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Long Non-coding RNA INE1 Induced Autophagy Promotes Sensitivity of Prostate Cancer Cells to Cisplatin

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Abstract

Prostate cancer is most prevalent malignancy of males in the world. In recent years, long non-coding RNAs (lncRNAs) were identified, and their functions are associated with prostate cancer initiation and progression. However, their molecular mechanisms still need to be elucidated before the clinical utility. In the present study, we identified the correlation of lncRNA inactivation escape 1 (INE1) with the characterization in prostate cancer patients, and detected the roles of INE1 in cell autophagy and apoptosis in prostate cancer cells. Our results showed that the lncRNA INE1 expression highly correlate with patients' survival times, tumor stage, biochemical recurrence, disease recurrence and Gleason pattern. High expression of INE1 was detected in prostate cancer cells, and knockdown INE1 by siRNA resulted in significant inhibition of cell viability. In addition, silencing INE1 induced early autophagy and pro-apoptosis, which augments cisplatin (CDDP)-induced cell apoptosis. Moreover, INE1 played an anti-apoptotic role by targeting the serine/arginine-rich splicing factor 2 (SRSF2).

Key words　prostate cancer, LncRNA, INE1, autophagy, apoptosis, cisplatin

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Introduction

Prostate cancer is most prevalent malignancy of the male reproductive system in the world. Based on GLOBOCAN 2020 estimates, 191,930 new cases of prostate cancer occurs worldwide in 2020, and approximately 11.6 percent of men will be diagnosed with prostate cancer at some point during their lifetime [1]. Prostate-specific antigen (PSA) testing at earlier stage in diagnosis and advances in treatments, there is a decline in the incidence of prostate cancer [1-3]. However, only PSA testing is not sufficient for the accurate diagnosis of prostate cancer [1, 4]. In recent years, the use of public database for cancer research provides enormous information for screening novel biomarkers and potential therapeutic targets. The Cancer Genome Atlas (TCGA) Research Network has profiled and analyzed large numbers of human tumors to discover molecular aberrations at the DNA, RNA, protein and epigenetic levels [5]. It is significant to combine bioinformatics analysis to identify new biomarkers or oncogenes based on genomic, transcriptomic, proteomic and epigenomic alterations, and clinical implications.

Long non-coding RNA (lncRNA) has been documented in TCGA database and has enormous potential in cancer research. It is a group of non-protein coding transcripts with ≥200 nucleotides, widely expressed (about 60,000 lncRNA) in the human genome and widespread both in nucleus and cytoplasm [6, 7]. In the past three decades, more than hundreds of cancer associated lncRNAs were identified and have been reported to play an important role in regulating cancer development [6, 8, 9], including prostate cancer [10, 11]. However, the functions of lncRNAs in prostate cancer initiation, progression, and their clinical utility in disease management are still infancy [12]. The lncRNA inactivation escape 1 (INE1) also known as non-protein coding RNA 10 is located on the human chromosome Xp11.3 and consists of 1014 bases and contains one exon. As an X chromosome gene, INE1 is intronless and was identified because its transcription escapes X inactivation in females [13]. Through a microarray analysis and an integrated functional gene map, lncRNA INE1 was identified as playing possible roles in pathogenesis of cerebral aneurysms and neurogenetic disorders [14, 15]. Recently, lncRNA INE1 was documented to play a critical role in the regulation of prostate cancer autophagy [16]. However, the function and mechanism of lncRNA INE1 in prostate cancer has not yet been elucidated.

The serine/arginine-rich splicing factors (SRSFs) belong to the serine arginine-rich protein family, which plays an important role in the recruit and assemble spliceosome to regulate the splicing process of precursor RNA. In tumors, SRSFs act as oncoproteins in disrupting both DNA stability and normal protein expression pattern, contributing to proliferation, migration and apoptosis resistance of tumor cells [17]. In prostatic cancer patients, SRSFs such as SRSF2, SRSF5, SRSF7 and SRSF8 were identified as negative prognostic biomarkers, due to their higher expression that correlated with poor overall survival [18]. The regulation of SRSFs by lncRNAs were shown to be involved in the progression of tumors. LncRNA PANDAR dictates the chemoresistance of ovarian cancer via regulating SFRS2-mediated p53 phosphorylation [19]. LncRNA LINC02580 suppresses the invasion of hepatocellular carcinoma by targeting SRSF1 [20]. SRSF1 splicing in gastric cancer was enhanced by lncRNA FENDRR [21]. However, the role of lncRNA on SRSFs in tumor progression still needs further evaluation.

In the present study, we identified the correlation of lncRNA INE1 with the survival times of patients with prostate cancer and the clinico-pathological features. In order to explore the role of INE1 in prostate cancer, we detected the cell viability, cell proliferation, autophagy and apoptosis in siRNA INE1 knockdown or cisplatin treated prostate cancer cells. Moreover, the role of lncRNA INE1 on splicing factor SRSF2 was detected in prostate cancer cells.

Materials and methods

Reagents

MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) was purchased from Solarbio (Beijing, China). The anti-SRSF2, Caspase-9, Caspase-3 and β-actin antibody were purchased from Cell Signaling Technology (Shanghai, China). The anti-Ki-67 antibody, Alexa Fluor 488 goat anti-mouse IgG and DAPI were purchased from Thermo Fisher Scientific (Shanghai, China). The terminal desoxynucleotidyl transferase (dUTP) nick end labeling (TUNEL) kit was purchased from Beyotime (Shanghai, China). RNA-to-cDNA kit and SYBR Green I kit were purchased from Vazyme (Nanjing, China). Mediums, FBS, penicillin, and streptomycin were purchased from Gibco (CA, USA). PVDF membranes and chemiluminescent reagent were purchased from Merck (Shanghai, China). Cisplatin (CDDP) was purchased from Master of Bioactive Molecules (Shanghai, China), Chloroquine (CQ) was purchased from Sigma (USA).

Cell culture and treatments

Prostate cancer cell lines DU145, LNCaP and PC-3, and normal human prostate stromal cell WPMY-1 were obtained from American Tissue Culture Collection (ATCC, Manassas, VA, USA). DU145 cells were cultured in MEM; LNCaP cells were cultured in RPMI 1640 (pH 7.4) medium; PC-3 cells were cultured in Ham'sF12k (pH 7.4); WPMY-1 cells were cultured in DMEM medium (pH 7.4), and supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin (pH 7.4) in a humidified atmosphere containing 5 % CO_2. For some experiments, the cells were treated with cisplatin (2, 5, 10 μM) for 24 h.

Cell migration detection

Cell migratory capacity was assessed using the wound-healing assay. Cells were seeded in 6-wells plate (2 x 10^5 cells/well) in 2 mL complete medium. Once reaching 90-95% confluence, a scratch was created manually through the confluent monolayer using a white tip (2-20 μL). The culture medium was then aspirated, and wells were washed with PBS to remove cellular debris. Fresh medium without FBS was then added along with the indicated treatments. Images were taken at baseline (0 h) and at the indicated time points by Olympus IX73 microscopy (Japan). Microscope software was used to measure the width of the scratch, which reflected the extent of cell migration. The distance migrated was measured in μm.

MTT assays

DU145 and PC-3 cells were seeded in 6-well and transfected with vector control, INE1 siRNA-2 and siRNA-3 for 24 hrs, cells were collected and seeded in 96-well with a density of 2×10^3 cells/well. In some experiments, the cells were treated with CDPP (2 μM) for 24 h with or without pretreatment of CQ (20 μM) 1 h. After culture for 24 hrs, the cell viability was measured by the MTT assay. In brief, cells were incubated in 100 μL MTT solution (0.5 mg/ml in MEM medium) in 96-well plate for 4 h before the end of incubation. The supernatant was then discarded, and 100 μL DMSO was added to dissolve the colored product. The absorbance was measured at 540 nm (690 nm as reference) using a Synergy H4 Hybrid Multi-Mode Microplate Reader (BioTek, USA).

Table 1. Correlation analysis of INE1 expression level with clinicopathological features of patients with prostate cancer.

Variable	Total (%)	Expression of INE1 level		P value
		Low	High	
Age	n=498			
≦60	223 (44.8%)	210	13	0.520
>60	275 (55.2%)	255	20	
Tumor Stage Code (AJCC)	n=491			
T1	0	0	0	
T2	187 (38.1%)	181	6	0.003*
T3	293 (59.7%)	271	22	
T4	11 (2.2%)	8	3	
Biochemical recurrence index	n=430			
No	371 (86.2%)	349	22	0.033*
Yes	59 (13.7%)	51	30	
Disease recurrence	n=492			
Disease Free	400 (81.3%)	379	21	0.045*
Recurred/Progressed	92 (18.7%)	82	10	
Lymph node metastasis (N)	n=406			
N0	325 (80.0%)	301	24	0.705
N1 or above	81 (20.0%)	76	5	
Gleason score	n=498			
Gleason ≦7	210 (42.2%)	200	10	0.014*
Gleason >7	288 (57.8%)	265	23	
PSA result	n=440	410	30	0.385
Lymph node count	n=423	391	32	0.873

Tumor Stage Code: American Joint Committee on Cancer Tumor Stage Code; Lymph Node metastasis (N): Positive Finding Lymph Node Hematoxylin and Eosin Staining Microscopy Count.

Real-time quantitative PCR (RT-qPCR) analysis

Cell total RNA was isolated by using Trizol (Life Technologies, MD, USA). All RNA isolations were performed as directed by the manufacturer. Gel electrophoresis and staining confirmed the purity and integrity of the samples. Quantification of RNA was based on spectrophotometric analysis at 260/280 nm. cDNA was generated from 10 μg total RNA using a RNA-to-cDNA kit. Real-time PCRs were carried out by ABI7300 system using SYBR Green I kit. Gene-specific primers for INE1-F: GGAAGGCCTGAGTTCTGCAA, INE1-R: CCCACCTCAGGATCTTTGCG, and β-Actin-F: CTCCATCCTGGCCTCGECTGT, β-Actin-R: GCTGTCACCTTCACCGTTCC were used. The occurrence of primer-dimers and secondary products was inspected using melting curve analysis and agarose gel electrophoresis. Control amplification was done either without reverse transcriptase or without RNA. The relative expression ratio of the target gene was calculated according to their threshold cycle Ct values.
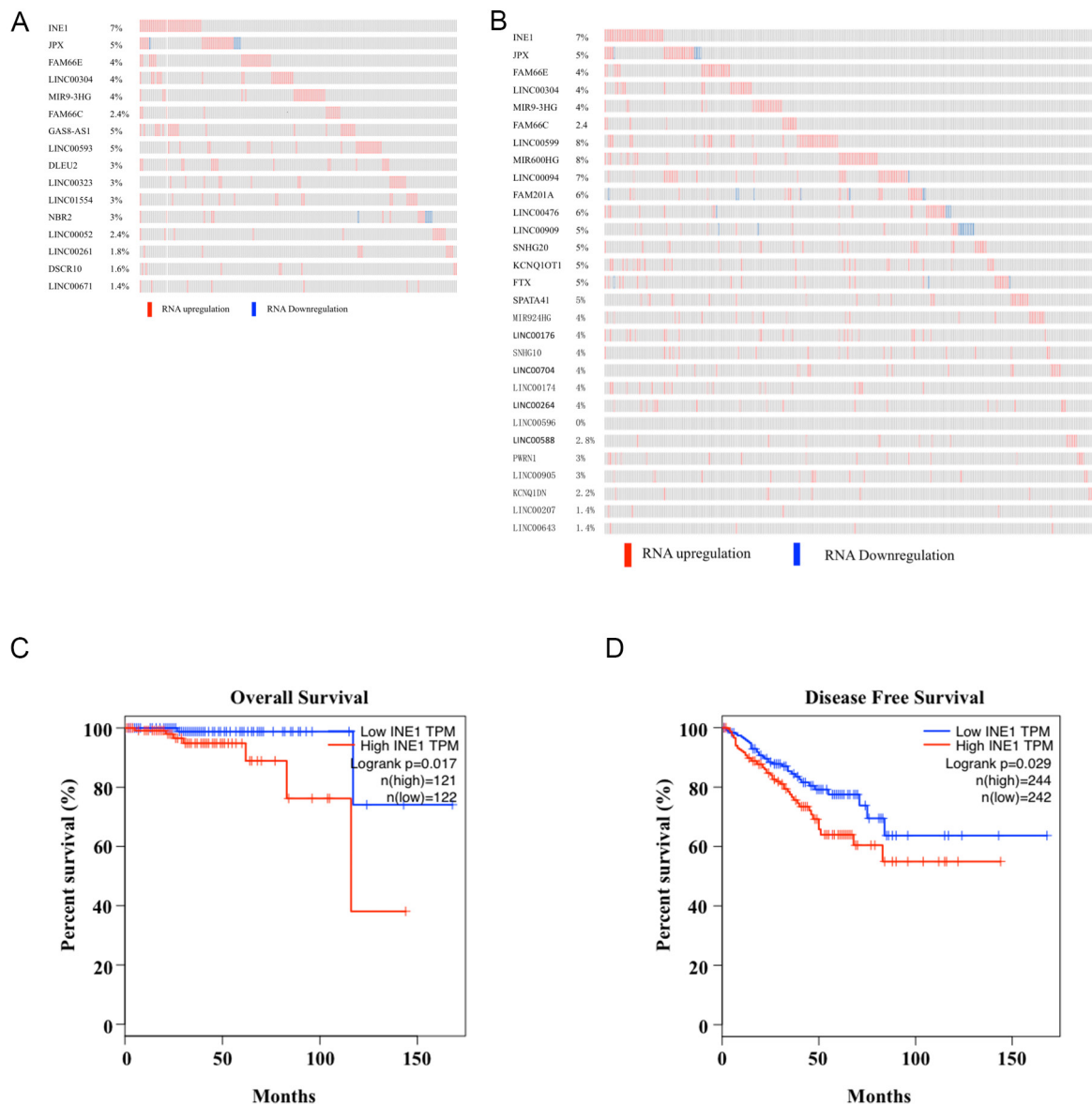
Knockdown of INE1 and SRSF2

Figure 1. Signature of INE1 gene expression with survival time of the prostate cancer patients. Signature of LncRNAs for Overall Survival (A) and Disease Free Survival (B) based on mRNA expression. GEPIA analysis shows Kaplan-Meier survival curves of Overall Survival (C) and Disease Free Survival (D) for prostate cancer patients compared high INE1 expression with low expression.

DU145 and PC-3 cells were seeded in 6-well with a density of 2×10^5 cells/well and cultured overnight. Then the cells were transfected with INE1 siRNAs packaged by Lipo2000 reagent according to manufacturer instructions (Thermo Fisher Scientific, WI, USA). At 24 hrs after transfection the cells were harvested for further evaluation. The siRNAs used in this study were INE1 siRNA-1: sense 5′- CGUGAGGUAUAUAGUUAAUTT-3′ and antisense 5′- AUUAACUAUAUACC UCACGTT-3′; INE1 siRNA-2: sense 5′-GGAGAAUGGUUUGUAGUAUTT-3′ and antisense 5′-AUACUACAAACCAUUCUCCTT-3′; INE1 siRNA-3: sense 5′-GUCC ACAGCUUCCCUUUAUTT-3′; and antisense 5′-AUAAAGGGAAGCUGUGGACT T-3′; SRSF2 siRNA-1: sense 5′-GCGGUGUCCUCUUAAGAAATT-3′ and antisense 5′-UUUCUUAAGAGGACACCGCUU -3′; SRSF2 siRNA-2: sense 5′-GAGCAGGU UUGUCUUUAAATT -3′ and antisense 5′-UUUAAAGACAAACCUGCUCTT-3′; and siRNA

control: sense 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense 5′-ACGUGACACGUUCGGAGAATT-3′ from GenePharm (Shanghai, China).

Western blot analysis

The cells were lysed in radioimmunoprecipitation assay (RIPA) buffer. After centrifugation at 13,000 g for 15 min at 4 ℃, the supernatant was collected and the total protein concentration was determined by BCA Assay (Protein Quantification Kit) (Thermo Fisher Scientific, USA). The protein lysates containing 40 μg total cellular protein in RIPA buffer were subjected to electrophoresis on 10% polyacrylamide gels. The gels were then blotted onto PVDF membranes. Western blotting was conducted using rabbit monoclonal antibodies against LC3B, P62, Caspase-9, Caspase-3, SRSF2, and β-actin antibody (1:1000), followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit antibody
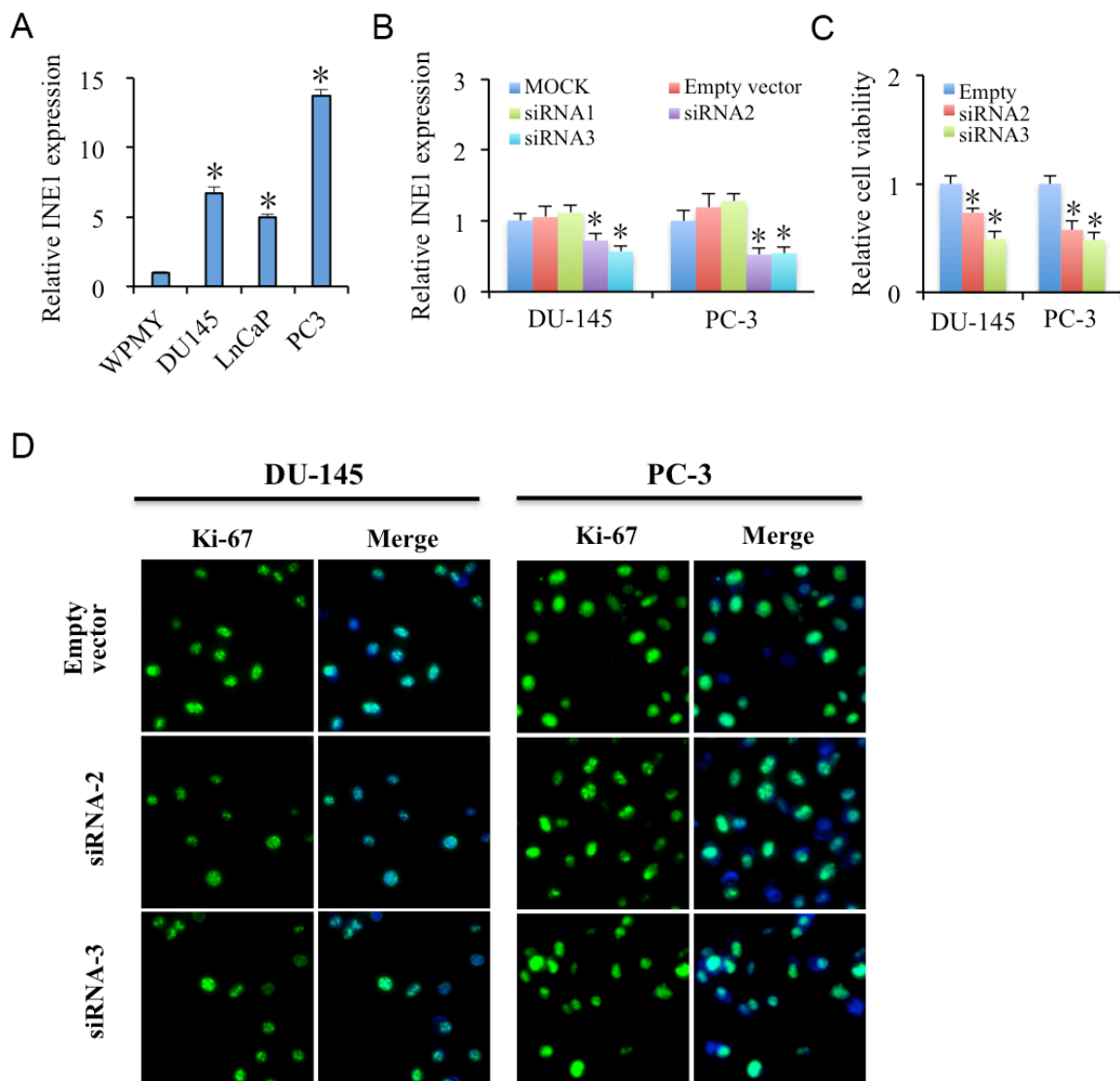
Figure 2. Knockdown INE1 inhibits cell viability in prostate cancer cells. (A) INE1 is highly expressed in prostate cancer cell lines DU145, LNCaP and PC-3 as compared to normal human prostate stromal cell WPMY-1 cells, determined by RT-PCR. Results are presented as mean ± SD (n=3). * P<0.05, using Student's t-test. (B) Expression of INE1 in knockdown DU-145 and PC-3 cells as compared to siRNA control. (C) INE1 knockdown in DU-145 and PC-3 cells suppresses cell growth as detected by MTT assays. Results are presented as mean ± SD (n=4). * Statistical significance between control and siRNA treatments, * P<0.05, using Student's t-test. (D) INE1 knockdown dose not affect cell proliferation in DU-145 and PC-3 cells as detected by Ki-67 immunofluorescence. Ki-67: Green, DAPI: Blue. Scalar bar: 50 μm.

(1:4000). Specific bands were visualized using chemiluminescent reagent, and the images were taken by Tanon-5200 Multi Automatic Chemiluminescence Image Analysis System. The relative protein levels were analyzed by Image J.

Immunofluorescence and fluorometric cell death detection

DU145 and PC-3 cells were seeded in 6-well and transfected with siRNA control, INE1 siRNA-2 and siRNA-3 for 24 hrs, with or without treatment of cisplatin (2 μM) for 24 hours in apoptosis detection experiments. Then the cells were fixed for 30 min in 4% Formaldehyde (FA, Sigma-Aldrich) and permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) in PBS for 20 min. After blocking with 3% normal goat serum, the cells were incubated with mouse anti-Ki-67 (1:100) antibody overnight at 4 °C, followed by 1 h

incubation with Alexa Fluor 488 goat anti-mouse IgG (1:200,). After washing twice with PBS, cell nucleus was stained by the DAPI for several minutes. The cells were washed in PBS for 10 min and the cells were mounted, then examined by Olympus IX73 microscopy (Japan). Fluorometric cell death was detected by TUNEL assay according to the manufacturer's instructions, and counterstained with DAPI as previously described. Then the images were taken by Olympus IX73 microscopy (Japan).

Statistical analysis of data

Prostate cancer dataset (TCGA) (https://www.genenames. org/) was searched where 2708 lncRNAs (http://www.genenames.org) and 2687 lncRNAs (http://www. cbioportal.org) were analyzed by two different software. We selected all 499 tumors samples
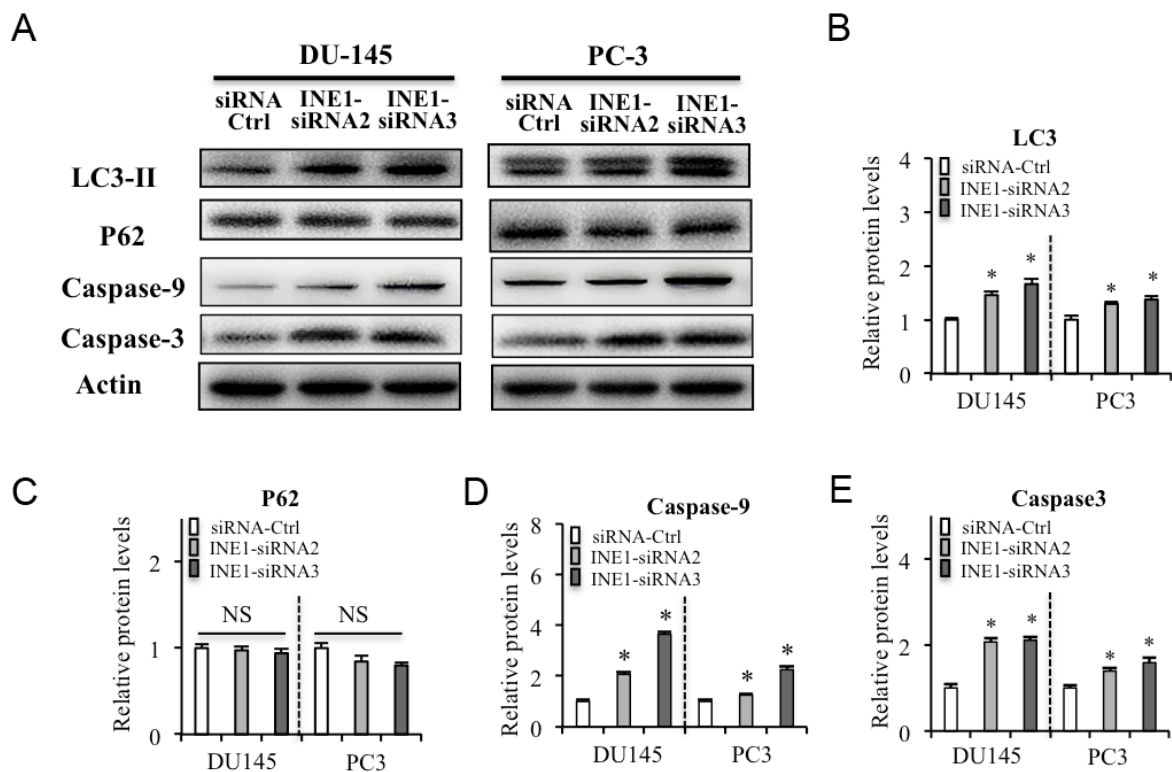
Figure 3. Silencing INE1 on autophagy and pro-apoptosis in prostate cancer cells. (A) Effects of INE1-siRNAs on the expressions of the autophagy biomarkers LC3-II, P62, and pro-apoptosis biomarkers Caspase-9 and Caspase-3 in DU-145 and PC-3 cells detected by western blotting. (B-E) Relative protein levels were analyzed by Image J. NS: no significant difference. *P<0.05, Statistical significance between indicated groups, using Student's t-test.

(498 patients) identified in the primary search. The primary search parameters included mutations, copy number alterations (CNA) from GISTIC and mRNA expression (RNA seq data) with the default setting. For the secondary search, we focused on RNA seq data and analyzed the correlation between their expressions and the survival time of prostate cancer patients. All available lncRNAs were sorted by alteration frequency at the cBioPortal. Those lncRNAs with significant log-rank p values were entered in the candidate pool to be considered for further selection. P values less than 5% were determined as significant. Association with the INE1 expression levels and clinic-pathological features were analyzed with the chi-square ($\chi 2$) test for categorical variables and the Wilcoxon test for continuous variables. The statistical analyses were performed using the SPSS 20.0 software analysis. The student t-test was used to compare the results and expressed as mean ± S.D. between control and treatment groups. P < 0.05 was considered statistically significant.

Results

Correlation of INE1 expression with clinical features of prostate cancer patients

Analysis revealed that the lncRNA INE1 is the top one related to both overall survival and disease free survival time (**Figure 1A, B**). According to the GEPIA analysis, the disease free survival time and overall survival time of patients with higher INE1 expression were significantly shorter than those with lower INE1 expression (P < 0.05) (**Figure 1C, D**). As shown in **Table 1**, there

was no significant statistical correlation between age, lymph node metastasis and count, PSA results with the expression of INE1 (P > 0.05), but it positively correlated with tumor stage, biochemical recurrence, disease recurrence and Gleason pattern (P < 0.05). It suggested that lncRNA INE1 might act as an oncogene promoting tumor malignancy and recurrence.

Higher INE1 expression in prostate cancer cells promotes cell viability

To identify the functions of INE1, firstly, we compared the mRNA expression of INE1 in prostate cell lines DU145, LNCaP and PC-3 with normal human prostate stromal cell WPMY-1 cells. It showed that INE1 expression in prostate cancer cell lines was significantly higher than in WPMY-1 cells (**Figure 2A**). To further clarify the roles of INE1 in prostate cancer growth, small interfering RNA was applied. The results of RT-PCR showed the second and third pair of siRNA had the higher efficiency on INE1 silencing in DU145 and PC-3 cells (**Figure 2B**). Furthermore, MTT assays indicated that siRNA silencing INE1 for 24 h resulted in significant inhibition of cell viability in both DU145 and PC-3 cells (**Figure 2C**). However, the immunofluorescence staining of cell proliferation biomarker Ki-67 showed that there was no difference of the expression of Ki-67 in INE1 knockdown cells compared to negative control in both cell lines (**Figure 2D**).

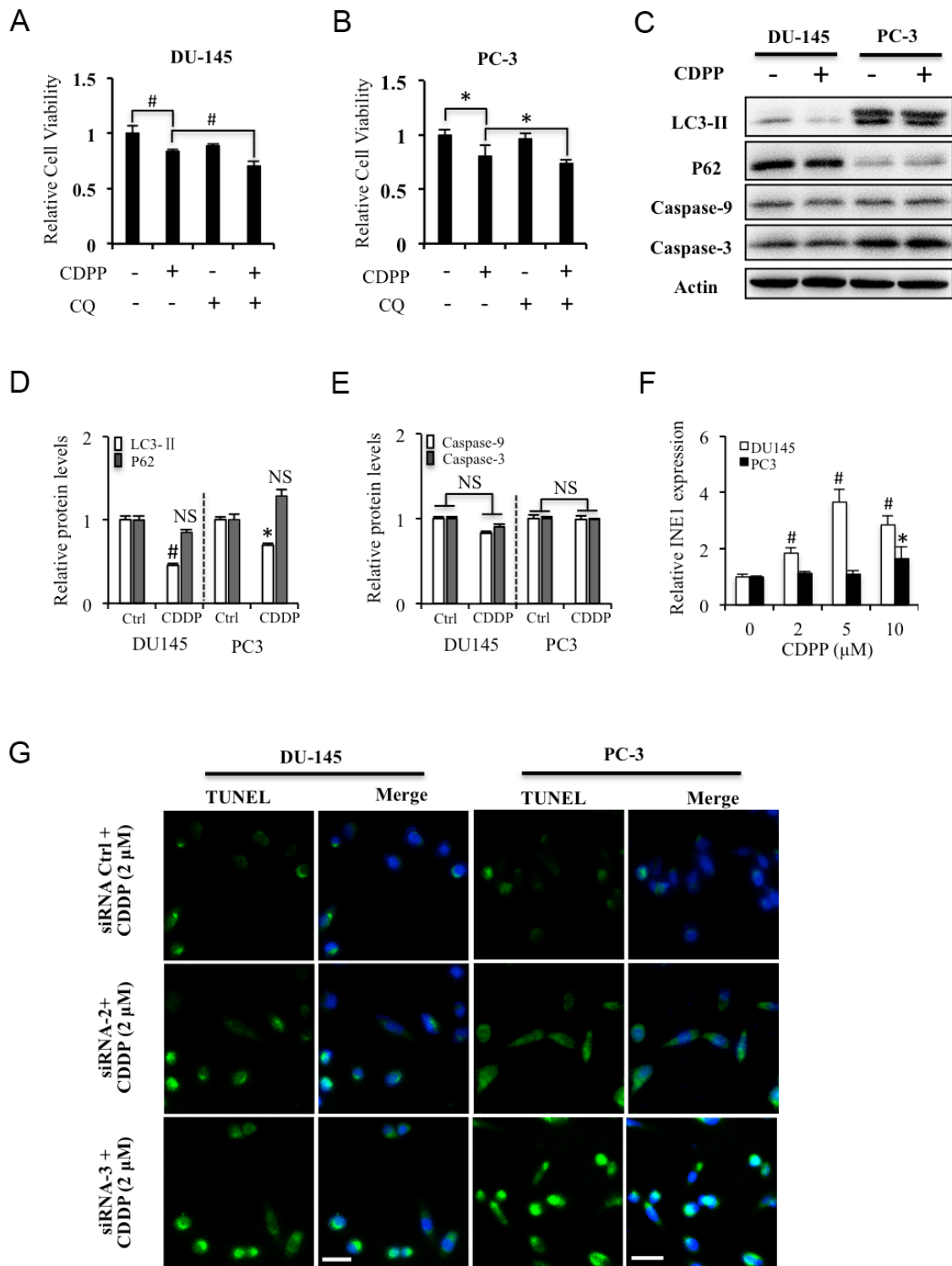*Silencing INE1 expression induces autophagy and pro-apoptosis of prostate cancer cells*

Figure 4. Silencing INE1 on cisplatin induced apoptosis in prostate cancer cells. (A, B) Effects of CDPP and CQ on the cell viability in DU-145 and PC-3 cells detected by MTT assay. (C) Effects of CDPP on the expressions of the autophagy biomarkers LC3-II, P62, and pro-apoptosis biomarkers Caspase-9 and Caspase-3 in DU-145 and PC-3 cells detected by western blotting. (D, E) Relative protein levels were analyzed by Image J. (F) Effects of CDPP on the expressions of INE1 expression detected by RT-qPCR. (G) Effects of CDPP on DNA damage in INE1 knockdown DU-145 and PC-3 cells detected by TUNEL staining. Green: TUNEL, Blue: DAPI. Scalar bar: 50 μm. CDPP: cisplatin, 2 μM. CQ: Chloroquine, 20 μM. NS: no significant difference. *P<0.05, # P<0.01, Statistical significance between indicated groups, using Student's t-test.

To further clarify INE1 promote cell viability, the expressions of the biomarkers of cell autophagy and apoptosis were detected by western blotting. Knockdown INE1 by siRNAs increased the expression of early autophagy inducer LC3-II but did not change the expression of p62 in both DU145 and PC-3 cells (**Figure 3A-C**). In addition, INE1-siRNAs increased the expression of
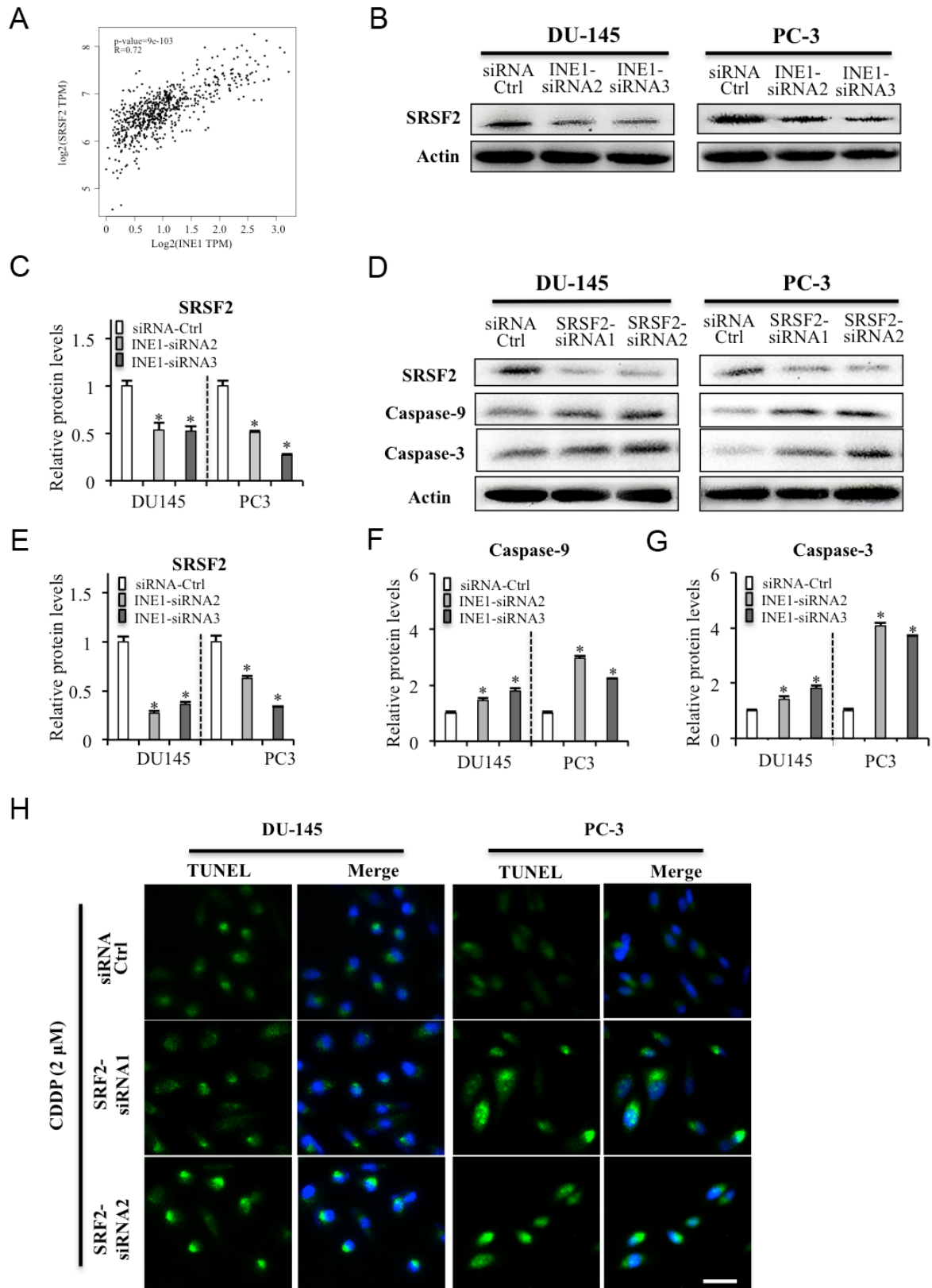
Figure 5. Roles of INE1 targeting SRSF2 on cell apoptosis in prostate cancer cells. (A) Correlation of INE1 with SRSF2. (B, C) Expression of SRSF2 in INE1 knockdown DU145 and PC-3 cells detected by western blotting. (D-G) Efficacies of SRSF2 siRNAs on the expression of apoptosis marker Caspase-9 and Caspase-3 as detected by western blotting. β-Actin was used as loading reference. Relative protein levels were analyzed by Image J. *P<0.05, Statistical significance between indicated groups, using Student's t-test. (H) CDDP induced cell apoptosis in silencing SRSF2 DU145 and PC-3 cells detected by TUNEL staining. CDDP: cisplatin, 2 μM. Green: TUNEL, Blue: DAPI. Scalar bar: 50 μm.
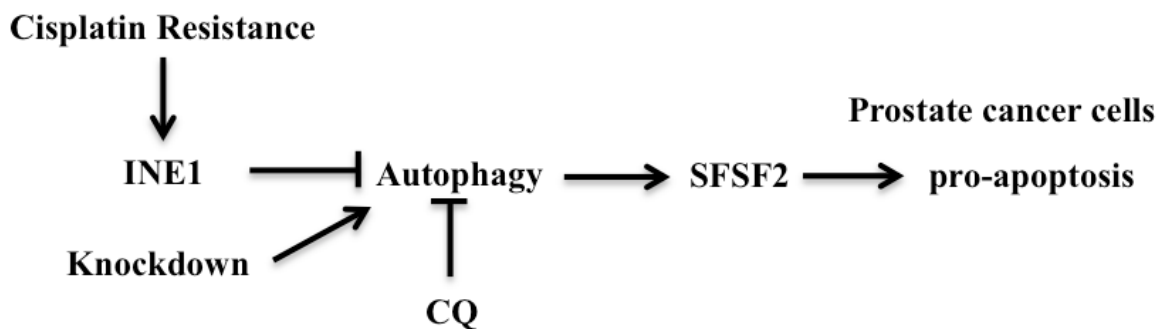
Figure 6. Schematic diagram to show the mechanism that relationship between LncRNA FAM66C and prostate cancer.

caspase-9 and caspase-3 in both DU145 and PC-3 cells (**Figure 3A, D, E**). These results indicated that silencing INE1 expression might induce autophagy signal and pro-apoptosis in prostate cancer cells.

Silencing INE1 promotes sensitivity of prostate cancer cells to cisplatin

To further identify lncRNA INE1-regulated autophagy in prostate cancer, the cells were treated with cisplatin and/or autophagy inhibitor CQ. The results of MTT assay showed that low dose of cisplatin (2 μM) inhibited cell viability of prostate cancer cells, which were augmented by autophagy inhibitor CQ (**Figure 4A, B**). While the results of Western blotting showed that low dose of cisplatin treatment significantly suppressed the expression of LC3-II, but did not significantly change the expression of p62, caspase-9 and caspase-3 in both DU145 and PC-3 cells (**Figure 4C-E**). The result of qRT-PCR showed that cisplatin induced the expression of INE1 in DU145 cells, and high dose of cisplatin induced its expression in PC-3 cells (**Figure 4F**). In addition, the result of TUNEL staining showed that INE1-siRNAs increased low dose of cisplatin treatment -induced nuclear DNA damage in both DU145 and PC-3 cells (**Figure 4G**). These results suggest that silencing lncRNA INE1-induced autophagy and apoptosis aggravated cisplatin-inhibited cell viability in prostate cancer.

INE1 plays an anti-apoptotic role by regulating the expression of the splicing factor SRSF2

To identify the anti-apoptotic role INE1 in prostate cancer cells, we detected the regulation of INE1 on SRSF2, which is well known splicing factor and plays anti-apoptotic role in cancer cells [22, 23]. The result of Western blotting showed that knockdown INE1 by siRNAs decreased the protein level of SRSF2 in both DU145 and PC-3 cells (**Figure 5A, B**). In addition, the results of Western blotting and TUNEL staining showed that knockdown SRSF2 by siRNA increased the expressions of apoptosis markers Caspase-9 and Caspase-3 and induce DNA damage (**Figure 5C-G**). These results suggest that INE1 play an anti-apoptotic role by targeting the expression of the splicing factor SRSF2 in prostate cancer cells.

Discussion

Long noncoding RNA (lncRNA) has been identified to play important roles in tumor risk and initiation, promotion, suppression, and treatment resistance [8-11, 24]. In the past three decades, more than hundreds of prostate cancer associated lncRNAs were identified [12]. Some of them can be utilized in clinical prostate cancer diagnosis, prognosis, and prediction, and might be therapeutic targets. The biological and molecular functions of lncRNA in cancers still need to be clarified. In the present study, after analysis of the prostate carcinoma dataset, we identified lncRNA INE1 expression significantly correlated with overall survival time and disease-free survival, and the clinicopathological features, including tumor stage, biochemical recurrence, disease recurrence and Gleason pattern. Furthermore, silencing INE1 expressions results in inhibition cell viability of prostate cancer cells.

Previous studies have explored that lncRNAs enhances drug resistance, promotes ferroptosis and bone metastasis in prostate cancer [25-28], as well as promote proliferation, migration, and invasion in prostate cancer cells [29]. It is also reported that lncRNAs regulates autophagy in prostate cancer cells. Upregulation of LncRNA IDH1-AS1 promotes tumor growth in prostate cancer by regulating ATG5-mediated autophagy [30]. Silencing lncRNA PRRT3-AS1 inhibits prostate cancer cell proliferation and promotes apoptosis and autophagy [31]. LncRNA BCAR4 enhances autophagy in prostate cancer cells [32]. Autophagy is a process that delivers cellular material to lysosomes for degrading, recycling, and providing energy and molecular precursors. It suggests that promoting autophagy might prevent cancer development in premalignant lesions, but both enhancing and inhibiting autophagy could be therapeutic strategies in advanced cancers [33]. INE1 was indicated as an autophagy-associated lncRNA in prostate cancer [16]. Our results suggest that silencing INE1 expression induce autophagy signal associated with pro-apoptosis in prostate cancer cells.

Cisplatin (CDDP) is a widely used chemotherapy agent for various types of cancers. It can also induce autophagy in cancer cells, including prostate cancer cells at high dose (5 μM). Low dose of CDDP suppressed the expression of autophagy-related molecules including LC3-II, P62, ATG5, and ATG7 in rat astrocytes in vitro [34]. Our results showed that low dose of CDDP (2 μM) reduced the expression of LC3-II, but did not change the expression of P62, as well as the pro-apoptosis biomarkers caspase-9 and caspase-3. It suggested that low dose of CDDP inhibited the early autophagy signal and could not induce cell apoptosis in prostate cancer cells. The regulation of autophagy could modulate the sensitivity of cancer cells to cisplatin. Ambra1 induces autophagy and desensitizes prostate cancer cells (DU145) to CDDP (10 μM) [35]. Knockdown of cystic fibrosis transmembrane conductance regulator (CFTR) enhances sensitivity of prostate cancer cells (LNCaP/CP) to CDDP (40 μM) via inhibition of autophagy [36]. It is also reported miR-205 impaired the autophagic flux and

enhanced CDDP (5, 10 μM) cytotoxicity in androgen-refractory prostate cancer cells (DU145 and PC-3) [37]. Circular RNA circCUL2 regulate cisplatin sensitivity through miR-142-3p/ROCK2-mediated autophagy activation in gastric cancer cells [38]. Artesunate impairs growth in cisplatin-resistant bladder cancer cells by cell cycle arrest, apoptosis and autophagy induction [39]. In present study, our results confirmed that autophagy inhibitor CQ could aggravate low dose of CDDP -inhibited cell viability, and silencing INE1 can aggravate low dose of CDDP-induced apoptosis of prostate cancer cells.

Precursor messenger RNA (pre-mRNA) splicing is an essential for gene expression from DNA to protein in all eukaryotes. Structural studies have proved that pre-mRNA is spliced by the spliceosome, which catalytic metals in the active site are coordinated by U6 small nuclear RNA and the catalytic triplex as a protein-directed metalloribozyme [40]. Aberrant mRNA splicing is prevalent in many cancers [41]. Dysregulation of spliceosome gene expression were investigated in advanced prostate cancer, such as SF1, HNRNPU, which are targeted by an RNA-binding transcriptional and splicing factor, splicing factor proline and glutamine-rich (PSF/SFPQ) [42]. LncRNA CTBP1-AS interacts with PSF and represses cell cycle regulators, and promotes castration-resistant prostate cancer growth [43]. It suggests that lncRNA-mediated association of RNA-binding proteins or transcription factors deregulates pre-mRNA splicing of spliceosome in prostate cancer progression, such as promoting cell proliferation, invasion, and drug resistance. In previous study, we demonstrated that lncRNA FAM66C activates epidermal growth factor receptor (EGFR)-extracellular signal-regulated kinase (ERK) signaling to promote cell proliferation by inhibiting proteasome pathway in prostate cancer [44]. Emerging research has also indicated that lncRNA acts as tumor oncogenes in human tumorigenesis by promoting anti-apoptosis of cancer cells [45, 46]. Our results suggest silencing INE1 increases apoptosis but does not affect cell proliferation in prostate cancer cell line DU145 and PC-3. SRSF2 is a splicing factor that binds to caspase-9 RNA [46], regulating the expression of its alternative spliced anti-apoptotic form [22, 23]. In particular, it has been reported that basal and cisplatin-induced caspase-9 activation and caspase-3 protein level is regulated by the splicing factor SRSF2 [47]. In the present study, silencing INE1 suppress the expression of SRSF2, which causes increasing the protein levels of caspase-9 and caspase-3, as well as cisplatin-induced DNA damage. Taken together, our study indicates that lncRNA INE1 plays an anti-apoptotic role by targeting the expression of the splicing factor SRSF2 in prostate cancer cell line DU145 and PC-3.

Conclusion

In summary, lncRNA INE1 expression highly correlated with survival times, cancer tumor stage, biochemical recurrence, disease recurrence and Gleason pattern of prostate cancer patients. Furthermore, higher expression of INE1 was detected in prostate cancer cells compared to normal cells, and silencing INE1 induce early autophagy and pro-apoptosis, which augments CDDP induced cell apoptosis. In addition, INE1 played an anti-apoptotic role by targeting the splicing factor SRSF2 (**Figure 6**). There are few limitations in present study, such as the mechanisms of knockdown lncRNA INE1-increased pro-apoptosis, and the therapeutic effect targeting on INE1 in prostate cancer, as well as its synergistic effects with the cisplatin treatment have not been further verified in in vivo models. This study indicates that the lncRNA INE1 could be a novel potential therapeutic target or overcoming drug resistance in prostate cancer.

Acknowledgements

Ethical policy

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Approval from institutional ethical committee was taken.

Availability of data and materials

The data sets supporting the conclusions of this study are included in this article and its additional images. Raw data are available on the main electronic data storage system of Affiliated Hospital of Jiangsu University, and access can be provided upon request to the authors.

Author contributions

HC and KL performed the research; HC, JG, YX and JM designed the research study and contributed essential reagents or tools; HC, KL, JG, WX, YX and JM analyzed and interpreted the data; HC, JG, WX, YX and JM wrote the manuscript. All authors reviewed the manuscript.

Competing interests

The authors declare no conflict of interest.

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