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ORIGINAL ARTICLE

Integrated Analysis of Transcriptomic Data Reveals Key Anti-CKD Targets and Anti-ESRD Targets

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SUMMARY

Background: **Chronic kidney disease (CKD) is a kidney disease in which there is gradual loss of kidney function over time and end-stage renal disease (ESRD) is the final stage of CKD. Both CKD and ESRD are worldwide health problems with a high economic cost to health systems. However, the molecular mechanisms of the development of CKD and ESRD remain poorly understood. This study aimed to systematically elucidate the molecular mechanisms of the development of CKD and ESRD.**
Methods: **Transcriptome data of CKD and ESRD were downloaded from the NCBI-GEO database. Differentially expressed genes between cases and controls (chronic kidney disease patients vs. controls, end-stage renal disease patients vs. controls) were calculated using the empirical Bayes algorithm. Gene set enrichment analysis (GSEA) was used for analyzing the KEGG pathway difference between cases and controls. Furthermore, CKD and ESRD target genes were obtained from the Thomson Reuters Integrity database. Tissue-specific gene interaction network analysis was performed using the GIANT web server.**
Results: **There were multiple damaged pathways in ESRD but only a few pathways were disturbed in CKD. Furthermore, we identified 9 dysregulated anti-ESRD genes but no dysregulated anti-CKD genes. Network analysis revealed that the NF-kB signaling pathway was essential for ESRD.**
Conclusions: **This study revealed several crucial anti-ESRD genes that are involved in the regulation of the NF-kB signaling pathway. This information may be helpful for the treatment of ESRD.**
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KEY WORDS

CKD, ESRD, gene expression, NF-kB, network, target genes

INTRODUCTION

Chronic kidney disease (CKD) is defined by the presence of kidney damage (GFR < 60) and associated with hypertension, diabetes, obesity, and primary renal disorders. The disease causes major health problems and 10% of the population is affected by CKD worldwide [1-3]. End-stage renal disease (ESRD) is regarded as the most serious outcome of CKD and more than 1,500 people per million population in countries including USA, Japan, and Taiwan have been affected [4,5]. With a high global prevalence, CKD is usually asymptomatic until later stages. ESRD almost always comes after CKD [6-8]. The outcome of ESRD is very poor and depends on the treatment. ESRD patients have two treatment options: transplantation and dialysis. Patients on dialysis have a 5-year survival rate of 35% and transplant recipients have a 3% mortality rate after 5 years [8]. Therefore, it is necessary to find the mechanism and treatment target for CKD and ESRD.

To date, there are insufficient reports on genes and pathways which play an important role in CKD and ESRD. Therefore, our study focused on improving the understanding of pathological mechanisms and clinical treatment in CKD and ESRD. Previous research has shown that Genome Wide Association Studies (GWAS) are widely used to define the contribution of genetic risk to kidney disease [9-13]. APOL1 is found to be associated with kidney disease in African Americans through GWAS [14]. FRMD3 and BMP pathway genes are in the presence of the risk allele in CKD [15]. Based on previous conclusions, most G-protein-coupled receptors (GPCRs) are expressed in different spatio-temporal patterns during kidney development and that altered GPCR expression is involved with CKD [16]. The results of a medical genomics study indicated that infection, injury or autoimmune factors lead to the activation of NF-kB in the pathogenesis of renal inflammation [17,18]. Thomson Reuters Integrity™ which contains much information on therapeutic drugs and gene targets for numerous human diseases is an accurate database for the research of the pharmaceutical industry, biology, and pharmacology (https://thomsonreutersintegrity.com). Nevertheless, there is no study on comparisons between the expression profiles of CKD and ESRD target genes. Thus, we performed a comprehensive analysis of gene expression datasets to find key targets for CKD and ESRD treatment. Gene expression profiling datasets of CKD and ESRD were download from the Gene Expression Omnibus of the National Center for Biotechnology Information which is repository of numerous high throughput gene expression data (NCBI-GEO; http://www.ncbi.nlm.nih.gov/geo) [17,18]. The molecular signaling pathways and biological functions of CKD and ESRD are reveled in our study by mRNA transcript analysis.

MATERIALS AND METHODS

Data collection

We retrieved and downloaded human chronic kidney disease, end-stage renal disease datasets from NCBI-GEO database (http://www.ncbi.nlm.nih.gov/geo, last accessed March 2018). Criteria for data filtering were: (1) all datasets were genome-wide; (2) the samples of each data set must include patients and controls; (3) non-cell line samples; and (4) raw data was available. If the number of samples is less than three in cases or controls, or samples were treated with drugs or other agents, these datasets will be excluded. According to above criteria, GSE97709 was finally obtained for our analysis. This dataset including eight chronic kidney disease, nine end-stage renal disease patients, and 12 controls. The sequencing reads were mapped to hg19 human genome using HISTA2 (v2.1.0) [19]. Then, we used Cufflinks (v2.2.1) to calculate the gene expression (FPKMs) of Ensembl annotated genes [20,21].

Differential expression genes analysis

Differential expression analysis was conducted using R v4.1 and Bioconductor Library. We used the empirical Bayes algorithm in "limma" package to detect differentially expressed genes between cases and controls (chronic kidney disease patients vs. controls, end-stage renal disease patients vs. controls) [22]. Regulated genes were considered as absolute value of logarithmic transformed fold-change ($|\log2FC| \geq 1$ and a false discovery rate (FDR) adjusted P value ≤ 0.05. We carried out the differential expression analysis in chronic kidney disease and end-stage renal disease.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) of KEGG pathways in two comparisons (chronic kidney disease patients vs. controls, end-stage renal disease patients vs. controls) were performed with javaGSEA desktop application v3.0 [23]. Gene sets with more than 15 genes or less than 500 genes were included. The remaining parameters were set as default. Pathways with normalized enrichment scores (NESs) > 0 were considered up-regulated, and NESs < 0 were considered down-regulated. False discovery rate (FDR) P values ≤ 0.05 were determined as statistically significant.

Renal disease target genes analysis

Renal disease target genes were collected from Thomson Reuters Integrity database (https://thomsonreutersintegrity.com). Then, we defined renal disease target genes as there were already marketed drugs or drugs under development that target this gene. A list of 25 renal disease target genes were obtained and then mapped

to our dataset. Finally, we got the union set of the differentially expressed target genes in chronic kidney disease and end-stage renal disease and showed their expression difference.

Gene network analysis

Genome-scale integrated analysis of gene networks in CKD and ESRD were achieved using Gene Networks in Tissues (GIANT) web server (http://giant.princeton.edu/) [24]. We used the differentially expressed target genes CKD and ESRD as input data to perform gene network analysis. The tissue in GIANT web server was chosen as "kidney". The server could generate a gene network of target genes and other genes that interacted with the target genes and perform biological function enrichment analysis of the genes in the network.

RESULTS AND DISCUSSION

Differentially expressed genes between control and CKD, control and ESRD

Significantly differentially expressed genes in controls, CDK, and ESRD were presented in Figure 1. There are 61 downregulated and 19 upregulated genes determined in CDK in contrast to control groups. Most of differentially expressed genes were downregulated in CDK and the control groups. Therefore, minor changes in gene expression of CDK and the control groups were presented (Figure 1A). However, 3,724 upregulated genes and 3,248 downregulated genes in the ESRD were found (Figure 1B). Our present study revealed dramatic changes between the gene expression pattern in ESRD and the control groups, which implied kidney function is significantly impaired in ESRD. As known to us, CKD is frequently asymptomatic until later stages [25-30]. The pronounced pathology and mechanism underlying its development were ambiguous [31-33]. We concluded that it might be explained by dramatic changes of gene expression in ESRD rather than CKD.

KEGG enrichment results

The gene set enrichment analysis (GSEA) of KEGG pathways in two comparisons (chronic kidney disease patients vs. controls, end-stage renal disease patients vs. controls) is presented in Figure 2. Differentially expressed genes were enriched in 20 KEGG Pathway classes and 41 KEGG Pathways. Among the 41 pathways, most are identified to be significantly enriched for ESRD, including cancers: “Specific types”, “Cell growth and death”, “Immune system”. Only one KEGG pathway in the category, namely “Basal transcription factors”, was enriched in both groups. Previous study demonstrated that transcription factors truly played a vital role in kidney disease [34]. As mentioned above, there are minor changes in gene expression of CDK and the control groups. We presented a hypothesis that different-expression gene of CDK and the control groups enriched in “Basal transcription factors” might result in a cascade effect, affecting development of CKD through basal transcription factors. Only 10 pathways are significantly enriched in CKD, because there are less differentially expressed genes in chronic kidney disease patients vs. controls than end-stage renal disease patients vs. controls. Several pathways were enriched in CKD rather than ESRD. Interestingly, almost all of the pathways were down-regulated in ESRD excepted “Basal cell carcinoma”, “Olfactory transduction”, “Taste transduction”, and “Neuroactive ligand-receptor interaction”. It may be concerned with loss of kidney function of ESRD. Also, several pathways have been reported to be associated with CKD or ESRD [35-39]. For instance, glycan has been reported to have an effect on kidney dysfunction [40]. Meanwhile, “Glycan biosynthesis and metabolism” pathways are down-regulated in ESRD in our result. “Olfactory transduction” and “Taste transduction” pathways are down-regulated in CKD and may account for loss of appetite which is common in children with CKD [41]. The downregulation of these pathways can help us further understand the development of CKD and ESRD.

Mapping of anti-ESRD target genes

We show the differentially expressed renal disease target genes in Figure 3. Significantly differentially expressed genes are marked with asterisks. The combined target gene set contained 17 genes. The log2 (fold-change) of these targets in each group is shown in Figure 3. None of these target genes is significantly differently expressed in CKD. However, six of 17 target genes were over-expressed in ESRD including arginine vasopressin receptor type 2 (AVPR2), melanocortin 1 receptor (MC1R), melanocortin 3 receptor (MC3R), melanocortin 4 receptor (MC4R), melanocortin 5 receptor (MC5R), and nuclear factor kB (NFKB2). AVPR2 has been reported to be a key stimulant for cyclic AMP production in the collecting duct and promotes pathologic kidney growth [42]. MC1R plays an important role in normal pigmentation. Glomerular expression of MC1R increases in renal glomerular diseases affecting the podocytes [43]. NF-kB mediates renal inflammation in different cell types, including renal cells, innate immune cells, and lymphocytes. Previous studies suggested targeting NF-kB signaling pathway represented an attractive therapeutic approach in disease treatment [44-47]. Otherwise, three of 17 target genes are were low-expressed in ESRD including Arginine Vasopressin Receptor 1A (AVPR1A), Protein Phosphatase 3 Regulatory Subunit B, Alpha (PPP3R1), and Thromboxane A2 receptor (TBXA2R). TBXA2R is a potent stimulator of platelet aggregation. In kidney, the binding of TXA2 to glomerular TP receptors causes intense vasoconstriction [48,49].

Gene network of anti-ESRD target genes

We performed genome-scale integrated analysis of nine deregulated renal disease target genes in Figure 4A. The gene interaction network of target genes (big circle)

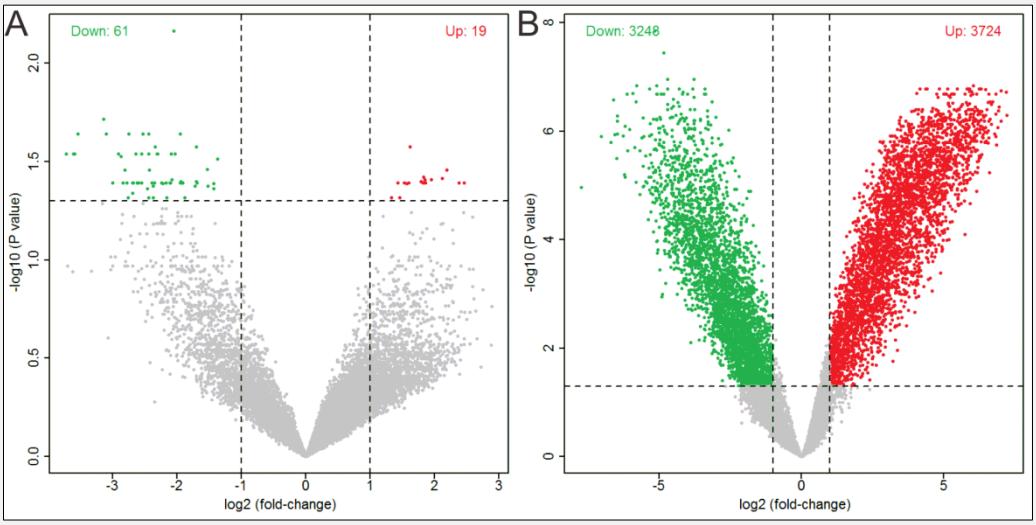


Figure 1. Volcano plot of differential expressed genes in (A) chronic kidney disease and (B) end-stage renal disease.

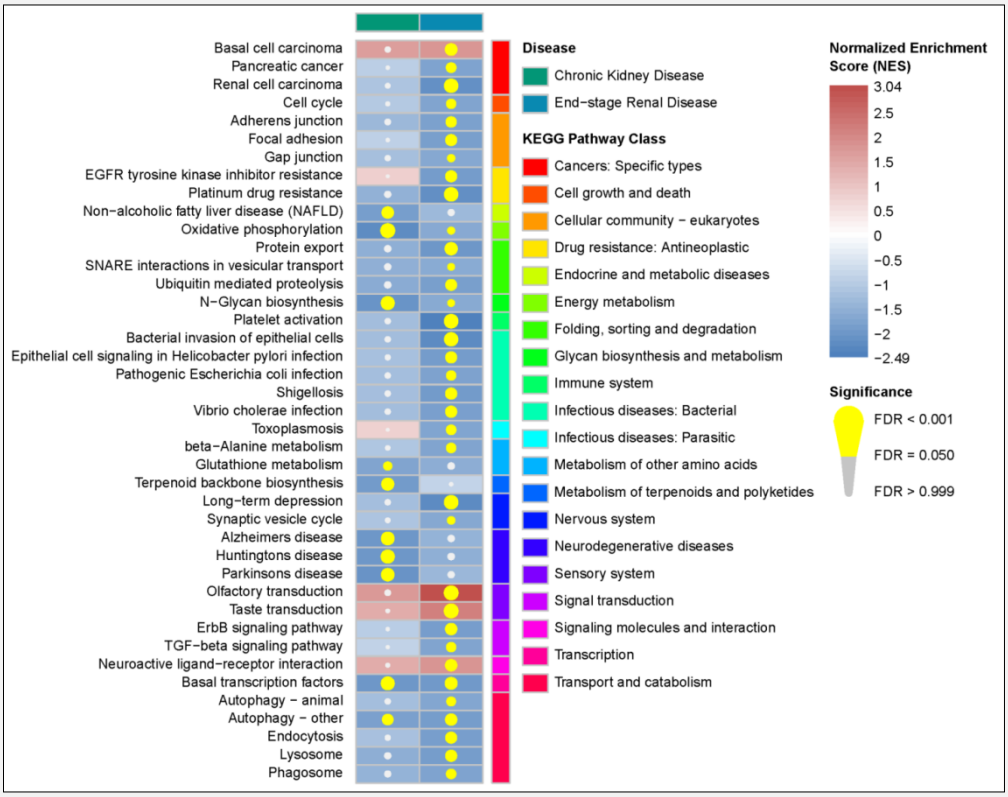


Figure 2. Gene set enrichment analysis of KEGG pathways. The red box represents the pathway is up-regulated and the blue box represents the pathway is down-regulated. The yellow circle indicates the pathway is significantly enriched.

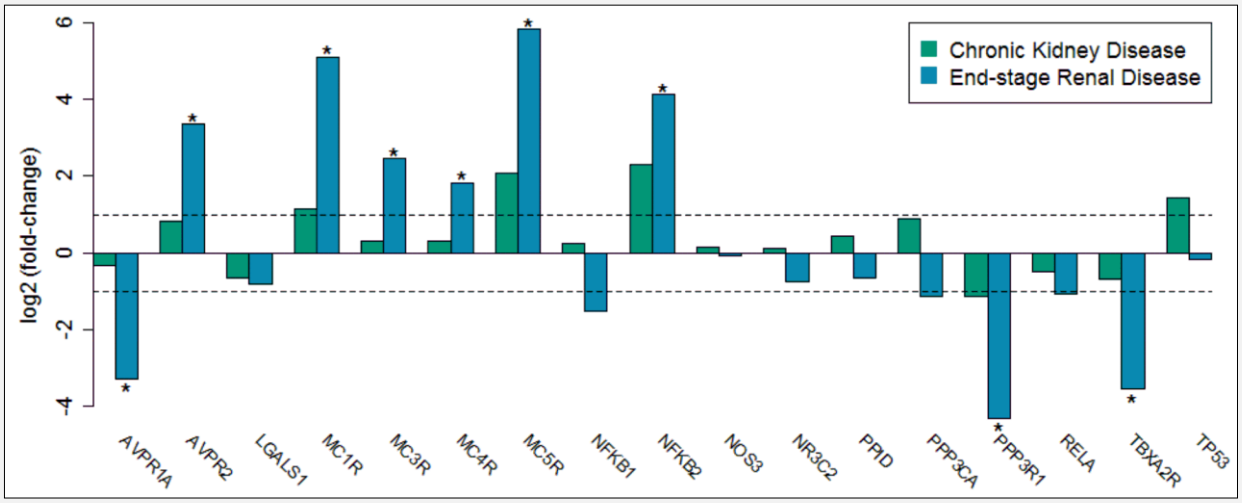
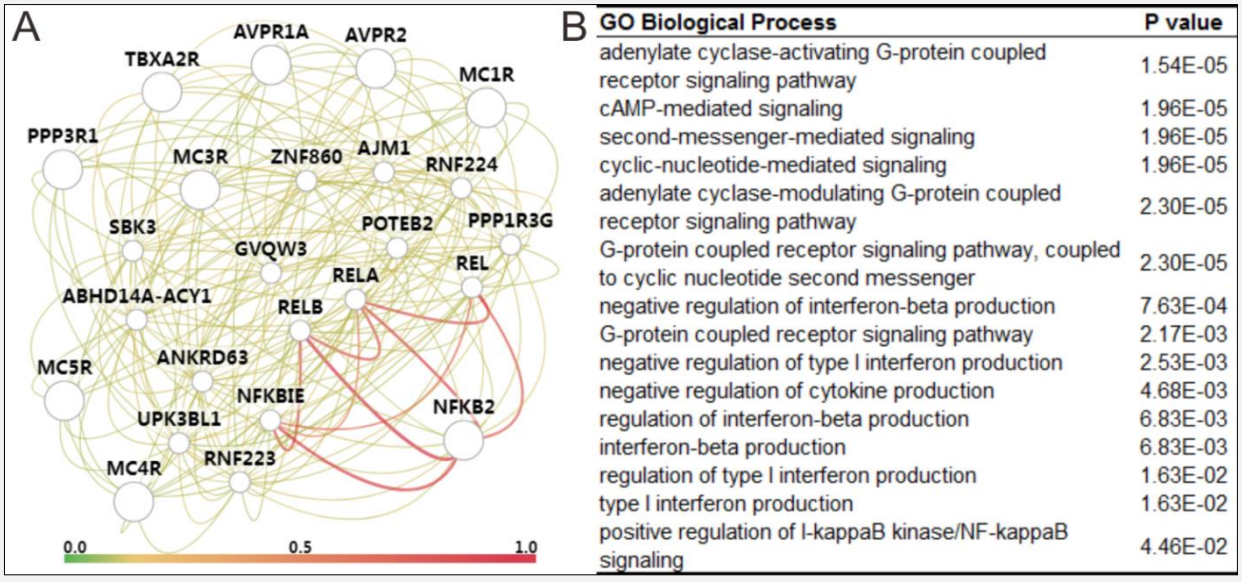


Figure 3. Expression difference of mapped renal disease target genes in chronic kidney disease and end-stage renal disease. Differentially expressed genes are marked with asterisks.



GO Biological Process	P value
adenylate cyclase-activating G-protein coupled receptor signaling pathway	1.54E-05
cAMP-mediated signaling	1.96E-05
second-messenger-mediated signaling	1.96E-05
cyclic-nucleotide-mediated signaling	1.96E-05
adenylate cyclase-modulating G-protein coupled receptor signaling pathway	2.30E-05
G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger	2.30E-05
negative regulation of interferon-beta production	7.63E-04
G-protein coupled receptor signaling pathway	2.17E-03
negative regulation of type I interferon production	2.53E-03
negative regulation of cytokine production	4.68E-03
regulation of interferon-beta production	6.83E-03
interferon-beta production	6.83E-03
regulation of type I interferon production	1.63E-02
type I interferon production	1.63E-02
positive regulation of I-kappaB kinase/NF-kappaB signaling	4.46E-02

Figure 4. Genome-scale integrated analysis of deregulated renal disease target genes. (A) The gene interaction network of target genes (big circle) and associated genes (small circle). (B) GO biological process of the genes in the network.

which is associated with many genes (small circle) that play essential roles in kidney disease is large. Among the genes, NFKB2 is widely known for its function in different types of kidney diseases [17]. A family of structurally related proteins (The structurally related protein family), including RelA, RelB, Rel, and

NFKBIE, which share extensive homology in a region known as Rel homology domain, are associated with NFKB2. RelB is upregulated several fold in diabetic murine kidney cortex [50]. Upregulation of RelA in tubular epithelial cells has a pathogenic role in mediating chronic inflammation in chronic kidney disease (CKD) [17,50]. Additionally, the enriched biological processes of these target genes and interacting genes were found to be mostly associated with cytokines, G-protein-coupled receptors, and NF-kappaB. G-protein-coupled receptors (GPCRs) play an active role in transcriptional regulation and contribute to the expression of cytokines. NF-kB is one of the transcription factors of these proinflammatory genes [51]. Our results suggested that GPCRs and NF-kB may have key functions in ESRD.

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Availability of Data and Materials:
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions:
Study Design: RCZ, HLY.
Data Collection: ZWS, LLL, RCZ.
Statistical Analysis: ZWS, JZT, YPY, YCG, RCZ.
Data Interpretation: ZWS, LLL, RCZ.
Manuscript Preparation: ZWS, LLL, RCZ.
Literature Search: ZWS, LLL.
Funds Collection: HLY.

Declaration of Interest:
The authors declare that they have no competing interests.

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