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Vimentin (VIM) predicts advanced liver fibrosis in chronic hepatitis B patients: A random forest-derived analysis

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Abstract. – OBJECTIVE: The crosstalk between Toll-like receptor 4 (TLR-4) and lipopolysaccharide (LPS) accounts for liver fibrosis progression. This study aimed to investigate the predictive performance of altered genes induced by TLR-4 and LPS challenge for advanced liver fibrosis.

MATERIALS AND METHODS: The overlapping differentially expressed genes (DEGs) of TLR-4 and LPS challenge models from the Gene Expression Omnibus (GEO) database were screened and included in the random forest analysis to identify potential candidates for predicting advanced liver fibrosis in the GSE84044 dataset. The roles of the identified candidates in liver injury development and activation of hepatic stellate cells (HSCs) were also addressed.

RESULTS: Among the overlapping DEGs in the GSE30485, GSE33446 and GSE166488 datasets, vimentin (VIM) was the most important gene for predicting advanced liver fibrosis (S ≥ 2) by the random forest model. In the GSE84044 dataset, VIM was positively correlated with liver fibrosis (r = 0.68, 95% CI = 0.57-0.76, p < 0.0001), and accurately predicted advanced liver fibrosis (AUC = 0.85, 95% CI = 0.78-0.91), both in males (AUC = 0.84, 95% CI = 0.76-0.92) and females (AUC = 0.87, 95% CI = 0.76-0.99). VIM was significantly upregulated in various liver diseases (cirrhosis, liver failure, chronic hepatitis B and fatty liver disease) and liver injury models (ANIT, BDL, CCl_4 and DMN). Additionally, VIM was correlated with HSC regulators (TGFβ, PDGF, CTGF and BMP7) and overexpressed in activated HSCs (p < 0.05). Enrichment analysis indicated that VIM-induced gene alterations were involved in the cytosolic DNA-sensing pathway, Toll-like receptor signaling pathway, etc.

CONCLUSIONS: VIM could predict advanced liver fibrosis in CHB patients and is mainly involved in the activation of HSCs and profibrotic signaling pathways.

Key Words:
TLR-4, Lipopolysaccharide, VIM, Liver fibrosis, Cirrhosis, Hepatic stellate cells, TGFβ.

Introduction

Liver fibrosis is the precursor to cirrhosis and liver cancer, which finally progress to liver failure and death[1,2]. A variety of chronic liver injuries, including virological, alcoholic and nonalcoholic, autoimmune, metabolic, and chemical-toxic liver diseases can lead to liver fibrosis[3]. Hepatic stellate cell (HSC) activation and excessive deposition of extracellular matrix (ECM) are the major pathophysiological changes during the development of liver fibrosis[4-6]. In addition, various signaling pathways are involved in liver fibrosis progression, such as transforming growth factor β (TGFβ) and nuclear factor-κB (NF-κB)[7,8]. Beyond this, intestinal bacterial microflora and a functional Toll-like receptor 4 (TLR4) are required for hepatic fibrogenesis, which can enhance TGFβ and NF-κB signaling pathways[9-11].

The TLR4/myeloid differentiation-2 (MD-2) heterodimer is a recognition molecule of lipopolysaccharide (LPS), which is a membrane component of Gram-negative bacteria[12,13]. The activated TLR4/MD-2/LPS complex triggers intracellular pathways resulting in the activation of nuclear transcription factors and promoting the production and accumulation of proinflammatory cytokines[14,15]. In wild type mice, LPS could markedly induce serum liver dysfunction and liver pathological changes, increase the levels of inflammatory cytokines, and upregulate TLR4 signaling cascade markers. Interestingly, these pathological changes are greatly alleviated in TLR4-knockout mice. LPS stimulation provokes the pathological responses in primary Kupffer cells isolated from wild type and TLR4-knockout mice[16-18]. Considering the crosstalk between LPS and TLR4, we assume that gene expression changes induced by LPS challenge and TLR4-mutant toxic liver inju-

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ries might contribute to the liver fibrosis progression and serve as predictive candidates.

Since liver fibrosis is the result of various chronic liver injuries and the precursor to end-stage liver damage, early diagnosis and assessment are critical for the reversal of this pathological stage[3]. In the present study, we aim to elucidate the gene alterations induced by LPS challenge and TLR4-mutant toxic liver injuries and investigate the correlations between these alterations and liver fibrosis progression, hoping that these data could provide novel diagnostic candidates, as well as useful insights into the pathogenesis and mechanisms of liver fibrosis.

Materials and Methods

Ethics Statement

All participants provided written informed consent. The protocol of the primary study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University, School of Medicine. The protocol of this secondary analysis was reviewed and approved by the Ethics Committee, Shanghai Public Health Clinical Center, Fudan University.

Patients

The GSE84044 dataset in the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database was used to screen candidates for predicting advanced liver fibrosis[19-21]. A total of 124 chronic hepatitis B (CHB) patients in this dataset who underwent liver biopsy were included. The age, sex, and histopathology stages of these participants were publicly available. As stated by Wang et al., the diagnosis of CHB patients was determined according to the criteria from the Asian Pacific Association for the Study of the Liver (APASL)[22]. Patients with the following conditions were excluded from the study: 1) Use of any antiviral therapies or immunosuppressive drugs within six months before sampling; 2) Infection with human immunodeficiency virus (HIV) or with a hepatitis virus other than hepatitis B virus (HBV), autoimmune liver disease, drug induced liver injury, alcoholic liver disease or hepatocellular carcinoma.

Outcome

The primary outcome was advanced liver fibrosis, which was defined as histological fibrosis staging $S \geq 2$. The histopathological diagnosis of all the liver biopsies of CHB patients was conducted by two experienced pathologists from the Pathology Department of Shanghai Fudan University, School of Medicine. The fibrosis staging (Scheuer S) and inflammation grading (Scheuer G) were calculated according to the Scheuer scoring system, namely, S 0-4 and G 0-4[23,24].

Differentially Expressed Genes (DEGs) Identification

This identification framework was applied to all GEO series included in this study. The affy package was used to normalize the microarray data of raw.cel files from each GEO dataset with the quantile method of robust multichip analysis (RMA)[25]. The limma package was used to compare gene expression[26]. Missing gene expression data were imputed with the k-nearest neighbor method by impute index[27]. The average gene expression was calculated when multiple probes of the genes existed. All platforms and samples of each microarray series were downloaded from the GEO database. DEGs were identified with the criterion of a $|\log2FC| > 1.0$ and adjusted p-value < 0.05.

The keywords "Toll-like receptor", "TLR4", "lipopolysaccharide", and "LPS" were used to search potential profiles for subsequent analysis in the GEO database. GSE33446, GSE30485, and GSE166488 were included in the analysis for the identification of overlapping DEGs. The GSE33446 and GSE30485 profiles compared the gene expression in the mouse liver induced by carbon tetrachloride (CCl_4) and diethylnitrosamine (DEN) in the TLR4 mutant models[28], and GSE166488 compared the gene expression in the mouse liver induced by LPS injection[29]. The details of these profiles are presented in the **Supplementary Materials**.

Enrichment Analysis

The Investigate Gene Sets tool of the Molecular Signatures Database (MSigDB) version 7.5.1 in the Gene Set Enrichment Analysis (GSEA, http://www.gsea-msigdb.org/) was used for functional enrichment of the DEGs[30,31]. Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome pathway analyses and Gene Ontology (GO) analyses were performed in the GSEA database. The enrichment terms with a false discovery rate (FDR) less than 0.05 were included. Human or mouse species were determined by the sample type.

Random Forest Model Establishment

The randomForest package[32] was used to screen potential candidates for predicting advanced liver fibrosis. The 124 CHB patients in the GSE84044 dataset were randomly divided into training and validation cohorts in a 5:5 ratio using the "caret" package. In the process of constructing the random forest model, the variable importance of the output results of the Gini coefficient method was calculated from the perspective of decreasing accuracy and decreasing mean square error. DEGs with an importance greater than 3 were identified as candidate genes for subsequent analysis[33].

Statistical Analysis

Differences in gene expression between the individual groups were analyzed using Student's *t*-test, the Mann-Whitney test or two-way ANOVA based on variable types by GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). The OptimalCutpoints package[34] in the R program was used to perform ROC analysis to evaluate the predictive values of potential candidates for the liver fibrosis stage. Correlation analysis was addressed by Spearman or Pearson methods. Stata software version 16.1 (Stata Corp LLC, TX, USA) was used for logistic regression and correlation analysis. A two-tailed $p < 0.05$ was considered significant.

Results

Overlapping DEGs of TLR4-Mutant Toxic and LPS-Induced Liver Injury Models

A total of 49 overlapping DEGs were identified among GSE33446, GSE30485, and GSE166485 (Figure 1A). As summarized in Figure 1B, enrichment analysis indicated that these DEGs were mainly involved in biological processes (BP) including defense response, programmed cell death, and regulation of apoptosis. These DEGs had the molecular function (MF) in Toll-like receptor 4 binding. Additionally, the cellular component (CC) of these overlapping DEGs was collagen containing extracellular matrix. Reactome analysis showed that these overlapping DEGs were significantly enriched in the innate immune system, neutrophil degranulation, etc. (Figure 1B). Since 15 DEGs (Al426330, AW112010, Ces1e, Gm2788, Hsd3b5, Ifi-27l2a, Ly6a, Mup5, Mup10, Slco1a1, Slfn4, Ugt2b1, 4921539H07Rik, 4933438K21Rik, and 9130409I23Rik) were not expressed in human species, the levels of CD163, CD52, CTSC, DYNLL1, ECSCR, EGR1, EVI2A, GPCPD1, ICAM1, IFI44, LCN2, LGALS3, ME1, MOXD1, NCF4, NUCB2, PBK, PROCR, S100A9, and VIM were significantly upregulated in CHB patients with liver fibrosis S stage ≥ 2 ($p < 0.05$, Figure 1C), while APOA4 and NUPR1 were significantly downregulated in this population ($p < 0.001$, Figure 1C).

Random Forest Model Establishment

The baseline characteristics of the subjects were described in Table I. All 34 genes presented in Figure 1C, together with age and sex, were input into the random forest classifier. The number of variables was as small as possible, and the out-of-band error was as low as possible. According to the relationship plot between the model error and the number of decision trees, 500 trees were selected as the parameter of the final model. When the number of trees was > 100, the error of the model was stable (Figure 2A). The variables included in the random forest model are presented in Figure 2B. Among these, VIM, with an importance greater than 3, was selected for the potential candidate gene for subsequent analysis (Figure 2B).

Predictive Values of VIM for Advanced Liver Fibrosis

In the GSE84044 dataset, the VIM levels were significantly correlated with liver fibrosis stage ($r = 0.68$, 95% CI = 0.57 – 0.76, $p < 0.0001$, Figure 3A), and the expression of VIM gradually increased with increasing liver fibrosis stage ($p < 0.05$, Figure 3A). ROC analysis indicated that VIM with a cutoff of 6.0 could accurately predict advanced liver fibrosis S ≥ 2 (AUC = 0.85, 95% CI = 0.78-0.91, Figure 3B), and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 0.79, 0.77, 0.78, and 0.79 respectively (Figure 3B). Subgroup analysis showed that VIM was a good predictor for liver fibrosis S ≥ 2 in males and females, respectively (for males, cutoff = 6.1, AUC = 0.84, 95% CI = 0.76-0.92; for females, cutoff = 6.1, AUC = 0.87, 95% CI = 0.76-0.99; Figure 3C and Figure 3D).

VIM Expression in Liver Diseases and Liver Injury Models

As shown in Figure 4, VIM was significantly upregulated in cirrhosis patients compared to normal controls ($p < 0.05$, GSE7741[35], GSE14323[36] and GSE103580[37], Figure 4A). The levels of VIM were significantly higher in patients with

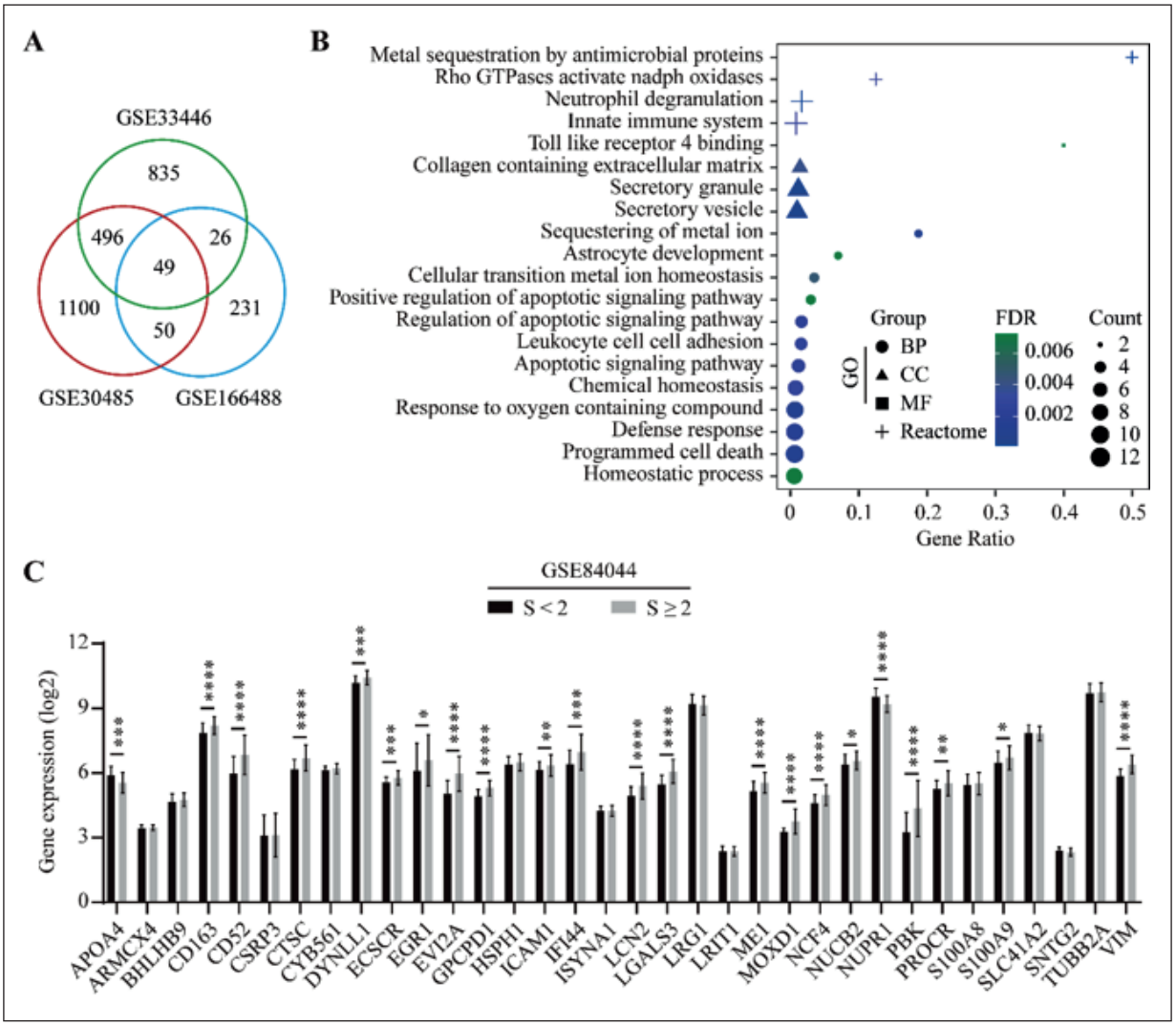

Figure 1. The Overlapping differentially expressed genes (DEGs) in the GSE33446, GSE30485 and GSE166488 datasets (**A**), functional enrichment of these DEGs (**B**) and the expression comparison between liver fibrosis S < 2 and S ≥ 2 in the chronic hepatitis B patients (**C**).

HBV-related liver failure than in healthy individuals ($p < 0.001$, GSE14668[38], GSE38941[39] and GSE96851[40], Figure 4B) and significantly elevated in HBV patients in the immune clearance stage compared to inactive carriers or immune tolerant patients ($p < 0.05$, GSE65359, Figure 4B). In addition, interferon therapy significantly decreased VIM expression in CHB patients ($p < 0.001$, GSE66698[41], Figure 4B). Similarly, VIM was significantly overexpressed in patients suffering from NAFLD or NASH compared to normal controls ($p < 0.05$, GSE35961[42], GSE59045[43] and GSE63067[44], Figure 4C), and it was also significantly upregulated in the high-fat-diet model ($p < 0.05$, GSE57425[45] and GSE23740, Figure 4C).

In addition, VIM was significantly upregulated in liver injury models, namely, ANIT ($p < 0.01$, GSE122184[46] and GSE72387[47], Figure 4D), BDL ($p < 0.0001$, GSE152494[48], Figure 4E), CCl_4 ($p < 0.05$, GSE167033[49], GSE122184[46] and GSE89147[50], Figure 4F), and DMN ($p < 0.001$, GSE122184[46], GSE68110[51], GSE58032[52] and GSE44783[53], Figure 4G) compared to normal livers. The levels of VIM were also significantly overexpressed in LPS-induced livers compared to normal controls in the GSE37546[54] and GSE55084[55] datasets ($p < 0.05$, Figure 4H).

Correlation Between VIM and HSCs

Since the activation of HSCs is the core process of liver fibrosis, the VIM levels in HSCs were in-

Table I. Baseline characteristics of CHB patients included in this study.

Variables	S < 2 (n = 62)	S ≥ 2 (n = 62)	p
Age, median (Interquartile range, IQR)	35.5 (31, 46)	43.5 (37, 55)	0.001
Male, n (%)	48 (77.4)	40 (64.5)	0.114
G grade, n (%)			< 0.001
0	30 (48.4)	7 (11.3)	
1	21 (33.9)	12 (19.4)	
2	10 (16.1)	24 (38.7)	
3	1 (1.6)	14 (22.6)	
4	0 (0)	5 (8.1)	

vestigated in four GEO series. As shown in Figure 4A, VIM was significantly upregulated in growing HSCs compared to senescent HSCs ($p < 0.05$, GSE11954[56], Figure 5A), myofibroblasts transited HSCs compared to quiescent HSCs ($p < 0.0001$, GSE34949[57], Figure 5A), HSCs compared to liver stem/progenitor cells, hepatocytes, and liver sinusoidal endothelial cells ($p < 0.01$, GSE49995[58] and GSE68000[59], Figure 5A), and activated HSCs compared to quiescent HSCs and reverted HSCs ($p < 0.05$, GSE68000[59] and GSE68001[60], Figure 5A).

TGFβ, PDGF, and CTGF are the main activators of HSCs, and BMP7 is an inhibitor of HSCs. The correlations between VIM and these HSC regulators in the GSE84044 dataset are presented in Figure 5B. The HSC regulators with correlation coefficient $|r| \geq$

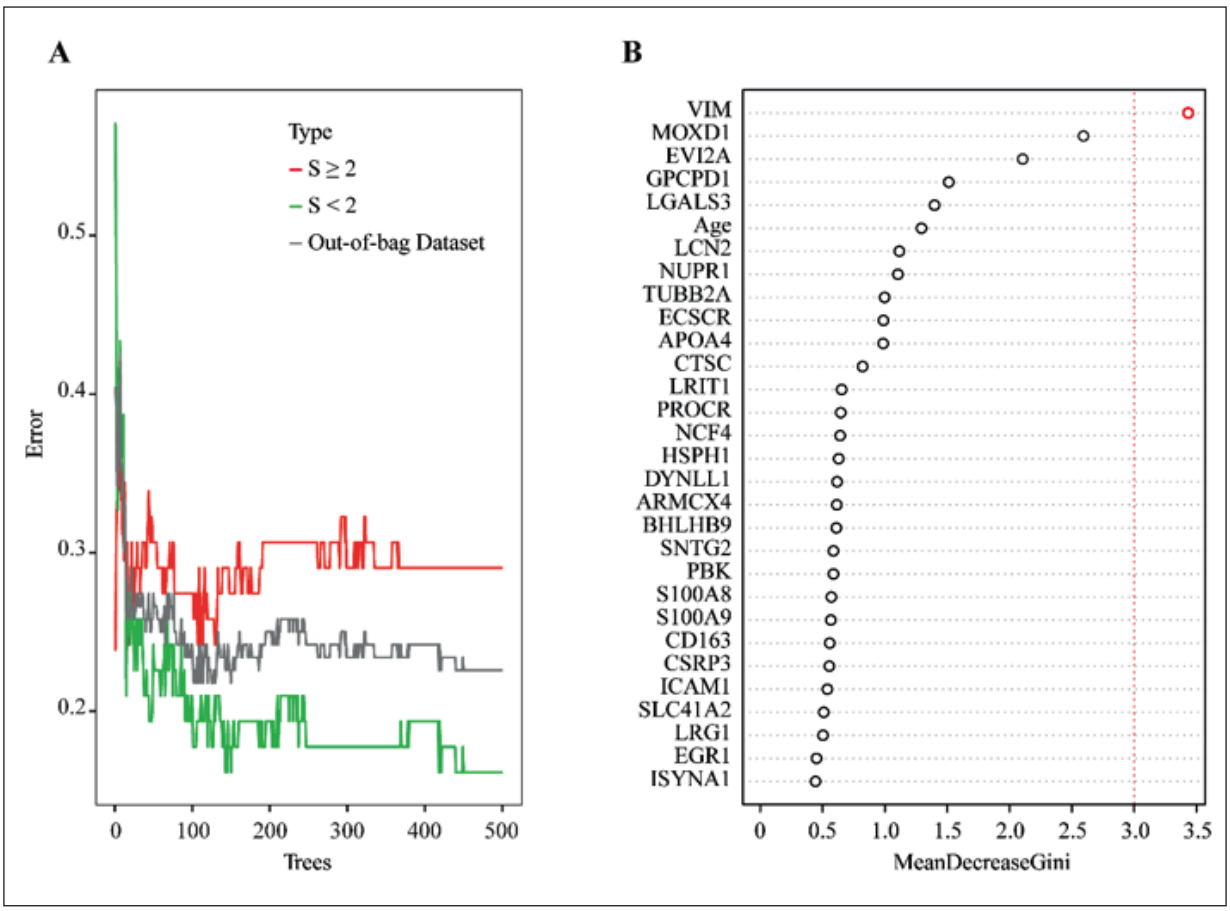

Figure 2. The establishment of random forest. The error of random forest trees (**A**), and the variable importance of the output results of the Gini coefficient method (**B**).

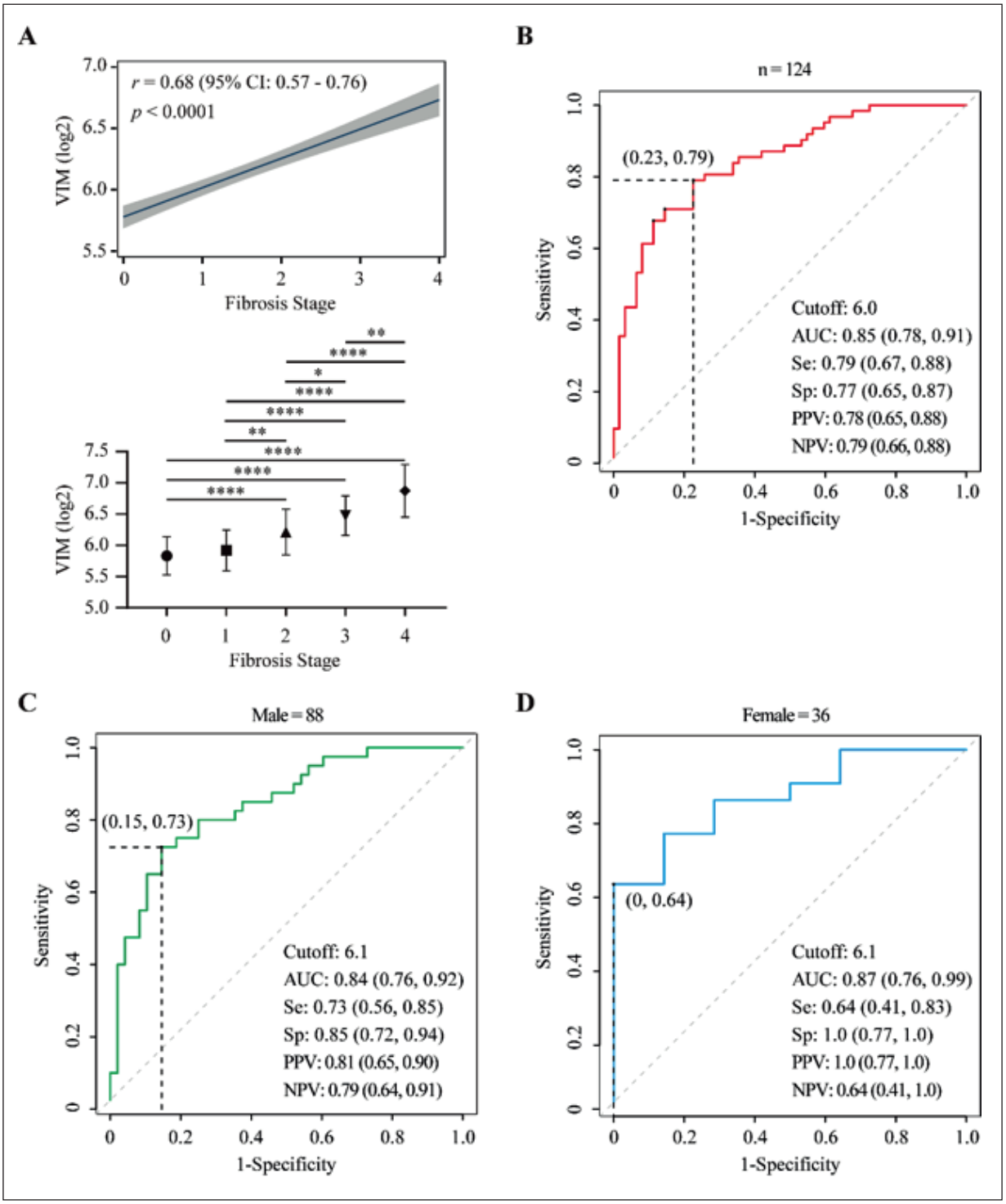

Figure 3. The correlation and expression of VIM in liver fibrosis (**A**), the ROC of VIM for predicting advanced liver fibrosis in CHB patients (**B**), and in male (**C**) and female (**D**) subgroups.

0.5 is detailed in Figure 5C. VIM was positively correlated with TGFβ2, PDGFA, PDGFD, and CTGF ($p < 0.0001$, Figure 5C) and negatively correlated with BMP7 ($p < 0.0001$, Figure 5C).

Signaling Pathways and GO Enrichment of VIM-Induced DEGs

We searched the GEO database with the keywords "VIM" and "vimentin", and the GSE63653

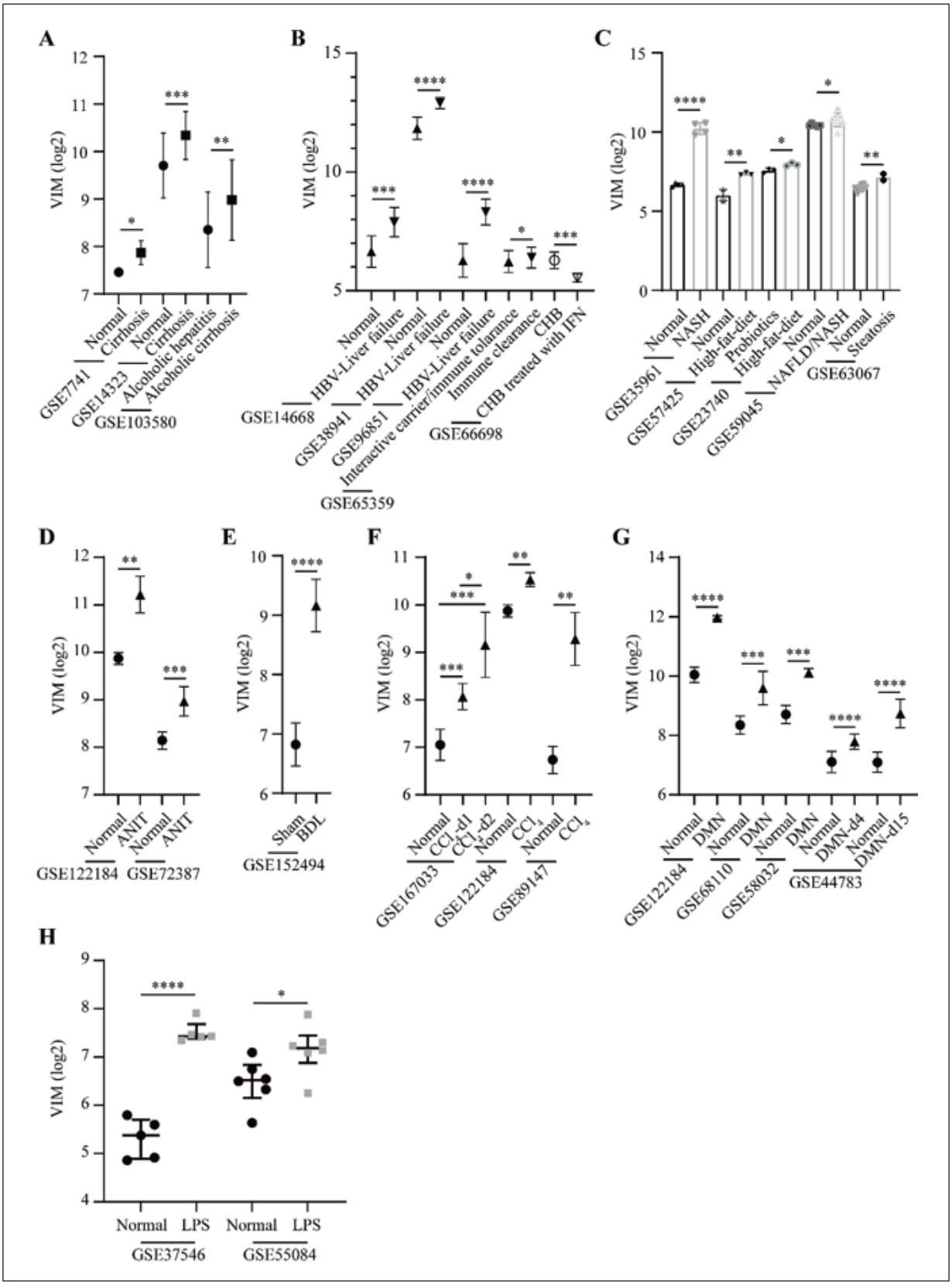

Figure 4. The VIM expression in cirrhosis (**A**), HBV infected patients (**B**), fatty liver diseases (**C**), ANIT (**D**), BDL (**E**), CCl4 (**F**), DMN (**G**), and LPS-induced liver injury (**H**) models.

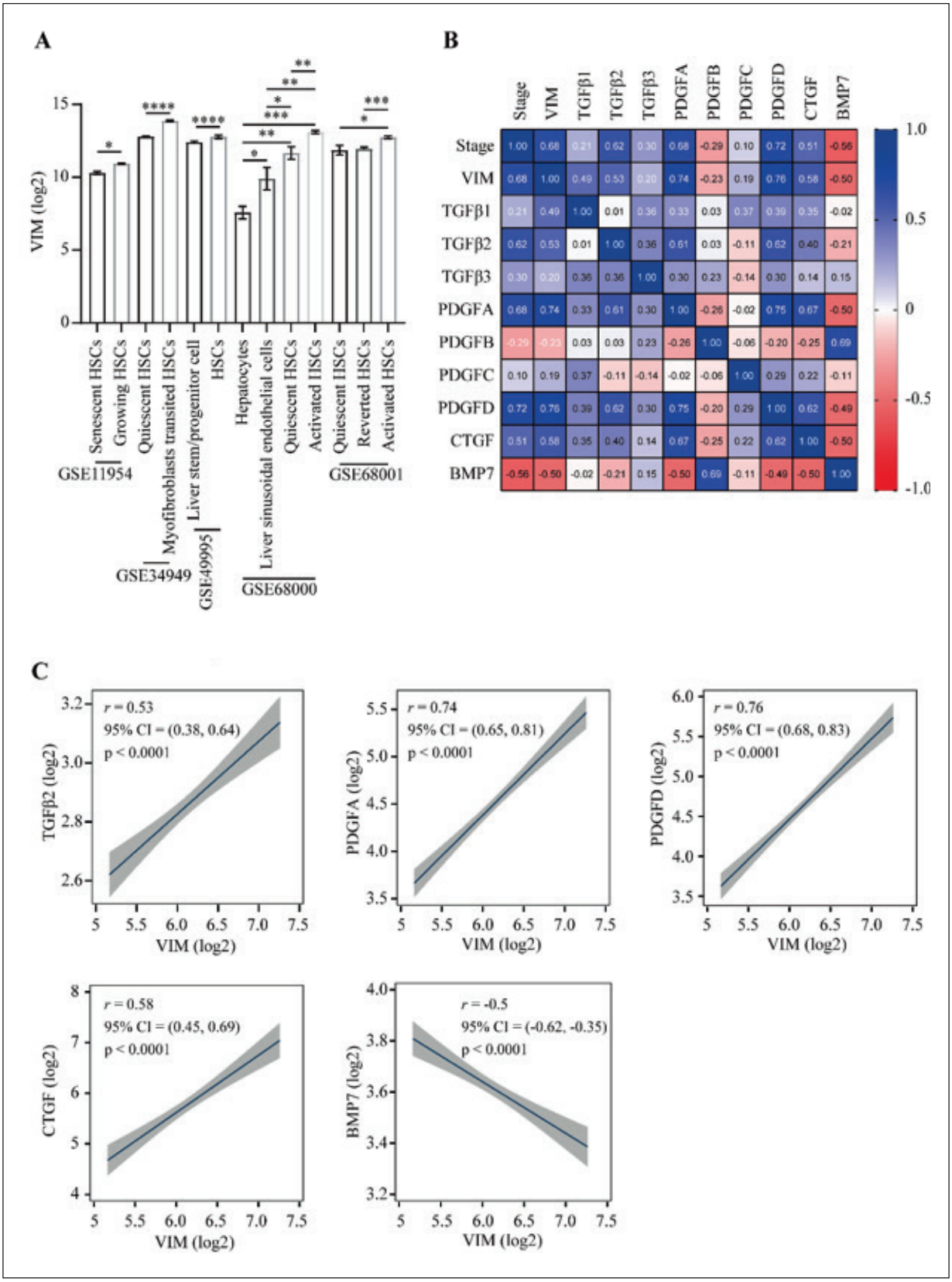

Figure 5. The VIM expression in hepatis stellate cells (**A**); the correlation coefficient between VIM and HSC regulators (**B**); the statistics of markers correlated with VIM with coefficient $|r| \geq 0.5$ (**C**).

dataset was included for subsequent analysis of functional enrichment. The global gene expression profiling of bone marrow-derived macrophages from vimentin-deficient or wild-type littermates on a C57BL/6 background was elucidated in the GSE63653 dataset. As shown in Figure 6A, 176 DEGs were identified with 140 upregulated and 36 downregulated (Figure 6A). GSEA indicated that these genes were significantly enriched in KEGG pathways, including the cytosolic DNA-sensing pathway, Toll-like signaling pathway, RIG-like receptor signaling pathway, and renin-angiotensin system ($p < 0.001$, Figure 6B). Reactome analysis showed that these DEGs mainly were involved in interferon signaling, cytokine signaling, and antiviral responses (all $p < 0.001$, Figure 6C). The top ten GO biological process, cellular component and molecular function terms of these DEGs induced by the VIM-deficient pattern are presented in Figure 6D.

Discussion

VIM is a type of intermediate filament protein displayed in normal stellate cells[61]. Emerging evidence indicates that VIM contributes to cell migration, adhesion, macrophage-like cell activation, inflammation and apoptosis[62-65]. Previous studies[66-68] revealed that VIM overexpression accounted for the activation of HSCs during liver fibrosis. Even though VIM was significantly increased in early-stage fibrosis in chronic hepatitis C and NAFLD patients compared to healthy individuals, no relevance was observed between VIM expression and disease severity[68]. Currently, the exact role of VIM in predicting liver fibrosis is not fully understood.

In this comprehensive analysis, we found that VIM is significantly upregulated in advanced liver fibrosis and increases gradually with disease severity. The ROC results showed that VIM is an accurate predictor for advanced liver fibrosis in CHB patients. VIM plays a role in stabilizing intracellular structure, wound healing and tissue regeneration, and is a widely recognized phenotypic marker of fibroblasts and of epithelial-mesenchymal transition[65,69]. VIM is a key regulator of fibrosis, and VIM knockout mice are widely used to explore the process of fibrosis, but not frequently in hepatic fibrosis[70]. VIM is expressed in mesenchymal cells with very high expression in myofibroblasts[71]. In the liver, VIM is predominantly expressed in HSCs and is also present in vascular smooth muscle cells and portal fibroblasts[72-74]. The expression of VIM in liver fibrosis is still controversial. Immunohistochemistry identified that VIM was positive in fibrotic areas in human liver fibrosis patients[75]. Endogenous VIM was remarkably expressed in the CCl_4 and BDL-induced liver fibrosis models[76], which is consistent with our results. In another report[77], VIM was upregulated in nonorthotopic liver transplantation (OLT) and post-LOT patients compared to nonfibrosis populations, but did not reach statistical significance. In CCl4-induced liver fibrosis, VIM was not observed in the hepatocytes, even upon stimulation with TGFβ1[78]. Our results may further explain the role of VIM in the progression of liver fibrosis. Given the previous reports and our results, we believe that VIM should be a promising predictor for advanced liver fibrosis in clinical practice.

Our results showed that VIM is involved in the activation of HSCs and might be linked to profibrotic signaling pathways, including TGFβ, TLR4, and NF-κB. VIM regulates the assembly of focal adhesions through collagen and affects signaling pathways that control extracellular matrix remodeling[69,79]. A previous report[80] revealed that CCl_4 and dextran sulfate sodium treatment could increase the expression of VIM, TGFβ, TLR4, and NF-κB p65, leading to activation of HSCs and the TLR4 signaling pathway. In addition, VIM mRNA in primary hepatocytes was downregulated in matrix metalloproteinase-19 (MMP-19) knockout mice, which showed a decreased response to TGFβ1 stimulation[81]. Unfortunately, we have not investigated the causality of VIM upregulation, profibrotic pathways, and HSC activation through experimental assays. Future research should focus on the clinical application and basic mechanisms of VIM in the development of liver fibrosis.

Some limitations existed in this study. First, this was a secondary analysis from a public database with inadequate clinical characteristics; for example, liver function tests, serum liver fibrosis parameters and virological markers were not obtained and adjusted, which may have resulted in biases in the predictive power of VIM for advanced liver fibrosis. Second, the serum VIM levels in liver fibrosis patients were not addressed, which limits the application of this candidate in clinical practice in the current situation. Third, the mechanisms of VIM in the progression of liver fi-

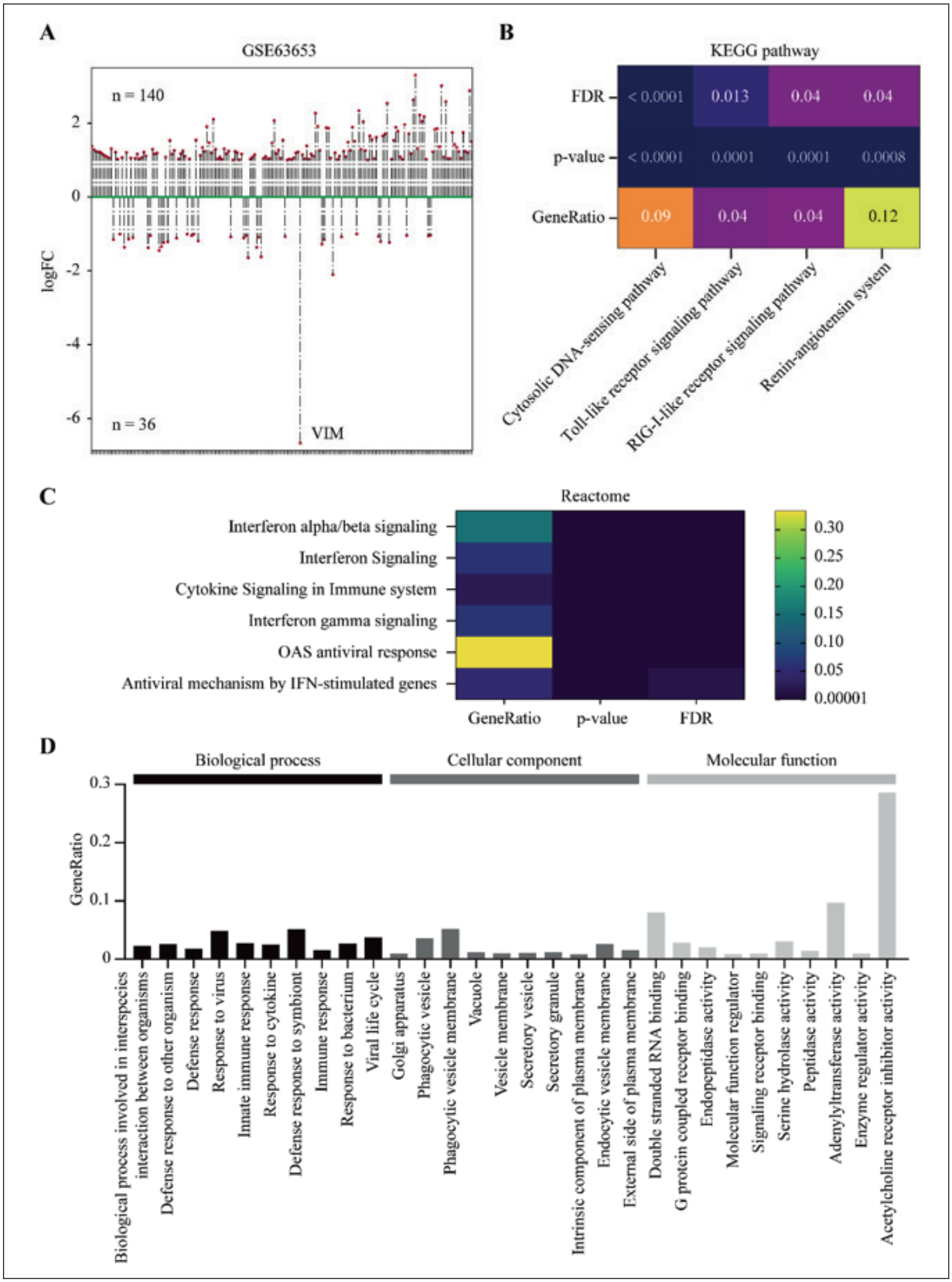

Figure 6. The DEGs induced by VIM knockout in macrophages in the GSE63653 (**A**), and the KEGG (**B**), Reactome (**C**) and GO enrichment (**D**) of these DEGs.

brosis were discussed superficially in this study, and no experimental clarifications were conducted *in vivo* and *in vitro*. Fourth, VIM might be a novel diagnostic candidate for liver fibrosis, but research on VIM as a therapeutic target still needs to go further. Considering the complicated mechanisms of liver fibrosis, genetic and epigenetic aspects of VIM in liver fibrosis progression should be investigated in depth.

Conclusions

In this study, we identified VIM as a promising predictor for advanced liver fibrosis in CHB patients. Additionally, VIM was widely upregulated in various liver injuries and was involved in the activation of HSCs and HSC regulators. Moreover, VIM might be linked to profibrotic signaling pathways, including TGFβ, TLR4, and NF-κB. Considering the current evidence, VIM should be used as a novel diagnostic candidate for liver fibrosis. More prospective studies should be conducted to evaluate the diagnostic values of serum VIM for liver fibrosis.

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Authors' Contributions

Z.Y. and W.Z. conceived and designed the study. W.W. wrote the manuscript. Z.Y., and W.Z. rewriting the manuscript. W.W., W.Z., and Z.Y. analyzed and interpreted the data. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declared no conflicts of interest in this work.

Data Availability

Datasets of the current study are available from the NCBI Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). All the datasets were available from Dr. Zongguo Yang upon reasonable request.

References

1) Brenner DA. Molecular pathogenesis of liver fibrosis. Trans Am Clin Climatol Assoc 2009; 120: 361-368.
2) Rockey DC, Bell PD, Hill JA. Fibrosis--a common pathway to organ injury and failure. N Engl J Med 2015; 372: 1138-1149.
3) Wang FD, Zhou J, Chen EQ. Molecular Mechanisms and Potential New Therapeutic Drugs for Liver Fibrosis. Front Pharmacol 2022; 13: 787748.
4)Chen ZK, Chen DZ, Cai C, Jin LL, Xu J, Tu YL, Huang XZ, Xu JL, Chen MZ, Xue FB, Lan XL, Wang XD, Ge YL, Sun HL, Chen YP. BMSCs attenuate hepatic fibrosis in autoimmune hepatitis through regulation of LMO7-AP1-TGFβ signaling pathway. Eur Rev Med Pharmacol Sci 2021; 25: 1600-1611.
5) Elpek GO. Cellular and molecular mechanisms in the pathogenesis of liver fibrosis: An update. World J Gastroenterol 2014; 20: 7260-7276.
6) Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008; 88: 125-172.
7) Peng D, Fu M, Wang M, Wei Y, Wei X. Targeting TGF-beta signal transduction for fibrosis and cancer therapy. Mol Cancer 2022; 21: 104.
8) Zhao J, Cheng J. [Research progress of signaling pathways in liver fibrosis]. Zhonghua Gan Zang Bing Za Zhi 2019; 27: 403-406.
9) Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 2007; 13: 1324-1332.
10) Ince MN, Elliott DE, Setiawan T, Blum A, Metwali A, Wang Y, Urban JF Jr, Weinstock JV. Heligmosomoides polygyrus induces TLR4 on murine mucosal T cells that produce TGFbeta after lipopolysaccharide stimulation. J Immunol 2006; 176: 726-729.
11) Khedr LH, Rahmo RM, Farag DB, Schaalan MF, El Magdoub HM. Crocin attenuates cisplatin-induced hepatotoxicity via TLR4/NF-kappaBp50 signaling and BAMBI modulation of TGF-beta activity: Involvement of miRNA-9 and miRNA-29. Food Chem Toxicol 2020; 140: 111307.
12) Kimoto M, Nagasawa K, Miyake K. Role of TLR4/MD-2 and RP105/MD-1 in innate recognition of lipopolysaccharide. Scand J Infect Dis 2003; 35: 568-572.
13) Park BS, Lee JO. Recognition of lipopolysaccharide pattern by TLR4 complexes. Exp Mol Med 2013; 45: e66.
14) Cochet F, Peri F. The Role of Carbohydrates in the Lipopolysaccharide (LPS)/Toll-Like Receptor 4 (TLR4) Signalling. Int J Mol Sci 2017; 18: 2318.
15) Zamyatina A, Heine H. Lipopolysaccharide Recognition in the Crossroads of TLR4 and Caspase-4/11 Mediated Inflammatory Pathways. Front Immunol 2020; 11: 585146.
16) Chen SN, Tan Y, Xiao XC, Li Q, Wu Q, Peng YY, Ren J, Dong ML. Deletion of TLR4 attenuates lipopolysaccharide-induced acute liver injury by inhibiting inflammation and apoptosis. Acta Pharmacol Sin 2021; 42: 1610-1619.

17) Guo W, Xiang Q, Mao B, Tang X, Cui S, Li X, Zhao J, Zhang H, Chen W. Protective Effects of Microbiome-Derived Inosine on Lipopolysaccharide-Induced Acute Liver Damage and Inflammation in Mice via Mediating the TLR4/NF-kappaB Pathway. J Agric Food Chem 2021; 69: 7619-7628.
18) Khan HU, Aamir K, Jusuf PR, Sethi G, Sisinthy SP, Ghildyal R, Arya A. Lauric acid ameliorates lipopolysaccharide (LPS)-induced liver inflammation by mediating TLR4/MyD88 pathway in Sprague Dawley (SD) rats. Life Sci 2021; 265: 118750.
19) Wang M, Gong Q, Zhang J, Chen L, Zhang Z, Lu L, Yu D, Han Y, Zhang D, Chen P, Zhang X, Yuan Z, Huang J, Zhang X. Characterization of gene expression profiles in HBV-related liver fibrosis patients and identification of ITGBL1 as a key regulator of fibrogenesis. Sci Rep 2017; 7: 43446.
20) Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013; 41: D991-995.
21) Wilhite SE, Barrett T. Strategies to explore functional genomics data sets in NCBI's GEO database. Methods Mol Biol 2012; 802: 41-53.
22) Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GKK, Locarnini S, Chronic Hepatitis B Guideline Working Party of the Asian-Pacific Association for the Study of the Liver. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2008; 2: 263-283.
23) Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994; 19: 1513-1520.
24) Goodman ZD. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol 2007; 47: 598-607.
25) Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20: 307-315.
26) Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.
27) Dong X, Lin L, Zhang R, Zhao Y, Christiani DC, Wei Y, Chen F. TOBMI: trans-omics block missing data imputation using a k-nearest neighbor weighted approach. Bioinformatics 2019; 35: 1278-1283.
28) Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabanian H, Adeyemi A, Bataller R, Lefkowitch JH, Bower M, Friedman R, Sartor RB, Rabadan R, Schwabe RF. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell 2012; 21: 504-516.
29) Holland CH, Ramirez Flores RO, Myllys M, Hassan R, Edlund K, Hofmann U, Marchan R, Cadenas C, Reinders J, Hoehme S, Seddek AL, Dooley S, Keitel V, Godoy P, Begher-Tibbe B, Trautwein C, Rupp C, Mueller S, Longerich T, Hengstler JG, Saez-Rodriguez J, Ghallab A. Transcriptomic Cross-Species Analysis of Chronic Liver Disease Reveals Consistent Regulation Between Humans and Mice. Hepatol Commun 2022; 6: 161-177.
30) Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst 2015; 1: 417-425.
31) Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545-15550.
32) Le TT, Simmons WK, Misaki M, Bodurka J, White BC, Savitz J, McKinney BA. Differential privacy-based evaporative cooling feature selection and classification with relief-F and random forests. Bioinformatics 2017; 33: 2906-2913.
33) Tian Y, Yang J, Lan M, Zou T. Construction and analysis of a joint diagnosis model of random forest and artificial neural network for heart failure. Aging (Albany NY) 2020; 12: 26221-26235.
34) López-Ratón M, Rodríguez-Álvarez MX, Cadarso-Suárez C, Gude-Sampedro F. OptimalCutpoints: An R package for selecting optimal cutpoints in diagnostic tests. J Stat Softw 2014; 61: 1-36.
35) Khalid SS, Hamid S, Siddiqui AA, Qureshi A, Qureshi N. Gene profiling of early and advanced liver disease in chronic hepatitis C patients. Hepatol Int 2011; 5: 782-788.
36) Mas VR, Maluf DG, Archer KJ, Yanek K, Kong X, Kulik L, Freise CE, Olthoff KM, Ghobrial RM, McIver P, Fisher R. Genes involved in viral carcinogenesis and tumor initiation in hepatitis C virus-induced hepatocellular carcinoma. Mol Med 2009; 15: 85-94.
37) Trépo E, Goossens N, Fujiwara N, Song WM, Colaprico A, Marot A, Spahr L, Demetter P, Sempoux C, Im GY, Saldarriaga J, Gustot T, Devière J, Thung SN, Minsart C, Sersté T, Bontempi G, Abdelrahman K, Henrion J, Degré D, Lucidi V, Rubbia-Brandt L, Nair VD, Moreno C, Deltenre P, Hoshida Y, Franchimont D. Combination of Gene Expression Signature and Model for End-Stage Liver Disease Score Predicts Survival of Patients With Severe Alcoholic Hepatitis. Gastroenterology 2018; 154: 965-975.
38) Farci P, Diaz G, Chen Z, Govindarajan S, Tice A, Agulto L, Pittaluga S, Boon D, Yu C, Engle RE, Haas M, Simon R, Purcell RH, Zamboni F. 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 U S A 2010; 107: 8766-8771.
39) Nissim O, Melis M, Diaz G, Kleiner DE, Tice A, Fantola G, Zamboni F, Mishra L, Farci P. Liver regeneration signature in hepatitis B virus (HBV)-associated acute liver failure identified by gene expression profiling. PLoS One 2012; 7: e49611.
40) Chen Z, Diaz G, Pollicino T, Zhao H, Engle RE, Schuck P, Shen CH, Zamboni F, Long Z, Kabat J, Battista DD, Bock KW, Moore IN, Wollenberg K, Soto C, Govindarajan S, Kwong PD, Kleiner

DE, Purcell RH, Farci P. Role of humoral immunity against hepatitis B virus core antigen in the pathogenesis of acute liver failure. Proc Natl Acad Sci U S A 2018; 115: E11369-E11378.
41) Wu HL, Hsiao TH, Chen PJ, Wong SH, Kao JH, Chen DS, Lu JY, Lu TP, Chen Y, Chuang EY, Tu HC, Liu CJ. Liver Gene Expression Profiles Correlate with Virus Infection and Response to Interferon Therapy in Chronic Hepatitis B Patients. Sci Rep 2016; 6: 31349.
42) Kita Y, Takamura T, Misu H, Ota T, Kurita S, Takeshita Y, Uno M, Matsuzawa-Nagata N, Kato KI, Ando H, Fujimura A, Hayashi K, Kimura T, Ni Y, Otoda T, Miyamoto K, Zen Y, Nakanuma Y, Kaneko S. Metformin prevents and reverses inflammation in a non-diabetic mouse model of nonalcoholic steatohepatitis. PLoS One 2012; 7: e43056.
43) du Plessis J, van Pelt J, Korf H, Mathieu C, van der Schueren B, Lannoo M, Oyen T, Topal B, Fetter G, Nayler S, van der Merwe T, Windmolders P, Gaal LV, Verrijken A, Hubens G, Gericke M, Cassiman D, Francque S, Nevens F, van der Merwe S. Association of Adipose Tissue Inflammation With Histologic Severity of Nonalcoholic Fatty Liver Disease. Gastroenterology 2015; 149: 635-648.e14.
44) Frades I, Andreasson E, Mato JM, Alexandersson E, Matthiesen R, Martínez-Chantar ML. Integrative genomic signatures of hepatocellular carcinoma derived from nonalcoholic Fatty liver disease. PLoS One 2015; 10: e0124544.
45) Lu Y, Liu X, Jiao Y, Xiong X, Wang E, Wang X, Zhang Z, Zhang H, Pan L, Guan Y, Cai D, Ning G, Li X. Periostin promotes liver steatosis and hypertriglyceridemia through downregulation of PPARα. J Clin Invest 2014; 124: 3501-3513.
46) Rao MS, Vleet TRV, Ciurlionis R, Buck WR, Mittelstadt SW, Blomme EAG, Liguori MJ. Comparison of RNA-Seq and Microarray Gene Expression Platforms for the Toxicogenomic Evaluation of Liver From Short-Term Rat Toxicity Studies. Front Genet 2019; 9: 636.
47) Church RJ, Otieno M, McDuffie JE, Singh B, Sonee M, Hall L, Watkins PB, Ellinger-Ziegelbauer H, Harrill AH. Beyond miR-122: Identification of MicroRNA Alterations in Blood During a Time Course of Hepatobiliary Injury and Biliary Hyperplasia in Rats. Toxicol Sci 2016; 150: 3-14.
48) Gijbels E, Vilas-Boas V, Annaert P, Vanhaecke T, Devisscher L, Vinken M. Robustness testing and optimization of an adverse outcome pathway on cholestatic liver injury. Arch Toxicol 2020; 94: 1151-1172.
49) Holland CH, Flores ROR, Myllys M, Hassan R, Edlund K, Hofmann U, Marchan R, Cadenas C, Reinders J, Hoehme S, Seddek AL, Dooley S, Keitel V, Godoy P, Begher-Tibbe B, Trautwein C, Rupp C, Mueller S, Longerich T, Hengstler JG, Saez-Rodriguez J, Ghallab A. Transcriptomic Cross-Species Analysis of Chronic Liver Disease Reveals Consistent Regulation Between Humans and Mice. Hepatol Commun 2022; 6: 161-177.
50) Zhang K, Han X, Zhang Z, Zheng L, Hu Z, Yao Q, Cui H, Shu G, Si M, Li C, Shi Z, Chen T, Han Y, Chang Y, Yao Z, Han T, Hong W. The liver-enriched lnc-LFAR1 promotes liver fibrosis by activating TGFβ and Notch pathways. Nat Commun 2017; 8: 144.
51) Römer M, Eichner J, Metzger U, Templin MF, Plummer S, Ellinger-Ziegelbauer H, Zell A. Cross-platform toxicogenomics for the prediction of non-genotoxic hepatocarcinogenesis in rat. PLoS One 2014; 9: e97640.
52) Ali MAE, Naka K, Yoshida A, Fuse K, Kasada A, Hoshii T, Tadokoro Y, Ueno M, Ohta K, Kobayashi M, Takahashi C, Hirao A. Association of a murine leukaemia stem cell gene signature based on nucleostemin promoter activity with prognosis of acute myeloid leukaemia in patients. Biochem Biophys Res Commun 2014; 450: 837-843.
53) Eichner J, Kossler N, Wrzodek C, Kalkuhl A, Toft DB, Ostenfeldt N, Richard V, Zell A. A toxicogenomic approach for the prediction of murine hepatocarcinogenesis using ensemble feature selection. PLoS One 2013; 8: e73938.
54) Herrema H, Derks TGJ, van Dijk TH, Bloks VW, Gerding A, Havinga R, Tietge UJF, Müller M, Smit GPA, Kuipers F, Reijngoud DJ. Disturbed hepatic carbohydrate management during high metabolic demand in medium-chain acyl-CoA dehydrogenase (MCAD)-deficient mice. Hepatology 2008; 47: 1894-1904.
55) Oshida K, Vasani N, Thomas RS, Applegate D, Gonzalez FJ, Aleksunes LM, Klaassen CD, Corton JC. Screening a mouse liver gene expression compendium identifies modulators of the aryl hydrocarbon receptor (AhR). Toxicology 2015; 336: 99-112.
56) Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, Miething C, Yee H, Zender L, Lowe SW. Senescence of activated stellate cells limits liver fibrosis. Cell 2008; 134: 657-667.
57) Chen Y, Choi SS, Michelotti GA, Chan IS, Swiderska-Syn M, Karaca GF, Xie G, Moylan CA, Garibaldi F, Premont R, Suliman HB, Piantadosi CA, Diehl AM. Hedgehog controls hepatic stellate cell fate by regulating metabolism. Gastroenterology 2012; 143: 1319-1329.e11.
58) Berardis S, Lombard C, Evraerts J, Taghdouini AE, Rosseels V, Sancho-Bru P, Lozano JJ, van Grunsven L, Sokal E, Najimi M. Gene expression profiling and secretome analysis differentiate adult-derived human liver stem/progenitor cells and human hepatic stellate cells. PLoS One 2014; 9: e86137.
59) El Taghdouini A, Sørensen AL, Reiner AH, Coll M, Verhulst S, Mannaerts I, Øie CI, Smedsrød B, Najimi M, Sokal E, Luttun A, Sancho-Bru P, Collas P, van Grunsven LA. Genome-wide analysis of DNA methylation and gene expression patterns in purified, uncultured human liver cells and activated hepatic stellate cells. Oncotarget 2015; 6: 26729-26745.
60) El Taghdouini A, Najimi M, Sancho-Bru P, Sokal E, van Grunsven LA. In vitro reversion of activated primary human hepatic stellate cells. Fibrogenesis Tissue Repair 2015; 8: 14.
61) Puche JE, Saiman Y, Friedman SL. Hepatic stellate cells and liver fibrosis. Compr Physiol 2013; 3: 1473-1492.

62) Benes P, Macecková V, Zdráhal Z, Konecná H, Zahradnícková E, Muzík J, Smarda J. Role of vimentin in regulation of monocyte/macrophage differentiation. Differentiation 2006; 74: 265-276.
63) Ivaska J, Pallari HM, Nevo J, Eriksson JE. Novel functions of vimentin in cell adhesion, migration, and signaling. Exp Cell Res 2007; 313: 2050-2062.
64) McInroy L, Maatta A. Down-regulation of vimentin expression inhibits carcinoma cell migration and adhesion. Biochem Biophys Res Commun 2007; 360: 109-114.
65) Paulin D, Lilienbaum A, Kardjian S, Agbulut O, Li Z. Vimentin: Regulation and pathogenesis. Biochimie 2022; 197: 96-112.
66) Geerts A, Eliasson C, Niki T, Wielant A, Vaeyens F, Pekny M. Formation of normal desmin intermediate filaments in mouse hepatic stellate cells requires vimentin. Hepatology 2001; 33: 177-188.
67) Miyata E, Masuya M, Yoshida S, Nakamura S, Kato K, Sugimoto Y, Shibasaki T, Yamamura K, Ohishi K, Nishii K, Ishikawa F, Shiku H, Katayama N. Hematopoietic origin of hepatic stellate cells in the adult liver. Blood 2008; 111: 2427-2435.
68) Vassiliadis E, Oliveira CP, Alvares-da-Silva MR, Zhang C, Carrilho FJ, Stefano JT, Rabelo F, Pereira L, Kappel CR, Henriksen K, Veidal SS, Vainer B, Duffin KL, Christiansen C, Leeming DL, Karsdal M. Circulating levels of citrullinated and MMP-degraded vimentin (VICM) in liver fibrosis related pathology. Am J Transl Res 2012; 4: 403-414.
69) Ostrowska-Podhorodecka Z, Ding I, Norouzi M, McCulloch CA. Impact of Vimentin on Regulation of Cell Signaling and Matrix Remodeling. Front Cell Dev Biol 2022; 10: 869069.
70) Ridge KM, Eriksson JE, Pekny M, Goldman RD. Roles of vimentin in health and disease. Genes Dev 2022; 36: 391-407.
71) Satelli A, Li S. Vimentin in cancer and its potential as a molecular target for cancer therapy. Cell Mol Life Sci 2011; 68: 3033-3046.
72) de Leeuw AM, McCarthy SP, Geerts A, Knook DL. Purified rat liver fat-storing cells in culture divide and contain collagen. Hepatology 1984; 4: 392-403.
73) Van Rossen E, Vander Borght S, van Grunsven LA, Reynaert H, Bruggeman V, Blomhoff R, Roskams T, Geerts A. Vinculin and cellular retinol-binding protein-1 are markers for quiescent and activated hepatic stellate cells in formalin-fixed paraffin embedded human liver. Histochem Cell Biol 2009; 131: 313-325.
74) Bhunchet E, Wake K. Role of mesenchymal cell populations in porcine serum-induced rat liver fibrosis. Hepatology 1992; 16: 1452-1473.
75) Forbes SJ, Russo FP, Rey V, Burra P, Rugge M, Wright NA, Alison MR. A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. Gastroenterology 2004; 126: 955-963.
76) Troeger JS, Mederacke I, Gwak GY, Dapito DH, Mu X, Hsu CC, Pradere JP, Friedman RA, Robert F Schwabe. Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. Gastroenterology 2012; 143: 1073-1083.e22.
77) Borg BB, Seetharam A, Subramanian V, Basha HI, Lisker-Melman M, Korenblat K, Anderson CD, Shenoy S, Chapman WC, Crippin JS, Mohanakumar T. Immune response to extracellular matrix collagen in chronic hepatitis C-induced liver fibrosis. Liver Transpl 2011; 17: 814-823.
78) Taura K, Miura K, Iwaisako K, Osterreicher CH, Kodama Y, Penz-Osterreicher M, Brenner DA. Hepatocytes do not undergo epithelial-mesenchymal transition in liver fibrosis in mice. Hepatology 2010; 51: 1027-1036.
79) Ostrowska-Podhorodecka Z, McCulloch CA. Vimentin regulates the assembly and function of matrix adhesions. Wound Repair Regen 2021; 29: 602-612.
80) Liu YF, Niu GC, Li CY, Guo JB, Song J, Li H, Zhang XL. Mechanism of Ulcerative Colitis-Aggravated Liver Fibrosis: The Activation of Hepatic Stellate Cells and TLR4 Signaling Through Gut-Liver Axis. Front Physiol 2021; 12: 695019.
81) Jirouskova M, Zbodakova O, Gregor M, Chalupsky K, Sarnova L, Hajduch M, Ehrmann J, Jirkovska M, Sedlacek R. Hepatoprotective effect of MMP-19 deficiency in a mouse model of chronic liver fibrosis. PLoS One 2012; 7: e46271.