

Gene Network Analysis and Key Genes Associated with Biological Pathways Involved in Docetaxel-Resistant Prostate Cancer Cells *In Silico*

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Abstract Resistance of drug has been a serious problem in cancer treatment and docetaxel is an anti-microtubule agent with antitumor activity in prostate cancer. Therefore, it is necessary to investigate key genes and understand the molecular mechanisms of prostate cancer cell resistance to docetaxel. In the present study, combined analysis of microarray data by R packages to identify the mechanisms behind resistant prostate cancer cells against docetaxel was achieved. The top-upregulated differentially expressed genes (DEGs) included IGFBP3, ABCB1, GSPT2, ROBO1, FSTL1, NID2, S100A4, CDH1 and top-downregulated DEGs were detected as CD24, ZNF587B, OCLN, ADGRG2, POP7. Common gene ontology (GO) in down and upregulated genes mainly related to regulation of biological process, cell death, stimulus response and process of apoptotic. The TFs related to upregulated genes mainly included SRF, TBPs, POLR2A, TAF1 and RREB and downregulated genes as IRFs, ZEB1, PRDM1, SPI1, JUNs, FOSs and NFKBs. According to protein-protein interaction (PPI) analysis, COL4A1, LOX, SMAD2, TIMP1 and FOS as top-upregulated hub genes and EXO1, RRM2, CDC45, KIAA0101, MCM10 and HMMR as top-downregulated hub DEGs were identified. In addition, top-upregulated consensus hub genes including LOX, SERPINE1, FOS and PTK2 and top-downregulated consensus hub genes including BRCA1, EGFR, IL6 and CXCL8 were identified by twelve methods. Accordingly, the upregulated hub genes mostly related to prognosis and invasion of tumors, apoptosis and oncogene. Moreover, the hub downregulated genes mainly associated to tumor progression, metastasis and proliferation of prostate cancer cells. Some key genes of the present study were compared with reported studies as validation based on external literature and experimental molecular tests including qRT-PCR in vitro and clinical validation are required. The identified key genes could be potential targets for improving the efficacy of prostate cancer treatment as well as understanding the molecular mechanisms in docetaxel-resistant prostate cancer cells.

Key Words Prostate Cancer, DEGs, Docetaxel Resistance, PPI

INTRODUCTION

Prostate cancer is the most common cancer diagnosed among men worldwide and the second most common cause of cancer death [1]. However, prostate cancer can be classified to castration-resistant and hormone-dependent prostate cancer. According to previous reports related to prostate cancer, apoptosis plays an important role in the chemotherapy response, suggesting a relationship between apoptosis induced by drug and treatment efficiency [2]. The resistance of drug is a critical issue for cancer treatment but its underlying mechanisms are unclear.

Docetaxel is an anti-microtubule drug with antitumor activity in different cancers, such as prostate cancer, which acts by binding to tubulin and disrupting the

balance between disassembly during mitosis and microtubule assembly [3]. It was previously reported which docetaxel downregulates some genes related to proliferation of cell, formation of mitotic spindle, transcription factors and oncogenesis and upregulates some genes associated with apoptosis induction and cell cycle arrest in prostate cancer cells [4]. The mechanism of resistant prostate cancer to docetaxel is related to different factors, such as expression of androgen receptor splice variant, β -tubulin expression alternations, multidrug resistance caused by unnatural expression of the ATP binding cassette (ABC) transporter family and unnatural expression of NF-k β /interleukin (IL) 6 and PI3K/AKT/mTOR signaling pathways [5].

CDH1 gene has identified in PC-3 and DU145 cells resistant to docetaxel and confirmed in prostate tumors from patients resistant to docetaxel [6]. According to the results of Jiang *et al* [7], mutation of the CDH1 gene is related to invasion and metastasis in various types of cancer, because it alters the transcriptional activity of epithelial cells. Previous research has shown that enhanced expression of the ABCB1 gene causes resistance to docetaxel and that ABCB1 expression is associated with prostate tumors [8].

Calcium-binding protein S100 A4 (S100A4) plays an important role in the cancer metastasis progression and has been reported to mediate migration, invasion and prostate cancer apoptosis and also plays a role in the extracellular and intracellular regions of tumors [9]. TSPAN1 is a novel member of the tetraspanin family and its role in the progression of tumor has been reported and it can enhance the proliferation and invasion of tumor cell in the laboratory and its expression is increased in human tumor cells [10].

Results from studies that combine different results in the form of meta-analysis are more reliable and provide a better answer than single results. In cancer-related studies, results from meta-analysis provide a better understanding of molecular mechanisms. Microarrays technology has allowed us to simultaneously examine the expression of more genes and identify the molecular mechanism involved in the resistance of prostate cancer cells to docetaxel. Therefore, the results from the integration of various studies could lead to the identification of important genes that are associated with the molecular mechanisms of prostate cancer cell resistance to docetaxel. Generally, this study provides information about regulatory genes, transcription factors (TFs) and gene ontology (GO) related to docetaxel resistance in prostate cancer.

METHODS

To investigate the expression of microarray genes associated with docetaxel-resistant prostate cancer cells were extracted through the Gene Expression Omnibus (GEO). The samples tested in this study after quality control are listed in the Table 1. The general methodology, from raw data extraction to identification of key genes and related work in this research, is presented in Figure 1.

Meta-Analysis and Differentially Expressed Genes (DEGs)

Microarray expression data were pre-analyzed using the R language package. Data control was done using boxplot and Heatmap (S2). The background correction and quantile normalization were performed on the unnormalized data. The Limma software package was used to perform various

analyses on the selected data and finally a linear model was drawn. An empirical model of simple Bayesian was applied for correct standard errors [11]. For unadjusted pooled p-values, the log-sum Fisher's exact approach was used and the 'fdr' method was used to apply the p.adjust function in meta-RNASeq to adjust the combined p-values for the microarray expression data (S3). The genes with p-values ≤ 0.01 and $\log_2\text{FoldChange} > 1$ were used as DEGs. The method and analyses used on each GSEs (for example GSE33455) to extract DEGs are shown in Supplementary file (S1).

GO and Biological Pathways

Enrichment analysis for GO was performed for genes with increased (up genes) and decreased (down genes) expression metaDEGs using g.profiler, (<https://biit.cs.ut.ee/gprofiler/gost>). Shared GO terms for up and downregulated genes (metaDEGs) were identified through the web tool Venny v2.1. Biological pathways related to metaDEGs for up and downgenes were identified through Kyoto Genes and Genomes Encyclopedia (KEGG) (<https://www.kegg.jp/kegg/pathway.html>).

Investigation of Motifs and TFs Related to Promoters

For the analysis TFs and motifs, the enrichment score threshold of 3.5 and a minimum similarity between orthologous genes of 0.05 were chosen. For this analysis, the obtained results related to DEGs of up and downregulated genes were analyzed using iRegulon through Cytoscape 3.6.1 software.

Identifying Hub Genes Through PPI

To analyze the Protein-Protein Interaction (PPI) of up and down regulated genes, the STRING (version db 12) was used and the PPI gene network was drawn using the CytoHubba plugin in Cytoscape 3.6.1. Gene network was drawn using different methods including EPC, Bottle Neck, Closeness, Degree, DMNC, EcCentricity, MNC, Betweenness, Stress, ClusteringCoefficient, Radiality and MCC for the identified up- and down-regulated genes and high-ranking genes (high degree) were selected as central genes. The CytoHubba plugin identified more relevant genes as central genes in the biological network using various methods [12].

Validation of DEGs for Up and Downregulated Genes Through Reported Studies

Based on the genes obtained from this study related to DEGs, the gene expression of eight genes (four upregulated and four downregulated genes) was compared with reported

Table 1: Docetaxel-Resistant Prostate Cancer Cell Samples Studied from a Meta-Analysis of Microarray Data

Platform	Accession number	Cell lines	Resistant:Sensitive	Status of cells selected for testing
GPL571	GSE36135	DU145 cell line 22Rv1 cell line	3:3 3:3	Cell lines such as DU-145 and 22RV1 were obtained from American Type Culture Collection (ATCC)
GPL6244	GSE47040	TaxR cell line C4-2B cell line	1:1	cells lines were obtained from the ATCC.
GPL26944	GSE158494	DU-145 cell line PC-3 cell line	3:3 3:3	Tumor samples from patients diagnosed with mCRPC treated with D or CZ.
GPL570	GSE33455	DU-145 cell line PC-3 cell line	3:3 3:3	Patients were given docetaxel-based therapy after removal of the obstruction through the urethra in a palliative manner or after biopsy after tumor spread.

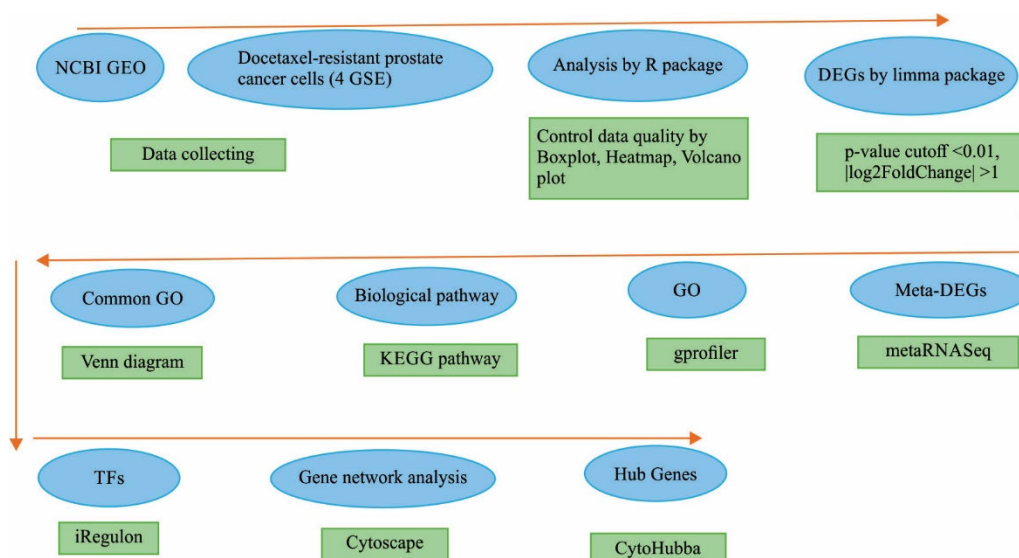


Figure 1: Meta-Analysis Scheme of Microarray Data

qRT-PCR studies. For final confirmation, it is suggested that the expression of these genes be experimentally confirmed and tested in the laboratory so that these genes can be introduced as key and effective genes associated with docetaxel-resistant prostate cancer cells.

RESULTS

Identify of DEGs

The present study showed 373 upregulated and 558 downregulated genes associated with prostate cancer cells resistant to docetaxel ($p\text{-value} \leq 0.05$ and $|\log_2\text{FoldChange}| > 1$). Moreover, top down and upregulated meta-DEGs ($|\log_2\text{FoldChange}| > 1.5$ and meta-pvalue ≤ 0.05) related to docetaxel-resistant prostate cancer cells are represented in Table 2. Accordingly, the upregulated genes involved in docetaxel-resistant prostate cancer cell lines mainly included IGFBP3, ABCB1, GSPT2, ROBO1, FSTL1, NID2, S100A4, CDH1 and ADAMTS1 and downregulated genes mainly related to CD24, ZNF587B, PARP2, MIR4271, OCLN, TSPAN1, ADGRG2, MPZL2 and POP7.

GO and biological pathways

To identify meaningful ($p < 0.05$ and $|\log_2\text{FoldChange}| > 1$) GO terms for biological processes related to resistant prostate cancer cells to docetaxel, 145 and 187 terms were enriched in down and upregulated genes, respectively (Figure 2). Shared GO terms in down and upregulated genes are mainly related to the regulation of biological processes, cell death, stimulus response and apoptotic process (Table 3). According to the top 15 GO, GO terms in the upregulated genes mostly associated with the regulation of cell differentiation, signal transduction, cell death and apoptotic process; and in the downregulated genes mainly belonged to response to chemicals, defense and immune system process, cell death and proliferation (Table 4).

According to our results, the down and up meta-DEGs by KEGG pathways mainly related to cancer pathways

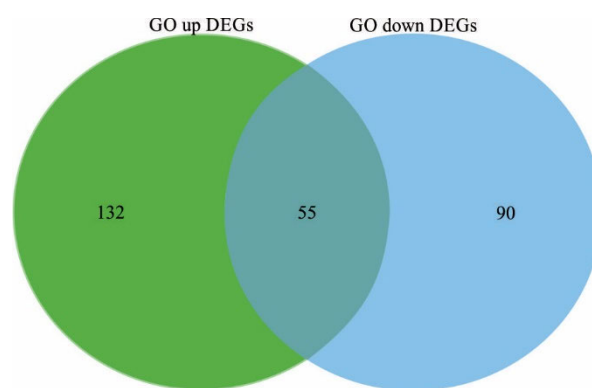


Figure 2: Common GO Term of Up and Downregulated Genes

(up genes such as HMOX1, GSTP1 and CEBPA and down gene such as CSF2RA), PI3K-Akt signaling pathway (up genes such as PTK2 and ITGAV and BRCA1 as down gene), cytokine-cytokine receptor interaction (up genes such as IL13RA2 and TNFSF4 and down gene such as IL15RA, CSF2RA and LTB), MAPK signaling pathway (up genes such as FOS and JUND and down gene such as FAS), cancer transcriptional misregulation (up genes such as ZEB1 and down gene such as PLAU and IL1R2), MicroRNAs in cancer (EZH2, GLS2 and KIF23 as down genes), apoptosis (up genes such as TUBA1A and LMNB1 and PARP2 as down genes), p53 signaling pathway (up genes such as TP53I3 and IGFBP3 and FAS and BID as down genes), prostate cancer (ZEB1 as up gene and PLAU and IL1R2 as down genes) and DNA replication (PRIM1 and RFC2/5 as down genes).

Identification of TFs

The motifs and TFs of down and up meta-DEGs in resistant prostate cancer cells to docetaxel are shown in S Table 1. The dominant TFs associated with upregulated genes

Table 2: Key Genes with High log2FC Related to Prostate Cancer Cells Resistant to Docetaxel

Upregulated genes					
Gene.ID	Gene Stable ID	Gene name	Log2FC	Meta-pvalue	Description
3486	ENSG00000146674	IGFBP3	2.97	0	Insulin like growth factor binding protein 3
5243	ENSG00000085563	ABCB1	2.89	1.71E-07	ATP binding cassette subfamily B member 1
23708	ENSG00000189369	GSPT2	2.66	0	G1 to S phase transition 2
5244	ENSG00000005471	ABCB4	2.63	3.11E-06	ATP binding cassette subfamily B member 4
6091	ENSG00000169855	ROBO1	2.61	0	Roundabout guidance receptor 1
11167	ENSG00000163430	FSTL1	2.23	0	Follistatin like 1
22795	ENSG00000087303	NID2	2.22	0	Nidogen 2
6275	ENSG00000196154	S100A4	2.15	0	S100 calcium binding protein A4
1010	ENSG00000154162	CDH1	2.07	0	Cadherin 1
9510	ENSG00000154734	ADAMTS1	2.06	0	ADAM metalloproteinase with thrombospondin
8277	ENSG00000007350	TKTL1	1.96	0	Transketolase like 1
5654	ENSG00000166033	HTRA1	1.91	0	HtrA serine peptidase 1
3398	ENSG00000115738	ID2	1.88	0	Inhibitor of DNA binding 2
5270	ENSG00000135919	SERPINE2	1.88	0	Serpin family E member 2
55076	ENSG00000181458	TMEM45A	1.83	0	Transmembrane protein 45A
2669	ENSG00000164949	GEM	1.81	0	GTP binding protein overexpressed in skeletal muscle
10962	ENSG00000213190	MLLT11	1.68	0	MLLT11 transcription factor 7 cofactor
57088	ENSG00000114698	PLSCR4	1.66	0	Phospholipid scramblase 4
4162	ENSG00000076706	MCAM	1.66	0	Melanoma cell adhesion molecule
2690	ENSG00000112964	GHR	1.66	0	Growth hormone receptor
668	ENSG00000183770	FOXL2	1.58	0	Forkhead box L2
79901	ENSG00000071967	CYBRD1	1.56	0	Cytochrome b reductase 1
8349	ENSG00000184678	H2BC21	1.53	0	H2B clustered histone 21
1287	ENSG00000188153	COL4A5	1.52	0	Collagen type IV alpha 5
Downregulated genes					
Gene.ID	Gene Stable ID	Gene name	Log2FC	Meta-pvalue	Description
100133941	ENSG00000272398	CD24	-3.32	0	CD24 molecule
100293516	ENSG00000269343	ZNF587B	-2.98	0	Zinc finger protein 587B
10038	ENSG00000129484	PARP2	-2.82	0	Poly (ADP-ribose) polymerase 2
100422952	ENSG00000264633	MIR4271	-2.66	0	microRNA 4271
100506658	ENSG00000197822	OCLN	-2.55	0	Occludin
10103	ENSG00000117472	TSPAN1	-2.42	0	Tetraspanin 1
10149	ENSG00000173698	ADGRG2	-2.30	0	Adhesion G protein-coupled receptor G2
10205	ENSG00000149573	MPZL2	-2.21	0	Myelin protein zero like 2
10248	ENSG00000172336	POP7	-2.15	0	POP7 homolog
10279	ENSG00000112812	PRSS16	-1.85	0	Serine protease 16
10282	ENSG00000105829	BET1	-1.74	0	Bet1 golgi vesicular membrane trafficking protein
10295	ENSG00000103507	BCKDK	-1.66	0	Branched chain keto acid dehydrogenase kinase
10329	ENSG00000118600	RXYLT1	-1.62	0	Ribitol xylosyltransferase 1
1033	ENSG00000100526	CDKN3	-1.58	0	Cyclin dependent kinase inhibitor 3
10360	ENSG00000107833	NPM3	-1.56	0	Nucleophosmin/nucleoplasmin 3
10371	ENSG00000075213	SEMA3A	-1.54	0	Semaphorin 3A

Table 3: Common GO Enrichment in Down and Up DEGs Involved in the Resistant Prostate Cancer Cells to Docetaxel

Common GO	Description	Common GO	Description
GO:0048731	System development	GO:0010648	Cell communication regulation
GO:0048856	Anatomical structure development	GO:0023057	Signaling regulation
GO:0007275	Multicellular organism development	GO:0008219	Cell death
GO:0009653	Anatomical structure morphogenesis	GO:0030334	Cell migration regulation
GO:0032502	Developmental process	GO:0023051	Signaling regulation
GO:0035239	Morphogenesis of tube	GO:0010646	Cell communication regulation
GO:0048513	Development of animal organ	GO:0048522	Cellular process regulation
GO:0035295	Development of tube	GO:0030855	Differentiation of epithelial cell
GO:0048523	Cellular process regulation	GO:0010033	Organic substance response
GO:0009888	Development of tissue	GO:0048583	Stimulus response regulation
GO:0051239	Multicellular process regulation	GO:0051716	Stimulus cellular response
GO:0008283	Cell population proliferation	GO:0070887	Chemical stimulus
GO:0042127	Proliferation of cell population	GO:2000145	Regulation of cell motility
GO:0051240	Multicellular process regulation	GO:0030512	Signalling pathway
GO:0048519	Biological process regulation	GO:0009719	Endogenous stimulus response
GO:0016477	Cell migration	GO:0006915	Process of apoptotic
GO:0032501	Process of multicellular	GO:0007154	Communication of cell

Table 3: Continued

Common GO	Description	Common GO	Description
GO:0050896	Stimulus response	GO:0050794	Cellular process regulation
GO:0042221	Chemical response	GO:0048732	Gland development
GO:0040011	Locomotion	GO:0035556	Signal transduction of intracellular
GO:0048870	Cell motility	GO:0051128	Cellular component organization regulation
GO:0010941	Cell death regulation	GO:0012501	Programmed cell death
GO:0009968	Signal transduction regulation	GO:0008284	Cell population proliferation regulation
GO:0048585	Stimulus response regulation	GO:0006935	Chemotaxis
GO:0009966	Signal transduction regulation	GO:0042330	Taxis
GO:0065009	Molecular function regulation	GO:0071310	Cellular response
GO:0060429	Epithelium development	GO:0050789	Biological process regulation
GO:0048518	Biological process regulation		

Table 4: Top 15 GO Annotation's Biological Process Involved in Docetaxel-Resistant Prostate Cancer Cells

Upregulate Genes					
GO	Gene.ID	Gene Stable ID	Gene.Symbol	adjusted p-value	Description
GO:0030154	2296	ENSG000000054598	FOXC1	8.82E-10	Cell differentiation
GO:0009888	3913	ENSG000000172037	LAMB2	4.13E-08	Tissue development
GO:0045597	2690	ENSG000000112964	GHR	8.26E-08	Cell differentiation regulation
GO:0045595	2737	ENSG000000106571	GLI3	1.17E-07	Cell differentiation regulation
GO:0050896	9734	ENSG000000048052	HDAC9	1.22E-07	Response to stimulus
GO:0009968	26575	ENSG000000091844	RGS17	2.52E-06	Signal transduction regulation
GO:0009966	81848	ENSG000000187678	SPRY4	7.57E-06	Signal transduction regulation
GO:0008219	143	ENSG000000102699	PARP4	4.55E-05	Cell death
GO:0060548	10979	ENSG000000073712	FERMT2	6.81E-05	Regulation of cell death
GO:0048598	2737	ENSG000000106571	GLI3	08.69E-05	Embryonic morphogenesis
GO:0042981	9748	ENSG000000065613	SLK	1.05E-04	Regulation of apoptotic process
GO:0048589	2737	ENSG000000106571	GLI3	1.32E-04	Developmental growth
GO:0006915	9821	ENSG000000023287	RB1CC1	6.97E-04	Apoptotic process
GO:0071363	90	ENSG000000115170	ACVR1	1.64 E-03	Cellular response to growth factor stimulus
GO:0012501	5654	ENSG000000166033	HTRA1	1.57 E-03	Programmed cell death
Downregulated genes					
GO	Gene.ID	Gene Stable ID	Gene.Symbol	adjusted_p_value	Description
GO:0050896	6742	ENSG000000012048	BRCA1	5.69E-11	Response to stimulus
GO:0071345	2633	ENSG000000107485	GATA3	2.02E-10	Response to cytokine stimulus
GO:0002376	317749	ENSG000000029993	HMGB3	3.63E-10	Immune system process
GO:0006260	6019	ENSG000000049541	RFC2	4.01E-09	DNA replication
GO:0019221	79023	ENSG000000115590	IL1R2	5.86E-09	Cytokine-mediated signalling pathway
GO:0033993	6821	ENSG000000072310	SREBF1	1.47E-08	Response to lipid
GO:0006974	6742	ENSG000000012048	BRCA1	3.21E-08	Cellular response to DNA damage stimulus
GO:0035556	55972	ENSG000000027075	PRKCH	4.79E-07	Intracellular signal transduction
GO:0006952	2633	ENSG000000107485	GATA3	2.1E-06	Defence response
GO:0030334	339166	ENSG000000041982	TNC	3.26E-05	Regulation of cell migration
GO:0030856	2633	ENSG000000107485	GATA3	1.05E-04	Regulation of epithelial cell differentiation
GO:0006915	3576	ENSG000000026103	FAS	3.07E-04	Apoptotic process
GO:0009725	2274	ENSG000000115641	FHL2	3.19E-03	Response to hormone
GO:0050678	6374	ENSG000000015475	BID	3.52E-03	Cell proliferation
GO:0008219	2013	ENSG0000000213853	EMP2	2.83E-02	Cell death

included SRF, TBP, POLR2A, TAF1 and RREB as well as downregulated genes included IRFs, ZEB1, PRDM1, SPI1, JUNs, FOSs and NFKBs. Moreover, common TFs in up and down meta-DEGs were identified as STATs, E2F1, MYB, FOXs, ZNFs and BCL6.

PPI Gene Network and Hub Genes

PPI gene network for key up and down genes (30 genes) was drawn separately using CytoHubba plugin in Cytoscape 3.6.1 based on level degrees and STRING was also used for the interaction between proteins (Figure 3). The string results for up and down genes are given in the supplementary file (S4). Based on the findings, COL4A1 (collagen type IV alpha 1 chain), LOX (lysyl oxidase), SMAD2 (SMAD family member 2), TIMP1 (TIMP metalloproteinase inhibitor

1) and FOS (Fos proto-oncogene) were identified as key upregulated hub genes. Generally, the hub genes of upregulated mostly associated with collagen types and metastasis-related factors. Next, EXO1 (exonuclease 1), RRM2 (ribonucleotide reductase regulatory subunit M2), CDC45 (cell division cycle 45), KIAA0101 (PCNA clamp associated factor), MCM10 (minichromosome maintenance 10 replication initiation factor) and HMMR (hyaluronan mediated motility receptor) were reported as key downregulated hub genes. Moreover, the hub genes of downregulated mainly related to molecular processes and cancer-related factors (Table 5).

Based on the results obtained from various methods (twelve methods) used to identify hub genes through Cytohubba plugin in Cytoscape 3.6.1, LOX (lysyl oxidase), SMAD2

Table 5: Key Hub Genes of Up and Downregulated Genes in The Resistant Prostate Cancer Cells to Docetaxel by MCC Method

Rank	Gene ID	Gene Stable ID	Degree	Gene.Symbol	Description
Upregulated genes					
1	1289	ENSG00000130635	18	COL5A1	Collagen type V alpha 1 chain
2	1282	ENSG00000187498	17	COL4A1	Collagen type IV alpha 1 chain
3	1284	ENSG00000134871	16	COL4A2	Collagen type IV alpha 2 chain
4	5351	ENSG00000083444	15	PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1
5	1287	ENSG00000188153	14	COL4A5	Collagen type IV alpha 5 chain
6	19316	ENSG00000117385	14	LEPRE1	Leucine proline-enriched proteoglycan (leprecan) 1
7	4015	ENSG00000113083	13	LOX	Lysyl oxidase
8	4087	ENSG00000175387	13	SMAD2	SMAD family member 2
9	5054	ENSG00000106366	12	SERPINE1	Serpin family E member 1
10	871	ENSG00000149257	11	SERPINH1	Serpin family H member 1
11	19317	ENSG00000090530	10	LEPREL1	Leprecan-like 1
12	4089	ENSG00000141646	9	SMAD4	SMAD family member 4
13	2200	ENSG00000166147	9	FBN1	Fibrillin 1
14	7076	ENSG00000102265	9	TIMP1	TIMP metalloproteinase inhibitor 1
15	2353	ENSG00000170345	9	FOS	Fos proto-oncogene, AP-1 TF subunit
16	4092	ENSG00000101665	7	SMAD7	SMAD family member 7
17	7077	ENSG00000035862	7	TIMP2	TIMP metalloproteinase inhibitor 2
18	6935	ENSG00000148516	7	ZEB1	Zinc finger E-box binding homeobox 1
19	6876	ENSG00000149591	7	TAGLN	Transgelin
20	25937	ENSG00000018408	7	WWTR1	WW domain containing transcription regulator 1
21	8850	ENSG00000114166	7	KAT2B	Lysine acetyltransferase 2B
22	5747	ENSG00000169398	6	PTK2	Protein tyrosine kinase 2
23	3685	ENSG00000138448	6	ITGAV	Integrin subunit alpha V
24	2034	ENSG00000116016	6	EPAS1	Endothelial PAS domain protein 1
25	11167	ENSG00000163430	6	FSTL1	Follistatin like 1
26	3486	ENSG00000146674	5	IGFBP3	Insulin like growth factor binding protein 3
27	467	ENSG00000162772	5	ATF3	Activating transcription factor 3
28	1050	ENSG00000245848	5	CEBPA	CCAAT enhancer binding protein alpha
29	6275	ENSG00000196154	5	S100A4	S100 calcium binding protein A4
30	1649	ENSG00000175197	3	DDIT3	DNA damage inducible transcript 3
Downregulated genes					
Rank	Gene ID	Gene Stable ID	Degree	Gene.Symbol	Description
1	9388	ENSG00000174371	29	EXO1	Exonuclease 1
2	6302	ENSG00000171848	29	RRM2	Ribonucleotide reductase regulatory subunit M2
3	837	ENSG00000093009	29	CDC45	Cell division cycle 45
4	9768	ENSG00000166803	29	KIAA0101	PCNA clamp associated factor
5	5557	ENSG00000065328	29	MCM10	Minichromosome maintenance 10 replication initiation factor
6	9601	ENSG00000137807	29	KIF23	Kinesin family member 23
7	10635	ENSG00000111247	28	RAD51AP1	RAD51 associated protein
8	3198	ENSG00000072571	28	HMMR	Hyaluronan mediated motility receptor
9	9111	ENSG00000198901	28	PRC1	Protein regulator of cytokinesis 1
10	6742	ENSG00000012048	28	BRCA1	BRCA1 DNA repair associated
11	55215	ENSG00000138180	28	CEP55	Centrosomal protein 55
12	55247	ENSG00000140525	28	FANCI	FA complementation group I
13	9914	ENSG00000184445	27	KNTC1	Kinetochore associated 1
14	79762	ENSG00000151725	27	CENPU	Centromere protein U
15	54898	ENSG00000146918	27	NCAPG2	Non-SMC condensin II complex subunit G2
16	439	ENSG00000148773	27	MKI67	Marker of proliferation Ki-67
17	11169	ENSG00000198554	26	WDHD1	WD repeat and HMG-box DNA binding protein 1
18	1033	ENSG00000100526	26	CDKN3	Cyclin dependent kinase inhibitor 3
19	54458	ENSG00000100479	26	POLE2	DNA polymerase epsilon 2
20	23397	ENSG00000121152	26	NCAPH	Non-SMC condensin I complex subunit H
21	3149	ENSG00000119969	25	HELLS	Helicase, lymphoid specific
22	2305	ENSG00000111206	25	FOXO1	Forkhead box M1
23	55612	ENSG00000198056	24	PRIM1	DNA primase subunit 1
24	81892	ENSG00000167513	24	CDT1	Chromatin licensing and DNA replication factor 1
25	10721	ENSG000000051341	23	POLQ	DNA polymerase theta
26	7134	ENSG00000167900	22	TK1	Thymidine kinase 1
27	2146	ENSG00000106462	21	EZH2	Enhancer of zeste 2 polycomb repressive complex 2
28	6296	ENSG00000167325	20	RRM1	Ribonucleotide reductase catalytic subunit M1
29	55312	ENSG00000109674	19	NEIL3	nei like DNA glycosylase 3
30	83451	ENSG00000159259	19	CHAF1B	Chromatin assembly factor 1 subunit B

(SMAD family member 2), SERPINE1 (serpin family E member 1) and FOS (Fos proto-oncogene, AP-1 transcription factor subunit) in eleven methods; PTK2 (protein tyrosine

kinase 2) in eight methods and TIMP1(TIMP metalloproteinase inhibitor 1) in five methods were identified as key hub genes related to upregulated genes (Figure 4).

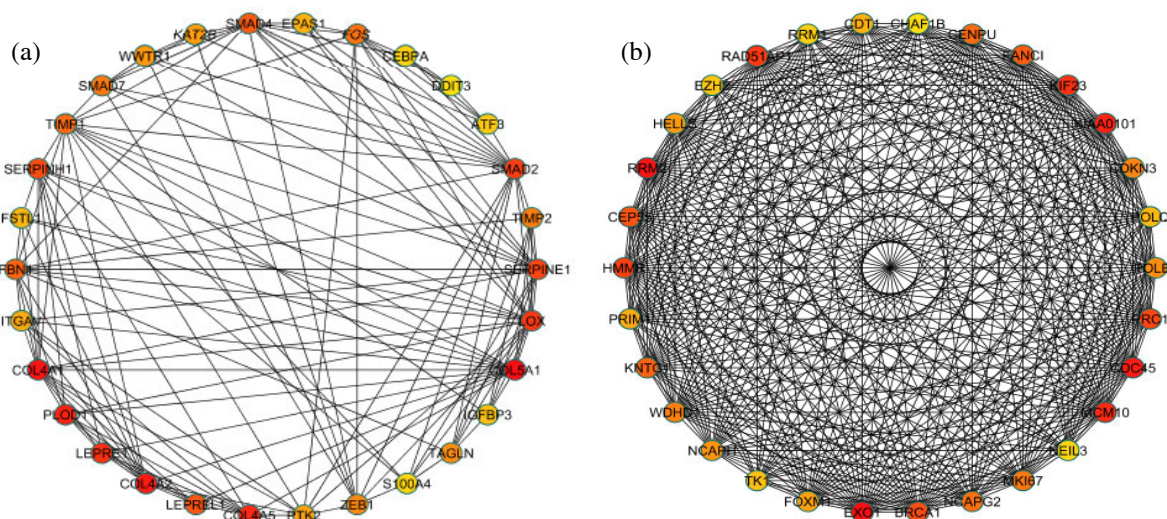


Figure 3: PPI Gene Network of (a) Key Hub Genes of Upregulated and (b) Key Hub Genes of Downregulated in Docetaxel-Resistant Prostate Cancer Cells via MCC Method

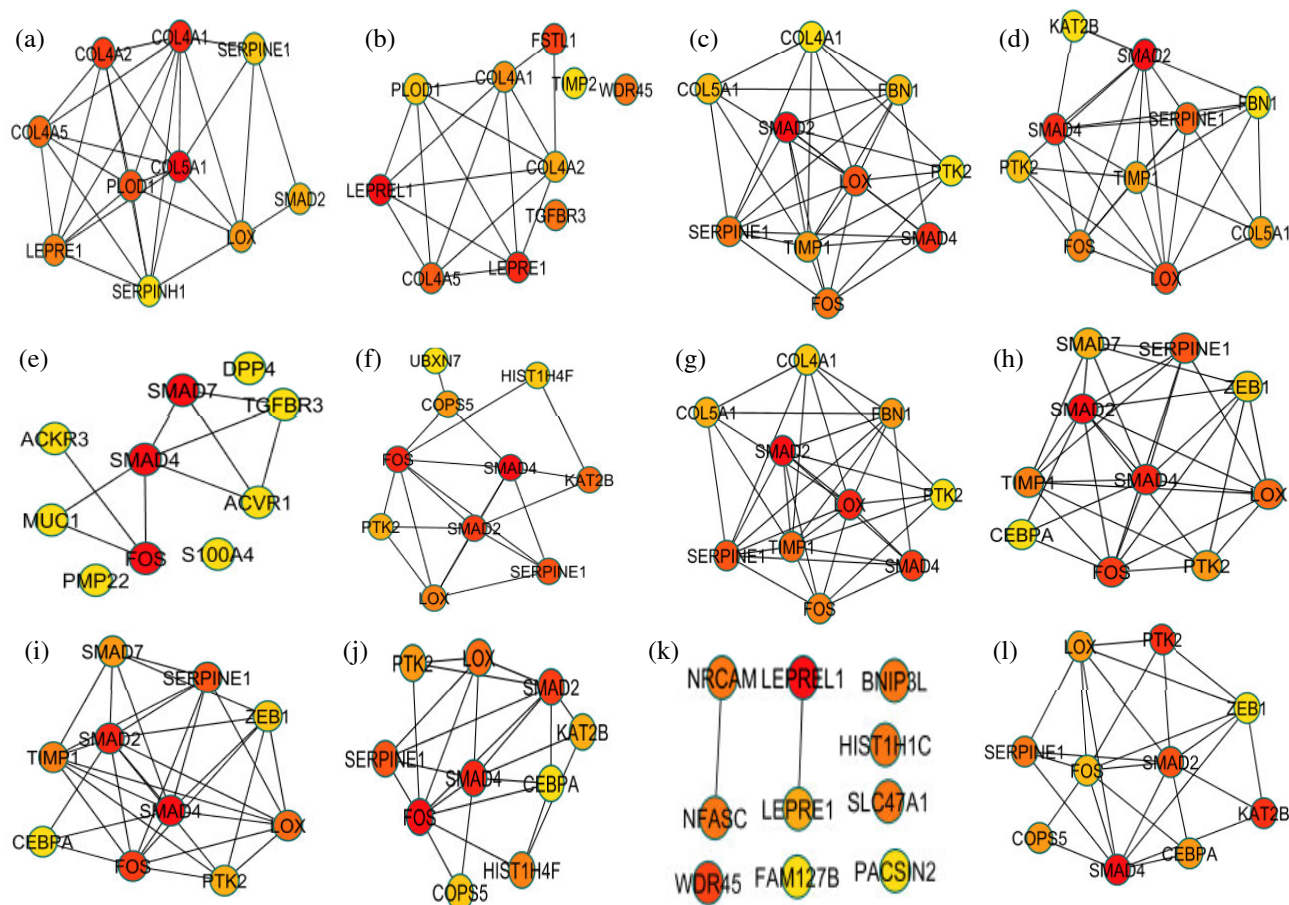


Figure 4: PPI Gene Network of Key Hub Genes (10 Genes) of Upregulated in Resistant Prostate Cancer Cells to Docetaxel Through Twelve Methods

Moreover, BRCA1 (BRCA1 DNA repair associated) in ten methods; EGFR (epidermal growth factor receptor) in eight methods; IL6 (interleukin 6) in seven methods; CXCL8 (C-X-C motif chemokine ligand 8) in six methods; EPRS (glutamyl-prolyl-tRNA synthetase 1), MKI67 (marker of

proliferation Ki-67) and EXO1 (exonuclease 1) in five methods were identified as key hub genes related to downregulated genes (Figure 5). The results obtained from the analysis of twelve methods to identify 10 key hub genes are shown in the Table 6.

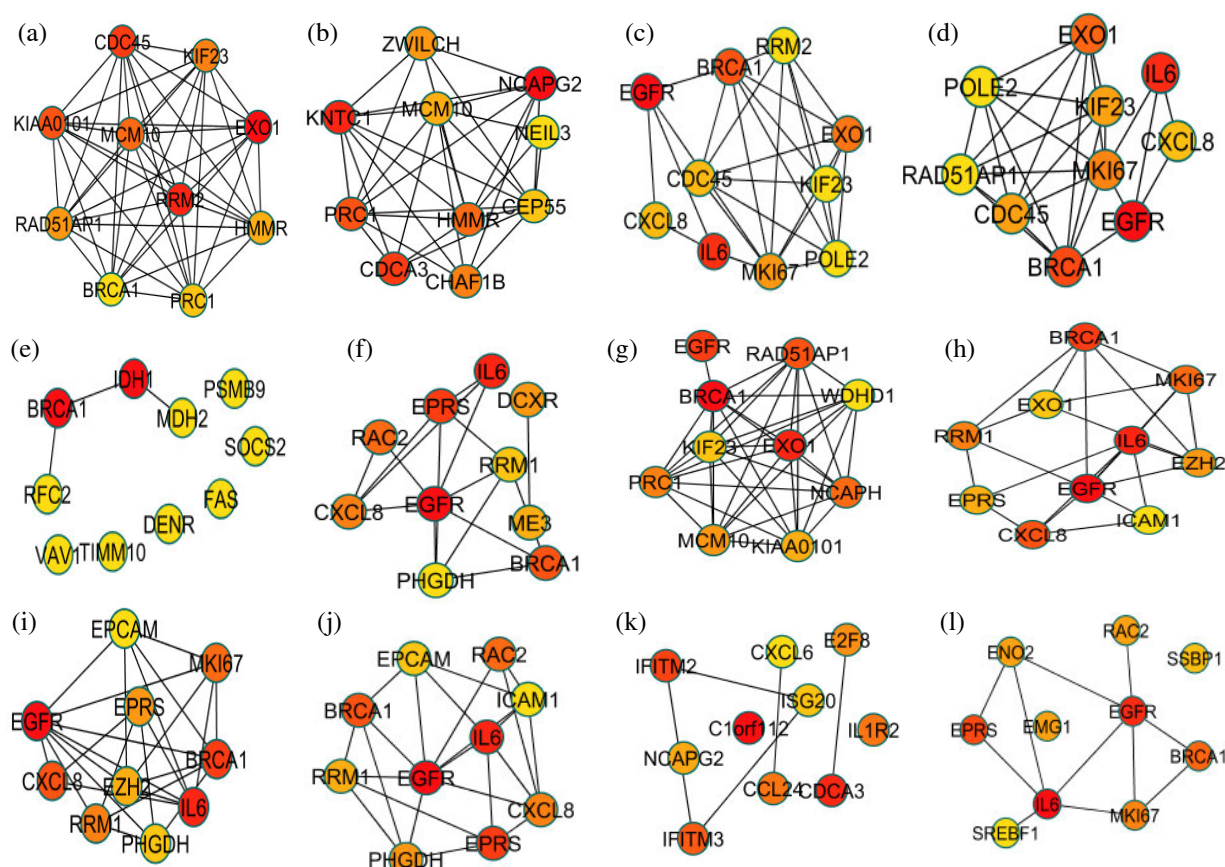


Figure 5: PPI Gene Network of Key Hub Genes (10 Genes) of Downregulated in Resistant Prostate Cancer Cells to Docetaxel Through Twelve Methods

Table 6: Key Hub Genes (10 Genes) of Up and Downregulated Genes by Twelve Topological Algorithms of PPI Gene Network Analysis Through CytosHubba

Methods	Upregulated genes
MCC	COL5A1, COL4A1, COL4A2, PLOD1, COL4A5, LEPRE1, LOX, SMAD2, SERPINE1 and SERPINH1
MNC	SMAD2, SMAD4, LOX, FOS, SERPINE1, TIMP1, FBN1, COL5A1, COL4A1 and PTK2
Degree	SMAD2, SMAD4, LOX, SERPINE1, FOS, TIMP1, COL5A1, PTK2, FBN1 and KAT2B
Closeness	SMAD2, SMAD4, FOS, SERPINE1, LOX, TIMP1, PTK2, SMAD7, ZEB1 and CEBPA
BottleNeck	SMAD4, PTK2, KAT2B, SMAD2, SERPINE1, LOX, COPS5, CEBPA, FOS and ZEB1
Stress	SMAD4, FOS, SMAD2, SERPINE1, KAT2B, LOX, COPS5, PTK2, HIST1H4F and UBXN7
DMNC	LEPREL1, LEPRE1, FSTL1, COL4A5, TGFB3, WDR45, COL4A1, COL4A2, PLOD1 and TIMP2
EcCentricity	FOS, SMAD7, SMAD4, PMP22, MUC1, DPP4, ACKR3, S100A4, ACVR1 and TGFB3
EPC	SMAD2, LOX, SMAD4, SERPINE1, TIMP1, FOS, FBN1, COL5A1, COL4A1 and PTK2
Radiality	SMAD4, SMAD2, FOS, SERPINE1, LOX, TIMP1, SMAD7, PTK2, ZEB1 and CEBPA
Betweenness	FOS, SMAD4, SMAD2, SERPINE1, LOX, HIST1H4F, PTK2, KAT2B, COPS5 and CEBPA
ClusteringCoefficient	LEPREL1, WDR45, NFASC, NRCAM, SLC47A1, HIST1H1C, BNIP3L, LEPRE1, FAM127B and PACSIN2
Methods	Downregulated genes
MCC	EXO1, RRM2, CDC45, KIAA0101, MCM10, KIF23, RAD51AP1, HMMR, PRC1 and BRCA1
MNC	EGFR, IL6, BRCA1, EXO1, MKI67, CXCL8, CDC45, KIF23, POLE2 and RRM2
Degree	EGFR, IL6, BRCA1, EXO1, MKI67, KIF23, CDC45, CXCL8, POLE2 and RAD51AP1
Closeness	EGFR, IL6, BRCA1, CXCL8, MKI67, RRM1, EZH2, EPRS, EXO1 and ICAM1
BottleNeck	IL6, EGFR, EPRS, BRCA1, MKI67, RAC2, EMG1, ENO2, SSBP1 and SREBF1
Stress	EGFR, IL6, EPRS, BRCA1, RAC2, CXCL8, DCXR, ME3, RRM1 and PHGDH
DMNC	NCAPG2, KNTC1, CDCA3, PRC1, HMMR, CHAF1B, ZWILCH, MCM10, CEP55 and NEIL3
EcCentricity	IDH1, BRCA1, SOCS2, PSMB9, DENR, RFC2, FAS, VAV1, TIMM10 and MDH2
EPC	BRCA1, EXO1, EGFR, RAD51AP1, NCAPH, PRC1, MCM10, KIAA0101, KIF23 and WDHD1
Radiality	EGFR, IL6, BRCA1, CXCL8, MKI67, RRM1, EPRS, EZH2, PHGDH and EPCAM
Betweenness	EGFR, IL6, EPRS, BRCA1, RAC2, CXCL8, PHGDH, RRM1, EPCAM and ICAM1
ClusteringCoefficient	C1orf112, CDCA3, IFITM2, IFITM3, IL1R2, CCL24, E2F8, NCAPG2, ISG20 and CXCL6

To prove the results obtained from this research in the first stage; our results were compared with the results of research reported by other researchers based on gene

expression data (fold-change expression) from molecular studies, including qRT-PCR results. The upregulated genes of this study including (CLU and DDIT3 [13], GSPT2 and

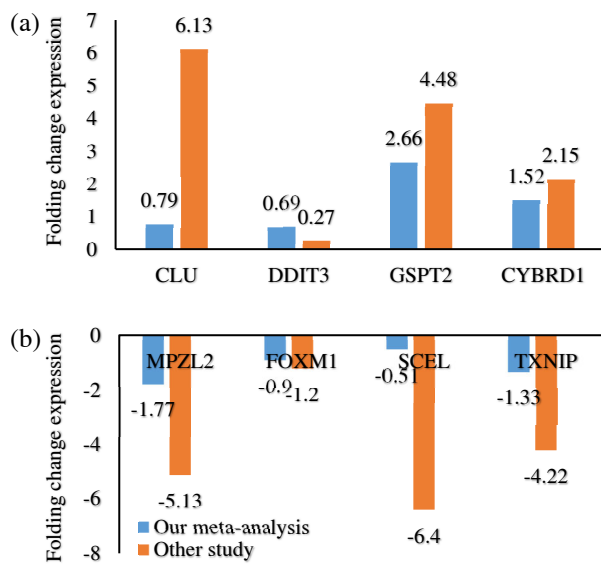


Figure 6: Comparison of the Present Results with the qRT-PCR Results of Reported Studies, (a) Up Regulated Genes and (b) Down Regulated Genes

CYBRD1 [14]) and the downregulated genes including (MPZL2 [15], FOXM1 [16], SCEL and TXNIP [14]) match and agree with the results of reported qRT-PCR studies (Figure 6). To confirm the results of this research, scientific confirmation in the laboratory is needed experimentally.

DISCUSSION

Microarray technologies have widely been used to simultaneously examine gene on large scale, providing an efficient method for examine the expression of thousands of genes. So, the integration and analysis of data related to microarray data supply precious information for the investigation of resistance prostate cancer cells to docetaxel [17]. Based on our results, 373 upregulated genes and 558 downregulated genes were identified. Accordingly, top-upregulated genes associated with docetaxel-resistant prostate cancer cells were mostly included IGFBP3, ABCB1, GSPT2, ROBO1, FSTL1, NID2, S100A4, CDH1 and ADAMTS1 (Table 2). Insulin-like growth factor binding protein 3 (IGFBP3) inhibits adhesion of cell, endometrial cancer invasion and prostate cancer metastasis [18]. According to previous reports, ROBO1 is expressed as a member of the immunoglobulin protein ROBO and its expression has been observed in primary tumors [19]. In cancer, FSTL1 may affect cancer cells by directly interacting with cells expressing TGF β /BMP family receptors and DIP2A protein and indirectly by affecting the immune system [20]. Next, in this study top-downregulated genes mainly related to CD24, ZNF587B, PARP2, MIR4271, OCLN, TSPAN1, ADGRG2, MPZL2 and POP7 (Table 2). CD24 encodes a glycosyl phosphatidyl inositol and is most abundantly expressed in hematopoietic cells as well as has demonstrated its' central role in tumor formation, progression and metastasis in prostate cancer [21].

ZNF587B is known as a member of the Krüppel-type zinc-finger proteins (KRAB-ZFPs) and has been shown to be associated with the regulating the proliferation of cell, cancer and apoptosis [22]. Accordingly, NF587B is a potential novel tumor suppressor for prostate cancer and may be a treatment target for this cancer. Occludin (OCLN) is a key tight junction protein which regulates remodeling of cytoskeletal and suppresses activation of the AKT/PI3K signaling pathway and proliferation of cell, thereby enhancing apoptosis of tumor in prostate cancer [23].

In the biological processes, GO enrichment in the upregulated genes mostly associated with the regulation of cell differentiation, signal transduction, cell death and process of apoptotic; and in the downregulated genes mainly belonged to response to chemicals, defense and immune system process, cell death and proliferation (Table 4). Moreover, meta-DEGs by KEGG pathways mostly associated with pathways in cancer, PI3K-Akt signaling pathway, interaction of cytokine-cytokine receptor, MAPK signaling pathway, transcriptional misregulation in cancer, MicroRNAs in cancer, apoptosis, p53 signaling pathway, prostate cancer and DNA replication. According to the KEGG analysis in prostate cancer, the DEGs were mostly related to cancer pathways, metabolic pathways, signaling pathway of PI3K-Akt, Jak-STAT signaling pathway, cancer proteoglycans and signaling pathway of NF-kb [24].

In this study, the TFs associated with upregulated genes mostly identified as SRF, TBPs, POLR2A, TAF1 and RREB and downregulated genes were detected as IRFs, ZEB1, PRDM1, SPI1, JUNs, FOSs and NFKBs as well as common TFs in down and up genes identified as STATs, E2F1, MYB, FOXs, ZNFs, BCL6 (S Table 1). Activation of STAT3 has been identified in most of prostate cancer cell lines and has been shown to increase cell survival, tumor growth and apoptosis resistant [25]. Serum response factor (SRF) is a member of the TFs MADS-box family and is one of the important-known DNA-binding proteins in the human proteome. In addition, SRF plays a role in promoting cell proliferation, resistance to cell death and induction of metastasis and invasion [26].

Based on the present study, COL4A1, LOX, SMAD2, TIMP1 and FOS were identified as key hub genes of upregulated (Table 5). COL4A1 encodes the alpha1 chain of the Col IV gene, that is a dominant structural component of the microenvironment of tumor [27]. COL4A1 enhances invasion of tumor by inducing of tumor budding in various cancer cells and also involved in the migration and proliferation cancers such as to prostate cancer [28]. LOX is involved in the cross-linking of collagen and elastin as key enzymatic step and it is a copper-dependent amine oxidase [29]. The role of LOX in prostate cancer is to either enhance or suppress tumorigenesis, cell type, location and status of transformation [30]. The Small Mothers Against Decapentaplegic (SMAD) is an intracellular signal transduction protein related to the prognosis and growth of various types of tumors. SMADs have been shown to play a role in proliferation of cell, apoptosis, migration and

regulation of cancer cells immune [31]. Based on our study, the hub genes of the upregulated DEGs mostly associated with collagen types and metastasis -related factors.

Additionally, EXO1, RRM2, CDC45, KIAA0101, MCM10 and HMMR were detected as key hub genes of downregulated genes (Table 5). Increased expression of EXO1 has been reported to be related to invasion of tumor and metastasis. EXO1 overexpression is associated with poor survival in prostate cancer patients and increases progression of tumor and metastasis [32]. RRM2 may lead to genome instability and enhanced mutation, thereby affecting the progression of tumor [7] and overexpression of RRM2 plays an important role in the proliferation of cell, metastasis and dependence of drug in prostate cancer [33]. CDC45 plays an important role in the replication of the CDC45-MCM-GINS helicase holoenzyme and activates MCM2-7, which leading to replication of DNA [34] and also, it inhibits replication of DNA and suppresses proliferation of cell in human cells [35]. Based on our study, the hub genes of downregulated genes mostly associated with DNA molecular processes and cancer-related factors.

To validate our research, this study was compared with the reported qRT-PCR studies through the log2 fold change score of some upregulated genes including CLU and DDIT3 ([13], GSPT2 and CYBRD1 [14]) and downregulated genes including MPZL2 [15], FOXM1 [16], SCEL and TXNIP [14] (Figure 6).

Cancer is initially caused by oncogenic changes in oncology, as well as by genes that are effective in tumor suppression [36]. Based on the reported results, no comprehensive report has been presented so far on the integration of microarray data from docetaxel-resistant prostate cancer cells for transcriptomic analyses. Accordingly, it was considered necessary to integrate and analyze data from different microarray experiments so that comprehensive results can be reported on the genes involved in docetaxel-resistant prostate cancer cells. According to this results, key upregulated consensus hub genes including LOX, SERPINE1, FOS and PTK2 and downregulated consensus hub genes including BRCA1, EGFR, IL6 and CXCL8 were identified. Based on KEGG pathways analysis, dominant biological pathways including cancer pathways, PI3K-Akt signaling pathway, cytokine-cytokine receptor interaction, MAPK signaling pathway, apoptosis, p53 signaling pathway were identified. The current confirmation is not sufficient for this research and for strong and robust validation in the future, molecular tests, including qRT-PCR are needed experimentally in the laboratory.

CONCLUSIONS

In conclusion, this study showed several hub genes associated with docetaxel resistance in prostate cancer cells by advanced bioinformatics analysis *in Silico* to understand the molecular mechanisms and can also suggested them as effective genes for treatment of docetaxel-resistance prostate cancer cells. To finally confirm this idea, the key genes obtained need to be experimentally validated in the laboratory.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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