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An extensive study of the mechanism of prostate cancer metastasis

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The study aimed to identify the pivotal genes and pathways involved in prostate cancer metastasis. Using the expression profile dataset GSE7930, downloaded from the Gene Expression Omnibus (GEO) database, differentially expressed genes (DEGs) between primary and highly metastatic prostate cell samples were screened, followed by functional analysis and tumor associated genes (TAG) screening. Protein-protein interaction (PPI) network of DEGs was constructed and module analysis was performed. The expression of DEGs and pathway related genes were evaluated by PCR analysis and the migration ability of prostate tumor cells was observed after *FABP4*-siRNA blocking. Upregulated *FABP4* and *GK* were significantly enriched in the PPAR signaling pathway, whereas downregulated *IGFBP3* and *THBS1* were involved in p53 signaling pathway. Among the identified DEGs, 4 downregulated genes (*IGFBP3*, *NPP4B*, *THBS1*, and *PCDH1*) and 2 upregulated genes (*GJA1* and *TUSC3*) were TAGs. The module was associated with focal adhesion, ECM-receptor interaction, p53 signaling, and gap junction pathways with the hub node GJA1. After FABP4 silencing by siRNAs in LNcap and metastatic DU-145 cells, the numbers of migrated cells were all significantly declined. The expressions of *IGFBP3*, *TP53* and *PPAR* were significantly lower in DU-145 cells than in LNcap cells. In conclusion, *FABP4*, *IGFBP3*, *THBS1*, and *GJA1* were determined to be potential markers of prostate cancer cell metastasis, and P53, PPAR and gap junction pathways were found to play important roles in prostate cancer cell metastasis. This study may provide helpful guidelines for clinical management.

Key words: metastasis, prostate cancer, microarray profile, differentially expressed genes, tumor associated genes

Prostate cancer is the second most common malignancy with 250,000 new deaths every year [1, 2]. It may show no apparent symptoms or mere urinary system disorders such as blood in the urine and difficulty in urinating. Moreover, serious symptoms such as bone pain in the vertebrae, spinal cord, pelvis, and ribs may occur due to metastasis of advanced prostate cancer to other organs of the body, especially the bones and lymph nodes, as well as invasion into local surrounding tissues including the rectum, bladder, and lower ureters [3, 4]. Determination of the stage of prostate cancer, a reflection of the extent of cancer metastasis, is essential for evaluating the prognosis of patients with prostate cancer. Although there are numerous approaches such as prostate cancer screening, prostate-specific antigen (PSA) blood testing, and digital rectal exams for detecting and preventing prostate cancer, the impact of those technologies on reducing incidence and morbidity of prostate cancer has proven controversial and may even result in unnecessary harm to patients [5]. Therefore, the major priority now is to identify promising biomarkers specific for predicting the probability of prostate cancer metastasis.

In recent years, some molecular pathways and genes have been reported to elucidate the molecular mechanism of prostate cancer metastasis. Some pathways play important role in the metastatic process of prostate cancer; for instance, Hedgehog (Hh) signaling has been reported to modulate prostate cancer metastasis [6]. Russell et al. reported that Stromal Cell-derived Factor-1/C-X-C Motif Chemokine Receptor 4 (SDF-1/CXCR4) pathway facilitates the metastasis of prostate cancer to bone [7]. Mitogen-activated protein kinase (MAPK) signaling pathway has been identified in metastatic prostate cancer samples [8], which promotes progression of the prostate cancer to advanced prostate cancer [9]. Besides, some genes have been found to play important role in prostate cancer metastasis. Secreted phosphoprotein 1 (*SPPI)* is reportedly involved in prostate

cancer bone metastasis [3]. Developmentally regulated GTP-binding protein 1 (*DRG1*) [10], Raf kinase inhibitor protein (RKIP) [11], mitogen-activated protein kinase kinase 4/stress-activated protein/Erk kinase 1 (*MKK4*/*SEK1*) [12] have been demonstrated to suppress prostate cancer metastasis. Despite these studies, the extensive molecular mechanisms underlying metastatic prostate cancer development are still poorly understood.

In an attempt to reveal the genes and pathways that specifically play key roles in prostate cancer metastasis, differentially expressed genes (DEGs) were identified between primary and highly metastatic prostate cancer samples. The specific biological functions of the identified DEGs were subsequently studied by Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, accompanied by screening the tumor associated genes (TAG) and TF (Transcription Factor) genes from the DEGs. Furthermore, a protein-protein interaction (PPI) network was established to evaluate interactions among the proteins encoded by DEGs, followed by extracting modules from the network to unearth the pivotal proteins showing multiple interactions with others.

Materials and methods

Microarray data and preprocessing. The expression profile dataset GSE7930 deposited by Wong et al. [13] and based on the Affymetrix Human Genome U133A Array platform, was downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo). Three primary prostate cancer subcutaneous tumor samples derived from the PC3-#78 cell line (GSM155023, GSM155024, GSM155025) and three highly metastatic prostate subcutaneous tumor tissues derived from the PC-#82 cell line (GSM155026, GSM155027, GSM155028) were used for expression profiling analysis. These prostate cancer cell lines were derived by the re-implantation of pMicro-1 cells into mice, which were obtained by implanting the human cell line PC-3 into mice.

Gene probes were assigned to corresponding gene names according to the Affymetrix genechip probe annotation profile provided by Brain Array Lab, and the expression value was averaged when numerous probes corresponded to the same gene. Gene expression data were sequentially subjected to preprocessing procedures including background correction, quantile normalization, and probe summarization using the robust multiarray analysis (RMA) of affy package in R language [14].

Differentially expressed gene (DEG) screening. After data preprocessing, DEGs were identified between primary prostate cancer cell lines and highly metastatic prostate cancer cell lines using the empirical Bayes method of Linear Models for Microarray Analysis (LIMMA) package in R language. A false discovery rate (FDR) <0.05 and $|\log2FC| \geq 1$ were regarded as the cut-off criteria.

GO and KEGG enrichment analysis for DEGs. For the purpose of providing an insight into exact biological functions related to the obtained DEGs, GO enrichment analysis was performed in terms of Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) based on Gene Ontology database [15], with $p<0.05$ as the strict cutoff. Besides, KEGG enrichment analysis based on KEGG database [16] was conducted to determine the signaling pathways with $p<0.05$, which were enriched by the acquired DEGs.

Gene function annotation. The transcription factor database (TRANSFAC) is publicly available and is rich in information about TFs, TF binding sites, and DNA-binding profiles [17]. Based on the TFs information from TRANSFAC, the DEGs encoding TFs were picked out from the identified DEGs. TAGs including oncogenes and tumor suppressor genes (TSG) were also extracted from DEGs based on the TAG database [18] and TSGene database [19].

Protein-Protein interaction (PPI) network construction and module extraction. The Search Tool for the Retrieval of Interacting Genes (STRING) [20] is a database containing all the known and predicted protein interactions and associations. By mapping DEGs in the STRING database, the PPI network was built and visualized using Cytoscape software [21], in which the combined score of pairwise interaction was ≥0.9. In the PPI network, the undirected link represented a pairwise interaction between proteins, which served as "nodes". "Degree" was defined as the number of interactions between a given protein and other proteins, and the protein with the highest degree was considered as a hub "node".

Subsequently, analysis of the constructed PPI network was performed to mine the module with a FDR <0.05 using the BioNet analytical tool that combines the data on transcriptome and functions with biological networks for an integrative network analysis, followed by KEGG enrichment analysis for module genes.

Quantitative reverse transcription-PCR (RT-PCR) analysis. The primary prostate tumor LNcap cells and metastatic prostate cancer DU-145 cells were respectively maintained in complete DMEM and MEM media (Gibco Laboratories, Grand Island, NY) at standard conditions. After culture, cells were collected by centrifugation at 1,000 rpm for 5min. The total RNA of cells was isolated by Trizol kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions.

Total 0.5 μg RNA was reversely transcribed to cDNA by using TaKaRa cDNA Synthesis Kit (TAKARA, Japan). The expressions of *IGFBP3, TP53* and *PPAR* were analyzed by PCR analysis. The PCR reaction volume was 20 μl, 10 μl SYBR Premix EX Taq (2x), 1 μl forward primer, 1 μl reverse primer, and 8 μl cDNA. The primer sequences of *IGFBP3, THBS1, GJA1, TP53* and *PPAR* are listed in Supplemental Table 1. PCR reaction was performed in Applied Biosystems ViiA7 PCR System as follows: 50 °C for 3 min followed by 40 cycles of 95 °C for 3 min, 95 °C for 10 s, 60 °C for 30 s. The same experiments were performed in triplicate.

FABP4 silencing by siRNA. LNcap and DU-145 cells were seeded in 12-well plates. Cells were cultured in antibiotic-free media until the density was up to 30–50%. Then, cells were incubated with 5 µl FABP4 siRNA (20 µM) in a final volume of 100 µl diluted by serum-free media, followed by transfection with lipofectamine 2000. FABP4-siRNA (sense-siFABP4: 5'-GGAUGUGAUCACCAUUAAA-3'; antisense-siFABP4 5'-UUUAAUGGUGAUCACAUCC-3') and non-target control siRNA (sense-siNC: 5'-UUCUCCGAACGUGUCACGUTT-3', antisense-siNC: 5'-ACGUGACACGUUCGGAGAATT-3') were synthesized by Shanghai Tuoran biotechnology co. LTD, Shanghai, China. The expression of *FABP4* in LNcap and DU-145 cells was observed after cells were cultured for 24 h and 48 h, respectively.

Cell migration assay. After FABP4 silencing, the cell migration ability was assessed by using a 24-well transwell plate according to the manufacturer's instructions (Corning, NY, USA). After FABP4 siRNA transfection, total 3×10^4 cell/well were seeded into the upper chamber in serum-free media. The lower chamber was filled with 700 µL media supplemented with 10% FBS. After cells were incubated for 24 h, cells that migrated through the surface of membrane were fixed with methanol and stained with hematoxylin for 15 min. Cells were counted by using CKX31 microscope (Olympµs, Lake Success, NY) at ×100 magnification from three random fields.

Statistical analysis. All the data were expressed as mean ± SEM (standard error of the mean) and analyzed by SPSS 22.0 software. Differences between groups were analyzed by t test. $p<0.05$ was considered as significant.

Results

DEGs analysis. We found 19 upregulated DEGs and 22 downregulated DEGs between the primary and highly metastatic prostate cell samples (Table 1).

Enrichment analyses for DEGs. The KEGG pathways enriched by the upregulated and downregulated DEGs are displayed in Table 2, showing that upregulated fatty acid binding protein 4 (*FABP4*) and glucokinase (*GK*) were significantly enriched in peroxisome proliferator-activated receptor (PPAR) signaling pathway (Supplemental Figure 1), whereas downregulated insulin-like growth factor-binding protein 3 (*IGFBP3*) and thrombospondin 1 (*THBS1*) were significantly enriched in p53 signaling pathway (Supplemental Figure 2). As shown in Table 3, downregulated DEGs were closely related to several biological processes, including magnesium ion response, apoptotic cell clearance, regulation of macrophage activation, peptide cross-linking, negative regulation of endopeptidase activity, and cell adhesion, whereas upregulated DEGs were significantly linked to the regulation of behavior, response to hormone stimulus, tube development, and cardiac muscle cell proliferation.

TFs and TAG analyses. A range of analyses were conducted to classify DEGs based on their functions in tumors. Based on the DEGs, downregulated genes like *IGFBP3*, natriuretic peptide 4B (*NPP4B*), *THBS1*, and protocadherin-1 (*PCDH1*), and upregulated genes like Gap junction alpha-1 protein

Table 1. DEGs screening.

Category	Gene name	FDR value	|log2FC| value
Upregulated DEGs	*CCDC6*	0.023673	1.031336
	DNM3	0.000476	3.209143
	FABP4	0.007272	1.353411
	GJA1	0.027124	1.399577
	GK	0.002738	1.154329
	GOLM1	0.022781	1.306406
	GPRC5C	0.006742	1.300801
	HAPLN1	0.000511	1.489024
	HSD17B2	0.002566	1.541932
	IVNS1ABP	0.009312	1.064485
	LMO4	0.024245	1.074291
	MDK	0.024245	1.766798
	NAP1L3	0.018714	1.309721
	PLA2G7	0.044923	1.132075
	RBP4	0.027222	1.018216
	SPARC	0.000476	2.047274
	TENM4	0.027387	1.252775
	TUSC3	0.02776	1.060124
	UTS2	0.000112	3.694131
Downregulated DEGs	*AMIGO2*	0.022781	1.01386
	ANO1	0.007021	1.23848
	ATP1B1	0.022781	1.2993
	CD70	0.011196	1.01488
	CEACAM5	0.006742	2.29386
	CEACAM6	0.018714	1.71073
	GPR87	0.000699	1.51978
	IGFBP3	0.024245	1.11962
	IGFBP6	0.024245	1.10129
	INPP4B	0.029642	1.26662
	KRT7	0.005608	1.14847
	MT1M	0.009713	1.12131
	PCDH1	0.026083	1.33697
	PI3	0.009312	1.49302
	RBPMS	0.000511	1.27176
	SCG5	0.019963	1.47413
	SLPI	0.000668	2.03183
	TFF2	0.024245	1.18254
	TGM2	0.022832	1.76294
	THBS1	0.002968	1.7228
	TNFAIP6	0.023673	1.10826
	TNFRSF11B	0.00199	2.32087

DEGs, differentially expressed genes

Table 2. KEGG pathway enrichment analysis.

Regulation	KEGG Pathway	Gene Counts	p-value	Gene List
Up	PPAR signaling pathway	2	0.003747	*FABP4,GK*
Down	p53 signaling pathway	2	0.002675	*IGFBP3,THBS1*

KEGG, Kyoto Encyclopedia of Genes and Genomes

Table 3. GO enrichment analysis.

Category	GO ID	Term	Gene Counts	p-value	Gene List
Down	GO:0032026	response to magnesium ion	2	0.0000682156	*THBS1, TNFRSF11B*
	GO:0043277	apoptotic cell clearance	2	0.000393871	*TGM2,THBS1*
	GO:0043030	regulation of macrophage activation	2	0.000473712	*TFF2,THBS1*
	GO:0018149	peptide cross-linking	2	0.000704749	TGM2,THBS1
	GO:0010951	negative regulation of endopeptidase activity	3	0.000743516	PI3,SLPI,THBS1
	GO:0046850	regulation of bone remodeling	2	0.001040601	INPP4B, TNFRSF11B
	GO:0007155	cell adhesion	6	0.001805051	AMIGO2, ATP1B1,PCDH1,TGM2, THBS1,TNFAIP6
Up	GO:0050795	regulation of behavior	3	0.000773064	*MDK, PLA2G7, UTS2*
	GO:0060038	cardiac muscle cell proliferation	2	0.001006527	RBP4,TENM4
	GO:0009725	response to hormone stimulus	5	0.001207102	GJA1,MDK,RBP4,SPARC,UTS2
	GO:0035295	tube development	4	0.002248938	GJA1, LMO4, RBP4,SPARC

GO, gene ontology

Table 4. TAG and TF analysis.

	TF counts	TAG counts	TAG genes
Down	0	4	*IGFBP3*, *NPP4B*, *THBS1*, *PCDH1*
Up	0	2	*GJA1*,*TUSC3*

TF, Transcription Factor; TAG, Tumor Associated Genes

(*GJA1*) and tumor suppressor candidate 3 (*TUSC3*) were determined to be TAGs (Table 4), whereas no DEG associated TF was identified.

PPI network and module analysis. As shown in Figure 1, the PPI network was constructed with DEGs, the degree of nodes (proteins) in the PPI network was calculated, and the nodes with degrees ≥10 were listed in descending order as follows: urotensin-2 (UTS2, degree = 47), secreted protein acidic and rich in cysteine (SPARC, degree = 32), estradiol 17-beta-dehydrogenase 2 (HSD17B2, degree = 29), FABP4 (degree = 29), THBS1 (degree = 28), GJA1 (degree = 27), lipoprotein-associated phospholipase A_2 (PLA2G7, degree = 19), IGFBP3 (degree = 17), inositol polyphosphate-4-phosphatase B (INPP4B, degree = 15), and LIM domain only 4 (LMO4, degree = 11).

The module extracted from the PPI network included 28 nodes with the hub "GJA1" (degree = 7, Figure 2), and subsequent KEGG analysis showed that the genes included in the module were significantly associated with focal adhesion, ECM-receptor interaction, p53 signaling pathway, melanoma, arrhythmogenic right ventricular cardiomyopathy (ARVC), and gap junction pathways (Table 5).

The expression of IGFBP3, TP53 and PPAR by RT-PCR analysis. The expression of *IGFBP3, TP53 and PPAR* genes was validated by RT-PCR analysis. Compared with the primary prostate cancer LNcap cells, the relative expression of *IGFBP3* was significantly declined in metastatic DU-145 cells (1.00 vs. 0.72; $p<0.001$). Similar findings were found in the expression of *TP53* and *PPAR* genes. The expression of *TP53* and *PPAR* was significantly lower in DU-145 cells than in LNcap cells ($p<0.01$, $p<0.001$) (Figure 3).

The expression of FABP4 and cell migration after siRNA transfection. After transfected with *FABP4*-siRNA at 24 h and 48 h, the expressions of *FABP4* were evaluated. As shown in Figure 4 A and B, the expression of *FABP4* was significantly declined in LNcap cells at 24 h and 48 h after *FABP4*-siRNA silencing ($p<0.01$). The inhibitive rate of *FABP4* expression in DU-145 cells was more significant. Compared with cells transfected with non-target siRNA, DU-145 cells showed a significant decline of *FABP4* expression after siRNA blocking ($p<0.001$, Figure 4C, 4D). Besides, after *FABP4*-siRNA transfection, the number of migrated cells was significantly declined in LNcap and DU-145 cells at 24 h and 48 h (all $p<0.001$) (Figure 4 E–H).

Discussion

Prostate cancer remains a threat to peoples' health. Far worse is its moderate metastatic propensity to spread to other organs of the body. We aimed to unravel the molecular mechanism underlying prostate cancer metastasis. In a screen for DEGs implicated in metastasis, 19 upregulated and 22 downregulated DEGs were acquired between the primary and highly metastatic prostate cancer samples. A range of bioinformatics approaches was utilized to analyze the exact function of DEGs in order to identify potential biomarkers of prostate cancer metastasis.

FABP4, also termed aP2 (adipocyte protein 2), is a carrier protein mainly residing in adipocytes and macrophages. Emerging studies have demonstrated that targeting this protein might be conducive to treat various diseases such as asthma, atherosclerosis, and diabetes [22–24]. A recent

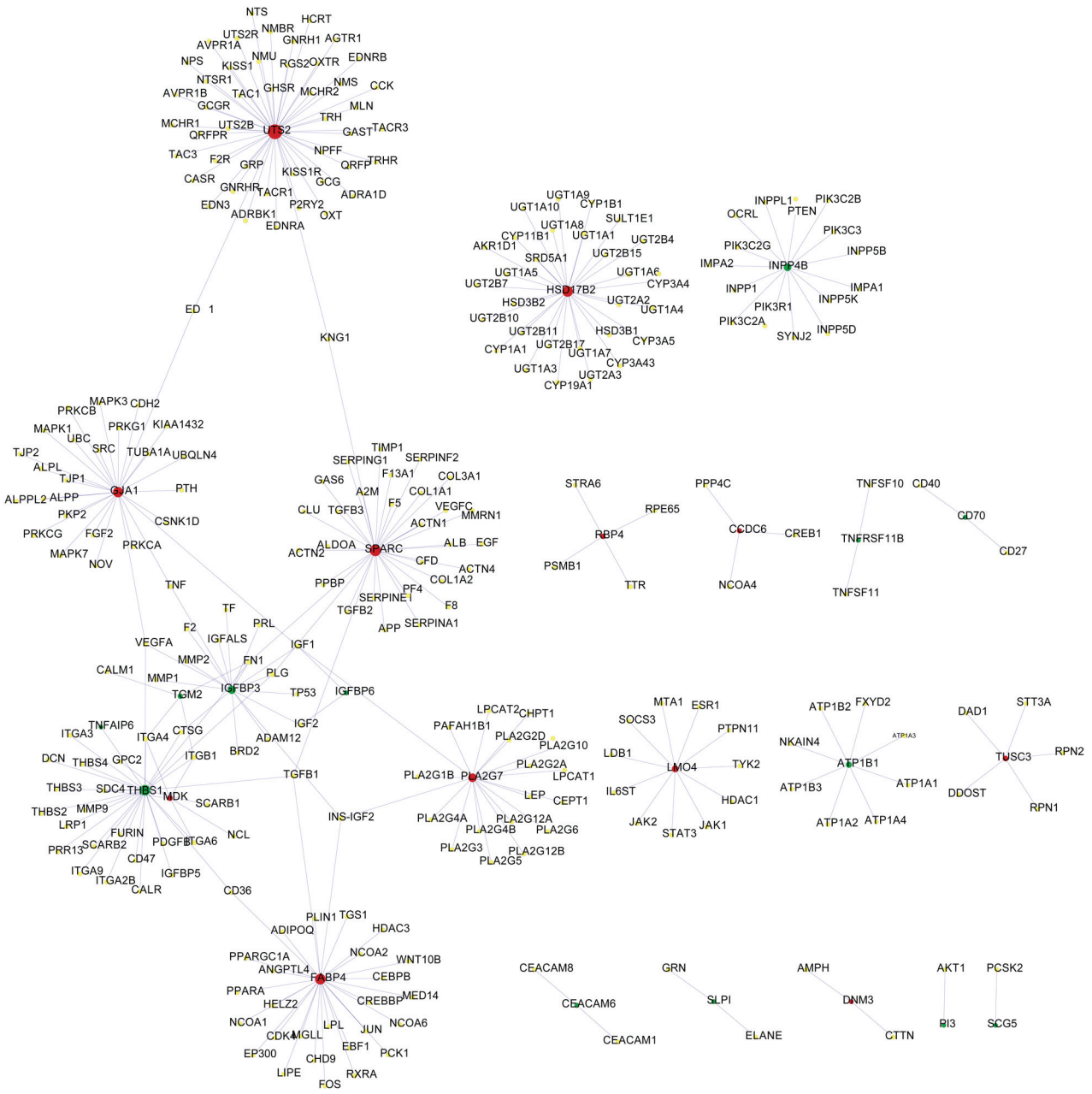

Figure 1. Protein-Protein interaction network analysis. Red node represents upregulated DEGs; green node represents downregulated DEGs; yellow node represents non-DEGs. DEGs, differentially expressed genes

study showed that *FABP4* was upregulated in bone metastasis samples from prostate cancer patients and that *FABP4* pathway might play an important role in the aggression of bone metastasis [25]. Similarly, the present study showed that *FABP4* expression was significantly increased in highly metastatic cells compared with that in the primary cell line, and was enriched in PPAR pathway, suggesting that *FABP4* might promote prostate cancer metastasis through the PPAR pathway. In order to validate the findings based on microarray data analysis, we performed the experimental validation. Our data showed that the migration ability of prostate tumor cells was significantly declined after *FABP4* silencing, which indicates the significant role of *FABP4* in mediating prostate cancer cell migration. However, in the present study, the expression of *PPAR* was significantly declined in DU-145 and LNcap cells. Whether *FABP4* plays a key role in the cell migration through PPAR pathway should be further analyzed.

Table 5. KEGG enrichment analysis for the module.

KEGG Pathway	Gene Counts	p-value	Upregulated genes	Downregulated genes
Focal adhesion	6	0.0000191	*MAPK1*, *COL3A1*	*FN1*, *THBS1*, *ITGA3*
ECM-receptor interaction	4	0.000107	*COL3A1*	*THBS1*, *ITGA3*
Bladder cancer	3	0.000258	*MAPK1*, *MMP1*	THBS1
Pathways in cancer	6	0.000294	*MAPK1*, *MMP1*	*FGF2*, *FN1*, *ITGA3*
p53 signaling pathway	3	0.001071		*IGFBP3*, *THBS1*
Melanoma	3	0.001215	*MAPK1*	*FGF2*
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	3	0.00137	*GJA1*	*CDH2*, *ITGA3*
Gap junction	3	0.002408	*TUBA1A*, *MAPK*, *GJA1*	
Regulation of actin cytoskeleton	4	0.003454	*MAPK1*	*FGF2*, *FN1*, *ITGA3*
Aldosterone-regulated sodium reabsorption	2	0.007112	*MAPK1*	

KEGG, Kyoto Encyclopedia of Genes and Genomes

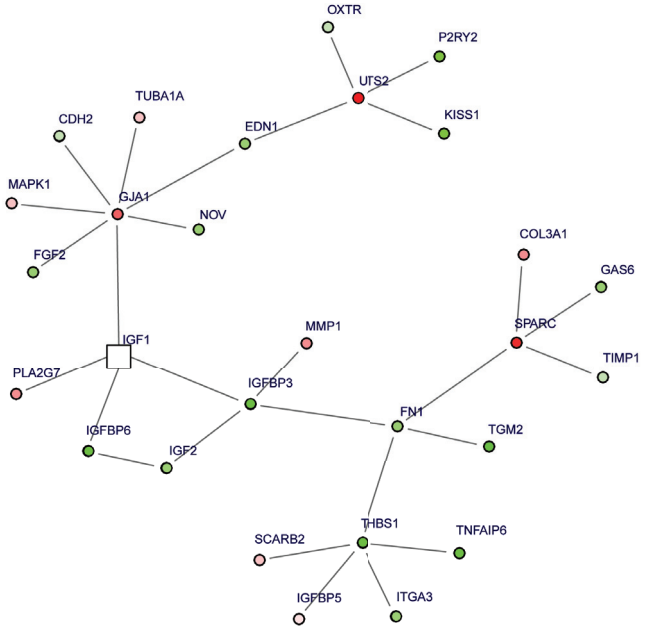

Figure 2. The module extracted from the Protein-Protein interaction network. The color depth of the node is positively associated with the fold change of the node gene. Red node, upregulated gene; green node, downregulated gene; white node, genes with significant changes in expression; square nodes, unimportant genes; round nodes, important genes.

IGFBP3 belongs to the *IGFBP3* family with the characteristic IGFBP domain, and often functions in a complex with insulin-like growth factor [26]. Accumulating evidences based on findings from epidemiological studies and *in vivo* studies have revealed that *IGFBP3* is a metastasis-suppressed gene in prostate cancer and could serve as a potential biomarker for its diagnosis and prognosis [27–29]. In the present study, IGFBP3 was found to be downregulated in highly metastatic cancer cells, which was determined by PCR analysis. PCR analysis showed that the expression of IGFBP3 was significantly lower in metastatic DU-145 cells than in the primary LNcap cells. Besides, IGFBP3 was identified as a

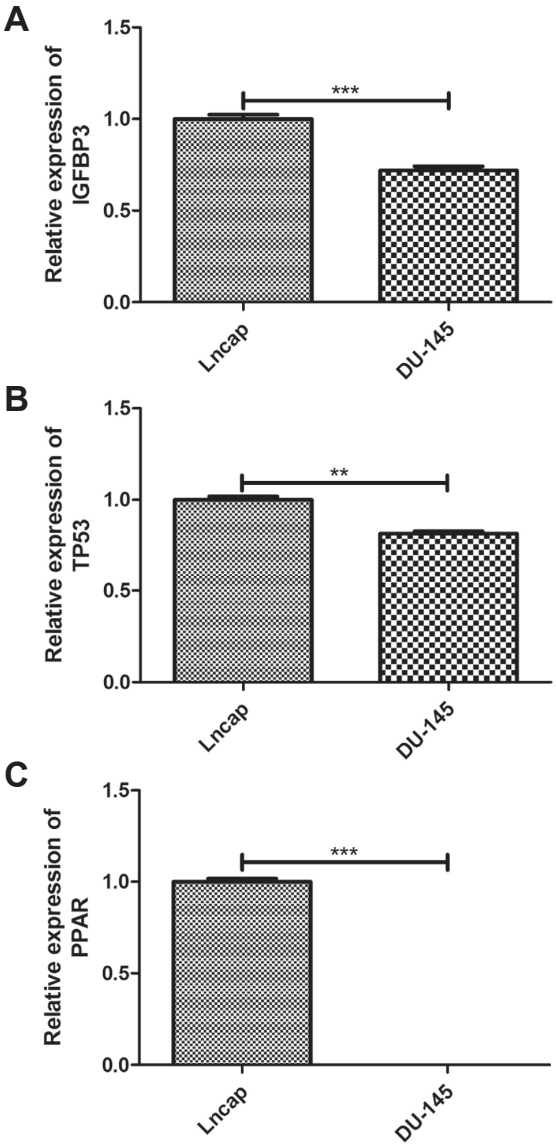

Figure 3. The expression of IGFBP3, TP53, and PPAR in primary LNcap and metastatic DU-145 cells; A: relative expression of IGFBP3; B: relative expression of TP53; C: relative expression of PPAR;p<0.01, ***p<0.001**

TAG, which indicated the inhibitory role of IGFBP3 in prostate cancer metastasis. Besides, the downregulated genes, *IGFBP3* and *THBS1*, were mainly enriched in the p53 pathway by bioinformatic analysis. The p53 signaling pathway has been considered a critical determinant of anti-tumor responses and expression of the p53 tumor suppressor gene is known to result in prostate cancer cell death through apoptotic and autophagic signaling pathways [30]. Inactivation of the p53 signaling pathway induced by mdm2 (murine double minute-2) overexpression could facilitate tumor progression in prostate cancer [31]. *THBS1*, encoding a matrix glycoprotein, is involved in interactions between cells, and between cells and the matrix. In consistence with the result of this study, *THBS1* is known to inhibit tumor-related angiogenesis by regulating p53 [32] and repress tumor cell growth by activating transforming growth factor β in tumor cells [33]. In this study, *THBS1* was a critical hub protein in the PPI network and was enriched in apoptotic cell clearance, regulation of macrophages, and the p53 signaling pathway, indicating that the downregulated THBS1 might inhibit the apoptotic and autophagic signaling pathway to facilitate growth of metastatic prostate cancer cells. Besides, *IGFBP3* has been found to induce apoptosis in metastatic prostate cancer cells [34]. *IGFBP3* was downregulated and also enriched in the p53 signaling pathway, indicating that the downregulated *IGFBP3* might inactivate the p53 apoptotic signaling pathway by inhibiting apoptosis in metastatic prostate cancer cells. TP53 gene is a tumor suppressor that encodes P53 protein and is mutated in various human tumors [35]. In the present study, PCR analysis showed that the expression of TP53 gene is significantly decreased in the metastatic DU-145 cells compared with the LNcap cells, suggesting p53 signaling pathway was inhibited in metastatic DU-145 cells.

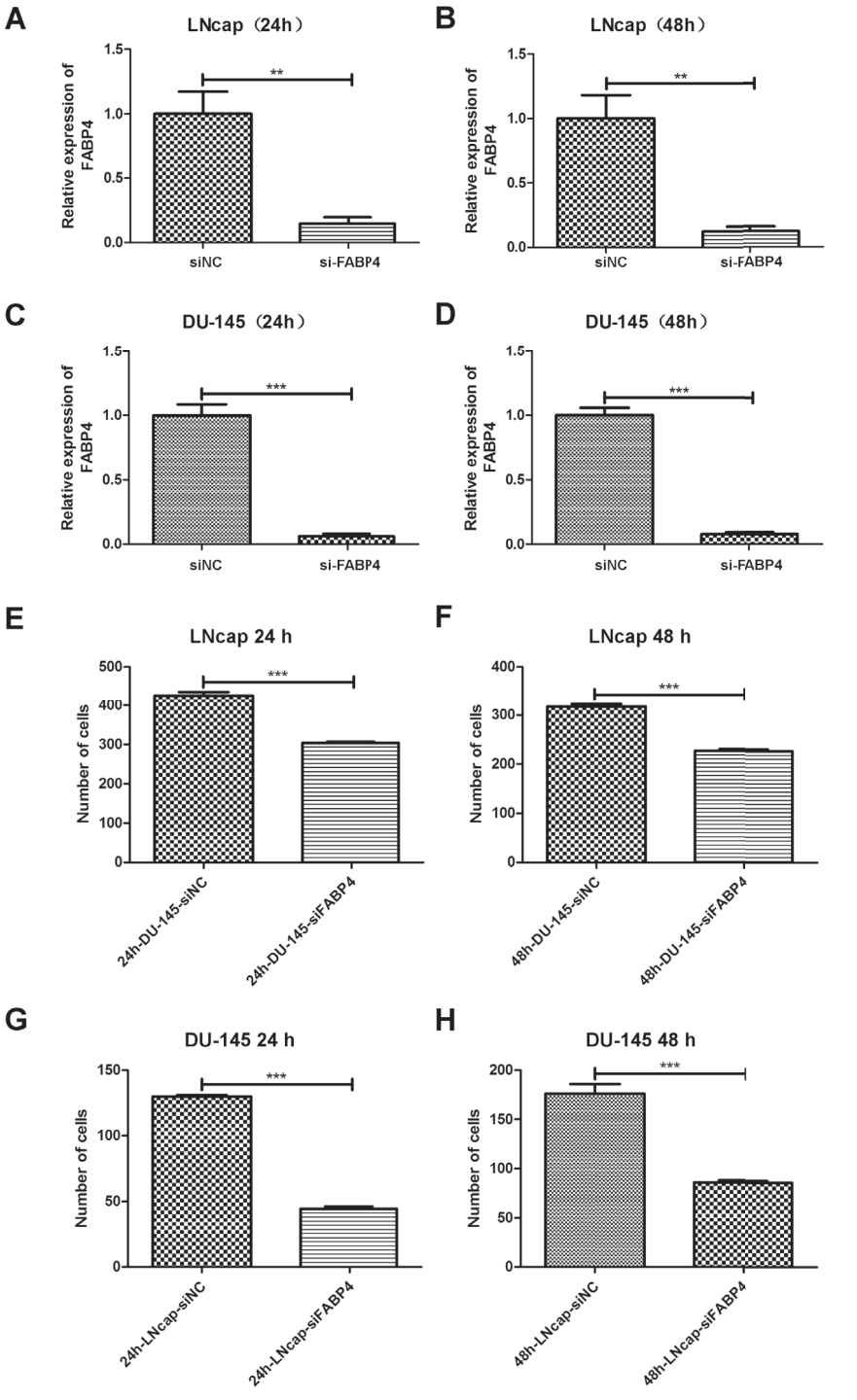

Figure 4. The expression of FABP4 and the migrated cells count after siRNA transfection A–D: the expression of FABP4 after siRNA transfection; E–H: the number of migrated cells after siRNA transfection; ***p<0.001.

From the PPI network constructed in this study, a module was extracted and further analyzed by GO analysis which showed that the module was closely associated with focal adhesion, ECM-receptor interaction, p53 signaling pathway, melanoma, arrhythmogenic right ventricular cardiomyopathy, and gap junction pathways,

indicating the critical roles of these pathways in prostate cancer metastasis. The hub gene of the module was upregulated *GJA1*, which was enriched in the gap junction pathway and identified as a TAG in the study. The protein encoded by *GJA1* termed as connexin 43, is a major gap junction component, and plays a role in tumor invasion and progression [36]. *GJAI* is reportedly involved in early cancer invasion in prostate cancer [37, 38], indicating that *GJAI* might facilitate progression of prostate cancer cells by mediating the gap junction pathway and might be a promising biomarker for prostate cancer metastasis treatment.

Although we have made efforts to review the Gene Expression Omnibus (GEO) database and The Cancer Genome Atlas (TCGA) database, we have not found any data about metastatic prostate tumor samples. Moreover, we face difficulties in collecting metastatic prostate tumor samples in the clinic. Therefore, more experiments based on human metastatic prostate tumor samples are needed to verify our findings**.**

In conclusion, *FABP4*, *IGFBP3*, *THBS1*, and *GJA1* might be potential biomarker candidates for diagnosing prostate cancer and potential targets for developing effective therapies to suppress cancer metastasis. P53, PPAR, and gap junction pathways could play an important role in prostate cancer invasion and metastasis. The findings from this study could contribute to a better understanding of the mechanism underlying prostate cancer metastasis and provide guidelines for its clinical management. Further experiments based on human metastatic prostate tumor samples are needed to verify our findings.

Supplementary information is available in the online version of the paper.

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