Identification and Validation of Glomerulotubular Crosstalk Genes Mediating IgA Nephropathy by Integrated Bioinformatics

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Abstract

Background

IgA nephropathy (IgAN), which has been reported as the most prevalent glomerulonephritis globally, is the major contributor to end-stage renal illness. This bioinformatics study aimed to explore glomeruli-tubulointerstitial crosstalk genes and dysregulated pathways relating to the pathogenesis of IgAN.

Methods

The microarray datasets from the Gene Expression Omnibus (GEO) database were searched. Weighted gene co-expression network analysis (WGCNA) and differentially expressed genes (DEGs) of both glomeruli and tubulointerstitial were conducted individually. The co-expression gene modules of tubulointerstitial and glomeruli were compared via gene function enrichment analysis. Subsequently, the crosstalk co-expression network was constructed via the STRING database and key genes were mined from the crosstalk network.

Results

583 DEGs and eight modules were identified in glomeruli samples, while 272 DEGs and four modules were in tubulointerstitial samples. There were 119 overlapping DEGs of the two groups. Among the distinctive modules, four modules in glomeruli and one module in tubulointerstitial were positively associated with IgAN. While four modules in glomeruli and two modules in tubulointerstitial were negatively associated with IgAN. The top ten key genes screened by CytoHubba were ITGAM, ALB, TYROBP, ITGB2, CYBB, HCK, CSF1R, LAPT5M, FN1 and CTSS. The above genes were all validated using another two datasets, and all of the key genes demonstrated possible diagnostic significance.

Conclusions

The crosstalk genes confirmed in this study may provide novel insight into the pathogenesis of IgAN. Immune-related pathways are associated with both glomerular and tubulointerstitial injuries in IgAN. The glomerulotubular crosstalk might perform a role in the pathogenesis of IgAN.

Background

Immunoglobulin A (IgA) nephropathy (IgAN), which is identified as the most prevalent primary glomerular disease in many countries, is the major contributor to kidney failure. Specifically, end-stage renal disease (ESRD) will develop in 20 percent to 40 percent of IgAN patients within 20 years after illness initiation, according to recent research.[1, 2]. This illness is predominantly prevalent among young individuals between the ages of 20 and 40 years and causes a great burden to individuals and societies[2]. Currently, scholars believe that genetic, environmental, and immune factors together determine the occurrence and development of IgA nephropathy[3], but the precise pathogenic mechanisms have not been elucidated. Even though IgAN has traditionally been thought of as a mesangial proliferative glomerular disorder,
recent research has revealed that tubulointerstitial damage might be more strongly associated with disease progression than glomerulonephritis. Tubular atrophy/interstitial fibrosis has been reported to independently serve as a risk indicator of IgAN progression among patients with this disease. Moreover, tubular injury makes the glomerulus sensitive to injury and decreases glomerular filtration through tubuloglomerular feedback in chronic kidney lesions[4]. However, the potential genes and signaling pathways through which glomeruli and tubulointerstitial influence each other and promote the occurrence and progression of IgAN are still unclear. Moreover, the treatments for IgAN are based mostly on nonspecific blockers of the renin-angiotensin system (RAS), which are not always effective[5]. The efficacy and safety of immunosuppressants have also long been controversial. Therefore, new therapeutic strategies aiming to interfere with tubuloglomerular crosstalk may shed new light on IgAN treatment.

A huge amount of data has been generated and preserved in publicly available databases in recent years as a result of the widespread use of genome transcriptome analysis, such as the Gene Expression Omnibus (GEO) database. The data has been generally applied and used in multiple disease research areas, especially in cancer[6]. Weighted gene co-expression network analysis (WGCNA) is an innovative and potent tool, especially in generating co-expression gene modules from mRNA microarray datasets[7]. The detected gene modules may be utilized for subsequent analyses, including biological functions and key genes identification. Although some bioinformatic studies have been applied to exploring potential molecular mechanisms and therapeutic targets in IgAN[8-10], few studies focus on the shared and different genes of glomeruli and tubulointerstitial of IgAN.

In this study, we aimed to compare similarities and differences between glomeruli and tubulointerstitial samples and revealed crosstalk genes underlying the molecular mechanism of IgAN. Firstly, gene expression datasets of glomeruli and tubulointerstitial from IgAN patients as well as corresponding controls were obtained from the GEO database. We then detected the Differentially expressed genes (DEGs) of two groups, and the overlapping part was screened. The co-expression networks of glomeruli and tubulointerstitial samples were constructed with the help of WGCNA. Then, functional enrichment analysis was performed on the DEGs in each co-expression gene module. Subsequently, the crosstalk network of glomeruli and tubulointerstitial was formed via String database and key genes were mined from the crosstalk network by CytoHubba in Cytoscape. Lastly, additional GEO datasets were utilized to verify the identified key genes (GSE37460 and GSE35487).

Methods

Ethical compliance

The research was carried out in strict accordance with the Declaration of Helsinki (2013). Neither animal experiments nor human clinical trials were conducted as part of our investigation. The raw datasets were available from the GEO database.

Acquisition and Processing of Data
Figure 1 depicts the flowchart of the present research. The GEO database was used to obtain datasets series matrix files comprising GSE104948, GSE104954, GSE37460, and GSE35487. GSE104948 and GSE104954 contained gene expression profiles from glomeruli and tubulointerstitial of IgAN patients and controls. GSE37460 and GSE35487 were used for validation of the key genes. Table 1 provides the details of each dataset. Platform document of GPL22945, GPL24120[11], GPL11670, GPL14663[12] and GPL 96[13] were downloaded to annotate the gene expression. Finally, the gene expression matrices were generated with column names as gene symbols and row names as group names for subsequent analysis.

Table 1 Microarray datasets information.

<table>
<thead>
<tr>
<th>Dataset ID</th>
<th>Platform</th>
<th>Sample Details</th>
<th>Sample IgAN vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSE104948</td>
<td>GPL22945</td>
<td>Glomeruli from renal biopsy</td>
<td>27 vs 21</td>
</tr>
<tr>
<td></td>
<td>GPL24120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSE104954</td>
<td>GPL22945</td>
<td>Tubulointerstitial from renal biopsy</td>
<td>25 vs 21</td>
</tr>
<tr>
<td></td>
<td>GPL24120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSE37460</td>
<td>GPL11670</td>
<td>Glomeruli from renal biopsy</td>
<td>27 vs 27</td>
</tr>
<tr>
<td></td>
<td>GPL14663</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSE35487</td>
<td>GPL 96</td>
<td>Tubulointerstitial from renal biopsy</td>
<td>25 vs 6</td>
</tr>
</tbody>
</table>

Screening of Differentially Expressed Genes (DEGs)

The limma package in R (version: 3.5.3) was performed to detect DEGs between glomeruli, tubulointerstitial and normal tissues. Genes with set $P < 0.05$ and $|\log_2(\text{Fold Change})| > 1.5$ were considered DEGs.

Co-expression Gene Modules Constructing

As a bioinformatics approach, WGCNA was utilized to create a scale-free network according to gene expression profiles[14]. All genes in a module are groups of genes with similar patterns of expression[15]. In this research, the WGCNA algorithm was conducted to construct the co-expression modules of glomeruli and tubulointerstitial individually, and then examine the association between the gene modules and disease.

Firstly, outlier samples were filtered using the WGCNA package, and correlation matrices were created afterward. In order to assess whether the genes exhibited comparable expression patterns, the Pearson correlation coefficient between them was computed and the screening cutoff value was used. Furthermore, the correlation matrices were transformed into a topological overlap matrix (TOM), which could measure the network connection of a gene with all other genes for network gene ratio. To divide
genes that have comparable expression patterns into gene modules, we performed the average link hierarchical clustering in accordance with the TOM-based dissimilarity measure using the least size of 20 (tubulointerstitial samples were set to 40) for the genes dendrogram. In addition, we combined modules with a distance of less than 0.6. Finally obtained eight co-expression modules (four modules in tubulointerstitial). It should be noted that the grey module is considered to be a set of genes that cannot be assigned to any module. Then, we computed the gene significance (GS) and module membership (MM) and performed Pearson correlation analysis to assess the association between modules and clinical traits (IgAN and control).

**Function Annotation Analysis of Co-expression Gene Modules**

For further insight into the potential mechanisms of co-expression gene modules, DEGs regarding each module (except grey module) were performed in gene ontology (GO) analysis to reveal their biological function via DAVID (http://ncifcrf.gov). The biological process (BP) of the Go term were focused (The cut-off was adjusted as count > 4, and $p < 0.05$).

**Crosstalk Network Construction and Key Genes Selection**

The crosstalk network of glomeruli and tubulointerstitial was constructed by mapping the hub genes regarding each module. The hub genes were defined as GS $> 0.6$. Then we used the STRING database (http://string-db.org) to construct the crosstalk network at the protein level. The CytoHubba plugin in Cytoscape software (version 3.9.0) was used to screen key genes from the crosstalk module.

**Validation of key genes**

GSE37460 and GSE35487 were downloaded to validate key genes. Firstly, gene expression matrices of the two datasets were combined, and the inter-batch difference was removed using the ComBat function of the sva package in R[16]. Then the expression of key genes was analyzed in the corrected dataset. We performed independent-group t-tests to determine whether there was a substantial difference in gene expression between the IgAN group and the control group. Once this was completed, a receiver operating characteristic (ROC) analysis was carried out in order to determine the possible diagnostic performance of the genes.

**Statistical analysis**

The statistical analyses were carried out utilizing SPSS (version: 19.0) and R (version: 3.5.3). Statistical significance was described as a p-value less than 0.05.

**Results**

**DEGs Screening**
In glomeruli samples, 583 DEGs were detected, which comprised 213 down-regulated genes and 370 up-regulated genes. Meanwhile, 272 DEGs were identified in tubulointerstitial samples, including 122 down-regulated genes and 150 up-regulated genes (Figure 2 a, b). Among these DEGs, 119 genes were overlapping in both groups (Figure 2 c).

**Co-expression Gene Modules Constructing**

We detected eight distinctive modules in glomeruli, and the four modules “blue” “yellow” “green-yellow” and “magenta” were positively associated with IgAN, while four modules “purple” “tan” “black” and “turquoise” were negatively associated with IgAN (Figure 3 a). Similarly, four gene modules in the tubulointerstitial group were detected (excluded a grey module assigning to no cluster). The “grey60” module was positively associated with IgAN, and two modules “black” and “dark-green” were negatively associated with IgAN (Figure 3 b). Figure 3 (c, d) depicts a heatmap of the association between each module and a clinical trait (IgAN and control).

**Functional Enrichment Analysis of Co-expression Gene Modules**

The DEGs in blue and green-yellow modules that had a positive correlation with IgAN in glomeruli were found to be enriched in the cellular response to tumor necrosis factor (TNF), type I interferon signaling pathway, and chemokine-mediated signaling pathway. The DEGs in the yellow module were enriched in vasculogenesis. The DEGs in the dark-green module negatively correlated with IgAN were enriched in ion transmembrane transport and drug metabolism (Table 2). In tubulointerstitial, the grey60 module positively correlated to IgAN was associated with complement activation and bacterial infection. The negatively correlated module dark-green was enriched in response to cAMP and hormone stimulus, skeletal muscle cell differentiation, and pathways inducing by viral infection (Table 3).

**Table 2.** GO-BP enrichment analysis of DEGs in co-expression modules of glomeruli.
<table>
<thead>
<tr>
<th>Modules</th>
<th>The Number of DEGs</th>
<th>Go-BP Terms*</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue Module</td>
<td>311</td>
<td>GO:0070098: chemokine-mediated signaling pathway</td>
<td>3.38E-09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0090026: positive regulation of monocyte chemotaxis</td>
<td>5.93E-06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0071356: cellular response to tumor necrosis factor</td>
<td>2.33E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0070374: positive regulation of ERK1 and ERK2 cascade</td>
<td>2.66E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0002755: MyD88-dependent toll-like receptor signaling pathway</td>
<td>2.52E-04</td>
</tr>
<tr>
<td>Yellow Module</td>
<td>28</td>
<td>GO:0000122: negative regulation of transcription from RNA polymerase II promoter</td>
<td>4.39E-06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0003151: outflow tract morphogenesis</td>
<td>3.74E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0035050: embryonic heart tube development</td>
<td>2.03E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0001570: vasculogenesis</td>
<td>0.003</td>
</tr>
<tr>
<td>Green yellow Module</td>
<td>19</td>
<td>GO:0060337: type I interferon signaling pathway</td>
<td>1.87E-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0009615: response to virus</td>
<td>4.54E-08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0051607: defense response to virus</td>
<td>3.45E-07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0032728: positive regulation of interferon-beta production</td>
<td>2.95E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0045087: innate immune response</td>
<td>0.007</td>
</tr>
<tr>
<td>Magenta Module</td>
<td>5</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Turquoise Module</td>
<td></td>
<td>GO:0035435: phosphate ion transmembrane transport</td>
<td>9.21E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0035725: sodium ion transmembrane transport</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0006817: phosphate ion transport</td>
<td>0.004</td>
</tr>
<tr>
<td>Black Module</td>
<td>25</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Tan Module</td>
<td>1</td>
<td>——</td>
<td>——</td>
</tr>
</tbody>
</table>
Table 3. GO-BP enrichment analysis of DEGs in co-expression modules of tubulointerstitia

<table>
<thead>
<tr>
<th>Modules</th>
<th>The Number of DEGs</th>
<th>Go-BP Terms*</th>
<th>( P )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey60 Module</td>
<td>158</td>
<td>GO:0006958: complement activation</td>
<td>1.55E-06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0019731: antibacterial humoral response</td>
<td>2.93E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0050829: defense response to Gram-negative bacterium</td>
<td>8.72E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0019882: antigen processing and presentation</td>
<td>8.72E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0033209: tumor necrosis factor-mediated signaling pathway</td>
<td>4.28E-04</td>
</tr>
<tr>
<td>Dark green Module</td>
<td>114</td>
<td>GO:0051591: response to cAMP</td>
<td>1.11E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0035914: skeletal muscle cell differentiation</td>
<td>2.74E-04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0032870: cellular response to hormone stimulus</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0071277: cellular response to calcium ion</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GO:0042493: response to drug</td>
<td>0.014</td>
</tr>
<tr>
<td>Black Module</td>
<td>0</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

*The top five terms were displayed according to p-value.

Crosstalk Network Construction and Key Genes Selection

The crosstalk network of glomeruli and tubulointerstitial was generated by String database (Figure 4a). The key genes were screened by the CytoHubba plugin in Cytoscape (Figure 4b).

Key Genes Validation

GSE37460 and GSE35487 were utilized to verify these key genes. The inter-batch difference was removed using the ComBat and the density plot was used to visualization the batch effect before and after correlation (Figure 5a). Using SPSS (version: 19.0), it was discovered that there were substantial
differences in the expression levels of key genes between the IgAN group and the control group (P<0.05) (Figure 5b). With the aid of ROC analysis, we revealed the possible diagnostic values of key genes. All of the genes demonstrated possible diagnostic values (P<0.05) (Figure 5c).

**Discussion And Conclusion**

IgAN, the most common glomerulonephritis worldwide, has a high risk of progression to ESRD[2]. The progression of IgAN is not completely related to glomerular lesions, for, in some patients with controlled glomerular lesions, the renal function continues to decline, which implies that tubulointerstitial injury may play a role. The Oxford MEST (interstitial fibrosis/tubular atrophy, segmental sclerosis, hypercellularity, endocapillary, and mesangial) histologic score in IgAN suggested that T, S, and M lesions are related to the prognosis of the disease[2, 17]. Although guidelines recommend histologic risk factors and clinical features so that disease prediction and therapy selection can be applied properly, specific genes related to glomerular and tubular damage are still poorly understood. The mechanisms of crosstalk between glomeruli and renal tubules in the pathogenesis of IgAN are lacking. Therefore, we performed integrated bioinformatics analysis to identify key genes and explore the correlation between glomeruli and renal tubules in the pathogenesis of IgAN.

In the present research, 583 DEGs were detected in glomeruli, comprising 213 down-regulated genes and 370 up-regulated genes. Meanwhile, 272 DEGs were detected in tubulointerstitial, including 150 up-regulated genes and 122 down-regulated genes. Among these DEGs, 119 genes were overlapping in both groups (Figure 2). The overlapping DEGs suggested that both glomerular and tubulointerstitial lesions may be caused by the same genes and pathways, which could explain that two lesions always occur together in IgAN nephropathy.

Based on WGCNA, eight and four co-expression modules were detected in glomeruli and tubulointerstitial samples (Figure 3), respectively. The positively related modules were “blue” “yellow” “green-yellow” and “magenta” in glomeruli and “grey60” module in tubulointerstitial. While the negatively related modules were “black”, “tan”, “turquoise” and “purple” modules in glomeruli and “dark-green” and “black” modules in tubulointerstitial.

Through gene functional enrichment analysis of the positively related gene modules, we found that both glomeruli and tubulointerstitial were involved in the adaptive and innate immune systems. Since bacterial or viral infection may trigger the occurrence of IgAN and recurrent infections may worsen the disease. Previous studies have shown that streptococcus may aggravate inflammatory damage in IgAN via the chemotaxis of Th22 cells[3]. The activation of Toll-like receptors (TLRs) might promote the production of IgA and elevate IgA glycosylation. Moreover, TLR 9 and TLR 4 were correlated with IgAN severity[18]. The yellow module in glomeruli was related to vasculogenesis, which suggested that vascular endothelial growth factor (VEGF) and other related inflammatory factors may lead to inflammation and proliferation of the mesangial cells and further cause glomerulosclerosis[19]. Plate-derived growth factor (PDGF) not only directly stimulates the proliferation of mesangial cells but also can lead to renal fibrosis[20]. The
grey60 module in tubulointerstitial positively correlated with IgAN was predominately enriched in the complement activation pathway. It has been confirmed that in human and rodent experiments, IgA (mainly poly IgA involved) can activate complement alternative pathway (AP) *in vivo* and *in vitro* [21]. The modules negatively related to IgAN indicated that the biological process of ion transmembrane transport has a protective effect in IgAN. The key genes screened from the crosstalk network were mainly associated with adaptive and innate immune, such as ITGAM, ITGB2, TYROBP, CSF1R, HCK, and LAPTM5. Integrin alpha M (ITGAM) encodes the integrin alpha M chain (CD11b) and belongs to the integrin family. CD11 is an important leukocyte differentiation antigen, which is widely expressed in a variety of immune cell subsets, such as dendritic cells, neutrophils, NK cells, and B cells. CD11 integrin participates in innate immunity, adaptive immunity, and inflammatory response, and plays an important role in regulating immune tolerance [22, 23]. A previous study based on the Chinese Han population suggested that ITGAM gene polymorphisms are correlated with IgAN [24]. ITGAM is proved to participate in the modulation of intestinal IgA-producing plasma cells in mice, which indicates the function of the intestinal immune in the pathogenic mechanism of IgAN [23]. Integrin subunit beta 2 (ITGB2) also belongs to the integrin family and is implicated in binding between endothelial cells and inflammatory cells, inflammatory cells chemotaxis [25]. However, the mechanisms of ITGB2 mediating kidney injuries are not clear, which needs further study. Transmembrane immune signaling adaptor (TYROBP) encodes a transmembrane signaling polypeptide which encompasses an immunoreceptor tyrosine-based activation motif in its cytoplasmic domain. TYROBP binds non-covalently to NK cell activity receptors and activates signal transduction. It has been previously reported that TYROBP is highly correlated with proteinuria in systemic lupus erythematosus (SLE) [26]. Cytochrome b-245 beta chain (CYBB) has been postulated as a major constituent of the phagocyte microbicidal oxidase system [27]. Fibronectin 1 (FN1) is a well-known protein that has great binding activity and is also the primary constituent of the extracellular matrix. The aggregation and chemotaxis of FN1 and collagen serve as critical building blocks for the proliferation of endothelial cells, mesangial cells, and fibroblasts. The expression of FN1 in the glomeruli indicates the presence of active mesangial cell growth and the progression of the lesion [28]. The previous study shows that cathepsin S (CTSS) expressed high in diabetic nephropathy and lupus nephritis [29]. CTSS participates in antigen presentation, cytokine secretion, and angiogenesis mediates the degradation of the extracellular matrix. It has been demonstrated that CTSS inhibits apoptosis and promotes cell proliferation through PI3K/Akt or MAPK pathway [30]. Hemopoietic cell kinase (HCK) plays an important part in regulating innate immune response, phagocytosis, cell survival and proliferation, cell adhesion, and migration [31]. HCK can activate TGF-β-mediated pro-fibrotic pathway, as well as other proliferation contributing factors, which are implicated in renal tubular cell damage and fibrosis and even the modulation of the immune system [32]. It has been demonstrated that lysosomal protein transmembrane 5 (LAPTM5) performs an instrumental function in the lysosomal disintegration of B cell and T cell antigen receptors (BCR/TCR) by transporting endosomes to lysosomes. Study shows that insufficient expression of LAPTM5 is associated with the pathogenic process of SLE and results in disease severity [33].
Among the crosstalk genes, some have been shown to perform a vital part in the pathogenic process of IgAN, such as ITGAM[34], ALB[35], FN1[36], CTSS[37], and CYBB[38]. However, there is little known about TYROBP, ITGB2, CSF1R, HCK, and LAPTM5, which need further research to reveal their mechanisms.

The limitation of this study. Because of the scarcity of clinical data in the GEO database, it is hard for us to link gene modules to specific clinical characteristics. In addition, the data of our study were obtained by bioinformatic analysis of microarray datasets; consequently, more in vivo and in vitro tests are required to validate the findings.

**Abbreviations**

IgAN: IgA nephropathy; DEG: differentially expressed genes; WGCNA: Weighted gene co-expression network analysis; NC: normal control; TLRs: toll-like receptors; LAPTM5: lysosomal protein transmembrane 5; PDGF: Plate-derived growth factor; VEGF: vascular endothelial growth factor; ITGAM: Integrin alpha M; ITGB2: Integrin subunit beta 2; TYROBP: Transmembrane immune signaling adaptor; SLE: systemic lupus erythematosus; CYBB: Cytochrome b-245 beta chain; FN1: Fibronectin 1; CTSS: cathepsin S; HCK: hemopoietic Cell Kinase; LAPTM5: lysosomal protein transmembrane 5

**Declarations**

**Acknowledgments**

We would like to thank the Sangerbox tools, a free online platform for data analysis (http://www.sangerbox.com/tool).

**Author Contributions**

Conception and design: Y Bai, C Ma; Administrative support: None; Provision of study materials or patients: None; Collection and assembly of data: Y Bai, Y Li, Y Xi, C Ma; Data analysis and interpretation: Y Bai; Manuscript writing: All authors; Final approval of manuscript: All authors.

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**Availability of data and materials**

These data were derived from the following resources available in the public domain: Gene Expression Omnibus(GEO) database http://www.ncbi.nlm.nih.gov/geo/.

**Ethical Statement**

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The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was not involved in the experiments of humans or animals.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

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**References**


Figures
Figure 1

Flow chart of our study.
Figure 2

Differential expression genes (DEGs) in glomeruli (Glom) and tubulointerstitial (Tub).

(a) The heat map and volcano plot of DEGs in glomeruli (|log2(Fold Change)| > 1.5; adjusted P < 0.05). Pie charts represent the numbers of genes found to be upregulated or downregulated. (b) The heat map and volcano plot of DEGs in tubulointerstitial (|log2(Fold Change)| > 1.5; adjusted P < 0.05). Pie charts
represent the numbers of genes found to be upregulated or downregulated. (c)Venn diagram shows overlapping DEGs in glomeruli and tubulointerstitial.

Figure 3

Weighted gene co-expression network analysis.

(a) The cluster dendrogram of co-expression genes in glomeruli. (b) The cluster dendrogram of co-expression genes in tubulointerstitia. (c) Module–trait relationships in glomeruli. Each cell contains the corresponding correlation and p-value. (d) Module–trait relationships in tubulointerstitia. Each cell contains the corresponding correlation and p-value.
Figure 4

Glomeruli and tubulointerstitial crosstalk network and key genes screened by CytoHubba. (a) Down-regulated genes were in blue while up-regulated genes were in red. (b) Key genes screened by CytoHubba.
Figure 5

Validation of key genes. (a) The density plot of the inter-batch difference of GSE35487 and GSE37460 is removed. (b) Key genes in datasets GSE35487 and GSE37460. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001 (c) ROC curves for key genes