Original Paper

Identification of Genes Related to the Early Stage of Angiotensin II-induced Acute Renal Injury by Microarray and Integrated Gene Network Analysis

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Key Words

Angiotensin II • Renal damage • Microarray • Gene expression profiles • Fabp1

Abstract

Background/Aims: Angiotensin II (Ang II) mediated signaling plays a key role in the development of chronic kidney damage that contributes to renal fibrosis. However, the gene expression changes regulated by Ang II in the early stage of acute renal injury remain unclear. **Methods:** C57BL/6 wild-type (WT) mice were injected with Ang II (1500 ng/kg/min) for 1, 3 and 7 days. A time series analysis of microarrays was performed to evaluate Ang II-induced differentially gene expression in the kidneys. The data of gene expression in the kidney was further dissected by ANOVA analysis, gene expression profiles, gene network construction and quantitative real-time RT-PCR. Ang II-induced renal inflammation and fibrosis in mice were confirmed by pathological examination. **Results:** Our microarray data showed that a total of 1,511 differentially expressed genes were identified in the kidneys at 1, 3 and 7 days after Ang II infusion. These genes function in multiple biological processes, including response to stimuli, immune response, cell adhesion, metabolic process, kidney development, regulation of blood pressure, and ion transport, which may play critical roles in the pathobiology of Ang II-induced acute renal injury at the early stage. Furthermore, among these genes, 20 genes were further selected for final investigation. The dynamic gene network analysis demonstrated that fatty acid binding protein 1 (Fabp1) localized in the core of the network. **Conclusions:** Our data suggests that genes involved in lipid metabolic process, especially Fabp1, may play a central role in the development of Ang II-induced acute renal injury at the early stage.

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Introduction

Hypertensive nephropathy is a major complication in hypertension, which causes disabilities and a high mortality rate in patients. Over time, hypertension can damage blood vessels and the tiny filtering units in the kidneys leading to renal injury, inflammation and fibrosis [1]. The mechanisms underlying the development of hypertensive nephropathy are extremely complex, however, the important role of the renin-angiotensin system (RAS) has been demonstrated. Angiotensin II (Ang II), the major peptide of the RAS, is involved in the pathogenesis of renal diseases via regulating inflammation and fibrosis [2, 3]. There are two receptors AT1 and AT2 for Ang II. AT1 mediates most of actions of Ang II through enhancing proliferation, inflammation and fibrosis thereby contributing to chronic diseases, such as hypertension and renal damage [2, 3]. Importantly, AT1 receptor blockers (ARB) or angiotensin-converting enzyme inhibitors (ACEI) have been suggested to ameliorate the progression of hypertensive renal injury [4]. Of interest, it has also been shown that angiotensin blockade has clinical benefits for patients who have hypertension and renal disease [4].

Numerous studies have demonstrated that Ang II-mediated renal inflammation and fibrosis are mediated via multiple mechanisms. Transforming growth factor- β 1 (TGF- β 1)/Smad, Rho/Rho kinase and NF- κ B are the main signaling pathways involved in Ang II-induced renal matrix production and recruitment of renal inflammatory cells [2, 3, 5]. Interestingly, inhibition of these pathways markedly suppresses the inflammatory and fibrotic processes [3, 6]. Despite its critical role in the regulation of renal injury, inflammation and fibrosis, the mechanism of how Ang II causes the early acute renal injury at a molecular level remains to be elucidated. Recently, microarray analysis has been widely used to investigate the global alterations of gene expression and identify genes that are important to cardiovascular and other diseases [7]. Therefore, microarray analysis may help us understand crucial factors that control the kidney damage and elucidate the precise molecular mechanisms of acute renal injury in Ang II-induced hypertension.

In this study, we investigated Ang II-mediated molecular events associated with the development of early renal injury by using microarray assay in a mouse model. Our results showed that after Ang II infusion at 1, 3 and 7 days, totally 1,511 genes with different expression variations were identified. These genes were then classified into 26 clusters based on expression pattern. Among these, five profiles (including No. 19, 21, 8, 22 and 6) of gene expression that contained a total of 814 genes were significant. Finally, the gene network analysis demonstrated that one gene *Fabp1* localized in the core of the network, and may play a key role in the processes of Ang II-induced acute renal injury and fibrosis.

Materials and Methods

Animals and Ang II administration

Eight to ten-week-old C57BL/6 male mice were implanted with osmotic pumps (Alzet Model 1007D; Cupertino, CA, USA) and infused with Ang II at 1500 ng/kg/min in Ringer's solution (0.01 mmol/L acetic acid in saline) for 1, 3, and 7 days [8-10]. Systolic blood pressure was measured by the tail-cuff method before starting treatment and again every day after Ang II infusion [8-10]. All mice were kept at least 1 wk at 22°C and 55% relative humidity in a 12-h day/night lighting environment with free access to food and water. All procedures were performed in accordance with the Animal Care and Use Committee of Capital Medical University. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Histology and immunohistochemistry

The kidney tissues were fixed in phosphate-buffered 4% formalin (pH 7.4) for 24 h and then embedded in paraffin. The sections (5 μ m) were used for staining with H&E, Masson's trichrome, and periodic acid-Schiff stain (PAS) [8-10]. Tubular injury including brush-border loss, tubule dilatation, tubule necrosis, and tubule cast formation after Ang II injection was evaluated in PAS-stained sections. For immunohistochemical

staining of Mac-2 (a marker for macrophages), tissue sections were deparaffinized and incubated with a Mac-2 primary antibody (Santa Cruz, CA, USA) as described [8-10]. The slides were evaluated and scored in a blinded fashion to the treatment by a renal pathologist. Images were viewed and captured using a Nikon Labophot 2 microscope (Nikon, Tokyo, Japan).

RNA isolation and microarrays

All mice were euthanized by an overdose of pentobarbital (100 mg/kg, i.p) on days 1, 3 and 7 of Ang II treatment. Total RNA was isolated with TRIzol (Invitrogen) from kidneys (n=2 per group) at each time point according to manufacturer's instructions. Gene expression profiling was performed using Affymetrix GeneChip Mouse Genome 430 2.0 Array according to the manufacturer's instructions (Affymetrix, Inc., Santa Clara, CA) [11, 12]. On the GeneChip Mouse Genome 430 2.0 Array, over 45,000 probe sets analyze the expression level of over 39,000 transcripts and variants from over 34,000 well characterized mouse genes. The in detail gene expression data are also available at the GEO website under accession number GSE59437: <http://www.ncbi.nlm.nih.gov/geo/>.

Analysis of microarray data

A comprehensive bioinformatics analysis was used to enrich the dataset for genes that were most likely to be associated with the development of Ang II-induced acute renal injury, including Gene ontology (GO) analysis, pathway analysis, clustering analysis, and dynamic gene network analysis.

Significant Differential Gene Analysis

The random-variance model (RVM) F-test was applied to filter the differentially expressed genes for the saline-treated control and Ang II-treated group (at days 1, 3, and 7) because the RVM F-test can raise degrees of freedom effectively in the cases of small samples. After the significant analysis and the false discovery rate (FDR) analysis, we selected the differentially expressed genes according to the *P*-value threshold [13].

Series test of cluster analysis

The series test of cluster (STC) algorithm of gene expression dynamics was used to profile the gene expression time series and to identify the most probable set of clusters generating the observed time series. This method explicitly took into account the dynamic nature of the temporal gene expression profiles during clustering and identified the number of distinct clusters. We selected differential expression genes at a logical sequence according to RVM corrective ANOVA. In accordance with different signal density change tendency of genes under different situations, we identify a set of unique model expression tendencies. The raw expression values were converted into log₂ ratio. Using a strategy for clustering short time-series gene expression data, we defined some unique profiles. The expression model profiles are related to the actual or the expected number of genes assigned to each model profile. Significant profiles have higher probability than expected by Fisher's exact test and multiple comparison test [14, 15].

Gene ontology (GO) analysis

GO analysis according to the Gene Ontology that is the key functional classification of NCBI, which can organize genes into hierarchical categories and uncover the gene regulatory network on the basis of biological process and molecular function. Specifically, two-side Fisher's exact test and χ^2 test were used to classify the GO category, and FDR was calculated to correct the *P*-value, the smaller the FDR, the smaller the error in judging the *P*-value [16, 17].

Pathway analysis

Pathway analysis was used to find out the significant pathway of the differential genes according to KEGG, Biocarta and Reatome. We turn to the Fisher's exact test and χ^2 test to select the significant pathway, and the threshold of significance was defined by *P*-value (*P*<0.05) and FDR [18].

Dynamic gene network analysis

The normalized expression value of significant differential genes from the five most significant profiles, namely profiles 19, 21, 8, 22 and 6, was used to build a co-expression network. In the network,

Table 1. Primer sequences

Gene symbol	Forward primer (5'-3')	Reverse primer (5'-3')
Fabp1	TGAAGGCAATAGGTCTGCCC	GTCATGGTCTCCAGTTTCGCA
Fgl1	GCTGGTGGTTTAAACAGGTGTC	CAGGTGTACCACACAACACCA
Rgn	GGATACGGTCAGCAATCAAG	TCCCAGTTCAAAGCACAGAA
Ccdc69	AAGCCTCGTTGCAGAACACC	TGGGCCAGGACTCCCATAT
BB144871	CTCCGGCCTTTCCTCTCTA	ATTTCAGGTGACCACACAGG
Slc36a2	CCCTACCTTGAGCTTGCCC	AATGTCTGGAACCCGGTTATG
OTTMUSG00000008561	ACACATCCCAGCTACCCAAC	TTTCCCATGCGCTCATTCCT
Tmem30a	ATCGGCATCTTCGTCACCTC	ACACATTGCCCTCAAATGACTG
Tmem25	TCACCAGCCGAGGTTTCATC	TGCCTCACCATCAAGCTCAG
Fads3	ACAAGTGGCTGGTCATCGAG	CGTGAAGGCATCCGTGG
Plagl1	TCCGGCTAGGGTAGGTTGTT	CCAGAGCACAATAGCTGGGT
Ace	GGAAGCTCCTGGCGGATG	GTGTTGGCGCCAGTTG
Acy1	AGACGTGGAGAGAGCAAGCAC	GCCAGGCACCACCTCTATTTT
Hk2	AGTGAACCCAGCTGTTTGA	ATGAAGTTGGCCAGGCATT
Hba-a1	GGAAGACAAAAGCAACATCAAG	CATCAAAGTGAGGGAAGTAGGT
Bcl2l11	GCTGCATTGTTCCCGTAGAG	GTTGGATGGCCACCTATCTG
Fkbp5	AGATTCAGGCGTTATCCGTAGA	ATCAAATGTCCTTCCACCACAG
GAPDH	TCCCAGAGCTGAACGGGAAG	TCA GTGCCCTCAGATGC

cycle nodes represent the genes, and edges between two nodes represent interactions between genes. All the nodes were marked with degree, which is defined as the link numbers one node has to the others. Genes with higher degrees occupied more central positions in the network and had a stronger capacity of modulating adjacent genes. In addition, k-core in graph theory was applied to describe the characteristics of the network. As network elements represent the ways in which genes may regulate other genes, large scale gene networks can be divided into certain subgraphs, and marked with different colors.

Validation of microarray data by qPCR analysis

Quantitative real-time PCR (qPCR) was used to verify the differential expression of 17 selected genes (n=4-6 per group) that were detected by microarray. The primers used are listed in Table 1. The first-strand cDNA was synthesized with moloney murine leukemia virus reverse transcriptase (Promega, Southampton, UK). qPCR was performed with an iCycler IQ system (Bio-Rad, USA) as described [8-10]. The cycling conditions consisted an initial, single cycle of 5 min at 95°C, followed by 40 cycles of 30 sec at 95°C, 30 sec at 54°C, 15 sec at 72°C, and fluorescence acquisition at 83°C for 1 sec. The gene expression levels were quantified relative to the expression of GAPDH.

Statistical analysis

All values were presented as mean $\pm$ S. E. M. Statistical differences between groups were analyzed by the non-parametric tests (Mann-Whitney) or by the parametric test one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test for group differences. $P < 0.05$ was considered significant.

Results

Angiotensin II induces renal inflammation and fibrosis in mice

Hypertensive mouse model was successfully induced by Ang II infusion. The blood pressure was markedly increased in a time-dependent manner (at days 1, 3 and 7) (Fig. 1A). Pathological examination indicated that continuous infusion of Ang II caused renal inflammation and fibrosis reflected by increased infiltration of Mac-2-positive macrophages and collagen deposition in the kidney tissues at days 3 and 7 (Fig. 1B). In contrast, PAS staining showed no significant effect of Ang II infusion on renal tubular injury (Fig. 1B).

Genes detected by microarray assay in Ang II-infused kidneys

To determine the gene expression changes in Ang II-induced acute renal injury at early stage, kidney tissues were taken from the mice at days 1, 3, and 7 after Ang II infusion, and a time series microarrays (n=2 per time point) were performed by using the Affymetrix probe dataset. Moreover, we found that total 1,511 genes that had a P -value and the false discovery

Fig. 1. Blood pressure and renal pathological examination in mouse model with acute renal injury induced by Ang II-infusion. (A) Wild-type mice were infused with Ang II (1500 ng/kg/min) for 1, 3 and 7 days. Systolic blood pressure was measured by the tail-cuff method ($n=6$ in each group). (B) Pathological examination was performed with H&E staining, immunohistochemical staining with anti-Mac-2 antibody, Masson's trichrome staining and PAS staining. Black arrows indicate Mac-2 positive cells. Data are shown as mean $\pm$ S. E. M. ($n=4$ per group). *** $P<0.0001$ vs. control.

rate (FDR) less than 0.05 were shown to be significantly expressed in Ang II-infused mice at least at one time point when compared to control ($P<0.05$). Furthermore, when compared with probes in the saline-treated control, 640 probes significantly increased and 434 probes decreased in the 1 d group; 380 probes markedly increased and 277 probes decreased in the 3 d group, and 224 probes increased and 190 probes decreased in the 7 d group. These results indicate that by time after Ang II infusion the number of different gene expression in the kidneys was decreased.

To confirm the reliability of microarray data, qPCR analysis was performed for 10 genes at each time points, including *Rgn*, *Ccdc69*, *Slc36a2*, *OTTMUSG00000008561*, *Tmem30a*, *Fads3*, *Ace*, *Acy1*, *Fkbp5* and *Bcl2l11*, which were differentially expressed in our microarray assay. As shown in Fig. 2A-C, scatter plot graphs of gene expressions showed a significant correlation between microarray and qPCR results at days 1, 3, and 7 after Ang II infusion.

Fig. 2. Scatter plot of relative changes in gene expression as determined by microarray analysis and by qPCR analysis. To confirm the results obtained with the microarray, qPCR was performed for 10 genes at each time points, including Rgn, Ccdc69, Slc36a2, OTTMUSG00000008561, Tmem30a, Fads3, Ace, Acy1, Fkbp5 and Bcl2l11. Quantification of gene expression by qPCR showed a significant correlation with microarray quantification.

Gene Ontology (GO) Analysis

GO analysis was used to analyze differentially expressed genes that had the main cellular functions during Ang II infusion. The most statistically significantly overrepresented GO terms ($P < 0.001$) were observed. As shown in Fig. 3, the high enrichment GO terms included response to stimuli (including drug, nutrient, glucocorticoid, lipopolysaccharide, hypoxia, peptide hormone, cAMP, mechanical, ethanol, cytokine, estradiol, hydrogen peroxide, cold, organic nitrogen and insulin), aging, organ regeneration, immune response, cell adhesion, metabolic process (including lipid, fatty acid, cholesterol and lipoprotein), kidney development, regulation of blood pressure, cellular processes (cell proliferation and differentiation), and ion transport, suggesting that these GO terms may play critical roles in the pathobiology of Ang II-induced early renal injury and fibrosis.

Pathway analysis

Biological pathway analysis yielded that 93 pathways ($P < 0.05$) were significantly modulated in the kidney tissues during Ang II infusion. Several key pathways associated with metabolic pathways (including PPAR, cytochrome P450, retinol, amino acids, fatty acid, nitrogen and lipid), immune/inflammatory process (including cytokine-cytokine receptor interaction, antigen processing and presentation, natural killer cell mediated cytotoxicity, chemokine signaling pathways, adipocytokine, leukocyte transendothelial migration and

Fig. 3. GO category based on biological process for differentially expressed genes. The significant Go category for differentially expressed genes. LgP is the logarithm of *P*-value.

TLR), ECM-receptor interaction, rennin-angiotensin system, MAPK, mTOR, Jak-STAT and Wnt signaling pathways as well as apoptosis were significantly overrepresented (Fig. 4). The importance of metabolic pathways, immune response and ECM-receptor interaction, and rennin-angiotensin system at the early phase of Ang II infusion was highlighted and could be considered to be important for Ang II-induced renal inflammation and fibrosis.

Cluster analysis of significant differential genes in Ang II-treated kidneys

After hierarchical cluster analysis, a total 1,511 genes were classified into 26 profiles based on expression patterns, and each cluster has similar expression patterns after Ang II infusion (Fig. 5). Among these profiles, we identified 5 significant profiles (including No. 19, 21, 8, 22 and 6) of gene expression that contained a total of 814 genes. Gene expressions in profiles No. 19, 21 that are comprised of 275 and 176 genes respectively, were increased at early time points (1 or 3 d) and then gradually decreased at later times (7 d); gene expressions in profile 22 were constantly increased at all time (1, 3, and 7 d); while the genes in profiles No. 8 and 6 had opposite effects compared with genes in profiles No. 19, 21 (Fig. 6). Among these patterns, one most significant pattern was profile 19 according to ascending *P*-values. The profile No. 19 contained 275 genes that were the most abundant and exhibited increased expression at 1 d and decreased expression at days 3 and 7, after Ang II infusion. Thus, this significant profile is considered to be the potential main expression profile in our experiment, with the tendencies consistent with or opposite to that occurred during hypertension and acute renal injury induced by Ang II (Fig. 1).

Dynamic Gene network analysis based on GO analysis

To further determine which gene or genes may play key roles in the early stage of acute renal injury induced by Ang II infusion, gene co-expression networks with the k-core algorithm were established from functional gene associations, and 814 genes selected in 5

Fig. 4. KEGG pathway analysis for differentially expressed genes. The significant pathway for differentially expressed genes associated with renal injury. LgP is the logarithm of *P*-value.

profiles were then analyzed. The correlations between genes were shown in Fig. 7. In the networks, cycle nodes represent genes, and edges between two nodes represent interactions between genes, which were quantified by degree. The k-core subnetwork with higher k-core level is considered to have a core status within a large scale gene network and is made up of differential 20 genes, which were from profile No. 19 (Table 2). Most of the genes were attributed to response to stimuli (including drug, nutrient, glucocorticoid, hypoxia), organ regeneration, metabolic process (including lipid, fatty acid, cholesterol, carbohydrate, steroid, amino acid), and ion transport. The core gene fatty acid binding protein 1 (Fabp1) appeared at the center of the large-scale networks and the 46 k-core subnetwork. It directly regulated 63 neighboring genes that interact according to their degrees. These interactions depended in large part on Fabp1 because the clustering coefficient of this gene was 0.85, which was lower than for other genes. In this network, Fabp1 was shown to play a direct role in the important processes of regulation of hydrolase activity, fatty acid beta-oxidation, intestinal absorption and cell proliferation. The result of Fabp1 up expression at 1 day may be related to development of early renal injury.

Verification of the gene expression by qPCR analysis

To further confirm the results from microarray results, qPCR was performed on the kidney tissues. As shown in Fig. 8, the relative expression levels of 17 differentially expressed genes from 5 significant profiles, including No. 19 (Fabp1, Fgl1, Rgn), No. 21 (Ccdc69, BB144871, Slc36a2), No.8 (OTTMUSG00000008561, Tmem30a, Tmem25, Fads3),

Fig. 5. The expression patterns of 1,511 genes by hierarchical cluster analysis. The expression patterns of 1,511 genes were classified into 26 profiles by hierarchical cluster analysis. Genes in the same box have a similar expression pattern. 5 significant profiles (including No. 19, 21, 8, 22 and 6) were identified ($P < 0.001$).

No. 22 (*Plagl1*), No. 6 (*Ace*, *Acy1*), and 4 non-significant profiles, including No. 15 (*Hk2*), No. 18 (*Hba-a1*), No. 24 (*Fkbp5*) and No. 26 (*Bcl2l11*), were assayed. 8 genes *Fabp1*, *Fgl1*, *Rgn*, *Ccdc69*, *BB144871*, *Slc36a2*, *Plagl1*, and *Hba-a1* were peaked at 1d, and decreased at 3 and 7 d; six genes *OTTMUSG00000008561*, *Tmem30a*, *Tmem25*, *Fads3*, *Ace*, and *Acy1* were decreased at all time points; two genes *Fkbp5* and *Hk2* increased at 1 and 3 d, and returned to baseline at 7 d; one gene *Bcl2l11* was constantly increased at 1, 3, and 7 days, indicating that these data were good consistent between the two experiments (Fig. 6).

Discussion

Due to the high morbidity and mortality of renal disease, early diagnosis followed by early effective prevention is the key for prognosis improvement. So far, the genes related to the very early stage of acute renal injury and fibrosis induced by Ang II infusion remain

Fig. 6. Gene expression of profiles No. 19, 21, 8, 22 and 6 detailed in expression graphs. Gene expressions in profiles No. 19, 21 (comprised of 275 and 176 genes respectively) were increased at early time points (1 or 3 d) and then decreased at the later time point (7 d); gene expressions in profile 22 were constantly increased at all time (1, 3, and 7 d); while genes in profiles No. 8 and 6 had opposite alterations compared with genes in profiles No. 19, 21.

unknown. In the present study, we conducted time series microarrays and integrated gene network analysis to identify differentially expressed genes, GO terms, and KEGG pathways. Our results suggest that 1,511 genes were involved in multiple biological processes, including metabolic process, immune response, cell adhesion, kidney development, and regulation of blood pressure at early stage of acute renal injury induced by Ang II. Finally, our work further identified one key gene fatty acid binding protein 1 (Fabp1) that may play a key role in the development of acute renal damage and fibrosis. Taken together, this work identifies significant expressed genes and provides novel evidence for the molecular events associated with the development of early renal injury.

Renal fibrosis is a main pathological feature of progressive kidney disease [19]. It is characterized by accumulation of fibroblasts and excessive matrix proteins along with a loss of functioning nephrons [20]. Numerous studies have suggested that progressive renal fibrosis is mediated by various mediators, including growth factors, cytokines, metabolic toxins, and stress molecules through multiple signaling pathways [21]. Ang II participates

Fig. 7. Gene co-expression network. 814 genes from the 5 significant profiles (including No. 19, 21, 8, 22 and 6) were analyzed with k-core algorithm and gene co-expression networks were established. Cycle nodes represent genes, and edges between two nodes represent interactions between genes which were quantified by degree. The higher the degree is of a gene, the more central position it occupies in the network.

in the pathogenesis of renal diseases via regulating two key processes inflammation and fibrosis [2]. Among them, Ang II plays a critical role in the pathogenesis of renal diseases through main AT1 and AT2 receptors. AT1 mediates most of the actions of Ang II, which activates several signaling pathways, including TGF- β 1/Smad, Rho/Rho kinase, MAPK, mTOR, and Jak-STAT [22]. Recently, microarray assay has been widely used to investigate the global changes of gene expression, and identify genes that are important to Ang II-induced response in smooth muscle cells, vascular, and kidney [23-25]. Despite its central role in the regulation of blood pressure and renal injury, the mechanism of Ang II actions in the kidney at a molecular level remains to be defined. In this study, we performed time series microarrays to identify significant differentially expressed genes, GO terms, and KEGG pathways. We found that a total 1,511 differentially expressed genes in the Ang II-treated kidney when compared to control. Of them, 1074 genes were markedly

altered at day 1, and 657 genes at day 3. Interestingly, at day 2 of Ang II infusion, 805 genes were differentially expressed (>1.5-fold), indicating that the data from Makhanova's study fits within the time-course of our results. Importantly, a lot of gene expression patterns in our results were similar with Makhanova's data [25]. Moreover, these genes participate in response to stimuli, organ regeneration, immune response, cell adhesion, metabolic process, kidney development, regulation of blood pressure, cellular processes, and ion transport. These observations were consistent with previous report by Makhanova, including response to stimuli, metabolism, transport, physiological processes, etc [25]. Moreover, the present data revealed that Ang II infusion significantly up-regulated metabolic pathways, immune/inflammatory process, ECM-receptor interaction, rennin-angiotensin system, MAPK, mTOR, Jak-STAT and Wnt signaling pathways (Fig. 4), which are main mechanisms involved in Ang II-induced matrix regulation and inflammation [22, 26, 27]. Together, the analysis of Gene Ontology and pathway enrichment of these differentially expressed genes have provided novel insight into the molecular pathogenesis of acute renal injury and gene regulation induced by Ang II.

Fabp1 (also known as L-Fabp) is a 14-kDa organic anion-binding cytoplasmic protein produced mainly in the liver, and can be filtered via glomeruli because of its small molecular size and reabsorbed in proximal tubule epithelial cells like other small proteins [28]. Immunohistochemical analysis indicates that Fabp1 is exclusively localized to the cytoplasm of the proximal tubule cells [29]. Several studies suggest that Fabp1 is not expressed in both mouse and rat kidney in normal and pathophysiological conditions [30]. However, a recent study indicates that Fabp1 is expressed in the kidney [31]. Importantly, several clinical studies demonstrated the significance of urinary Fabp1 in kidney diseases. Fabp1 is superior to blood urea nitrogen (BUN) and urinary N-acetyl-beta-D-glucosaminidase (NAG) for early and accurate detection of acute tubular necrosis in different models of animal acute kidney injury. Thus, urinary Fabp1 is considered as a novel renal biomarker to predict the severity of kidney diseases, such as diabetic nephropathy [29]. Interestingly, Fabp1 has been reported to play an important role in the processes of fatty acid uptake, transport,

Table 2. 20 genes identified by gene co-expression network with k-core algorithm

Gene symbol	Clustering Coefficient	Degree	k-core
Fabp1	0.84946237	63	46
Fgl1	0.87890005	62	46
Rgn	0.87890005	62	62
Slc38a4	0.87890005	62	46
Ang	0.87149656	62	46
Baat	0.87043892	62	46
Lcn2	0.86567953	62	46
Cpn2	0.82072977	62	46
Alb	0.89672131	61	46
Apoa1	0.89672131	61	46
Apoa2	0.89672131	61	46
Cyp2c29	0.89672131	61	46
Cyp3a11	0.89672131	61	46
Cyp3a13	0.89672131	61	46
Hamp	0.89672131	61	46
LOC100046946	0.89672131	61	46
Serpinc1	0.89672131	61	46
Tdo2	0.89672131	61	46
Ugt2b36	0.89672131	61	46
Ugt2b5	0.89672131	61	46

Fig. 8. Validation of microarray data by quantitative real-time PCR. Expression fold changes of 17 differentially expressed genes from both significant and non-significant profiles were verified by qPCR, including Fabp1, Fgl1, Rgn, Ccde69, BB144871, Slc36a2, OTTMUSG0000008561, Tmem30a, Tmem25, Fads3, Plagl1, Ace, Acy1, Hk2, Hba-a1, Fkbp5, Bcl2l11. Data were presented as mean $\pm$ S.E.M. (n=4-6 per group).

metabolism and fibrin clot formation. Fabp1 can bind selectively to free fatty acid (FFA) and is thought to be involved in energy production/metabolism in renal tubule cells [29]. Moreover, several recent studies suggest that Fabp1 plays a critical role in protecting renal injury through regulating oxidative stress, FFA metabolism. For example, human Fabp1 overexpression in proximal tubules reduces oxidative stress and tubulointerstitial damage induced by Ang II or unilateral ureteral obstruction [32, 33]. In the present study, cluster analysis indicated that Fabp1 expression peaked at 1 day and decreased at day 3 and 7 after Ang II infusion. Gene network analysis further demonstrated that Fabp1 localized in the core of the gene network, indicating Fabp1 may contribute to initiation and development of acute renal damage.

In this study, we investigated Ang II-mediated molecular events associated with the development of early renal injury by using microarray assay in a mouse model. We found that after Ang II infusion at 1, 3 and 7 days, 1511 genes were differently expressed in the kidney compared with control. Through an integrative analysis that combines changes in gene expression with gene function within a genetic network, we identified one gene *Fabp1* that may be related to the very early stage of Ang II-induced renal injury and fibrosis.

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