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miR-197 Expression in Peripheral Blood Mononuclear Cells from Hepatitis B Virus-Infected Patients

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Background/Aims: This study aimed to investigate the microRNA (miRNA) expression profiles in peripheral blood mononuclear cell (PBMC) of hepatitis B virus (HBV)-infected patients with different clinical manifestations and to analyze the function of miR-197. **Methods:** PBMC miRNA expression profiles in 51 healthy controls, 70 chronic asymptomatic carriers, 107 chronic hepatitis B patients, and 76 HBV-related acute on chronic liver failure patients were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). miR-197 mimic and inhibitor were transfected in THP-1 cells. qRT-PCR and ELISA for interleukin (IL)-18 mRNA and protein levels were performed, respectively. **Results:** The microarray analysis revealed that 17 PBMC miRNA expression profiles (12 miRNAs downregulated and five miRNAs upregulated) differed significantly in HBV-induced liver disease patients presenting with various symptoms. The qRT-PCR results suggested that the PBMC miR-197 levels regularly decreased as the severity of liver disease symptoms became aggravated. IL-18, a key regulator in inflammation and immunity, was inversely correlated with miR-197 levels. Bioinformatic analysis indicated that IL-18 was a target of miR-197. Exogenous expression of miR-197 could significantly repress IL-18 expression at both the mRNA and protein levels in THP-1 cells. **Conclusions:** We concluded that multiple PBMC miRNAs had differential expression profiles during HBV infection and that miR-197 may play an important role in the reactivation of liver inflammation by targeting IL-18. **(Gut Liver 2013;7:335-342)**

Key Words: microRNAs; Hepatitis B virus; Liver failure; miR-197; Interleukin-18

INTRODUCTION

Chronic hepatitis B virus (HBV) infection causes a wide spectrum of clinical manifestations, including chronic asymptomatic carriers (ASCs), variable chronic hepatitis activity, and even liver failure. HBV-related acute on chronic liver failure (ACLF) is a serious liver disease associated with significant morbidity and mortality. Despite recent advances in antiviral treatment and artificial liver support treatment, the majority of patients have poor outcomes. The pathogenesis of ACLF is associated with HBV replication and host immune response, and abnormal immune response caused by virus is likely to substantially contribute to the pathogenesis of ACLF.[1]

A new category of noncoding RNA, microRNA (miRNA), has been found to be involved in diverse biological processes, such as cell differentiation, development, and apoptosis. miRNAs are endogenous 21- to 22-nucleotide RNAs that play important regulatory roles in gene expression by interacting with the 3' untranslated region (UTR) of target genes.[2] Studies have revealed that many miRNAs are also involved in the immune responses. miRNAs have been shown to modulate innate immune responses through Toll-like receptors and cytokine signaling pathway. In addition to regulating innate immune responses, miRNAs participate in adaptive immune responses by influencing antigen presentation and modulating T cell receptors signaling.[3] Considering the effect of miRNAs on the immune system, the role of miRNA in HBV has increased attention. The current researches mainly focus on the regulation of virus replication (e.g., miR-122, miR-1, and so on)[4,5] and the progression of HBV-induced liver disease (e.g., miR-223, miR-224, and so on).[6,7] But it is poorly understood about the relation between miRNA and the development of ACLF.

In present study, we investigated miRNA expression profiles

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in peripheral blood mononuclear cell (PBMC) of HBV-infected patients with different clinical manifestations employing microarray and quantitative real-time polymerase chain reaction (qRT-PCR), studied the correlation between miRNA expression profiles and the severity of HBV-induced liver disease, and analyzed the function of miR-197.

MATERIALS AND METHODS

1. Subjects

PBMC isolated from four ASC and four ACLF patients were used for the microarray experiment. The PBMC of the second cohort for the qRT-PCR experiment was composed of 253 patients with hepatitis B surface antigen (HBsAg) positive for at least 12 months and 51 healthy controls (HCs). All of the participants were recruited from the Xiangya Hospital, Central South University (Changsha, China), the Second Xiangya Hospital, Central South University (Changsha, China), and the Teaching Hospital of Hunan University (Changsha, China) from 2008 to 2010. The patients in the second cohort were classified into three groups: group I, 70 ASC; group II, 107 chronic hepatitis B (CHB) patients; and group III, 76 ACLF patients. The diagnostic criteria were based on the guideline of prevention and treatment for CHB and diagnosis and treatment for liver failure issued by Chinese Medical Association, respectively.[8,9] The ACLF patients were recruited in the early phase of the disease. All ACLF patients with previously diagnosed chronic HBV infection had gastrointestinal dysfunction, jaundice (total bilirubin, ≥171 μmol/L), and coagulopathy (prothrombin activity, ≤40%), but there were no any complications.

All of the subjects were not received antiviral and immunomodulatory therapy. There was no evidence for any type of bacterial infection (with normal blood routine, erythrocyte sedimentation rate, C-reactive protein, chest radiograph, and abdominal ultrasound), tumor, hepatitis C virus, hepatitis D virus, human immunodeficiency virus 1/2, mycobacterium tuberculosis infection, and metastatic or inflammatory autoimmune diseases.

The clinical characteristics of the subjects are showed in Table 1. Ficoll-Hypaque gradient centrifugation (Haoyang, Tianjin, China) was used to isolate PBMC from 10 mL heparin anticoagulant venous blood samples that were collected from all of the subjects.

This study was approved by the institutional ethical committee of the Xiangya Hospital, and written informed consent was obtained from each patient in the study.

2. Microarray for detecting miRNA expression

The PBMC obtained from the first cohort was immediately lysed by adding 1.0 mL Lysis Buffer (Ambion, Austin, TX, USA). The RNA from the PBMC was purified using a mirVana RNA Isolation Kit (Ambion) according to the manufacture's instructions. All of the RNA samples were quality controlled by measuring the optical density at 260 and 280 nm and by analyzing

Table 1. The Clinical Characteristics of the Subjects Used for the Validation Analysis

Clinical variable	HC (n=51)	ASC (n=70)	CHB (n=107)	ACLF (n=76)	p-value
Age, yr	32.0±11.75	36.01±13.9	29.06±10.12	38.02±10.44	0.189
Sex, male/female	35/16	48/22	79/28	61/15	0.352
PTA, %	87.96±19.2	89.67±22.7	79.83±20.10	35.41±9.42	<0.05
TBIL, μmol/L	11.80±2.49	15.67±6.49	20.60±6.50	407.31±151.07	<0.05
ALT, U/L					
Median	21.90	25.40	114.50	576.75	<0.05
Range	17.20–25.35	21.00–34.20	101.25–143.50	200.98–1,059.40	
AST, U/L					
Median	21.85	23.80	82.30	327.50	<0.05
Range	15.02–26.37	18.30–29.10	58.95–120.00	131.70–756.95	
HBV DNA, copies/mL*					
Median	NA	2.06×10^{3}	3.02×10^{7}	8.58×10^{4}	<0.05
Range	NA	0–6.62×10^{4}	3.95×10^{6}–1.36×10^{8}	0–1.53×10^{6}	
HBeAg, +/-	NA	12/58	93/14	33/43	<0.001
HBsAg, IU/mL					
Median	NA	325.64	29,871.00	1,924.40	<0.001
Range	NA	89.88–2,072.40	6,886.10–50,024.00	984.69–6,920.70	

HC, healthy control; ASC, chronic asymptomatic carrier; CHB, chronic hepatitis B; ACLF, HBV-related acute on chronic liver failure; PTA, prothrombin activity; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; NA, not applicable; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.
*HBV DNA <500 was treated as 0.

an aliquot of the RNA preparation on an Agilent 2100 Bioanalyzer using RNA 6000 Nano chips (Agilent Technologies, Santa Clara, CA, USA). Human miRNA V3 microarray and miRNA Complete Labeling and Hyb Kit (Agilent Technologies) were used for the RNA labeling and hybridization. The processing steps and fluorescence scanning were performed by a commercial service provider (Agilent Technologies).

3. Microarray data analysis

The data were analyzed by using extracted fluorescence intensity values (Agilent Feature Extraction software version 10.5 and GeneSpring GX software; Agilent Technologies). The intensities were normalized using average factors scaled to the median array intensities over the entire array using the median array as a reference. The miRNA expression levels were described by quantitative $\log_2$ metrics. The miRNAs that were differentially expressed between ASC and ACLF patients were identified using two-sided Student's t-test ($p<0.05$) combined with the $\log_2$ absolute value of signal difference multiple modulus ($|\log_2|>1$).

4. qRT-PCR for detecting miRNA and mRNA expression

The total PBMC RNA from the second cohort was extracted using Trizol total RNA isolation reagent (Invitrogen; Life Technologies, Carlsbad, CA, USA) following the manufacture's instructions. The RNA concentration and purity were measured using an ultraviolet spectrophotometer on samples diluted 1/50 with RNase-free water (Tiangen Biotech, Beijing, China). The RNA yield was 1.15±0.43 mg/mL, and the purity was 1.94±0.18 (A_{260}/A_{280}).

The miRNA expression profiles were conducted by two-step RT-PCR. Purified RNA (1 μg) was immediately used to synthesize cDNA according to the miScript Reverse Transcription Kit (QIAGEN, Hilden, Germany). The RT was performed in the model AG22331 GeneAmp PCR system (Eppendorf, Hamburg, Germany) for 60 minutes at 37°C, followed by 5 minutes at 95°C. The cDNA was diluted 1/100 for the subsequent qRT-PCR.

The miR-150, miR-197, miR-574-3p, and miR-30a expressions were detected and quantified using miScript SYBR Green PCR kit (QIAGEN). The 50 μL PCR reactions consisted of 25 μL of 2× QuantiTect SYBR Green PCR Master Mix, 5 μL of 10× miScript Universal Primer, 5 μL of 10× miScript Primer Assay, 2 μL of template cDNA, and 13 μL of RNase-free water. The miRNA amplification was conducted on an Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA, USA) using suitable cycling conditions. To verify that the amplified product was only mature miRNA, a dissociation analysis of PCR products from 65°C to 95°C was performed after thermo cycling. Mammalian U6B small nuclear RNA in the PBMC was used to normalize the miRNA expression level. The miRNAs and U6B primes were purchased from QIAGEN, Germany. The relative interleukin-18 (IL-18) expression level was identified with 5'-TGGCTGCTGAACCAGTAGAAG-3' and 5'-TTCCAGGTTTTCATCATCTTCA G-3' primes and normalized to glyceraldehyde phosphate dehydrogenase. The PCR amplification was performed with a volume of 20 μL containing 10 μL SYBR qPCR Mix (Toyobo, Osaka, Japan).

All of the qPCR reactions, including the no-template controls, were performed in triplicate wells. Automatic baseline and threshold cycle settings were used throughout the analysis. The SDS Relative Quantification 7500 software version 2.0.1 (Applied Biosystems) was used to miRNA date analysis. The relative expression of each miRNA was calculated by the $2^{-\triangle\triangle Ct}$ method.[10]

5. MiRNA target gene prediction

The possible target genes for each miRNA were identified using TargetScan 5.2, DIANA-microT 3.0, and Pictar target prediction program. These target prediction programs use an algorithm-based on conserved and nonconserved recognition elements. TargetScan 5.2 provided a possible interaction region for the miRNA and the target gene, and DIANA-microT 3.0 reported a precision score to help evaluate the value of the predicted results.

6. Cell culture and transfection

THP-1 cell line (obtained from Shanghai Institute for Biological Science, China) was cultured in RPML 1640 (Gibco, Grand Island, NY, USA) with 10% FBS (Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C with 5% carbon dioxide. The has-miR-197 mimic, has-miR-197 inhibitor, and unrelated sequence positive control (miR-C) and negative control (anti-miR-C) were purchased from GeneCopoeia, Germantown, MD, USA. Cells were transfected using HiPerFect Transfection Reagent (QIAGEN) and harvested 48 hours later. The expression levels of miR-197 and IL-18 were measured in THP-1 cells by qRT-PCR, as described above. The protein expression levels of IL-18 were measured in supernatant by ELISA (R&D Systems, Minneapolis, MN, USA).

7. Statistical analysis

The SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Date are presented as mean±SD. Differences between two groups were determined by the two-tailed Student's t-test, and multiple comparisons were determined by the ANOVA. The correlation between the miRNA and the patients' clinical characteristics was examined by the Pearson's correlation. The p-values of less than 0.05 were considered to be statistically significant for all of the tests.

RESULTS

1. The PBMC miRNA profiles of the ASC and ACLF patients differed

To identify the miRNA that may have been correlated with the severity of HBV-induced liver disease, we first used micro-

array to investigate the miRNA expression profiles in PBMC from four ASC and four ACLF patients. Overall, we profiled 347 human miRNA species. The microarry analysis showed that 17 PBMC miRNA expressions differed significantly between the ASC and ACLF patients. A detailed analysis revealed that the levels of five miRNAs (miR-30a, miR-1246, miR-1305, miR-193a-3p, and miR-196b) increased with the liver disease severity. The levels of 12 miRNAs, including miR-150, miR-223, miR-574-3p, miR-197, and miR-328, simultaneously decreased (Table 2 and Fig. 1).

2. The expressions of miR-150, miR-197, miR-30a, and miR-574-3p in PBMC from HBV-infected patients with different clinical manifestations

To identify the miRNAs potentially associated with HBV symptom severity and the availability of the differentially expressed miRNA, the expression profiles of four PBMC miRNAs (miR-150, miR-197, miR-574-3p, and miR-30a) from HC and HBV-infected patients presenting with various symptoms (ASC, CHB, and ACLF) were confirmed by qRT-PCR. We choosed two downregulated miRNAs (miR-197 and miR-574-3p) and one upregulated miRNA (miR-30a) based on the p-values obtained from the microarray analysis. miR-150 was selected based on the literature. To evaluate the amplification efficiency, standard curves were generated using 10-fold serial dilution of the reverse transcribed cDNA.

The initial miRNA microarray profiles identified three miRNAs (miR-150, miR-197, and miR-574-3p) that were significantly downregulated, and miR-30a was significantly upregulated. The miR-197, miR-574-3p, and miR-30a profiles were confirmed by qRT-PCR. However, the qRT-PCR profiles of miR-150 were inconsistent with the previous results. We observed that the PBMC miR-197 levels gradually decreased as the severity of liver disease symptoms aggravated. The expression levels in HC, the ASC, the CHB patients, and the ACLF patients were 1.07±0.16, 0.90±0.12, 0.72±0.10, and 0.55±0.10, respectively. Furthermore, a comparative analysis between two randomly selected groups showed statistical significance. On the other hand, the results indicate that the miR-150 expression of the ACLF patients was strongly upregulated compared with that of CHB patients ($p<0.05$), but the difference among the HC, ASC, and CHB patients was not significant. The miR-574-3p expression was lower in the ACLF patients than in the HC ($p<0.01$), but it did not differ significantly among the ASC, CHB patients, and ACLF patients. The miR-30a expression was noticeably higher in the HC than in the ACLF patients ($p<0.01$), but no significant difference was observed among the ASC, CHB patients, and ACLF patients (Fig. 2A).

3. Correlation between the miR-197 expression levels and the patients' clinical characteristics

The serum HBV DNA levels reflect the HBV replication in liver tissue. To comprehend the relationship between the HBV DNA levels and miRNAs, the correlation between the HBV DNA

Table 2. The Differentially Expressed Peripheral Blood Mononuclear Cell miRNAs from the Chronic Asymptomatic Carriers Compared with Those from the Acute on Chronic Liver Failure Patients

miRNA	Expression	Fold change	p-value
miR-1246	Up	4.19	<0.05
miR-30a	Up	3.11	<0.05
miR-1305	Up	2.39	<0.05
miR-193a-3p	Up	2.03	<0.05
miR-196b	Up	1.61	<0.05
miR-223	Down	-4.18	<0.05
miR-574-3p	Down	-3.04	<0.01
miR-150	Down	-2.90	<0.05
miR-486-5p	Down	-2.39	<0.05
miR-197	Down	-2.05	<0.01
miR-328	Down	-1.87	<0.01
miR-766	Down	-1.86	<0.05
miR-326	Down	-1.74	<0.05
miR-185	Down	-1.73	<0.05
miR-483-3p	Down	-1.33	<0.05
miR-301a	Down	-1.12	<0.05
miR-30d	Down	-1.01	<0.05

miRNA, microRNAs.

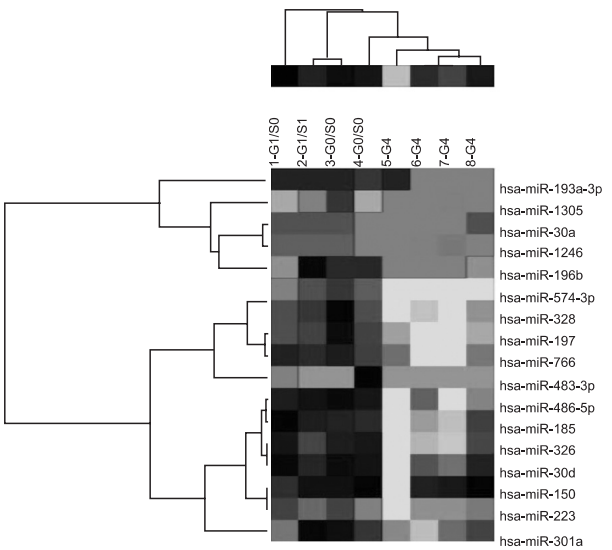

Fig. 1. The microRNA (miRNA) signatures in peripheral blood mononuclear cell (PBMC) differentiates chronic asymptomatic carriers (ASCs) from acute on chronic liver failure (ACLF) patients. This hierarchically clustered heat map illustrates the changes in miRNA expression profiles between ASCs and ACLF patients. The map shows the (log_2) PBMC miRNA expression differences between the ASCs and ACLF patients. The significantly expressed miRNA cluster was identified using the Student's t-test. The red and green sections represent increases and decreases in miRNA expression, respectively.

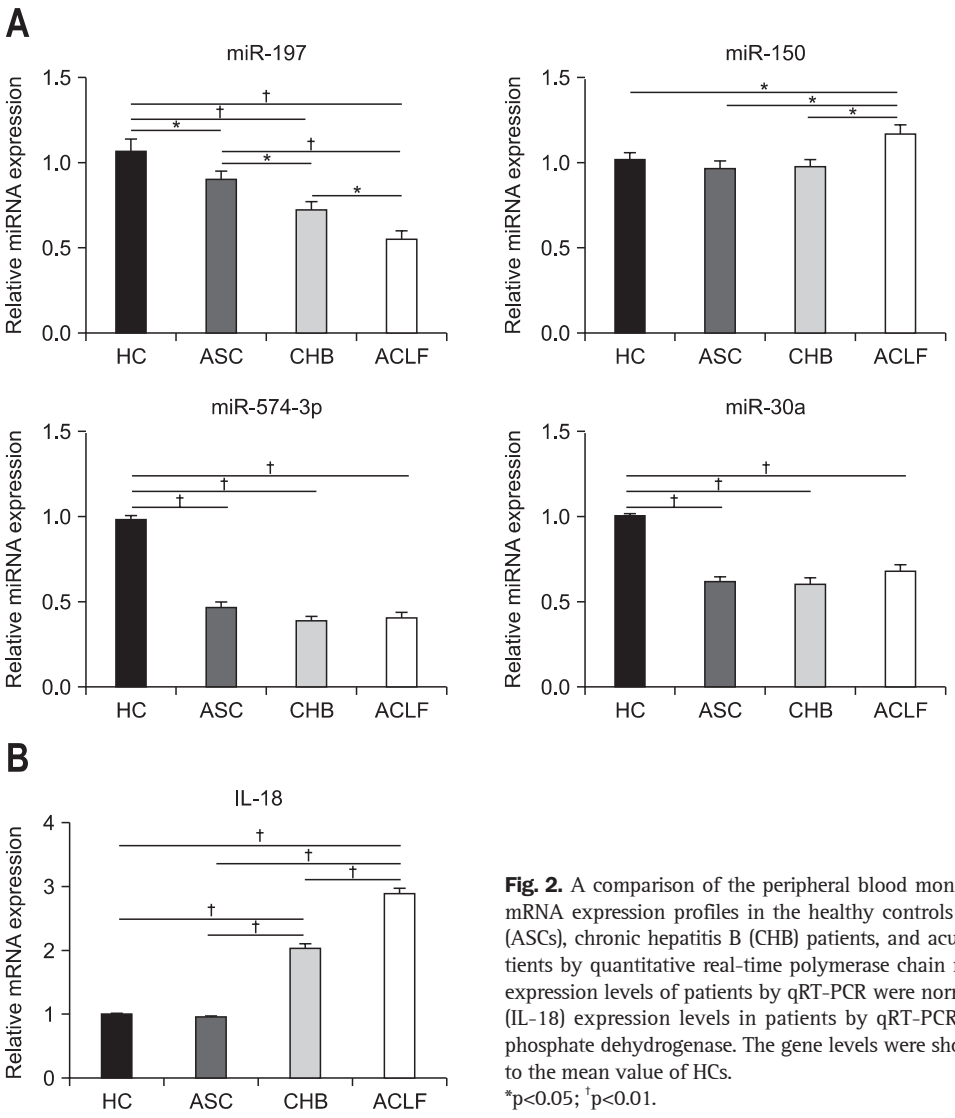

Fig. 2. A comparison of the peripheral blood mononuclear cell microRNA (miRNA) and mRNA expression profiles in the healthy controls (HCs), chronic asymptomatic carriers (ASCs), chronic hepatitis B (CHB) patients, and acute on chronic liver failure (ACLF) patients by quantitative real-time polymerase chain reaction (qRT-PCR). (A) The 4 miRNA expression levels of patients by qRT-PCR were normalized to U6B. (B) The interleukin-18 (IL-18) expression levels in patients by qRT-PCR were normalized to glyceraldehyde phosphate dehydrogenase. The gene levels were shown as the mean fold changes relative to the mean value of HCs.
*$p<0.05$; †$p<0.01$.

titre and the PBMC miR-197 expression levels in ASC, CHB, and ACLF patients were analyzed. However, no significance correlation was observed. The serum hepatitis B e antigen (HBeAg) shows positive when the virus replicates in liver tissue, so we analyzed the PBMC miR-197 expression levels in HBeAg positive patients and HBeAg negative patients in ASC, CHB, and ACLF group. But there was no significant difference. At the same time, the correlation between the levels of miR-197 and HBsAg levels or age was not reached statistical significance.

4. IL-18 was one of the possible target genes for miR-197

Using the DIANA-microT 3.0, we found that IL-18 was one of the possible target genes for miR-197. This result was verified by an additional target prediction program, TargetScan 5.2, which identified the possible interaction regions between miR-197 and IL-18 (Fig. 3A).

Consistent with earlier studies, the PBMC IL-18 expression levels were gradually increased from the ASC, the CHB patients, to the ACLF patients. But, its mRNA levels were not significantly altered in the HC and ASC ($p=0.72$) (Fig. 2B). This result was in stark contrast to miR-197 results.

5. IL-18 expression after transfection with miR-197 mimic/inhibitor

As shown in Fig. 3B and C, the miR-197 level was strongly increased in 48 hours after miR-197 mimic tranfection, and it was strongly decreased in 48 hours after miR-197 inhibitor tranfection. To determine the effect of miR-197, the IL-18 mRNA and protein levels were measured. The mRNA and protein expression levels of IL-18 were significantly downregulated in THP-1 cells transfected with miR-197 mimic compared with cells transfected with miR-C and blank-C, and these were significantly upregulated in cells transfected with miR-197 inhibitor compared with cells transfected with anti-miR-C and blank-C. Meanwhile, there was no significant difference among cells that were transfected with miR-C, anti-miR-C, and blank-C.

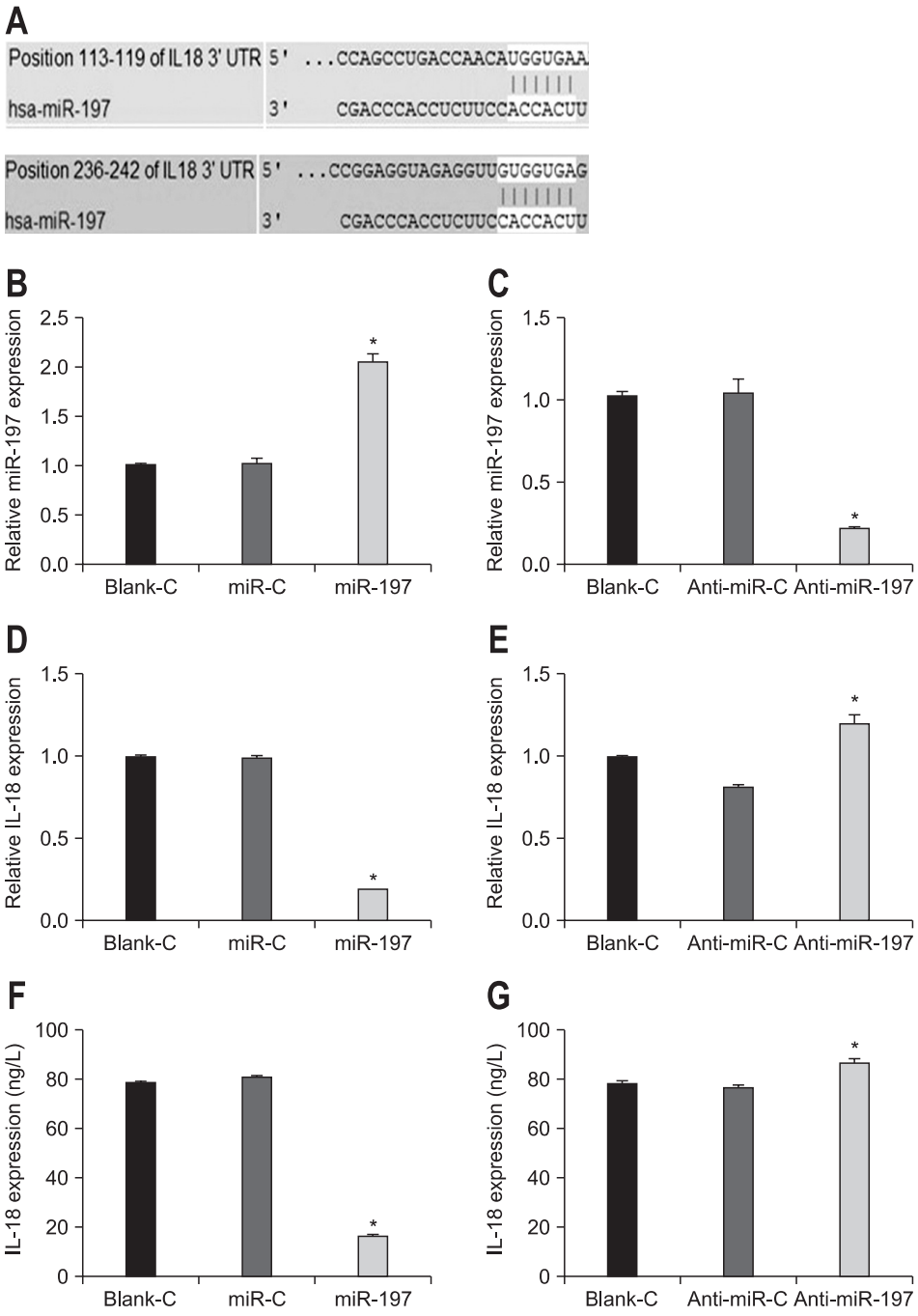

Fig. 3. Interleukin-18 (IL-18) expression after transfection with miR-197 mimic/inhibitor. (A) miR-197 is predicted to target IL-18, with a sequence of miR-197 and a sequence of IL-18. (B) Quantitative real-time polymerase chain reaction (qRT-PCR) results showed increased miR-197 expression levels increased in THP-1 cells after miR-197 mimic transfection. (C) The qRT-PCR results indicated that the miR-197 expression levels decreased in THP-1 cells after miR-197 inhibitor transfection. (D) The IL-18 mRNA expression levels were significantly downregulated in THP-1 cells after miR-197 mimic transfection by qRT-PCR. (E) The IL-18 mRNA expression levels were significantly upregulated in THP-1 cells after miR-197 inhibitor transfection by qRT-PCR. (F) The IL-18 protein expression levels were significantly downregulated in THP-1 cells after miR-197 mimic transfection by ELISA. (G) The IL-18 protein expression levels were significantly upregulated in THP-1 cells after miR-197 inhibitor transfection by ELISA. The results were obtained from three independent experiment. *p<0.01.

DISCUSSION

Results from recent researches have suggested that PBMC miRNAs are potential biomarkers for various diseases, including multiple sclerosis, coronary artery disease, and the clinical outcome of interferon (IFN) therapy in HBV-infected patients.[11-13] In the present study, we investigated the PBMC miRNA expression profiles of the ASC and ACLF patients using microarray first. In order to reduce the bias of microarray experiment, we then expanded the sample cases to 304 in qRT-PCR experiment. Several miRNAs showed differential expressions in the PBMC of the HBV-infected patients presenting with various symptoms. These results demonstrated that PBMC miRNAs may have a clinicopathological influence on HBV-induced liver disease. These exciting results also increased our understanding of clinical characterization and pathogenesis of HBV infection.

HBV is a typically noncytopathic virus that dose not directly damage infected cells. The pathogenesis is largely mediated by the immune response following HBV infection. Recent studies have shown the importance of miRNAs in the immune responses. These miRNAs affect immune cell development and differentiation and the outcome of the immune responses to infection in HBV-induced liver disease. MiR-155 has been demonstrated to play a significant role in regulating IFN and tumor necrosis

factor production during the innate immune response.[14] Huang *et al*.[15] found that miR-142-3p restricted cAMP production in CD4+CD25- T cells and CD4+CD25+ TREG cells. In 2007, researches showed that miR-150 exerted critical regulation on B cell development.[16] While miR-223 has shown its apparent importance for progenitor cell proliferation and granulocyte function.[17] The present study found that differently expressed miRNA in PBMC from patients with HBV-induced liver disease was high related to the immune responses. This study showed that PBMC miR-150 level was markedly higher in ACLF patients than in CHB patients. Surprisingly, Ji *et al*.[18] showed that miR-150 is upregulated in ACLF patients by extracting RNA from the serum.

The correlation between miR-197 and follicular thyroid carcinoma and human male breast cancer has been the focused of the several previous researches.[19,20] In 2010, Estep and coworkers[21] have noted that miR-197 is significantly associated with pericellular fibrosis in nonalcoholic fatty liver disease, suggesting a potential role of this miRNA in liver disease. Other research has provided evidence that miR-197 expression in human HepG2 cells is modulated by proanthocyanidins.[22] The present study is the first time to find that miR-197 in PBMC is associated with HBV-induced liver disease. In particular, examining the differential miRNA expression showed that miR-197 levels strikingly decrease as the severity of liver disease symptom aggravated.

IL-18, a member of IL-1 cytokine family, has already been reported to be elevated in patients with hepatic failure.[23,24] A study on the effects of IL-18 on PBMC derived from CHB suggested that IL-18 can induce IFN-γ secretion and that it plays a key role in modulating both innate and adaptive immunity.[25] For these reasons, IL-18 profiles were measured in the PBMC of all the subjects. Consistent with earlier studies, the PBMC IL-18 levels of ACLF patients were dramatically higher than that of patients with other HBV-induced liver disease and HC (Fig. 2B). The present data indicated that the expression of IL-18 was inversely correlated with the miR-197 expression in HBV-infected patients.

miRNAs act mainly via regulation of their target genes. Using computational methods, we predicted that miR-197 is highly likely to interact with the 3' UTR of IL-18 (Fig. 3A). To confirm the miR-197 regulatory role in IL-18 expression, we transfected mimic and inhibitor of miR-197 in THP-1 cells. Our results showed that exogenous expression of miR-197 could significantly repress the IL-18 expression at both the mRNA and protein levels (Fig. 3D and F), whereas miR-197 inhibitor had the opposite effects on the IL-18 expression (Fig. 3E and G). These data suggested that miR-197 may be an effective strategy for regulating the expression of IL-18. However, the directly interacted sequence of miR-197 with the 3' UTR of IL-18 requires further study.

Overall, our results are the first demonstration that the PBMC miR-197 expression levels were associated with the severity of HBV-induced liver disease symptoms. In addition, our results suggested that miR-197 downregulated IL-18 expression. We believe that our findings represent a valuable resource for understanding of clinical characterization and pathogenesis of HBV infection.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Chisari FV, Isogawa M, Wieland SF. Pathogenesis of hepatitis B virus infection. Pathol Biol (Paris) 2010;58:258-266.
2. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281-297.
3. Bi Y, Liu G, Yang R. MicroRNAs: novel regulators during the immune response. J Cell Physiol 2009;218:467-472.
4. Qiu L, Fan H, Jin W, et al. miR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. Biochem Biophys Res Commun 2010;398:771-777.
5. Zhang X, Zhang E, Ma Z, et al. Modulation of hepatitis B virus replication and hepatocyte differentiation by microRNA-1. Hepatology 2011;53:1476-1485.
6. Ura S, Honda M, Yamashita T, et al. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. Hepatology 2009;49:1098-1112.
7. Murakami Y, Yasuda T, Saigo K, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene 2006;25:2537-2545.
8. Chinese Society of Hepatology, Chinese Medical Association; Chinese Society of Infectious Diseases, Chinese Medical Association. The guidelines of prevention and treatment for chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi 2005;13:881-891.
9. Liver Failure and Artificial Liver Group, Chinese Society of Infectious Diseases, Chinese Medical Association; Severe Liver Diseases and Artificial Liver Group, Chinese Society of Hepatology, Chinese Medical Association. Diagnostic and treatment guidelines for liver failure. Zhonghua Gan Zang Bing Za Zhi 2006;14:643-646.
10. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402-408.
11. Otaegui D, Baranzini SE, Armañanzas R, et al. Differential micro

RNA expression in PBMC from multiple sclerosis patients. PLoS One 2009;4:e6309.
12. Hoekstra M, van der Lans CA, Halvorsen B, et al. The peripheral blood mononuclear cell microRNA signature of coronary artery disease. Biochem Biophys Res Commun 2010;394:792-797.
13. Pan XB, Ma H, Jin Q, Wei L. Characterization of microRNA expression profiles associated with hepatitis B virus replication and clearance in vivo and in vitro. J Gastroenterol Hepatol 2012;27:805-812.
14. Tili E, Michaille JJ, Cimino A, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol 2007;179:5082-5089.
15. Huang B, Zhao J, Lei Z, et al. miR-142-3p restricts cAMP production in CD4+CD25- T cells and CD4+CD25+ TREG cells by targeting AC9 mRNA. EMBO Rep 2009;10:180-185.
16. Zhou B, Wang S, Mayr C, Bartel DP, Lodish HF. miR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. Proc Natl Acad Sci U S A 2007;104:7080-7085.
17. Johnnidis JB, Harris MH, Wheeler RT, et al. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature 2008;451:1125-1129.
18. Ji F, Yang B, Peng X, Ding H, You H, Tien P. Circulating microRNAs in hepatitis B virus-infected patients. J Viral Hepat 2011;18:e242-e251.
19. Weber F, Teresi RE, Broelsch CE, Frilling A, Eng C. A limited set of human microRNA is deregulated in follicular thyroid carcinoma. J Clin Endocrinol Metab 2006;91:3584-3591.
20. Lehmann U, Streichert T, Otto B, et al. Identification of differentially expressed microRNAs in human male breast cancer. BMC Cancer 2010;10:109.
21. Estep M, Armistead D, Hossain N, et al. Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2010;32:487-497.
22. Arola-Arnal A, Bladé C. Proanthocyanidins modulate microRNA expression in human HepG2 cells. PLoS One 2011;6:e25982.
23. Shinoda M, Wakabayashi G, Shimazu M, et al. Increased serum and hepatic tissue levels of interleukin-18 in patients with fulminant hepatic failure. J Gastroenterol Hepatol 2006;21:1731-1736.
24. Roth GA, Faybik P, Hetz H, et al. Pro-inflammatory interleukin-18 and caspase-1 serum levels in liver failure are unaffected by MARS treatment. Dig Liver Dis 2009;41:417-423.
25. Sun Y, Chen HY, Xin SJ. Effect of IL-18 on peripheral blood mononuclear cells of chronic hepatitis B and hepatitis B virus DNA released by HepG2.2.15 cell lines. Hepatobiliary Pancreat Dis Int 2004;3:230-234.