Identification of Potential Biomarkers in Human Prostate Cancer Using Bioinformatics Analysis

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Abstract

Prostate Cancer (PCa) is one of the leading causes of cancer-related deaths among the men around the world. In this study, we aim to identify candidate biomarkers in PCa using bioinformatics analysis combined with the analysis of the common database of tumors and uncover possible mechanisms. The gene expression profiles of GSE55945 including 13 PCa samples (with Gleason score of 6 or 7) and 8 normal prostate samples were downloaded from GEO database. Firstly, Differentially Expressed Genes (DEGs) were obtained using "limma" R package followed by pre-procession of raw expression data. A total of 581 genes, including 204 up-regulated genes and 377 down-regulated genes, were screened out in PCa tissues compared with normal prostate tissues with the cut-off criteria $p<0.05$ and $|log2FC|>1$. Secondly, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analyses were performed using DAVID database. Thirdly, protein-protein interaction (PPI) network of the DEGs was constructed by Cytoscape software. Modules in PPI network were screened using Molecular Complex Detection (MCODE). At last, 7 hub genes, ANXA1, CHRM3, UTS2, PROK1, AGT, CCK and EDN3 were identified from the modules of PPI network, and then validated by Oncomine database and Protein atlas database. In conclusion, our study suggested that the identified DEGs and hub genes promote our understanding of the molecular mechanisms underlying the development of PCa, and might reveal preliminary information with regard to carcinogenesis of prostate cancer.

Keywords: Prostate cancer; Bioinformatic analysis; microarray; PPI

Introduction

Prostate Cancer (PCa) is one of the leading causes of cancer-related deaths among the men around the world [1]. Approximately 900,000 men were estimated to have been diagnosed with PCa in 2008 [2]. As to PCa of early stages, androgen-ablative therapy is considered as an effective therapeutic treatment. However, in those of later stages, PCa becomes androgen-independent and more invasive, with a resistance to androgen-ablative therapy and other chemotherapies [3,4]. Therefore, it is urgently needed to find effective biomarkers for diagnosis and treatment of PCa in order to improve patient survival rates.

Currently, the microarrays are widely used in molecular biology and viewed as a powerful tool with extensive applications, such as molecular classification of cancers, prediction of diagnosis biomarkers and discovery of new drug targets for cancer [5-7]. To date, gene expression profiling by microarray has been performed in recent years to uncover molecular variations between various tumor types vs. other tissue groups, revealing that hundreds of Differentially Expressed Genes (DEGs) enriched in various pathways and biological processes [8-10]. However, the studies related to comparative analysis of the DEGs between prostate cancer and normal prostate tissues were fewer. Therefore, in order to figure out the dysregulated mRNAs and their biological processes in PCa progression, we conducted microarray analysis using bioinformatics method to achieve this goal.

In the present study, microarray dataset GSE55945 was obtained from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) for subsequent analysis. This dataset included 13 prostate cancer samples (with Gleason score of 6 or 7) and 8 normal prostate samples. Firstly, we performed a comparison between PCa samples (with Gleason score of 6 or 7) and normal
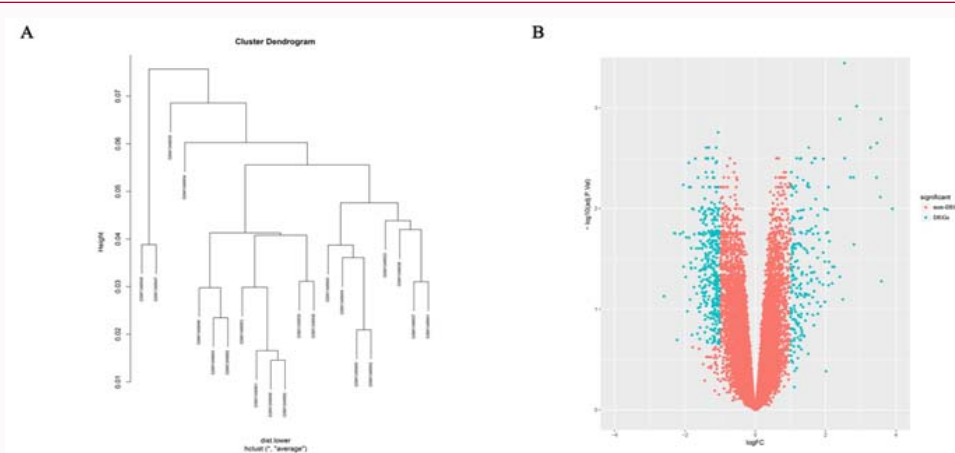
Figure 1: Samples clustering and identification of DEGs in PCa tissues. (A) Samples clustering of GSE55945 to detect outliers. (B) The volcano plot of all DEGs.
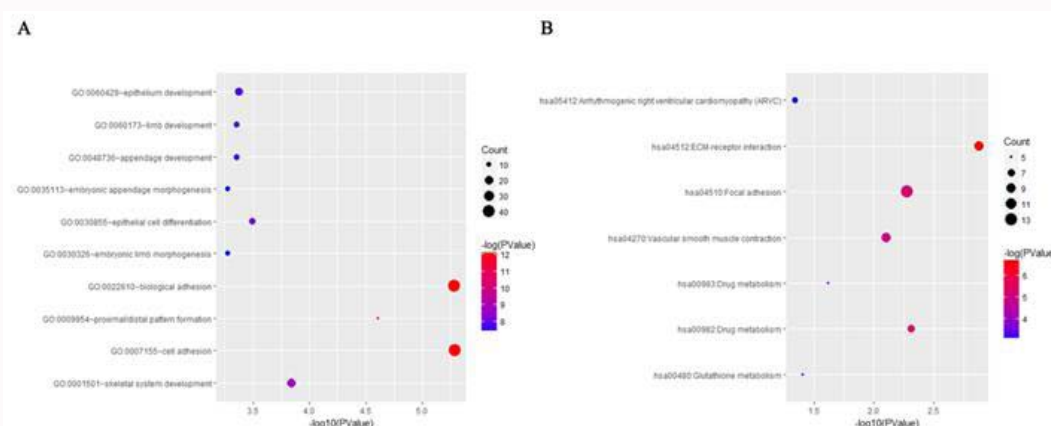

Figure 2: Bioinformatical analysis of DEGs. (A) GO analysis and (B) KEGG pathway enrichment of DEGs in PCa tissues.

prostate samples to identify the Differently Expressed Genes (DEGs). Secondly, After the DEGs were screened using R software, we the conducted Gene Ontology (GO) and pathway enrichment to analyze their biological functions and related pathways. Moreover, to assess the interrelationships of DEGs, we used STRING database for analysis and Cytoscape software for visualization. In addition, hug genes were also identified and validated. The aim of study was to provide valuable information for PCa progression at molecular level and identifies the possible diagnostic and therapeutic biomarkers for PCa.

Materials and Methods

Data collection

Gene expression profile was downloaded from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). Dataset GSE55945 was used to screen differentially expressed genes in this study. This dataset included 13 prostate cancer samples (with Gleason score of 6 or 7) and 8 normal prostate samples.

Data preprocessing

Raw expression data were calculated following the pre-processing procedures: RMA background correction, $\log_2$ transformation, quantile normalization and median polish algorithm summarization using the "affy" R package. Probes were annotated by the Affymetrix annotation files. Microarray quality was assessed by sample clustering according to the distance between different samples in Pearson's correlation matrices. No samples were removed from subsequent
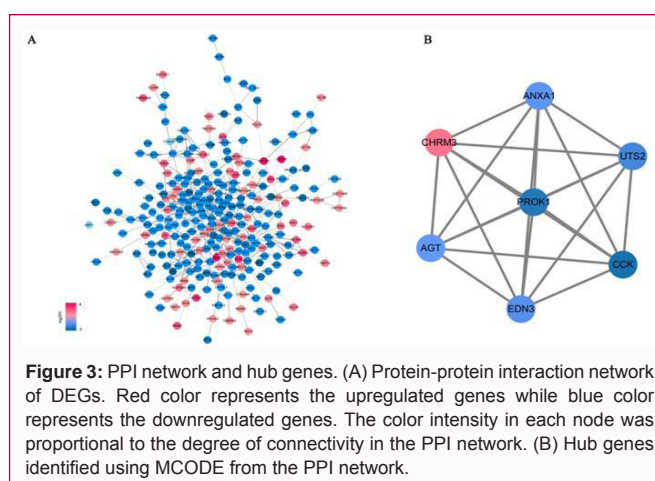

Figure 3: PPI network and hub genes. (A) Protein-protein interaction network of DEGs. Red color represents the upregulated genes while blue color represents the downregulated genes. The color intensity in each node was proportional to the degree of connectivity in the PPI network. (B) Hub genes identified using MCODE from the PPI network.

analysis in the two datasets (Figure 1A).

Differentially expressed genes (DEGs) screening

We use the "limma" R package to screen the DEGs between prostate cancer samples and normal prostate samples. The p value < 0.05 and |log2fold change (FC)| >1 were chosen as the cut-off criteria.

Functional and pathway enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/) is an online

program providing a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes [11]. Enriched biological themes of DEGs, particularly GO terms and visualization of those on KEGG pathway maps were performed using DAVID database, p <0.05 was set as the cut-off criterion.

PPI network construction

We used Search Tool for the Retrieval of Interacting Genes (STRING) Database (STRING) (http://www.string-db.org/) to assess protein-protein interaction (PPI) information. In addition, to evaluate the interrelationships of DEGs, we used STRING database for analysis and Cytoscape software for visualization. Confidence score >0.4 was set as significant.

Hub module selection and validation

We used plug-in Molecular Complex Detection (MCODE) to select hub modules of PPI network in Cytoscape. Meanwhile, degree = 5, node score = 0.2, k-core = 2, and max. Depth = 100 were used as cut-off criteria. Then genes in hub module were validated by Onocomine database (http://www.oncomine.org/) and Protein atlas database (http://www.proteinatlas.org/).

Results

Identification of DEGs in PCa tissues

The gene expression profiling of GSE55945 including 13 PCa tissues (with Gleason score of 6 or 7) and 8 normal prostate tissues were analyzed. Using "limma" package of R software, selecting p <0.05 and |log2fold change (FC)| > 1 as the cut-off criteria, 581 DEGs were identified, of which, 204 were up-regulated and 377 were downregulated. The volcano plot of all DEGs is shown in Figure 1B.

Functional and pathway enrichment analysis

To obtain further insight into the function of DEGs of PCa, the DEGs were uploaded to the DAVID database. GO analysis results showed that the DEGs were significantly enriched in Biological Process (BP), including epithelium development, limb development, appendage development, embryonic appendage morphogenesis, epithelial cell differentiation, embryonic limb morphogenesis, biological adhesion, proximal/distal pattern formation, cell adhesion and skeletal system development (Figure 2A). Moreover, seven KEGG pathways were overrepresented in DEGs, including Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), ECM–receptor interaction, focal adhesion, vascular smooth muscle contraction, 2 drug metabolism pathway and glutathione metabolism (Figure 2B).

PPI network construction

Based on the string profile obtained from STRING, the PPI network of DEGs consisted of 276 nodes and 538 edges, including 78 up-regulated genes and 198 down-regulated genes (Figure 3A).

Hub module selection and validation

Degree cut-off = 5, node score cut-off = 0.2, k-core = 2, and max. depth = 100 as the criterion, a significant module was selected, of which the score was 7 and had 7 nodes by using plug-in MCODE. The members in hub module includes Annexin A1 (ANXA1), Cholinergic Receptor Muscarinic 3 (CHRM3), Urotensin 2 (UTS2), Prokineticin 1 (PROK1), Angiotensinogen (AGT), Cholecystokinin (CCK), Endothelin 3 (EDN3) (Figure 3B). Validation of the hub genes was performed by Oncomine database and Protein atlas database (Figure 4).

Discussion

Prostate cancer is biologically heterogeneous and has a variable clinical course; therefore, it is essential to understand the molecular mechanism for better diagnosis and treatment of PCa. In this study, we investigated the gene expression profile of GSE55945, including 13 PCa samples (with Gleason score of 6 or 7) and 8 normal prostate samples to explore the molecular mechanism of PCa and find some biomarkers, as a tumor suppressor or oncogene of PCa which could be helpful therapeutic targets using bioinformatics analysis.

Results show that expressions of total 581 genes were altered between normal prostate tissues and prostate cancer tissues at a p-value <0.05. Among the 581 DEGs, 204 were up-regulated and 377 were down-regulated. GO analysis results showed that the DEGs were significantly enriched in Biological Process (BP), including epithelium development, limb development, appendage development, embryonic appendage morphogenesis, epithelial cell differentiation, embryonic limb morphogenesis, biological adhesion, proximal/distal pattern formation, cell adhesion and skeletal system development. As to pathway enrichment, we found that those DEGs were significantly enriched in 7 pathways, related to signal transduction and tumorigenesis.

Then, we constructed the PPI network with DEGs and list the top degree hub genes: Annexin A1 (ANXA1), Cholinergic Receptor Muscarinic 3 (CHRM3), Urotensin 2 (UTS2), Prokineticin 1 (PROK1), Angiotensinogen (AGT), Cholecystokinin (CCK), and Endothelin 3 (EDN3). Annexin A1 (ANXA1), is a member of a family of calcium-dependent phospholipid-binding proteins, possessing a wide range of physiological and pathological functions, some of whom have correlation to tumorigenesis. Paweletz CP "et al." [12] firstly reported that ANXA1 expression was altered in prostate cancer by molecular profiling studies of human prostate cancer samples. In addition, Kang JS "et al." [13] demonstrated that ANXA1 expression of prostatic intraepithelial lesions and early stage prostate cancer was decreased in >90%. Inokuchi J "et al." [14] reported that the decreased ANXA1 expression plays a critical role in prostate carcinogenesis and enhancing tumor aggressiveness via the upregulation of IL-6 expression and activity *in vitro* and Bizzarro V "et al." [15] found that ANXA1 may be a key mediator of hypoxia-related metastasis-associated processes in PCa.

Other studies suggested that ANXA1 might play an important role in acquisition and maintenance of a stem cell-like/aggressive phenotype in prostate cancer cells [16,17]. We also observed the differential expression of Angiotensinogen (AGT), the precursor of angiotensin I, involved in tumor angiogenesis and associated with the pathogenesis of coronary atherosclerosis. Choi JH "et al." [18] reported the role played by AGT in endothelial progenitor cells (EPCs) in tumor progression and metastasis in angiotensinogen knockout mice. Urotensin 2 (UTS2), is a cyclic heptapeptide with a most potent vasoconstrictor activity, which has been documented in various tumors [19-23]. Grieco P "et al." [24] reported that the receptor of Urotensin 2 (UTR) is involved in the regulation of motility of prostate adenocarcinoma cells and predicts the clinic outcome of PCa patients, which could be considered as a prognostic marker in human prostate adenocarcinoma patients. Cholinergic receptor muscarinic 3 (CHRM3), as a member of a large family of G protein-coupled receptors, could cause smooth muscle contraction and glandular secretion. Wang N "et al." [25] found that autocrine activation of CHRM3 promotes prostate cancer growth and castration
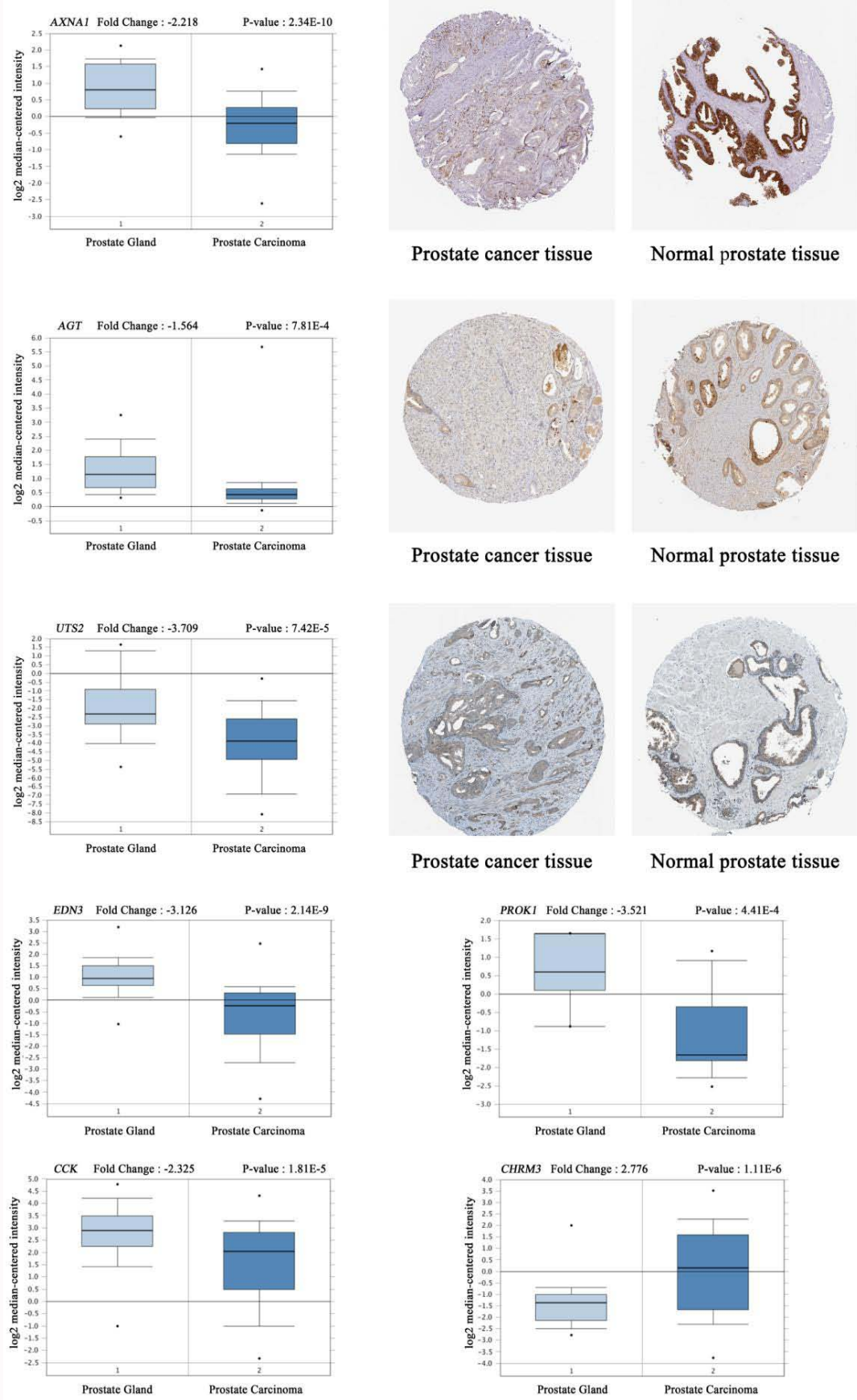
Figure 4: Validation of hub genes. ANXA1, AGT and UTS2 expression at mRNA and protein levels were validated by Oncomine database and Protein atlas database, respectively. EDN3, CHRM3, CCK and PROK1 were also validated using Oncomine database.

resistance via CaM/CaMKK-mediated Phosphorylation of Akt *in vivo* and *in vitro*. Prokineticin 1 (PROK1), as an angiogenic growth factor that is expressed merely in endocrine cells, including the adrenal gland, ovary, and testis. Pasquali D "et al." [26] discovered that the expression levels of prokineticins and their receptors increased with prostate malignancy. Many studies had revealed that PROK1 was a growth factor for cancer cells, an angiogenic and a chemotactic factor for pro-inflammatory neutrophils, participating actively in carcinogenesis process [27,28]. Cholecystokinin (CCK), is a classic gut hormone, whose receptor plays a vital role in various of cancers [29,30]. Song Y "et al." [31] reported that Mir-148b suppresses cell growth by targeting cholecystokinin-2 receptor in colorectal cancer *in vivo* and *in vitro*, which should be further evaluated as a biomarker and therapeutic tool against colorectal cancer. Endothelin 3 (EDN3), as a family of endothelin, plays a crucial role in cell differentiation, proliferation and migration processes [32,33]. Granchi S "et al." [34] suggested that Endothelin-1 production by prostate adenocarcinoma cells is down-regulated by androgens and up-regulated by factors leading to tumor progression.

Overall, by using bioinformatics analysis, we have illustrated 581 genes which may be involved in the progress of PCa, were differentially expressed in prostate cancer samples compared with normal controls. However, further molecular biological experiments are needed to confirm the function of the candidate biomarkers in PCa.

Acknowledgment

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