

Construction of chronic glomerulonephritis-related lncRNA-mRNA regulatory network and lncRNA-miRNA-mRNA ceRNA network by bioinformatics analysis

XING-XING ZHUANG^{1,2*}, TAO LIU^{2*}, LIANG-BING WEI² and JIA-RONG GAO²

¹Department of Pharmacy, Chaohu Hospital of Anhui Medical University, Chaohu, Anhui 238000; ²Department of Pharmacy, The First Affiliated Hospital of Anhui University of Chinese Medicine, Hefei, Anhui 230012, P.R. China

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Abstract. Long non-coding RNAs (lncRNAs) are ncRNA transcripts >200 nucleotides that are important genetic regulators. LncRNAs can directly regulate mRNA through a lncRNA-mRNA regulatory mode and can also regulate mRNA through competitive binding to micro (mi)RNA, which is generally known as the competitive endogenous RNA (ceRNA) network. The present study evaluated the functional roles and regulatory networks of lncRNAs in chronic glomerulonephritis (CGN). The proliferative ability of mouse glomerular mesangial cells (GMCs) induced by different concentrations of lipopolysaccharide (LPS) was assessed using the Cell Counting Kit-8 assay, and RNA sequencing (RNA-seq) was performed to identify differentially expressed lncRNAs in LPS-induced GMCs. Based on the sequencing results, six lncRNAs were selected for validation using reverse transcription-quantitative PCR (RT-qPCR). Furthermore, the lncRNA-mRNA regulatory network and the lncRNA-miRNA-mRNA ceRNA network were constructed to assess the role and mechanism of CGN-related lncRNAs. To elucidate the biological functions of lncRNAs, Gene Ontology (GO) biological process term enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed on all mRNAs involved in the lncRNA-mRNA regulatory network and in the ceRNA network. A total of 1,532 differentially expressed lncRNAs, including 594 upregulated lncRNAs and 938 downregulated lncRNAs, were identified using RNA-seq.

The results of RT-qPCR validation were consistent with RNA-seq results. An lncRNA-mRNA regulatory network, including 236 lncRNAs and 556 mRNAs, and a ceRNA network, including 6 lncRNAs, 18 miRNAs and 419 mRNAs, were successfully constructed. The GO biological process term enrichment and KEGG pathway enrichment analyses demonstrated that those lncRNAs were often related to inflammatory response and substance metabolism. The present study identified key CGN-related lncRNAs in LPS-induced GMCs, and further demonstrated a global view of the lncRNA-mRNA regulatory network and ceRNA network involved in CGN. These results offered novel insights into the roles of lncRNAs in the pathogenesis of CGN and identified potential diagnostic biomarkers.

Introduction

Chronic glomerulonephritis (CGN) is an autoimmune glomerulopathy, characterized by the excessive proliferation of mesangial cells, accumulation of extracellular matrix and infiltration of circulating inflammatory cells (1). Glomerular mesangial cells (GMCs), distributed in the mesangial matrix of the glomerulus, constitute the mesangial region of the glomerulus together with the mesangial matrix. GMCs have multiple physiological functions, including stabilizing the structure of glomerular capillaries, maintaining mesangial matrix homeostasis, regulating filtration surface area, phagocytosis of apoptotic cells and immune complexes (2). Under the influence of certain pathological factors such as hyperglycemia and inflammation, GMCs can proliferate abnormally, increase intracellular protein synthesis and, increase the secretion of the extracellular matrix (3-4). Therefore, excessive proliferation of GMCs is an important pathological feature of certain human kidney diseases, including chronic glomerulonephritis and diabetic nephropathies. Knowledge of the responses of GMCs to pathological stimuli is crucial to the understanding of the pathogenesis of chronic glomerulonephritis. Thus, a deeper understanding of the excessive proliferation of GMCs is required to devise more effective prevention and therapies for CGN.

Long non-coding RNAs (lncRNAs) are generally considered as non-coding transcripts >200 nucleotides that are important genetic regulators. LncRNAs have been previously

Correspondence to: Professor Jia-Rong Gao, Department of Pharmacy, The First Affiliated Hospital of Anhui University of Chinese Medicine, 117 Meishan Road, Hefei, Anhui 230012, P.R. China
E-mail: zyfygjr2006@ahtcm.edu.cn

*Contributed equally

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reported to be crucial determinants of epigenetic regulation and serve a key role in regulation of chromatin structure, scaffolding or the decoy function of mRNAs, and post-transcriptional regulation of mRNAs (5). The regulatory role of lncRNA can be summarized as cis-action on neighboring genes or as trans-action through effects on mRNA stability, mRNA translation or the regulation of microRNA-mRNA interactions and RNA binding proteins (6-7). For example, Gao *et al* (8) reported that lncRNA NONRATG001910.2 promoted CTNNB1 expression by targeting miR-339-3p in lipopolysaccharide (LPS)-stimulated rat mesangial cells, which suggested that NONRATG001910.2 may be a potential biomarker for CGN. Moreover, Zhou *et al* (9) suggested that lncRNA NORAD directly regulated RUNX2 transcription and promoted the proliferation, migration and invasion of breast cancer cells. Competitive endogenous (ce)RNA is the most frequently reported mechanism of lncRNAs.

Materials and methods

GMC culture conditions. Mouse SV40-MES-13 GMCs were purchased from BNCC Biological Technology and cultured in DMEM (Beijing Solarbio Science & Technology Co., Ltd.) supplemented with 10% FBS (Biological Industries Sartorius AG) and penicillin/streptomycin at 37°C in a humidified, 5% CO₂ atmosphere. GMCs stimulated with 3.0 µg/ml LPS (MilliporeSigma) (10-11) were defined as the model group (n=3) and unstimulated GMCs were defined as the control group (n=3).

Cell proliferation assay. Cell proliferation rates were assessed using the Cell Counting Kit-8 (CCK-8) assay (cat. no. BB19071X; Shanghai Besto Biological Technology Co., Ltd.). GMCs were seeded (5.0x10⁴ cells/well) into 96-well tissue culture plates with DMEM medium and treated with CCK-8 solution (10 µl/well) for 1 h. The optical density (absorbance at 450 nm) was assessed using an RT-6100 ELISA reader (Rayto Life and Analytical Sciences Co., Ltd.).

lncRNA sequence analysis. To ensure the quality of paired-end sequencing reads, FastQC software (version 0.10.1) was used to evaluate the quality of the original sequencing data. The input reads were considered good data quality when the Q20 base percentage in 'Reads' was ≥90% and the Q30 base percentage in 'Reads' was ≥80%.

High-throughput RNA-seq was performed by Genesky Bio-tech Co., Ltd. RNA fragmentation was performed using Bioruptor Pico (cat. no. B01060001; Diagenode SA) sonication in RNase-free water. RNA integrity was detected by denaturing gel electrophoresis and quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). The purified RNA fragments were then used to construct libraries using the TruSeq RNA Sample Prep Kit (cat. no. RS-122-2002; Illumina, Inc.). Libraries underwent quality control and were quantified using an Agilent 2100 bioanalyzer system (Agilent Technologies, Inc.). Paired-end 150 bp sequencing was performed using an Illumina HiSeq 2500 (Illumina, Inc.).

Isolation of RNA and reverse transcription-quantitative (RT-qPCR). The expression levels of NONMMUG036949.2, NONMMUG089165.1, NONMMUG030447.2,

NONMMUG028702.2, NONMMUG039651.2 and NONMMUG032587.2 were assessed using RT-qPCR. Total RNA was extracted from the cells using TRIzol® (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Primers (Table I) were designed based on the cDNA sequences, which were accessed from the National Center for Biotechnology Information (NCBI) and tested using NCBI Primer BLAST, using Primer Premier 5 (Premier Biosoft International), and synthesized by Sangon Biotech Co., Ltd. The concentration and purity of the isolated RNA were determined using an OD1000+ Ultra Micro Spectrophotometer (WuYi Technologies), and reverse transcription was performed using the PrimeScript™ RT Reagent Kit with gDNA Eraser (cat. no. RR047A; Takara Biotechnology Co., Ltd.). qPCR was performed using a StepOne Plus fluorescence quantitative PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR Green qPCR Master Mix (cat. no. G3322-05; Wuhan Servicebio Technology Co., Ltd.). β-actin was used as the internal control; for quantitative results, the expression of lncRNA was expressed as fold change using the 2^{-ΔΔCq} method (12).

Construction of the lncRNA-mRNA regulatory network. LncTar (<http://www.cuilab.cn/lncTar>) was used to predict lncRNA-mRNA interactions utilizing free energy minimization (13-14). LncTar utilizes a variation on the standard 'sliding' algorithm approach to calculate the normalized binding free energy (ndG) and predicts the minimum free energy joint structure. ndG ≤0.1 was regarded as the cutoff to determine the paired RNAs as interacting, ndG >0.1 was considered to indicate that paired RNAs did not interact. Subsequently, Cytoscape 3.8.1 was used to visualize the lncRNA-mRNA network after screening the target mRNAs of differentially expressed lncRNAs.

Construction of the lncRNA-associated ceRNA network. Interactions between miRNAs and lncRNAs were predicted using the miRNA target prediction software, miRanda (<http://www.miranda.org>) (15), and the three miRNAs of the highest confidence that can bind to lncRNAs were chosen. The target mRNAs of miRNAs were predicted using TargetScan 8.0 (https://www.targetscan.org/vert_80/) (16), and mRNAs with high confidence (cumulative weighted context score cutoff level <0.8) bound to the miRNAs were screened out (17). Cytoscape 3.8.1 was then used to delineate the lncRNA-miRNA-mRNA ceRNA network.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. To evaluate the role of differentially expressed lncRNAs in LPS-induced GMCs, GO biological process (BP) term enrichment and KEGG pathway enrichment were assessed (18). GO terms with P<0.05 were considered statistically significant. KEGG pathway enrichment analysis was used to identify significantly enriched signal transduction pathways or metabolic pathways (P<0.05). GO and KEGG enrichment analysis were performed using the free online data analysis platform OmicShare tools (<https://www.omicshare.com/tools>).

Protein-protein interaction (PPI) network construction and core gene screening. The PPI network of the mRNA involved

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Amplicon size, bp	Sequence (5'-3')
NONMMUG036949.2	185	F: TTCTCCAGGACACTCACCAC R: AGAGAGCCAGGCATAGTGTG
NONMMUG089165.1	140	F: AGACCACGACGCCCTTAAGTA R: GAATGGGAGTCCGAATGCAG
NONMMUG030447.2	73	F: GCGTCGCTAACCGGTCC R: GGGACAAGAAGGTCATCGGT
NONMMUG028702.2	181	F: ACTGATACCATGCACCTCTCA R: GCATGTCACTTCAGCCTCTG
NONMMUG039651.2	160	F: TCCCTGCTGCAGTGTCAATA R: AGCAAAGCTCCCTTGTCTCT
NONMMUG032587.2	175	F: CAAGCCTGGATGTTCCATCG R: AGGGCACACCCTTCAAAGAT

F, forward; R, reverse

in the ceRNA network was generated using the STRING online database tool (Version 11.5, <https://string-db.org>) (19); interaction pairs with a confidence value >0.4 were deemed significant and were retained. The top 10 genes in the PPI network were evaluated using the MCC, MNC and Degree algorithms through the CytoHubba plug-in for Cytoscape (20). The final hub genes were identified as those that were identified by all three of the algorithms.

GEO public dataset analysis. The public datasets (GSE104066) utilized in this study were obtained from the GEO database (<https://www.ncbi.nlm.nih.gov/geo>) and were initially screened based on disease (CGN), organism (*Homo sapiens*) and experiment type (expression profiling by array). Ultimately, the GSE104066 dataset was selected for use in CGN analysis.

Statistical analysis. Statistical analysis was performed using SPSS 22.0 software (IBM Corp.), and data are reported as the mean + SD. The data were subjected to one-way ANOVA with Tukey's post hoc multiple comparison tests. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Optimal concentration and treatment time of LPS were determined using the CCK-8 assay. The present study evaluated the proliferative ability of GMCs induced by different concentrations of LPS (0.5, 1.0, 3.0, 5.0 and 10.0 $\mu\text{g/ml}$) using the CCK-8 assay at 24 and 48 h following treatment. The relationship between cell proliferation, LPS concentration and intervention time were assessed (Fig. 1). The results of the CCK-8 proliferation assay demonstrated that LPS could effectively induce cell proliferation in GMCs, with the highest absorbance (optical density 450 nm) observed at a concentration of 3.0 $\mu\text{g/ml}$ (Table SI), which indicated that 3.0 $\mu\text{g/ml}$ was the optimal concentration of LPS for the induction of cell proliferation in GMCs. Furthermore, there was no significant

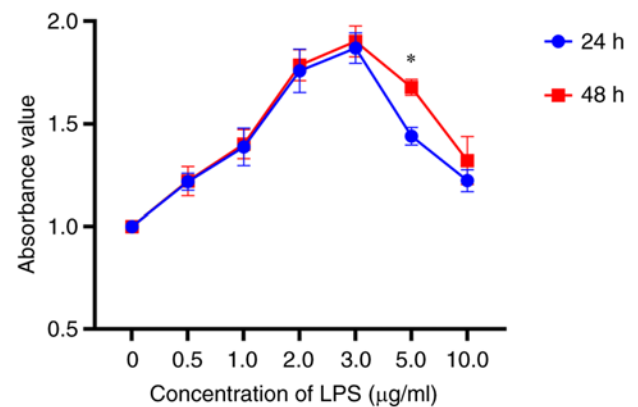


Figure 1. The optimal concentration and treatment time with LPS was assessed using the Cell Counting Kit-8 assay. * $P < 0.05$ vs. 24 h. LPS, lipopolysaccharide.

difference demonstrated between the treatment times of 24 and 48 h ($P = 0.62$) at a concentration of 3.0 $\mu\text{g/ml}$ LPS. Consequently, the concentration of 3.0 $\mu\text{g/ml}$ and the treatment time of 24 h were selected for use in subsequent experiments.

Characteristics of differentially expressed lncRNAs. In the present study, six groups of cells were used in subsequent experiments, including three groups of LPS-induced GMCs as the model group (LPS1-3) and three groups of normal GMCs as the control group (CON1-3).

After deduplication, quality trimming and quality filtering, the sequencing data at both ends of the R1 and R2 paired reads were assessed as being of good quality (Q20 base and Q30 base were both >95%). The proportion of 'clean reads' retained after cleaning was >95% for each of the six samples which met the pre-determined quality requirements for sequencing. The quality control results of the sequencing data were presented (Table II; Table SII).

The violin plot demonstrated the relative abundance of lncRNAs in each sample (Fig. 2A). The violin plot demonstrated

Table II. The quality control results for the sequencing data.

Sample	R1		R2		Clean reads, % of total
	Q20, %	Q30, %	Q20, %	Q30, %	
CON-1	98.1	95.3	98.5	95.9	97.7
CON-2	98.1	95.2	98.5	97.5	97.5
CON-3	98.1	95.3	98.5	97.5	97.5
LPS-1	98.1	95.2	98.2	97.7	97.7
LPS-2	98.2	95.4	98.3	96.9	96.9
LPS-3	98.1	95.3	98.4	97.3	97.3

CON, control; LPS, lipopolysaccharide.

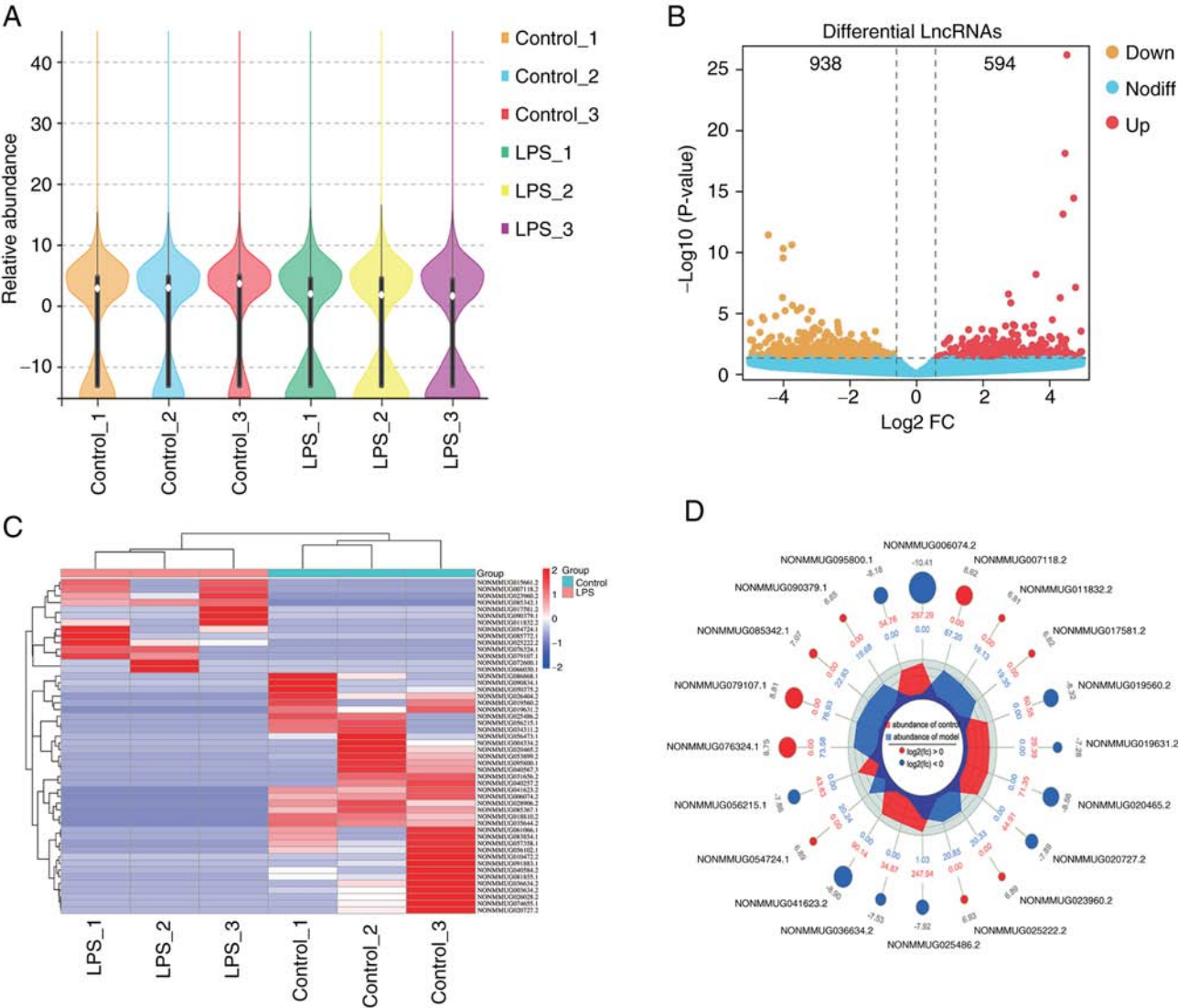


Figure 2. Features of differentially expressed lncRNAs. (A) Violin plot of the relative abundance of lncRNAs in each sample. (B) Differentially expressed lncRNAs. (C) Heat map of the top 50 differentially expressed lncRNAs. (D) Radar map of the top 10 lncRNAs with upregulated and downregulated expression. lncRNA, long non-coding RNA; LPS, lipopolysaccharide; No diff, no significant difference.

that there were significant differences in the expression of lncRNAs in LPS-induced GMCs compared with control GMCs. The threshold for differentially expressed lncRNAs

was set at absolute fold change $|FC| \geq 1.5$ and $P < 0.05$ (21-22). A total of 1,532 differentially expressed lncRNAs, including 594 upregulated lncRNAs and 938 downregulated lncRNAs,

Table III. Top 10 upregulated and downregulated expressed log non-coding RNAs.

Gene ID	Log ₂ (FC)	P-value	Regulation
NONMMUG011832.2	6.8068	0.0013	Up
NONMMUG017581.2	6.8226	0.0052	Up
NONMMUG090379.1	6.8473	0.0049	Up
NONMMUG054724.1	6.8894	0.001	Up
NONMMUG023960.2	6.895	0.0001	Up
NONMMUG025222.2	6.9314	0.0001	Up
NONMMUG085342.1	7.0671	<0.0001	Up
NONMMUG007118.2	8.6184	0.0274	Up
NONMMUG076324.1	8.7489	0.0252	Up
NONMMUG079107.1	8.8134	0.0241	Up
NONMMUG006074.2	-10.4113	<0.0001	Down
NONMMUG041623.2	-8.8981	<0.0001	Down
NONMMUG020465.2	-8.5609	0.0285	Down
NONMMUG019560.2	-8.3242	0.0332	Down
NONMMUG095800.1	-8.1796	0.0364	Down
NONMMUG025486.2	-7.9163	<0.0001	Down
NONMMUG020727.2	-7.8928	0.0435	Down
NONMMUG056215.1	-7.8581	0.0444	Down
NONMMUG036634.2	-7.5271	<0.0001	Down
NONMMUG019631.2	-7.2817	<0.0001	Down

FC, fold change.

were identified from a total of 46,879 lncRNAs (Fig. 2B; Table SIII). A heat map of the top 50 differentially expressed lncRNAs was presented (Fig. 2C). The top 10 upregulated and downregulated lncRNAs are presented in Table III, and the radar map in Fig. 2D presents the top 10 upregulated and downregulated lncRNA expression levels in LPS-induced GMCs compared with control GMCs.

Construction of the lncRNA-mRNA regulatory network. To evaluate the functions of differentially expressed lncRNAs, 556 target mRNAs of the 236 lncRNAs were predicted using LncTar (Table SIV). The highly coordinated expression between lncRNAs and target mRNAs may be due to complementary base pairing between lncRNA and mRNA (23). The lncRNA-mRNA regulatory network presented in Fig. 3 shows the interaction relationship between lncRNA and mRNA. For example, lncRNA NONMMUG029023.2 may regulate Stoml2 mRNA expression. Ptdss2 may be affected by both lncRNA NONMMUG039651.2 and lncRNA NONMMUG095401.1.

To elucidate the biological functions of differentially expressed lncRNAs in LPS-induced GMCs, GO BP term enrichment and KEGG pathway analyses were performed on all mRNAs in the lncRNA-mRNA regulatory network (Fig. 4). Within the GO BP classification, 'cellular macromolecule metabolic process', 'negative regulation of biological process' and 'nitrogen compound metabolic process' were the top three over-represented terms (Fig. 4A). Within the GO cellular components (CC) classification, 'intracellular', 'intracellular part' and 'membrane-bounded organelle'

were the top three over-represented terms (Fig. 4B). Within the GO molecular function (MF) classification, 'galactoside 2- α -L-fucosyltransferase activity', ' α -(1,2)-fucosyltransferase activity' and 'chromatin insulator sequence' binding were the top three over-represented terms (Fig. 4C).

The KEGG metabolic pathway enrichment analysis demonstrated that 'GnRH secretion', 'Melanoma', 'Ras signaling pathway', 'Glycosphingolipid biosynthesis-globo and isoglobo series' and ' β -Alanine metabolism' were the top five most significantly enriched KEGG pathways (Fig. 4D). In addition, inflammation-related signaling pathways, such as the 'Rap1 signaling pathway' and 'Ras signaling pathway', and substance metabolism pathways, such as 'Riboflavin metabolism', ' β -Alanine metabolism' and 'Histidine metabolism' were also significantly enriched. These results suggested that inflammation and substance metabolism disorder may be the underlying CGN pathogenesis.

RT-qPCR validation. To verify the reliability of the sequencing data and to support more clinically meaningful follow-up research, RT-qPCR validation of certain highly conserved lncRNAs between humans and mice was performed. The conservation information of lncRNAs was obtained from the NONCODE database (<http://www.noncode.org>). The conservation analysis of lncRNAs was assessed using the E-value, where a threshold of E-value $<1 \times 10^{-5}$ was used (24). After conservation analysis among species (Table IV), NONMMUG036949.2, NONMMUG089165.1, NONMMUG030447.2, NONMMUG028702.2, NONMMUG039651.2 and NONMMUG032587.2 were selected for RT-qPCR validation (25). The RT-qPCR results demonstrated the same expression trend as the RNA-Seq results, with the six selected lncRNAs all significantly upregulated in LPS-induced GMCs compared with the control group (Fig. 5; Table SV).

Construction of the lncRNA-associated ceRNA network. In addition to the lncRNA-mRNA regulatory network, lncRNAs can also serve a role in other gene networks that regulate diverse biological processes, such as the ceRNA network (26). To construct an lncRNA-associated ceRNA network, the aforementioned six highly conserved and RT-qPCR-validated lncRNAs were used to construct ceRNA networks. The ceRNA network consisted of miRNAs of the highest confidence with lncRNAs, and mRNAs with a high confidence (cumulative weighted context score cutoff level <0.8) (27) bound to the miRNAs, which included 6 lncRNAs, 18 miRNAs and 419 mRNAs (Fig. 6; Tables SVI and SVII). lncRNAs can be competing targets of shared miRNAs with other mRNAs and form a complex regulatory ceRNA network. For example, the lncRNA NONMMUG089165.1 may act as a sponge for mmu-miR-3960 to affect EVX1 expression. PARD3B could be affected by both the lncRNA NONMMUG039651.2/mmu-miR-7081-5P axis and the lncRNA NONMMUG028702.2/mmu-miR-7044-5P axis at the same time.

To evaluate the biological functions of the lncRNAs in the ceRNA network, GO BP and KEGG pathway analysis was performed on all mRNAs involved in the ceRNA network (Fig. 7). Within the GO BP classification, 'system development', 'animal organ development' and 'multicellular

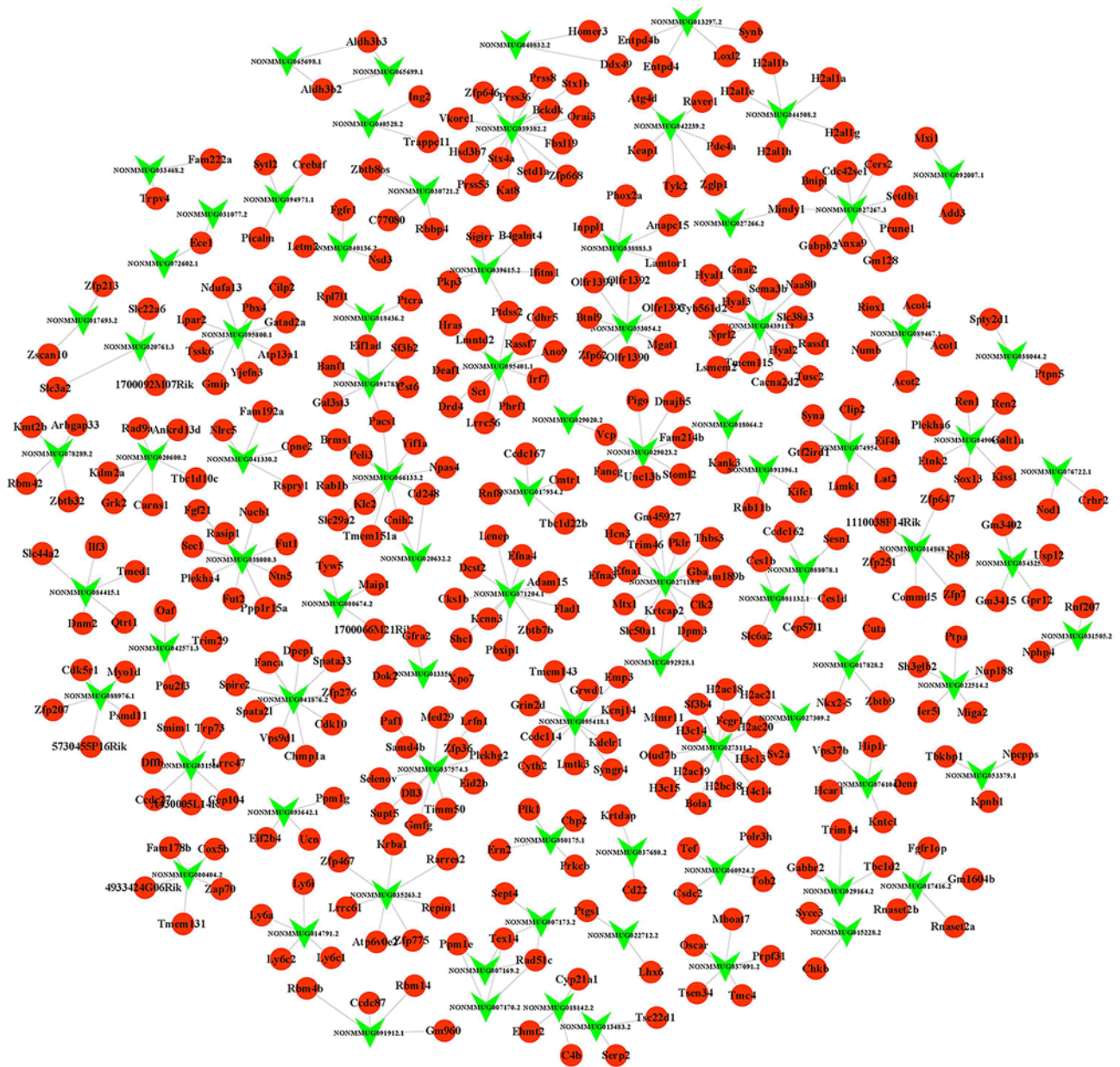


Figure 3. lncRNA-mRNA regulatory networks. The red circles represent mRNAs, and the green arrows represent lncRNAs. lncRNA, long non-coding RNA.

organism development' were the top three over-represented terms (Fig. 7A). Within the GO CC classification, 'neuronal cell body membrane', 'cell body membrane' and ' α - β T cell receptor complex' were the top three over-represented terms (Fig. 7B). Within the GO MF classification, 'DNA-binding transcription activator activity, RNA polymerase II-specific', ' α -1,6-mannosylglycoprotein 6- β -N-acetylglucosaminyltransferase activity' and 'DNA-binding transcription factor activity' were the top three over-represented terms (Fig. 7C). The KEGG metabolic pathway enrichment analysis demonstrated that 'Collecting duct acid secretion', 'Circadian rhythm', 'Proteoglycans in cancer', 'Pathways in cancer' and 'Oxytocin signaling pathway' were the top five most significantly enriched KEGG pathways (Fig. 7D). Furthermore, inflammation-related signaling pathways, such as 'MAPK signaling pathway', 'mTOR signaling pathway' and 'Rap1 signaling pathway' were significantly enriched KEGG pathways. These

results suggested that inflammation may be the underlying CGN pathogenesis.

Screening of PPI core genes and validation on GEO dataset.

The PPI network of all the mRNAs involved in ceRNA was successfully constructed, including 396 nodes and 260 edges (Fig. 8A). The top 10 genes were evaluated in the PPI network using the MCC, MNC and Degree algorithms; the results of the three algorithms were cross-compared to identify five core genes, including IKAROS family zinc finger 1 (IKZF1), CD3 ϵ subunit of T-cell receptor complex (CD3E), synapsin 1 (SYN1), glutamate ionotropic receptor NMDA subtype 2B (GRIN2B) and SPI1 (Fig. 8B). According to the ceRNA hypothesis, lncRNAs compete with miRNAs, to bind to the target genes, which increases their mRNA expression. Public dataset GSE104066 from the GEO database was used to validate the expression of IKZF1, CD3E, SYN1, GRIN2B

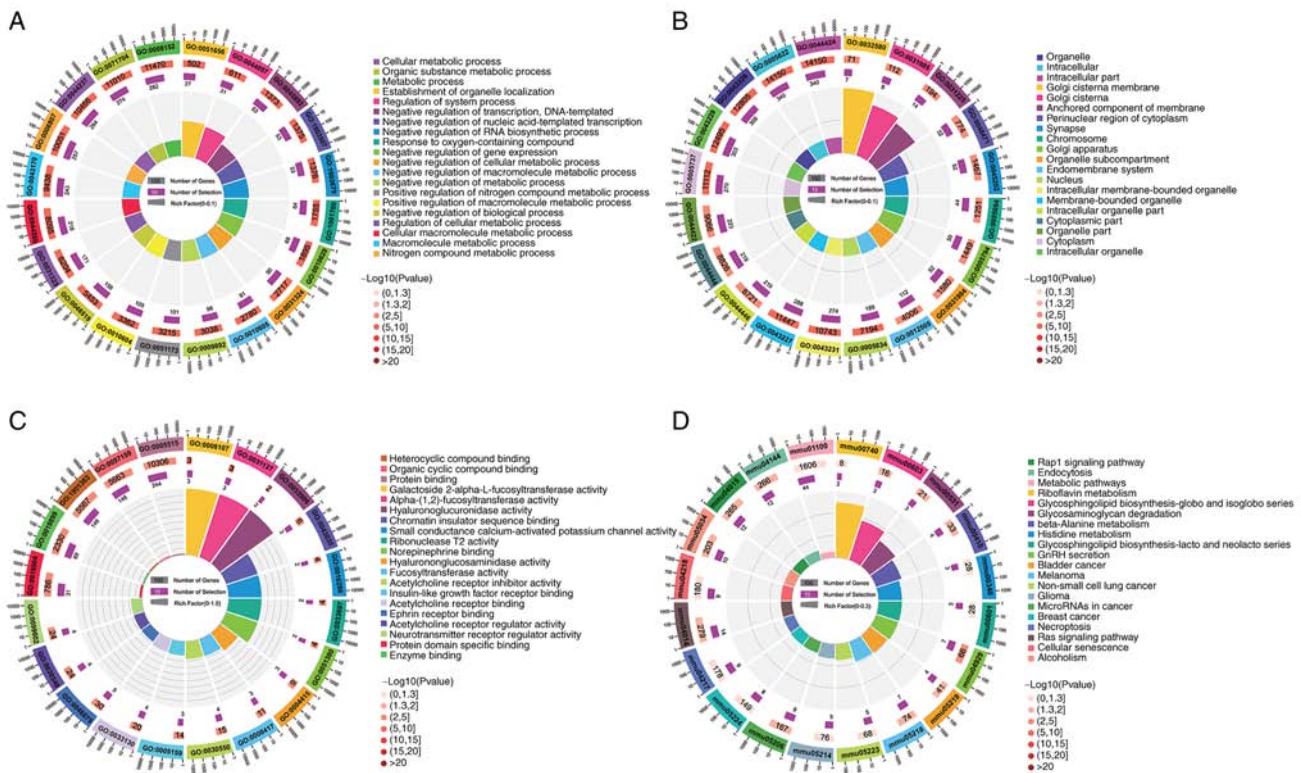


Figure 4. GO and KEGG pathway and enrichment analysis of mRNA in the long non-coding RNA-mRNA regulatory network. GO term enrichment categories (A) biological process, (B) cellular component and (C) molecular function. (D) KEGG pathway enrichment analysis. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

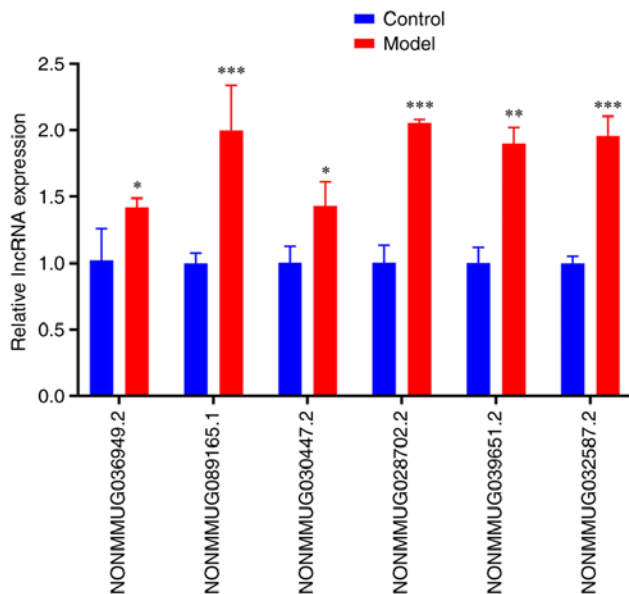


Figure 5. RNA expression levels of six highly conserved lncRNAs were all upregulated in lipopolysaccharide-induced glomerular mesangial cells. RNA expression levels of six highly conserved lncRNAs assessed using reverse transcription-quantitative PCR. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. Control. lncRNA, long non-coding RNA.

and SPI1 in CGN. The data demonstrated that IKZF1, CD3E, SYN1 and GRIN2B were significantly upregulated; however, no significant difference was demonstrated for SPI1 (Fig. 8C; Table SVIII).

Discussion

Chronic inflammation is an essential factor in the occurrence and development of CGN, which may activate cell proliferation and induce the deregulation of cells (28-29). Excessive proliferation of GMCs, commonly observed in glomerulonephritis, is an important pathological basis of kidney disease (30-31). LPS is a major constituent of the outer membrane of gram-negative bacteria that can stimulate the activation of inflammatory factors in cells, leading to systemic inflammatory response and activation of the immune system. LPS has frequently been used as an inducer of cell proliferation in numerous previous studies. For example, it has been previously reported that LPS can induce THP-1 cell (32) and naive B cell (33) proliferation. Our research group has previously used LPS-induced GMCs as an *in vitro* model of CGN (34); therefore, in the present study, RNA-seq was used to evaluate CGN-related lncRNAs in LPS-induced GMCs, and an lncRNA-mRNA regulatory network and an lncRNA-miRNA-mRNA ceRNA network were constructed to elucidate the possible molecular mechanism of CGN.

The ceRNA theory was first proposed by Salmena (35) in 2011; it provided a new perspective for studying the role of RNA biological behavior in the occurrence and development of disease. According to the ceRNA theory, RNA transcripts, including non-coding RNAs, circular RNAs and pseudogene transcripts, could function as miRNA sponges and so regulate miRNA expression. Previous studies have reported that lncRNAs could affect mRNA stability through a ceRNA theory. For example, it has been previously reported that

Table IV. Conservative information for the six selected long non-coding RNAs.

Gene	Chromosome	log ₂ (FC)	P-value	Alignment	Score, bits	E-value
NONMMUG036949.2	6	1.2899	0.0476	NONHSAG058656.2	60	6.00x10 ⁻⁷
NONMMUG089165.1	12	1.9608	0.0054	NONHSAG048570.2	283	3.00x10 ⁻⁷⁴
NONMMUG030447.2	4	1.4135	0.0250	NONHSAG056378.1	232	7.00x10 ⁻⁵⁹
NONMMUG028702.2	4	1.2152	0.0287	NONHSAG038858.2	56	2.00x10 ⁻⁵
NONMMUG039651.2	7	1.0544	0.0353	NONHSAG063259.1	168	3.00x10 ⁻³⁹
NONMMUG032587.2	5	1.3938	0.0013	NONHSAG088273.1	66	2.00x10 ⁻⁸

FC, fold change.

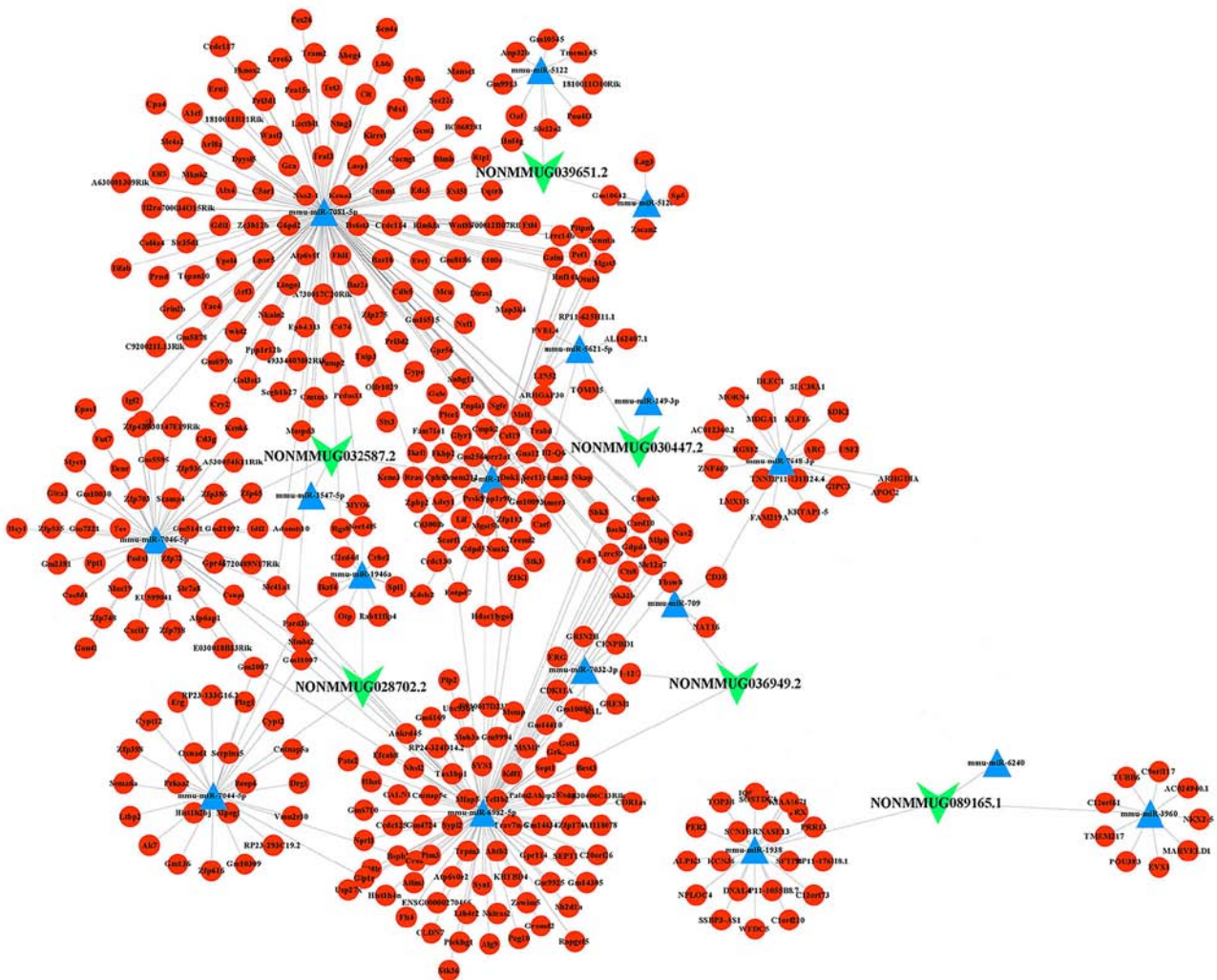


Figure 6. lncRNA-miRNA-mRNA competitive endogenous RNA network. The red circles represent mRNAs, the blue triangles represent miRNAs and the green arrows represent lncRNAs. lncRNA, long non-coding RNA; miRNA, microRNA.

lncRNA linc00673 acts as a ceRNA by sponging miR-150-5p and thus regulating ZEB1 expression; as such, it was suggested that linc00673 served an essential role in the regulation of non-small cell lung cancer proliferation, migration and invasion (36). Previous studies have reported on the ceRNA theory and the link between lncRNAs and miRNAs (37). Therefore, the construction of a lncRNA-associated ceRNA network in CGN could have important research significance.

The enrichment analysis of the GO terms and KEGG pathways of lncRNA-targeted genes presented a preliminary depiction of the lncRNA function under investigation. The present study performed a comprehensive analysis of the GO terms and KEGG pathways for all mRNAs involved in the lncRNA-mRNA regulatory network and lncRNA-associated ceRNA network. The results of the two KEGG pathway analyses indicated significant enrichment of classical inflammatory

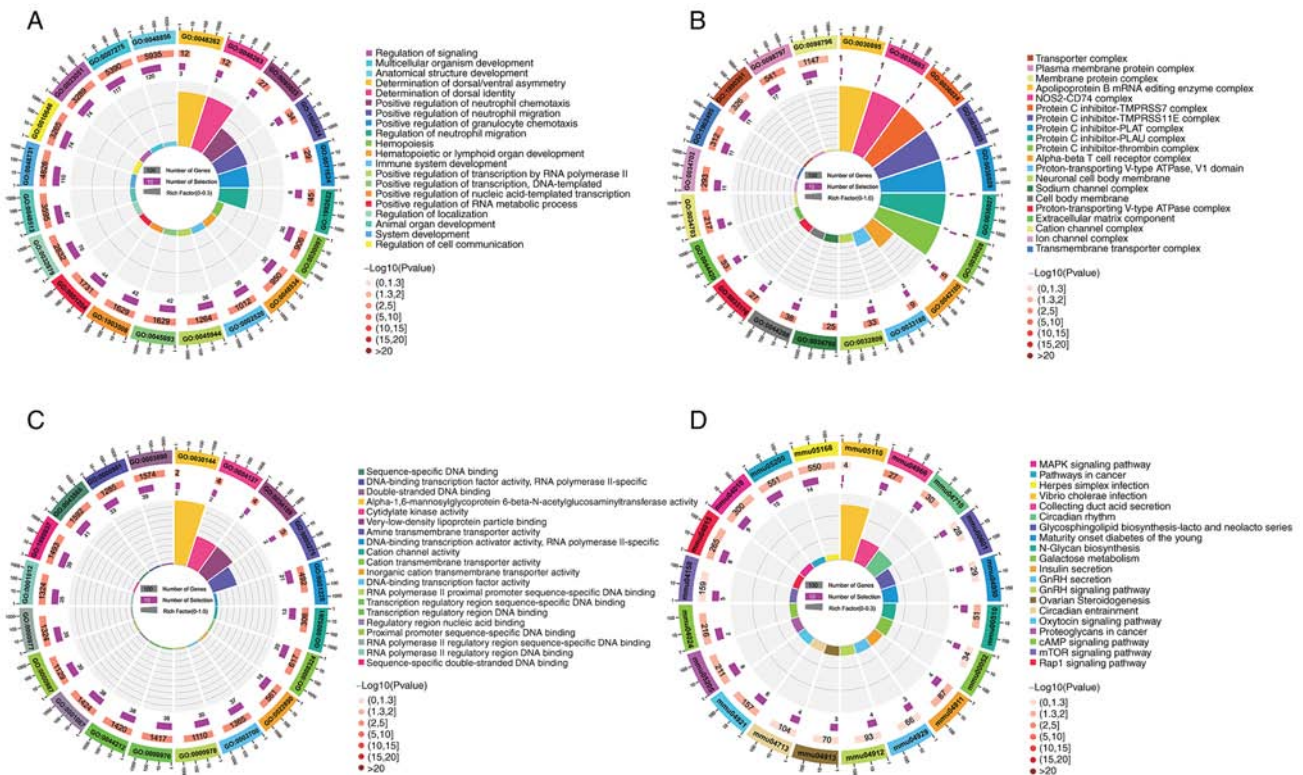


Figure 7. GO and KEGG pathway and enrichment analysis of mRNA in competitive endogenous RNA network. GO term enrichment categories (A) biological process, (B) cellular component and (C) molecular function. (D) KEGG pathway enrichment analysis. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long non-coding RNA.

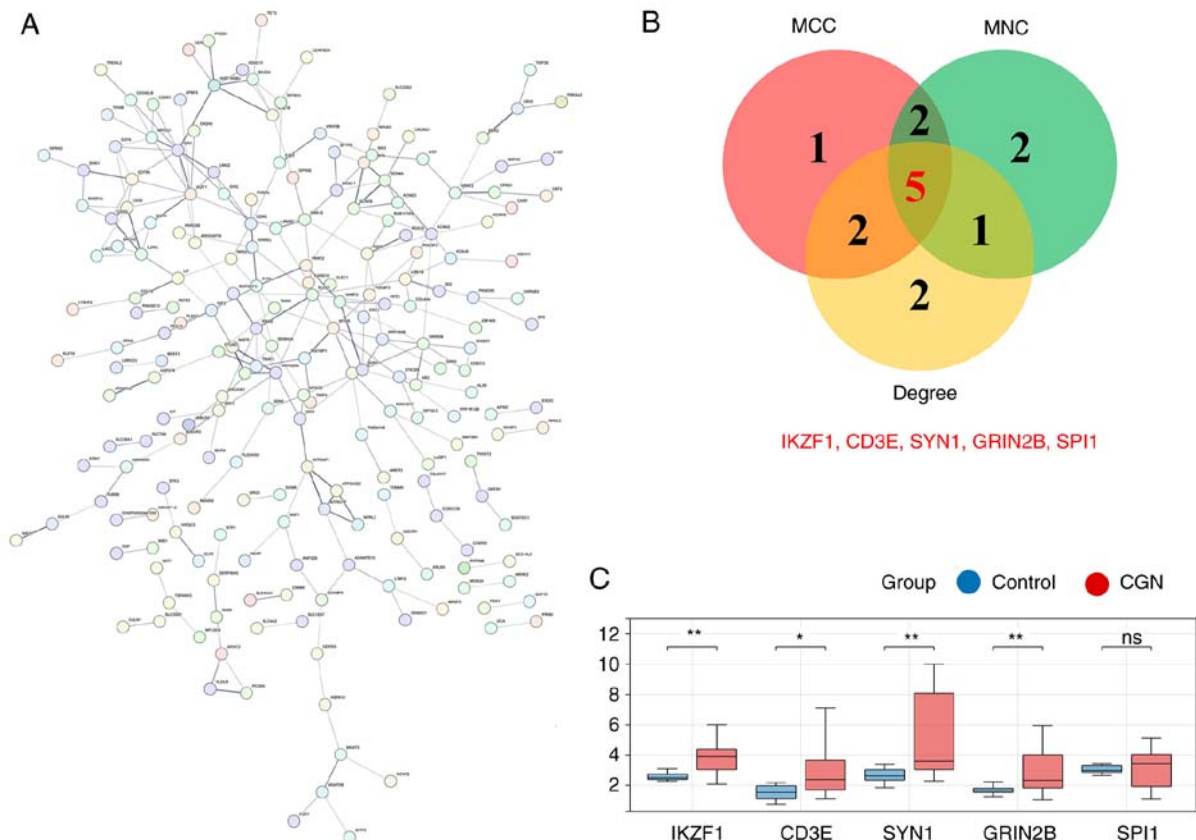


Figure 8. Screening of PPI core genes and validation on GEO dataset. (A) PPI network diagram. (B) Screened core genes identified using the MCC, MNC and Degree algorithms. (C) The expression levels of IKZF1, CD3E, SYN1, GRIN2B and SPI1 in the GEO GSE104066 dataset. * $P < 0.05$ and ** $P < 0.01$. CD3E, CD3 ϵ subunit of T-cell receptor complex; GRIN2B, glutamate ionotropic receptor NMDA subtype 2B; IKZF1, IKAROS family zinc finger 1; ns, not significant; PPI, protein-protein interaction; SYN1, synapsin 1.

signaling pathways, including MAPK, Rap1, Ras and mTOR. In instances of renal dysfunction, the timely clearance of pro-inflammatory cytokines and small to medium-sized molecular toxins is hindered, which leads to an elevation in inflammatory cytokines. Furthermore, the accumulation of these toxins within the body can stimulate the production of additional inflammatory cytokines (38), as evidenced by the enrichment of numerous inflammatory signaling pathways in the present study.

Furthermore, the metabolism of certain substances appeared to be affected, with pathways such as 'N-Glycan biosynthesis', 'Glycosphingolipid biosynthesis', 'Galactose metabolism', 'Glycosaminoglycan degradation', ' β -Alanine metabolism', 'Riboflavin metabolism' and 'Histidine metabolism' being significantly enriched in the KEGG enrichment analysis of the lncRNA-mRNA regulatory network. The kidneys are vital organs responsible for the elimination of metabolic waste products from the bloodstream and the regulation of the homeostatic levels of electrolytes and metabolites, while simultaneously eliminating harmful toxins from the body. In instances where kidney function is compromised, the metabolic equilibrium of specific substances within the body is disrupted (39). Previous studies have reported notable variances in the plasma amino acid profile of individuals with chronic kidney disease in comparison with those of healthy individuals, which is typically characterized by alterations in the levels of endogenous and essential amino acids (40,41).

There were certain limitations in the present study. Firstly, the number of samples tested in RNA-seq was small and should be increased in future studies to reduce possible bias in the sequencing results. Secondly, both the lncRNA-miRNA and miRNA-mRNA regulatory relationships were only predicted using bioinformatics analysis software, and all hypotheses and relevant mechanisms need to be verified by further experimental molecular studies.

In conclusion, 1,532 differentially expressed lncRNAs, including 594 upregulated lncRNAs and 938 downregulated lncRNAs, were identified using RNA-seq in LPS-induced GMCs. Furthermore, the lncRNA-mRNA regulatory network including 236 lncRNAs and 556 mRNAs, and the lncRNA-miRNA-mRNA ceRNA network including 6 lncRNAs, 18 miRNAs and 419 mRNAs were constructed. KEGG pathway analysis demonstrated that certain classical inflammatory signaling pathways and substances metabolism were significantly enriched. The present study demonstrated a global view of the lncRNA-associated ceRNA network, and may offer novel insights into the roles of lncRNAs in the pathogenesis of CGN as well as in identifying promising diagnostic biomarkers.

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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. The raw RNA sequencing data were deposited in the National Genomics Data Center (accession number PRJCA017179; <https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA017179>).

Authors' contributions

XXZ, TL, LBW and JRG wrote the manuscript. JRG conceived and designed the experiment. XXZ performed the cell experiment and acquisition of data. TL and LBW interpreted and analyzed the sequencing data. LBW and JRG revised the manuscript critically for important intellectual content. XXZ, TL, LBW and JRG confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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