

Identification of genes associated with renal cell carcinoma using gene expression profiling analysis

TING YAO^{1*}, QINFU WANG^{2*}, WENYONG ZHANG³, AIHONG BIAN⁴ and JINPING ZHANG⁵

¹Physical Examination Center; Departments of ²Chronic Non-Communicable Diseases Control and Prevention, ³Health Education, ⁴Health Inspection and ⁵Communicable Diseases Control and Prevention, Laiwu Center for Disease Control and Prevention, Laiwu, Shandong 271100, P.R. China

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Abstract. Renal cell carcinoma (RCC) is the most common type of kidney cancer in adults and accounts for ~80% of all kidney cancer cases. However, the pathogenesis of RCC has not yet been fully elucidated. To interpret the pathogenesis of RCC at the molecular level, gene expression data and bio-informatics methods were used to identify RCC associated genes. Gene expression data was downloaded from Gene Expression Omnibus (GEO) database and identified differentially coexpressed genes (DCGs) and dysfunctional pathways in RCC patients compared with controls. In addition, a regulatory network was constructed using the known regulatory data between transcription factors (TFs) and target genes in the University of California Santa Cruz (UCSC) Genome Browser (<http://genome.ucsc.edu>) and the regulatory impact factor of each TF was calculated. A total of 258,0427 pairs of DCGs were identified. The regulatory network contained 1,525 pairs of regulatory associations between 126 TFs and 1,259 target genes and these genes were mainly enriched in cancer pathways, ErbB and MAPK. In the regulatory network, the 10 most strongly associated TFs were FOXC1, GATA3, ESR1, FOXL1, PATZ1, MYB, STAT5A, EGR2, EGR3 and PELP1. GATA3, ERG and MYB serve important roles in RCC while FOXC1, ESR1, FOXL1, PATZ1, STAT5A and PELP1 may be potential genes associated with RCC. In conclusion, the present study constructed a regulatory network and screened out several TFs that may be used as molecular biomarkers of RCC. However, future studies are needed to confirm the findings of the present study.

Introduction

Kidney cancer that forms in tissues of the kidney is not a single disease, instead it comprises a number of different types of cancer of which renal cell carcinoma (RCC) is the most common type in adults, responsible for ~80-90% of cases (1,2). The diagnosis of RCC is a challenging and difficult task, and RCC is one of the most therapy-resistant types of cancer. RCC is regarded as a highly lethal cancer and ~35% of patients succumb to the disease after 5 years (3).

Understanding the pathogenesis and biological mechanism of RCC may improve the current diagnosis, treatment and prognosis of RCC. However, the pathogenesis of RCC is extremely complex and remains to be fully elucidated. Smoking, obesity and mutations in specific genes increase the risk of developing RCC (4-6). The best characterized oncogenic gene in human RCC is the tumor suppressor gene von Hippel-Lindau (VHL). VHL along with elongin B, elongin C and cullin 2 form a E3 ubiquitin-ligase complex, and are considered to serve an important role in RCC (7,8). The VHL complex targets hypoxia-inducible factor (HIF) for ubiquitin-mediated degradation (9). Mutations in VHL can result in the over accumulation of HIF, and its target genes such as VEGF (vascular endothelial growth factor), PDGF (platelet derived growth factor) and EGRF (epidermal growth factor receptor), thus resulting in carcinogenesis (10-12). Mutations of either tuberous sclerosis 1 (TSC1) or TSC2 are associated with aberrant activation of mammalian target of rapamycin (mTOR) pathway, which increases the risk of RCC (13,14). Owing to the above reasons, VEGF, PDGF, EGRF and mTOR may serve as potential target molecules for the treatment of RCC. In addition, dysregulation of membrane MHC class I chain-related gene A (MICA), cyclooxygenase-1 (COX1), TGF- β -activated Kinase-1 (TAK1), and cell division cycle 25B (CDC25B) serve critical roles in RCC progression (15-18). Previous studies have predominantly focused on single genes, and paid little attention to the dysregulation of transcription factors or differentially coexpressed genes; therefore the present study aimed to address this issue to provide novel insights into RCC.

DNA microarray has previously been used to identify gene expression patterns in RCC (19,20). In order to achieve a more comprehensive understanding of the molecular mecha-

Correspondence to: Dr Qinfu Wang, Department of Chronic Non-Communicable Diseases Control and Prevention, Laiwu Center for Disease Control and Prevention, 58 Luzhong East Avenue, Laicheng, Laiwu, Shandong 271100, P.R. China
E-mail: qfwang1395@163.com

*Contributed equally

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nisms underlying RCC, gene expression profiling analysis was applied to identify DCGs and regulatory network analysis was used to identify potential associations.

Materials and methods

Affymetrix microarray data. The gene expression profile dataset GSE6344 (21), including 10 RCC samples and 10 patient-matched normal control samples, was obtained from the Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) database. The probe-level data was converted into the corresponding gene symbol using the annotation information derived from platform GPL96. For genes corresponding with multiple probe sets that had multiple expression values, the mean expression values of those probe sets were obtained. As a result, 12,754 genes were obtained from this dataset.

Construction of regulatory network. The data of TF binding sites of human genome hg18 and the coordinates of genes were downloaded from UCSC (<http://genome.ucsc.edu>). TF binding sites within the area from 1 kb upstream to 0.5 kb downstream of the transcription initiation site of target genes were selected to construct the regulatory network.

Differentially coexpressed genes analysis. For any pair of genes (X,Y), the absolute difference (Diff) of the Pearson's correlation coefficient in the normal state (P-normal) and in the cancer state (P-tumor) was calculated. The pair of genes was regarded as DCGs only when their Diff was >1. Diff value >1 indicated that the pair of genes had a negative correlation (P-normal $\in [-1,0]$) and a positive correlation (P-tumor $\in [0,1]$) under the normal and tumor state respectively, or vice versa. The difference was calculated using the following equation 1: $\text{Diff} = \text{abs}(r1_{ij} - r2_{ij})$. In this equation, $r1_{ij}$ and $r2_{ij}$ indicate the Pearson correlation coefficients between gene i and gene j under the normal state and the state of cancer, respectively.

Measurement of RIF. Regulatory impact factors (RIF) (22), which is a robust and effective methodology to identify the regulatory impact factor of TF, was applied to identify the TF with the largest contribution to differential expression of genes in two biological conditions. RIF was calculated using the following equation 2:

$$RIF_i = \frac{1}{n_{de}} \sum_{j=1}^{j=n_{de}} [(e1_j \times r1_{ij})^2 - (e2_j \times r2_{ij})^2]$$

In this equation, n_{de} is the number of DEGs; $e1_j$ and $e2_j$ indicate the expression value of the DEG in conditions 1 and 2, respectively; $r1_{ij}$ and $r2_{ij}$ indicate the correlation coefficient for the i TF and the j DEG in conditions 1 and 2, respectively.

Pathway enrichment analysis. For functional analysis of the large gene lists in the regulatory network, the DCGs were inputted into Database for Annotation, Visualization and Integrated Discovery (DAVID) (23) for Kyoto Encyclopedia of Genes and Genomes (KEGG) (24) pathway enrichment analysis. By calculating the hypergeometric test P-value for

Table I. Part of the differentially co-expressed genes.

Gene1	Gene2	Diff
AAGAB	A1CF	1.031645
ABCD4	A1CF	1.0116908
ACCN2	A1CF	1.071472
ACTR5	A1CF	1.039394
ADAM22	A1CF	1.014619
AHCTF1	A1CF	1.194273
AIP	A1CF	1.130951
AK2	A1CF	1.069488
ALKBH1	A1CF	1.034613
AMD1	A1CF	1.083589
AMELX	A1CF	1.278415
AMH	A1CF	1.040918
ANKRD12	A1CF	1.137963

Diff indicates the absolute difference of Pearson's correlation coefficient. AAGAB, α - and γ -adaplin binding protein; ABCD4, adenosine triphosphate binding cassette subfamily D member 4; ACCN2, acid-sensing (proton-gated) ion channel 1; ACTR5, ARP5 actin-related protein 5 homolog (yeast); ADAM22, ADAM metalloproteinase domain 22; AHCTF1, AT-hook containing transcription factor 1; AIP, aryl hydrocarbon receptor interacting protein; AK2, adenylate kinase 2; ALKBH1, AlkB homolog 1, histone H2A dioxygenase; AMD1, adenosylmethionine decarboxylase 1; AMELX, amelogenin, X-Linked; AMH, anti-mullerian hormone; ANKRD12, ankyrin repeat domain 12; A1CF, APOBEC1 complementation factor.

probability of random association between a given list of genes and a pathway, DAVID identifies canonical pathways associated with this set of genes. FDR <0.05 was used as the cutoff criteria.

Results

Identification of differentially coexpressed genes in RCC. The gene expression profile dataset GSE6344 was downloaded from the GEO database and formula 1 was used to identify DCGs with Diff >1 between 10 RCC samples and 10 control samples. Finally, a total of 2,580,427 DCGs were screened out (Table I).

Construction of regulatory network. Based on the known regulatory data from UCSC, TFs and their corresponding target genes from DCGs were selected to construct a regulatory network. The network contained a total of 1,525 pairs of regulatory associations between 126 TFs and 1,259 target genes. Using Cytoscape (25), the regulatory associations were integrated and visualized in Fig. 1.

KEGG pathway enrichment. The DCGs with FDR <0.05 were inputted into DAVID for KEGG pathway enrichment analysis. The results are presented in Table II, from which it was identified that DCGs were predominantly enriched in cancer pathways, ErbB, mitogen-activated protein kinase (MAPK) and other important pathways.

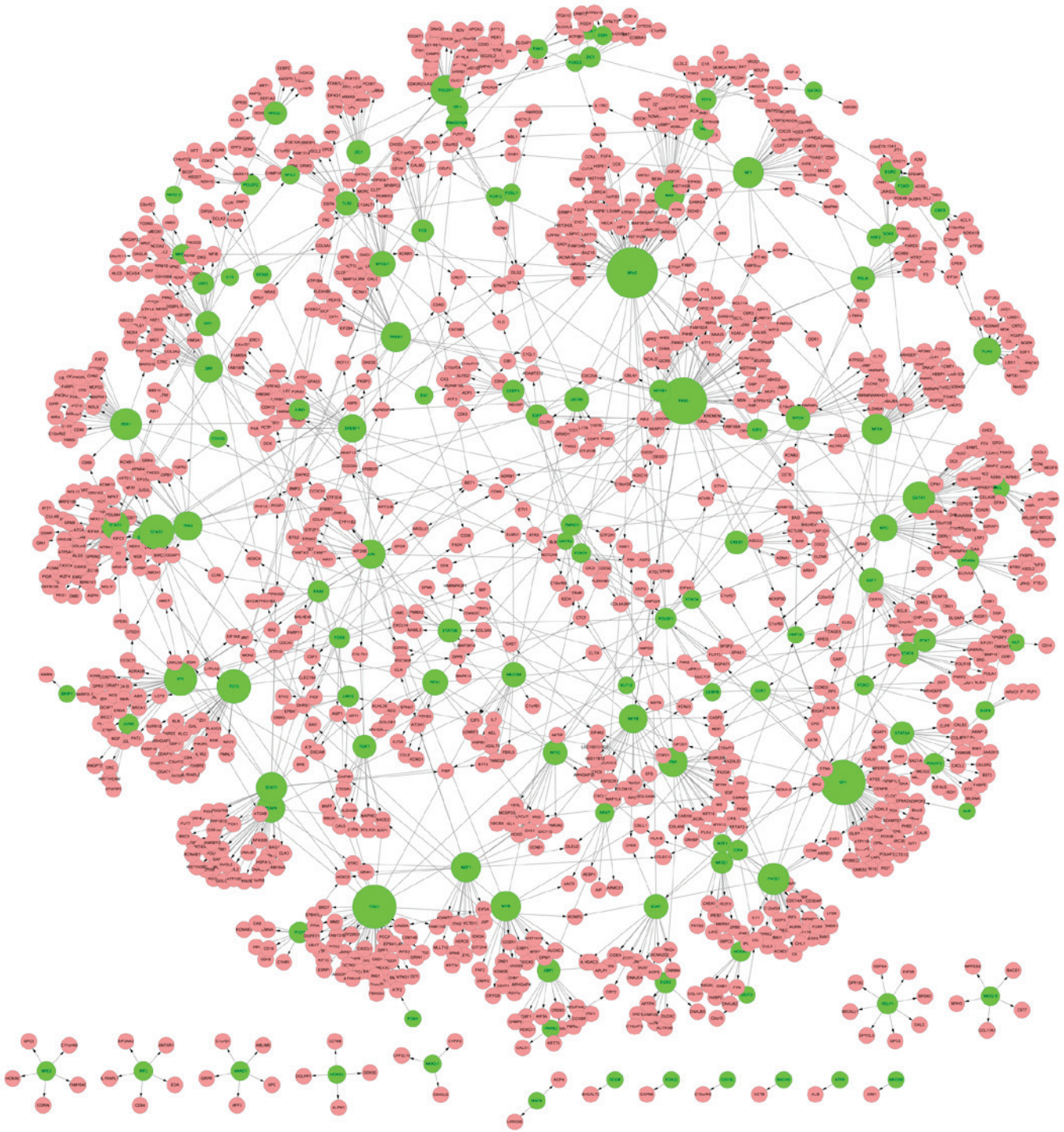


Figure 1. Regulatory network among TFs and their target genes. The green nodes indicate TF. The pink nodes indicate target genes. The lines indicate regulatory associations. TF, transcription factors.

Analysis of transcription factor impact. First, total 4,793 differentially expressed genes (DEGs) with FDR <0.05 were identified between normal and tumor samples by linear models for microarray data (limma) method (26). Subsequently, 469 overlapping DEGs were collected by comparing these 4,793 DEGs with the 1,259 target genes in the network. To further investigate which TFs were significant, the RIF of each TF targeting to the overlapping DEGs was targeted. The top 10 were forkhead box C1 (FOXC1), GATA-binding protein 3 (GATA3), estrogen receptor 1 (ESR1), FOXL1, POZ

(BTB) and AT hook containing zinc finger 1 (PATZ1), v-myb avian myeloblastosis viral oncogene homolog (MYB), signal transducer and activator of transcription 5A (STAT5A), early growth response 2 (EGR2), EGR3 and proline, glutamate and leucine rich protein 1 (PELP1) (Table III). Of these TFs, GATA3, MYB, EGR2, and EGR3 have previously been identified to be associated with RCC and the regulatory associations of them with their targets are presented in Fig. 2. Occurrence of RCC is likely caused by the abnormal changes of these regulatory associations.

Table II. The enriched KEGG pathways.

Category	Term	FDR (%)
KEGG PATHWAY	has05200:Pathways in cancer	0.012504
KEGG PATHWAY	has05215:Prostate cancer	0.028298
KEGG PATHWAY	has04710:Circadian rhythm	0.147888
KEGG PATHWAY	has04012:ErbB singling pathway	0.185805
KEGG PATHWAY	has05220:Chronic myeloid leukemia	0.192832
KEGG PATHWAY	has05221:Acute myeloid leukemia	0.218097
KEGG PATHWAY	has05222:Small cell lung cancer	0.323675
KEGG PATHWAY	has05212:Pancreatic cancer	1.018255
KEGG PATHWAY	has04010:MAPK singling pathway	1.268065
KEGG PATHWAY	has05214:Glioma	1.84753
KEGG PATHWAY	has05213:Endometrial singling pathway	2.33947
KEGG PATHWAY	has04062:Chemokine singling pathway	3.258893
KEGG PATHWAY	has05120:Epithelial cellsignaling in <i>Helicobacter pylori</i> infection	4.078908

KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table III. The top 10 ranked TFs.

TF	RIF score	RIF rank
FOXC1	7.804438725	1
GATA3	6.908779522	2
ESR1	6.32301186	3
FOXL1	4.242514268	4
PATZ1	3.800727043	5
MYB	3.507929833	6
STAT5A	3.467483166	7
EGR2	3.361578969	8
EGR3	3.337751915	9
PELP1	2.818195935	10

TF represents the transcription factor in the regulatory network. RIF represents the regulatory impact factor of TF. Rank represents the impact rank of TF. TF, transcription factor; FOXC1, forkhead box C1; GATA3, GATA-binding protein 3; ESR1, estrogen receptor 1, FOXL1, forkhead box L1; PATZ1, POZ (BTB) and AT hook containing zinc finger 1; MYB, v-myb avian myeloblastosis viral oncogene homolog, STAT5A, signal transducer and activator of transcription 5A, EGR2/3, early growth response 2 or 3; PELP1, proline, glutamate and leucine rich protein 1.

Discussion

Molecular biomarkers are useful to improve diagnosis, clinical predictive capability and novel therapeutic efficacy. Because the emergence of microarray technology makes it possible to investigate the expression levels of thousands of genes simultaneously (27), it has been widely used in discovery of disease biomarkers (28-30). However, the majority of previous studies are based on a single gene differential expression analysis. In the current study, differentially coexpressed genes analysis was used to find the dysregulated gene pairs in RCC. The differ-

entially coexpressed genes analysis provides novel analytical perspective to studies including identification of differentially coexpressed genes and marker genes of disease, construction of differential coexpression network and analysis of module (31,32). Besides, a regulatory network was constructed and the RIF score of each TF targeting to the DEGs with FDR <0.05 was calculated. Finally, the top 10 ranked TFs were identified which were FOXC1, GATA3, ESR1, FOXL1, PATZ1, MYB, STAT5A, EGR2, EGR3 and PELP1. They may be potential molecular biomarkers of RCC.

FOXC1 is one of the forkhead transcription factor family, which is characterized by a unique DNA-binding domain. Although the specific function of this gene has not yet been determined, it is known that the gene is associated with the occurrence of a number of types of cancer, such as breast cancer, lung cancer, prostate cancer, bile duct cancer (33-36). FOXC1 appears to be a cancer-associated gene. FOXL1 and FOXC1 belong to the same family and have similar functions. So these genes may serve important roles in the pathogenesis of RCC.

The transcription factor encoding by GATA3 belongs to the GATA family, and it contains two GATA-type zinc fingers which serve an important role in regulating T cell development and the biology of endothelial cell. Cooper *et al* (37) demonstrated that GATA3 was methylated in clear cell RCC patients and its mRNA expression level was downregulated in all stages of clear cell RCC (37), which indicated the critical role of GATA3 in RCC.

As an estrogen receptor, ESR1, which contains a number of important structural domains such as the DNA-binding domain, transcriptional activation domain and hormone binding domain, is a TF activated by its corresponding ligand. It is known that ESR1 serves an important role in breast cancer (38). Although it has not yet been reported that this gene is associated with RCC at present, it is likely to be a potential factor because its RIF ranks third.

Among PATZ1, MYB, STAT5A, EGR2/EGR3 and PELP1, it is known that MYB and EGR2/EGR3 (39) are RCC associ-

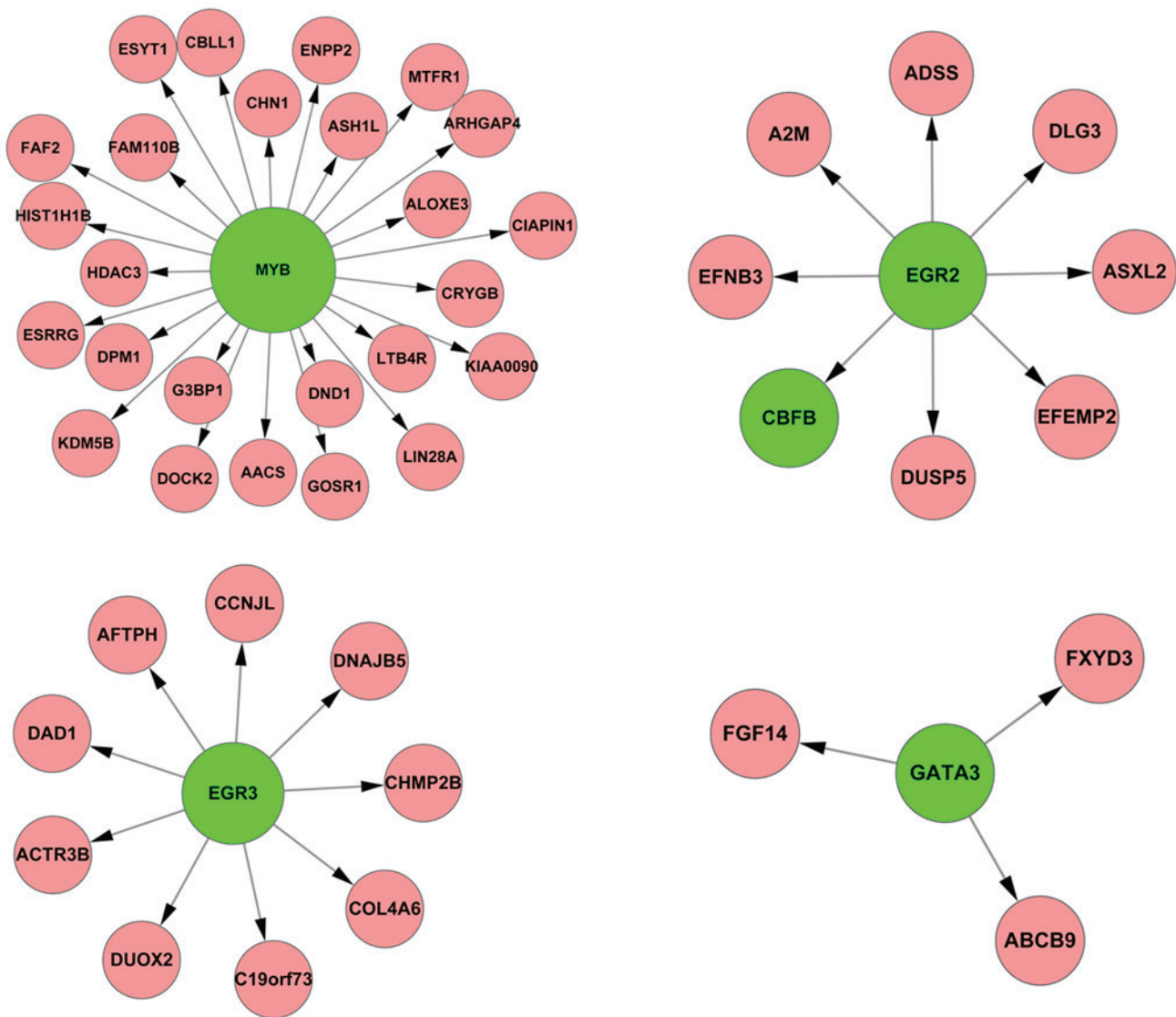


Figure 2. The regulatory associations between the 4 TFs associated with RCC and their target genes. The green nodes indicate TFs and the red nodes indicate their target genes. TF, transcription factors; RCC, renal cell carcinoma.

ated genes, and the PATZ1, STAT5A and PELP1 are hormone associated genes. The latest article reported that hormones may inhibit the occurrence of renal carcinoma (40), so that PATZ1, STAT5A and PELP1 may serve as potential renal cancer-associated genes.

Table II identifies genes of the regulatory network which were enriched in various cancer pathways, just as the above discussion that the top 10 TFs were involved in a number of types of cancers. In addition, ErbB and MAPK pathways were overrepresented. The MAPK signaling pathways serve vital roles in cell proliferation and differentiation. Recently, Huang *et al* (41) reported that suppression of one or more MAPK signaling pathways by inhibitor of MAPK kinases (MKKs) reduced RCC cell proliferation *in vitro* and inhibited RCC growth *in vivo*. The ErbB protein family is a family containing 4 structurally associated receptor tyrosine kinases, ErbB1 (also termed EGFR), ErbB2, ErbB3 and ErbB4. ErbB signaling pathway has been implicated in the development of a wide variety of types of tumor, including RCC (42,43). Further analysis of these pathways will

contribute to an improved understanding of the roles of the differentially expressed genes and the underlying molecular mechanism of RCC.

In conclusion, the present study used microarray gene expression profiling and regulatory network analysis to explore the molecular mechanism of RCC. The top 10 ranked TFs were identified, which were FOXC1, GATA3, ESR1, FOXL1, PATZ1, MYB, STAT5A, EGR2, EGR3 and PELP1. GATA3, ERG and MYB are considered RCC associated genes while FOXC1, ESR1, FOXL1, PATZ1, STAT5A and PELP1 may also be potential genes associated with RCC. The present study indicates that the above TFs may be used as biomarkers of RCC for accurate diagnosis, prognosis or as predictive markers for treatment efficiency. However, further experiments are needed to confirm these result.

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