Pass H and Miyamoto S: Proteomic profiling of lung adenocarcinoma indicates heightened DNA repair, antioxidant mechanisms and identifies LASP1 as a potential negative predictor of survival. *Clin Proteomics* 13: 31, 2016.
14. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
15. Corrêa S, Pizzatti L, Du Rocher B, Menciaha A, Pinto D and Abdelhay E: A comparative proteomic study identified LRPPRC and MCM7 as putative actors in imatinib mesylate cross-resistance in lucena cell line. *Proteome Sci* 10: 23, 2012.
16. Corrêa S, Pizzatti L, Du Rocher B and Abdelhay E: Comparative proteomic study of multidrug resistance in chronic myeloid leukemia. *Eur J Cancer Supplements* 8: 204, 2010.
17. Li X, Lv L, Zheng J, Zhou J, Liu B, Chen H, Liang C, Wang R, Su L, Li X and Fan D: The significance of LRPPRC overexpression in gastric cancer. *Med Oncol* 31: 818, 2014.
18. Li X, Zhou J and Li X: Reversal of multidrug resistance in gastric cancer cells by LRPPRC downregulation. *J Digest Dis* 15: 84, 2014.
19. Michaud M, Barakat S, Magnard S, Rigal D and Baggetto LG: Leucine-rich protein 130 contributes to apoptosis resistance of human hepatocarcinoma cells. *Int J Oncol* 38: 169-178, 2011.
20. Choi HS, Kim YK and Yun PY: Upregulation of MDR- and EMT-related molecules in cisplatin-resistant human oral squamous cell carcinoma cell lines. *Int J Mol Sci* 20: E3034, 2019.
21. Januchowski R, Sterzyńska K, Zaorska K, Sosińska P, Klejewski A, Brązert M, Nowicki M and Zabel M: Analysis of MDR genes expression and cross-resistance in eight drug resistant ovarian cancer cell lines. *J Ovarian Res* 9: 65, 2016.