Original Paper

Gene Expression Analysis in Tubule Interstitial Compartments Reveals Candidate Agents for IgA Nephropathy

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Key Words

IgA nephropathy • Gene ontology • Gene pairs • Network • Small molecules

Abstract

Background/Aims: Our aim was to explore the molecular mechanism underlying development of IgA nephropathy and discover candidate agents for IgA nephropathy. **Methods:** The differentially expressed genes (DEGs) between patients with IgA nephropathy and normal controls were identified by the data of GSE35488 downloaded from GEO (Gene Expression Omnibus) database. The co-expressed gene pairs among DEGs were screened to construct the gene-gene interaction network. Gene Ontology (GO) enrichment analysis was performed to analyze the functions of DEGs. The biologically active small molecules capable of targeting IgA nephropathy were identified using the Connectivity Map (cMap) database. **Results:** A total of 55 genes involved in response to organic substance, transcription factor activity and response to steroid hormone stimulus were identified to be differentially expressed in IgA nephropathy patients compared to healthy individuals. A network with 45 co-expressed gene pairs was constructed. DEGs in the network were significantly enriched in response to organic substance. Additionally, a group of small molecules were identified, such as doxorubicin and thapsigargin. **Conclusion:** Our work provided a systematic insight in understanding the mechanism of IgA nephropathy. Small molecules such as thapsigargin might be potential candidate agents for the treatment of IgA nephropathy.

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Introduction

IgA nephropathy is a common form of glomeruli inflammation, which is characterized by the deposition of glomerular IgA antibody in kidney accompanied with a heterogeneous clinical course [1]. The presence of glomerular IgA deposits often resulted in various diseases

including dermatitis herpetiformis, celiac disease and systemic or immunologic disorders (systemic lupus erythematosus; cryoimmunoglobulinemia) [2]. Although IgA nephropathy is considered to be benign in early stage of patients with primary glomerular disease, many cases progress to later stage of renal disease resulting from IgA nephropathy [3, 4]. The incidence of primary IgA nephropathy was reported to be the highest in Asia [2] and there was an increasing trend for the prevalence of IgA nephropathy in United States [5]. IgA nephropathy has been a healthy concern throughout the world.

Numerous studies have made contributions to explore the mechanism and therapeutic method for IgA nephropathy, since it was first documented in 1968 [6]. The best option for treating the end-stage renal disease is renal transplantation. Although the survival of IgA nephropathy patients by renal transplantation is excellent [7], the recurrence is common in patients (20-60%) which results in the renal function failure and graft loss [8]. Therapies in preserving renal functions and preventing substantial progress are necessary for patients with IgA nephropathy. The agents capable of reversing IgA nephropathy are mainly angiotensin-converting-enzyme inhibitors, corticosteroids, and n-3 polyunsaturated fatty acids. But there is no clear evidence that among the 3 classifications of drugs which is the better treatment [2]. In recent years, it is reported that mycophenolate mofetil (MMF) combined with prednisone showed advantage in the remission of severe IgA nephropathy [9]. However, there is no promising therapy available to treat the patients with IgA nephropathy by now.

In the present study, we utilized the biological informatics methods to assess the gene expression profiles derived from patients with IgA nephropathy and normal subjects. We aimed to potential mechanism underlying IgA nephropathy and discover the candidate effective agents for patients with IgA nephropathy.

Materials and Methods

Affymetrix microarray data

The microarray data of GSE35488 deposited by Heather N. Reich et al [10] was downloaded from GEO (Gene Expression Omnibus) database (<http://www.ncbi.nlm.nih.gov/geo/>). Total 31 specimens were used for the development of the gene expression profiles, which derived from tubule interstitial compartments of patients with IgA nephropathy (n=25) and normal living donors (n=6). The eligible patients including 18 males and 7 females aged from 19 to 84 years old (mean=36). Subjects with IgA nephropathy had a wide range in proteinuria (trace to 10g/day) and renal function (normal to stage 4/5 chronic kidney disease) [10]. The raw data and the annotation files were downloaded for further study, based on the platform of GPL96 (Affymetrix Human Genome U133A Array).

Data preprocessing and differential expression genes analysis

All the array data was preprocessed using Robust Multichip Average (RMA) method [11]. Normalization was performed at probe level. If multiple probes corresponding to the same gene, the mean value was calculated as the gene expression value for this gene. The differentially expressed genes (DEGs) in IgA nephropathy patients were analyzed by R multtest package [12] with reference to the data from normal subjects. The multiple testing correction was carried out to control the false discovery rate (FDR) with the application of BH (Benjamini-Hochberg) procedure [13]. Fold change (FC) of the expression of individual gene was also analyzed for DEGs analysis. DEGs with FDR<0.05 and |logFC|>1 were considered to be significant.

Gene-annotation enrichment analysis

Gene Ontology (GO) database is a collection of a large number of gene annotation terms. DAVID (The Database for Annotation, Visualization and Integrated Discovery) Gene Functional Classification Tool has been developed for relating the functional terms with gene lists by clustering algorithm. We performed GO enrichment analysis of the DEGs using the DAVID functional classification tool. The multiple testing correction were carried out and FDR<0.05 was defined as the cutoff value.

Co-expressed gene pairs and gene-gene interaction network construction

The genes with similar expression profiles are considered to have the common regulatory function and high degree of correlation [14]. R/EBcoexpress package applies empirical Bayesian approach to examine the correlations of gene pairs [15].

In this paper, we identified the co-expressed gene pairs using R/EBcoexpress in R statistical programming language with the changes at mean expression level. The gene pairs were displayed with a co-expression value. The co-expression value > 0.6 was defined as the cutoff

Table 1. Differential expression genes between patients with IgA nephropathy and controls

Gene.symbol	adj.P.Val	logFC	Gene.symbol	adj.P.Val	logFC
ADM	2.24E-02	-1.06055041	KLF10	7.09E-06	-1.42782542
ALB	2.38E-02	-1.77184817	KLF4	9.02E-06	-1.38334165
ATF3	6.36E-15	-2.7028136	KLF6	3.78E-06	-1.93120425
CCL2	4.75E-04	-1.60417775	KLF9	2.81E-04	-1.07153542
CDH6	2.04E-02	1.12792679	LDLR	6.13E-07	-1.67135544
CFB	9.64E-03	1.2471012	MAFF	2.34E-08	-3.92158659
CLIC5	7.77E-03	-1.44615453	MCL1	3.77E-04	-1.0099153
CLU	4.25E-02	1.23643911	NDNF	4.04E-03	-1.25784278
CTGF	1.21E-04	-1.31155107	NEDD9	2.60E-08	-1.09462535
CYP27B1	3.77E-03	-1.12546917	NPHS2	3.05E-02	-1.2010379
CYR61	2.89E-04	-1.87788833	NR4A3	2.27E-10	-1.44543878
DUSP1	6.37E-09	-2.4652777	PCOLCE2	2.59E-04	-1.12480373
DUSP5	1.10E-07	-1.10377021	PKD4	1.73E-05	-1.72640231
DUSP6	9.05E-04	-1.05313771	PHLDA1	7.77E-03	-1.11416127
EGR1	2.50E-05	-2.32136726	PHLDA2	4.79E-03	-1.23489186
EMP1	1.92E-02	-1.21803616	PTPRO	3.70E-02	-1.0914533
FGF1	1.01E-02	-1.13997719	RCAN1	9.57E-07	-1.22683885
FOS	2.16E-04	-1.85498171	RGS2	4.05E-04	-1.53410687
FOSB	1.23E-16	-2.48403917	RGS3	5.19E-03	-1.15382489
GOS2	4.66E-03	-1.08082859	RHOB	1.21E-06	-1.38133201
GADD45B	2.61E-07	-2.28301071	SERPINE1	3.21E-09	-1.42754893
GDF15	6.52E-03	-1.85593406	SGK1	2.10E-07	-1.24389669
GEM	2.61E-07	-1.65243169	SOCS2	9.78E-06	-1.06279136
HBEGF	2.52E-09	-1.57106108	TRIB1	6.91E-05	-1.18699205
IER3	3.22E-04	-1.38408794	TSC22D2	1.06E-06	-1.07913854
JUN	4.61E-05	-1.74887525	ZBTB16	4.11E-02	-1.04837005
JUNB	3.14E-06	-1.25499411	ZFP36	2.52E-09	-1.43225319
JUND	4.23E-10	-1.32931508			

value. Then the gene-gene interaction network with significant gene pairs was visualized using Cytoscape software [16]. The function annotation for genes in the network was performed with BiNGO (Biological Networks Gene Ontology tool) [17]. The multiple testing correction was performed to adjust p values according to BH procedure. FDR (adj p value) < 0.01 was set as the threshold value.

Identification of candidate small molecules

Connectivity Map (cMap) database collects the gene expression profiles from cultured human cells treated with small molecules. cMap is widely used to identify small molecules sharing the mechanism of disease and drugs [18].

The gene set enrichment analysis (GSEA) was performed for the up-regulated DEGs and down-regulated DEGs compared with the data from cMap database. The query small molecules were output with a connectivity score from +1 to -1. The higher negative score indicated that the query small molecules suppressed disease, while the higher positive score revealed that the corresponding molecules induced the disease. A connectivity score nearer to "0" indicated no effect from query small molecules on disease. A p value for enrichment was also displayed. Candidate agents were identified with p value < 0.05 and |connectivity score| > 0.9.

Results

Identification of differential expression genes

After data preprocessing, we obtained 12001 probes of 12029 probes in raw gene expression profiles. Compared with the expression profiles from normal subjects, total of 55 genes were assessed to be differentially expressed in IgA nephropathy patients, among which, 3 genes were up-regulated and 52 ones were down-regulated. The DEGs with FDR < 0.05 and |logFC| > 1 were listed in Table 1.

Function analysis for DEGs

To analysis the DEGs in the function level, we performed GO enrichment analysis for the DEGs. With $FDR < 0.05$, all the DEGs were classified into 12 categories such as transcription factor activity, response to steroid hormone stimulus, doub-

Table 2. Function analysis of the differentially expressed gene sets

Term	Function	Count	FDR
GO:0010033	response to organic substance	17	9.43E-06
GO:0003700	transcription factor activity	16	4.29E-04
GO:0048545	response to steroid hormone stimulus	9	9.64E-04
GO:0003690	double-stranded DNA binding	7	0.00101
GO:0009991	response to extracellular stimulus	9	0.00269
GO:0043566	structure-specific DNA binding	7	0.01052
GO:0009725	response to hormone stimulus	10	0.01454
GO:0031667	response to nutrient levels	8	0.01473
GO:0032496	response to lipopolysaccharide	6	0.01697
GO:0002237	response to molecule of bacterial origin	6	0.02914
GO:0008201	heparin binding	6	0.02926
GO:0009719	response to endogenous stimulus	10	0.03191

Fig. 1. Function categories of DEGs. The significance of the function categories declines from dark blue to white. DEG: differentially expressed genes.

le-stranded DNA binding, response to hormone stimulus and response to endogenous stimulus (Figure 1, Table 2). The most enriched GO term was revealed to be response to organic substance (Table 2).

Co-expressed gene pairs and gene-gene interaction network

In order to investigate the interactions of DEGs, we performed co-expressed gene pairs analysis. Total 45 gene pairs with co-expression value > 0.6 were identified to be significant. With the application of Cytoscape, the gene-gene interaction network was established with the 45 co-expressed gene pairs (Figure 2). Then the functions of the genes in the network were further analyzed. As shown in Table 3, the significant function of the co-expression gene network was response to organic substance. Other functions included response to chemical stimulus, response to stress, organ development and system development.

Identification of candidate active small molecules

In order to identify the candidate agent for patients with IgA nephropathy, we applied the information of cMap database and verified with p value and enrichment scores. Total

of 5 potential small molecules were obtained with $p < 0.05$ and $|\text{connectivity score}| > 0.9$, including doxorubicin, atovaquone, mitoxantrone, podophyllotoxin and thapsigargin. As shown in Table 4, the candidate small molecules of doxorubicin (connectivity score=0.991) and thapsigargin (connectivity score=-0.966) had highest positive score and negative score, respectively.

Discussion

IgA nephropathy is generally recognized as the most common form of glomerulonephritis worldwide [19, 20]. IgA nephropathy is always accompanied with other numerous diseases which increased the risk of death. Although a large number of studies have been conducted to explore the pathogenesis of IgA nephropathy, the mechanism underlying IgA nephropathy progression has not been fully elucidated. The development of biological informatics approach for gene expression profiles advanced our ability to study human diseases [21, 22]. Accord-

ingly, the purpose of our study was to explore the potential mechanism underlying IgA nephropathy.

Our results showed that 55 genes were identified to be differentially expressed in tubule cells between IgA nephropathy patients and normal ones. GO analysis revealed that several function items were statistically over-represented by the DEGs, which mainly related with diverse biological process such as response to

organic substance, transcription factor activity, response to steroid hormone stimulus and response to extracellular stimulus (Table 2). In order to better understand the interactions of disturbed genes, we established the network with significant gene pairs. The function analysis indicated response to organic substance was the most significant function of the network, which was corresponding to the results of GO analysis.

Fig. 2. Network of gene interaction pairs. The downward triangle represents down-regulated gene, while the upward triangle represents the up-regulated gene.

Table 3. Function annotation of genes in network

GO-ID	corr p-value	x	Description
10033	3.52E-06	10	response to organic substance
42221	8.18E-05	10	response to chemical stimulus
6950	2.97E-04	10	response to stress
48513	3.02E-04	10	organ development
48731	1.09E-04	12	system development
48856	2.27E-04	12	anatomical structure development
48522	8.54E-07	14	positive regulation of cellular process
48518	1.79E-06	14	positive regulation of biological process
7275	2.31E-05	14	multicellular organismal development
32502	4.13E-05	14	developmental process
50896	1.31E-04	14	response to stimulus
32501	7.41E-04	14	multicellular organismal process
50794	2.31E-05	18	regulation of cellular process
50789	3.52E-05	18	regulation of biological process
65007	7.75E-05	18	biological regulation

Table 4. Candidate small molecules

cMap name	Score	P value
doxorubicin	0.991	0.00002
atovaquone	0.949	0.00016
mitoxantrone	0.91	0.00154
podophyllotoxin	-0.911	0.0001
thapsigargin	-0.966	0.0001

There is a hypothesis that the exposure to organic solvents may be close associated with the pathogenesis of glomerulonephritis [23]. Previous evidence showed that subjects with occupational solvent exposure to paint, glue and styrene had higher risk for chronic glomerulonephritis compared with the ones with non-occupational exposure to solvent [24]. Recently, cigarette smoking was also proved to be a risk factor for chronic kidney disease in IgA nephropathy [25]. Nicotine, an organic compound in cigarette smoke was implied to modulate the endothelial dysfunction and accelerate the progression of kidney disease. Potent evidence showed that human mesangial cells possess the neuronal nicotinic acetylcholine receptors (nAChRs) [26]. The expression of nAChRs subunits promotes the nicotine effect on activating pro-fibrotic pathways, reducing glomerular filtration rate and glomerular filtration rate [27]. Accordingly, response to organic substance is a significant biological process in the development of kidney disease. Similarly, other clustered function by GEGs in this work may also play key roles in IgA nephropathy. Getting knowledge of the functions of disturbed genes is a better way to understand the pathogenesis.

Another aim of our study was to uncover the candidate small molecules that play key roles in IgA nephropathy development. Doxorubicin and thapsigargin were identified to be the significant small molecules in the development of IgA nephropathy. Doxorubicin, also named as adriamycin, is an anthracycline class of antibiotic and has been primarily used in the treatment of ovarian cancer [28] and others such as bladder cancer [29], breast cancer [30] and lung cancer [31]. Adriamycin is used restrictedly in clinical application for its toxicity which may result in cardiomyopathy and nephropathy development [32, 33]. Ariamycin can induce the development of hyperlipidaemia, glomerulosclerosis and tubulointerstitial fibrosis in normal subjects [34]. So it is widely used to construct nephropathy models in rats and mice. Although the mechanism of ariamycin induced nephropathy is far from being clear, ariamycin shows close association with IgA nephropathy development.

Furthermore, thapsigargin is served as non-competitive inhibitor of a class of enzymes such as endoplasmic reticulum (ER) Ca^{2+} ATPase [35] and is a tumor promoter in mammalian cells [36]. Tumorigenesis initiated by acute responses in cells. Thapsigargin induced the release of intracellular stored Ca^{2+} and rapidly increased the cytosolic free Ca^{2+} concentration by the potent inhibition of ER Ca^{2+} ATPase [37]. It is also reported that thapsigargin induces autophagy arrest and in turn increases the ER stress which ultimately leads to cell death. As outlined in recent studies, thapsigargin is available in experimental examining related with ER stress [38]. The clinical application of thapsigargin is mentioned in posterior capsule opacification treatment [39]. However, the evidence concerning the drug application of thapsigargin in IgA nephropathy is rare. Our work showed that thapsigargin had the highest negative score (connectivity score=-0.966) (Table 4), implying the capable of reversing disease. Therefore, attentions on the drug potential of thapsigargin may open vistas for developing novel therapy for IgA nephropathy treatment.

Although bioinformatics technologies have the potential to indentify and validate the candidate agents for serious diseases, many challenges still remain in this study. First, we downloaded the microarray data from GEO database instead of that developed by our research team. Second, for the specially designed platform of the microarray, we only obtained the array data from small number of samples. Besides, the results obtained in our paper were not validated by further experimental studies. Therefore, a large number of studies should be conducted to confirm the results in the future.

Conclusion

In summary, the biological informatics methods provide a systematic perspective to understand the progression of IgA nephropathy. The dysfunctions of significant gene sets may contribute to the development of IgA nephropathy, such as response to organic substance. Thapsigargin may be candidate agents for improving therapeutic effect on IgA nephropathy. The identification of active small molecules opened a novel insight to discover

the candidate agents for IgA nephropathy. However, the clinical applications of the candidate agents warrant further considerations.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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