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FULL LENGTH ARTICLE

Bioinformatic identification of key genes and pathways that may be involved in the pathogenesis of HBV-associated acute liver failure

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Module analysis;
Protein–protein interaction network

Abstract In order to explore the molecular mechanisms behind the pathogenesis of acute liver failure (ALF) associated with hepatitis B virus (HBV) infection, the present study aimed to identify potential key genes and pathways involved using samples from patients with HBV-associated ALF. The GSE38941 array dataset was downloaded from the Gene Expression Omnibus database, and differentially expressed genes (DEGs) between 10 liver samples from 10 healthy donors and 17 liver specimens from 4 patients with HBV-associated ALF were analyzed using the Linear Models for Microarray Data package. Gene Ontology and KEGG pathway enrichment analyses of the DEGs were performed, followed by functional annotation of the genes and construction of a protein–protein interaction (PPI) network. Subnetwork modules were subsequently identified and analyzed. In total, 3142 DEGs were identified, of which 1755 were upregulated and 1387 were downregulated. The extracellular exosome, immune response, and inflammatory response pathways may potentially be used as biomarkers of ALF pathogenesis. In total, 17 genes (including *CCR5*, *CXCR4*, *ALB*, *C3*, *VGEFA*, and *IGF1*) were identified as hub genes in the PPI network and may therefore be potential marker genes for HBV-associated ALF.

Abbreviations: HBV, Hepatitis B Virus; ALF, acute liver failure; DEGs, differentially expressed genes; GEO, Gene Expression Omnibus; GO, Gene Ontology; CC, cell components; MF, molecular functions; BP, biological processes; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein–protein interaction; STRING, the Search Tool for the Retrieval of Interacting Genes; OLT, orthotopic liver transplantation; HSPC, hepatic stem/progenitor cells.

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Introduction

Acute liver failure (ALF), also known as fulminant hepatic failure, is a rare but life-threatening critical clinical syndrome marked by the sudden loss of hepatic function in a person with no prior history of liver disease. The spontaneous survival rate for ALF is <50% and, unfortunately, there are no effective and noninvasive treatments for this condition yet. Liver transplantation is the only therapy of proven benefit, but both the rapidity of ALF progression and its variable course limit the use of the procedure. The causes of ALF include viral hepatitis, drug-induced and toxin-induced liver diseases, metabolic errors, and ischemia, as well as other miscellaneous rare causes.[1–3] Hepatitis B virus (HBV) infects more than 300 million people worldwide and is a common cause of liver disease and liver cancer.[4] ALF occurs in approximately 1% of patients with acute hepatitis B and jaundice.[5] Severe reactivation of a chronic HBV infection can also lead to acute or subacute liver failure.[6] However, the dramatic clinical course of ALF and the difficulties in obtaining liver samples are major limitations to elucidating the molecular mechanisms behind the pathogenesis of this condition in individuals without evidence of chronic liver disease.[7] Therefore, many research groups have instead focused on HBV-related acute-on-chronic liver failure and rarely studied ALF without chronic liver disease. Consequently, there is a need for greater efforts to clarify the molecular mechanisms underlying HBV-associated ALF and to identify other potential avenues for its treatment.

Gene expression analysis based on microarray technology is a widely used, high-throughput, and powerful research method for studying the pathogenesis of complex diseases. However, studies that have performed gene expression profiling of HBV-associated ALF in humans are very rare. Nissim et al[7] designed a study, involving 4 patients with HBV-associated ALF who had undergone orthotopic liver transplantation (OLT), to investigate the molecular mechanisms of liver regeneration in humans. Their results demonstrated that this medical condition is associated with the expression of genes related to hepatic stem/progenitor cell (HSPC) and hepatic stellate cell activation and fibrogenesis. The aims of our present study were to identify differentially expressed genes (DEGs) between patients with HBV-associated ALF and individuals with healthy livers, as well as potential key genes and pathways closely related to this condition, through Gene Ontology (GO) term enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and protein–protein interaction (PPI) network analyses. A total of 3142 DEGs (including 17 potential hub genes) and some pathways were identified, which may be candidate biomarkers for HBV-associated ALF.

Materials and methods

Affymetrix microarray data

The microarray data obtained from the gene expression profiling for HBV-associated ALF were downloaded from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/gds/). The ALF-associated dataset GSE38941, based on the GPL570 ([HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array) platform, was provided by Nissim et al[7] This dataset consists of the results from 27 gene chips, developed using 10 liver samples from 10 healthy donors and 17 liver specimens from 4 patients with HBV-associated ALF (patients 241, 31, 219, and 32). All 4 patients had undergone OLT for HBV-associated ALF, and 4 liver specimens (5 in the case of patient 219) were obtained from each of them at the time of the procedure. Each liver specimen was divided into 2 pieces, one of which was snap-frozen for microarray gene expression profiling, and the other fixed in formalin for liver histological and immunohistochemical analyses. Total RNA was extracted from the frozen liver specimens and subjected to amplification, and then standard Affymetrix protocols were used to hybridize the labeled RNA with the Affymetrix Human Genome U133 Plus 2.0 arrays.

Screening for DEGs

The statistical software R (ver. 3.4.1, https://www.r-project.org/) and the Bioconductor package (http://www.bioconductor.org/) were used to analyze the significance of the DEGs between the HBV-associated ALF samples and healthy liver samples. First, the raw CEL files were normalized and background adjusted using the robust multichip average algorithm in the Affymetrix package.[8] Then, the k-nearest neighbor algorithm within the impute package was applied for missing values. An empirical Bayes method was used to select significant DEGs, based on the "limma" package of Bioconductor.[9] To improve the reliability of the DEGs, probe sets with an adjusted $P < 0.001$ and $|\log FC| > 1$ (where FC stands for fold change) between 2 comparison groups were defined as significant DEG sets. Finally, the DEGs were annotated using the ''annotate" package.

GO term and KEGG pathway enrichment analyses

Enrichment of the DEGs into the Cell Components (CC), Molecular Functions (MF), and Biological Processes (BP) GO terms was performed using the online gene function annotation tools (ver. 6.8) on the DAVID website (http://david.ncifcrf.gov/home.jsp). The relevant signaling pathways

were analyzed using the KEGG database.[10] The statistical significance threshold level for all GO enrichment and KEGG pathway analyses was $P < 0.05$.

PPI network analysis and hub gene identification

The DEGs were uploaded to the Search Tool for the Retrieval of Interacting Genes (STRING) database analysis platform[11] to obtain a PPI map. Thereafter, Cytoscape software was used to construct the PPI network. The CytoHubba plug-in[12] in Cytoscape 3.5.1[13] was used to identify hub genes. To calculate the hub scores of the PPI network, we used 12 built-in centrality indices; namely, Maximal Clique Centrality, Density of Maximum Neighborhood Component, Maximum Neighborhood Component, Degree, Edge Percolated Component, Bottleneck, Eccentricity, Closeness, Radiability, Betweenness, Stress, and Clustering Coefficient. The top 50 genes ranked by each centrality index were considered to be highly ranked hub gene candidates. Among the 12 lists of potential hub genes ranked by different hub scores, we applied a strict filter criterion that only genes within the intersection of $\geq$6 lists were considered to be high-confidence hub genes with potential biological significance. A p value of $<$0.05 was considered significantly significant.

Results

Screening for DEGs

In total, 3142 DEGs were identified by screening, of which 1387 were downregulated (Table 1) and 1755 were upregulated genes (Table 2). Of these, 828 DEGs ($|\log FC| > 2$) comprising 424 downregulated and 404 upregulated genes were selected for subsequent bioinformatic analysis. Nissim et al[7] conducted their multivariate permutation analysis with a false discovery rate of $<$1% and identified 2533 differentially expressed unique genes, 1385 of which were upregulated and 1148 were downregulated. In that study, the genes associated with HSPC, liver fibrogenesis, cell growth and proliferation, and tumorigenesis were overexpressed in the patients with ALF relative to the levels in healthy liver donors. A complete list of the down- and upregulated genes is reported in Tables S1 and S2.

GO enrichment analysis

The top 3 clustering groups obtained by GO enrichment analysis are shown in Table 3. The downregulated DEGs were enriched in the BP terms associated with oxidation–reduction processes and negative regulation of endopeptidase activity; those under CC were mainly related to the extracellular exosome and extracellular regions, whereas those under MF were mostly related to oxidoreductase activity and serine-type endopeptidase activity. The upregulated DEGs were largely enriched in BPs associated with immune and inflammatory responses, those under CC were mainly related to the plasma membrane and extracellular exosome, and those under MF were mostly for actin binding and extracellular matrix structural constituents.

Table 1 The genes that were down-regulated by a logFC < -1 and with a value of $p < 0.001$ (top 30).

Gene symbol	Gene ID	Fold-change	p-value
C9	735	0.008501541	9.80948E-09
AVPR1A	552	0.011617178	2.93874E-20
CFHR4	10877	0.012302981	9.37099E-12
F9	2158	0.013980439	3.46268E-06
CFHR3	10878	0.014787324	9.39989E-17
HSD17B6	8630	0.016711275	2.88368E-08
ORM1	5004	0.017566409	5.72594E-08
APOF	319	0.018146199	4.90698E-12
LPA	4018	0.019704092	2.47068E-21
GNMT	27232	0.024196979	8.22188E-21
AMDHD1	144193	0.025157795	2.45226E-10
CFHR5	81494	0.026036783	5.29434E-08
C8B	732	0.029256683	7.12359E-10
C8A	731	0.031092852	3.06658E-07
SLC38A4	55089	0.031553736	4.21503E-07
SLC13A5	284111	0.031932472	1.34728E-08
TFR2	7036	0.032498528	1.76399E-08
SERPINA10	51156	0.032850761	2.94065E-06
SERPINC1	462	0.034076133	0.000146525
MBL2	4153	0.034551883	1.56039E-05
HPX	3263	0.035363715	3.81807E-07
FGB	2244	0.035591625	0.000165435
PON1	5444	0.035625126	8.85678E-06
AOX1	316	0.036363375	2.27557E-07
HAO2	51179	0.03750449	2.05172E-14
PLG	5340	0.037571827	1.45562E-05
CDO1	1036	0.037948273	4.15547E-07
KNG1	3827	0.038057783	2.94455E-05
ACSM2A	123876	0.038810052	1.8805E-05
SLC17A2	10246	0.039033711	7.7752E-07

KEGG signaling pathway analysis

The top 5 results of the pathway enrichment analysis are shown in Table 4. The downregulated DEGs were enriched in metabolic, complement and coagulation cascade, and chemical carcinogenesis pathways, whereas the upregulated DEGs were enriched in cell adhesion molecule and chemokine signaling pathways.

PPI network analysis

A PPI network of the DEGs, consisting of 787 nodes and 5292 edges, was constructed by Cytoscape software on the basis of the STRING database (Fig. 1), and the CytoHubba plug-in in Cytoscape was used to identify hub genes. In total, 17 genes were selected as hub genes of HBV-associated ALF (Table 5). The plug-ins MCODE and BiNGO were used to carry out module analysis in Cytoscape. The top 4 significantly enriched gene modules were response to wounding, cell division, drug metabolism processes, and cytolysis (Fig. 2).

Discussion

Microarray analysis has become one of the most popular molecular profiling methods used for the study of cancer

Table 2 The genes that were up-regulated by a logFC>1 and with a value of $p < 0.001$ (top 30).

Gene symbol	Gene ID	Fold-change	*p*-value
MMP7	4316	54.40400488	1.03364E-13
KRT19	3880	49.77361201	2.45483E-15
AKR1B10	57016	35.15666241	2.97559E-06
IGHM	3507	28.91711368	3.92216E-14
KRT7	3855	23.32938433	1.1416E-16
CD52	1043	19.26424013	1.84291E-17
IGLC1	3537	19.09196975	2.98106E-15
KRT23	25984	18.94877375	4.45862E-18
TACSTD2	4070	18.23029878	1.5754E-15
C1QB	713	16.11947001	1.03643E-14
HLA-DQA1	3117	16.0144232	3.54936E-17
PLA2G7	7941	15.49627982	9.81681E-20
GZMB	3002	15.30271169	2.83016E-18
IGLL5	100423062	14.54548913	1.01885E-19
CXCL5	6374	13.8143893	1.27858E-13
RNASE6	6039	13.51691099	5.25033E-15
VMO1	284013	13.43633136	1.37802E-12
MZB1	51237	13.20579024	5.57179E-16
CXCR4	7852	12.88352813	7.74864E-09
GZMA	3001	12.86152786	1.82921E-15
HLA-DMA	3108	12.73049193	1.58567E-14
IGLV1-44	28823	12.58714194	1.71684E-13
EPCAM	4072	12.161157	9.66236E-14
GPNMB	10457	11.89306056	2.0122E-15
CCL5	6352	11.80515223	3.29201E-18
C1QA	712	11.79159439	3.06465E-14
VSIG4	11326	11.67315243	2.44347E-13
HLA-DPA1	3113	11.59526685	7.80962E-19
SPP1	6696	11.4922757	2.92676E-12
CXCL6	6372	11.04837791	2.48988E-13

Table 4 KEGG pathway enrichment analysis for the down-regulated and up-regulated DEGs.

Term	Biological process/pathway	count	*p*-value
Down-regulated DEGs			
hsa01100	Metabolic pathways	108	7.92E-65
hsa04610	Complement and coagulation cascades	32	1.61E-37
hsa00982	Drug metabolism - cytochrome P450	17	1.89E-17
hsa04976	Bile secretion	17	2.85E-17
hsa00830	Chemical carcinogenesis	17	2.32E-16
Up-regulated DEGs			
hsa04514	Cell adhesion molecules (CAMs)	22	1.30E-18
hsa04145	Phagosome	21	5.58E-17
hsa05166	HTLV-I infection	21	6.19E-13
hsa05152	Tuberculosis	20	8.87E-15
hsa04062	Chemokine signaling pathway	20	1.89E-14

and other complex diseases. Bioinformatic analysis of microarray data plays a key role in such studies. However, the dramatic progress of ALF and the difficulties in obtaining liver samples have limited the use of bioinformatic analysis for studying this disease, and thus the validation of large independent datasets for ALF is still rare. The GSE38941 dataset was provided by Nissim et al,[7] who aimed to study the molecular pathogenesis of liver regeneration in humans. They chose 4 patients who had ALF due to HBV infection and who had undergone OLT within one week of clinical onset. Using a combination of immunohistochemistry with gene expression profiling, those authors successfully demonstrated that liver regeneration in

Table 3 GO functional enrichment analysis for the down-regulated and up-regulated DEGs.

category	term	count	*p*-value	Fold enrichment	FDR
Down-regulated DEGs					
GOTERM_CC_DIRECT	extracellular exosome	131	2.74E-19	2.2	3.51E-16
GOTERM_CC_DIRECT	extracellular region	97	3.68E-21	2.8	4.72E-18
GOTERM_CC_DIRECT	extracellular space	88	2.43E-21	3.1	3.11E-18
GOTERM_BP_DIRECT	oxidation-reduction process	54	2.75E-18	4.1	4.70E-15
GOTERM_BP_DIRECT	negative regulation of endopeptidase activity	21	2.47E-12	7.7	4.22E-09
GOTERM_BP_DIRECT	platelet degranulation	19	1.18E-11	8.3	2.02E-08
GOTERM_MF_DIRECT	oxidoreductase activity	27	2.40E-13	6.2	3.65E-10
GOTERM_MF_DIRECT	receptor binding	27	6.43E-08	3.5	9.78E-05
GOTERM_MF_DIRECT	serine-type endopeptidase activity	25	1.84E-09	4.5	2.80E-06
Up-regulated DEGs					
GOTERM_CC_DIRECT	plasma membrane	137	1.00E-09	1.6	1.34E-06
GOTERM_CC_DIRECT	extracellular exosome	108	5.11E-11	1.9	6.84E-08
GOTERM_CC_DIRECT	membrane	78	1.73E-06	1.7	0.002315
GOTERM_BP_DIRECT	signal transduction	52	2.70E-07	2.2	3.84E-08
GOTERM_BP_DIRECT	immune response	51	5.46E-24	5.8	9.21E-07
GOTERM_BP_DIRECT	inflammatory response	40	1.00E-16	5.1	0.046362
GOTERM_MF_DIRECT	actin binding	19	1.50E-05	3.3	0.021708
GOTERM_MF_DIRECT	extracellular matrix structural constituent	10	7.68E-06	7.4	0.011150
GOTERM_MF_DIRECT	MHC class II receptor activity	7	2.79E-07	23.0	4.05E-04

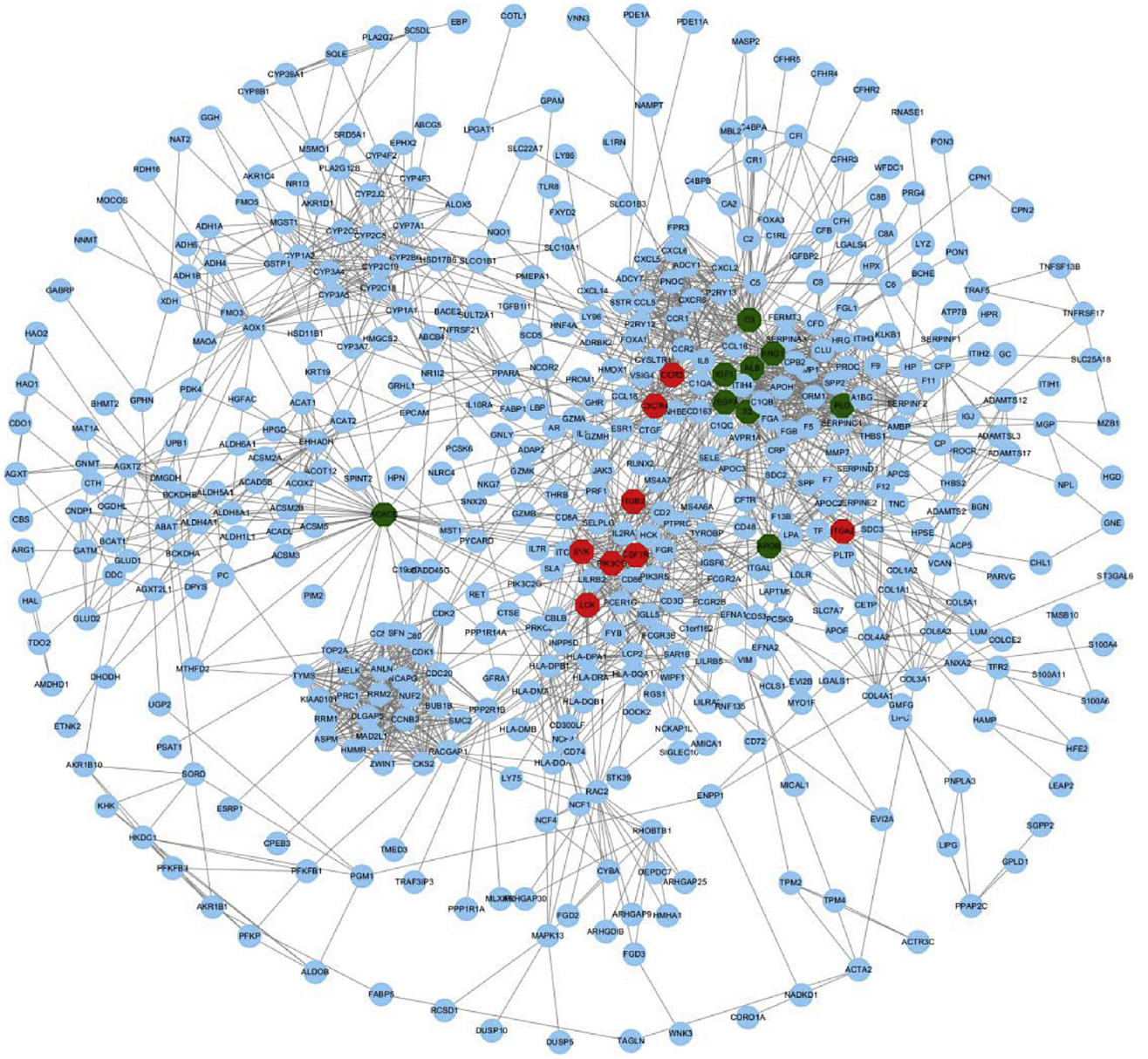


Figure 1 The protein—protein interactions of DEGs, red nodes represent up-regulated hub genes and green nodes represent down-regulated hub genes.

ALF is characterized by a prominent ductular reaction associated with extensive fibrogenesis to repair damaged liver tissue. They also found that the genes overexpressed in the patients versus in healthy liver donors were associated with HSPC, liver fibrogenesis, cell growth and proliferation, and tumorigenesis. In our present study, we aimed to conduct a comprehensive bioinformatic analysis of the molecular mechanisms underlying HBV-associated ALF. To achieve this goal, we employed several bioinformatic tools; namely, DEG screening, GO term enrichment analysis, KEGG pathway analysis, and PPI network analysis. By significance analysis using microarray data and the statistical software R, we identified 3142 DEGs ($|\log FC| > 1$) between the liver samples from the 4 patients and those from the 10 healthy donors. We chose 828 DEGs with a $|\log FC| > 2$ to identify potential hub genes and pathways that exert considerable effect on HBV-associated ALF initiation and progression from various aspects.

According to GO term enrichment analysis, both upregulated and downregulated genes were enriched in the extracellular exosome category. Previous studies have reported that exosomes play an important role in immune regulation and antiviral responses during viral infections.[14] Kapoor et al[15] elucidated that the viral HBx protein may impact liver homeostasis through intercellular communication via exosomes. Chen et al[16] observed the anti-apoptotic capacity of menstrual fluid-derived exosomes in acute liver failure. Wang et al[17] demonstrated that mesenchymal stem cell (MSC)-derived extracellular exosomes exhibited striking therapeutic benefits against liver

Table 5 17 Hub genes of HBV-associated ALF.

Gene symbol	Full name	Degree	logFC	*p*-value
ALB	albumin	57	−3.57	6.8171E-05
KNG1	kininogen 1	52	−4.71	2.94455E-05
VEGFA	vascular endothelial growth factor A	39	−2.06	3.32681E-11
C3	complement C3	38	−2.13	3.25594E-08
PLG	plasminogen	36	−4.73	1.45562E-05
ACACB	acetyl-CoA carboxylase beta	36	−2.40	8.43004E-17
IGF1	insulin like growth factor 1	34	−3.89	4.0507E-19
F2	coagulation factor II	33	−4.23	1.58304E-06
CXCR4	C-X-C motif chemokine receptor 4	31	3.69	7.74864E-09
CCR5	C−C motif chemokine receptor 5	29	2.25	7.46533E-13
SYK	spleen associated tyrosine kinase	29	2.26	1.80793E-13
ITGB2	integrin subunit beta 2	27	2.38	9.95784E-10
APOB	apolipoprotein B	27	−2.17	4.51921E-07
LCK	LCK proto-oncogene, Src family tyrosine kinase	26	2.77	3.83339E-17
PIK3CG	phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit gamma	25	2.16	2.1593E-11
ITGA2	integrin subunit alpha 2	21	2.94	7.03805E-11
CSF1R	colony stimulating factor 1 receptor	19	2.80	1.34257E-13

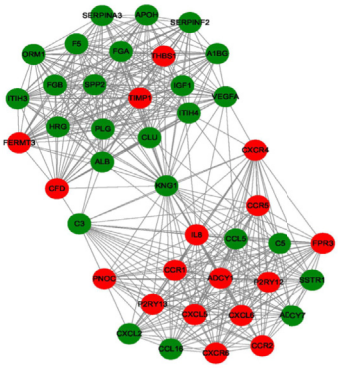
failure. These past findings were consistent with our results, demonstrating that extracellular exosomes may play an important role in HBV-associated ALF.

In our present comprehensive study, all of the bioinformatic analysis results pointed to inflammatory- or immune response-related genes and pathways. Specifically, GO term enrichment analysis showed that 51, 34, 19, and 16 upregulated DEGs were significantly enriched in the categories of immune response, innate immune response, regulation of immune response, and adaptive immune response, respectively, whereas 40 upregulated genes were significantly enriched in the inflammatory response term. KEGG pathway analysis indicated that 22, 21, and 20 upregulated DEGs were significantly enriched in the cell adhesion molecules, chemokine signaling pathway, and cytokine−cytokine receptor interaction categories, respectively. Immune dysregulation is now recognized to be central to the pathogenesis of ALF,[18,19] and HBV infection is thought to be an immune-mediated liver disease.[20] Furthermore, Woolf et al[21] suggested that a prompt host immune response plays a major role in fulminant hepatic failure due to HBV infection. With deeper study of the pathogenesis of HBV-associated ALF, there is increasing evidence that activation of the systemic immune responses plays a pivotal role in the disease pathogenesis and outcome. Upon exposure to HBV, individuals with a vigorous immune response to the virus will develop an acute, self-limited infection, which may result in ALF,[22] and massive production of IgG and IgM antibodies specific for the HBV core antigen was observed in the liver of patients suffering from HBV-associated ALF.[23,24] Furthermore, the secondary immune response to hepatocyte death was thought to drive many of the extrahepatic features of ALF, including hemodynamic disturbance and multiple organ failure.[25] It is well known that the inflammatory response is the basis of such disturbance and is common in ALF, resulting in tissue damage and organ failure. It was reported that ALF is due to massive leukocyte-dependent inflammation and the extensive apoptosis of hepatocytes.[26] Additionally, chemokines and cytokines are important for both immune and inflammatory responses and are related to many infectious diseases, including ALF.[27,28] In our study, the upregulation of genes related to immune and inflammatory responses and their related pathways supports the aforementioned findings. Furthermore, our PPI network and module analyses identified that the first gene module was significantly enriched in inflammatory response activity, and the fourth gene module was significantly enriched in immune response-related pathways. These results provide more powerful evidence to support the theory that immune and inflammatory responses play very important roles in HBV-associated ALF.

PPI networks usually serve an important role in identifying novel protein functions[29] and in examining the associations between protein network structures and functions.[30] In the present study, the PPI network analysis identified a significant module that was mainly enriched in the cell division, cell cycle process, cell cycle phase, and nuclear division categories, all genes of which were upregulated. This indicated that cell proliferation and differentiation could be one of the molecular mechanisms underlying HBV-associated ALF. Our further use of PPI analysis thus validates the results described by Nissim et al.[7]

We selected 17 DEGs as potential hub genes, using a strict filter. Among these hub genes, C-X-C motif chemokine receptor 4 (*CXCR4*) and C−C motif chemokine receptor 5 (*CCR5*) showed the highest node degrees of 31 and 29, respectively. From our results, these 2 chemokine receptors were enriched in chemokine signaling and cytokine−cytokine receptor interaction pathways as well as in other chemokine-related pathways. Hepatocyte damage induced by liver failure stimulates the secretion of various cytokines and chemokines, which are involved in mediating the process of tissue repair. *CXCR4* and *CCR5*, as receptors of chemokines, play important roles in the immune responses in ALF by regulating the migration of lymphocytes to sites of inflammation. There are a number of reports suggesting that the expression of *CCR5* and *CXCR4* is markedly increased in the liver in ALF.[31,32] *CXCR4* overexpression was shown to strengthen the homing of

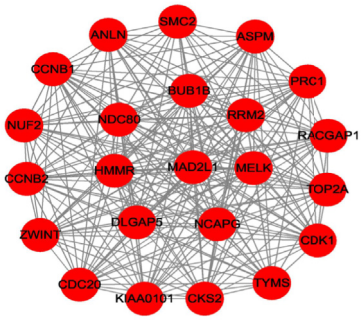
a



b

GO-ID	P-value	Description	Genes in the set
9611	5.2701E-27	response to wounding	CFD | ITIH4 | SERPINA3 | CXCL6 | ORM1 | CXCR4 | THBS1 | CXCL2 | CLU | KNG1 | C3 | C5 | CCL5 | CCR5 | CCL16 | CCR2 | FGB | P2RY12 | CCR1 | FGA | SERPINF2 | IGF1 | F5 | HRG | FERMT3
6954	2.0621E-20	inflammatory response	CFD | CCR1 | ITIH4 | SERPINA3 | CXCL6 | ORM1 | SERPINF2 | CXCR4 | THBS1 | CXCL2 | CLU | KNG1 | C3 | C5 | CCL5 | CCR5 | CCL16 | CCR2
6950	8.2607E-17	response to stress	CFD | ITIH4 | SERPINA3 | CXCL6 | ORM1 | CXCR4 | THBS1 | CXCL2 | CLU | KNG1 | C3 | C5 | CCL5 | CCR5 | CCL16 | CCR2 | FGB | P2RY12 | CCR1 | FGA | SERPINF2 | IGF1 | F5 | VEGFA | ALB | HRG | FERMT3
6952	3.4169E-15	defense response	CFD | CCR1 | ITIH4 | SERPINA3 | CXCL6 | ORM1 | SERPINF2 | CXCR4 | THBS1 | CXCL2 | CLU | KNG1 | C3 | C5 | CCL5 | CCR5 | CCL16 | CCR2

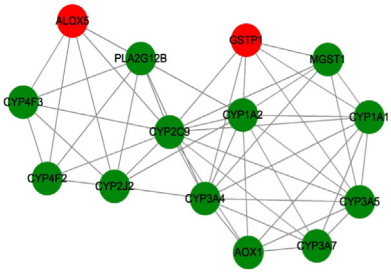
c



d

GO-ID	P-value	Description	Genes in the set
51301	5.2097E-22	Cell division	NCAPG | NDC80 | ZWINT | SMC2 | CDC20 | CCNB2 | ANLN | CCNB1 | RAP1 | PRC1 | NUF2 | CDK1 | CKS2 | MAD2L1
279	4.9114E-19	M phase	NCAPG | NDC80 | ZWINT | SMC2 | CDC20 | CCNB2 | ANLN | CCNB1 | PRUF2 | CDK1 | CKS2 | MAD2L1
22402	3.2535E-18	Cell cycle process	NCAPG | NDC80 | ZWINT | SMC2 | CDC20 | CCNB2 | ANLN | CCNB1 | RAP1 | PRC1 | NUF2 | CDK1 | CKS2 | MAD2L1
22403	8.2098E-18	Cell cycle phase	NCAPG | NDC80 | ZWINT | SMC2 | CDC20 | CCNB2 | ANLN | CCNB1 | PRUF2 | CDK1 | CKS2 | MAD2L1

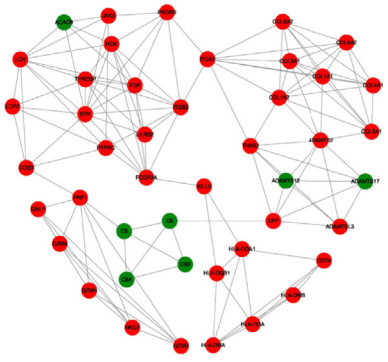
e



f

GO-ID	P-value	description	Genes in the set
17144	1.9943E-9	Drug metabolic process	CYP2C9 | CYP1A2 | CYP1A1 | CYP3A4
55114	2.4534E-9	Oxidation reduction	CYP2J2 | CYP2C9 | ALOX5 | CYP4F2 | CYP1A2 | CYP1A1 | A4 | CYP3A7
16098	6.7555E-9	Monoterpenoid metabolic process	CYP2C9 | CYP1A2 | YP3A4
6721	1.2333E-8	Terpenoid metabolic process	CYP2C9 | CYP1A2 | CYP1A1 | CYP3A4

g



h

GO-ID	P-value	Description	Genes in the set
19835	1.3116E-16	cytolysis	C6 | C9 | GZMA | PRF1 | GZMB | C8B | C8A | GZMH
2376	1.1743E-12	Immune system process	CD74 | SYK | GZMA | ITGB2 | LILRB2 | C8B | CFP | C8A | CD3D | HLA-DMA | HLA-DMB | C6 | PTPRC | LCK | C9 | LCP2 | HLA-DOA | HLA-DQA1 | HLA-DQB1
2504	5.8534E-12	Antigen processing and presentation of peptide	CD74 | HLA-DMA | HLA-DMB | HLA-DOA | HLA-DQA1 | HLA-DQB1
6955	4.3540E-11	Immune response	CD74 | GZMA | LILRB2 | C8B | CFP | C8A | HLA-DMA | HLA-DMB | C6 | PTPRC | C9 | LCP2 | HLA-DOA | HLA-DQA1 | HLA-DQB1

Figure 2 Module analysis of PPI network. Red nodes represent up-regulated genes and green nodes represent down-regulated genes. a. module 1; b. GO enrichment analysis of module 1; c. module 2; d. GO enrichment analysis of module 2; e. module 3; f. GO enrichment analysis of module 3; g. module 4; h. GO enrichment analysis of module 4.

systemically delivered MSCs, enhancing their engraftment in the damaged liver and improving liver regeneration.[33] On the other hand, *CCR5* deficiency has been shown to promote the development of ALF.[34] According to our results, these two genes and their respective proteins were upregulated, likely due to the OLT, which was helpful for liver regeneration.

To the best of our knowledge, no previous research has studied the relationships between ALF and the other upregulated hub genes, including *CSF1R*, *ITGA2*, *ITGB2*, *LCK*, *PIK3CG*, and *SYK*, but these genes may play key roles in the pathogenesis of HBV-associated ALF. Most of these genes are crucial components of immune or inflammatory responses; for example, the spleen associated tyrosine kinase (*SYK*) and LCK proto-oncogene, Src family tyrosine kinase (*LCK*) genes have important roles in the B-cell receptor signaling pathway,[35,36] as was supported by our study, where both were identified as hub genes and as being enriched in this same pathway. Additionally, these 2 genes were significant in module 4, and thus potentially have crucial roles in the pathogenesis of HBV-associated ALF through immune and inflammatory responses. Further studies are needed to elucidate the association and biological functions of these genes in HBV-associated ALF or in other kinds of ALF.

Among the downregulated hub genes, albumin (*ALB*) had the highest node degree of 57 from the PPI network analysis. *ALB* is regarded as a marker of liver function after bone marrow MSC transplantation,[37] and in another study, this gene was regarded as a good prognostic biomarker of hepatocellular carcinoma (HCC) because of its greater prognostic value than that of Child-Pugh grading in patients with HCC.[38] *ACACB* and *KNG1* are rarely expressed in the liver and have never been reported in ALF. Nevertheless, both are regarded as key genes in other diseases.[39,40] Complement complement C3 (*C3*) is activated by the classical complement pathway, which is fully involved in immune reactions operating in acute infections in fulminant hepatic failure.[41] The complement activation product C3a is essential for liver regeneration, mediated through its effects on the cell signaling processes involved in hepatocyte proliferation,[42] although the liver has inherent tissue-repairing activities. In ALF, when acute tissue loss overwhelms the liver's regenerative capacity, hepatic function becomes impaired, leading to systemic inflammation, multiple organ failure, and sudden death.[43,44] *F2*, *PLG*, and *KNG1* were also enriched in the complement and coagulation cascade pathways in our results. Vascular endothelial growth factor A (*VEGFA*), another growth factor functioning in the liver, is the focus of attention in liver disease research because of its various actions, and the mechanism behind its protective effects in fulminant hepatic failure has been assessed.[45] Insulin like growth factor 1 (*IGF1*) is used to assess liver function and liver regeneration after liver transplantation.[46] All of these DEGs were downregulated in the patients with ALF, which might explain the basis of the poor prognoses.

In conclusion, the present study provides a comprehensive bioinformatic analysis of DEGs that may be involved in HBV-associated ALF. Our results should contribute to knowledge on the underlying molecular mechanisms related to the occurrence, development, and prognosis of this medical condition. The extracellular exosome, immune response, and inflammatory response pathways may potentially be used as biomarkers of ALF pathogenesis. Among the associated hub genes, *CCR5*, *CXCR4*, *ALB*, *C3*, *VEGFA*, and *IGF1* may have significant roles in HBV-associated ALF. However, further genetic and experimental studies on these potential key genes and pathways are required to further confirm the findings of the present study.

Conflicts of interest

The authors declare that no conflict of interest exists.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.gendis.2018.02.005.

References

1. Bernal William, Wendon Julia. Acute liver failure. *N Engl J Med*. 2013;369(26):2525—2534.
2. Ostapowicz George, Fontana Robert J, Schiødt Frank V, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med*. 2002;137(12):947—954.
3. Lee William M, Squires Jr Robert H, Nyberg Scott L, et al. Acute liver failure: summary of a workshop. *Hepatology*. 2008;47(4):1401—1415.
4. Jake Liang T, Hepatitis B. The virus and disease. *Hepatology*. 2009;49(5Suppl):S13—S21.
5. Berk PD, Popper H. Fulminant hepatic failure. *Am J Gastroenterol*. 1978;69:349—400.
6. Wang Yu-Ming, Tang Ying-Zi. Antiviral therapy for hepatitis B virus associated hepatic failure. *Hepatobiliary Pancreat Dis Int*. 2009;8(1):17—24.
7. Nissim Oriel, Melis Marta, Diaz Giacomo, et al. Liver regeneration signature in hepatitis B virus (HBV)-Associated acute liver failure identified by gene expression profiling. *PLoS One*. 2012;11:e49611.
8. Gautier Laurent, Cope Leslie, Bolstad Benjamin M, et al. Affy-analysis of Affmetrix GeneChip data at the probe level. *Bioinformatics*. 2004;20(3):307—315.
9. Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol*. 2004;3(1):1—26.
10. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28(1):27—30.
11. Szklarczyk Damian, Franceschini Andrea, Wyder Stefan, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. 2015;43:D447—D452.
12. Lin Chung-Yen, Chin Chia-Hao, Wu Hsin-Hung, et al. Hubba: hub objects analyzer-a framework of interactome hubs identification for network biology. *Nucleic Acids Res*. 2008;36:438—443.

13. Shannon Paul, Markiel Andrew, Ozier Owen, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;11:2498—2504.
14. Alenquer Marta, Amorim Maria João. Exosome biogenesis, regulation, and function in viral infection. *Viruses*. 2015;7:5066—5083.
15. Kapoora Neetu Rohit, Chadhaa Radhika, Kumarb Saravanan, et al. The HBx gene of hepatitis B virus can influence hepatic microenvironment via exosomes by transferring its mRNA and protein. *Virus Res*. 2017;240:166—174.
16. Chen Lu, Xiang Bingyu, Wang Xiaojun, et al. Exosomes derived from human menstrual blood-derived stem cells alleviate fulminant hepatic failure. *Stem Cell Res Ther*. 2017;8:9.
17. Wang Jie, Cen Panpan, Chen Jiajia, et al. Role of mesenchymal stem cells, their derived factors, and extracellular vesicles in liver failure. *Stem Cell Res Ther*. 2017;8:137.
18. Antoniades Charalambos Gustav, Berry Philip A, Wendon Julia A, et al. The importance of immune dysfunction in determining outcome in acute liver failure. *J Hepatol*. 2008;49:845—861.
19. Krenkel Oliver, Mossanen Jana C, Tacke Frank. Immune mechanisms in acetaminophen-induced acute liver failure. *Hepatobiliary Surg Nutr*. 2014;6(3):331—343.
20. Wang Yadong, Wang Wei, Shen Chuan, et al. NKG2D modulates aggravation of liver inflammation by activating NK cells in HBV infection. *Sci Rep*. 2017;7:88.
21. Woolf IL, El Sheikh N, Cullens H, et al. Enhanced HBsAb production in pathogenesis of fulminant viral hepatitis type B. *Br Med J*. 1976;2:669—671.
22. Oketani M, Uto H, Ido A, Tsubouchi H. Management of hepatitis B virus-related acute liver failure. *Clin J Gastroenterol*. 2014;7:19—26.
23. Farcia Patrizia, Diazb Giacomo, Chena Zhaochun, et al. B cell gene signature with massive intrahepatic production of antibodies to hepatitis B core antigen in Hepatitis B Virus-associated acute liver failure. *Proc Natl Acad Sci*. 2010;107(19):8766—8771.
24. Wu Weimin, Chen Zhaochun, Cheng Naiqian, et al. Wingfield and Alasdair C. Steven. Specificity of an anti-capsid antibody associated with Hepatitis B Virus-related acute liver failure. *J Struct Biol*. 2013;181:53—60.
25. Donnelly Mhairi C, Hayes Peter C, Simpson Kenneth J. Role of inflammation and infection in the pathogenesis of human acute liver failure: clinical implications for monitoring and therapy. *World J Gastroenterol*. 2016;22(26):5958—5970.
26. Pathil Anita, Warth Arne, Chamulitrat Walee, et al. The synthetic bile acid-phospholipid conjugate ursodeoxycholyl lysophosphatidylethanolamide suppresses TNFα-induced liver injury. *J Hepatol*. 2011;54:674—684.
27. Szabo Gyongyi, Petrasek Jan. Inflammasome activation and function in liver disease. *Gastroenterol Hepatol*. 2015;12,387—340.
28. Rottman JB. Key role of chemokines and chemokine receptors in inflammation, immunity, neoplasia, and infectious disease. *Vet Pathol*. 1999;36:357—367.
29. Yook SH, Oltvai ZN, Barabási AL. Functional and topological characterization of protein interaction networks. *Proteomics*. 2004;4:928—942.
30. Horvath S, Dong J. Geometric interpretation of gene coexpression network analysis. *PLoS Comput Biol*. 2008;4(8):e1000117.
31. Tuncer Ceren, Oo Ye H, Murphy Nick, et al. The regulation of T-cell recruitment to the human liver during acute liver failure. *Liver Int*. 2013;33:852—863.
32. Liepelt Anke, Tacke Frank. Stromal cell-derived factor-1 (SDF-1) as a target in liver diseases. *Am J Physiol Gastrointest Liver Physiol*. 2016;311:G203—G209.
33. Ma Hu-Cheng, Shi Xiao-Lei, Ren Hao-Zhen. Targeted migration of mesenchymal stem cells modified with CXCR4 to acute failing liver improves liver regeneration. *World J Gastroenterol*. 2014;40:14884—14894.
34. Ajuebor Maureen N, Aspinall Alex I, Zhou Feng, et al. Lack of chemokine receptor CCR5 promotes murine fulminant liver failure by preventing the apoptosis of activated CD1d-restricted NKT cells. *J Immunol*. 2005;174:8027—8037.
35. Seda Vaclav, Mraz Marek. B-cell receptor signalling and its crosstalk with other pathways in normal and malignant cells. *Eur J Haematol*. 2015;94:193—205.
36. Talab Fatima, Allen John C, Thompson Victoria, et al. LCK is an important mediator of B-cell receptor signaling in chronic lymphocytic leukemia cells. *Mol Cancer Res*. 2013;11:541—554.
37. Yuan Shufang, Jiang Tao, Zheng Rongjiong, et al. Effect of bone marrow mesenchymal stem cell transplantation on acute hepatic failure in rats. *Exp Therapeut Med*. 2014;8:1150—1158.
38. Li Mu-xing, Zhao Hong, Bi Xin-yu, et al. Prognostic value of the albumin-bilirubin grade in patients with hepatocellular carcinoma: validation in a Chinese cohort. *Hepatol Res*. 2017;47:731—741.
39. Li Ling, Wang Guangyu, Li Ning, et al. Identification of key genes and pathways associated with obesity in children. *Exp Therapeut Med*. 2017;14:1065—1073.
40. Schrödter Sarah, Braun Martin, Syring Isabella, et al. Identification of the dopamine transporter SLC6A3 as a biomarker for patients with renal cell carcinoma. *Mol Cancer*. 2016;15:1—10.
41. Irshad M, Acharya SK, Joshi YK, et al. Role of fibronectin and complement in immunopathogenesis of acute and subacute hepatic failure. *J Gastroenterol Hepatol*. 1994;9:355—360.
42. Markiewski Maciej M, DeAngelis Robert A, Strey Christoph W, et al. The regulation of liver cell survival by complement. *J Immunol*. 2009;182(9):5412—5418.
43. Miyajima A, Tanaka M, Itoh T. Stem/progenitor cells in liver development, homeostasis, regeneration, and reprogramming. *Cell Stem Cell*. 2014;14:561—574.
44. Riordan SM, Williams R. Mechanisms of hepatocyte injury, multiorgan failure, and prognostic criteria in acute liver failure. *Semin Liver Dis*. 2003;23(03):203—215.
45. Tanaka Yoichi, Sohda Tetsuro, Matsuo Katsuhiko, et al. Vascular endothelial growth factor reduces Fas-mediated acute liver injury in mice. *J Gastroenterol Hepatol*. 2008;23:e207—e211.
46. Jara Maximilian, Schulz Antje, Malinowski Maciej, et al. Growth hormone/insulin-like growth factor 1 dynamics in adult living donor liver transplantation. *Liver Transpl*. 2014;20:1118—1126.