Identification of Novel Biomarkers and Targets for Natural Products in Inhibiting Prostate Cancer

Wenji Li (✉ wenji_li@yzu.edu.cn)

Yangzhou University <https://orcid.org/0000-0003-0406-9489>

Wei Xu

Yangzhou University

Kai Sun

Yangzhou University

Fujun Wang

Yangzhou University

Tin Wui Wong

Universiti Teknologi MARA

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Abstract

Background: Prostate cancer (PCa) is a common urinary system malignancy. The lack of specific and sensitive biomarkers for the diagnosis and prognosis of PCa makes it important to seek alternatives. Meanwhile, targeted PCa inhibitors are limited. Natural products that potentially target PCa may offer a useful approach.

Methods: Expression profile datasets about PCa from GEO were analyzed. Core differential genes were identified by String and Cytoscape. GEPIA and HPA were utilized to further validate the key genes. The targets of natural products were obtained from the Drugbank, Therapeutic Target Database, BindingDB, PubChem, and ChEMBL databases, and PCa therapeutic targets were generated from the GeneCards, OMIM, and PharmGkb databases. Cytoscape was also used to screen the core modules and disease-drug targets. Construction of molecular docking models of drug-core targets was performed by Autodock to confirm the accuracy of the targets.

Results: Four identified biomarkers, CENPF, TPX2, TK1 and CCNB1 were verified by HPA. Five novel PCa biomarkers, RRM2, UBE2C, TOP2A, BIRC5 and ZWINT were also identified. All the nine markers indicated poor prognosis for PCa patients were verified by GEPIA. PCa carcinogenesis is found to be mainly associated with hepatic fibrosis pathway, ILK signaling, NRF2-mediated oxidative stress response and many others. Four key PCa targets for curcumin (EP300, RELA, EGFR, NFkB1), seven for taxol (PTEN, EGFR, ERBB2, TP53, KRAS, AR, AKT1) and two for ursolic acid (GSK3B, RELA) were identified by Cytoscape combined KEGG and verified by Autodock.

Conclusions: The novel identified biomarkers in our study would be valuable for the diagnosis and prognosis of PCa. Key targets of curcumin, paclitaxel, and ursolic acid in PCa could lay a solid foundation for precise treatment and molecularly targeted therapy for PCa.

1. Background

The incidence of prostate cancer (PCa) is on the rise globally(1). The prevalence of PCa and associated deaths in China has steeply risen in recent years due to rising living standards, changing diets and an aging population(2). Prostate-specific antigen (PSA) is the most common marker used to identify an increased risk of PCa, but PSA level is not a highly accurate indicator and can be influenced by many other factors such as alcohol consumption and inflammation(3). According to the *Chinese expert consensus on genomic testing of PCa patients (2020 edition)* and *Role of Genetic Testing for Inherited PCa Risk:Philadelphia PCa Consensus Conference 2017*, the number of biomarkers that can be used in early diagnosis of PCa is relatively small, and their specificity and sensitivity are low(4, 5). Therefore, it is particularly important to screen novel, highly specific and sensitive biomarkers for the diagnosis and prognosis of PCa.

Common treatment options for PCa include surgical resection, radiotherapy(6), endocrine therapy, and immunotherapy(7), however the number of adverse side effects and frequent progression to refractory

PCa often have a significant impact on the health and quality of life of PCa patients(8). Molecular targeted therapy (MTT) refers to the inhibition of tumor growth and development by interfering with specific molecules that increase proliferation and migration of tumor cells(9). Currently there are only a few molecularly targeted drugs for PCa(10), and since none of them are very specific, it is essential to identify alternatives as well.

Natural products are certain chemicals obtained from naturally growing organisms(11). Curcumin is extracted from turmeric and is an important active ingredient in traditional Chinese medicine. Curcumin has good antioxidant, anti-pathogenic microorganism, anti-rheumatism and anti-tumor activities, and can also improve the function of the immune, cardiovascular, digestive and nervous systems(12). Paclitaxel is a natural secondary metabolite isolated and purified from the bark of the Pacific yew(13). Paclitaxel and its synthetic variants are known to have anti-cancer properties and have been used extensively in the clinic, but their use for treatment of PCa is less frequent and has been described primarily in laboratory studies(14). Ursolic acid is extracted from fruit rind and has sedative and anti-inflammatory, anti-bacterial, anti-diabetic, anti-ulcer effects and antioxidant properties(15). Recent studies have shown that all the three natural products have significant inhibitory effects on cancer, but their mechanisms of action and targets in PCa have not been thoroughly investigated. Therefore, this study aimed to identify targets of these three natural products in PCa in order to provide theoretical support for their use in MTT of PCa.

In our study, genes associated with the diagnosis and prognosis of PCa were screened using bioinformatics software, in the meanwhile key targets of natural products that act on PCa were analyzed as well. A molecular model of natural product docking with PCa targets was constructed based on network pharmacology to confirm the accuracy of the PCa-natural products targets. This study would provide solid support for the development of MTT of PCa.

2. Methods

2.1 Raw data selection

Differences in gene expression between PCa and normal tissues were analyzed using the GEO module on the Assistant for Clinical Bioinformatics (Aclbi) website (www.Aclbi.com), by selecting datasets in the Dataset Screening and Differential Gene Analysis sub-modules. Gene expression data for both GSE3325(16) and GSE46602(17) were based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array). The GSE3325 dataset contains information from 6 benign prostate tissues and 13 PCa tissues; and the GSE46602 dataset contains information from 14 benign prostate tissues and 36 PCa tissues (Additional file : Table. S1). (The GEO data in Aclbi is updated every 3 months and the latest updated version on May 17, 2021 was utilized.)

2.2 Data processing and screening of differentially expressed genes(DEGs)

The selected data were processed using the GEO-Differential Gene Module of the Aclbi. The website is based on R software (v4.0.3) core to run automatic data processing, including normalization of data, de-batching effects and other related processing to ensure validity and accuracy. The data obtained from the Aclbi were further processed using Ingenuity Pathways Analysis (IPA, Qiagen 2021) software to improve the accuracy of the data. The data obtained from the Aclbi for GEO were analyzed using the Core-Analysis module in the IPA software with a cutoff value of $|\log FC| > 1$, $p < 0.05$ to obtain the final DEGs.

2.3 GO enrichment analysis and IPA pathway analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) database (<https://david.ncifcrf.gov/>)(18) was used for analysis of DEGs by Gene Ontology (GO)(19) enrichment methods. GO enrichment analysis focuses on describing the biological processes (BPs), cellular components (CCs) and molecular functions (MFs) associated with DEGs. The pathway analysis of DEGs was performed by IPA. A cutoff value of $p < 0.05$ was used. The hiplot database (<https://hiplot.com.cn/>), a comprehensive web platform for scientific data visualization, was used to visualize the enrichment results.

2.4 Protein-Protein Interactions (PPI) network construction, core genes and modules screening

Interrelationships between the DEGs were constructed using the String(20) database (<https://www.string-db.org/>) (version 11.5), with interrelationship scores calculated from text mining, experiments, databases, co-expression neighborhood, gene fusion, and co-occurrence, while hiding unrelated nodes in the network with a confidence score ≥ 0.4 . The Cytohubba(21) plugin of Cytoscape(22) software(version 3.8.2), which is based on the maximal clique centrality algorithm, was used to identify the connected genes most closely associated with the development of PCa. The top 30 scored genes were selected. In addition, the molecular complex detection (MCODE)(23) app in Cytoscape was used to check modules of the PPI network (degree cutoff = 2, max. Depth = 100, k-core = 2, and node score cutoff = 0.2). The enrichment analysis of core modules was performed by the DAVID and Hiplot.

2.5 Validation of the expression of core genes and survival analysis

The Gene Expression Profiling Interactive Analysis (24) (GEPIA; <http://gepia.cancer-pku.cn/>) database, launched in 2017 and now cited in a large number of articles, is an efficient, accurate and convenient database for gene expression analysis based on tumor and normal samples from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases. GEPIA was used to further validate expression of core genes screened by Cytoscape, by setting $|\log FC| > 1$ and $p < 0.01$ as the cutoff value, with matched tumor–normal tissue data from the TCGA and GTEx databases respectively. In addition, the survival analysis of core genes was performed by GEPIA database as well. The protein expression of key genes in PCa and normal tissues was analyzed by the human protein atlas(HPA,

<https://www.proteinatlas.org/>) (Version: 20.1)(25). The pan-cancer analysis for core genes was performed in Timer2(<http://timer.cistrome.org/>) database(26).

2.6 PCa treatment targets

Validated PCa targets were identified in the GeneCards(27) (<https://www.genecards.org/>), Online Mendelian Inheritance in Man(28) (OMIM,<https://omim.org/>) and PharmGkb(29) (<https://www.pharmgkb.org/>) databases. Targets with relevance scores > 1 in the GeneCards database were considered to be significant. The targets retrieved from the three databases were combined with the core differential genes while duplicate values were removed, resulting in the set of therapeutic targets for PCa.

2.7 Natural product targets

The DrugBank(30) (<https://go.drugbank.com/>), Therapeutic Target Database (TTD)(31) (<http://db.idrblab.net/ttd/>), BindingDB(32) (<http://www.bindingdb.org/bind/index.jsp>), PubChem(33) (<https://pubchem.ncbi.nlm.nih.gov/>), and ChEMBL(34) (<https://www.ebi.ac.uk/chembl/>) databases were used to identify the targets of curcumin/paclitaxel/ursolic acid. The targets identified in the five databases were combined while duplicate values were removed.

2.8 Disease-natural product targets

Intersections of the PCa and curcumin/paclitaxel/ursolic acid targets were identified in Excel following Duilio's method(35), and the resulting targets were designated pharmacodynamic targets of curcumin/paclitaxel/ursolic acid in PCa.

2.9 Construction of PPI networks of PCa-natural product targets and screening of core modules

Information about PCa-natural products was imported into the String database to construct a network of interactions between targets, and interactions with a confidence score of 0.4 or more were retained. The inter-target interaction scores were also imported into Cytoscape software for core module screening by using the CytoNCA(36) plugin, and the median of the Betweenness Centrality (BC), Closeness Centrality (CLC), Degree Centrality (DC), Eigenvector Centrality (EC), Local Average Connectivity-based method (LAC) and Network Centrality (NC) scores was used as the basis for screening of genes to obtain their core motifs.

2.10 GO and KEGG enrichment analysis of disease-natural product targets

DAVID database was used to provide data for the GO and KEGG analysis of disease-natural product targets, when the p-value was set as 0.05 or less. The Hplot online database was used for enrichment analysis of disease-natural product targets for mapping with a p-value < 0.05 and q-value < 0.05.

2.11 Construction of molecular docking models

Protein receptors were identified based on the KEGG enrichment results of the disease-natural product targets. The small-molecule ligands were curcumin/paclitaxel/ursolic acid. 2D structures of curcumin/paclitaxel/ursolic acid were downloaded from the PubChem database and converted into 3D structures by ChemOffice software(version 14.0.0.117), and the structure was adjusted to minimize the binding energy, where the minimum RMS gradient = 0.01. Protein receptor 3D structures were obtained from the Protein Data Bank(37) (PDB, <https://www1.rcsb.org/>). In PyMoL (version 2.4.0) software(38), the water molecules and the original small-molecule ligand were removed from the 3D structure. The 3D protein structure was hydrogenated using AutoDockTools software(version 1.5.6)(39), and a grid box was added to the protein structure with different reference values depending on the protein receptors. Molecular docking was performed in AutodockVina by setting the energy range to 5. Targets with binding energy lower than - 5 kcal/mol are retained, and the lower the binding energy, the affinity between small molecules and targets. The resulting models were displayed using PyMoL software.

3. Results

In IPA, $|\log FC| > 1$ and a p-value < 0.05 were set as the cutoff value to obtain DEGs from the GEO module. A total of 582 DEGs were obtained after analysis of the GEO dataset, of which 201 were up-regulated and 381 were down-regulated(Fig. 1,Table 1).

Table 1
GEO differentially expressed genes

Category	Genes
Up-regulated DEGs names	HOXC6, OR51E2, CRISP3, AMACR, RRM2, LUZP2, ZIC2, DLX1, GLYATL1, HPN, CGREF1, HOXC4, COL2A1, DNAH5, GDF15, FAM222A, TMEM178A, MS4A8, F5, ERG, ACSM1, INSM1, TFF3, TRPM4, SMIM22, TWIST1, GJB1, MIPEP, CTHRC1, C4B, FOXD1, TDRD1, TOP2A, GCNT1, THBS4, STX19, CYP2J2, MB, AGR2, BICD1, SIM2, RGS17, NETO2, KCNG3, ASPN, PCDHGA4, GREM1, CXCL14, KCNH8, NUSAP1, TMTC4, EZH2, RPL22L1, SFRP4, NEK5, AGR3, INHBA, TPX2, FZD8, B3GAT1, ITGBL1, SLC43A1, TMEM45B, STIL, TOX3, MELK, CACNA1D, RAB17, MARCKSL1, SRM, REPS2, ELL3, ARHGEF26, PPM1E, PLA1A, DUS1L, MYO6, CAMKK2, PCDHB2, PTTG1, SPON2, COMP, SLC27A2, ZNF577, APOF, ENC1, UHRF1, RET, NCAPG, UAP1, TMSB15A, ZNF30, CHRM3, BUB1B, PKIB, TTLL12, UCK2, HLCS, TUBB2A, RAP1GAP2, SDK1, FOXM1, GPR160, FBP1, MTHFD2, PTP4A3, ERVH48-1, ST6GALNAC1, ZDHHC23, KIF20A, PPP1R9A, GMDS, COL12A1, SPIN4, COL28A1, SBK1, SLC12A8, NKAIN1, GJB2, CLDN7, MICAL2, MRPL17, POPDC3, CLDN8, FABP5, CCNB1, ATP8A2, EIF4EBP1, MMP26, ECT2, TGM3, BIRC5, ALDH3B2, CHMP4C, MNX1, RAB39B, ABRACL, RELN, ZWINT, CKS2, EPCAM, MEOX1, UGT2B4, SCIN, NPIP15, RCC1, TMC5, PABPC1L2A, KLK12, PDCD2L, BEND4, HLA-DMB, RNF44, VSTM2L, AURKA, TK1, SOX4, PIGW, KNSTRN, COL9A2, CENPF, ENTPD5, ZNF511, SLC44A5, TNNT1, OCLN, CNTD1, ELAVL2, HMMR, PCDHB10, PRR15L, PCTP, CDCA5, MAL2, LMNB1, CLDN3, F2RL1, MND1, ZDHHC11, CDKN3, PLEKHB1, CHTF18, BAMBI, LRRC8D, KIF4A, YEATS4, ESRP2, DVL1, NAA60, SLC38A11, SMOC2, CRACR2B, CPLX1, MMP10, SAMD5, NME1, ARHGAP32, FASN, UBE2C, RACGAP1, CCNA2

Category	Genes
Down-regulated DEGs names	SEMA3E, KIF1A, PHYHIPL, TRIM22, SPON1, CFL2, DEFB132, PGM5, INPP1, JAZF1, NR3C1, CASQ2, ALDH2, DDX19B, BHLHE40, GBP1, HCAR3, PLA2G4A, FLNC, TSPYL2, RBP7, CAPNS2, HOXA13, CWH43, GBP2, INMT, LURAP1, CPA3, LPCAT2, KRT19, CAVIN1, SYNPO2, TMEM252, CCDC8, AMOTL1, FERMT2, LXN, ZNF394, CD69, MAML2, MXRA7, EHD2, DNAJB4, AMIGO2, PLPP3, MT1F, MT1E, ELF4, SOWAHA, UPK3A, PIK3R1, LIX1, PER2, HOXD11, GNAL, SH3BGRL2, CCN1, INSIG1, UBA7, SHROOM4, P2RY1, RARRES2, LMOD1, ANKRD35, SFMBT2, SCGB3A1, NR4A2, SCRNI, GPM6B, PRRG4, ASPA, GSTM5, SGCG, TGFB3, PRDM8, SERPINB1, SYNGR1, PDE4B, ANXA1, TRIP6, FXVD6, SOX7, RECQL4, FOXF1, SULT2B1, EDNRB, CACNA2D1, TUBB6, ETV5, KRT13, BASP1, ANGPT1, PTN, PARP6, TSHZ3, TMEM237, CLIC6, ZNF662, ZNF334, ACADL, AMT, TGFB3, SYNM, DUOXA1, SLC16A5, LYPD6B, CTSB, BMP4, ST6GALNAC2, PDGFC, CPVL, MYOCD, SMOC1, PPP1R14A, LRCH2, S100A16, NEU1, THSD4, KANK4, CYTL1, CACNB4, TMEM79, MT1H, KCNJ8, PDLIM1, ATP1A2, ANO5, GMPR, KRT222, KCNJ3, AKR1B1, ANKH, APOBEC3C, ATF3, ABCG2, MT1X, SNX7, RFPL2, COL9A1, JAKMIP1, BMP5, WFDC1, EVA1C, EDN3, ACTG2, CSRP2, DPP4, GPRASP1, BEX5, PENK, KCNJ2, ZHX2, PROM1, P3H2, PGR, ZNF185, CAV2, ANXA2, BNIPL, HLF, SFN, LPAR3, EMCN, GSTO2, LPAR1, TMEM121B, TIMP4, FADS1, TENT5B, ENPP2, DST, PRIMA1, DEFB1, MYH11, PLP2, MYZAP, NRG4, RAB34, PNMA8A, CXCL13, EFEMP1, HSPB1, ID4, CPXM1, TCF7L1, ZBTB20, PTGS1, OGN, LMAN1L, KCNK3, TMEM132C, FAM83B, ZNF532, NR4A3, OAT, PKP1, OLFML3, MALL, BST2, SEMG1, IGF1, CYP27A1, CNN1, SOD3, SPATA6, CXCL12, TRAF3IP2, LGALS3BP, ECRG4, DMKN, ETS2, EML5, ACTC1, NXPE4, GPR87, CHRDL1, S100A4, IRS1, AJUBA, CPA6, NSG1, CITED1, NINL, ACSF2, ZNF827, ATP8B4, ZNF711, HSPB6, VSIG2, EFS, CLIP4, RGN, CDC42EP5, ALDH1A2, TENM1, UPK1A, CDH3, ADD3, GPX3, ID1, AZGP1, GJA1, SPRY2, CAV1, CARTPT, GMNN, DNER, SLC2A5, GAS1, CPAMD8, MSMO1, NDRG2, IER3, FRMD6, TENM2, SCGB2A1, RND3, SERPINF1, PROK1, PCP4L1, GSTP1, SALL3, GBP3, CRISPLD2, SCN7A, COL4A6, RNASE4, APCDD1, CHRM1, HOXD10, TFCP2L1, PARM1, TNS4, AFAP1L2, MPZL2, ACSS3, PDE8B, EFHD1, PDK4, SELE, CYP4X1, SDC1, NTF4, TGM4, PDE11A, ANTXR2, PEBP4, AVPI1, OLFM4, ANG, DPYS, PAK1IP1, MYOF, SRD5A2, SPOCK3, CYP4B1, NBL1, FAM107A, CCL2, NPY, ADAMTS1, GPD1L, COL17A1, NELL2, ITGB6, PAGE4, GATA3, VWA5A, NTN4, POTEM, DSC3, CRYAB, CFD, SLC39A2, KLK11, NDNF, PPARGC1A, TP63, ADIRF, BCL11A, ARMCX1, FHDC1, CPM, MCC, FOSB, RCAN3, GSTM1, MEIS2, PTGDS, SUPT3H, ZNF655, LGR6, MPPED2, DPT, FHL2, ALOX15B, TCEAL2, GSTM2, FBXO17, SNAI2, PI15, PTGS2, MSMB, RLN1, SCUBE2, LSAMP, NIPAL3, CES1, CCK, CAPG, GCNT2, FOXQ1, MUC3A, BEX1, CPLX3, FLRT3, VEGFA, CD38, VSNL1, CYP3A5, AOX1, TRIM29, S100A2, SLC18A2, GABRE, AOC1, SERPINB11, WIF1, SCGB1A1, KIAA1210, KRT14, CSTA, NTNG2, KRT23, MME, LAMB3, KRT15, KRT5, SLC14A1, NEFH, CD177

3.1 GO and IPA pathway analysis

After analysis of the information in the DAVID database, GO analysis revealed that biological processes BPs were enriched in muscle system process, cell junction assembly, epithelial cell proliferation, muscle contraction, extracellular matrix organization, regulation of epithelial cell proliferation, extracellular structure organization, regulation of actin filament-based process, muscle cell differentiation, and others. (Fig. 2a). MFs were enriched in receptor ligand activity, signaling receptor activator activity, sulfur compound binding, actin binding, enzyme inhibitor activity, glycosaminoglycan binding, heparin binding, tubulin binding, extracellular matrix structural constituent and others.(Fig. 2b). CCs were enriched in collagen-containing extracellular matrix, cell-cell junction, cell-substrate junction, focal adhesion, membrane raft, membrane microdomain, membrane region, contractile fiber, myofibril, microtubule, sarcomere, cell leading edge, apical part of cell (Fig. 2c). IPA pathway analysis indicated the major pathways associated with carcinogenesis of PCa includes: hepatic fibrosis/hepatic stellate cell

activation, ILK signaling, xenobiotic metabolism PXR signaling pathway, xenobiotic metabolism AHR signaling pathway, LPS/IL-1 mediated inhibition of RXR function, xenobiotic metabolism CAR signaling pathway, SPINK1 pancreatic cancer pathway, aryl hydrocarbon receptor signaling, NRF2-mediated oxidative stress response, MSP-RON signaling in cancer cells pathway, osteoarthritis pathway, agranulocyte adhesion and diapedesis, nicotine degradation II, human embryonic stem cell pluripotency, amyotrophic lateral sclerosis signaling, MSP-RON signaling pathway, WNT/ β -catenin signaling, endocannabinoid cancer inhibition pathway and others.(Fig. 2d).

3.2 PPI network construction, core genes and modules screening

As indicated in Fig. 3a, by using the String database, we constructed a protein relationship network graph for DEGs, in which there were 582 nodes and 1869 edges after removing the unlinked nodes. Thirty genes with the highest scores, namely NCAPG, UBE2C, AURKA, BUB1B, TPX2, CENPF, MELK, RACGAP1, NUSAP1, HMMR, TOP2A, CCNA2, CCNB1, ZWINT, RRM2, CDKN3, BIRC5, CDCA5, KIF20A, KIF4A, FOXM1, PTTG1, ECT2, CKS2, EZH2, TK1, UHRF1, MND1, GMNN, and STIL, were obtained using the Cytohubba plugin in Cytoscape software (Fig. 3b). Two modules with the highest scores were screened. Module 1(Fig. 3c, 3e), with 31 nodes and 434 edges, scored 28.933, in the GO analysis revealed that BPs were enriched in nuclear division, organelle fission, mitotic nuclear division, chromosome segregation, nuclear chromosome segregation, sister chromatid segregation, mitotic sister chromatid segregation, etc, MFs were enriched in microtubule binding, tubulin binding, protein kinase regulator activity, kinase regulator activity, etc, and CCs were enriched in spindle, chromosomal region, chromosome-centromeric region, condensed chromosome, condensed chromosome-centromeric region, midbody, microtubule, etc. The KEGG pathway analysis was mainly concentrated in cell cycle, progesterone-mediated oocyte maturation, oocyte meiosis, cellular senescence, human T-cell leukemia virus 1 infection, etc. Module 2(Fig. 3d, 3f), with 15 nodes and 33 edges, scored 4.714, in the GO analysis revealed that BPs were enriched in muscle system process, cell junction assembly, epithelial cell proliferation, muscle contraction, extracellular matrix organization, regulation of epithelial cell proliferation, extracellular structure organization, regulation of actin filament-based process, muscle cell differentiation, etc, MFs were enriched in efflux transmembrane transporter activity, sulfur compound transmembrane transporter activity, E-box binding, amide transmembrane transporter activity, peroxidase activity, etc, and CCs were enriched in membrane raft, membrane microdomain, membrane region, apical plasma membrane, apical part of cell, etc. KEGG analysis indicated no gene enrichment in possible pathways.

3.3 Validation of core prognosis genes by GEPIA

The expression levels of the DEGs were further validated in the GEPIA database using a cutoff value of $|\log FC| > 1$ and $p < 0.01$. Then 9 key candidate genes were screened out, which may be closely related to

the occurrence and development of PCa, namely UBE2C, TPX2, CENPF, TOP2A, CCNB1, ZWINT, RRM2, BIRC5, and TK1 (Fig. 4a). According to GEPIA's disease free survival analysis, all nine genes were closely related to the poor prognosis of PCa (Fig. 4b). Survival analysis showed that the high-risk values of all validated genes were greater than 1, indicating that they were all high-risk genes for PCa with poor prognosis outcomes.

3.4 Validation of key diagnosis genes by HPA

According to the results of GEPIA expression analysis, these 9 key genes were highly expressed in PCa and low in normal cancer tissues. HPA database, which contains plenty of clinical immunohistochemical (IHC) sample data, can be used as a tool to validate the key diagnosis genes. From HPA analysis, CENPF, TPX2, TK1 and CCNB1 were found to be the most extensively studied diagnosis genes from the 9 candidates (Fig. 5a), which were only expressed in prostate tumor tissues but not in normal prostate tissues. RRM2 was not expressed in both normal and tumor tissues. UBE2C, TOP2A and BIRC5 were expressed in both normal tissues and tumor tissues with no significant difference. ZWINT has no data in this database. After validation by HPA, CENPF, TPX2, TK1 and CCNB1 were found to be more certain in the carcinogenesis of PCa and can be used as diagnosis biomarkers. The other 5 genes, RRM2, UBE2C, TOP2A, BIRC5 and ZWINT would be potential diagnosis markers for PCa and need further studies to validate. The pan-cancer analysis in TisID(40) database indicated the expression of all the 9 genes were significantly upregulated in most cancers including PCa, which suggest their potency of being the diagnosis markers of PCa (Fig. 5b).

3.5 Disease-Drug targets initial identification

The GeneCards, OMIM and PharmGkb databases were used to search for PCa targets. 10,055 targets were found in the GeneCards database, 403 in the OMIM database and 411 in the PharmGkb database. After combining the analysis with the DEGs and removing duplicate targets, a total of 10,366 targets were identified for PCa (Fig. 6, Additional file: Table. S2).

3.5.1 PCa-curcumin initial targets

A total of 141 curcumin targets were found in the DrugBank, TTD, BindingDB, PubChem and ChEMBL databases after removing duplicate targets (Additional file: Table. S3), and a total of 114 intersections of the initial PCa and curcumin targets were obtained. (Table 2)

3.5.2 PCa-paclitaxel initial targets

After searching the above 5 databases, 121 targets of paclitaxel were obtained after removing duplicate targets (Additional file: Table. S3), and 105 targets were obtained after taking the intersections (Table 2) with PCa targets, which constituted the initial PCa-paclitaxel targets.

3.5.3 PCa-ursolic acid initial targets

In the same five databases, a total of 59 targets (Additional file: Table. S3) were obtained after removal of duplicate targets, and 41 initial PCa-ursolic acid targets (Table 2) were obtained from the intersections with PCa targets.

3.6 Construction of PPI network of secondary disease-drug targets and core motif screening

3.6.1 PCa-curcumin secondary targets

A PPI network graph of PCa-curcumin targets was constructed from the String database. 109 nodes and 623 edges were obtained in the network graph after removing unconnected nodes (Fig. 7a). The PPI network was analyzed in Cytoscape software by using the CytoNCA plugin, and again using R software. The targets were screened according to the median of the BC, CLC, DC, EC, NC and LAC scores, and targets with scores larger than the median were retained. The core motifs of these targets were calculated. The first screening, calculated by CytoNCA and R software, was based on BC: 55.12646418; CLC: 0.397058824; DC: 10; EC: 0.03516328; LAC: 3.75; and NC: 5.047619048. Then we obtained 26 nodes with 180 edge secondary core motifs (Fig. 7b), and the same calculations and filtering were performed for this motif. The second filtering values were based on BC: 10.48737374; CLC: 0.684210526; DC: 14; EC: 0.175377861; LAC: 8.545454545; and NC: 10.77973138; resulting in a key motif containing 9 nodes and 36 edges (Fig. 7c, 7d). Finally, the secondary core PCa-curcumin targets were identified as STAT3, PTGS2, RELA, PPARG, EGFR, IL1B, NFKB1, SRC and EP300.

3.6.2 PCa-paclitaxel secondary targets

A PPI network graph of PCa-paclitaxel targets was constructed from the String database. 100 nodes and 951 edges were obtained after removing unconnected nodes (Fig. 8a). The PPI network was analyzed in Cytoscape software by using the CytoNCA plugin, while the data analyzed by Cytoscape was analyzed again with the R software. The targets were screened according to the median of the BC, CLC, DC, EC, NC and LAC scores, and all targets with scores larger than the median were retained. The core motifs of these were obtained by calculation. The first screening, calculated by CytoNCA and R software, was based on BC: 40.031635475; CLC: 0.4817546765; DC: 16; EC: 0.0590513925; LAC: 9.4142857145; and NC: 11.252039625. Then we obtained a secondary core motif with 28 nodes and 267 edges (Fig. 8b), and the same calculation and filtering were performed again for this motif. The second filtering values were based on BC: 4.953907041; CLC: 0.782773109; DC: 19.5; EC: 0.1917769165; LAC: 15.67777778; and NC: 17.37566557; resulting in a key motif containing 13 nodes and 78 edges (Fig. 8c, 8d). Finally, the secondary core PCa-paclitaxel targets were identified as SRC, ATM, PTEN, CASP3, CDKN2A, NOTCH1, EGFR, ERBB2, TP53, KRAS, AR, AKT1 and BRCA1.

3.6.3 PCa-ursolic acid secondary targets

A PPI network graph of PCa-ursolic acid targets was constructed from the String database. The PPI graph with 38 nodes and 78 edges was obtained after removing unconnected nodes (Fig. 9a) The PPI were analyzed in Cytoscape software using the CytoNCA plugin, and the data analyzed by Cytoscape was

analyzed again with R software. The targets were screened according to the median of the BC, CLC, DC, EC, NC and LAC scores, and all genes with scores larger than the median were retained. The core motifs were obtained by calculation. The targets were screened based on BC: 17.015873015; CLC: 0.359223301; DC: 3; EC: 0.054310229; LAC: 1; NC: 1.5833333335 using CytoNCA and R software. The core motifs of 7 nodes and 11 edges were obtained afterward (Fig. 9b, 9c), and the secondary core PCa-ursolic acid targets were identified as PTGS2, PLA2G1B, PPARA, RELA, HIF1A, GSK3B and HDAC1.

3.7 Functional enrichment results for disease-drug targets

GO and KEGG enrichment for disease-drug targets was performed using the DAVID database and analyzed and mapped on the Hiplot website to explore the most relevant pathways to the PCa carcinogenesis and to find the PCa pathway highly associated targets.

3.7.1 Functional analysis of PCa-curcumin targets

As shown in Fig. 10a, GO analysis revealed that BPs were enriched in positive and negative regulation of transcription from the RNA polymerase II promoter, negative regulation of apoptosis, responses to drugs, negative regulation of transcription, DNA-templates, signal transduction, and oxidation-reduction processes. MFs were enriched in protein binding, zinc ion binding, enzyme binding, ATP binding, and transcription factor binding. CCs were enriched in cytoplasm, nucleus, cytosol, plasma membrane, extracellular exosomes, and nucleoplasm. In terms of KEGG pathway enrichment, the targets were focused on viral carcinogenesis, pathways in cancer, gap junctions, alcoholism, pathogenic *Escherichia coli* infection, and phagosomes. Based on KEGG pathway enrichment results, 7 targets were enriched in the PCa pathway, namely GSK3B, CREBBP, MDM2, EP300, RELA, EGFR, NFKB1.

3.7.2 Functional analysis of PCa-paclitaxel targets

In Fig. 10b, GO analysis revealed that BPs were enriched in positive regulation of transcription from the RNA polymerase II promoter, cell proliferation, positive and negative regulation of apoptosis, positive and negative regulation of transcription, DNA-templates, apoptosis, positive and negative regulation of cell proliferation, protein phosphorylation, response to drugs, microtubule-based processes, peptidyl-tyrosine phosphorylation, protein autophosphorylation, MAPK cascade, and intracellular signal transduction. MFs were enriched in protein binding, ATP binding, enzyme binding, protein kinase activity, protein homodimerization and heterodimerization, identical protein binding, structural constituents of the cytoskeleton, GTPase activity, GTP binding, protein kinase binding, protein tyrosine kinase activity, kinase activity and CCs. In terms of KEGG pathway enrichment, the targets were focused on pathways in cancer, microRNAs in cancer, PI3K-Akt signaling pathway, hepatitis B, gap junction, proteoglycans in cancer, prostate cancer, HTLV-I infection, melanoma, phagosomes, MAPK signaling pathway, pathogenic *Escherichia coli* infection, focal adhesions, central carbon metabolism in cancer, glioma, pancreatic cancer, ErbB signaling pathway, FOXO signaling pathway, bladder cancer, colorectal cancer, chronic myeloid leukemia, chemical carcinogenesis, viral carcinogenesis, Rap1 signaling pathway, non-small cell lung cancer, metabolism of xenobiotics by cytochrome P450, estrogen signaling pathway, thyroid

hormone signaling pathway, neurotrophin signaling pathway, regulation of the actin cytoskeleton, and the Ras signaling pathway. Based on KEGG pathway enrichment results, 16 targets were enriched in the PCa pathway, namely PDGFRA, CDKN1A, PTEN, EGFR, PIK3CG, AR, PIK3CA, ERBB2, CDK2, E2F1, BCL2, AKT1, KRAS, RAF1, TP53, FGFR1.

3.7.3 Functional analysis of PCa-ursolic acid targets

As shown in Fig. 10c, GO analysis revealed that BPs were enriched in histone H3 deacetylation, oxidation-reduction processes, protein deacetylation, peptidyl-tyrosine dephosphorylation, negative regulation of myotube differentiation, regulation of transcription from the RNA polymerase II promoter in response to hypoxia, negative regulation of the insulin receptor signaling pathway, response to hypoxia, peptidyl-proline hydroxylation to 4-hydroxy-L-proline, positive regulation of receptor biosynthetic process, histone deacetylation, and inflammatory responses. MFs were enriched in NAD-dependent histone deacetylase activity (H3-K14 specific), histone deacetylase binding, protein deacetylase activity, histone deacetylase activity, protein tyrosine phosphatase activity, transcription factor binding, enzyme binding, peptidyl-proline 4-dioxygenase activity, NF-kappaB binding, repression of transcription factor binding, and protein kinase binding. CCs were enriched in the histone deacetylase complex, nucleus, cytosol, nucleoplasm, protein complexes, cytoplasm, transcriptional repressor complexes, Golgi apparatus, and extracellular exosomes. In terms of KEGG pathway enrichment, the targets were focused on viral carcinogenesis, insulin resistance, HIF-1 signaling pathway, pathways in cancer, arachidonic acid metabolism, renal cell carcinoma, insulin signaling pathway, galactose metabolism, starch and sucrose metabolism, alcoholism, thyroid hormone signaling pathway, regulation of lipolysis in adipocytes, and steroid hormone biosynthesis. Based on the enrichment results of the KEGG pathway, 3 targets were enriched in the PCa pathway: GSK3B, RD5A2 and RELA.

3.8 Construction of molecular docking models

The molecular docking was utilized to determine the tightness of binding between the target proteins and the natural products, which will be used to confirm the final PCa-natural products' targets.

3.8.1 Curcumin-receptor protein molecular docking models

Four final PCa-curcumin targets were identified from intersections with the secondary core targets and 7 targets from KEGG pathway enrichment results, namely EP300, RELA, EGFR, and NFKB1. The four targets were used as protein receptors in the molecular docking models with curcumin as the small-molecule ligand to confirm the final PCa-curcumin targets. When EP300 was used as the protein receptor, the spacing of the grid box in the AutodockTools was set to 1, the number of points in the x-, y-, and z-dimensions was set to 40, and the offset values of the Center Grid Box were: x center: -40.097; y center: 97.454; and z center: 194.199. Molecular docking was performed using Vina, which resulted in a model located at the center of the active site with the lowest binding energy, where the binding energy was - 6.9 kcal/mol. PyMol was used to obtain the model (Fig. 11a). The construction of molecular docking models for the other three genes was completed by using the same method. For RELA, the spacing of the grid box

in the AutodockTools was set to 1, the number of points in the x-, y-, and z-dimensions was set to 40, and the offset values of the Center Grid Box were: x center: 62.502; y center: 11.629; and z center: 37.997. The binding energy of the model was – 6.9 kcal/mol. For EGFR, the spacing of the grid box in AutodockTools was set to 1, the number of points in the x-, y-, and z-dimensions was set to 40, and the offset values of the Center Grid Box were: x center: –0.112; y center: –0.618; and z center: 0.111. The binding energy of the model was – 5.3 kcal/mol. For NFKB1, the spacing of the grid box in AutodockTools was set to 1, the number of points in x-, y-, and z-dimensions was set to 40, and the offset values of the Center Grid Box were: x center: –0.743; y center: –3.335; and z center: –0.161. The binding energy of the model was – 5.8 kcal/mol (Fig. 11b, 11c, 11d). The binding energies of the four targets to curcumin were lower than – 5 kcal/mol, indicating that the binding was stable and the affinity was strong, so all the four targets were confirmed as the final PCa-curcumin targets .

3.8.2 Paclitaxel-receptor protein molecular docking models

Seven final PCa- Paclitaxel targets were found from the intersections with the secondary core targets and 16 targets from pathway enrichment results, namely PTEN, EGFR, ERBB2, TP53, KRAS, AR, and AKT1. These seven targets were used as protein receptors in the molecular docking models with paclitaxel as the small-molecule ligand. When using PTEN as the protein receptor, the spacing of the grid box in AutodockTools was set to 1, the number of points in the x-, y-, and z-dimensions was set to 40, and the offset values of the Center Grid Box were: x center: 36.248; y center: 82.41; and z center: 31.742. The model with the lowest binding energy was obtained using Vina for molecular docking, where the binding energy was – 7.4 kcal/mol. PyMoL was used to obtain the model (Fig. 12a). Construction of molecular docking models for the other six genes was completed using the same method. We set all the values of spacing, number of points in the x-, y-, and z-dimensions to the same value as PTEN. For EGFR, the offset values of the Center Grid Box were: x center: –0.112; y center: –0.618; and z center: 0.111; the binding energy was – 5.5 kcal/mol. For ERBB2, the offset values of the Center Grid Box were: x center: 0.017; y center: 0.054; and z center: 0.005; the binding energy was – 6.8 kcal/mol. For TP53, the offset values of the Center Grid Box were: x center: –21.948; y center: 0.61; and z center: 22.886; the binding energy was – 9.2 kcal/mol. For KRAS: the offset values of the Center Grid Box were: x center: 52.69; y center: 108.574; and z center: 30.638; the binding energy was – 9.0 kcal/mol. For AR: the offset values of the Center Grid Box were: x center: 21.912; y center: 5.289; and z center: 10.612; the binding energy was – 6.6 kcal/mol. For AKT1: the offset values of the Center Grid Box were: x center: 13.902; y center: –0.811; and z center: 3.298; the binding energy was – 6.9 kcal/mol (Fig. 12b – g). The binding energies of the seven targets to paclitaxel were lower than – 5 kcal/mol, indicating that the binding was stable and the affinity was strong, so all the seven targets were confirmed as the final PCa-Paclitaxel targets.

3.8.3 Ursolic acid-receptor protein molecular docking models

Two final PCa-ursolic targets were found from the intersections with the secondary core targets and 3 targets from KEGG pathway enrichment results, namely GSK3B and RELA, which were also used as the protein receptors in the molecular docking models when ursolic acid was used as the small-molecule

ligand. When GSK3B was used as the protein receptor, the spacing of the grid box in AutodockTools was set to 1, the number of points in the x-, y- and z-dimensions was set to 40, and the offset values of the Center Grid Box were: x center: 0.296; y center: 3.935; and z center: -4.831. The model with the lowest binding energy was obtained using Vina for molecular docking, where the binding energy was -7.4 kcal/mol. When RELA was used as the protein receptor, the spacing of the grid box in AutodockTools was set to 1, the number of points in the x-, y- and z-dimensions was set to 40, and the offset values of the Center Grid Box were: x center: 62.502; y center: 11.629; and z center: 37.997. The model with the lowest binding energy was obtained after analysis using Vina for molecular docking, where the binding energy was -8.1 kcal/mol. PyMOL was used to obtain the model (Fig. 13a, 13b). The binding energies of the two targets to ursolic acid were lower than -5 kcal/mol, indicating that the binding was stable and the affinity was strong, so both targets were confirmed as the final PCa-Ursolic acid targets.

4. Discussion

4.1 PCa biomarkers analysis

According to HPA results, the 9 PCa associated genes can be classified into 2 groups, the well-studied biomarkers and the relatively less-studied biomarkers. CENPF, TPX2, TK1 and CCNB1 were only expressed in prostate tumor tissues but not in normal prostate tissues by the clinical IHC results (Fig. 5a), while there is no significant difference for the expression of RRM2, UBE2C, TOP2A, BIRC5 and ZWINT in PCa and normal prostate tissues. However, the lack of significant difference between PCa and normal samples may probably due to the relatively limited samples stored in HPA. In addition, from the pan-cancer analysis, it demonstrates that the expression of all the 9 genes are significantly upregulated in most cancers including PCa, which suggest the value of studying the 9 genes as potential diagnosis markers of PCa (Fig. 5b).

4.1.1 The well-studied PCa biomarkers

The identified well-studied PCa diagnosis markers from our bioinformatics study indicate the rationality of the study design.

Mitotic-specific cyclin-B1 (CCNB1) played an important stabilization role of mitotic process in the G2 to M phase of the cell cycle(41). Through IHC experiments, CCNB1 was found to be overexpressed in PCa specimens, and acted as a prognostic marker of PCa chemotherapy(42). It is found that prostate cancer cells with overexpressed CCNB1 were more sensitive to chemically induced apoptosis(43).

Centromeric protein F (CENPF) is the basic element of the kinetochore complex and plays an important role in the chromosome separation mechanism of mitosis(44). CENPF was confirmed to be a prognostic marker in PCa and its expression was found up-regulated in more severe PCa patients with higher Gleason score, later pathological stage, and lymph node metastasis(45).

Thymidine kinase-1 (TK-1) has been extensively studied as a diagnostic biomarker for various tumors including PCa(46). It was found that the serum level of TK1 was significantly higher in PCa patients than in normal subjects, and the expression levels were significantly different between PCa patients in different stages(47). Increased TK1 in cancer patients after surgery or chemotherapy treatment are associated with a worse prognosis(48).

Targeting protein for *Xenopus* kinesin-like protein 2 (TPX2) is a microtubule-associated protein that plays an important role in the mechanism of chromosome segregation in mitosis(49). It is a good biomarker for the diagnosis and prognosis of PCa. PanHW et al. found in the IHC samples of human normal prostate tissues, PCa tissues and PCa cells, that TPX2 was not expressed in normal tissues but highly expressed in PCa, which was consistent with our findings.(50). TPX2 can be used as prognosis biomarkers as well. It shows that the time to biochemical recurrence-free was shorter in the TPX2 high expression group, which indicating a poor prognosis(51).

4.1.2 The relatively less-studied PCa biomarkers

Though lack of validation by HPA database, the identified relatively less-studied PCa biomarkers from our bioinformatics study would provide novel and valuable insights in the diagnosis and prognosis of PCa.

Baculoviral IAP repeat-containing protein 5(BIRC5) is multitasking protein with dual roles in promoting cell proliferation and preventing apoptosis(52). Its role in the carcinogenesis and prognosis of PCa is not quite clear. According to Adisetiyo et al.'s finding, the expression of BIRC5 is directly proportional to the tumor volume in a mouse model of PCa (53). According to a latest large clinical study, BIRC5 was found increased in PCa especially advanced cases but still not clear whether it is an independent PCa biomarker(54). These results indicate consistence with our findings, that BIRC5 is highly expressed in PCa and is a high-risk gene for the prognosis of PCa.

Ribonucleotide reductase regulatory subunit M2 (RRM2) is an enzyme that regulate DNA synthesis and repair, which is important in carcinogenesis(55). RRM2 was highly expressed in patients with poorly differentiated PCa or advanced PCa ($p < 0.05$), and RRM2 would be a prognostic marker for the risk of recurrence in patients with low-risk PCa(56). In patients with Gleason score 4–7 and no invasion of the tumor into the prostate capsule, Cox proportional hazards analysis revealed that the risk of recurrence was positively correlated with RRM2 protein expression levels(56). In a recent preclinical and clinical study, RRM2 was found to be a driven factor for poor PCa prognosis outcomes and knockdown RRM2 could prohibit the PCa development(57). Although RRM2 was not found in either PCa or normal prostate IHC samples in HPA database, RRM2 is high likely to be a vital diagnosis and prognosis biomarker of PCa based on our analysis and recent findings.

DNA topoisomerase 2-alpha(TOP2A) encodes topoisomerase IIa, which controls DNA topology as well as cell cycle progression(58). According to Labbé et al., TOP2A can be a marker for PCa metastasis, and they found that TOP2A levels were highly expressed in patients with metastatic PCa by cohort studies, in IHC staining of tissue sections from patients with different stages of PCa, and in mice PCa cells (59).

According to Resende et.al., TOP2A can exist as a prognostic marker in PCa (60). In PCa patients with high Gleason scores (i.e., poorly differentiated PCa), the levels of TOP2A were significantly higher. And biochemical recurrence-free survival was significantly shorter in patients with high TOP2A expression ($P = 0.001$)(60). Our results consistently found TOP2A was highly expressed in PCa patients, and was a high-risk gene for PCa prognosis.

Ubiquitin-conjugating enzyme E2 C(UBE2C) is an anaphase-promoting complex/cyclosome (APC/C) - specific ubiquitin-conjugating enzyme(61). Currently, there are few studies on the mechanism of UBE2C in PCa. It has been confirmed that UBE2C levels regulated by miR-381-3p are positively correlated with the proliferation of PCa cells(62). UBE2C exists as a target gene for Androgen receptor (AR), and in castration resistant PCa(CRPC), the expression of UBE2C positively correlates with the level of Androgen receptor splice variant 7 and promotes the progression of CRPC(63). According to the study from ours and the peers, UBE2C would probably be a promising biomarker for the diagnosis and prognosis of PCa.

ZW10 interactor(ZWINT) refers to a protein involved in kinetochore function(64). There are less clinical proves to illustrate the mechanistic link between ZWINT and PCa. In a bioinformatics analysis, ZWINT was found to be increased in PCa and was negatively correlated with miR-1(65). In a study of PCa microarray data, ZWINT was also found to be upregulated in PCa and correlated with the PCa grade(66). In our study, ZWINT is highly expressed in PCa and is positively correlated with poor prognosis, which can be used as a potential biomarker for prostate cancer diagnosis and prognosis. However more follow-up experiments are needed to verify and discover its role in PCa.

4.2 Targets of natural products for treatment of PCa

Our study identified four potential curcumin targets (EP300, EGFR, RELA, NFKB1), 7 paclitaxel targets (PTEN, EGFR, ERBB2, TP53, KRAS, AR, AKT1), and 2 ursolic acid targets (GSK3B, RELA) that could be used for PCa treatment. Peer research results suggest they may be potential PCa-natural products targets.

4.2.1 Targets of curcumin for treatment of PCa

E1A-associated protein P300(EP300) plays an important role in cell proliferation, cell cycle regulation, apoptosis, and DNA damage repair(67). It is highly expressed in advanced PCa such as CRPC and is accepted as targets for PCa treatment(68, 69). Though there is no direct evidence, EP300 could probably be a potential PCa target for curcumin's inhibition. It is found that Curcumin could prohibit the acetyltransferase function of EP300 in Human osteosarcoma cells(70).By a silico docking analysis, curcumin analogue was found to molecularly target EP300 and inhibit its acetyltransferase ability(71).

Epidermal growth factor receptor (EGFR) is actively involved in the carcinogenesis processes such as tumor cell proliferation, angiogenesis, tumor invasion, metastasis and apoptosis(72). Curcumin may exert its anti-PCa effects through targeting EGFR. Co-delivery of curcumin and docetaxel by EGFR ligand nanoparticles have shown therapeutic effects on PCa(73). Synergetically administration of curcumin and

phenylethyl isothiocyanate were found to inhibit the growth of PCa PC-3 cells most probably through targeting EGFR, Akt and NF-kappaB pathways(74).

Transcription factor p65(RELA) is an isoform of the nuclear factor kappa-B protein, also known as p65, which can regulate inflammation, cell differentiation, proliferation and apoptosis(75). Only one reference was found indicating curcumin could inhibit PCa through RELA. In analyzing human PCa cell lines (PC3, DU145, LNCap), it was found that curcumin could inhibit the growth of androgen-independent PCa cells through p65 inhibition (76). Our network pharmacology analysis highly suggests the value of studying RELA as a novel potential PCa-curcumin target.

Nuclear factor NF-kappa-B p105 subunit(NFKB1) is another isoform of the kappa-B protein, also called p105/p50, which plays an important role in modulating innate and adaptive immunity, cell proliferation and apoptosis(77). Activation and translocation of NFKB1 is correlated with the progression of PCa(78). In human PCa cells, curcumin was found to inhibit the growth of LNCaP and PC3 cells by blocking the transactivation of NFKB1(78, 79).

4.2.2 Targets of paclitaxel for treatment of PCa

Phosphatase and tensin homolog deleted on chromosome ten(PTEN) plays an important role in carcinogenesis and tumor metastasis suppression(80). Combination application of Naringin and paclitaxel were found to inhibit growth of DU145 PCa cells by increasing PTEN and decreasing NFKB1(81). Paclitaxel could induce more apoptosis in PTEN-positive 22Rv1 PCa cells than these cells with PTEN knockdown (82). These results probably demonstrate that PTEN can be used as a target for paclitaxel treatment of PCa.

EGFR is a common potential target for both curcumin and paclitaxel in the treatment of PCa. Co-administration of EGFR inhibitor and paclitaxel indicated significant inhibition in the tumor growth and metastasis in human PCa transplanted nude mice model(83). Upregulated EGFR and actin level were found to be the causes of paclitaxel resistance to PCa cells(84).

Erb-b2 receptor tyrosine kinase 2 (ERBB2 or HER2) is a member of the EGFR family, which is overexpressed in PCa and bone metastasis of PCa(85). Co-administration of paclitaxel and anti-HER2 emulsion (10mg/kg) every week for 3 weeks significantly inhibited the PCa growth in PCa transplanted nude mice model(86).

Tumor protein 53 (TP53) is a tumor suppressor gene which is mainly involved in regulation of apoptosis and cell cycles (87). In an interactome and topology comparison experiments in PC-3 PCa cells, TP53 was identified as one of the vital pathways for paclitaxel in anti-PCa(88).

Kirsten rat sarcoma viral oncogene homolog (KRAS) is one of the three members of the RAS family(KRAS, HRAS and NRAS) with the highest probability of mutation in human tumors among them(89). Mutations of KRAS are associated with a variety of malignant tumors, including lung cancer, pancreatic cancer, and colorectal cancer(90). It is still unknown whether paclitaxel exert its anti-PCa

effects via KRAS. However, though not commonly mutated in PCa, KRAS was believed to play an important role in bone metastasis of PCa(91). It is worthwhile to do further study to verify the hypothesis.

Androgen receptor (AR) is highly expressed in primary and metastatic PCa, which can regulate the proliferation, apoptosis, and metastasis of PCa(92). Jiang et al. found that paclitaxel was able to target and inhibit the transcriptional activity of AR in the 22Rv1 PCa cell line, a CRPC cell model(93). In another CRPC cell line 22RV1, paclitaxel was found to inhibit AR transcription by inducing nuclear accumulation of FOXO1(94).

AKT serine/threonine kinase 1 (AKT1), a family member of AKT (protein kinase B) and core component of the PI3K/AKT signaling pathway, plays an important role in PCa carcinogenesis(95). Co-administration of AKT inhibitors and paclitaxel are very powerful in treating triple negative breast cancer with mutated AKT1(96, 97). However, the correlation between AKT1, paclitaxel and PCa is still unknown. Our findings may provide novel direction in the future study.

4.2.3 Targets of ursolic acid for treatment of PCa

Glycogen synthase kinase 3 β (GSK3B) is involved in biological processes such as energy metabolism, inflammation, and apoptosis(98). Only 1 report so far indicates the correlation between ursolic acid and PCa and GSK3B. Ursolic acid was found to induce apoptosis to PC-3, LNCaP and DU145 PCa cells with enhanced phosphorylation GSK3B and the apoptosis effects can be reversed by GSK3B inhibitor SB216763(99). In HepG2 liver cancer cells, ursolic acid can also induce apoptosis by targeting and regulating the phosphorylation of GSK3B(100). Hence GSK3B is a probably a novel potential target for ursolic acid in the treatment of prostate cancer.

Transcription factor p65(RELA) is a common potential PCa therapeutic target of curcumin and ursolic acid, which deserves further studies in the mechanism of action. The direct proof of ursolic acid inhibit PCa through RELA is limited. Shanmugam et al. found ursolic acid could induce apoptosis in DU145, LNCaP PCa cells in a dose-dependent manner through inhibiting the activity of NF- κ B and phosphorylation of p65(RELA)(101).

5. Conclusions

In this study, by analyzing two PCa datasets from the GEO database and validated by GEPIA and HPA, 9 genes were identified to be closely associated with the development and prognosis of PCa. All the nine markers indicated poor prognosis for PCa patients verified by GEPIA. The 4 identified biomarkers verified by HPA (CENPF, TPX2, TK1 and CCNB1) indicate the rationality of our study design and the identified 5 relatively less-studied PCa biomarkers (RRM2, UBE2C, TOP2A, BIRC5 and ZWINT) would provide novel and valuable insights in the in the diagnosis and prognosis of PCa. The carcinogenesis of PCa is found to be mainly associated with hepatic fibrosis pathway, ILK signaling, NRF2-mediated oxidative stress response and many others. By network pharmacology, after the integration of data on small molecule natural products acting on PCa, 4 targets of curcumin (EP300, RELA, EGFR, NFKB1), 7 targets of

paclitaxel (PTEN, EGFR, ERBB2, TP53, KRAS, AR, AKT1), and 2 targets of ursolic acid (GSK3B, RELA) were identified.

Our study reveals potential key biomarkers for the diagnosis and prognosis of PCa and demonstrates three valuable natural products for molecular targeting of PCa, which laid a promising foundation for further study of novel biomarkers and molecularly targeted drugs for PCa.

Abbreviations

Abbreviations	Full name
Aclbi	Assistant for Clinical Bioinformatics
AR	Androgen receptor
BC	Betweenness Centrality
BPs	Biological processes
CCs	Cellular components
CLC	Closeness Centrality
CRPC	Castration Resistant Prostate Cancer
DAVID	The Database for Annotation, Visualization and Integrated Discovery
DC	Degree Centrality
DEGs	differentially expressed genes
EC	Eigenvector Centrality
GEO	Gene Expression Omnibus
GEPIA	Gene Expression Profiling Interactive Analysis
GO	Gene Ontology
GTE _x	The Genotype-Tissue Expression
HPA	The Human Protein Atlas
IHC	Immunohistochemical
IPA	QIAGEN Ingenuity Pathway Analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes
LAC	Local average connectivity-based method
MFs	Molecular functions
NC	Network Centrality
OMIM	Online Mendelian Inheritance in Man
PCa	Prostate Cancer
PDB	Protein Data Bank
PI3K	Phosphatidylinositol 3 kinase
PPI	Protein-Protein Interaction
PRAD	Prostate Adenocarcinoma

Abbreviations	Full name
PSA	prostate-specific antigen
TCGA	The Cancer Genome Atlas
TTD	Therapeutic Target Database

Declarations

Ethics statement

Our research was approved by the Ethics Committee of Medical College at Yangzhou University, and followed the principles of the Declaration of Helsinki. All datasets analyzed were obtained from publicly available databases, hence written informed consent was not applicable.

Consent for publication

Not applicable

Availability of data and materials

All data involved in the study are included in this manuscript and its supplementary files.

Competing Interests

The authors declare that there are no conflicts of interest.

Author Contribution

WL designed the project, analyzed the data, drafted and revised the manuscript. WX did most of the bioinformatics analysis and modified the manuscript. KS, FW and TWW performed the literature search and revised the manuscript.

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Tables

Due to technical limitations, table 2 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Identification of DEGs. (a) Volcano plots for both the GSE3325 and GSE46602 datasets. Red indicates up-regulated genes, blue indicates down-regulated genes, and grey indicates non-significant genes. (b) Heatmap for both the GSE3325 and GSE46602 datasets. Each column represents one dataset and each row represents one gene. Color changes from blue to red represents a transition from downregulation to upregulation of expression (first 100 targets displayed).

Figure 2

GO enrichment analysis and IPA pathway analysis of DEGs. (a) Biological processes(BPs) analysis of GO. (b) Molecular functions(MFs) analysis of GO . (c) Cellular components (CCs) analysis of GO. (d) IPA pathway analysis of DEGs.

Figure 3

(a) PPI network map of DEGs constructed from the String database. (b) 30 genes with the highest scores of protein interaction calculated using Cytoscape software. (c) Module 1: module with the highest score. (d) Module 2: the second-ranked module. (e) GO and KEGG enrichment analysis of Module 1. (f) GO and KEGG enrichment analysis of Module 2.

Figure 4

Results of validation and survival analysis for 30 genes. (a) With $|\log FC| > 1$ and $p < 0.01$ as the cutoff value, 30 genes are verified in the GEPIA database. (b) Survival analysis results of 30 genes in the GEPIA database.

Figure 5

(a) HPA database verification results of 4 key genes,including CCNB1, CENPF, TK1,TPX2. (b) The pan-cancer analysis results of 9 key genes in the TCGA database. Red box represents the tumor group, blue box represents the normal group, and*: p-value < 0.05; **: p-value <0.01; ***: p-value <0.001.

Figure 6

Concatenation of PCa targets from the GeneCards, OMIM, PharmGkb databases and DEGs.

Figure 7

PPI network diagram of PCa-curcumin targets and the screening process. (a) PPI network diagram of PCa-curcumin targets. (b) The yellow part of the diagram shows the groups after the first screening by Cytoscape. (c) Substrates after the first screening. The yellow part of the diagram shows the groups after the second screening. (d) Second screened moiety.

Figure 8

PPI network diagram of PCa-paclitaxel targets and the screening process. (a) PPI network diagram of PCa-paclitaxel targets. (b) The yellow part of the diagram shows the groups after the first screening by Cytoscape. (c) Substrates after the first screening. The yellow part of the diagram shows the groups after the second screening. (d) Second screened moiety.

Figure 9

PPI network diagram of PCa-ursolic acid targets and the screening process. (a) PPI network diagram of PCa-ursolic acid targets. (b) The yellow part of the diagram shows the groups after screening. (c) Substrates after screening.

Figure 11

Curcumin was used as the small-molecule ligand and the target as the macromolecular receptor for molecular docking models. (a) Molecular docking model of curcumin and EP300. (b) Molecular docking model of curcumin and RELA. (c) Molecular docking model of curcumin and EGFR. (d) Molecular docking model of curcumin and NFKB1.

Figure 12

paclitaxel was used as the small-molecule ligand and the target as the macromolecular receptor for molecular docking models. (a) Molecular docking model of paclitaxel and PTEN. (b) Molecular docking model of paclitaxel and EGFR. (c) Molecular docking model of paclitaxel and ERBB2. (d) Molecular docking model of paclitaxel and TP53. (e) Molecular docking model of paclitaxel and KRAS. (f) Molecular docking model of paclitaxel and AR. (g) Molecular docking model of paclitaxel and AKT1.

Figure 13

Ursolic acid was used as the small-molecule ligand and the target as the macromolecular receptor for molecular docking models. (a) Molecular docking model of ursolic acid and GSK3B. (b) Molecular docking model of ursolic acid and RELA.

Supplementary Files

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