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Identification of candidate genes that may contribute to the metastasis of prostate cancer by bioinformatics analysis

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Abstract. To screen for marker genes associated with to the metastasis of prostate cancer (PCa), *in silico* analysis of the Gene Expression Omnibus dataset GSE27616, which included 4 metastatic and 5 localized PCa tissue samples, was performed. Differentially expressed genes (DEGs) were identified. Their potential functions were identified by Gene Ontology and Kyoto Encyclopedia of Gene Genomes pathway enrichment analyses. Furthermore, protein-protein interaction (PPI) networks for DEGs were constructed using Cytoscape. Module analysis of the PPI networks was performed with Cluster ONE. A total of 561 DEGs were screened, including 208 upregulated and 353 downregulated genes. Proliferating cell nuclear antigen (*PCNA*) and cluster of differentiation 4 (*CD4*) exhibited the highest degrees of connectivity in the PPI networks for up- and down-regulated DEGs, respectively. The DEGs in module A, including *CD58*, *2*, *4* and major histocompatibility complex, class II DP-β1 were enriched in 'cell adhesion molecules'. Anaphase promoting complex subunit 4, cell division cycle 20 and cell division cycle 16 in module B were primarily enriched in 'cell cycle'. The DEGs, including *CD4*, *PCNA* and baculoviral IAP repeat containing 5, may have critical roles in PCa metastasis and could thus be used as novel biomarker candidates for metastatic PCa. However, further studies are required to verify these results.

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Abbreviations: CAMs, cell adhesion molecules; DAVID, database for annotation visualization and integrated discovery; DEGs, differentially expressed genes; FDR, false discovery rate; GEO, gene expression omnibus; GO, Gene Ontology; HIF, hypoxia inducible factor; HPRD, Human Protein Reference Database; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; PCa, prostate cancer; PSA, prostate specific antigen; TRP, transient receptor potential

Key words: prostate cancer, metastasis, differentially expressed genes, protein-protein interaction, module

Introduction

Prostate cancer (PCa) is the most common malignancy among men worldwide (1) as well as the second leading cause of cancer-associated mortality (2,3). According to a previous study, PCa resulted in ~256,000 incidences of mortality in 2010 globally (4). This type of cancer is caused by a combination of and environmental factors, including age, ethnicity and diet, and genetic causes (5). Although PCa is curable in the early stages by radiation therapy or surgical resection, the majority of patients with locally advanced or metastatic PCa lack a curative treatment option (6).

Metastasis, the most common cause of cancer-associated mortality, represents the final and the most devastating stage of tumor progression (7). The majority of cancer types originate from epithelial tissues and, upon metastasis, leave the primary tumor and invade the adjacent tissue (8). PCa cells may thus spread from the prostate to other regions of the body. Pelvic lymph node involvement is the first indication of metastasis in the majority of cases of PCa, followed by transfers to other organs, including bones, lungs and liver (9). The metastasis of PCa to regional lymph nodes is a frequent early event that is associated with poor clinical treatment (10). Aurora kinase A is overexpressed in metastatic PCa and is associated with tumor development and progression (11). In addition, the expression of extracellular signal-regulated kinase, p38 mitogen-activated protein kinase and c-Jun-N-terminal kinase were demonstrated to be significantly reduced in metastatic PCa tissue compared with primary PCa (12). At present, prostate specific antigen (PSA), encoded by the prostate-specific gene kallikrein-3 (*KLK3*), is a serum biomarker used for the detection of PCa (13). However, the prognostic value of PSA is limited due to the variation in its specificity and sensitivity (14). Therefore, it is urgent to screen for valid biomarkers for metastatic PCa.

The present study investigated the global gene expression profile of localized and metastatic PCa, and identified the differentially expressed genes (DEGs) between localized and metastatic PCa tissue samples. Protein-protein interaction (PPI) networks were constructed for the DEGs, and pathway analyses of the PPI networks were performed. Finally, a set of genes associated with PCa metastasis was identified. The present study aimed to identify key genes that were involved in the metastasis of PCa and provide clues for the treatment of PCa, by bioinformatics methods.

Materials and methods

Affymetrix microarray data. Gene expression profiles (identification no., GSE27616) from the study of Kim *et al* (15), which were deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) were extracted, including 4 metastatic and 5 localized PCa tissue sample expression profiles. Raw data had been collected using the Agilent-014850 Whole Human Genome Microarray 4x44K G4112F array platform (Agilent Technologies, Inc., Santa Clara, CA, USA).

DEGs analysis. The original expression data from all conditions was scaled using the robust multi-array average (RMA) method (16), with the default settings in Bioconductor (http://bioconductor.org/help/search/index.html?q=AgiMicroRna) (17) and a linear model was constructed. Linear Models for Microarray Data package (18) was applied to identify DEGs. To reduce the likelihood of false positive results, the Benjamini-Hochberg method (19) was used to adjust the raw P-value. Finally, DEGs with the cut-off criteria [$\log_2$ Fold change (FC)]>1 and P<0.05 were selected.

Gene ontology (GO) and pathway enrichment analysis. The Database for Annotation, Visualization and Integrated Discovery (DAVID; version 6.7, http://david.abcc.ncifcrf.gov/) is an annotation tool that allows for the extraction of biological meaning from a large list of genes (20). In the present study, DAVID was used to identify over-represented GO terms in the biological process category based on the hypergeometric distribution with a false discovery rate (FDR) <0.05 and gene count >2. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was applied to identify the main functional and metabolic pathways enriched in DEGs from PCa metastasis. P<0.05 was selected as the cut-off criterion.

PPI network construction. To demonstrate potential PPIs, PPI data from the Human Protein Reference Database (HPRD) (21) and the Biological General Repository for Interaction Database (BioGRID) (22) were used to identify interactions among the DEGs. On the basis of this dataset, PPI networks of the DEGs were constructed in the Search Tool for the Retrieval of Interacting Gene database (23) using Cytoscape (version 3.2.0; http://www.cytoscape.org/release_notes_3_2_0.html) software (24).

Screening and analyses of relevant regulatory network modules. The functional modules of the network for downregulated DEGs were investigated with Cluster ONE in Cytoscape (25). The sub-modules were screened with a criterion of P<0.01. Finally, the two most significant sub-modules from the modularity analysis were selected for GO functional enrichment analysis and KEGG pathway enrichment analysis.

Results

DEGs between metastatic and localized PCa cells. To identify DEGs between metastatic and localized PCa samples, the publicly available microarray dataset GSE27616 was obtained from the GEO database. DEGs with a ($\log_2$ FC)>1 and P<0.05 were identified. A total of 561 DEGs were obtained, including 208 upregulated genes and 353 downregulated genes.

KEGG pathway enrichment and GO analysis of DEGs. KEGG pathway enrichment analyses were performed for upregulated and downregulated DEGs. The results revealed that the upregulated DEGs were enriched in 'renal cell carcinoma' (P=0.00848), 'cell cycle' (P=0.01438) and 'homologous recombination' (P=0.04125; Table I). The downregulated DEGs were enriched in 'vascular smooth muscle contraction' (P=0.00889), 'calcium signaling pathway' (P=0.03116) and 'dilated cardiomyopathy' (P=0.0436; Table II).

To investigate whether DEGs share specific functional features, the online biological classification software DAVID was used to identify overrepresented GO categories in biological process. Gene count >2 and P<0.05 were selected as threshold values. There were 48 categories enriched in upregulated DEGs and 94 categories enriched in downregulated DEGs that met these criteria. The most frequent GO functions of upregulated DEGs included 'response to hypoxia' ($P=2.72\times10^{-8}$), 'response to oxygen levels' ($P=4.81\times10^{-8}$) and 'cell cycle phase' ($P=6.60\times10^{-4}$; Table III). The GO functions of downregulated DEGs included 'response to organic substance' ($P=3.50\times10^{-5}$), 'response to cytokine stimulus' ($P=2.70\times10^{-4}$) and 'response to hypoxia' ($P=3.07\times10^{-4}$; Table IV).

Construction of an integrated PPI network. PPI interaction data were obtained from HPRD and BioGRID, and PPI networks were constructed for upregulated and downregulated DEGs (Figs. 1 and 2). In the PPI network for upregulated DEGs, the top ten nodes with the highest connectivity were proliferating cell nuclear antigen (*PCNA*), cell division cycle associated 8 (*CDCA8*), cell division cycle 20 (*CDC20*), baculoviral IAP repeat containing 5 (*BIRC5*), DNA polymerase δ-1, chromatin licensing and DNA replication factor 1, kinesin family member 2C, checkpoint kinase 1, aurora kinase B (*AURKB*) and thymidine kinase 1. In the PPI network for downregulated DEGs, the top ten nodes with the highest connectivity were *CD4*, *CCR5*, *LCK*, C-X-C motif chemokine ligand 12 (*CXCL12*), fibromodulin, fibroblast growth factor 2, *CXCL9*, *CD2*, *CD69* and *CCL5*. The connectivity degree of these proteins was >10 (Table V).

Analysis of modules. Module A ($P=3.029\times10^{-5}$) and module B ($P=2.901\times10^{-4}$; Fig. 3) were isolated from the PPI network of the downregulated DEGs using Cluster ONE software. GO and KEGG pathway analysis were then performed to analyze the modules.

In module A, genes including *CCL2*, *CCR5*, *CXCL9*, *CXCL11* and *CCL5* were enriched in 'chemokine signaling pathway' ($P=4.77\times10^{-4}$) and 'cytokine-cytokine receptor interaction' (P=0.00171); genes including *CD58*, *CD2*, *CD4* and major histocompatibility complex, class II, DP-β1 (*HLA-DPB1*) were enriched in 'adhesion molecules' (P=0.00242); genes including *CD38*, *2* and *4* were enriched in 'hematopoietic cell lineage' (P=0.01408); genes including *CXCL9*, *CXCL11* and *CCL5* were enriched in 'toll-like receptor signaling pathway' (P=0.01912).

In module B, genes including anaphase promoting complex subunit 4 (*ANAPC4*), protein phosphatase 3 catalytic

Table I. Enriched Kyoto Encyclopedia of Genes and Genomes pathways for the upregulated differentially expressed genes.

Term	Count	P-value	Genes
hsa05211: Renal cell carcinoma	5	0.00848	*BRAF*, *CREBBP*, *EGLN3*, *EGLN1*, *FLCN*
hsa04110: Cell cycle	6	0.01438	*CDKN2D*, *CREBBP*, *PKMYT1*, *CHEK1*, *CDC20*, *PTTG1*
hsa03440: Homologous recombination	3	0.04125	*POLD1*, *RAD52*, *RAD54L*

Table II. Enriched Kyoto Encyclopedia of Genes and Genomes pathways for the downregulated differentially expressed genes.

Term	Count	P-value	Genes
hsa04270: Vascular smooth muscle contraction	8	0.00889	*KCNMA1*, *ACTG2*, *PLA2G4A*, *PLCB4*, *ACTA2*, *PPP1R12B*, *MYLK*, *ITPR2*
hsa04020: Calcium signaling pathway	9	0.03116	*CD38*, *SLC8A1*, *PLCB4*, *PHKB*, *PLN*, *GRPR*, *PPP3CB*, *MYLK*, *ITPR2*
hsa05414: Dilated cardiomyopathy	6	0.04364	*SLC8A1*, *DES*, *PLN*, *DMD*, *TPM2*, *CACNA2D2*

Table III. Top ten enriched GO functions of upregulated differentially expressed genes.

GO term	Count	P-value	Genes
0001666: Response to hypoxia	13	2.72×10^{-8}	*ALDOC*, *CREBBP*, *EGLN3*, *EGLN1*, *UBE2B*, *ADA*, *DDIT4*, *ADM*, *PLOD2*, *SERPINA1*, *SCNN1B*, *ANGPTL4*, *MT3*
0070482: Response to oxygen levels	13	4.81×10^{-8}	*ALDOC*, *CREBBP*, *EGLN3*, *EGLN1*, *UBE2B*, *ADA*, *DDIT4*, *ADM*, *PLOD2*, *SERPINA1*, *SCNN1B*, *ANGPTL4*, *MT3*
0022403: Cell cycle phase	14	6.60×10^{-4}	*PKMYT1*, *CDC20*, *CHEK1*, *PTTG1*, *RCC1*, *RAD52*, *RAD54L*, *KIF2C*, *CDCA8*, *CDKN2D*, *POLD1*, *STMN1*, *CIT*, *FANCA*
0010033: Response to organic substance	19	9.65×10^{-4}	*F12*, *EIF2C2*, *AQP9*, *ALDOC*, *TAF9B*, *DUOX1*, *HSPA1A*, *UBE2B*, *SDC1*, *HSF1*, *ADM*, *SLC25A36*, *ENO2*, *HSPA6*, *HSPB1*, *SERPINA1*, *CA2*, *ABCC5*, *SPP1*
0000279: M phase	12	0.00104	*KIF2C*, *CDCA8*, *PKMYT1*, *CHEK1*, *CDC20*, *PTTG1*, *STMN1*, *CIT*, *RAD52*, *RCC1*, *RAD54L*, *FANCA*
0006260: DNA replication	8	0.00508	*RFC4*, *POLD1*, *CDKN2D*, *CHTF18*, *CHEK1*, *C16ORF75*, *TK1*, *CDT1*
0007049: Cell cycle	18	0.00517	*CKS1B*, *PSRC1*, *PKMYT1*, *CHEK1*, *CDC20*, *PTTG1*, *RAD52*, *RCC1*, *RAD54L*, *CDT1*, *KIF2C*, *CDCA8*, *POLD1*, *CDKN2D*, *CHTF18*, *STMN1*, *CIT*, *FANCA*
0042060: Wound healing	8	0.00522	*F12*, *FGG*, *SDC1*, *MST1*, *ENO3*, *SERPINA1*, *SCNN1B*, *TM4SF4*
0010035: Response to inorganic substance	8	0.00761	*FGG*, *SDC1*, *AQP9*, *DUOX1*, *SERPINA1*, *CA2*, *ADA*, *MT3*
0000278: Mitotic cell cycle	11	0.00785	*KIF2C*, *CDCA8*, *POLD1*, *CDKN2D*, *PKMYT1*, *CHEK1*, *CDC20*, *PTTG1*, *STMN1*, *CIT*, *RCC1*

GO, Gene Ontology.

subunit-β, *CDC20* and *CDC16* were enriched in 'oocyte meiosis' ($P=3.88\times10^{-5}$); genes including *ANAPC4*, *CDC20* and *CDC16* were enriched in 'cell cycle' (P=0.003482) and 'ubiquitin mediated proteolysis' (P=0.004173; Table VI).

The enriched GO analysis results revealed that the enriched GO functions for genes in module A were predominantly enriched in 'immune response' ($P=6.77\times10^{-10}$), 'taxis' ($P=3.07\times10^{-5}$) and 'chemotaxis' ($P=3.07\times10^{-5}$). The enriched GO functions for genes in module B were mainly enriched in 'mitotic cell cycle' ($P=1.08\times10^{-11}$), 'cell cycle phase' ($P=2.39\times10^{-11}$) and 'mitosis' ($P=1.19\times10^{-10}$; Table VII).

Table IV. Top ten enriched GO functions of downregulated differentially expressed genes.

GO term	Count	P-value	Genes
0010033: Response to organic substance	28	3.50×10^{-5}	*CCL2*, *SNCA*, *ADH5*, *PDE3B*, *PTEN*, *ASAH1*, *STAT6*, *SORBS1*, *BCL2*, *PPP3CB*, *SRD5A2*, *GNG4*, *EIF2B4*, *GHR*, *KCNMA1*, *BSG*, *SLC8A1*, *SP100*, *SOCS2*, *FBP1*, *LIFR*, *COLEC12*, *SELS*, *CYP7B1*, *CD38*, *PLA2G4A*, *SMPD1*, *WFDC1*
0034097: Response to cytokine stimulus	8	2.70×10^{-4}	*STAT6*, *CD38*, *SP100*, *BCL2*, *SNCA*, *LIFR*, *PPP3CB*, *GHR*
0001666: Response to hypoxia	10	3.07×10^{-4}	*KCNMA1*, *CD38*, *SLC8A1*, *SMAD9*, *CCL2*, *BCL2*, *PSEN2*, *CABC1*, *DPP4*, *ITPR2*
0009266: Response to temperature stimulus	8	3.67×10^{-4}	*PLA2G4A*, *CCL2*, *TRPM8*, *DIO2*, *BCL2*, *CIRBP*, *GMPR*, *EIF2B4*
0070482 Response to oxygen levels	10	4.47×10^{-4}	*KCNMA1*, *CD38*, *SLC8A1*, *SMAD9*, *CCL2*, *BCL2*, *PSEN2*, *CABC1*, *DPP4*, *ITPR2*
0007610 Behavior	19	5.65×10^{-4}	*KCNMA1*, *CCL2*, *SNCA*, *CXCL9*, *ATP1A2*, *FOSB*, *CXCL11*, *PTEN*, *CXCL12*, *SLIT2*, *PTGDS*, *CCR5*, *BCL2*, *PSEN2*, *GRPR*, *PTN*, *PBX3*, *FGF2*, *IL1RAPL1*
0006955 Immune response	24	7.18×10^{-4}	*GBP5*, *SP100*, *CCL2*, *GZMA*, *IGJ*, *SNCA*, *TUBB2C*, *GPR65*, *CXCL9*, *COLEC12*, *CXCL11*, *CXCL12*, *CLEC10A*, *IGSF6*, *CCR5*, *BCL2*, *PSEN2*, *MS4A2*, *HLA-DRB5*, *HLA-DPB1*, *SPON2*, *IL1RAPL1*, *ERCC1*, *RAB27A*
0009725 Response to hormone stimulus	16	8.63×10^{-4}	*KCNMA1*, *BSG*, *CCL2*, *SOCS2*, *FBP1*, *PDE3B*, *PTEN*, *CD38*, *PLA2G4A*, *SORBS1*, *BCL2*, *WFDC1*, *SRD5A2*, *GNG4*, *EIF2B4*, *GHR*
0042110 T-cell activation	9	9.58×10^{-4}	*BCL2*, *LCK*, *PSEN2*, *PPP3CB*, *CD2*, *CXCL12*, *DPP4*, *RAB27A*, *RHOH*
0048511 Rhythmic process	9	0.00106	*KCNMA1*, *CYP7B1*, *HLF*, *PLA2G4A*, *EGR3*, *PTGDS*, *BCL2*, *LFNG*, *EIF2B4*

GO, Gene Ontology.

Discussion

PCa is a relatively common cancer in men, with a subset ultimately developing metastatic disease to other regions of the body (25). The severity of the disease and its clinical heterogeneity, combined with the lack of effective diagnostic markers and therapeutic strategies, make the treatment of PCa a major challenge (26,27). Therefore, it is urgent to screen key genes that are associated with the metastasis of PCa with the aim of improving its treatment. The present study used bioinformatics methods to identify the DEGs between metastatic and localized PCa expression profiles. The results revealed that the expression of 561 genes (including 208 upregulated and 353 downregulated genes) was altered in metastatic PCa, compared with localized PCa. PPI networks were then constructed to reveal the associations among these genes. Furthermore, two potentially important modules were selected from the downregulated DEG set and their functions were determined by GO and KEGG pathway analyses.

The upregulated DEGs were significantly enriched in 'response to hypoxia' and 'response to oxygen levels'. Hypoxia increases the likelihood of tumor invasion and metastasis by activating relevant gene expression through the expression of hypoxia-inducible factor (HIF) (28). HIF is a transcription factor that responds to changes in the available oxygen in the cell (29). A previous study demonstrated that hypoxia may regulate each step in the process of tumor metastasis, from the initial epithelial-mesenchymal transition to the final organotropic colonization (28). Hypoxia also increases the mRNA expression of lysyl oxidase, which is associated with the early and later stages of metastasis (30). It also has been reported that hypoxia promotes cell invasion in PCa cells (31). Thus, these genes may be associated with the metastasis of PCa.

In addition, it was identified that the downregulated DEGs were enriched in 'calcium signaling pathway'. Calcium, as a ubiquitous second messenger, is an important signaling molecule that is involved in numerous fundamental physiological functions, including the cell cycle, apoptosis and migration (32). Certain human diseases have been associated with the dysregulation of calcium homeostasis, including hypertension, diabetes, Alzheimer's disease, cardiovascular disease and cancer (33,34). Calcium signaling in cancer cells has been demonstrated to be associated with events during tumor progression, including migration, invasion and

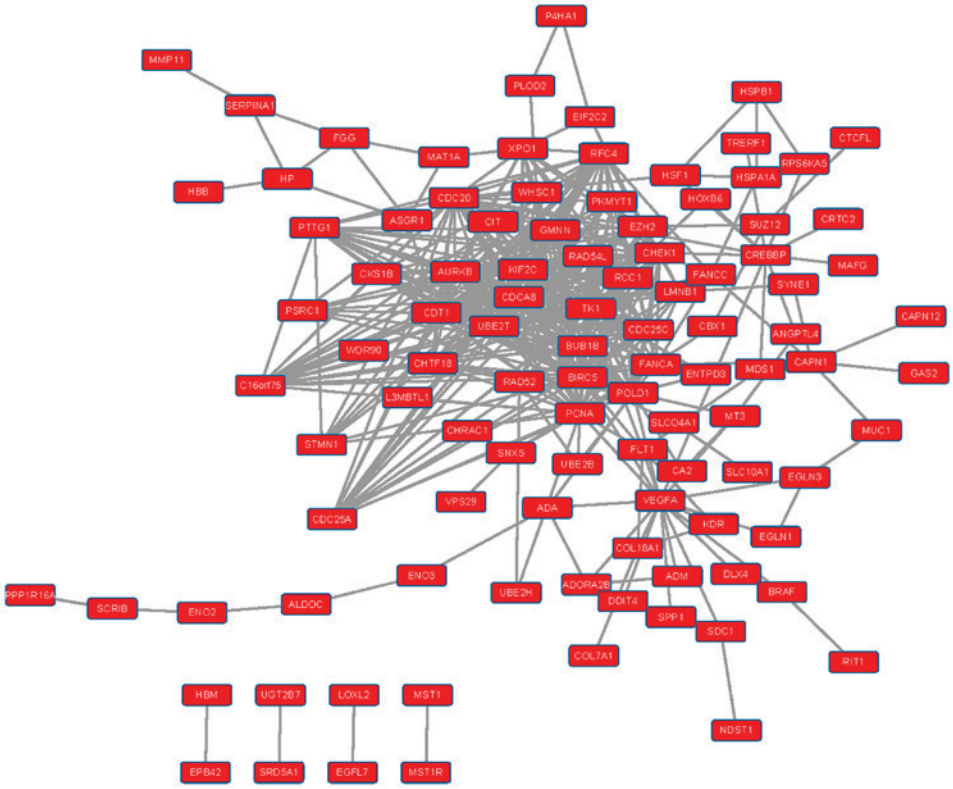

Figure 1. Protein-protein interaction network of the upregulated differentially expressed genes between metastatic and localized prostate cancer tissue samples.

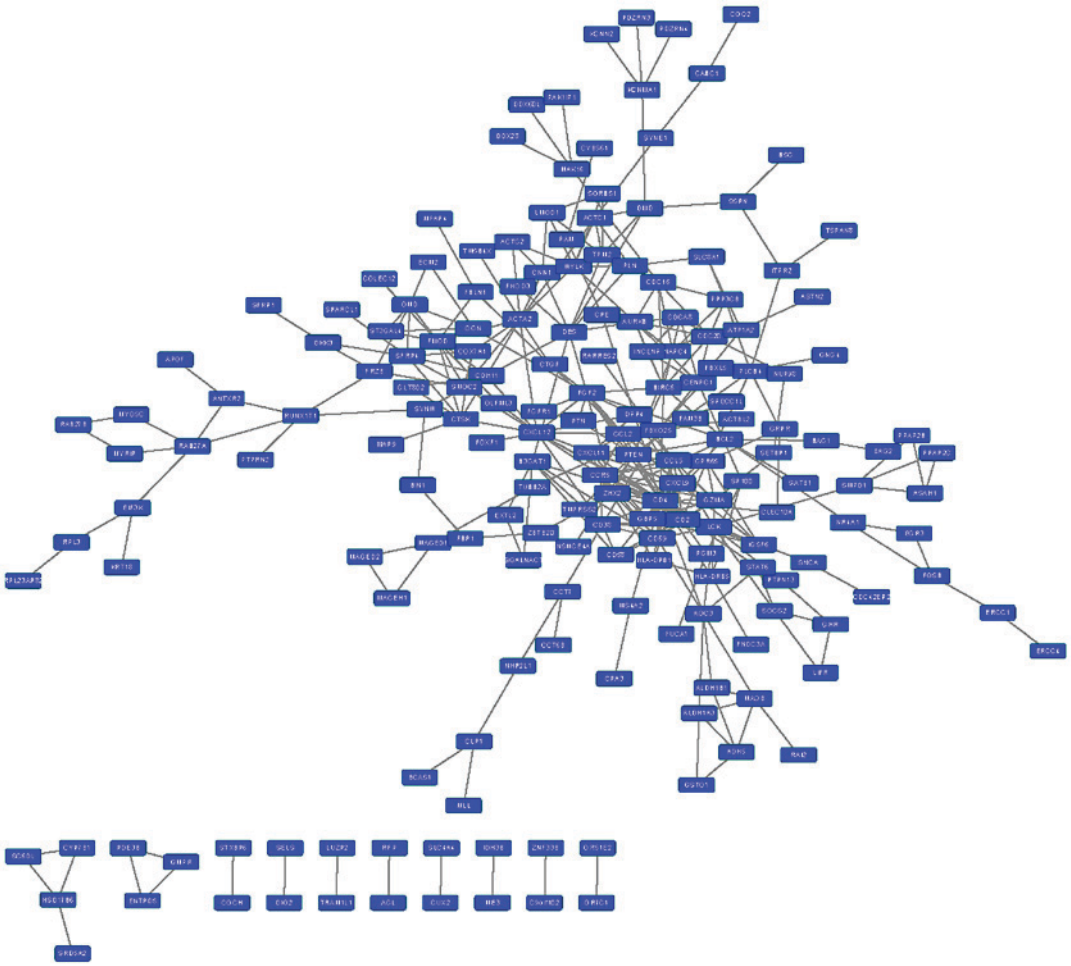

Figure 2. Protein-protein interaction network for downregulated differentially expressed genes between metastatic and localized prostate cancer tissue samples.

Table V. Differentially expressed genes with the top-20 highest connectivity degree in the protein-protein interaction network.

Status	Gene symbol	Degree
Upregulated	*PCNA*	34
	CDCA8	30
	CDC20	30
	BIRC5	30
	POLD1	29
	CDT1	29
	KIF2C	28
	CHEK1	28
	AURKB	28
	TK1	27
	RFC4	27
	BUB1B	27
	RAD54L	25
	UBE2T	22
	CDC25C	21
	GMNN	20
	C16orf75	19
	PTTG1	19
	EZH2	18
	CDC25A	18
Downregulated	*CD4*	25
	CCR5	15
	LCK	15
	CXCL12	14
	FMOD	13
	FGF2	13
	CXCL9	12
	CD2	12
	CD69	12
	CCL5	12
	PTEN	11
	ACTA2	11
	GZMA	11
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	CCL2	9
	DES	9
	CDC20	9
	AURKB	9
	SFRP4	9
	MYLK	9

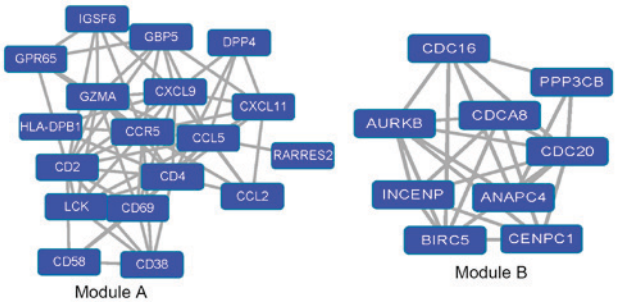

Figure 3. Module A and module B of the protein-protein interaction network for downregulated differentially expressed genes between metastatic and localized prostate cancer tissue samples.

metastasis (35). Several membrane-bound Ca^{2+} channels have been reported to have critical roles in regulating cancer cell migration and malignant metastasis (32,36). Cationic channels of the transient receptor potential (TRP) family are considered to be key players in calcium homoeostasis (37). The expression of transient receptor potential melastain 8 (TRPM8), part of the TRP channel subfamily, has been demonstrated to reduce the migration of PCa PC-3 cells (37,38). TRP vanilloid 2, another TRP channel, enhances PCa cell migration and invasion (39). Therefore, the DEGs enriched in 'response to hypoxia' and 'calcium signaling pathway' may contribute to the metastasis of PCa.

Based on the result of a PPI network construction from the DEGs, it was identified that a number of DEGs may be linked with others. *CD4*, *LCK* and *CCR5* had high degrees of connectivity in the PPI network and were associated with 'immune system'. It has been reported that a range of T-cell subsets found in solid tumors are associated with tumor progression and metastasis (40). *CD4* encodes a T-lymphocyte membrane glycoprotein and functions as an adhesion molecule that binds to non-polymorphic regions of major histocompatibility complex class II antigens (41,42). $CD4^+$ cells are involved in the pulmonary metastasis of mammary carcinoma (43). It has been reported that the infiltrating $CD4^+$ T cells may promote PCa metastasis (44). Furthermore, *CD4* was also associated with 'cell adhesion molecules (CAMs)' in module A. CAMs, which belong to the family of membrane receptors that mediate cell-matrix and cell-cell interactions, are essential for transducing intracellular signals, which are responsible for facilitating cell adhesion, invasion, migration and metastasis (45). *LCK* is a member of the Src tyrosine kinase family expressed primarily in T lymphocytes and natural killer cells (46). LCK localizes to the surface of the plasma membrane and binds to transmembrane receptors, including CD4 (47). CCR5, a protein on the surface of white blood cells, has been demonstrated to alter the proliferation of PCa cells (47). In the present study, *LCK* and *CCR5* were connected to *CD4* in the PPI network. Therefore, we hypothesize that *CD4*, *LCK* and *CCR5* could have important roles in the metastasis of PCa.

PCNA exhibited a high degree of connectivity in the PPI network for upregulated DEGs. *PCNA* belongs to the family of DNA sliding clamps, is essential for DNA replication and is associated with DNA repair, chromatin remodeling and epigenetics (48). The phosphorylation of PCNA is a frequent event in the development of prostate cancer (49). It was demonstrated that PCNA was highly expressed in triple-negative breast cancer and associated with axillary lymph node metastasis (50). Although the regulation of PCNA function in prostate cancer cells has not been fully characterized, the data of the present study indicated that *PCNA* may have a critical role in PCa metastasis.

GO analysis of module B revealed that the DEGs were enriched in terms associated with the cell cycle.

Table VI. Enriched Kyoto Encyclopedia of Genes and Genomes pathways for genes in module A and module B.

Module	Term	Count	P-value	Genes
A	hsa04062: Chemokine signaling pathway	5	4.77×10^{-4}	*CCL2*, *CCR5*, *CXCL9*, *CXCL11*, *CCL5*
	hsa04060: Cytokine-cytokine receptor interaction	5	0.00171	*CCL2*, *CCR5*, *CXCL9*, *CXCL11*, *CCL5*
	hsa04514: Cell adhesion molecules	4	0.00242	*CD58*, *CD2*, *CD4*, *HLA-DPB1*
	hsa04640: Hematopoietic cell lineage	3	0.01408	*CD38*, *CD2*, *CD4*
	hsa04620: Toll-like receptor signaling pathway	3	0.01912	*CXCL9*, *CXCL11*, *CCL5*
B	hsa04114: Oocyte meiosis	4	3.88×10^{-5}	*ANAPC4*, *PPP3CB*, *CDC20*, *CDC16*
	hsa04110: Cell cycle	3	0.003482	*ANAPC4*, *CDC20*, *CDC16*
	hsa04120: Ubiquitin mediated proteolysis	3	0.004173	*ANAPC4*, *CDC20*, *CDC16*

Table VII. Top ten enriched GO functions for genes in module A and module B.

Module	GO term	Count	P-value	Genes
A	0006955 Immune response	11	6.77×10^{-10}	*IGSF6*, *GBP5*, *CCL2*, *CCR5*, *GZMA*, *GPR65*, *CXCL9*, *CD4*, *HLA-DPB1*, *CXCL11*, *CCL5*
	0042330 Taxis	5	3.07×10^{-5}	*CCL2*, *CCR5*, *CXCL9*, *CXCL11*, *CCL5*
	0006935 Chemotaxis	5	3.07×10^{-5}	*CCL2*, *CCR5*, *CXCL9*, *CXCL11*, *CCL5*
	0006874 Cellular calcium ion homeostasis	5	5.19×10^{-5}	*CD38*, *CCL2*, *CCR5*, *LCK*, *CCL5*
	0055074 Calcium ion homeostasis	5	5.77×10^{-5}	*CD38*, *CCL2*, *CCR5*, *LCK*, *CCL5*
	0006875 Cellular metal ion homeostasis	5	6.79×10^{-5}	*CD38*, *CCL2*, *CCR5*, *LCK*, *CCL5*
	0007166 Cell surface receptor linked signal transduction	10	7.77×10^{-5}	*IGSF6*, *CCL2*, *CCR5*, *LCK*, *GPR65*, *CD2*, *CXCL9*, *CD4*, *CXCL11*, *CCL5*
	0055065 Metal ion homeostasis	5	8.08×10^{-5}	*CD38*, *CCL2*, *CCR5*, *LCK*, *CCL5*
	0030005 Cellular di-, tri-valent inorganic Cation homeostasis	5	1.20×10^{-4}	*CD38*, *CCL2*, *CCR5*, *LCK*, *CCL5*
	0055066 Di-, tri-valent inorganic cation homeostasis	5	1.46×10^{-4}	*CD38*, *CCL2*, *CCR5*, *LCK*, *CCL5*
B	0000278 Mitotic cell cycle	8	1.08×10^{-11}	*CDCA8*, *INCENP*, *ANAPC4*, *PPP3CB*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0022403 Cell cycle phase	8	2.39×10^{-11}	*CDCA8*, *INCENP*, *ANAPC4*, *PPP3CB*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0007067 Mitosis	7	1.19×10^{-10}	*CDCA8*, *INCENP*, *ANAPC4*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0000280 Nuclear division	7	1.19×10^{-10}	*CDCA8*, *INCENP*, *ANAPC4*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0000087 M phase of mitotic cell cycle	7	1.33×10^{-10}	*CDCA8*, *INCENP*, *ANAPC4*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0048285 Organelle fission	7	1.52×10^{-10}	*CDCA8*, *INCENP*, *ANAPC4*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0022402 Cell cycle process	8	2.14×10^{-10}	*CDCA8*, *INCENP*, *ANAPC4*, *PPP3CB*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0051301 Cell division	7	7.03×10^{-10}	*CDCA8*, *INCENP*, *ANAPC4*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0000279 M-phase	7	1.36×10^{-9}	*CDCA8*, *INCENP*, *ANAPC4*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*
	0007049 Cell cycle	8	1.99×10^{-9}	*CDCA8*, *INCENP*, *ANAPC4*, *PPP3CB*, *BIRC5*, *CDC20*, *CDC16*, *AURKB*

GO, Gene Ontology.

For example, *BIRC5*, *CDCA8*, inner centromere protein, *ANAPC4*, *CDC20*, *CDC16* and *AURKB* were enriched in 'mitosis', 'nuclear division', 'M phase of mitotic cell cycle' and 'organelle fission'. Cell cycle deregulation is a common feature of tumors (51). *BIRC5* is a member of the inhibitor of apoptosis protein family, which has a critical role in the occurrence and progression of tumors (52). *BIRC5* has been reported to be associated with colorectal cancer tumorigenesis and progress (53). BIRC5, as a component of the chromosomal passenger complex, is involved in the microtubule-kinetochore attachment that ensures cohesion between sister chromatids and centrosome aggregation (54). The regulation of centrosome coalescence may link mitosis to cell adhesion (55). Therefore, *BIRC5* may be associated with PCa cell metastasis.

There are certain limitations to the present study; due to the *in silico* nature of the analysis of the PCa expression profiles, the obtained results were only forecasted with contrived criteria. Therefore, a number of important genes may have been ignored. The absence of experiments verifying the expression of DEGs was also a limitation in the present study. The limited sample size may also represent a limitation and restrict the ability to draw a valid conclusion. Despite these caveats, the results identified in the present study may provide a novel stimulus for the further experimental study of PCa metastasis.

The present study analyzed the gene expression profiles of localized and metastatic PCa tissue using bioinformatics analysis. It was identified that DEGs, including *CD4*, *PCNA* and *BIR5C*, may serve roles in driving the metastasis of PCa. These genes may be novel potential biomarkers and/or therapeutic targets for patients with PCa. However, further research is required to confirm these results.

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