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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 extrated and subjected to Agilent Rat 4 × 44 K whole genome microarray. KEGG, Gene Ontology (GO) analyse, 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, inmmune 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.

Abbreviations list

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; intercellular adhesion molecule-1, ICAM-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.

Keywords: CGN; Gene expression; microarray; pathogenesis; molecular mechanism

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 noval 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 analysis, in order to reveal the key genes involved in the CGN, analyze the biological functions and interactions, and finaly 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℃ 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 $mg \cdot kg^{-1}$ adriamycin should be given on the first day and 3.0 $mg \cdot kg^{-1}$ on the fourteenth day. On twenty-first day, all rats were put into the metabolism cages and urine was collected in 24 hours to determine the urinary protein. Urinary protein more than 50mg/kg (in 24 hours) 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℃.

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 colour, 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 (Figure 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 experimetal model group. Additionaly, 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 (Figure 2).

3.3 Screening of differentially expressed genes

Fluorescence signaling in the microarray hybridization signal scanning was strong and uniform, quality control was qualified. The noise ratio of signal was low, and the signal intensity reflected the RNA expression of the samples. All the raw data was analyzed by Agilent Feature Extraction Software and was standardized by robust quantile. LIMMA software with P value <0.05 and fold change ≥ 2 as a standard was applied in screening genes. Results showed that there were 2,334 significantly differentially expressed genes, including 1294 up-regulated genes and 1040 down-regulated genes. Volcano Plot analysis was performned to represent the differentially expressed mRNA between the control group and experimental model group (Figure 3). Hierarchical cluster analysis of differentially expressed genes showed that the genes were well distinguished between the normal and experimental groups (Figure 4A).

3.4 Biological function analysis of differentially expressed genes

In order to further identify the specific and new biological pathways or functional gene groups that performed differentially and associated with CGN, GO and KEGG analysis were applied. In the case of up-regulated biological processes (BP), the top-ten significant BP were showed in the Figure 5A and the most significant BP was the regulation of immune system process, which played a vital role in the pathogenesis of CGN, and indicated the considerable roles of inflammation in the initiation and perpetuation of CGN. The main concentration of down-regulation BP was metabolic processes, as 6 out of 10 top significant down-regulated (BP) were involved in metabolism (Figure 5B).

Differentially expressed genes were depicted in interaction networks. Generank was based on the premise that nodes with more multiple of differentially expression and joint-edges have more important biological function (Luscombe et al., 2004; Khalyfa et al.,

2010). Therefore, genes with the more advanced rankings were more biologically vital in the process of CGN. The 2334 differentially expressed genes were analyzed using String online software, with the high confidence level of 700. Results showed that there existed interactions among 760 nodes with a total of 6,494 joint-edges, and there were 239 nodes with ≥ 10 joint-edges, including 4575 joint-edges, accounting for 70.45% of the total, which indicated that the 239 genes may have significant functions in CGN (Figure 6). 27 were either ranked in the top 100 up-regulated genes or the top 100 down-regulated genes in Generank rank among these 239 nodes, which included 14 up-regulated genes and 13 down-regulated genes. They were marked in the hierarchical cluster analysis (Figure 4B), which represented the most outstanding pathogenesis of CGN. 2 hub genes were selected to construct gene networks (Figure 7) so that we could clearly determine the hard-core roles of the hub genes.

Annotation and analysis results of GCBI and pathway showed that the up-regulated genes were mainly related to cell cycle, cell proliferation, cytokine-cytokine receptor interaction, immune response, inflammatory response, whereas the down-regulated genes were mainly related to various kinds of metabolic process (Table 2).

3.5 Real-time RT- PCR analysis

Real-time PCR results showed that trends in expression change for the selected five genes were consistent with the microarray results. Fos, Syk showed a high expression level in experimental model group, and low expression in CYP1a1, Ugt2b15, Hsd3b6 (Figure 8), verifying that the microarray results were accurate and reliable.

4. Discussion

In our study, glomerular tissues from nomal and experimental rats were analyzed based on Agilent Rat 4 × 44 K whole genome microarray, the accumulation of high-throughput gene expression profile data has greatly promoted the application of genome microarray in functional gene studies and in which 2,334 differentially expressed genes were screened. However, a single gene cannot account for complex biological functions, as certain biological processes are clear-cut by the cooperation of a series of genes. Our results manifested that multiple genes and pathways involved in pathogenesis of CGN. Hierarchical clustering analysis of the 2,334 differentially expressed genes showed that they were exsited obviously distinguished between control group and experimental model group, in particular, Fos

(degree=69), Myc (degree=65), Aldh2 (degree=51), Dpyd (degree=50), Mthfd1 (degree=48), Kng1 (degree=43), Rac2 (degree=41) exhibited higher degrees of connectivity in interaction network of differentially expressed genes, which suggested that these genes may play an important role in the process of CGN.

Cell cycle promoted differentially expressed genes

Hub gene, the Fos gene family, was consisted by 4 members: Fos, FOSB, FOSL1, and FOSL2 (Jeong et al., 2015). These genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1. Prior researches showed that AP-1 as a transcription factor can regulate the expression of TNF-α, IL-6, IL-8, MIP-1α, MCP-1, ICAM-1 (intercellular adhesion molecule-1), and VCAM-1 by phosphorylation of MAPK, T and B cell receptor signaling pathway(Wu et al., 2015). As such, Fos gene family has been implicated as regulators of cell proliferation, differentiation, transformation and inflammation, which participates in the inflammation of CGN (Glover et al., 2015). Myc is a highly pleiotropic transcription factor whose deregulation promotes multiple diseases, not only can promote cell proliferation but also activate the transcription of chemokines (Hofmann et al., 2015). Rac2, a ras-related guanosine triphosphatase mainly expressed in hematopoietic cells, is a crucial molecule regulating a diversity of functions of mast cell, macrophage, and neutrophil. Recent research demonstrates that in some diseases, such as cancer, inflammation, Myc and Rac2 can induce inflammation factor response, which can raise mastocyte (Sima et al., 2014).

Phosphatidylinositol 3-kinase phosphorylates the inositol ring of phosphatidylinositol at the 3-prime position. PIK3R1, a regulatory subunit of phosphoinositide-3-kinase, mediates binding to a subset of tyrosine-phosphorylated proteins through its SH2 domain, which involved in T cell receptor signaling pathway, PI3K-AKT signaling pathway, and promoting cell multiplication, differentiation, inhibiting apoptosis. Meanwhile, it can up-regulate the TNF-α and activate inflammatory cells. Researches have shown that PI3K-AKT signaling pathway can also enhance NF-κB signaling pathway, which regulates the expression of IL-1, IL-6, IL-8, ICAM-1 and NO (Costa and Engelman, 2014; Chapman et al., 2015), participates in inflammatory immune cells multiplication and aggravates the development of inflammation in CGN. Upregulation of Fos, Myc, ICAM-1, Rac2, PIK3R1 were observed in

this study, which was consistent with the previous studies. We presumed that CGN might up-regulate the expression of Fos, Myc, ICAM-1, Rac2, PIK3R1, subsequently inducing the changes in the stability of the genome, as well as inducing B and T lymphocyte cell transformation and abnormal proliferation.

Upregulation of several cell cycle related genes were observed in this study, including Anxa1, Lgals3. Annexins are a family of structurally related proteins whose common property is calcium-dependent binding to phospholipids. There are at least ten different annexins in mammalian species. Annexins do not contain signal peptides, yet some annexins (A1, A2 and A5) appear to be secreted in a physiologically regulated fashion. Annexin a1 (Anxa1) is a calcium/phospholipid-binding protein which promotes membrane fusion and involves in endocytosis. Additionly, it is an effector of the resolution of inflammation, and the expression of anxa1 was up-regulated by cytokine IL-6. As such, IL-1β was found to be regulated by anxa1 in the cells sensitive to anxa1 (Bruschi et al., 2014; Locatelli et al., 2014). Galectin-3 is a member of the lectin family, of which 14 mammalian galectins have been identified. Galectin-3 is approximately 30 kDa and, like all galectins, contains a carbohydrate-recognition-binding domain (CRD) of about 130 amino acids that enable the specific binding of β-galactosides. Galectin-3 is encoded by a single gene (Arar et al., 1998). Lgals3, located on chromosome 14, locus q21–q22. It is expressed in the nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space. This protein has been shown to be involved in the following biological processes: cell adhesion, cell activation and chemoattraction, cell growth and differentiation, cell cycle, and apoptosis. Given galectin-3's broad biological functions, it has been demonstrated to be involved in cancer, inflammation and fibrosis(Hu et al., 2011; Han et al., 2014). We presumed that during the process of CNG, a series of cell cycle related genes might be up-regulated, thus inducing the development of inflammation.

Inflammatory response promoted differentially expressed genes

HK as the expression product of High-molecular-weight kininogen (Kng) was involved in intrinsic coagulation pathway, for instance, its activation products HKa and BK both have pro-inflammatory role. BK and HKa as the inflammatory factors, which can promote the vasodilatation, increase vascular permeation, stimulate monocytes to secreting cytokines and

chemotactic factor, such as IL-1β, TNF-α, IL-8, MCP-1, activate inflammation cell expression of adhesion molecules, like Ptprc, Itgb7, and recruite leukocytic aggregation (Sainz et al., 2005; Langhauser et al., 2012). Egr1 as an important nuclear transcription factor is involved in a large number of the developmet of inflammatory diseases. Recent studies showed that in the hepatic injured rats model, Egr1 can induce the increasing of inflammatory factors, and then lead to the inflammation of hepatocytes and hepatocellular damage (Zhang et al., 2013; Ponti et al., 2014). We hypothesize that the regultion of different pathogenic factors through specific signaling pathways in the inflammatory diseases, such as CGN, Egr1 may be the most important pivot of the complex signal-network. Egr1 serves as an activator that assembles signals from upstream pathways and effects the expression of downstream target genes that related to the inflammation and further magnifies inflammatory response.

The development and functions of B lymphocyte are adjusted by a number of signaling pathways, some emanating from the B-cell antigen receptor (BCR) (Campa et al., 2016). Hub gene, the spleen tyrosine kinase (Syk), plays a central role in the activation of the BCR, its expression in epithelial cells could indeed induce cell proliferation by activating the phosphoinositide 3-kinase (PI3K)–Akt–NF-κB survival pathway, and regulate the expression of IL-8, IL-2, 1L-6, VCAM, ICAM-1 (Zeng et al., 2014; Hossen et al., 2015). NF-κB signaling pathway which has been shown not only can regulate cell apoptosis and immune response (Xiao et al., 2014), but also induce the expression of IL-8, that would cause catabolism increasing of heparan sulfate proteoglycan (HSPG) in the glomerular basement membrane and subsequent damage of glomerulus static barrier. Furthermore, plasma-albumin was leakaged from glomerular basement membrane and finally lead to proteinuria. Therefore, we hypothesize that during the process of CGN, up-regulation of Syk could induce the inflammatory reaction and proteinuria.

RUNX1 (Runt-Related Transcription Factor 1) is a protein coding gene. Diseases associated with RUNX1 including platelet disorder, familial, myeloid malignancy and familial platelet disorder with propensity to acute myelogenous leukemia. RUNX1 is a transcriptional factor of SLC22A4, however, function of SLC22A4 remians unclear. It is reported that SLC22A4 might be related to inflammation and immunoreaction, when it was expressed in leukocyte and erythrocyte(Orozco et al., 2006). Chemokine (C-C motif) ligand 6

(CCL6) is a small cytokine belonging to the CC chemokine family. In mice, CCL6 is secreted by neutrophil and macrophage lineages. It could recruit numerous inflammatory cells to the lesion by which contributes to inflammatory response (Ma et al., 2004). The high expression of Kng1, Ptprc, Itgb7, Egr1, Syk, Runx1 and Ccl6 in this study suggested that CGN is caused by various inflammation responses via multiple signal pathways.

Anti-inflammatory response and metabolism function hindered differentially expressed genes

The activation of peroxisome proliferators is thought to be mediated via specific receptor, so called Ppar-αs, which belongs to the steroid hormone receptor superfamily. Ppar-αs affects the expression of target genes involved in cell proliferation, cell differentiation and in immune and inflammation responses (Yamamoto-Furusho et al., 2014). Three closely related subtypes (alpha, beta/delta, and gamma) have been identified. These gene encode the subtype Ppar-α, which is a nuclear transcription factor (Tyagi et al., 2011). Currently, the role of Ppar-α in the inflammation and its regulation mechanisms have attracted increasing attention. It could inhibited the expression and secretion of quite a number of cytokines by restraining the NF-κB, STAT, C/EBP and AP-1 pathways and interdicting adhesion and migration of inflammatory cells, thus it plays an important role in the development of anti-inflammatory and anti-proliferation (Zhang et al., 2014). The low expression of Ppar-α in this study suggested that inhibition of Ppar-α could hinder the normal immune surveillance and clearance of the anti-inflammatory cytokine with multifarious factors through multiple ways in CGN.

Kidney function is to excrete metabolic products, adjust the balance of water, electrolyte and acid-base, and secret active substances to maintain homeostasis. Prior researches have demonstrated that there was a certain degree disorder of metabolism in the later period of CGN with injured kidney functions (Kushnereva et al., 1976; Litovkina et al., 2014; Pulin et al., 2014). For instance, cells and tissues were suppressed to utilize the carbohydrate, additionally, disordered amino acid and fat metabolism were also observed. Accordingly, all of these resulted in hematuresis, azotemia, generation of edema and increasing of serum creatinine, blood urea nitrogen. Furthermore, aperture of glomerular filtration membrane was enlarged, subsequently, the breakage and static electricity protective screen were weakened on account of metabolism disordered, finally plasma protein filter out that generate to proteinuria.

Gene function annotation showed that down-regulated genes, such as Aldh2, Dpyd, Mthfd1, Gldc, Igf1, Cyp1a1, et al., which were mainly involved in metabolism of small molecule, organic acid, carboxylic acid, glycine, serine and threonine. We found that these genes were significantly affected in CGN. Hence, we speculated that the inhibited expression of these genes would hinder the normal metabolism, which may ultimately induce the typical pathological features in the CGN.

5. Conclusion

In summary, CGN was a complicated process with many genes and pathways involved. Global gene expression profile analysis showed that there existed significant differences in the gene expression patterns between the nomal and experimental glomerular tissues of rats. The pathogenesis of CGN may be related to promotion of cell cycle and mitosis, dysregulation of cytokine secretion and inflammatory response, inhibition of anti-inflammatory response and abnormal metabolism function. Furthermore, highly represented genes are hub genes, Fos, Syk. Our present results indicated 27 key regulatory genes. The function and specific effect of these key regulatory genes in CGN were screened and needed to be further studied. This study revealed crucial information on the molecular mechanisms of CGN and laid a foundation for subsequent gene validation and functional studies, which could contribute to the development of novel diagnostic markers and provide new therapeutic targets for the clinic treatment of CGN.

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Authors' contributions and conflicts

Jia-rong Gao conceived and designed the study. Jun-mei Song and Shuang-zhi Xu performed the experiments. Xiu-juan Qin wrote the paper. Hui Jiang and Ting Wang reviewed and edited the manuscript. All authors read and approved the manuscript. The authors declare that they have no conflicts of interest.

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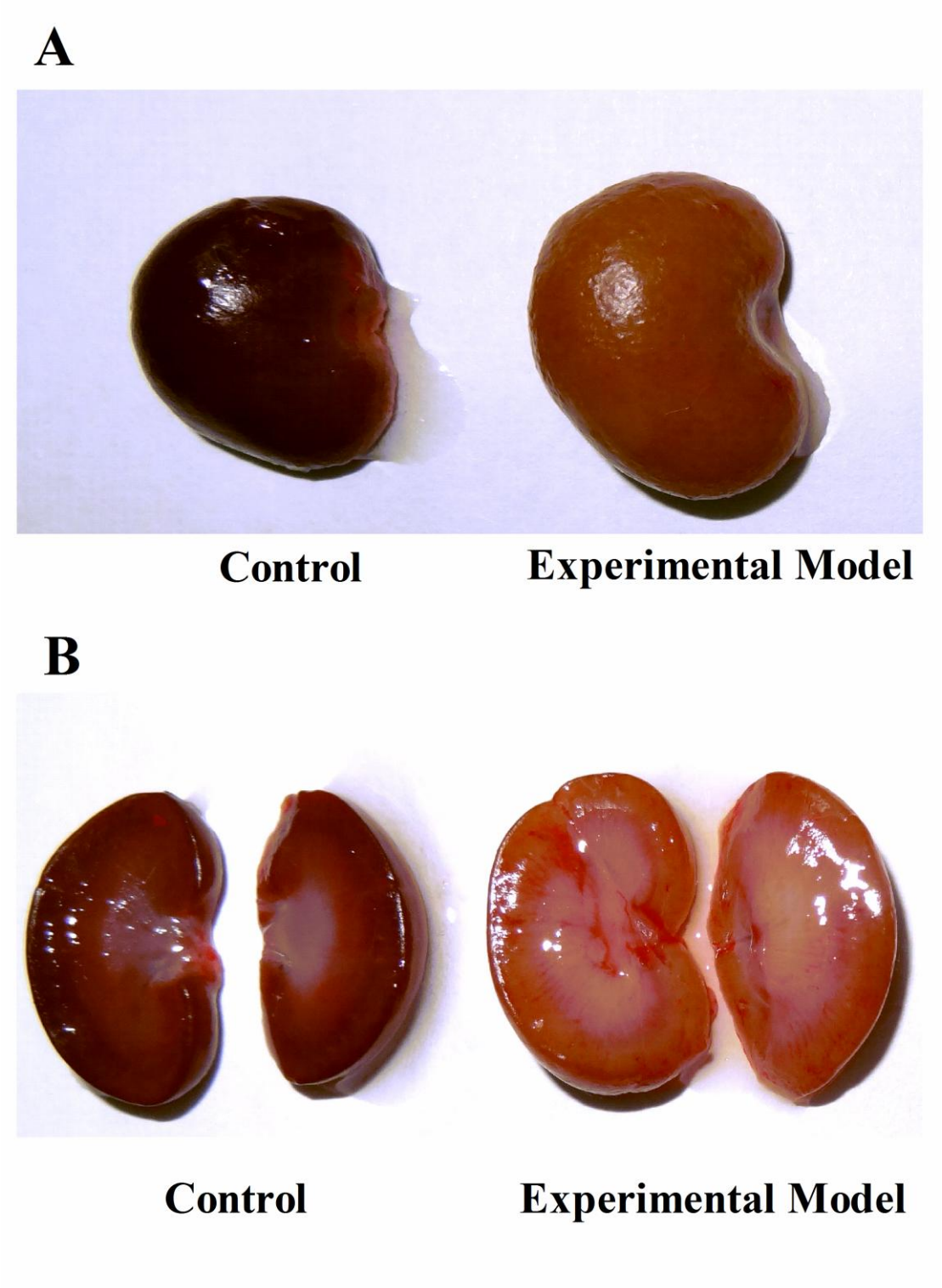


Fig. 1

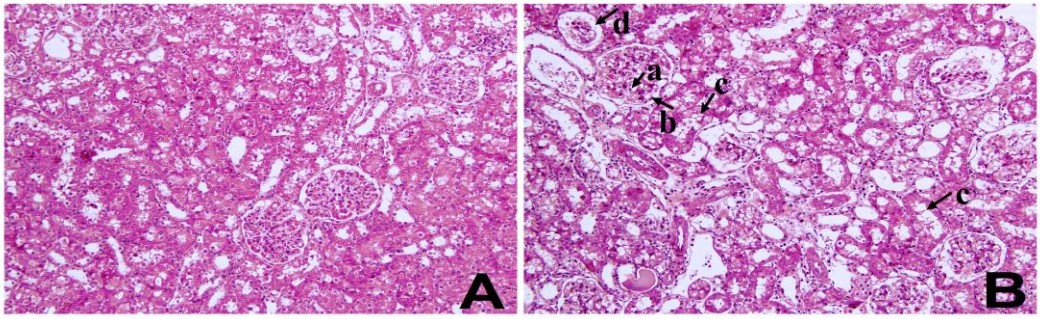
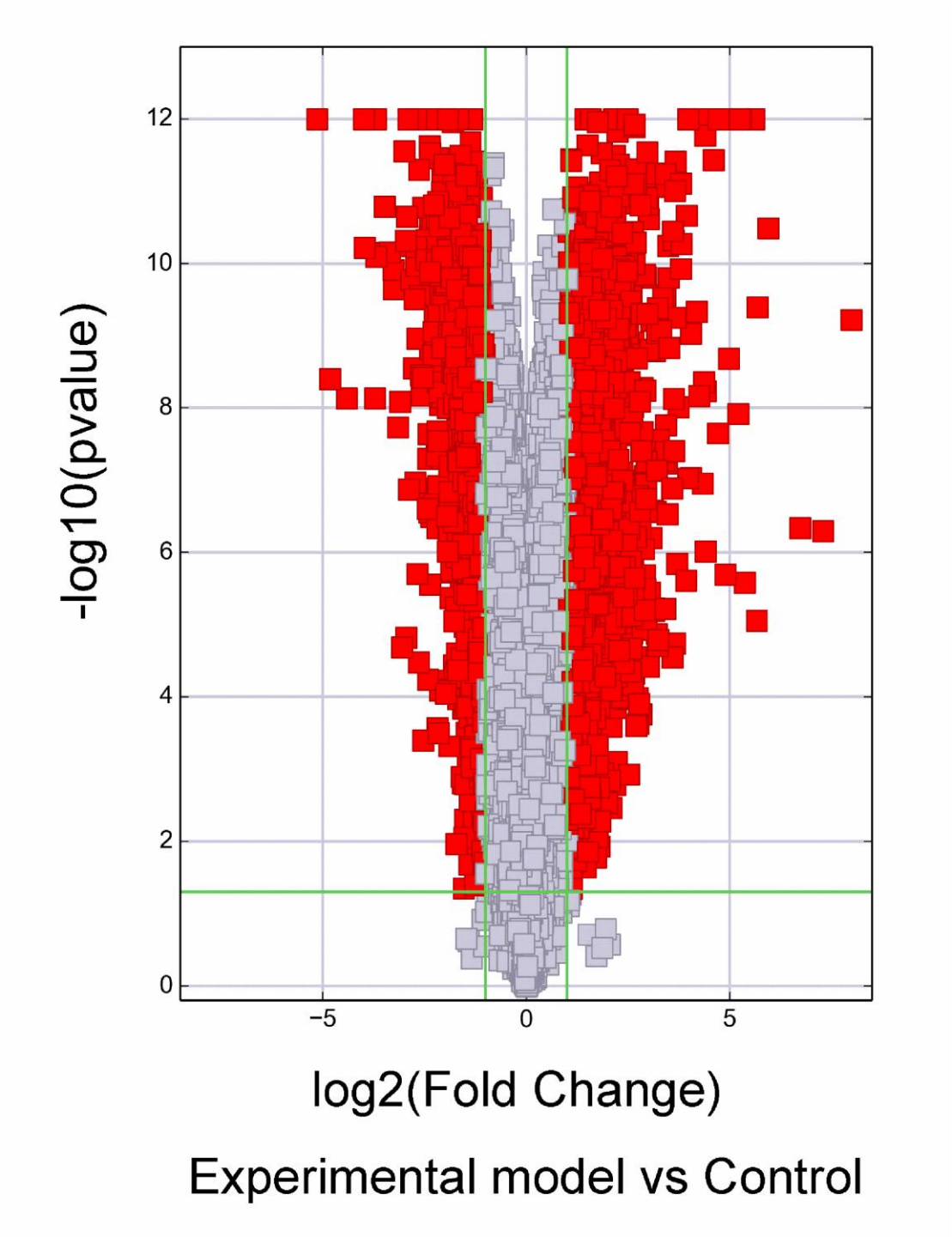


Fig. 2



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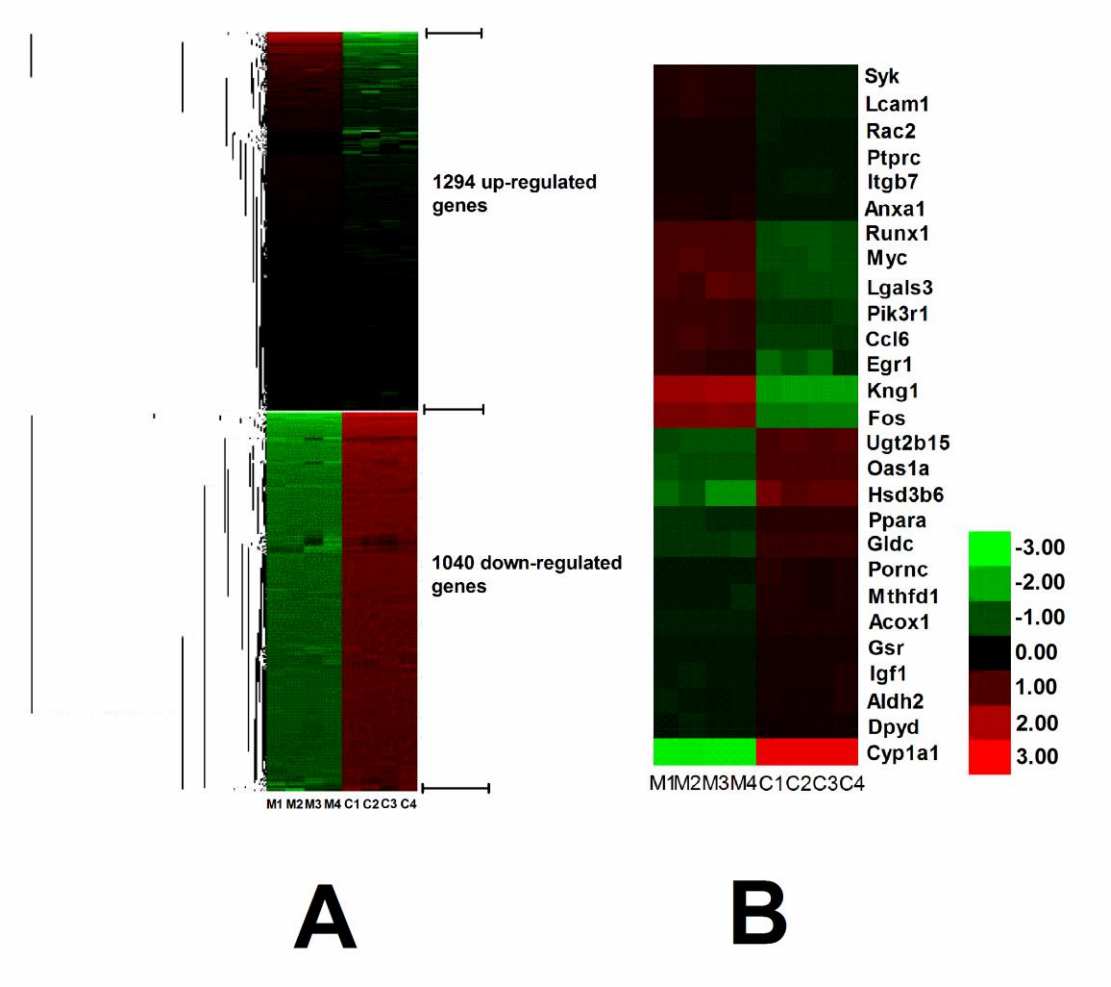


Fig. 4

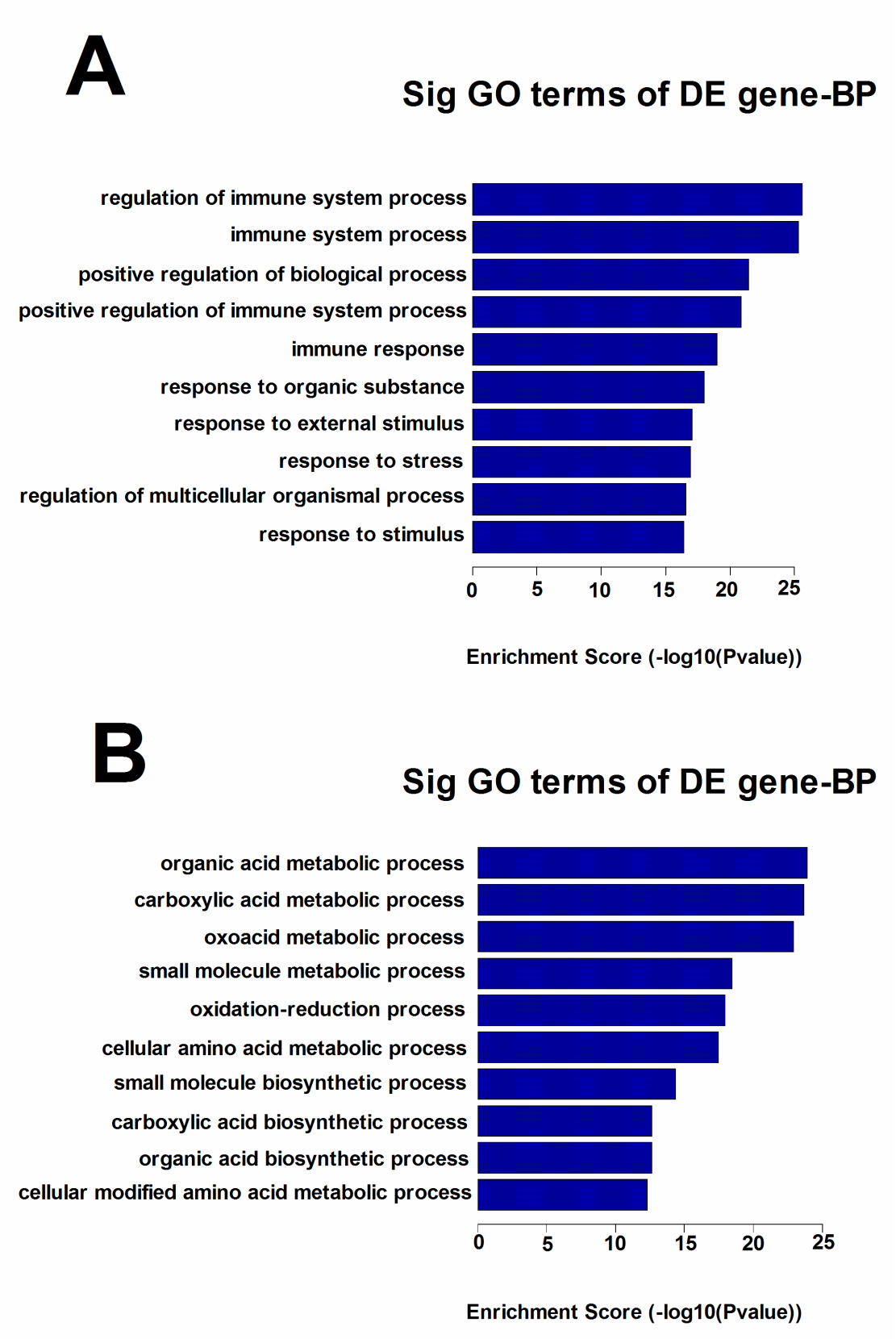
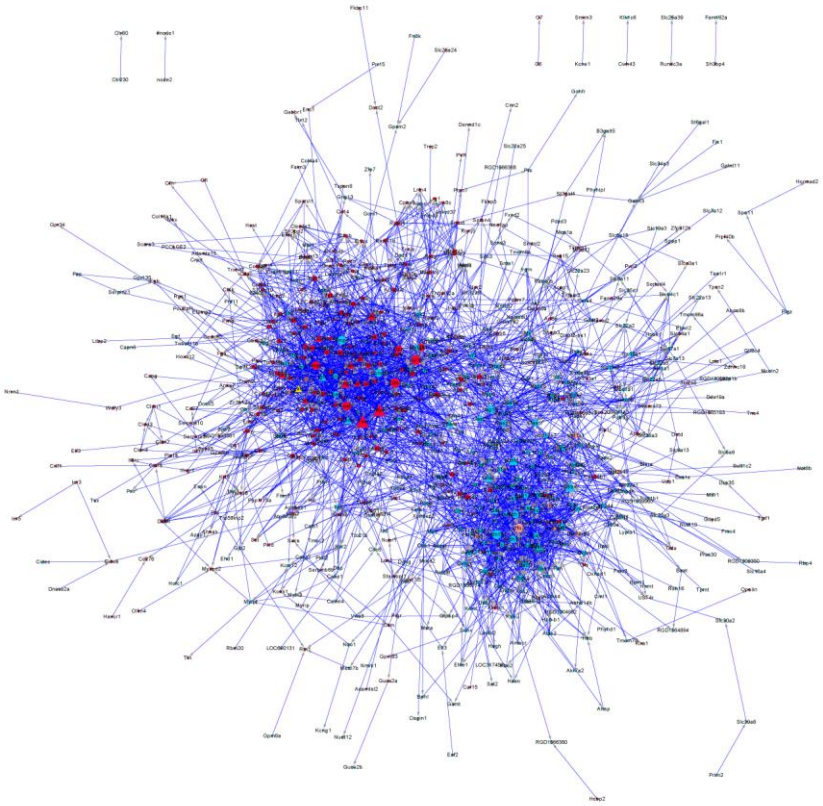


Fig. 5



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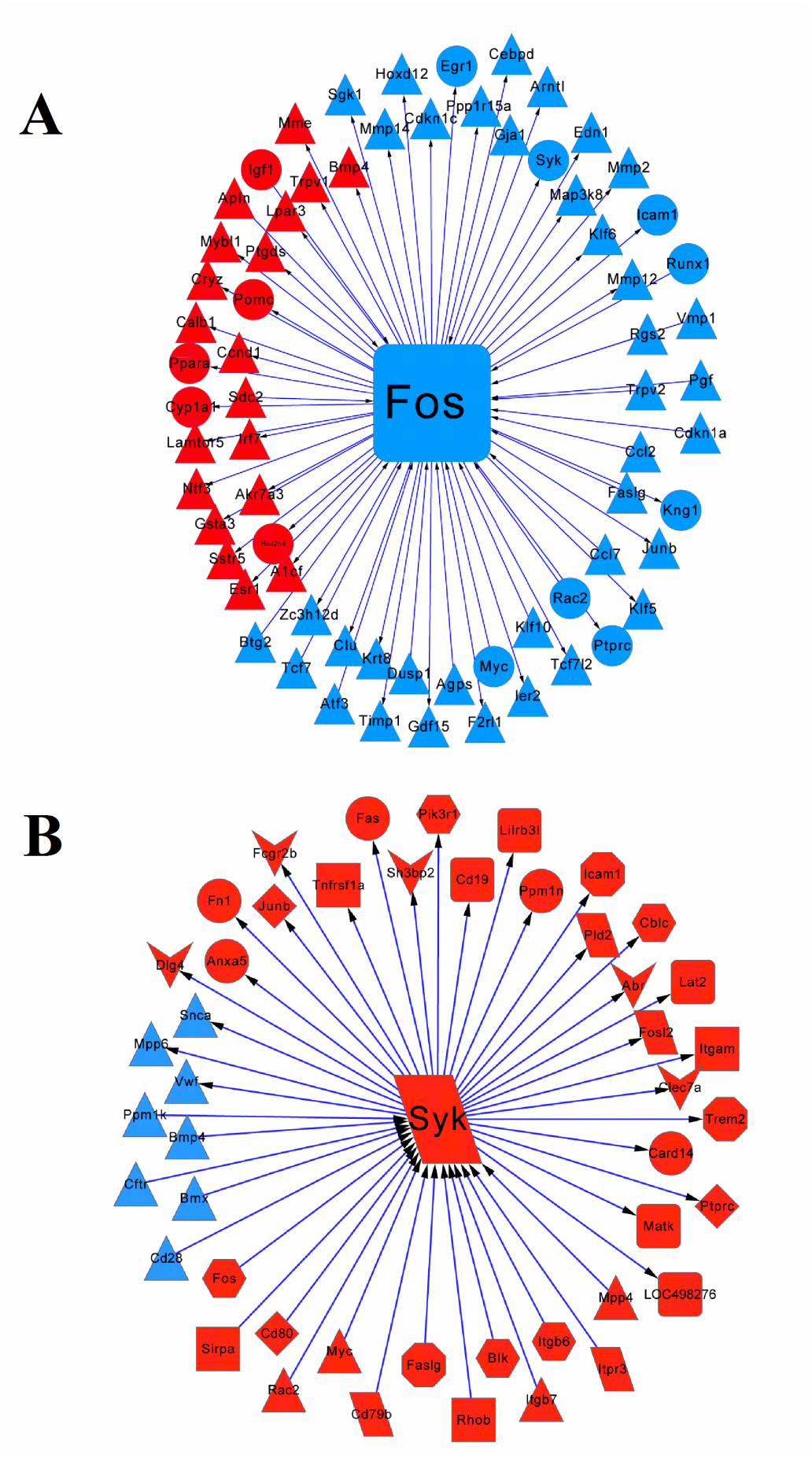

Fig. 7

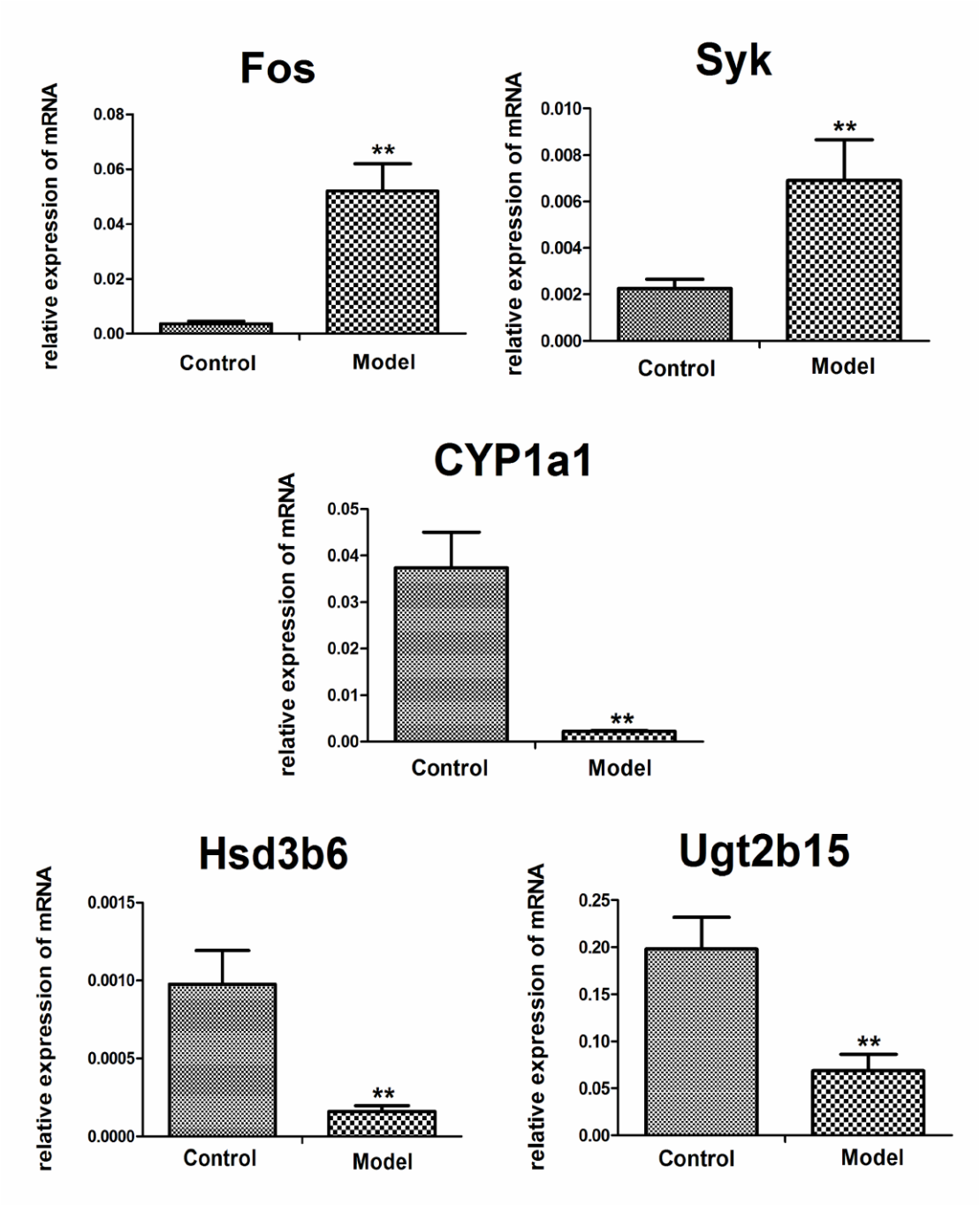

Fig. 8

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.

Table 2. Functional annotation of 27 differentially expressed genes

Notes	Fold change	Edges	Gene Annotation
Up-regulated genes			
Fos	8.0297296	69	involved in AP-1 and BCR/TCR signal pathway
Myc	4.1438585	65	Cell cycle
Kng1	12.1121263	43	inflammatory response
Rac2	2.0969104	41	positive regulation of cell proliferation
Pik3r1	3.2847732	33	involved in TNF signaling pathway, PI3K-Akt signaling pathway and responsible for a range of cell functions
Egr1	4.7316341	32	positive regulation of glomerular metanephric mesangial cell proliferation
Icam1	2.4268932	32	cell adhesion molecules (CAMs)
Syk	2.4019165	32	regulation of immune response
Anxa1	2.2180252	31	regulation of G1/S transition of mitotic cell cycle
Lgals3	4.1048343	31	cell differentiation
Ptprc	2.0039476	30	cell adhesion molecules (CAMs)
Runx1	4.2135894	27	pathways in cancer
Itgb7	2.1661526	27	Cell adhesion molecules (CAMs) and ECM-receptor interaction
Ccl6	3.6788905	22	cytokine-cytokine receptor interaction and immune response
Down-regulated genes			
Aldh2	2.0629359	51	small molecule metabolic process
Dpyd	2.0000327	50	organic acid metabolic process
Mthfd1	2.3394123	48	carboxylic acid metabolic process
Gldc	3.1550011	41	Glycine, serine and threonine metabolism
Ppara	2.7997117	37	PPAR signaling pathway
Igf1	2.3847533	37	organic acid metabolic process
Pomc	2.2407415	35	oxidation-reduction process
Oas1a	4.0912425	33	small molecule metabolic process
Gsr	2.1289038	31	Glutathione metabolism
Acox1	2.3054001	26	Biosynthesis of unsaturated fatty acids
Cyp1a1	35.2260987	24	Tryptophan metabolism
Ugt2b15	4.511544	18	Pentose and glucuronate interconversions
Hsd3b6	6.4022918	14	Steroid hormone biosynthesis

Note: Annotation and analysis results showed that the up-regulated genes were mainly related to cell cycle, cell proliferation, cytokine-cytokine receptor interaction, immune response, inflammatory response and etc.; the down-regulated genes were mainly related to various kinds of metabolic process.

Highlights

- It was the first time to explor pathogenesis of CGN use whole genome microarray.
- 27 genes may be the key controlled genes in the pathogenesis of CGN.
- Fos and Syk were considered as potent hub genes.
- Pathogenesis of CGN may be related to cell proliferation, disordered inflammatory response, ect.