



Original article

The regulatory role of microRNA-mRNA co-expression in hepatitis B virus-associated acute liver failure

Kanda Pan^{a,1}, Yunchao Wang^{a,1}, Ping Pan^b, Guanhua Xu^a, Lujiao Mo^a, Lijia Cao^a, Channi Wu^{c,*}, Xiaoyuan Shen^a^a Department of Intensive Care Unit (ICU), The First People's Hospital of Xiaoshan District, Hangzhou, Xiaoshan District, Hangzhou, China^b Department of General Medicine, The First People's Hospital of Xiaoshan District, Hangzhou, Xiaoshan District, Hangzhou, China^c Department of Gastroenterology, Zhejiang Xiaoshan Hospital, Xiaoshan District, Hangzhou, China

ARTICLE INFO

Article history:

Received 27 November 2018

Accepted 25 June 2019

Available online 21 August 2019

Keywords:

Acute liver failure

MicroRNA

Microarray

Bioinformatics analysis

ABSTRACT

Introduction and objectives: Acute liver failure (ALF) is a dramatic disorder requiring intensive care. MicroRNAs (miRNAs) have been identified to play important roles in ALF. This study was performed to identify miRNA-mRNA co-expression network after ALF to investigate the molecule mechanism underlying the pathogenesis of ALF.

Materials and methods: The microarray dataset GSE62030 and GSE62029 were downloaded from Gene Expression Omnibus database. Overlapping differentially expressed miRNAs (DEmiRNAs) and genes (DEGs) were identified in liver tissues from patients with hepatitis B virus (HBV)-associated ALF in comparison with normal tissues from donors. Gene enrichment analysis was performed. Key pathways associated with the DEGs were identified. The miRNA-mRNA regulatory network was constructed.

Results: Total 42 DEmiRNAs and 523 DEGs were identified in liver tissues from patients with HBV-associated ALF. Gene ontology and pathways enrichment analysis showed upregulated DEGs were related to immune responses, inflammation, and infection, and downregulated DEGs were associated with amino acids, secondary metabolites and xenobiotics metabolism. In miRNA-mRNA co-expression network, DEGs were regulated by at least one DEmiRNA and transcription factor. Further analysis showed DEmiRNAs, including has-miR-55-5p, has-miR-193b-5p, has-miR-200b-3p, and has-miR-3175 were associated with amino acid metabolism, drug metabolism and detoxication, and signaling pathways including mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/AKT, Ras, and Rap1.

Conclusions: These miRNA-mRNA pairs and changed profiles were associated with and might be responsible for the impairment of detoxification and metabolism induced by HBV-associated ALF.

© 2019 Published by Elsevier España, S.L.U. on behalf of Fundación Clínica Médica Sur, A.C. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Acute liver failure (ALF) is a rare, dramatic and life-threatening disorder often with a fatal outcome and requires intensive care. ALF is a multiorgan clinical syndrome with various clinical features, including hepatic dysfunction, coagulopathy, severe cardiovascular dysfunction, hypoglycemia, hepatic encephalopathy, and multiorgan failure [1–3]. The overall 21-day survival rate of patients with ALF was approximately 75%, and the 21-day transplant-free sur-

vival rate was approximately 50% [4]. Up until now it has not effective disease-specific or general treatment for ALF. The lack of broad-spectrum or disease-specific anti-ALF therapeutic agents, which are badly in need, might contribute to ALF-related mortality.

ALF is widely known to be caused by various and uncertainly reasons, the most common reason includes viral hepatitis or drug toxicity [1,3]. MicroRNAs (miRNAs) play important roles, protective or harmful, in the onset, development, and recovery of ALF [5–7]. It has been reported that ALF is associated with the overexpression of miR-125b-5p, which was downregulated in ALF patients, in mouse liver could prevent ALF development [5]. John et al. showed that the upregulations of miR-221 and miR-21 were beneficial for the spontaneous recovery from ALF [7].

* Corresponding author.

E-mail address: wuchanni1022@163.com (C. Wu).¹ Equal contribution, first authors.

The predication or identification of co-expression/regulatory network of miRNA-mRNA at transcriptional level promotes the understanding of miRNA-related disease pathogenesis, development, treatment and recovery [9–11]. This study was performed to identify the differentially expressed miRNA-mRNA co-expression profiles at the transcriptional level in patients with ALF. The miRNA-mRNA co-expression network in ALF was identified and bioinformatics analyses were performed to understand the molecule mechanism underlying the pathogenesis of ALF.

Fig. 1. The heatmap of differentially expressed items. (A and B) The heatmap of differentially expressed miRNAs (DEmiRNAs) and genes (DEGs) between 13 ALF liver samples and 10 normal liver tissues from donors, respectively. Red and green notes up and down regulated expression level, respectively.

Table 1

GO biological processes and KEGG pathways related to the up- and down-regulated DEGs.

Term	Count	p Value	Bonferroni
Up-regulated DEGs			
<i>GO biological processes</i>			
GO:0006955~immune response	31	4.88E–15	5.61E–12
GO:0006954~inflammatory response	22	7.51E–09	8.63E–06
GO:0019886~antigen processing and presentation of exogenous peptide antigen via MHC class II	12	1.48E–08	1.70E–05
GO:0002504~antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	7	3.31E–08	3.80E–05
GO:0031295~T cell costimulation	11	3.45E–08	3.96E–05
GO:0030574~collagen catabolic process	10	7.47E–08	8.58E–05
GO:0050776~regulation of immune response	14	2.73E–07	3.14E–04
GO:0045087~innate immune response	21	3.05E–07	3.50E–04
GO:0019882~antigen processing and presentation	9	3.09E–07	3.55E–04
GO:0007155~cell adhesion	19	1.31E–05	0.0149
GO:0002250~adaptive immune response	11	1.34E–05	0.0152
GO:0007229~integrin-mediated signaling pathway	9	2.75E–05	0.0311
GO:0030198~extracellular matrix organization	12	2.83E–05	0.0320
<i>KEGG pathways</i>			
hsa05150:Staphylococcus aureus infection	13	2.83E–11	4.07E–09
hsa05323:Rheumatoid arthritis	13	1.08E–08	1.55E–06
hsa05332:Graft-versus-host disease	9	3.18E–08	4.58E–06
hsa04672:Intestinal immune network for IgA production	10	3.88E–08	5.59E–06
hsa05330:Allograft rejection	9	8.40E–08	1.21E–05
hsa04940:Type I diabetes mellitus	9	2.40E–07	3.46E–05
hsa05310:Asthma	8	3.22E–07	4.64E–05
hsa04514:Cell adhesion molecules (CAMs)	14	3.23E–07	4.66E–05
hsa05152:Tuberculosis	15	6.74E–07	9.70E–05
hsa04512:ECM–receptor interaction	11	9.94E–07	1.43E–04
hsa05320:Autoimmune thyroid disease	9	1.34E–06	1.93E–04
hsa05140:Leishmaniasis	10	1.54E–06	2.22E–04
hsa05145:Toxoplasmosis	12	2.31E–06	3.33E–04
hsa05416:Viral myocarditis	9	2.76E–06	3.97E–04
hsa04612:Antigen processing and presentation	10	2.76E–06	3.98E–04
hsa04145:Phagosome	13	4.89E–06	7.04E–04
hsa05322:Systemic lupus erythematosus	12	8.04E–06	0.00116
hsa05321:Inflammatory bowel disease (IBD)	8	6.29E–05	0.00902
hsa04974:Protein digestion and absorption	9	7.14E–05	0.0102
hsa05164:Influenza A	12	9.23E–05	0.0132
Down-regulated DEGs			
<i>GO biological processes</i>			
GO:0002576~platelet degranulation	20	1.52E–15	2.12E–12
GO:0010951~negative regulation of endopeptidase activity	20	3.31E–14	4.51E–11
GO:0055114~oxidation–reduction process	38	3.74E–13	5.10E–10
GO:0006953~acute-phase response	13	3.80E–13	5.18E–10
GO:0042730~fibrinolysis	10	1.08E–11	1.47E–08
GO:0030449~regulation of complement activation	11	1.47E–11	2.00E–08
GO:0019373~epoxygenase P450 pathway	9	1.10E–10	1.50E–07
GO:0006805~xenobiotic metabolic process	14	1.87E–10	2.55E–07
GO:0042632~cholesterol homeostasis	13	2.27E–10	3.09E–07
GO:0006957~complement activation, alternative pathway	8	3.04E–10	4.14E–07
GO:0017144~drug metabolic process	9	4.95E–09	6.75E–06
GO:0051918~negative regulation of fibrinolysis	6	1.99E–07	2.71E–04
GO:0008202~steroid metabolic process	9	2.61E–07	3.56E–04
GO:0007596~blood coagulation	15	1.04E–06	0.001414
GO:0006631~fatty acid metabolic process	9	1.20E–06	0.001639
GO:0006958~complement activation, classical pathway	11	2.99E–06	0.00407
GO:0055085~transmembrane transport	16	6.10E–06	0.008276
GO:0007597~blood coagulation, intrinsic pathway	6	6.11E–06	0.008288
GO:0097267~omega-hydroxylase P450 pathway	5	6.56E–06	0.008897
GO:0031639~plasminogen activation	5	6.56E–06	0.008897
GO:0008203~cholesterol metabolic process	9	9.55E–06	0.012939
GO:0006699~bile acid biosynthetic process	6	1.40E–05	0.018851
GO:0042157~lipoprotein metabolic process	7	2.32E–05	0.031106
<i>KEGG pathways</i>			
hsa04610:Complement and coagulation cascades	29	1.83E–28	2.67E–26
hsa01100:Metabolic pathways	77	6.43E–18	9.39E–16
hsa00830:Retinol metabolism	15	2.03E–10	2.97E–08
hsa05204:Chemical carcinogenesis	16	3.60E–10	5.25E–08
hsa00140:Steroid hormone biosynthesis	12	7.84E–08	1.15E–05
hsa00980:Metabolism of xenobiotics by cytochrome P450	13	1.22E–07	1.79E–05
hsa00982:Drug metabolism – cytochrome P450	12	4.31E–07	6.29E–05
hsa04976:Bile secretion	12	5.02E–07	7.33E–05
hsa00650:Butanoate metabolism	7	3.08E–05	0.00449
hsa05150:Staphylococcus aureus infection	8	2.49E–04	0.0357

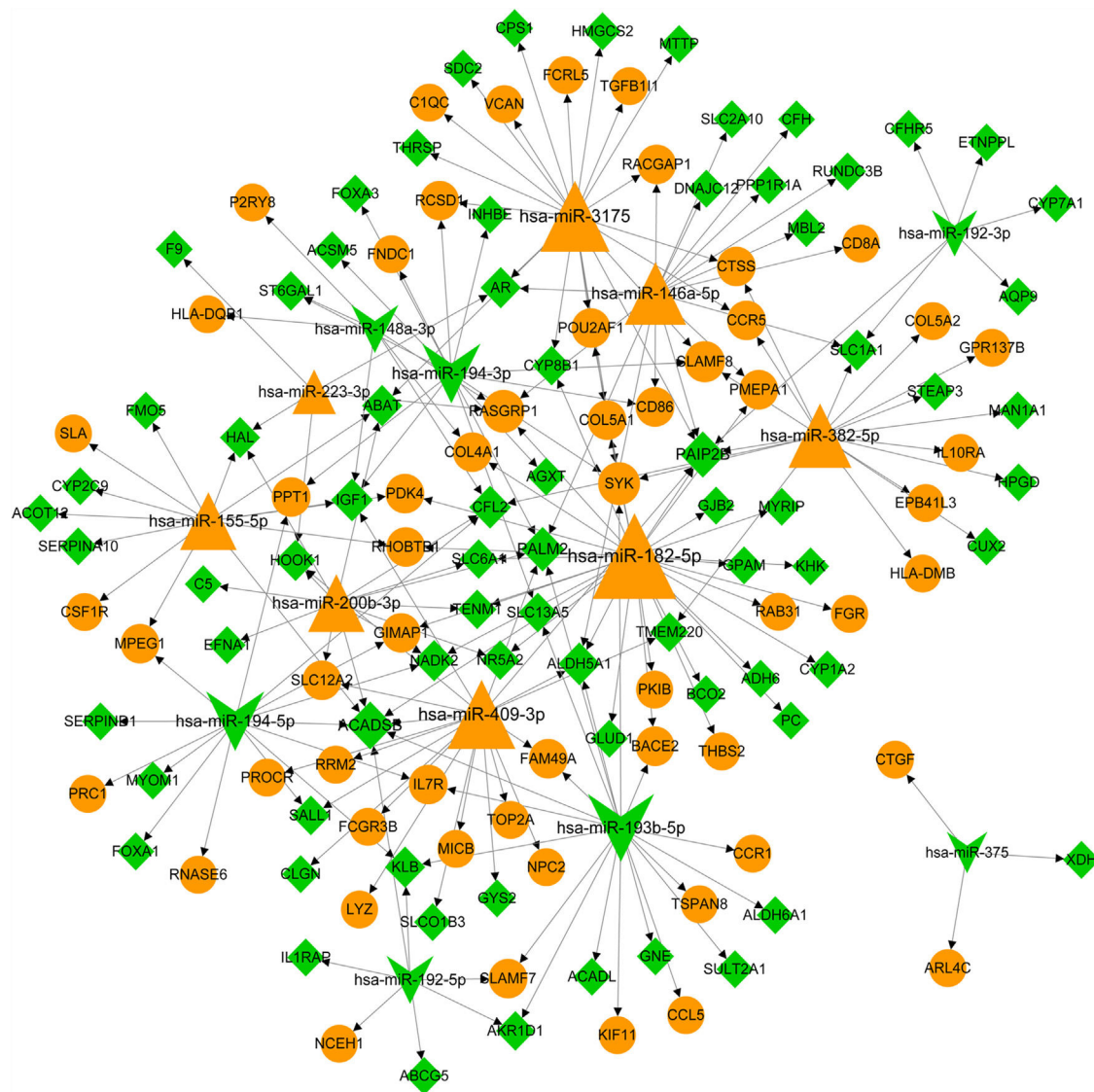


Fig. 2. The miRNA-target network of top 10 miRNAs and differentially expressed genes (DEGs). Yellow triangle notes upregulated miRNA. Green yellow indicates down-regulated miRNA. Yellow circle represents upregulated DEG. Green rhombus note downregulated DEG. Arrows represent the regulatory direction. The larger the node, the higher the interaction degree.

2.4. MiRNA target prediction and annotation

The targets of DE miRNAs were predicted using miRWalk2.0 (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>) [17]. The common mRNA targets in 5 of 8 databases, including miR-Walk, miRanda, miRDB, miRMap, miRNAmap, RNA22, Targetscan and mirbridge, were identified and selected as candidate miRNA targets. In addition, the overlaps between candidate targets of DE miRNAs and identified DEGs (DE miRNA-targeted DEGs) in microarray data were checked and used for further analysis. The GO biological processes and KEGG pathways associated with DE miRNA-targeted DEGs were predicated as previously reported. The R package clusterprofiler (v2.4.3, <http://bioconductor.org/packages/3.2/bioc/html/clusterProfiler.html>) was used for the KEGG pathway annotation with the same criteria.

2.5. Network analysis

The construction of miRNA-gene regulatory network was performed using Cytoscape (v3.2.0, <http://www.cytoscape.org/>) [18]. The interactions among the DE miRNAs and DEGs were

predicted using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; version 10.0, <http://string-db.org/>) [19]. The protein–protein interaction (PPI) network was constructed and visualized using Cytoscape. The significant modules in the PPI network were identified using the Cytoscape MCODE plugin, with the criteria of score >10.

2.6. Transcription factors (TFs) identification and regulatory network analysis

TFs among DE miRNAs-targeted DEGs were predicted using webgestal (<http://www.webgestal.org/option.php>) using the Overrepresentation Analysis methods with the threshold of p value <0.05 [20]. The TF-DEGs pairs and networks were visualized using Cytoscape.

3. Results

3.1. Identification of DE miRNAs and DEGs

A total of 43 DE miRNAs (including 27 upregulated and 16 down-regulated DE miRNAs) and 523 DEGs (including 241 upregulated

and 282 downregulated DEGs) were identified from GSE62030 and GSE62029 dataset, with the threshold of adjusted p -value <0.05 and $|\log 2FC| > 1$, respectively (Fig. 1). The DEmiRNAs and DEGs are listed in Table S1.

3.2. Enrichment analysis for DEGs

Table 1 shows the GO biological processes and KEGG pathways significantly related to the up- and down-regulated DEGs. We found the upregulated DEGs were associated with biological processes of immune and inflammatory systems, including inflammatory response (GO:0006954) and immune response (GO:0006955). The upregulated DEGs were enriched into KEGG pathways including *Staphylococcus aureus* infection (hsa05150), rheumatoid arthritis (hsa05323), and intestinal immune network for IgA production (hsa04672). The downregulated DEGs were associated with biological processes including platelet degranulation (GO:0002576), epoxide P450 pathway (GO:0019373), oxidation–reduction process (GO:0055114), fibrinolysis (GO:0042730), and acute-phase response (GO:0006953); and were related to pathways including Retinol metabolism (hsa00830), metabolism of xenobiotics by cytochrome P450 (hsa00980), metabolic pathways (hsa01100), and *Staphylococcus aureus* infection (hsa05150).

3.3. Predication of DEmiRNA targets in DEGs

The predicative targets of top 10 up- and down-regulated DEmiRNAs were identified using miRWalk2.0 database. A total of 11,543 targets, including 131 DEGs, were predicted. The DEmiRNA-DEG regulatory network was comprised of 15 DEmiRNAs (including 8 up- and 7 down-regulated DEmiRNAs) and 131 DEGs (including 57 up- and 74 down-regulated DEGs) and 205 miRNA-target pairs (Fig. 2). We noted that some DEGs were regulated by more than one

miRNA. For instance, insulin-like growth factor 1 (IGF1, down) was co-regulated by has-miR-155-5p (up), hsa-miR-148a-3p (down), hsa-miR-194-3p (down) and hsa-miR-409-3p (up); cathepsin S (CTSS, up) and β -chemokine receptor 5 (CCR5) were co-regulated by hsa-miR-382-5p (up) and hsa-miR-3175 (up); colony stimulating factor 1 receptor (CSF1R, up) and forkhead box A1 (FOXA1) gene was only regulated by upregulated hsa-miR-155-5p and has-miR-194-5p, respectively. The interaction degree of the miRNA-target pairs in Fig. 2 is listed in Table S2.

3.4. Enrichment analysis for DEmiRNA-targeted DEGs

Using DAVID tool, we identified that DEmiRNA-targeted DEGs were significantly enriched into one biological process of immune response (GO:0006955; Table 2). The top 20 biological processes associated with DEmiRNA-targeted DEGs included bioluminescence (GO:0008218), antigen processing and presentation (GO:0019882), cholesterol homeostasis (GO:0042632), and antigen processing and presentation of exogenous peptide antigen via MHC class II (GO:0019886, Table 2). The DEmiRNA-targeted DEGs were significantly enriched in one KEGG pathway *S. aureus* infection [hsa05150, including leukocyte antigen (HLA) class II genes HLA-DQB1, HLA-DMB, Fc γ -receptor-IIIB gene, and mannose-binding lectin gene]. Other 11 KEGG pathways associated with DEmiRNA-targeted DEGs included alanine, aspartate and glutamate metabolism [hsa00250, including succinate semialdehyde dehydrogenase gene (ALDH5A1) and glyoxylate aminotransferase gene (AGXT)], and bile secretion [hsa04976, organic anion transporting polypeptide 8 encoding gene, ATP-cassette binding proteins G5 and hydroxysteroid sulfotransferase (SULT2A1)]. The detail information of biological processes and KEGG pathways related to DEmiRNA-targeted DEGs is shown in Table S3.

Table 2

The list of GO biological processes and KEGG pathways related to DEmiRNA-targeted DEGs.

Term	Count	p Value	Bonferroni
<i>GO biological processes</i>			
GO:0006955~immune response	14	9.90E–06	0.009889
GO:0008218~bioluminescence	3	1.51E–04	0.140878
GO:0055114~oxidation–reduction process	14	3.15E–04	0.271473
GO:0019882~antigen processing and presentation	5	6.39E–04	0.473377
GO:0042632~cholesterol homeostasis	5	0.00113	0.679352
GO:0007267~cell–cell signaling	8	0.00233	0.903754
GO:0006957~complement activation, alternative pathway	3	0.00375	0.977043
GO:0032355~response to estradiol	5	0.00411	0.983987
GO:0019886~antigen processing and presentation of exogenous peptide antigen via MHC class II	5	0.00427	0.98642
GO:0006631~fatty acid metabolic process	4	0.00609	0.997845
GO:0002407~dendritic cell chemotaxis	3	0.00642	0.998447
GO:0006541~glutamine metabolic process	3	0.00884	0.999866
GO:0019835~cytolysis	3	0.00973	0.999946
GO:0006699~bile acid biosynthetic process	3	0.00973	0.999946
GO:0006637~acyl-CoA metabolic process	3	0.0106	0.999979
GO:0030574~collagen catabolic process	4	0.0108	0.999982
GO:0006935~chemotaxis	5	0.0114	0.999999
GO:0009749~response to glucose	4	0.0127	0.999999
GO:1903225~negative regulation of endodermal cell differentiation	2	0.0142	0.999999
GO:0009450~gamma-aminobutyric acid catabolic process	2	0.0142	0.999999
<i>KEGG pathways</i>			
hsa05150: <i>Staphylococcus aureus</i> infection	7	4.67E–05	0.00698
hsa00250:Alanine, aspartate and glutamate metabolism	5	8.35E–04	0.117767
hsa01100:Metabolic pathways	28	0.00118	0.161851
hsa04610:Complement and coagulation cascades	6	0.00152	0.203582
hsa04976:Bile secretion	6	0.00152	0.203582
hsa00650:Butanoate metabolism	4	0.00425	0.471777
hsa00120:Primary bile acid biosynthesis	3	0.0180	0.934848
hsa00280:Valine, leucine and isoleucine degradation	4	0.0197	0.949763
hsa05322:Systemic lupus erythematosus	6	0.0243	0.974866
hsa04151:PI3K-Akt signaling pathway	10	0.0252	0.978316
hsa04514:Cell adhesion molecules (CAMs)	6	0.0302	0.989
hsa04145:Phagosome	6	0.0397	0.997

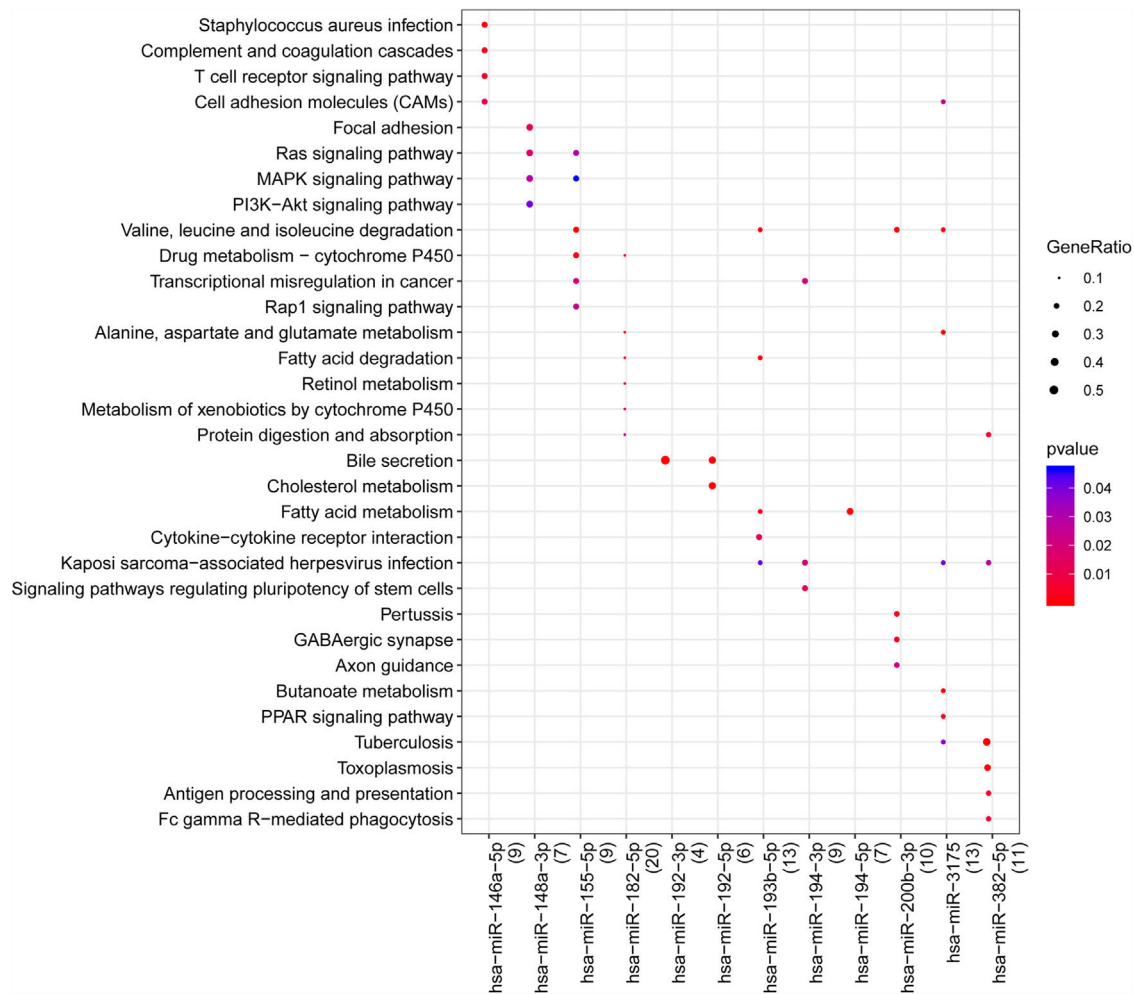


Fig. 3. The KEGG pathways related to the top 20 differentially expressed miRNAs. KEGG pathways were predicted using R package clusterprofiler with the criteria of $p < 0.05$ and gene count ≥ 2 . The larger the circle, the higher the gene number.

With the enrichment analysis using R package clusterprofiler, we found hsa-miR-146a-5p was associated with *S. aureus* infection, T cell receptor signaling pathway, cell adhesion molecules (CAMs), and complement and coagulation cascades; hsa-miR-148a-3p was related to mitogen-activated protein kinase (MAPK) signaling pathway, Ras signaling pathway, MAPK signaling pathway and phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway; hsa-miR-155-5p was associated with valine, leucine and isoleucine degradation, drug metabolism-cytochrome P450, Rap1, Ras and MAPK signaling pathways; hsa-miR-192-5p was associated with bile secretion and cholesterol metabolism; hsa-miR-382-5p and hsa-miR-3175 were related to tuberculosis, kaposi sarcoma-associated herpes virus infection and viral carcinogenesis. In addition, we found hsa-miR-192-5p and hsa-miR-192-3p was related to the secretion of bile (Fig. 3).

3.5. PPI network analysis

We further constructed the PPI network of the DEGs targeted by the top 10 up- and down-regulated DE miRNAs. The PPI network was consisted of 882 miRNA-gene pairs (lines) and 120 nodes (gene products, including 54 up- and 66 down-regulated DEGs, Fig. 4A). Using MCODE plugin, we identified a significant module (score = 17.032) that was consisted of 264 miRNA-gene pairs and 32 nodes, including CSF1R (degree = 45), IGF1 (degree = 42), CTSS

(degree = 37), and SULT2A1 (degree = 28, Fig. 4B). The degree of the 32 nodes in the module is listed in Table 3.

3.6. Identification of TFs in top 20 DE miRNAs-targeted DEGs

Using webgestal, we identified there were 15 TFs among the top 10 up- and down-regulated DE miRNAs-targeted DEGs (Fig. 5). A total of 54 DEGs (including 20 up- and 30 down-regulated DEGs) were regulated by these 15 TFs. Fig. 5 shows TFs including TATA, lymphoid enhancer factor 1 (LEF1) and chicken ovalbumin upstream promoter (COUP) regulate more than 10 DEGs; We found the expression of IGF1 was regulated by TFs like TATA, TATA-binding protein (TBP), and GATA binding protein 3 (GATA3); FOXA1 gene was co-regulated by 7 TFs including LEF1, TATA, COUP, GATA3, and HNF4. These results suggested the complex mechanism related to these DEGs.

4. Discussion

ALF is a rare and life-threatening disorder requiring intensive care and heavy spending [2]. MiRNAs play crucial roles in the pathogenesis of HBV-induced hepatocellular injury and viral persistence [21]. In current study, we identified the co-expression network of miRNA-mRNA in liver tissues from patients with HBV-associated ALF. We identified these HBV-infection-induced DE miRNAs and DEGs in liver tissues from patients with ALF were

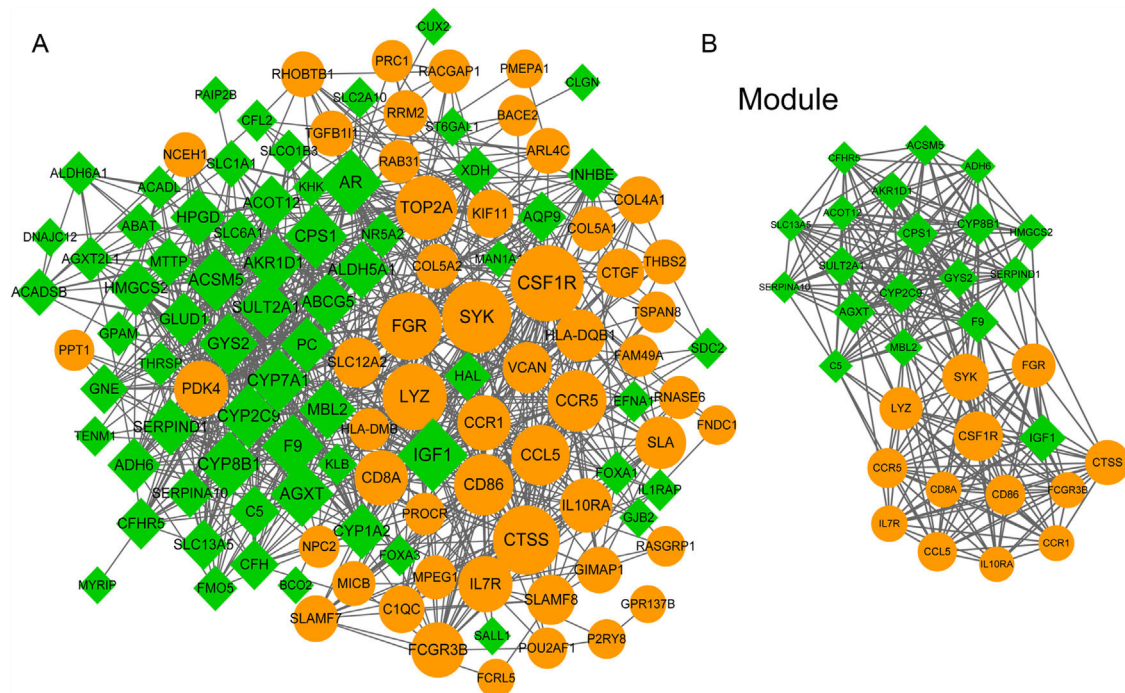


Fig. 4. The protein–protein interaction (PPI) network of differentially expressed genes (DEGs) targeted by the top 10 up- and down-regulated differentially expressed miRNAs (DEmiRNAs). (A) The whole PPI network of top 10 up- and down-regulated DEmiRNAs-targeted DEGs. (B) The significant module (score = 17.032) in PPI network. Yellow and green nodes indicate up- and down-regulated DEGs, respectively. The larger the node, the higher the interaction degree.

Table 3

The list of 32 nodes' degree in the module of protein–protein interaction network.

Module					
Nodes	Description	Degree	Nodes	Description	Degree
CSF1R	Up-gene	45	CCL5	Up-gene	27
IGF1	Down-gene	42	AKR1D1	Down-gene	27
SYK	Up-gene	40	MBL2	Down-gene	26
CTSS	Up-gene	37	HMGC52	Down-gene	23
FGR	Up-gene	35	SERPIND1	Down-gene	23
LYZ	Up-gene	34	IL7R	Up-gene	22
CYP8B1	Down-gene	34	FCGR3B	Up-gene	21
CPS1	Down-gene	33	ACOT12	Down-gene	21
AGXT	Down-gene	33	ADH6	Down-gene	21
CYP2C9	Down-gene	32	CCR1	Up-gene	21
F9	Down-gene	32	CD8A	Up-gene	21
ACSM5	Down-gene	30	IL10RA	Up-gene	20
GYS2	Down-gene	29	C5	Down-gene	18
CCR5	Up-gene	28	CFHR5	Down-gene	17
SULT2A1	Down-gene	28	SLC13A5	Down-gene	16
CD86	Up-gene	28	SERPINA10	Down-gene	14

related to immune responses, inflammation, herpesvirus infection, amino acid metabolism and metabolism of xenobiotics by cytochrome P450. These results suggested that these miRNAs and genes might be associated with ALF via regulating immune, infection and metabolism system.

It has been reported that the causes of ALF-related deaths include infection, hemorrhage and multiorgan failure [1]. Hepatitis A, B and E virus infections are rare but important common reasons of ALF in many countries, especially hepatitis E virus (HEV) infection in Europe and developing countries [2,4,22,23]. HEV infection is related to various hepatic diseases including acute hepatitis, hepatic dysfunction and ALF [24,25]. HEV infection rate among adults has increased in central Europe [23]. It has been reported that the HEV infection-related ALF is approximate 20–40% in developing countries [26]. Our current study identified that the DEmiRNA miR-382-5p and hsa-miR-3175 were related to KEGG pathways of

herpes virus, Epstein–Barr virus (EBV), human immunodeficiency virus 1 (HIV-1) and human cytomegalovirus (HCMV) infections and viral carcinogenesis. We identified that both miR-382-5p and hsa-miR-3175 were upregulated in liver tissues from patients with HBV-associated ALF comparing with normal liver tissues. In addition, the expression of the common target of miR-382-5p and hsa-miR-3175, CTSS, was upregulated. CTSS plays important roles in vacuolar cross-presentation of TAP-Independent MHC Class I molecules and formation of mature epitope [27]. The lack of CTSS in antigen-presenting cells reduced cell-associated antigens, and thus lacking crosspriming to virus [27]. By contrast, the administration of CTSS peptides rescued these above changes [27]. Another cytokine CCR5 was related to the infection by HIV-1 and HBV [28,29]. Choe et al. showed that the expression of CCR5, along with the expression of HIV-1 receptor CD4, eliminated the resistance to HIV-1 in [28]. Stevens et al. showed that the CCR5 loss or deficiency

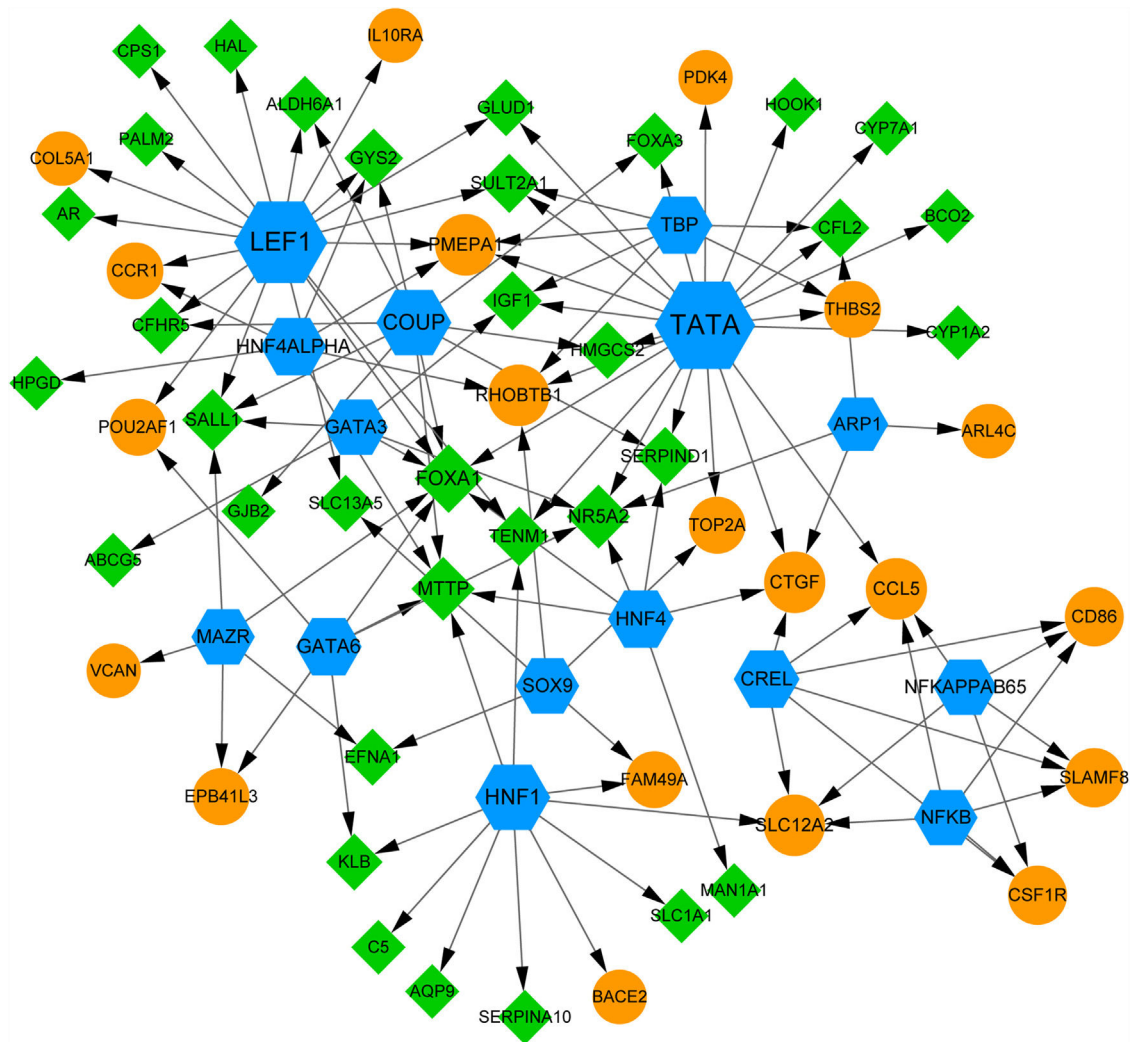


Fig. 5. The transcription factor (TF)-target regulatory network. Blue hexagons are TFs. Yellow circles and green rhombuses are up- and down-regulated differentially expressed genes (DEGs), respectively. The larger the node, the higher the interaction degree.

enhanced early NK cell, hepatic innate immune cell and neutrophil recruitment and increased hepatic inflammation [29,30]. We identified that both CTSS and CCR5 were upregulated by over 4 times fold changes in liver tissues from patients with HBV-associated ALF comparing with normal liver tissues. One study has reported that the miRNA profiles could be influenced by HBV infection for permitting the replication of HBV virus and the onset and progression of hepatocellular injury [21]. In addition, we also identified that the upregulated CD86, which is related to immune surveillance and virus infection including HBV [31–33], was co-regulated by upregulated has-miR-146a-5p (associated with T cell receptor signaling pathway) and downregulated has-miR-194-3p. However, there was no direct evidence showing the association of these genes with HBV-infection related ALF up till now. These results in our present study suggested that CTSS, CCR5 and CD86 expression were HBV-associated and the related miRNA-mRNA regulatory networks might be responsible for the immune responses, spontaneous recovery from or deteriorate of ALF.

Our study showed that the two DE miRNAs (including upregulated hsa-miR-148a-3p, downregulated hsa-miR-155-5p) were associated with the KEGG pathways including MAPK, PI3K-Akt, Ras and Rap1 signaling pathways. These pathways were essential for the proliferation, differentiation and apoptosis of various cells, including hepatocytes [34,35]. The activation of Ras promoted

Rap1-mediated activation of cyclic adenosine monophosphate (cAMP)/extracellular signal-regulated kinase (ERK) [36]. The cAMP-ERK axis was crucial for activation of MAPK signaling pathway [37,38]. Previous study had shown that hsa-miR-155-5p was inducible by hypoxia and hypoxia-inducible factor (HIF)-1 α [39,40]. hsa-miR-155-5p targets to TF ELK3 [40]. The IGF1 pathway is related to various conditions, including cancer, aging and diabetes via regulating cell growth, survival and differentiation [41,42]. Carotti et al. showed that the impairment of growth hormone-induced IGF-1 axis, reduction or inhibition exactly, was negatively associated with chronic hepatitis and liver damage level [43]. IGF1 is also essential for hepatocyte proliferation [44]. In addition, we identified that IGF1 (downregulated) was a common target of hsa-miR-155-5p (upregulated), hsa-miR-148a-3p (downregulated), hsa-miR-194-3p (downregulated) and hsa-miR-409-3p (upregulated). IGF1 was predicted to be regulated by three TFs including TATA, TBP and GATA3. These results showed that the dysregulation of these miRNAs and their target mRNAs were associated with the ALF via modulating cell growth and apoptosis through a variety of signaling pathways or axes. These might also be responsible for the multiorgan dysfunction after ALF.

We also found a DEG related to neurons and degradation of γ -aminobutyric acid (GABA), ALDH5A1, was downregulated. The deficiency or mutations of ALDH5A1 is found in neurometabolic

disease by impairing the GABA degradation [45]. We predicted that ALDH5A1 is a common target of hsa-miR-146a-5p, hsa-miR-182-5p, hsa-miR-409-3p and hsa-miR-193b-5p, and is regulated by two TFs including COUP and LEF1. These results were consistent with the reported fact that ALF induced hepatic encephalopathy.

In addition to the impairment in immune and infection response and multiorgan function, the body's metabolic systems were impaired by ALF. We demonstrated that most of the downregulated DEGs were associated with biological processes and pathways including amino acids and secondary metabolite metabolism, biosynthesis and degradation. For instance, hsa-miR-155-5p, hsa-miR-182-5p, hsa-miR-193-5p, hsa-miR-200b-3p and hsa-miR-3175 were associated with 'valine, leucine and isoleucine degradation', 'fatty acid degradation' and/or 'drug metabolism-cytochrome P450'. Most of the degradation of amino acids were conducted in liver. AGXT is a gene widely reported to be associated with primary hyperoxaluria type 1 (PH1) in children and accumulation of kidney stones [46]. Both PH1 and kidney stone are resulted from the excessive accumulation of oxalate which is induced by abnormal amino acid metabolism in liver. Mutation or reduced expression of AGXT in liver blocks the conversion from glyoxylic acid to glycine and enhances its transfer to oxalic acids, and the accumulation of oxalate in plasma and urine [46,47]. SULT2A1 is an enzyme for many drugs and xenobiotic detoxification and homeostasis of hydroxysteroid [48,49]. SULT2A1 catalyzes degradation of drugs and xenobiotics, including xenobiotic alcohols, phenols, and amines [49,50]. In our present study, we found SULT2A1 and AGXT were downregulated in liver tissues from patients with HBV-associated ALF comparing with normal tissues. In addition, we identified that AGXT was targeted by downregulated has-miR-194-3p, and SULT2A1 was targeted by downregulated has-miR-193-5p, respectively. These facts revealed these miRNA-mRNA pairs played important roles in detoxification and metabolism in liver. The dysregulations of these miRNA-mRNA pairs were associated and might be responsible for the ALF-related multisystem failure.

5. Conclusions

In summary, we concluded these DE miRNAs and DEGs in liver tissues from patients with HBV-associated ALF comparing with normal tissues were associated with the pathogenesis of ALF. These genetic factors and regulatory networks might be responsible for the HBV-induced impairment in detoxification and metabolism in liver as well as in HBV-induced multiorgan dysfunction. This study provided new insights of miRNA-mRNA co-expression network into the molecular mechanism underlying HBV-associated ALF.

Abbreviations

AGXT	glyoxylate aminotransferase gene
ALDH5A1	succinate semialdehyde dehydrogenase
CAMs	cell adhesion molecules
cAMP	cyclic adenosine monophosphate
CCR5	β-chemokine receptor 5
COUP	chicken ovalbumin upstream promoter
CSF1R	colony stimulating factor 1 receptor
CTSS	cathepsin S
DAVID	database for annotation; visualization; and integrated discovery
DEGs	differentially expressed genes
DE miRNAs	differentially expressed miRNAs
FC	fold change
FOXA1	forkhead box A1
GABA	γ-aminobutyric acid
GO	gene ontology
HCMV	human cytomegalovirus
HIF	hypoxia-inducible factor

HIV-1	human immunodeficiency virus 1
IGF1	insulin-like growth factor 1
IL	interleukin
KEGG	Kyoto Encyclopedia of Genes and Genomes database
LEF1	lymphoid enhancer factor 1
MAPK	mitogen-activated protein kinase
miRNAs	microRNAs
PH1	primary hyperoxaluria type 1
PI3K	phosphatidylinositol-3-kinase
PPI	protein-protein interaction
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
SULT2A1	hydroxysteroid sulfotransferase
TBP	TATA-binding protein
TFs	transcription factors

Author participation

Pan K.D., Pan P., and Wu C.N.: study design, data interpretation. Wang Y.C., Xu G.H., and Mo L.J.: data analysis. Pan K.D. and Cao L.J.: manuscript drafting. Wu C.N. Pan P., and Wang Y.C.: revision for important intellectual content. All authors have read and approved the final version of manuscript.

Funding

None.

Conflict of interest

The authors have no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.aohp.2019.07.007](https://doi.org/10.1016/j.aohp.2019.07.007).

References

- [1] Lee WM. Acute liver failure. *Semin Respir Crit Care Med* 2012;33:36–45, <http://dx.doi.org/10.1055/s-0032-1301733>.
- [2] Bernal W, Wendon J. Acute liver failure. *New Engl J Med* 2013;369:2525–34.
- [3] Hillman L, Gottfried M, Whitsett M, Rakela J, Schilsky M, Lee W, et al. Corrigendum: clinical features and outcomes of complementary and alternative medicine induced acute liver failure and injury. *Am J Gastroenterol* 2016;111:958.
- [4] Reuben A, Tillman H, Fontana RJ, Davern T, McGuire B, Stravitz RT, et al. Outcomes in adults with acute liver failure between 1998 and 2013: an observational cohort study. *Ann Int Med* 2016;164:724–32.
- [5] Yang D, Yuan Q, Balakrishnan A, Bantel H, Klusmann JH, Manns MP, et al. MicroRNA-125b-5p mimic inhibits acute liver failure. *Nat Commun* 2016;7:11916.
- [6] Roy S, Bantel H, Wandrer F, Schneider AT, Gautheron J, Vucur M, et al. miR-1224 inhibits cell proliferation in acute liver failure by targeting the antiapoptotic gene Nf1b. *J Hepatol* 2017;67:966–78.
- [7] John K, Hadem J, Krech T, Wahl K, Manns MP, Dooley S, et al. MicroRNAs play a role in spontaneous recovery from acute liver failure. *Hepatology* 2014;60:1346–55.
- [8] Tomar S, Nagarkatti M, Nagarkatti NPS. 3,3'-Diindolylmethane attenuates LPS-mediated acute liver failure by regulating miRNAs to target IRAK4 and suppress Toll-like receptor signalling. *Br J Pharmacol* 2015;172:2133–47.
- [9] Diaz G, Zamboni F, Tice A, Farci P. Integrated ordination of miRNA and mRNA expression profiles. *BMC Genom* 2015;16:767.
- [10] Enerly E, Steinfeld I, Kleivi K, Leivonen SK, Aure MR, Russnes HG, et al. miRNA-mRNA integrated analysis reveals roles for miRNAs in primary breast tumors. *PLoS ONE* 2015;6:e16915.
- [11] Allantaz F, Cheng DT, Bergauer T, Ravindran P, Rossier MF, Ebeling M, et al. Expression profiling of human immune cell subsets identifies miRNA-mRNA regulatory relationships correlated with cell type specific expression. *PLoS ONE* 2012;7:e29979.
- [12] Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003;4:249–64, <http://dx.doi.org/10.1093/biostatistics/4.2.249>.

- [13] Smyth GK. limma: linear models for microarray data. In: Gentleman R, et al., editors. Bioinformatics and computational biology solutions using R and bioconductor. New York, NY: Springer; 2005. p. 397–420.
- [14] Kolde R, Kolde MR. Package 'pheatmap'. In Version 2015.
- [15] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. *Nat Genet* 2000;25:25–9, <http://dx.doi.org/10.1038/75556>.
- [16] Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 2000;28:27–30, <http://dx.doi.org/10.1093/nar/28.1.27>.
- [17] Dweep H, Gretz N. miRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nat Meth* 2015;12, <http://dx.doi.org/10.1038/nmeth.3485>, 697–697 <http://www.nature.com/nmeth/journal/v12/n8/abs/nmeth.3485.html#supplementary-information>.
- [18] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;13:2498–504.
- [19] Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, et al. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Res* 2011;39:561–8.
- [20] Zhang B, Kirov S, Snoddy J. WebGestalt: an integrated system for exploring gene sets in various biological contexts. *Nucleic Acids Res* 2005;33:W741–8, <http://dx.doi.org/10.1093/nar/gki475>.
- [21] Singh A, Rooge S, Varshney A, Vasudevan M, Bhardwaj A, Venugopal SK, et al. Global microRNA expression profiling in the liver biopsies of hepatitis B virus-infected patients suggests specific microRNA signatures for viral persistence and hepatocellular injury. *Hepatology* 2018;67:1695–709.
- [22] Fontana RJ, Engle RE, Scaglione S, Araya V, Shaikh O, Tillman H, et al. The role of hepatitis E virus infection in adult Americans with acute liver failure. *Hepatology* 2016;64:1870.
- [23] Manka P, Bechmann LP, Coombes JD, Thodou V, Schlattjan M, Kahraman A, et al. Hepatitis E virus infection as a possible cause of acute liver failure in Europe. *Clin Gastroenterol Hepatol* 2015;13:1836–42, e2.
- [24] Zhang J, Zhang XF, Huang SJ, Wu T, Hu YM, Wang ZZ, et al. Long-term efficacy of a hepatitis E vaccine. *New Engl J Med* 2015;372:914–22.
- [25] Adlhoch C, Avellon A, Baylis SA, Ciccaglione AR, Couturier E, de Sousa R, et al. Hepatitis E virus: assessment of the epidemiological situation in humans in Europe 2014/15. *J Clin Virol Off Publ Pan Am Soc Clin Virol* 2016;82:9–16.
- [26] Acharya SK, Panda SK, Saxena A, Gupta SD. Acute hepatic failure in India: a perspective from the East. *J Gastroenterol Hepatol* 2010;15:473–9.
- [27] Shen L, Sigal LJ, Boes M, Rock KL. Important role of cathepsin s in generating peptides for TAP-independent MHC class I crosspresentation in vivo. *Immunity* 2004;21:155–65.
- [28] Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, et al. The β -chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 1996;85:1135–48.
- [29] Stevens K, Thio C, Osburn W. CCR5 deficiency enhances hepatic innate immune cell recruitment and inflammation in a murine model of acute hepatitis B infection. *Immunol Cell Biol* 2019;97:317–25.
- [30] Stevens K, Thio C, Osburn W. Loss of CCR5 signaling results in early NK cell and neutrophil recruitment into the liver and increased hepatic inflammation in a mouse model of acute hepatitis B. *J Immunol* 2017;198(Suppl. 1).
- [31] Fuse S, Obar JJ, Bellfy S, Leung EK, Zhang W, Usherwood EJ. CD80 and CD86 control antiviral CD8⁺ T-cell function and immune surveillance of murine gammaherpesvirus 68. *J Virol* 2006;80:9159.
- [32] Lumsden JM, Roberts JM, Harris NL, Peach RJ, Ronchese F. Differential requirement for CD80 and CD80/CD86-dependent costimulation in the lung immune response to an influenza virus infection. *J Immunol* 2000;164:79–85.
- [33] Said EA, Al-Reesi I, Al-Riyami M, Al-Naamani K, Al-Sinawi S, Al-Balushi MS, et al. Increased CD86 but not CD80 and PD-L1 expression on liver CD68⁺ cells during chronic HBV infection. *PLoS ONE* 2016;11:e0158265.
- [34] Guan X, Wang N, Cui F, Liu Y, Liu P, Zhao J, et al. Caveolin-1 is essential in the differentiation of human adipose-derived stem cells into hepatocyte-like cells via an MAPK pathway-dependent mechanism. *Mol Med Rep* 2016;13:1487–94.
- [35] Mao Y, Wang J, Yu F, Li Z, Li H, Guo C, Fan X. Ghrelin protects against palmitic acid or lipopolysaccharide-induced hepatocyte apoptosis through inhibition of MAPKs/iNOS and restoration of Akt/eNOS pathways. *Biomed Pharmacother* 2016;84:305–13.
- [36] Li Y, Dillon TJ, Takahashi M, Earley KT, Stork PJ. Protein kinase A-independent Ras protein activation cooperates with Rap1 protein to mediate activation of the extracellular signal-regulated kinases (ERK) by cAMP. *J Biol Chem* 2016;291:21584–95.
- [37] Liu D, Huang Y, Bu D, Liu AD, Holmberg L, Jia Y, et al. Sulfur dioxide inhibits vascular smooth muscle cell proliferation via suppressing the Erk/MAP kinase pathway mediated by cAMP/PKA signaling. *Cell Death Dis* 2014;5:e1251.
- [38] Khan K, Pal S, Yadav M, Maurya R, Trivedi AK, Sanyal S, et al. Prunetin signals via G-protein-coupled receptor, GPR30(GPER1): stimulation of adenylyl cyclase and cAMP-mediated activation of MAPK signaling induces Runx2 expression in osteoblasts to promote bone regeneration. *J Nutr Biochem* 2015;26:1491–501.
- [39] Abebayehu D, Spence AJ, Qayum AA, Taruselli MT, McLeod JJ, Caslin HL, et al. Lactic acid suppresses IL-33-mediated mast cell inflammatory responses via hypoxia inducible factor (HIF)-1 α -dependent miR-155 suppression. *J Immunol* 2016;197:2909.
- [40] Robertson ED, Wasyluk C, Tao Y, Jung AC, Wasyluk B. The oncogenic microRNA Hsa-miR-155-5p targets the transcription factor ELK3 and links it to the hypoxia response. *PLoS ONE* 2014;9:e113050.
- [41] O'Neill HS, O'Sullivan J, Porteous N, Ruiz-Hernandez E, Kelly HM, O'Brien FJ, et al. A collagen cardiac patch incorporating alginate microparticles permits the controlled release of hepatocyte growth factor and insulin-like growth factor-1 to enhance cardiac stem cell migration and proliferation. *J Tissue Eng Regen Med* 2017;12.
- [42] Waraky A, Aleem E, Larsson O. Downregulation of IGF-1 receptor occurs after hepatic lineage commitment during hepatocyte differentiation from human embryonic stem cells. *Biochem Biophys Res Commun* 2016;478:1575–81.
- [43] Carotti S, Guarino M, Valentini F, Porzio S, Vespasiani-Gentilucci U, Perrone G, et al. Impairment of GH/IGF-1 axis in the liver of patients with HCV-related chronic hepatitis. *Hormone Metab Res* 2017;50:145–51.
- [44] Matsuo R, Ohkohchi N, Murata S, Ikeda O, Nakano Y, Watanabe M, et al. Platelets strongly induce hepatocyte proliferation with IGF-1 and HGF in vitro. *J Surg Res* 2008;145:279–86.
- [45] Liu N, Kong XD, Kan QC, Shi HR, Wu QH, Zhuo ZH, et al. Mutation analysis and prenatal diagnosis in a Chinese family with succinic semialdehyde dehydrogenase and a systematic review of the literature of reported ALDH5A1 mutations. *J Perinatal Med* 2016;44:441–51.
- [46] Cui W, Lu J, Lang Y, Liu T, Wang X, Zhao X, et al. Two novel AGXT mutations identified in primary hyperoxaluria type-1 and distinct morphological and structural difference in kidney stones. *Sci Rep* 2016;6:33652.
- [47] Penniston KL. Nutritional management of hyperoxaluria. Springer International Publishing; 2015.
- [48] Huang C, Zhou T, Chen Y, Sun T, Zhang S, Chen G. Estrogen-related receptor ERR α regulation of human hydroxysteroid sulfotransferase (SULT2A1) gene expression in human Caco-2 cells. *J Biochem Mol Toxicol* 2014;28:32–8.
- [49] Ekuase EJ, Tj VTE, Rahaman A, Robertson LW, Duffel MW, Luthe G. Mechanistic insights into the specificity of human cytosolic sulfotransferase 2A1 (hSULT2A1) for hydroxylated polychlorinated biphenyls through the use of fluoro-tagged probes. *Environ Sci Pollut Res* 2016;23:1–9.
- [50] James MO, Ambadapadi S. Interactions of cytosolic sulfotransferases with xenobiotics. *Drug Metab Rev* 2013;45:401–14.