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Research paper

Screening and functional analysis of differentially expressed genes in chronic glomerulonephritis by whole genome microarray

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ABSTRACT

Background: Chronic glomerulonephritis (CGN) is the most common form of the glomerular disease with unclear molecular mechanisms, which related to immune-mediated inflammatory diseases. The aim of this study was to characterize differentially expressed genes in the normal and adriamycin-induced CGN rats by microarray analysis, and to determine the potential molecular mechanisms of CGN pathogenesis.
Methods: For the gene expression analysis, fresh glomerular tissues from both normal and adriamycin treated rats ($n = 4$, respectively) were collected. Total RNA was extracted and subjected to Agilent Rat 4×44 K whole genome microarray. KEGG, Gene Ontology (GO) analyze, LIMMA, String and Cytoscape software were applied to screen and analyze differentially regulated genes. In addition, the Real-time polymerase chain reaction (RT-PCR) was performed to verify the selected genes.
Results: 2334 differentially regulated genes were identified including 1294 up-regulated genes and 1040 down-regulated genes. According to the results of Generank, String and Cytoscape analyses, 27 genes may be key controlled genes in the pathogenesis of CGN, including 14 up-regulated genes (Fos, Myc, Kng1, Rac2, Pik3r1, Egr1, Icam1, Syk, Anxa1, Lgals3, Ptprc, Runx1, Itgb7, Ccl6) and 13 down-regulated genes (Aldh2, Dpyd, Mthfd1, Gldc, Ppar-α, Igf1, Pomc, Oas1a, Gsr, Acox1, Cyp1a1, Ugt2b15, Hsd3b6), which primarily contribute to biological processes such as, cell cycle, cell proliferation, inflammatory response, immune response, metabolic process, and so on. Fos and Syk were considered as potent hub genes.
Conclusions: Global gene expression profile analysis showed that the molecular mechanism of CGN pathogenesis may be related to the promotion of cell cycle and mitosis, dysregulation of cytokine secretion and disordered inflammatory response as well as abnormal metabolism.

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1. Introduction

Chronic glomerulonephritis (CGN), the most common form of glomerular disease, is related to immune-mediated inflammatory diseases and characterized by proteinuria, hematuria, hypertension and edema, which accompanied by renal dysfunction and frequently led to the end-stage of renal disease (Chebotareva et al., 2015; Satirapoj et al., 2015). A number of pathogenic factors may induce this disease, however, the molecular mechanisms of this disease still remain unknown (Dudnyk et al., 2015; Hule et al., 2015). Therefore, it is vitally important to identify the molecular characteristics of CGN, which could contribute to the understanding of the pathogenesis of CGN and development of the novel diagnostic markers.

The DNA microarray is performed on a chip which is made of silicon, plastic or glass with fixed gene probes. Thousands of genes can be examined simultaneously and information of all the samples could be harvested by computer after hybridization. Moreover, this technology has been extensively used to discover novel molecular diagnostic markers, gene functions, DNA re-sequence, therapeutic targets (Serizawa et al., 2004; Sokolov et al., 2006; Yasuike et al., 2016), and explore biochemical pathways to achieve better understanding of the pathogenesis of diversity diseases (Liang et al., 2011).

In this study, adriamycin-induced CGN rats were used as experimental model to identify the differentially expressed genes compared with the normal rats via Agilent Rat 4×44 K whole genome microarray

Abbreviations: CGN, chronic glomerulonephritis; GO, gene ontology; RT-PCR, real-time polymerase chain reaction; IP, intraperitoneal injection; H&E, hematoxylin and eosin; SPSS, Statistic Package for Social Science; GBMT, glomerular basement membrane thickness; ICAM-1, intercellular adhesion molecule-1; Anxa1, annexin a1; CRD, carbohydrate-recognition-binding domain; Kng, kininogen; BCR, B-cell antigen receptor; Syk, spleen tyrosine kinase; HSPG, heparan sulfate proteoglycan; Runt-Related Transcription Factor 1, RUNX1; CCL6, Chemokine (C-C motif) ligand 6.

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analysis, in order to reveal the key genes involved in the CGN, analyze the biological functions and interactions, and finally illuminate the potential pathogenesis of CGN.

2. Material and methods

2.1. Ethics statement

Male Sprague-Dawley rats (200 ± 20 g, 7 weeks old, SPF grade) were purchased from the Experimental Animal Center of Anhui Medical University. The protocol was approved by the Committee on the Ethics of Animal Experiments of Anhui university of Chinese medicine (Permit Number: 2012AH-036-03). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Chemicals

Adriamycin was obtained from Pfizer Pharmaceuticals Ltd. (Wuxi, China); sodium pentobarbital was obtained from Shanghai chemical reagent company (Shanghai, China).

2.3. Animals experiments and samples collection

All the rats were allowed free access to food and water and housed individually in a facility at 18–22 °C and 40–60% humidity. After acclimatization for 1 week, the rats were randomly divided into the control ($n = 10$) and experimental model ($n = 10$) groups. The rat from experimental mode group were injected with adriamycin twice via tail intravenous injection, 3.5 mg kg^{-1} adriamycin should be given on the first day and 3.0 mg kg^{-1} on the fourteenth day. On twenty-first day, all rats were put into the metabolism cages and urine was collected in 24 h to determine the urinary protein. Urinary protein >50 mg kg^{-1} (in 24 h) was considered as successful experimental model. Rats were anesthetized intraperitoneally with sodium pentobarbital (2 mL/kg, intraperitoneal injection, IP) and glomerular tissues were harvested, subpackaged, sealed in freezing tubes and stored at −80 °C.

2.4. Pathological examination

Rats were anesthetized intraperitoneally with sodium pentobarbital (2 mL/kg, IP) and sacrificed. Blood was removed by cutting the abdominal aorta. Subsequently, kidneys were harvested for pathological examination such as color, luster, texture and surface flatness, etc.

2.5. Histological analysis

Rats were anesthetized intraperitoneally with sodium pentobarbital (2 mL/kg, IP). The kidneys of each rat were cut and 4-μm-thick of glomerular specimens were obtained. Specimens were fixed in 10% neutral formalin and 2.5% glutaraldehyde stationary liquid in order to examine the pathological changes after hematoxylin and eosin (H&E) staining.

2.6. Differentially expressed genes analysis

Total RNAs were extracted from 8 cases of glomerular tissues (4 cases from the control group and 4 cases from the experimental model group). Quantity and quality of RNA were determined by NanoDrop ND-1000 and then marked with dihydroxyfluorane after reverse transcription and hybridized with Agilent Rat 4×44 K whole genome microarray, which was performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology). These experiments were provided and completed by Kangchen Bio-tech Inc. (Shanghai, China). Differentially expressed genes with statistical significance between the two groups were identified through Volcano Plot filtering. Differentially expressed genes between two groups were identified through fold change filtering (an fold change of ≧2) and p values of the *t*-test (a p value of ≦0.05). Hierarchical Clustering was performed using the R scripts. GO and Pathway analysis were performed for gene function analysis in the standard enrichment computation method. String and Cytoscape software were used to draw genetic interaction network.

2.7. Real-time PCR verification

Real-time PCR was performed with β-actin as the internal control to verify the microarray results. The expression of the following genes was analyzed: Fos, Syk, CYP1a1, Ugt2b15 and Hsd3b6. The primers are listed in Table 1. Both glomerular tissues from control and experimental model groups were verified for eight samples, and each experiment was repeated three times.

2.8. Statistical analysis

Quantitative data was presented as means ± SD. Statistical analysis was analyzed by one-way analysis of variance with Student-Newman-Keul's test using the Statistic Package for Social Science (SPSS) 17.0 software (SPSS, Chicago, USA). The results of the animal experiments and real-time PCR were analyzed by Statistical Analysis System (SAS) 9.2 software (SAS Institute Inc. USA). $p < 0.05$ was considered statistically significant.

3. Results

3.1. Pathological analysis

Results showed that kidney tissues from rats from control group were crimson, soft, and the surface was smooth and lustrous. The cross sections showed that corticomedullary demarcations were clearness. Compared with control group, kidney tissues from experimental rats were light red, hardened, fragile and non-lustrous. Additionally, the cortex of cross sections showed that corticomedullary demarcations were unclearness (Fig. 1).

3.2. Histopathology

H&E staining showed that rats from control group represented normal glomerulus structure and glomerular basement membrane thickness (GBMT), convoluted tubular, clear Bowman's capsule structure, and opened capillary loops. Compared with control group, incrassation of capillary loops and Bowman's capsule showed expansion of convoluted tubular of kidney in the rats of experimental model group. Additionally, degeneration of renal tubule epithelial cells, occurrence casts (protein) in the lumen, infiltration of inflammatory cells, hyperemia and edema of renal interstitium were also observed (Fig. 2).

Table 1
Primer sequence.

Gene name	Forward sequence and reverse sequence	Product length (bp)
β-actin	F:5′ CGAGTACAACCTTCTTGCAGC3′ R:5′ ACCCATACCCACCATCACAC3′	202
Fos	F:5′ TGCGTTGCAGACCGAGATT3′ R:5′ GGTAGCCTCAGGCAGACCC3′	163
Syk	F:5′ CACCGTGTCCTTCAATCCCTAT3′ R:5′ AGTTGCCAGAGCCCAGTTCAT3′	197
Cyp1a1	F:5′ GGGTGTAGCACCTTCATTTAC3′ R:5′ GTTCAGAGGCAACTTGGACTA3′	206
Ugt2b15	F:5′GCATAGCTTCTTTGTAATTTTGTAC3′ R:5′ GGTCACATATCAGAAGCCTCAG3′	68
Hsd3b6	F:5′ GCTCCTGGTTGGGACTACTGAT3′ R:5′ GTCACCTTGATGCTTGTCCCT3′	274

Note: we used β-actin as an internal control.

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