着率が有意に高率であった。またPBMCを移入したマウスでは有意にHBV DNAレベルが低下していた。さらに、組織学的検討では、PBMC移入マウスでは肝臓内へのリンパ球の集簇が認められ、肝細胞の細胞死も認められた。これらのことから移入したヒトPBMCによってHBV感染肝細胞を傷害しHBV DNA量が低下したと考えられた。次に、血清中の各種サイトカインの産生レベルを調べたところPBMC移入マウスではコントロールマウスに比較して有意にGranzyme A、IFN-γレベルが上昇していた。さらに本モデルマウスにおけるGranzyme A、IFN-γ産生細胞は何か調べるためにフローサイトメトリーにより肝臓内に浸潤したリンパ球の表現型を検討した。その結果、HBV感染マウスでは非感染マウスに比較してヒトPBMCの生着率が2.6倍程度上昇しており、またPBMC移入マウスでのみCD8陽性、テトラマー陽性のHBV特異的CTLが検出された。これらのことから本モデルにおける肝障害はHBV特異的CTLが主役であると考えられた。そこで、有効な治療法が確立していないB型劇症肝炎に対する有効な治療法していないためT細胞を標的とした薬剤であるCTLA4Igを用いてT細胞の活性化を抑制することでHBV感染肝細胞の傷害が抑制できるか否かについて検討を行った。PBMC移入前にCTLA4Igを投与したところヒトアルブミン値の減少やHBV DNA量の減少は認められずCTLA4IgによってHBV感染肝細胞の傷害が阻害された。

D. 考察

ヒト肝細胞キメラマウスにB型急性重症肝炎治癒後の患者から得られたPBMCを移入することで、HBV特異的なCTLによるヒトの劇症肝炎を再現するモデルを作製した。

E. 結論

本モデルマウスにより免疫学的機序によるウイルス排除によるHBVの持続感染から治癒を目指す治療を開発することが可能となった。

G. 研究発表

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2. 学会発表

なし

H. 知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得

提出中

2. 実用新案登録

なし

3. その他

なし

Ⅲ. 研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表

雑誌

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Ⅳ．　研究成果の刊行物・別刷

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Human microRNA hsa-miR-1231 suppresses hepatitis B virus replication by targeting core mRNA

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SUMMARY. Pathogen-specific miRNA profiles might reveal potential new avenues for therapy. To identify miRNAs directly associated with hepatitis B virus (HBV) in hepatocytes, we performed a miRNA array analysis using urokinase-type plasminogen activator (uPA)–severe combined immunodeficiency (SCID) mice where the livers were highly repopulated with human hepatocytes and human immune cells are absent. Mice were inoculated with HBV-infected patient serum samples. Eight weeks after HBV infection, human hepatocytes were collected from liver tissues, and miRNAs were analysed using the Toray 3D array system. The effect of miRNAs on HBV replication was analysed using HBV-transfected HepG2 cells. Four miRNAs, hsa-miR-486-3p, hsa-miR-1908, hsa-miR-675 and hsa-miR-1231 were upregulated in mouse and human livers with HBV infection. These miRNAs were associated with immune response pathways such as inflammation mediated by chemokine and cytokine signalling. Of these miRNAs, hsa-miR-1231, which showed high homology with HBV core and HBx sequences, was most highly upregulated. In HBV-transfected HepG2 cells, overexpression of hsa-miR-1231 resulted in suppression of HBV replication with HBV core reduction. In conclusion, a novel interaction between hsa-miR-1231 and HBV replication was identified. This interaction might be useful in developing new therapeutic strategies against HBV.

Keywords: HB core, hepatitis B virus, hsa-miR-1231, human hepatocyte chimeric mouse, microRNA.

INTRODUCTION

Hepatitis B virus (HBV) is a member of the *Hepadnaviridae* family, which contains a group of hepatotropic small DNA viruses that infect their respective animal hosts [1–3]. Once HBV infects human hepatocytes, the HBV genome translocates into the nucleus. Some genome copies are converted into a covalently closed circular DNA (cccDNA) form and organized into a minichromosome with histone and nonhistone proteins [4–8]. HBV cccDNA utilizes the cellular transcriptional machinery to produce all viral RNAs including the pregenomic RNA [9], and these gene products regulate viral replication and pathogenesis by regulating host gene expression [10,11].

MicroRNAs (miRNAs) are small noncoding RNAs of 21-25 nucleotides in length, processed from hairpin-shaped transcripts [12]. MiRNAs can bind the 3′-untranslated regions (UTRs) of messenger RNAs and downregulate gene expression by cleaving messenger RNA or inhibiting translation. Several miRNAs associated with HBV infection, HBV replication and hepatocarcinogenesis have recently been identified [13–19]. However, the direct influence of HBV infection on miRNA expression is still unclear.

MicroRNAs are currently being investigated for their therapeutic potential in antiviral therapy. As several studies have demonstrated that hsa-miR-122, which is specifically and abundantly expressed in hepatocytes, supported hepatitis C virus (HCV) replication by improving RNA

Abbreviations: HBc, hepatitis B core; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; miRNA, microRNA; RI, replication intermediates.

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stability [20–24]. small molecules or siRNAs which are able to knock down miR-122 expression have been explored as a new therapeutic agent for HCV eradication.

A similar microRNA-based antiviral approach is also sought for the treatment of chronic hepatitis B, as it is difficult to eradicate HBV genomes converted into cccDNA or minichromosomes under present antiviral therapies. To develop new strategies for complete eradication of the viral genome from hepatocytes, it is important to clarify the direct associations between hepatic miRNAs and HBV infection.

In this study, miRNA microarray analysis was performed using human hepatocyte chimeric mouse livers to assess the direct impact of HBV infection on miRNA expression. We successfully demonstrated that HBV infection attenuated the expression of miRNAs under immunodeficient conditions to protect early viral propagation. A novel interaction between hsa-miR-1231 and HBV replication was identified.

MATERIALS AND METHODS

Human serum inoculum

Serum samples were obtained from a carrier infected with HBV genotype C after obtaining written informed consent for the donation and evaluation of blood samples. Inoculum was positive for HBs and HBe antigens with high-level viremia (HBV DNA: 7.1 log copies/mL). The experimental protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Hiroshima University Hospital ethical committee (Approval ID: D08-9).

Human hepatocyte chimeric mice experiments

Human hepatocyte chimeric mice (PXB mice), in which human hepatocytes were transplanted into $uPA^{+/+}/SCID^{+/+}$ mice, were purchased from Phoenix Bio (Hiroshima, Japan). Mouse experiments were performed in accordance with the guidelines of the local committee for animal experiments at Hiroshima University.

Six chimeric mice, in which more than 90% of the liver tissue was replaced with human hepatocytes, were divided into two experimental groups. Group A contained three uninfected mice. Group B consisted of three mice that were inoculated via the mouse tail vein with human serum containing 6×10^6 copies of HBV. Serum HBV DNA titres were quantified every 2 weeks by real-time PCR, and human albumin levels were measured using the Human Albumin ELISA Quantitation kit (Bethyl Laboratories Inc., Montgomery, TX, USA) as described previously [25]. Eight weeks after inoculation, all three infected mice were sacrificed. Infection, extraction of serum samples and sacrifice were performed under ether anaesthesia as described previously [26].

miRNA microarray analysis

Human hepatocytes were finely dissected from the mouse livers and stored in liquid nitrogen after submerging in RNA*later*® solution (Applied Biosystems, Foster City, CA, USA). Experimental sample RNAs were isolated using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and analysed using TORAY 3-D Gene Chip human miRNA ver. 12.1 (TORAY, Chiba, Japan).

Data analysis

Gene expression profiles were analysed using GeneSpring GX 10.0.2 software (Tomy Digital Biology, Tokyo, Japan). Expression ratios were normalized per chip to the 50th percentile. To determine whether there were miRNAs differentially expressed among samples, we performed two Welch's *t*-tests ($P < 0.01$) on this prescreened list of miRNAs with Benjamini and Hochberg's correction. Complete linkage hierarchical clustering analysis was applied using Euclidean distance.

Pathway analysis

The miRNA target genes were predicted by the online database miRWalk (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/index.html). Target prediction was performed using 3′-UTR sequences of mRNAs, and the probability distributions were calculated using the Poisson distribution [27]. The mRNAs with *P* values <0.01 were considered significant. To improve the accuracy of target gene selection, the predicted genes were screened using other prediction programs, including miRanda (August 2010 release), miRDB (April 2009 release) and TargetScan version 5.1 (Whitehead Institute for Biomedical Research, Cambridge, MA, USA). Genes that were predicted by at least two alternate programs were selected. Pathway analysis was performed by PANTHER version 8.1 (http://www.pantherdb.org/) to determine the effects of the predicted target genes on pathways.

Quantification of miRNAs

Small RNAs were extracted from liver tissues or HepG2 cells with mirVana™ miRNA Isolation Kit (Applied Biosystems) and reverse-transcribed according to the manufacturer's instructions. The selected miRNAs were quantified with TaqMan® MicroRNA Assays (Applied Biosystems) using the 7300 Real-Time PCR System (Applied Biosystems), and the expression of RNU6B served as a control.

Quantification of mRNAs

Total RNA was extracted from HepG2 cells transfected with control miRNA or miR-1231 expression plasmid using

RNeasy Mini Kit and reverse-transcribed (RT) using ReverTra Ace (TOYOBO, Osaka, Japan) with random primer according to the manufacturer's instructions. The selected cDNAs were quantified by real-time PCR. Differences between groups were examined for statistical significance using Student's *t*-test. The primer sequences were as follows: GAPDH forward 5′-ACAACAGCCTCAAGATCATCAG-3′ and reverse 5′-GGTCCACCACTGACACGTTG-3′; Mx1 forward 5′- TTCGGCTGTTTACCAGACTCC-3′ and reverse 5′- CAAAGCCTGGCAGCTCTCTAC-3′; 2′–5′ oligoadenylate synthetase 1 (OAS1) forward 5′- ACCTGGTTGTCTTCCTCA GTCC-3′ and reverse 5′- GAGCCTGGACCTCAAACTTCAC-3′; double stranded RNA dependent protein kinase (PKR) forward 5′- TGGCCGCTAAACTTGCATATC-3′ and reverse 5′- AGTTGCTTTGGGACTCACACG-3′; and SOCS1 forward 5′-ACGAGCATCCGCGTGCACTT-3′ and reverse 5′-AAGAGG CAGTCGAAGCTCTC-3′.

Plasmid construction

The construction of wild-type HBV 1.4 genome length, pTRE-HB-wt, was described previously [25]. The nucleotide sequence of the cloned HBV genome was deposited into GenBank AB206817. The HBc and HBx genes, amplified from pTRE-HB-wt, were cloned into pcDNA3 and p3xFLAG-CMV10 vectors and designated pcDNA-HBc and p3FLAG-HBx, respectively. The human miR-1231 precursor expression plasmid (HmiR0554-MR04) and the control miRNA plasmid (CmiR0001-MR01), which was a miRNA-scrambled control clone, were commercially produced (GeneCopoeia™, Rockville, MD, USA).

Transfection of HepG2 cell lines with the plasmids

The HBV expression plasmid was transfected into HepG2 cells with control miRNA or miR-1231 expression plasmid using *Trans*IT-LT1 (Mirus, Madison, WI, USA) reagent according to the manufacturer's instructions. 24–48 h after transfection, core-associated HBV DNA and HBV RNA were extracted and quantified by real-time PCR or RT real-time PCR, respectively [28]. For identifying targets within the HBV genome, HBc or HBx expression plasmids were transiently transfected with miR-1231 expression plasmid into HepG2 cells. Twenty-four hours after transfection, the cells were harvested to perform Western blot analysis.

Analysis of HBV replication intermediates

Quantitative analysis of HBV replication intermediates was performed as described previously [29]. The HBV-specific primers used for amplification were 5′-TTTGGGCATGGAC-ATTGAC-3′ and 5′-GGTGAACAATGTTCCGGAGAC-3′. The lower detection limit of this assay was 300 copies.

Western blot analysis

Cell lysates, prepared with RIPA like buffer [50 mM Tris-HCl (pH 8.0), 0.1% SDS, 1% NP-40, 150 mM sodium chloride, and 0.5% sodium deoxycholate] containing protease inhibitor cocktail (Sigma-Aldrich, Tokyo, Japan), were separated on 5–20% (wt/v) SDS-polyacrylamide gels (Bio-Rad Laboratories, Inc., Tokyo, Japan). Immunoblotting was performed with anti-FLAG M2 monoclonal antibody (Sigma-Aldrich) or anti-HBV core monoclonal antibody HB91 (Advanced Life Science Institute Inc., Saitama, Japan) or anti β-actin monoclonal antibody (Sigma-Aldrich) followed by incubation with horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin (GE Healthcare, Buckinghamshire, UK). Expression of HBc protein was quantified based on the densities of the immunoblot signals by Quantity One® software (Bio-Rad Laboratories, Inc.).

RESULTS

miRNA expression alterations associated with HBV infection

To analyse the influence of HBV infection on human hepatocytes, miRNA microarray expression profiles were compared between groups A (mice without HBV infection) and B (mice with HBV infection). Among the 900 miRNAs on the microarray, 10 miRNAs showed a more than 2.0-fold change with HBV infection. Five of the 10 miRNAs were upregulated, and the remaining five were downregulated (Fig. S1). Because immunity was severely suppressed in the chimeric mice, changes in miRNA expression are thought to be closely associated with HBV infection, and the upregulated miRNAs might play a protective role against HBV infection. Thus, we focused on these 5 upregulated miRNAs.

Comparison of expression of the 5 upregulated miRNAs in human liver tissues

To verify the microarray results, quantitative analysis of miRNAs was performed using liver tissues from the chimeric mice. Three of the 5 miRNAs were significantly upregulated by HBV infection (Fig. 1). Expression changes in the other 2 miRNAs (hsa-miR-675 and hsa-miR-1908) showed a similar trend but were not significant due to individual variation. Therefore, further quantitative analysis was performed using human liver tissues. Nine liver tissue samples were obtained from patients with chronic hepatitis B ($N = 3$), chronic hepatitis C ($N = 2$) or alcoholic liver dysfunction ($N = 4$), and miRNA expression levels were compared. Expressions of all miRNAs except for miR-886-5p were significantly higher in liver tissues with chronic hepatitis B than in those with other liver diseases (Fig. 2).

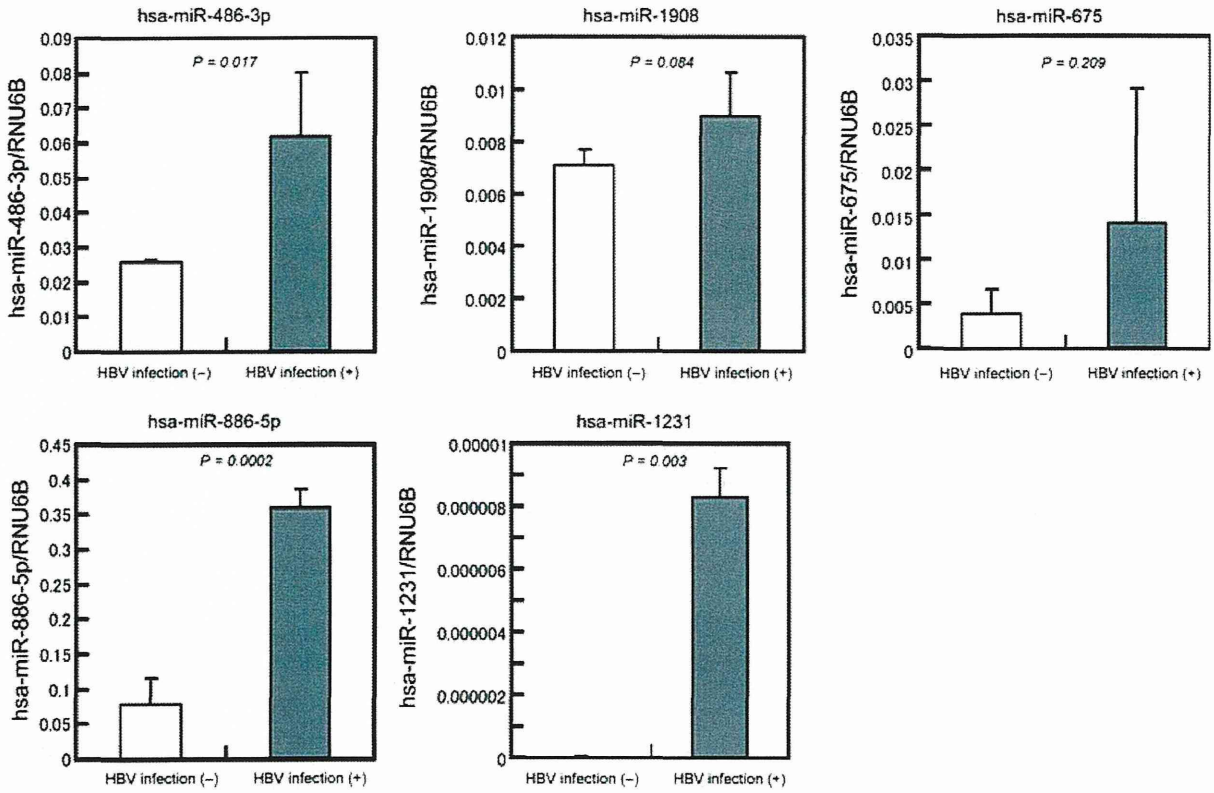

Fig. 1 Upregulation of microRNA by HBV infection. Signal intensities of five upregulated miRNAs were compared between HBV-infected and noninfected mouse livers. All 5 miRNAs were significantly upregulated by HBV infection. *P* values were calculated by the Mann–Whitney *U*-test.

Associations between signalling pathways and the upregulated miRNAs

To analyse the influence of miRNA upregulation on signalling pathways, pathway analysis was performed. However, there are several obstacles in analysing the association between miRNAs and pathways, such as the lack of reliable miRNA target prediction algorithms, differences in the results among target prediction systems, and the small number of validated target genes. To improve the reliability of the targets, we performed the pathway analysis in combination with four prediction tools (miRWalk, TargetScan, miRanda and miRDB). After this operation, 482 targets were predicted (hsa-miR-1231: 203 targets, hsa-miR-1908: 3 targets, hsa-miR-486-3p: 251 targets, hsa-miR-675: 25 targets), and these 482 targets were submitted to the PANTHER classification system for pathway analysis. As shown in Table 1, several immunological pathways such as inflammation mediated by chemokine and cytokine signalling pathway, and the interleukin signalling pathway were identified, but it was difficult to identify characteristic pathways.

Suppression of HBV replication with miR-1231 overexpression

Because hsa-miR-1231 was most the highly upregulated among these four miRNAs and had a high homology with the HBV genome, we focused on hsa-miR-1231. Using GENETYX ver. 8.2.1 (GENETYX, Tokyo, Japan), the hsa-miR-1231 sequence was predicted to hybridize at the HB core and X regions of the HBV genome (Fig. 3). To analyse the influence of hsa-miR-1231 on HBV replication, changes in HBV replication intermediates were evaluated using an *in vitro* HBV replication model. As shown in Fig. 4a, HBV replication intermediates were significantly reduced by hsa-miR-1231 overexpression, and the suppression of HBV RNA and HBc proteins were also observed by hsa-miR-1231 overexpression (Figs 4b,c). Thus, HBV replication was concluded to be inhibited by hsa-miR-1231 at the post-transcriptional level.

Specific regulation of HBV-related protein levels with hsa-miR-1231 overexpression

As the preceding results indicated an association between the production of HBV-related protein or HBV particles and

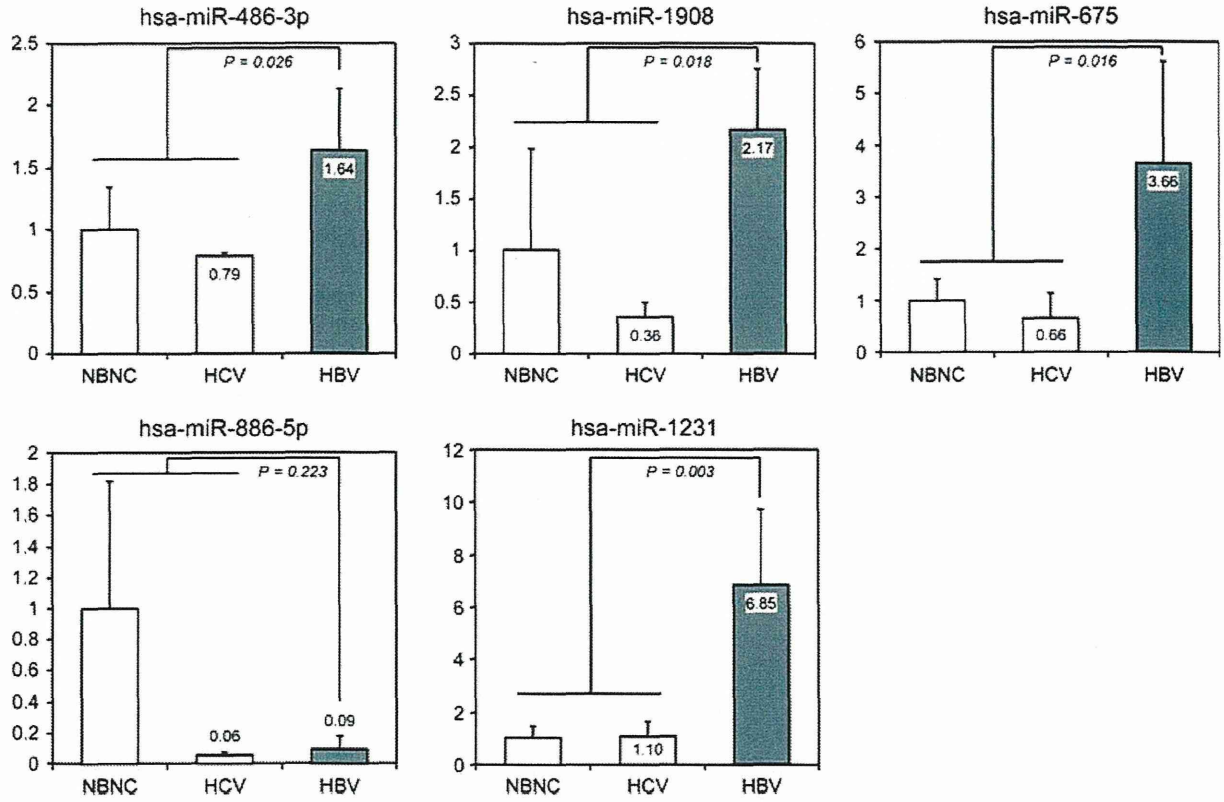

Fig. 2 Comparison of microRNA expression in clinical liver tissues. Quantification of miRNAs was performed by real-time PCR using nine human liver tissues obtained from the patients who had chronic hepatitis B (N = 3), C (N = 2) or alcoholic liver dysfunction (N = 4). Expression levels of four miRNA were significantly higher in the chronic hepatitis B patients than in those of other liver diseases. The results of miR-886-5p levels were not statistically significant. P values were assessed by Mann–Whitney U-test.

hsa-miR-1231 expression, further analysis was performed to identify the region hybridized by hsa-miR-1231. As shown in Fig. 5, HBc protein expression was remarkably reduced by hsa-miR-1231 expression, but no reduction in HBx protein was observed. These results indicate that hsa-miR-1231 might interact with HBV core mRNA and suppress HBV replication by inhibiting HBV core protein production.

The effects of hsa-miR-1231 on the expression of interferon-stimulated genes

Alternatively, hsa-miR-1231 might suppress HBV replication through activation of the interferon signalling pathway. We thus evaluated mRNA expression of interferon-stimulated genes (ISGs) with or without hsa-miR-1231 overexpression. None of the examined ISGs (MxA, PKR, OAS-1 and SOCS1) were regulated by hsa-miR-1231 expression (Fig. S3). These results suggest that hsa-miR-1231 suppresses HBV replication at the post-transcriptional level but not through the activation of interferon signalling.

DISCUSSION

Previously, we have demonstrated that human hepatocyte chimeric mice can be chronically infected with hepatitis B and C viruses [25,30,31]. This mouse model facilitates analysis of the effect of viral infection under immunodeficient conditions. In the present study, we performed miRNA array analysis using this mouse model and obtained miRNA expression profiles reflecting the direct influence of HBV infection on human hepatocytes. Furthermore, we found a novel mechanism for HBV replication mediated by hsa-miR-1231.

To avoid contamination with mouse tissue, human hepatocyte chimeric mice were used in which liver tissue was largely (>90%) replaced by human hepatocytes. Although it is feasible to use microarray analysis in this chimeric mouse model [32], signals from miRNA array analysis may be influenced by cross-hybridization with mouse miRNA from a small amount of contaminated mouse-derived cells because of the high homology between the human and mouse genomes. To compensate

Table 1 Pathways associated with the 4 miRNAs upregulated by HBV infection

Pathway	Number of gene hits	Ratio of genes %
Inflammation mediated by chemokine and cytokine signalling pathway (P00031)	11	2.60
Angiogenesis (P00005)	10	2.30
Integrin signalling pathway (P00034)	9	2.10
Gonadotropin releasing hormone receptor pathway (P06664)	7	1.60
Wnt signalling pathway (P00057)	7	1.60
Parkinson disease (P00049)	7	1.60
EGF receptor signalling pathway (P00018)	7	1.60
Alzheimer's disease–presenilin pathway (P00004)	6	1.40
PDGF signalling pathway (P00047)	6	1.40
B-cell activation (P00010)	6	1.40
Interleukin signalling pathway (P00036)	5	1.20
Huntington disease (P00029)	5	1.20
FGF signalling pathway (P00021)	5	1.20
Cadherin signalling pathway (P00012)	5	1.20
VEGF signalling pathway (P00056)	4	0.90
Toll receptor signalling pathway (P00054)	4	0.90
T-cell activation (P00053)	4	0.90
Ras pathway (P04393)	4	0.90
Heterotrimeric G-protein signalling pathway-Gi alpha and Gs alpha-mediated pathway (P00026)	4	0.90
Endothelin signalling pathway (P00019)	4	0.90

for contamination, mice that were negative for HBV infection were set up as negative controls.

Only 5 miRNAs showed more than 2.0-fold upregulation with HBV infection under miRNA array analysis using chimeric mouse livers (Fig. S1). Comparing these results with our previous study using patient sera, only hsa-miR-486-3p showed a similar change in sera from chronic hepatitis B patients, but no upregulation of the other 4 miRNAs was observed [15]. These results suggest that miRNA expression in sera from chronic hepatitis B patients might be regulated not only by HBV infection but also by human immune responses. In addition, it might be difficult to analyse changes in expression of miRNAs that are expressed at low levels in human hepatocytes, including hsa-miR-1231, using human serum.

To identify targets of miR-1231, we searched using four prediction systems. Although 632 target genes were identified (data not shown), and involvement of a number of pathways was indicated (Table S1), critical targets associated with human immunity or HBV replication could not be identified. Interferon signalling was also a potential mechanism of HBV suppression, but several ISG mRNAs were not induced by hsa-miR-1231 overexpression *in vitro* (Fig. S2). Therefore, we concluded that hsa-miR-1231 does not suppress HBV replication via interferon signalling.

To examine the possibility that miR-1231 directly regulates HBV replication by interacting with HBV-related mRNAs, we searched for hsa-miR-1231-binding motifs and found two candidate sequences in the HBV core and X genes (Fig. 3). As shown in Fig. 5, one target in the HBV core region could hybridize with hsa-miR-1231, and HBc expression was found to be suppressed by hsa-miR-1231 overexpression. The hsa-miR-1231-binding motif in the HBV core region was conserved in more than 90% of the HBV sequences in GenBank, regardless of HBV genotype (data not shown). Thus, we speculate that hsa-miR-1231 binds to the HBc target region and suppresses HBc production to inhibit HBV replication.

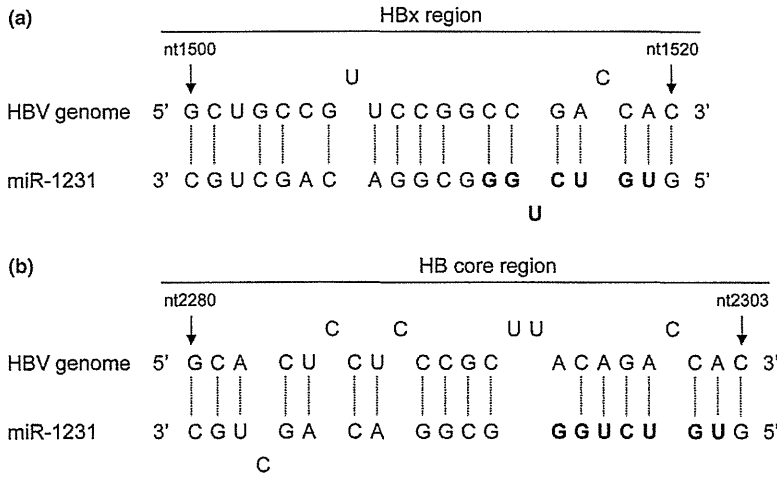

Fig. 3 Alignment of hsa-miR-1231 to HBV genome. Alignment of hsa-miR-1231 to the HBV genome was performed. MiR-1231 sequence was predicted to hybridize at the HBV core (a) and HBV X region (b).

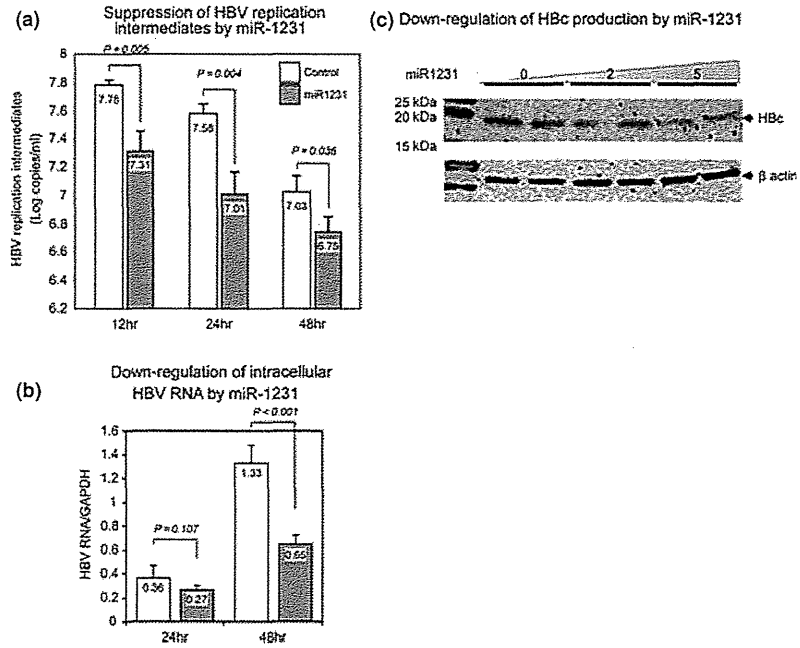

Fig. 4 Suppression of HBV replication by miR-1231. HBV replication intermediates were measured using an *in vitro* HBV replication model. (a) Production of HBV replication intermediates was significantly suppressed in cells transfected with both HBV and miR-1231 expression plasmids. (b, c) The levels of HBV RNA and HBc protein were also reduced by miR-1231 expression at 24 and 48 h after transfection.

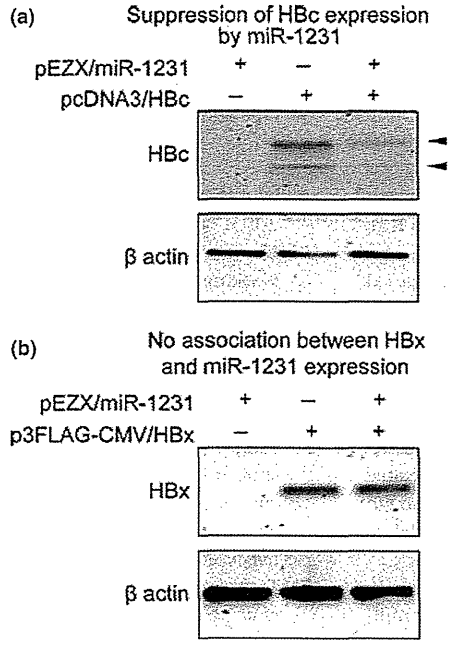

Fig. 5 Identification of miR-1231 target region in HBV genome. To determine the target for miR-1231, HBc or HBx expression plasmid was transfected into HepG2 cells with miR-1231 expression plasmid, and changes in protein levels were analysed by Western blot. HBc protein levels were reduced by miR-1231 expression (a), but HBx protein levels were not reduced (b).

To confirm the association between hsa-miR-1231 and HBV replication, we also tried to suppress hsa-miR-1231 expression using a miRNA inhibitor *in vitro*. However, no significant effects of miR-1231 inhibition on HBV replication were observed *in vitro*. As mentioned previously, expression levels of hsa-miR-1231 are quite low in HepG2 cells and human hepatocytes, and therefore, significant effects of hsa-miR-1231 inhibition could not be observed. The level of hsa-miR-1231 activity was also a factor. As shown in Fig. 4, HBV replication intermediates and HBc expression were significantly suppressed by hsa-miR-1231 overexpression, but the reduction rate was quite small even when 5-fold volume of hsa-miR-1231 plasmid and a volume of HBV expression plasmid were transfected into HepG2 cells. Therefore, it was difficult to observe changes in HBV replication by miRNA inhibition when HBV was replicating vigorously.

In conclusion, we performed miRNA array analysis using human hepatocyte chimeric mice and were able to analyse the direct effects of HBV infection without the confounding effects of the lymphocyte immunological response. We obtained evidence that hsa-miR-1231 was upregulated in response to HBV infection in human hepatocytes, whereupon hsa-miR-1231 suppressed replication of HBV.

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FINANCIAL DISCLOSURE

Kohno T, Tsuge M, Murakami E, Hiraga N, Abe H, Miki D, Imamura M, Takahashi S, Ochi H, Hayes CN, Chayama K: None to declare.

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SUPPORTING INFORMATION

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

Figure S1: HBV infection regulated expression of several microRNAs. Complete linkage hierarchical clustering analysis was performed using Euclidean distance. Among the 900 miRNAs, 10 miRNAs showed more than 2.0-fold change between groups. Five of the 10 miRNAs were upregulated by HBV, and the other five were downregulated.

Figure S2: No effect of miR-1231 expression on IFN signalling. To analyse the influence of miR-1231 expression on interferon signalling, four interferon-stimulated genes (ISGs) were quantified by real-time PCR. None of the four ISGs (MxA, PKR, OAS-1 and SOCS1) were suppressed by miR-1231 expression.

Table S1: Pathway analysis of miR-1231 target genes.

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Original article

On-treatment low serum HBV RNA level predicts initial virological response in chronic hepatitis B patients receiving nucleoside analogue therapy

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Background: Serum HBV RNA is detectable during nucleoside/nucleotide analogue therapy as a result of unaffected RNA replicative intermediates or interrupted reverse transcription. We studied the predictive value of serum HBV RNA for initial virological response during nucleoside analogue therapy.
Methods: Serum HBV RNA was quantified before and at 12 and 24 weeks of lamivudine or entecavir therapy. Serum HBV DNA was measured every 4–12 weeks during treatment to define initial virological response.
Results: Serum HBV RNA was detectable in 21 of 52 (40%) consecutive patients with a mean of 5.2 log copies/ml (male/female 35/17, mean age of 60 years with a range of 31–82, 44% HBeAg-positive, and 26 with lamivudine and 26 with entecavir) before treatment. Serum HBV RNA level at week 12 in patients with an interval from detectable to undetectable serum HBV DNA level <16 weeks was significantly lower than those with an interval ≥16 weeks (3.8 ±3.8 versus 6.6 ±3.5 log copies/ml, P=0.013). After adjustment for serum HBV DNA level at week 12, serum quantatitive HBsAg level at week 12 and pretreatment ALT level, low serum HBV RNA level at week 12 predicted a shorter interval to undetectable serum HBV DNA level (adjusted hazard ratio =0.908, 95% CI 0.829, 0.993, P=0.035).
Conclusions: Low serum HBV RNA level at week 12 of nucleoside analogue therapy independently predicts initial virological response in treated chronic hepatitis B patients. Serum HBV RNA levels may thus be useful for optimizing treatment of chronic hepatitis B.

Introduction

Although effective vaccines against HBV infection have been available for more than three decades, HBV infection remains a global health problem. It is estimated that more than 350 million people are chronic carriers of HBV worldwide [1,2]. In the United States, 1.2 million individuals have chronic HBV infection [3]. HBV infection causes a wide spectrum of clinical manifestations, ranging from acute or fulminant hepatitis to various forms of chronic liver disease, including inactive carrier state, chronic hepatitis, cirrhosis and even hepatocellular carcinoma [2,4,5].