Heliyon 10 (2024) e28329

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

CCL20 and *CD8A* as potential diagnostic biomarkers for HBV-induced liver fibrosis in chronic hepatitis B

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ARTICLE INFO

Keywords:
Chronic hepatitis B
Hepatitis B virus
Liver fibrosis
Diagnostic biomarkers
Immune infiltration

ABSTRACT

Background: The main cause of the liver fibrosis (LF) remains hepatitis B virus (HBV) infection, especially in China. Histologically, liver fibrosis still occurs progressively in chronic hepatitis B (CHB) patients, even if HBV-DNA is negative or undetectable. The diagnosis of LF is beneficial to control the development of it, also it may promote the reversal of LF. Although liver biopsy is the gold standard of diagnosis in LF at present, it isa traumatic diagnosis. There are no diagnostic biomarkers as yet for the condition. It is badly in need of biomarkers clinically, which is simple to test, minimally invasive, highly specific, and sensitive. Early detection of HBV-LF development is crucial in the prevention, treatment, and prognosis prediction of HBV-LF. Cytokines are closely associated with both immune regulation and inflammation in the progression of hepatitis B virus associated-liver fibrosis (HBV-LF). In this bioinformatic study, we not only analyzed the relationship between HBV-LF and immune infiltration, but also identified key genes to uncover new therapeutic targets.

Objectives: To find potential biomarkers for liver fibrosis in the development of chronic hepatic B patients.

Materials and methods: We obtained two sets of data including CHB/healthy control and CHB/HBV-LF from the Integrated Gene Expression (GEO) database to select for differential expression analysis. Protein-protein interaction (PPI) network was also generated, while key genes and important gene modules involved in the occurrence and development of HBV-LF were identified. These key genes were analyzed by functional enrichment analysis, module analysis, and survival analysis. Furthermore, the relationship between these two diseases and immune infiltration was explored.

Results: Among the identified genes, 150 were individually associated with CHB and healthy control in the differential gene expression (DGE) analysis. While 14 with CHB and HBV-LF. It was also analyzed in the Robust rank aggregation (RRA) analysis, 34 differential genes were further

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https://doi.org/10.1016/j.heliyon.2024.e28329
Received 23 June 2023; Received in revised form 14 March 2024; Accepted 15 March 2024
Available online 25 March 2024

identified by Cytohubba. Among 34 differential genes, two core genes were determined: *CCL20* and *CD8A*. *CCL20* was able to predict CHB positivity (area under the receiver operating characteristic curve [AUC-ROC] = 0.883, 95% confidence interval [CI] 0.786–0.963), while HBV-LF positivity ([AUC-ROC] = 0.687, 95% confidence interval [CI] 0.592–0.779). And *CD8A* was able to predict CHB positivity ([AUC-ROC] = 0.960, 95% confidence interval [CI] 0.915–0.992), while HBV-LF positivity ([AUC-ROC] = 0.773, 95% confidence interval [CI] 0.680–0.856). Relationship between *CCL20* gene expression and LF grades was $P < 0.05$, as well as *CD8A*.
Conclusion: *CCL20* and *CD8A* were found to be potential biomarkers and therapeutic targets for HBV-LF. It is instructive for research on the progression of LF in HBV patients, suppression of chronic inflammation, and development of molecularly targeted-therapy for HBV-LF.

1. Introduction

Chronic liver disease is one of the major concerns for public health worldwide [1]. About 2 billion people are infected with HBV around the world, of which 248 million are affected as they test positive for hepatitis B surface antigen (HBsAg). With 15–25% of chronic liver diseases patients died from cirrhosis or liver cancer [2]. Furthermore, HBV poses an enormous global economic and health burden, and geographic region and possibly other factors (gender, race, socioeconomic status) can influence the prevalence of chronic liver disease greatly. For instance, the high-intermediate to high endemic countries with prevalence rates of about 5–8% are in the sub-Saharan Africa and the Western Pacific region, as high as 15% in some countries. In other countries, such as in the Americas and Western Europe, prevalence rates generally do not exceed 2% [3,4]. In China, there are about 20–30 million CHB patients, and the mortality rate of HBV-related diseases is higher than the average worldwide [5].

Similarly, liver fibrosis and cirrhosis are the major causes of morbidity and mortality of patients with chronic liver diseases which are mainly caused by chronic infection by hepatotropic viruses (like hepatitis B and hepatitis C virus, both worldwide distributed), excess alcohol consumption (alcoholic liver disease), non-alcoholic fatty liver disease, autoimmune liver diseases, and hereditary diseases [6]. Viral infections however remain the most common cause, especially HBV is the most common risk factor in Asia [7,8]. Liver fibrogenesis is a dynamic, highly integrated molecular, cellular and tissue process responsible for driving the progressive excess accumulation of extracellular matrix components. And it is sustained by the activation of hepatic myofibroblasts, which is a heterogeneous population of proliferative, migratory and profibrogenic cells that also modulate inflammatory/immune response and angiogenesis [9]. Almost liver fibrosis represents critical characteristics in the progression of any form of chronic liver diseases to liver cirrhosis, hepatic failure and hepatocellular carcinoma [10,11]. Therefore, it is important for the early diagnosis of liver fibrosis. Histological staging of LF ranges from F0 to F4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with rare septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis). Currently, the gold standard for LF stages diagnosis is liver biopsy, while it comes with limitations, such as operation-related complications, sampling error, inter-observer variation, and the difficulty of popularization. Serological indicators are mainly used to screen for cirrhosis, but they cannot diagnose early LF [12].

As for CHB, the pathogenesis of the disease has not been fully elucidated. It is nonetheless influenced by both HBV and host immune system [13]. Interferon and nucleotide analogues suppress HBV DNA replication and improve liver inflammation and fibrosis, which do not completely cure chronic liver disease. Because of the low cure rate, it has to be treated indefinitely for majority of patients. When treatment is stopped, the infection may recur [14]. Oral antivirals drugs which can ameliorate complications also have limitations [15]. As of today, there are no effective treatments for LF other than eliminating of the underlying cause or undergoing liver transplant [16]. The effects of many anti-fibrosis drugs are limited with lack of clinical trials, although there are some strong effects have been shown in experimental animal models [6].

In studies of hepatitis, LF, and cirrhosis, Gene Expression Omnibus database (GEO) has been widely used [17–19]. In this paper, we analyzed gene expression profiles from GEO database (GSE83148 and GSE84044), with 252 patients included. We aimed to determine hub genes and investigate the relationship between their expression and immune infiltration. This study may provide the basis for further research in HBV-LF, so as to find novel therapeutic targets and discover new anti-fibrosis drugs (Fig. 1).

2. Materials and methods

2.1. Data acquisition and preprocessing

Gene expression microarray datasets related to CHB and HBV-LF were searched with the search strategy was "chronic hepatitis B″ and "chronic hepatitis B-associated liver fibrosis" [20]. The microarrays included the following criteria: (1) human liver specimens (2) samples infected with HBV (3) LF caused by HBV, excluding alcoholic liver, nonalcoholic fatty liver disease, and other viral hepatitis, etc. (4) no less than 30 samples per dataset.

2.2. Screening of differentially expressed genes (DEGs)

DEGs were assessed using limma package [21]. DEGs with an adjusted $p < 0.05$ and $|\log_2 \text{fold change}| > 1$ were defined as significantly differentially expressed probe sets. We performed differential analysis on the two datasets separately looking for differential genes between healthy control (HC) and CHB as well as CHB and HBV-LF.

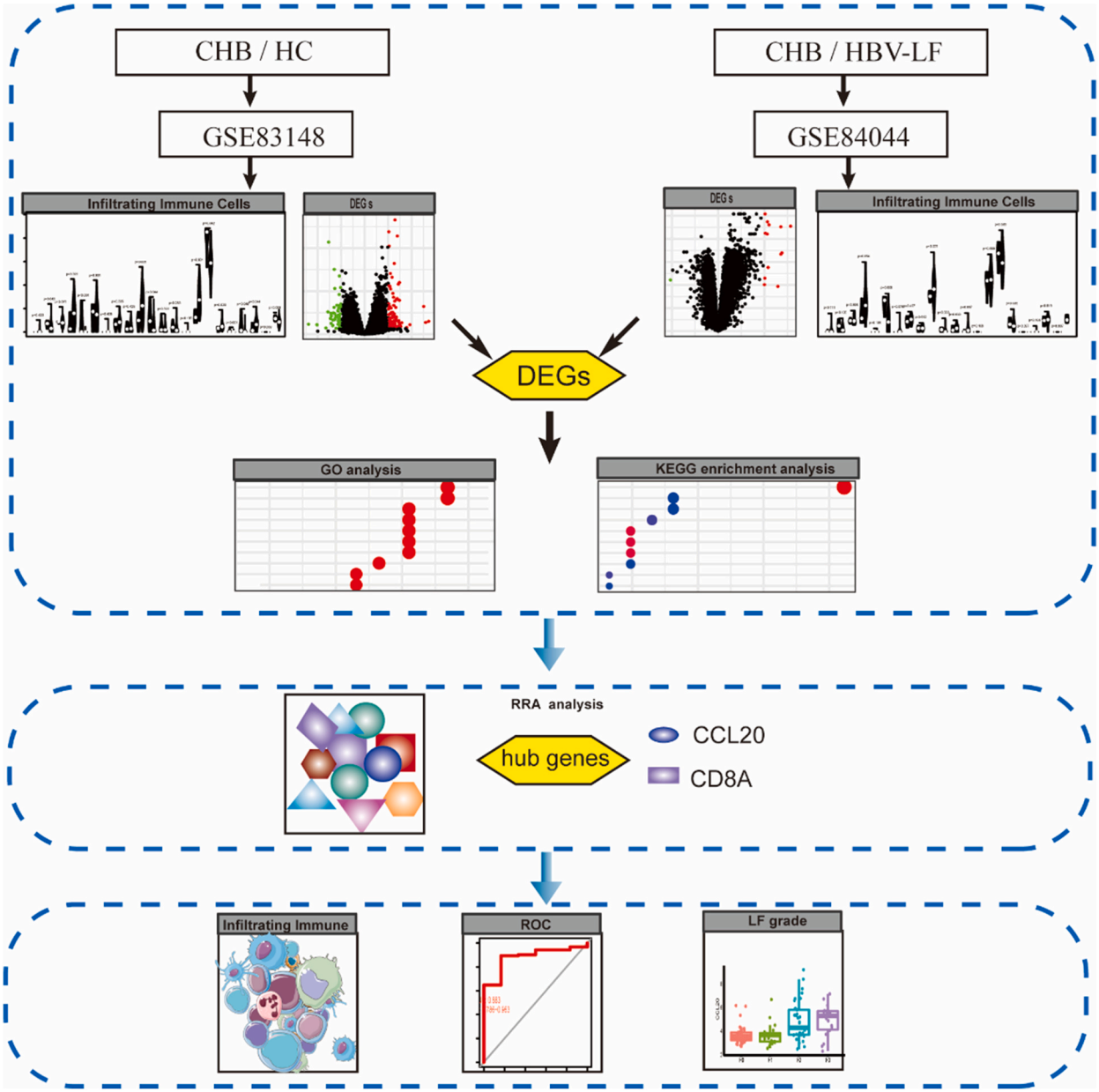

Fig. 1. Flow chart.

Robust rank aggregation (RRA) analysis was then performed to integrate these 2 microarray datasets and obtain DEGs of normal individuals infected with HBV progressing to HBV-LF, which is the standard method for minimizing bias and error between multiple datasets [22].

Prior to RRA analysis, upregulated and downregulated genes were ranked by their fold change in each dataset. Robust DEGs was then obtained based on the ranked genes in the 2 datasets using the RobustRankAggreg R package [22].

2.3. Functional correlation analysis

ClusterProfiler, org.Hs.eg.db, and enrichplot R packages were used in functional enrichment analysis [23], including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

2.4. Immune cell infiltration analysis

We used the CIBERSORT algorithm (http://cibersort.stanford.edu) [24] to transform the normalized gene expression matrix into 22 immune cell matrices. The number of permutations was set to 100, and immune cell matrices were screened for $p < 0.05$. The relative expression of the 22 immune cells in liver tissues from CHB and HC, as well as CHB and HBV-LF, was identified using the R packages. Principal component analysis (PCA) was used to determine differences in immune infiltration between CHB and HC, which was also used between CHB and HBV-LF.

2.5. Protein–protein interaction network construction and module analysis

We uploaded the robust DEGs to the STRING online database (https://cn.string-db.org/), with a confidence level >0.4 and hid the disconnected nodes as the filtering criterion. Image processing was performed with Cytoscape (Version 3.9.0) software, and filtering of significant modules in protein-protein interaction (PPI) networks was done with plugin-MCODE (Version 2.0.0). Here, MCODE was used to screen the modules of the PPI network identified by degree cutoff = 2, node score cutoff = 0.2, K core = 2 and a maximum depth = 100. These genes are thought to be hub genes.

2.6. Identification of key genes

The plugin-CytoHubba provides various topological analysis algorithms such as degree, edge permeability component (EPC), maximum neighborhood component (MNC), etc., which can be used to identify centrality of genes [25]. Based on the gene centrality scores, the intersection of the top overexpressed 10 genes from 10 algorithms be considered key genes.Correlation analysis between hub genes and infiltrating immune cells.

Correlations between hub genes and infiltrating immune cells were explored using Spearman's rank correlation analysis in R software.

2.7. Diagnostic value of Feature biomarkers in HBV-LF

The area under the ROC curve (AUC) value was calculated to determine the diagnostic validity of differentiating CHB and HC, also it was used between CHB and HBV-LF, respectively. The relationship between these 2 genes and the LF stage was verified in GSE84044.

2.8. Statistical analysis

All statistical analyses were performed using R (version 4.1.0). Group comparisons were performed for continuous variables, using the student's *t*-test for normally distributed variables and the Mann-Whitney test for abnormally distributed variables. For continuous variables, one-way analysis of variance (ANOVA) was used to assess differences between three or more groups. $P < 0.05$ would be set to significance level.

Then, we performed receiver operating characteristic (ROC) curve analyses of the biomarkers to evaluate the sensitivity and specificity of these diagnostic biomarkers in patients.

3. Results

3.1. Retrieved microarray datasets data related to CHB and HBV-LF

According to the search criteria, GSE83148 [26] and GSE84044 [8] were included in this study, with GEO accession number, sample size, platform, and description shown in Table 1. GSE83148 contained 6 HC cases and 122 CHB. GSE84044 contained 43 CHB without HBV-LF cases (LF grade F0), 71 patients with HBV-LF (LF grade F1: 20 cases, F2: 33, and F3: 18), and 10 patients with F4 (cirrhosis).

Compared with HC samples, 97 differential genes associated with CHB occurrence were upregulated and 52 were downregulated (Fig. 2a). In the differential analysis of CHB and HBV-LF, 13 DEGs were upregulated and 1 DEG was downregulated (Fig. 2b). A total of 34 robust DEGs were then identified, including 30 upregulated and 4 downregulated genes. Based on the values of robustness, we assigned 34 DEGs in the visual heat map (Fig. 2c). Table S1 showed the details of the selected DEGs in the 2 datasets. These genes were used in further enrichment analysis.

Table 1
| Detailed information of the 2 microarray datasets used in the present study.

GSE Acc. No.	No. of Samples	Platform	Description
GSE83148	122 vs. 6	GPL570	CHB (without LF) and HC (healthy control)
GSE84044	43 vs. 71	GPL570	CHB (43 F0) and HBV-LF (20 F1, 33 F2, 18 F3, and 10 F4)

Identification of DEGs.

Volcano plots of DEGs distribution in GSE83148 (a) and GSE84044 (b) red and green dots represented upregulated and downregulated genes, respectively. (c) Heat map of the 30 upregulated robust genes and 4 downregulated robust genes identified by the RRA method. Red represents highly expressed robust DEGs while green represents low expressed robust DEGs.

3.2. Functional correlation analysis

GO analysis of DEGs in HC infected with HBV showed that cellular component (CC), biological process (BP), and molecular function (MF) were significantly enriched in different GO terms (Fig. 3a). BP analysis was mostly clustered to cell migration and chemokine-mediated signaling pathway. CC analysis was clustered in the outer part of plasma membrane, late endosome, and secretory granule lumen. MF function was enriched in cytokine activity, cytokine receptor binding, and G protein-coupled receptor binding. The most enriched terms in KEGG analysis were cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine

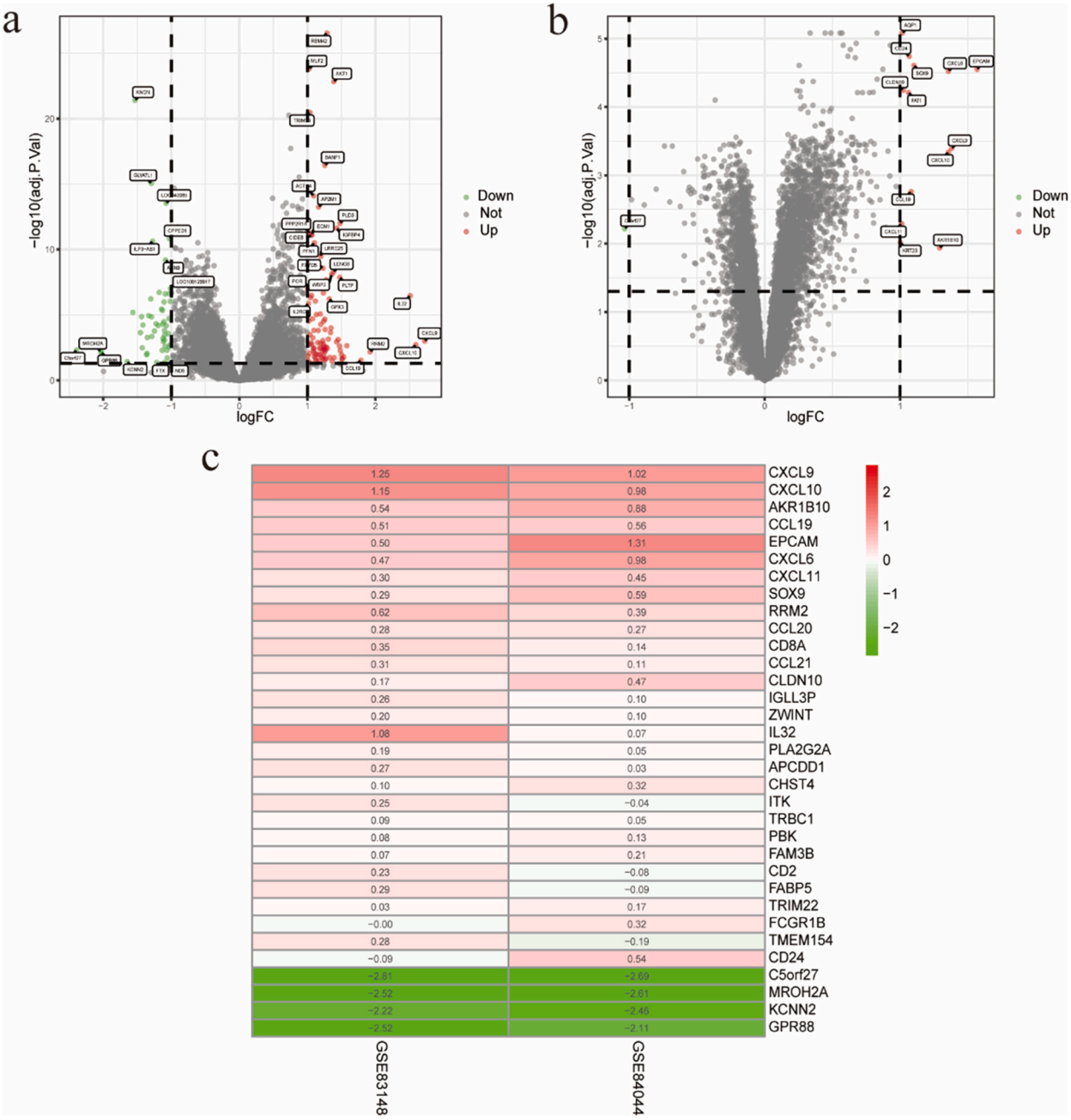

Fig. 2. Identification of DEGs and robust DEGs.

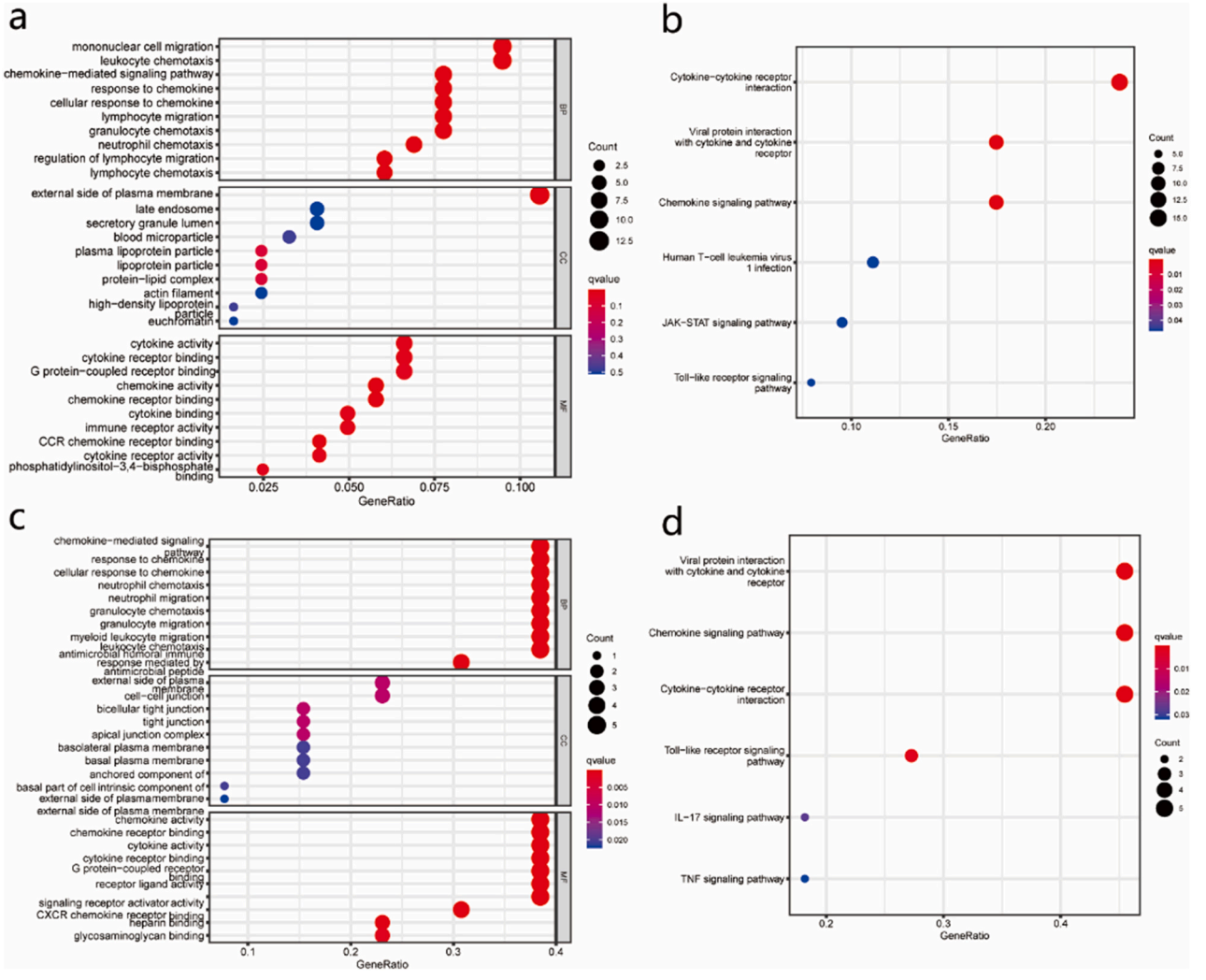

Fig. 3. Functional enrichment analysis of DEGs. (a) GO analysis of DEGs in the GSE83148 data set. (b) KEGG analysis of DEGs in the GSE83148 data set. (c) GO analysis of DEGs in the GSE84044 data set. (d) KEGG analysis of DEGs in the GSE84044 data set.

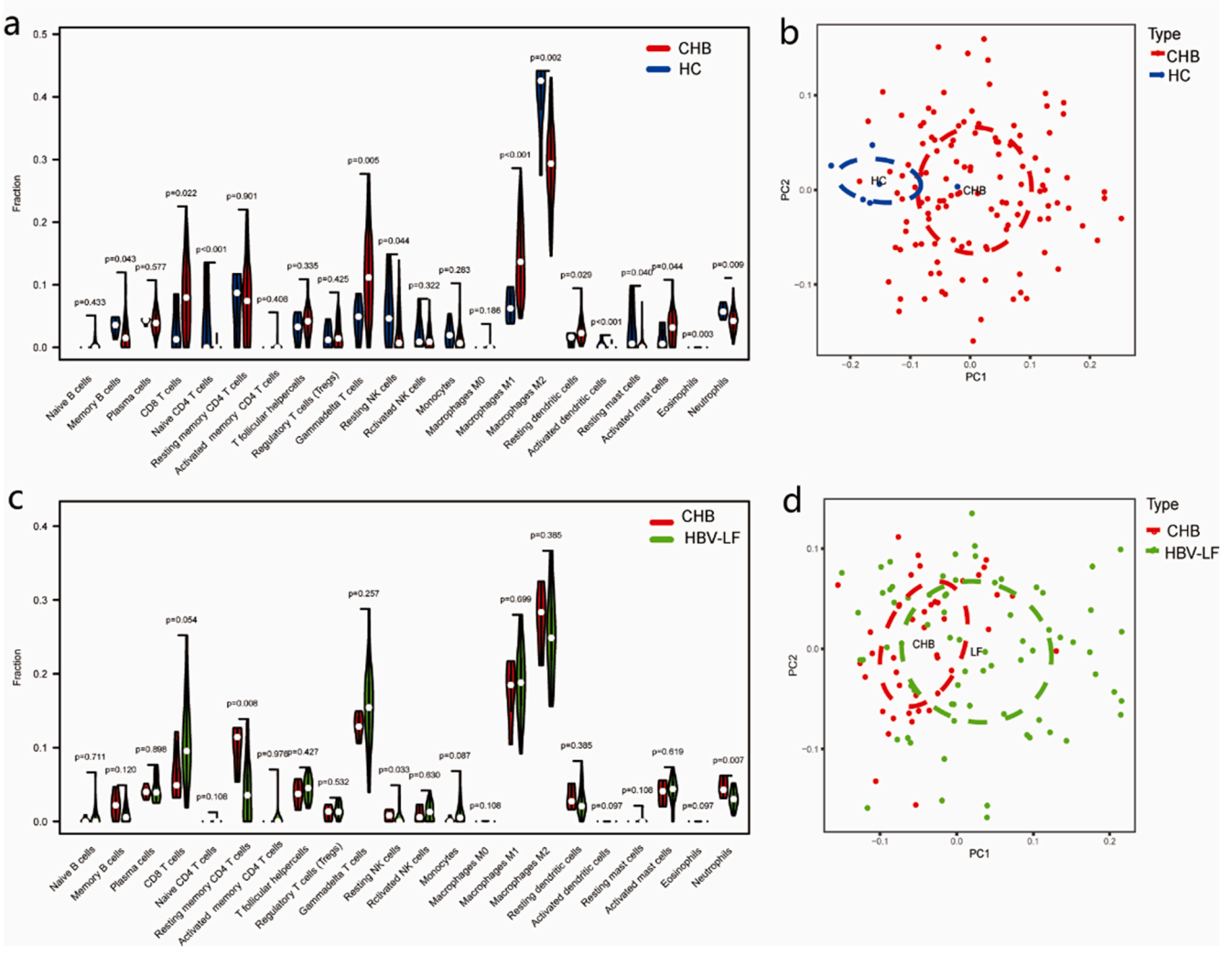

Fig. 4. Immune cell infiltration analysis. (a) Violin plot visualizing differential infiltration of 22 immune cell species in CHB and HC liver tissues. (b) PCA was performed on all CHB and HC. (c) Violin plot visualizing differential infiltration of 22 immune cell species in liver tissues in CHB and HBV-LF. (d) PCA was performed on all CHB and HBV-LF.

receptor, and chemokine signaling pathway (Fig. 3b).

The results showed that BP terms were enriched in chemokine-mediated signaling pathway. CC were mostly enriched in external side of plasma membrane, cell-cell junction, and bicellular tight junction. MF were enriched in chemokine activity, chemokine receptor binding, and cytokine activity (Fig. 3c). The most enriched terms in KEGG analysis were viral protein interaction with cytokine and cytokine receptor, chemokine signaling pathway, and cytokine-cytokine receptor interaction (Fig. 3d). A detailed analysis of GO and KEGG was shown in Table S2 and Table S3.

3.3. Immune cell infiltration analysis

The infiltration immune cells in CHB were shown in Fig. 4a and Table S4. Memory B cells, resting memory CD4 T cells, naive CD4 T cells, resting NK cells, macrophages M2, activated dendritic cells, mast cells, and neutrophils count decreased ($p < 0.05$). In contrast, CD8 T cells, gammadelta T cells (γδ T cells), macrophages M0, resting dendritic cells, and activated mast cells were all elevated ($p < 0.05$). PCA of immune infiltration in Fig. 4b showed significant individual differences between CHB and HC.

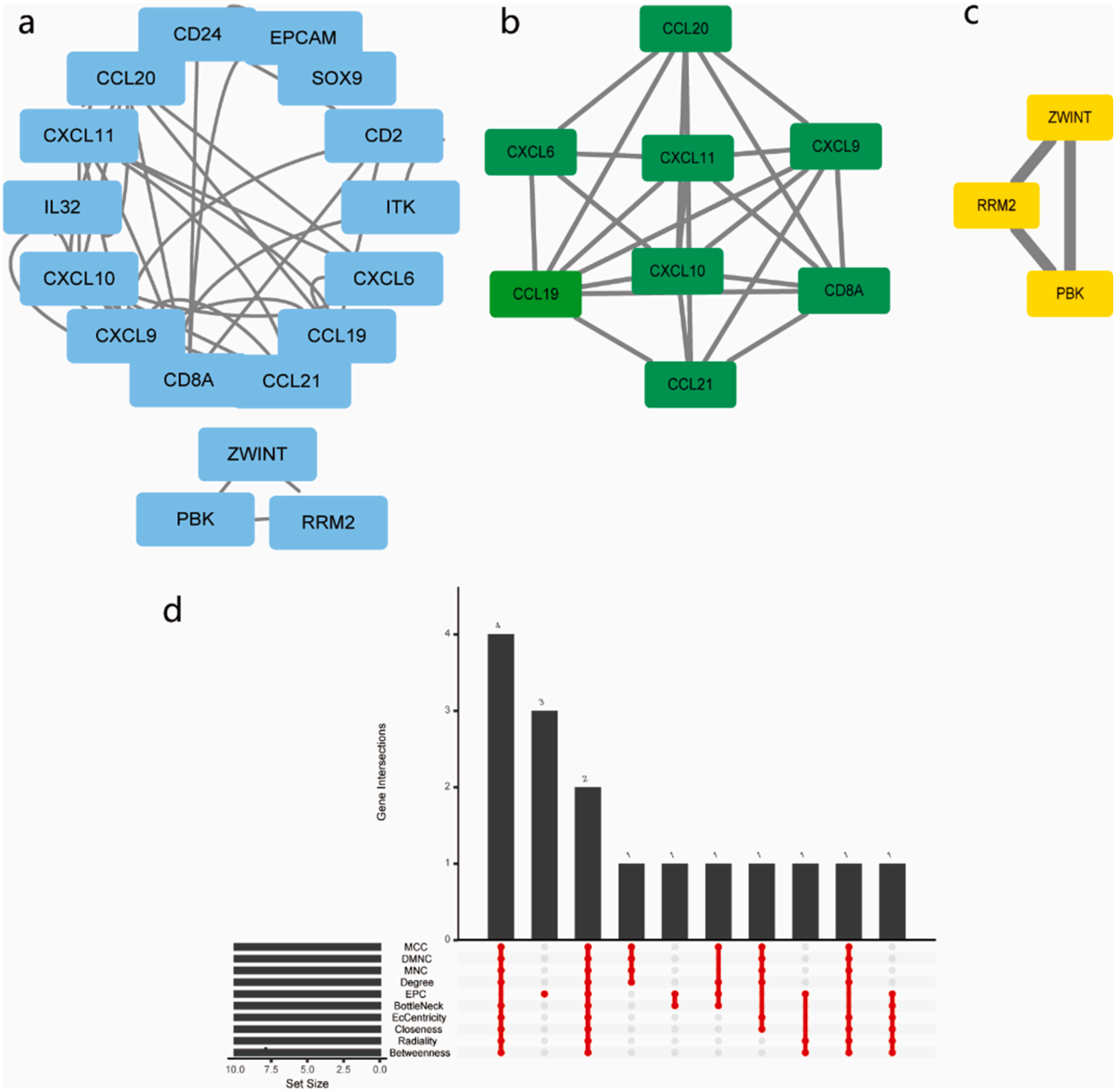

Fig. 5. Identification of hub genes. (a) The whole PPI network. (b) PPI network of module 1. (c) PPI network of module 2. (d) Intersection of 10 genes from 10 algorithms to identify key genes.

Fig. 4c showed the infiltration of 22 immune cells in CHB and HBV-LF, where memory B cells, resting memory CD4 T cells, resting NK cells, and neutrophils were suppressed during the transition from CHB to LF ($p < 0.05$). The PCA of immune infiltration showed individual differences between CHB and HBV-LF (Fig. 4d).

3.4. Protein-protein interaction network construction and hub gene identification

PPI network was visualized with the final network comprising 18 nodes and 42 edges, including 18 upregulated genes (Fig. 5a). By using the MCODE plugin, 2 key modules were filtered out from the whole network (Fig. 5b and c). The intersection of the top overexpressed 10 genes from 10 algorithms revealed 2 hub genes: *CCL20* and *CD8A* (Fig. 5d and Table S5). Description of these 2 genes, including full names, synonyms and main functions, can be found in Table 2.

3.5. Correlation analysis between CCL20, CD8A, and infiltrating immune cells

We investigated the correlation of *CCL20* expression and immune infiltration in CHB patients (Fig. 6a), and between *CD8A* expression and immune infiltration in CHB patients respectively (Fig. 6b). Correlation analysis revealed positive correlation between *CCL20* expression and macrophages M1 ($r = 0.647$, $p < 0.001$), γδ T cells ($r = 0.565$, $p < 0.001$), naive B cells ($r = 0.296$, $p < 0.001$), resting dendritic cells ($r = 0.282$, $p = 0.001$), activated mast cells ($r = 0.231$, $p = 0.009$), macrophages M0 ($r = 0.220$, $p = 0.013$), and T follicular helper cells ($r = 0.215$, $p = 0.015$). On the other hand, *CCL20* negatively correlated with resting memory CD4 T cells ($r = -0.224$, $p = 0.011$), resting NK cells ($r = -0.555$, $p < 0.001$), macrophages M2 ($r = -0.538$, $p < 0.001$), memory B cells ($r = -0.447$, $p < 0.001$), neutrophils ($r = -0.384$, $p < 0.001$), T regulatory cells (Tregs) ($r = -328$, $p < 0.001$) (Fig. 6a).

As for the second hub gene, *CD8A* expression positively correlated with γδ T cells ($r = 0.807$, $p < 0.001$), macrophages M1 ($r = 0.733$, $p < 0.001$), activated memory CD4 T cells ($r = 0.305$, $p < 0.001$), T follicular helper cells ($r = 0.255$, $p = 0.004$), CD8 T cells ($r = 0.238$, $p = 0.007$), naive B cells ($r = 0.235$, $p = 0.008$), activated mast cells ($r = 0.216$, $p = 0.014$), and macrophages M0 ($r = 0.203$, $p = 0.021$). Moreover, *CD8A* expression negatively correlated with memory B cells ($r = -0.478$, $p < 0.001$), resting memory CD4 T cells ($r = -0.284$, $p = 0.001$), Tregs ($r = -0.461$, $p < 0.001$), resting NK cells ($r = -0.641$, $p < 0.001$), monocytes ($r = -0.294$, $p < 0.001$), macrophages M2 ($r = -0.656$, $p < 0.001$), activated dendritic cells ($r = -0.201$, $p = 0.023$), resting mast cells ($r = -0.290$, $p < 0.001$), and neutrophils ($r = -0.542$, $p < 0.001$) (Fig. 6b).

In addition, we analyzed the relationship between *CCL20* expression and immune infiltration in patients with HBV-LF (Fig. 6c), and the relationship between *CD8A* expression and immune infiltration in patients with HBV-LF (Fig. 6d). Correlation analysis showed that *CCL20* expression positively correlated with macrophages M1 ($r = 0.432$, $p = 0.004$), activated memory CD4 T cells ($r = 0.392$, $p = 0.009$), γδ T cells ($r = 0.377$, $p = 0.013$), and activated mast cells ($r = 0.353$, $p = 0.020$). *CCL20* negatively correlated with memory B cells ($r = -0.338$, $p = 0.027$), resting NK cells ($r = -0.390$, $p = 0.010$), neutrophils ($r = -0.426$, $p = 0.005$), and macrophages M2 ($r = -0.532$, $p < 0.001$). Correlation analysis also showed that *CD8A* expression positively correlated with γδ T cells ($r = 0.832$, $p < 0.001$), activated memory CD4 T cells ($r = 0.496$, $p < 0.001$), and macrophages M1 ($r = 0.302$, $p = 0.049$). On the other hand, *CD8A* negatively correlated with resting NK cells ($r = -0.568$, $p < 0.001$), resting memory CD4 T cells ($r = -0.576$, $p < 0.001$), macrophages M2 ($r = -0.621$, $p < 0.001$), and neutrophils ($r = -0.672$, $p < 0.001$) (Table S6).

3.6. Validation of the diagnostic value of CCL20 and CD8A

The biomarkers of *CCL20* and *CD8A* were of high diagnostic value for CHB vs. HC, CHB vs. HBV-LF. The AUC value of *CCL20* in the diagnosis of CHB was 0.883 (95% CI 0.786–0.963), and the AUC value of *CCL20* in the diagnosis of HBV-LF was 0.687 (95% CI 0.592–0.779) (Fig. 7a). The AUC value of *CD8A* in the diagnosis of CHB was 0.960 (95% CI 0.915 to 0.992), and the *CD8A* value of *CCL20* in the diagnosis of HBV-LF was 0.773 (95% CI 0.680 to 0.856) (Fig. 7c).

GSE84044 detected that the expression levels of *CCL20* ($p < 0.001$) and *CD8A* ($p < 0.001$) significantly increased in advanced HBV-LF stages, and the detailed expression of the two hub genes in the different stages of LF (Fig. 7b and d). This result indicated that *CCL20* and *CD8A* gene expression played a vital role in the process of HBV-LF.

4. Discussion

Studies have shown that the replication of the virus in liver cells led to immune-mediated hepatocyte damage and liver injury in patients with CHB [27].The degrees of liver injury are related to immune response level and HBV variation, especially cellular immunity and complement system [28]. Myofibroblasts are activated in response to liver injury, while they are not present in the healthy

Table 2
| Description of the 2 hub genes.

Gene	Full name	Synonyms	Function
CCL20	C–C Motif Chemokine Ligand 20	MIP-3α, LARC, Exodus-1	chemotactic effect on lymphocytes and dendritic cells
CD8A	T-cell surface glycoprotein CD8 alpha chain	Ly-2, Ly-B, Ly-35, Lyt-2	immune response and serves multiple functions in responses against both external and internal offenses.

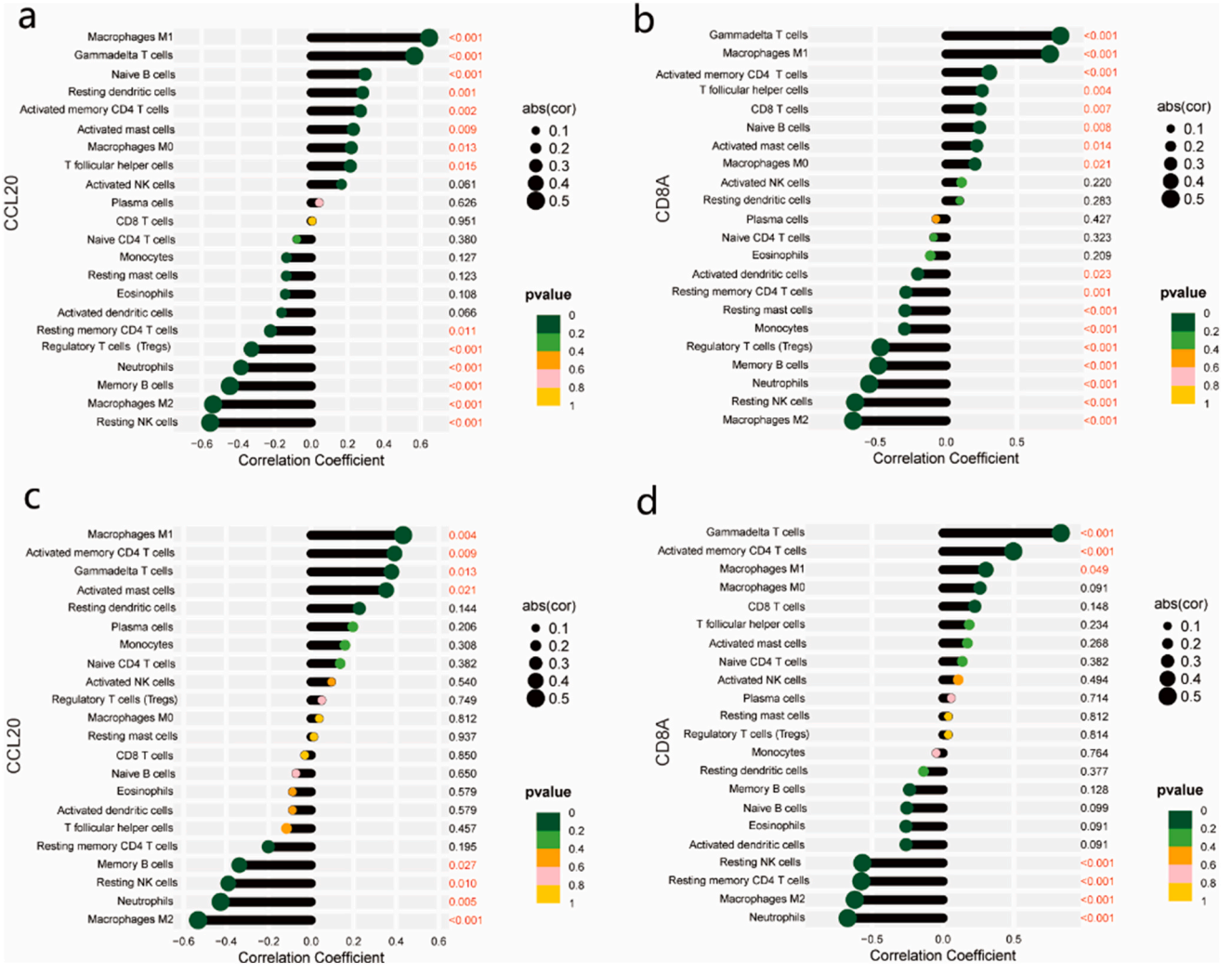

Fig. 6. Correlation of hub genes with immune cell infiltration Liver tissue *CCL20* (a) and *CD8A* (b) with immune cell infiltration in CHB patients. Correlation of liver tissue *CCL20* (c) and *CD8A* (d) with immune cell infiltration in HBV-LF patients.

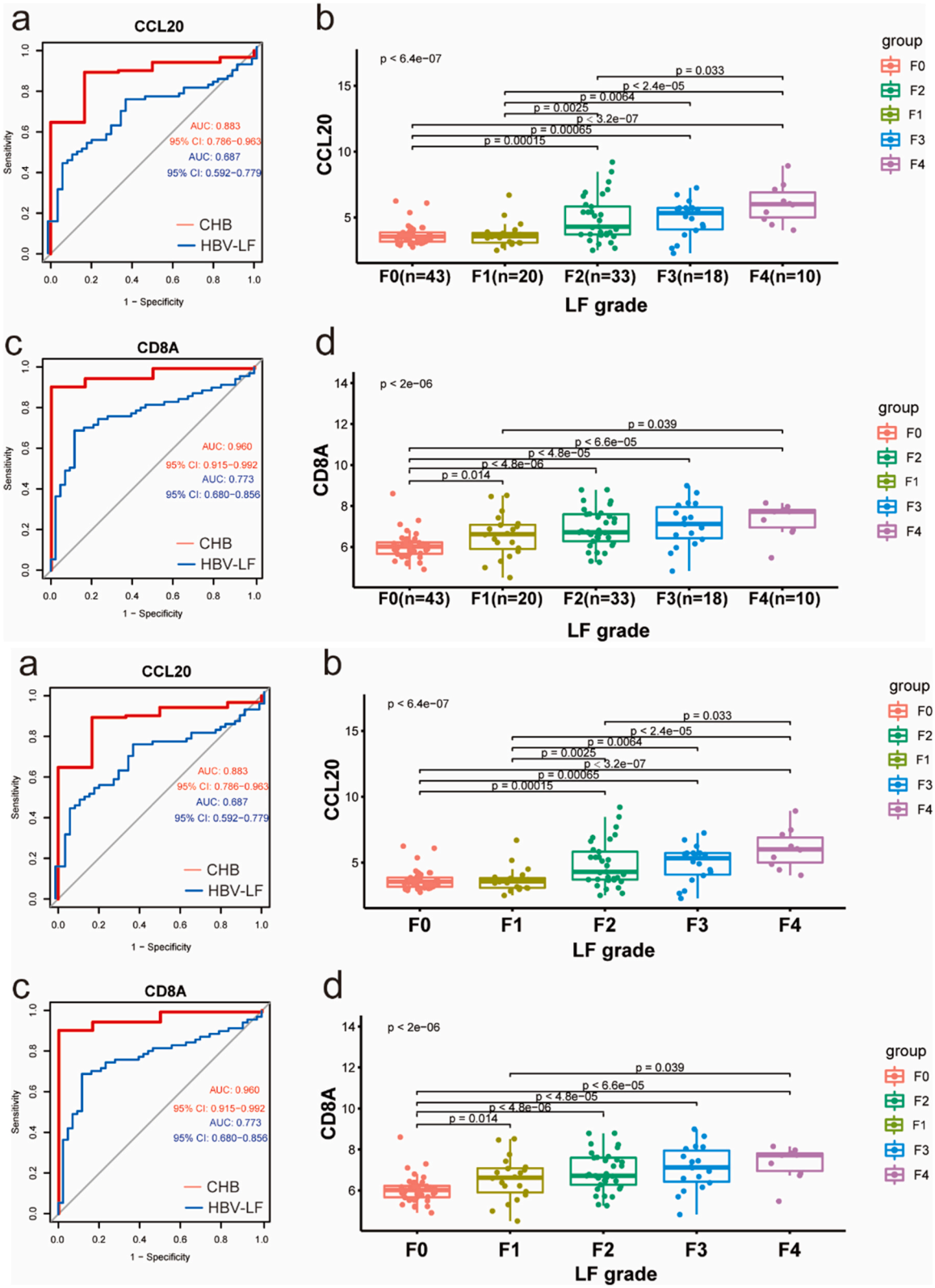

Fig. 7. Validation of the diagnostic value of *CCL20* and *CD8A*. (a) ROC curve of *CCL20* for the diagnosis of CHB and HBV-LF. (b) Relationship between *CCL20* gene expression and LF grades. (c) ROC curve of *CD8A* for the diagnosis of CHB and HBV-LF. (d) Relationship between *CD8A* gene expression and LF grades.

liver. Chronic liver injury leads to activation of hepatic stellate cells, which are the main source of the fibrous scar in liver fibrosis. The continuous replication of HBV in hepatocytes leads to the disorder of immune response and inflammatory response, then it causes chronic liver damage and fibrosis. Disorder of immune response is an important risk factor for viral replication in patients with HBV-cirrhosis. In fact, excessive apoptosis of immune cells will change the function of immune cell and lead to disorders of immune response.

Also, host genetic factors may influence the curative effect in the treatment of CHB patients. Anti-HBV treatment may be related to chemokine-mediated immune responses and immune cell infiltration in the liver microenvironment [29]. Although CHB patients lack effector cells, they have an inadequate immune response. There is evidence that the virus can be cleared through activation of the immune system [30]. The point is to research on HBV infection about understanding chronic disease and immune control preferably. Ideal antiviral therapy not only reduces the replication of HBV, but also clears and eliminates HBsAg or at least controls viral covalently closed circular DNA (cccDNA). These therapeutic actions may need to be coordinated with an adaptive immune response [27].

In addition, parenchymal cells, including immune cells and hepatic stellate cells (HSC), play a crucial role in the progression and regression of LF in the injured liver [31]. Endogenous Kupffer cells (KC), infiltrated monocytes and lymphocytes can promote inflammatory responses by responding to intracellular components released by impaired liver cells (HCs) and secreting a range of cytokines, which leads to LF [32,33]. Multiple immune cell subpopulations, including endogenous KC and infiltrated monocytes and lymphocytes, regulate LF through combined action in the impaired liver [33]. Stellate cells have been shown to be central modulators of liver inflammation and immunity, rather than merely passive targets of inflammatory cytokines. Especially, a growing number of chemokines and their homologous receptors have the dual function of further stimulating fibrogenesis, while interacting with inflammatory cells to modify the immune response during injury [34–36]. Therefore, the development of new therapeutic strategies targeting immunoregulation is becoming a potential treatment of CHB and HBV-LF.

It was reported that 30% of cirrhosis and 45% of liver cancer patients are due to CHB worldwide. At present, it is believed that the diagnosis and staging of HBV-LF are fundamental in evaluating the degree of chronic liver disease and developing antiviral treatment of HBV. They also play a role in delaying the progression of liver cirrhosis and liver cancer [37]. In the development of HBV-LF, cytokines are secreted by the intrinsic and adaptive immune cells involved in the control of the virus. As intercellular intermediary substances, they play an important role in cell activation, intracellular signaling and intercellular communication, and are closely related to immune regulation and inflammation [38,39].

In the study, we focused on the differential genes and immune infiltration to find hub genes based on the gene expression data of CHB and HBV-LF. Differential genes for CHB and HC, CHB and HBV-LF were enriched in GO and KEGG analysis, respectively. The RRA dataset was used for joint analysis to identify 34 differential genes. The expression of *CCL20* and *CD8A* is positively correlated with HBV-LF, which plays an important role in HBV-LF process. Nonetheless, further study is needed to elucidate the relationship between *CCL20* and *CD8A* in CHB and HBV-LF.

Chemokines can regulate leukocytes, lymphocytes, tumor cells and other cells through chemotaxis. That is, when the body is stimulated by inflammation, chemokines exert immune response mainly by inducing and promoting inflammatory cells to reach the immune response site, as well as by activating them. This process may play a regulatory role in tissue fibrosis, organ sclerosis, tumor growth and angiogenesis [40]. Chemokines such as *CXCL9*, *CXCL10* and *CXCL11* can regulate the chemotactic activities of inflammatory cells and inflammatory factors, and then regulate the occurrence of LF. Indeed, it was shown that the mRNA levels of *CXCL9* and *CXCL11* in the liver were significantly upregulated in patients with severe LF [41]. In addition, mRNA level of *CXCL10* was also significantly upregulated in liver tissues of patients with chronic liver diseases, such as CHB and cirrhosis, which indicates a certain correlation between *CXCL10* and the occurrence of LF [42].

CC chemokines are involved in the occurrence of liver inflammation through chemotaxis and activation of inflammatory cells such as KC and lymphocytes. They can also regulate the occurrence and inhibition of LF. *CCL20,* an important component of *CC* family, has been proved to be constitutively expressed in liver tissues [43]. Here, serum *CCL20* protein levels were significantly elevated in patients with LF. *CCL20* is the main ligand of *CC* chemokine receptor 6 (*CCR6*) and was shown to produce many inflammatory molecules that mediate LF as a chemokine molecule in immature dendritic cells [44]. On the other hand, the expression of *CCL20* is significantly downregulated during active HBV infections, which may affect the body's immune response. Moreover, *CCL20* could promote the progression of HCC by transforming epithelial cells into mesenchymal cells. *CCL20* may act as a chemical attractant for immune cells and an autocrine factor for driving the fibrosis process, which may also be a potential diagnostic and/or therapeutic target [45]. In addition, it has been revealed that *CCL20* and IL-1β play an essential role on promoting ILC3-dependent antitumor immunity and enhancing tumor sensitivity to immunotherapy [46].

CD8A, as one of the immune cell marker genes, is mainly involved in cell-mediated immune defense and T-cell development [47,48]. Also, it can serve as a diagnostic and progressive biomarker for many diseases, including tumors and inflammatory diseases [49–51]. CD8 gene loci represent probably one of the most distinctive gene loci in T cells [52], and T-cell sensitivity to antigen can be profoundly affected by modulation of CD8 coreceptor levels [53].What's more, changes in CD8 expression are associated with different methylation patterns in and around the *CD8A* gene [53]. It has demonstrated that CD8 tissue-resident memory CD8 T (CD8 Trm) cells have a direct role in resolving liver fibrosis [54]. In addition, the expressions of CD8A was shown to be positively correlated with the degree of liver inflammation and fibrosis [55]. Our study also suggested that CD8A may play a pivotal role in LF and its inflammatory damage.

Due to the limitations and potential risks of liver biopsy, the search for non-invasive techniques has become a research hotspot in the field of liver fibrosis. Serologic markers and imaging techniques are included for the diagnosis of HBV-LF at present. Serum markers including a single serum marker or combinations of them are now established in clinical use, as Hyaluronan [56], AST-Platelet Ratio Index (APRI) [57], and fibrosis 4 sore (FIB-4) [58]. However, they are usually affected by a variety of factors, the differentiation of the

degree of hepatic fibrosis is not precise enough, and the prognostic value is not clear yet [59]. Conventional ultrasound, CT, and MRI have no specific findings for the diagnosis of mild-to-moderate liver fibrosis and provide a more accurate diagnosis only in patients with cirrhosis [60,61]. Transient elastography (TE) is easily interfered with by the patient's abdominal fat level, intercostal space width, ascites and other factors, limiting its use [62]. In addition, CT, MRI and TE are expensive and usually require an appointment in advance, which does not allow for timely examinations. In support, we conducted a series of bioinformatics analyses on DEGs and immunity between CHB and HBV-LF samples in the study. We identified the key genes and immune infiltration which were related to CHB and HBV-LF. We found that *CCL20* and *CD8A* were significantly up-regulated in HBV and HBV-LF samples, increasing gradually with the stage of histological fibrosis. ROC curve analysis was performed to determine the diagnostic value of these two genes for diseases. Therefore, we speculate that *CCL20* and *CD8A* play an important role in the pathological progression of chronic hepatitis B virus infection. In addition, enrichment analysis showed that *CCL20* and *CD8A* may promote LF through related signaling pathways. These results suggest that they could serve as effective diagnostic biomarkers for HBV-LF.

But, there are several limitations associated with our study. First, it would be better to have additional samples to verify the current findings especially clinically relevant information. Second, we just applied bioinformatics methods for data analysis, and further studies will be crucial to determine whether *CCL20* and *CD8A* could be used as predictive biomarkers for the diagnosis of HBV infection-induced liver fibrosis development. It also needs further study for elucidating the mechanism how the genes acting in the prevention and treatment of CHB and HBV-LF.

5. Conclusion

Recent studies revealed that immune response plays an crucial role in the pathological process of CHB [39], HBV infection induces various immune responses within the liver microenvironment, altering the liver "immune niche", and infiltration of immune cells is essential for the progression of HBV-LF [63]. In the study, we found that CCL20 and CD8A were significantly up-regulated in HBV and HBV-LF samples, increasing gradually with the stage of histological fibrosis. Our results suggest that CD8A may be involved in the progression of CHB via regulation of immune functions. ROC curve analysis was performed to determine the diagnostic value of these two genes for diseases. Therefore, we speculate that CCL20 and CD8A play an important role in the pathological progression of chronic hepatitis B virus infection. In addition, enrichment analysis showed that CCL20 and CD8A may promote LF through related signaling pathways. These results suggest that they could serve as effective diagnostic biomarkers for HBV-LF. It is instructive for research on the progression of LF in HBV patients, suppression of chronic inflammation, and development of molecularly targeted-therapy for HBV-LF.

Funding

This work was supported by the major project of Shanghai Municipal S and T Commission (no. 19401972300), Shandong Province Key R&D Program (Major Science and Technology Innovation Project, 2021CXGC010509), Shanghai Key Laboratory of Traditional Chinese Clinical Medicine, Key Disciplines of Liver and Gall Bladder Diseases, and Key Laboratory of Chronic Deficiency Liver Disease of the State Administration of Traditional Chinese Medicine of the People's Republic of China (20DZ2272200), Hangzhou Health and Health Commission (A20230658), Sub project supported by Shanghai Municipal Commission of Science and Technology (22YJ1900608), TCM prevention and treatment capacity building project for infectious diseases by Shanghai Municipal Health Commission (ZYYB-FRMZ-08).

Ethical approval

There were no ethical issues with our research. The human samples we used were obtained from public databases, and the patients had obtained ethical approval who were involved in the GEO database.

Consent to participate

Not applicable.

Consent for publication

All the authors agreed to publish the manuscript in the European Journal of Medical Research.

Data availability statement

Publicly available datasets were analyzed in this study. These data can be found at: GSE83148: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = GSE83148; GSE84044: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE84044.

CRediT authorship contribution statement

Jingru Song: Writing – original draft. **Lu Liu:** Methodology, Investigation, Conceptualization. **Zheng Wang:** Formal analysis. **Dong Xie:** Visualization, Software. **Nisma Lena Bahaji Azami:** Conceptualization. **Lu Lu:** Visualization. **Yanping Huang:**

Visualization. **Wei Ye:** Visualization. **Qin Zhang:** Visualization. **Mingyu Sun:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28329.

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