Identification of key genes and pathways in IgA nephropathy using bioinformatics analysis

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1. Introduction

Immunoglobulin A nephropathy (IgAN) identified by Jacques Berger 50 years ago[1] was once considered as an uncommon variant of mesangial proliferative glomerular disease, but was actually the leading primary glomerulonephritis worldwide.[2,3] The International Kidney Biopsy Survey on glomerular disease frequency involving over 42,000 renal biopsies in 4 continents showed IgAN was identified in 22% and 39% of all glomerular diseases in Europe and Asia respectively.[4] Despite its generally benign course in clinic, finally 15% to 20% of the patients within 10 years and 30% to 40% within 20 to 30 years after the first onset will develop to end-stage renal disease.[5]

IgAN is a complex disease with variable clinical and pathological features, but its exact pathogenesis is only partially known. The causes and development of IgAN are ascribed to genetic factors given its varying incidence among ethnicities and its high familial aggregation. Multiple genes and signaling pathways have been suggested to take part in the initiation and development of IgAN. Thus, studying the molecular mechanism or pathology of IgAN is extremely important in order to find out more efficient diagnostic, therapeutic, and prognostic methods. So far, the microarray technique plus bioinformatics has facilitated the analysis of DNA or RNA expression changes amid the variation and prognosis of IgAN. We can also explore the interactions among differentially-expressed genes (DEGs) and study the enrichment pathways and functional annotation and in the interaction network.

In this study based on bioinformatics, we investigated DEGs, the enrichment of GO terms or pathways, and protein-protein interaction (PPI) in IgAN by using the samples of GSE93798 in order to predict potential targets. We also analyzed the modules and their functions in each PPI network and sought to determine possible hub genes using cytoHubba and a network analyzer. Finally, the common genes of the 5 methods were selected as the hub genes.

Abstract

Background: IgA nephropathy (IgAN) is the most frequent type of primary glomerulonephritis globally and the leading cause of end-stage renal disease in young adults. Its pathogenesis is not fully known, but is largely attributed to genetic factors. This study was aimed to explore the prognostic values of key genes in IgAN.

Methods: The gene expression profile GSE93798 of 20 IgAN samples and 22 normal samples using glomeruli from kidney biopsy was adopted. Totally 447 upregulated and 719 downregulated differentially expressed genes were found in IgAN patients on the R software. The Gene Ontology enrichment and the Kyoto Encyclopedia of Gene and Genomes pathway were investigated on DAVID, and the protein-protein interaction network and the top 13 hub genes of the differentially expressed genes were built via the plug-in molecular complex detection and cytoHubba of Cytoscape.

Results: From the protein-protein interaction network, of the top 13 hub genes, FOS, EGFR, SIRT1, ALB, TFRC, JUN, IGF1, HIF1A, and SOCS3 were upregulated, while CTNN, ACTR2, CREB1, and CTNNB1 were downregulated. The upregulated genes took part in the HIF-1 signaling pathway, Choline metabolism in cancer, Pathways in cancer, Amphetamine addiction, Estrogen, TNF, and FoxO signaling pathways, and Osteoclast differentiation, while the downregulated genes were involved in Pathogenic Escherichia coli infection, Bacterial invasion of epithelial cells, prostate cancer, and melanogenesis.

Conclusion: This study based on the Gene Expression Omnibus database updates the knowledge about the mechanism of IgAN and may offer new treatment targets.

Abbreviations: DEGs = differentially expressed genes, ECM = extracellular matrix, IgAN = IgA nephropathy, KEGG = Kyoto Encyclopedia of Genes and Genomes, MCODE = molecular complex detection, PPI = protein-protein interaction, PTC = peritubular capillary.

Keywords: bioinformatics, IgA nephropathy, key genes, pathways

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2. Materials and methods

2.1. Microarray data

The Gene Expression Omnibus (free on www.ncbi.nlm.nih.gov/geo) provides original submitter-offered records and curated datasets. The microarray profile dataset GSE93798 in IgAN (including 20 IgAN kidney biopsy samples and 22 normal samples) was obtained from the Affymetrix GPL22945 platform (Affymetrix Human Genome U133 Plus 2.0 Array), which was uploaded by Liu et al in January 18, 2017 and updated in October 13, 2017. Data were pretreated and DEGs were identified using the Bioconductor in R software and the original data were preprocessed using the Affy package. Firstly, the raw data of intensity were treated by background calibration, log2 transformation, and quantile normalization. Then the potential batch impacts of the pooled datasets were detected via the principal component analysis. Finally, the normalized data were fitted by a linear model to form an expression measure set on the qualified dataset. The DEGs for each disease were chosen using the empirical Bayes method and the significant DEGs were set at \( P < 0.05 \) after adjustment by the Benjamini–Hochberg method. Fold change of the expression of individual gene was also observed for differential expression test. The DEGs with false discovery rate (FDR) < 0.05 and \(|\log \text{fold change}| > 1.5\) were considered to be significant.

2.2. GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The GO enrichment and KEGG pathway of DEGs were analyzed at the function level on DAVID 6.7 (david.ncifcrf.gov), which offers a complete set of gene function annotation tools. \( P < .05 \) was regarded as significant difference.

2.3. PPI network analysis

The functional interactions among these DEGs were further explored using a PPI network. The DEGs were plotted via the tool STRING (www.string-db.org) and the interactions validated at a combined score of \( >0.5 \) were chosen. Then the PPI network was built and visualized on Cytoscape 3.4.0 and the modules of the network were identified using the plug-in Molecular Complex Detection (MCODE) as per the criteria of MCODE score ≥4 and node number >4. The function enrichment of DEGs in the top module was analyzed with DAVID.

2.4. Identification of hub genes

To balance between the core genes and avoid the missing of the key gene, we extracted the hub genes using cytoHubba and through the cytoHubba plugin, obtained 12 topological analysis methods. The top 25 hub-forming genes/proteins were identified based on MCC, MNC, degree, closeness, and betweenness separately. Then the overlapping genes were chosen as the hub genes. Finally, the common genes were found using Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/).

2.5. Ethical statement

All the data of this paper was obtained from the open-access database, we did not get these data from patients or animals directly, nor intervene these patients. So the ethical approval was not necessary.

3. Results

3.1. The DEGs

A total of 1166 DEGs were finally screened out from the IgAN samples and compared with the normal samples, including 447 upregulated and 719 downregulated DEGs. The heat map of the DEGs and the volcano plot are displayed in Figure 1A and B, respectively.

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Figure 1. (A) Heatmap and (B) volcano plot for DEGs. DEGs = differentially expressed genes.
3.2. Go term enrichment

The DEGs were imported to the DAVID for GO and KEGG pathway analysis. As for the bioprocesses, the upregulated DEGs were considerably enriched in positive modulation of smooth muscle cell growth, drug reaction, skeletal muscle cell division, fat cell division, and xenobiotic metabolism, while the downregulated DEGs were concentrated in cell-cell adhering, vascular endothelial growth factor receptor signaling pathway, actin cytoskeleton organization, peptidyl-serine phosphorylation and protein localization to cytoplasmic stress granule (Table 1).

3.3. KEGG pathway analysis

The KEGG pathways of the DEGs were analyzed using DAVID (Table 2). The upregulated DEGs were mainly involved in tyrosine metabolism, drug metabolism-cytochrome P450, Valine, leucine and isoleucine degradation, and prostate cancer and pathways in cancer (Table 2).

Table 1
GO analysis of upregulated and downregulated differentially expressed genes in biological processes.

| A, Upregulated Term | Function | Count | P-value |
|---------------------|----------|-------|---------|
| GO:0048661          | Positive regulation of smooth muscle cell proliferation | 8 | 5.22 × 10⁻⁴ |
| GO:0042493          | Response to drug | 18 | 9.84 × 10⁻⁴ |
| GO:0035914          | Skeletal muscle cell differentiation | 7 | .001008892 |
| GO:0045444          | Fat cell differentiation | 8 | .001692024 |
| GO:0006805          | Xenobiotic metabolic process | 8 | .002482084 |

| B, Downregulated Term | Function | Count | P-value |
|-----------------------|----------|-------|---------|
| GO:0098609            | Cell-cell adhesion | 33 | 2.42 × 10⁻⁸ |
| GO:0048010            | Vascular endothelial growth factor receptor signaling pathway | 14 | 3.65 × 10⁻⁶ |
| GO:0000306            | Actin cytoskeleton organization | 18 | 1.22 × 10⁻⁵ |
| GO:0018105            | Peptidyl-serine phosphorylation | 17 | 2.86 × 10⁻⁵ |
| GO:1903608            | Protein localization to cytoplasmic stress granule | 5 | 7.31 × 10⁻⁵ |

Table 2
KEGG pathway analysis of upregulated and downregulated differentially expressed genes. Top 5 terms were selected according to P-value when more than 5 terms enriched terms were identified in each category.

| A, Upregulated Pathway ID | Name | Count | P-value |
|---------------------------|------|-------|---------|
| hsa00350                  | Tyrosine metabolism | 5 | .012844 |
| hsa00982                  | Drug metabolism- cytochrome P450 | 6 | .032779 |
| hsa00280                  | Valine, leucine and isoleucine degradation | 5 | .034349 |
| hsa05215                  | Prostate cancer | 6 | .081613 |
| hsa00630                  | Retinol metabolism | 5 | .087275 |

| B, Downregulated Pathway ID | Name | Count | P-value |
|-----------------------------|------|-------|---------|
| hsa04722                    | Neurotrophic signaling pathway | 20 | 2.17 × 10⁻⁶ |
| hsa04510                    | Focal adhesion | 27 | 2.64 × 10⁻⁶ |
| hsa05200                    | Pathways in cancer | 39 | 1.03 × 10⁻⁵ |
| hsa04611                    | Platelet activation | 19 | 2.72 × 10⁻⁵ |

GO = gene ontology.

KEGG = Kyoto Encyclopedia of Genes and Genomes.
Table 3

| Cluster | Score | Nodes | Edges | Node IDs |
|---------|-------|-------|-------|----------|
| 1       | 14.772| 58    | 421   | UBA6, CST2T, SRS1, RN1F14B, SRS3, TFC, RN1F4, AGTR1, YBX1, FBXO30, VAMP2, HUWE1, SGP1, SH3GL2, FBXO9, ITCH, SYT2, STAM2, FCH2, UL53, UBE2H, FBXL20, RPL14, SEC61B, DNAJ6, RPS27L, TRAM1, SRRP68, SRP72, DDX2, OAK, AAK1, DDX9, CRPSF2, SNRNP, SNRNP, RNF6, SCAR2B, KHL5, HECTD1, RPL27A, RABSC, RPL31, SF3B4, CDC5L, EGRF, DDX46, RPL37A, FBXN11, DNAJC8, SRRM2, RPL38, FZD4, APCC, PRP40A, RPL22, SCS2, UFL1 |
| 2       | 11.222| 37    | 202   | TAOK1, PRKAR2B, JUN, PIM1, HAU53, HAU65, AKT2, DYNCL12, CREB1, STAG2, TCLN2, CC2D2A, SIRT1, EIF3M, ALB, CTNNB1, SDHC, AKT3, EIF4E, ACTR1A, RANBP2, SMC3, MDM2, YWHAE, EIF2S3, BUB3, SKA2, NSL1, SMCA1, HSPA4, IGFI, FOS, CEP76, BCL2L1, EGRI, HIF1A, VEGFA |
| 3       | 7     | 7     | 21    | VAV3, FCGR2A, BAIV2, NCOAP1, WASF2, ABI2, BRK1 |
| 4       | 6.286 | 36    | 110   | RB1, MRPS15, NTRK2, SOD2, RYR1A, GSK3B, DNAJ4B, P4H2, GT2A1, HSPA14, CDK9, GFM1, DNAJ2, FSTL1, SNAP33, TAB5, SPT4H1, FGFR2, DNAJ2, SDC2, GSK3B, B102, SHC1, ELL2, SPP4A1, SUSD1, DUSP1, TAF13, FGA, RHA2, CCN2, ORP51, GFBP1, HSPH1, VCAN |
| 5       | 4.8   | 36    | 84    | HIST3H2A, DNAH9, HIST1H4H, ATP6V2, DNA1I1, TPR, SNAP23, DYNCL12, KAT2B, BAG1L1, CD274, HST1H4E, DNAJC13, FCER1G, CYBA, ULRB2, RAP2C, WDR60, PDGFA, PDGFC, TAF9B, NOO1, SUPT6H, MGT1, GAF1, ATP11B, PCGF2, LAMP2, TADA2B, MSYM1, KDM4B, CMTM6, NG4, PSMD2, USP22, EEFD1 |

Cluster Score Nodes Edges Node IDs

Five modules from the protein-protein interaction network satisfied the criteria of MCODE scores ≥4 and number of nodes >4.

4. Discussion

With the 20 IgAN samples and 22 normal samples from GSE93798, totally 447 upregulated genes and 719 downregulated genes were found. As for GO enrichment, the upregulated DEGs were mainly related to cell division and cell cycle, vascular endothelial growth factor (VEGF) receptor pathway, actin cytoskeleton organization, peptidyl-serine phosphorylation and protein positioning to cytoplasmic stress granule. Furthermore, the KEGG pathways were tyrosine metabolism, drug metabolism-cytochrome P450, VEGF receptor pathway, actin cytoskeleton organization, peptidyl-serine phosphorylation and protein positioning to cytoplasmic stress granule. The KEGG pathways were tyrosine metabolism, drug metabolism-cytochrome P450, valine, leucine and isoleucine degradation, prostate cancer and retinol metabolism. The downregulated DEGs were mainly involved in actin cytoskeleton, neurotrophin pathway, focal adhesion, pathways in cancer and platelet activation.

4.1. PPI network construction and module analysis

A total of 5 modules from the PPI network were identified with MCODE score ≥4 and node number >4 (Table 3) and the top 3 modules were chosen (Fig. 2). As for KEGG pathway enrichment, the 3 modules were mainly involved in modulating actin cytoskeleton, HTLV-I infection, cancer pathways, PI3K-Akt pathway, and HIF-1 pathway.

4.2. Hub gene selection

The hub genes were screened out by overlapping the genes according to 5 ranked methods in cytoHubba (Fig. 3A). Thirteen hub genes were selected, including 9 with upregulation and 4 with downregulation. The details are shown in Table 4 and visualized in Figure 3B. Results show the functions of the 13 hub genes and their probable role in IgAN, indicating they may be novel therapeutic target genes.

4.3. GO enrichment

With the 20 IgAN samples and 22 normal samples from GSE93798, totally 447 upregulated genes and 719 downregulated genes were found. As for GO enrichment, the upregulated DEGs were considerably concentrated in positively regulating smooth muscle cell proliferation, response to drug, skeletal muscle cell division, fat cell division, and xenobiotic metabolism, and the downregulated DEGs were mainly involved in cell-cell adhesion, vascular endothelial growth factor (VEGF) receptor pathway, actin cytoskeleton organization, peptidyl-serine phosphorylation and protein positioning to cytoplasmic stress granule. Furthermore, the KEGG pathways were tyrosine metabolism, drug metabolism-cytochrome P450, Valine, leucine and isoleucine degradation, prostate cancer, and retinol metabolism for the upregulated DEGs, while were actin and isoleucine degradation, prostate cancer, and retinol metabolism for the downregulated DEGs. Among these DEGs, 13 hub genes were selected in the PPI network by cytoHubba, including FOS, EGFR, SIRT1, ALB, TFRC, JUN, IGF1, HIF1A, SOC3, ACTR2, CREB1, CTNNB1, and CTNN. The first 9 genes were upregulated and the latter 4 were downregulated in IgAN patients.

Among the FOS family (including FOS, FOSB, FOSL1, and FOSL2), FOS is involved in the molecular mechanisms of cell growth, division, apoptosis, and migration.[6] Some proto-oncogenes were overexpressed glomerularly in IgAN patients.[7,8,9] FOSL1 may promote the progression of podocyte foot affacement in IgAN to induce glomerular injury, suggesting FOSL1 may be associated with IgAN severity.[10] FOS is connected with DNA destruction, telomere injury-causing aged phenomena, and neurophil actions, which regulate the initiation and evolution of IgAN.[11]

Growth hormone and insulin-like growth factor IGF-(1) can considerably affect the kidney growth, actions and structural conservation and are associated with extracellular matrix (ECM) reshaping, and podocyte and mesangial cell growth.[12] These findings indicate the role of IGF-1 in the pathogenesis of IgAN. IGF-1 can be generated and released by mesangial cells and activate downstream signaling molecules (eg, phosphatidylinositol 3 [PI3] kinase and extracellular signal-regulated protein kinase [ERKs]). These activities are seemingly related with the renewing and components of glomerular ECM, leading to the occurrence of kidney diseases.[13–15] IGF-1 is largely associated with pathology,[16] and the altered IGF-1/1R function may affect the development of glomerular sclerosis or interstitial fibrosis. The rs1520220 and rs2195239 variants were related with the development of glomerular sclerosis or interstitial fibrosis.

Hypoxia is one of the key causes of kidney damage. Though acutely-impaired kidneys may benefit from the positive effects of HIF-modulated bioprocesses,[18–20] partial HIF-1α-mediated chronic hypoxia can intensify ECM production and epithelial-mesenchymal transition, which may facilitate renal fibrosis and kidney diseases.[21–23] Among various kidney diseases, progressive interstitial fibrosis is related to peritubular capillary (PTC) loss around the renal tubules and consequently renal dysfunction.[24] PTC loss in chronic tubulointerstitial damage is also connected with the expression alteration of VEGF, an inducer that regulates capillary growth and vessel formation in several
Figure 2. Top 3 modules from the protein-protein interaction network. (A) module 1, (B) the enriched pathways of module 1, (C) module 2, (D) the enriched pathways of module 2, (E) module 3, (F) the enriched pathways of module 3.

Figure 3. (A) Overlapping DEGs among cytoHubba of the 5 methods; (B) PPI network of the 13 hub genes. DEGs = differentially expressed genes.
organ systems. In the early stages of IgAN, the VEGF expression increases at least in part by local tissue hypoxia through an HIF-1α-relying pathway to maintain the number of PTC, but this salvage mechanism in reaction to tissue hypoxia fails in the advanced stages. MiR-29c can be down-regulated by renal interstitial fibrosis in IgAN, and up-regulated by HIF-1α activation to alleviate fibrosis.

The new soluble transferrin receptor (TfR or CD71) of IgA1 is expressed on mesangial cells. IgA1 precipitation is connected with higher expression of CD71 and is first linked with CD71 expression. Double-labeled research with confocal microscopy shows that the IgA deposits mostly localize together with CD71 mesangially.

In conclusion, some key genes closely related with the bioprocesses and signaling pathways in IgAN initiation and progression were screened out. We identified 9 novel genes that were not reported before and may be imperative in IgAN. These genes can be potentially used to molecularly diagnose or cure IgAN. Nevertheless, further research is needed to explore the mechanisms of these genes in IgAN.

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