Am J Transl Res 2023;15(9):5730-5746
www.ajtr.org /ISSN:1943-8141/AJTR0151402

Original Article

Identification of lncRNA-miRNA-mRNA ceRNA network as biomarkers for acute kidney injury

Chengxiao Hu, Jianfei Li

Department of Nephrology, Liuzhou People's Hospital, Liuzhou, Guangxi, China

Received May 24, 2023; Accepted July 25, 2023; Epub September 15, 2023; Published September 30, 2023

Abstract: Objective: Acute kidney injury (AKI) is a global problem due to its high morbidity and mortality. The aim of this study was to identify the key RNAs involved in the ischemia/reperfusion (I/R) or cisplatin (CIS) induced AKI. Methods: Gene Expression Omnibus database was used to download the microarray dataset GSE106993, GSE130814 and GSE98622. Differentially expressed lncRNAs (DE-lncRNAs) and DE-mRNAs were identified in I/R and CIS induced AKI. The target miRNAs of DE lncRNAs were predicted from miRDB, and the miRNA of lncRNA target mRNAs were predicted form StarBase dataset. The ceRNA regulatory networks, GO and KEGG enrichment analysis, and protein-protein interaction (PPI) of I/R and CIS induced AKI specific genes were constructed. The CIBESORT was applied to infer the proportion of 22 immune infiltration cells based on gene expression profiles of I/R and CIS induced AKI. Results: Totally, 2 DE-lncRNAs and 375 DE-mRNAs were identified in I/R and CIS induced AKI. The common ceRNA network was constructed between CIS group and I/R induced AKI group, which contained 2 lncRNAs (Platr7 and Gm15611), 65 mmu-miRNAs and 167 mRNAs. The 167 common mRNAs were enriched in the biological process of transcription regulation, metabolic process, cell proliferation, the cellular component (CC) of extracellular region and space, the molecular function of DNA binding, and transcription regulator activity in CIS and IRI induced AKI. The common 167 mRNAs involved in the MAPK signaling pathway and JAK-STAT signaling pathway were identified. Protein-Protein Interaction (PPI) Network of ceRNAs network expressed gene was constructed, including 81 nodes, which contained 3 upregulated genes and 78 downregulated genes. Among them, mitochondrial apoptosis-related genes Pmaip1 and Nptx1 showed significantly high expression in the GSE98622 and GSE106993 data sets. The investigation to the connection between the gene expression profiles and immune cell infiltration showed considerable differences in immune cell percentage between AKI group and normal group. Conclusion: Novel lncRNAs and mRNAs were identified, which may serve as potential biomarkers to predict the diagnostic and therapeutic targets for AKI patients based on a large-scale sample. More importantly, the ceRNA network of I/R or CIS induced AKI was constructed, which provides valuable information to further explore the molecular mechanism underlying onset and progression of AKI.

Keywords: LncRNAs, ceRNAs, mRNAs, miRNAs, mitochondrial apoptosis, immune infiltration

Introduction

Acute kidney injury (AKI) is the most common acute diseases, which is characterized by rapid decline in renal function and eventually leads to acute renal failure and other organ failure [1]. AKI is initiated in various ways, including ischemia/reperfusion (I/R), application of surgical contrast agents, improper administration of drugs, rhabdomyolysis, infection, etc. [2, 3]. In recent years, the incidence of AKI is increasing, the incidence of AKI in hospitalized patients is 1-5%, and the mortality rate is up to 50% [4]. I/R injury is the main cause of AKI in clinic, accounting for 60% of all causes, and is also the primary cause of acquired AKI in domestic hospitals [5]. The mechanism of AKI induced by I/R is very complex, including oxidative stress, cell apoptosis, inflammatory response, and other factors [6]. Cisplatin (CIS) is an effective drug in the treatment of solid tumors. Noteworthy, its serious toxic and side effects limit its clinical application. Nephrotoxicity is the most common side effect, occurring in 25% to 40% of patients receiving CIS therapy [7]. To date, no effective targeted therapies are available to treat AKI. As a result, it is critical to find promising biomarkers and novel therapeutic

targets for more precise AKI diagnosis and treatment.

Recently, researchers paid attention to innate and adaptive immune responses which have a role in modulating AKI recovery and injury to renal tubular cells. Injuries to the kidney are caused by dendritic cells, neutrophils, monocytes/macrophages, T lymphocytes, and B lymphocytes. Due to increased production and slower clearance of cytokines as well as immune cell malfunction, AKI itself increases the chance of contracting an infection. The most common type of cell that AKI renders dysfunctional is neutrophils. On the other hand, M2 macrophages and regulatory T cells are crucial for reducing inflammation and healing tissues after kidney injury [8]. It was reported that the $CD4^+$ T cell was a major pathogenic factor in ischemic acute renal failure [9]. $CD4^+$ and $CD8^+$ T cells provide protection against AA-induced acute tubular necrosis [10]. There is growing evidence that the tumor microenvironment contains an imbalance of pro-inflammatory and anti-inflammatory cells which play an important role in the development of malignancies [11]. However, to date, little is known regarding the complex immune microenvironment in AKI.

It is well known that mitochondria are important organelles in cells, which play vital roles in metabolic modulation, adenosine triphosphate (ATP) synthesis, reactive oxygen species (ROS) generation, and cell differentiation and death. Recent studies have shown that mitochondrial dysfunction has been recognized as a contributor in AKI. Renal microvascular loss, oxidative stress, apoptosis, and eventually renal failure are all exacerbated by persistent dysregulation of mitochondrial homeostasis in AKI [12]. Thus, understanding the molecular processes that control mitochondrial activities and the pathophysiology in AKI is crucial to find new therapeutic approaches.

Over the past decades, non-coding RNAs (ncRNA) have been shown to play an important role in a variety of cellular physiological and pathological processes, including cell proliferation, cell death, inflammation, etc. [13]. Long noncoding RNA (LncRNA) is a type of non-coding RNA with a transcript length greater than 200 nt, which is transcribed by RNA polymerase II. Abnormally expressed lncRNAs often play a role in promoting or inhibiting the development of AKI [14-16]. Recent studies have found that lncRNAs regulate the key microRNAs (miRNAs) in AKI through the mechanism of competing with endogenous RNA (ceRNA), which plays an important role in the occurrence and development of AKI [17]. ceRNA networks, as a new mechanism for regulating genes at the post-transcriptional level with competitive endogenous regulation, can be applied to the systematic study of biological processes and provide a new method for the investigation and treatment of diseases [18]. The potential lncRNAs associated with ceRNA pairs involved in CIS or I/R induced AKI stills remain unclear.

In this study, differentially expressed lncRNAs and mRNAs were explored in I/R and CIS induced AKI. The target miRNAs of DE lncRNAs and the miRNA of lncRNA target mRNAs were predicted. lncRNA-miRNA-mRNA ceRNA network was constructed in I/R and CIS induced AKI. We further analyzed Gene Ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of I/R and CIS-induced deferentially expressed genes in ceRNA network. A protein-protein interaction (PPI) network of I/R and CIS-induced specific genes in AKI was construct. Finally, the correlation between mRNA and immune cell infiltration in the tumor microenvironment was evaluated based on the CIBERSORT algorithm. Our findings may shed light on a novel mechanism of AKI etiology and provide novel diagnostic or prognostic markers and therapeutic target.

Materials and methods

Gene expression omnibus dataset

The genes expression profile of AKI was obtained from Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo). The GSE106993 dataset contains 3 normal group and 3 CIS-induced AKI group samples in mice, and the GSE130814 includes 2 normal group and 6 CIS-induced AKI group samples in mice. The GSE106993 was used to analyze the DE lncRNA, and the GSE130814 was used to analyze the DE mRNA in CIS-induced AKI group and normal group. The GSE98622 dataset, which contains 9 normal group and 31 ischemia reperfusion injury (IRI) induced AKI group

sample in mice, was used to analyze DE mRNAs and DE lncRNAs between IRI-induced AKI group and normal group.

Identification of DE mRNAs and lncRNAs

After the expression data of normal group and AKI group were normalized, the differentially expressed lncRNAs and mRNAs were analyzed using Limma package in R software based on *P*-value < 0.05 and log2|fold change (FC)| ≥ 1. The gene ID was transformed into gene symbol by using the gene annotation file of mice in GENCODE (https://www.gencodegenes.org/). The 'pheatmap' package was used to exhibit the heat map in R software [20].

Prediction of targets

The ceRNA network of AKI was then developed step-by-step according to the following procedures. First, the file of potential interactions of DE lncRNA-miRNA was downloaded from miRDB (http://mirdb.org) [21]. Next, the highly reliable online miRNA reference databases, StarBase dataset (http://starbase.sysu.edu.cn/) [22] were used to retrieve experimentally-validated or predictive miRNA targeted mRNAs. The targeted mRNAs were further compared with differentially expressed RNAs identified between normal group and AKI group, and the intersected mRNAs were retained to develop the ceRNA network.

Construction of lncRNA-miRNA-mRNA ceRNA network

The CIS-specific lncRNA-miRNA-mRNA ceRNA network, IRI induced AKI-specific lncRNA-miRNA-mRNA ceRNA network and common ceRNA network were constructed. Cytoscape software (San Diego, CA, USA) was used to visually display.

GO and KEGG analysis

GO functional and KEGG pathway enrichment analyses of CIS-induced specific genes, IRI-induced specific genes, and common genes in ceRNA network were enriched by using DAVID database (https://david.ncifcrf.gov/) [23]. The cellular component (CC), molecular function (MF), and biological process (BP) were analyzed. GO terms with *p* values less than 0.05 were considered significantly enriched.

Protein-protein interaction (PPI) network construction

PPI network of CIS-induced specific genes, IRI-induced specific genes and common genes in AKI was constructed with the STRING database (https://string-db.org/) and visualized using Cytoscape software [24]. STRING database is a functional protein association network, assembling all known and predicted proteins. Multiple protein names were put into the list box, with one name per line. PPI network interaction file with medium confidence scores ≥ 0.4 was downloaded.

Immune infiltration analysis

Normalized gene expression data were uploaded to TIMER2.0 (http://timer.cistrome.org/), a website that provides comprehensive evaluation and analysis of tumor-infiltrating immune cells, to calculate the proportion of the 22 immune cells in each sample based on the CIBERSORT algorithm. The Wilcoxon rank-sum test was used to assess the variations in immune cell infiltration between the IR/CIS-induced group and normal group.

Statistical analysis

P-value < 0.05 was considered statistically significant. Data processing and analysis were performed using Microsoft Excel and R software (R software, version 3.5.1).

Results

Identification of differentially expressed (DE) lncRNAs and mRNAs in AKI

To explore the difference of RNA expression in AKI tissue and normal kidney tissues, DE-lncRNAs and DE-mRNAs were screened out by microarray data analysis. *P*-value < 0.05 and fold change ≥ 1 was used as the threshold of screening DE-lncRNAs and DE-mRNAs. It was reported that CIS induced AKI to change lncRNAs and mRNA expression level. As shown in the Volcano plot (**Figure 1A** and **1B**), a total of 26 DE-lncRNAs were identified between normal group and CIS group from GSE106993 and a total of 1627 DE-RNAs were identified between normal group and CIS group from GSE130814; red indicates upregulated lncRNA and mRNA, and blue indicates downregulated lncRNA and mRNA. The heatmap (**Figure 1C** and **1D**) shows the DE-lncRNA expression level

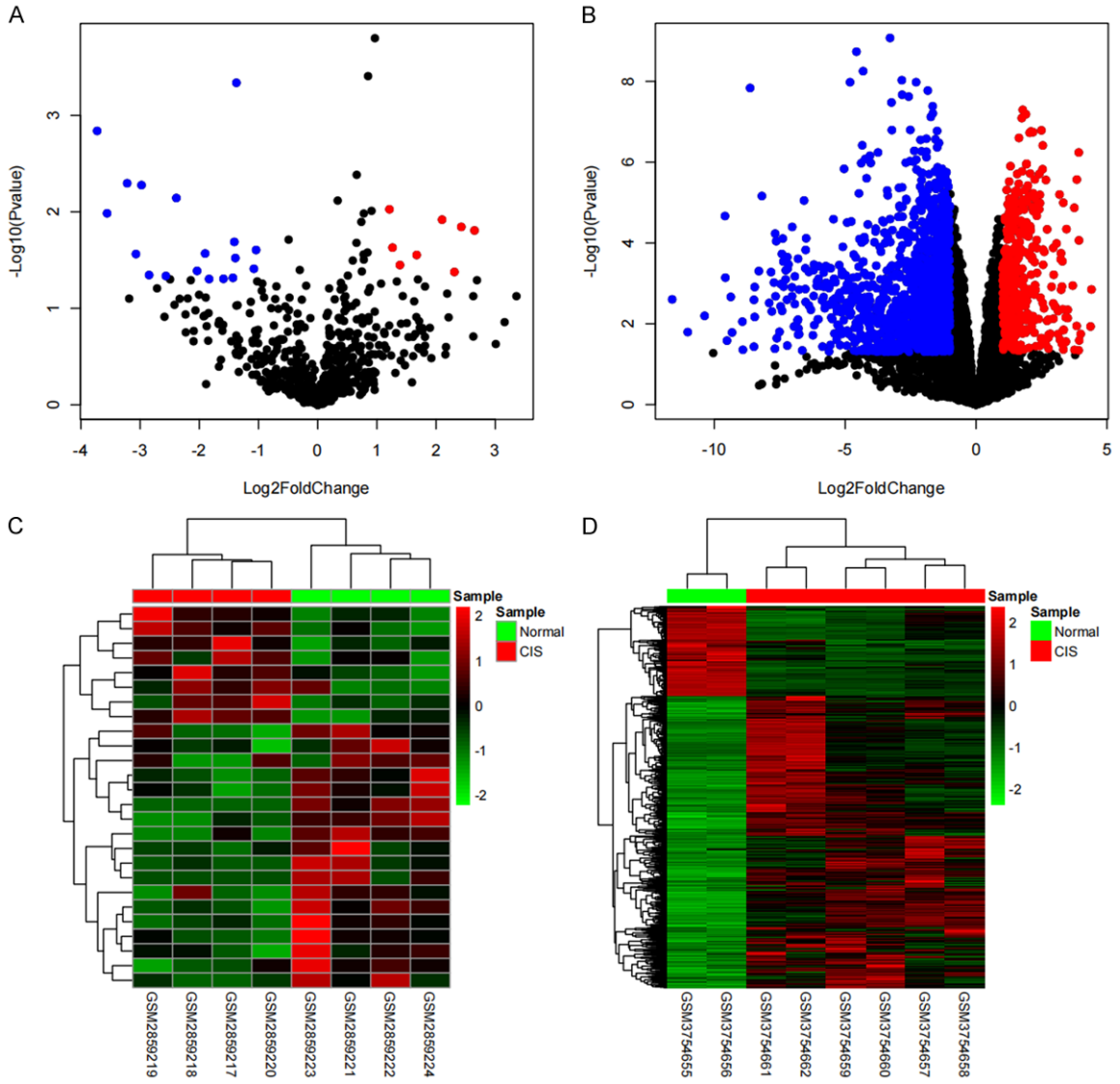


Figure 1. Identification of DE-lncRNAs and DE-mRNAs in CIS induced AKI. A. Volcano map of DE-lncRNAs in GSE106993. B. Volcano map of DE-mRNAs in GSE130814. Blue represents downregulated genes, and red represents upregulated genes. C. The heatmap shows the expression level of DE-lncRNAs in normal group and CIS group. D. The heatmap shows the expression level of DE-mRNAs in normal group and CIS group. AKI, acute kidney injury.

in 3 normal group and 3 CIS-induced AKI group samples and the DE-mRNA expression level in 2 normal group and 6 CIS-induced AKI group samples in mice.

In IRI induced AKI, the Volcano plot shows that a total of 321 DE-lncRNAs (**Figure 2A**) and 2635 DE-mRNA (**Figure 2B**) were identified between normal group and IRI induced group. Next, the heatmap shows the DE-lncRNAs expression level (**Figure 2C**) and DE-mRNAs expression level (**Figure 2D**) in 9 normal group and 31 IRI induced AKI group samples. Subsequently, we performed Venn analysis to obtain more credible DE-lncRNAs and DE-mRNAs. Next, Venn diagram shows that there were 2 common DE-lncRNAs in the GSE98622 of CIS group and GSE106993 of IRI group (**Figure 3A**). We further found that there were 375 common DE-mRNAs in the GSE98622 of CIS group and GSE106993 of IRI group (**Figure 3B**).

In addition, the differentially expressed mitochondrial apoptosis-related genes in the two datasets are shown in **Figure 4A** and **4B**. Among them, mitochondrial apoptosis-related genes Pmaip1 and Nptx1 showed signifi-

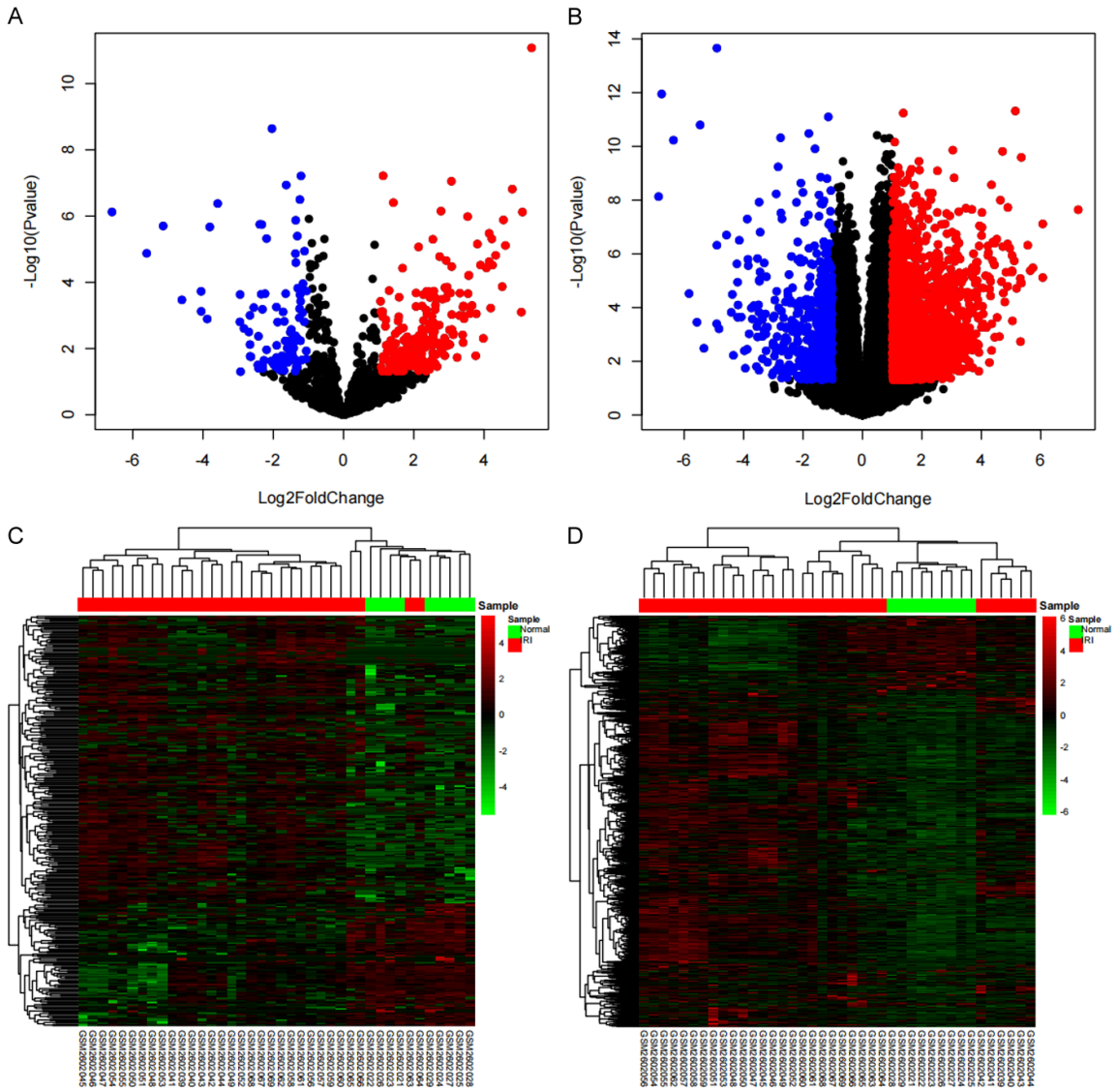


Figure 2. Identification of DE-lncRNAs and DE-mRNAs in IRI induced AKI. Volcano map of DE-lncRNAs (A) and DE-mRNAs (B) in GSE98622. (C) The heatmap shows the expression level of DE-lncRNAs (C) and DE-mRNAs (D) in normal group and IR group. AKI, acute kidney injury.

cantly high expression in the GSE98622 and GSE106993 datasets as shown in the expression box plot (**Figure 4C** and **4D**). This result indicates that mitochondrial apoptosis and abnormal expression of related genes may play an important role in AKI.

Construction of lncRNA-miRNA-mRNA ceRNA network

miRanda was used to predict the target miRNA of DE-lncRNA, and StarBase dataset was used to predict the target mRNA of miRNA. In CIS induced AKI group, the CIS-specific ceRNA network contained 18 lncRNA (**Figure 5A**). In IRI induced AKI group, the IRI-specific ceRNA network contained 15 lncRNA (**Figure 5B**). Next, the common ceRNA network was constructed between CIS group and IRI group, which contained 2 lncRNA, 65 miRNAs and 167 mRNAs (**Figure 5C**). Previous analysis found that many mitochondrial apoptosis-related genes were abnormally expressed. Among them, the fold difference of Pmaip1 and Nptx1 was the two most significant. Therefore, in order to explore its mechanism of action on AKI, we constructed a ceRNA regulatory network map for Pmaip1 and Nptx1 (**Figure 5D**).

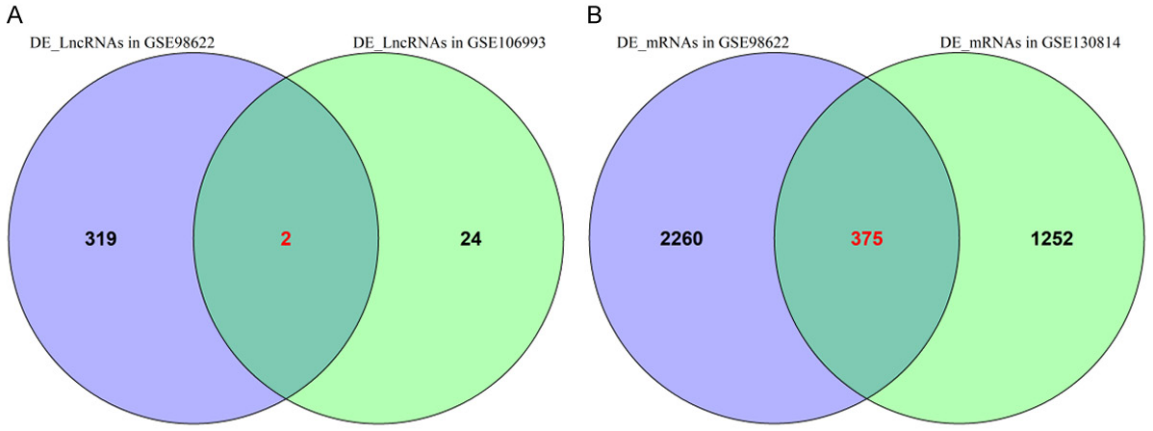


Figure 3. The common DE-lncRNAs and DE-mRNAs in CIS and IRI induced AKI. A. Venn diagram of the 2 common DE-lncRNAs in GSE98622 and GSE106993. B. Venn diagram of the 375 common DE-mRNAs in GSE98622 and GSE130814. AKI, acute kidney injury.

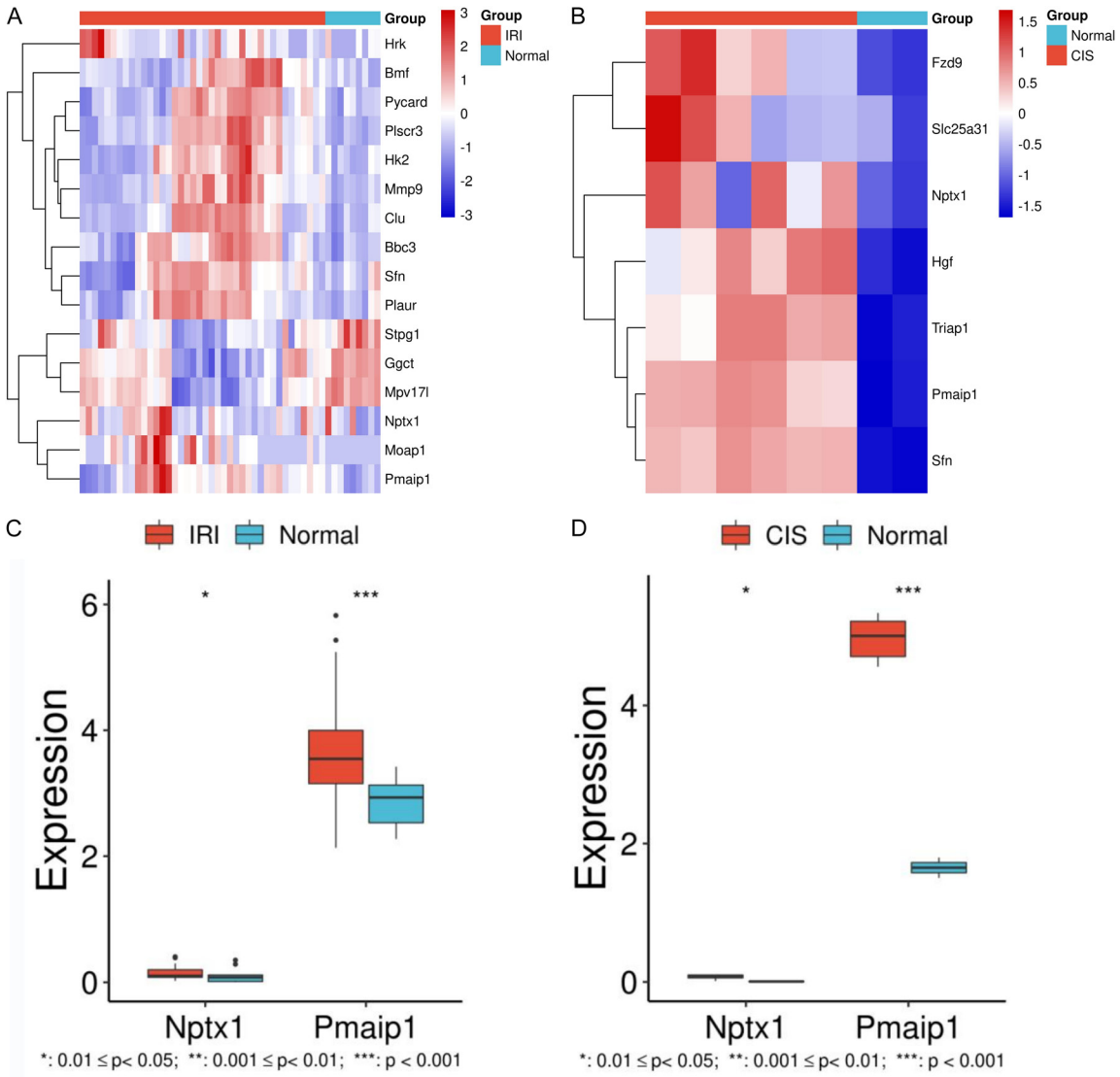
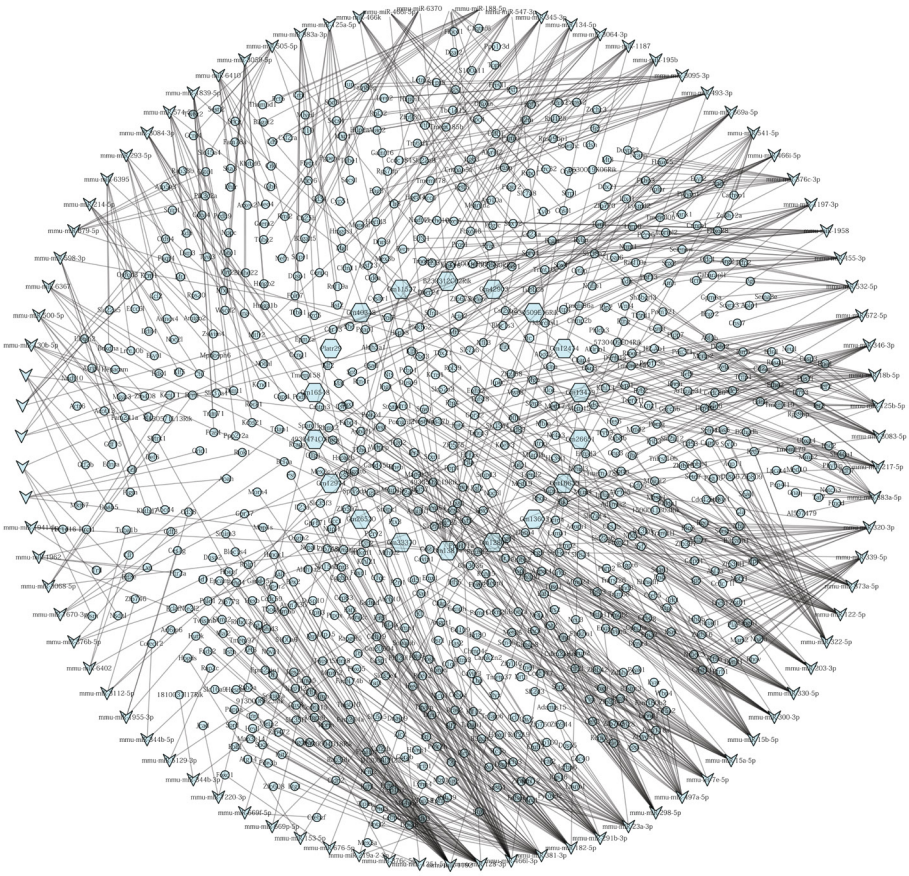
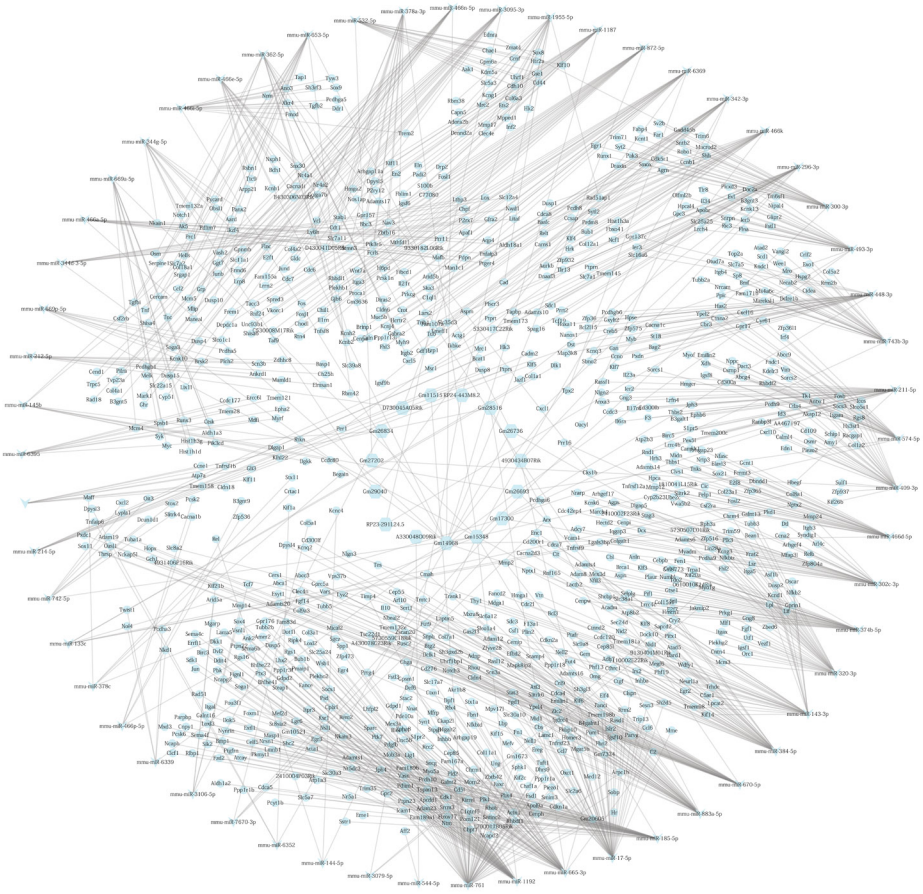


Figure 4. Mitochondrial apoptosis-related DEGs in CIS and IRI induced AKI. The heatmap shows the expression level of mitochondrial apoptosis related DEGs in GSE98622 (A) and GSE130814 (B). The expression pattern of Nptx1/Pmaip1 in GSE98622 (C) and GSE130814 (D). AKI, acute kidney injury.

A



B



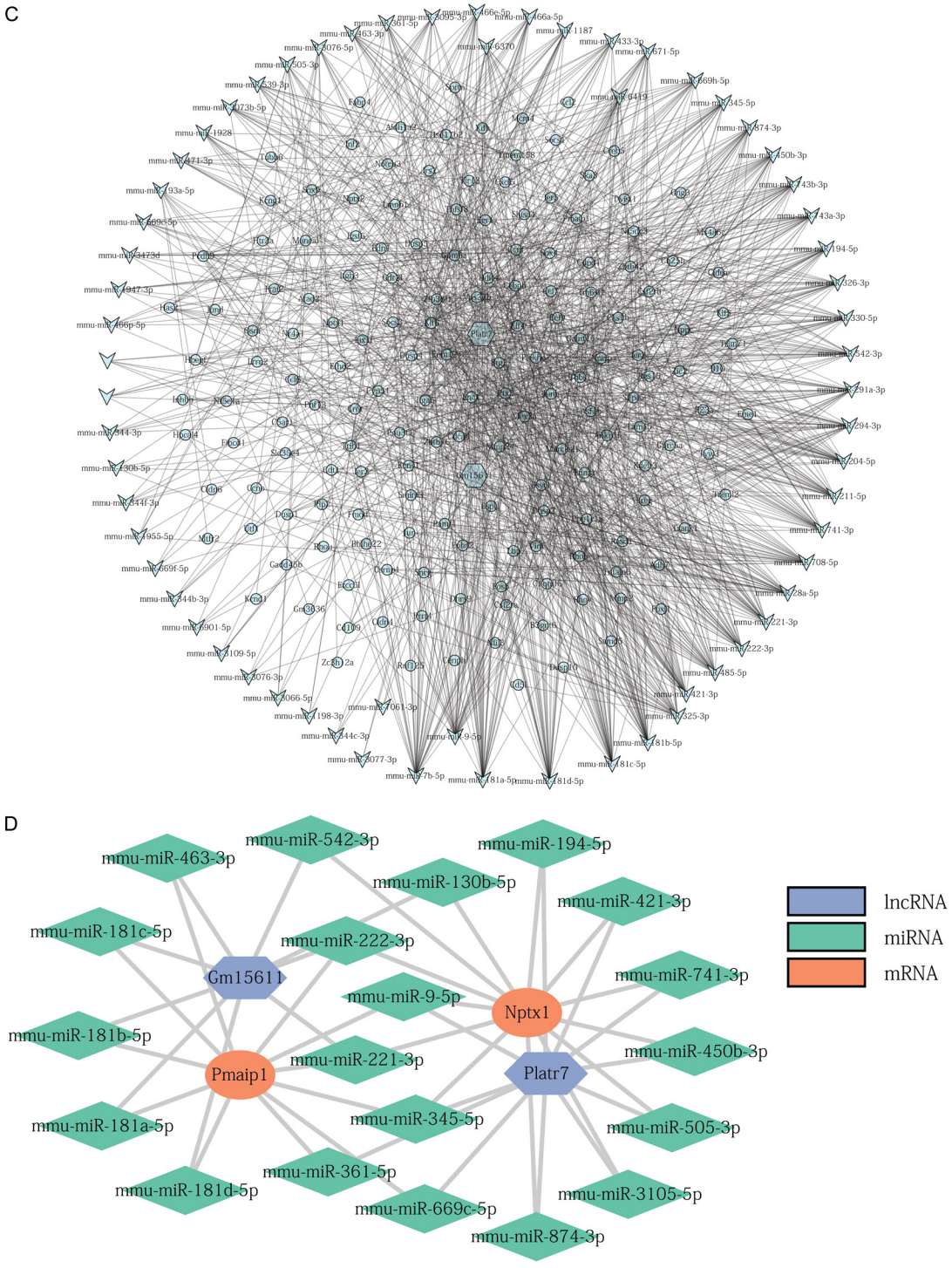


Figure 5. The construction of ceRNAs network. A. ceRNA network in CIS induced AKI. B. ceRNA network in IRI induced AKI. C. The common DE-lncRNAs ceRNA network in CIS and IRI induced AKI. D. Mitochondrial apoptosis-related ceRNA network in CIS induced AKI. AKI, acute kidney injury.

GO functional and KEGG pathway analyses of DE mRNAs

To understand the underlying biological functions of CIS-induced specific genes, IRI-induced specific genes, and common genes in ceRNAs network, GO functional and KEGG pathway analyses were performed. As shown in **Figure 6A**, the CIS-induced specific genes were involved in biological process (BP) of cell prolif-

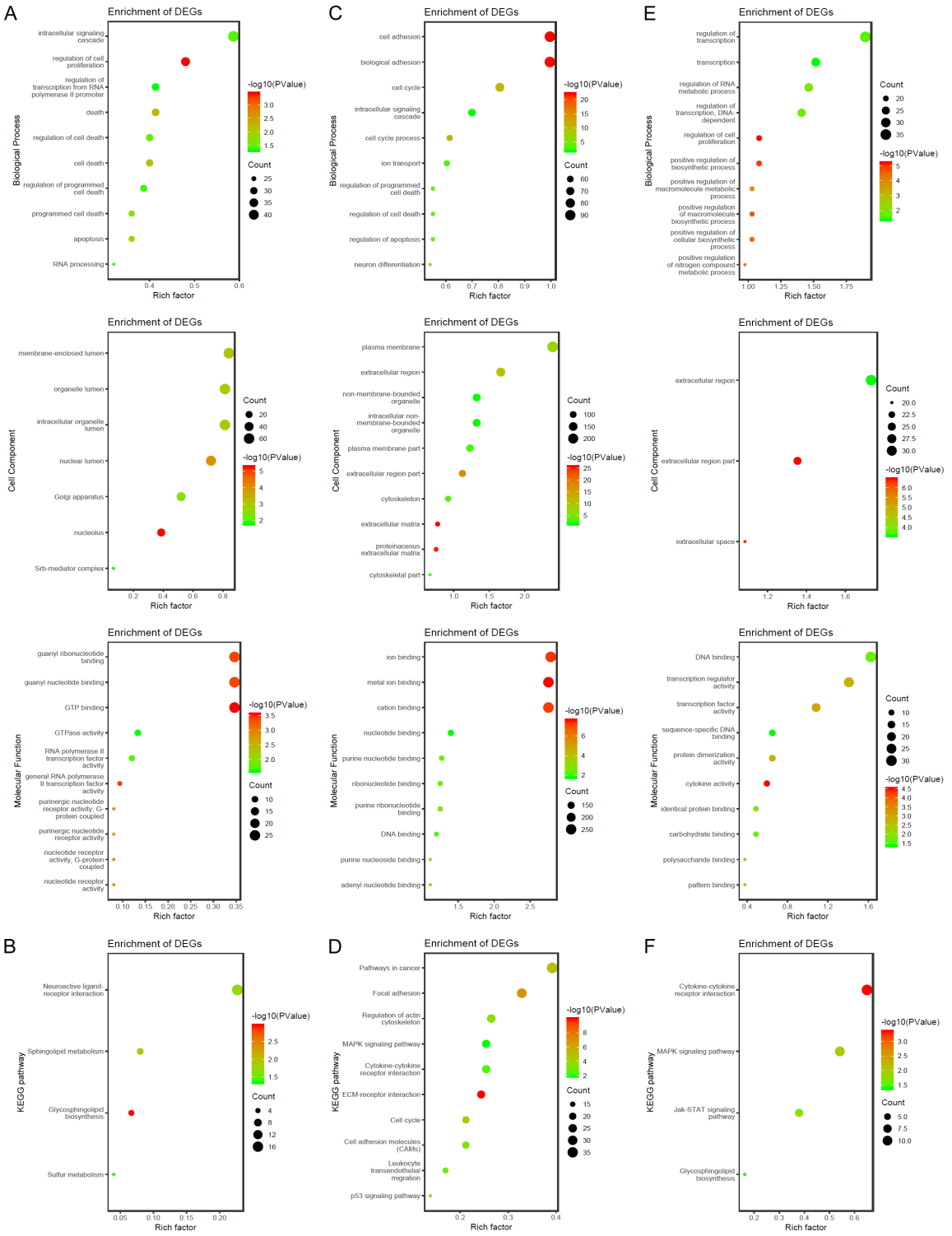


Figure 6. The GO and KEGG pathway enrichment of all mRNAs involved in ceRNA network. A. The GO analysis of CIS-induced specific genes. B. The KEGG pathway enrichment of CIS-induced specific genes. C. The GO analysis of IRI-induced specific genes. D. The KEGG pathway enrichment of IRI-induced specific genes. E. The GO analysis of common genes in CIS and IRI induced AKI. F. The KEGG pathway enrichment of common genes in CIS and IRI induced AKI. AKI, acute kidney injury.

eration, cell death, cell component (CC) analysis of membrane-enclosed lumen, organelle lumen, and molecular function (MF) category of guanyl nucleotide binding, GTP binding and

GTPase activity. Most of these functions are involved in AKI cell death process through regulating protein binding and enzyme activity. In addition, the KEGG pathway analysis indicated that CIS-induced specific genes were involved the neuroactive ligand receptor interaction, sphingolipid metabolism, glycosphingolipid biosynthesis, etc. (**Figure 6B**). As shown in **Figure 6C**, the IRI induced specific genes were enriched in the BP of cell adhesion, cell cycle, and cell death, the CC of plasma membrane, extracellular region, and cytoskeleton, the MF of ion binding, and nucleotide binding. The KEGG pathway analysis was involved in MAPK signaling pathway, cell cycle, extracellular matrix (ECM)-receptor interaction (**Figure 6D**). The 167 common mRNAs were enriched in the BP of regulation of transcription, metabolic process, cell proliferation, the CC of extracellular region and space, and the MF of DNA binding, transcription regulator activity, sequence-specific DNA binding and protein dimerization activity in CIS and IRI induced AKI (**Figure 6E**). The 167 common mRNAs were involved in the MAPK signaling pathway and JAK-STAT signaling pathway etc. (**Figure 6F**).

PPI network construction and analysis

The PPI network shows 292 nodes and 728 edges. There were 62 upregulated genes and 230 downregulated genes in CIS induced AKI (**Figure 7A**). In IRI induced AKI, the PPI network shows 391 nodes and 1885 edges. There were 362 upregulated gens and 29 downregulated genes (**Figure 7B**). Besides, a total of 81 node, which contained 3 upregulated genes and 78 downregulated gens in CIS group and IRI group (**Figure 7C**), including Jun, Btg2, Fosb, CCl9, etc.

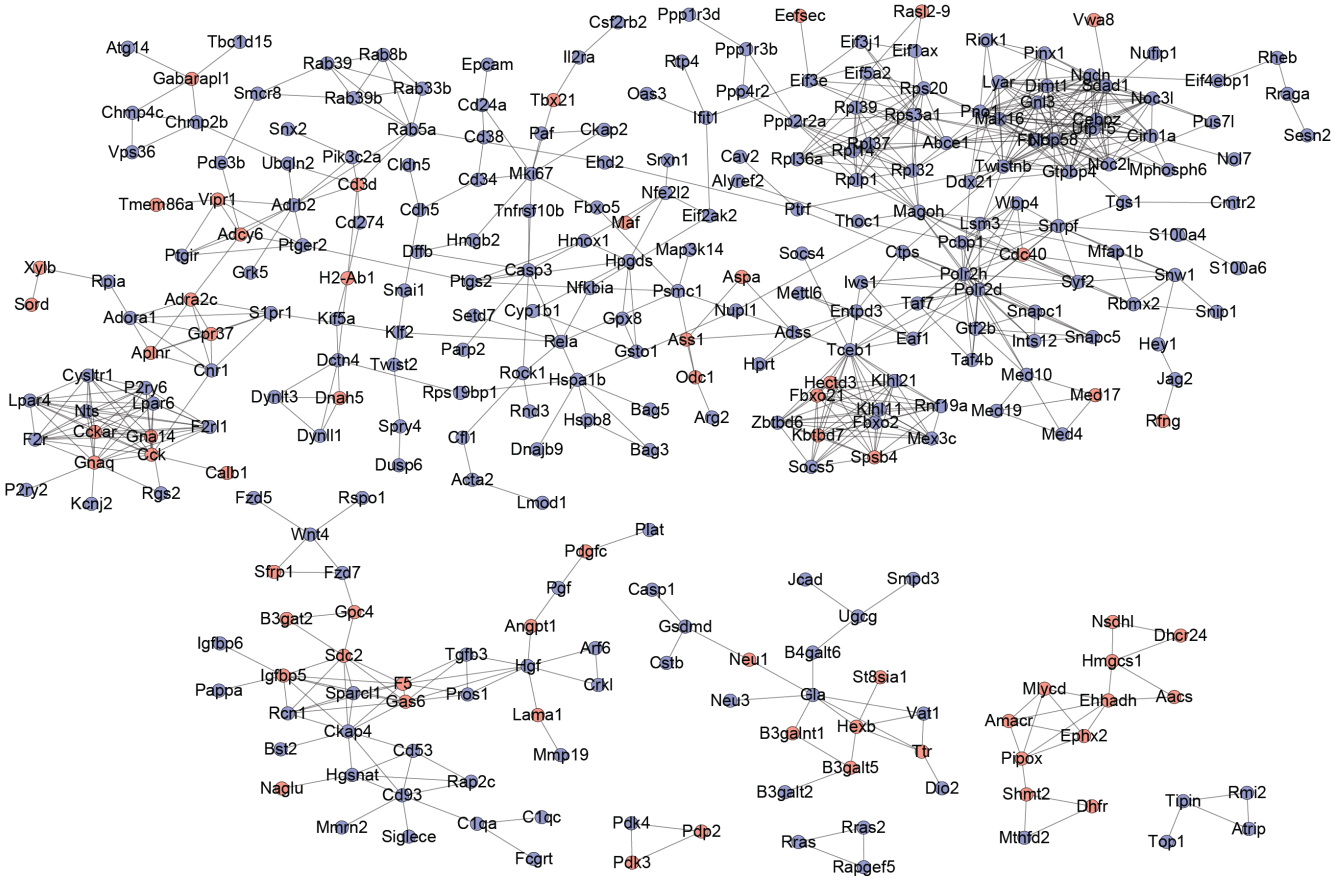
The landscape of immune infiltration in AKI

We first disclosed the landscape of 22 immune cell infiltrating subpopulations in CIS/IRI in order to explore the landscape of immune infiltration in AKI. Then, using the CIBERSORT algorithm, we looked at the differences between the CIS/IRI group and the normal group. The findings demonstrated that there were significant differences in immune cell fractions across the groups (**Figure 8A**). In addition, compared with normal group, the CIS/IR group contained a great number of NK cells and $CD8^+$ T cells, while $CD4^+$ T cells were relatively lower (**Figure 8B**). Moreover, $CD8^+$ T cells and $CD4^+$ T cells showed the strongest positive correlation ($P < 0.001$, $r > 0.5$), while B cells and macrophages showed the strongest negative correlation ($P < 0.001$, $r < -0.5$) in GSE130814 (**Figure 8C**). Altogether, these results reveal that the immune responses in AKI act as an intricate network and proceed in a tightly regulated way.

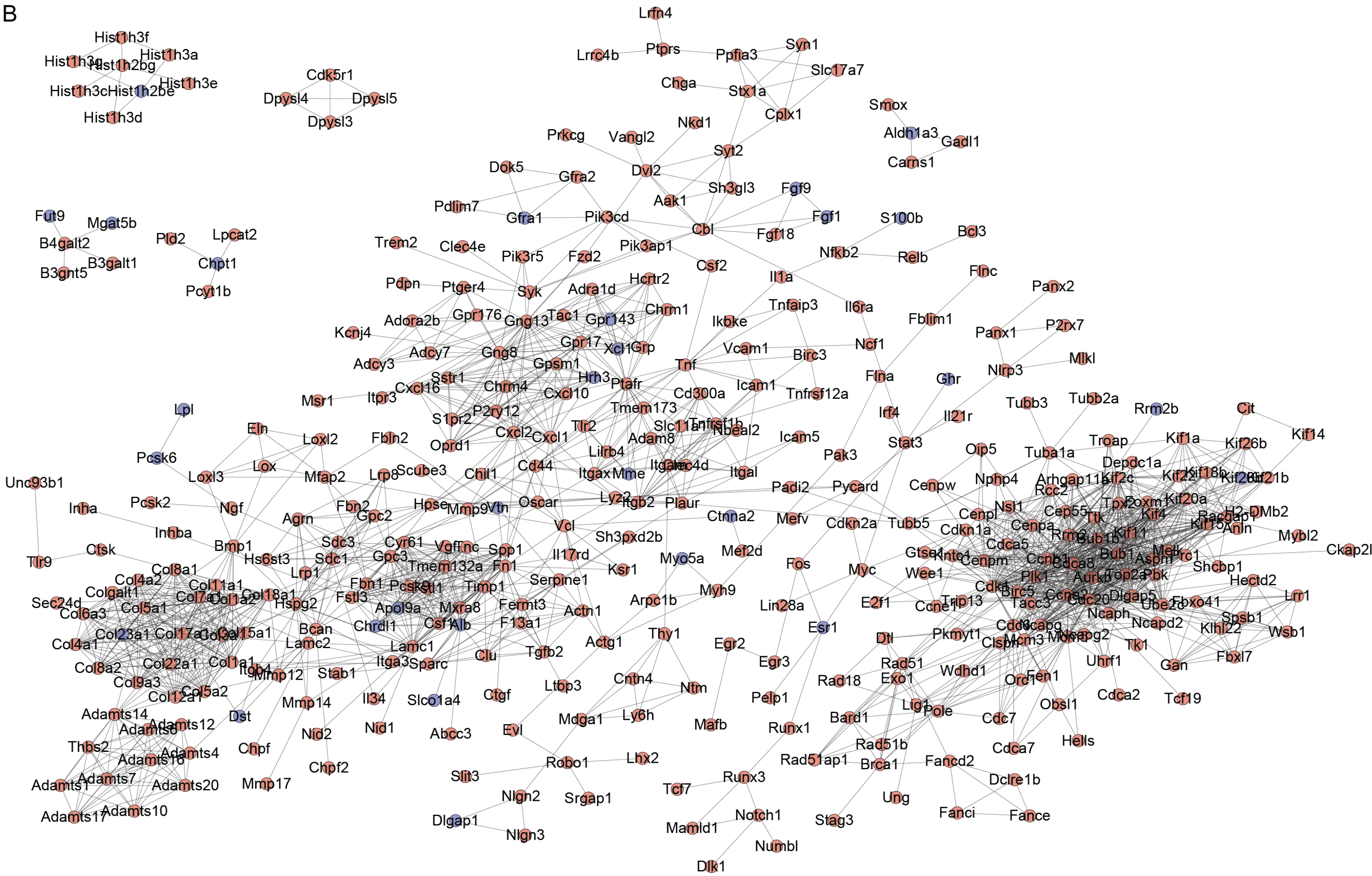
Discussion

AKI is an immediate syndrome characterized by a sudden drop-in glomerular filtration rate, a rapid increase in serum creatinine (SCr), and decreased urine volume, with high morbidity and mortality rate. Due to the high incidence, high mortality rate and high medical treatment cost, AKI has become a global public health problem. At present, the pathogenesis and treatment of AKI is the focus of research, which has important clinical and social significance. Recent studies have indicated a potential link between CIS induced AKI, IRI induced AKI and circRNA, lncRNA, mRNA [25, 26]. For example, 368 DE-circRNAs, 618 mRNAs and 98 lncRNAs were identified between CIS group and normal group [27]. The expression levels of lncRNAs (PRNCR1 and OIP5-AS1) were significantly decreased in CIS-induced AKI mice [28, 29]. Neat1 was upregulated in IRI induced AKI [30]. In this study, we found that a total of 26 DE-lncRNAs and 1627 DE-RNAs were identified between normal group and CIS-induced AKI group, while a total of 321 DE-lncRNAs and 2635 DE-mRNA were identified between normal group and IRI-induced AKI group. The 2 common DE-lncRNAs and 375 common DE-mRNAs were identified in the CIS- and IRI-induced AKI. Previous analysis found that many mitochondrial apoptosis-related genes were abnormally expressed. Among them, the fold difference of Pmaip1 and Nptx1 was the two most significant. Pmaip1 belongs to a pro-apoptotic subfamily within the BCL-2 protein family, also referred as the BCL-2 homology domain 3 (BH3)-only subfamily, which determines whether a cell commits to apoptosis. In response to death-inducing stimuli, BH3-only members inhibit the anti-apoptotic BCL-2 family members, which keep the multi-BH domain proteins BAX and BAK under steady-state conditions, in an inactive state. As a downstream target of the AKT pathway, NPTX1 inhibits pro-

A


Csf2rb2
Ppp1r3d
Eefsec
Rasl2-9
Vwa8
Atg14
Tbc1d15
Ppp1r3b
Eif3j1
Eif1ax
Rick1
Pinx1
Nufip1
Rheb
Il2ra
Rtp4
Gabarapl1
Rab39
Rab8b
Epcam
Eif3e
Eif5a2
Rps20
Lyar
Dimt1
Ngdn
Sdad1
Eif4ebp1
Smcr8
Rab39b
Rab33b
Oas3
Ppp4r2
Gnl3
Noc3l
Rraga
Chmp4c
Chmp2b
Cd24a
Tbx21
Ifit1
Rpl39
Rps3a1
Ppa1
Mak16
Cebpz
Pus7l
Sesn2
Snx2
Rab5a
Cd38
Raf
Ckap2
Ppp2r2a
Ftsj3
Dtl
Cirh1a
Vps36
Pde3b
Ubqln2
Pik3c2a
Cldn5
Ehd2
Srxn1
Cav2
Rpl36a
Rpl14
Rpl37
Abce1
Fblbp56
Nol7
Cd3d
Cd34
Mki67
Alyref2
Rplp1
Rpl32
Twistnb
Gtpbp4
Noc2l
Mphosph6
Tmem86a
Vipr1
Adrb2
Cd274
Cdh5
Tnfrsf10b
Fbxo5
Nfe2l2
Maf
Eif2ak2
Ptrf
Thoc1
Magoh
Ddx21
Wbp4
Tgs1
Cmtr2
Adcy6
Ptger2
Ptgir
Dffb
Hmgb2
Hmox1
Map3k14
Hpgds
Socs4
Ctps
Pcbp1
Lsm3
Snrpf
S100a4
Cdc40
Mfap1b
Xylb
Rpia
Grk5
H2-Ab1
Casp3
Ptgs2
Snai1
Nfkbia
Aspa
Psmc1
Iws1
Polr2h
Polr2d
Syf2
Snw1
S100a6
Sord
Adra2c
Adora1
S1pr1
Kif5a
Setd7
Cyp1b1
Nupl1
Mettl6
Entpd3
Taf7
Snapc1
Rbmx2
Snip1
Gpr37
Klf2
Gpx8
Adss
Gtf2b
Ints12
Snapc5
Aplnr
Parp2
Rela
Gsto1
Ass1
Eaf1
Tceb1
Taf4b
Hey1
Cysltr1
P2ry6
Cnr1
Dctn4
Twist2
Rock1
Hspa1b
Odc1
Hprt
Med10
Jag2
Lpar4
Nts
Lpar6
Dynlt3
Dnah5
Rps19bp1
Hectd3
Klhl21
Fbxo21
Klhl11
Rnf19a
Med17
F2r
Cckar
Gra14
F2rl1
Dynll1
Spry4
Rnd3
Hspb8
Bag5
Arg2
Zbtbd6
Fbxo2
Med19
Rfng
Gnaq
Cck
Calb1
Cfl1
Dnajb9
Bag3
Kbtbd7
Mex3c
Med4
Dusp6
Socs5
Spsb4
P2ry2
Kcnj2
Rgs2
Acta2
Lmod1
Fzd5
Rspo1
Wnt4
Plat
Pdgfc
Sfrp1
Fzd7
Pgf
Casp1
Jcad
Smpd3
Ugcg
B3gat2
Gpc4
Angpt1
Gsdmd
Nsdhl
Dhcr24
Igfbp6
Sdc2
Tgfb3
Hgf
Aff6
Cstb
Neu1
B4galt6
Hmgcs1
Igfbp5
St8sia1
Pappa
Sparcl1
F5
Gas6
Pros1
Crkl
Neu3
Gla
Mlycd
Ehhadh
Aacs
Rcn1
Lama1
Vat1
Amacr
Ckap4
B3galnt1
Hexb
Ephx2
Bst2
Cd53
Mmp19
Ttr
Pipox
B3galt5
Hgsnat
Rap2c
Naglu
Cd93
B3galt2
Dio2
Shmt2
Dhfr
Tipin
Rmi2
Mmrn2
Siglece
C1qa
C1qc
Fcgrt
Pdk4
Pdp2
Pdk3
Rras
Rras2
Rapgef5
Mthfd2
Top1
Atrip

B


Hist1h3f
Hist1h3a
Hist1h2bg
Hist1h3e
Hist1h3d
Cdk5r1
Dpysl4
Dpysl5
Dpysl3
Fut9
Mgat5b
B4galt2
B3gnt5
B3galt1
Pld2
Lpcat2
Chpt1
Pcyt1b
Lrfn4
Ptprs
Lrrc4b
Ppfia3
Syn1
Slc17a7
Chga
Stx1a
Cplx1
Smox
Aldh1a3
Gadl1
Carns1
Nkd1
Prkcg
Vangl2
Dvl2
Syt2
Dok5
Gfra2
Sh3gl3
Fgf9
Aak1
Pdlim7
Gfra1
Pik3cd
Cbl
Fgf1
Fgf18
S100b
Bcl3
Nfkb2
Relb
Trem2
Clec4e
Pik3r5
Fzd2
Pik3ap1
Csf2
Il1a
Flnc
Pdpn
Ptger4
Syk
Adra1d
Hcrtr2
Tnfaip3
Il6ra
Panx2
Fblim1
Kcnj4
Adora2b
Gpr176
Gng13
Tac1
Gpr143
Chrm1
Ikbke
Panx1
P2rx7
Adcy7
Gng8
Gpr17
Xcl1
Grp
Vcam1
Ncf1
Birc3
Adcy3
Gpsm1
Hrh3
Tnf
Flna
Ghr
Nlrp3
Mlkl
Msr1
Itpr3
Cxcl16
Sstr1
Chrm4
Cxcl10
Ptafr
Cd300a
Icam1
Tnfrsf12a
Tubb3
Tubb2a
Lpl
S1pr2
P2ry12
Tmem173
Irf4
Il21r
Rrm2b
Cit
Oprd1
Cxcl2
Cxcl1
Tlr2
Adam8
Nbeal2
Stat3
Kif14
Eln
Loxl2
Fbln2
Lilrb4
Icam5
Olp5
Tuba1a
Troap
Kif1a
Kif26b
Pcsk6
Lox
Scube3
Cd44
Pak3
Depdc1a
Unc93b1
Loxl3
Mfap2
Lrp8
Chil1
ItgaxMme
Itgal
Padi2
Pycard
Cenpw
Nphp4
Arhgap11a
Kif2c
Kif21b
Inha
Pcsk2
Ngf
Hpse
Mmp9
Vtn
Oscar
Lyz2
Itgb2
Plaur
Ctnna2
Nsl1
Cep55
Foxm1
Kif20a
Mybl2
Agrn
Fbn2
Gpc2
Mefv
Cdkn2a
Tubb5
Cenpl
Kif4
Inhba
Vcl
Sh3pxd2b
Cdkn1a
Cenpa
Anln
Ckap2l
Ctsk
Bmp1
Cyr61
Sdc3
Il17rd
Mef2d
Gtse1
Kntc1
Cdca5
Bub1
Tlr9
Hs6st3
Sdc1
Gpc3
Spp1
Myo5a
Wee1
Cenpm
Ccnb1
Aspm
Shcbp1
Col8a1
Lrp1
Fn1
Serpine1
Ksr1
Fos
Myc
Plk1
Aurka
Top2a
Pbk
Hectd2
Col4a2
Colgalt1
Col11a1
Tmem132a
Timp1
Arpc1b
Myh9
E2f1
Ccne1
Trip13
Cdk1
Birc5
Dlgap5
Fbxo41
Lrr1
Sec24d
Col6a3
Col5a1
Col18a1
Hspg2
Fstl3
Fbn1
Mxra8
Fermt3
Actn1
Lin28a
Ube2c
Ncaph
Spsb1
Chrdl1
Csf1
Alb
F13a1
Esr1
Ncapg
Ncapd2
Klhl22
Wsb1
Col4a1
Col23a1
Col15a1
Bcan
Lamc2
Lamc1
Actg1
Thy1
Egr2
Dtl
Pkmyt1
Mcm3
Ncapg2
Tk1
Col8a2
Col22a1
Col1a1
Sparc
Clu
Tgfb2
Egr3
Rad51
Uhrf1
Gan
Fbxl7
Col9a3
Mmp12
Stab1
Itga3
Cntn4
Ntm
Rad18
Exo1
Wdhd1
Orc1
Fen1
Cdca2
Tcf19
Col12a1
Col5a2
Il34
Slco1a4
Ctgf
Ltbp3
Pelp1
Adamts14
Adamts12
Mmp14
Mdga1
Ly6h
Mafb
Lig1
Pole
Obsl1
Dst
Nid1
Abcc3
Evl
Runx1
Bard1
Cdc7
Hells
Thbs2
Adamts4
Chpf
Nid2
Rad51b
Cdca7
Adamts7
Adamts20
Chpf2
Robo1
Lhx2
Rad51ap1
Brca1
Fancd2
Dclre1b
Adamts1
Mmp17
Slit3
Tcf7
Runx3
Ung
Adamts17
Adamts10
Srgap1
Nlgn2
Notch1
Stag3
Fanci
Fance
Dlgap1
Mamld1
Numbl
Nlgn3
Dlk1

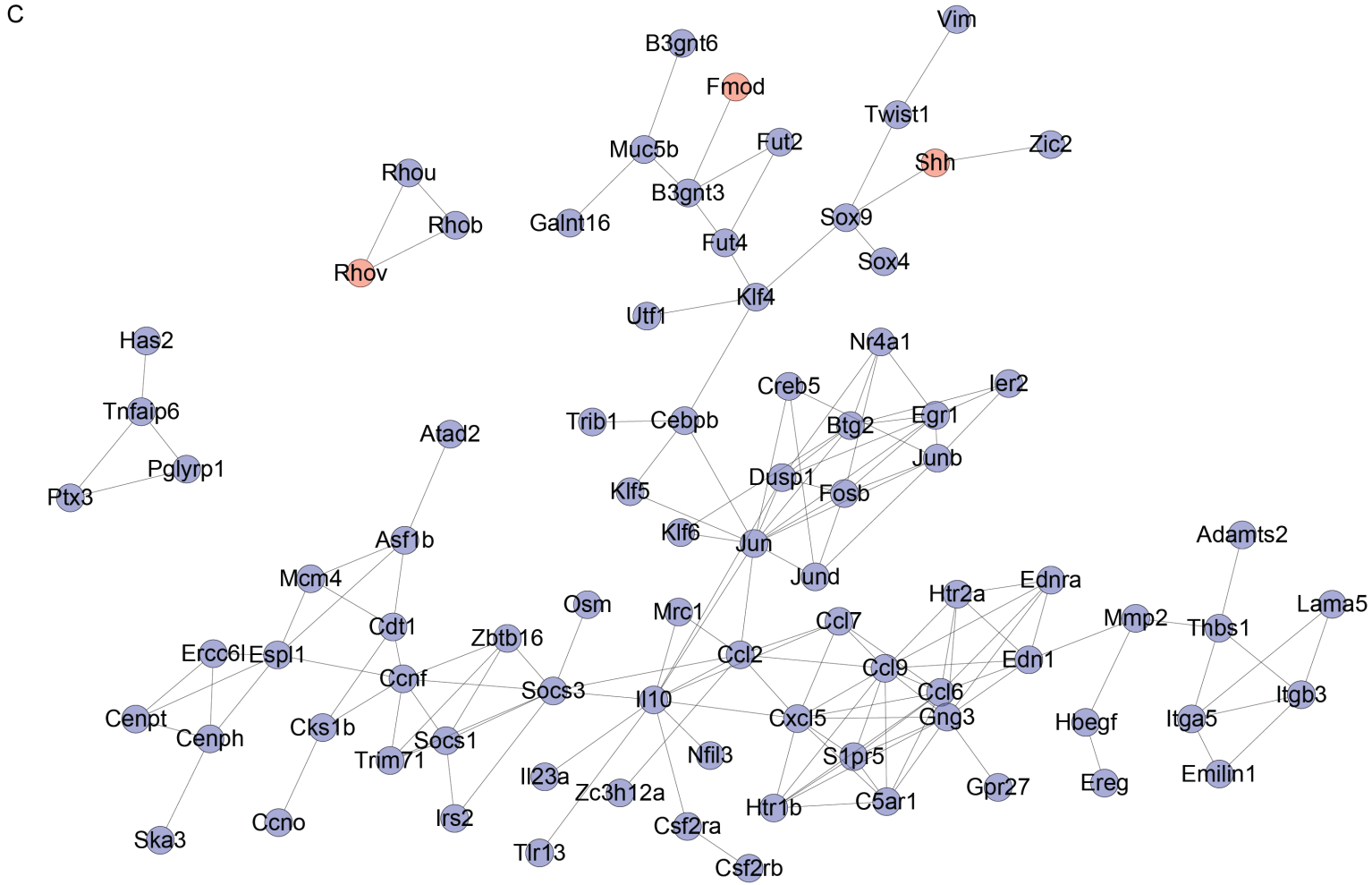


Figure 7. PPI network construction of ceRNA network expressed genes. A. The PPI network construction of CIS-induced specific genes. B. The PPI network construction of IRI induced specific genes. C. The PPI network construction of common mRNAs in ceRNA network. Red nodes indicate high-expressed mRNAs, and blue nodes indicate low-expressed mRNAs. PPI, protein-protein interaction.

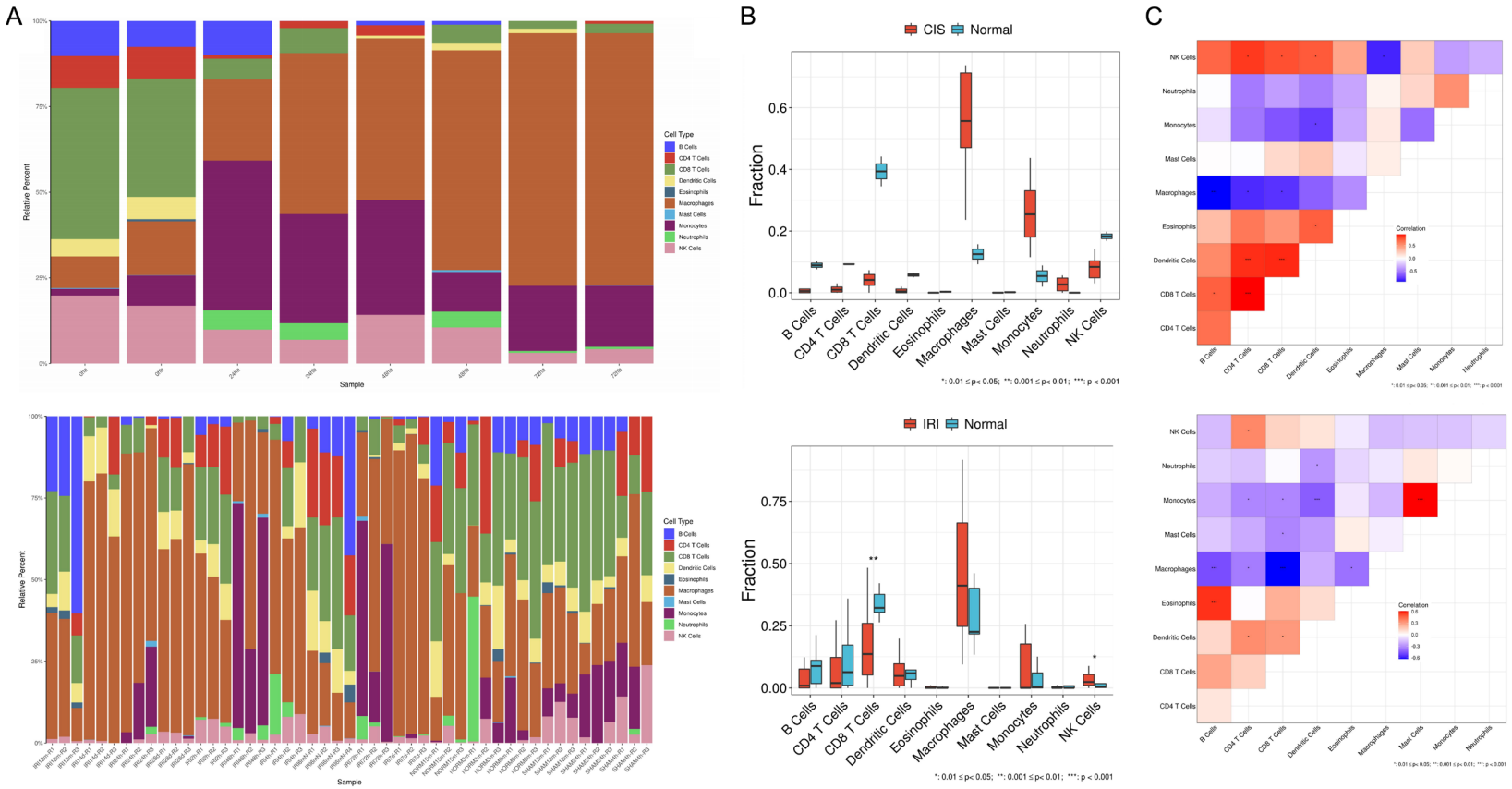


Figure 8. Immune infiltration analysis. A. The landscape of immune infiltration in AKI and difference of immune infiltration between AKI group and normal group in GSE98622 and GSE130814. B. Violin plot visualizing the differentially infiltrated immune cells. C. Correlation heatmap depicting correlations between infiltrated immune cells in AKI. AKI, acute kidney injury.

liferation and promotes apoptosis in hepatocellular carcinoma [31]. As a downstream target of the AKT pathway, NPTX1 inhibits proliferation and promotes apoptosis in hepatocellular carcinoma. NPTX1 inhibits pancreatic cancer cell proliferation and migration and enhances chemotherapy sensitivity by targeting RBM10. However, the underlying mechanism of AKT and NPTX1 involved in apoptosis still need to be explored.

The potential lncRNAs - ceRNA pairs involved in CIS/IRI-induced AKI still remain unclear. CeRNA networks, as a new mechanism for regulating genes at the post-transcriptional level with competitive endogenous regulation, can be applied to the systematic study of biological processes and provide a new method for the study and treatment of diseases [13]. Previous studies found that lncRNA HCG18 as a ceRNA inhibited miR-16-5p and increased Bcl-2 to alleviate AKI in mice with IRI [32]. lncRNA can play a role in AKI through the ceRNA mechanism [33]. Here, we found that the common ceRNA network was constructed between CIS group and IRI group, which contained 2 lncRNAs, 65 mmu-miRNAs and 167 mRNAs. The hub lncRNAs (Platr7 and Gm15611) might act as the novel therapeutic targets for AKI patients.

Many signaling pathways play important regulatory roles in inflammation, apoptosis, cell death, oxidative stress, cell cycle and metabolism in the processes of AKI [34, 35]. lncRNA regulated AKI related signaling pathways through the mechanism of ceRNA [19]. For example, lncRNA H19 promoted kidney fibrosis by regulating Wnt/β-catenin signaling pathway [36]. The downregulated Panx1 improved IRI-induced AKI by attenuating MAPK/ERK activation [37]. Previous studies found that the inhibition of JAK/STAT signaling pathway could protect against CIS-induced AKI [38]. GO and KEGG analysis were enriched in the BP of regulation of transcription, metabolic process, cell proliferation, the CC of extracellular region and space, and the MF of DNA binding, transcription regulator activity, sequence-specific DNA binding and protein dimerization activity of ceRNA network genes in CIS- and IRI-induced AKI. The 167 common mRNAs in ceRNA network were involved in the MAPK signaling pathway and JAK-STAT signaling pathway, etc.

There are still some limitations in the current investigation. First, this is a bioinformatic study without animal experiment verification. Furthermore, it is unknown how the two mitochondrial apoptosis-related genes play a role in AKI. In addition, the biological functions and specific molecular mechanisms of DE-lncRNAs in the pathogenesis of AKI warrant further exploration. At last, the findings in this study should be verified by experiments.

In conclusion, by means of comprehensive analysis of mRNA, lncRNA and miRNA expression profiles of AKI in GEO database, we screened out DE-lncRNAs and DE-mRNAs in AKI patients and identified I/R and CIS induced AKI-specific lncRNAs and miRNAs, which might be involved in the onset, progression and prognosis of AKI. More importantly, the ceRNA network was successfully constructed and enriched in MAPK signaling pathway and JAK-STAT signaling pathway. Our data might lay a foundation for further functional research of lncRNAs in AKI and suggest that specific lncRNAs and miRNAs could be valuable for diagnosis and treatment of AKI.

Disclosure of conflict of interest

None.

Address correspondence to: Jianfei Li, Department of Nephrology, Liuzhou People's Hospital, No. 8 Wenchang Road, Chengzhong District, Liuzhou, Guangxi, China. Tel: +86-13633060108; E-mail: lijianfei25223@163.com

References

[1] Ronco C, Bellomo R and Kellum JA. Acute kidney injury. Lancet 2019; 394: 1949-64.
[2] Zhao M, Wang Y, Li L, Liu S, Wang C, Yuan Y, Yang G, Chen Y, Cheng J, Lu Y and Liu J. Mitochondrial ROS promote mitochondrial dysfunction and inflammation in ischemic acute kidney injury by disrupting TFAM-mediated mtDNA maintenance. Theranostics 2021; 11: 1845-63.
[3] Radi ZA. Kidney pathophysiology, toxicology, and drug-induced injury in drug development. Int J Toxicol 2019; 38: 215-27.
[4] Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A and Anders HJ. Acute kidney injury. Nat Rev Dis Primers 2021; 7: 52.
[5] Pressly JD and Park F. DNA repair in ischemic acute kidney injury. Am J Physiol Renal Physiol 2017; 312: F551-F555.

[6] Nieuwenhuijs-Moeke GJ, Pischke SE, Berger SP, Sanders JSF, Pol RA, Struys MMRF, Ploeg RJ and Leuvenink HGD. Ischemia and reperfusion injury in kidney transplantation: relevant mechanisms in injury and repair. J Clin Med 2020; 9: 253.
[7] Holditch SJ, Brown CN, Lombardi AM, Nguyen KN and Edelstein CL. Recent advances in models, mechanisms, biomarkers, and interventions in cisplatin-induced acute kidney injury. Int J Mol Sci 2019; 20: 3011.
[8] Bonavia A and Singbartl K. A review of the role of immune cells in acute kidney injury. Pediatr Nephrol 2018; 33: 1629-1639.
[9] Burne MJ, Daniels F, El Ghandour A, Mauiyyedi S, Colvin RB, O'Donnell MP and Rabb H. Identification of the CD4+ T cell as a major pathogenic factor in ischemic acute renal failure. J Clin Invest 2001; 108: 1283-1290.
[10] Baudoux T, Husson C, De Prez E, Jadot I, Antoine MH, Nortier JL and Hougardy JM. CD4+ and CD8+ T cells exert regulatory properties during experimental acute aristolochic acid nephropathy. Sci Rep 2018; 8: 5334.
[11] Coussens LM and Werb Z. Inflammation and cancer. Nature 2002; 420: 860-867.
[12] Zhang X, Agborbesong E and Li X. The role of mitochondria in acute kidney injury and chronic kidney disease and its therapeutic potential. Int J Mol Sci 2021; 22: 11253.
[13] Liu Z, Wang Y, Shu S, Cai J, Tang C and Dong Z. Non-coding RNAs in kidney injury and repair. Am J Physiol Cell Physiol 2019; 317: C177-C188.
[14] Ma T, Jia H, Ji P, He Y and Chen L. Identification of the candidate lncRNA biomarkers for acute kidney injury: a systematic review and meta-analysis. Expert Rev Mol Diagn 2021; 21: 77-89.
[15] Bridges MC, Daulagala AC and Kourtidis A. LNCcation: lncRNA localization and function. J Cell Biol 2021; 220: e202009045.
[16] Qian X, Zhao J, Yeung PY, Zhang QC and Kwok CK. Revealing lncRNA structures and interactions by sequencing-based approaches. Trends Biochem Sci 2019; 44: 33-52.
[17] Shen J, Liu L, Zhang F, Gu J and Pan G. LncRNA TapSAKI promotes inflammation injury in HK-2 cells and urine derived sepsis-induced kidney injury. J Pharm Pharmacol 2019; 71: 839-48.
[18] Cheng Q and Wang L. LncRNA XIST serves as a ceRNA to regulate the expression of ASF1A, BRWD1M, and PFKFB2 in kidney transplant acute kidney injury via sponging hsa-miR-212-3p and hsa-miR-122-5p. Cell Cycle 2020; 19: 290-9.
[19] Li GM, Zhang CL, Rui RP, Sun B and Guo W. Bioinformatics analysis of common differential genes of coronary artery disease and ischemic cardiomyopathy. Eur Rev Med Pharmacol Sci 2018; 22: 3553-69.
[20] Chen Y and Wang X. miRDB: an online database for prediction of functional microRNA targets. Nucleic Acids Res 2020; 48: D127-D131.
[21] Tan Q, Tan Z, Liu J, Mo Y and Liu H. Integrated bioinformatic analysis of competing endogenous RNA network of choriocarcinoma. Med Sci Monit 2021; 27: e931475.
[22] Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC and Lempicki RA. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 2003; 4: P3.
[23] Legeay M, Doncheva NT, Morris JH and Jensen LJ. Visualize omics data on networks with omics visualizer, a cytoscape App. F1000Res 2020; 9: 157.
[24] Sun Z, Wu J, Bi Q and Wang W. Exosomal lncRNA TUG1 derived from human urine-derived stem cells attenuates renal ischemia/reperfusion injury by interacting with SRSF1 to regulate ASCL4-mediated ferroptosis. Stem Cell Res Ther 2022; 13: 297.
[25] Jiang S, Zhang H, Li X, Yi B, Huang L, Hu Z, Li A, Du J, Li Y and Zhang W. Vitamin D/VDR attenuate cisplatin-induced AKI by down-regulating NLRP3/caspase-1/GSDMD pyroptosis pathway. J Steroid Biochem Mol Biol 2021; 206: 105789.
[26] Li CM, Li M, Ye ZC, Huang JY, Li Y, Yao ZY, Peng H and Lou TQ. Circular RNA expression profiles in cisplatin-induced acute kidney injury in mice. Epigenomics 2019; 11: 1191-207.
[27] Li CM, Li M, Zhao WB, Ye ZC and Peng H. Alteration of N6-methyladenosine RNA profiles in cisplatin-induced acute kidney injury in mice. Front Mol Biosci 2021; 8: 654465.
[28] Li J, Fan X, Wang Q, Gong Y and Guo L. Long noncoding RNA PRNCR1 reduces renal epithelial cell apoptosis in cisplatin-induced AKI by regulating miR-182-5p/EZH1. Kidney Blood Press Res 2021; 46: 162-72.
[29] Chang S, Chang M, Liu G, Xu D, Wang H, Sun R and Feng M. LncRNA OIP5-AS1 reduces renal epithelial cell apoptosis in cisplatin-induced AKI by regulating the miR-144-5p/PKM2 axis. Biomed J 2022; 45: 642-653.
[30] Ma T, Li H, Liu H, Peng Y, Lin T, Deng Z, Jia N, Chen Z and Wang P. Neat1 promotes acute kidney injury to chronic kidney disease by facilitating tubular epithelial cells apoptosis via sequestering miR-129-5p. Mol Ther 2022; 30: 3313-3332.
[31] Zhao Y, Yu Y, Zhao W, You S, Feng M, Xie C, Chi X, Zhang Y and Wang X. As a downstream target of the AKT pathway, NPTX1 inhibits proliferation and promotes apoptosis in hepatocellular carcinoma. Biosci Rep 2019; 39: BSR20181662.

[32] Zhao W, Zhang Y, Zhang M, Zhi Y, Li X and Liu X. Effects of total glucosides of paeony on acute renal injury following ischemia-reperfusion via the lncRNA HCG18/miR-16-5p/Bcl-2 axis. Immunobiology 2022; 227: 152179.
[33] Cheng W, Li XW, Xiao YQ and Duan SB. Non-coding RNA-associated ceRNA networks in a new contrast-induced acute kidney injury rat model. Mol Ther Nucleic Acids 2019; 17: 102-12.
[34] Li N, Wang Y, Wang X, Sun N and Gong YH. Pathway network of pyroptosis and its potential inhibitors in acute kidney injury. Pharmacol Res 2022; 175: 106033.
[35] Ren Q, Guo F, Tao S, Huang R, Ma L and Fu P. Flavonoid fisetin alleviates kidney inflammation and apoptosis via inhibiting Src-mediated NF-kappaB p65 and MAPK signaling pathways in septic AKI mice. Biomed Pharmacother 2020; 122: 109772.
[36] Dong X, Cao R, Li Q and Yin L. The long noncoding RNA-H19 mediates the progression of fibrosis from acute kidney injury to chronic kidney disease by regulating the miR-196a/Wnt/beta-catenin signaling. Nephron 2022; 146: 209-19.
[37] Su L, Jiang X, Yang C, Zhang J, Chen B, Li Y, Yao S, Xie Q, Gomez H, Murugan R and Peng Z. Pannexin 1 mediates ferroptosis that contributes to renal ischemia/reperfusion injury. J Biol Chem 2019; 294: 19395-404.
[38] Tsogbadrakh B, Ryu H, Ju KD, Lee J, Yun S, Yu KS, Kim HJ, Ahn C and Oh KH. AICAR, an AMPK activator, protects against cisplatin-induced acute kidney injury through the JAK/STAT/SOCS pathway. Biochem Biophys Res Commun 2019; 509: 680-686.