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ORIGINAL RESEARCH

Identification of Critical Pathways and Hub Genes in LanCL1-Overexpressed Prostate Cancer Cells

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Background: Prostate cancer is one of the most common malignancies in urology, especially in developed countries. Our previous studies showed that Lanthionine synthase C-like protein 1 (*LanCL1*) can promote the proliferation of prostate cancer cells and protect cells from oxidative stress. Also, *LanCL1* protects cells by inhibiting the *JNK* signaling pathway after H_2O_2 treatment.

Materials and Methods: In our study, we analyzed the data of RNA-seq to identify the DEGs after *LanCL1* overexpression. We performed a functional enrichment analysis with gene set enrichment analysis (GSEA) and a database for annotation, visualization, and integrated discovery (DAVID). We also identified the critical hub gene correlated with disease prognosis by Cox regression analysis.

Results: A total of 8928 DEGs were identified. Through the analysis of GO and KEGG, we found that DEGs are significantly enriched in categories related to metabolism, cancer-related signaling pathways, and inflammation. The top 15 hub genes were then identified and ranked by degree from the protein–protein interaction network. Survival analysis showed 4 hub genes related to disease prognosis and *ICAM1* expression is an independent risk factor for the prognosis.

Conclusion: Our results suggest the critical genes and pathways that might play key roles after *LanCL1* overexpression in prostate cancer. We also provide candidate gene targets that might play important roles in prostate cancer development.

Keywords: prostate cancer, RNA sequencing, *LanCL1*

Introduction

Prostate cancer remains the most common cause of cancer-related death in men, particularly in developed countries.[1] Up to now, many studies have found out multiple critical genes, cellular processes, and signaling pathways that contribute to disease progression. In previous study, we focused on *LanCL1* and explored whether it contributes to prostate cancer disease progression. As a result, we determined that *LanCL1* expression correlated with the progression of human prostate cancer and that *LanCL1* could also promote prostate cancer proliferation. Moreover, *LanCL1* protects prostate cancer cells from reactive oxygen species (ROS) via suppression of *JNK* pathway.[2]

Lanthionine synthase C-like protein 1 (also known as P40 or GRP69A) is a mammalian member of the LanC-like protein superfamily. It contains highly differentiated peptide-modifying enzymes found in bacteria and plants (LanCs).[3] Multiple studies reveal the role of *LanCL1* in relieving oxidative stress in neurons during normal development by binding zinc ion and GSH. In addition, *LanCL1* can

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catalyze the formation of thioether products in neurons, thereby protecting them from oxidative stress.[4–6] In breast cancer, *LanCL1* expression is related to breast cancer survival, making it a marker of aging.[7] In prostate cancer, we have preliminarily explored the role played by *LanCL1* in the disease process. However, further mechanisms are still unclear. As we all know, a gene or protein usually exerts its function by affecting the signaling pathways downstream. In this study, we attempt to further study and explore the mechanism underlying to discover new potential targets for disease diagnosis and treatment.

Materials and Methods

Constructs

We generated pPB-CAG-ires-Pac vector as previously described.[2,8] We inserted full-length *LanCL1* into the multiple-cloning sites (MCS) of pPB-CAG-ires-Pac to generate pPB-CAG-*LanCL1*-ires-Pac vector.

Cell Lines and Cell Culture

LNCaP and PC-3 cells (ATCC) were maintained in RPMI1640 supplemented with 10% FBS. LNCaP and PC-3 cells were co-transfected with pPB-CAG-*LanCL1* and pCMVPBase to obtain control and *LanCL1* stable overexpressing LNCaP/PC-3 cells. We used 2 μg/mL puromycin (Amresco) to screen for 2 weeks for selecting the stable cell lines. Stable cell lines were identified by Western blotting. All cells were added with antibiotic-antifungal solution (100 units/mL penicillin, 0.1 mg/mL streptomycin, and 0.25 mg/mL amphotericin B), and under standard cell culture conditions (5% CO2, 95% humidity) grow at 37°C.

RNA Extraction

According to the manufacturer's instructions (Invitrogen), total RNA was extracted from the tissue using TRIzol® reagent and genomic DNA was removed using DNase I (TaKara). RNA quality was then determined by 2100 Bioanalyser (Agilent) and quantified using ND-2000 (NanoDrop Technologies). Only high-quality RNA samples (OD260/280 = 1.8–2.2, OD260/230 ≥ 2.0, RIN ≥ 6.5, 28S: 18S ≥ 1.0, >10 μg) were used to construct the sequencing library.

Library Preparation, and Illumina Hiseq4000 Sequencing

RNA-seq was performed according to the manufacturer's guidelines (C-10,365; Life Technologies) and previous research.[9] RNA-seq transcriptome library was prepared following TruSeq™ RNA sample preparation Kit from Illumina (San Diego, CA) using 5μg of total RNA. Libraries were size selected for cDNA target fragments of 200–300 bp on 2% Low Range Ultra Agarose followed by PCR amplified using Phusion DNA polymerase (NEB) for 15 PCR cycles. After quantified by TBS380, paired-end RNA-seq sequencing library was sequenced with the Illumina HiSeq 4000 (2 × 150 bp read length). RNA-seq was performed on three biological replicates.

Analysis of RNA-Seq and Differentially Expressed Genes (DEG)

RNA-seq was performed according to the manufacturer's guidelines (C-10,365; Life Technologies) and previous research.[9] We then used EdgeR, an R package to examine differential expressed genes as previously described.[10,11] DEG was determined using the following criteria: | Fold Change (FC) | ≥ 2 and P value < 0.05. DEG is used for further bioinformatics analysis.

Gene Set Enrichment Analysis (GSEA)

GSEA v3.0 was used to analyze the mRNA expression level of biological function annotations and pathways after *LanCL1* overexpression. The results indicate the effects of *LanCL1* overexpression on various biological functional gene sets. The number of permutations is set to 100. In the analysis, we used the following values as cut-off values: the P-value cut-off value is 0.05 and the false discovery rate (FDR q-val) is <0.25.

Functional Annotation and Pathway Enrichment Analysis

We uploaded the top 2000 DEGs and the top 2000 downgraded DEGs to the database, respectively, for annotation, visualization, and integrated discovery (DAVID) website for GO analysis and KEGG analysis,[12] while specifying a P-value <0.05 for statistical significance.

Integration of Protein–Protein Interaction (PPI) Networks and Module Analysis

We used STRING database, which provides a critical assessment and integration of PPI, to analyze the protein interaction.[13] To evaluate the interactive relationships among the DEGs above, we uploaded the top 1000 upregulated and top 1000 downregulated DEGs together to STRING. Then, only experimentally validated interactions

(total score > 0.4) were selected as meaningful. The PPI network was then used for module screening by Molecular Complex Detection (MCODE) in Cytoscape (scores > 3, nodes > 4).[14]

Publicly Available Gene Expression and Clinical Datasets for Prognosis Analysis

We download the gene expression data with corresponding prognostic profiles of 140 prostate cancer patients from online dataset GSE21032 for prognosis analysis.[15] The Kaplan–Meier method using Log-rank test was used to calculate the clinical results between different gene expression groups by Graphpad. Values of P <0.05 were considered statistically significant.

Statistical Analysis

The Kaplan–Meier method using Log-rank test was used to calculate the clinical results by Graphpad. Multivariate Cox regression analyses were used to investigate the association between prognosis and hub genes expression/clinical factors. The FDR in GSEA was tested several times using the Benjamini–Hochberg program to control the FDR.[16,17] Values of P <0.05 were considered statistically significant. All statistical analyses were performed using SPSS v19, Graphpad, and R 3.3.0.

Results

RNA-Seq and Identification of DEGs

To further explore the critical signaling pathways or the regulatory network downstream, we obtained the *LanCL1* stably overexpressed LNCaP cell line. Western blotting showed upregulation of *LanCL1* in stable cell lines (Figure 1A). We then performed RNA-seq on a stable cell line with *LanCL1* overexpression or an empty vector as a control. RNA-seq was performed on three biological replicates.

Based on computer analysis, we used P < 0.05 and Fold Control (FC)≥ 2.0 or ≤0.5 as criteria, and identified 8928 genes as DEGs, of which 4755 were upregulated and 4173 were downregulated. The volcano map of DEGs is shown in Figure 1B, while the heat map of DEGs expression for the first 100 genes is shown in Figure 1C.

GO and KEGG Analyses of DEGs

To further analyze the DEGs at the functional level, we submitted the top 2000 upregulated and top 2000 downregulated DEGs, respectively, online for further analyses using DAVID. In the upregulation group, the GO-BP (Biological Process) analysis of DEGs (Figure 1D) suggested significant enrichment in extracellular matrix organization, collagen fibril organization, proximal/distal pattern formation, cell adhesion, peptidyl-tyrosine phosphorylation, axon guidance, anterior/posterior pattern specification, chemical synaptic transmission, regulation of ion transmembrane transport, ureteric bud morphogenesis, angiogenesis, negative regulation of neuronal apoptosis, ureteric bud development, calcium ion-regulated exocytosis of neurotransmitter, and positive regulation of synapse assembly. We also showed the results of GO-CC (Cellular Component) and GO-MF (Molecular Function) in Supplementary Figures 1A and B. While KEGG pathway analysis showed enrichment mainly in focal adhesion, ECM–receptor interaction, *PI3K-AKT* signaling pathway, arrhythmogenic right ventricular cardiomyopathy (*ARVC*), axon guidance, pathways in cancer, Ras signaling pathway, cell adhesion molecules (CAMs), amoebiasis, cAMP signaling pathway, hypertrophic cardiomyopathy (HCM), protein digestion and absorption, proteoglycans in cancer, platelet activation, and Rap1 signaling pathway (Figure 1E).

In downregulation group, in GO-BP (Biological Process) analysis, DEGs were significantly enriched in oxidation-reduction process, homophilic cell adhesion via plasma membrane adhesion molecules, inflammatory response, establishment of skin barrier, nucleosome assembly, phosphatidylserine acyl-chain remodeling, acute-phase response, angiogenesis, phosphatidylinositol acyl-chain remodeling, positive regulation of cell adhesion, phosphatidylethanolamine acyl-chain remodeling, phospholipid metabolic process, negative regulation of viral genome replication, positive regulation of inflammatory response, and transmembrane transport (Figure 1F). The results of GO-CC (Cellular Component) and GO-MF (Molecular Function) were shown in Supplementary Figures 1C and D. KEGG pathway analysis showed enrichment mainly in arachidonic acid metabolism, alcoholism, systemic lupus erythematosus, linoleic acid metabolism, alpha-Linolenic acid metabolism, drug metabolism – cytochrome P450, chemical carcinogenesis, influenza A, glutathione metabolism, mineral absorption, ether lipid metabolism, transcriptional misregulation in cancer, Aldosterone-regulated sodium reabsorption, Hepatitis C, and Bladder cancer (Figure 1G).

GSEA

To determine the effect of LanCL1 overexpression on prostate cancer progression, we then analyzed various

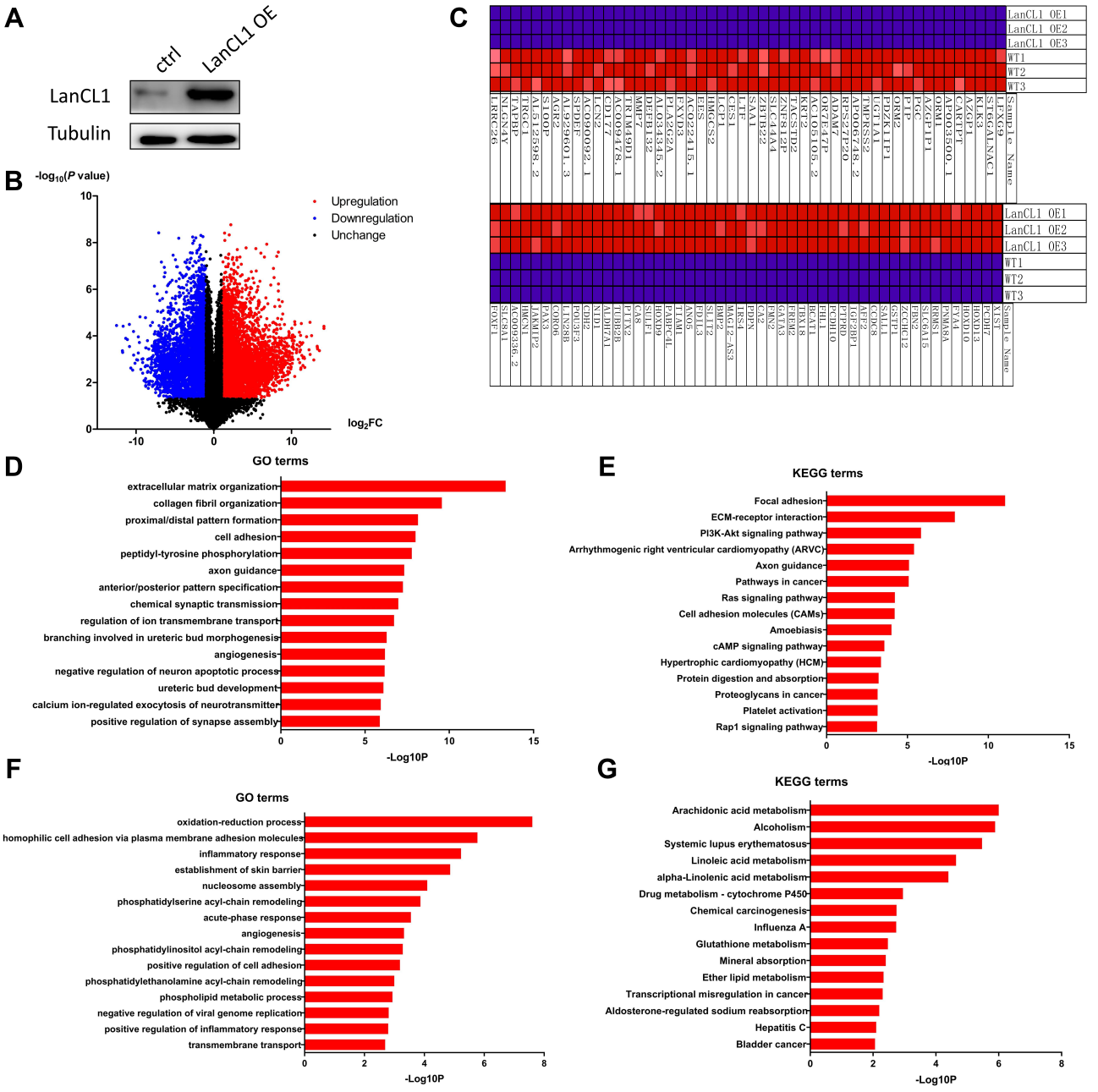


Figure 1 Differentially expressed genes (DEGs) and functional enrichment results of differentially expressed genes. DEG was determined using the following criteria: | Fold Change (FC) | ≥ 2 and P value <0.05. For enrichment analysis, a P-value <0.05 is taken to indicate statistical significance. (**A**) Western blotting result of *LanCL1* overexpression in LNCaP cells. (**B**) Volcano plot for differentially expressed genes. (**C**) Heat map of the top 100 differentially expressed genes (50 upregulated genes and 50 downregulated genes). Red: upregulation; purple: downregulation. (**D**) The GO- BP (Biological Process) enrichment terms of upregulated genes. (**E**) The KEGG pathway analysis of upregulated genes. (**F**) The GO- BP (Biological Process) enrichment terms of downregulated genes. (**G**) The KEGG pathway analysis of downregulated genes.

gene sets by GSEA. As shown in Figure 2 and Supplementary Figure 2, the GSEA results indicate biological processes including UV response to *DN*, *WNT* beta-catenin signaling, Hedgehog signaling, hypoxia, myogenesis, angiogenesis, apical junction, *KRAS* signaling, complement, *IL2-STAT5* signaling, epithelial–mesenchymal transition, allograft rejection, pancreas beta cells, and mitotic spindle were significantly enriched.

PPI Network Analysis

To investigate the protein–protein interaction and find out the hub genes, we then uploaded the top 1000 upregulated DEGs and 1000 downregulated DEGs to the

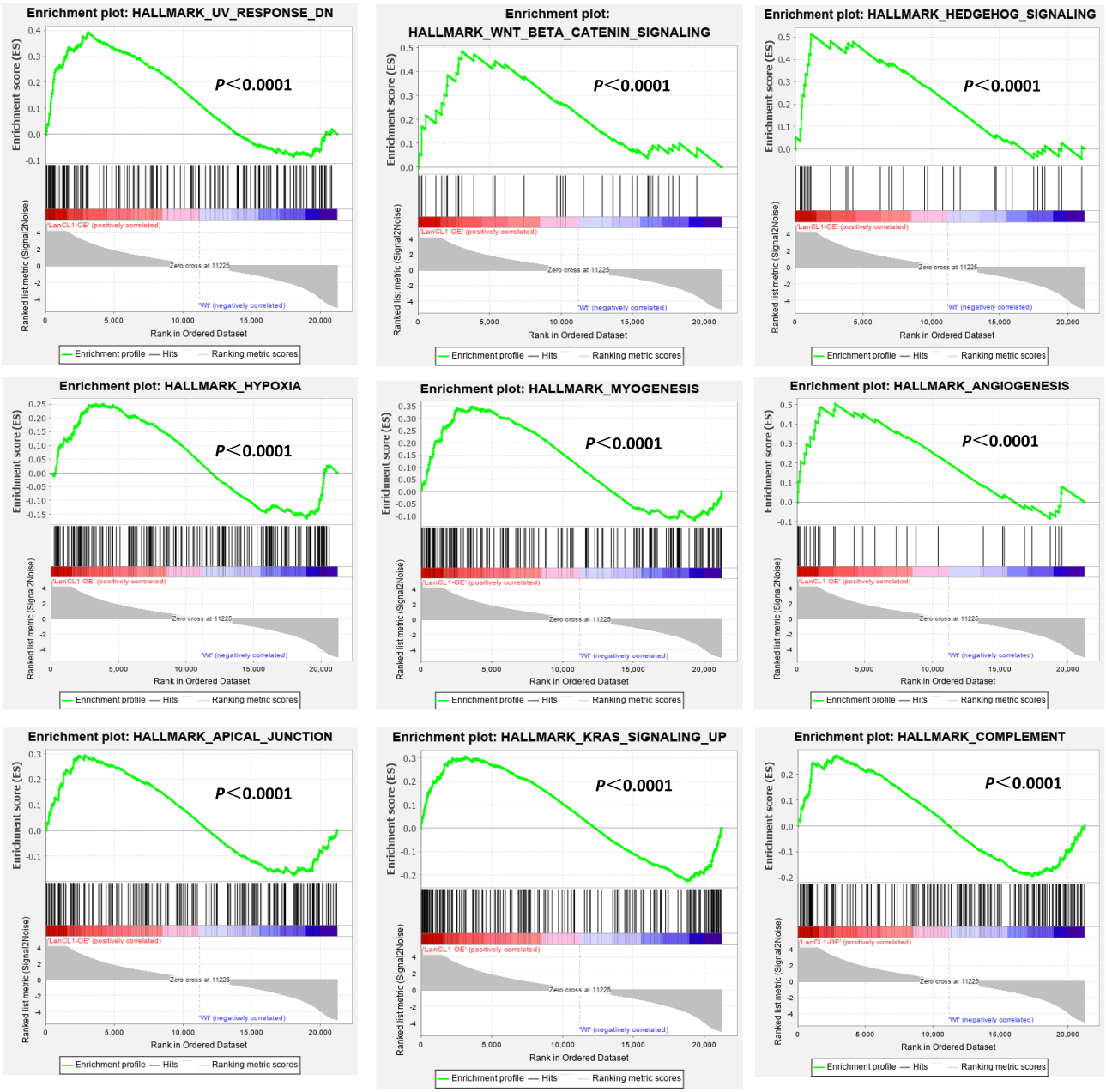


Figure 2 Gene set enrichment analysis (GSEA) results of *LanCL1* overexpression in prostate cancer cells (Top1–9). In the analysis, we used the following values as cut-off values: the P-value cut-off value is 0.05 and the false discovery rate (FDR q-val) is <0.25.

website to screen the information in the STRING database. The top 15 genes ranked by degree were identified as hub genes. These hub genes included *TNF, FGF2, BMP4, CXCL8, BDNF, ESR1, IGF1, KDR, ICAM1, MMP2, KNG1, NCAM1, CDH2, KIT, and APOE* (Table 1). TNF is the top hub gene with the highest degree of 137. Then, we identified the gene modules in the PPI network through MCODE. The top three modules were selected, and we performed GO-BP and KEGG pathway enrichment analysis (Figure 3). Functional annotations in module 1 genes are richer in G protein-coupled receptor signaling pathways, chemotaxis, positive regulation of cytosolic calcium ion concentration, chemokine signaling pathways, neuroactive ligand–receptor interactions, and Cytokine–cytokine receptor interaction.

Enrichment analysis showed that the genes in module 2 were mainly associated with interferon-gamma-mediated signaling pathway, type I interferon signaling pathway, collagen catabolic process, protein digestion and absorption, ECM–receptor interaction, and herpes simplex

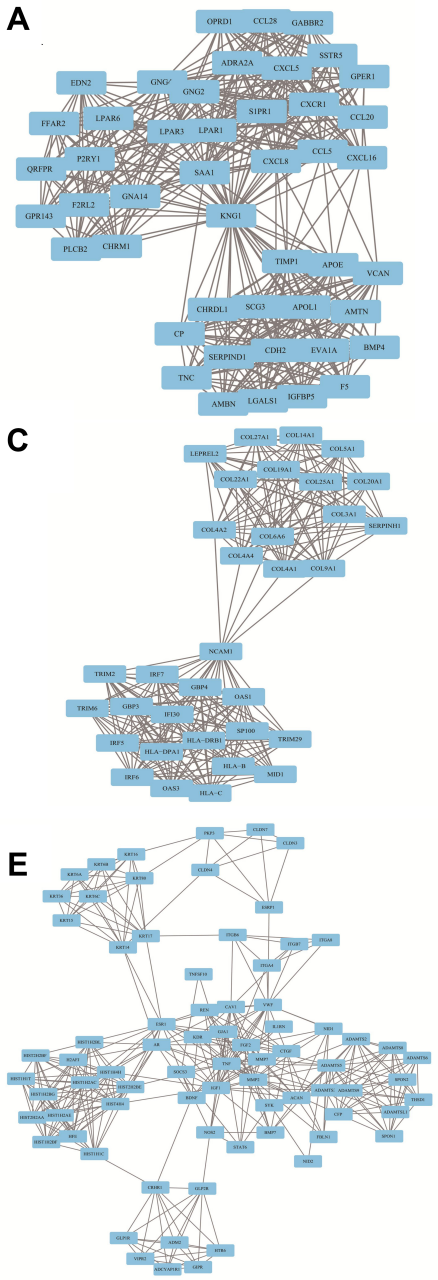
Table 1 Functional Roles of the 15 Hub Genes

No.	Gene	Full Name	Function
1	*TNF*	*Tumor necrosis factor*	Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR.
2	*FGF2*	*Fibroblast growth factor 2*	Plays an important role in the regulation of cell survival, cell division, angiogenesis, cell differentiation and cell migration.
3	*BMP4*	*Bone morphogenetic protein 4*	Induces cartilage and bone formation. Also act in mesoderm induction, tooth development, limb formation and fracture repair.
4	*CXCL8*	*Interleukin-8*	IL-8 is a chemotactic factor that attracts neutrophils, basophils, and T-cells, but not monocytes.
5	*BDNF*	*Brain-derived neurotrophic factor*	During development, promotes the survival and differentiation of selected neuronal populations of the peripheral and central nervous systems.
6	*ESR1*	*Estrogen receptor*	Nuclear hormone receptor.
7	*IGF1*	*Insulin-like growth factor 1*	The insulin-like growth factors, isolated from plasma, are structurally and functionally related to insulin but have a much higher growth-promoting activity.
8	*KDR*	*Vascular endothelial growth factor receptor 2*	Tyrosine-protein kinase that acts as a cell-surface receptor for VEGFA, VEGFC and VEGFD.
9	*ICAM1*	*Intercellular adhesion molecule 1*	ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin alpha-L/beta-2).
10	*MMP2*	*72 kDa type IV collagenase*	MMP2 is a widely distributed metalloprotease that involves multiple functions, such as remodeling of the vascular system, angiogenesis, tissue repair, tumor infiltration, inflammation, and rupture of atherosclerotic plaques.
11	*KNG1*	*Kininogen-1*	Kininogens are inhibitors of thiol proteases.
12	*NCAM1*	*Neural cell adhesion molecule 1*	This protein is a cell adhesion molecule involved in neuron-neuron adhesion, neurite fasciculation, outgrowth of neurites.
13	*CDH2*	*Cadherin-2*	Cadherins are calcium-dependent cell adhesion proteins.
14	*KIT*	*Mast/stem cell growth factor receptor Kit*	Tyrosine-protein kinase that acts as cell-surface receptor for the cytokine Stem Cell Factor (SCF) and plays an essential role in the regulation of cell survival and proliferation, hematopoiesis, stem cell maintenance, gametogenesis, mast cell development, migration and function, and in melanogenesis.
15	*APOE*	*Apolipoprotein E*	Mediates the binding, internalization, and catabolism of lipoprotein particles.

infection. In module 3, genes were mainly enriched in extracellular matrix organization, nucleosome assembly, integrin-mediated signaling pathway, systemic lupus erythematosus, alcoholism, and hypertrophic cardiomyopathy (HCM).

Clinical Outcome and Hub Genes

To select the hub genes that are closely related to clinical prognosis, we use the data and clinical information of GSE21032.[15] Figure 4 and Table 2 show that 4 of the 15 hub genes strongly associated with clinical outcomes, including *APOE, FGF2, ICAM1, and KIT*. Lower *APOE* expression level correlated with better outcome, while higher *FGF2, ICAM1*, and *KIT* correlated with better outcome. Then, we reorganized the clinical data of GSE21032 and found that it contains two clinical factors, Gleason score and Tumor stage (Table 3). To investigate the association between prognosis and these clinical two factors, and to evaluate whether the expressions of the 4 hub genes above are independently associated with disease recurrence, we first did Kaplan-Meier analysis. The results suggest that both Gleason score and Tumor stage significantly correlated with the prognosis of the disease (Table 2). Then, we conducted a multivariate COX regression analysis. Results showed that only the expression of *ICAM1* in the four genes, as well as Gleason score and T stage, is an independent risk factor for the prognosis (Table 4). At last, to confirm our results, we investigated the expression of the 4 critical hub genes after *LanCL1* overexpression in another prostate cancer cell line, PC-3, which revealed the same results as in LNCaP cells (Figure 5). These



B

Term	Count	PValue	Genes
GO:0007186~G-protein coupled receptor signaling pathway	20	3.77E-13	QRFPR, CXCL5, FFAR2, CXCR1, CXCL8, GABBR2, GPER1, LPAR1, CCL5, GPR143, SSTR5, S1PR1, CCL20, APOE, CHRM1, P2RY1, ADRA2A, GNG2, GNG4, OPRD1
GO:0006935~chemotaxis	9	1.06E-09	S1PR1, CXCL5, CCL20, CXCL16, CXCR1, CXCL8, SERPIND1, CCL5, CCL28
GO:0007204~positive regulation of cytosolic calcium ion concentration	8	6.20E-08	KNG1, SAA1, EDN2, P2RY1, LPAR3, LPAR1, GPER1, CCL28
hsa04062:Chemokine signaling pathway	10	1.40E-07	CXCL5, CCL20, CXCL16, CXCR1, CXCL8, GNG2, CCL5, GNG4, PLCB2, CCL28
hsa04080:Neuroactive ligand-receptor interaction	11	3.88E-07	F2RL2, SSTR5, S1PR1, LPAR6, CHRM1, P2RY1, ADRA2A, LPAR3, GABBR2, LPAR1, OPRD1
hsa04060:Cytokine-cytokine receptor interaction	7	9.07E-04	CXCL5, CCL20, CXCL16, CXCR1, CXCL8, CCL5, CCL28

D

Term	Count	PValue	Genes
GO:0060333~interferon-gamma-mediated signaling pathway	13	3.52E-22	NCAM1, SP100, HLA-DRB1, IRF5, IRF6, IRF7, OAS3, IFI30, HLA-C, OAS1, HLA-DPA1, HLA-B, MID1
GO:0060337~type I interferon signaling pathway	8	9.26E-12	SP100, IRF5, IRF6, IRF7, OAS3, HLA-C, OAS1, HLA-B
GO:0030574~collagen catabolic process	8	9.26E-12	COL4A4, COL4A2, COL4A1, COL19A1, COL6A6, COL3A1, COL25A1, COL5A1
hsa04974:Protein digestion and absorption	10	1.57E-12	COL4A4, COL9A1, COL4A2, COL14A1, COL4A1, COL6A6, COL27A1, COL3A1, COL22A1, COL5A1
hsa04512:ECM-receptor interaction	7	1.60E-07	COL4A4, COL4A2, COL4A1, COL6A6, COL27A1, COL3A1, COL5A1
hsa05168:Herpes simplex infection	8	7.13E-07	SP100, HLA-DRB1, IRF7, OAS3, HLA-C, OAS1, HLA-DPA1, HLA-B

F

Term	Count	PValue	Genes
GO:0030198~extracellular matrix organization	12	6.72E-10	VWF, FBLN1, TNF, ITGB7, ITGA8, ITGB6, ACAN, NID1, NID2, ITGA4, FGF2, KDR
GO:0006334~nucleosome assembly	9	4.11E-08	HIST1H1T, HIST4H4, HIST1H1C, HIST1H2BL, HIST1H2BF, HIST2H2BE, HIST2H2BF, HIST1H2BG, HIST1H4H
GO:0007229~integrin-mediated signaling pathway	8	2.15E-07	FBLN1, CTGF, ITGB7, ITGA8, ITGB6, ITGA4, ADAMTS3, SYK
hsa05322:Systemic lupus erythematosus	11	7.94E-09	HIST1H2AC, HIST4H4, TNF, HIST1H2BL, HIST1H2BF, HIST2H2BE, HIST2H2BF, HIST1H2BG, HIST1H2AE, H2AFJ, HIST1H4H
hsa05034:Alcoholism	11	1.15E-07	HIST1H2AC, HIST4H4, BDNF, HIST1H2BL, HIST1H2BF, HIST2H2BE, HIST2H2BF, HIST1H2BG, HIST1H2AE, H2AFJ, HIST1H4H
hsa05410:Hypertrophic cardiomyopathy (HCM)	6	1.27E-04	TNF, ITGB7, ITGA8, ITGB6, IGF1, ITGA4

Figure 3 Top three modules from the protein–protein interaction (PPI) network. (**A**) PPI network of module 1. (**B**) GO-BP (Biological Process) and KEGG analyses of module 1. (**C**) PPI network of module 2. (**D**) GO-BP (Biological Process) and KEGG analyses of module 2. (**E**) PPI network of module 3. (**F**) GO-BP (Biological Process) and KEGG analyses of module 3.

results suggested that these 4 genes, especially *ICAM1*, might be potential key genes for further research.

Discussion

Prostate cancer is still one of the most common malignant tumors in male urinary system all around the world, especially in developed countries. In-depth research on the downstream pathways and regulatory networks of key genes involved in disease progression is of great significance for understanding the disease and finding new therapeutic targets. In our previous study, we revealed the role of *LanCL1* as an oncogene in prostate cancer to promote cell proliferation and protect cell from attack of ROS via *JNK* pathway. However, important downstream genes and regulatory signaling pathways are still not clear.

Here in this study, we overexpressed *LanCL1* gene in LNCaP cells and did RNA-Seq analysis. By selecting DEGs and performing GO and KEGG analysis on the

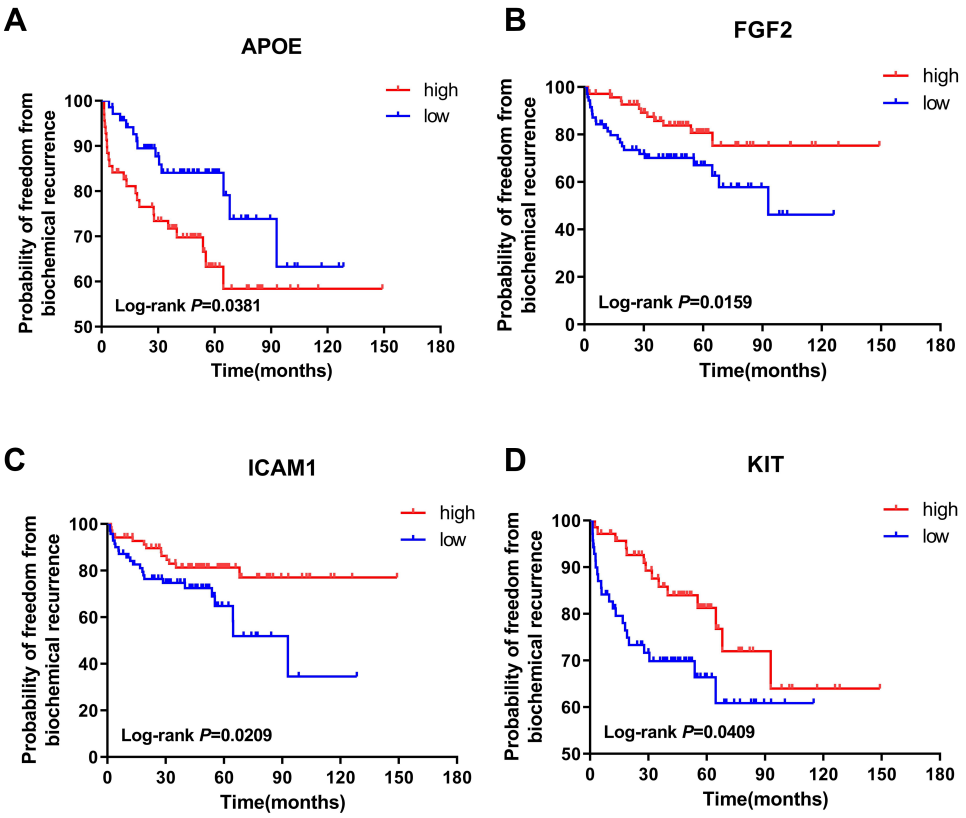


Figure 4 Prognosis analyses of the hub genes. (**A**–**D**) Four hub genes that significantly related to prognosis by analyzing the data from GSE21032. The Kaplan-Meier method using Log-rank test was used to calculate the clinical results. Values of P <0.05 were considered statistically significant.
Abbreviations: *APOE*, apolipoprotein E; *FGF2*, fibroblast growth factor 2; *ICAM1*, intercellular adhesion molecule 1; *KIT*, KIT proto-oncogene.

top 4000 DEGs, we found that *LanCL1* overexpression was significantly associated with the metabolism, axon and synaptic function, and several signaling targets or pathways, such as cAMP signaling pathway, Ras signaling pathway, *PI3K-AKT-mTOR* signaling pathway, and *Rap1* signaling pathway. Previous researches have shown that *Rap1* (ras-related protein 1) signaling pathway and Ras signaling pathway are related to *JNK* and participate in cellular oxidative stress and the production and elimination of ROS in a variety of cellular backgrounds.[18–21] In addition, the cAMP signaling pathway is also involved in the production, metabolism, and clearance of ROS in the body, which has the same important role as the *JNK*-related pathway.[22,23] These results suggest that *LanCL1* plays an important role in assisting cells in processing ROS, which is consistent with our previous findings. Besides, because the oxidative stress state and related signaling pathways in the cells will change after H_2O_2 treatment, there may be significant differences in the signaling pathways between treated and untreated cells. This might be the main reason why *JNK* pathway was not enriched in the RNA-seq results of untreated cells in this study. *JNK* pathway is the main mechanism by which *LanCL1* protects prostate cancer cells from oxidate stress in H_2O_2 treated cells. Our results here also show that the mechanism of *LanCL1* in dealing with ROS is more complicated. It also validates our previous experiments, that is, changing the activity of *JNK* pathway in rescue experiments only partially restored the ROS-related phenotype.

In addition to oxidative stress such as ROS, we also found that many pathways related to tumorigenesis and progression appeared enriched after *LanCL1* overexpression, mainly including *WNT* beta-catenin signaling, *PI3K-AKT* signaling pathway, and *KRAS* signaling. These pathways play important roles in the pathogenesis and progression of various tumors, including prostate cancer.

In prostate cancer, *WNT/β-catenin* and *AR* signals inhibit each other. *β-catenin* activation and *PTEN* deletion synergistically drive non-*AR* independent castration-resistant prostate cancer.[24–26] In most cancer types, the PI3K signaling pathway is activated and promotes

Table 2 Disease Free Survival and Clinicopathological Affairs/ Hub Genes Expression

Characteristics	Overall Survival,%	*P*
	10-Year Probability	
Gleason score		**<0.0001**
<7	70.73	
7	54.54	
>7	15.23	
Tumor stage		**<0.0001**
T2	69.45	
T3-T4	44.7	
FGF2 expression		**0.0159**
High	75.3	
Low	46.22	
ICAM1 expression		**0.0209**
High	77.03	
Low	34.56	
KIT expression		**0.0409**
High	63.96	
Low	60.84	
APOE expression		**0.0381**
High	58.41	
Low	63.29	

Notes: P<0.05 was considered statistically significant: values shown in bold. Kaplan–Meier analysis was used.

tumorigenesis by regulating nutrient metabolism, cell proliferation, survival, migration, and angiogenesis.[26] The potential mechanism of *PI3K/AKT* activation is mainly due to the deletion or mutation of its key negative regulatory gene, *PTEN*.[27] In fact, it is known that the mutual interference between *PI3K/AKT* and other pathways (such as *AR, WNT,* and *ERK* signaling pathways) plays a vital role in the disease progression and drug resistance of prostate cancer, making co-targeting the *PI3K/AKT* signaling pathway and its synergistic pathways critical to improve the anticancer efficacy of *PI3K/AKT* inhibitors.[28,29] Our results indicate that *LanCL1* may contribute to prostate cancer by influencing these important tumor regulatory signal pathways or signal networks, especially its promotion effect on prostate cancer cell proliferation. *LanCL1* also may affect one of these pathways. As for the specific regulatory mechanism, further molecular biology experiments are needed to verify it.

Table 3 Summary of the Clinical Characteristics of Patients with Prostate Cancer in GSE21032

Characteristics	Case, N (%)
Gleason score	
<7	78 (55.7%)
7	49 (35%)
>7	13 (9.3%)
Tumor stage	
T2	86 (61.4%)
T3–T4	54 (38.6%)

Table 4 Multivariate Cox Regression Analyses Investigating the Association Between Prognosis and Hub Genes Expression/ Clinical Factors

Variables	Hazard Ratio	*P*	95.0% CI
Gleason score		**0.0200**	
GS<7	1.00	–	Referent
GS=7	0.26	**0.0063**	0.09–0.681
GS>7	0.41	**0.0403**	0.17–0.96
Tumor stage	4.98	**0.0001**	2.24–11.08
FGF2	2.18	0.0808	0.91–5.21
ICAM1	2.61	**0.0167**	1.19–5.74
KIT	0.70	0.4415	0.28–1.718
APOE	0.53	0.1016	0.25–1.13

Notes: P<0.05 was considered statistically significant: values shown in bold. Multivariate Cox regression analysis was used.

In the PPI network analysis, we identified the top 15 central genes with the highest degree of interaction. *TNF* (tumor necrosis factor) is the top hub gene, which is mainly secreted by macrophages. *TNF* can bind to its receptors *TNFRSF1A/TNFR1* and *TNFRSF1B/TNFBR* to exert its function. *TNF* could involve in the development of tumors by regulating inflammatory and immune responses. Among the 15 hub genes, we also identified 4 hub genes that strongly associated with clinical outcomes in prostate cancer, including *APOE, FGF2, ICAM1*, and *KIT*. We used data from a classic and authoritative prostate cancer RNA-seq database GSE21032 to analyze. These 4 genes might play critical roles in the development of prostate cancer and are closely related to the prognosis of the disease. Then, we conducted a multivariate COX regression analysis and found that only the expression of *ICAM1* is an independent risk factor for the prognosis. This result emphasizes the important potential role of *ICAM1* in prostate cancer initiation and development. *ICAM1* (Intercellular adhesion molecule 1) is a cell surface glycoprotein expressed on endothelial cells and immune system cells. It binds to *CD11a/CD18* or *CD11b/CD18* type integrins and is also used by rhinovirus as a receptor.[30,31] Previous studies have shown that *ICAM1* may facilitate breast and lung.[32–34] Researchers

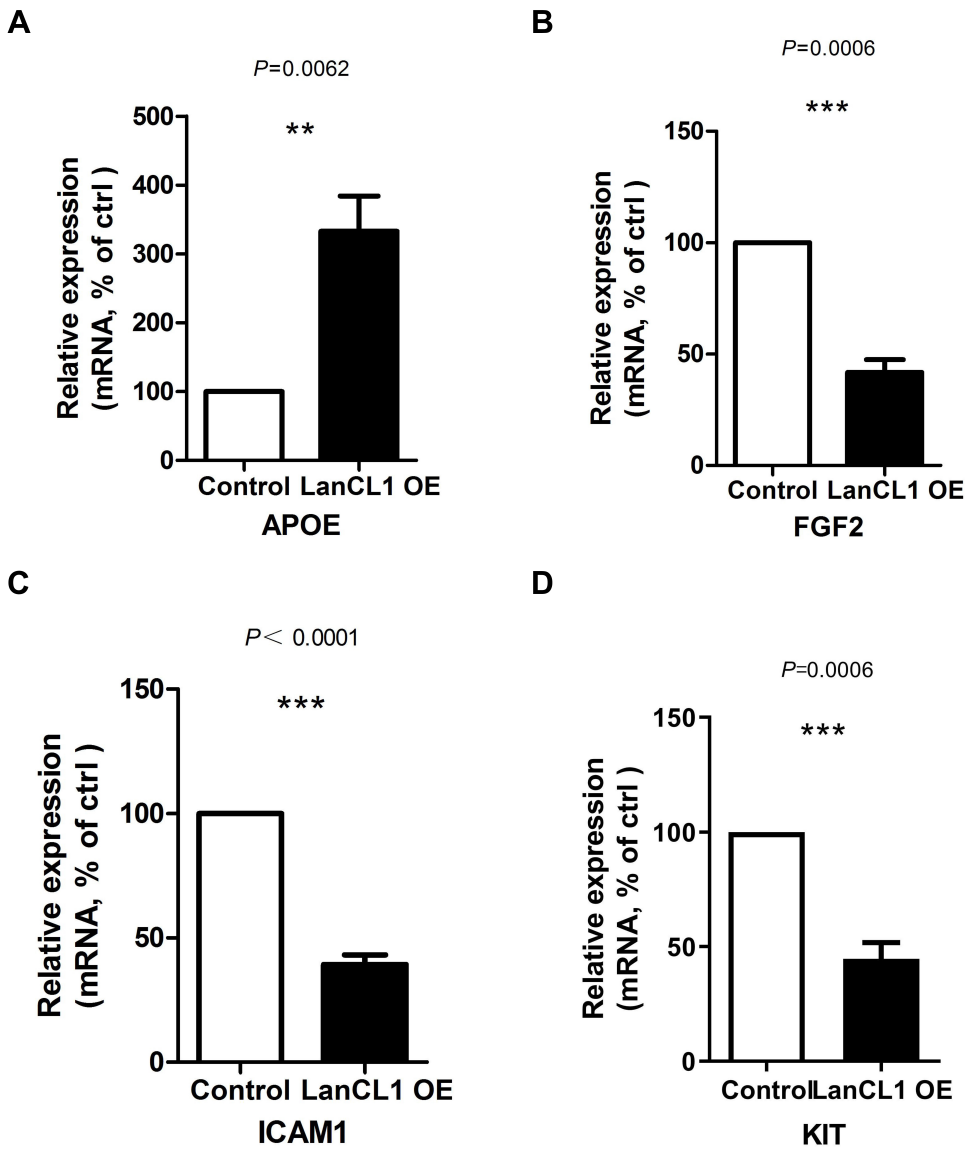


Figure 5 The expression levels of the 4 hub genes in PC-3 *LanCL1* overexpression cells. (**A**–**D**) The mRNA expression levels of Apolipoprotein E (*APOE*, (**A**)), Fibroblast Growth Factor 2 (*FGF2*, (**B**)), Intercellular Adhesion Molecule 1 (*ICAM1*, (**C**)) and KIT Proto-Oncogene (*KIT*, (**D**)) in *LanCL1* overexpressed PC-3 cells. N=5 and a P-value <0.05 is taken to indicate statistical significance. ***P*<0.01, ****P*<0.001.

reported that *CCN3-ICAM1* and MiRNA-296-3p-*ICAM1* axes could influence cell migration in prostate cancer cells.[35,36] However, the role and mechanism of *ICAM1* in prostate cancer are still largely unknown. *ICAM1* may be further studied as key a gene to find more clues about the initiation and progression of prostate cancer disease. As for *APOE*, *FGF2*, and *KIT*, many previous studies have investigated their roles in prostate cancer, and our results further confirmed these findings.

Our study contained one limitation. In our study, we analyzed the data of RNA-seq to identify the DEGs after *LanCL1* overexpression and figured out the critical genes and pathways that might play key roles after *LanCL1* overexpression in prostate cancer using bioinformatics analysis. The mechanism and validation of *LanCL1* overexpression in prostate cancer still need further research in clinical and molecular biology experiments.

Conclusions

In conclusion, our results revealed the critical genes and pathways that might play key roles after *LanCL1* overexpression in prostate cancer. We also provide 4 critical hub genes that could play critical roles in the development of prostate cancer and are closely related to the prognosis of the disease,

which may help in developing prostate cancer treatment strategies.

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Disclosure

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