Identification of Key Genes and Molecular Mechanisms Associated With Docetaxel Resistance in Castration Resistant Prostate Cancer Based on Bioinformatics Analysis

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Research

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Abstract

Background

Castration resistant prostate cancer (CRPC) is one of the most common solid tumor with high mortality and limited therapeutic options, and docetaxel is the first-line chemotherapy for patients. However, the long-term use of docetaxel has limited its clinical applications. The aim of this study was to identify docetaxel-resistant key genes and molecular mechanisms.

Results

TUBB4A (Class IVa beta-tubulin), SRPX (Sushi repeat containing protein, X chromosome) and CSRP2 (Cysteine and glycine rich protein 2) were finally identified as the key genes tightly related to docetaxel resistance. TUBB4A and CSRP2 may participate in docetaxel resistance by E2F transcription factor and MYC proto-Oncogene in the process of cell cycle, and SRPX may participate in docetaxel resistance by epithelial–mesenchymal transition (EMT) and P53 pathway.

Conclusion

TUBB4A, SRPX and CSRP2 may be the key genes associated with docetaxel resistance, which could be prognostic biomarkers for docetaxel resistance in CRPC.

Background

Prostate cancer, an androgen-dependent solid tumor, is the second leading cause of cancer death among men [1, 2]. And androgen-deprivation therapy (ADT) is currently the primary approach for the treatment of prostate cancer [3]. Typically, androgen-dependent prostate cancer progresses to castration-resistant prostate cancer (CRPC) quickly within 2-3 years of initiation of ADT [4]. In current years, docetaxel-mediated chemotherapy is regarded as the standard first-line treatment for CRPC, and it has significantly improved the overall survival of CRPC patients [5, 6]. However, docetaxel resistance in patients limits the clinical option of it [7, 8]. Thus, there is an urgent need to develop novel strategies to reverse the docetaxel resistance.

Although docetaxel-based chemotherapy is the standard first-line treatment for CRPC, about half of patients are resistant to docetaxel [9]. Thus, various mechanisms have been studied. It is reported that docetaxel resistance is main related to decreased cellular drug accumulation, altered expression of microtubule-associated proteins, changes to microtubules induced by interactions with other cytoskeletal proteins and defects in apoptotic pathways [10]. However, docetaxel resistance has not been reported to be effectively reversed. Therefore, we further explored the mechanisms of docetaxel resistance and identified novel biomarkers for docetaxel resistance in CRPC.

As an innovative and high-throughput research method, bioinformatics analysis of microarray data has been widely used in new drug target discovery, molecular diagnosis, and the molecular mechanisms of

drug resistance [11, 12]. Based on the bioinformatics analyses, You and Gao [13] found neuromedin U is the key gene conferring the alectinib resistance in Non-small cell lung cancer and Li, Wang [14] identified 8 hub genes and 4 molecular complex detections for progesterone resistance in endometrial cancer.

In this work, bioinformatics methods were performed to analyze the microarrays GSE33455 and GSE55945. The differentially expressed genes (DEGs) of CRPC were identified via R language. Gene Ontology analysis (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were applied to further annotate DEGs. In total, 50 DEGs were identified. Kaplan-Meier survival analysis and GEPIA were further used to screen the real key genes. Finally, TUBB4A, SRPX and CSRP2 were screened as the real key genes, which were further functionally analyzed by Gene set enrichment analysis (GSEA) and GeneMANIA online analysis. We found that TUBB4A and CSRP2 may participate in the docetaxel resistance by E2F transcription factor and MYC proto-Oncogene in the process of cell cycle, and SRPX may participate in the docetaxel resistance by EMT and P53 pathway. TUBB4A, SRPX and CSRP2 could be novel prognostic biomarkers for docetaxel resistance in CRPC.

Materials And Methods

Affymetrix microarray data

Gene expression profiles of GSE33455 and GSE55945 based on the platform of GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array) were collected from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). GSE33455 consists of docetaxel-sensitive cell lines (DU-145 and PC-3) and docetaxel-resistant cell lines (DU-145R and PC-3R). GSE55945 consists of 13 human prostate malignant tissue samples and 8 benign tissue samples.

Processing of microarray data and differential expression gene analysis

R software (version 3.5.3) and Bioconductor packages were used to analyze original array data. Firstly, the original CEL file and annotation files of Affymetrix platform were downloaded, followed by background correction and quantile normalization using RMA method. Afterwards, Bioconductor package affyPLM was adopted for GSE33455 and GSE55945. The DEGs between two group samples were then analyzed by the paired t-test based on the limma package. Finally, the genes with $|\log_2(\text{fold change})| > 1$ were considered to be significant. Adj.p.value < 0.05 was set as the cut-off criterion.

Functional and pathway enrichment analysis

In order to investigate the potential biological functions associated with upregulated DEGs and downregulated DEGs, R package clusterProfiler [15] was used for GO analyses and KEGG pathway analysis. GO analyses include biological process (BP), molecular function (MF), and cellular component (CC). $P < 0.05$ was considered as the threshold.

Kaplan-Meier survival analysis

To evaluate the influence of the key genes on overall survival, UCSC Xena (https://xena.ucsc.edu/) was applied. According to the expression level of genes, prostate cancer patients from 497 TCGA samples were separated into high and low expression groups, and the overall survival was then analyzed. Log-rank test statistic and P value was calculated.

Expression level analysis with GEPIA

To confirm the expression level of the key genes, GEPIA [16], a web server for cancer and normal gene expression profiling and interactive analyses, was applied based on TCGA samples. Genes with $|\log_2(\text{Fold change})| > 1$ and $P < 0.01$ were considered significant.

Gene set enrichment analysis (GSEA)

In all, 6 samples from docetaxel-resistant cell lines were divided into two groups according to the median values of the expression of the gene (high vs low expression). GSEA was applied to explore the potential functions of TUBB4A, SRPX and CSRP2. The annotated gene sets of h.all.v6.2.symbols.gmt is the reference gene sets. Nominal $P < 0.05$, FDR < 0.05 and |Normalized Enrichment Score (NES)| > 1 were set as the cut-off criterion.

Analysis of TUBB4A, SRPX, CSRP2 by GeneMANIA

GeneMANIA (https://www.genemania.org/), a user-friendly web interface for generating hypotheses about gene function [17], was applied to predict potential functions. Choosing Homo sapiens from the 9 optional organisms and entering the gene into the search bar, then the results were collected.

Results

Identification and functional characterization of DEGs in GSE33455 and GSE55945

GEO microarray (GSE33455) was used to screen DEGs using limma package. Volcano plots displayed the distribution of the 20482 expressed genes ($|\text{Log}_2\text{FC}| > 1$ and adj.p.value < 0.05 as the cutoff criteria, Figure 1A). A total of 540 DEGs were identified, of which 99 genes were upregulated and 441 genes were downregulated (Figure 1B). To further functionally analyze DEGs, GO term enrichment analysis including BP, CC and MF ontologies and KEGG pathway enrichment analysis were performed. For the upregulated genes, the DEGs were mainly enriched in SMAD protein signal transduction, BMP signaling pathway and regulation of transmembrane receptor protein serine/threonine kinase signaling pathway in the BP group. The KEGG results revealed that the DEGs were mainly enriched in adherens junction, TGF-beta signaling pathway, systemic lupus erythematosus, signaling pathways regulating pluripotency of stem cells and mitophagy–animal (Figure 1C; Table S1). For the downregulated genes, the DEGs were mainly concentrated in regulation of epidermal cell differentiation, response to virus, epidermis development, cellular response to hydrogen peroxide and cellular response to antibiotic in the BP group. The KEGG results revealed that the DEGs were mainly concentrated in NOD-like receptor signaling pathway,

rheumatoid arthritis, IL-17 signaling pathway, NF-kappa B signaling pathway and TNF signaling pathway (Figure 1D; Table S2).

To screen out key genes contributing to docetaxel resistance, the GSE55945 dataset, consisted of 13 human prostate malignant tissues and 8 benign tissues, was used. A total of 1362 DEGs, including 80 upregulation and 1282 downregulation, were identified to be candidate key genes in prostate cancer tissues (Figure 2A, B). The results of GO terms of each ontologies and KEGG pathway enrichment analysis were shown in Supplementary Table 3, 4 and the top 5 were exhibited in Figure 2C, D. For the upregulated genes, the DEGs were mainly enriched in forebrain neuron fate commitment, cerebral cortex gabaergic interneuron differentiation, camera-type eye development, regulation of peroxisome proliferator activated receptor signaling pathway and negative regulation of oligodendrocyte differentiation in the BP group. The KEGG results revealed that the DEGs were mainly enriched in insulin secretion, linoleic acid metabolism, ferroptosis, arachidonic acid metabolism and calcium signaling pathway. For the downregulated genes, the DEGs were mainly concentrated in muscle system process, cell-substrate adhesion and regulation of blood pressure in the BP group. The KEGG results revealed that the DEGs were mainly enriched in cGMP-PKG signaling pathway, vascular smooth muscle contraction, hypertrophic cardiomyopathy, proteoglycans in cancer and Dilated cardiomyopathy. As we listed, the DEGs were involved in multiple biological processes and pathways, this revealed the possible mechanisms contributing to docetaxel resistance and tumorigenesis in CRPC, and it deserves further study.

Identification of key genes associated with docetaxel resistance in CRPC

Key gene candidates were identified through Venn diagram analysis. 1 overlapping upregulated gene and 49 overlapping downregulated genes were obtained in the intersection of the GSE33455 DEGs and the GSE55945 DEGs (Figure 3A and B). To further identify the key genes, we analysis the overall survival of the total 50 genes (overlapping genes of the upregulated gene and the downregulated genes) on UCSC Xena online analysis (497 samples from TCGA prostate cancer) (Figure S1; Figure 3C). We found that TUBB4A expression was negatively associated with the overall survival, and the expression level of SRPX and CSRP2 were positively associated with the overall survival (Figure 3C). In addition, we further verified the expression level of the key genes on GEPIA online analysis, and the results revealed that TUBB4A expression was significantly upregulated compared with normal prostate tissues, while the expression level of SRPX and CSRP2 were downregulated (Figure 3D). Altogether, we confirmed TUBB4A, SRPX and CSRP2 as the real key genes contributing to docetaxel resistance and tumorigenesis.

GSEA and validation of the function of TUBB4A, SRPX and CSRP2

To clarify the biological functions of TUBB4A, SRPX and CSRP2, GSEA was performed. Under the cut-off criteria Nominal $P < 0.05$, $FDR < 0.05$ and $|NES| > 1$, a total of 41 functional gene sets were enriched (Figure 4A; Table S5, S6, S7). The top 4 TUBB4A-regulated gene sets were “E2F targets”, “G2M checkpoint”, “MYC targets v1” and “MITOTIC spindle”. The top 4 SRPX-regulated gene sets were “epithelial mesenchymal transition”, “P53 pathway”, “hypoxia” and “estrogen response early”.

Interestingly, the top 4 CSRP2-regulated gene sets were also “E2F targets”, “G2M checkpoint”, “MYC targets v1” and “MITOTIC spindle”.

Protein/gene interactions of TUBB4A, SRPX, CSRP2

To investigate the potential functions of TUBB4A, SRPX and CSRP2 in CRPC, GeneMANIA online analysis were employed. As shown in the GeneMANIA network, the 20 proteins/genes were highly associated with the key genes. Among them, PAICS, TUBA3E, HSPA5 and TUBB4B were the most related genes of TUBB4A. TNXB, CCDC80, KCTD13 and SRPX2 were the most related genes of SRPX. KAT14, AGPS, PIAS1 and ATF2 were the most related genes of CSRP2 (Figure 5A).

Discussion

Castration resistant prostate cancer (CRPC) is one of the most common tumors in male. Docetaxel is the first line treatment for CRPC patients, but the long-term treatment of docetaxel has limited its clinical applications due to docetaxel resistance. It is of great value to identify the key genes associated with docetaxel resistance in CRPC. Currently, the combination of gene expression profiling and bioinformatics analysis is becoming more valuable in screening potential key genes and molecular mechanisms underlying drug resistance [18]. Therefore, we carried out a series of bioinformatics analyses to explore the key genes conferring to docetaxel resistance in CRPC [19].

In the present study, we integrated two gene expression profile datasets (GSE33455 and GSE55945) and analyzed it using R software and bioinformatics analysis. The results identified 50 DEGs, including 1 upregulated gene and 49 downregulated genes. Among these genes, TUBB4A, SRPX and CSRP2 were identified as the key genes due to the survival probability and expression in prostate cancer. Next, we performed GSEA to clarify the gene function of TUBB4A, SRPX and CSRP2. Both TUBB4A and CSRP2 are enriched in “E2F targets”, “G2M checkpoint”, “MYC targets v1” and “MITOTIC spindle”. E2F transcription factors play a crucial role in the control of cell cycle and action of tumor suppressor proteins [20], which modulate epirubicin response and resistance in breast cancer by regulating FOXM1 expression [21]. MYC proto-oncogene is reported as an oncogenic transcription factor, promotion of the cell cycle is the major oncogenic mechanism [22], and it has been reported that N-Myc (one of the MYC family) differentially regulating miR-421/ATM pathway contributes to ADT and enzalutamide resistance [23]. The “G2M checkpoint” and “MITOTIC spindle” are gene sets involved in cell cycle [24, 25]. These results indicate that TUBB4A and CSRP2 may participate in docetaxel resistance by E2F transcription factor and MYC proto-oncogene in the process of cell cycle. SRPX is enriched in “EMT” and “P53 pathway”. EMT is known to play an important role in cancer progression, metastasis and drug resistance [26], it has been reported that promoting EMT could induce cisplatin resistance in non-small cell lung cancer cells in an AKT signaling pathway-dependent manner [27]. Additionally, P53 pathway is a key biological process inducing chemotherapy resistance in multiple cancers. Ma, Guo [28] reported that p53 repression leads to doxorubicin resistance in Hepatocellular Carcinoma. Gan, Wang [29] revealed that P53 pathway participate in docetaxel resistance by regulating the cell cycle and apoptosis in prostate cancer.

Therefore, we supposed that SRPX may participate in docetaxel resistance by EMT and P53 pathway in CRPC.

Finally, gene interactions were constructed to explore the function of the key genes on GeneMANIA online analysis. Top 20 genes related to the key genes were identified in the network. As the most related genes of the key genes, HSPA5 [30, 31], SRPX2 [32], AGPS [33], ATF2 [34] have been reported to participate in drug resistance in multiple cancers. And PAICS [35], TUBB4B [36], CCDC80 [37], PIAS1 [38] have been reported in tumorigenesis and tumor progression of multiple cancers. The results revealed that TUBB4A, SRPX and CSRP2 may participate the occurrence of CRPC and docetaxel resistance through a similar mechanism of the related genes. Further studies should test this hypothesis.

Conclusion

In this study, 50 DEGs of CRPC were identified in the intersection of the microarrays GSE33455 and GSE55945. According to the results of Kaplan-Meier survival analysis and GEPIA analysis, we identified that TUBB4A, SRPX and CSRP2 are the real key genes associated with docetaxel resistance in CRPC. Moreover, GSEA and GeneMANIA online analysis were applied to further analyze the function of the key genes. The results of GSEA demonstrated that TUBB4A and CSRP2 may participate in docetaxel resistance by E2F transcription factor and MYC proto-oncogene in the process of cell cycle, and SRPX may participate in docetaxel resistance through EMT and P53 pathway. Altogether, TUBB4A, SRPX and CSRP2 may be the key genes associated with docetaxel resistance in CRPC. This study would deepen the understanding of docetaxel resistance and provide prognostic biomarkers for docetaxel resistance docetaxel resistance in CRPC.

Abbreviations

Full name	Abbreviation
Androgen-deprivation therapy	ADT
Biological process	BP
Cellular component	CC
Castration resistant prostate cancer	CRPC
Cysteine and glycine rich protein 2	CSRP2
Differentially expressed genes	DEGs
Epithelial–mesenchymal transition	EMT
Kyoto Encyclopedia of Genes and Genomes	KEGG
Fold change	FC
Gene Ontology analysis	(GO)
Gene set enrichment analysis	GSEA
Gene Expression Omnibus	GEO
Kyoto Encyclopedia of Genes and Genomes	KEGG
Molecular function	MF
Class IVa beta-tubulin	TUBB4A
Sushi repeat containing protein, X chromosome	SRPX

Declarations

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available in the GEO database (https://www.ncbi.nlm.nih.gov/geo).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YL analyzed the data and wrote the manuscript. CPH collected the data. YZ and RZ reviewed the manuscript. All authors read and approved the manuscript.

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Not applicable.

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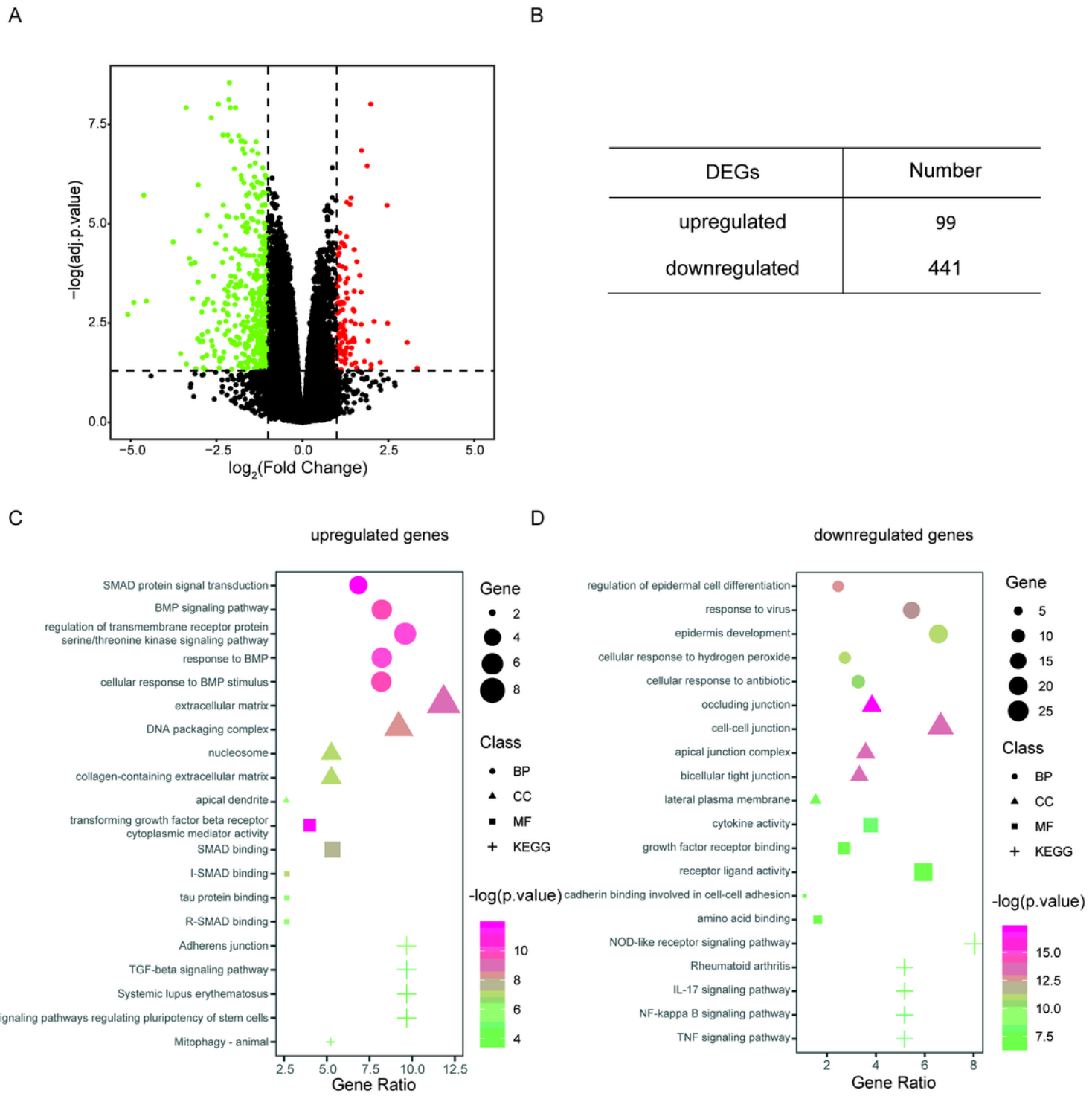
Figures



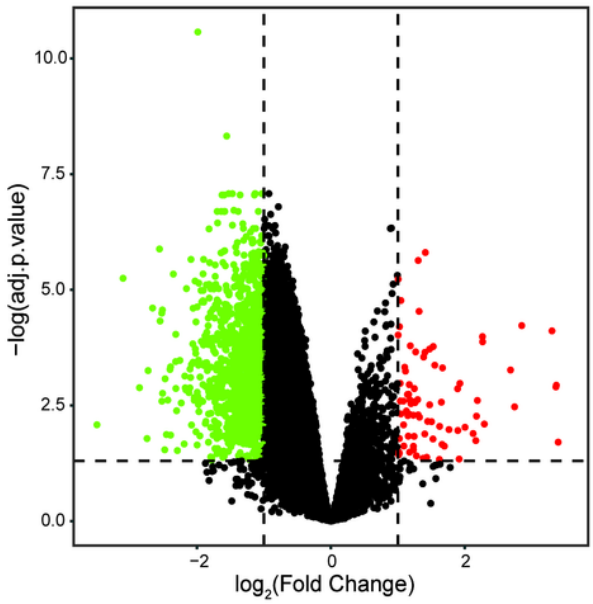
DEGs	Number
upregulated	99
downregulated	441

Figure 1

Identification and functional characterization of DEGs from GSE33455 dataset. (A) Volcano plot of DEGs between docetaxel-resistant CRPC cell lines and the parental cell line. Red dots represent significantly upregulated DEGs in docetaxel-resistant CRPC cell lines; green dots represent significantly downregulated DEGs in docetaxel-resistant CRPC cell lines; black dots represent no significant difference (P<0.01 and |logFC|⩾1 as the threshold cutoff). (B) The distribution of significant DEGs in docetaxel-resistant CRPC

cell lines. (C) Top 5 enriched GO terms under ‘BP’, ‘CC’ and ‘MF’ and KEGG pathways of significantly upregulated DEGs in docetaxel-resistant CRPC cell lines. (D) Top 5 enriched GO terms under ‘BP’, ‘CC’ and ‘MF’ and KEGG pathways of significantly downregulated DEGs in docetaxel-resistant CRPC cell lines. BP, biological process; CC, cellular component; MF, molecular function.

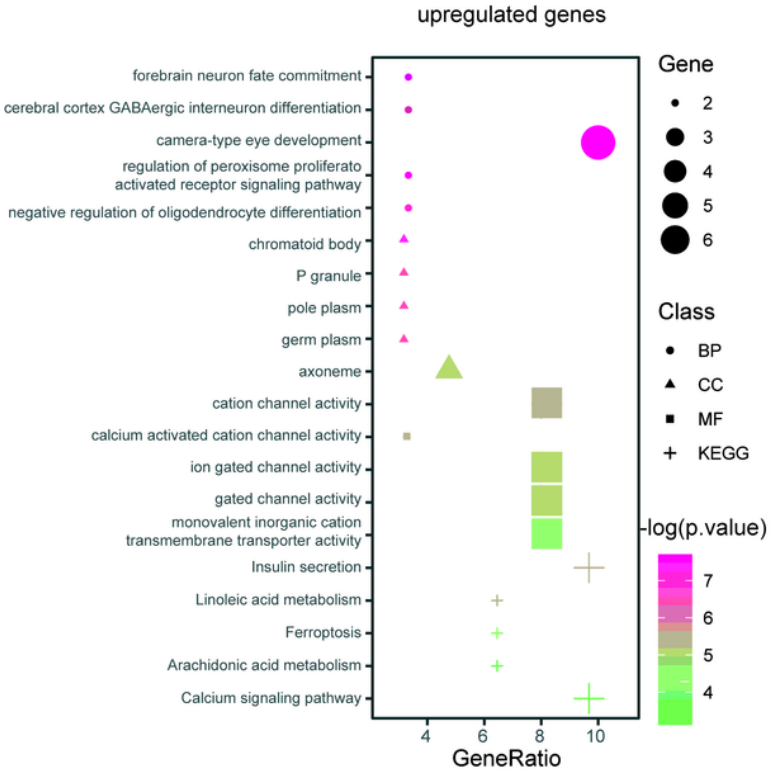
A



B

DEGs	Number
upregulated	80
downregulated	1282

C



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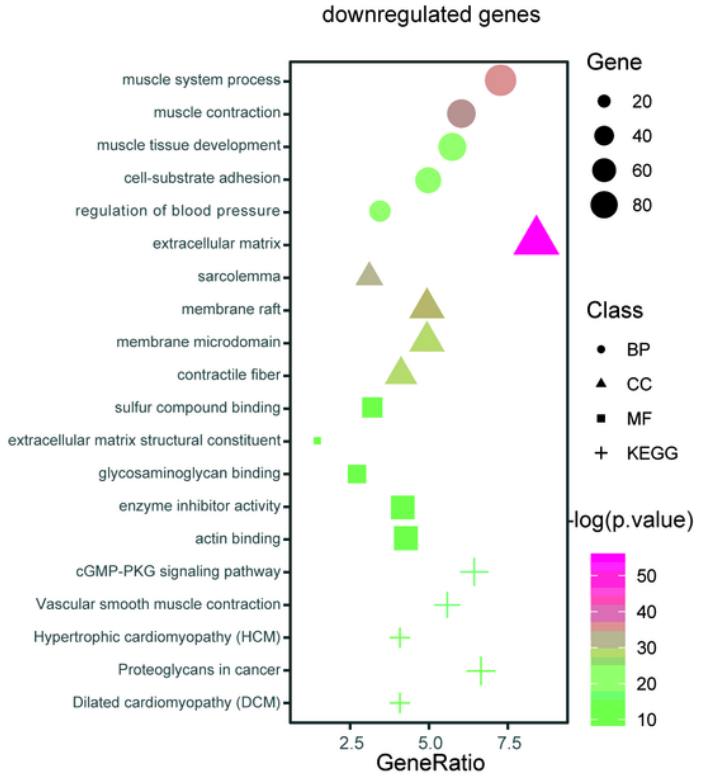
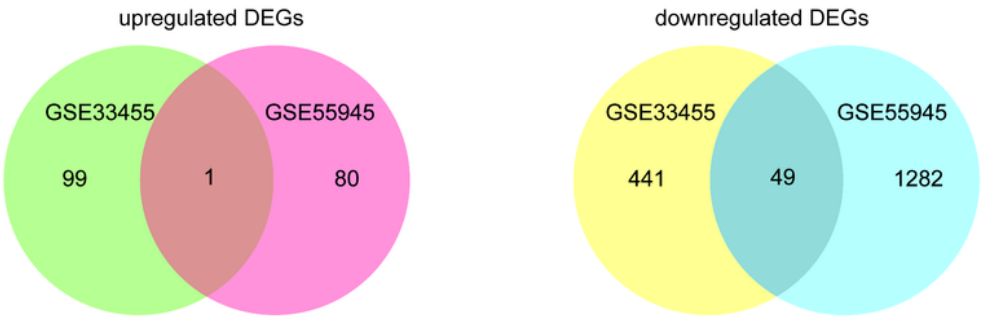


Figure 2

Identification and functional characterization of DEGs from GSE55945 dataset. (A) Volcano plot of DEGs between prostate malignant tissues and benign tissues. Red dots represent significantly upregulated DEGs in prostate malignant tissues; green dots, represent significantly downregulated DEGs in prostate malignant tissues; black dots represent no significant difference ($P<0.05$ and $|logFC| \geq 1$ as the threshold cutoff). (B) The distribution of significant DEGs in prostate malignant tissues. (C) Top 5 enriched GO terms under ‘BP’, ‘CC’ and ‘MF’ and KEGG pathways of significantly upregulated DEGs in prostate malignant tissues. (D) Top 5 enriched GO terms under ‘BP’, ‘CC’ and ‘MF’ and KEGG pathways of significantly downregulated DEGs in prostate malignant tissues. BP, biological process; CC, cellular component; MF, molecular function.

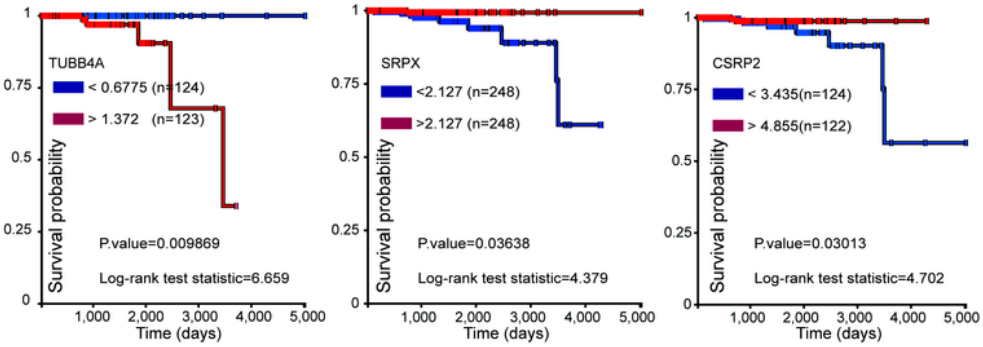
A



B

Upregulated	TUBB4A
Downregulated	SRPX CSRP2 FAM169A CXCL6 GATA3 TNC KRT16 C11orf70 C11orf45 LINC00595 ACOX2 LAMC2 LAMB3 PPP1R14C BEX1 CPED1 IL18R1 ETS2 MISP CALD1 TMEM132E SMIM6 MPZL2 PRSS16 DMKN HSPB8 RPL39L RBMS3 MOCOS FHL2 TNS4 MAL CORO2B LIPG BST1 MDFIC SEMA3C TMEM154 FHOD3 HS3ST3A1 MIR205 TSPAN2 MME EFEMP1 AMIGO2 CCBE1 PLAU GPR87 CDC42EP3

C



D

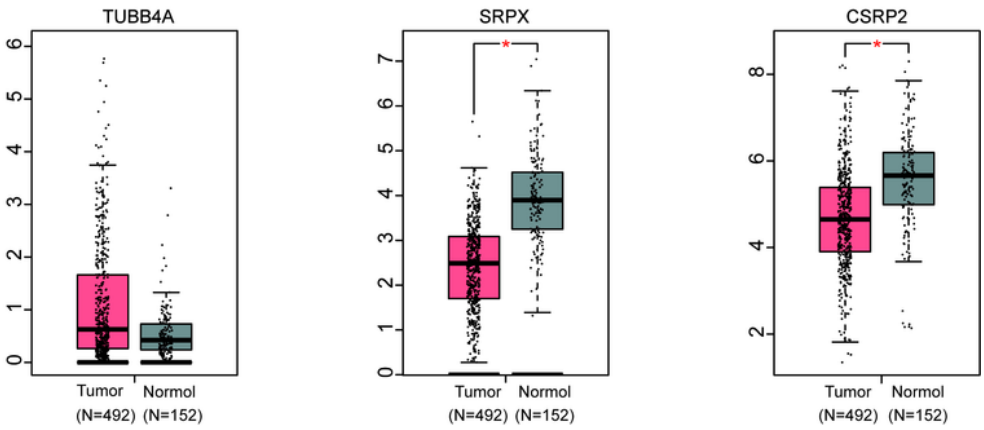


Figure 3

(A) Venn diagram of significantly upregulated DEGs and downregulated DEGs between GSE33455 and GSE55945. (B) Significantly regulated genes both in GSE33455 and GSE55945 DEGs. (C) Survival analysis of TUBB4A, SRPX and CSRP2 obtained using UCSC Xena. The red curve represents samples with high-expressed genes and the dark blue curve represents with low-expressed genes. (D) The expression level of TUBB4A, SRPX and CSRP2 based on TCGA database analyzed by GEPIA. The red plot

represents tumor tissues and the grey plot represents normal tissues. P < 0.01 and |log FC| > 1 were set as the criteria.

A

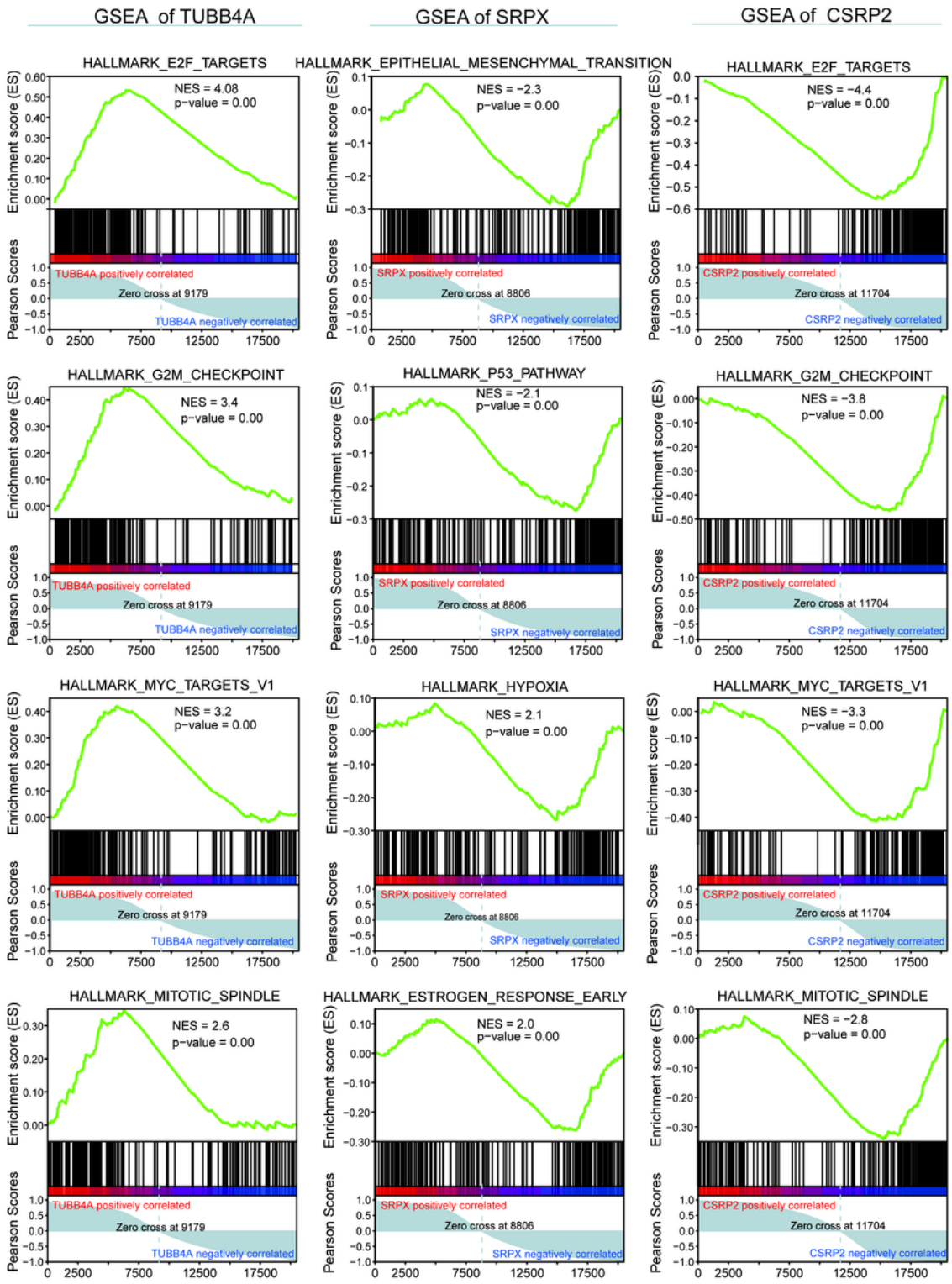


Figure 4

Gene set enrichment analysis (GSEA) for top 4 functional gene sets of TUBB4A, SRPX and CSRP2, respectively.

A

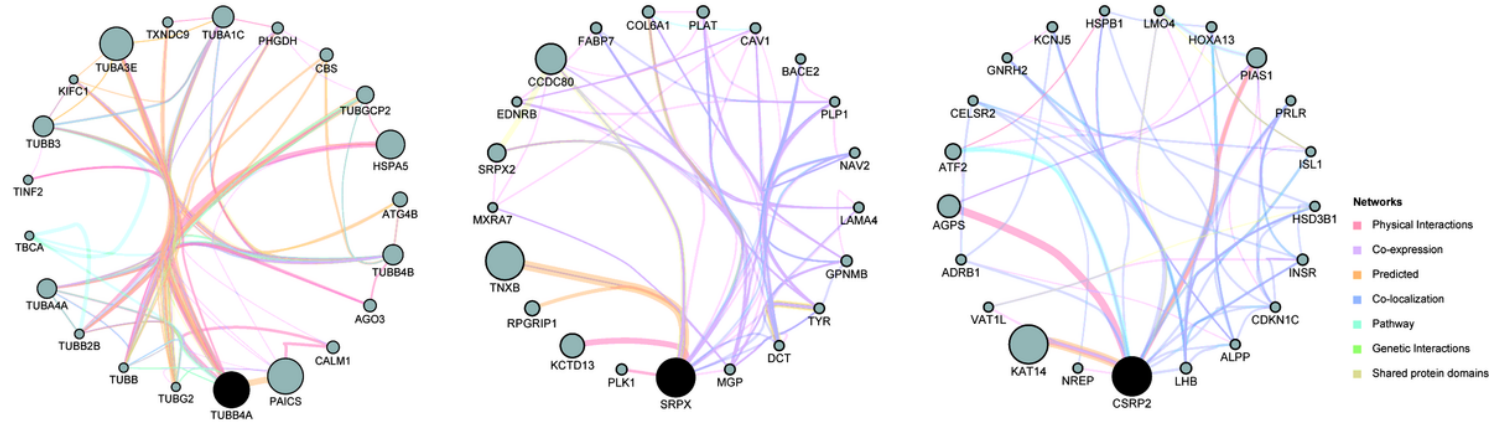


Figure 5

(A) Protein/gene interaction networks of TUBB4A, SRPX and CSRP2 performed by GeneMANIA online analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.docx