

Whole Transcriptome Analysis on Sepsis Caused by Pyogenic Liver Abscess

Ding Zhao

Jilin University First Hospital

Jia Liu

Jilin University First Hospital

Dong-feng Han

Jilin University First Hospital

Xing-liang Li

Jilin University First Hospital

Jian Chen

Shanghai TCM-Integrated hospital, shanghai University of TCM

Wei Li

Jilin University First Hospital

Ji-hong Xing

Jilin University First Hospital

Lichao Sun (✉ sunlichao@jlu.edu.cn)

the first hospital of Jilin University

Research

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Abstract

Background

Pyogenic liver abscess (PLA) is a common clinical disease throughout the world with remaining high mortality and morbidity. This study sought to investigate key genes and non-coding RNAs (ncRNAs) associated with the pathogenesis and treatment of sepsis caused by PLA (SLA).

Methods

We constructed the expression profiles of RNAs by RNA sequencing in the peripheral blood of healthy subjects, SLA and seven day therapy of SLA patients. Then the sequencing data was analysed through integrated bioinformatics strategy.

Results

4549 mRNAs, 9808 lncRNAs, 467 circRNAs and 292 miRNAs were differentially expressed (DE) between SLA and control samples. 3199 mRNAs, 6018 lncRNAs, 161 circRNAs and 132 miRNAs were differentially expressed between SLA after 7 days therapy and SLA control subjects. Moreover, DE mRNAs of the former two comparisons were both related to immunity and T cell-mediated immune response; DE lncRNAs were both associated with B cell receptor signaling pathway and Osteoclast differentiation; DE circRNAs were both related to Chagas disease and B cell receptor signaling pathway; DE miRNAs exerts its specific role in the process of SLA by MAPK signaling pathway and Estrogen signaling pathway compared to DE lncRNAs, circRNAs and mRNAs. Finally, lncRNA/circRNA-miRNA-mRNA network was constructed to explore the internal regulating relationships of these ncRNAs and mRNAs.

Conclusions

Our results may provide a new perspective on differential RNA expression involved in the pathogenesis and treatment of SLA and accelerate the development of novel treatments for this disease.

1. Introduction

Pyogenic liver abscess (PLA) is a common infectious disease of the liver, which can be divided into amoebic, bacterial, fungal and hydatid liver abscesses[1]. Among them, bacterial liver abscess is the most common, accounting for 80% of the incidence of liver abscess[2]. The incidence of PLA has been on the rise in recent years. PLA has a rapid onset, severe disease and rapid development[3]. The mortality rate has increased significantly after the progression to sepsis [4]. There are few studies on PLA-induced sepsis, and there is a lack of biomarkers for early recognition of liver abscess progression to sepsis.

Non-coding RNA (ncRNA) cannot be translated into protein, but it can regulate protein expression[5]. MicroRNAs (miRNAs) are a family of ncDNAs with 21–25 nucleotides, which play an important role in regulating protein expression by binding to specific target mRNA for cleavage or translation inhibition[6]. Long noncoding RNA (LncRNA) is a kind of ncRNA that accounts for the majority of mammalian ncRNAs, with a length of more than 200 nucleotides, and regulates gene expression through a variety of mechanisms[7]. Circular RNA (circRNA) is an endogenous ncRNA with a closed circular structure, which can regulate linear RNA transcription, downstream gene expression and protein production[8]. Some researchers have shown that mRNA, miRNA, lncRNA and circRNA can interact as competitive endogenous RNA to form ceRNA regulatory networks[9, 10]. LncRNA and circRNA can act as ceRNA molecules and have the function of miRNA sponge[11]. They regulate gene transcription and translation by competitively binding to RNA binding protein sites on miRNA, which plays a role in multiple physiological and pathological processes [12]. However, so far, SLA-related RNA molecules are still rarely studied in SLA, and their specific role in the pathogenesis and treatment of SLA is not clear. Therefore, comprehensive detection and analysis of ncRNAs in the development of SLA are critical for the prevention and treatment of SLA.

In the present study, we used whole transcriptome sequencing analyses to construct RNA expression profiles in peripheral blood of samples. Two comparisons (SLA samples vs. healthy controls, 7 days therapy of SLA vs. SLA) were applied to screen differentially expressed mRNAs, miRNAs, lncRNAs, and circRNAs, and their functions were predicted by bioinformatics analysis. The analysis flow of this study is shown in Fig. 1. The findings facilitate our understanding of regulatory mechanisms, discovering new targets and providing assistance for the treatment of sepsis caused by liver abscess.

2. Materials And Methods

2.1 Ethical approval.

This research was approved by the Ethics Committee of the first hospital of Jilin University (20K019-002). All subjects, enrolled from the first hospital of Jilin University, agreed to participate in the study after fully understanding the purpose and procedure of the experiment. Full inclusion and exclusion criteria are listed in Table 1. The subjects were divided into three groups: SLA (M0), SLA after 7 days therapy (T7) and the healthy subjects (C). Subsequently, the fasting venous blood of all subjects were obtained and stored at – 80°C until analysis.

2.2 RNA isolation, library preparation & RNA sequencing

Total RNA was extracted using Trizol reagent (Life Technologies, MD, USA), and the quality and quantity were assessed by real-time quantitative PCR (RT-PCR) and Agilent 2100 bioanalyzer (Thermo Fisher Scientific, MA, USA). Ribo-Zero™ rRNA Removal Kits (Epicentre, WI, USA) were applied for rRNA depletion. A total 1 µg RNA of each sample was used for constructing cDNA library. This library was sequenced by Illumina HiSeq4000 sequencer. The raw reads were saved as FASTQ format and processed for quality control. After removing low-quality reads and adaptor sequences, clean data were obtained and used for

all subsequent analyses. The fragments per kilobase per million values were used to estimate the level of gene expression. Differential expression of circRNAs, mRNAs, miRNAs and lncRNAs with statistical significance (DEGs) was identified by DESeq package, based on cut-off criteria of fold change (FC) > 2 and corrected p-value < 0.05.

2.3 Gene ontology and pathway enrichment analyses.

To understand the biological significance of these DE transcriptome, we performed functional enrichment (KEGG pathway and Gene ontology enrichment) analyses through the clusterProfiler package[13]. And those with $P < 0.05$ were significantly enriched. R language ggplot2 and GOplot package were used to draw the bubble plot of the top 5 enriched GO terms in each category (biological process, molecular function, and cellular component).

2.4 PPI network construction and sub-network identification.

To discover the co-differentially genes in the two comparisons (SLA samples vs. healthy controls, 7 days therapy of SLA vs. SLA), we conducted venn analysis of the differential expressed mRNAs (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). Then, the Search Tool for the Retrieval of Interacting Genes (STRING) database[14] was used to construct the protein-protein interaction (PPI) network of the common and opposite differential expressed mRNAs between the two comparisons. The interaction pairs with the PPI combined score > 0.4 were selected as significant and the resulting network was performed with Cytoscape(version 3.2.1)[15]. The MCODE can effectively find densely connected regions of a molecular interaction network [16]. MCODE was used to discover the PPI network function module, which used a cut-off value for the connectivity degree of nodes (proteins in the network) greater than 3 and score > 4.

2.5 ceRNA analysis.

Cytoscape was used to construct the lncRNA/circRNA-miRNA-mRNA ceRNA regulatory network based on the DE transcriptome in the blood between SLA vs. healthy samples and 7 days therapy of SLA vs. SLA patients. The screening criteria of ceRNA were as follows: 1. differentially expressed mRNAs, lncRNAs, circRNAs and miRNAs; 2. a targeted relationship and negative correlation between miRNA and lncRNA/circRNA (lncRNA-miRNA-mRNA: $pvalue < 0.05$ and $cor \leq -0.7$, circRNA-miRNA-mRNA: $pvalue < 0.05$ and $cor \leq -0.6$); 3. a targeted relationship and negative correlation between miRNA and mRNA ($pvalue < 0.05$ and $cor \leq -0.7$); 4. a significant correlation between lncRNA and mRNA targeted to the same miRNA (lncRNA-miRNA-mRNA: $pvalue < 0.05$ and $cor \geq -0.7$, circRNA-miRNA-mRNA: $pvalue < 0.05$ and $cor \geq 0.6$). Then, to discover the co-differentially lncRNA/circRNA-miRNA-mRNA in the two comparisons (SLA samples vs. healthy controls, 7 days therapy of SLA vs. SLA), we conducted venn analysis of the differential expressed ceRNA regulatory network. Finally, ceRNA was further constructed using the two intersections (up-regulated ceRNA in SLA patients and down-regulated after therapy, down-regulated ceRNA in SLA patients and up-regulated after therapy)

2.6 Statistical Analysis.

This study used SPSS software (version 20.0) and GraphPad Prism 8 (GraphPad Software, CA) for the statistical evaluations. The values were expressed as mean \pm standard deviation. Statistical differences were performed with two-tailed Student's t-tests (two groups) and One-way ANOVA (three groups). Differences with $p \leq 0.05$ were indicated significant.

3. Results

3.1 Clinical characteristics of the participants.

In this study, eight healthy subjects, eight SLA patients and eight SLA patients after 7 days therapy were enrolled. All patients fulfilled the diagnostic criteria for SLA. There was no significant difference in age and gender between the SLA and healthy control groups. The characteristics of all subjects are shown in Table 1. Compared with the SLA patients, the SLA patients after 7 days therapy exhibited significant increases in platelets level and decreases in procalcitonin and CPR levels.

3.2 Quality assessments and mapping results

To construct expression profiles of miRNAs, lncRNAs, circRNAs, and mRNAs of the three groups, transcriptome data sets were generated by whole-transcriptome sequencing. Subsequently, quality control and mapping analysis were performed for the sequencing output (Supplementary Table 1). For miRNA clean data, the base percentage of Q20 was $> 97\%$, Q30 was $> 92.3\%$. For total RNA clean data, the base percentage of Q20 was $> 97.2\%$, Q30 was $> 92.3\%$, and the average mapping rate of the 24 samples was 97.73% . These results could indicate the high quality of transcriptome sequencing data with suitable mapping.

3.3 Differentially expressed mRNAs, lncRNAs, circRNAs and miRNAs.

4549 mRNAs (including 2281 up-regulated and 2268 down-regulated), 9808 lncRNAs (including 3794 up-regulated and 6014 down-regulated), 467 circRNAs (including 293 up-regulated and 174 down-regulated) and 292 miRNAs (including 183 up-regulated and 109 down-regulated) were differentially expressed between SLA samples and control samples with $p < 0.05$ and $|\log_2\text{foldchange}| \geq 1$. 3199 mRNAs (including 1851 up-regulated and 1348 down-regulated), 6018 lncRNAs (including 4029 up-regulated and 1989 down-regulated), 161 circRNAs (including 73 up-regulated and 98 down-regulated) and 132 miRNAs (including 43 up-regulated and 89 down-regulated) were differentially expressed between SLA after 7 days therapy samples and SLA control samples with $p < 0.05$ and $|\log_2\text{foldchange}| \geq 1$. Four heatmaps of hierarchical clustering of DE mRNAs, lncRNAs, circRNAs or miRNAs was generated to visualize the overall pattern of gene expression (Fig. 2). Moreover, the PCA loading plot showed that DE mRNAs, lncRNAs, circRNAs or miRNAs can be independently used to distinguish SLA after 7 days therapy samples, SLA and healthy control samples (Fig. 3).

3.4 Functional and pathway enrichment analyses of DEGs.

For the DE transcriptome in SLA vs. healthy samples, the GO terms (Fig. 4) showed that DE mRNAs were mainly related to neutrophil activation, neutrophil mediated immunity, and secretory granule membrane et al. (Supplementary Table 2); DE lncRNAs were significantly related to neutrophil activation, secretory granule membrane, and transcription factor complex et al. (Supplementary Table 3); DE circRNAs were significantly related to ubiquitin-like protein transferase activity, secretory granule membrane, and protein polyubiquitination et al. (Supplementary Table 4); DE miRNAs were significantly related to vacuolar membrane, NAD-dependent histone deacetylase activity, and acetylcholine receptor binding et al. (Supplementary Table 5). On the other hand, the top 5 enriched KEGG pathways (Fig. 5) terms of DE mRNAs were NF-kappa B signaling pathway, primary immunodeficiency, hematopoietic cell lineage, T cell receptor signaling pathway, and Chagas disease (Supplementary Table 6); DE lncRNAs were significantly related to PD-L1 expression and PD-1 checkpoint pathway in cancer, B cell receptor signaling pathway, osteoclast differentiation, human T-cell leukemia virus 1 infection, and Chagas disease (Supplementary Table 7); DE circRNAs were significantly related to Fc gamma R-mediated phagocytosis, neurotrophin signaling pathway, proteoglycans in cancer, endocytosis, and axon guidance (Supplementary Table 8); DE miRNAs were significantly related to Rap1 signaling pathway, Calcium signaling pathway, MAPK signaling pathway, GnRH signaling pathway, and aldosterone synthesis and secretion (Supplementary Table 9).

For the DE transcriptome in 7 days therapy of SLA vs. SLA patients, the GO terms showed that DE mRNAs were mainly related to neutrophil activation, neutrophil mediated immunity, and secretory granule membrane et al.; DE lncRNAs were significantly related to methyltransferase activity, membrane lipid metabolic process, and transcription factor complex et al.; DE circRNAs were significantly related to amyloid precursor protein metabolic process, secretory granule membrane, and cadherin binding et al.; DE miRNAs were significantly related to vacuolar membrane, NAD-dependent histone deacetylase activity, and regulation of metal ion transport et al. (Fig. 4). On the other hand, the top 5 enriched KEGG pathways terms of DE mRNAs were primary immunodeficiency, osteoclast differentiation, hematopoietic cell lineage, NF-kappa B signaling pathway and PD-L1 expression and PD-1 checkpoint pathway in cancer; DE lncRNAs were significantly related to Glycosphingolipid biosynthesis-lacto and neolacto series, B cell receptor signaling pathway, central carbon metabolism in cancer, Glycosaminoglycan biosynthesis-keratan sulfate, and various types of N-glycan biosynthesis; DE circRNAs were significantly related to transcriptional misregulation in cancer, proteoglycans in cancer, PD-L1 expression and PD-1 checkpoint pathway in cancer, Legionellosis, and axon guidance; DE miRNAs were significantly related to 2-Oxocarboxylic acid metabolism, oxytocin signaling pathway, glycosaminoglycan biosynthesis-chondroitin sulfate, human immunodeficiency virus 1 infection, and GnRH signaling pathway (Fig. 5).

3.5 Construction and analysis of PPI networks.

PPI networks were constructed through a STRING database to explore the functional relationship between the SLE therapy and SLE differential genes. Cytoscape was used to analyze PPI networks of the 364 dysregulated genes, including 243 U2D and 121 D2U genes. The results showed that there were 269 nodes and 696 edges (Fig. 6). MCODE was used to discovery the PPI network function module and four

significant modules (nodes > 3 and score > 4) presented (Fig. 7). Module 1 (score: 12.5) consisted of 13 nodes and 75 edges. The seed gene was CYSTM1; Module 2 (score: 7.931) consisted of 30 nodes and 115 edges, seed gene was IRAK2; module 3 (score: 4.5) consisted of 5 nodes and 9 edges, and the seed gene was S100A8; Module 4 (score: 4.5), consisted of 5 nodes and 9 edges, and the seed gene was ACER3. These four seed genes were all up-regulated in SLE compared to healthy controls, and down-regulated after therapy.

3.6 ceRNA analysis.

As the method described, we constructed the ceRNA regulatory network of M0 vs. C and T7 vs. M0, respectively. The lncRNA-miRNA-mRNA network contained 68275 pairs in M0 vs. C and 1632 pairs in T7 vs. M0. A total of 1031 common and opposite pairs were screened (Fig. 8). Specifically, 459 ceRNAs were upregulated in M0 vs. C (lncRNA_down-miRNA_up-mRNA_down) and down-regulated in T7 vs. M0 (lncRNA_up-miRNA_down-mRNA_up), including 176 lncRNAs (lnc-RP9-2, lnc-RWDD2B-3, STX18-AS1 et.al), one miRNAs (miR-671-5p), and four mRNAs (CRIP2, ATP10A, DAGLA and OXNAD1). 572 ceRNAs were downregulated in M0 vs. C (lncRNA_up-miRNA_down-mRNA_up) and up-regulated in T7 vs. M0 (lncRNA_down-miRNA_up-mRNA_down), including 191 lncRNAs (GK-AS1, UVRAG-DT, RARA-AS1, KLF7-IT1 et.al), three miRNAs (miR-150-5p, miR-134-5p and miR-150-3p), and eight mRNAs (GLT1D1, GPR27, C1RL, OPRL1, et. al).

The circRNA-miRNA-mRNA network contained 2420 pairs in M0 vs. C and 70 pairs in T7 vs. M0. A total of 25 common and opposite pairs were screened (Fig. 9). Specifically, 7 ceRNAs were upregulated in M0 vs. C (circRNA_down-miRNA_up-mRNA_down) and down-regulated in T7 vs. M0 (circRNA_up-miRNA_down-mRNA_up), including two circRNAs (chr16:68121987|68126610|NFATC3 and chr4:143437999|143440382|GAB1), two miRNAs (miR-27a-5p and miR-671-5p), and seven mRNAs (CD6, CD96, DAGLA, TRIM46, et. al). 18 ceRNAs were downregulated in M0 vs. C (circRNA_up-miRNA_down-mRNA_up) and up-regulated in T7 vs. M0 (circRNA_down-miRNA_up-mRNA_down), including seven circRNAs (chr11:128758115|128782023|FLI1, chr6:18212488|18213781|KDM1B, chr13:113520616|113539507|TMCO3 et.al), four miRNAs (miR-150-3p, miR-150-5p, miR-370-3p and miR-134-5p), and eleven mRNAs (CUTC, CTCFL, ASPH, GPR27 et.al).

4. Discussion

In this study, we used whole transcriptome sequencing to investigate the significantly differentially expressed mRNAs, circRNAs, lncRNAs, and miRNAs in the PBMC of patients with SLA. Then, these differentially expressed RNAs were to predict their potential biological functions through GO and KEGG pathway analysis. In addition, we also performed PPI network and circRNA/lncRNA-miRNA regulatory network. Our results showed that ncRNAs may participate in the pathogenesis of SLE and provide some potential targets for the treatment of SLA.

In this study, 243 genes were upregulated in SLA patients and downregulated after seven days therapy, and 121 genes were downregulated in SLA patients and upregulated after seven days therapy. And the

common GO terms of these two comparisons included neutrophil activation, neutrophil mediated immunity, T cell activation, et.al; the common KEGG terms contained NF-kappa B signaling pathway, Th1 and Th2 cell differentiation, T cell receptor signaling pathway. Consistent with our results, previous study has found that circulating Th1 and Th2 subset accumulation kinetics was related to immunosuppression and prognosis in sepsis[17]. It is known that sepsis is the result of host immune response to infection, and the original site of infection might be the main driver of secondary organ injury or even death[18]. Moreover, another study shows that circulating Th1 and Th2 subset accumulations differ in septic patients with different infection sites[19]. Therefore, these results indicated that the pathogenesis and treatment of SLE may be related to immunity and T cell-mediated immune response.

LncRNA, a mRNA-like transcript, functions in various biological process [20]. Through its colocated and coexpressed protein-coding genes, we predicted the biological function of lncRNA. The common GO terms of the two comparisons (T7 vs. M0 and M0 vs. C) included transcription factor complex and nuclear transcription factor complex; the common KEGG terms contained Chagas disease, B cell receptor signaling pathway, Osteoclast differentiation, RNA degradation and Central carbon metabolism in cancer. Previous study has demonstrated that B-cell receptor-associated protein 31 (BAP31) played a critical role in mitochondrial function and ER homeostasis in sepsis-related myocardial injury[21]. A transcriptomic meta-analysis study reveals that osteoclast differentiation was upregulated in human septic shock [22]. Therefore, DE lncRNAs appear to function in the pathogenesis and therapy of SLA by regulating B cell receptor signaling pathway and Osteoclast differentiation. In our future work, the specific role of lncRNAs of these two functions needs to be further studied.

CircRNA expression was significantly altered in PBMCs of sepsis patients [23]. To identify a benchmark map of circRNA expression dynamics in the therapy of SLA patients, we identified DE circRNAs in two comparisons (T7 vs. M0 and M0 vs. C). The common GO terms were secretory granule membrane and inclusion body; the common KEGG terms contained Chagas disease, B cell receptor signaling pathway, Axon guidance, Transcriptional misregulation in cancer, PD-L1 expression and PD-1 checkpoint pathway in cancer, and Proteoglycans in cancer. Interestingly, Chagas disease and B cell receptor signaling pathway were significantly dysregulated between lncRNA and circRNA functional enrichment, suggesting that these two pathways may function in SLA through lncRNA and circRNA.

DE miRNAs in the therapy of SLA patients were performed by functional enrichment analyses of predicted target genes. The common GO terms of the two comparisons (T7 vs. M0 and M0 vs. C) included vacuolar membrane, NAD-dependent histone deacetylase activity and NAD-dependent protein deacetylase activity; the common KEGG terms contained MAPK signaling pathway, Rap1 signaling pathway, Oxytocin signaling pathway, Estrogen signaling pathway, GnRH signaling pathway et.al.. MAPK, a serine-threonine kinase, plays a critical role in regulating stress response, inflammation, cell proliferation and apoptosis [24]. A recent study showed that inhibition of MAPK pathway could ameliorate sepsis-induced cardiac injury in AT1R-knockdown rats [25]. It is known that estrogen counteracts inflammation and clinical symptoms in various diseases including chronic liver injury [26], neurovascular/neuroimmune disease [27], and aging [28]. A previous study revealed that estrogen could

protect sepsis-related disorder through decreasing inflammation [29]. Moreover, a recent study proved that estrogen significantly ameliorated sepsis-induced liver injury through blocking oxidative stress mediated activation of pyroptosis signaling pathway [30]. Interestingly, these common functional terms were different from those of lncRNA, circRNA and mRNA. Taken together, the results revealed that DE miRNAs exerts its specific role in the process of SLA by MAPK signaling pathway and Estrogen signaling pathway compared to DE lncRNAs, circRNAs and mRNAs.

In recent years, the role of ceRNA in sepsis has become increasingly prominent. Fang et al. proved that lncRNA-H19 increase the expression of AQP1 through competitive binding miRNA-874 in sepsis, restoring dysregulated inflammatory responses[31]. Circular RNA VMA21 ameliorated sepsis-associated acute kidney injury via regulating miR-9-39/SMG1/inflammation axis and oxidative stress[32]. In this study, lncRNA/circRNA-miRNA-mRNA network was also constructed to discovery the regulatory relations of these ncRNAs and mRNAs. In the lncRNA-miRNA-mRNA network, miR-150-5p was downregulated in SLA patients compared to healthy control, and recover after 7 days therapy. Consisting with our results, Zhu et al. [33]found that miR-150-5p protected sepsis-induced myocardial depression by alleviating apoptosis. Interestingly, miR-150-5p show the same trend in circRNA-miRNA-mRNA network, suggesting that miR-150-5p may have a key role in the therapy of SLA through lncRNA/circRNA-miRNA-mRNA network. In addition, many researches revealed several miR-370-3p related ceRNA in sepsis progression, including lnc-MALAT1/miR-370-3p/HMGB1[34], lnc-NEAT1/miR-370-3p/ Irak2 [35], lnc-NEAT1/miR-370-3p/TSP-1[36], which is consistent with our results of circRNA-miRNA-mRNA network. However, the mechanism of ncRNAs is complicated and we will further explore these predicted ceRNA mechanisms. Finally, it will help to discovery the therapeutic targets and internal mechanism of SLA.

5. Conclusion

In conclusion, we used whole transcriptome sequencing analysis to explore the expression profile of RNAs in the peripheral blood of seven days therapy of SLA, SLA and healthy subjects. DE mRNAs, lncRNAs, circRNAs and miRNAs were screened, and their predicted biological functions were performed via bioinformatics analysis. These results may provide new perspectives on the pathogenesis of SLA and accelerate the development of new therapeutic approaches on SLA.

Declarations

Acknowledgements

Not applicable.

Ethical Approval and Consent to participate

This research was approved by the Ethics Committee of the first hospital of Jilin University (20K019-002). All subjects, enrolled from the first hospital of Jilin University, agreed to participate in the study after fully understanding the purpose and procedure of the experiment.

Authors' contributions

LCS and JHX conceived and designed the study, DZ collected samples, JL processed samples, XLL analyzed samples, WL analyzed data, JC wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Availability of supporting data

Author(s) declare the data are available.

Consent for publication

Authors are consent for publication.

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References

1. Meddings L, Myers RP, Hubbard J, Shaheen AA, Laupland KB, Dixon E, Coffin C, Kaplan GG: **A population-based study of pyogenic liver abscesses in the United States: incidence, mortality, and temporal trends.** *The American journal of gastroenterology* 2010, **105**(1):117-124.
2. Tian LT, Yao K, Zhang XY, Zhang ZD, Liang YJ, Yin DL, Lee L, Jiang HC, Liu LX: **Liver abscesses in adult patients with and without diabetes mellitus: an analysis of the clinical characteristics, features of the causative pathogens, outcomes and predictors of fatality: a report based on a large population, retrospective study in China.** *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2012, **18**(9):E314-330.
3. Jepsen P, Vilstrup H, Schonheyder HC, Sorensen HT: **A nationwide study of the incidence and 30-day mortality rate of pyogenic liver abscess in Denmark, 1977-2002.** *Alimentary pharmacology & therapeutics* 2005, **21**(10):1185-1188.
4. Qu TT, Zhou JC, Jiang Y, Shi KR, Li B, Shen P, Wei ZQ, Yu YS: **Clinical and microbiological characteristics of Klebsiella pneumoniae liver abscess in East China.** *BMC infectious diseases* 2015, **15**:161.
5. Hon CC, Ramilowski JA, Harshbarger J, Bertin N, Rackham OJ, Gough J, Denisenko E, Schmeier S, Poulsen TM, Severin J *et al*: **An atlas of human long non-coding RNAs with accurate 5' ends.** *Nature* 2017, **543**(7644):199-204.

6. Hausser J, Zavolan M: **Identification and consequences of miRNA-target interactions—beyond repression of gene expression.** *Nature reviews Genetics* 2014, **15**(9):599-612.
7. Ransohoff JD, Wei Y, Khavari PA: **The functions and unique features of long intergenic non-coding RNA.** *Nature reviews Molecular cell biology* 2018, **19**(3):143-157.
8. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE: **Circular RNAs are abundant, conserved, and associated with ALU repeats.** *Rna* 2013, **19**(2):141-157.
9. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP: **A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?** *Cell* 2011, **146**(3):353-358.
10. Wu J, Huang H, Huang W, Wang L, Xia X, Fang X: **Analysis of exosomal lncRNA, miRNA and mRNA expression profiles and ceRNA network construction in endometriosis.** *Epigenomics* 2020, **12**(14):1193-1213.
11. Li X, Yu S, Yang R, Wang Q, Liu X, Ma M, Li Y, Wu S: **Identification of lncRNA-associated ceRNA network in high-grade serous ovarian cancer metastasis.** *Epigenomics* 2020, **12**(14):1175-1191.
12. Tay Y, Rinn J, Pandolfi PP: **The multilayered complexity of ceRNA crosstalk and competition.** *Nature* 2014, **505**(7483):344-352.
13. Yu G, Wang LG, Han Y, He QY: **clusterProfiler: an R package for comparing biological themes among gene clusters.** *Omics : a journal of integrative biology* 2012, **16**(5):284-287.
14. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP *et al.* **STRING v10: protein-protein interaction networks, integrated over the tree of life.** *Nucleic acids research* 2015, **43**(Database issue):D447-452.
15. Kohl M, Wiese S, Warscheid B: **Cytoscape: software for visualization and analysis of biological networks.** *Methods in molecular biology* 2011, **696**:291-303.
16. Bader GD, Hogue CW: **An automated method for finding molecular complexes in large protein interaction networks.** *BMC bioinformatics* 2003, **4**:2.
17. Xue M, Xie J, Liu L, Huang Y, Guo F, Xu J, Yang Y, Qiu H: **Early and dynamic alterations of Th2/Th1 in previously immunocompetent patients with community-acquired severe sepsis: a prospective observational study.** *Journal of translational medicine* 2019, **17**(1):57.
18. Leligdowicz A, Matthay MA: **Heterogeneity in sepsis: new biological evidence with clinical applications.** *Critical care* 2019, **23**(1):80.
19. Xue M, Tang Y, Liu X, Gu M, Xie J, Liu L, Huang Y, Guo F, Yang Y, Qiu H: **Circulating Th1 and Th2 Subset Accumulation Kinetics in Septic Patients with Distinct Infection Sites: Pulmonary versus Nonpulmonary.** *Mediators of inflammation* 2020, **2020**:8032806.
20. Lee JT: **Epigenetic regulation by long noncoding RNAs.** *Science* 2012, **338**(6113):1435-1439.
21. Zhang J, Wang L, Xie W, Hu S, Zhou H, Zhu P, Zhu H: **Melatonin attenuates ER stress and mitochondrial damage in septic cardiomyopathy: A new mechanism involving BAP31 upregulation and MAPK-ERK pathway.** *Journal of cellular physiology* 2020, **235**(3):2847-2856.

22. Mukhopadhyay S, Thatoi PK, Pandey AD, Das BK, Ravindran B, Bhattacharjee S, Mohapatra SK: **Transcriptomic meta-analysis reveals up-regulation of gene expression functional in osteoclast differentiation in human septic shock.** *PloS one* 2017, **12**(2):e0171689.
23. Khan HN, Brands X, Aufiero S, Hoogendijk AJ, Klarenbeek AM, van Engelen TSR, Haak BW, van Vught LA, Horn J, Schultz MJ *et al.*: **The circular RNA landscape in specific peripheral blood mononuclear cells of critically ill patients with sepsis.** *Critical care* 2020, **24**(1):423.
24. He S, Hou X, Xu X, Wan C, Yin P, Liu X, Chen Y, Shu B, Liu F, Xu J: **Quantitative proteomic analysis reveals heat stress-induced injury in rat small intestine via activation of the MAPK and NF-kappaB signaling pathways.** *Molecular bioSystems* 2015, **11**(3):826-834.
25. Zhang T, Yin YC, Ji X, Zhang B, Wu S, Wu XZ, Li H, Li YD, Ma YL, Wang Y *et al.*: **AT1R knockdown confers cardioprotection against sepsis-induced myocardial injury by inhibiting the MAPK signaling pathway in rats.** *Journal of cellular biochemistry* 2020, **121**(1):25-42.
26. Jiang M, Klein M, Zanger UM, Mohammad MK, Cave MC, Gaikwad NW, Dias NJ, Selcer KW, Guo Y, He J *et al.*: **Inflammatory regulation of steroid sulfatase: A novel mechanism to control estrogen homeostasis and inflammation in chronic liver disease.** *Journal of hepatology* 2016, **64**(1):44-52.
27. Maggioli E, McArthur S, Mauro C, Kieswich J, Kusters DHM, Reutelingsperger CPM, Yaqoob M, Solito E: **Estrogen protects the blood-brain barrier from inflammation-induced disruption and increased lymphocyte trafficking.** *Brain, behavior, and immunity* 2016, **51**:212-222.
28. Gomez CR, Plackett TP, Kovacs EJ: **Aging and estrogen: modulation of inflammatory responses after injury.** *Experimental gerontology* 2007, **42**(5):451-456.
29. Zhao C, Li J, Cheng M, Shi J, Shen J, Gao T, Xi F, Yu W: **Estrogen Maintains Skeletal Muscle in Septic Rats Associated with Altering Hypothalamic Inflammation and Neuropeptides.** *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* 2017, **49**(3):221-228.
30. Xu Z, Mu S, Liao X, Fan R, Gao W, Sun Y, Wu W, Jia Q: **Estrogen protects against liver damage in sepsis through inhibiting oxidative stress mediated activation of pyroptosis signaling pathway.** *PloS one* 2020, **15**(10):e0239659.
31. Fang Y, Hu J, Wang Z, Zong H, Zhang L, Zhang R, Sun L: **LncRNA H19 functions as an Aquaporin 1 competitive endogenous RNA to regulate microRNA-874 expression in LPS sepsis.** *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2018, **105**:1183-1191.
32. Shi Y, Sun CF, Ge WH, Du YP, Hu NB: **Circular RNA VMA21 ameliorates sepsis-associated acute kidney injury by regulating miR-9-3p/SMG1/inflammation axis and oxidative stress.** *Journal of cellular and molecular medicine* 2020, **24**(19):11397-11408.
33. Zhu XG, Zhang TN, Wen R, Liu CF: **Overexpression of miR-150-5p Alleviates Apoptosis in Sepsis-Induced Myocardial Depression.** *BioMed research international* 2020, **2020**:3023186.
34. Xu L, Hu G, Xing P, Zhou M, Wang D: **Paclitaxel alleviates the sepsis-induced acute kidney injury via Inc-MALAT1/miR-370-3p/HMGB1 axis.** *Life sciences* 2020, **262**:118505.

35. Xiao T, Sun C, Xiao Y, Li Y: **lncRNA NEAT1 mediates sepsis progression by regulating Irak2 via sponging miR-370-3p.** *Biology open* 2020, **9**(6).
36. Wu XY, Fang Y, Zheng FX, Zhang YZ, Li QL: **LncRNA NEAT1 facilitates the progression of sepsis through up-regulating TSP-1 via sponging miR-370-3p.** *European review for medical and pharmacological sciences* 2020, **24**(1):333-344.

Tables

Table 1
Characteristics of study subjects.

Characteristics	Healthy control	SLA patients	SLA patients after 7 days therapy	P _{M0C}	P _{T7M0}
Number	8	8	8		
Male/female	3/5	3/5	3/5	1.00	
Age (year)	57.88 ± 12.80	55.50 ± 4.36	55.50 ± 4.36	0.627	
Procalcitonin(ng/mL)		32.25 ± 35.80	1.805 ± 2.869		0.031
WBC(×10 ⁹ /L)		13.30 ± 10.54	9.578 ± 3.268		0.356
Platelets(×10 ⁹ /L)		99.88 ± 31.94	249.4 ± 90.36		< 0.001
CPR(mg/L)		232.6 ± 64.65	38.85 ± 19.81		< 0.001
SLA: sepsis caused by pyogenic liver abscess, P _{M0C} : student independent t-test was performed between SLA and healthy samples, P _{T7M0} : student independent t-test was performed between SLA and SLA after 7 days therapy samples.					

Figures

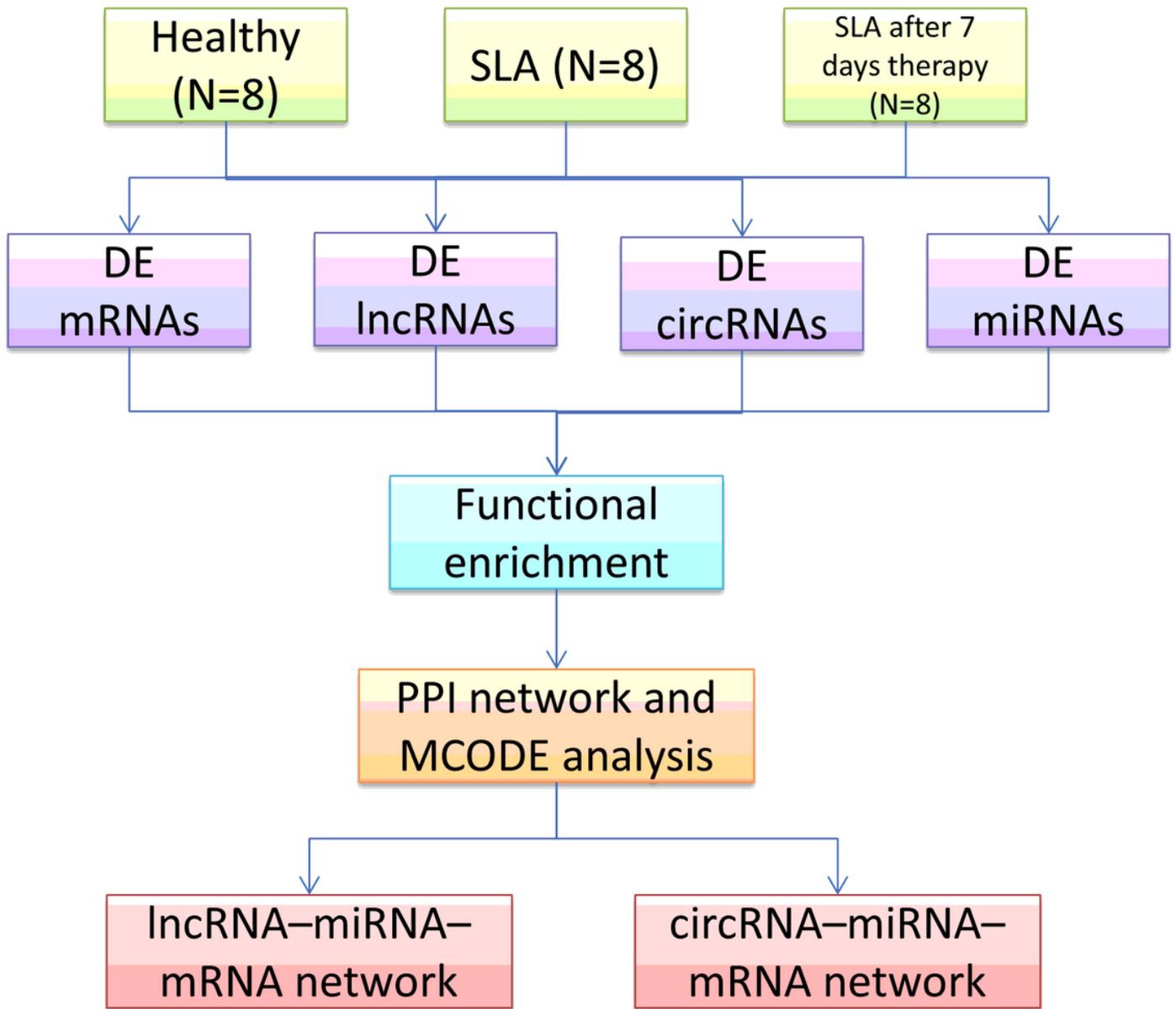


Figure 1

Analysis flow chart of this study

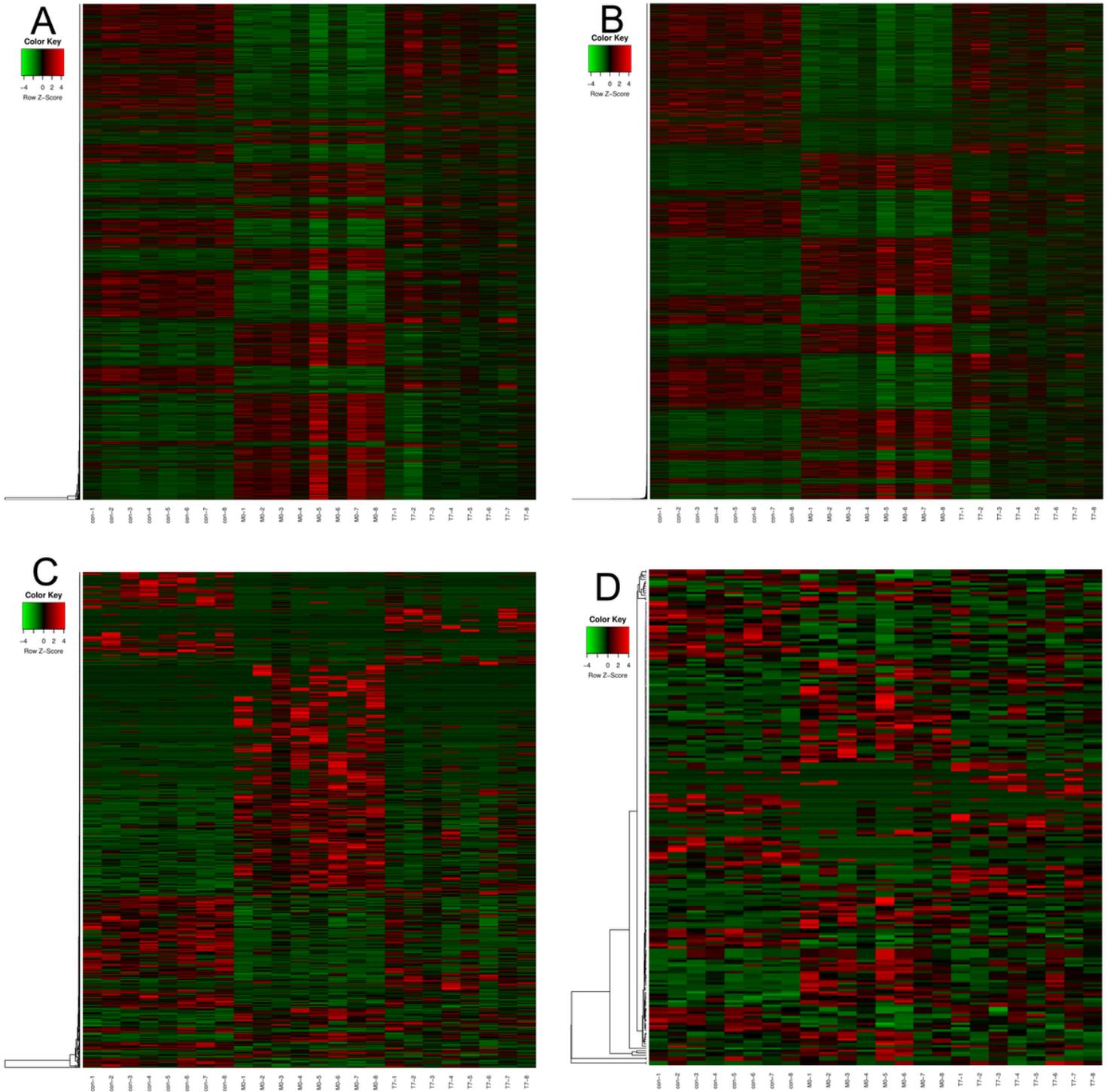


Figure 2

The expression profiling changes of RNAs in peripheral blood sample of SLA patients. The heatmap plot of differentially expressed mRNAs (A), lncRNAs(B), circRNAs (C) and miRNAs (D).

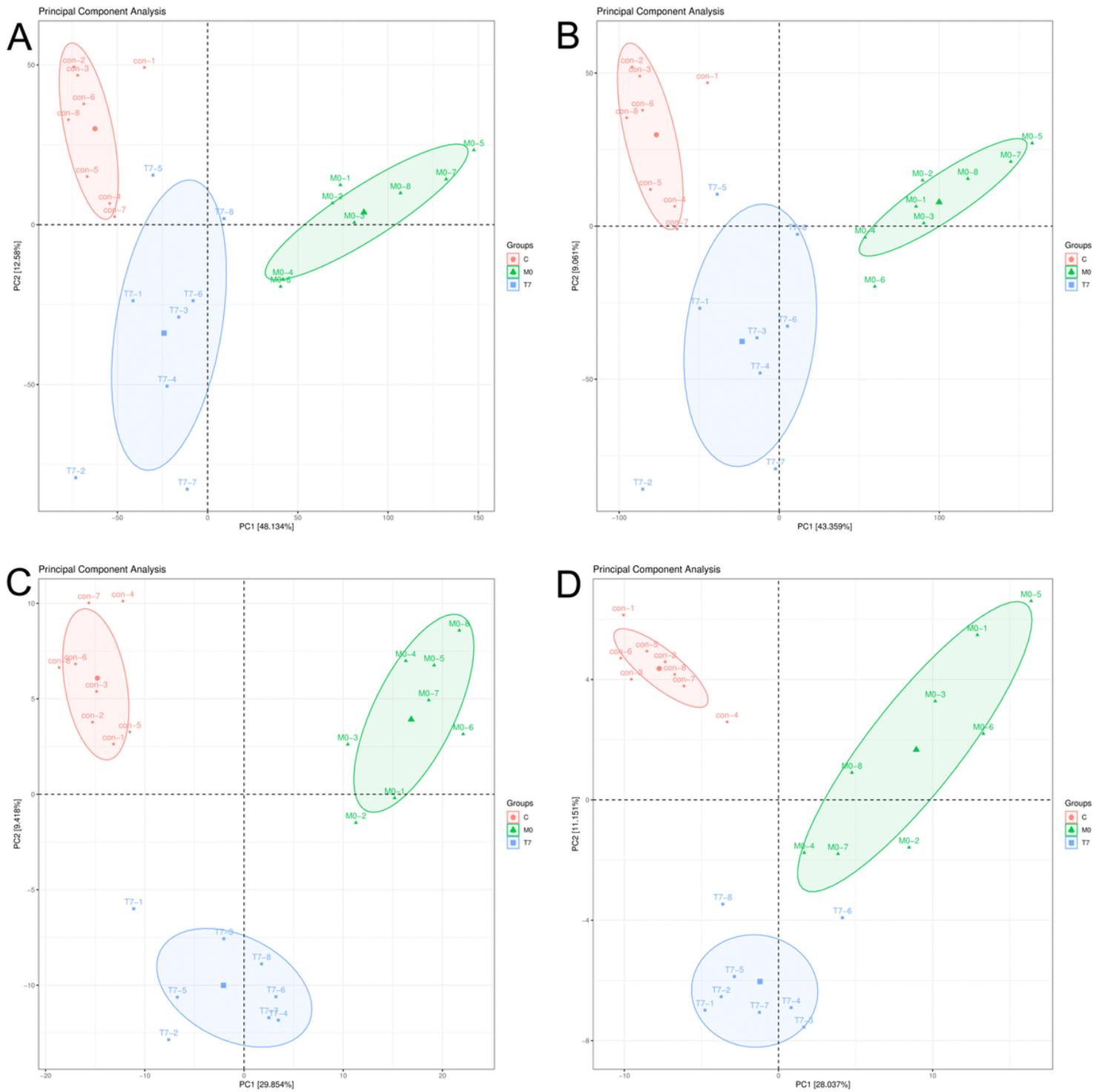


Figure 3

The PCA plot of differentially expressed mRNAs (A), lncRNAs (B), circRNAs (C) and miRNAs (D) in peripheral blood sample of SLA patients. C: healthy control, M0: SLA patients, T7: SLA patients after 7 days therapy.

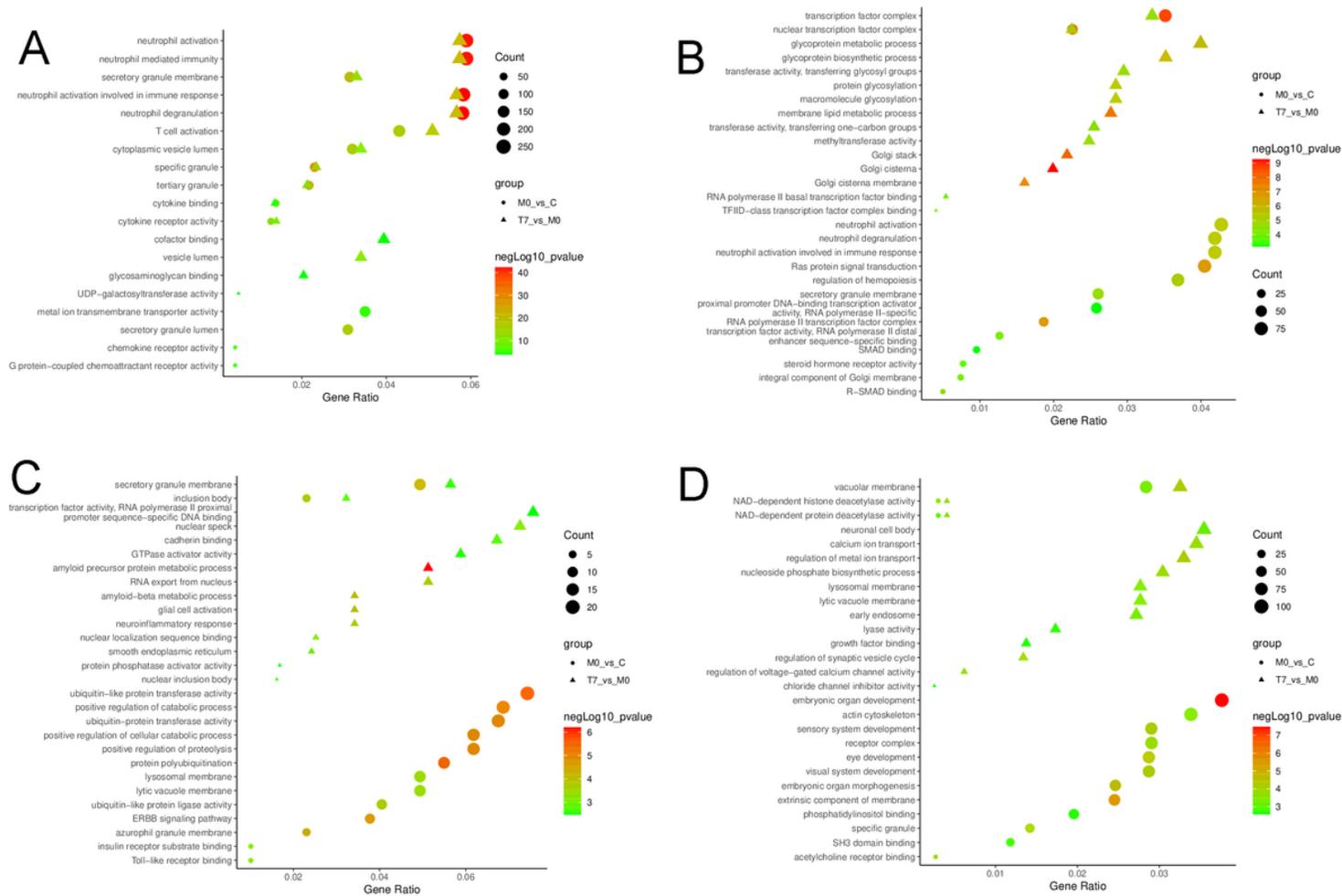


Figure 4

Bubble plot of the GO analysis of the two comparisons. mRNAs (A), lncRNAs(B), circRNAs (C), miRNAs (D). C: healthy control, M0: SLA patients, T7: SLA patients after 7 days therapy.

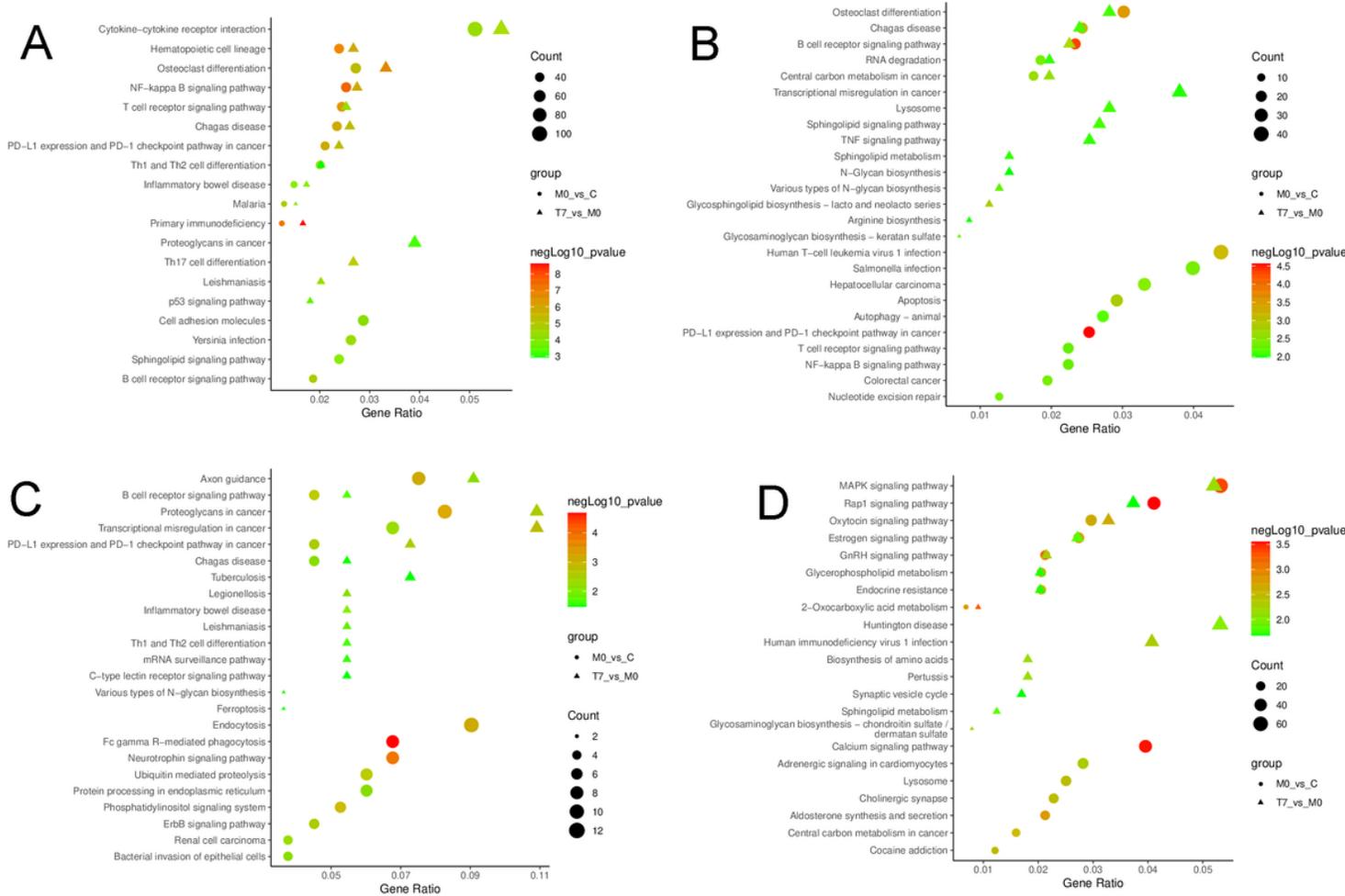


Figure 5

Bubble plot of the KEGG analysis of the two comparisons. mRNAs (A), lncRNAs(B), circRNAs (C), miRNAs (D). C: healthy control, M0: SLA patients, T7: SLA patients after 7 days therapy.

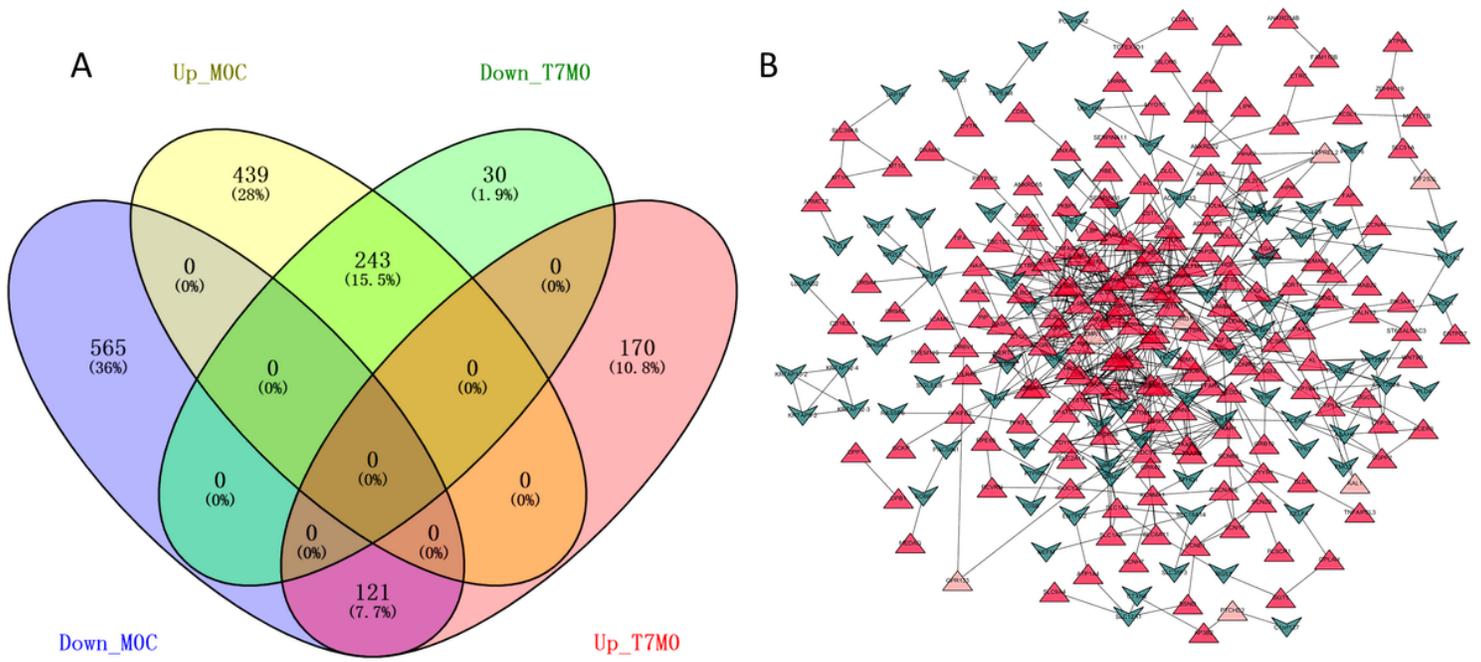


Figure 6

PPI network analysis. A: venn analysis of the two comparison, B: PPI network, red: genes were upregulated in SLA patients (M0) and downregulated after seven days therapy (T7), green: genes were downregulated in SLA patients and upregulated after seven days therapy.

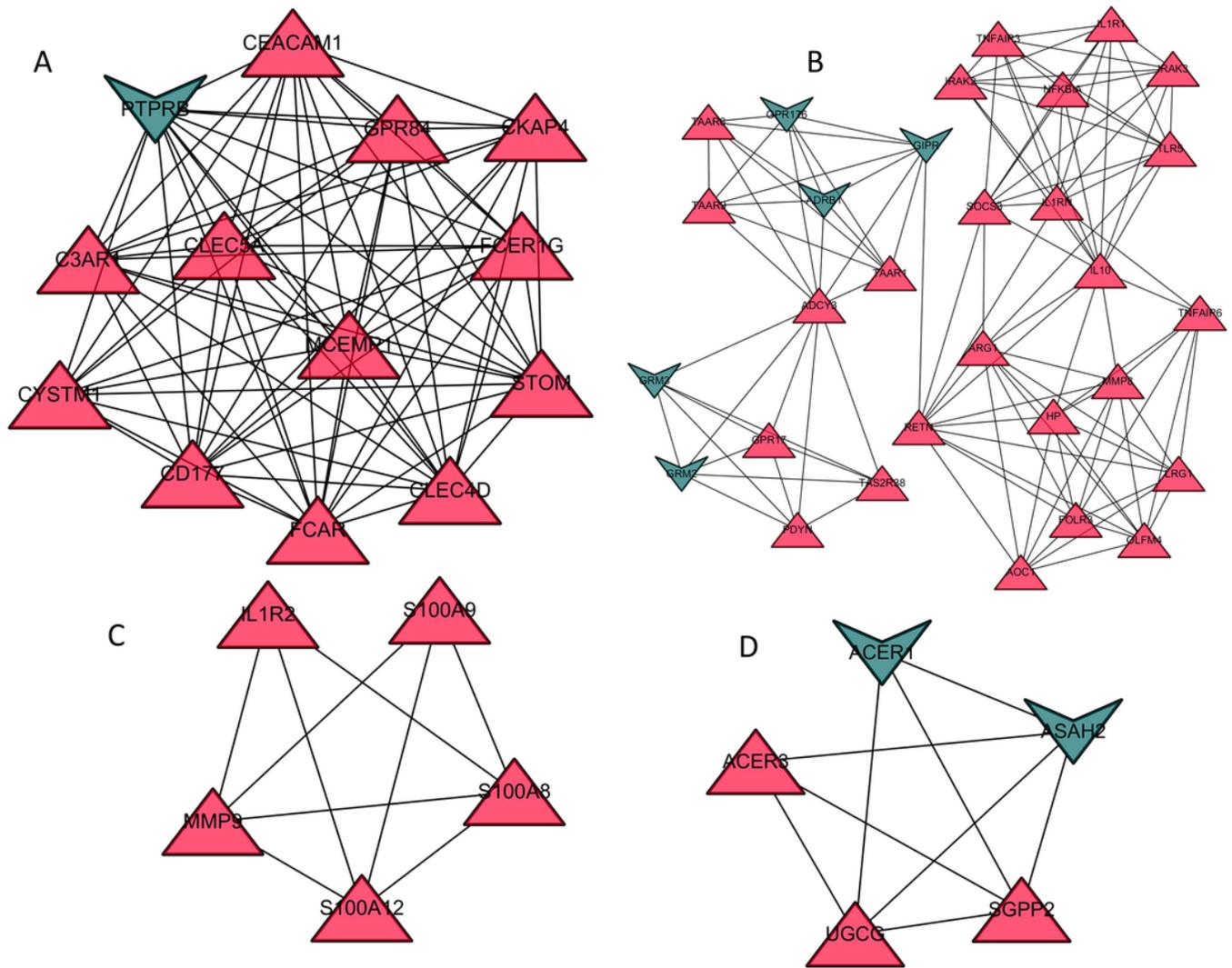


Figure 7

MCODE analysis of the PPI network. red: genes were upregulated in SLA patients and downregulated after seven days therapy, green: genes were downregulated in SLA patients and upregulated after seven days therapy.

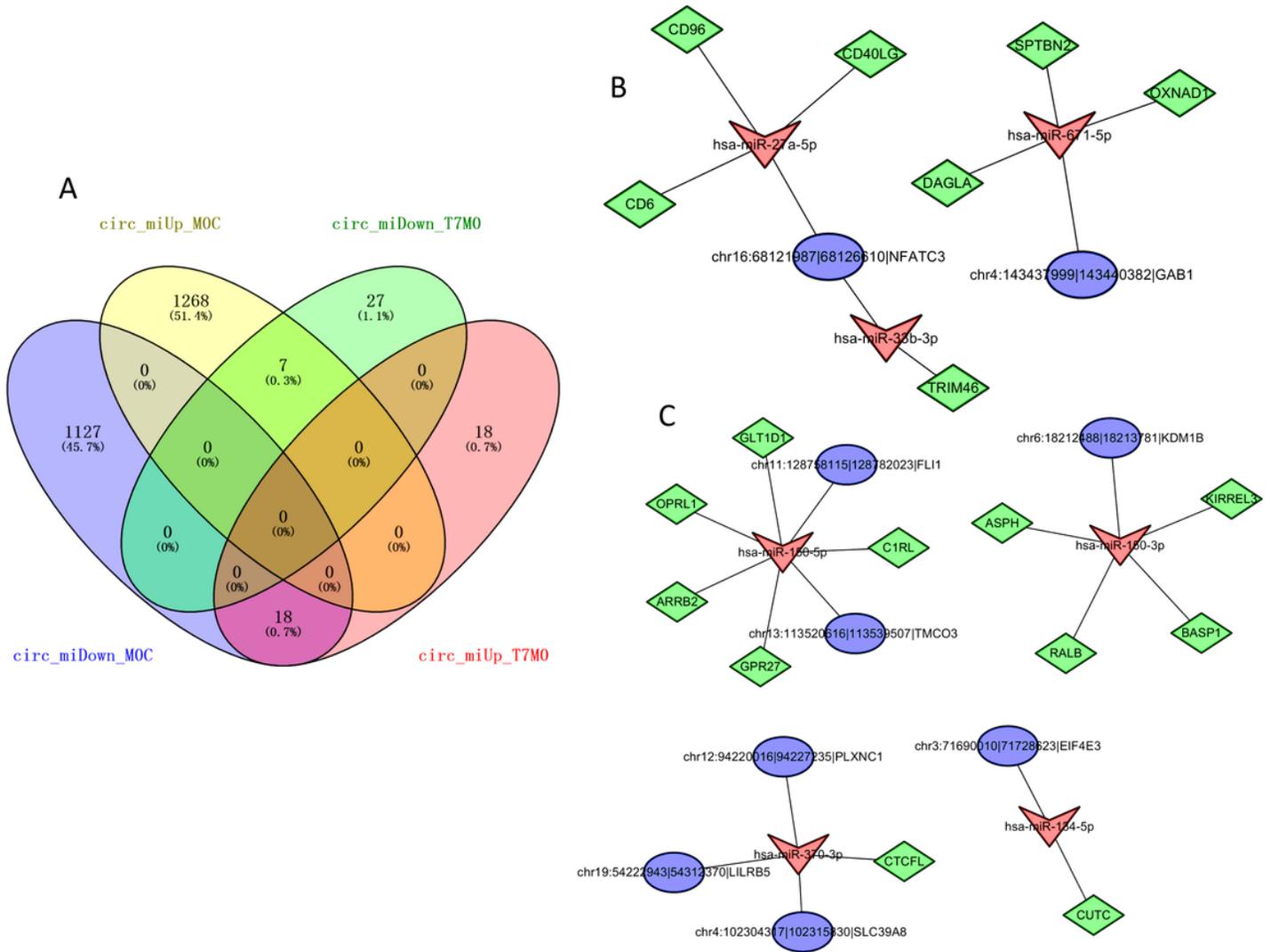


Figure 9

lncRNA-miRNA-mRNA analysis. A: venn analysis of the two comparisons (T7 vs. M0 and M0 vs. C), B: lncRNA(up)-miRNA(down)-mRNA(up) network, C: lncRNA(down)-miRNA(up)-mRNA(down) network red: miRNA, green: mRNA, blue: lncRNA.

Supplementary Files

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