Comprehensive Analysis of Urinary lncRNA And mRNA Expression Profiles In Patients With lupus Nephritis

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Abstract

Background: To explore long-non-coding RNA (lncRNAs),messenger RNAs (mRNAs) expression profiles and biological functions in the urine samples in lupus nephritis (LN) patients.

Methods: Three LN patients and three healthy controls(con) were recruited,whose midstream morning urine was collected.A microarray of mRNA and lncRNA was applied to explore total RNA expression variation.Then venn analysis was applied to screen out specific gene expression in the LN group compared to the con group.Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to reveal the gene functions of the dysregulated lncRNA-associated with LN;STRING online website and Cytoscape software network analysis were applied to construct protein interaction network (PPI) and screen out the Hub Gene.Drug online websites were conducted as drug prediction websites.

Results:A total of 273 mRNAs and 549 lncRNAs were differentially expressed in LN patients' urine compared with the con group.GO analysis of LN revealed that mRNAs from the lncRNA-mRNA network were enriched in terms of antigen-negative regulation of immune response.KEGG analysis of hub genes related to the LN lncRNA-mRNA network highlights their critical role in protein processing in the endoplasmic reticulum,P53 signaling pathway.Co-expression and PPI network analysis suggested that high-degree nodes are clustered in apoptosis,autophagy, reactive oxygen species pathway.Drug prediction indicated that the targeted drugs mainly included antioxidant activity,phosphorylative mechanism.

Conclusion: Our findings indicated that the differential expressed urinary lncRNAs(

DELs) possessed considerable clinical value in the diagnosis and potential therapeutic applications of drugs in LN patients.

Background

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with various clinical manifestations affecting different tissues.The deposition of immune complexes characterized by the deposition of immune complexes accounted for by the widespread loss of immune tolerance to nuclear self-antigens and excessive prion inflammatory cytokine production and damage to multiple organ systems[1]. Almost all patients with SLE have pathological changes in renal tissue[2].Lupus nephritis (LN)is characterized by alternation between remission and recurrence. Pathological types can change from one type to another, and other subtypes of the same immunological type can also be transformed into each other dynamically. If a patient’s condition suggests that the renal lesion is aggravated, renal biopsy should be repeated to confirm the extent of LN[3], but it is invasive and often inconvenient. Looking for non-invasive clinical indicators to predict pathological types has become a hot research topic over recent years.

Long non-coding RNAs (lncRNAs) are endogenous transcript RNA molecules with a length of more than 200 nucleotides and have no protein-encoding capacity [4].LncRNAs have emerged as potentially powerful regulators involved in various biological processes through a multitude of mechanisms.According to their genomic proximity to protein-coding genes,lncRNAs can be classified into six categories: intronic antisense, intronsense overlapping, natural antisense, exonsense overlapping, bidirectional, and intergenic[5]. A lot of lncRNAs have been confirmed to have vital functions and act as local regulators[6]. LncRNAs could also promote or repress the translation of mRNAs in the cytoplasm. Accumulating studies have demonstrated that lncRNAs play an important role in the pathogenesis of a variety of autoimmune diseases, such as SLE, ankylosing spondylitis(AS) and rheumatic arthritis(RA)[7-10]. In the nucleus, lncRNAs can act in cis to control local allele-specific functions or in trans at one or more genomic loci to regulate gene expression. However, no research about urinary LN-related lincRNAs was reported. This study aimed to detect the levels of urinary lncRNAs in LN patients. Some factors are reported in partaking in the pathogenesis of LN: genetic, environmental, epigenetic and hormonal. But the exact pathogenesis of LN remains largely unknown. In this study, integrative lncRNA-mRNA microarray analysis was determined to demonstrate the expression profiles of lncRNAs in LN samples and normal ones. Since urine has been identified as an optimal non-invasive source of proteins,DNA and RNA[11]. Most urinary biomarkers have been developed to monitor bladder tumor recurrences after standard therapy, and accordingly, surveillance-related[12]. The urine of LN patients may also directly reflect the associated pathological alterations of SLE to some extent, we collected urine of LN patients and further investigated their alterations and interaction among mRNAs and lncRNAs in this study. We also used the microarray technology and bioinformatics databases to integrally analyze the mRNAs and lncRNAs expression network for LN patients'urine.

We aim to highlight critical functional modules and signaling pathways of urine sample analysis of DELs, which will improve our understanding of LN pathogenesis mechanism, and will provide the potential drug targets for LN.

Results

1. Aberrant lncRNAs/mRNA expression in the urine of LN vs con:

We examined the lncRNA/mRNA profiling in the urine from 3 LN patients and 3 con.We compared the lncRNA/mRNA expression between the two groups.

Expression profile of lncRNAs and mRNAs Hierarchical clustering revealed systematic variations between LN patients (n=3) and control groups (n=3) in the expression of lncRNAs and mRNAs(Fig 2 A,B,C).Compared to the con group,159 mRNAs/318 lncRNAs were observed to be up-regulated,while 114 mRNAs/231 lncRNAs were down-regulated in LN patients.Top5 of up-regulated lncRNA is RTCA-AS1,LINC01006,LINC01239,SCOC-AS1,NAP1L1;while top5 of down-regulated lncRNA is PCAT4,UCA1,LMO7DN-IT1,RRP36(Table 2.3).Barplot and heatmap of the differential expression of

mRNAs/lncRNAs between the two groups were shown in Fig.2D,E.

3.GO and KEGG analysis of differentially expressed mRNA of the urinary sample in LN.

The GO and pathway analysis are performed to illuminate the top 10 molecular functions(MF),biological processes(BP),cellular components(CC).

To predict DEMs'functions,we adopted a previously described method demonstrated in the paper[13].The GO enrichment analysis for DEMs in LN patients showed that the dysregulated genes were mainly involved in regulation of transcription, intrinsic apoptotic signaling pathway in response to DNA damage, regulation of mitophagy(Fig 2 F); KEGG enrichment analyses found that DEMs were mainly enriched in the p53 pathway, apoptosis,endocytosis signaling pathway(Fig 2 G).These results suggest that LN may be related to apoptosis signaling pathway（sup fig.1).

4.Construction and functional analysis of lncRNAs-mRNAs co-expression and PPI network in the urine sample of LN patients.

To uncover possible modulating mechanisms of lncRNAs,the lncRNA-mRNA co-expression network was constructed to identify hub regulatory factors associated with LN vs con group.The absolute value of the correlation coefficient was> 0.8 and p <0.05.A total of 21183 lncRNA-mRNA co-expression relationship pairs ,including 451 lncRNA and 224 mRNA were identified.Co-expression network analysis of these 156 lncRNAs and relevant 122 mRNAs.Our co-expression network implied a complex regulating relationship between lncRNAs and mRNAs.Among them,gene PARK2(Parkin) was related with lncRNA NONHSAG017222.3(p=0.0275,down-regulated)(sup fig.2).PARK2 is a RING domain-containing E3 ubiquitin ligase involved in proteasome-dependent degradation of proteins[14]. Ubiquitination regulates cell survival and death through NF-κB and apoptosis signaling pathways.

Dysfunction of some ubiquitin ligases is involved in SLE[15].PPI network construction for DEMs in the urine sample of LN patients. Cluster analysis in LN patients of the PPI network of genes with significantly altered expression (FC>1.2 and P<0.05) was delineated using the STRING database[16].The PPI network of DEMs contained 55 nodes and 63 edges in 141 up-regulated (PPI enrichment p-value:2.02e-05);while down-regulated one contained 33 nodes and 69 edges in 141 up-regulated (PPI enrichment p-value:5.39e-07) (Fig.3A and Fig.3E).

Furthermore, the cytoHubba plugin was used to select the top 10 hub genes from the PPI network using the MCC method. The top 10 hubs = genes in up-regulated genes were CENPE, ATP6VID, ATP6VIH, KIF20A, KIF11, AURKA, TPX2, ATP6-V1C2, ATP6VIE2, ATP6V1E1; While the top 10 hub genes in down-regulated genes were PARK2, UBB, RPS27A, UBC, UBA52, PINK1, SQSTM1, MFN2, MFN1, SNCA; Top 2 modules were produced using MCODE plug-in of Cytoscape based on the PPI network information. The top 2 up-regulated and down-regulated modules were generated separately: up-regulated module 1 consisting of 7 nodes and 21 edges (Fig.3B), module 2 of 7 nodes and 21 edges (Fig.3C),module 3 of 6

nodes and 15 edges (Fig.3D). The highest degree nodes in up-regulated modules 1 and 2 are ATP6VIH and CENPE, respectively.

The functional annotation indicated in these modules is mainly involved in oxidative phosphorylation,mTOR signaling pathway.For down-regulated DEMs, module 1 contains 11 nodes and 48 edges (Fig.3F),module 2 contains 5 nodes and 10 edges (Fig.3G).The highest degree nodes in modules 1 and 2 are PARK2 and SNCA, respectively.The functional annotation indicated in these modules is mainly involved in mitophagy,NOD-like receptor ,P53 signal pathway.Co-expression and PPI network analyses suggest that high-degree nodes are clustered in apoptosis,autophagy, reactive oxygen species pathway and metabolic pathway(Fig.4).

5.Drug prediction of DEMs of LN patients.

Since drug-target interactions play an important role in drug discovery.We used four drug prediction databases: DrugBank, genecards, DGIdb, UniProt, which aimed to combine the known associations among protein, drugs, DEMs and LN and other autoimmune diseases(table.4). Four targets including UDP-glucuronosyl-Transferase 2B1, Kappa-casein, V-type proton ATPase subunit G and apolipoprotein A-IV were predicted.Nine approved drugs respond to these 4 treatment targets. Including Losartan, tamoxifen, gemfibrozil, Dextromethorphan, Fostamatinib, Tiludronic acid, Copper, Zinc and Zinc Acetate. Among these drugs, V-type proton ATPase subunit G was a Catalytic subunit of the peripheral V1 complex of vacuolar ATPase (V-ATPase). As we described above that the top 10 hub genes in up-regulated genes including several V-Type ATPases. V-ATPase is responsible for acidifying a variety of intra-cellular compartments in eukaryotic cells. It has been described that V-ATPase facilitated the development of murine thymocytes progressing toward the $CD4^+$ and $CD8^+$ αβ T cell lineages[17]. The effect of V-ATPase on T development indicated V-ATPase is a potential target to combat autoimmune disease. Drug prediction indicated that the targeted drugs mainly included antioxidant activity, phosphorylative mechanism. It may provide insight into new therapies for LN patients.

Discussion

Systemic lupus erythematosus(SLE) is a chronic systemic autoimmune disease with various clinical manifestations affecting different tissues[18].LN is one of the most severe complications of SLE.More investigation of the molecular mechanisms for LN and discovering of new therapeutic targets with reduced adverse effects and improved curative efficacy[19].Immune tolerance deficiency is relevant to nuclear auto-antigens and produces antinuclear antibodies(ANAs) in LN patients.

Long non-coding RNAs (lncRNAs) have recently been identified to be tightly linked to diverse biological functions in plenty of human diseases [20-22].The relationship between LN and lncRNAs remains limited[23].

LncRNAs might contribute to a new layer of the molecular regulation of LN.Some researches indicate that the DELs are involved in the pathogenesis of LN,including organ damage,clinical manifestations and

clinical parameters.Zhang et al.found that NEAT1,an early LPS response gene,plays a role in regulating the expression of inflammatory chemokines involved in LN and was positively associate to SLE disease activity by mitogen-activated protein kinase (MAPK) and type I interferon(IFN) signal pathway[19].

There is an urgent need to explore the molecular mechanisms to identify novel diagnostic markers and mechanism-driven therapeutics for LN[22].Renal biopsy remains the gold standard for the diagnosis and management of LN,but the invasive nature and associated risks have limited its use,especially during follow-up[24,25] Currently,urine cytology,as the most widely used non-invasive approach,has high specificity for the detection and monitoring of bladder cancer[26].For example LncRNA PCA3 was considered to be a well-established urine biomarker for the detection of prostate cancer (PCa)[27].Urine test is an ideal specimen to detect intrarenal changes and accessible conveniently.One study showed that urinary lncRNA biomarkers could be used to noninvasively separate bladder cancer patients and urocystitis patients[28].

LncRNAs have emerged as the most informative urinary biomarkers, especially for genitourinary tumors.In a case of kidney injury(especially acute renal allograft rejection),lncRNAs can be successfully isolated from urine and quite stable[29].

In this study,we systematically analyzed the lncRNA-involved regulatory networks through microarray technology of urine from LN patients.This is the first paper exploring a comprehensive molecular event leading to the pathogenesis of formation and discovered functional RNAs regulatory networks in LN patients.

One research found 8,868 DELs and 6,876 DEMs in PBMCs in SLE patients using microarray assessment,which indicated that the lncRNA expression profile in SLE was significantly changed[30].We found 273 mRNAs and 549 lncRNAs differentially expressed in urine between LN patients and the controls in the present study. The dysregulated expression of lncRNAs may imply their potential application as biomarkers for LN screening.

Furthermore,83 down-regulated /141 up-regulated DEMs was found uniquely in LN,which indicates that urine sample can distinguish potential molecular markers.The results of further GO and KEGG pathway analyses indicated that lncRNAs were involved mainly in pathways with crucial pathobiological relevance.The DEMs in LN were significantly enriched in regulation of transcription, intrinsic apoptotic signaling pathway in response to DNA damage,regulation of mitophagy.The analysis of the KEGG signaling pathway included 30 abnormally expressed genes in LN patients.The pathogenesis of LN was found to be reacted to the following :endoplasmic reticulum pathway,P53 signaling pathway,apoptosis signaling pathway.

The endoplasmic reticulum (ER) is a cellular organelle and continuous membrane system that forms a series of flattened sacs within the cytoplasm of eukaryotic cells and serves multiple functions,being important particularly in the synthesis,folding, modification,and transport of proteins .The endoplasmic reticulum (ER) in protein folding and in sustaining calcium equilibrium[31].A study suggests that LN

serum may activate pathways known to be involved in ERS.Peroxisome proliferator- activated receptors(PPARβ/δ) activation may be an important target to control ERS.

The three signaling pathways of ERS activate NF-κB and accelerate stress-induced apoptosis in LN[32].LN induced kidney damage and dysfunction,which was associated with podocyte autophagy Activation of ER stress might affect inducing autophagy in LN[32,33].

P53 plays an important role in the progression of autoimmunity and the production of autoantibodies in lpr lupus mice[34]. Several cytokines(IL-10,TNF-a) may interfere with the p53 oncogene product,and induce the survival of autoreactive lymphocytes,which facilitating the characteristic features of LN[35].Apoptosis in LN occurs via two pathways, an extrinsic pathway involving transduction of some apoptotic signals following aggregation of a death receptor, and an intrinsic pathway that signals through the mitochondria and is regulated by the Bcl-2 family[36]. Lentivirus-mediated lincRNA-p21-overexpressed cells revealed enhanced apoptosis with up-regulated downstream PUMA/Bax expression in LN mice glomerular pathology[37].Apoptosis activates the immune system and causes inflammation and aberrant apoptosis seems to contribute to lupus nephritis[38].In this study,our co-expression and PPI net-work revealed some key hub genes in LN and found that the top 10 hub key genes were clustered in apoptosis,autophagy, reactive oxygen species pathway and metabolic pathway.The functional annotation indicated in these 5 modules(including 3 up-regulated modules and 2 down-regulated modules) conducted with MCODE plug-in of cytoscape are mainly involved in oxidative phosphorylation,mTOR signaling pathway,mitophagy,NOD-like receptor.

Oxidative phosphorylation (OXPHOS) produces more than 95% of a cell's energy in the form of ATP mainly from mitochondrial one.Research found the association of common variants of the adenosine triphosphate 6q(ATP6) genes with SLE,which raises the possibility of a shared mitochondrial genetic background of LN[39].The mTOR mentioned above forms two distinct multiprotein complexes, mTORC1and mTORC2.mTOR is a serine/threonine kinase and expresses a phosphoinositide 3-kinase (PI3K)-related kinase family.The mTOR signaling pathway is closely associated with immune cells,the abnormalities of autophagy and OS,and plays an important role in the pathogenesis of LN.A clinical study found that rapamycin/sirolimus(mTOR Inhibitor),a drug currently approved to prevent organ transplant rejection,may offer a safe and effective treatment for lupus by blocking T cell function and decreasing mitochondrial dysfunction by activating mitophagy[40].The process is regulated by the autophagy-related gene family, While the atg5 gene has been linked to the development of LN by epigenetic studies.

mTOR pathway guides the development of novel and effective therapies for patients with LN[41].The mitophagy pathway is an important form of autophagy for the selective removal of dysfunctional or redundant mitochondria.Proficient mitophagy may have therapeutic effects in LN,and drugs that induce mitophagy,such as rapamycin works in exploration as therapeutic strategies to enhance the clearance of injury-promoting fragmented mitochondria and accelerate recovery from LN flares[42,43].

The NOD-like receptors (NLRs) are a group of intracellular receptors that represent a key component of the host's innate immune system.Nucleotide-binding domain(NOD) like receptor protein 3(NLRP3) inflammasome plays key role in inflammation and autoimmunity diseases.Numerous studies have revealed their significance in LN.The activation of the NLRP3 inflammasome contributed to LN development and thus became a potential therapeutic target[44,45].

Thus,we conducted this study which might provide a non-invasive way to reveal the pathogenesis and novel biomarker in LN.

Currently,pharmacological therapies for SLE range from antimalarials to glucocorticoids,immunosuppressive agents or biologics if needed. However, current research and development of novel drugs would take a long time and cost a lot of money.At present,the drugs applied to SLE/LN were still limited.Herein,we used three drug prediction databases to express detailed drug data.As showed in table 4,four targets were associated with SLE/LN,including UDP-glucuronosyltransferase 2B17,Kappa-casein,V-type proton ATPase subunit G and apolipoprotein A-IV.

Among them,apolipoprotein A-IV precursor was up-regulated in PBMC of LN patients;vacuolar ATPase inhibitor-FR177995 was found anti-immunoinflammatory effects in adjuvant-induced arthritic rats.Nine approved drugs that respond to these 4 treatment targets were applied for the treatments of diversified diseases varied from cardiovascular diseases to tumors.

Most of the predictions of the drugs in our study are not in clinical use for LN, but they might be applying for subsequent prospect investigations in pharmacologic therapies .The majority of these drugs focused on anti-immunoinflammatory,regulate metabolism,tumor suppressor function ,limiting tissue damage and fibrosis,potential target to combat autoimmune disease,et al.The integration of multiple histological techniques is becoming more and more important in the era of precision medicine for autoimmune diseases. These molecules have the potential role to be used as disease biomarkers in personalized medicine.

This study also has several limitations. Because the sample size was small and from single center, the results of the study cannot yet be extended to all LN patients. Secondly, the mechanism and the possible effects of DELs and DEMs in urine sample of LN has not been explored through a cohort study within different treatments. Thirdly, the specificity of the constructed urinary lncRNA panel for LN diagnosis was not very clear. As this study recruited only LN patients, it would be desirable to include more patients with other types of rheumatoid diseases in urinary tract. Therefore, it is necessary to expand the sample size to conduct a prospective longitudinal multi-center cohort study to validate our current findings.

Conclusions

In conclusion,we conducted research to reveal lncRNA and mRNA expression levels in urine from LN samples using microarrays,which may directly reflect the role of integrated identification of key genes,providing novel non-invasive potential biomarkers for early diagnosis and prognosis in a special

specimen from LN patients.Further investigations are required to evaluate the signaling modules pathways identified in the GO and KEGG analyzes concerning to their role in the development and progression of LN.

Methods

The human ethics committee approved the Shenzhen Futian Hospital for Rheumatic Diseases (Shenzhen,Guangdong,China).All patients signed informed consent before the study (No.FS201903007).

Patients and samples

The samples and clinical data were collected from the Department of Rheumatology,Shenzhen Futian Hospital for Rheumatic Diseases (Shenzhen, Guangdong,China) from October 2018 to December 2019.A total of 3 patients with LN and 3 age and sex matched healthy control people were enrolled in this study(Table 1.).The diagnosis of LN was established by the American College of Rheumatology classification criteria for SLE)[46];All participants were taken from the Han Chinese population.Study flow was shown in Fig 1.

Urine RNA extraction

Total RNA was extracted from urine using mirVana™PARIS™ Kit (Ambion-1556,USA) according to the company's protocol.Total RNAs were quantified by the NanoDrop ND-2000 (Thermo Scientific,USA) and the RNAs integrity from urine was assessed using Agilent Bioanalyzer 2100 (Agilent Technologies, QIAGEN, Germany). The concentration of extracted RNA was about 200–2000 pg/μl. The sample labeling,microarray hybridization and washing were performed based on the manufacturer's standard protocols. Briefly,total RNAs were transcribed to double-strand cDNAs and then synthesized cRNAs. Next, 2nd cycle cDNAs were synthesized from cRNAs. Followed fragmentation and biotin labeling, the 2nd cycle cDNAs were hybridized onto the microarray.After washing and staining,the arrays were scanned by the Affymetrix Scanner 3000(Affymetrix).

Microarray analysis

Affymetrix GeneChip Command Console (version 4.0,Affymetrix) software was used to extract raw data.Next,Expression Console (version1.3.1,Affymetrix) software offered RMA normalization for both gene and exon level analysis.Then the gene expression analysis and alternative splice analysis proceeded separately.Microarray analyses were performed by Oebiotech Corporation (Shanghai,China).Hierarchical clustering analysis and volcano maps were carried out using the R platform's plots and heatmap packages.

1 Gene expression analysis

GeneSpring software (version 14.9; Agilent Technologies) was employed to finish the basic analysis.Differentially expressed genes were then identified through fold change as well as *P* value calculated with a t-test.The threshold set for up-and down-regulated genes was a fold change>= 2.0 and a P value<= 0.05.Afterward,GO analysis and KEGG analysis were applied to determine the roles these DEMs played in these GO terms or pathways.

2 Construction of lncRNA-mRNA co-expression network and PPI network. We drew the co-expression networks using the Cytoscape software network (http://www.cytoscape.org;VERSION 3.6.1). To estimate the relative significance of a gene or lncRNA in the network,we calculated the core degree of each gene/lncRNA defined as the number of directly linked genes or lncRNAs.The bigger the degree it has,the more significant it is.Pearson correlation coefficient of mRNA-lncRNA pairs was calculated according to their expressive value.The co-expressed mRNA-lncRNA pairs with the absolute value of Pearson correlation coefficient> 0.8 were selected and the co-expression network was established using Cytoscape software.

The identified DEMs of LN were input into STRING online website (https://string-db.org)[47],the Cytoscape MCODE,Genemania and CytoHubba plug-in (version 3.6.1) were applied to construct protein interaction network (PPI) and visual analysis,and then screen out the core genes (Hub Gene).Maximal Clique Centrality (MCC) was used to identify the top 10 hub genes.DAVID's pathway analysis of genes in each MOCDE(15)□with score ≥ 5□ module was performed[48].

3 Drugs prediction of DEMs

DrugBank(http://www.drugbank.ca),genecards(https://www.genecards.org), DGIdb (http://www.dgidb.org), UniProt (https://www.uniprot.org/) databases were used to identify novel drug targets and the comparison of drug structures with potential mechanisms of action[49]. The DEMs of LN were put into three databases mentioned above to examine their association with potential targeted drugs. The purpose of the present study was to explain the rationale of current drug therapy used for LN and to explore additional potential target genes for future drug development.

Abbreviations

lncRNA:long-non-coding RNA (lncRNAs)

mRNAs:messenger RNAs (mRNAs)

LN:lupus nephritis (LN)

GO:Gene ontology (GO)

KEGG:Kyoto Encyclopedia of Genes and Genomes (KEGG)

PPI□protein interaction network (PPI)

DELs：differential expressed urinary lncRNAs(DELs)

SLE:Systemic lupus erythematosus (SLE)

MF:molecular functions(MF)

BP:biological processes(BP)

CC:cellular components(CC)

ANAs:antinuclear antibodies(ANAs)

MAPK:mitogen-activated protein kinase (MAPK)

IFN:type I interferon(IFN)

PCa:prostate cancer (PCa)

ER:endoplasmic reticulum (ER)

PPARβ/δ:Peroxisome proliferator-activated receptors(PPARβ/δ)

OXPHOS:Oxidative phosphorylation (OXPHOS)

PI3K:phosphoinositide 3-kinase (PI3K)

NLRs:NOD-like receptors (NLRs)

Declarations

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The datasets analyzed in this manuscript are not publicly available. Requests to access the datasets should be directed to yezhizhong0506@163.com

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This study was approved by Shenzhen FutianHospital Ethics Committee Shenzhen Futian Hospital for Rheumatic Diseases((No.FS201903007)).

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Not applicable.

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The authors declare that they have no competing interests.

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Tables

Table 1 Clinical characteristics of study population

	LN(n=3)	HCs(n=3)
Sex,male/female(number)	1/2	1/2
Age(years)	29.7	29.0
Disease duration (months)	76.0	-
SLEDAI score*	25.4	-
BMI	28.8	25.4
Anti-dsDNA, no. positive/negative	3/0	-
Sex,male/female(number)	1/2	1/2
Age(years)	29.7	29.0
Disease duration (months)	76.0	-
SLEDAI score*	25.4	-

*SLE Disease Activity Index (SLEDAI): 0–4 inactive, 5–9 mild active, 10–14 moderate active, >14 severe active.

Table 2 Top 5 up and down regulated mRNAs between LN and con group

Gene ID	Fold Change	P-val	Gene Symbol	Trend
TC0600014318.hg.1	-12.37	0.044	FAM46A	down
TC1700007571.hg.1	-7.47	0.0057	ZNF830	down
TC0100016018.hg.1	-7.47	0.0102	CRABP2	down
TC0600014096.hg.1	-6.35	0.0497	TRIM39-RPP21	down
TC0800011072.hg.1	-6.05	0.02	farvorbu	down
TC1000008116.hg.1	19.1	0.0354	pleyskey	up
TC0600011112.hg.1	17.33	0.0233	SLC17A1	up
TC1900011251.hg.1	14.19	0.0224	NKG7	up
TC1700007636.hg.1	11.55	0.007	PIGW	up
TC1100012308.hg.1	10.93	0.0443	CRYAB	up

Table 3 Top 5 up and down regulated lncRNA between LN and con group

Gene ID	Fold Change	P-val	Gene Symbol	Trend
TC0400007952.hg.1	-57.49	0.0167	PCAT4	down
TC0400009004.hg.1	-27.46	0.0484	snygy	down
TC1900007242.hg.1	-16.96	0.0475	UCA1	down
TC1200012843.hg.1	-6.47	0.0142	ARPC3	down
TC0600014140.hg.1	-3.33	0.03	PRIM2	down
TC1100011184.hg.1	33.23	0.015	SF1	up
TC0800012363.hg.1	9.87	0.0105	KHDRBS3	up
TC0700013638.hg.1	7.83	0.0074	LINC01006	up
TC0200013734.hg.1	4.65	0.0314	LINC01158	up
TC0800012361.hg.1	2.7	0.0012	NCRNA00250	up

Table 4 The predicted drugs of DEGs in LN patients

Targets	Drugs	Accession Number	Guoups	Drug Description
Apolipoprotein A-IV	Copper	DB09130	Approved, Investigational	A transition metal and a trace element in the body. It is important to the function of many enzymes.
	Zinc	DB01593	Approved, Investigational	A metallic element forms an essential part of many enzymes, and plays an important role in protein synthesis and in cell division.
	Zinc Acetate	DB14487	Approved, Investigational	-
V-type proton ATPase subunit G	Tiludronic acid	DB01133	Approved, Investigational, Vet approved	A first generation of bisphosphonate similar to etidronic acid and clodronic acid, which were developed to mimic the action of pyrophosphate, a regulator of calcification and decalcification.
Kappa-casein	Fostamatinib	DB12010	Approved, Investigational	Fostamatinib has been investigated for the treatment and basic science of Rheumatoid Arthritis and Immune Thrombocytopenic Purpura (ITP).
UDP-glucuronosyltransferase 2B17	Losartan	DB00678	Approved	Belonging to angiotensin-converting enzyme (ACE) inhibitors,which are commonly prescribed to treat high blood pressure, heart problems and other conditions.
	Tamoxifen	DB00675	Approved	Tamoxifen blocks the actions of estrogen, a female hormone as well as prevent the incidence of breast cancer in high risk populations. Since certain types of breast cancer require estrogen to grow .
	Gemfibrozil	DB01241	Approved	Gemfibrozil is a fibric acid agent, similar to clofibrate, used to treat Type IIb, IV, and V hyperlipidemias.
	Dextromethorphan	DB00514	Approved	Dextromethorphan is a levorphanol derivative and codeine analog commonly used as a cough suppressant and also a drug of abuse. Although similar in structure to other opioids, it has minimal interaction with opioid receptors.
	Copper	DB09130	Approved, Investigational	A transition metal and a trace element in the body. It is important to the function of many enzymes.

Figures

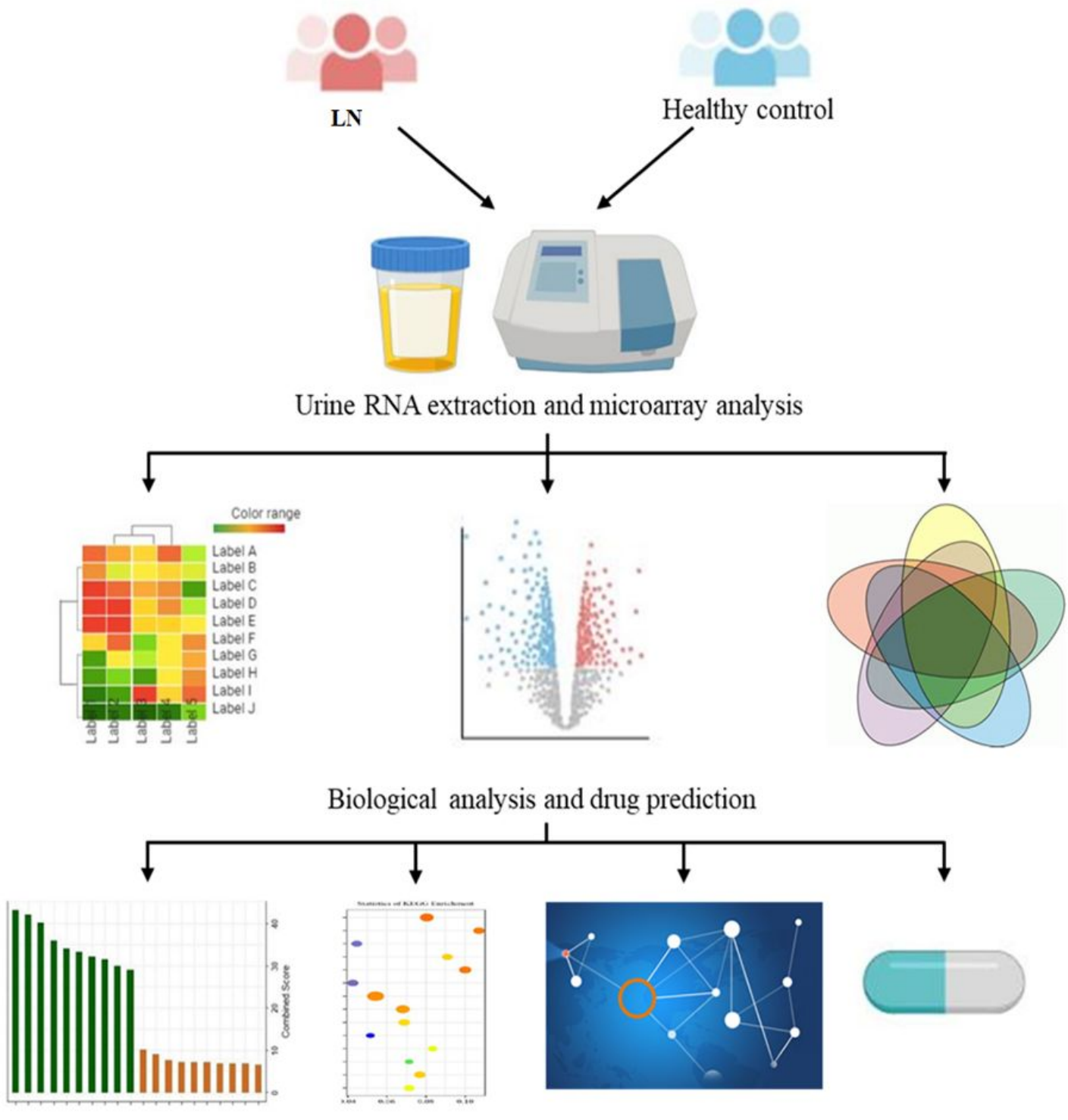

Figure 1

All participants were taken from the Han Chinese population.Study flow was shown in Fig 1.

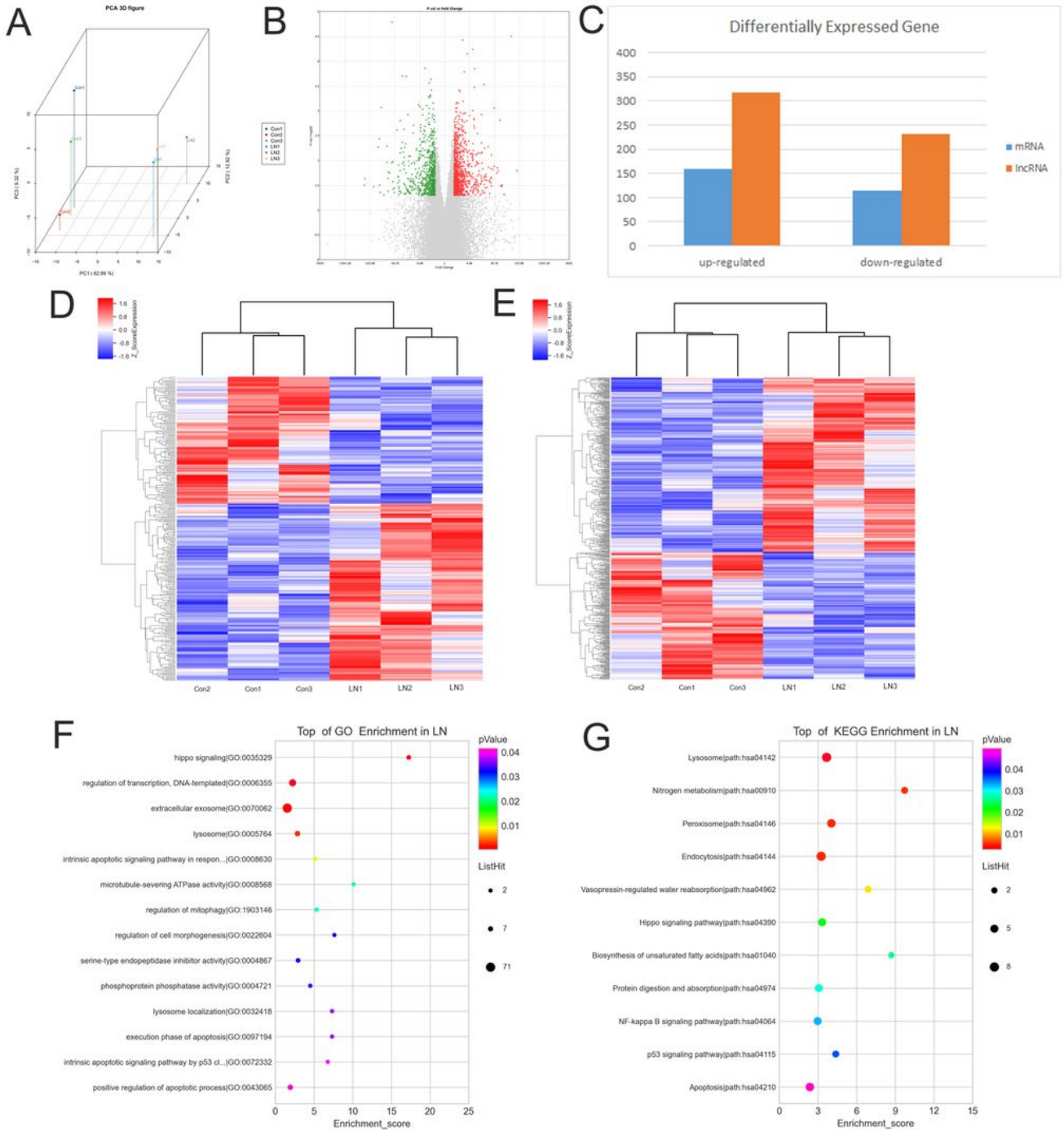

Figure 2

Expression profile of lncRNAs and mRNAs Hierarchical clustering revealed systematic variations between LN patients (n=3) and control groups (n=3) in the expression of lncRNAs and mRNAs(Fig 2 A,B,C). Barplot and heatmap of the differential expression of mRNAs/lncRNAs between the two groups were shown in Fig.2D,E. The GO enrichment analysis for DEMs in LN patients showed that the dysregulated genes were mainly involved in regulation of transcription, intrinsic apoptotic signaling pathway in response to DNA

damage, regulation of mitophagy(Fig 2 F); KEGG enrichment analyses found that DEMs were mainly enriched in the p53 pathway, apoptosis,endocytosis signaling pathway(Fig 2 G).

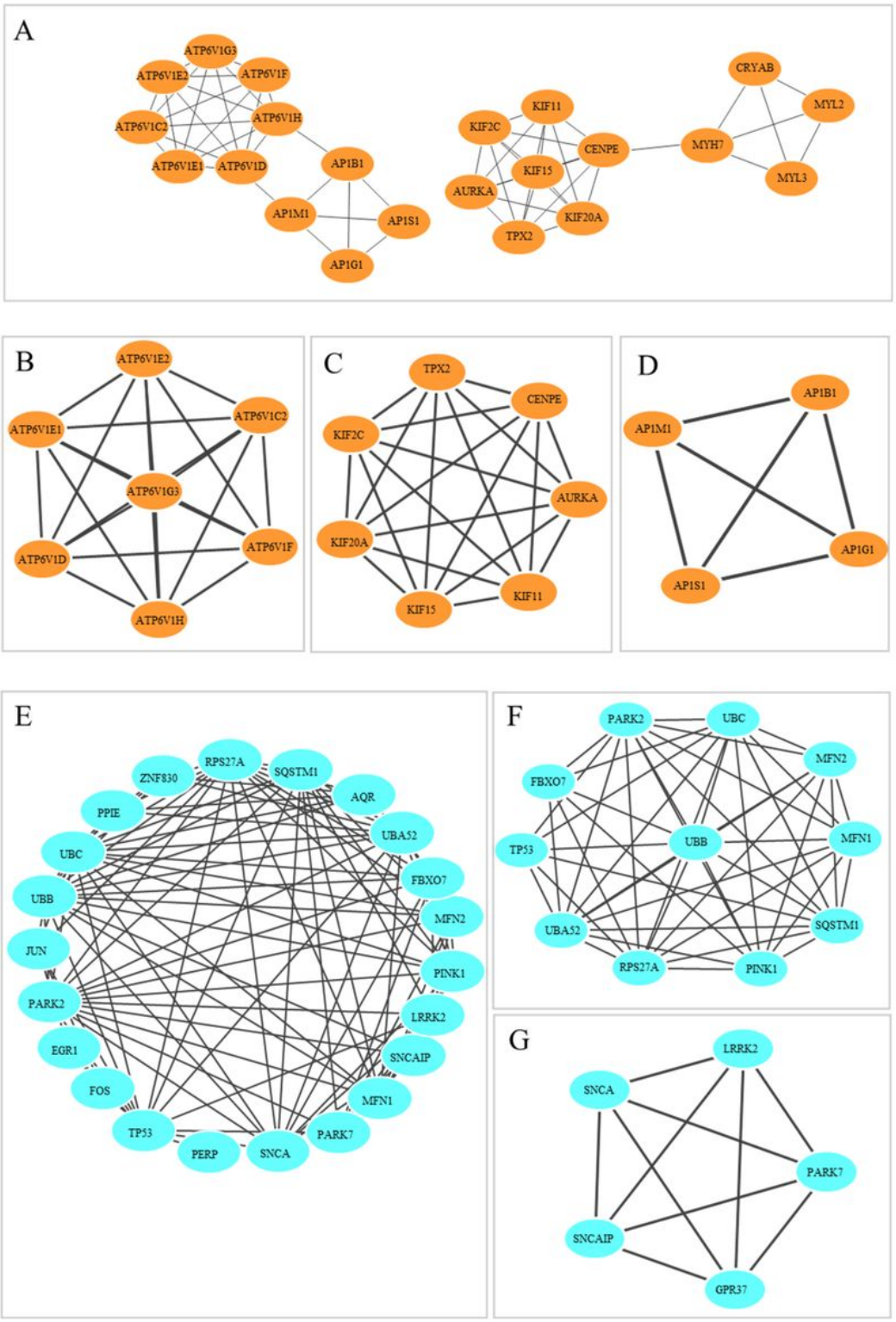

Figure 3

The PPI network of DEMs contained 55 nodes and 63 edges in 141 up-regulated (PPI enrichment p-value:2.02e-05);while down-regulated one contained 33 nodes and 69 edges in 141 up-regulated (PPI enrichment p-value:5.39e-07) (Fig.3A and Fig.3E).

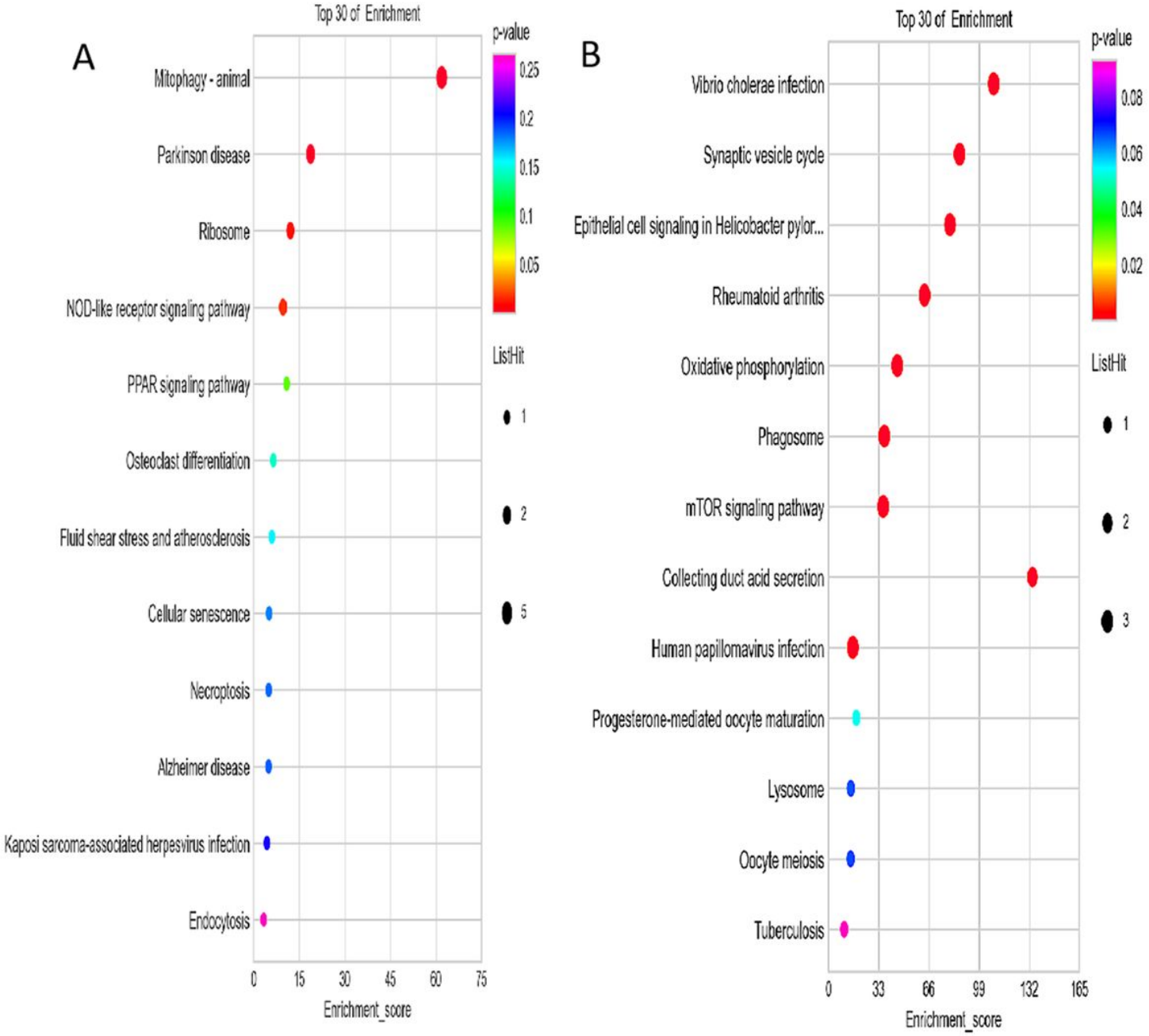

Figure 4

Co-expression and PPI network analyses suggest that high-degree nodes are clustered in apoptosis,autophagy, reactive oxygen species pathway and metabolic pathway(Fig.4).

Supplementary Files

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