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LUZP2 Inhibition Sensitizes Prostate Cancer to Chemotherapy by Suppressing RAD50

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Abstract

Background: Prostate cancer is the most frequent malignancy in the worldwide male population. The expression of LUZP2 in human tissues has marked tissue specificity, mainly in the brain, adrenal gland and prostate. However, the chemosensitivity of LUZP2 in prostate cancer cells to drugs is unclear. In this study, the role of LUZP2 in paclitaxel resistance in prostate cancer was therefore investigated.

Methods: LUZP2 and RAD50 protein expression in DU145 or PC3 cells was examined by western blotting. The rates of cell apoptosis were measured by an Annexin V-FITC/PI Apoptosis Detection Kit. Cell proliferation was measured by CCK8 and colony formation assay. Cell migration ability was tested by wound healing assay.

Results: LUZP2 overexpression diminished the chemosensitivity of prostate cancer cells to paclitaxel, inhibition LUZP2 expression enhanced the chemosensitivity of prostate cancer cells to paclitaxel. Mechanistically, the expression of LUZP2 was positively correlated with RAD50 level, and LUZP2 regulated the mRNA and protein levels of RAD50. In addition, RAD50 knockdown also increased the chemosensitivity of prostate cancer cells to paclitaxel, and RAD50 overexpression reversed the promoting effect of LUZP2 inhibition on the chemosensitivity of prostate cancer cells to paclitaxel.

Conclusion: Our results demonstrated that LUZP2 knockdown could increase the chemosensitivity of prostate cancer cells to paclitaxel by suppressing RAD50.

Keywords: Prostate cancer; LUZP2; RAD50; Chemotherapy

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Introduction

Prostate Cancer (PCa) is one of the most common malignancies that seriously harms the health of men [1]. Androgen can stimulate prostate cancer cells growth, so Androgen Deprivation Therapy (ADT) as part of the standard therapeutic regimen was usually utilized to treat advanced prostate cancers and shows good disease control in the beginning [2,3]. However, patients who received these treatments are more likely to progress into Castration-Resistant Prostate Cancer (CRPC) in the end [4-6]. Although chemotherapy drug could improve the overall survival in metastatic Castration-Resistant Prostate Cancer (mCRPC), many patients almost relapsed and developed chemoresistant into castration-resistant state [7], and eventually progressed without a cure [8]. Therefore, it is urgent to find novel drug-resistant targets and develop new therapeutic strategies for advanced prostate cancer.

The human Leucine Zipper Protein 2 (LUZP2) is located on Chr11p13–11p14 and encodes a leucine zipper protein. LUZP2 knockout by gene targeting in mice showed no obvious abnormal phenotypes [9]. LUZP2 has been reported to play a crucial role in nervous system extracellular matrix development and metabolic pathways and serves as an important clinical biomarker for Low-Grade Glioma (LGG) patients [10]. Feng et al. identified distinct senescence-related molecular subtypes and critical genes among PCa patients undergoing Radical Prostatectomy (RP) or Radical Radiotherapy (RT), including LUZP2 [11]. Feng et al. also reported that LUZP2 serves as a prognostic biomarker for prostate cancer, and LUZP2 expression was negatively correlated with the tumor immune environment in prostate cancer patients who have received RP or RT [12]. Furthermore, inhibition of LUZP2 expression impaired the growth of enzalutamide-resistant C4-2 cells [13]. However, the chemosensitivity of LUZP2 in prostate cancer cells to drugs are still unclarified.

In this research, we explored the features of LUZP2 in chemotherapy resistant prostate cancer cells. Our results showed that overexpressed LUZP2 increased the chemo-resistance of prostate cancer cells to paclitaxel; LUZP2 was positively associated with the expression of RAD50, which is a DNA double strand break repair protein. Overexpression of RAD50 reversed the increased chemosensitivity caused by LUZP2 knockdown. Our findings suggested that LUZP2 might be a potential drug-resistant target in prostate cancer, and provide a new direction for further revealing the pathogenesis of prostate cancer.

Methods

Cell lines and Cell culture

Human Embryonic Kidney cells (HEK293T), human PCa cell lines (DU145 and PC3) were purchased from American Type Culture Collection (ATGC). HEK293T and DU145 cells were cultured in DMEM (Gibco, Cat#12800017). PC3 cells were cultured in Kaighn's Modification of Ham's F-12 Medium (HyClone, Cat#SH30526.01). All medium were supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. All cell lines were cultured at 37°C with 5% CO_2. No mycoplasma contamination was detected in any of the cell lines.

Antibodies

Primary antibodies: FLAG Tag Monoclonal Antibody (Sigma, Cat#F3165); GAPDH Monoclonal antibody (Proteintech, Cat#60004-1-Ig); RAD50 Polyclonal antibody (Proteintech, Cat#29390-1-AP); BAX Polyclonal antibody (Proteintech, Cat#50599-2-Ig); Anti-BCL2 (Phospho-S70) Antibody (Cohesion Biosciences, Cat#CPA7145).

Secondary antibodies: Goat anti-Mouse IgG (H+L) HRP Secondary Antibody (Invitrogen, Cat#31430); Goat anti-Rabbit IgG (H+L) HRP Secondary Antibody (Invitrogen, Cat#31460).

Plasmids, Lentivirus Production and Infection

For gene overexpression experiments, human full-length LUZP2 amplified by PCR was cloned to pLVX-mCMV-ZsGreen1-puro vector with Flag-tag. Human full-length RAD50 was cloned to pLVX-mCMV-ZsGreen1-puro vector. For knockdown experiments, LUZP2 and RAD50 targeting shRNA designed using shRNA sequence designer software and the oligonucleotide were commercially synthesized and then cloned to the pLKO.1-TRC vector, respectively.

For lentivirus packaging, HEK293T cells were seeded per well of a 6-well plate the day before transfection. The Opti-MEM was used to dilute plasmids and lentivirus packaging vectors (psPAX2 and pMD2.G), as well as lipofectamine 2000, respectively. The above components were mixed and incubated for 15 min before being added to the cell medium. The medium was changed 6 h to 8 h after transfection. At 48 h and 72 h after the initial transfection, the virus-containing supernatant was collected and filtered with a 0.45 μM filter. Then the lentivirus was incubated with target PCa cells with 4 μg/mL (Sigma, Cat#TR1003) polybrene to establish individual stable cells. About 48 h after being infected with the lentivirus, the cells containing the integrated virus were selected with 1 mg/mL puromycin (Sigma, Cat#P9620) until uninfected control cells were totally dead.

Western Blot analysis

Western blot was performed as described previously [14]. Briefly, cells were lysed in lysis buffer (20 mM Tris-HCl, pH 7.4; 0.5 M NaCl; 1% NP-40; 5 mM EDTA, pH 8.0; 1 mM DTT;) with protease inhibitor, followed by brief sonication and then centrifugation to remove insoluble material. Protein concentration was detected by BCA Protein Assay Kit (Thermo Fisher Scientific, Cat#23227). Protein samples were separated on SDS-PAGE and transferred to PVDF membranes (Millipore, Cat#ISEQ00010). The membranes were blocked with 5% nonfat milk at room temperature for 1 h, then incubated with primary antibodies at 4°C overnight. After washing off the primary antibodies, the membranes were then incubated with secondary antibodies for 1 h at room temperature. Immunoreactive bands on blots were visualized using ECL Western Blotting Substrate (Thermo Fisher Scientific, Cat#32209).

RNA extraction and RT-qPCR

Total RNA was extracted from cell lines using Trizol (Takara, Cat#9109) according to the manufacturer's instructions. Then extracted RNA was reversely transcribed into cDNA using ABScript II cDNA First-Strand Synthesis Kit (ABclonal, Cat#RK20400). The obtained cDNA samples were diluted and used for RT-qPCR using SYBR Green qPCR Master Mix (TargetMol, Cat#C0006). Gene specific primers with sequences listed in Supplementary Table 1 were used for PCR amplification and detection. RT-qPCR data were normalized to Actin and presented as fold changes of gene expression in the test sample compared to the control.

Cell viability and Proliferation assays

Cell viability and proliferation were detected by CCK-8 assays following the manufacturer's instructions. In brief, the cells were seeded in 96-well plates with approximately 5×10^3 cells and 100 μL medium in each well. After cell adherence, different doses of paclitaxel were added respectively. Each well was incubated with 10 μL CCK-8 solution (TargetMol, Cat#C0005) for 4 h before measuring the absorbance at 450 nm.

Wound healing assay

Cells were seeded into 12-well plates and scratched when reached nearly 90% confluence. Cells were washed with medium to remove detached cells and photographed with an inverted microscope. The rate of migration was measured as the percentage of invaded area with respect to the initial wound area using ImageJ.

Cell apoptosis assay

Cells were seeded into 6-well plates with 12 h serum starvation and the rates of apoptosis were measured by an Annexin V-FITC/PI Apoptosis Detection Kit (UElandy, Cat#A6030L). According to the manufacturer's instructions, cells were harvested and washed twice with cold PBS. After being resuspended in 500 μL of binding buffer, cells were incubated with Fluorescein Isothiocyanate (FITC)-Annexin V and Propidium Iodide (PI) for 15 min in the dark. Cells were analyzed by analytical flow cytometry (CytoFLEX S, CHINA). All data were analyzed using FlowJo.

Colony formation assay

For colony formation assay, cells were seeded at a density of 500 cells per well in 12-well plates with medium changed every 2 to 3 days. After two weeks, the plates were harvested, and the colonies were treated with 3.7% formaldehyde for 15 min. Following this, a stain of 0.1% crystal violet was applied for 30 min, and the colonies were counted using ImageJ software. All results are representative of three independent experiments. Statistical significance was calculated by the two-tailed t test.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9 software and statistical significance was determined by $p<0.05$. Data were presented as means with SD unless otherwise specified. Statistical comparisons were performed using unpaired Student's t test for two-tailed p values unless otherwise specified (*$p<0.05$, **$p<0.01$, ***$p<0.001$). Survival analysis was measured using the Kaplan-Meier method.

Results

LUZP2 overexpression enhanced the proliferation ability of prostate cancer cells

In order to test the effect of LUZP2 on the prostate cancer cell proliferation and migration, we firstly constructed the LUZP2 overexpressed stable cell lines DU145 and PC3. The expression levels of LUZP2 were confirmed by western blot and real-time PCR. The results showed that LUZP2 overexpression lentivirus could significantly increase the LUZP2 protein levels and mRNA levels relative to the control cells in DU145 and PC3 cells (Figure 1A-1D). CCK-8 assay showed that overexpression of LUZP2 could markedly enhance cell viability (Figure 1E, 1F) and colony formation ability (Figure 1G-1I) in DU145 and PC3 cells as compared with control cells. In addition, we also explored the effect of LUZP2 overexpression on migration abilities of PCa cells using *in vitro* wound healing. The results suggested that overexpression of LUZP2 had no effect on migration abilities of DU145 and PC3 cells (Figure 1J-1M). Collectively, these results indicated that LUZP2 could play critical role in prostate cancer proliferation.

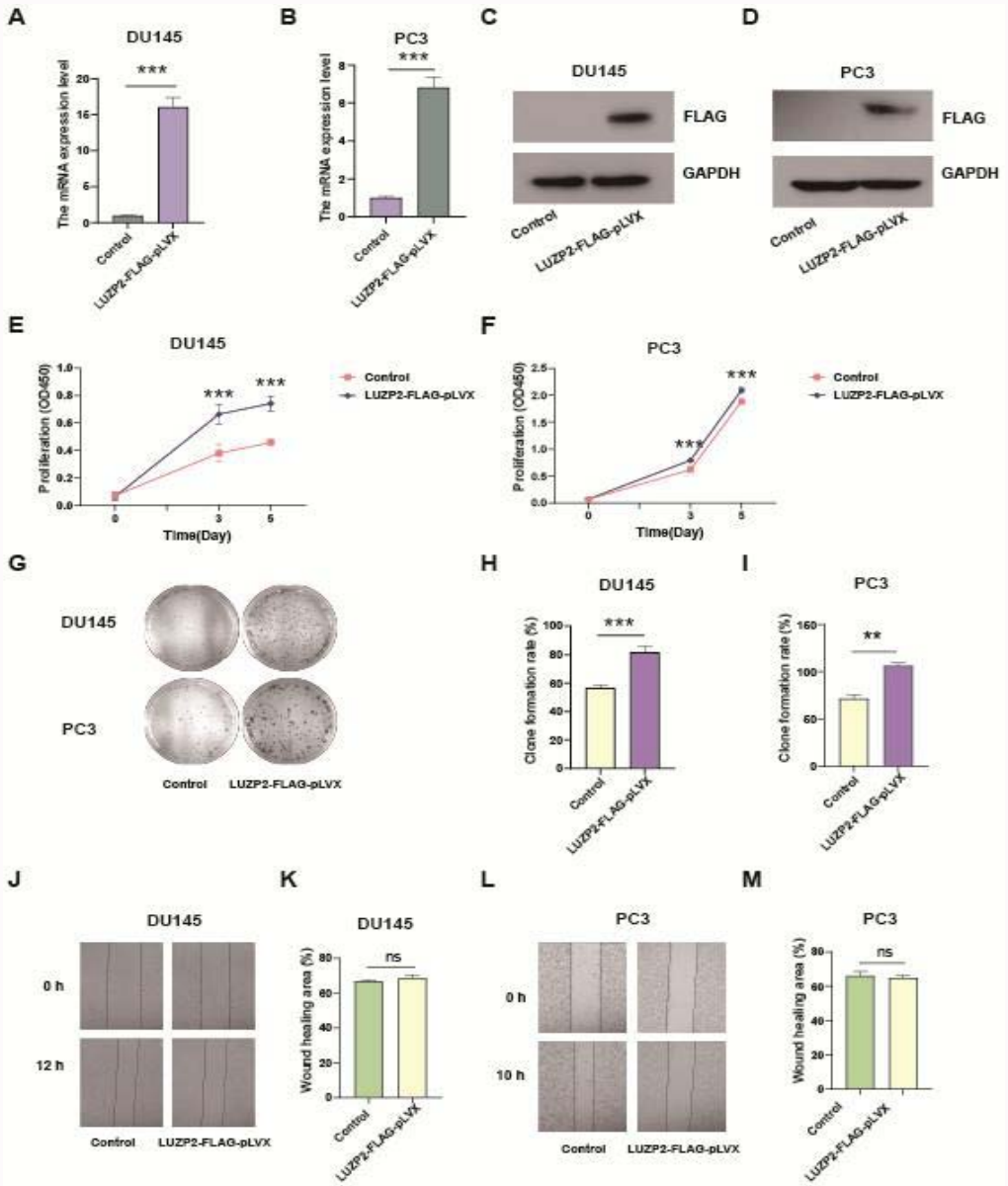

Figure 1: Overexpression of LUZP2 in PCa cells promoted the proliferation, cloning formation ability of prostate cancer cells. A,B) The mRNA level of LUZP2 was detected by RT-qPCR in LUZP2 overexpression DU145 (A) or PC3 (B) cells, respectively. C, D) The protein level of LUZP2 was detected by Western blot in LUZP2 overexpression DU145 (C) or PC3 (D) cells, respectively. E, F) Cell viability was measured in LUZP2 overexpression DU145 (E) or PC3 (F) cells by CCK-8 assay. G-I) The effect of LUZP2 overexpression on cell proliferation in DU145 or PC3 cells was determined by clonal formation assay. J-M) The effect of LUZP2 overexpression on cell migration in DU145 (J-K) or PC3 (L-M) cells was examined by Wound Healing Assays. **Control:** Negative control group; LUZP2-FLAG-pLVX: LUZP2 overexpression group. Values are shown as mean ± SD from triplicate experiments. *$P<0.05$; **$P<0.01$; ***$P<0.001$

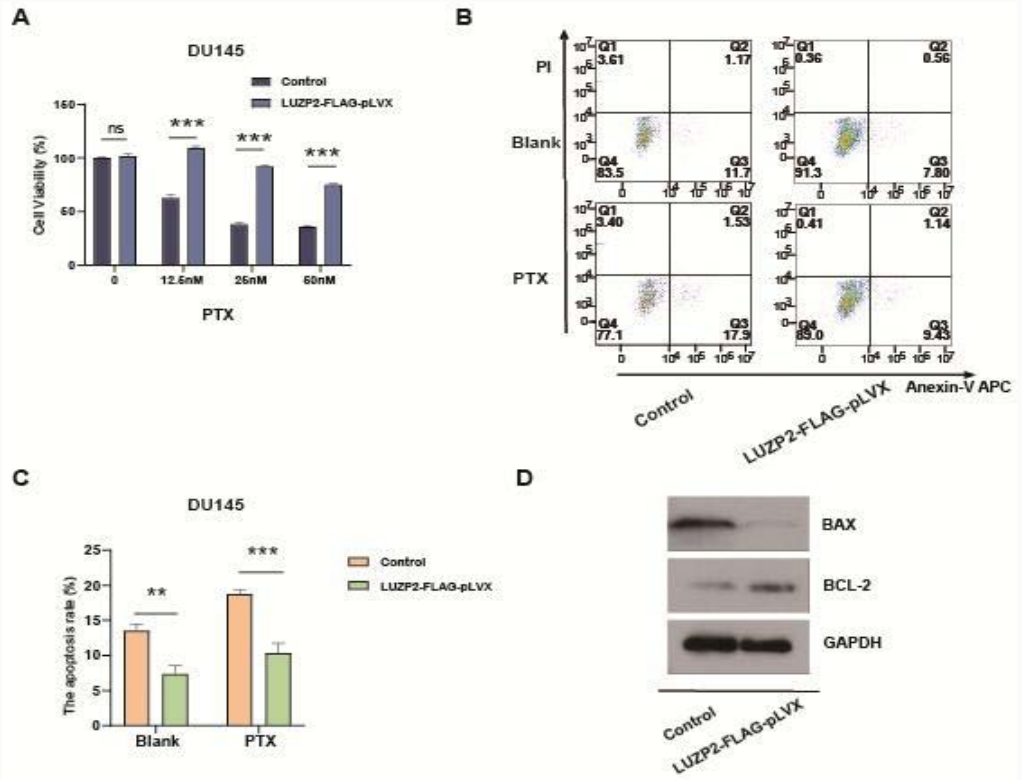

Figure 2: Overexpression of LUZP2 diminished the chemosensitivity of prostate cancer cells to paclitaxel. A) The cell viability was detected in LUZP2 overexpression DU145 cells treated with different concentrations of paclitaxel for 48 h. B, C) LUZP2 overexpression DU145 cells were treated with 25 nM paclitaxel for 24 h, the cell apoptosis was detected by flow cytometry. D) Western blot was used to detect the expression levels of BAX and BCL-2 in LUZP2 overexpression DU145 cells stimulated by paclitaxel for 24 h, using GAPDH as the internal reference.
Control: Negative control group; LUZP2-FLAG-pLVX: LUZP2 overexpression group. Values are shown as mean ± SD from triplicate experiments. **P*<0.05, ***P*<0.01, ****P*<0.001

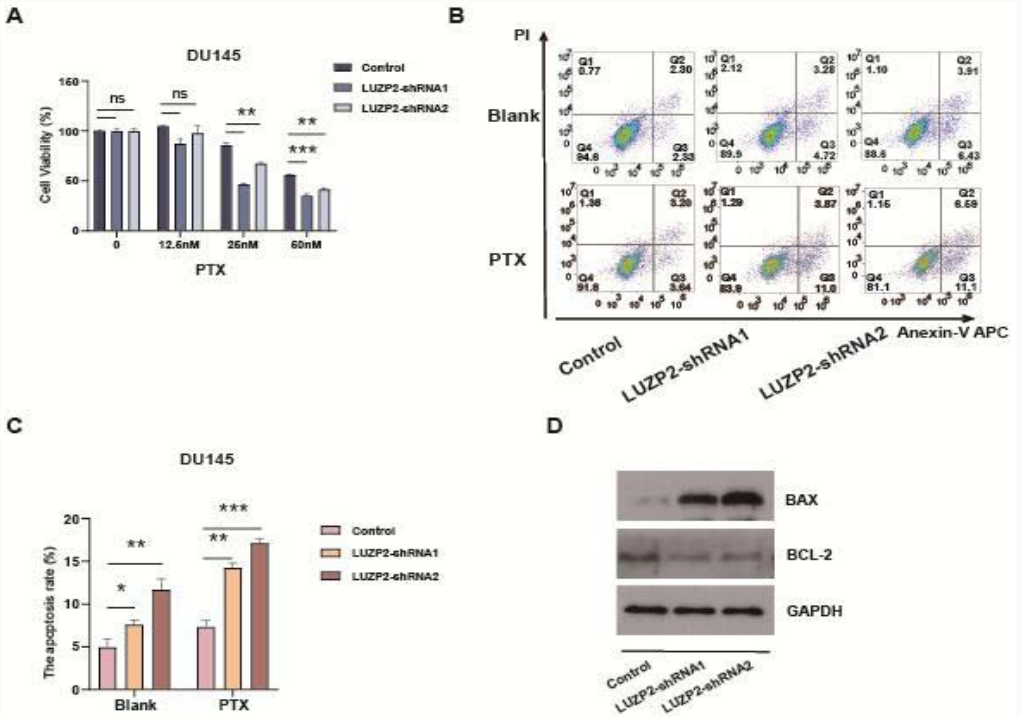

Figure 3: Knockdown of LUZP2 enhanced the chemosensitivity of prostate cancer cells to paclitaxel. A) The cell viability was detected in LUZP2 knockdown DU145 cells treated with different concentrations of paclitaxel for 48 h. B, C) LUZP2 knockdown DU145 cells were treated with 25 nM paclitaxel for 24 h, the cell apoptosis was detected by flow cytometry. D) Western blot was performed to determine the protein levels of BAX and BCL-2 in LUZP2 knockdown DU145 cells stimulated by paclitaxel for 24 h, using GAPDH as the internal reference.
Control: Negative control group; LUZP2-shRNA1, LUZP2-shRNA2: LUZP2 knockdown group. Values are shown as mean ± SD from triplicate experiments. **P*<0.05, ***P*<0.01, ****P*<0.001

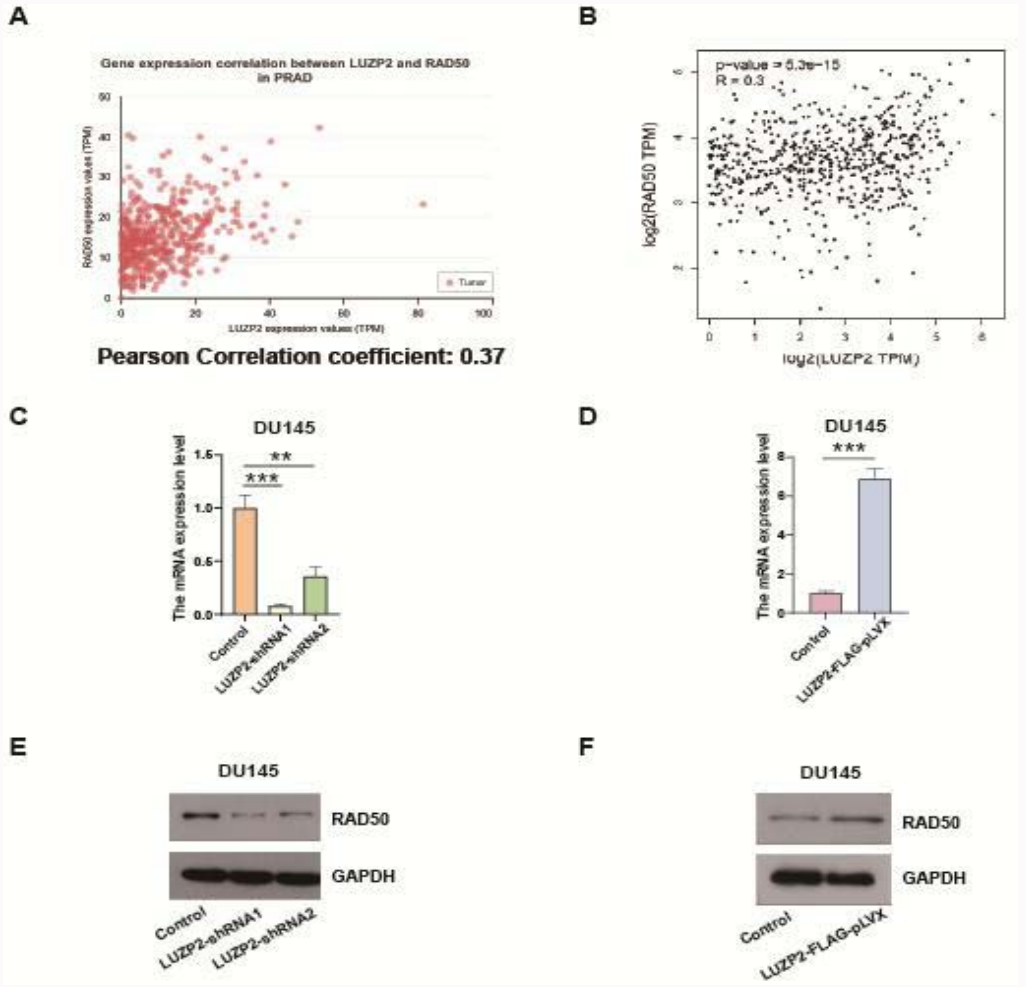

Figure 4: LUZP2 was correlated with RAD50. A, B) The correlation between the expression of LUZP2 and RAD50 in TCGA cohort. C, D) RT-qPCR was used to detect the mRNA level of RAD50 in DU145 cells after LUZP2 knockdown (C) or overexpression (D). E, F) The protein level of RAD50 in LUZP2 knockdown (E) or overexpression (F) DU145 cells was detected by Western blot, using GAPDH as the internal reference.
Control: Negative control group; LUZP2-shRNA1, LUZP2-shRNA2: LUZP2 knockdown group. LUZP2-FLAG-pLVX: LUZP2 overexpression group. Values are shown as mean ± SD from triplicate experiments. *P<0.05, **P<0.01, ***P<0.001

LUZP2 effected the chemosensitivity of prostate cancer cells to paclitaxel

To further confirm the effect of LUZP2 on the chemosensitivity of prostate cancer cells to paclitaxel, LUZP2 overexpression DU145 cells were treated with different concentrations of paclitaxel for 48 h, respectively. Then, the cell viability was tested by CCK-8 assay. The data suggested that LUZP2 overexpression could attenuate the chemosensitivity of prostate cancer cells to paclitaxel (Figure 2A). Furthermore, LUZP2 overexpression DU145 cells were treated with 25 nM paclitaxel for 24 h. The effect of LUZP2 expression on paclitaxel-induced apoptosis of prostate cancer cells was detected by flow cytometry. The results showed that paclitaxel could promote PCa cell apoptosis, LUZP2 overexpression cells could apparently inhibit paclitaxel-induced apoptosis of prostate cells compared with control cells (Figure 2B, 2C). In addition, western blot was used to detect the protein levels of pro-apoptotic gene BAX and anti-apoptotic gene BCL-2, respectively. The results showed that LUZP2 overexpression could significantly decline the protein level of BAX and augment the protein level of BCL-2 (Figure 2D). Collectively, LUZP2 overexpression attenuated the chemosensitivity of prostate cancer cells to paclitaxel.

To further validate the effect of LUZP2 on the chemosensitivity of prostate cancer cells to paclitaxel, LUZP2 shRNA or control lentivirus were transduced into PCa cells. LUZP2 knockdown DU145 cells were treated with different concentrations of paclitaxel for 48 h. Then, the cell activity was tested by CCK-8 assay. The data suggested that LUZP2 knockdown could increase the chemosensitivity of prostate cancer cells to paclitaxel (Figure 3A). Moreover, LUZP2 inhibited DU145 cells were treated with 25 nM paclitaxel for 24 h. The effect of LUZP2 expression on paclitaxel-induced apoptosis of prostate cancer cells was detected by flow cytometry. The results showed that paclitaxel could promote apoptosis of prostate cancer cells, knockdown of LUZP2 could significantly promote paclitaxel-induced apoptosis of prostate cells compared with control cells (Figure 3B, 3C). In addition, the protein expression levels of pro-apoptotic gene BAX and anti-apoptotic gene BCL-2 were detected by western blot, respectively. The results showed that LUZP2 knockdown strongly increased the expression level of BAX and decreased the protein level of BCL-2 (Figure 3D). Taken together, these results revealed that LUZP2 enhances the chemo-resistance of prostate cancer cells to paclitaxel.

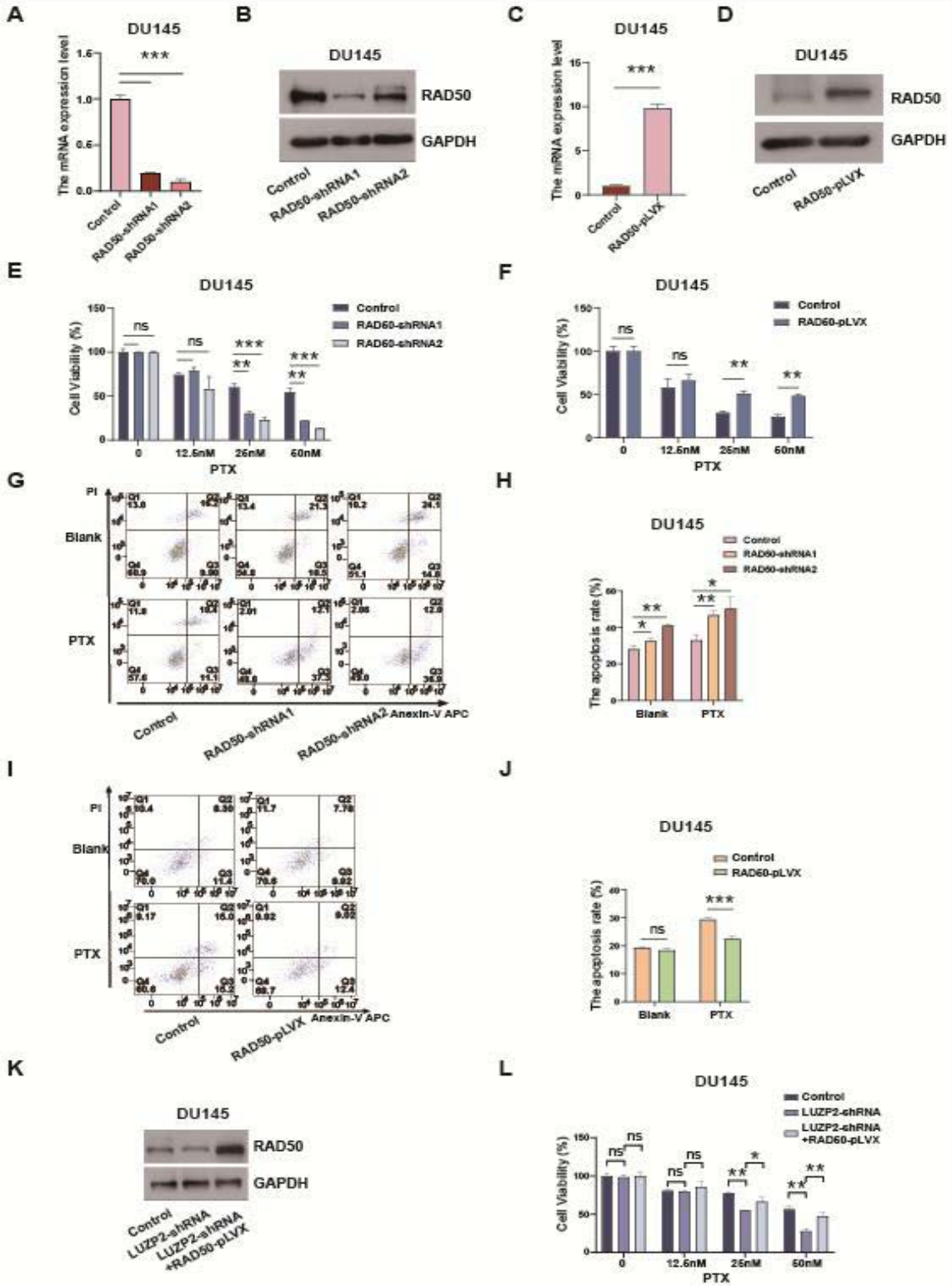

Figure 5: RAD50 overexpression reversed the promoting effect of LUZP2 knockdown on the chemosensitivity of prostate cancer cells to paclitaxel. A, B) The mRNA and protein levels of RAD50 were detected by RT-qPCR (A) and Western blot (B) in RAD50 knockdown DU145 cells, respectively. C, D) The mRNA and protein levels of RAD50 were detected by RT-qPCR (C) and Western blot (D) in RAD50 overexpression DU145 cells, respectively. E) The cell viability was detected in RAD50 knockdown DU145 cells treated with different concentrations of paclitaxel for 48 h. F) The cell viability was detected in RAD50 overexpression DU145 cells treated with different concentrations of paclitaxel for 48 h. G, H) RAD50 knockdown DU145 cells were treated with 25 nM paclitaxel for 48 h, the cell apoptosis was detected by flow cytometry. I, J) RAD50 overexpression DU145 cells were treated with 25 nM paclitaxel for 48 h, the cell apoptosis was detected by flow cytometry. K) RAD50 was overexpressed in LUZP2 knockdown DU145 cells, and the protein levels of RAD50 were detected by western blot. L) The cell viability was detected in LUZP2 knockdown DU145 cells with RAD50 overexpression stimulated with different concentrations of paclitaxel for 48 h.

Control: Negative control group; LUZP2-shRNA: LUZP2 knockdown group; RAD50-shRNA1, RAD50-shRNA2: RAD50 knockdown group; RAD50-pLVX: RAD50 overexpression group. Values are shown as mean ± SD from triplicate experiments. *P<0.05, **P<0.01, ***P<0.001

Supplementary Table 1: Gene specific primers with sequences listed in below were used for PCR amplification and detection.

Primer	Sequence
LUZP2-shRNA1-F	CCGGGGAATTAGGACAGAAACAAAGCTCGAGCTTTGTTTCTGTCCTAATTCCTTTTTG
LUZP2-shRNA1-R	AATTCAAAAAGGAATTAGGACAGAAACAAAGCTCGAGCTTTGTTTCTGTCCTAATTCC
LUZP2-shRNA2-F	CCGGGACAAAGACATCAAGAGAACTCTCGAGAGTTCTCTTGATGTCTTTGTCTTTTTG
LUZP2-shRNA2-R	AATTCAAAAAGACAAAGACATCAAGAGAACTCTCGAGAGTTCTCTTGATGTCTTTGTC
RAD50-shRNA1-F	CCGGAGATTCGTGATCAGATTACACTCGAGTGTAATCTGATCACGAATCTTTTTTG
RAD50-shRNA1-R	AATTCAAAAAAGATTCGTGATCAGATTACACTCGAGTGTAATCTGATCACGAATCT
RAD50-shRNA2-F	CCGGCCGACCATCATTGAATGTCTAACTCGAGTTAGACATTCAATGATGGTCGTTTTTG
RAD50-shRNA2-R	AATTCAAAAACCGACCATCATTGAATGTCTAACTCGAGTTAGACATTCAATGATGGTCG
LUZP2-FLAG-F	CCGGAATTCCATGAAGTCTCTTCAGGAG
LUZP2-FLAG-R	CGCGGATCCTTACAGTATTTTTTCTTC
LUZP2-FLAG-pLVX-F	TCGAGCTCAAGCTTCGAATTCATGGACTACAAAGACGATGACGAC
LUZP2-FLAG-pLVX-R	GCAAATACGCGTCGCGGATCCTTACAGTATTTTTTCTTCTCTAGCTGCC
RAD50-pLVX-F	TCGAGCTCAAGCTTCGAATTCATGTCCCGGATCGAAAAGATG
RAD50-pLVX-R	GCAAATACGCGTCGCGGATCCTTAATGAACATTGAATCCCAGGG
LUZP2-RT-F	AGCAGCTCTTGACAGGGAGT
LUZP2-RT-R	GTGGGAGTAACATCCGAGTTG
β-Actin-F	AGAAAAATCTGGCACCACACC
β-Actin-R	AGAGGCGTACAGGGATAGCA

LUZP2 was positively correlated with RAD50

To investigate the mechanism of LUZP2 on the chemosensitivity of prostate cancer cells to paclitaxel, we analyzed the PCa dataset and found that *LUZP2* mRNA level was positively correlated with *RAD50* (Figure 4A, 4B). We further knocked down or overexpressed LUZP2 in DU145 cells. The expression of *RAD50* mRNA was detected by RT-qPCR after LUZP2 knockdown or overexpression in DU145 cell lines. The results showed that knockdown of LUZP2 reduced *RAD50* mRNA level, and overexpression of LUZP2 increased the mRNA level of *RAD50* (Figure 4C, 4D). We also detected the effect of LUZP2 on the protein levels of RAD50. The results demonstrated that LUZP2 knockdown significantly diminished the protein level of RAD50 and LUZP2 overexpression enhanced the protein levels of RAD50 (Figure 4E, 4F). These results suggested that RAD50, a DNA double strand break repair protein, might plays important roles in paclitaxel resistant of prostate cancer.

RAD50 overexpression reversed the promoting effect of LUZP2 inhibition on the chemosensitivity of PCa cells to paclitaxel

To investigate the effect of RAD50 on the chemosensitivity of prostate cancer cells to paclitaxel, RAD50 was knocked down or overexpressed in DU145 cells. The mRNA expression level of *RAD50* was detected by RT-qPCR, and the protein expression level was detected by Western blot. As shown in (Figure 5A, 5B), the RAD50 shRNA lentivirus could significantly decrease the endogenous RAD50 mRNA levels or protein expression level relative to the control cells. The results showed that RAD50 overexpression lentivirus could apparently increase the RAD50 protein levels and mRNA levels relative to the control cells in DU145 cells (Figure 5C, 5D). Then, the RAD50 knockdown or overexpression cells were treated with different concentrations of paclitaxel for 48 h, respectively, and the cell activity was tested by CCK-8 assay. The data suggested that RAD50 knockdown could increase the chemosensitivity of prostate cancer cells to paclitaxel; RAD50 overexpression could accordingly attenuate the chemosensitivity of prostate cancer cells to paclitaxel (Figure 5E, 5F). Furthermore, the RAD50 knockdown or overexpression cells were treated with 25 nM paclitaxel for 48 h, respectively. The effect of RAD50 expression on paclitaxel-induced apoptosis of prostate cancer cells was detected by flow cytometry. The results showed that paclitaxel induced apoptosis of prostate cancer cells, RAD50 knockdown promoted paclitaxel-induced apoptosis of prostate cells compared with control cells (Figure 5G, 5H) and RAD50 overexpression inhibited paclitaxel-induced apoptosis of prostate cells compared with control cells (Figure 5I, 5J).To further explore the role of RAD50 in LUZP2 deletion PCa cells, RAD50 was overexpressed in LUZP2 knockdown DU145 cells, and the protein levels of RAD50 were detected by western blot (Figure 5K). Then, the cell viability was detected in LUZP2 knockdown DU145 cells with RAD50 overexpression stimulated with different concentrations of paclitaxel for 48 h. The results showed that RAD50 overexpression reversed the promoting effect of LUZP2 knockdown on the chemosensitivity of prostate cancer cells to paclitaxel (Figure 5L).

Discussion

It was reported that the *LUZP2* mRNA level was significantly up-regulated in Prostate Adenocarcinoma (PRAD) compared with normal samples from a pan-cancer analysis, and the lower mRNA expression of *LUZP2* was closely associated with poor prognosis [15]. However, the function and mechanisms of LUZP2 in prostate cancer are still illusive. In the present study, we investigated the role of LUZP2 in the chemosensitivity of prostate cancer cells to paclitaxel for the first time. The results raised the possibility that LUZP2 overexpression contributes to the growth and paclitaxel resistance of prostate cancer cells by increasing RAD50 expression level. Our findings suggested that LUZP2 might be a potential drug-resistant target in prostate cancer.

Previous studies have shown that knockdown of LUZP2 diminished the growth of enzalutamide-resistant C4-2 cells [13].

Bortezomib was found to be a potentially sensitive drug to the LUZP2 [12]. These data suggested that LUZP2 was probably associated with chemosensitivity. In our study, we found that LUZP2 knockdown could significantly promote the chemosensitivity of prostate cancer cells to paclitaxel, with increased the protein level of BAX and decreased the BCL-2 protein level; LUZP2 overexpression could attenuate the chemosensitivity of prostate cancer cells to paclitaxel, with decreased the protein level of BAX and increased the BCL-2 protein level. Our findings expanded the knowledge of LUZP2 about chemoresistance in prostate cancer.

RAD50 was reported to be involved in regulating the DNA damage repair, and associated with tumor prognosis. RAD50 overexpression was associated with prostate cancer aggressiveness and poor survival in PCa patients [15]. It was reported that combination cisplatin with mutant RAD50 therapy led to tumor cytotoxicity with increased DNA damage and telomere shortening. The combination therapy resulted in tumor regression with increased apoptosis in cisplatin-resistant human squamous cell cancer xenografts in nude mice. These findings suggested that the targeted RAD50 disruption is a novel chemosensitizing approach for cancer therapy in the context of chemoresistance [16]. Furthermore, combinations of cisplatin with doxorubicin and paclitaxel drugs produced synergistic effects in early cell death of RAD50-deficient breast cancer cells. RAD50 inhibition could weaken DNA damage response and make breast cancer cells sensitive to cisplatin-combined therapies [17]. In our study, we found that LUZP2 could regulate the mRNA and protein level of RAD50, and LUZP2 expression was positively correlated with RAD50 level. RAD50 knockdown significantly promoted the chemosensitivity of prostate cancer cells to paclitaxel; RAD50 overexpression attenuated the chemosensitivity of prostate cancer cells to paclitaxel. Furthermore, RAD50 overexpression could reverse the effect of LUZP2 on inhibiting the chemosensitivity of prostate cancer cells to paclitaxel. Whether LUZP2 can affect the chemosensitivity of prostate cancer cells to cisplatin or other drugs need to be further investigated.

Conclusion

LUZP2 contributes to the growth of prostate cancer cells and enhance the paclitaxel resistance of prostate cancer cells. It effects the expression level of RAD50 to regulate the chemo resistant of prostate cancer cells to paclitaxel. Our findings indicated that LUZP2 might be a potential drug-resistant target in prostate cancer.

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References

1. Rosellini M, Santoni M, Mollica V, Rizzo A, Cimadamore A, Scarpelli M, et al. Treating prostate cancer by antibody-drug conjugates. Int J Mol Sci. 2021;22:1551.
2. Rizzo A, Santoni M, Mollica V, Fiorentino M, Brandi G, Massari F. Microbiota and prostate cancer. Semin Cancer Biol. 2022;86:1058-65.
3. Katzenwadel A, Wolf P. Androgen deprivation of prostate cancer: Leading to a therapeutic dead end. Cancer Lett. 2015;367:12-7.
4. Wong YNS, Ferraldeschi R, Attard G, de Bono J. Evolution of androgen receptor targeted therapy for advanced prostate cancer. Nat Rev Clin Oncol. 2014;11:365-76.
5. Zhang Y, Liao Y, Luo M, Ye Y, Xu Z, Hou W, et al. IU1 and enzalutamide combination yields synergistic effects on castration-resistant prostate cancer. Prostate. 2023;83:1446-57.
6. Wu G, Sun Y, Xiang Z, Wang K, Liu B, Xiao G, et al. Preclinical study using circular RNA 17 and micro RNA 181c-5p to suppress the enzalutamide-resistant prostate cancer progression. Cell Death Dis. 2019;10:37.
7. Denmeade SR, Sena LA, Wang H, Antonarakis ES, Markowski MC. Bipolar androgen therapy followed by androgen receptor inhibition as sequential therapy for prostate cancer. Oncologist. 2023;28:465-73.
8. Chen Y, Gera L, Zhang S, Li X, Yang Y, Mamouni K, et al. Small molecule BKM1972 inhibits human prostate cancer growth and overcomes docetaxel resistance in intraosseous models. Cancer Lett. 2019;446:62-72.
9. Wu M, Michaud EJ, Johnson DK. Cloning, functional study and comparative mapping of Luzp2 to mouse chromosome 7 and human chromosome 11p13-11p14. Mamm Genome. 2003;14:323-34.
10. Li Y, Deng G, Qi Y, Zhang H, Jiang H, Geng R, et al. Downregulation of LUZP2 Is Correlated with Poor Prognosis of Low-Grade Glioma. Biomed Res Int. 2020;2020:9716720.
11. Feng D-C, Zhu W-Z, Shi X, Xiong Q, You J, Wei Q, et al. Identification of senescence-related molecular subtypes and key genes for prostate cancer. Asian J Androl. 2023;25:223-9.
12. Feng D, Zhu W, Shi X, Wei W, Han P, Wei Q, et al. Leucine zipper protein 2 serves as a prognostic biomarker for prostate cancer correlating with immune infiltration and epigenetic regulation. Heliyon. 2022;8:e10750.
13. Zhao J, Zhao Y, Wang L, Zhang J, Karnes RJ, Kohli M, et al. Alterations of androgen receptor-regulated enhancer RNAs (eRNAs) contribute to enzalutamide resistance in castration-resistant prostate cancer. Oncotarget. 2016;7:38551-65.
14. Gao P, Hao J-L, Xie Q-W, Han G-Q, Xu B-B, Hu H, et al. PELO facilitates PLK1-induced the ubiquitination and degradation of Smad4 and promotes the progression of prostate cancer. Oncogene. 2022;41:2945-57.
15. Feng D, Shi X, Zhu W, Zhang F, Li D, Han P, et al. A pan-cancer analysis of the oncogenic role of leucine zipper protein 2 in human cancer. Exp Hematol Oncol. 2022;11:55.
16. Abuzeid WM, Jiang X, Shi G, Wang H, Paulson D, Araki K, et al. Molecular disruption of RAD50 sensitizes human tumor cells to cisplatin-based chemotherapy. J Clin Invest. 2009;119:1974-85.
17. Flores-Pérez A, Rafaelli LE, Ramírez-Torres N, Aréchaga-Ocampo E, Frías S, Sánchez S, et al. RAD50 targeting impairs DNA damage response and sensitizes human breast cancer cells to cisplatin therapy. Cancer Biol Ther. 2014;15:777-88.