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Identification and construction of lncRNA-associated ceRNA network in diabetic kidney disease

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Abstract

Diabetic kidney disease (DKD) has become the major contributor to end-stage renal disease with high incidence and mortality. The functional roles and exact mechanisms of long noncoding RNA (lncRNA)-associated competing endogenous RNA (ceRNA) network in DKD are still largely unknown. This study sought to discover novel potential biomarkers and ceRNA network for DKD.

The candidate differentially expressed genes (DEGs), lncRNAs and microRNAs (miRNAs) in human glomerular and tubular tissues derived from Gene Expression Omnibus database were systematically selected and analyzed. Functional enrichment analysis and protein-protein interaction network analysis were conducted to identify hub genes and reveal their regulatory mechanisms involved in DKD. Following this, the integrated ceRNA network was constructed by bioinformatics methods.

A total of 164 DEGs, 6 lncRNAs and 18 miRNAs correlated with DKD were finally filtered and identified. It is noteworthy that the global lncRNA-associated ceRNA network related to DKD was constructed, among which lnc-HIST2H2AA4-1, VCAN-AS1 and MAGI2-AS1 were identified as the 3 key lncRNAs, and VCAN, FN1, CCL2, and KNG1 were identified as the predominant genes. Consistent with that observed in the training set, 3 of the key genes also showed significant differences in the 2 validation datasets. Integrating with functional enrichment analysis results, these key genes in the ceRNA network were mainly enriched in the immune and inflammation-related pathways.

This study first identified key lncRNAs, miRNAs and their targets, and further revealed a global view of lncRNA-associated ceRNA network involved in DKD by using whole gene transcripts analysis.

Abbreviations: ceRNA = competing endogenous RNA, DEGs = differentially expressed genes, DKD = diabetic kidney disease, lncRNA = long noncoding RNA.

Keywords: competing endogenous RNA, diabetic kidney disease, long noncoding RNA

1. Introduction

Diabetic kidney disease (DKD) is the major microvascular implication of diabetes mellitus, as approximately 30% to 40% of patients with diabetes mellitus will develop DKD.[1,2] In addition, DKD has become the major contributor to end-stage renal disease (ESRD) with high incidence and mortality especially in developed countries.[3]Although certain advances associated with pathological mechanism of DKD have been made to date, our understanding about this multifactorial and complicated disease remains limited, and current treatments are still not completely effective in preventing or delaying progression to ESRD. Consequently, identification of novel potential diagnostic and therapeutic biomarkers and exploration of the pathogenic mechanisms of DKD is urgently needed to prevent the occurrence and progression of this debilitating disease.

Emerging evidence has existed to highlight the key role for certain specific non-coding RNAs in the pathogenesis and therapeutic interventions of DKD.[4–6] microRNAs (miRNAs), a class of short non-codingRNAs, are approximately composed of 19 to 25 nucleotides, which function in the regulation of cellular activities and gene expression by binding to the selected sequences of target mRNAs.[7] Regarding kidney diseases, previous studies showed that miRNAs play a key role in renal fibrosis and glomerular and tubular dysfunction.[8,9] At present,

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YW and JT contributed equally to this paper.

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The datasets generated during and/or analyzed during the current study are publicly available.

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several important miRNAs have been identified that are involved in DKD, including miR-21, miR-192, miR-29a, miR-137 and miR-let-7d.[10–12] In addition, long noncoding RNAs (lncRNAs), another kind of non-coding RNAs which are a class of transcripts longer than 200 nucleotides, are reported to be centrally involved in diverse biological processes, including mRNA transcription, DNA methylation, chromatin modification, and regulation of cellular signaling.[13] Although more and more lncRNAs remain to be characterized, evidence suggests that some specific lncRNAs may serve as key early diagnostic and therapeutic biomarkers for DKD, such as EA15, LINC00472, and lncRNA HOTAIR.[14,15] More importantly, recent studies have revealed that lncRNAs can function as competing endogenous RNAs (ceRNAs) via competing with the specific miRNAs, thus altering the expression of the downstream key genes and participating in posttranscriptional regulatory networks in the development of disease. Such lncRNA-associated ceRNA regulatory networks have been found in some important biological processes in the initiation and progression of certain tumors.[16,17] However, the functional roles and exact mechanisms of their involvement in DKD are still largely unknown, and it remains some deficiencies with no lncRNA or miRNA matures, verifiable repeatability, risk assessment and model validation in the context of DKD. Moreover, single lncRNA or miRNA molecule is rarely the only reason of this complication, but the combined effect of multiple key non-coding RNAs signatures and their ceRNA regulatory networks based on statistically robust analysis should be probed and emphasized to further elucidate the biological mechanisms of DKD. Combined with the above, regulating specific non-coding RNAs especially lncRNA-miRNA-mRNA ceRNA networks are expected to open up attractive avenues for preventing and treating DKD.

In the present study, we investigated, verified and assessed key lncRNAs, miRNAs, mRNAs and their ceRNA regulatory networks in DKD by using whole genetranscripts analysis. The candidate differentially expressed mRNAs, lncRNAs and miRNAs in human glomerular and tubular tissues derived from Gene Expression Omnibus (GEO) database were systematically selected and analyzed. Functional enrichment analysis as well as protein-protein interaction (PPI) network analysis were conducted to identify hub genes and reveal their potential biological functions and regulatory mechanisms in DKD. Following this, the integrated lncRNA-associated ceRNA network involved in DKD was constructed by bioinformatics methods. Using these comprehensive analyses, this study aimed to discover novel potential biomarkers and lncRNA-associated ceRNA network for DKD and provide novel evidence for the molecular mechanisms of DKD.

2. Materials and methods

2.1. Chip data resources

The chip data used for bioinformatics analysis was downloaded from the online NCBI Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). We used "diabetic kidney disease," "diabetic nephropathy," and "Homo sapiens" as search terms to retrieve datasets. After careful screening, the independent chip dataset GSE96804 was selected as the training set for subsequent analysis.[18] The dataset GSE96804 consisted a total of 61 human glomeruli tissue samples. The glomeruli samples were from 41 DKD patients (DKD group) and 20 healthy controls (Control group). In addition, 2 more human chip datasets GSE104948 and GSE30122 in both glomerular and tubular tissues were used as the validation sets.[19,20] The detection platforms used in this study were Affymetrix Human Transcriptome 2.0 Array and Affymetrix Human Genome U133A 2.0 Array for the training and validation sets respectively. Ethical approval was unnecessary in this study because the chip data was downloaded from GEO database and we do not conduct new experiments in patients or animals.

2.2. Identification of differentially expressed genes (DEGs) in DKD

Raw data was pre-processed, normalized and background adjusted by using the Limma package.[21] The DEGs between DKD and the control group were identified based on the fold change and adjusted P value. The aforementioned criteria for significant DEGs selection were corrected fold change ≥ 1.5 and adjusted P value $<.05$. In addition, the pheatmap package (https://mirror.lzu.edu.cn/CRAN/) were utilized to visualize these expression differences.

2.3. Functional enrichment analyses

The functional enrichment analyses of the overlapping DEGs were assessed by using the online functional annotation tools of gene ontology (GO) database and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database, in combination with the STRING, DAVID database and the q value R language package.[22,23] The statistically significant cutoff values for functional enrichment analyses were gene count ≥ 2 and q value $<.05$.

2.4. Construction of the PPI network

The PPI network based on the STRING database[22] was constructed to probe into the potential interactions of the overlapping DEGs at the protein level. The parameters for the PPI network establishment were medium confidence ≥ 0.4. Score of a node in the PPI network was calculated and those nodes with high degrees were considered as the hub nodes, and their results were visualized by using the Cytoscape software (http://cytoscape.org/). Then, the PPI network relationship was clarified and the hub genes in the network were identified using the Cytoscape and cytoHubba Cytoscape plug-in software (http://cytoscape.org/).

2.5. Validation of the hub genes

To validate the hub genes identified in the training set, 2 more human chip datasets GSE104948 and GSE30122 were used subsequently. To testify the accuracy and stability of our results, 25 renal glomerular tissue samples (7 DKD patients vs 18 healthy controls) from GSE104948 and 34 renal tubular tissue samples (10 DKD patients vs 24 healthy controls) from GSE30122 were selected for validation analysis.[19,20] After batch effects removal, the differential expression analysis of the hub genes using the same method above was carried out in the 2 validation sets.

2.6. Identification of lncRNAs and miRNAs and construction of ceRNA regulatory network

The candidate differentially expressed lncRNAs and miRNAs between DKD and the control group were identified based on the

fold change and *P* value using the same method above. The aforementioned criteria were corrected fold change ≥1.5 and adjusted *P* value <.05, and the pheatmap package (https://mirror.lzu.edu.cn/CRAN/) were utilized to visualize these expression differences. MiRcode database (http://www.mircode.org/) was used to predict the lncRNA-miRNA interactions, and another 4 databases including TargetScan, miRDB, miRTarbase and StarBase were used to predict the interactions between miRNAs and the hub genes related to DKD. Furthermore, the primary lncRNA-miRNA-mRNA ceRNA regulatory networks in the progression of DKD were integrated and constructed by combining these results with the findings of KEGG pathway analysis through the qvalue R language package.[24] Pathway with a q value <.05 and *P* value <.05 was considered significantly enriched for the hub genes targeted by the miRNAs and lncRNAs signatures. The Cytoscape software (http://cytoscape.org/) was utilized to merge and visualize the ceRNA regulatory networks.

3. Results

3.1. Identification of DEGs in DKD

Following pre-processing of chip data, the DEGs were then identified from GSE96804 according to the filter criteria. The DEGs between 2 groups were shown in volcano plot (Fig. 1) and clustered and illustrated in heatmap (Fig. 2). After intersecting the above DEGs in DKD group compared with control group, and removing potential irrelevant genes, a total of 164 candidate DEGs were finally filtered and selected in the GSE96804, of which 83 were up regulated and 81 were down regulated (Fig. 2).

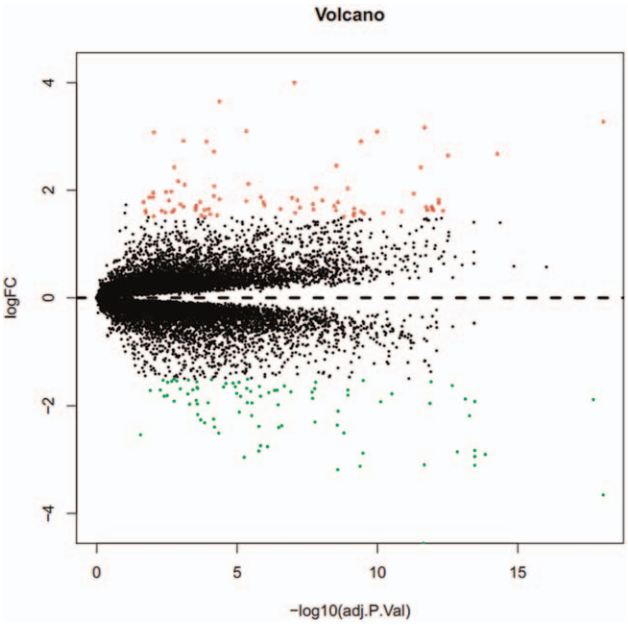

Figure 1. Volcano plot of the differential expressed genes (DEGs) between diabetic kidney disease (DKD) and control group in the training dataset GSE96804. x axis represents –log10 adjusted *P* value, and y axis represents transformed fold change (logFC).

3.2. Functional enrichment analyses of the DEGs

To probe into the biological functions of these candidate DEGs from GSE96804 and the possible regulatory mechanisms they may be involved in DKD, GO functional enrichment analysis as well as KEGG pathway analysis were conducted on these DEGs associated with DKD. The GO functional enrichment analysis of the DEGs in the GSE96804 was conducted from the aspects of molecular functions (MF), biological processes (BP), and cell components (CC). The GO enrichment analysis results revealed that there were a total of 320 functional items, including 64 MF, 237 BP, and 19 CC. As shown in Figure 3A, in the MF analysis, the top 5 enrichment items were organic acid binding, antioxidant activity, fatty acid binding, monocarboxylic acid binding, and RAGE receptor binding. In the BP analysis, the top 5 significant items were cellular oxidant detoxification, receptor-mediated endocytosis, cellular detoxification, cellular response to toxic substance, and neutrophil migration as shown in Figure 3B. Meanwhile, the GO CC analysis results revealed that the top 5 enrichment items were blood microparticle, collagen-containing extracellular matrix, collagen trimer, secretory granule lumen, and cytoplasmic vesicle lumen (Fig. 3C). Whereas in KEGG pathway enrichment results, 32 significant pathways were enriched for the DEGs, among which the top 5 were prion diseases, staphylococcus aureus infection, glycolysis/gluconeogenesis, phenylalanine metabolism, and IL-17 signaling pathway as shown in Table 1.

3.3. PPI network of the hub genes

Using the filter criteria, a PPI network was then constructed, containing 102 nodes and 273 interactions as shown in Figure 4A. In selected modules, the DEGs from the GSE96804 were ranked by using the abovementioned formula and the top 10 nodes with high degrees in PPI network were identified as the hub genes, including 3 up regulated genes (FN1, CCL2, and C3) and 7 down regulated genes (ALB, KNG1, EGF, PTGS2, CXCR2, FOS, and CXCR1) (Table 2 and Fig. 4B).

3.4. Validation of the hub genes related to DKD

To further validate the hub genes identified from the training set, we then compared them with the DEGs in the GSE104948 and GSE30122 datasets which were used as the validation sets. The box plots of the hub genes were presented in Figure 5, among which C3, CCL2, EGF, FN1, and VCAN simultaneously showed significant differences again in the 2 validation datasets, consistent with that observed in the training set.

3.5. Identification of lncRNAs and miRNAs signatures in DKD

The candidate lncRNAs and miRNAs signatures which may regulate the DEGs were finally identified from GSE96804 respectively based on the differential expressions. The differentially expressed lncRNAs and miRNAs between 2 groups were clustered and illustrated in heatmap (Fig. 2). Our results revealed that a total of 6 lncRNAs (lnc-HIST2H2AA4-1, LOC100190986, MAGI2-AS1, BRE-AS1, LOC100505985, and VCAN-AS1) and 18 miRNAs (MIR4521, MIR4256, MIR548AD, MIR548D2, MIR548AI, MIR1254-1, et al) were finally identified respectively, indicating their vital roles in the pathogenesis of DKD (Table 3).

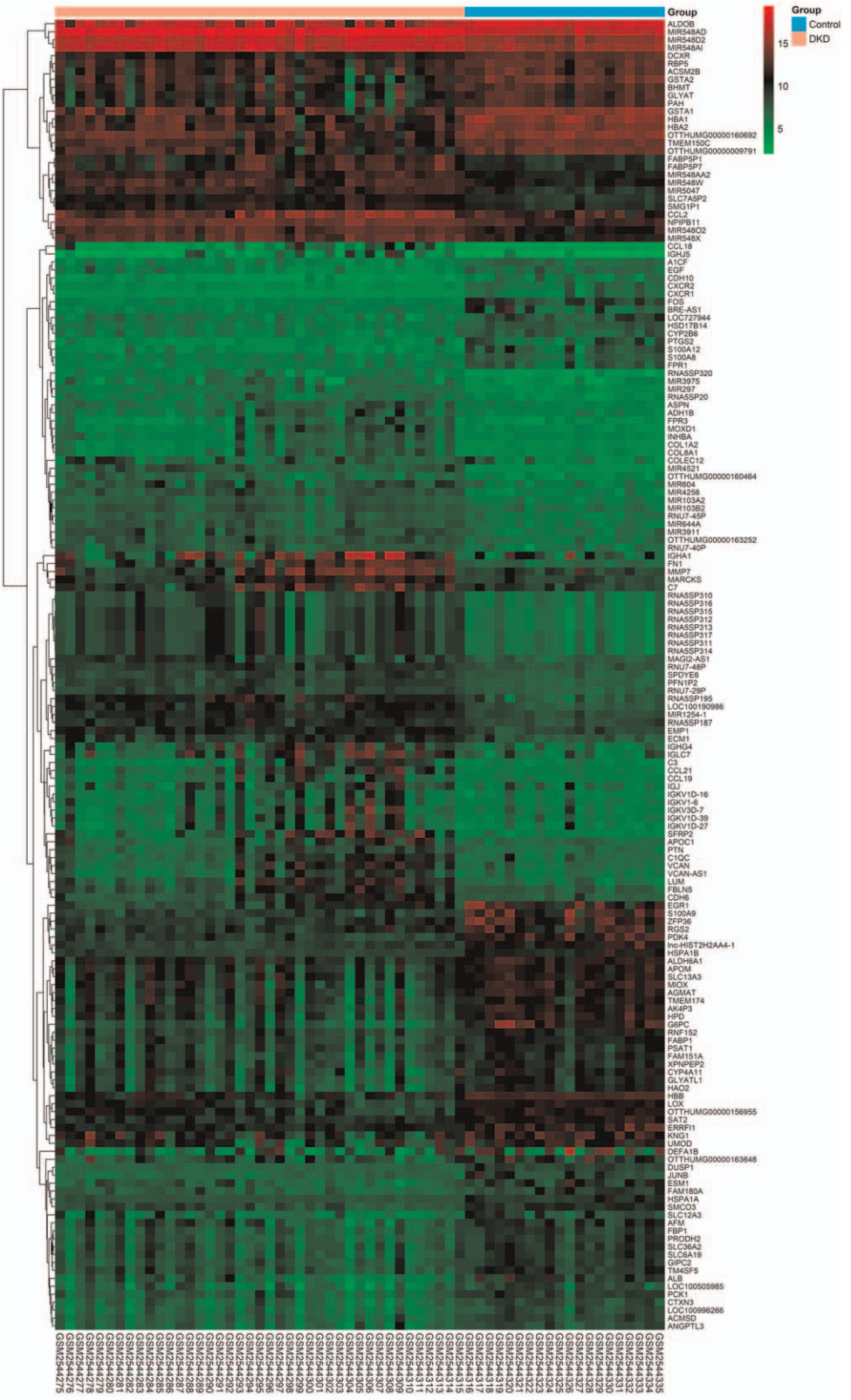

Figure 2. Heatmap of the expression profiles including DEGs, lncRNAs, and miRNAs between DKD and control group in the training dataset GSE96804.

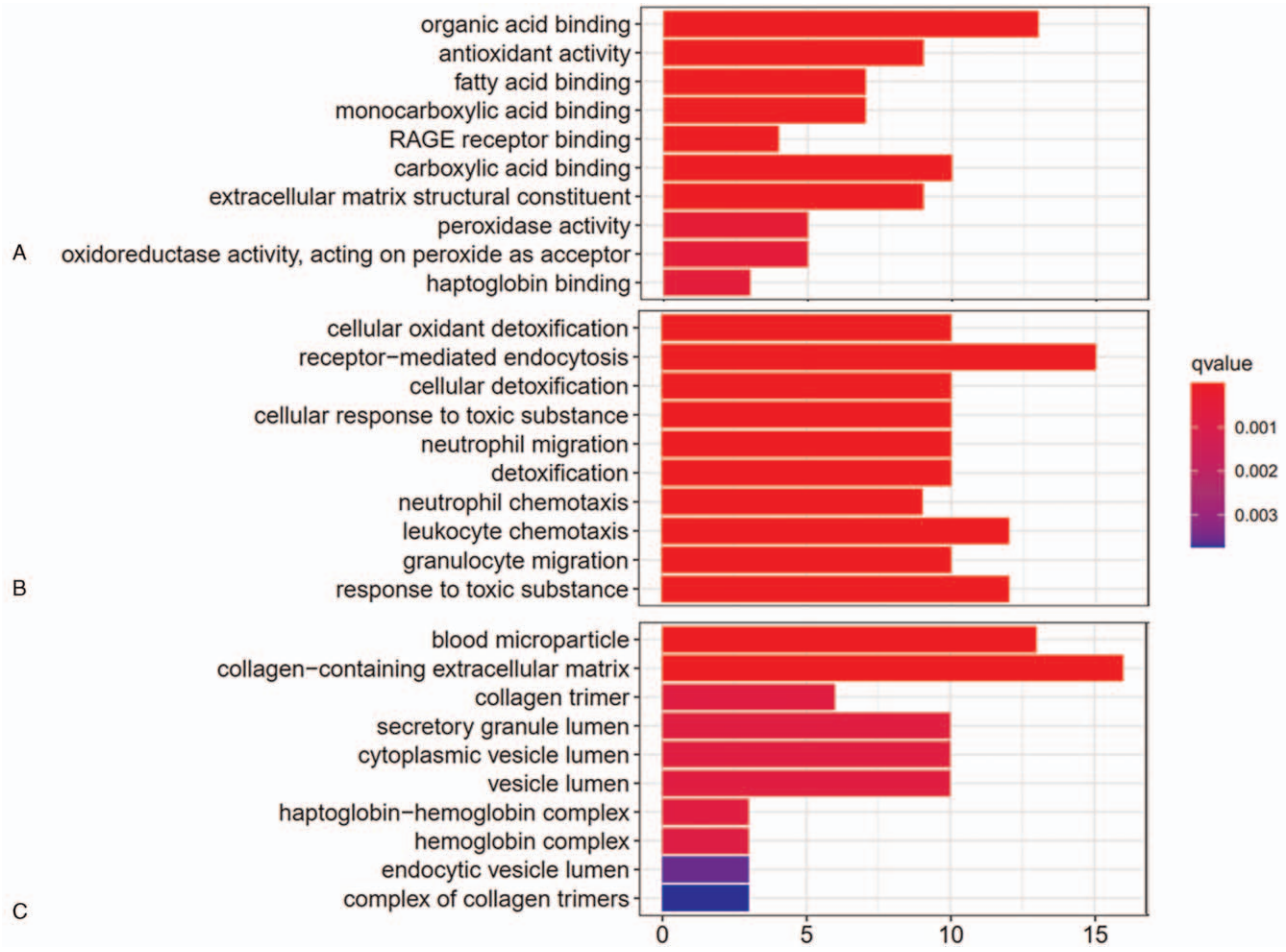

Figure 3. The gene ontology (GO) enrichment analysis of the DEGs in DKD in the training dataset GSE96804. The GO analysis of the DEGs from the aspects of MF (A), BP (B), and CC (C) with significant enrichment (top 10). Color of the bar chart indicates q value, and x axis indicates count of the DEGs in the GO function items. BP = biological processes, CC = cell components, MF = molecular functions.

Table 1

KEGG pathway analysis of the DEGs.

ID	Term	*P* value	DEGs
hsa05020	Prion diseases	.000256006	EGR1,C7,HSPA1A,C1QC
hsa05150	Staphylococcus aureus infection	.000349668	C3,FPR1,FPR3,DEFA1B,C1QC
hsa00010	Glycolysis/Gluconeogenesis	.000349668	G6PC,ADH1B,ALDOB,PCK1,FBP11
hsa00360	Phenylalanine metabolism	.000429976	PAH,GLYAT,HPD
hsa04657	IL-17 signaling pathway	.001458378	CCL2,FOS,PTGS2,S100A9,S100A8
hsa00980	Metabolism of xenobiotics by cytochrome P450	.004299684	CYP2B6,ADH1B,GSTA2,GSTA1
hsa05204	Chemical carcinogenesis	.006187546	ADH1B,GSTA2,GSTA1,PTGS2
hsa04062	Chemokine signaling pathway	.007173479	CXCR1,CCL21,CXCR2,CCL2, CCL19, CCL18
hsa05418	Fluid shear stress and atherosclerosis	.008143607	DUSP1,GSTA2,GSTA1,CCL2,FOS
hsa00330	Arginine and proline metabolism	.00946077	SAT2,PRODH2,AGMAT
hsa04933	AGE-RAGE signaling pathway	.012269268	EGR1,COL1A2,FN1,CCL2
hsa03008	Ribosome biogenesis in eukaryotes	.012688633	SNORD3B-2,SNORD3B-1 SNORD3C, SNORD3A
hsa00983	Drug metabolism	.015881308	CYP2B6,ADH1B,GSTA2,GSTA1
hsa04060	Cytokine-cytokine receptor interaction interaction	.016421793	CXCR1,CCL21,CXCR2,CCL2, A;CCL19;CCL18 INHBA, CCL19, CCL18
hsa04668	TNF signaling pathway	.016877923	CCL2,FOS,PTGS2,JUNB

DEGs = differentially expressed genes, KEGG = Kyoto Encyclopedia of Genes and Genomes.

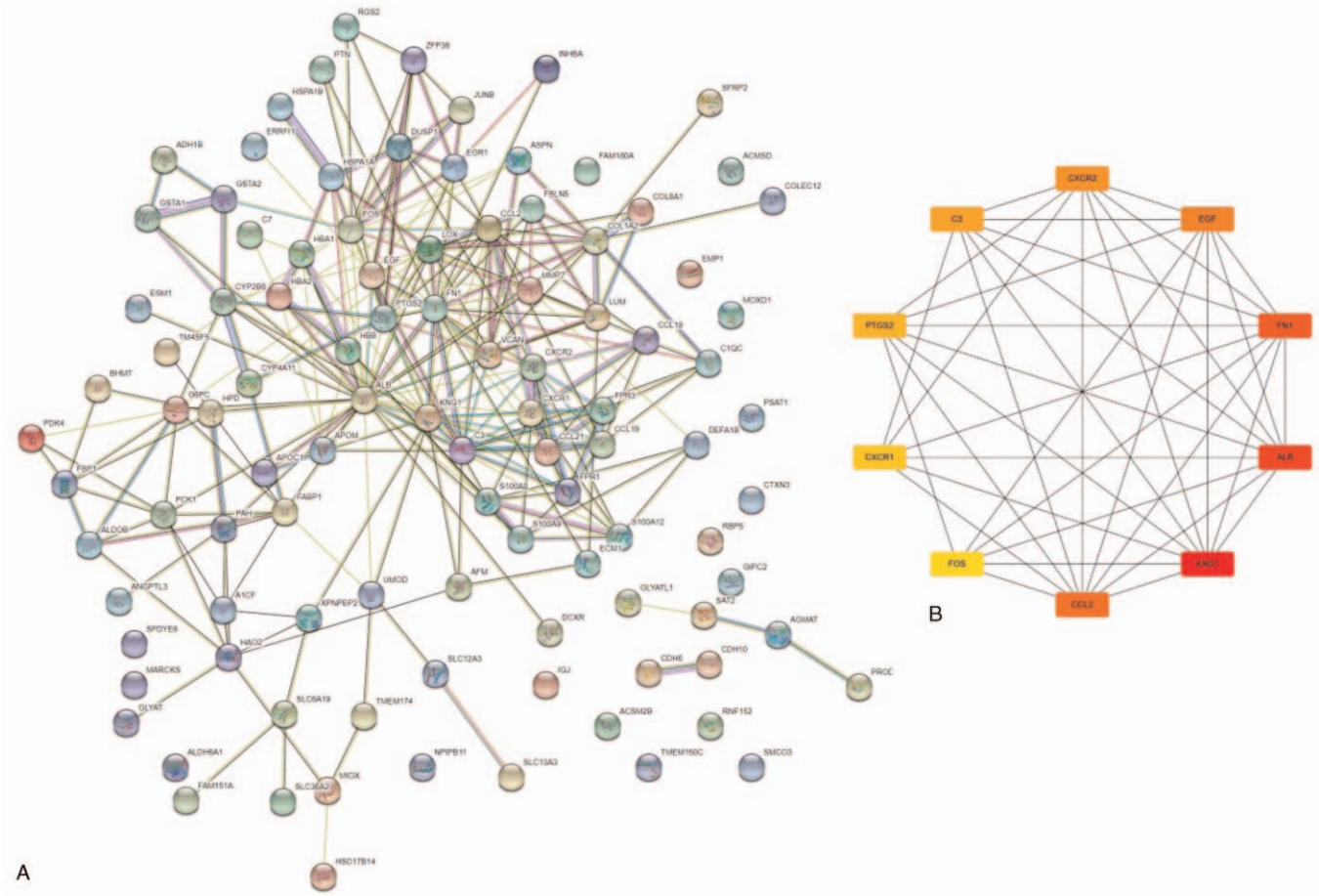

Figure 4. The protein-protein interaction (PPI) network analysis in the training dataset GSE96804. (A) The PPI network of the DEGs in DKD. (B) The PPI network of the hub genes in DKD. Network nodes indicate proteins, and edges indicate PPI. Darker color represents a higher level of interaction, and lighter color represents a lower level of interaction.

3.6. Construction of the integrated lncRNA-associated ceRNA network related to DKD

The integrated lncRNA-associated ceRNA regulatory network related to DKD was constructed, which revealed that single lncRNA could target multiple miRNAs and their target genes, and that single miRNA or gene was regulated by multiple lncRNAs or miRNA separately. As indicated in such integrated regulatory network, lnc-HIST2H2AA4-1, VCAN-AS1 and MAGI2-AS1 were identified as the 3 key lncRNAs, and each regulatory network contained at least 1 identified lncRNA which formed close connections with other miRNAs as well as mRNAs (Fig. 4A). Among this, there were 2 miRNAs connected with lnc-HIST2H2AA4-1, and 12 miRNAs linked with VCAN-AS1. At the same time, there were 5 miRNAs related to MAGI2-AS1 in the integrated regulatory network. It is of note that VCAN, FN1, CCL2, and KNG1 were identified as the predominant genes in

Table 2

The hub genes of DKD.

Regulation	Gene Symble sSymbleSymble	Gene Title	Degree	logFC	*P* value
Upregulation	FN1	Fibronectin 1	21	4.00264072	9.10E-08
	CCL2	C-C Motif Chemokine Ligand 2	17	1.71435445	.000284852
	C3	Complement C3	15	1.97677666	.002204951
Downregulation	ALB	Albumin	35	−2.75887	8.31E-07
	KNG1	Kininogen 1	23	−1.91301	.00175948
	EGF	Epidermal Growth Factor	17	−1.50573	4.27E-06
	PTGS2	Prostaglandin-Endoperoxide Synthase 2Synthase 2	15	−2.50535	1.57E-09
	CXCR2	C-X-C Motif Chemokine Receptor 2Receptor 2	15	−1.91982	3.42E-14
	FOS	Fos Proto-Oncogene, AP-1 Transcription Factor Subunit	15	−3.65627	9.07E-19
	CXCR1	C-X-C Motif Chemokine Receptor 1	14	−1.95441	1.35E-12

DKD = diabetic kidney disease.

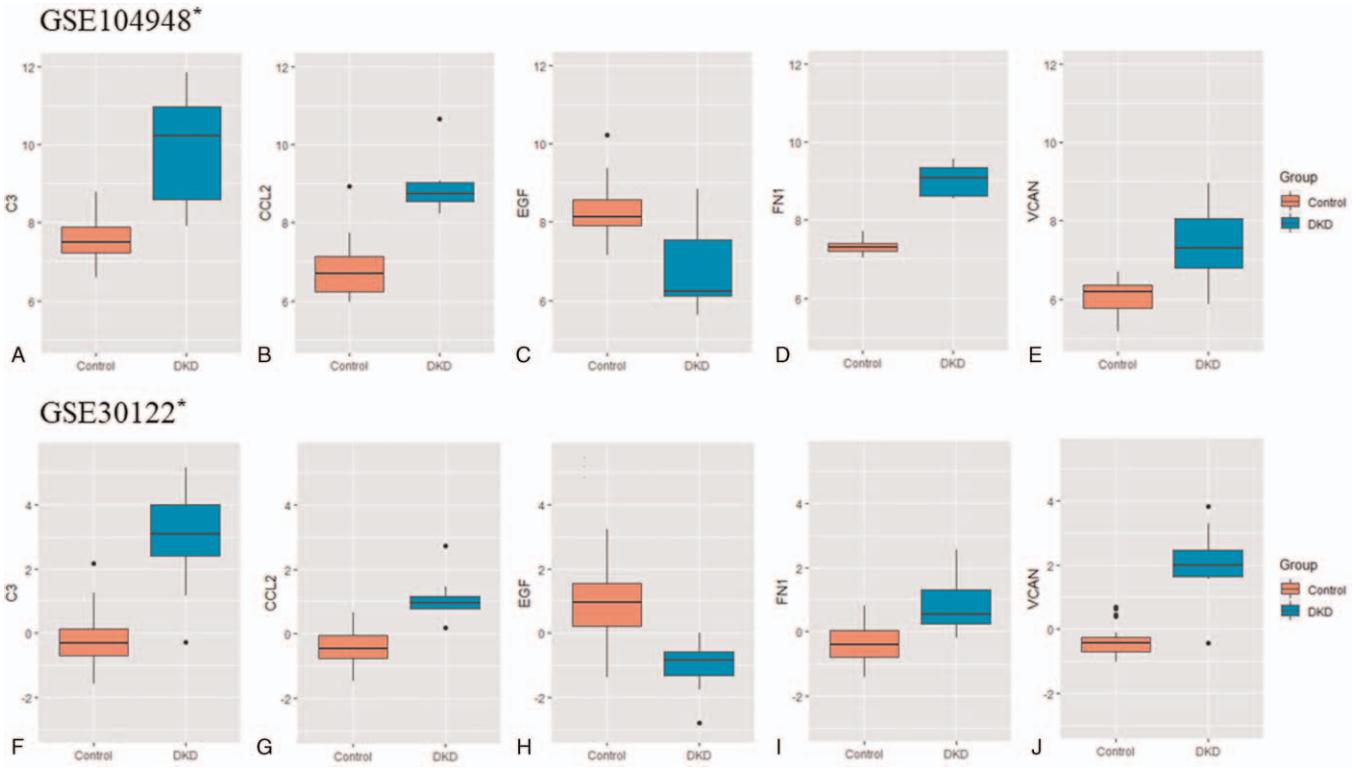

Figure 5. Validation of the hub genes related to DKD. The box plots of the key hub genes (C3, CCL2, EGF, FN1 and VCAN) showed significant differences between DKD and control group in the 2 validation datasets GSE104948 and GSE30122. Up: GSE104948. Down: GSE30122. *corrected $P < .05$.

this lncRNA-associated ceRNA network, indicating their pivotal roles in regulation of DKD (Fig. 6). Integrating with the KEGG pathway enrichment results, these key hub genes were mainly enriched in the immune and inflammation-related pathways, including IL-17 signaling pathway, TNF signaling pathway, cell adhesion molecules, complement and coagulation cascades, chemokine signaling pathway, and cytokine-cytokine receptor interaction. Furthermore, AGE-RAGE signaling pathway was intensively enriched, suggesting a vital role in the occurrence and progression of DKD (Fig. 7).

Table 3

The differentially expressed lncRNAs and miRNAs (top 6) in DKD.

Regulation	lncRNA/miRNA	logFC	*P* value
lncRNA			
Upregulation	LOC100190986	2.42579516	2.86E-12
	MAGI2-AS1	1.87166756	.00000141
	VCAN-AS1	1.5936535	.002055793
Downregulation	lnc-HIST2H2AA4-1	−2.1844679	
−2.1844679			
	5.27E-14		
	BRE-AS1	−2.3814417	.00000167
	LOC100505985	−1.9642697	.00026086
miRNA			
Upregulation	MIR4521	3.27223852	9.06E-19
	MIR4256	2.67496149	5.36E-15
	MIR548AD	1.61659718	4.59E-13
	MIR548D2	1.76061403	6.51E-13
	MIR548AI	1.63952248	1.14E-12
	MIR1254-1	1.69400033	1.17E-12
Downregulation			
	None		

lncRNA = long noncoding RNAs, miRNAs = microRNAs, DKD = diabetic kidney disease.

4. Discussion

At present, DKD has become the major cause of ESRD with high incidence and mortality, seriously affecting the survival quality of patients.[3] Although certain advances associated with the pathological mechanism of DKD have been made to date, our understanding about this complicated disease remains limited, and current treatments are still not completely effective in preventing or delaying progression to ESRD. Urinary microalbumin has been considered to be an early basic diagnostic marker for DKD up to now, yet its sensitivity is still not enough.[2,5] Therefore, identification of novel potential diagnostic and therapeutic biomarkers with high sensitivity and specificity is of great significance.

In this study, a total of 164 DEGs correlated with DKD were finally filtered and selected from the independent human chip dataset GSE96804 in GEO, and the hub genes were further identified and selected in all comparative conditions, including 3 upregulated genes (FN1, CCL2, and C3) and 7 down regulated genes (ALB, KNG1, EGF, PTGS2, CXCR2, FOS, and CXCR1). Furthermore, the candidate lncRNAs and miRNAs signatures that may regulate the DEGs associated with DKD were finally identified respectively based on the differential expressions, including 6 lncRNAs (lnc-HIST2H2AA4-1, LOC100190986, MAGI2-AS1, BRE-AS1, LOC100505985, and VCAN-AS1)

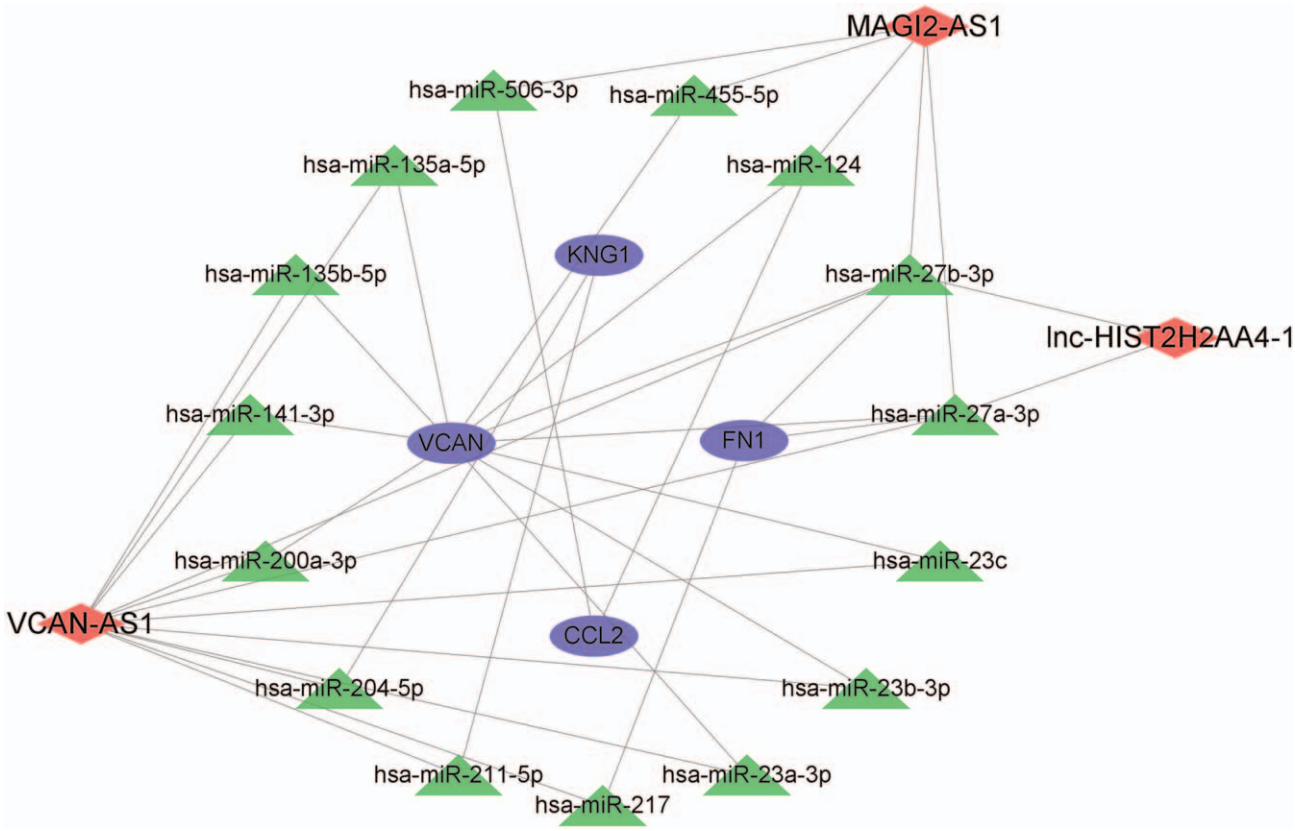

Figure 6. The lncRNA-associated ceRNA regulatory networks in DKD. Red color represents the key lncRNA, green color represents miRNA, and bule color represents the predominant genes. miR = miRNA, lncRNA = long noncoding RNA.

and 18 miRNAs (MIR4521, MIR4256, MIR548AD, MIR548D2, MIR548AI, MIR1254-1, et al), indicating their vital roles in DKD. It is noteworthy that since previous studies have mostly focused on biological function of either a single type of markers or limited number of molecules, but the combined effect of the integrated lncRNA-miRNA-mRNA-signaling pathways should be further emphasized in the pathogenesis of DKD. Strikingly, another important finding in this study is that the lncRNA-associated ceRNA regulatory network related to DKD was constructed, reflecting a comprehensive understanding of molecular interactions in key lncRNA-miRNA-mRNA-signaling pathways involved in DKD. Among which, lnc-HIST2H2AA4-1, VCAN-AS1 and MAGI2-AS1 were identified as the 3 key lncRNAs, suggesting that the above lncRNAs might be potential biomarkers for DKD. On the other hand, VCAN, FN1, CCL2, and KNG1 were identified as the predominant genes in this lncRNA-associated ceRNA network, indicating their pivotal roles in regulation of DKD. What is worth noticing is that consistent with that observed in the training set, 3 of the key genes (VCAN, FN1, and CCL2) also showed significant differences in the 2 validation datasets in both glomeruli and tubules, suggesting the robustness and stability of these differences among datasets and renal tissues. Integrating with the KEGG pathway enrichment results, these key hub genes were mainly enriched in the immune and inflammation-related pathways. Combined with the above, since lncRNA was reported to be a new target for managing inflammation response in some diseases,[26,27] this study once again supported and emphasized that certain specific lncRNA-associated ceRNA regulatory networks were involved in immune and inflammation processes of DKD, which may be expected to provide us promising novel targets for the diagnosis and treatment of DKD.

In terms of the key lncRNAs we identified, VCAN-AS1, located in 5q14.3, has been found to be upregulated in gastric cancer (GC) tissues and cell lines which contributed to the progression of GC via a p53-dependent pathway.[28] Nevertheless, the involvement and underlying biological mechanisms of VCAN-AS1 in DKD remains unclear. Additionally, these lncRNAs are just only the tip of iceberg, and lnc-HIST2H2AA4-1 and MAGI2-AS1 are also two novel lncRNAs that has never been identified and explored before. To our knowledge, this is the first study to identify the above novel lncRNAs and reveal a global view of integrated lncRNA-associated ceRNA regulatory network in DKD based on whole gene transcripts analysis. This study makes a significant contribution to the identification of novel lncRNAs, miRNAs, and their ceRNA regulatory network in DKD and provide novel evidence for the underlying molecular mechanisms of DKD.

On the other hand, 1 limitation existed in the present study was that it lacked further experimental validation of the lncRNA-associated ceRNA regulatory networks to confirm their functional roles in DKD. Along with the progress of genome-sequencing technology, elucidation of the precise molecular mechanisms of their involvement in DKD progression will be performed in our future study.

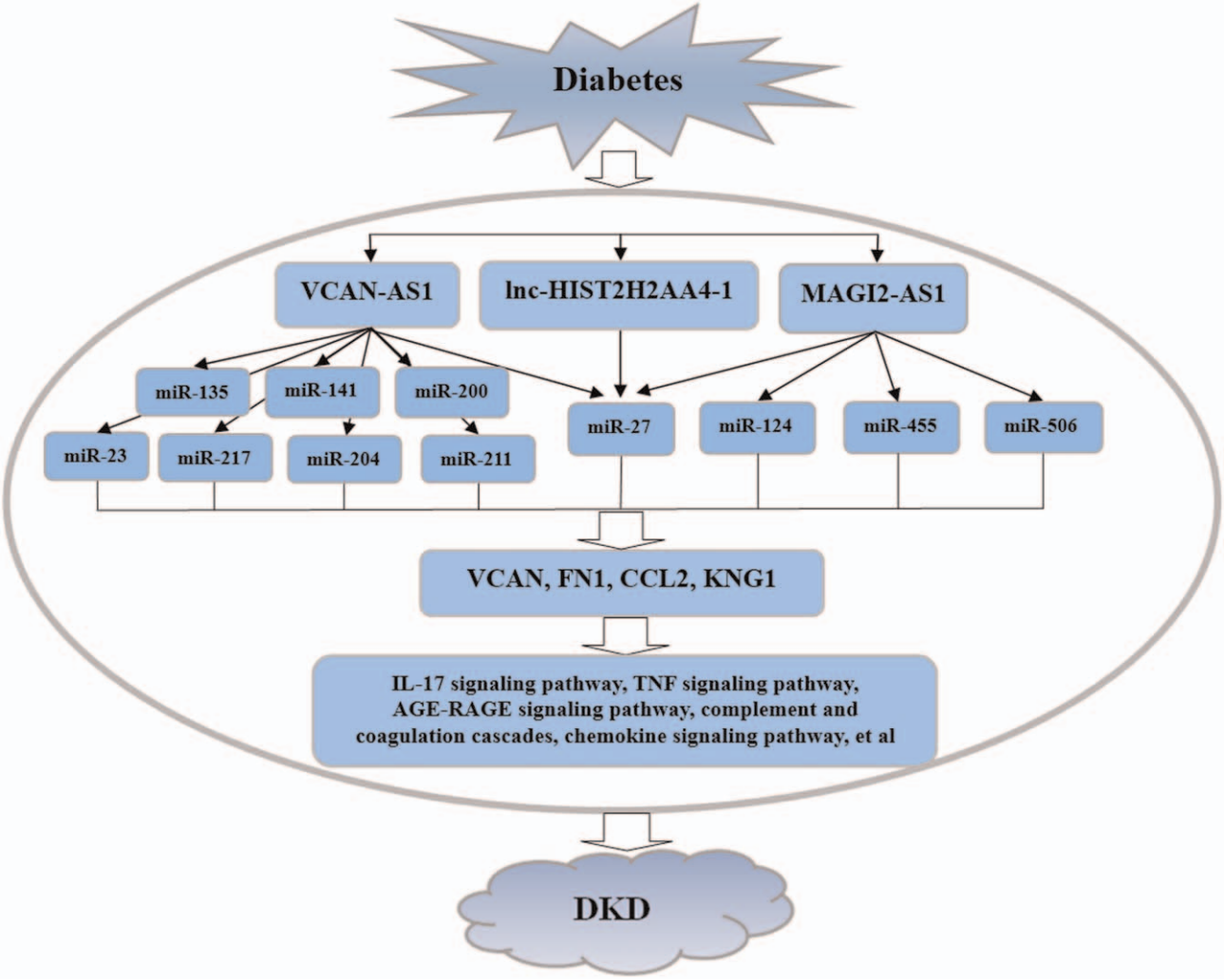

Figure 7. Schematic diagram of the integrated lncRNA-associated ceRNA regulatory networks in DKD. miR = miRNA, ceRNA = competing endogenous RNA.

5. Conclusions

In summary, this study identified and validated key lncRNAs, miRNAs and their targets, and further revealed a global view of lncRNA-associated ceRNA regulatory network involved in DKD by using whole gene transcripts analysis. These findings have significant value for early diagnosis and therapy of DKD and may provide new insights into the pathogenesis of DKD. New scientific discoveries and molecular biological experiments are needed to confirm the function of these novel lncRNAs, miRNAs and their ceRNA regulatory network responsible for DKD and further translate these findings to clinical application.

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Validation: Youshan Zhang.
Writing – original draft: Ya Wang.
Writing – review & editing: Ya Wang, Ying Xiong, Cunjian Yi.

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