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Original Article

Expression profile and clinical significance of miRNAs at different stages of chronic hepatitis B virus infection

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Abstract: Objective: To study the expression profile and clinical significance of microRNAs (miRNAs) at different stages of chronic hepatitis B virus (HBV) infection. Methods: The miRNA expression profiles of peripheral blood mononuclear cells (PBMCs) at different stages of chronic HBV infection were screened using miRNA microarray and validated using real-time quantitative polymerase chain reaction (qPCR). Results: Significant differences in miRNA expression profiles of PBMCs were observed between patients in IA and IT phases of CHB. Expression was significantly down-regulated in the former but up-regulated in the latter group. No significant differences in inactive hepatitis B surface antigen carriers were observed. Changes in expression of six miRNAs determined by real-time qPCR were consistent with those determined by microarray. Areas under the receiver operation characteristic curve of the six miRNAs distinguishing immune tolerance and clearance of chronic HBV infection were 99.4%, 98.4%, 96.7%, 100%, 100%, and 99.6%. Positive correlation was found between the levels of hsa- miR-146a and ALT (r = 0.56, P < 0.01) while negative correlation was found between the levels of hsa-miR-548ah-5p and HBV DNA (r = -0.73, P < 0.01). Conclusions: Abnormal expression of miRNAs and the resulting gradual decline in the various immune states of patients with chronic HBV infection may play important roles in maintenance of the immune homeostatic mechanisms of chronic HBV infection. Hsa-miR-548ah-5p, hsa-miR-3191-5p and hsa-miR-4711-3p can be used as potential molecular markers to distinguish among different stages of chronic HBV infection.

Keywords: Chronic hepatitis B virus infection, miRNA, immune tolerance, immune activation

Introduction

Hepatitis B virus (HBV) infection remains a serious public health problem worldwide despite wide utilization of the HBV vaccine. More than 350 million people are chronically infected with HBV, and approximately 20% to 40% of them develop liver cirrhosis and liver cancer due to chronic repeated episodes of inflammation [1]. Several studies have suggested that HBV is not directly cytopathogenic to infected hepatocytes. The host immune response, particularly the virus-specific T cell response, plays an important role in viral clearance and pathogenesis of liver diseases. Patients with chronic hepatitis B virus infection have weak and limited T cell response, whereas those with acute hepatitis have vigorous and polyclonal T cell response that completely clears the virus [2, 3]. Interferon-a and nucleotide/nucleotide analogues have been used widely for treatment of chronic hepatitis B patients with good efficacy [4]. However, use of these drugs is limited by factors such as drug resistance, recurrence and severe adverse reactions. A combined therapeutic strategy with both viral suppression and enhancement of antiviral immune response is needed for effective long-term clearance and cure of chronic HBV infection [5]. Therefore, elucidating the pathogenesis of HBV and development of new methods for diagnosis and treatment of chronic HBV infection are highly urgent.

MicroRNAs (miRNAs) are endogenous non-coding small-molecule RNAs that modulate gene expression at the post-transcriptional level. They play vital roles in many physiological processes in cells and are also implicated in various diseases, such as infection and cancer [6, 7]. Recent studies have shown that miRNAs may also have important roles in the development and differentiation of immune cells and regulation of immune responses [8, 9]. Some miRNAs function in negative feedback loops of

Table 1. Baseline data of group detected by microarray

	IT of CHB	IA of CHB	IASC	NC
Sex (m/f)	4/8	3/9	4/8	2/7
Age (yr)	29 ± 7	33 ± 8	46 ± 12	30 ± 6
ALT (U/L)	25.8 ± 7.1	216.5 ± 273.5	29.4 ± 8.6	24.1 ± 6.2
HBV DNA	6.8 ± 1.4	5.7 ± 1.5	-	-

(Log^{10}copies/ml).

Table 2. Baseline data of group detected by RT-qPCR

	IT of CHB	IA of CHB	IASC	NC
Sex (m/f)	10/13	6/15	7/13	8/13
Age (yr)	29 ± 8	32 ± 7	44 ± 10	28 ± 7
ALT (U/L)	25.2 ± 6.5	187.5 ± 212.1	28.7 ± 7.9	23.4 ± 5.7
HBV DNA	7.2 ± 1.6	5.9 ± 1.3	-	-

(Log^{10}copies/ml).

the immune system, whereas others help amplify immune response by repressing response inhibitors. Several studies have shown that abnormal expression of certain miRNAs is related to pathogenesis and disease progression of chronic HBV infection [10, 11]. Furthermore, several miRNAs have been found in the blood in cell-free form. Some reports suggest that circulating miRNAs may serve as potential molecular markers to predict liver injury resulting from chronic hepatitis B [12-14]. Consistent with this idea, Zhang et al. found that plasma miRNA profiles can indeed be used as a predictor of early virological reponse to interferon treatment in chronic hepatitis B patients [15].

Chronic HBV infection can be divided into the following three stages based on the characteristics of immune response: immune tolerant (IT), immune active (IA), and immune control (IC) [16]. The level of virus in the body, the degree of liver damage, and the immune characteristics vary across the three stages of chronic HBV infection. This suggests that the expression profiles of miRNAs may also be different among the three stages. However, the expression profile and clinical significance of miRNAs at different stages of chronic HBV infection have not been investigated to date. In this study, miRNA expression at different stages of chronic HBV infection was screened using miRNA microarray and validated using real-time quantitative polymerase chain reaction (qPCR). Furthermore, their significance for clinical diagnostics was analyzed.

Methods

Subjects

Blood samples were collected from 36 cases of chronic HBV infection and 9 healthy controls at the Taizhou People's Hospital from 2011 to 2012. Written informed consent was obtained from all subjects. Among the 36 cases of chronic HBV infection, 12 had CHB in the IT phase, were HBeAg-positive, and had normal ALT levels but elevated levels of HBV DNA (20,000 IU/mL).

Another 12 cases had CHB in the IA phase with elevated ALT and HBV DNA level above 2000 IU/mL. These patients were either HBeAg-positive or HBeAg-negative/anti-HBe-positive. The remaining 12 were inactive hepatitis B surface antigen carriers (IASCs) characterized by the absence of HBeAg and the presence of anti-HBe, normal ALT levels, and HBV DNA < 200 IU/mL. The baseline data of the four groups are shown in **Table 1**. The quantitative PCR validation group consisted of 64 cases of chronic HBV infection, of which 36 were from the microarray group. Among the 64 cases of chronic HBV infection, 23 had CHB in the IT phase, 21 had CHB in the IA phase, and 20 were IASCs. The healthy subjects consisted of 21 normal controls, of which 9 were from the microarray group. The baseline data of the four groups are shown in **Table 2**. The experimental protocol was approved by the ethical commission of Taizhou People's Hospital. The diagnostic criteria were based on the 2010 Chronic Hepatitis B Prevention Guide of China [17]. All patients were negative for antibodies against hepatitis A, C, D, and E viruses, as well as human immunodeficiency virus. All patients with history and clinical features of drug-induced liver injury, alcoholic hepatitis, and steatohepatitis as well as those treated with nucleotide/nucleotide analog antiviral or immunomodulatory drugs in the previous 6 months were excluded.

PBMC separation, RNA extraction and miRNA microarray

Peripheral blood mononuclear cell (PBMC) separation, RNA extraction and labeling, Array hybridization and Data analysis as described [18].

Table 3. RT primer sequence of cDNA synthesis

Gene name	RT primer
U6	5'CGCTTCACGAATTTGCGTGTCAT3'
hsa-miR-29b-3p	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAACACTG3'
hsa-miR-146a	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAACCCAT3'
hsa-miR-548ah-5p	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACCAAACA3'
hsa-miR-3191-5p	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTGGAAG3'
hsa-miR-4711-3p	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACATCAAG3'
hsa-miR-5704	5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGCATAA3'

Table 4. Primer sequence of qPCR

Gene name	Primer sequence	Product (bp)
U6	F: 5'GCTTCGGCAGCACATATACTAAAAT3' R: 5'CGCTTCACGAATTTGCGTGTCAT3'	89
hsa-miR-29b-3p	GSP: 5'GGGTAGCACCATTTGAAA 3' R: 5'TGCGTGTCGTGGAGTC3'	63
hsa-miR-146a	GSP: 5'GGGTGAGAACTGAATTCC3' R: 5'TGCGTGTCGTGGAGTC3'	62
hsa-miR-548ah-5p	GSP: 5'GGGGGAAAAGTGATTGCAG3' R: 5'GTGCGTGTCGTGGAGTCG3'	63
hsa-miR-3191-5p	GSP: 5'GGCTCTCTGGCCGTCTAC3' R: 5'CAGTGCGTGTCGTGGAGT3'	62
hsa-miR-4711-3p	GSP: 5'GAGGCGTGTCTTCTGGC3' R: 5'CAGTGCGTGTCGTGGAGT3'	62
hsa-miR-5704	GSP: 5'GGTTACGCCATCATCCCA3' R: 5'GTGCGTGTCGTGGAGTCG3'	62

Detection of miRNA molecules using real-time quantitative PCR

1. cDNA synthesis. All primers were synthesized by Shanghai Invitrogen Biotechnology Co., Ltd. The primer sequences are shown in **Table 3**. For cDNA synthesis, 2 μl of 2.5 mM deoxyribonucleotide triphosphate, 2 μl of 10× reverse-transcriptase (RT) buffer, 0.3 μl of 1 μM RT-specific primers, 400 ng total RNA, 0.2 μl of 200 U/μl Moloney murine leukemia virus RT, 0.3 μl of 40 U/μl RNAse inhibitor, and RNA enzyme were mixed with water to a total volume of 20 μl. The reaction condition for reverse transcription was as follows: 16°C for 30 min, 42°C for 40 min, and 85°C for 5 min.

2. Real-time quantitative PCR. All primers for real-time quantitative PCR (shown in **Table 4**) were synthesized by Shanghai Invitrogen Biotechnology Co., Ltd. A total of 5 μl of 2× Master Mix, 0.5 μl of 10 μM specific primer F, and 0.5 μl of 10 μM specific primer R were mixed with water to a total volume of 8 μl. Subsequently, each 8 μl mixture was added to a hole in a 384-well PCR plate, which was followed by addition of 2 μl cDNA in each hole. The plate was carefully sealed with parafilm and centrifuged briefly. The 384-PCR plate was placed in a real-time PCR instrument for analysis. The reaction condition was as follows: 95°C for 10 min followed by 40 cycles of 95°C for 10 s and 60°C for 60 s. Levels of U6 small nuclear RNA were used as the internal control.

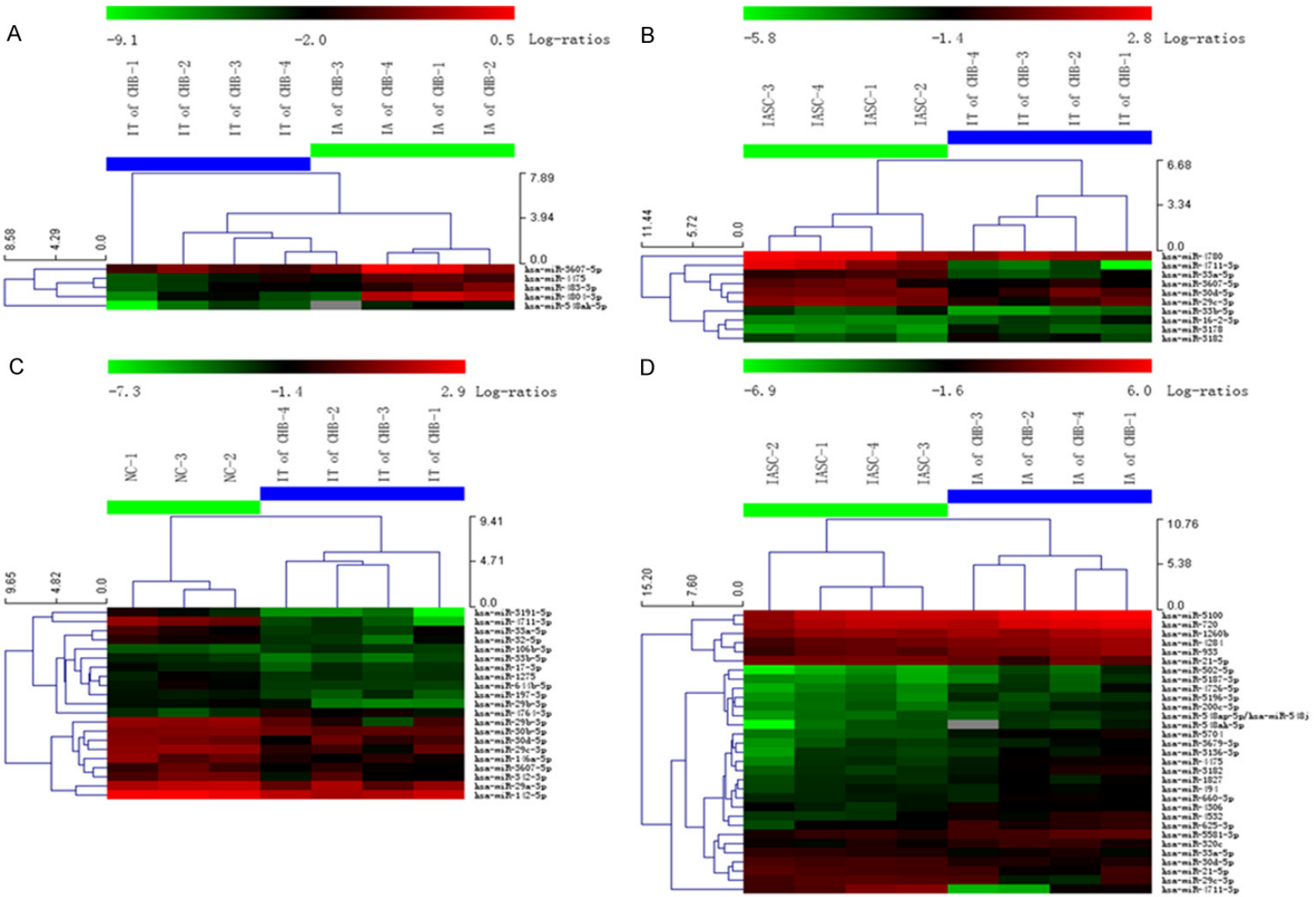
3. Data analysis. Data were analyzed and fold difference in gene expression level was measured by the $2^{-\Delta\Delta CT}$ method.

Detection of the main clinical indicators

Quantitative detection of HBV DNA was performed using an ABI7300-type quantitative PCR instrument (Applied Biosystems, USA). HBV markers were detected by enzyme-linked immunosorbent assay (Beijing YuanPingHao Biological Company Limited, China). Biochemical indicators were detected using an automatic biochemical analyzer (Hitachi Ltd., Japan).

Statistical analysis

Data are expressed as mean ± standard deviation. T-test was used for comparison between two groups, whereas one-way ANOVA and SNK-q tests were used for multiple comparisons. Data with skewed distribution are expressed as median ± interquartile range. Mann-Whitney and Kruskal Wallis H tests were used for comparison of two or more groups. The receiver operation characteristic (ROC)


A
-9.1
-2.0
0.5 Log-ratios
IT of CHB-1
IT of CHB-2
IT of CHB-3
IT of CHB-4
IA of CHB-3
IA of CHB-4
IA of CHB-1
IA of CHB-2
7.89
3.94
0.0
8.58
4.29
0.0
hsa-miR-3607-5p
hsa-miR-1275
hsa-miR-183-3p
hsa-miR-200b-3p
hsa-miR-548ak-5p
B
-5.8
-1.4
2.8 Log-ratios
IASC-3
IASC-4
IASC-1
IASC-2
IT of CHB-4
IT of CHB-3
IT of CHB-2
IT of CHB-1
6.68
3.34
0.0
11.44
5.72
0.0
hsa-miR-1280
hsa-miR-1911-5p
hsa-miR-33a-5p
hsa-miR-3607-5p
hsa-miR-504-5p
hsa-miR-574-3p
hsa-miR-33b-5p
hsa-miR-16-2-3p
hsa-miR-3178
hsa-miR-5182
C
-7.3
-1.4
2.9 Log-ratios
NC-1
NC-3
NC-2
IT of CHB-4
IT of CHB-2
IT of CHB-3
IT of CHB-1
9.41
4.71
0.0
9.65
4.82
0.0
hsa-miR-3191-5p
hsa-miR-1911-5p
hsa-miR-33a-5p
hsa-miR-32-5p
hsa-miR-106b-3p
hsa-miR-33b-5p
hsa-miR-17-5p
hsa-miR-1275
hsa-miR-611b-5p
hsa-miR-197-3p
hsa-miR-29b-3p
hsa-miR-376b-3p
hsa-miR-29b-3p
hsa-miR-30b-5p
hsa-miR-30d-5p
hsa-miR-29c-3p
hsa-miR-146a-5p
hsa-miR-3607-5p
hsa-miR-342-5p
hsa-miR-29a-3p
hsa-miR-142-5p
D
-6.9
-1.6
6.0 Log-ratios
IASC-2
IASC-1
IASC-4
IASC-3
IA of CHB-3
IA of CHB-2
IA of CHB-4
IA of CHB-1
10.76
5.38
0.0
15.20
7.60
0.0
hsa-miR-5100
hsa-miR-720
hsa-miR-1260b
hsa-miR-3201
hsa-miR-933
hsa-miR-21-5p
hsa-miR-502-5p
hsa-miR-5187-3p
hsa-miR-1226-5p
hsa-miR-5196-3p
hsa-miR-200c-3p
hsa-miR-548ap-5p/hsa-miR-548j
hsa-miR-548ak-5p
hsa-miR-5701
hsa-miR-3679-5p
hsa-miR-3136-3p
hsa-miR-1275
hsa-miR-1827
hsa-miR-191
hsa-miR-660-3p
hsa-miR-1306
hsa-miR-532
hsa-miR-625-3p
hsa-miR-5581-3p
hsa-miR-320c
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hsa-miR-1911-5p

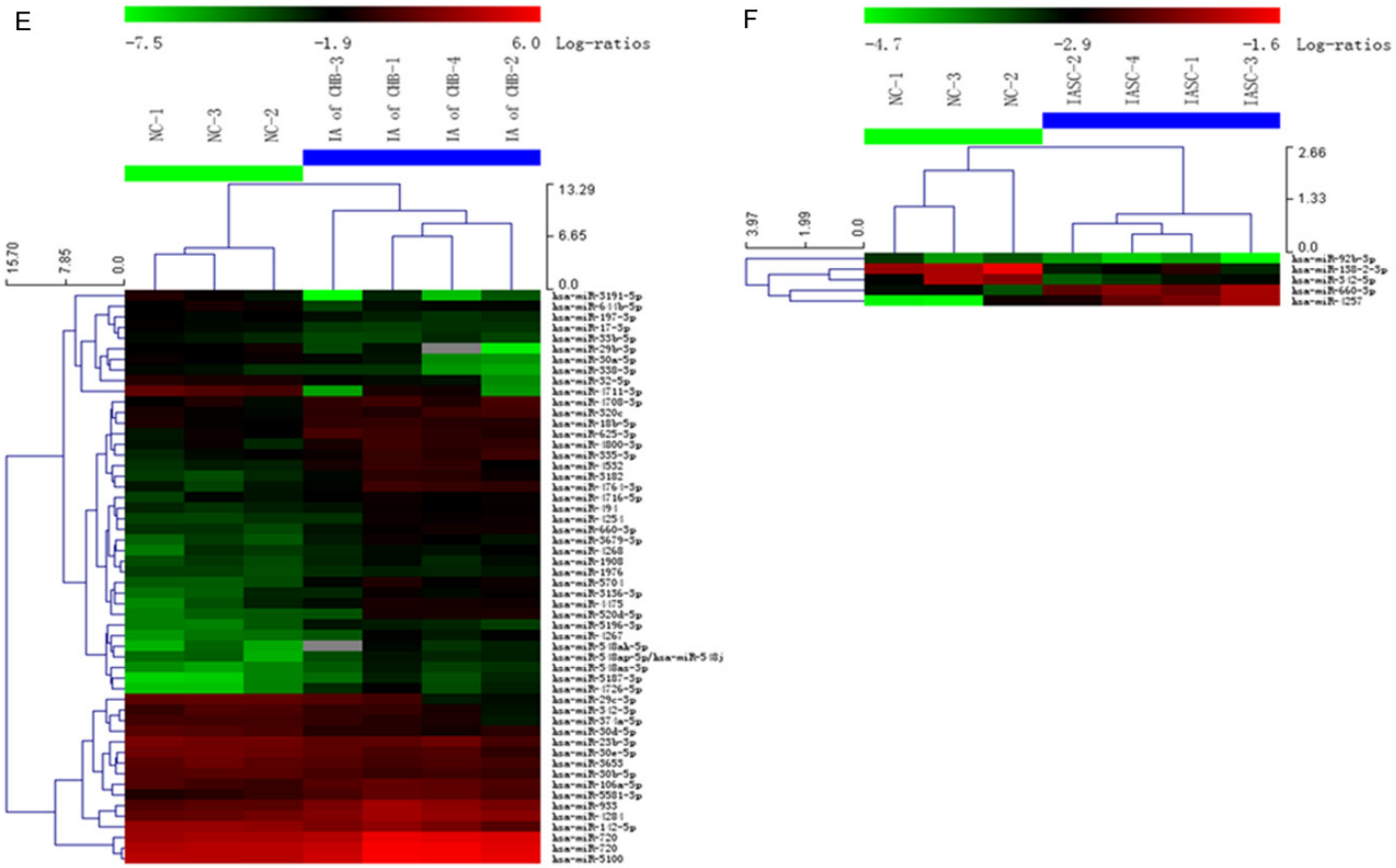

Figure 1. Hierarchical cluster analysis using total miRNA of PBMC in different stages of Chronic HBV infection. A: IT of CHB vs IA of CHB; B: IT of CHB vs IASC; C: IT of CHB vs NC; D: IA of CHB vs IASC; E: IA of CHB vs NC; F: IASC vs NC. Note: 1. Red represents that the upregulation of miRNA; Green represents a downregulation of miRNA. 2. The PBMCs of three subjects from the same group were mixed to a detected sample in the microarray groups.

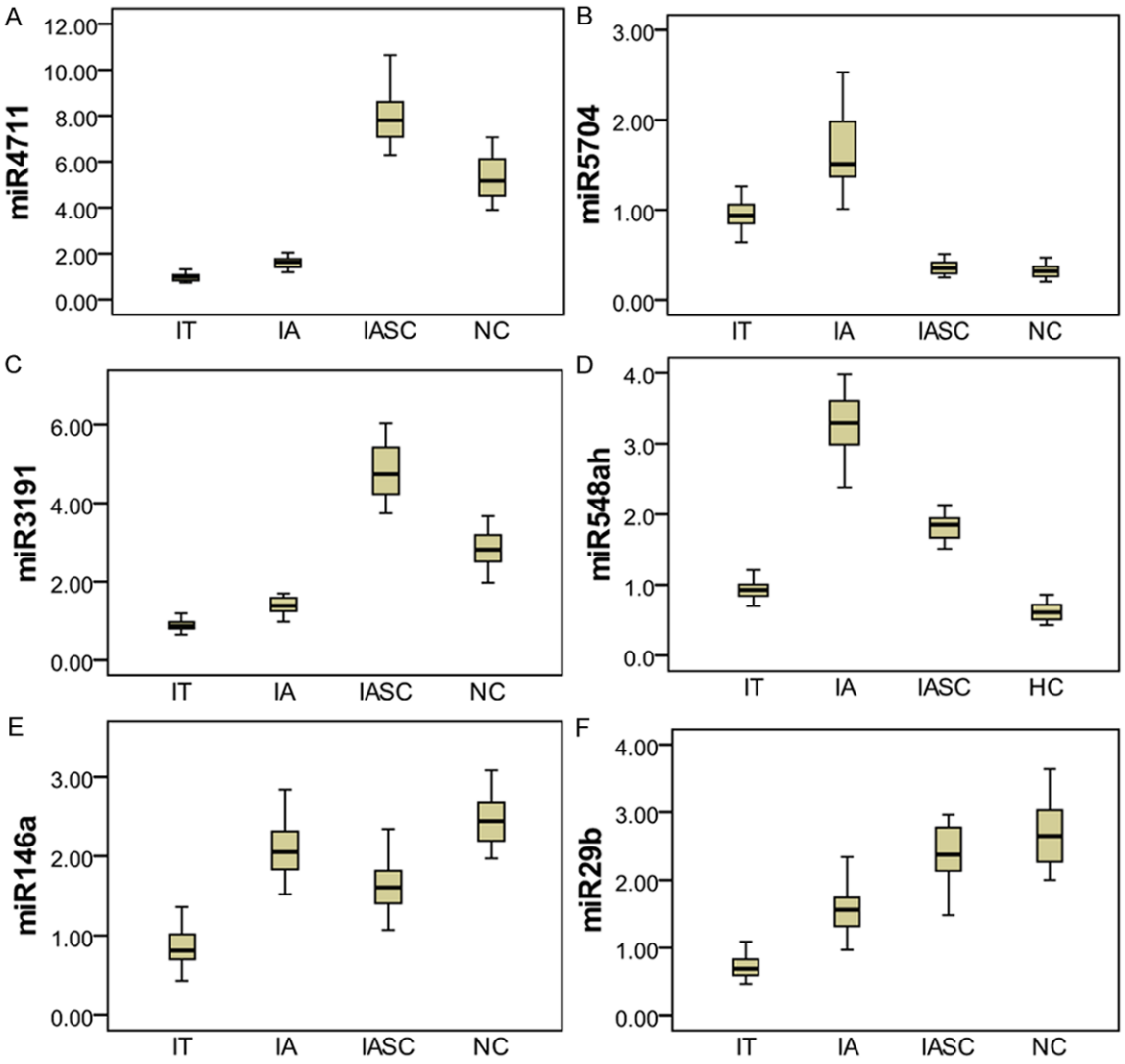

Figure 2. Detection of 6 miRNA molecules by real-time quantitative PCR. A: miR-4711; B: miR-5704; C: miR-3191; D: miR-548ah; E: miR-146a; F: miR-29b.

curve was constructed to evaluate the diagnostic significance of miRNAs. The Pearson correlation was used between variables. Data analysis was performed using SPSS 17.0 (SPSS, Inc.). $P < 0.05$ was considered to indicate statistically significant difference.

Results

Differential expression of miRNAs in PBMCs at different stages of chronic HBV infection

A miRNA microarray was used to detect miRNA expression in PBMCs at different stages of chronic HBV infection. Hierarchical cluster analysis was performed to analyze the resulting data (**Figure 1A-F**). Compared with the controls, 2 significantly up-regulated and 18 significantly down-regulated miRNAs were identified in PBMCs in the IT phase of CHB; 33 significantly up-regulated and 19 significantly down-regulated miRNAs were identified in PBMCs in the IA phase of CHB; and 2 significantly up-regulated and 3 significantly down-regulated miRNAs were identified in PBMCs in IASCs. However, the degree of up- or down-regulation was relatively small. Compared with the IA phase of CHB, 5 significantly down-regulated miRNAs were identified in PBMCs in the IT phase of CHB, with hsa-miR-548ah-5p and hsa-miR-4804-3p showing the largest changes (5.1 and 5.9 times, respectively). A total of 3 significantly up-regulated and 7 significantly down-regulated miRNAs were identified between the IT phase of CHB and IASC. Compared with IASC, 24 significantly up-regulated and 5 significantly

Table 5. ROC curve analysis of 6 miRNA molecules distinguishing different stages of chronic HBV infection

Group	miR-29b-3p	miR-146a-5p	miR-548ah-5p	miR-3191-5p	miR-4711-3p	miR-5704
IT of CHB	100	100	95.2	100	100	100
IA of CHB	96.1	78.1	100	100	100	100
IASC	66.3	96.3	100	100	96.5	63.9

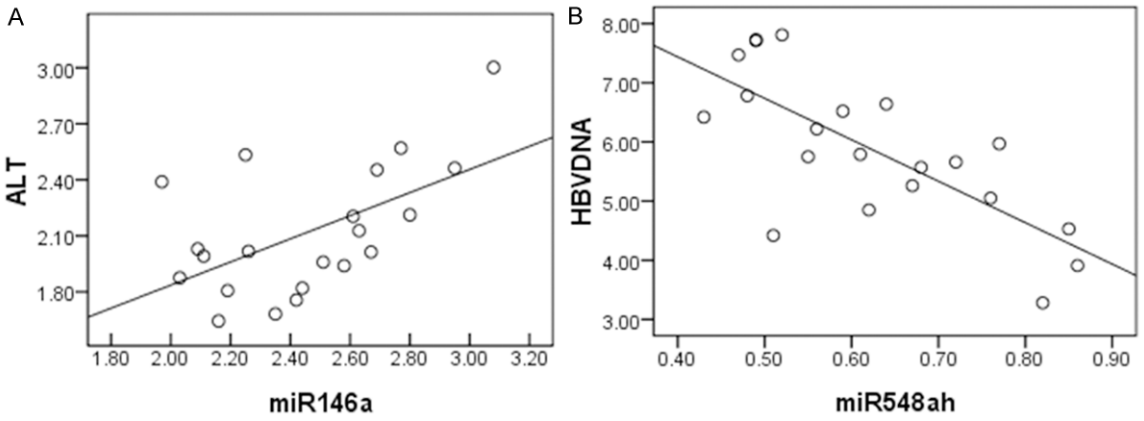

Figure 3. Scatter diagram of correlation between the miR-548ah, miR-146a and ALT and HBV DNA of CHB patients. A: hsa-miR-146a and ALT (r = 0.56, $P < 0.01$); B: hsa-miR-548ah-5p and HBV DNA (r = -0.73, $P < 0.01$).

down-regulated miRNAs were identified in PBMCs in the IA phase of CHB.

Detection of miRNAs by real-time quantitative PCR

Six miRNAs with abnormal expression (more than 5-fold up- or down-regulation), namely hsa-miR-29b-3p, hsa-miR-146a-5p, hsa-miR-548ah-5p, hsa-miR-3191-5p, hsa-miR-4711-3p, and hsa-miR-5704, were selected and analyzed by real-time quantitative PCR in order to validate the microarray results (**Figure 2A-F**). Compared with the controls, the expression levels of hsa-miR-3191-5p and hsa-miR-4711-3p were significantly down-regulated, whereas that of hsa-miR-5704 was significantly up-regulated in patients in the IA and IT phases of CHB (X^2 = 77.56, 78.19, 71.09, respectively; $P < 0.01$). Expression level of hsa-miR-548ah-5p in patients in the IA phase of CHB was significantly up-regulated, whereas that of hsa-miR-146a-5p in patients in the IT phase of CHB was significantly down-regulated (X^2 = 76.14, F = 104.88, $P < 0.01$). Significantly down-regulated expression was observed for hsa-miR-29b-3p in the three stages of chronic HBV infection (X^2 = 67.32, $P < 0.01$).

ROC curve analysis of six miRNAs at different stages of chronic HBV infection

ROC curve analysis was performed to evaluate the diagnostic significance of six miRNAs at different stages of chronic HBV infection. The results of ROC curve analysis of a single miRNA at different stages of chronic HBV infection and in the control are shown in **Table 5**. The areas under the ROC curve of hsa-miR-3191-5p, hsa-miR-548ah, and hsa-miR-4711 were more than 95%, whereas those of hsa-miR-29b-3p, hsa-miR-146a-5p, hsa-miR-548ah-5p, hsa-miR-3191-5p, hsa-miR-4711-3p, and hsa-miR-5704 in the IT and IA phases of CHB were 99.4%, 98.4%, 96.7%, 100%, 100%, and 99.6%, respectively. The sensitivity and specificity for hsa-miR-29b-3p, hsa-miR-146a-5p, hsa-miR-548ah-5p, hsa-miR-3191-5p, hsa-miR-4711-3p, and hsa-miR-5704 were 95.2% and 100%, 100% and 100%, 100% and 100%, 90.5% and 100%, 95.2% and 100%, and 90.5% and 91.3% at cut-off values of 1.10, 1.44, 1.79, 1.20, 1.33, and 1.18, respectively.

Correlation of six miRNAs with clinical indicators of patients with CHB

The ALT level of 21 patients with CHB was 2.1 ± 0. 3 (logarithm values) and the HBV DNA level

was 5.9 ± 1.3 (logarithmic values). Significant positive correlation was found between the levels of hsa-miR-146a and ALT (r = 0.56, P < 0.01). The levels of hsa-miR-548ah-5p were negatively correlated with that of HBV DNA (r = -0.73, P < 0.01), as shown in **Figure 3A** and **3B**. No significant correlation was found among the levels of hsa-miR-29b-3p, hsa-miR-548ah-5p, hsa-miR-3191-5p, hsa-miR-4711-3p, hsa-miR-5704 and ALT. Likewise, no significant correlation was found among the levels of hsa-miR-29b-3p, hsa-miR-146a-5p, hsa-miR-3191-5p, hsa-miR-4711-3p, hsa-miR-5704 and HBV DNA.

Discussion

The immune system plays an important role in the pathogenesis and prognosis of chronic HBV infection. Since miRNAs are known to regulate the immune function of the host, investigating the expression profiles and roles of miRNAs may pave the way for elucidating the pathogenesis of chronic HBV infection and for determining new therapeutic molecular targets. Winther et al. found a relationship between abundance of circulating miRNAs and immunological stages in the natural course of CHB. In addition, certain miRNAs have been suggested to contribute to the establishment and maintenance of CHB in children [19]. In this study, the expression profiles of miRNAs in PBMCs of different stages of chronic HBV infection were detected using miRNA microarray. Our results revealed significant differences in expression profiles of miRNAs between the IT and IA stages of CHB and healthy controls. Overall miRNA expression levels were down-regulated in the IT phase of CHB. This may be attributed to inhibition of miRNA expression and dysfunction of organism-specific immune response resulting from high HBV levels. Significant up- and down-regulation of miRNA molecules were observed in the IA phase of CHB, which may indicate partial recovery of immune function. No significant differences were observed between inactive HBsAg carriers and healthy controls. This result may be attributed to blockade of HBV replication in inactive HBsAg carriers. Thus, expression of miRNAs shows a trend of gradual decline across various immune states of patients with chronic HBV infection, i.e. from immune clearance to immune tolerance and immune control. Our results suggest that abnormal expression levels of miRNAs play important roles in maintaining the immune homeostatic mechanisms of chronic HBV infection.

The significant up-regulation of hsa-miR-548 ah-5p and hsa-miR-4804-3p (5.1 times and 5.9 times, respectively) in the IA phase of CHB in comparison with the IT phase of CHB observed in this study are particularly noteworthy. The human miR-548 belongs to a large gene family that consists of 69 members. Functional enrichment analysis based on the predicted target mRNAs of hsa-miR-548 has shown that the miR-548 gene family plays important roles in multiple biological processes, including various human diseases [20]. Li et al. found that members of the hsa-miR-548 family are highly associated with impaired IFN signaling in chronic hepatitis B [21]. Consistent with this, the expression level of hsa-miR-548 ah-5p was found to be significantly increased in the IA phase of CHB in the current study. HBV DNA is an important biomarker of HBV replication activity. The levels of hsa-miR-548 ah-5p were found to be negatively correlated with HBV DNA in our study. Recent studies have shown that liver-specific miR-122 may down-regulate HBV replication and contribute to persistent/chronic HBV infection [22]. Our results suggest that up-regulation of hsa-miR-548 ah-5p may be closely related to immune control of HBV replication. Hsa-miR-4804 is one of the newly discovered microRNAs and is located in Chromosome 5. The function of hsa-miR-4804 and its role in the pathogenesis of chronic hepatitis B is unclear at present and needs further study.

Murakami et al. reported that miRNA expression pattern in exosome-rich fractionated serum showed high potential as a biomarker for diagnosing the grade and stage of liver diseases [12]. In the present study, the expression levels of six miRNAs in PBMCs were assayed by real-time quantitative PCR. Our results revealed significant differences in the expression levels of these six miRNAs among different stages of chronic HBV infection and healthy controls. ROC curve analysis was performed to explore the diagnostic significance of the six miRNAs at different stages of chronic HBV infection. Results showed that the areas under the ROC curves of miRNAs that distinguished the different stages of chronic HBV infection were all

greater than 95%. Expression of hsa-miR-29b-3p and hsa-miR-5704 in IASC and that of hsa-miR-146a-5p in CHB patients were all less than 80%. The areas under the ROC curves of the six miRNAs in the IT and IA phases of CHB were all higher than 95% at 99.4%, 98.4%, 96.7%, 100%, 100%, and 99.6%, respectively. These results suggest that miRNAs can be used to distinguish the IA and IT phases of CHB. Notably, significant differences in hsa-miR-548ah-5p, hsa-miR-3191-5p and hsa-miR-4711-3p levels among the three phases of chronic hepatitis B infection were observed in both microarray and real-time qPCR results. Hence, hsa-miR-548ah-5p, hsa-miR-3191-5p and hsa-miR-4711-3p may serve as excellent molecular markers for evaluation of immune function of CHB patients.

Several other studies have investigated the function of hsa-miR-146a-5p and hsa-miR-29b-3p in different biological contexts. Taganov et al. found that hsa-miR-146a plays a vital role in negative regulation of the toll-like receptor-mediated inflammatory response [23]. Lu et al. found that hsa-miR-146a plays an important role in the control of Treg cell-mediated Th1 response by targeting signal transducer and activator transcription 1 (Stat-1) [10]. The present study showed that hsa-miR-146a expression was significantly down-regulated in the IT phase of CHB, which may be related to immune tolerance. However, a positive correlation was also found between the levels of hsa-miR-146a and ALT in patients in the IA phase of CHB. Several factors may account for this discrepancy: a) sample sizes were small, b) different means of detection were used, c) expression of different sets of miRNAs was altered. Several studies have shown that the hsa-miR-29 family is implicated in liver fibrosis via regulation of cellular differentiation or translation of extracellular matrix components [24, 25]. Ma et al. found that hsa-miR-29b can directly bind with the 3'-untranslated region of interferon-gamma (IFN-γ) mRNA and thereby reduce IFN-γ expression [26]. The results of the current study show that hsa-miR-29b expression in the IA and IT phases of CHB are significantly down-regulated. This may be associated with immune dysfunction that occurs during chronic HBV infection. However, the functions of hsa-miR-3191-5p, hsa-miR-4711-3p, and hsa-miR-5704 or their roles in chronic HBV infection are not clear and require further investigation.

In summary, our results show that gradual decline in expression of miRNAs with change in immune state of patients with chronic HBV infection may play important roles in the maintenance of immune homeostatic mechanisms of chronic HBV infection. Furthermore, our findings indicate that differential expression of miRNAs can be used to distinguish the different stages of chronic HBV infection. Thus, our findings provide novel insights into the pathogenesis of CHB and provide new candidate molecular markers for evaluating the immune functions of chronic HBV infection patients.

Disclosure of conflict of interest

None.

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