HEPATOLOGY

Characterization of microRNA expression profiles associated with hepatitis B virus replication and clearance in vivo and in vitro
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Key words
hepatitis B virus, HepG2, interferon, microRNA, peripheral blood mononuclear cell.

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Abstract

Background and Aims: Alpha interferon (IFN-α) is an approved treatment for chronic hepatitis B (CHB). MicroRNA (miRNA) are currently known as a part of IFN-mediated antiviral defense. We aimed at characterizing the miRNA expression associated with hepatitis B virus (HBV) replication and IFN-α-mediated HBV clearance.

Methods: We investigated the expression patterns of cellular miRNA induced by HBV replication and/or IFN-α treatment in HepG2 cells, and also analyzed the miRNA response in peripheral blood mononuclear cells in CHB patients on IFN-α treatment. The differentially expressed miRNA were verified using quantitative real-time polymerase chain reaction and an miRNA expression pattern was classified based on the final virological response.

Results: A total of 223 miRNA were differentially expressed (>1.5 folds) between the HepG2.2.15 and HepG2 cells, including 24 highly differentially expressed miRNA (>5 folds). With 12 h of IFN-α treatment, 23 totally differentially expressed miRNA were identified in HepG2 cells; whereas only five miRNA were identified in HepG2.2.15 cells. Similar amounts of the miRNA were regulated in patients with HBeAg or non-HBeAg seroconversion; whereas levels of eight miRNA were significantly differentially expressed between the two groups.

Conclusions: HBV replication alters miRNA expression profiles and impairs IFN-inducible miRNA response in HepG2 cells. The miRNA expression pattern of peripheral blood mononuclear cells in CHB patients with IFN therapy can be associated with their therapeutic outcome.

Introduction

Hepatitis B virus (HBV) is a prototypic member of the hepadnavirus family and a major cause of liver diseases. A significant subset of them progress to chronic liver disease, eventually evolving into cirrhosis and hepatocellular carcinoma.1 Alpha interferon (IFN-α), recently in the pegylated (PEG) form, is an approved treatment for CHB, whereas seroconversion of hepatitis B e antigen (HBeAg) can be induced in only 30% of patients with PEG-IFN-α treatment.2,3 The molecular mechanisms responsible for the ineffectiveness of IFN-α treatment in CHB remain unclear.4,5

MicroRNA (miRNA) are small RNA that can bind to the 30 untranslated region (UTR) of target mRNA and either positively or negatively regulate their translation.6,7 Recent observations indicated that miRNA are a part of IFN-mediated innate antiviral defense and act by directly targeting the viral RNA, including hepatitis C virus (HCV), herpes simplex virus (HSV), human cytomegalovirus (HCMV), Epstein–Barr virus (EBV), Simian vacuolating virus 40 (SV40) and HIV etc., or by regulating the innate immune signal pathway.8,9,10 Evidence also suggested that miRNA may directly regulate IFN-β protein expression and contribute significantly to the regulation of IFN in innate immune responses.11,12

In the present study, using miRNA microarray, we investigated the global expression profiles of cellular miRNA induced by HBV replication and/or IFN-α treatment in HepG2, and miRNA response in peripheral blood mononuclear cells (PBMC) from CHB patients on IFN-α treatment. These analyses allowed us to find potential characteristics of miRNA associated with HBV replication and clearance in vivo and in vitro.

Methods

Cell lines and cell culture

HepG2 cells were maintained in complete Dulbecco’s modified Eagle’s medium (DMEM, Gibco, Grand Island, NY, USA). HepG2.2.15 cells, which derived from HepG2 cells transfecte...
with a plasmid containing HBV DNA, were maintained in complete DMEM supplemented with antibiotics and 250 μg/mL of G418 (Sigma, St. Louis, MO, USA), at 37°C in a humidified incubator at 5% CO2. The cells were seeded at a density of 1 × 10^4 cells/well in a 6-well plate. After 24 h of seeding, the cells were treated with 250 IU/mL of human IFN-α 2b (Sigma) for 12 h.

**Patients for study**

Patients met the following criteria: adults (18–70 years old); positive for HBsAg for more than 6 months; positive for HBeAg; HBV DNA > 20 000 IU/mL; elevated serum alanine aminotransferase (ALT) value two to 10 times the upper limit of the normal range (ULN); granulocyte count ≥ 1.5 × 10^9/L; platelet count ≥ 80 × 10^9/L; and negative urine pregnancy test (for women of childbearing potential). Patients with causes of liver diseases other than CHB, pregnant or/and breast-feeding women, individuals who had used immune regulators within the previous 6 months, individuals who had received antiviral therapy within the previous 6 months, those with uncompensated or compensated cirrhosis, those with antibodies against human immunodeficiency virus, and those with a history of renal dialysis and/or organ transplantation were all excluded. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. This study was conducted in agreement with the Ethics Committee of Peking University People’s Hospital. Written informed consent was obtained from each patient.

**Treatment protocol and definitions**

Twenty-nine patients were enrolled in our study. Ten patients received PEG-IFNα-2b 1 μg/kg for 24 weeks. Nine patients received 1.5 μg/kg for 24 weeks. Ten patients received 1.5 μg/kg for 48 weeks, once weekly. All patients completed the treatment phase. One patient treated with 1.5 μg/kg for 48 weeks dropped out during follow up. The efficacy was assessed at the end of the treatment and after 24 weeks of follow up, including normalization of ALT, suppression of HBV DNA levels to < 12 IU/mL (Cobas Taqman; Roche, Mannheim, Germany), ≥ 2 log IU/mL decrease in HBV DNA level from baseline, and HBeAg seroconversion to anti-HBe (MEIA, Abbott, Abbott Park, IL, USA). Five milliliters of blood was collected at the time-points of pretreatment and the 8th week. PBMC were isolated using standard density gradient centrifugation on Ficoll-Paque (Amersham Biosciences, Freiburg, Germany) and frozen at –80°C for miRNA microarray analysis and quantitative real-time polymerase chain reaction (PCR) detection.

**RNA isolation**

Total cellular RNA from the HepG2, HepG2.2.15 cells and PBMC was extracted using TRIzol RNA reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Low-molecular-weight RNA was isolated using the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA).

**miRNA microarray analysis**

miRNA microarray analysis was performed using miRMAX microarray at Kanchen Bio-tech Corporation (Shanghai, China). A total of 1408 miRNA were examined and subjected to unsupervised hierarchical clustering (Cluster 3.0).

**Quantitative real-time PCR for miRNA verification**

Six differentially expressed miRNA were randomly selected for verification. Expression of these mature miRNA was assayed using stem-loop RT followed by quantitative real-time PCR analysis as previously described. Briefly, quantitative real-time PCR was performed on cDNA generated from 300 ng of total RNA using the protocol of the mirVana qRT-PCR miRNA detection kit (Ambion). A total of 30 ng of cDNA, 200 nmol/L of both sense and antisense primers and SYBR Green PCR Master Mix (PE Applied Biosystems, Foster, CA, USA) in a final volume of 25 μl were used for PCR. Amplification and detection of specific products were performed with the Roter-Gene 3000 real-time PCR (Corbett Research, Sydney, Australia). The relative amount of miRNA was normalized against an internal control U6 snRNA, and the fold change for each miRNA was calculated by the 2-ΔΔCt method. The primers used for stem-loop RT–PCR are shown in Table 1.

**Statistical analysis**

All results are expressed as the mean ± standard deviation. Differentially expressed miRNA were selected by the Significance Analysis of Microarrays (SAM, version 2.1, Stanford University, Stanford, CA, USA). Statistical analysis was done with the Student’s t-test for comparison of two groups. Differences with P < 0.05 were considered statistically significant.

**Results**

**HBV replication effects on miRNA expression in HepG2 cells**

To evaluate the effect of persistent HBV replication on miRNA expression of HepG2 cells, we analyzed the differential expression of miRNA profiles between Hep2.2.15 and the parent HepG2 cells. The miRNA array showed that there were a total of 1408 miRNA were examined and subjected to unsupervised hierarchical clustering (Cluster 3.0).
expression of miRNA between the HepG2.2.15 cells and HepG2 cells treated with IFN-α2b. As shown in Table 3, we identified a total of 23 differential expressing miRNA, including 13 upregulated miRNA and 10 downregulated miRNA, in HepG2 cells; however, only five miRNA were differentially expressed in HepG2.2.15 cells. Among the differentially expressed miRNA in HepG2 cells, a targeted pathway search (miRNA database, http://www.mirdb.org) showed that has-miR-365, hsa-miR-147, hsa-let-7i, has-miR-600 and has-miR-193b were related with the signal pathway of inflammation and cell cycle; hsa-miR-23a was related with the signal pathway of IFN-γ, interleukin, B cell activation, DNA replication and de novo purine biosynthesis.
Clinical efficacy in patients

At the end of the treatment, ALT normalization occurred in 11 patients, suppression of HBV DNA levels to <12 IU/mL occurred in one patient, ≥2 log IU/mL decrease in HBV DNA level from baseline occurred in 12 patients, six patients achieved HBeAg seroconversion, and one patient achieved HBsAg seroconversion. At the end of the 24-week follow up, ALT normalization occurred in eight patients, suppression of HBV DNA levels to <12 IU/mL occurred in two patients, ≥2 log IU/mL decrease in HBV DNA level from baseline occurred in 10 patients, and HBeAg seroconversion occurred in eight patients. One patient maintained HBsAg seroconversion. The clinical data of patients are listed in Table 4.

Characterization of miRNA expression of PBMC on IFN therapy

Based on the outcome at the end of the 24-week follow up, sixteen PBMC samples (eight HBeAg seroconversion vs. eight non-HBeAg seroconversion) were selected for miRNA expression detection. The pre-treatment expression levels of miRNA were defined as the baseline and compared with the levels of that treated for 8 weeks. The results showed that there were similar amounts of the differential expression miRNA in the HBeAg seroconversion and non-HBeAg seroconversion groups (82.25 ± 15.84 vs. 86.75 ± 46.70, P = 0.4306). However, the individual miRNA expression analysis revealed that the levels of five miRNA (hsa-miRPlus-E1063, hsa-miRPlus-E1236, evb-miR-BHRF1-2, hsa-miR-876-3p, hsa-miR-760) were significantly different between the two groups. The detailed results are listed in Table 3.1 and Table 3.2.

Table 3-1 Differential expression of miRNA induced by interferon in HepG2 cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold</th>
<th>Sequence (5’→3’)</th>
<th>Predicted target pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-876-3p</td>
<td>1.9659</td>
<td>uggugguucaaaaguauuca</td>
<td>Unknown</td>
</tr>
<tr>
<td>hsa-miR-760</td>
<td>1.9652</td>
<td>cccggcuuggucugggaga</td>
<td>Unknown</td>
</tr>
<tr>
<td>hsa-miR-99a*</td>
<td>1.7361</td>
<td>ccaacucgcuaauuaagcug</td>
<td>Unknown</td>
</tr>
<tr>
<td>hsa-miR-800</td>
<td>1.7285</td>
<td>acauacacagaaagugucuc</td>
<td>p53 pathway, heteromeric G-protein signaling pathway-Gq alpha and Go alpha-mediated pathway</td>
</tr>
<tr>
<td>hsa-miR-199b</td>
<td>1.6770</td>
<td>uguauauuuaauauuagugg</td>
<td>Unknown</td>
</tr>
<tr>
<td>eiv-miR-BART12</td>
<td>1.6704</td>
<td>cccggcuuggucugggga</td>
<td>EBV life cycle</td>
</tr>
<tr>
<td>hsa-miR-585</td>
<td>1.6169</td>
<td>ggggguaucauauacucua</td>
<td>Unknown</td>
</tr>
<tr>
<td>hsa-miR-611</td>
<td>1.5835</td>
<td>cggcaggccuccggggcug</td>
<td>Unknown</td>
</tr>
<tr>
<td>hsa-miR-487b</td>
<td>1.5705</td>
<td>acucacucacacaucaacucu</td>
<td>Unknown</td>
</tr>
<tr>
<td>hsa-miR-688-6p</td>
<td>1.5523</td>
<td>uguauauuuaauauuagugg</td>
<td>KSHV latent infection</td>
</tr>
<tr>
<td>hsa-miR-147</td>
<td>1.5131</td>
<td>cgggguaucauauacucucu</td>
<td>Cell cycle, inflammation, insulin/ISG, interleukin, TGF-beta</td>
</tr>
<tr>
<td>hsa-miR-149*</td>
<td>1.5056</td>
<td>cggggguaucauauacucucu</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Table 3-2 Differential expression of miRNA induced by interferon in HepG2.2.15 cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold</th>
<th>Sequence (5’→3’)</th>
<th>Predicted target pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-98</td>
<td>2.1257</td>
<td>ugggguaucaaaaguauuca</td>
<td>Apoptosis, Inflammation, TGF-beta, Wnt</td>
</tr>
<tr>
<td>hsa-miR-876-3p</td>
<td>1.5938</td>
<td>ugggguaucaaaaguauuca</td>
<td>Unknown</td>
</tr>
<tr>
<td>hmrv-miR-US25-2-5p</td>
<td>1.5843</td>
<td>cgggguaucauauacucua</td>
<td>Unknown</td>
</tr>
<tr>
<td>hsa-miR-135*</td>
<td>1.5843</td>
<td>cgggguaucauauacucua</td>
<td>Circadian clock system</td>
</tr>
<tr>
<td>eiv-miR-BART14</td>
<td>1.5049</td>
<td>cgggguaucauauacucua</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

EBV, Epstein–Barr virus; KSHV, Kaposi’s sarcoma-associated herpesvirus; PDGF, platelet-derived growth factor; TGF, transforming growth factor.

The sequences and predicted target pathways of miRNA were searched from miRNA database at website: http://mirdb.org/wiki/index.php?title=Main_Page.

TGF, transforming growth factor.
miRPlus-A1098 and hsa-miRPlus-E1138) in the non-HBeAg seroconversion group were significantly higher than that in the HBeAg seroconversion group, whereas the levels of three miRNA (hsa-miR-618, hsa-miR-1267 and hsa-miRPlus-E1163) in the non-HBeAg seroconversion group were significantly lower (Table 5).

Unsupervised hierarchical clustering based on all the miRNA spotted on the chip, revealed a marked, very distinct separation according to the patients’ final response to the IFN therapy (Fig. 1).

Validation of the microarray result by quantitative real-time PCR

The miRNA hsa-miR-98, ebv-miR-BART12 and hsa-miR-149* (IFN-induced miRNA in HepG2 or HepG2.2.15 cells), and the miRNA hsa-miR-618, hsa-miR-1267 and ebv-miR-BHRF1-2 (differentially expressed between HBeAg seroconversion and non-HBeAg seroconversion groups), were verified using stem-loop-based quantitative real-time PCR. The results of quantitative real-time PCR corresponded with the results of microarray analysis (Fig. 2).

Discussion

Recent evidences have shown that the several IFN-induced miRNA in PBMC or liver tissue in subjects may represent an important determinant of the clinical outcome of IFN therapy in HCV infection.18–20 Although there is a wide difference in the virological features between HBV and HCV, infection of the

Table 4  Baseline characteristics comparison of patients with HBeAg or non-HBeAg seroconversion at the end of 24-week follow up

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HBeAg seroconversion</th>
<th>Non-HBeAg seroconversion</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean ± SD</td>
<td>22.75 ± 2.06</td>
<td>29.52 ± 9.29</td>
<td>0.164</td>
</tr>
<tr>
<td>Sex (male : female)</td>
<td>6:2</td>
<td>17:4</td>
<td>0.326</td>
</tr>
<tr>
<td>ALT (U/L), mean ± SD</td>
<td>247.75 ± 104.33</td>
<td>158.40 ± 71.12</td>
<td>0.037</td>
</tr>
<tr>
<td>HBV DNA (IU/mL), median (range)</td>
<td>7.6 x 10^6 (2.2 x 10^4 - 1.3 x 10^6)</td>
<td>1.1 x 10^6 (5.84 x 10^4 - 1.1 x 10^6)</td>
<td>0.048</td>
</tr>
<tr>
<td>Genotype (B : C)</td>
<td>4.4</td>
<td>9.12</td>
<td>0.823</td>
</tr>
</tbody>
</table>

*P < 0.05.

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus DNA.

Table 5  Differential expression of miRNA in PBMC in patients with HBeAg or non-HBeAg seroconversion

<table>
<thead>
<tr>
<th>miRNA</th>
<th>HBeAg seroconversion (Mean ± SD)</th>
<th>Non-HBeAg seroconversion (Mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miRPlus-E1063</td>
<td>2.0756 ± 0.6428</td>
<td>0.8556 ± 0.1362</td>
<td>0.0050</td>
</tr>
<tr>
<td>hsa-miRPlus-E1236</td>
<td>2.5157 ± 0.6116</td>
<td>0.7810 ± 0.3568</td>
<td>0.0014</td>
</tr>
<tr>
<td>ebv-miR-BHRF1-2</td>
<td>1.6773 ± 0.3435</td>
<td>0.8388 ± 0.2087</td>
<td>0.0029</td>
</tr>
<tr>
<td>hsa-miRPlus-A1098</td>
<td>1.9986 ± 0.3801</td>
<td>0.7660 ± 0.1286</td>
<td>0.0004</td>
</tr>
<tr>
<td>hsa-miRPlus-E1138</td>
<td>2.1732 ± 0.6139</td>
<td>1.1439 ± 0.1364</td>
<td>0.0035</td>
</tr>
<tr>
<td>hsa-miR-618</td>
<td>1.2736 ± 2.0169</td>
<td>3.7507 ± 1.1015</td>
<td>0.0372</td>
</tr>
<tr>
<td>hsa-miR-1267</td>
<td>0.9575 ± 0.6393</td>
<td>2.8705 ± 1.3531</td>
<td>0.0216</td>
</tr>
<tr>
<td>hsa-miRPlus-E1163</td>
<td>0.9474 ± 0.5700</td>
<td>5.2430 ± 3.8600</td>
<td>0.0350</td>
</tr>
</tbody>
</table>

HBeAg, hepatitis B e antigen; PBMC, peripheral blood mononuclear cells; SD, standard deviation.
human liver with these two distinct viruses induces similar disease profiles and IFN treatment is a common therapeutic. Previous reports described several miRNA associating with HBV replication, hepatocarcinogenesis and cell migration in HepG2 cells.\textsuperscript{21–25}

In the present study, using miRNA microarray analysis, we mainly investigated the miRNA expression profiles related to IFN-mediated anti-HBV therapy \textit{in vivo} and \textit{in vitro}.

\textbf{HBV replication alerts miRNA expression in HepG2 cells}

HepG2.2.15 cells produce infectious HBV particles and thus can reveal the initial effects induced by HBV components. Our microarray analyses showed that miRNA expression in the HBV-transfected cell line HepG2.2.15 differed from that in parental HepG2 cells. Of the 1408 miRNA investigated, 44 miRNA were upregulated and 179 miRNA were downregulated in HepG2.2.15 cells. Of these, 24 miRNA were highly differentially expressed (\textgreek{g} 5 folds), including 15 upregulated and nine downregulated miRNA. As shown in Table 2, most of these miRNA are predicted to target the signal pathways of angiogenesis, apoptosis, oxidative stress response, p53, Fas, interleukin, IFN-\textgamma and inflammation. These findings are consistent with previous studies that suggest that HBV replication modulates the process of carcinogenesis, apoptosis and oxidative stress response of the cells.\textsuperscript{36–39}

Except for these highly differentially expressed miRNA, we also found that several of the low/medium differentially expressed miRNA (supplemental Table S1) changed similarly to those in previous studies. For example, miR-181a (3.589), miR-196b (3.114), miR-146a (4.043) and miR-17 (0.416) had similar changes, as previously reported.\textsuperscript{21} Let-7a was downregulated (0.316) in HepG2.2.15 and it had been demonstrated that let-7a is downregulated by hepatitis B virus x protein and targets signal transducer and activator of transcription 3.\textsuperscript{33} miR-29a was upregulated (1.966) in HepG2.2.15, and the upregulation is supposed to enhance hepatoma cell migration by targeting phosphatase and tensin homolog (PTEN) in a cell culture model.\textsuperscript{25} Several different changes were also observed, such as miR-16, which was upregulated (4.299) in HepG2.2.15 cells in our study; but another study showed that it was downregulated;\textsuperscript{21,24} and no obvious changes were observed for miR-181c, miR-181d and miR-378, which were reported to be changed before.\textsuperscript{21}

\textbf{HBV replication impairs IFN-\alpha induced miRNA response in HepG2 cells}

Consistent with previous reports,\textsuperscript{20–22} our detection showed that supernatant HBV DNA and intracellular replicative mediators were not obviously inhibited by IFN-\alpha treatment at 250 IU/mL even for 3 days, except that the cells were treated with IFN-\alpha at more than 1000 IU/mL, which is associated with inhibition of cell growth and cytotoxicity (data not shown).

Previous studies demonstrated that expression of IFN-inducible genes was impaired by HBV replication in HepG2 cells. For example, a domain of HBV polymerase associates with the failure to respond to IFN therapy.\textsuperscript{33} Precore/core proteins specifically inhibit the expression of the IFN-inducible gene, MxA by direct interaction reduces the antiviral activity of IFN in HepG2.2.15 cells.\textsuperscript{34–36} More recently, the surface proteins and/or the x protein is supposed to inhibit the methylation of the IFN-\alpha signal transducer STAT1 and then suppress the IFN response.\textsuperscript{31,37,38} Guan et al. demonstrated that the impaired IFN response in HepG2.2.15 cells is partially restored by inhibiting HBV replication.\textsuperscript{30} In the present work, we found that miRNA expression was differentially induced in HepG2.2.15 and HepG2 by IFN. A total of 23 differentially expressed miRNA were induced in HepG2 while only five were induced in HepG2.2.15 cells. These observations suggest that HBV replication likely impairs the IFN-stimulated antiviral miRNA response in HepG2 cells. On the contrary, further investigation is needed to verify whether the weakened miRNA response plays a role in the null IFN-mediated inhibition of HBV DNA.

\textbf{miRNA expression pattern of PBMC predicts therapeutic outcome of IFN-\alpha treatment}

We evaluated the value of early differential expression of miRNA in PBMC for prediction of outcome of IFN therapy in CHB patients. There was no significant difference in the total amounts of differentially expressed miRNA between the HBeAg and non-HBeAg seroconversion groups. As shown in Table 5, we found that eight miRNA were significantly differentially expressed; particularly, six miRNA, such as hsa-miRPlus-E1063, hsa-miRPlus-E1236, evb-miR-BHRF1-2, hsa-miRPlus-A1098, hsa-miR-1267 and hsa-miRPlus-E1163, were regulated contrarily in HBeAg and non-HBeAg seroconversion groups. However, no individual IFN-inducible miRNA in PBMC overlaps the miRNA that was induced...
in HepG2 or HepG2.2.15 culture cells. We hypothesized that PBMC and HepG2/HepG2.215 cells belong to different cell genera and the experiments were performed in vivo and in vitro, respectively. Presumably, response for no overlapping induced miRNA profiles between these groups. Although larger-scale studies are required to gain a better understanding of these differentially expressed miRNA in CHB patients treated with IFN, we believe that our obtaining miRNA pattern is a potential marker for predicting the drug response.

**IFN-induced ubiquitous infectious virus-related miRNA**

Interestingly, among these differentially expressed miRNA, several virus-related miRNA, such as ebv-miR-BART12, ebv-miR-BART14, hcmv-miR-US25-2-5p, kshv-miR-K12-6-3p and ebv-miR-BHRF1-2, were also regulated by IFN in vivo or in vitro experiments (Tables 3 and 5). Among of them, Cai et al. demonstrated that miRNA ebv-miR-BART12 plays important roles in the viral lifecycle.\(^{39,40}\) kshv-miR-K12-6-3p has been hypothesized to play a critical role in the establishment and/or maintenance of Kaposi’s sarcoma-associated herpesvirus (KSHV)-latent infection and KSHV-induced oncogenesis.\(^{1,42}\) ebv-miR-BHRF1-2 relates to EBV latency and after induction of replication and B lymphocytes transformation.\(^{43,44}\)

EBV and HCMV are ubiquitous infections in adults and the viral infections persist in carriers as a lifelong, mostly asymptomatic infection.\(^{45,46}\) KSHV infection is associated with immune suppression, such as uncontrolled AIDS and immunosuppression from medications used in organ transplantation or chemotheraphy for tumor.\(^{47}\) These viruses might infect many different cell types in vivo, including epithelial cells, connective tissue cells, hepatocytes, various leukocyte populations and vascular endothelial cells. However, EBV and HCMV only infect human primary human B cells and primary fibroblasts in vitro, respectively, and there is no truly permissive tissue culture system for KSHV currently.\(^{48,49}\) Owing that HepG2 cells resistant to infection of these viruses in vitro and PBMC were isolated for analysis without a procedure of cell culture, we deduced that these ubiquitous, infectious virus-related miRNA most likely originated from the host infected with the viruses.

The IFN-induced differential expression of the viral miRNA suggests that IFN impacts on the viral life cycle of these viruses. The association between expression of ebv-miR-BHRF1-2 and HBeAg seroconversion in PBMC implies the predictive value of ebv-miR-BHRF1-2 on therapeutic outcome of CHB IFN treatment, although the miRNA unlikely intervene in the HBV life cycle or IFN signal pathway in a direct way.

In conclusion, our present study shows that HBV replication alters miRNA expression and impairs the IFN-inducible miRNA response in HepG2 cells. Although larger-scale and further studies are indeed needed to clarify the connection between miRNA expression and patient response to IFN therapy, miRNA expression pattern in PBMC may associate with clinical outcome of IFN-α therapy in CHB.

**Acknowledgments**

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**References**

microRNA expression and hepatitis B virus


Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Low/medium differential expression of miRNA in HepG2.2.15 and HepG2 cells

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