Candidate gene and mechanism investigations in congenital obstructive nephropathy based on bioinformatics analysis

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Abstract. The aim of the present study was to explore the candidate genes, chemicals and mechanisms of congenital obstructive nephropathy (CON). The gene expression profiles of GSE48041, including 24 kidney tissue samples from megabladder (mgb-/-) mouse were downloaded from the Gene Expression Omnibus database. Samples were divided into 4 groups: Control, mild, moderate and severe. Differentially expressed genes (DEGs), protein-protein interaction network, Kyoto Encyclopedia of Genes and Genomes pathways and transcription factor (TF)-target gene analyses were performed on Set 1 (mild, moderate and severe groups), while Gene Ontology (GO) function enrichment analysis and chemical investigation were performed on Set 2 (severe group). A total of 187 and 139 DEGs were obtained in Set 1 and Set 2, respectively. Chemical carcinogenesis [enriched by genes such as *Carbonyl reductase 1* (*CBR1*)] was one of the most prominent pathways in Set 1. GO analysis for Set 2 revealed that DEGs were mainly assembled in functions such as cellular response to interleukin-1 and cellular response to tumor necrosis. Furthermore, genes such as *Fos Proto-Oncogene* (*FOS*) were co-regulated by TFs including *RNA polymerase II subunit A (Polr2a)* and *serum response factor (Srf)*. Chemical cyclosporine served the most important role in Set 2 by targeting several DEGs in Set 2. DEGs such as *CBR1* and *FOS*, TFs including *Polr2a* and *Srf,* and pathways such as chemical carcinogenesis may serve important roles in the process of CON. Interleukin-1 and tumor necrosis function may be novel targets for CON gene therapy. Furthermore, cyclosporine may be a promising option for future CON therapy.

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Introduction

Congenital obstructive nephropathy (CON) is a main cause of kidney insufficiency in child and infant (1). It is associated with various diseases, such as prune belly syndrome (2). As a result, CON generates a huge social burden especially in the morbidity and mortality (2). The different challenges associated with CON in areas like diagnose and therapy highlight the importance of the molecular mechanism of CON (3). Thus, the emerging theories of the biology of CON suggest new targets for therapeutic interventions (4).

Identification of novel biomarkers provides prognostic value for this process remains a major goal in the study of kidney disease (5). In renal and urinary tract, previous study showed that disruption of *angiotensin type 2 receptor* (*AGTR2*) induced a huge scale of anomaly (6). Meanwhile, the *Monocyte chemotactic protein 1 (MCP-1)* as well as the *Epidermal growth factor (EGF)* seem to be involved in the pathogenesis of tubulointerstitial damage of CON, and their urine excretion may serve as a powerful prognostic marker for this form renal disease (7). Not only gene, but also pathways enriched by differentially expressed genes (DEGs) are closed related with CON. Previous study demonstrated that TGF-β signaling pathway plays an important role in regulating kidney injury process following by obstruction (8). In a rat model, Hermens *et al* (9) show that there is an age-dependent role of Wnt signaling pathway in the pathophysiology of CON. Despite of revealing the potential genes or pathways, understanding the molecular mechanism of CON also brings a breakthrough for the novel treatment of CON (10). The development of biomarkers contribute to molecular therapies of CON (11). However, the molecular mechanisms underlying these histologic changes are still poorly defined.

To better understand the pathophysiology of CON, Brian Becknell and his colleagues evaluated the global transcription in kidneys with graded hydronephrosis in the megabladder (mgb2/2) mouse (12). Based on normal, mild, moderate, and severe mouse models, they prove that the development of progressive hydronephrosis can result in renal adaptation, including significant changes in the morphology and potential functionality of the renal urothelium. However, the co-regulated gene in all models, transcription factor (TF) associated with DEGs, gene expression in severe disease status, as well as the potential chemicals for CON treatment are still unclear. In the

current study, a bioinformatics research was designed based on the gene expression profile provided by Becknell *et al* (12). Then, investigations of DEGs, function and pathway enrichment, protein-protein interaction network (PPI) analyses, as well as TF-target gene regulatory network were performed in all disease status. Furthermore, the chemical-gene interaction network in severe models were also investigated. We aimed to explore the potential pathoenesis of CON and provided information about novel gene targets, as well as chemicals for CON treatment.

Materials and methods

Microarray data. Expression data of GSE48041 (12), sequenced on the platform of GPL10787 Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray, were obtained from the Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/). A total of 24 kidney tissue samples from control and megabladder (mgb-/-) mouse aged 23-30 days were included in this dataset, and hydronephrosis was induced by renal ultrasound as previously reported (13). According to the hydronephrosis degrees (www.sfu-urology.org/sfu_hydrone_grading.cfm), animals were divided into 4 groups: Control (n=6), mild (n=7), moderate (n=5) and severe (n=6) according to their disease stages.

Data preprocessing. The preprocessing were performed based on robust multi-array average (RMA) method (14) in Affy package (15) in R (v.3.10.3, bioconductor.org/biocLite.R). Contents of processing included background correct microarray expression intensities, normalize the expression within each array, and expression calculation. The probe ID was convert to the gene symbol based on the chip platform annotation files.

DEGs analysis. Classical Bayes method in Linear Models for Microarray Data (limma) package (16) of R was used to reveal DEGs by comparing expression value among samples from three comparisons: Mild vs. control, moderate vs. control, and severe vs. control. P-value <0.05 and $|\log_2$ log fold change (FC)$|\geq 1$ were selected as the cut-off criteria for DEG screening.

VennPlot analysis of DEGs. VENNY (v.2.1) (17) is an online tool used for Venn diagram analysis based on gene expression value. The number of up-regulated, down-regulated, and contra-regulated genes can be marked by VennPlex. In the present study, VennPlex software was used to investigate the difference of DEGs among 3 comparative groups. Then, the co-regulated DEGs (Set 1) and severe group DEGs (Set 2) were selected for further investigation.

Enrichment analysis of DEGs. The Database for Annotation, Visualization, and Integrated Discovery (DAVID; v.6.8) (18) provides a synthetic set of functional annotation tools for users for revealing the biological significances among huge scale of genes. Gene Ontology (GO) function enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis were performed using DAVID online tool (19). GO functional categories including molecular function (MF), biological process (BP), and cellular component (CC) (20). In the present study, GO functions assembled by DEGs in severe group, as well as KEGG pathways enriched by the co-regulated DEGs were investigated. P-value <0.05 and enriched gene numbers (count) ≥2 were considered as cut-off values.

PPI network investigation. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; v.10.0) is an online tool for the predication of PPI based on a large scale of biological database (21). In the present study, STRING was used to predict the interaction relations between DEGs corresponding coding proteins. PPI were revealed based on the STRING database with score (median confidence)=0.4, and nodes represent DEGs in the PPI interaction. The degree was defined as the number of the connections for the target proteins. Cytoscape software (v.3.2.0) (22,23) was used for the visualization of PPI network obtained above. Then, the CytoNCA software (v.2.1.6, apps.cytoscape.org/apps/cytonca) (24) was used for the nodes topological analysis (Parameter: Without weight). Furthermore, the sub-networks with score >4.5 were identified by MOCDE (v.1.4.2; apps.cytoscape.org/apps/MCODE) (23) plugin in Cytoscape software.

TF-target gene regulatory network construction. TF is an important class of participators in the regulation of gene expression. Analysis of TF binding sites is of great significance for the study of gene regulation system. In the current research, the TFs-co-regulated DEGs network was constructed with iRegulon (25) (v.1.3, apps.cytoscape.org/apps/iRegulon). The Parameters of this analysis were set as: Minimum identity between orthologous genes=0.05, and maximum false discovery rate (FDR) on motif similarity=0.001. TF-target gene relations with normalized enrichment score (NES) >4 were considered as the meaningful results.

Chemical-DEGs interaction network construction. Comparative Toxicogenomics Database (CTD) provides relationships, such as chemical-gene/protein interactions, chemical-disease as well as gene-disease (26). Relationship between disease (Nephrosis, congenital) associated chemical-gene or interactions was revealed by CDT. Then Chemical-DEGs interaction network was constructed by Cytoscape software.

Results

DEGs investigation in mild, moderate and severe groups. Considering a huge amount of calculation in gene expression profile, the original data was analyzed and filtered. The results revealed that totally 220 up-regulated DEGs and 225 down-regulated DEGs were obtained in the mild group. In the moderate group, there were 375 up-regulated DEGs and 142 down-regulated DEGs screened out. Furthermore, a total of 484 up-regulated DEGs and 280 down-regulated DEGs uncovered in the severe group.

VennPlot analysis. VennPlot for DEGs in the mild, moderate, and severe groups were investigated in the present

Table I. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis for common DEGs in the mild, moderate and severe groups.

Regulation	Pathway ID	Pathway name	Count	P-value
ALL	mmu00980	Metabolism of xenobiotics by cytochrome P450	9	$2.84x10^{-7}$
	mmu00830	Retinol metabolism	10	$3.16x10^{-7}$
	mmu05204	Chemical carcinogenesis	10	$4.21x10^{-7}$
	mmu00982	Drug metabolism-cytochrome P450	8	$5.09x10^{-6}$
	mmu00053	Ascorbate and aldarate metabolism	5	$1.64x10^{-4}$
	mmu01100	Metabolic pathways	27	$4.04x10^{-4}$
	mmu00040	Pentose and glucuronate interconversions	5	$5.13x10^{-4}$
	mmu00860	Porphyrin and chlorophyll metabolism	5	$8.48x10^{-4}$
	mmu00983	Drug metabolism-other enzymes	5	$1.93x10^{-3}$
	mmu00140	Steroid hormone biosynthesis	6	$2.12x10^{-3}$
	mmu00590	Arachidonic acid metabolism	6	$2.34x10^{-3}$
	mmu04668	TNF signaling pathway	6	$5.60x10^{-3}$
	mmu04976	Bile secretion	5	$6.42x10^{-3}$
	mmu00071	Fatty acid degradation	4	$1.45x10^{-2}$
	mmu05140	Leishmaniasis	4	$2.93x10^{-2}$
	mmu04610	Complement and coagulation cascades	4	$4.53x10^{-2}$
UP	mmu04668	TNF signaling pathway	6	$3.59x10^{-4}$
	mmu05140	Leishmaniasis	4	$5.65x10^{-3}$
	mmu04010	MAPK signaling pathway	6	$1.39x10^{-2}$
	mmu05204	Chemical carcinogenesis	4	$1.52x10^{-2}$
	mmu05152	Tuberculosis	5	$1.74x10^{-2}$
	mmu05166	HTLV-I infection	6	$2.01x10^{-2}$
	mmu05142	Chagas disease (American trypanosomiasis)	4	$2.06x10^{-2}$
	mmu04978	Mineral absorption	3	$2.26x10^{-2}$
DOWN	mmu00830	Retinol metabolism	8	$1.36x10^{-7}$
	mmu00053	Ascorbate and aldarate metabolism	5	$6.48x10^{-6}$
	mmu00980	Metabolism of xenobiotics by cytochrome P450	6	$1.03x10^{-5}$
	mmu00982	Drug metabolism-cytochrome P450	6	$1.20x10^{-5}$
	mmu01100	Metabolic pathways	18	$1.73x10^{-5}$
	mmu00040	Pentose and glucuronate interconversions	5	$2.11x10^{-5}$
	mmu00860	Porphyrin and chlorophyll metabolism	5	$3.57x10^{-5}$
	mmu00140	Steroid hormone biosynthesis	6	$4.64x10^{-5}$
	mmu05204	Chemical carcinogenesis	6	$6.07x10^{-5}$
	mmu00983	Drug metabolism-other enzymes	5	$8.52x10^{-5}$
	mmu04146	Peroxisome	4	$6.63x10^{-3}$
	mmu00071	Fatty acid degradation	3	$2.17x10^{-2}$

Count values indicate the number of genes enriched in a pathway. $P<0.05$ and the gene number (count) ≥2 were considered the threshold values for significant differences. DEGs, differentially expressed genes; UP, upregulated; DOWN, downregulated; ALL, up- and downregulated.

study (Fig. 1). The results revealed 187 co-regulated DEGs in Set 1, including 87 up-regulated DEGs and 100 down-regulated DEGs. In Set 1, *Serpina6*, *Hpd*, and *Nr4a1* were the 3 most outstanding up-regulated genes, while *Proz, 4122401K19Rik*, and *Nudt19* were the 3 most outstanding down-regulated genes. Meanwhile, there were 139 DEGs in Set 2, including 121 up-regulated DEGs and 18 down-regulated DEGs. In Set 2, *Sprr2f*, *Lcn2* and *Saa2* were the 3 most outstanding up-regulated gene, while *Symbol*, *Vps8* and *Ang2* were the 3 most down-regulated gene.

KEGG and GO enrichment analysis. The KEGG analysis of DEGs in Set 1 showed that a total of 16 pathways were significantly enriched (Table I). The up-regulated DEGs were mostly enriched in TNF signaling pathway (mmu04668, $P=3.59x10^{-4}$), while the down-regulated DEGs were mainly assembled in Retinol metabolism (mmu00830, $P=1.36x10^{-7}$). Among these pathways, Chemical carcinogenesis (mmu05204, Gene: *CBR1*, *UGT2B38*, *GSTA2*, *UGT2B37* and *UGT2B36*) was the common pathway in all 3 group of Set 1. The detail pathway map of Chemical carcinogenesis was showed in Fig. 2.

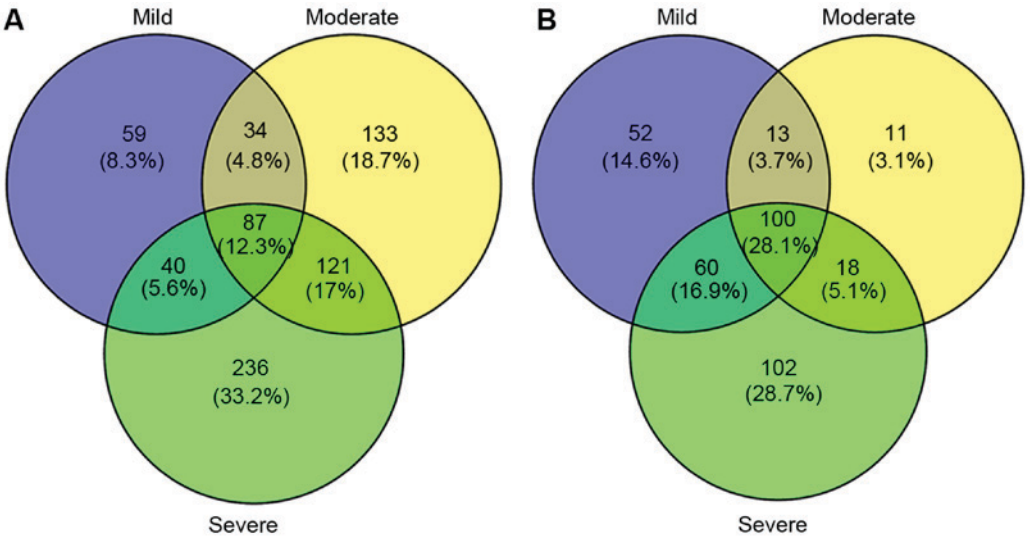

Figure 1. Colorful VennPlot showing the intersection of significant down- and upregulated genes among the mild, moderate and severe group. (A) VennPlot for the upregulated DEGs. (B) VennPlot for the downregulated DEGs. Purple represents the mild group; yellow represents the moderate group; and green represents the severe group. DEGs, differentially expressed genes.

GO functional analysis was performed in the severe group DEGs. With count number >10, the top 10 of BP, CC, and MF functions were revealed, respectively. The DEGs in the severe group was mainly assembled in functions like inflammatory response (BP, GO: 0006954, P=1.43x10^{-3}), cellular response to interleukin-1 (BP, GO: 0071347, P=8.87x10^{-3}), cellular response to tumor necrosis (BP, GO: 0071356, P=6.34x10^{-4}), extracellular region (CC, GO: 0005576, P=3.53x10^{-7}), and cytokine activity (MF, GO: 0005125, P=7.63x10^{-5}). The detail information was showed in Fig. 3.

PPI network and modules analysis. To dig out the potential interactions of the DEGs, PPI network and related modules were constructed based on the protein interactions of DEGs. A total of 102 nodes (*Jun, Fos, Ptgs2, Ugt2b37, Junb, Atf3, Cyp4a12a, Adh1, Ugt2b5, Egr1,* etc.) and 320 interactions were identified in the PPI network of Set 1. With score >4.5, 2 modules were further obtained from the Set 1 PPI network. There were 13 nodes (*Jun, Fos, Ptgs2, Junb, Atf3, Egr1, Dusp1, Nr4a1, Zfp36, Btg2,* etc.) and 60 interactions in module a (score=10), while 8 nodes (*Ugt2b5, Cyp2j13, Acsm3, Cyp4a12b, Cyp4a14, Cyp4b1 Slc22a30* and *Slc7a13*) and 20 interactions contained in module b (score=5.714) (Fig. 4). Furthermore, there were a total of 73 nodes (*Ccl2, Timp1, Acta2, Spp1, Actg2, Thbs1, Edn1, Aldh1a1, Mmp3, Aldh1a7,* etc.) and 143 interactions included in the PPI network of Set 2. With score >4.5, one module was further obtained from the Set 2 PPI network. A total of 5 nodes (*Aldh1a1, Aldh1a7, Aifm3, Hbb-b1* and *Acad9*) and 9 interactions were revealed in the module of Set 2 (Fig. 5).

TF-target gene regulatory network analysis. By using the iRegulon software, 2 TFs (*Polr2a* and *Srf*) were obtained from the Set 1 PPI network. To further investigate the relationship between TFs and their target genes, the TF-target gene network was visualized by cytoscape software. With NES >4, the results showed that there were totally 52 regulatory relations contained in this network. Some genes like *FOS* were co-regulated by both *Polr2a* and *Srf*. The detail information was showed in Fig. 6.

Chemical-gene interaction network analysis. The present study revealed totally 12012 chemical-gene interactions related to Nephrosis or congenital. Among these interactions, totally 60 interactions were matched with the 139 DEGs in Set 2. The results showed that Cyclosporine was the most important chemical that target with either the most number of up-regulated or down-regulated DEGs in Set 2. Detailed information was listed in Table II.

Discussion

CON is the leading cause of chronic kidney disease in child. However, the molecular mechanisms underlying disease progression are still poorly defined. To reveal the potential genes or pathways associated with CON, the present bioinformatics study was performed on mild, moderate, and severe CONs. The results showed that totally 187 and 139 co-regulated DEGs were obtained in Set 1 and Set 2, respectively. KEGG analysis for Set 1 showed that DEGs were mainly enriched in pathways like chemical carcinogenesis. Meanwhile, the GO analysis for Set 2 showed that DEGs were mainly assembled in functions like cellular response to interleukin-1 and cellular response to tumor necrosis. Two modules in Set 1 and one module in Set 2 were revealed based on PPI network analysis. Furthermore, the genes like *FOS* were co-regulated by TFs like *Polr2a* and *Srf*. Cyclosporine was the most important chemical that target with DEGs in Set 2.

Based on the Set 1 (including all disease status like mild, moderate and severe), KEGG pathway analysis, PPI network analysis, and TF-target investigation were carried out in this study to provide a potential mechanism understanding for the process of CON. Chemical carcinogenesis is a process that the cells need genetic and epigenetic variations based on elements including oncogenes and tumor suppressors (27,28). *Carbonyl reductase 1* (*CBR1*) metabolized by the body many

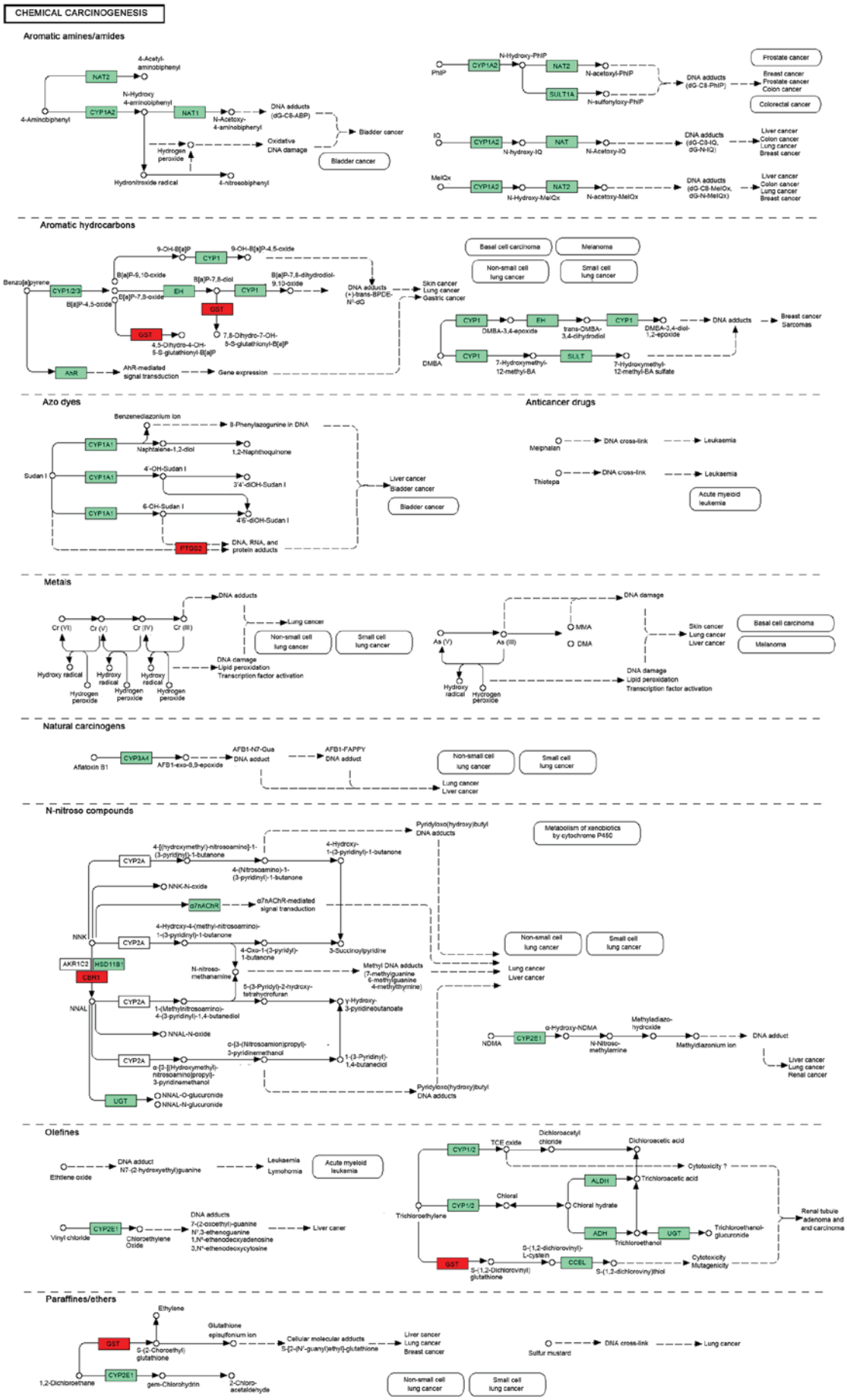

Figure 2. Kyoto Encyclopedia of Genes and Genomes pathway map for chemical carcinogenesis. Red represents the upregulated genes, while green represents the downregulated genes.

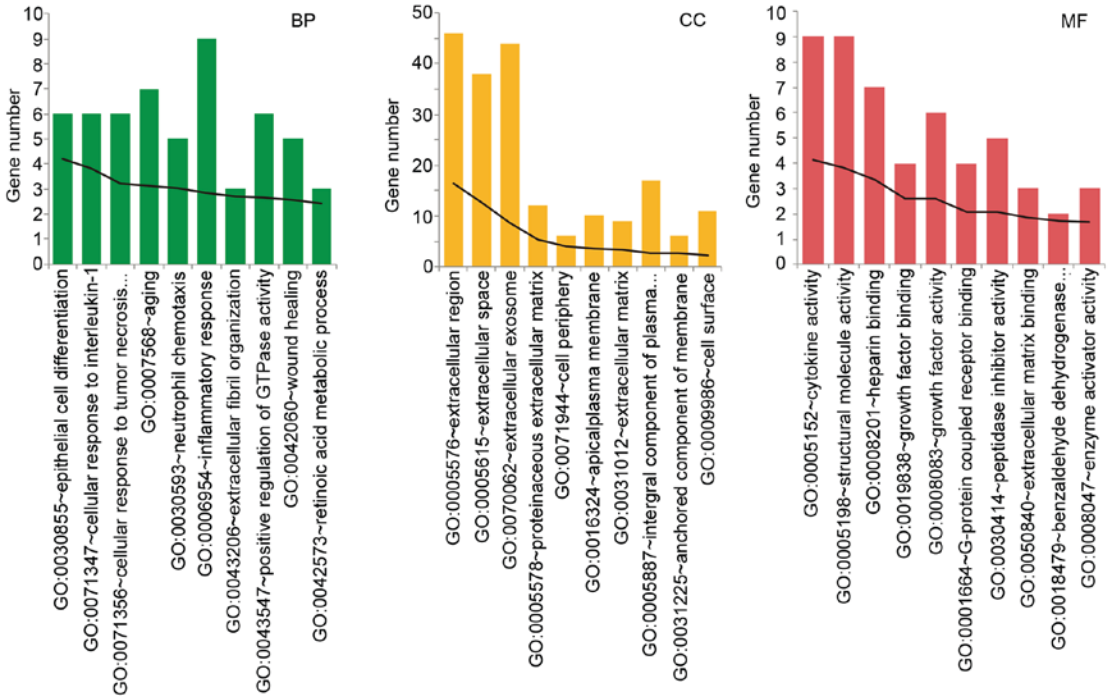

Figure 3. GO enrichment analysis for DEGs in the severe group. The top 10 functions associated with BP. The top 10 functions associated with CC. The top 10 functions associated with MF. The y-axis represents the count number of functions. Fold line represents the -log10 (P-value). GO, gene ontology; DEGs, differentially expressed genes; BP, biological process; CC, cellular component; MF, molecular function.

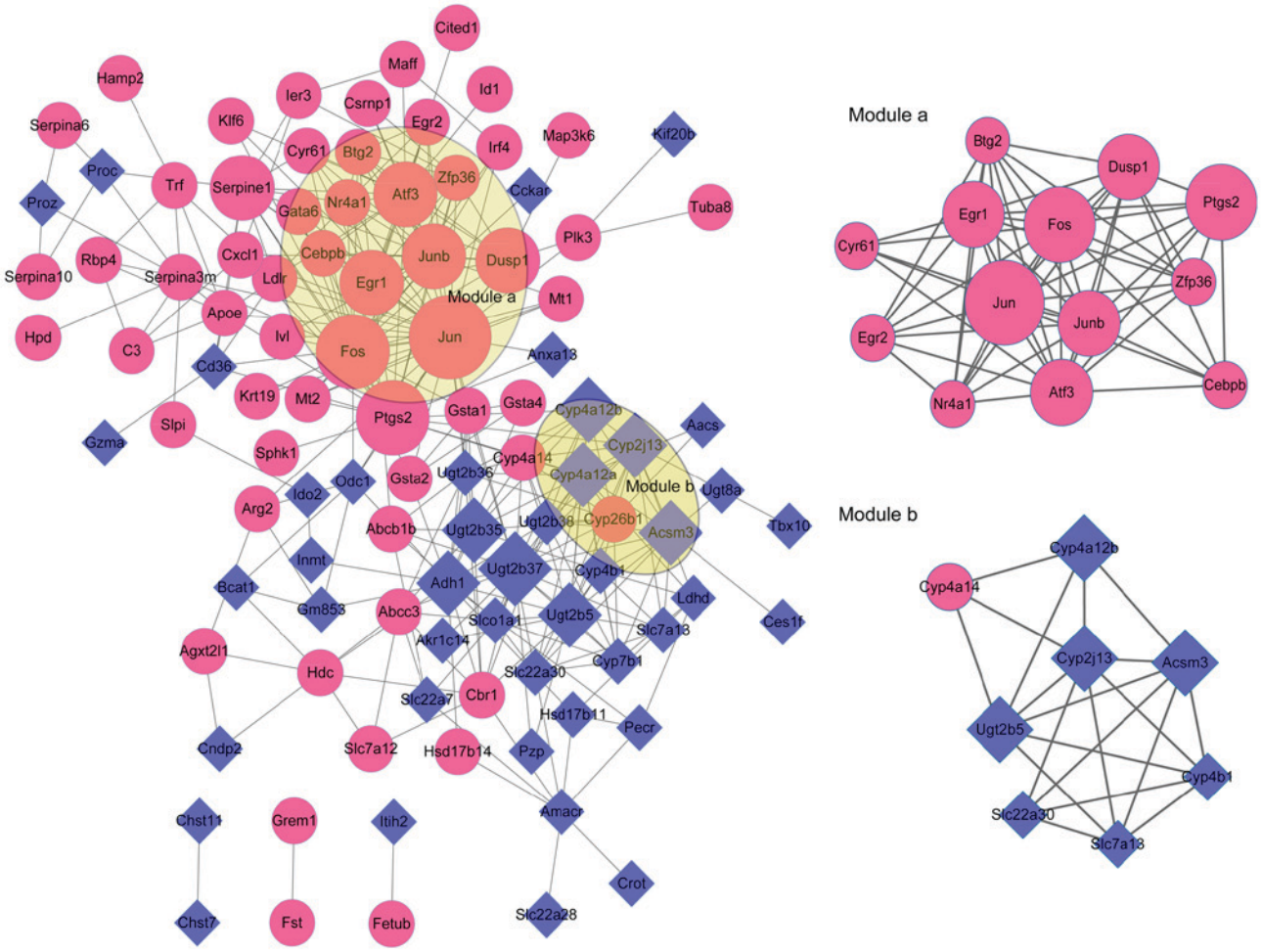

Figure 4. PPI network and modules of common DEGs in the mild, moderate and severe group. Red circles represent the upregulated genes and purple diamonds represent the downregulated genes. A bigger node indicates a higher degree. PPI, protein-protein interaction; DEGS, differentially expressed genes.

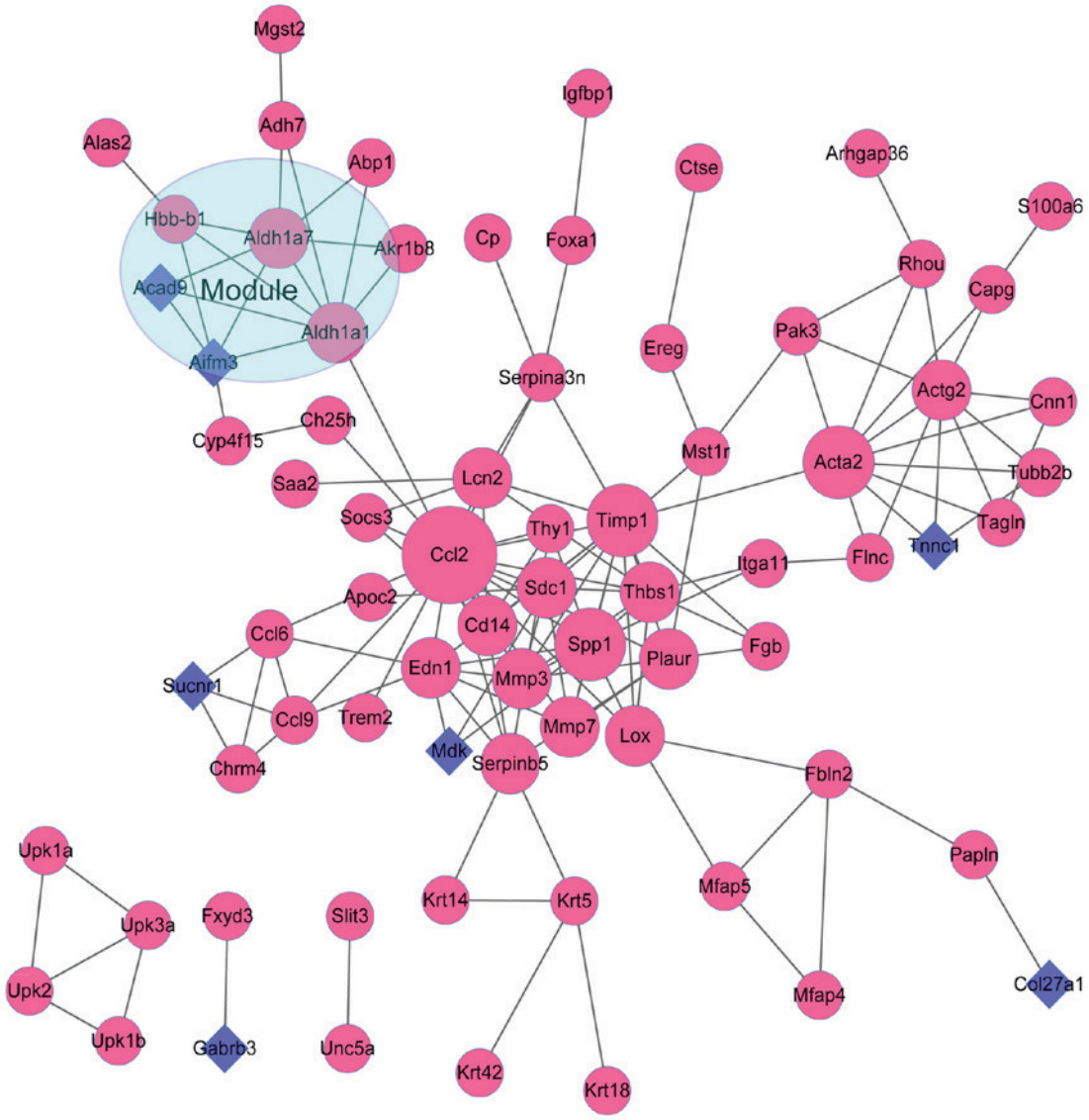

Figure 5. PPI network and modules of DEGs in the severe group. Red circles represent the upregulated genes and purple diamonds represent the downregulated genes. A bigger node indicates a higher degree. PPI, protein-protein interaction; DEGS, differentially expressed genes.

toxic environmental quinones and pharmacological relevant substrates, such as the anticancer doxorubicin (27). Previous study has shown that *CBR1* is vital for the cell protection in functions like oxidative stress and apoptosis (29). Pathway analysis showed that the chemical carcinogenesis was the common pathway enriched by both up- and down-regulated DEGs (especially *CBR1* in Set 1). Thus, we speculated that DEGs like CBR1 in chemical carcinogenesis pathway might be the potential pathogenesis of CON. However, the related study associated with chemical carcinogenesis pathway about *CBR1* and *CON* is very rare. Furthermore, c-Fos (*FOS*) gene has been considered as the regulators of cell proliferation, differentiation, transformation, and apoptotic cell death (30,31). Otsuka *et al* (32) indicates that the expression of *FOS* is potentially an early indicator of late radiation damage to the kidney. In rats model, previous study indicated that the renoprotective effects of drugs partly through inhibiting the upregulation of *FOS* mRNA and protein in renal cortices (33). In the present study, the PPI network analysis showed that *FOS* was one of the most outstanding DEGs in Set 1. Interestingly, the TF-target gene network in this study showed that *FOS* was the co-target gene of *Polr2a* and *Srf*. Thus, we speculated that DEGs like *FOS* might play an important role in the process of CON via TFs like *Polr2a* and *Srf*. However, the related study associated with *FOS* and CON is very rare. Thus, an additional research with a wide scale of gene expression analysis is necessary to confirm this speculation.

Based on Set 2 (only severe group), GO function analysis and chemical-gene interaction network investigation in this study were performed and intended to provide a potential therapy strategy for CON. Interleukin-1 family plays a vital role in the immune and inflammatory responses regulation (34). Previous study showed that the protective effect of interleukin-1 in renal injury might be induced by reducing the induction of the nuclear factors (35). Actually, Pindjakova *et al* (36) indicate that the production of interleukin-1 based on the resident dendritic cells enhances the situation of acute kidney injury. Moreover, tumor necrosis factor (TNF) is a superfamily of cytokines that can lead to cell death, and it is a potent pyrogen that can be caused by stimulation of interleukin-1 secretion (37).

Table II. Chemical-target gene interaction network.

Gene	Chemical name	Description	Gene	Chemical name	Description	Gene	Chemical name	Description
ACTA2	Cyclosporine	UP	EREG	Cyclosporine	UP	RGS16	Cyclosporine	UP
ACTG2	Cyclosporine	UP	FGB	Cyclosporine	UP	RHOU	Cyclosporine	UP
AIFM3	Cyclosporine	DOWN	FOXA1	Cyclosporine	UP	S100A6	Cyclosporine	UP
ALDH1A1	Cyclosporine	UP	FOXQ1	Cyclosporine	UP	SAA2	Cyclosporine	UP
APOC2	Cyclosporine	UP	GDF15	Cyclosporine	UP	SAMD5	Cyclosporine	UP
BCAS1	Cyclosporine	UP	GPR87	Cyclosporine	UP	SDC1	Cyclosporine	UP
BCL2L14	Cyclosporine	UP	IGFBP1	Cyclosporine	UP	SERPINA3N	Cyclosporine	UP
CAPG	Cyclosporine	UP	IGFBP6	Cyclosporine	UP	SLC16A3	Cyclosporine	UP
CCL2	Cyclosporine	UP	ITGA11	Cyclosporine	UP	SLC17A3	Cyclosporine	DOWN
CCL6	Cyclosporine	UP	LCN2	Cyclosporine	UP	SLC39A4	Cyclosporine	UP
CD14	Cyclosporine	UP	LOX	Cyclosporine	UP	SPP1	Cyclosporine	UP
CES1E	Cyclosporine	DOWN	LTBP2	Cyclosporine	UP	TACSTD2	Cyclosporine	UP
CLDN4	Cyclosporine	UP	MDK	Cyclosporine	DOWN	TAGLN	Cyclosporine	UP
CNN1	Cyclosporine	UP	MFAP5	Cyclosporine	UP	TGFB3	Cyclosporine	UP
COL27A1	Cyclosporine	DOWN	MGST2	Cyclosporine	UP	THBS1	Cyclosporine	UP
CP	Cyclosporine	UP	MMP3	Cyclosporine	UP	TIMP1	Cyclosporine	UP
CPE	Cyclosporine	UP	MMP7	Cyclosporine	UP	TRPV6	Cyclosporine	UP
CTSE	Cyclosporine	UP	OSMR	Cyclosporine	UP	TUBB2B	Cyclosporine	UP
EDN1	Cyclosporine	UP	PAK3	Cyclosporine	UP	UCHL1	Cyclosporine	UP
ENTPD3	Azathioprine	UP	PLAUR	Cyclosporine	UP	UPK3A	Cyclosporine	UP

UP, upregulated; DOWN, downregulated.

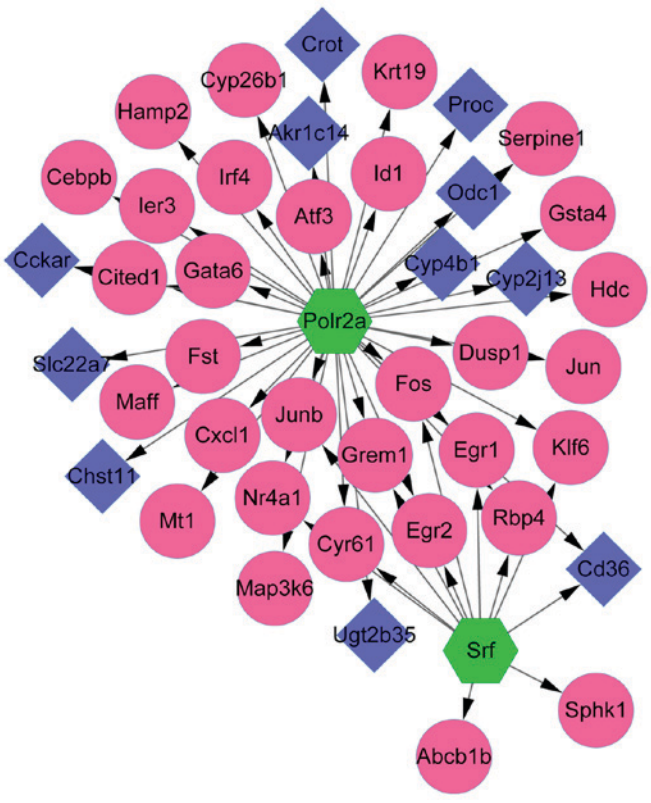

Figure 6. TF-target gene regulatory network. Green hexagons represent TFs; red circles represent the upregulated genes; purple diamonds represent the downregulated genes; and arrows indicate the direction of regulation. TF, transcription factor.

Meldrum *et al* (38) prove that TNF-α mediates renal tubular cell apoptosis in the process of obstructive uropathy. Recent study indicates that the production of TNF-α by macrophages take part in the progression of renal injury (39). Actually, interleukin-1 and TNF-α gene polymorphism are associated with the susceptibility, disease activity and long-term outcome in human nephropathy (40). In the current research, the results of GO analysis showed that cellular response to interleukin-1 and cellular response to tumor necrosis is two of most outstanding function assembled by DEGs. Thus, we speculated that these two functions might be vital for the process of severe CON. The deteriorated or even canceration might take place in severe CON, which needed an early therapy intervention. Furthermore, cyclosporine, also spelled ciclosporin or cyclosporin, is an immunosuppressant medication and natural product (41). As an immunosuppressant with therapeutic indications in various immunological diseases, the use of cyclosporine is reported to be associated with chronic nephropathy (42). In the present study, cyclosporine was the most outstanding chemical associated with DEGs identified in severe CON. Thus, cyclosporine might still be the preferences for the therapy of CON. However, due to the negative effects of cyclosporine in CON clinical treatment, a more effective drug with less adverse reaction is needed.

However, there were still some limitations in this study. First, findings in the present study were all analyzed by bioinformatics methods, and experimental validations were deficient. Second, parameters used in *in silico* analyses were set manually, and some results might be missed or

overemphasized. Therefore, further experimental validations in both *in vivo* and *in vitro* were required in the future.

In conclusion, the DEGs like *CBR1* and *FOS*, TFs like *Polr2a* and *Srf*, as well as pathways like chemical carcinogenesis might play important roles in the process of CON. Interleukin-1 and tumor necrosis function were associated with the deteriorate of CON, and might be novel targets for CON treatment. Furthermore, a more effective drug with less adverse reaction for severe CON is needed.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

GX contributed to the conceptualization of the research. GX and RC acquired the data and performed the analysis. RC and XZ interpreted the data. GX wrote the manuscript, and XZ revised the manuscript for important intellectual content.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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