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ORIGINAL ARTICLE

Integrated Bioinformatics Analysis of Potential Biomarkers for Prostate Cancer

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Abstract The aim was to expound the pathogenesis of prostate cancer and to identify the potentially biomarkers for prostate cancer (PC). DNA methylation microarray data GSE38240 containing 8 prostate cancer metastases and 4 normal prostate samples as well as gene expression profile data GSE26910 containing 6 prostate primary tumors and 6 normal samples were used. Differentially expressed genes (DEGs) and differently methylated sites of PC were screened and the regulatory network was constructed with DEGs-related transcription factors (TFs). The obtained hub genes were subjected to protein-protein interaction network analysis. Enrichment analysis of down-regulated DEGs were performed. Total 351 DEGs including 190 down-regulated and 161 up-regulated genes and 3234 differently methylated sites were identified. In total 69 DEGs-related TFs were found. Regulatory network contained 1301 nodes and 2527 connection pairs and that FOXA1 (forkhead box A1), BZRAP1-AS1 (benzodiazapine receptor associated protein 1 antisense RNA 1) and KRT8 (keratin 8) were the top three nodes of it. The enriched GO terms were mainly biological activity of the blood and cells-related. Total 29 DEGs (such as AGTR1, angiotensin II receptor, type 1) and 57 none-DEGs involved in the PPI network. Biological functions in blood circulation and the involved AGTR1 may play important roles in PC by gene-methylation. Besides, BZRAP1-AS1 may be novel biomarker related with PC.

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Keywords Prostate cancer · Differentially expressed genes · Methylation · Network

Introduction

Prostate cancer (PC), the leading cancer in males in developed countries, is the result of accumulated mutations in oncogenes and tumor suppressor genes and is metastasized preferentially to bone [7]. For the aggressive variant and health hazards of it, extensive research efforts were carried out and some achievement are forwarded.

Apart from radical prostatectomy and radiation etc., androgen ablation therapy is effective and is commonly used in the treatment of PC [13]. One of the reasons is that PC is mostly androgen-dependent and then androgens activate androgen receptor which finally involved in the cellular growth or apoptosis of PC cells. However, how to use androgen ablation therapy properly still remains controversial and the conversion from androgen-dependent to none androgen-dependent of PC is another challenge. Therefore, cancer genes, such as BCL2 [2], p21 [27] and p53 [28] are widely researched. In addition, gene transcription which showed abnormal in cancer is partly regulated by DNA methylation, which are ubiquity in a variety of tumors as well as in development [4]. With regard to PC, for example, basonuclin 1 is frequently methylated and related with the inactivation of gene expression [10]; glutathione S-transferase methylation has a strong association with mortality of PC patients [20]. Gene transcription is also under control of numerous transcription factors. For instance, over-expression of ETV1, the ETS-related transcription factor can initiate neoplastic transformation of prostate [16]. While, despite these efforts, largely mechanisms underlying these are still poorly understood, besides, very few biomarkers of PC are truly serviceable.

In order to continue the study of PC, two microarray datasets were used in the current study. Differentially expressed genes (DEGs) and differently methylated sites between prostate cancer and normal samples were screened. Besides, several bioinformatics tools were employed for the functional analysis of DEGs, differently methylated sites and DEGs-related transcription factors (TFs). We aimed to further expound the pathogenesis of PC and expect to identify the potentially biomarkers for it.

Material and Methods

Affymetrix Microarray Data

Microarray data used in the current study were obtained from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database. The DNA methylation microarray data numbered GSE38240 [1] included 8 prostate cancer metastases and 4 normal prostate samples. These data were detected based on the platform of GPL13534 Illumina Human Methylation 450 BeadChip. The gene expression profile data were numbered GSE26910 [17], and included 12 samples of breast cancer and 12 samples of prostate cancer. Thereinto, 6 samples of stroma surrounding invasive prostate primary tumors and 6 normal stroma matched samples were used. The based platform was GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array.

Preprocess of Microarray Data and Screen of DEGs and Differently Methylated Sites

GenomeStudio [5] was applied to preprocess methylation microarray data. The affy [8] package in R/Bioconductor was employed to preprocess gene expression profile data, including normalization and background correction.

Limma package in R/Bioconductor used to screen DEGs and differently methylated sites with Bonferroni corrected t tests. Differences of genes were considered significant if adj.p value <0.05. Differently methylated sites were screened with adj.$p < 1 \times 10^{-5}$.

Identification of DEGs-related Transcription Factors and Construction of Regulatory Network

All of the known regulatory relationship between transcription factors and target genes were provided by the UCSC (University of California at Santa Cruz) (https://genome.ucsc.edu/) [9] genome browser. Then with map of DEGs to the regulatory relationships, the DEGs-related TFs were obtained.

Downstream genes of differently methylated sites were potentially affected in the transcriptional regulation. Regulatory network were constructed to show the effect of TFs and methylation on downstream genes with Cytoscape [21] for visualization. Topological structure analysis of regulatory networks was executed to acquire nodes with more degree.

Gene Ontology Analysis of Down-Regulated DEGs

DNA methylation can silence or down-regulated genes. Therefore, GO (Gene Ontology) enrichment analysis of down-regulated DEGs was conducted with DAVID (database for annotation, visualization, and integrated discovery) [22] (http://david.abcc.ncifcrf.gov/). GO terms with $p < 0.05$ and contained at least 5 genes were selected under default parameter values.

Identification of Hub Genes and Construction of Protein-Protein Interaction Network

Based on the regulatory network, hub genes (degree ≥5) regulated by TFs and methylation were selected. Then PPI network were constructed by these hub genes with STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) [24] database. Cytoscape [21] was then used to visualize this network. Hub genes contained in the PPI network were compared with those in the regulatory network.

Results

Statistical Analysis of Differentially Expressed Genes and Differently Methylated Sites

There were 485,577 methylation sites before prerecession, and after it, 435,248 were left. Meanwhile, there were 54,675 probe symbol before prerecession, and after it, 21,960 gene symbols were left.

Total 351 DEGs including 190 down-regulated and 161 up-regulated genes were identified. Meanwhile, 3234 differently methylated sites were obtained. The significantly up- and down-regulated DEGs were PRAC1 (prostate cancer susceptibility candidate 1) ($\log_2$FC = 4.004) and CXCL13 (chemokine (C-X-C motif) ligand 13) ($\log_2$FC = −4.645).

DEGs-Related Transcription Factors and the Regulatory Network

In total 69 TFs forming 914 pair-correlations with DEGs were found. Besides, there were 946 differently methylated sites located in the chromosomal binding region of TFs and formed 1616 connection pair between them. The regulatory network among differently methylated sites, DEGs and TFs contained 1301 nodes and 2527 regulatory relationship (Fig. 1). All 69 TFs and 286 DEGs were involved in it. The network also

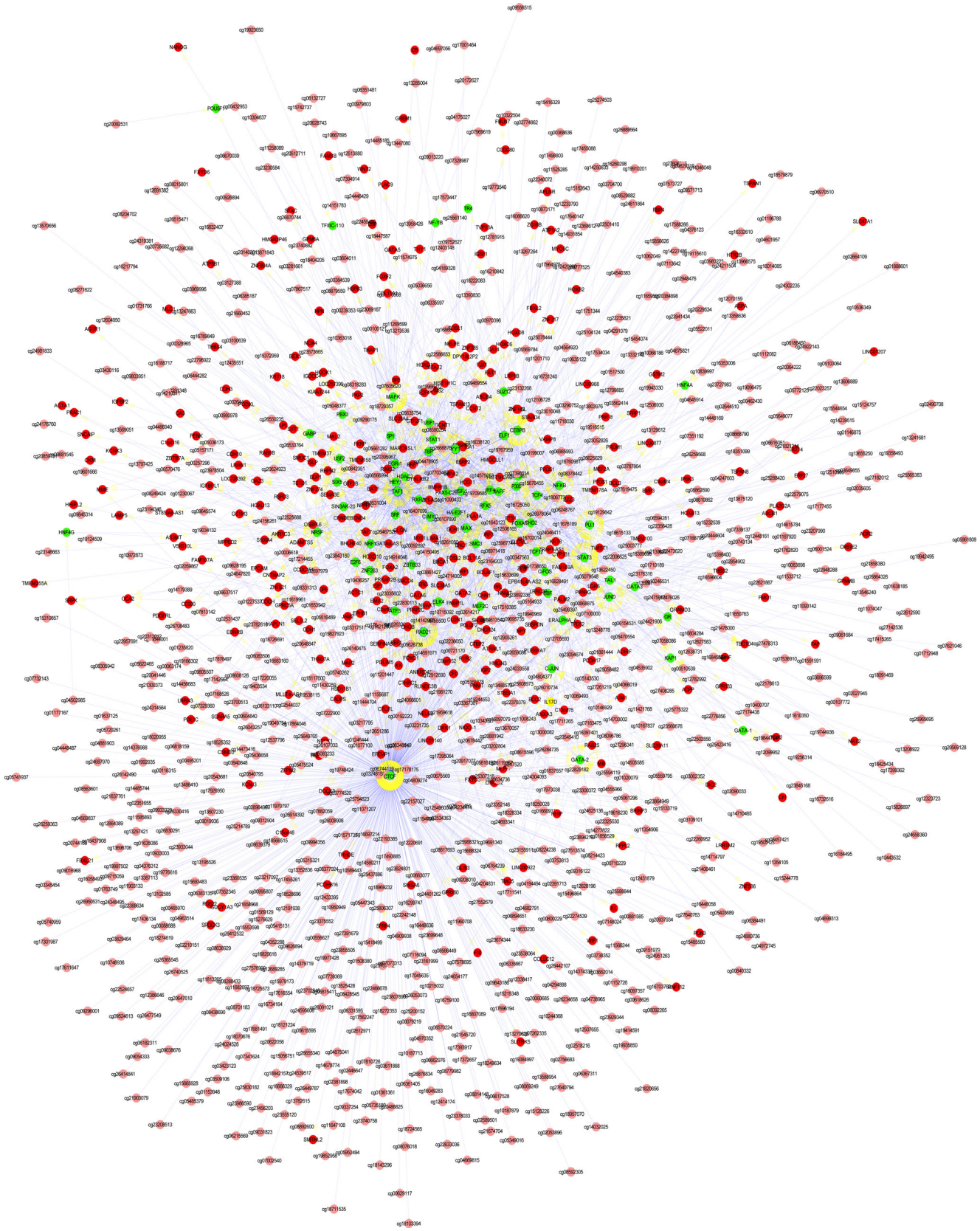

Fig. 1 Transcriptional regulatory network among differently methylated sites, differentially expressed genes (DEGs) and transcription factors. The green, crimson and pink dots represent transcription factors, DEGs and differently methylated sites, respectively. The lines represent interactions between nodes

Table 1 Top 10 Gene Ontology terms of down-regulated DEGs

Category	GO term	Gene Number	P value
BP	blood circulation	9	9.19E-05
BP	circulatory system process	9	9.19E-05
BP	regulation of blood pressure	7	1.19E-04
CC	extracellular region	31	3.41E-04
CC	extracellular region part	19	5.38E-04
BP	regulation of blood vessel size	5	7.23E-04
BP	regulation of tube size	5	7.23E-04
BP	vascular process in circulatory system	5	0.001017
BP	response to abiotic stimulus	10	0.002094
CC	cell fraction	19	0.002139

Bp biological process; CC cellular component

included 946 differently methylated sites. Among DEGs, *FOXA1* (forkhead box A1) had the most degree (n = 86), and the followed ones were *BZRAP1-AS1* (benzodiazapine receptor associated protein 1 antisense RNA 1) (n = 14) and *KRT8* (keratin 8) (n = 13).

Enrichment Analysis of Down-Regulated Genes

Top 10 GO terms were shown in Table 1. Top three GO-BP (biological process) terms respectively were blood circulation, circulatory system process and regulation of blood pressure, which mainly focused in biological activity of the blood and cells. There were more genes in BP than in CC (cellular component). Extracellular region is the significantly enriched term of CC.

Hub Genes and PPI Network Analysis

From the regulatory network, total 61 DEGs with degree equal or greater than 5 were selected and used to construct the PPI network. There were 86 nodes (29 DEGs and 57 none-DEGs) and 398 connection pairs in it (Fig. 2). The top 5 hub DEGs

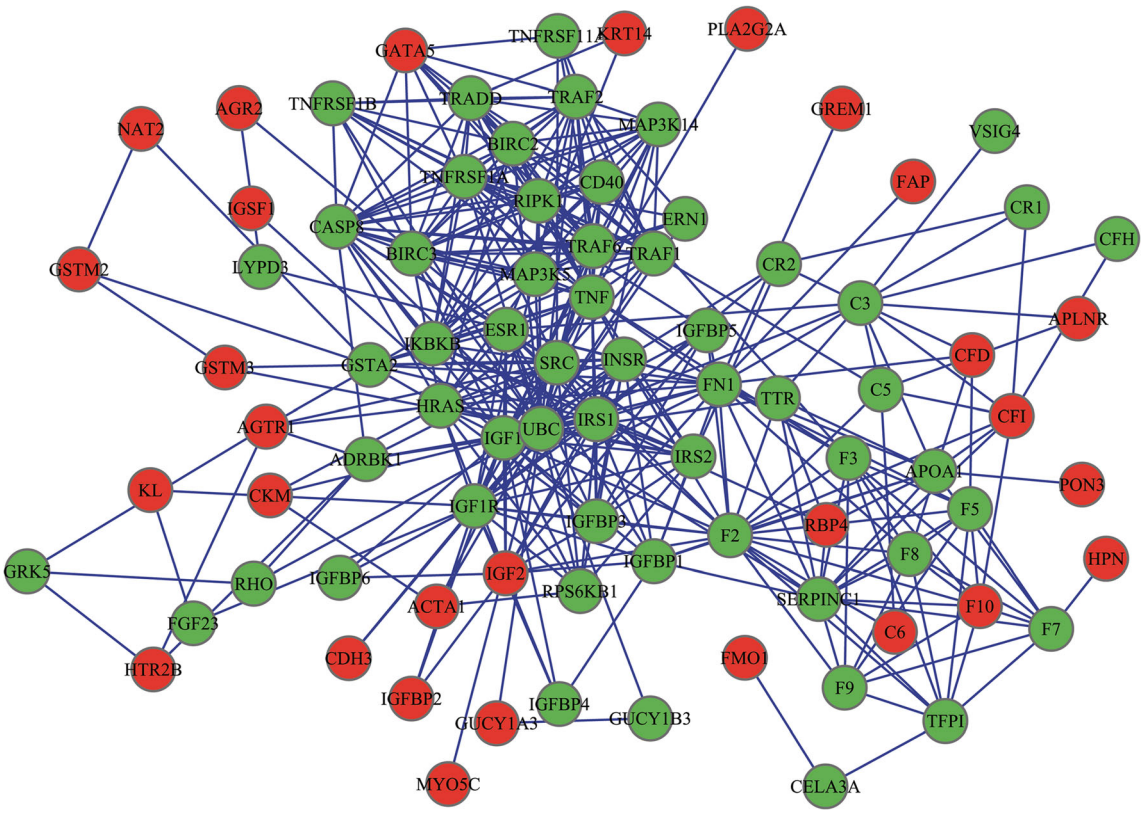

Fig. 2 Protein-protein interaction network of hub genes. The red and green dots represent differentially expressed genes (DEGs) and none-DEGs, respectively. The lines represent interactions between nodes

were IGF2 (insulin-like growth factor 2) (n = 13), GATA5 (GATA binding protein 5) (n = 9), F10 (coagulation factor X) (n = 9), CFI (complement factor I) (n = 8) and AGTR1 (angiotensin II receptor, type 1) (n = 6).

Discussion

Nowadays, PC remains essentially incurable for the unstated pathological features as well as pathogenesis of PC. Total 351 DEGs including 190 down-regulated and 161 up-regulated genes were identified from the GSE38240 and GSE26910 database. Regulatory network contained 1301 nodes and 2527 connection pairs. FOXA1, BZRAP1-AS1and KRT8 were the top three DEGs nodes of it. Besides, the significantly enriched GO-BP terms were mainly focused in biological activity of the blood and cells. Hub genes of the regulatory network were selected and conducted with PPI network. Total 86 nodes including 29 DEGs and 57 none-DEGs were involved in it. IGF2, GATA5, F10, CFI and AGTR1 were hub genes of this network. Some screened DEGs were proved the relationship with PC, while some other were not. Recognizing the importance of these genes in PC may help to diagnose or prevent this disease.

Tjensvoll et al. [25] suggested that circulating tumor cells in blood is responsible for the development of progression of multiple cancers including PC. Lin et al. [12]. referred that proteins secreted by cancer cells and important molecular targets most likely to enter the blood circulation. In the current study, down-regulated DEGs were mainly associated with blood circulation. Moreover, methylation is one of the main reason for down-regulation of genes. AGTR1 is an important effecter controlling blood pressure and blood volume in the circulation system [3] and was widely enriched in all the blood circulation-related GO terms. In a subset of breast cancer, AGTR1 is over-expressed and it is restricted to estrogen receptor-positive tumors [19]. For example, AGTR1 is down-regulated in thyroid tumors [18] and also found in PC of our current study. AGTR1 was previously described to interfere with cell proliferation by inactivating signal-regulated protein kinase signaling in neuroblastoma cell [6]. In addition, there are CpG islands at promoters of AGTR1 and that its expression is correlated with methylation [14]. Therefore, genes methylation may impact occurrence of PC through the influence on biological functions in blood circulation. Besides, AGTR1 may be closely associated with PC.

BZRAP1-AS1 encodes antisense RNA 1 of the benzodiazapine receptor associated protein 1. There is little known about this gene but mir-142, has a closely connection with cancers [11, 29], located in an intron of this non-coding gene [23]. In the current study, it was down-regulated and under control of 14 TFs. For the gene-specific transcriptional regulation of antisense RNA [15], expression of BZRAP1 may be affected. Mitochondrial BZRs have already been reported as therapeutic targets in photodynamic tumor therapy [26]. In addition, since that TFs were regulated by numerous methylation, BZRAP1-AS1 may also regulated by them. On account of the complicated regulation and vital role of BZRAP1-AS1 may be important for PC occurrence and development.

Conclusions

In the present study, hub nodes were selected from the regulatory and PPI network with the degree no less than 5, which may miss PC-related genes with less degree. However, numerous known PC-related genes which proved the correctness of our results were found. In conclusion, biological functions in blood circulation and the involved AGTR1 genes may play important roles in PC by methylation. Besides, BZRAP1-AS1 may be novel gene related with PC. Certainly, these results need more experimental verifications.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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