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Research Article

Comprehensive expression analysis reveals upregulated LUZP2 in prostate cancer tissues

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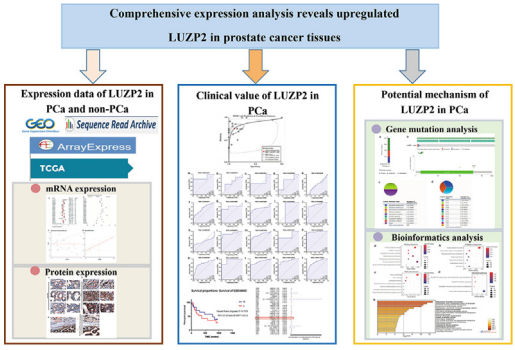
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Leucine zipper protein 2 (LUZP2) is a vital gene encoding leucine zipper protein. It is of great importance in the incidence and progress of several human cancers. However, little is known about the role and clinical effects of LUZP2 in prostate cancer (PCa). Therefore, it is crucial to unravel the clinicopathological value of LUZP2 in PCa. In all, 1467 PCa and 549 non-prostate cancer (non-PCa) prostate samples were collected from mRNA chip and RNA-sequencing datasets. The protein levels of LUZP2 were verified in 91 prostate gland tissues by in-house immunohistochemistry (IHC). The standardized mean difference (SMD) was calculated to analyze LUZP2 expression. Survival analysis was also conducted to explore the prognostic significance of LUZP2 in PCa. R software was employed to identify the upregulated differently expressed genes (up-DEGs) and coexpressed genes (CEGs) of LUZP2. Additionally, we explored the prospective molecular mechanism of CEGs of LUZP2 through GO and KEGG pathway analyses.
Results: Compared with non-PCa, LUZP2 showed predominantly higher expression in PCa (SMD = 1.05, AUC = 0.88). IHC indicated the protein expression level of LUZP2 was consistently upregulated in PCa tissues (SMD = 2.23, 95%CI: 1.67–2.79). LUZP2 upregulation had an AUC of 0.88 (95%CI: 0.85–0.90) to distinguish PCa from non-PCa tissues. KEGG pathway analysis showed that the pathways of amino sugar and nucleoside sugar metabolism were chiefly enriched with the LUZP2 CEGs in PCa.

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Conclusions: LUZP2 upregulation might play a promoting function in the occurrence of PCa.

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1. Introduction

Prostate cancer (PCa) occurs in the glandular epithelial cells of the prostate. Currently, PCa has the second-highest incidence rate among male malignant tumors worldwide. It is also a common cause of cancer-related death [1,2,3,4]. Recent molecular-level mechanism studies have shown promise in human PCa cancer [5]. Studies have shown that the progress of PCa is related to androgen-related signaling pathways or regulatory axes [6], various inflammasomes [7], oncogenic fusion genes [8], and ubiquitin ligase [9]. The TP53 gene promotes the proliferation of PCa by affecting the cell cycle [10]. SLC25A17 and SLC27A6 genes regulate the proliferation and migration of PCa cells [11]. Although progress has been made in research on PCa mechanisms, the specific mechanism of the development of PCa has not yet been unified. In this context, LUZP2 could become an important potential target for studying the molecular mechanism of PCa. This study explored the characteristics of LUZP2 expression and its coexpressed genes (CEGs) in PCa to assess its suitability for studying the mechanism of PCa.

Leucine zipper protein (LUZP) is a newly identified protein with three leucine zipper motifs at the amino-terminal [12,13]. Recent studies have shown that excess or mutation of LUZP plays an important role in cancer progression [12,14]. In the LUZP family, leucine zipper protein 2 (LUZP2) exists on Chr 11p13-11p14 and encodes LUZP [15,16]. Existing reports have suggested that the protein encoded by LUZP2 is generally limited in expression in the brain and spinal cord [15], and studies have confirmed that LUZP was expressed in the cerebral cortex and hippocampus [17]. Some studies have shown that it is also expressed in embryonic stem cells [18]. Current research on LUZP2 has focused mainly on its role in the nervous system. Polymorphic variants of LUZP2 are associated with late-onset Alzheimer's disease [19], neurodegenerative diseases, and neuropsychiatric diseases [19]. Another study indicated the coexpressed genes of LUZP2 to be involved in the development and metabolic pathway of the nervous system [15]. Recently, it was found that LUZP2 plays a part in the incidence and progress of human malignancies. Lack of LUZP2 expression was reported in patients with WAGR syndrome, including Wilms tumor, leading to intellectual disability [16]. Other studies have found that LUZP2 was regulated by mir-142-5p in glioma, and its expression decreased with the increase in tumor grade ($p < 0.05$) [15]. Thus, studies have shown that LUZP2 is a key molecule facilitating deep exploration of the relevant mechanisms in PCa.

At present, research on the biological functional characteristics of LUZP2 in PCa is still limited. Only one study concluded that the expression of LUZP2 mRNA in PCa was increased significantly compared with normal prostate tissue, while its expression was downregulated in metastatic castration-resistant PCa. The study revealed that the downregulation of LUZP2 induced the cytotoxicity of drug-resistant C4-2 castration-resistant PCa cells [20]. However, the exact mechanism of LUZP2 in the progression of PCa was not reported, and the number of included cases (87 cases) was limited, with only one detection method employed. In contrast, the current study had a significant advantage in its larger sample size (2016 cases). This enabled us to elucidate the potential role of LUZP2 in the occurrence and development of PCa.

In this study, mRNA chip, RNA-sequencing datasets, and in-house immunohistochemistry (IHC) were applied to explore the expression of LUZP2 in PCa and its relationship with clinicopathological parameters. Increasing numbers of studies have reported that cancer might be related to genetic changes. We therefore also performed mutation analysis of LUZP2 using online databases. Functional enrichment analysis and KEGG analysis of the intersection of the upregulated differently expressed genes (up-DEGs) and CEGs were further used to determine possible LUZP2-associated pathways. This study lays the foundation for in-depth comprehension of the underlying mechanism of LUZP2 in the progress of PCa.

2. Materials and methods

2.1. mRNA expression data of LUZP2 in PCa and non-PCa

Gene Expression Omnibus (GEO), ArrayExpress, the Cancer Genome Atlas (TCGA), Sequence Read Archive (SRA), and the Oncomine databases were searched. The screening procedure was performed according to the following criteria.

1. Inclusion criteria were as follows: (1) taking "prostate" and "cancer" as keywords; (2) extracting untreated human PCa mRNA data after 2005; (3) study samples were human tissues and body fluids; (4) tumor samples and normal samples in each dataset were $\geq$3; (5) expression profile data were complete; and (6) data containing study indicators.
2. Exclusion criteria were as follows: (1) no data, or lack of information on whether the data were related to PCa; (2) no mRNA expression profile data; (3) repeated and/or incomplete data; (4) unqualified number of samples; or (5) dataset without LUZP2 expression profile.

2.2. Protein expression data of LUZP2 in PCa and non-PCa

IHC was used to determine the protein level of LUZP2 in PCa and non-PCa controls. Two tissue chips (PRC481, PRC961) from Fanpu Biotech, Inc (Guilin, P.R.China) and the tissue samples of our institute (First Affiliated Hospital, Guangxi Medical University) were used. All patients signed informed consent and the study was approved by the Ethics Committee of Guangxi Medical University. It included 60 PCa tissues from patients aged 47–88 years and 31 non-PCa tissues from patients aged 54–84 years. Polyclonal antibody of LUZP2 was the first antibody (ThermoFisher SCIENTIFIC, PA5-61168, Shanghai, P.R. China). The first antibody was diluted 100 times. IHC analysis was carried out according to the manufacturer's instructions. The intensity and percentage of positive cells were evaluated, and the immunoreactive scores were calculated for each sample [21,22,23,24].

2.3. Screening the up-DEGs of PCa

We used R software (version 3.6.3) to screen the differential genes from the gene chip or RNA-seq data included from various databases by using the limma package. Because LUZP2 was observed to be highly expressed in PCa, after the duplicate genes were removed, the screening conditions of differential genes were

as follows: up-DEGs were screened according to the principle $p < 0.05$ and log2 FC > 1.

2.4. Screening the CEGs of LUZP2 in PCa

We used R (version 3.6.3) to screen the CEGs of LUZP2 in PCa according to the conditions of cor > 0.3, $p < 0.05$. Finally, in the CEG group with cor of >0.3, genes that appeared in not less than half of the total number of chips included (≥10 times) were considered to be CEGs of LUZP2.

2.5. Survival and prognosis analysis of LUZP2 in PCa

To study the association between LUZP2 level and the prognosis of patients with PCa, we used the "Survival" module of GEPIA to obtain information for the effect of LUZP2 on overall survival (OS) and disease-free survival (DFS), based on TCGA RNA-seq data. At the same time, the GEO data set GSE46602 was used for prognostic verification after comprehensive screening. We also used the UCSC Xena database to perform Kaplan–Meier analysis on the race, age, gleason score, and pathologic grade of patients with PCa.

2.6. Gene mutation analysis of LUZP2 in PCa

The cBioPortal database (https://www.cbioportal.org/) integrates data from large tumor genome studies such as TCGA and ICGC, covering DNA copy number, mRNA and microRNA expression, protein and phosphoprotein levels (RPPA), clinical data, and DNA methylation. The occurrence of cancer depends on the accumulation of gene mutations. Gene mutation analysis provides a comprehensive, detailed, and in-depth understanding of the cancer cell genome. We investigated the mutation frequency and mutation sites of LUZP2 in PCa with the help of the cBioPortal database. We chose "Prostate Adenocarcinoma (TCGA, Firehose Legacy)" (TCGA Prostate Adenocarcinoma and source data from GDAC Firehose). In order to systematically describe the genetic and genomic characteristics of the PCa cell line, we further used COSMIC (including COSMIC, COSMIC-3D, Cancer Gene Census, Cancer Mutation Census, and Actionability) to study the mutation type and alternative mutation type of LUZP2 in PCa. For understanding the significance of LUZP2 mutations in PCa, we used the cBioPortal database to view the OS and DFS results of LUZP2 mutations in patients with PCa.

2.7. GO, KEGG, PPI network analysis of LUZP2-related CEGs in PCa

A Venn diagram was drawn to display the intersection genes for up-DEGs and CEGs in PCa. STRING is an online database used for exploring protein–protein interactions. It was applied to build the protein–protein interaction (PPI) network, which was helpful for determining the functional relationship between these genes in biological systems and their relationship with PCa. In addition, we used DAVID to implement Gene Ontology (GO). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was also used to study possible related pathways to predict the obtained intersection genes' biological functions and potential molecular mechanisms as previously reported [25,26,27,28].

2.8. Statistical analysis

The expression of LUZP2 mRNA in gene chips was collected, and log2 conversion was performed. SPSS 22.0 software was used for independent *t*-test to study the differences in expression between PCa and non-PCa tissues. The receiver operating characteristic curve (ROC) and summary receiver operating characteristics

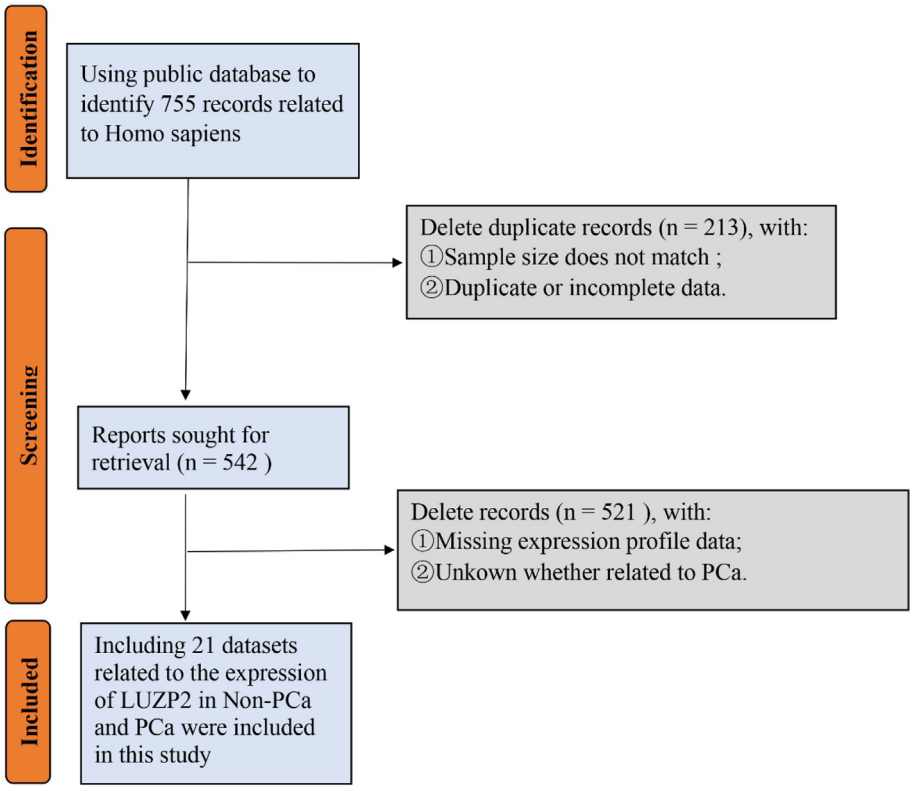

Fig. 1. Flow chart for obtaining LUZP2 expression datasets in PCa. LUZP2: leucine zipper protein 2; PCa: prostate cancer.

(sROC) were drawn. The AUC value was calculated to detect the ability of LUZP2 to discriminate between PCa and non-PCa samples. In addition, we used STATA 14.0 software to calculate the standardized mean difference (SMD) and its 95% confidence interval. Next, we used the *t*-test to detect the relevancy between LUZP2 level and clinicopathological parameters in PCa. The Kaplan–Meier method was leveraged to verify the prognostic value of LUZP2 expression in PCa.

Table 1
Different LUZP2 expression values between PCa and non-PCa prostate tissues based on 21 platforms.

Study	Patient sources	Year	Sample type	PCa			Non-PCa		
				N	M	SD	N	M	SD
GSE104749	China	2017	tissue	4	8.271	0.582	4	6.907	0.564
GSE60329	Italy	2014	tissue	108	0.004	1.219	28	−0.065	0.472
GSE69223	Germany	2015	tissue	15	1.166	1.520	15	−1.204	1.280
GSE94767	UK	2017	tissue	185	8.989	1.187	33	8.055	0.987
GSE88808	USA	2016	tissue	49	10.828	0.690	49	8.568	0.979
GSE72220	USA	2015	tissue	57	0.349	0.412	90	−0.158	0.195
GSE46602	Denmark	2013	tissue	36	7.476	2.338	14	3.687	1.740
GSE38043	USA	2012	body fluid	3	16.532	9.405	3	13.905	12.940
GSE32571	Germany	2011	tissue	59	6.270	0.240	39	6.097	0.155
GSE32982	Finland	2011	tissue	6	5.763	0.354	3	5.423	0.093
GSE35988	USA	2012	tissue	76	1.505	2.460	12	0.286	1.391
GSE28204	China	2011	tissue	4	10.033	1.526	4	8.756	1.826
GSE32448	USA	2011	tissue	40	7.893	1.474	40	7.745	1.360
GSE26910	Italy	2011	tissue	6	2.989	0.171	6	2.932	0.149
GSE12378	UK	2008	tissue	36	9.417	1.032	3	8.196	1.487
GSE134051	Germany	2019	tissue	216	6.198	0.511	39	5.561	0.146
GSE134073	Germany	2019	tissue	56	11.324	1.934	8	7.902	1.741
GSE27616	USA	2011	tissue	9	1.139	3.179	4	−0.116	2.383
GSE73397	USA	2015	tissue	3	9.526	0.424	3	9.923	0.555
TCGA+GTEx	NA	NA	tissue	499	2.075	1.000	152	0.888	0.816

LUZP2: leucine zipper protein 2; PCa: prostate cancer; N: number; M: mean (The expression profile is processed by log2); SD: standard deviation; TCGA: The Cancer Genome Atlas; GTEx: The Genotype-Tissue Expression.

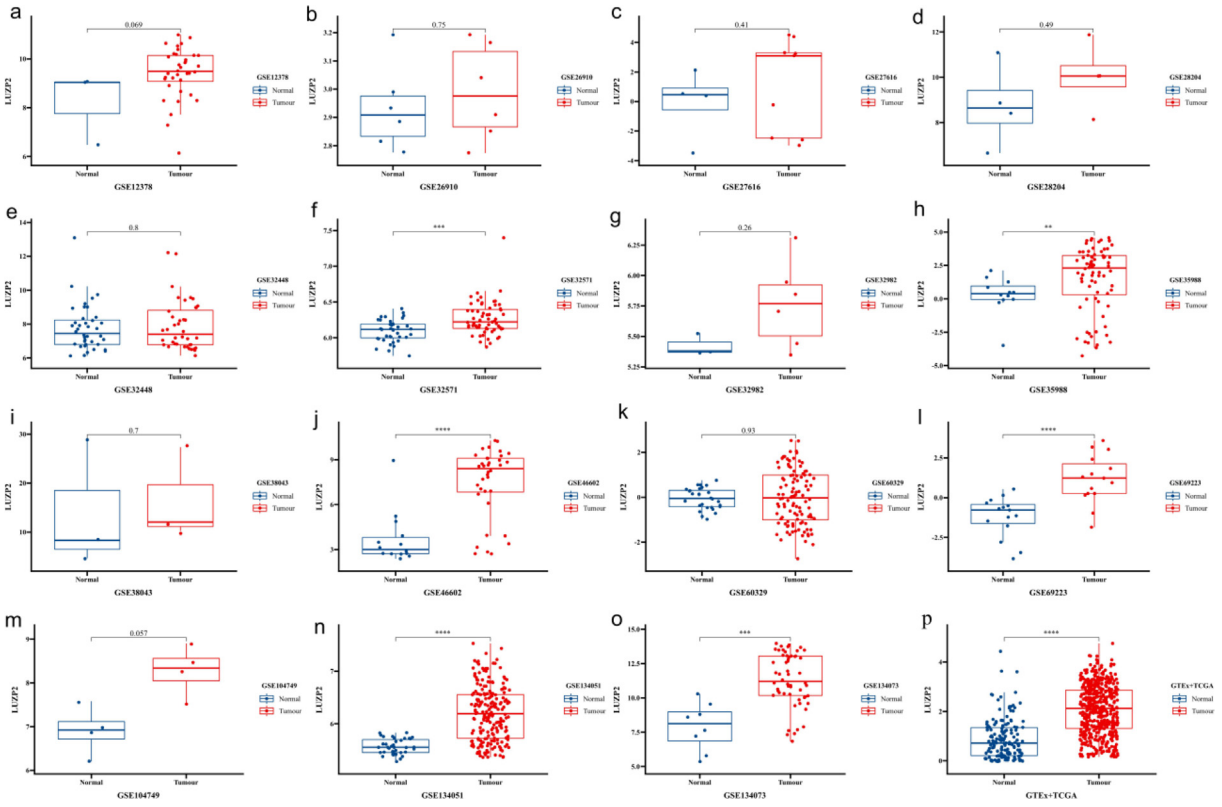

Fig. 2. Distinct expression levels of LUZP2 mRNA between PCa and Non-PCa prostate samples. The number in bold represents P value summary and one or more "*" represent significant difference, "ns" represents $P > 0.05$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). LUZP2: leucine zipper protein 2; PCa: prostate cancer; GTEx: Genotype-Tissue Expression Description; TCGA: The Cancer Genome Atlas.

3. Results

3.1. Expression of LUZP2 mRNA in PCa

This study was carried out in accordance with the research ideas in Fig. S1. We included 21 datasets that met the criteria (Fig. 1). The differences in the expression of LUZP2 in PCa and non-PCa in each dataset are shown in a box diagram (Table 1, Fig. 2). In order to evaluate the reliability of LUZP2 in differentiating PCa and non-PCa, the ROC curve is shown in Fig. 3.The level of LUZP2 in PCa was significantly upregulated in most datasets. To comprehensively analyze the expression status of LUZP2, we calculated the SMD. Overall, the SMD was 1.05 (95%CI: 0.72–1.38), indicating LUZP2 was evidently expressed more highly in PCa (1467) compared with non-PCa (549) (Fig. 4a). The sensitivity analysis showed that no individual studies significantly impacted the overall study (Fig. 4b). The Begg test and Egger test indicated no publication bias (Deeks' Funnel Plot results were $p > 0.10$) (Fig. 4c-d, f). The sROC, used to evaluate the accuracy of LUZP2 overexpression, showed that AUC = 0.88 (CI: 0.85–0.90) (Fig. 4e), suggesting that higher expression of LUZP2 reliably distinguished PCa from non-PCa.

In-house IHC analysis showed that the level of LUZP2 protein in PCa was clearly increased compared with that in the non-PCa controls ($p < 0.001$) (Table 2, Fig. 5a–c, Fig. 6a-b). Interestingly, in most of the cases, both non-PCa with weaker staining signaling and PCa tissues with much stronger staining signaling could be shown in the same slide, and even in the same field of the microscopic image. An example is displayed in Fig. 6c. The SMD of LUZP2 protein level in patients with PCa was 2.23 (CI: 1.67, 2.79) (Fig. 5d). The ROC curve, drawn based on the IHC analysis results of three tissue microarrays, verified the excellent discrimination ability of overexpressed LUZP2 in PCa (all AUC > 0.90; Fig. 5e–g).

3.2. Prognostic value of LUZP2 in PCa

The survival analysis with the GEPIA database suggested that there were no significant correlations between the expression of LUZP2 and either OS or DFS, while the same result was obtained in verification using GSE46602 (Fig. S2). The risk ratio analysis using pan-cancers data in the STRING database was not statistically significant (Fig. S2d). In addition, the results of subgroup survival analysis based on race, gleason score, and tumor grade (TNM) showed that the expression of LUZP2 had a significant difference for the OS of patients with PCa in gleason score ($p < 0.001$) and pathologic T ($p < 0.05$) (Fig. S3). The above results suggested that higher expression of LUZP2 was associated with advanced T clinical stage of PCa.

3.3. Gene changes of LUZP2 in PCa and its impact on prognosis

Based on the close relationship between cancer and genetic changes, we evaluated the change frequency of LUZP2 in 499 patients with PCa in cBioPortal. Among them, 0.8% of patients

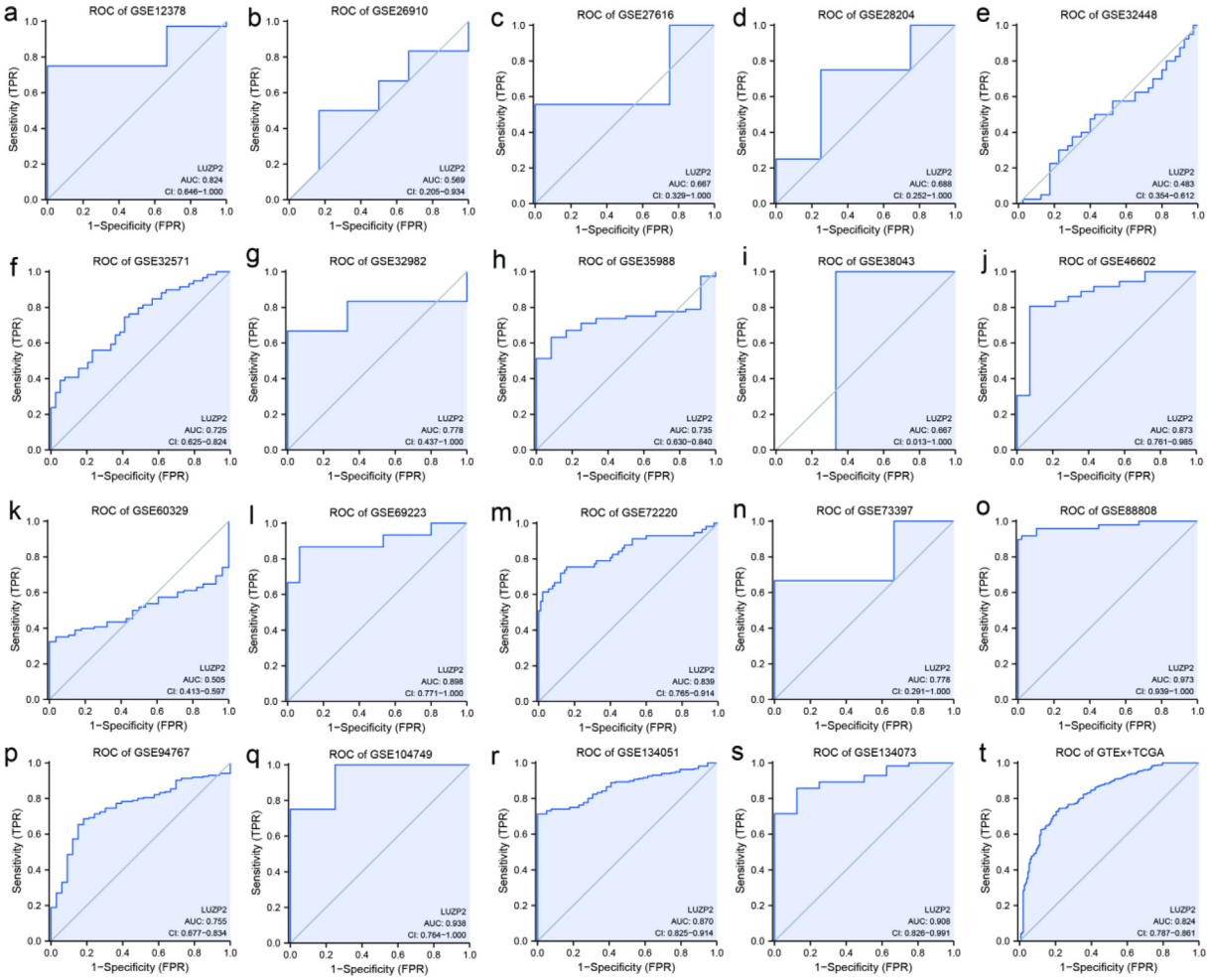

Fig. 3. The ROC curves of LUZP2 overexpression for distinguishing PCa from non-PCa prostate samples. LUZP2: leucine zipper protein 2; PCa: prostate cancer; AUC: area under the curve; ROC: receiving operator characteristic; GTEx: Genotype-Tissue Expression Description; TCGA: The Cancer Genome Atlas.

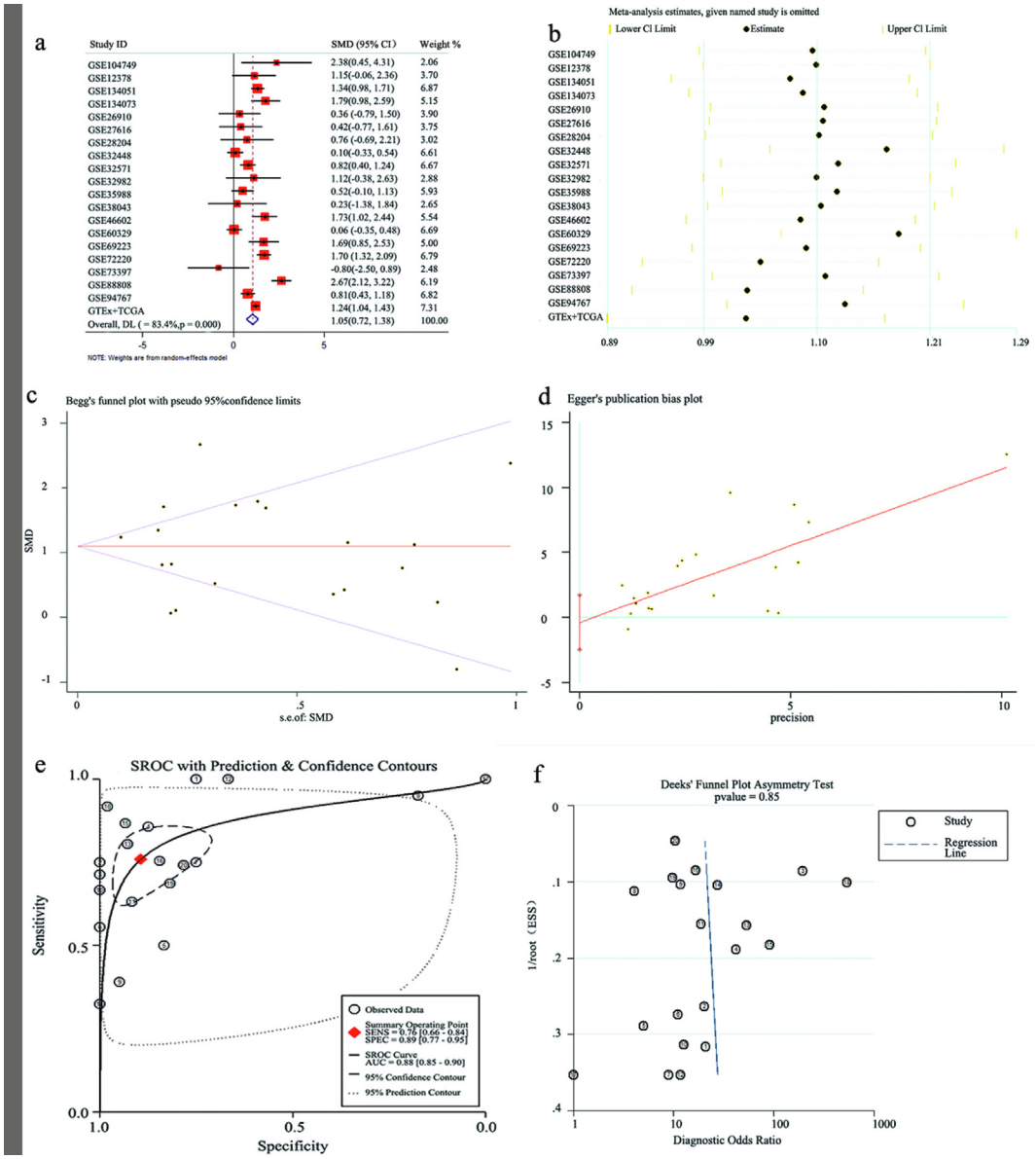

Fig. 4. The comprehensive expression of LUZP2 mRNA in PCa. (a) Forest plot. (b) Sensitivity analysis plot. (c) Begg's funnel plot of the publication bias. (d) Egger's publication bias plot. (e) SROC curve. (f) Deeks' funnel plot test. SMD: standardized mean difference; CI: confidence interval; AUC: area under the curve; SROC: summary receiver operating characteristics; LUZP2: leucine zipper protein 2; PCa: prostate cancer; TCGA: The Cancer Genome Atlas.

Table 2
Association between LUZP2 protein expression and clinicopathological parameters in PCa samples based on in-house immunohistochemistry.

Clinicopathological parameters	N	LUZP2 expression M	LUZP2 expression SD	T-test T-value	T-test P-value
Group					
Non-cancer	31	4.355	1.872	9.459	***P* < 0.001*****
Cancer	60	9.033	2.400		
Age (years)					
<60	9	6.889	3.480	0.740	0.462
≥60	64	7.719	3.104		
Pathological T-stage					
T1 + T2	9	8.222	2.333	5.763	***P* < 0.001*****
T3 + T4	4	12.000	0.000		
Gleason score					
≤7	32	8.938	2.382	0.328	0.744
8≥	28	9.143	2.460		

The number in bold represents *P* value summary, and one or more "*" represent significant difference (*** $p < 0.001$) (N-stage was not included because of N 1 had only 1 case; M-stage was not included because of lack of data for M1). M: mean; N: number; PCa: prostate cancer; SD: standard deviation; LUZP2: leucine zipper protein 2.

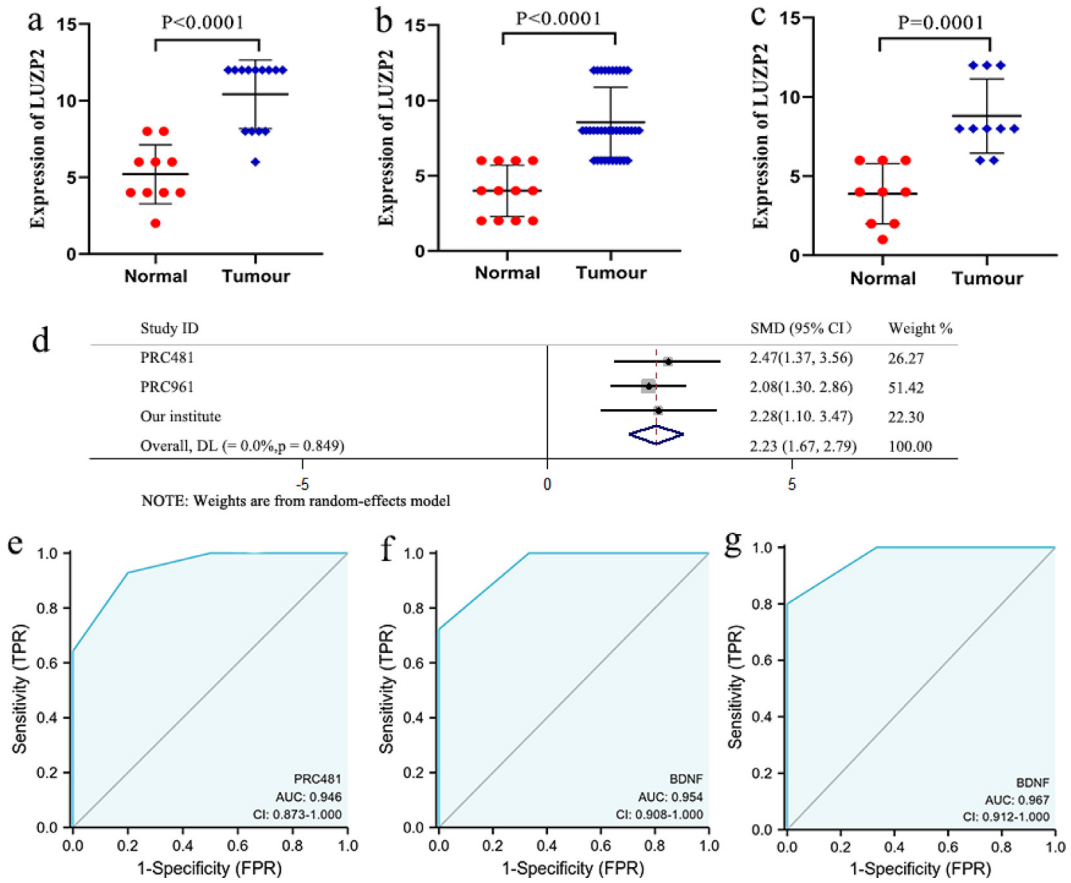

Fig. 5. The expression of LUZP2 protein detected by in-house IHC. (a) Tissue chip (No. PRC481). (b) Tissue chip (No. PRC961). (c) Samples collected from our institute. (d) Forest plot. (e–g) The ROC curves of PRC481, PRC961 and our institute. SMD: standardized mean difference; CI: confidence interval; AUC: area under the curve; LUZP2: leucine zipper protein 2; PCa: prostate cancer; IHC: immunohistochemistry.

had alteration changes in LUZP2, including mutation, amplification, multiple changes, and deletion (Fig. 7a-b). Among the 284 PCa samples in COSMIC, the mutation types and alternative mutation types of LUZP2 included missense substitution (1.03%) and others accounting for 1.06% (Fig. 7c). In 3 samples, substitution mutations mainly occurred in A > C (33.33%), A > T (33.33%), and T > A (33.33%) (Fig. 7d). No remarkable difference was found in the probability of OS (log-rank test, $p = 0.845$) or DFS (log-rank test, $p = 0.441$) between the changed group and the unchanged group in cBioPortal (Fig. S4).

3.4. Potential mechanism of LUZP2 in PCa

According to the results, LUZP2 was overexpressed in PCa. To interpret the possible molecular underpinnings of LUZP2 in PCa, 5456 up-DEGs and 630 CEGs of LUZP2 were screened. Then, 267 intersection genes were collected (Fig. 8a).

Regarding the results of GO and KEGG analysis, GO-BP showed that 267 CEGs of LUZP2 were enriched mainly in the cellular response to drug, copper ion import, and regulation of synapse assembly ($p < 0.05$) (Fig. 9a). GO-CC suggested that these genes tend mainly to be enriched in extracellular exosome, perinuclear region of cytoplast, and basolateral plasma membrane ($p < 0.05$) (Fig. 9b). GO-MF analysis indicated that they tend mainly toward metal ion binding, act binding, calmodulin binding, and transfer activity ($p < 0.05$) (Fig. 9c). KEGG pathway analysis is a method used to systematically analyze the metabolic pathway of gene products in cells. Our KEGG pathway analysis pinpointed that the above genes were closely related to the amino sugar and nucleoside sugar metabolism pathway (Fig. 9d). Second, to determine the correlation between a given gene set (including 267 genes) and tumor progression, DisGeNET showed that LUZP2 expression was related mainly to metastatic prostate carcinoma, metastasis from malignant tumor of the prostate, prostatic intraepithelial neoplasias, etc. (Fig. 9e). This suggested that LUZP2 may affect the progress of PCa with its CEGs and may even be related to malignant development, such as metastasis.

4. Discussion

In this study, we conducted a more in-depth exploration of the clinical role of LUZP2 in PCa than was previously reported [20]. Both high-throughput data (including gene chips and RNA-seq) and in-house IHC verified the higher expression of LUZP2 (mRNA expression and protein level) in PCa. In addition, the up-DEGs and CEGs of LUZP2 in PCa were identified by processing expression profile data. We found that these CEGs may play a role through the amino sugar and nucleoside sugar metabolism, which provides a new basis for the diagnosis and mechanism of PCa.

Previously, in the only study mentioned, the researchers compared 28 cases of normal prostate tissue with 59 cases of PCa and found that LUZP2 mRNA was highly expressed in PCa [20]. Through the analysis of 2016 samples (549 cases of non-PCa, 1467 cases of PCa), we found that the highly expressed LUZP2 mRNA in PCa has a moderate discriminating ability (SMD = 1.05, AUC = 0.88), which was consistent with the existing research results. Moreover, the large sample size enhanced the reliability of the results of our study. In the mentioned study, the researchers

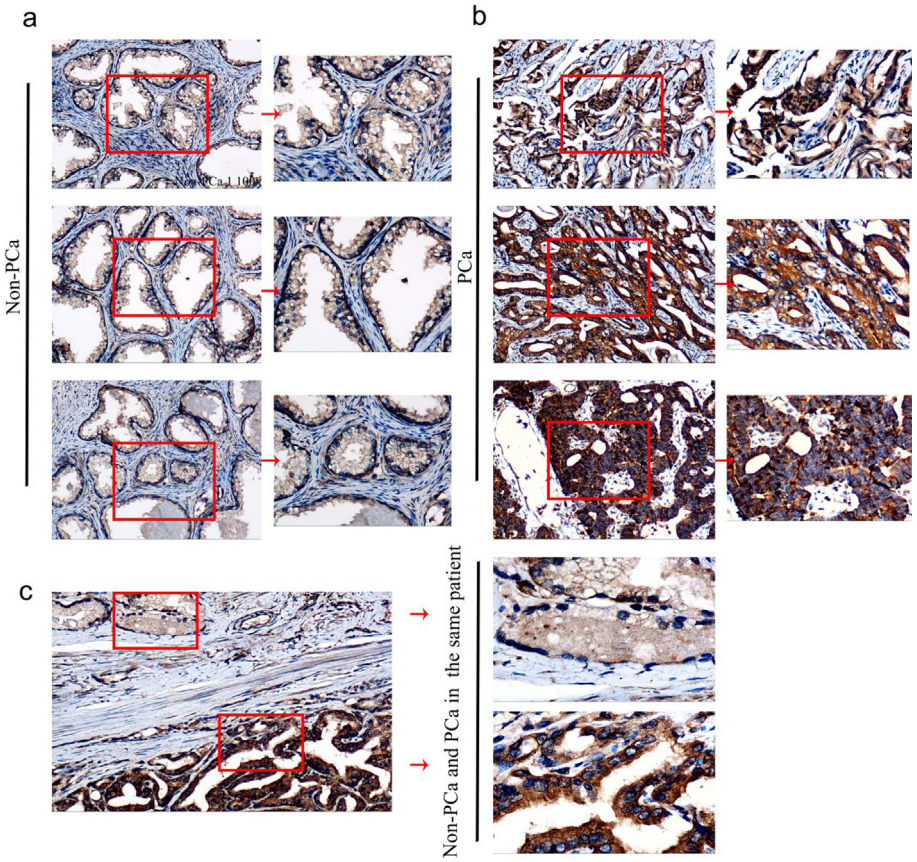

Fig. 6. The protein expression of LUZP2 in non-PCa and PCa assessed by IHC. (a) The expression level of LUZP2 protein was lower in non-PCa prostate tissue (Normal Case 1 [100X and 200X]; Normal Case 2 [100X and 200X]; Normal Case 3 [100X and 200X]). (b) The expression level of LUZP2 protein was higher in PCa (PCa Case 1 [100X and 200X]; PCa Case 2 [100X and 200X]; PCa Case 3 [100X and 200X]). (c) The different expression of LUZP2 protein between Non-PCa and PCa as shown in the same field (Case of Non-PCa [100X and 400X]; Case of PCa [100X and 400X]). LUZP2: leucine zipper protein 2; PCa: prostate cancer; IHC: immunohistochemistry.

only reported a single layer of LUZP2 expression, e.g. the mRNA level [20]. It is noteworthy that the protein expression of LUZP2 was further verified in our study. The study results were confirmed with 31 non-PCa samples and 60 PCa samples in IHC analysis (SMD = 2.23). Therefore, we had sufficient evidence that LUZP2 was significantly upregulated in PCa, with mRNA and protein levels. We suspect that LUZP2 might play a cancer-promoting effect in PCa.

It was of great significance to clarify the clinical value of LUZP2 in the progression of PCa. In the analysis of clinical parameters of IHC, the expression level of LUZP2 increased according to T-stage (T3 + T4). Additionally, although LUZP2 was not a prognostic factor in PCa, because of its correlation with clinicopathological features, we believed that the high expression of LUZP2 has clinical value for patients with PCa. However, due to the limitation of the number of included samples, the results needed to be further explored.

Genetic changes affect the occurrence and development of cancer. A previous study demonstrated that gene mutations in prostate cells could cause abnormal prostate hyperplasia and cancerous transformation [29]. The detection of 4399 PCa cases revealed that the mutation-carrying rates of HOXB13, BRCA2, ATM, and CHEK2 were notably elevated relative to the controls [30]. Another study detected four coding sequence changes, three silent mutations, and one missense mutation of LKB1 in 50 samples of benign prostatic hyperplasia, suggesting that LKB1 may be related to benign prostate transformation [31]. Given the important impact of the aforementioned genetic mutations on the progress of PCa, we evaluated the genetic changes and the types of mutations and substitution mutations of LUZP2 in PCa. In the analysis of 499 patients with PCa, 0.8% had mutation, amplification, and deletion involving LUZP2. However, the findings of the prognostic analysis of these gene changes of LUZP2 were not statistically significant. Our study suggested that overall change of LUZP2 was not a key factor affecting the prognosis of patients with PCa.

Analysis of the 267 CEG pathways of LUZP2 indicated that it is likely to participate in the occurrence and development of PCa through the amino sugar and nucleotide sugar metabolism pathway. Metabolic pathway and cell energy imbalance have recently been considered as affecting the growth of tumor cells and could be used as the main initiating factors and markers of cancer [32]. Aberrant carbohydrate metabolism could lead to a higher likelihood of the morbidity of pancreatic carcinoma [33]. Amino sugar and nucleoside sugar metabolism affect tumor progression by activating carbohydrate metabolism in tumor cells [34,35]. The decreased levels of multiple metabolites in the amino sugar and nucleoside sugar metabolism pathway are related to ovarian cancer cell growth inhibition and migration [36]. Thus, amino sugar and nucleoside sugar metabolism offer a novel aspect for understanding the molecular mechanism of PCa development. The DisGeNET enrichment analysis of this gene set also showed that they were related mainly to the progression of PCa and the advanced malignant performance of PCa. Therefore, we speculated that amino sugar and nucleoside sugar metabolism were involved in the role of LUZP2 in PCa.

In our study, IHC was used to evaluate the expression of LUZP2 in PCa. Mutation analysis and functional enrichment analysis of LUZP2 were also carried out. All these provided strong evidence

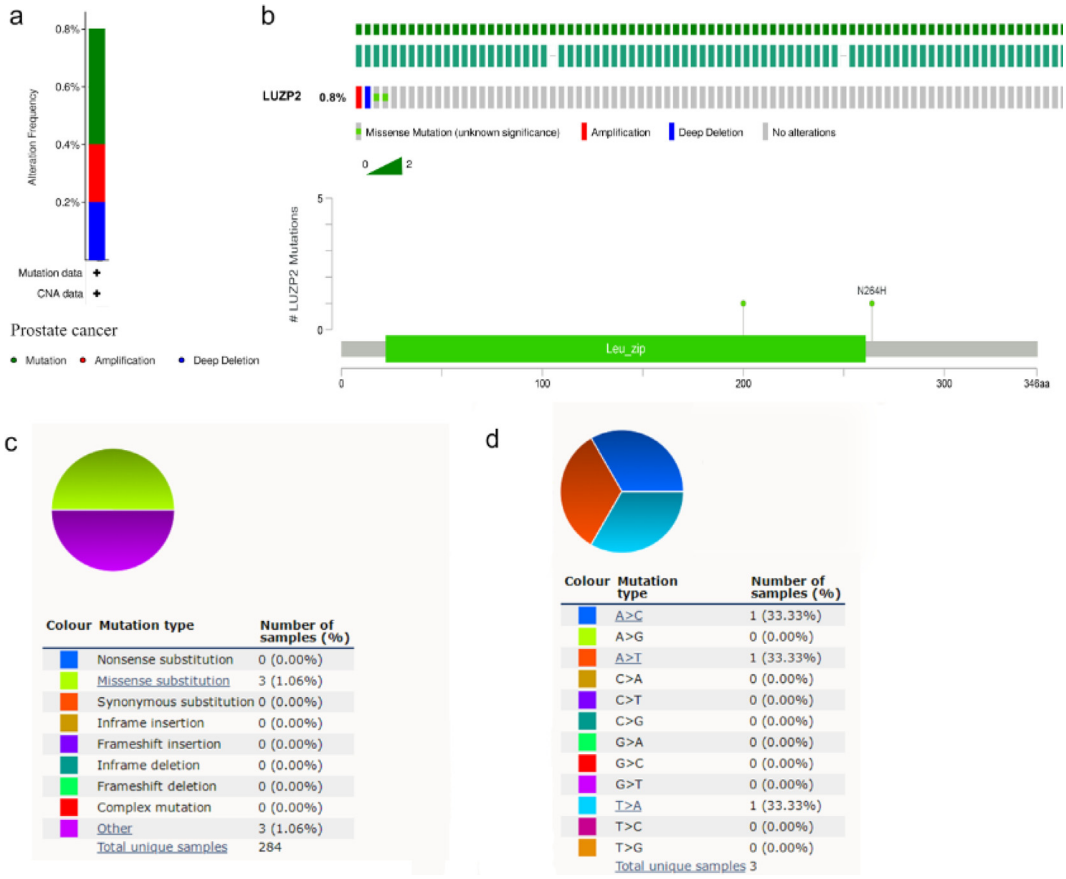

Fig. 7. Gene expression and mutation analysis of LUZP2 in PCa. (a) LUZP2 mutation in PCa (cBioPoral). (b) Detailed information on the gene mutation of PCa (cBioPoral). (c) The overview of mutation types of LUZP2 in COSMIC database. (d) The overview of substitutional mutation types of LUZP2 in the COSMIC database. COSMIC: the Catalogue of Somatic Mutations in Cancer; LUZP2: leucine zipper protein 2; PCa: prostate cancer.

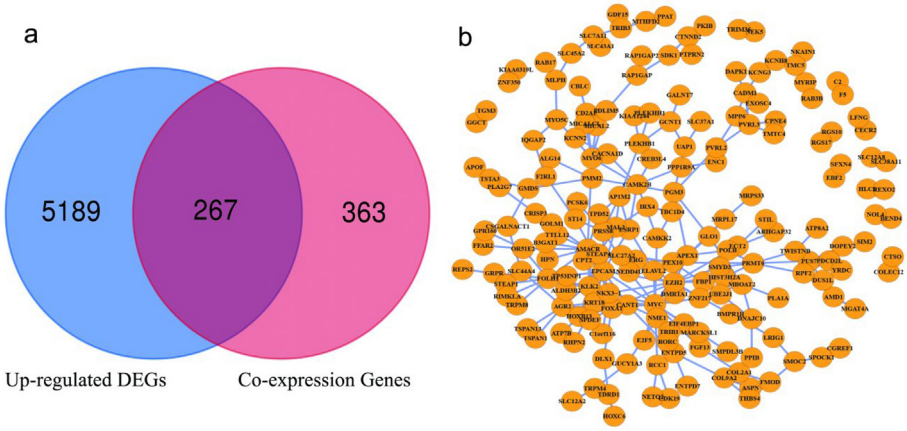

Fig. 8. The PPI network analysis of LUZP2 in PCa. (a) The Venn diagrams of LUZP2. (b) The PPI network analysis. DEGs: differently expressed genes; LUZP2: leucine zipper protein 2; PCa: prostate cancer; PPI: Protein–Protein Interaction Networks.

for the need to explore the molecular mechanism and clinical significance of LUZP2 in PCa. However, the research also had limitations. First, due to the lack of included sample information, the study was not able to use survival data with IHC to analyze the prognosis of LUZP2 in PCa. Moreover, the lack of clinical information parameters for the IHC samples restricted the analysis of clinical parameters of the N, M-stage, which limited the evaluation of the clinical value of LUZP2 for patients with PCa, to a certain extent. Finally, although our study conducted a preliminary analysis of the mutation of LUZP2, whether the mutation of LUZP2

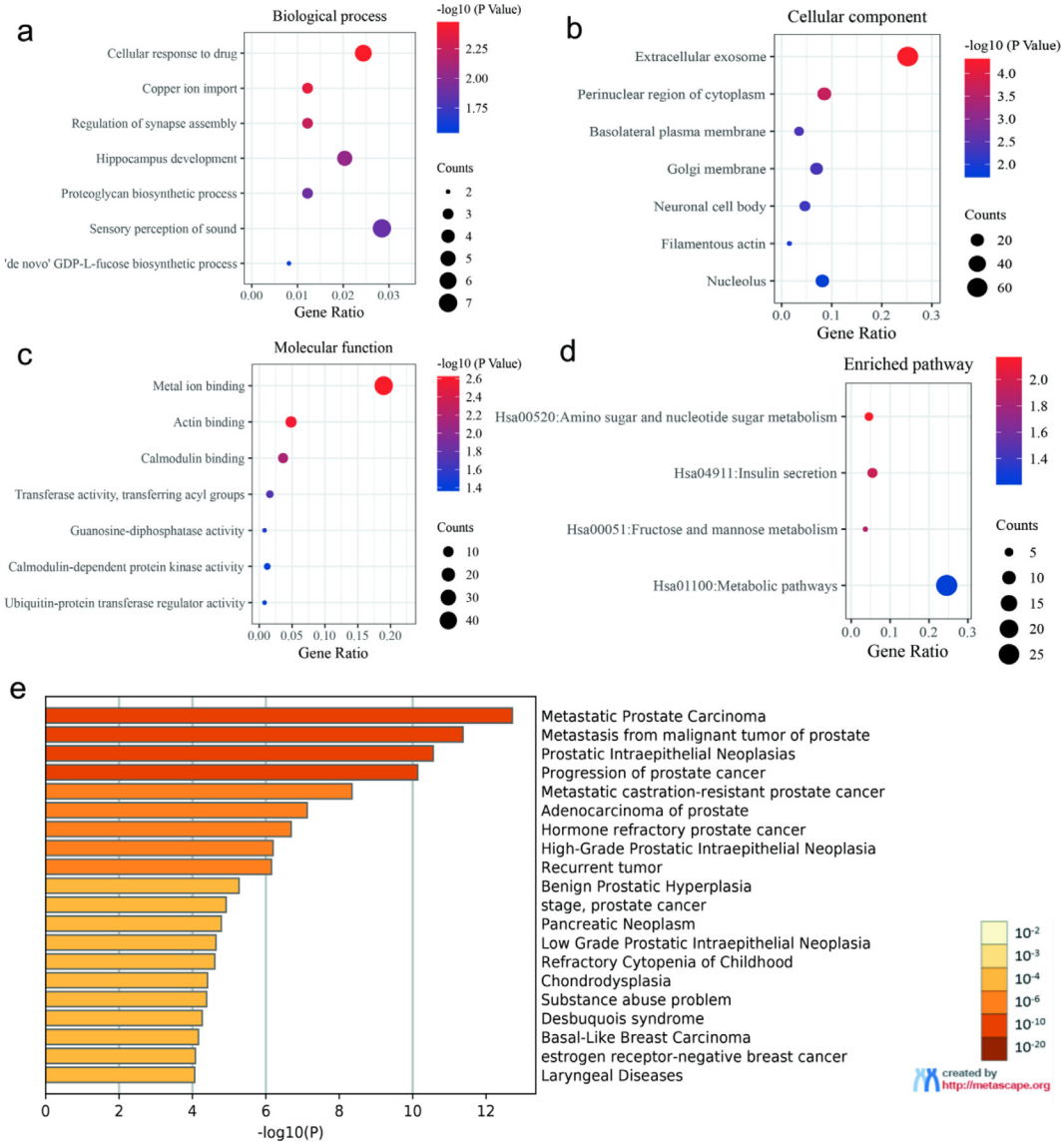

Fig. 9. The GO and KEGG pathway analyses of co-expressed genes in PCa. (a) Biological process (BP). (b) Cellular component (CC). (c) Molecular function (MF). (d) KEGG pathway analysis. (e) Summary of enrichment analysis in DisGeNET (in metascape). DEGs: differently expressed genes; PCa: prostate cancer; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants; DEGs: differently expressed genes; LUZP2: leucine zipper protein 2; PCa: prostate cancer.

affects the process of PCa and the specific mechanism need to be further studied. In any event, we put forward new ideas for the mechanism of LUZP2 in PCa.

5. Conclusion

In summary, LUZP2 is markedly upregulated in PCa tissues, which may affect the occurrence of PCa. Three highlights of this paper are: (1) We used a variety of methods (gene chip, RNA-sequencing, immunohistochemistry, and data integration) to show and confirm the promoting effect of highly expressed LUZP2 in the occurrence and growth of PCa. (2) Such clinical significance was proved based on a large size of samples, in which the mRNA level contained 1467 cases of PCa and 549 cases of control, while the protein level had 60 cases of PCa and 31 cases of control. (3) More importantly, the discovery of our study was based on the multi-centered samples, including 21 high-throughput datasets, covering the populations with PCa from seven countries, such as China, Italy, Germany, UK, USA, Denmark, and Finland, which proved that over-expression of LUZP2 in PCa could be a universal phenomenon in the world without racial and geographical specificity. Further, LUZP2 may participate in the progress of PCa through the amino sugar and nucleotide sugar metabolism pathway. The specific mechanism of LUZP2 needs to be further explored and elaborated in the future.

Ethical approval

The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, and all patients involved signed informed consent.

Author contributions

-Study conception and design: S-H Li; Z-G Huang.
-Data collection: Y-P Yang; R-Q He, G-L Zhang.

-Analysis and interpretation of results: S-H Li; Y-P Yang; R-Q He.

-Draft manuscript preparation: J He; X Feng; J-W Cheng.

-Revision of the results and approved the final version of the manuscript: X-X Yu; J Li; G Chen; Y-X Yao.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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Supplementary material

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References

[1] Che B, Zhang W, Xu S, et al. Prostate microbiota and prostate cancer: a new trend in treatment. Front Oncol 2021;11. https://doi.org/10.3389/fonc.2021.805459. PMid: 34956913.

[2] Su Y, Yu C, Xue P, et al. Research progress on the treatment of advanced prostate cancer with Olaparib. Neoplasma 2021;68(6):1132–8. https://doi.org/10.4149/neo_2021_210627N856. PMid: 34779643.

[3] Hosseindokht M, Bakherad H, Zare H. Nanobodies: a tool to open new horizons in diagnosis and treatment of prostate cancer. Cancer Cell Int 2021;21(1):580. https://doi.org/10.1186/s12935-021-02285-0. PMid: 34717636.

[4] Taheri M, Najafi S, Basiri A, et al. The role and clinical potentials of circular RNAs in prostate cancer. Front Oncol 2021;11. https://doi.org/10.3389/fonc.2021.781414. PMid: 34804984.

[5] Hu W, Afradiasbagharani P, Lu R, et al. Morphometric analysis of rat prostate development: roles of MEK/ERK and Rho signaling pathways in prostatic morphogenesis. Biomolecules 2021;11(12):1829. https://doi.org/10.3390/biom11121829. PMid: 34944473.

[6] Shui X, Xu R, Zhang C, et al. Advances in neuroendocrine prostate cancer research: from model construction to molecular network analyses. Lab Invest 2022;102:332–40. https://doi.org/10.1038/s41374-021-00716-0. PMid: 34937865.

[7] Xu Z, Wang H, Qin Z, et al. NLRP3 inflammasome promoted the malignant progression of prostate cancer via the activation of caspase-1. Cell Death Discovery 2021;7(1):399. https://doi.org/10.1038/s41420-021-00766-9. PMid: 34930938.

[8] Gupta S, Jea J, Yen L. RNA-driven JAZF1-SUZ12 gene fusion in human endometrial stromal cells. PLoS Genet 2021;17(12). https://doi.org/10.1371/journal.pgen.1009985. PMid: 34928964.

[9] Yan Y, Zhou B, Lee Y, et al. BoxCar and shotgun proteomic analyses reveal molecular networks regulated by UBR5 in prostate cancer. Proteomics 2021;22(4). https://doi.org/10.1002/pmic.202100172. PMid: 34897998.

[10] Marei H, Althani A, Afifi N, et al. p53 signaling in cancer progression and therapy. Cancer Cell Int 2021;21(1):703. https://doi.org/10.1186/s12935-021-02396-8. PMid: 34952583.

[11] Kushwaha P, Verma S, Shankar E, et al. Role of solute carrier transporters SLC25A17 and SLC27A6 in acquired resistance to enzalutamide in castration-resistant prostate cancer. Mol Carcinog 2021;61(4):397–407. https://doi.org/10.1002/mc.23383. PMid: 34939235.

[12] Dong X, Zhang P, Liu L, et al. The Circ_0001367/miR-545-3p/LUZP1 axis regulates cell proliferation, migration and invasion in glioma cells. Front Oncol 2021;11. https://doi.org/10.3389/fonc.2021.781471. PMid: 34869035.

[13] Yang S, Park J, Hwang S, et al. Metformin-inducible small heterodimer partner interacting leucine zipper protein ameliorates intestinal inflammation. Front Immunol 2021;12. https://doi.org/10.3389/fimmu.2021.652709. PMid: 34211461.

[14] Boldbaatar J, Gunarta I, Suzuki R, et al. Protective role of c-Jun NH-terminal kinase-associated leucine zipper protein (JLP) in curcumin-induced cancer cell death. Biochem Biophys Res Commun 2020;522(3):697–703. https://doi.org/10.1016/j.bbrc.2019.11.154. PMid: 31787236.

[15] Li Y, Deng G, Qi Y, et al. Downregulation of LUZP2 is correlated with poor prognosis of low-grade glioma. Biomed Res Int 2020;2020. https://doi.org/10.1155/2020/9716720. PMid: 32695826.

[16] Wu M, Michaud E, Johnson D. Cloning, functional study and comparative mapping of *Luzp2* to mouse chromosome 7 and human chromosome 11p13-11p14. Mamm Genome 2003;14(5):323–34. https://doi.org/10.1007/s00335-002-2248-6. PMid: 12856284.

[17] Lee M, Chang A, Sun D, et al. Restricted expression of LUZP in neural lineage cells: a study in embryonic stem cells. J Biomed Sci 2001;8(6):504–11. https://doi.org/10.1007/BF02256613. PMid: 11702014.

[18] Hsu C, Chang N, Lee M, et al. LUZP deficiency affects neural tube closure during brain development. Biochem Biophys Res Commun 2008;376(3):466–71. https://doi.org/10.1016/j.bbrc.2008.08.170. PMid: 18801334.

[19] Goes F, McGrath J, Avramopoulos D, et al. Genome-wide association study of schizophrenia in Ashkenazi Jews. Am J Med Genetics Part B, Neuropsych Genet 2015;168(8):649–59. https://doi.org/10.1002/ajmg.b.32349. PMid: 26198764.

[20] Zhao J, Zhao Y, Wang L, et al. Alterations of androgen receptor-regulated enhancer RNAs (eRNAs) contribute to enzalutamide resistance in castration-resistant prostate cancer. Oncotarget 2016;7(25):38551–65. https://doi.org/10.18632/oncotarget.9535. PMid: 27221037.

[21] Li G, Yang L, Chen G, et al. Laryngeal squamous cell carcinoma: clinical significance and potential mechanism of cell division cycle 45. Cancer Biother Radiopharm 2021;37(4):300–12. https://doi.org/10.1089/cbr.2020.4314. PMid: 34672813.

[22] Zhang H, Chen G, Chen S, et al. Overexpression of cyclin-dependent kinase 1 in esophageal squamous cell carcinoma and its clinical significance. FEBS Open Bio 2021;11(11):3126–41. https://doi.org/10.1002/2211-5463.13306. PMid: 34586751.

[23] Huang W, Chen G, Chen S, et al. The indication of poor prognosis by high expression of ENO1 in squamous cell carcinoma of the lung. J Oncol 2021;2021. https://doi.org/10.1155/2021/9910962. PMid: 34504528.

[24] He R, Li J, Du X, et al. *LPCAT1* overexpression promotes the progression of hepatocellular carcinoma. Cancer Cell Int 2021;21(1):442. https://doi.org/10.1186/s12935-021-02130-4. PMid: 34419067.

[25] Wu H, Li Q, Liang L, et al. Prognostic alternative splicing signature in cervical squamous cell carcinoma. IET Syst Biol 2020;14(6):314–22. https://doi.org/10.1049/iet-syb.2019.0095. PMid: 33399095.

[26] Liu C, Guo W, Peng J, et al. Clinical significance of *CCNE2* protein and mRNA expression in thyroid cancer tissues. Adv Med Sci 2020;65(2):442–56. https://doi.org/10.1016/j.advms.2020.09.001. PMid: 33059229.

[27] Yang D, Huang W, Chen G, et al. Clinical significance of transcription factor RUNX2 in lung adenocarcinoma and its latent transcriptional regulating mechanism. Comput Biol Chem 2020;89. https://doi.org/10.1016/j.compbiolchem.2020.107383. PMid: 33032037.

[28] Liang L, Zhu J, Chen G, et al. Prognostic values for the mRNA expression of the ADAMTS family of genes in gastric cancer. J Oncol 2020;2020. https://doi.org/10.1155/2020/9431560. PMid: 32884571.

[29] Zarei A, Javid H, Sanjarian S, et al. Metagenomics studies for the diagnosis and treatment of prostate cancer. Prostate 2021;82(3):289–97. https://doi.org/10.1002/pros.24276. PMid: 34855234.

[30] Wei J, Yang W, Shi Z, et al. Observed evidence for guideline-recommended genes in predicting prostate cancer risk from a large population-based cohort. Prostate 2021;81(13):1002–8. https://doi.org/10.1002/pros.24195. PMid: 34254341.

[31] Koseoglu H, Celebi A, Galamiyeva G, et al. No tumor suppressor role for LKB1 in prostate cancer. DNA Cell Biol 2021;40(9):1222–9. https://doi.org/10.1089/dna.2021.0274. PMid: 34370601.

[32] Cao G, Song Z, Hong Y, et al. Large-scale targeted metabolomics method for metabolite profiling of human samples. Anal Chim Acta 2020;1125:144–51. https://doi.org/10.1016/j.aca.2020.05.053. PMid: 32674760.

[33] Hu C, Tien S, Hsieh P, et al. High glucose triggers nucleotide imbalance through O-GlcNAcylation of key enzymes and induces KRAS mutation in pancreatic cells. Cell Metab 2019;29(6):1334–1349.e10. https://doi.org/10.1016/j.cmet.2019.02.005. PMid: 30853214.

[34] Gan B, Zhang L, Gao L, et al. Downregulation of miR-224-5p in prostate cancer and its relevant molecular mechanism via TCGA, GEO database and in silico analyses. Oncol Rep 2018;40(6):3171–88. https://doi.org/10.3892/or.2018.6766.

[35] Brunner J, Finley L. SnapShot: Cancer metabolism. Mol Cell 2021;81 (18):3878–3878.e1. https://doi.org/10.1016/j.molcel.2021.06.021. PMid: 34547243.

[36] Gupta R, Yang Q, Dogra S, et al. Serine hydroxymethyl transferase 1 stimulates pro-oncogenic cytokine expression through sialic acid to promote ovarian cancer tumor growth and progression. Oncogene 2017;36(28):4014–24. https://doi.org/10.1038/onc.2017.37. PMid: 28288142.