Identification and Validation of Four Novel Biomarkers Involved in Docetaxel Resistant Prostate Cancer

Ruiji Liu
Southeast University Zhongda Hospital
Shuying Li
Sichuan Cancer Hospital and Research Institute: Sichuan Cancer Hospital and Institute
Liquan Liu
meishan city people's hospital
Bin Xu
Southeast University Zhongda Hospital
Ming Chen (mingchenseu@126.com)
Southeast University Zhongda Hospital https://orcid.org/0000-0002-3572-6886

Research Article

Keywords: CRPC, docetaxel resistance, prognosis, GEO

Posted Date: March 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-298854/v1

Abstract

Background: Prostate cancer (PCa) is one of the most common malignancies affecting men's health. Docetaxel chemotherapy has been utilized in castration resistant prostate cancer (CRPC). However, docetaxel resistance further reduces the survival of PCa patients. Thus, it is necessary to explore effective biomarkers for docetaxel chemotherapy resistant PCa (DCRPC).

Methods: A comprehensive bioinformatic analysis was performed to identify differentially expressed genes (DEGs) between DCRPC and docetaxel chemotherapy sensitive PCa (DCSPC) cell. DEGs were selected between GSE33455 and GSE36135 obtained from the Gene Expression Omnibus (GEO) database. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were applied for functional enrichment and pathway analysis. The STRING database were used to construct protein-protein interaction (PPI) networks. Diseases free survival (DFS) was used for prognostic analysis via GEPIA.

Results: A total of 89 DEGs from GSE33455 and GSE36135 were screened. GO functional analysis showed that these DEGs are mainly enriched in the extracellular exosome, cell adhesion molecule binding, ATP binding and cellular response to tumor necrosis factor. KEGG pathway analysis showed that these DEGs are mainly involved in the TNF signaling pathway, chemokine pathway, and nicotinate and nicotinamide metabolism. 30 closely related DEGs were used for PPI network construction. Finally, four prognostic relative genes (RHOF, ADCY7, DOCK2, LMO7) were identified.

Conclusion: The present study identified prognostic hub genes and related signaling pathways, which may help improve understanding of the molecular mechanisms of DCRPC. These hub genes can potentially serve as therapeutic and prognostic biomarkers for DCRPC.

1. Introduction

Prostate cancer (PCa) is one of the most common malignancies and remains the second most deadly disease in men worldwide. In 2013, more than 230,000 men were diagnosed with PCa in the United States, and about 12% of them died from cancer-related causes [1]. It is well known that the growth and progression of PCa is dependent on androgens, and androgen deprivation therapy (ADT), whether chemical or surgical deprivation, can achieve good results in the early stages of treatment [2–4]. However, due to multiple molecular mechanisms leading to reactivation of the androgen receptor (AR) signaling pathway, such as AR mutations, AR overexpression/amplification, and AR splicing variants, castration resistant prostate cancer (CRPC) is inevitable after approximately 2 years of treatment [5–8]. Compared to early, localized cases, CRPC has much shorter of median survival time and poorer quality of life [9, 10].

When CRPC occurs, radiotherapy, bone-seeking isotopes therapy, and cytotoxic chemotherapy are viable options. Chemotherapy can reduce serum prostate-specific-antigen (PSA) levels in patients with CRPC, and in some cases can reduce pain [11]. Docetaxel is an m-phase cycle specific drug that promotes tubules aggregation to form stable microtubules and inhibits their depolymerization by binding β-tubulin, thereby significantly reducing the number of tubules and destroying the microtubule reticular structure [12]. Two prospective phase III trials (The TAX 327 and SWOG 99–16), had demonstrated a survival benefit of docetaxel in patients with CRPC patients, with a median survival benefit approximately 2.5-months [11, 13]. In 2004, the US FDA approved docetaxel as a new standard treatment for metastasis CRPC. However, some cases initially respond t poorly to docetaxel-based therapy, and eventually all patients will develop docetaxel-resistance. Therefore, understanding the molecular mechanisms underlying the development of resistance in DCRPC patients and analyzing new biomarkers can help identify new therapeutic targets to prolong the survival and improve the quality of life in patients with DCRPC

Currently, microarrays and bioinformatics analysis are being used to screen for differentially expressed genes (DEGs) in tumorigenesis and epigenetic variations. Researchers can access high-throughput microarray and next-generation

sequence functional genomic data from the international public repository Gene Expression Omnibus (GEO), and download them for free [14]. Multiple biomarkers are highly expressed in CRPC, but the specific markers in DCRPC are unknown. In this study, we downloaded the microarray datasets of GSE33455 and GSE36135 comparing docetaxel-sensitive and resistant cell lines were downloaded from the GEO database [15, 16]. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were applied for functional enrichment and pathway analysis. A protein-protein interaction (PPI) network was constructed using the STRING database. Diseases free survival (DFS) was used for prognostic analysis via GEPIA. Finally, RHOF, ADCY7, DOCK2 and LMO7 are the prognostic related hub genes. These findings contribute to the understanding of the molecular mechanism underlying the development of DCRPC and provide new gene targets for future studies.

2. Methods

2.1 Systematic search and data selection

Using “docetaxel resistant, prostate cancer” as keywords, a systematic search was conducted for human expression profile data in the GEO databases (http://www.ncbi.nlm. nih.gov/geo/) [17]. Finally, series matrix files and data tables of GSE33455 and GSE36135 were downloaded. GSE33455, updated on Mar 12, 2020, is based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array) using two prostate cancer cell lines (PC3 and Du145) and compared six docetaxel-sensitive samples and six docetaxel-resistant samples[15]. GSE36135, updated on Mar 25, 2019, is based on the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array) and GPL571 platform (Affymetrix Human Genome U133A 2.0 Array) using two prostate cancer cell lines (22Rv1 and Du145) and compared six docetaxel-sensitive samples and six docetaxel-resistant samples [16].

2.2 Identification of DEGs

The two microarray datasets were merged together via Perl and batch normalized using the “sva” R package. Then, the integrated file of DEGs were analyzed using the “limma” R package. The screening conditions were set to $|\log_2 FC| > 1$, $P < 0.05$. The “heatmap” R package was used to display the DEGs.

2.3 GO functional enrichment and KEGG pathway analysis

GO analysis is widely used for gene functional classification and gene annotation including biological process (BP), cellular component (CC), and molecular function (MF) [18]. KEGG is a database resource that integrates genomic, chemical and systemic functional information [19, 20]. GO functional enrichment and KEGG pathway analysis of DEGs were investigated through the DAVID website (https://david.ncifcrf.gov/). $P < 0.05$ was considered statistically significant, and the analysis was visualized using the “clusterProfiler” R package.

2.4 PPI network

All DEGs were imported into STRING (https://string-db.org/) to construct a protein-protein interaction network. Protein pair score < 0.4 and non-interacting DEGs will be removed.

2.5 Survival analyses of hub genes

Gene Expression Profile Interactive analysis (GEPIA) (http://gepia.cancer-pku.cn/) is a newly developed web server based on the TCGA and the GTEx projects and contains 9,736 tumor and 8,587 normal samples of RNA sequencing expression data [21]. In this study, DFS analysis of hub gene was performed, and the hazard ratio (HR) P value < 0.05 was considered as prognostic hub genes.

2.6 Software and versions

Perl (version strawberry-perl-5.32.1.1-64bits.msi) was used to merge the data (https://www.perl.org); R software (x64, version 4.0.3) was used for statistical calculations and graphs (https://www.r-project.org/).

3. Results

3.1 Identification of DEGs

A total of 89 DEGs were identified based on GSE33455 and GSE36135, of which 47 genes were up-regulated and 42 genes were down-regulated. The information of these DEGs were shown in Table 1. Volcano plot distribution map and cluster heatmap of these DEGs were shown in Fig. 1a and Fig. 1b respectively.

Table 1
Overlap of DEGs between GSE33455 and GSE36135 datasets.

DEGs	Genes
Up-regulated	ADAM22, TMEM135, FGD6, SPAG9, CDCP1, PBLD, MAGEA5, SMOX, AKAP12, FUT8, RBPMS, MITF, ITGB1, RNF170, NAMPT, RHOF, ERAP2, PCNX2, ZMAT3, CLEC2B, RALGPS1, TAPBP, MCTP1, CRISPLD2, GATM, DSG2, ADAM22, WWP2, VSIG10, RHBDL2, RPS6KA5, CELF2, GPRIN2, SRGN, TMEM135, ADAM22, OSMR, RBPMS, SRGN, WFDC2, CXADR, DYNC2LI1, LMO7, ATF6B, SCEL, RBAK, ATF3
Down-regulated	SIK2, NT5E, LEPR, ADCY7, HACD1, CCL5, IGSF3, MYO10, GNB5, HDAC4, PDS5B, PDS5B, TRAM2, JRKL, GNB5, UBE2W, SYNJ2, PLEKHS1, ATF2, ATP11B, NAV3, CCSER2, SYNCRIP, ZMYND11, NMNAT2, ZBED1, C11orf63, SAMSN1, USP36, DOCK2, CCL5, SERPINB8, PIGK, PLD1, THADA, TAGLN, UGGT2, JMJD6, SOHLH2, ARHGEF2, MVB12B, GRK5

DEGs, differentially expressed genes.

3.2 GO functional enrichment and KEGG pathway analysis of DEGs

GO functional analysis of these DEGs showed that up-regulated DEGs were mainly enriched in viral entry into host cell, antigen processing and presentation of endogenous peptide via MHC class I, integrin-medicated signaling pathway, germ cell migration, positive regulation of transcription from RNA polymerase II promoter in response to endoplasmic reticulum stress, intercalated disc, the extracellular exosome, cell adhesion molecule binding, integrin binding 3 and down-regulated DEGs in chemotaxis, positive regulation of smooth muscle cell migration, protein binding, ATP binding and cellular response to tumor necrosis factor, Rac guanyl-nucleotide exchange factor activity. Barplots of GO enrichment analysis were shown in Fig. 2a and Fig. 2b ($P < 0.05$).

KEGG pathway analysis showed that these DEGs were mainly involved in the TNF signaling pathway; chemokine pathway; nicotinate and nicotinamide metabolism; human cytomegalovirus infection; parathyroid hormone synthesis, secretion and action. Barplot and dotplot of KEGG analysis were shown in Fig. 3a and Fig. 3b respectively ($P < 0.05$).

3.3 Protein-protein interaction (PPI) network

A total of 89 DEGs were imported into STRING to construct the protein-protein interaction network. After removing 59 non-interacting genes, there were 30 genes in the final PPI network finally (Fig. 4). Number of adjacent nodes in PPI network was shown in Fig. 5.

3.4 Identification of prognostic hub gene

In order to investigate the correlation between DEGs expression and prognosis in PCa patients, we performed survival analysis using GEPIA in 492 prostate adenocarcinoma samples. Compared to the low expression group, the high expression group had poorer DFS for three genes and better DFS for one gene (Fig. 6). High expression of RHOF (HR = 1.9, $P < 0.01$), ADCY7 (HR = 1.7, $P = 0.012$) and DOCK2 (HR = 1.6, $P = 0.029$) were risk factor for DFS, while high expression of LMO7 (HR = 0.54, $P < 0.01$) was a protective factor.

3.5 Re-analysis of prognostic hub gene via GO and KEGG

Subsequent GO functional enrichment analysis showed that these four genes were enriched in small GTPase mediated signal transduction, intracellular and cytoskeleton; while KEGG pathway analysis showed that these genes were enriched in chemokine signaling pathway, regulation of lipolysis in adipocytes, adherens junction, GnRH signaling pathway, endocrine resistance and purine metabolism (Table 2) with $P < 0.05$ being a statistically significant difference.

Table 2
Functional and pathway enrichment analysis of hub DEGs.

Category	ID	Term	Count
BP	GO:0007264	small GTPase mediated signal transduction	RHOF, DOCK2
CC	GO:0005622	intracellular	RHOF, DOCK2, ADCY7
CC	GO:0005856	cytoskeleton	RHOF, DOCK2
KEGG	hsa04062	Chemokine signaling pathway	ADCY7, DOCK2
KEGG	hsa04923	Regulation of lipolysis in adipocytes	ADCY7
KEGG	hsa04520	Adherens junction	LMO7
KEGG	hsa04912	GnRH signaling pathway	ADCY7
KEGG	hsa01522	Endocrine resistance	ADCY7
KEGG	hsa00230	Purine metabolism	ADCY7

DEGs, differentially expressed genes *GO*, gene ontology; *BP*, biological process; *CC*, cellular component; *KEGG*: Kyoto Encyclopedia of Genes and Genomes.

Discussion

For PCa, the primary treatment is ADT. When it comes to CRPC, docetaxel chemotherapy is a standard approach, showing significant symptomatic and survival benefits[11, 13]. However, docetaxel resistance in CRPC patients usually occurs after approximately six months of systemic therapy. Multiple mechanisms have been reported to be involved in drug resistance, such as limiting intracellular drug concentrations, impaired drug-induced microtubules stability, and neutralizing cytotoxic effects [22–26]. Therefore, targeting drug-resistance and prognosis-related genes has the potential to improve chemotherapy and survival. The aim of this study was to identify and analyze the functions of hub prognostic genes to help understand the molecular mechanisms underlying the development of DCRPC and to provide novel gene targets for future studies.

After a systematic search, two microarray datasets were included. In GSE33455 and GSE31635, PCa cell lines were converted to docetaxel-resistant cells to compare the gene expression profile of sensitive and resistant cells. To improve the reliability of our results, we performed batch normalization on both datasets before identifying DEGs. Finally, 89 DEGs were identified, of which 47 genes were up-regulated and 42 genes were downregulated. In order to further investigate the functions of these DEGs, we performed a series of bioinformatic analyses.

The cellular mechanism of drug resistance can be generally divided into two categories: inhibition of chemotherapeutic drug delivery to tumor cells and increased genetic and epigenetic alterations affecting drug sensitivity [27]. GO functional analysis had showed that these DEGs were mainly enriched in the extracellular exosome, cell adhesion molecule binding, ATP binding and cellular response to tumor necrosis factor (TNF). Exosomes can serve as effective carriers of chemotherapeutic drugs [28, 29]. However, in drug-resistant cancer cells, this mechanism is blocking, which may lead sensitive cells to drug-resistance [30]. Several studies had identified a role for exosomes in chemotherapy resistance through induction of epithelial mesenchymal transformation (EMT); promotion of antiapoptotic pathways; drug efflux or sequestration; alerted signal transduction and immune cell regulation [31–36]. Cell adhesion molecules (CAMs) are in involved in tumor progression, metastasis. Expression of CD44, a member of the CAMs family, could increase drug efflux and lead to drug-resistance [37]. Targeting CAMs has been reported to inhibit metastasis and drug-resistance [38]. In addition, ATP-binding cassette (ABC) transporters have been reported to be drug-resistance proteins. P-glycoprotein (P-gp), a product of the ABCB1 gene, is an ATP dependent efflux pump that increases drug efflux when substrates are bound to transmembrane regions [39]. TNF plays an important role in cellular autophagy. Previous study found that TNF signaling could promote paclitaxel-resistance in ovarian cancer by inducing autophagy [40]. In addition, TNF inhibitor was also reported to overcome drug-resistance to anti-PD-1 treatment [41].

KEGG pathway analysis revealed that these DEGs are mainly involved in the TNF signaling pathway, chemokine pathway, and nicotinate and nicotinamide metabolism. Several chemokines were found to be highly overexpressed in oxaliplatin-resistant in the HTOXAR3 cell line, however, gene silencing resulted in a slight reversal of resistance [42]. Furthermore, targeting CXCL2 (a chemokine receptor) in taxane-exposed mCRPC model resulted in PCa cells being sensitive to cisplatin [43]. Nicotinamide (NAM) is a vitamin B3 derivative and a precursor of nicotinamide adenine dinucleotide (NAD+). Nicotinamide metabolism is essential for a variety of biological functions in human cells, such as: cellular metabolic processes and DNA repairing [44, 45]. Nicotinamide derivatives have been reported to be effective in reversing P-gp-induced drug-resistance by inhibiting the efflux function [46, 47].

In this study, we identified four prognostic hub genes, RHOF, ADCY7, DOCK2 and LMO7. RHOF is a member of the Rho GTPase family, an oncogene that plays an important role in tumor cell invasion and migration. High expression of RHOF is closely associated with tumor progression and poor survival of patients [48, 49]. Yang et al. found that targeting RHOF could improve the sensitivity of pancreatic cells to gemcitabine treatment through the EMT pathway [49].

Adenylate cyclase 7 (ADCY7) is a member of enzymes involved in ATP metabolism and cAMP production. Although the molecular mechanism of ADCY7 in drug-resistance is not clear. However, ADCY7 has been reported to be involved in inflammatory responses and TNF-α signaling pathway [50], which could be a potential association between ADCY7 and chemotherapy resistance.

DOCK2 (dedicator of cytokinesis 2) is a Rac-specific guanine nucleotide exchange factor for Rho GTPases. Previous studies had demonstrated the role of DOCK2 in cell proliferation, migration and invasion and DNA damage response [51, 52]. Wu et al. found that targeting DOCK2 improved the sensitivity of leukemia cells to chemotherapy by modulating DNA repairing pathway [53].

LIM domain only protein 7 (LMO7) is a fibrous actin-binding protein that belongs to PDZ and LIM domain-containing protein family and functions as protein–protein recognition modules [54]. LMO7 was found to be located in cell membrane, cytoskeleton and intracellular nucleus and is involved in cell invasion and migration [55, 56]. LMO7 deficiency leads to enhanced TGF-β signaling pathway and exacerbates extracellular matrix (ECM) deposition [57]. In mice model, lung cancer was found in 22% of LMO7(-/-) mice compared to only 13% of LMO7(+/-) mice [54]. Previous study had also shown that targeting LMO7 lead to drug-resistance [58].

In summary, GO and KEGG enrichment analysis confirmed the functions and pathways of DEGs. In addition, our study identified RHOF, ADCY7, DOCK2 and LMO7 as prognostic hub gene. Although several studies had been conducted to investigate these gens, the mechanism of CRPC docetaxel-resistance is not clear. The present study will contribute to the understanding of the molecular mechanism of DCRPC development and provide new gene targets for future studies.

Declarations

ETHICS APPROVAL

This article is based on public data. Therefore, ethics and informed consent of patients are not involved.

CONSENT FOR PUBLICATION

All authors approved the final version and its publication.

AVAILABILITY OF DATA AND MATERIALS

Series matrix files and data tables of GSE33455 and GSE36135 were downloaded from GEO databases (http://www.ncbi.nlm. nih.gov/geo/).

CONFLICTS OF INTEREST

All authors have no conflict of interest.

FUNDING

This study was funded by The National Natural Science Foundation of China (No. 81872089, 81370849, 81672551, 81300472, 81070592, 81202268, 81202034); Six talent peaks project in Jiangsu Province, Jiangsu Provincial Medical Innovation Team (CXTDA2017025); Natural Science Foundation of Jiangsu Province (BK20161434, BL2013032, BK20150642 and BK2012336).

AUTHOR CONTRIBUTIONS

RJL and SYL contributed equally to this manuscript. Study design, data extraction and analysis: RJL and SYL. Manuscript writing: RJL, SYL, LQL and BX. Manuscript reviewed and revised: RJL, BX and MC.

ACKNOWLEGEMENTS

We thank all the study authors for the support in this study.

References

1. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer Statistics, 2017.* CA Cancer J Clin, 2017. **67(1)**(1542-4863 (Electronic)): p. 7-30.
2. Huggins, C., *EFFECT OF ORCHIECTOMY AND IRRADIATION ON CANCER OF THE PROSTATE.* Ann Surg., 1941. **115(6)** (0003-4932 (Print)): p. 1192-200.
3. Karantanos, T., T.C. Corn Pg Fau - Thompson, and T.C. Thompson, *Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches.* Oncogene., 2013. **32(49)** (1476-5594 (Electronic)): p. 5501-11.
4. Ruizeveld de Winter, J.A., et al., *Androgen receptor status in localized and locally progressive hormone refractory human prostate cancer.* Am J Pathol, 1994. **144(4)**(0002-9440 (Print)): p. 735-46.

5. Chen, Y., H.I. Sawyers Cl Fau - Scher, and H.I. Scher, *Targeting the androgen receptor pathway in prostate cancer.* Curr Opin Pharmacol, 2008. **8(4)**(1471-4892 (Print)): p. 440-8.
6. Dehm, S.M., et al., *Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance.* Cancer Res., 2008. **68(13)**(1538-7445 (Electronic)): p. 5469-77.
7. Gottlieb, B., et al., *The androgen receptor gene mutations database (ARDB): 2004 update.* Hum Mutat., 2004. **23(6)** (1098-1004 (Electronic)): p. 527-33.
8. Sun, S., et al., *Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant.* J Clin Invest., 2010. **120(8)**(1558-8238 (Electronic)): p. 2715-30.
9. Inoue, T., et al., *Prevalence of skeletal complications and their impact on survival of hormone refractory prostate cancer patients in Japan.* Urology, 2009. **73(5)**(1527-9995 (Electronic)): p. 1104-9.
10. Kato, I., A.G. Severson Rk Fau - Schwartz, and A.G. Schwartz, *Conditional median survival of patients with advanced carcinoma: surveillance, epidemiology, and end results data.* Cancer, 2001. **C92(8)**(0008-543X (Print)): p. 2211-9.
11. Tannock, I.F., et al., *Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer.* N Engl J Med, 2004. **351(15)**(1533-4406 (Electronic)): p. 1502-12.
12. Fabbri, F., et al., *Mitotic catastrophe and apoptosis induced by docetaxel in hormone-refractory prostate cancer cells.* J Cell Physiol, 2008. **217(2)**(1097-4652 (Electronic)): p. 494-501.
13. Petrylak, D.P., et al., *Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer.* N Engl J Med, 2004. **351(15)**(1533-4406 (Electronic)): p. 1513-20.
14. Barrett, T., et al., *NCBI GEO: archive for functional genomics data sets--update.* Nucleic Acids Res. , 2013(1362-4962 (Electronic)).
15. Marín-Aguilera, M., et al., *Identification of docetaxel resistance genes in castration-resistant prostate cancer.* Mol Cancer Ther, 2012. **11(2)**(1538-8514 (Electronic)): p. 329-39.
16. Domingo-Domenech, J., et al., *Suppression of acquired docetaxel resistance in prostate cancer through depletion of notch- and hedgehog-dependent tumor-initiating cells.* Cancer Cell 22(3), 2012(1878-3686 (Electronic)): p. 373-88.
17. Clough, E. and T. Barrett, *The Gene Expression Omnibus Database.* Methods Mol Biol, 2016. **1418**(1940-6029 (Electronic)): p. 93-110.
18. Ashburner, M., et al., *Gene ontology: tool for the unification of biology. The Gene Ontology Consortium.* Nat Genet, 2000. **25(1)**(1061-4036 (Print)): p. 25-9.
19. Kanehisa, M. and S. Goto, *KEGG: kyoto encyclopedia of genes and genomes.* Nucleic Acids Res, 2000. **.28(1)**(0305-1048 (Print)): p. 27-30.
20. Ogata, H., et al., *KEGG: Kyoto Encyclopedia of Genes and Genomes.* Nucleic Acids Res, 1999. **27(1)**(0305-1048 (Print)): p. 29-34.
21. Tang, Z., et al., *GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses.* Nucleic Acids Res, 2017. **45(W1)**(1362-4962 (Electronic)): p. W98-W102.
22. Mahon, K.L., et al., *Cytokine profiling of docetaxel-resistant castration-resistant prostate cancer.* Br J Cancer, 2015. **112(8)**(1532-1827 (Electronic)): p. 1340-8.
23. West, K.A., P.A. Castillo Ss Fau - Dennis, and P.A. Dennis, *Activation of the PI3K/Akt pathway and chemotherapeutic resistance.* Drug Resist Updat, 2002. **5(6)**(1368-7646 (Print)): p. 234-48.
24. Fizazi, K., et al., *Randomised phase II study of siltuximab (CNTO 328), an anti-IL-6 monoclonal antibody, in combination with mitoxantrone/prednisone versus mitoxantrone/prednisone alone in metastatic castration-resistant prostate cancer.* Eur J Cancer, 2012. **48(1)**(1879-0852 (Electronic)): p. 85-93.
25. Duran, G.E., et al., *Mechanisms of resistance to cabazitaxel.* Mol Cancer Ther, 2015. **14(1)**(1538-8514 (Electronic)): p. 193-201.

26. Galletti, G., et al., *ERG induces taxane resistance in castration-resistant prostate cancer.* Nat Commun, 2014. **5**(2041-1723 (Electronic)): p. 5548.
27. Gottesman, M.M., S.E. Fojo T Fau - Bates, and S.E. Bates, *Multidrug resistance in cancer: role of ATP-dependent transporters.* Nat Rev Cancer, 2002. **2(1)**(1474-175X (Print)): p. 48-58.
28. Munagala, R., et al., *Bovine milk-derived exosomes for drug delivery.* Cancer Lett, 2016. **371(1)**(1872-7980 (Electronic)): p. 48-61.
29. Saari, H., et al., *Microvesicle- and exosome-mediated drug delivery enhances the cytotoxicity of Paclitaxel in autologous prostate cancer cells.* J Control Release, 2015. **220(Pt B)**(1873-4995 (Electronic)): p. 727-37.
30. Corrado, C., et al., *Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications.* Int J Mol Sci, 2013. **14(3)**(1422-0067 (Print)): p. 5338-66.
31. Wang, M., et al., *Paclitaxel-resistant gastric cancer MGC-803 cells promote epithelial-to-mesenchymal transition and chemoresistance in paclitaxel-sensitive cells via exosomal delivery of miR-155-5p.* Int J Oncol, 2019. **54(1)**(1791-2423 (Electronic)): p. 326-338.
32. Liu, T., et al., *Exosomes containing miR-21 transfer the characteristic of cisplatin resistance by targeting PTEN and PDCD4 in oral squamous cell carcinoma.* Acta Biochim Biophys Sin (Shanghai), 2017. **49(9)**(1745-7270 (Electronic)): p.:808-816.
33. Binenbaum, Y., et al., *Transfer of miRNA in Macrophage-Derived Exosomes Induces Drug Resistance in Pancreatic Adenocarcinoma.* Cancer Res, 2018 **78(18)**(1538-7445 (Electronic)): p. 5287-5299.
34. Hu, Y.B., et al., *Exosomal Wnt-induced dedifferentiation of colorectal cancer cells contributes to chemotherapy resistance.* Oncogene, Oncogene. 2019 Mar;38(11):1951-1965. doi: 10.1038/s41388-018-0557-9. **38(11)**(1476-5594 (Electronic)): p. 1951-1965.
35. Hellwinkel, J.E., et al., *Glioma-derived extracellular vesicles selectively suppress immune responses.* Neuro Oncol, 2016. **18(4)**(1523-5866 (Electronic)): p. 497-506.
36. Mashouri, L., et al., *Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance.* Mol Cancer, 2019. **18(1)**(1476-4598 (Electronic)): p. 75.
37. Hoofd, C., et al., *CD44 promotes chemoresistance in T-ALL by increased drug efflux.* Exp Hematol, 2016. **44(3)**(1873-2399 (Electronic)): p. 166-71.
38. Sun, L., et al., *miR-302a Inhibits Metastasis and Cetuximab Resistance in Colorectal Cancer by Targeting NFIB and CD44.* Theranostics, 2019 **9(26)**(1838-7640 (Electronic)): p. 8409-8425.
39. Sauna, Z.E. and S.V. Ambudkar, *Evidence for a requirement for ATP hydrolysis at two distinct steps during a single turnover of the catalytic cycle of human P-glycoprotein.* Proc Natl Acad Sci U S A, 2000. **97(6)**(0027-8424 (Print)): p. 2515-20.
40. Zhang, S.F., et al., *TXNDC17 promotes paclitaxel resistance via inducing autophagy in ovarian cancer.* Autophagy, 2015. **11(2)**(1554-8635 (Electronic)): p. 225-38.
41. Bertrand, F., et al., *TNFα blockade overcomes resistance to anti-PD-1 in experimental melanoma.* Nat Commun, 2017. **8(1)**(2041-1723 (Electronic)).
42. Ruiz de Porras, V., et al., *Curcumin mediates oxaliplatin-acquired resistance reversion in colorectal cancer cell lines through modulation of CXC-Chemokine/NF-κB signalling pathway.* Sci Rep. **6**(2045-2322 (Electronic)): p. 24675.
43. Ruiz de Porras, V., et al., *Taxane-induced Attenuation of the CXCR2/BCL-2 Axis Sensitizes Prostate Cancer to Platinum-based Treatment. LID - S0302-2838(20)30778-8 [pii] LID - 10.1016/j.eururo.2020.10.001 [doi].* Eur Urol, 2020. **S0302-2838(20)**(1873-7560 (Electronic)): p. 30778-8.
44. Fang, E.F., et al., *NAD(+) in Aging: Molecular Mechanisms and Translational Implications.* Trends Mol Med, 2017. **23(10)**(1471-499X (Electronic)): p. 899-916.

45. Weinreb, O., et al., *Effect of long-term treatment with rasagiline on cognitive deficits and related molecular cascades in aged mice.* Neurobiol Aging, 2015. **36(9)**(1558-1497 (Electronic)): p. 2628-36.
46. Qiu, Q., et al., *Exploration of 2-((Pyridin-4-ylmethyl)amino)nicotinamide Derivatives as Potent Reversal Agents against P-Glycoprotein-Mediated Multidrug Resistance.* J Med Chem, 2017. **60(7)**(1520-4804 (Electronic)): p. 2930-2943.
47. Mi, Y.J., et al., *Apatinib (YN968D1) reverses multidrug resistance by inhibiting the efflux function of multiple ATP-binding cassette transporters.* Cancer Res, 2010. **70(20)**(1538-7445 (Electronic)): p. 7981-91.
48. Li, S., et al., *RHOF promotes hepatocellular carcinoma metastasis by altering the metabolic status of cancer cells via RAB3D. LID - 10.1002/hep.31641 [doi].* Hepatology, 2020 (1527-3350 (Electronic)).
49. Yang, R.M., et al., *miR-3656 expression enhances the chemosensitivity of pancreatic cancer to gemcitabine through modulation of the RHOF/EMT axis.* Cell Death Dis, 2017. **8(10)**(2041-4889 (Electronic)): p. e3129.
50. Luo, Y., et al., *Exploring the genetic architecture of inflammatory bowel disease by whole-genome sequencing identifies association at ADCY7.* Nat Genet, 2017. **49(2)**(1546-1718 (Electronic)): p. 186-192.
51. Hasan, M.K., et al., *Wnt5a induces ROR1 to recruit DOCK2 to activate Rac1/2 in chronic lymphocytic leukemia.* Blood, 2018. **132(2)**(1528-0020 (Electronic)): p. 170-178.
52. Fukui, Y., et al., *Haematopoietic cell-specific CDM family protein DOCK2 is essential for lymphocyte migration.* Nature, 2001. **412(6849)**(0028-0836 (Print)): p. 826-31.
53. Wu, M., et al., *FLT3-ITD cooperates with Rac1 to modulate the sensitivity of leukemic cells to chemotherapeutic agents via regulation of DNA repair pathways.* Haematologica, 2019. **104(12)**(1592-8721 (Electronic)): p. 2418-2428.
54. Tanaka-Okamoto, M., et al., *Increased susceptibility to spontaneous lung cancer in mice lacking LIM-domain only 7.* Cancer Sci, 2009 **100(4)**(1349-7006 (Electronic)): p. 608-16.
55. Ooshio, T., et al., *Involvement of LMO7 in the association of two cell-cell adhesion molecules, nectin and E-cadherin, through afadin and alpha-actinin in epithelial cells.* J Biol Chem, 2004. **279(30)**(0021-9258 (Print)): p. 31365-73.
56. Hu, Q., et al., *LMO7 mediates cell-specific activation of the Rho-myocardin-related transcription factor-serum response factor pathway and plays an important role in breast cancer cell migration.* Mol Cell Biol, 2011 **31(16)**(1098-5549 (Electronic)): p. 3223-40.
57. Xie, Y., et al., *LMO7 Is a Negative Feedback Regulator of Transforming Growth Factor β Signaling and Fibrosis.* Circulation, 2019. **139(5)**(1524-4539 (Electronic)): p. 679-693.
58. Wu, H., et al., *Circulating exosomal microRNA-96 promotes cell proliferation, migration and drug resistance by targeting LMO7.* J Cell Mol Med, 2017. **21(6)**(1582-4934 (Electronic)): p. 1228-1236.

Figures

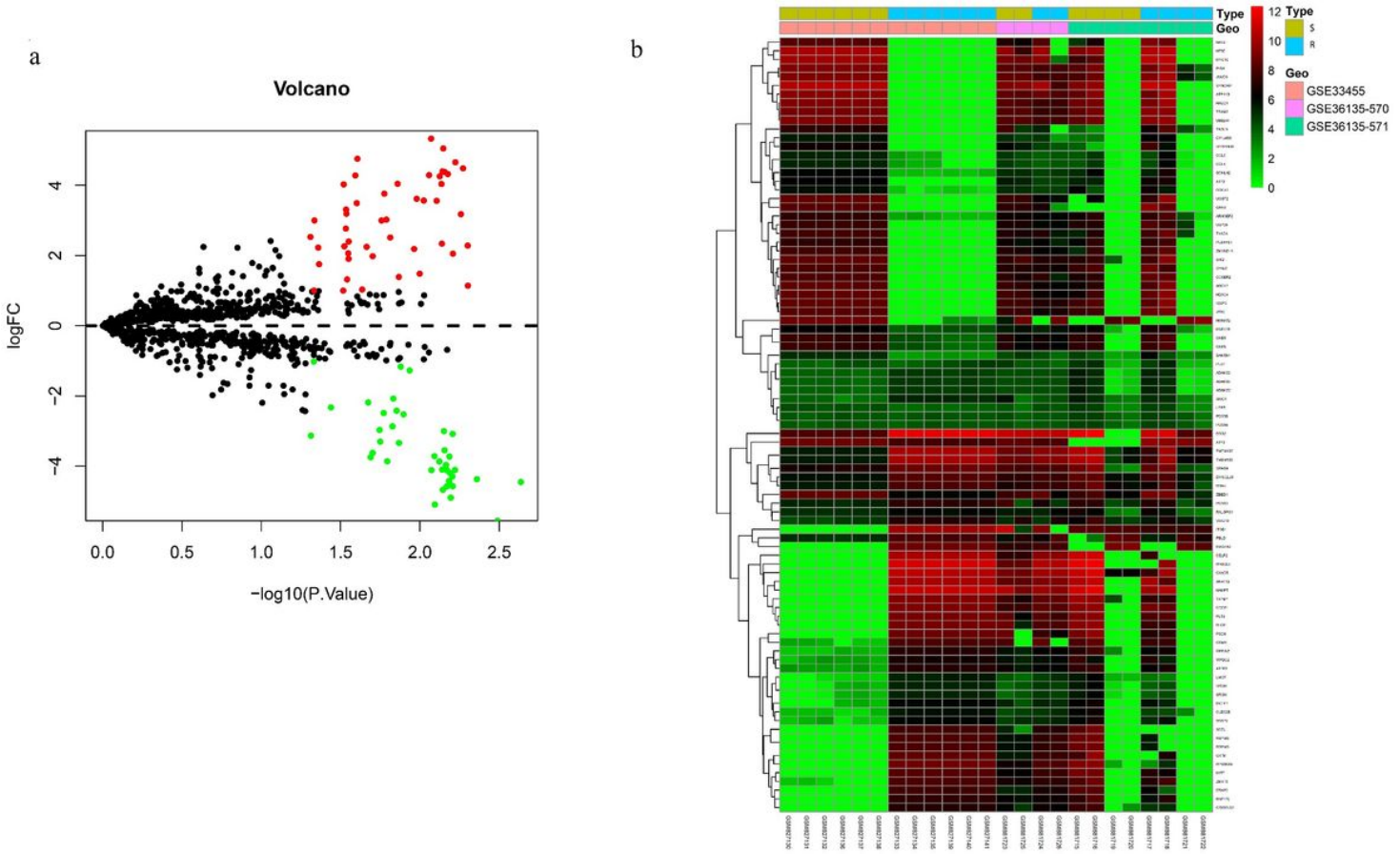

Figure 1

Volcano plot distribution and heatmap of the DEGs. The (a) volcano plot of GSE33455 and GSE36135. The red points indicate up-regulated DEGs, the green points indicate down-regulated DEGs, and the gray points with no significant difference; (b) DEGs heatmap of GSE33455 and GSE36135. From red to green, the expression level of the gene in the samples gradually decreases. All DEGs are screened based on P value <0.05, |log2 FC |> 1. (DEGs, differentially expressed genes; S, docetaxel-sensitive; R, docetaxel-resistant)

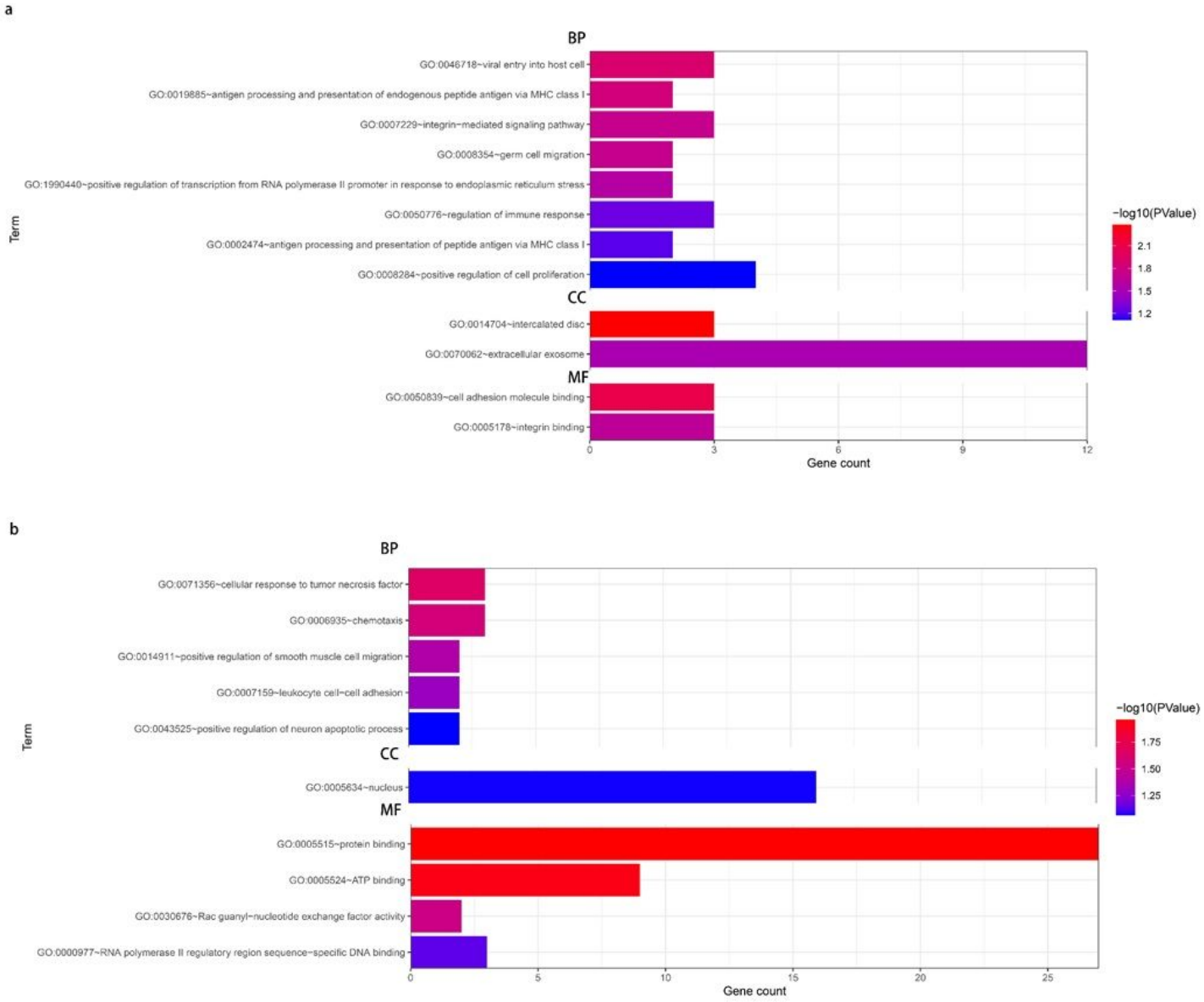

Figure 2

Functional enrichment analysis of (a) up-regulated and (b) down-regulated differentially expressed genes. BP, biological process; CC, cellular component; MF, molecular function.

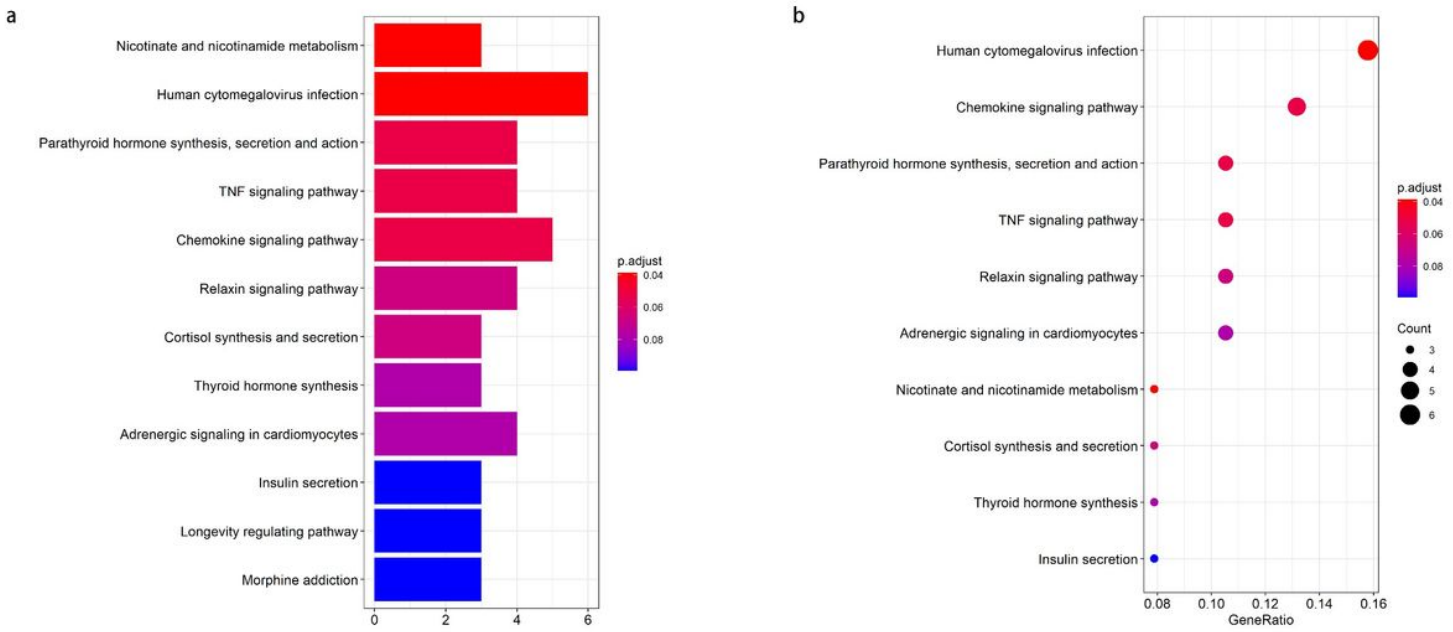

Figure 3

KEGG pathway analysis (a) barplot and (b) dotplot of DEGs (DEGs, differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes).

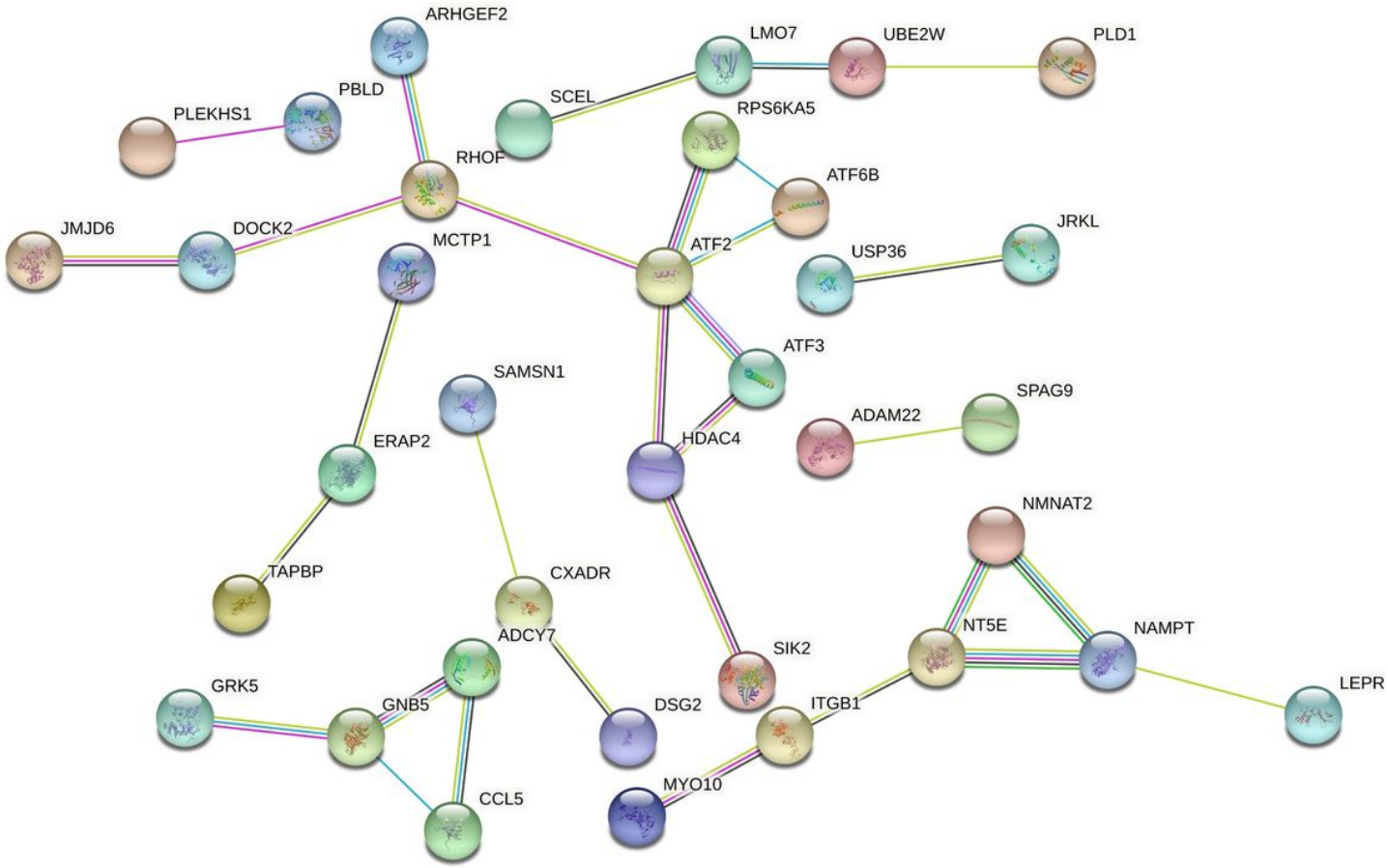

Figure 4

Protein-Protein Interaction (PPI) network of differentially expressed genes (DEGs)

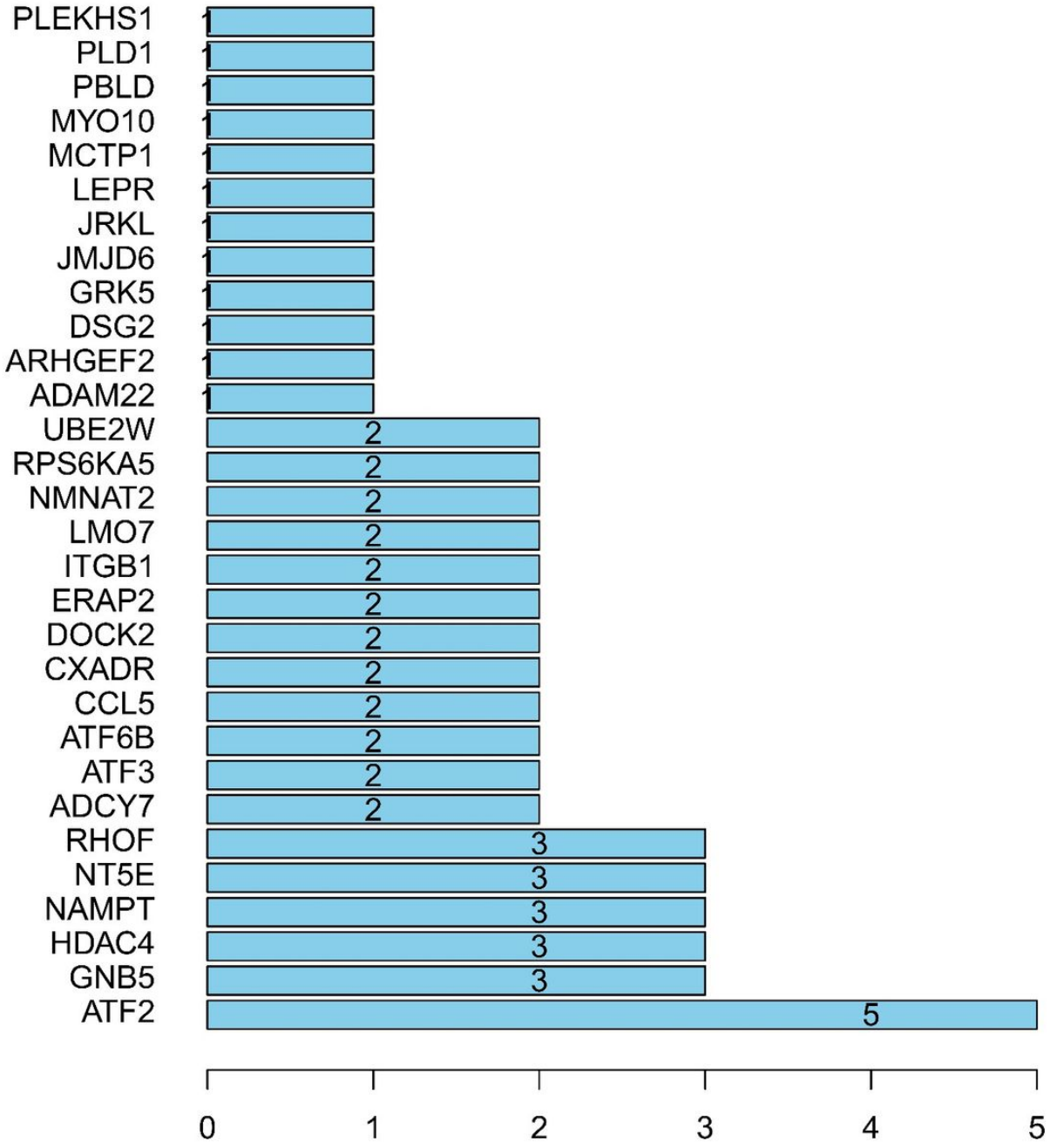

Figure 5

Number of adjacent nodes in Protein-Protein Interaction (PPI) network.

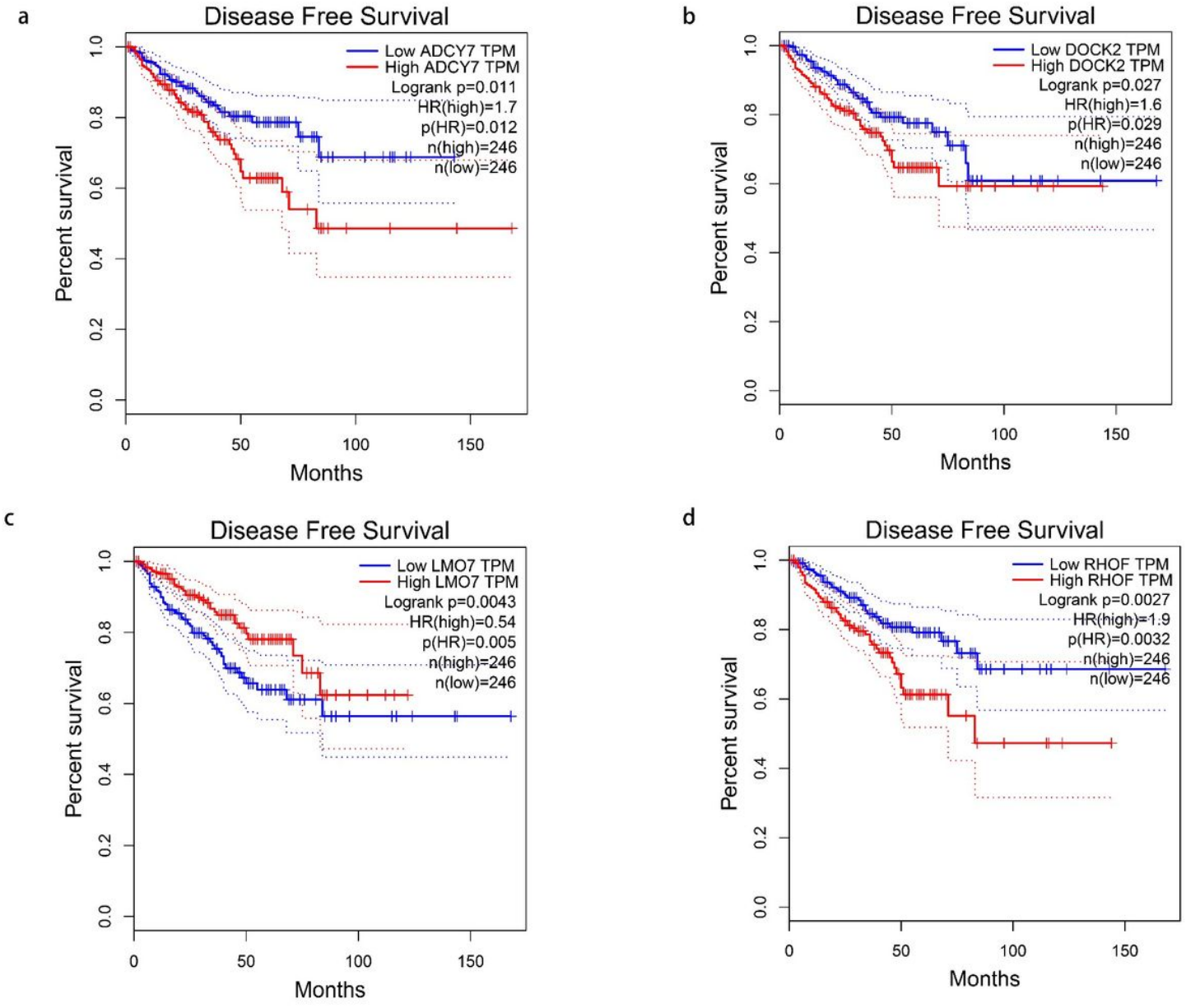

Figure 6

Diseases free survival (DFS) analysis of hub differentially expressed genes (DEGs). DFS analysis of (a) ADCY7, (b) DOCK2, (c) LMO7, (d) RHOF in prostate cancer. $P < 0.05$ was considered statistically significant.