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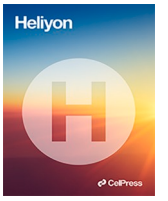

Research article

Transcriptome analysis reveals PRKCA as a potential therapeutic target for overcoming cisplatin resistance in lung cancer through ferroptosis

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ABSTRACT

Cisplatin-based chemotherapy is the current standard care for lung cancer patients; however, drug resistance frequently develops during treatment, thereby limiting therapeutic efficacy.The molecular mechanisms underlying cisplatin resistance remain elusive. In this study, we conducted an analysis of microarray data from the Gene Expression Omnibus (GEO) database under the accession numbers GSE21656, which encompassed expression profiling of cisplatin-resistant H460 (DDP-H460)and the parental cells (H460). Subsequently, we calculated the differentially expressed genes (DEGs) between DDP-H460 and H460. Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs demonstrated significant impact on the Rap1, PI3K/AKT and MAPK signaling pathways. Moreover, protein and protein interaction (PPI) network analysis identified PRKCA, DET1, and UBE2N as hub genes that potentially contribute predominantly to cisplatin resistance. Ultimately, PRKCA was selected for validation due to its significant prognostic effect, which predicts unfavorable overall survival and disease-free survival in patients with lung cancer. Network analysis conducted on The Cancer Genome Atlas (TCGA) database revealed a strong gene-level correlation between PRKCA and TP53, CDKN2A, BYR2, TTN, KRAS, and PIK3CA; whereas at the protein level, it exhibited a high correlation with EGFR, Lck, Bcl2, and Syk. The in vitro experiments revealed that *PRKCA* was upregulated in the cisplatin-resistant A549 cells (DDP-A549), while knockdown of PRKCA increased DDP-A549 apoptosis upon cisplatin treatment. Moreover, we observed that PRKCA knockdown attenuated DDP-A549 proliferation, migration and invasion ability. Western blot analysis demonstrated that PRKCA knockdown downregulated phosphorylation of PI3K

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expression while upregulated the genes involved in ferroptosis signaling. In summary, our results elucidate the role of PRKCA in acquiring resistance to cisplatin and underscore its potential as a therapeutic target for cisplatin-resistant lung cancer.

1. Introduction

Neoplasm is a global public health challenge characterized by a substantial incidence (2.0 million) and mortality rate (1.7 million) in 2016 [1]. In China, lung and bronchus cancer represents one of the most prevalent malignancies, exhibiting the highest incidence among males and the second among females. It stands as the primary cause of cancer-related fatalities in both genders [2]. Surgery and radiotherapy are the main treatment modalities in early-stage cases, whereas advanced-stage patients usually receive a combination of therapies, including systemic chemotherapy, radiotherapy, and targeted therapy [3]. Nevertheless, these therapeutic approaches often encounter limitations due to the emergence of drug resistance.

Cisplatin, one of the most effective anticancer agents, has long been established as the first-line regimen for lung cancer patients for several decades [4–6]. The cytotoxic effects of cisplatin rely on its interaction with DNA, inducing DNA damage response and interfering with DNA repair. Additionally, cisplatin exerts its action on mitochondrial DNA and various cytoplasmic targets to induce cell death. Cisplatin resistance arises due to alterations in the expression level of plasma membrane transporters, increased nucleotide excision repair (NER) system activation, and regulation of pro- or anti-apoptotic signals [7,8]. Therefore, investigating genetic changes in drug-resistant cancers may unveil biomarkers and targets for cancer diagnosis and treatment.

PRKCA and its encoding protein, Protein kinase C alpha (PKCα), primarily regulate proliferation, differentiation, as well as anti-apoptotic signals and, are therefore considered pivotal factors in tumor formation and chemoresistance [9]. However, their role in cancer remains controversial. PKCα is up-regulated in certain cancers (eg., bladder and breast cancer), it is down-regulated in others (eg., colorectal tumors, lung cancer, and malignant renal cell carcinomas). Consequently, it can function as a tumor promoter or suppressor [9]. Additionally, studies have proposed that PKCα activation is required for doxorubicin-resistance in lung cancer [10]. In breast cancer, inhibition of PKCα can reverse multidrug resistance (MDR) by reducing drug efflux [11]. Meanwhile, PKCα also caused an anti-apoptosis response in a breast cell line [12]. Therefore, unraveling the precise role of PKCα and its coding gene PRKCA in tumor formation and drug resistance is imperative.

RNA sequencing (RNA-Seq) and microarray technologies are very useful tools for genetic analysis, extensively employed in numerous studies to investigate the involvement of many genes, RNAs, and proteins in lung cancer initiation, progression, and recurrence [13,14]. In lung cancer, the heterogeneity of genes and somatic mutations, such as TP53, KRAS, PIK3CA, and BRAF, have been associated with drug efficiency [15]. In addition, Sun Y et al. reported that IGFBP3 expression was decreased in cisplatin-resistant lung cancer cells, which increased activation of IGF-1R signaling, contributing to resistance to cisplatin and radiation [16]. Therefore, advances in RNA technology have facilitated comprehensive exploration of genetic changes and altered signaling pathways in drug-resistant tumor cells.

In this study, RNA microarray data was used to investigate the underlying mechanisms of cisplatin resistance. Our findings provide compelling evidence that PRKCA serves as a pivotal hub gene contributing to cisplatin resistance in lung cancer and exhibits significant correlations with the migration, invasion and proliferation. Moreover, inhibition of PRKCA promotes ferroptosis in tumor cells, suggesting that targeting PRKCA may overcome cisplatin resistance in lung cancer patients.

2. Materials and methods

2.1. Data sorting

The transcription profile of GSE21656 which includes the expression profiling of cisplatin-resistant lung cells derived from the H460 lung cell line and the parental cells, was downloaded from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database. The platform information was GPL6244: [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]. In total, 6 samples were retrieved, including 3 parental H460 cancer cell lines and 3 cisplatin-resistant cell lines. The pre-processed level 3 RNA-seq data and corresponding lung cancer clinical information were downloaded from The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov)

2.2. DEGs screening and clustering

The limma package was utilized for the identification of DEGs in drug-resistant cell lines and parental cell lines. The DEGs with an adjusted value of $p < 0.05$ were selected for further analysis. Hierarchical clustering of the DEGs was based on the Euclidean method, which calculates the distance and complete linkage for the tree construction using R package heatmap.

2.3. Functional enrichment analysis of DEGs

The Gene Set Enrichment Analysis (GSEA) was conducted using GSEA v3.0 software with 1000 gene set permutations. The gene sets used in the article were obtained from the Molecular Signatures Database v4.0. The threshold of FDR q value was set to 0.25 based on

the previously reported description and interpretation of the GSEA methodology [17,18]. The GO enrichment analysis and KEGG pathway analysis were performed by using the R packages "clusterProfiler". Terms and pathways with p and q values < 0.05 indicated significantly enriched terms and pathways.

2.4. Gene and protein interaction, mutually exclusive and co-occurrence analysis

Somatic mutations, copy number variations, and mRNA sequencing data of pan-lung cancer were obtained from The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/). Part of the analysis of TCGA data was based on the online software cBioPortal and GEPIA. The protein expression data of a part of TCGA pan-lung cancer were obtained [19], and the protein network was generated by The Cancer Proteome Atlas (TCPA) based on Reverse-phase protein array (RPPA) [20–23]. Mutually exclusive and co-occurrence analysis was performed as described by Ciriello et al. [19].

2.5. Cell lines

The human lung cancer cell line A549 was purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China). Cisplatin-resistant A549 cells (DDP-A549) were also obtained. All cell lines were cultured in DMEM high glucose (Sigma-Aldrich, St. Louis, MO, USA) containing 10 % FBS (Lonsera, Uruguay), 100 U/mL penicillin, and 100 μg/mL streptomycin without mycoplasma at 37 °C in a humidified 5 % CO2 incubator.

2.6. RNA interference

For the transient knockdown assay, small-interfering RNA (siRNA)-PRKCA and negative control were designed and purchased from Gene Pharma Company (Shanghai, China); the siRNA sequences are shown in Supplementary table 2. The siRNAs (SiPRKCA-1: GAAGGGTTCTCGTATGTCA, SiPRKCA-2: GGACTGGGATCGAACAACA, and negative control, SiPRKCA-NC) were diluted in diethyl pyrocarbonate water at a final concentration of 20 μM according to the manufacturer's protocol. A549 and DDP-A549 cells were plated in six-well plates at a density of 5×10^5 cells per well. After reaching approximately 60 % confluency, the cells were transfected with the siRNA or negative control (2 μM/well) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA,#L3000001) for 48 h. Subsequently, the cells were collected for WB or treated with cisplatin.

2.7. Flow cytometry for apoptosis analysis and lipid peroxidation measurement

After 48 h of treatment with cisplatin, the supernatant was harvested, and tumor cells were washed twice with ice-cold phosphate-buffered saline (PBS). The cells were suspended in Annexin-V binding buffer (BioLegend, San Diego, CA, #422201) at a final concentration of 10^6 cells/mL. 100 μL of cell suspension was transferred into a 1.5 mL centrifuge tube and incubated with 5 μL of Alexa Fluor 647 Annexin-fluorescein isothiocyanate (BioLegend, San Diego, CA, #640906) and RNase (Thermo Fisher Scientific, USA, #AM2286) for 15 min at 4 °C in the dark. After incubation, the samples were treated with propidium iodide (5 μL) (Sigma, Santa Clara, CA, USA, #P4170) and immediately analyzed by flow cytometry (BD, San Diego, CA, USA, FACSCanto II) with BD FACSDiva software. BODIPY-C11 (BODIPYTM581/591C11, D3861, Thermo, USA) was used to measure lipid peroxidation. The final concentration was 5 μmol, and cells were stained for 15mins and analyzed immediately.

2.8. Western blot analysis

Radioimmunoprecipitation assay (RIPA) buffer (Solarbio, China, #R0010) mixed with protease and phosphatase inhibitors was used to isolate total proteins from cells. The protein concentration was measured using the bicinchoninic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific, Massachusetts, #A53226), according to the manufacturer's instructions. Primary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA): PI3K110α (C73F8), β-Tubulin, phospho-Akt (Ser 473), phosphor-S6 (Ser 235/236), PKCα, PARP (46D11), Cleaved PARP (Asp 214), Cleaved Caspase 3 (Asp 175), NRF2 (D1Z9C) and SLC7A11 (D2M7A). Horseradish peroxidase (HRP)-labeled goat anti-rabbit/mouse IgG (1:3000 dilution, ZSGB-BIO, Beijing, China, #ZB2301) were used as secondary antibodies. Enhanced chemiluminescence (ECL) substrate (CWBIO, Beijing, China, #CW00495). FluorChem E System (Protein Simple, USA) was used to evaluate protein expression. Image J software was used to quantify the level of proteins.

2.9. Cell proliferation assays

Cell proliferation was evaluated using a cell counting kit-8 (CCK8) assay (Dojindo, Japan). 3×10^3 cells/100 μL were plated in each well of a 96-well plate 24h at 37 °C before treatment. Then at 0, 24, 48 and 72 h, the supernatant of each well was discarded, and 10 μL CCK-8 regent and 100 μL fresh serum-free DMEM were added to each well. Also, be careful not to generate bubbles during the sample addition process. After 1 h of incubation at 37 °C, 5 % CO2, the absorbance of each well at 450 nm was detected by a Microplate Reader (Thermo Fisher Scientific). The experiment was repeated 3 times.

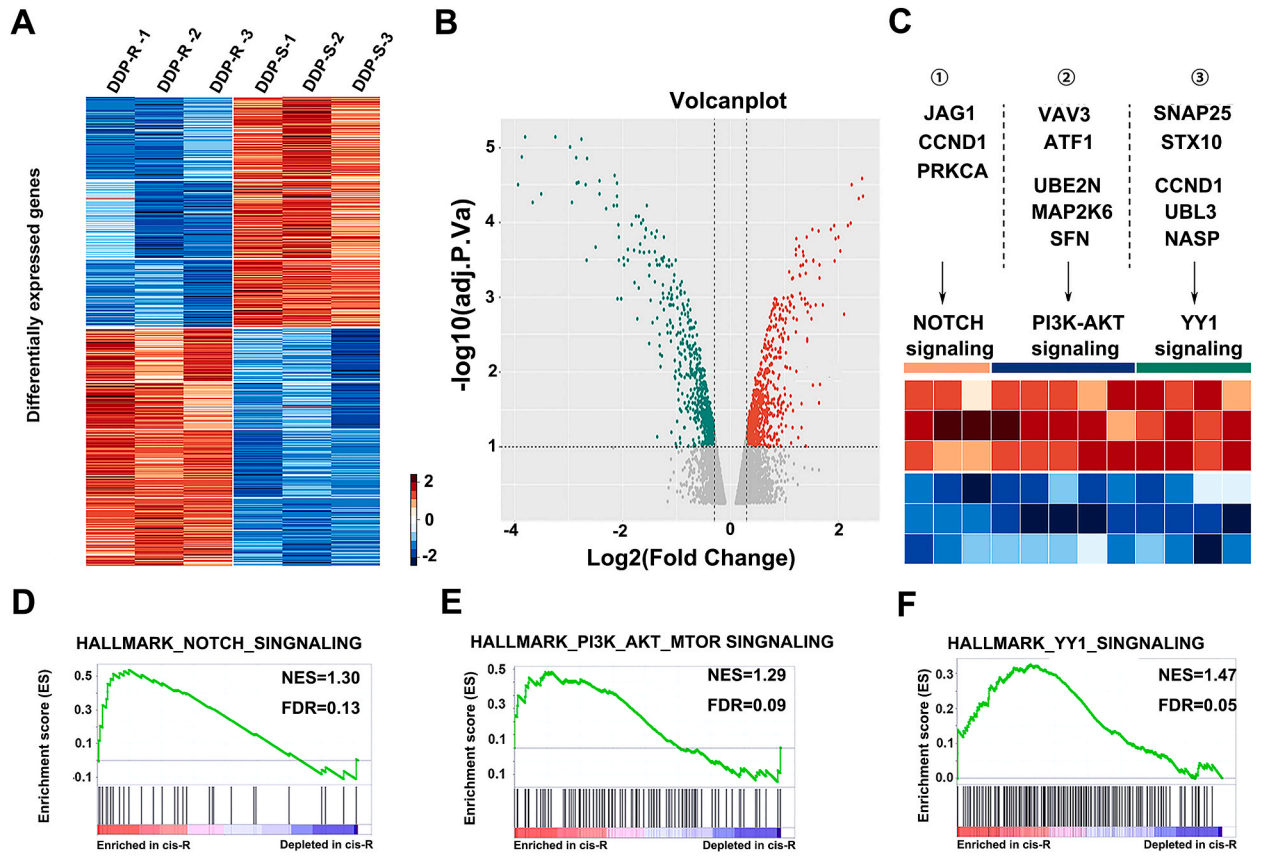

Fig. 1. RNA microarray analysis revealed the difference of genes and signaling pathways between DDP-H460 and H460. (A) Two-way clustering analysis of DEGs; (B) Volcano plot of DEGs; (C) Expression of three signaling in DDP-H460 and H460; (D–F) Enrichment plots show the three gene signatures (NOTCH signaling, PI3K-AKT signaling, and YY1 targets) analyzed by GSEA of DDP-H460 and H460. FDR: false discovery rate q-value. NES: normalized enrichment score.

2.10. Transwell assay

Cell migration ability was assessed by Transwell assay. 1×10^5 cells were plated in the upper chamber of a 24-well plate with 8-μm polycarbonate membranes (Corning) filled with 200 μL serum-free DMEM medium. 600 μL DMEM containing 10 % FBS (as a chemoattractant) was added to the lower chamber. After 24 h of incubation at 37 °C, 5 % CO2, the cells remaining in the upper chamber were removed with a cotton swab, and cells migrated to the lower surface were fixed with 4 % paraformaldehyde for 30 min and then stained with 0.1 % crystal violet for 20 min. Cells were counted under a microscope (x200) in five random visual fields. Each experiment was independently repeated three times.

2.11. Wound healing assay

Wound healing assays were performed in 12-well plates with 2×10^5 seeded cells. After cells were grown to full confluence in a complete medium, a 10 μL pipette tip was used to perform the monolayer of cells (record 0 h) and then washed thrice with PBS to remove the detached cells. 1 mL serum-free DMEM was then added. After incubation for 24h in an incubator, the wounded areas were imaged by inverted microscopy. Five randomly chosen fields were used to calculate the percentage of wound closure using the ImageJ software. All experiments were independently repeated three times.

2.12. Statistical analysis

GraphPad Prism 7 (version 5.0; San Diego, CA) was used to analyze the data, and the results were presented as mean ± SD. A two-tailed unpaired *t*-test was used to analyze the proliferation ratio, apoptosis ratio and migrated tumor cells. Each experiment has at least three biological replicates. A *p-value* less than 0.05 was considered statistically significant.

Table 1
The top 10 upregulated and downregulated differently expressed genes.

	Gene symbol	*P* value	Log FC	Function
Upregulated DEGs	GJA5	4.51E-5	2.4517	Gap junction
	CNTN1	2.58E-5	2.4368	Cell adhesion
	COL3A1	4.84E-5	2.3774	Pro-alpha 1 chains of type III collagen
	ATP11A	3.16E-5	2.2456	Integral membrane ATPase
	ADARB1	1.10E-4	2.1768	Pre-mRNA edition
	SLPI	3.68E-4	1.6520	Epithelial tissue protection
	IGF2BP1	5.59E-4	1.6445	Translation regulation
	TGM2	1.29E-4	1.6289	Crosslink of protein
	ADGRG3	3.30E-4	1.5320	G-protein coupled receptor
	SNAP25	3.20E-4	1.5297	Vesicle fusion
Downregulated DEGs	NTS	3.16E-5	−3.9405	Gut structure and function maintenance , fat metabolism
	SERPINB11	1.35E-5	−3.8713	Serine protease inhibition
	SLFN11	7.29E-5	−3.8085	Sensitive to DNA damage agents
	FAM216B	5.42E-5	−36716	Unknown
	ACSS3	9.90E-5	−2.9742	Fatty acid metabolism
	TMPRSS15	1.37E-5	−2.8615	Convert trypsinogen into trypsin
	MCAM	2.95E-5	−2.8147	Cell adhesion
	SCN3A	7.81E-5	−2.7671	sodium channel alpha subunit gene family
	KCNK2	2.16E-4	−2.4322	two-pore-domain background potassium channel family

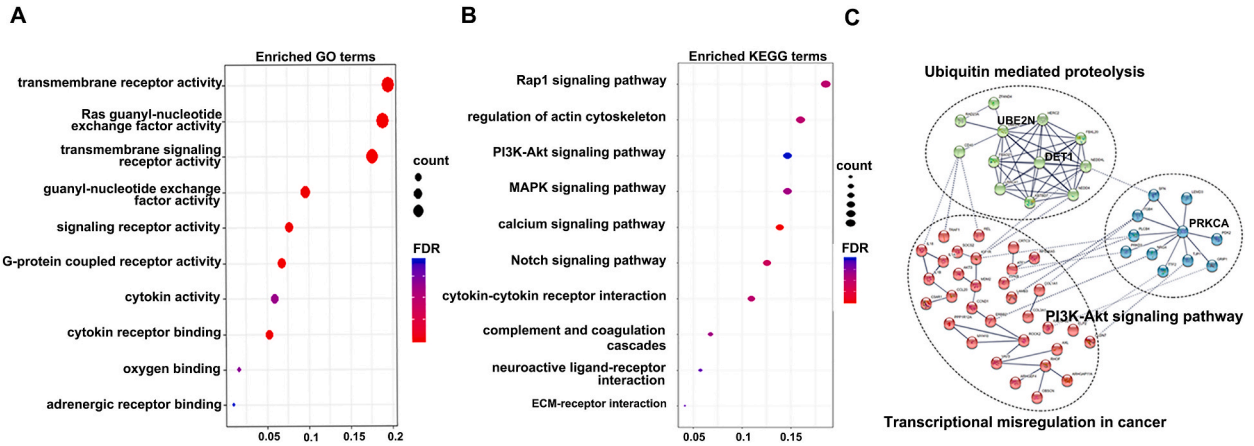

Fig. 2. Enrichment analysis of DEGs. (A) GO enrichment analyses of DEGs; (B) KEGG pathway analysis of DEGs; (C)PPI network of DEGs identified three hub genes and signaling signature.

3. Results

3.1. RNA microarray analysis revealed the altered signaling pathway in cisplatin-resistant lung cancer cells

To investigate cisplatin resistance, the R package limma was used to identify DEGs between the DDP-H460 and H460. Two-dimensional hierarchical clustering analysis and volcano plot analysis revealed a significant distinction in the clustering pattern of the unique DEGs between DDP-H460 and H460 (Fig. 1A and B). A total of 665 significant DEGs (FDR<0.05) were identified for further analysis, consisting of 331 upregulated genes and 334 downregulated genes. The top ten upregulated and downregulated genes are listed in Table 1. To gain insight into the development of chemoresistance, unsupervised clustering was used to explore coherent gene signatures that were preferentially co-expressed in DDP-H460. A manual review discovered three upregulated gene signatures (NOTCH signaling, PI3K-AKT signaling, and YY1 targets) in DDP-H460 (Fig. 1C). which are involved in the regulation of cisplatin resistance in various tumors [24–26]. Subsequently, GSEA was performed to evaluate the enriched gene signatures related to canonical signaling pathways in cancer cells. The results showed that the three signaling signatures were all significantly enriched in DDP-H460 (Fig. 1D–F).

3.2. Functional enrichment analysis

GO enrichment analysis was performed to investigate the interactions among DEGs. The results revealed that the DEGs were associated with receptor activities, including transmembrane receptor activity, cytokine receptor activity, G-protein coupled receptor

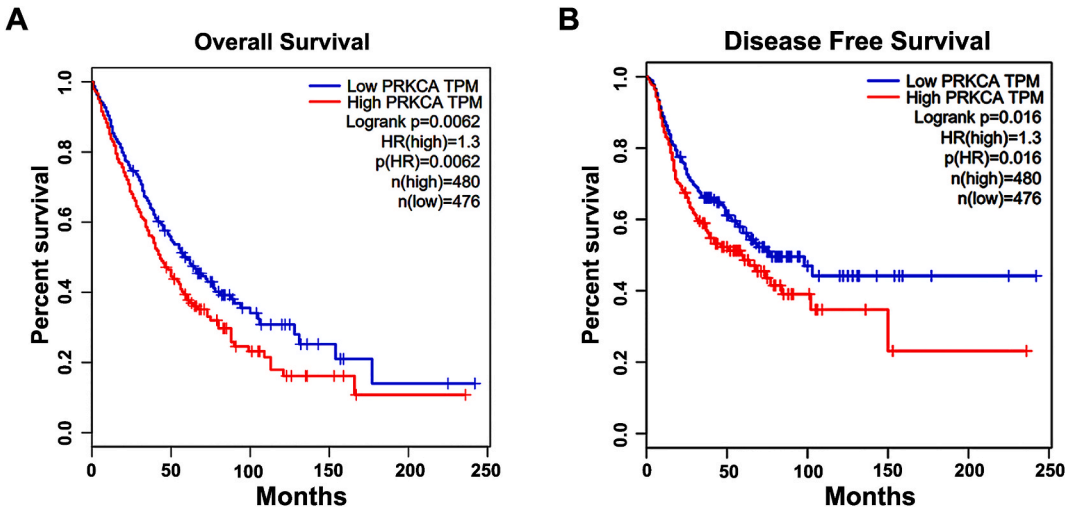

Fig. 3. Kaplan–Meier analysis was used to evaluate the prognostic role of PRKCA in TCGA database. (A) High expression PRKCA was correlated with unfavorable overall survival in lung cancer patients. (B) High expression PRKCA predicted poor disease-free survival in lung cancer patients.

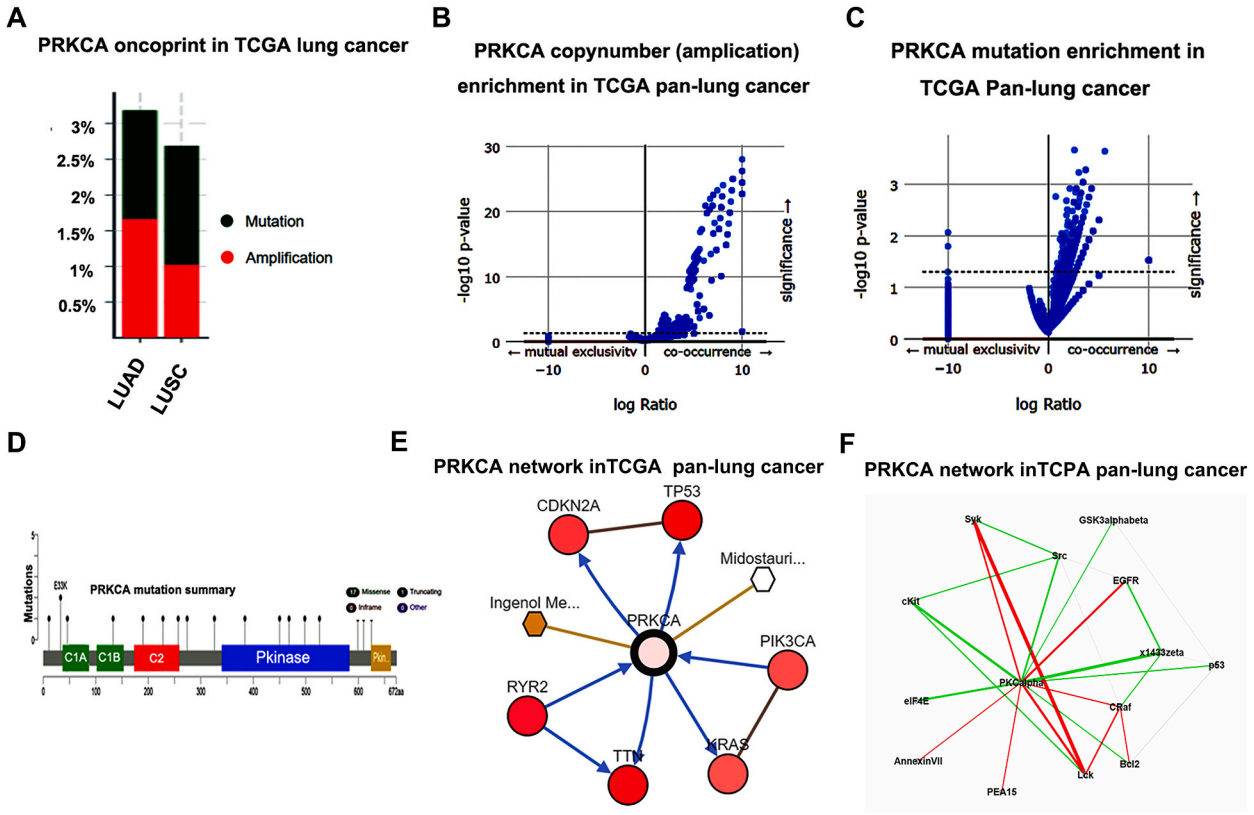

Fig. 4. Gene alterations of PRKCA in pan-lung cancer in TCGA database. (A) PRKCA oncoprint status (amplification and mutation) in TCGA pan-lung cancer. (B) PRKCA co-occurrence and mutual exclusivity amplification in pan-lung cancer. (C) PRKCA co-occurrence and mutual exclusivity mutation in pan-lung cancer. (D) PRKCA mutation site analyzed in the TCGA database. (E) PRKCA network analysis at the gene level in pan-lung cancer. (F) PRKCA network analysis at the protein level in pan-lung cancer.

activity and adrenergic receptor binding. These findings suggest that tumor cells may exhibit increased responsiveness molecules in the tumor microenvironment acquiring cisplatin resistance (Fig. 2A). In addition, KEGG pathway analysis was carried out to identify the key pathways that these DEGs were involved in. The most significant pathway was the Ras-proximate 1 (Rap1) signaling pathway.

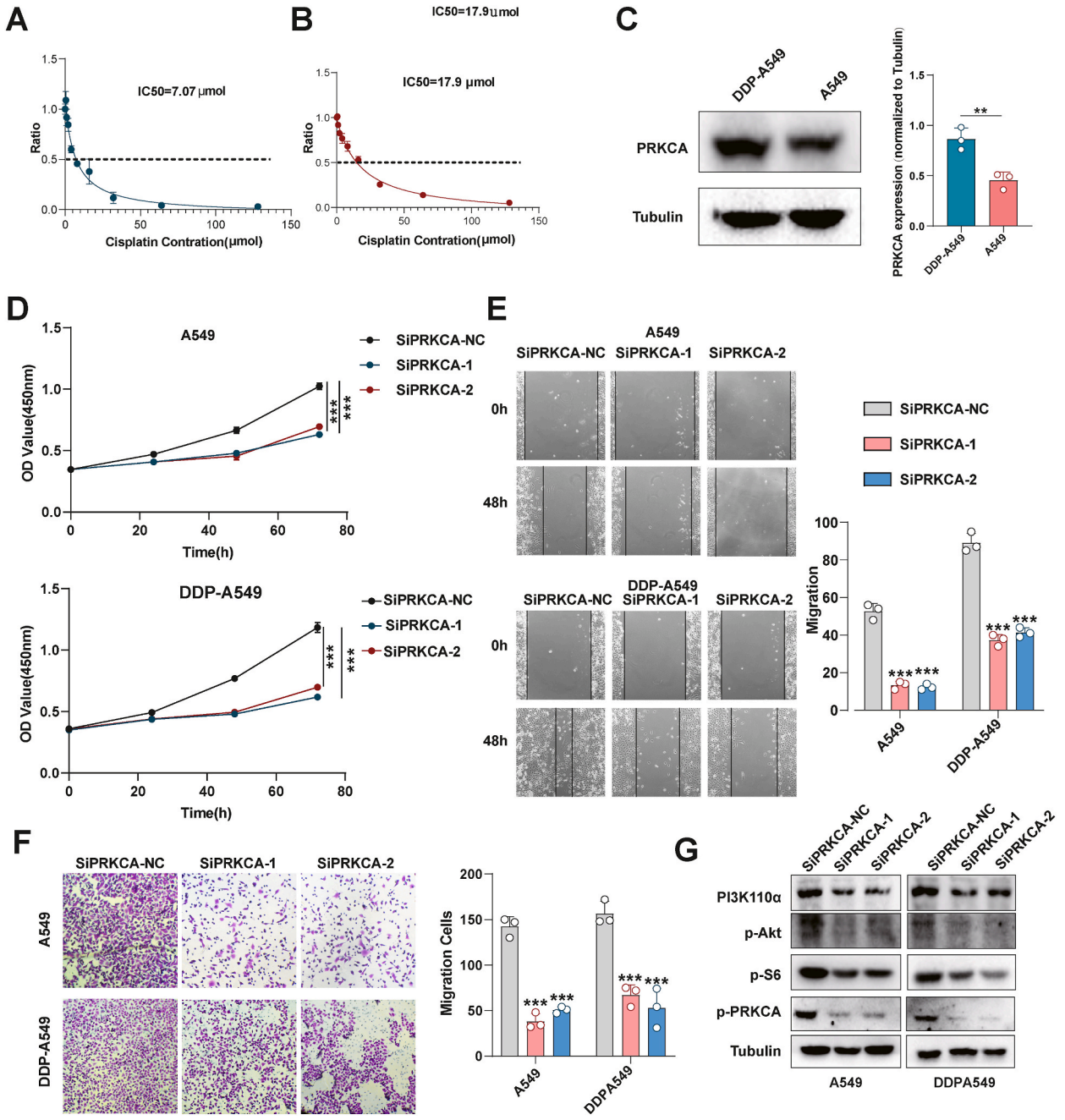

Fig. 5. Validation of PRKCA and signaling pathways in vitro experiments. (A)CCK8 analysis calculate the IC50 of Cisplatin in A549. (B) CCK8 analysis calculate the IC50 of Cisplatin in DDP-A549. (C) The expression of PRKCA in A549 and DDP-A549 was detected by Western blot. (D)PRKCA inhibition decreased the proliferation ability of A549 cells and DDP-A549 cells. (E) The wound-healing assay showed the reduced ability of A549 and DDP-A549 cells due to PRKCA inhibition. (F) Transwell assay analysis of A549 and DDP-A549 cells after transfected with si-PRKCA or control. (G) The expression of PI3K signaling pathway was examined by Western blot assay in A549 and DDP-A549 cells after transfected with si-PRKCA or control.

Furthermore, some well-known signaling pathways which mediate drug resistance were also enriched, including the PI3K/AKT, MAPK and Notch signaling pathways (Fig. 2B). These results confirm the reliability of our data for further analysis. Proteins play a pivotal role as key mediators in cell biology, and the analysis of protein-protein interaction (PPI) networks serves as a potent tool for identifying central protein-coding genes. In this study, we utilized the STING database to construct protein-encoding gene interaction networks. Centrality refers to genes that promote communication between nodes in a biological network. Therefore, hub genes were identified based on centrality analysis. These genes were mainly involved in the PI3K-AKT signaling pathway, ubiquitin-mediated proteolysis

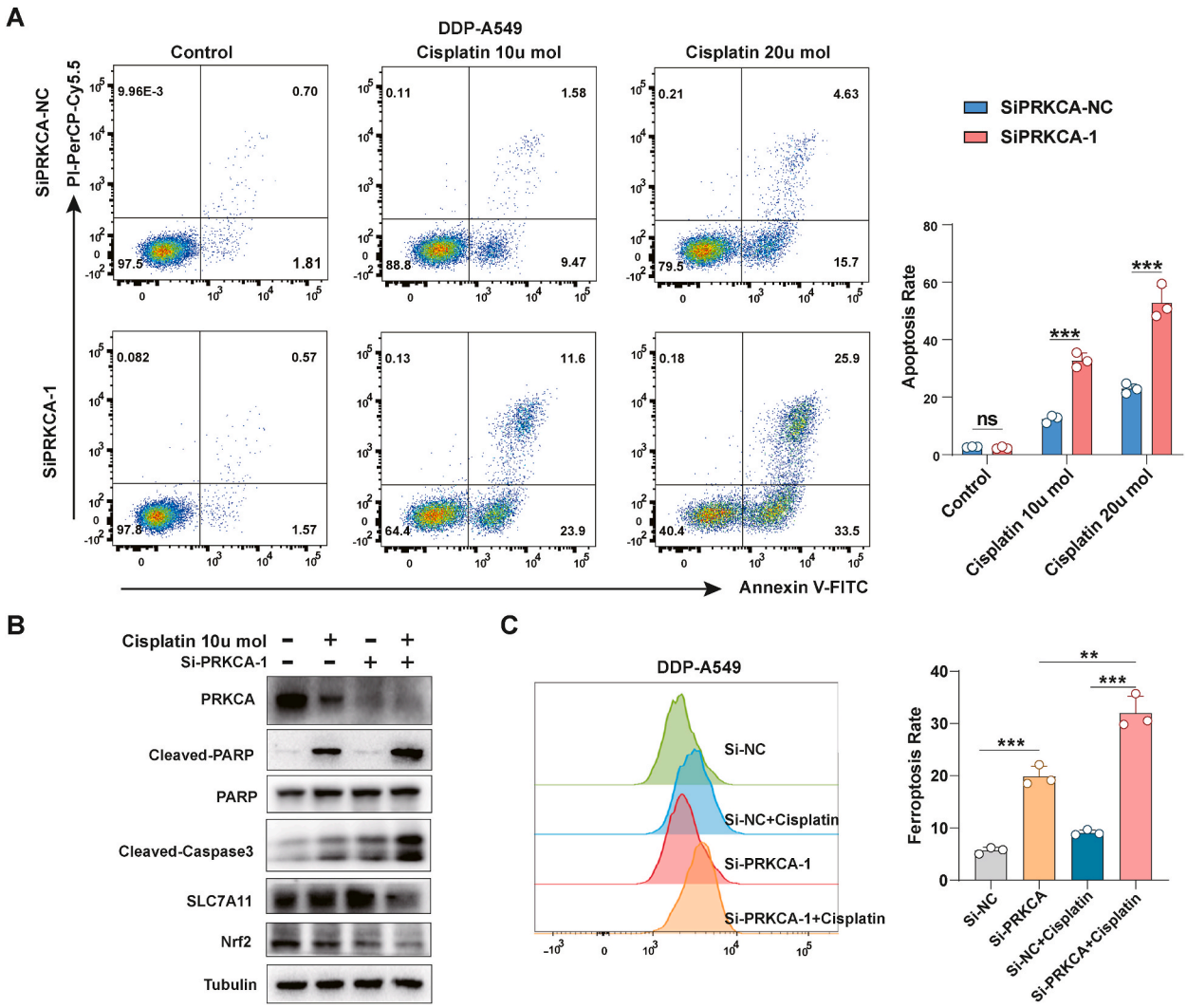

Fig. 6. Inhibition PRKCA can reverse the cisplatin resistance in DDP-A549 cells. (A)Cisplatin-induced apoptosis increased in PRKCA inhibited DDP-A549 cells. (B) Apoptosis-related and ferroptosis-related markers were detected by Western blot.
(C)Cisplatin combined with PRKCA inhibition can induce ferroptosis in DDP-A549 cells.

and transcriptional misregulation in cancer. Additionally, PRKCA, DET1, and UBE2N were identified as hub genes, which have more connections with other protein-coding genes (Fig. 2C).

3.3. PRKCA was correlated with an unfavorable outcome in lung cancer

The PPI network analysis identified three hub genes associated with cisplatin resistance, and the impact of these genes on patient survival was assessed. For this purpose, the lung cancer patients were stratified into two groups based on the mRNA expression of these hub genes in the TCGA dataset. Kaplan–Meier analysis was performed to analyze the correlation between gene expression and survival. Our findings revealed that only PRKCA predicted an unfavorable overall survival (Fig. 3A) and disease-free survival (Fig. 3B). These results suggest that high PRKCA expression in tumor tissues predicts malignant features and mostly contributes to cisplatin resistance.

3.4. Comprehensive analysis of PRKCA in lung cancer

The above findings have demonstrated the potential significance of PRKCA in mediating drug resistance, thus necessitating a comprehensive understanding of its multifaceted role in tumorigenesis. Genomic alterations frequently occur during tumor development, and genomic changes of a specific gene often affect the expression of interacting genes or genes in the same pathway. In this regard, the amplification and mutation changes of PRKCA in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) were analyzed in the TCGA database. Notably, similar alterations were observed in LUAD, whereas LUSC exhibited a higher prevalence

of mutations (Fig. 4A). In addition, we observed a significantly mutually co-occurring genes with PRKCA, including BCAS3, CA4, and HEATR6, while no significantly mutually exclusive genes were found (Fig. 4B). Furthermore, significantly mutually co-occurring mutated genes such as NF1, HMCN1, TP63 and TP53, and mutually exclusive mutated genes such as PRDM9, MUC5B, and TMEM132D were observed (Fig. 4C). Subsequent analysis revealed that the PRKCA mutations mainly occurred in the pkinase domain in lung cancer (Fig. 4D). Next, the molecular relationship between PRKCA and other molecules were analyzed at the gene and protein levels. The results showed a high correlation between PRKCA and *TP53*, *KRAS*, and *PIK3CA* in the TCGA RNAseq dataset (Fig. 4E) and PRKCA was related to EGFR, P53, LCK, Syk at the protein level in the TCPA dataset (Fig. 4F). These results indicate significant differences between the mutual regulation at the gene and protein levels, suggesting post-transcriptional modification is important for the regulatory network of a particular gene. Meanwhile, PRKCA correlated with tumor oncogene and suppressor gene, suggesting the potential two-sided role of PRKCA in tumor progression.

3.5. Validation of signaling pathways in lung cancer cell in vitro experiments

A549 and DDP-A459 tumor cells were used to validate the role of PRKCA and key signaling pathways in lung cancer. Firstly, the IC50 of cisplatin in A549 (7.07 μmol/L) and DDP-A549 (17.9 μmol/L) was determined (Fig. 5A and B). Additionally, PRKCA was also upregulated in DDP-A549 cells (Fig. 5C, Supplementary F ig. 1A). To further confirm the involvement of PRKCA in drug resistance, PRKCA knockdown was performed with small RNA interference, which decreased the migration, invasion and proliferation of tumor cells. However, knocking down PRKCA in DDP-A549 resulted in a more significant change (Fig. 5D–F). Given the enrichment of the PI3K/AKT signaling pathway in cisplatin-resistant tumor cells and its correlation with PRKCA, the PI3K110α protein levels were detected by Western blot. It has been reported that decreased phosphorylation of PI3K expression might overcome cisplatin resistance in some human cancer cells [25,27]. The results showed that PRKCA knockdown decreased PI3K110α expression both in DDP-A549 and A549 (Fig. 5G,Supplementary fig. 1B), thereby indicating the ability of PRKCA to activate PI3K signaling in tumor cells.

In addition, PRKCA knockdown increased apoptosis and the expression of apoptosis-related biomarkers in DDP-A549 cells when treated with cisplatin (Fig. 6A and B, Supplementary fig. 1C). Interestingly, the lipid peroxidation levels were measured in DDPA549 cells treated with PRKCA inhibition or cisplatin or both. Increased lipid peroxidation levels were found in cisplatin-treated cells, which was promoted by PRKCA inhibition, and resulted in increased ferroptosis (Fig. 6C). Recent reports have suggested that upregulation of NRF2 and SLC7A11 might attenuate ferroptosis in human cancers [28–30]. The changes in NRF2 and SLC7A11 expression confirmed that PRKCA might also influence ferroptosis in lung cancer cells (Fig. 6B).

4. Discussion

Despite the emergence of novel therapeutic strategies, such as targeted therapy and immune therapy, cisplatin-based systematic chemotherapy remains the primary treatment modality for lung cancer patients. However, acquired drug resistance is a recurrent issue plaguing many cancer treatments. Genomic alterations and the activation of concomitant signaling pathways leading to the reduction of cellular drug accumulation is one of the most consistently known features of cisplatin-resistant lung cancer patients [31,32].

With the advent of next-generation sequencing technology, the identification of somatic mutations and abnormally activated signaling pathways promoted the generation of new targeted therapeutic strategies.The present study observed the upregulation of well-established signaling pathways associated with drug resistance, such as the PI3K/AKT (KEGG:$p < 0.05$ and $q < 0.05$), MAPK (KEGG:$p < 0.05$ and $q < 0.05$), and Notch signaling pathways (KEGG:$p < 0.05$ and $q < 0.05$), in DDP-H460. These findings are consistent with previous studies [33–35]. Rap1 signaling was the most significantly altered pathway in our study. Rap1 is a small GTPase consisting of two isoforms, Rap1a and Rap1b. Its role in cancer is mainly related to the proliferation and invasion of tumor cells, which are partially dependent on MAPK activation, also upregulated in our study [36]. Similarly, AKT is also a plausible downstream effector of Rap1. Moreover, recent work has confirmed that cytoplasmic Rap1 activates NF-κB and Bcl2 expression to mediate cisplatin resistance of non-small cell lung cancer [37], revealing that Rap1 signaling may be a starting signal and activate downstream signaling to initiate cisplatin drug resistance.

PPI networks revealed that PRKCA, DET1 and UBE2N act as hub genes for interaction with other molecules. DET1 is a key partner of E3 ubiquitin ligase constitutive photomorphogenic 1 (COP1) within the ubiquitylation complex. The co-expression of COP1 and DET1 is responsible for P53 and c-Jun degradation [38], and DET1 is also involved in regulating ERK transcriptome and sensitivity to MAPK inhibitors [39]. UBE2N, an E2 ubiquitin-conjugating enzyme, facilitates the formation of monomeric p53 and results in its cytoplasmic translocation and subsequent loss of function. Inhibition of UBE2N shows a potent anti-tumor effect in neuroblastoma cell lines and orthotopic xenografts via activating P53 and JNK pathways [40]. These findings suggest a potential synergistic impact of DET1 and UBE2N on tumorigenesis, but their role in cisplatin resistance remains unknown.

PRKCA was identified as the major contributor to cisplatin resistance based on PPI network analysis. Despite its association with an unfavorable outcome in lung tumor patients, the role of PRKCA and its encoding protein PKCα in tumor development has been a subject of controversy over the past decades. Low PKCα expression was observed in lung cancer tissue, and tumor formation was suppressed through the p21cip1 and KRAS-mediated pathway [41,42]. In contrast, PKCα has also been proved to mediate doxorubicin and erlotinib resistance [43,44], and confers resistance against cisplatin-induced apoptosis to lung adenocarcinoma through FAK-Ras signaling [45]. Chemotherapy drugs such as cisplatin exert their effects by selectively targeting rapidly proliferating cells. One hypothesis explaining the controversial role of PRKCA is that low PRKCA expression during tumor development promotes tumor cell proliferation. Conversely, PRKCA expression is upregulated in cisplatin treatment to arrest tumor cell proliferation, demonstrating dormancy and slow-cycling against the disruption of the drugs. These findings suggest that selective PRKCA activation is crucial for

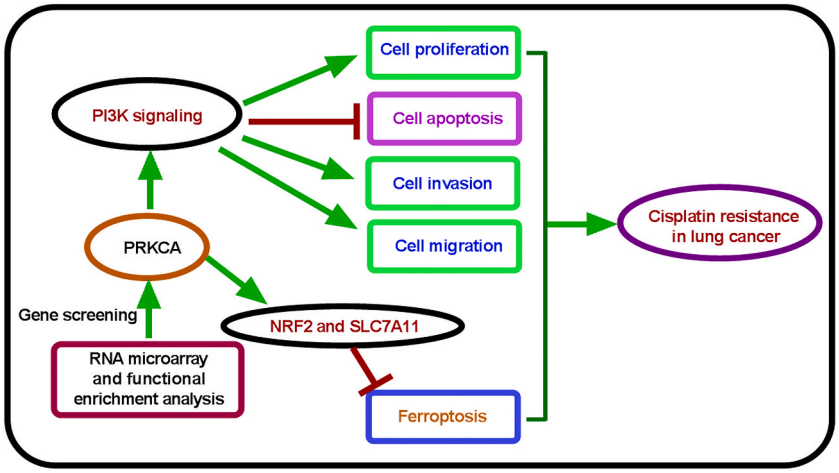

Fig. 7. Based on RNA microarray and functional enrichment analysis, PRKCA was selected to study cisplatin resistance in lung cancer. PRKCA might promote cell proliferation, migration, invasion, and block apoptosis by modulating PI3K signaling, and repress ferroptosis by regulating NRF2 and SLC7A11 expression, which ultimately contribute to cisplatin resistance in lung cancer.

tumor formation and drug resistance. Our results proved that PRKCA was upregulated in cisplatin-resistant tumor cells and may promote tumor survival under cisplatin treatment. Additionally, we also found that PRKCA increased the migration, invasion and proliferation ability of tumor cells. However, a phase III study of combination PKCα antisense oligonucleotide, aprinocarsen, with gemcitabine and cisplatin showed no significant benefit for NSCLC patients [46]. PRKCA and its encoding protein PKCα may still act as a potential target for reversing cisplatin resistance in lung cancer. It has been reported that induction of ferroptosis might alleviate cisplatin resistance in different cancers [47]. As a ferroptosis-related differentially expressed protein, PKCα has been confirmed to participate in the regulation of ferroptosis by targeting different proteins [48,49]. Furthermore, some researchers have suggested that the overexpression of NRF2 and SLC7A11 might relieve ferroptosis in various tumors [29,30]. Herein, our data displayed that the blockade of PKCα can inhibit ferroptosis by regulating NRF2 or SLC7A11 in cisplatin-treated lung cancer cells. Further understanding of the functions of PRKCA (PKCα) and its interacting molecules is required for potential clinical application.

In this study, genomic alterations (amplification and mutation) of PRKCA in pan-lung cancer were first explored. Our findings revealed a higher of frequency mutation than amplification in LUSC, while the two genomic alterations were almost equal in LUAD, suggesting distinct functional roles of PRKCA in the development of these two lung cancer subtypes. Meanwhile, it also provides evidence for the personalized treatment of tumors. Further mutual co-occurrence and exclusive analysis identified the PRKCA co-amplified and co-mutated genes, and co-mutation was observed between PRKCA and neurofibromatosis-1(NF1). NF1 functions as a tumor suppressor gene, and its mutations are commonly found in lung cancer. Furthermore, PRKCA and NF1 can regulate Ras signaling, which is also upregulated in cisplatin-resistant lung cancer cell lines [45,50]. Therefore, targeting PRKCA and NF1 may be a potential strategy for patients with cisplatin resistance. Next, a network analysis of PRKCA was performed at the gene level and protein level. A potent correlation was found between PRKCA and TP53, PIK3CA and KRAS. Although heterogeneity in these genes is common in all stages of lung cancer, they have been proved to participate in and influence drug efficacy and resistance. The mutation of TP53 increases genomic heterogeneity, thus promoting the mutation of other genes. Co-mutation of TP53 and PIK3CA increased lung cancer progression. Notably, KRAS is one of the most mutated oncogenes and the main oncogenic driver in lung cancer. The interaction of KRAS and TP53 contributes to multidrug response [15]. However, the association between PRKCA and these genes is rarely studied. Co-mutation of PRKCA and TP53 has been observed in this study. Previous studies also identified P53 as a substrate of PKCα [51], which further proved the interaction of PRKCA and P53 both at the gene level and protein level. Hill et al. revealed that PKCα could suppress KRAS-mediated lung tumor formation. Furthermore, PKCα may regulate cell proliferation by interacting with P53 and the KRAS signaling pathway. Additionally, PKCα was also correlated with Bcl2, which is a well-known gene mediating drug resistance. Villar J.et al. proved that PKCα induced Bcl2 phosphorylation and stabilized Bcl2 expression [46,52]. These findings further confirmed our results. Nevertheless, the effect of these co-mutated genes remains largely unknown. Moreover, mutually exclusive co-mutated genes, such as PRDM9, and MUC5B, have not been reported in lung cancer resistance. Further experiments are required to verify the exclusive mutations between PRKCA and these genes. Nevertheless, there are still some limitations in this research. For example, we only concentrated on the functional effect of PRKCA in vitro. Animal models should be considered in following studies to validate our findings. Furthermore, more clinical tests need to be performed in the future. In addition, compared with prior studies, our findings not only comprehensively demonstrated that PRKCA is an important regulator in cisplatin-resistant lung cancer, but also and enriched the PRKCA complex regulatory network.

In conclusion, the present study identified the key signaling pathways involved in cisplatin resistance in lung cancer. *PRKCA* was characterized as a potential therapeutic target. Strategies that block these key signaling pathways, target PRKCA may offset cisplatin resistance (Fig. 7). The comprehensive analysis revealed that *PRKCA* might promote tumor progression by interacting with *TP53*, *KRAS*, *bcl2*, and *PIK3CA*. Our study provides information about the role of PRKCA in tumor formation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

CRediT authorship contribution statement

Ting Sun: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Penghua Zhang:** Visualization, Validation, Investigation, Formal analysis, Data curation. **Qingyi Zhang:** Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis. **Binhui Wang:** Visualization, Software, Investigation, Data curation. **Qitai Zhao:** Software, Methodology, Data curation. **Fenghui Liu:** Software, Funding acquisition. **Xiaohua Ma:** Formal analysis. **Chunling Zhao:** Methodology. **Xiaolei Zhou:** Software. **Ruiying Chen:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Data curation, Conceptualization. **Songyun Ouyang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30780.

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