Identification of Core Genes and Key Pathways via Integrated Analysis of Gene Expression and DNA Methylation Profiles in Bladder Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCE Yongzhen Zhang
F Liang Fang
D Yuanwei Zang
AG Zhonghua Xu

Department of Urology, Qilu Hospital, Shandong University, Jinan, Shandong, P.R. China

Corresponding Author: Zhonghua Xu, e-mail: xuzhonghua1963@163.com

Background:
Bladder cancer (BC) is the most common urological malignant tumor. In BC, aberrant DNA methylation is believed to be associated with carcinogenesis. Therefore, the identification of key genes and pathways could help determine the potential molecular mechanisms of BC development.

Material/Methods:
Microarray data on gene expression and gene methylation were downloaded from the Gene Expression Omnibus (GEO) database. Abnormal methylated/expressed genes were analyzed by GEO2R and statistical software R. Gene Ontology term enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using the DAVID database and KOBAS 3.0. STRING and Cytoscape software were used to construct protein–protein interaction (PPI) networks and analyze modules of the PPI network.

Results:
A total of 71 hypomethylated/upregulated genes were significantly enriched in cell–cell adhesion and blood vessel development. KEGG pathway analysis highlighted p53 signaling and metabolic pathways. Five core genes in the PPI network were determined: CDH1, DDOST, CASP8, DHX15, and PTPRF. Additionally, 89 hypermethylated/downregulated genes were found. These genes were enriched mostly in cell adhesion and signal transduction. KEGG pathway analysis revealed enrichment in focal adhesion. The top 5 core genes in the PPI network were GNG4, ADCY9, NPY, ADRA2B, and PENK. We found most of the core genes were also significantly altered in the Cancer Genome Atlas database.

Conclusions:
Abnormal methylated/expressed genes and key signaling pathways involved in BC were identified through integrated bioinformatics analysis. In the future, these genes may serve as biomarkers for diagnosis and therapeutic targets in BC.

MeSH Keywords:
Computational Biology • Signal Transduction • Urinary Bladder Neoplasms

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Background

Bladder cancer (BC) is a common malignant urologic cancer. There are 2 major subsets of BC: non-muscle-invasive and muscle-invasive BC. BC is the fifth most common malignancy in the United States, with an estimated 76 960 new cases and 16 390 deaths in 2016 [1]. Chronic infection, smoking, and occupation are the main contributory factors of BC [2]. Aberrant DNA methylation, an epigenetic change, is believed to be associated with carcinogenesis [3–5]. The functions of key genes, including tumor suppressor genes and cell trans-formation genes, can be affected by changes in methylation, explaining how aberrant DNA methylation is involved in various processes of cancer development [3]. Aberrant DNA methylation has also been found in BC. Unlike genetic alterations, DNA methylation is reversible. Therefore, regulation of DNA methylation may represent a new strategy for cancer treatment [6–8]. Although some clinical results exist, such as anemia being a prognostic factor of BC [9], molecular and genetic biomarkers are considered as promising diagnostic options [10]. A few studies on have shown altered DNA methylation in cancer, but the roles of key differentially methylated genes (DMGs) and differentially expressed genes (DEGs) in BC remain unclear.

Recent technological advances in microarray analysis have provided an ideal and reliable method to identify important genetic or epigenetic modifications in cancer development. Thus, there have been many studies on DEGs in BC identified by gene expression profiling using microarrays [11,12]. Because of limited numbers of samples, individual studies are often not sufficient to demonstrate the underlying mechanism in caner progression. At present, we can obtain more reliable and accurate results by advanced bioinformatics analysis. To obtain novel abnormal methylated/expressed genes and key signaling pathways in BC, we extracted and analyzed information from both microarray-based gene expression and methylation profiling conducted in BC.

Material and Methods

Microarray data

We extracted gene expression (GSE3167 and GSE65635) and methylation (GSE37816 and GSE33510) profiling data from the Gene Expression Omnibus (GEO) database at the National Center for Biotechnology Information.

The BC-associated dataset GSE3167 submitted by Lars Dyrskjot based on the GPL96 platform (Affymetrix Human Genome U133A Array) was obtained from the GEO database and includes 51 BC samples and 9 normal samples. The BC-associated dataset GSE65635 submitted by Maria Vladimirovna Suntsova based on the GPL14951 platform (Illumina HumanHT-12 WG-DASL V4.0 R2 Expression BeadChip) was obtained from the GEO database and includes 8 BC samples and 4 normal samples. Regarding microarray-based gene methylation profiling, GSE37816 includes 18 BC samples and 6 normal samples, while GSE33510 includes 149 BC samples and 7 normal samples. Both of these methylation microarray analyses used the GPL8490 platform (Illumina HumanMethylation27 BeadChip).

Data processing

GEO provides users with a useful tool called GEO2R that can be used to analyze microarray data. We used GEO2R to identify DEGs and statistical software R (version 3.3.2, *http://www.r-project.org/*) with the minfi R package to identify DMGs. We set $P<0.05$ and $|t|>2$ as the thresholds to identify DEGs and DMGs. First, we identified up- and downregulated genes from the 2 gene expression profiling datasets (GSE3167 and GSE65635). Second, we identified hypo- and hypermethylated genes from GSE37816 and GSE33510. Finally, we identified hypomethylated/upregulated genes via overlapping the hypomethylated and overexpression gene lists and identified hypermethylated/downregulated genes via overlapping the hypermethylated and low-expression gene lists. Statistical software R and the pheatmap R package were used for bidirectional hierarchical clustering.

Functional and pathway enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, *https://david.ncifcrf.gov/*) provides a set of data-mining tools and thus was used to perform Gene Ontology (GO) term enrichment analysis. GO term enrichment analysis includes biological process, cellular component, and molecular function. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using KOBAS 3.0. $P<0.05$ was set as the screening criterion.

Protein–protein interaction (PPI) network construction and module analysis

PPI analysis is used to search core genes and gene modules related to carcinogenesis. In this study, PPI network analysis of the hypomethylated/upregulated genes and hypermethylated/downregulated genes were performed using the Search Tool for the Retrieval of Interacting Genes (STRING) database. The interaction score was set at 0.4. Subsequently, modules within the PPI network were screened by Molecular Complex Detection (MCODE) in Cytoscape software. An MCODE score >3 and number of nodes ≥3 were set as the screening criteria.
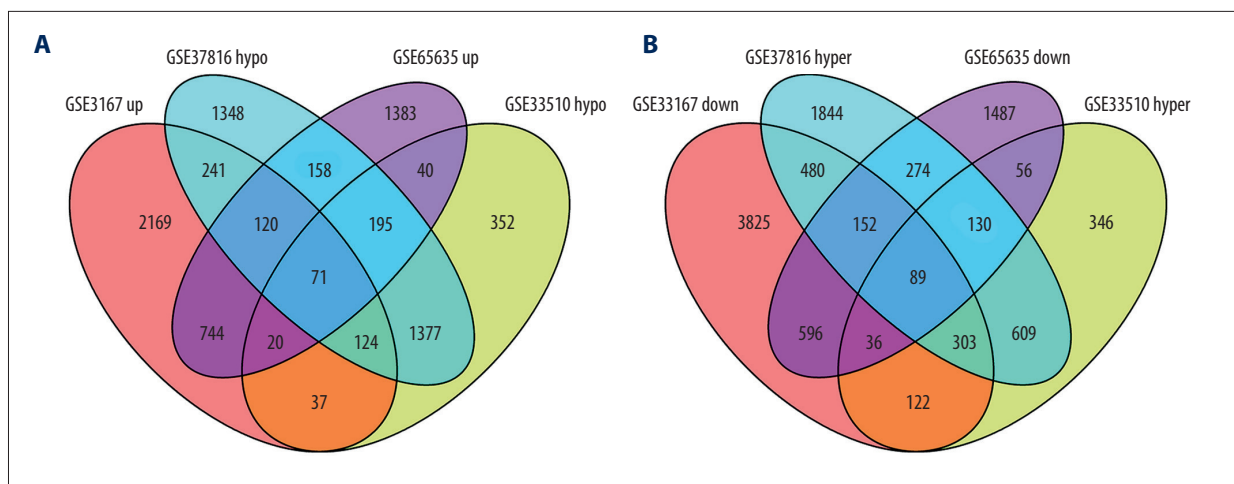
Figure 1. Identification of aberrantly methylated differentially expressed genes in gene expression datasets (GSE3167 and GSE65635) and gene methylation datasets (GSE37816 and GSE33510). (**A**) hypomethylation and upregulated genes. (**B**) hypermethylation and downregulated genes.

Validation of core genes in The Cancer Genome Atlas (TCGA) database

The TCGA database, which contains a tremendous amount of data on various types of cancers, was used to confirm our results. Most of the core genes selected by PPI analysis were verified in TCGA database.

Results

Identification of DEGs and DMGs in BC

After obtaining DEGs and DMGs, 955 overlapping upregulated genes (3526 in GSE3167 and 2731 in GSE65635) and 873 overlapping low-expression genes (5603 in GSE3167 and 2820 in GSE65635) were found from the gene expression microarray analysis. There were 1131 overlapping hypermethylated genes (3881 in GSE37816 and 1691 in GSE33510) and 1767 overlapping hypomethylated genes (3634 in GSE37816 and 2216 in GSE33510) identified by the gene methylation microarray analysis. Further analysis of overlapping genes revealed 71 hypomethylated/upregulated genes and 89 hypermethylated/downregulated genes (Figure 1A, 1B; Supplementary Tables 1, 2). Visualization of part of aberrantly expressed genes (GSE65635) was illustrated by heat map (Figure 2).

GO term analysis

GO enrichment analysis results are presented in Table 1. Regarding the biological processes (BP), the hypomethylated/upregulated genes were significantly enriched in cell–cell adhesion, positive regulation of cell growth, and blood vessel development. In the molecular function (MF), the hypomethylated/

high-expression genes were enriched in response to poly (A) RNA binding, KDEL sequence binding, and cadherin binding involved in cell–cell adhesion. In the cellular component (CC), the analysis revealed that enrichment mainly occurred at endoplasmic reticulum membrane and cytosol.

The biological processes enriched by the hypermethylated/low-expression genes included positive regulation of cell proliferation, cell adhesion, and signal transduction, and the molecular function included scaffold protein binding, transcription factor activity, and sequence-specific DNA binding. In the cellular component, the hypermethylated/low-expression genes were enriched in integral component of plasma membrane and proteinaceous extracellular matrix.

KEGG pathway analysis

According to KEGG analysis (Table 2), the hypomethylated/high-expression genes were significantly enriched in the fructose and mannose metabolism, p53 signaling, and metabolic pathways. Hypermethylated/low-expression genes were enriched in calcium signaling, insulin secretion, cAMP signaling pathway, and focal adhesion pathways.

PPI network analysis

PPI network analysis was performed by STRING database. The PPI network of hypomethylated/upregulated genes is shown in Figure 3A. We screened the data and found 3 modules via MCODE in Cytoscape software (Figure 3C). KOBAS 3.0 was used for module analysis and demonstrated functions related to *Vibrio cholerae* infection in Module 1, protein export in Module 2, and proteoglycans in cancer Module 3 (Table 3). The top core genes were chosen: CDH1, DDOST, CASP8, DHX15,
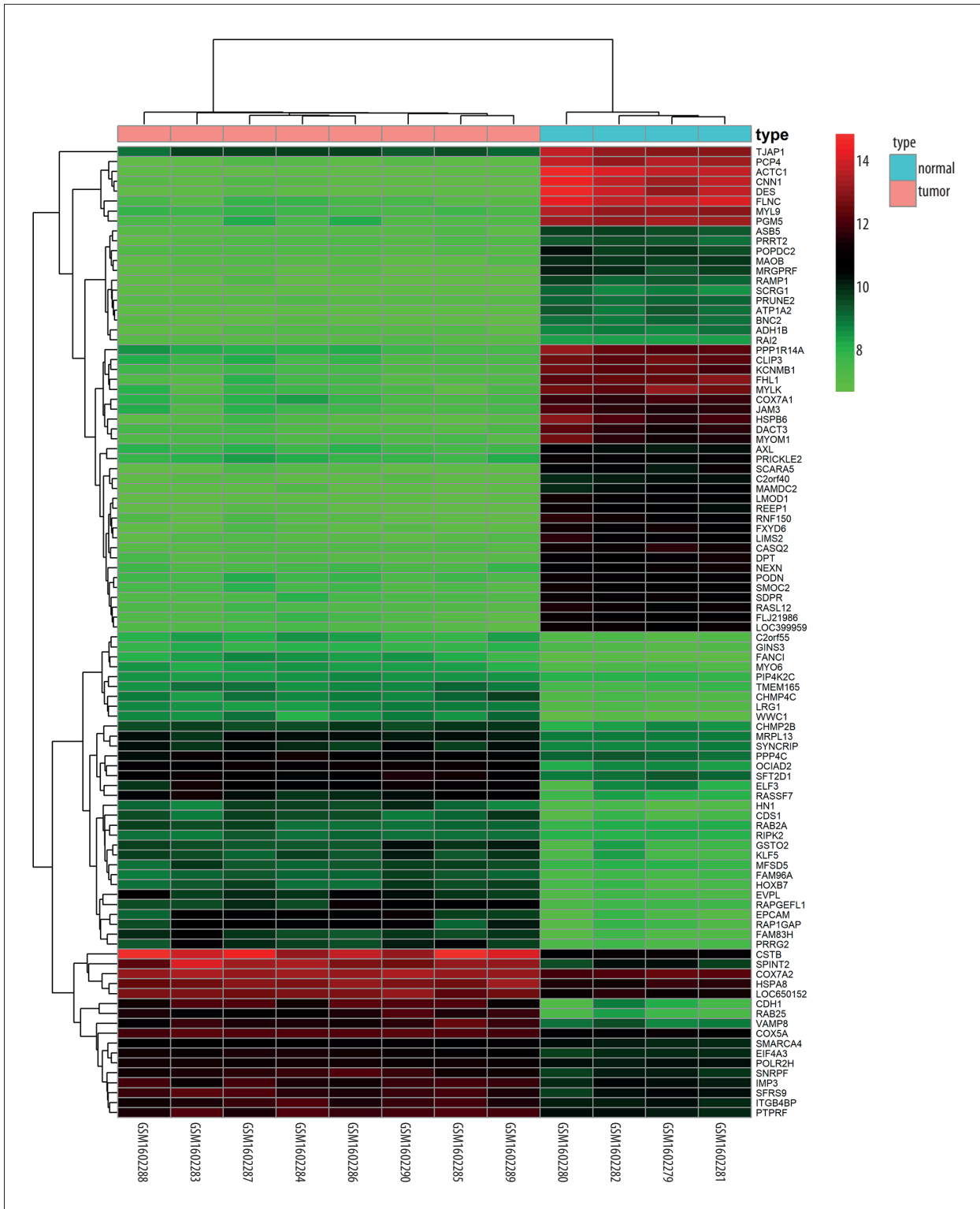
Figure 2. Representative heat map of the top 100 differentially expressed genes in dataset GSE65635 (50 upregulated genes and 50 downregulated genes). Red indicates upregulation and green indicates downregulation.

Table 1. Gene ontology analysis of aberrantly methylated-differentially expressed genes in bladder cancer.

Category	Term	Count	%	P value
Hypomethylation and high expression				
GOTERM_BP_DIRECT	GO: 0098609~cell-cell adhesion	5	7.0423	0.027
GOTERM_BP_DIRECT	GO: 0008284~positive regulation of cell proliferation	7	9.8592	0.013
GOTERM_BP_DIRECT	GO: 0001568~blood vessel development	3	4.2254	0.011
GOTERM_BP_DIRECT	GO: 0009636~response to toxic substance	5	7.0423	0.0004
GOTERM_BP_DIRECT	GO: 0051056~regulation of small GTPase mediated signal transduction	4	5.6338	0.018
GOTERM_MF_DIRECT	GO: 0044822~poly(A) RNA binding	10	14.085	0.043
GOTERM_MF_DIRECT	GO: 0005046~KDEL sequence binding	2	2.8169	0.008
GOTERM_MF_DIRECT	GO: 0098641~cadherin binding involved in cell-cell adhesion	6	8.4507	0.007
GOTERM_MF_DIRECT	GO: 0016853~isomerase activity	3	4.2254	0.003
GOTERM_MF_DIRECT	GO: 0005515~protein binding	49	69.014	0.003
GOTERM_CC_DIRECT	GO: 0005789~endoplasmic reticulum membrane	11	15.493	0.0016
GOTERM_CC_DIRECT	GO: 0043005~neuron projection	6	8.4507	0.0021
GOTERM_CC_DIRECT	GO: 0005829~cytosol	22	30.986	0.011
GOTERM_CC_DIRECT	GO: 0005739~mitochondrion	12	16.901	0.012
GOTERM_CC_DIRECT	GO: 0031234~extrinsic component of cytoplasmic side of plasma membrane	3	4.2254	0.028
Hypermethylation and low expression				
GOTERM_BP_DIRECT	GO: 0007155~cell adhesion	10	11.236	0.00035
GOTERM_BP_DIRECT	GO: 0008284~positive regulation of cell proliferation	8	8.9888	0.007
GOTERM_BP_DIRECT	GO: 0007165~signal transduction	12	13.483	0.023
GOTERM_BP_DIRECT	GO: 0030198~extracellular matrix organization	5	5.618	0.015
GOTERM_BP_DIRECT	GO: 0006936~muscle contraction	4	4.4944	0.015
GOTERM_MF_DIRECT	GO: 0031697~beta-1 adrenergic receptor binding	2	2.2472	0.014
GOTERM_MF_DIRECT	GO: 0097110~scaffold protein binding	3	3.3708	0.023
GOTERM_MF_DIRECT	GO: 0003700~transcription factor activity, sequence-specific DNA binding	10	11.236	0.046
GOTERM_CC_DIRECT	GO: 0008076~voltage-gated potassium channel complex	6	6.7416	0.00005
GOTERM_CC_DIRECT	GO: 0005886~plasma membrane	31	34.831	0.005
GOTERM_CC_DIRECT	GO: 0005887~integral component of plasma membrane	13	14.607	0.031
GOTERM_CC_DIRECT	GO: 0005578~proteinaceous extracellular matrix	5	5.618	0.036
GOTERM_CC_DIRECT	GO: 0008328~ionotropic glutamate receptor complex	2	2.2472	0.036

and PTPRF (Figure 3B). The PPI network of hypermethylated/downregulated genes was constructed (Figure 4A) and 2 key modules were found (Figure 4C). Module 1 demonstrated functions of GABAergic synapse and Module 2 functions of vascular smooth muscle contraction (Table 3). The top core genes were revealed: GNG4, ADCY9, NPY, ADRA2B, and PENK (Figure 4B).

Validate the identified genes in TCGA database

To confirm our results, the identified core genes were validated in the TCGA database and results are shown in Table 4. In TCGA, the methylation and expression status of the core genes were also significantly altered in BC samples. These findings suggest that our results were reliable.

Discussion

Aberrant methylation is an important molecular mechanism in BC initiation and development. In the present study, 71 hypomethylated/upregulated genes and 89 hypermethylated/downregulated genes were identified by a variety of bioinformatics analysis. These genes enriched certain pathways and affected by abnormal methylation. Knowledge of the aberrantly

Table 2. KEGG pathway analysis of aberrantly methylated-differentially expressed genes in bladder cancer.

Pathway name	ID	Gene num	P-value	Genes
Hypomethylation and high expression				
p53 signaling pathway	hsa04115	3	0.00029	SFN, ATP1B1, SERPINB5
Metabolic pathways	hsa01100	8	0.00171	TSTA3, DHCR7, B4GALT4, AHCY, IDH1, TUSC3, HSD17B4, DDOST
Fructose and mannose metabolism	hsa00051	2	0.00179	TSTA3, ENOSF1
Hypermethylation and low expression				
Insulin secretion	hsa04911	5	1.88E-06	ABCC8, ADCY9, KCNMA1, RYR2, VAMP2
Calcium signaling pathway	hsa04020	6	4.02E-06	RYR2, P2RX1, ADCY9, PTGFR, PDE1C, MYLK
cAMP signaling pathway	hsa04024	5	9.80E-05	ADCY9, GHSR, VIPR2, RYR2, NPY
Focal adhesion	hsa04510	5	0.00011	RELN, ITGA8, MYLK, CCND2, THBS4

Figure 3. PPI network and the top 3 modules of hypomethylation/high-expression genes. (**A**) PPI network. (**B**) Core genes and the number of gene cords. (**C**) Top modules 1–3.

Table 3. Module analysis of the protein–protein interaction network.

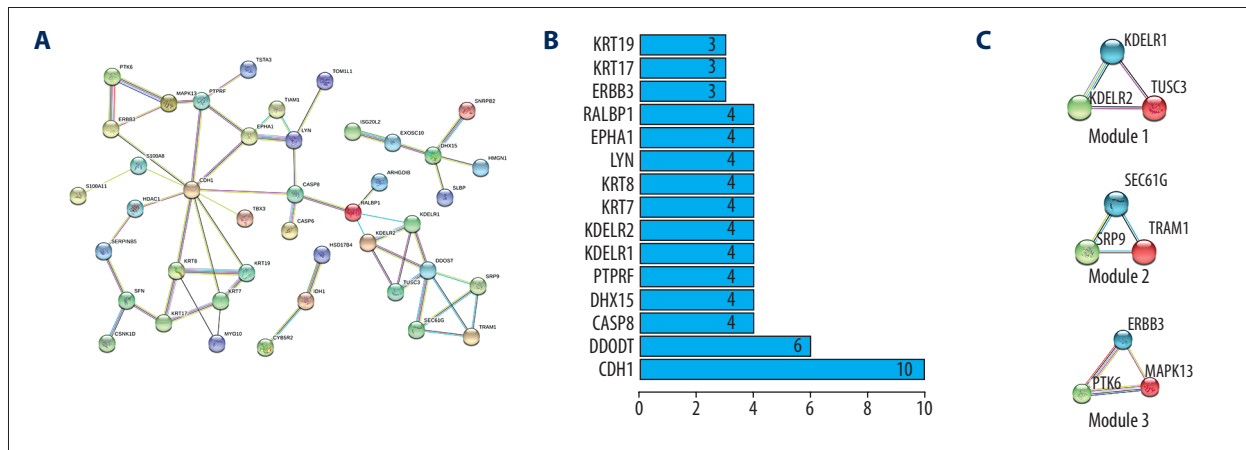| Category	Module	Function description	FDR	Nodes	Genes
Hypomethylation and high expression					
	1.	Vibrio cholerae infection	2.09E-05	3	KDELR2, KDELR1, TUSC3
	2.	Protein export	4.55E-06	3	SEC61G, SRP9, TRAM1
	3.	Proteoglycans in cancer	0.00346724	3	PTK6, ERBB3, MAPK13
Hypermethylation and low expression					
	1.	GABAergic synapse	0.000879043	6	ADRA2B, PENK, ADCY9, DRD4, NPY, GNG4
	2.	Vascular smooth muscle contraction	9.22E-07	4	RASL12, ACTA2, MYLK, ACTG2
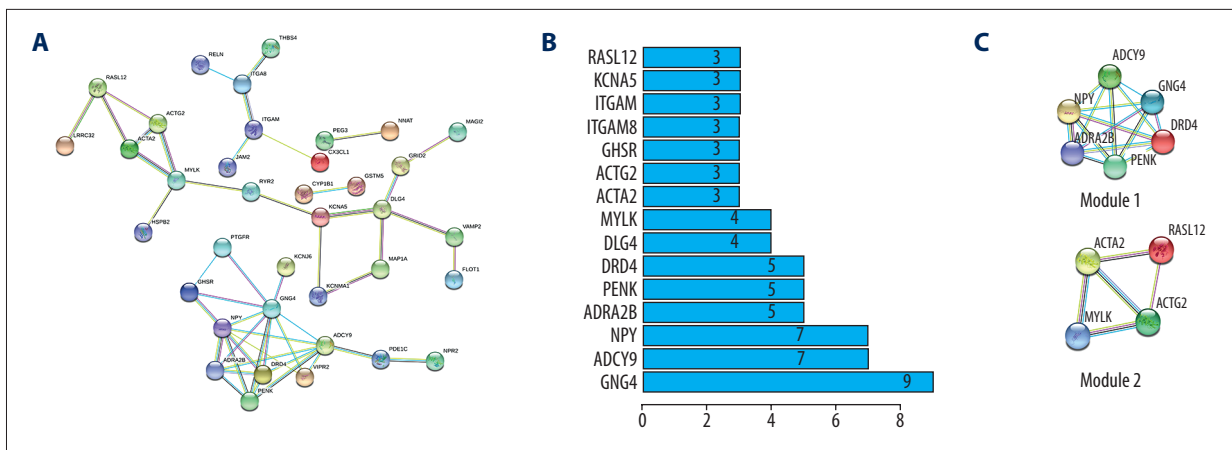
Figure 4. PPI network and the top 2 modules of hypermethylation/low-expression genes. (**A**) PPI network. (**B**) Core genes and the number of gene cords. (**C**) Top modules 1–2).

Table 4. Validation of the hub genes in TCGA database.

Category	Hub gene	Methylation status	P value	Expression status	P value
Hypomethylation and high expression					
	CDH1	Hypomethylation	9.26E-08	Up-regulated	0.007185916
	DDOST			Up-regulated	2.45E-11
	CASP8	Hypomethylation	9.13E-12	Up-regulated	0.001398258
	DHX15				
	PTPRF			Up-regulated	0.005353039
Hypermethylation and low expression					
	GNG4	Hypermethylation	9.09E-09		
	ADCY9	Hypermethylation	3.21E-06	Down-regulated	8.54E-13
	NPY	Hypermethylation	9.48E-11		
	ADRA2B	Hypermethylation	6.15E-11	Down-regulated	0.00036077
	PENK	Hypermethylation	1.40E-07	Down-regulated	2.98E-05

methylated/expressed key genes in BC would provide novel insights into its diagnosis, treatment, and prognosis.

In the GO analysis, we found hypomethylated/upregulated genes were significantly enriched in biological processes including cell–cell adhesion, positive regulation of cell growth, and blood vessel development. Regarding molecular function, enrichment of poly (A) RNA binding, KDEL sequence binding, and cadherin binding involved in cell–cell adhesion was found. Cell adhesion is an important factor in cancer dissemination and development of multiple types of cancers, including BC [13–16]. Cell growth and blood vessel growth are indispensable for the development of BC. RNA binding-related proteins, such as IGF2BP3 and MSI2, were shown to contribute to cancer cell malignancy and cancer progression [17,18]. The regulation of hypomethylation for poly (A) RNA binding

genes might be associated with the occurrence of BC. KEGG pathway analysis demonstrated that the hypomethylated/upregulated genes significantly enriched in pathways included fructose and mannose metabolism, p53 signaling, and metabolic pathways. Acute myeloid leukemia cells can efficiently utilize fructose, and this promotes the malignant progression of leukemia [19]. D-Mannose was suggested as a novel biomarker for early detection of colorectal cancer [20]. The p53 signaling pathway has been shown to play an important role in BC [21,22]. Aberrant metabolism of BC is correlated with bladder tumorigenesis [23]. In brief, the dysregulation of fructose and mannose metabolism, p53 signaling, and metabolic pathways might affect BC progression from multiple aspects.

For the hypomethylated/upregulated genes, PPI network analysis was performed. The top 5 core genes were: CDH1, DDOST,

CASP8, DHX15, and PTPRF. The CDH1 protein is a subunit of the APC/C complex, which is related to the regulation of cell division. The inactivation of CDH1 *in vivo* results in tumor cell death [24]. Maruyama et al. [25] first reported that the methylation status of CDH1 was significantly correlated with poor survival in BC. Some drugs induce apoptosis in BC by regulating CASP8 [26,27]. DHX15 plays multiple roles in tumor progression. In prostate cancer, DHX15 enhances transcriptional activity of the androgen receptor and promotes prostate cancer progression [28]. Additionally, DHX15 suppresses tumor proliferation by downregulating the expression of NF-κB in glioma [29]. PTPRF is considered a potential biomarker candidate for prostate cancer and non-small cell lung cancer [30,31]. These results suggest that these core genes play an important role in BC development. Thus, the functions and methylation status of these top core genes in BC warrant additional research.

In the PPI network, module analysis of hypomethylated/high-expression genes suggested that *Vibrio cholerae* infection, protein export, and proteoglycans in cancer are related to BC development. Various proteoglycans, which are key molecular effectors located at the cell surface, perform multiple functions in tumor cell growth, the tumor microenvironment, metastasis, and angiogenesis [32]. In addition, important alterations of small leucine-rich proteoglycans in BC have been reported [33]. In our study, PTK6, ERBB3, and MAPK13 were correlated with function of proteoglycans in cancers (Table 3). PTK6 has been demonstrated to be a potential predictive protein in bladder cancer patients [34]. ERBB3 was reported to be involved in treatment resistance [35]. In high-grade serous ovarian cancer, MAPK13 can serve as a potential predictive protein [36]. These results all suggest that PTK6, ERBB3, and MAPK13 genes can serve as potential biomarkers in BC.

In the GO analysis, we found that the hypermethylated/low-expression genes enriched in BP included positive regulation of cell proliferation, cell adhesion, and signal transduction. Cancer is normally considered a genetic disease. Aberrant expression of genes related to positive regulation of cell proliferation, cell adhesion, and signal transduction can affect tumor development and metastasis. Our findings suggest that these hypermethylated/low-expression genes represent promising targets for BC treatment or diagnosis. Regarding molecular functions, hypermethylated/low-expression genes were enriched in scaffold protein binding, transcription factor activity, and sequence-specific DNA binding. Scaffold proteins are an important regulatory factor in many key signaling pathways [37,38]. There are various scaffold proteins exerting different functions in carcinogenesis. Grp1-associated scaffold protein promotes the function of p53 tumor suppressor genes and inhibits cancer development [39]. In our study, we found that many hypermethylated/low-expression genes were enriched in scaffold protein binding. Additional research is needed to determine the function of these hypermethylated/low-expression genes enriched in scaffold protein binding. KEGG analysis revealed enrichment of the genes in the calcium signaling pathway, insulin secretion, cAMP signaling pathway, and focal adhesion, many of which are associated with BC. Calcium signaling is associated with carcinoma *in situ* in the bladder [40]. Activation of the cAMP/PKA signaling pathway may inhibit BC cell invasion [41]. Focal adhesion kinase (FAK) is a non-receptor protein-tyrosine kinase that is triggered by some growth factors and integrin. Some findings suggest that FAK is involved in oncogenic signaling of invasion and migration in BC [42]. In addition, inhibition of FAK induces apoptosis in bladder cancer cells. FAK may function as an important regulator of extracellular signaling-mediated apoptosis in bladder cancer and could be used as a novel therapeutic target in the treatment of BC [43]. Our results suggest that these signaling pathways are candidate research targets in BC.

We performed PPI network analysis on hypermethylated/down-regulated genes. The top 5 core genes were: GNG4, ADCY9, NPY, ADRA2B, and PENK. ADCY9 is an enzyme that modulates signal transduction, and its expression is correlated with colon cancer TNM staging [44]. MiR-181b promotes cell proliferation and reduces apoptosis by downregulating ADCY9 expression in cervical cancer cells [45]. GNG4 is a hypermethylated/low-expression gene identified in glioblastoma, and overexpression of GNG4 inhibited the proliferation of glioblastoma cells [46]. NPY is a 36-amino acid peptide that acts via G-protein-coupled receptors (Y1–Y6). Peripherally, NPY affects smooth muscle contraction of blood vessels, blood pressure, and atherogenic processes [47]. Activation of the Y2 receptor stimulated angiogenesis in various animal models [48]. The hypermethylation status of PENK has been validated in BC [49]. In multiple types of cancers, PENK serves as a potential diagnostic marker [50,51]. We found that all top core genes were deeply involve in cancer proliferation or progression, suggesting that these core genes may serve as prognostic biomarkers or therapeutic targets in BC.

According to core modules analysis in the PPI network, hypermethylated/downregulated genes were closely related to GABAergic synapse and vascular smooth muscle contraction. We found that all top 5 core genes with hypermethylated/low-expression were involved in the function of GABAergic synapse. This suggests hypermethylated/low-expression genes related to GABAergic synapse might play an important role in BC development. Until now, the effects of GABAergic synapse in BC and its regulation by aberrant methylation have been unclear.

Several limitations to this investigation should be acknowledged. The clinical application value of those core genes in BCs need further evaluation. Moreover, we only chose related data from the TCGA database as a validated cohort. Our study results need to be validated in more databases.

Conclusions

In conclusion, our research identified some aberrantly expressed genes and pathways potentially regulated by abnormal methylated in BC by integrated analysis of gene expression and methylation microarrays. An analysis of multiple microarray data makes our results more reliable and precise than individual microarray data analysis. This provided a better understanding of the molecular mechanisms underlying the occurrence and progression of BC. Core genes such as CDH1, DDOST, CASP8, DHX15, PTPRF, GNG4, ADCY9, NPY, ADRA2B, and PENK may became new markers in accurate diagnosis and therapy of BC. Further work is needed to determine the role of these identified genes in BC progression.

Acknowledgements

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Conflict of interest

None.

Supplementary Tables

Supplementary Table 1. The complete list of the 89 hypermethylated/downregulated genes.

Hypermethylated/low-expression genes									
NPR2	HLF	RYR2	ACTA2	LY6H	CCND2	TBX4	ZCCHC14	PEG3	ZNF219
CHST3	RNF122	STXBP6	SEMA3B	PHYHIP	SSBP3	CYP1B1	MYLK	EIF5A2	EMILIN1
POU6F1	RUSC2	NPY	PDLIM4	ITGAM	FLOT1	RAX	LCAT	THBS4	SPARCL1
DLG4	P2RX1	ARMC4	DRD4	AOX1	PLEKHA4	SPON1	NOVA1	LRRC32	ST6GALNAC5
RARB	SYDE1	ADCY9	NNAT	GNG4	PTGFR	VAMP2	HS1BP3	PHF1	RASL12
KCNMA1	ADRA2B	MAP3K14	CUL3	ZNF154	HSF4	KCNA5	STC2	GYPC	BHMT2
GSTM5	VIPR2	ROBO4	CKMT2	HAND2	NCAM2	NEFH	ABCC8	ITGA8	GHSR
PDE1C	HPSE2	HSPB2	BLZF1	ARHGEF4	FEZ1	CX3CL1	GRID2	LTC4S	MAGI2
HAAO	MAP1A	KCNJ6	FAM50B	ACTG2	PENK	CDO1	RELN	JAM2	

Supplementary Table 2. The complete list of the 71 hypomethylated/upregulated genes.

Hypomethylated/high-expression genes									
HMGN1	KRT7	SRP9	VAMP8	ARHGEF18	LYPD3	ENOSF1	TOM1L1	CASP6	TBX3
SLBP	CCDC59	SNRPB2	KRT17	MRPS15	MARCKSL1	SPINT1	KRT20	ERBB3	SERPINB5
KDELR2	EXOSC10	CDH1	KRT8	B4GALT4	MAPK13	CASP8	LYN	ID3	HSD17B4
TRAM1	MKNK2	CCDC47	RALBP1	ARHGDIB	TSTA3	TRAK1	KDELR1	FXYD3	S100A11
DDOST	HDAC1	ISG20L2	SLC25A5	KTN1	SFN	KRT19	CYB5R2	IDH1	BPHL
CSNK1D	AHR	AHCY	SEC61G	FAM3C	SPINT2	TIAM1	PTK6	EPHA1	GNA15
GRSF1	DHX15	TUSC3	GDF15	PTPRF	S100A8	MAP7	DHCR7	SH2D3A	MYO10
SCAP									

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