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Hindawi Publishing Corporation
Gastroenterology Research and Practice
Volume 2014, Article ID 351396, 6 pages
http://dx.doi.org/10.1155/2014/351396

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Research Article

An Increased Ratio of Glycated Albumin to HbA1c Is Associated with the Degree of Liver Fibrosis in Hepatitis B Virus-Positive Patients

Hirayuki Enomoto,[1] Nobuhiro Aizawa,[1] Hideji Nakamura,[2] Yoshiyuki Sakai,[1] Yoshinori Iwata,[1] Hironori Tanaka,[1] Naoto Ikeda,[1] Tomoko Aoki,[1] Yukihisa Yuri,[1] Kazunori Yoh,[1] Kenji Hashimoto,[1] Akio Ishii,[1] Tomoyuki Takashima,[1] Kazunari Iwata,[1] Masaki Saito,[1] Hiroyasu Imanishi,[1] Hiroko Iijima,[1] and Shuhei Nishiguchi[1]

[1] *Division of Hepatobiliary and Pancreatic Disease, Department of Internal Medicine, Hyogo College of Medicine, Mukogawa-cho 1-1, Nishinomiya, Hyogo 663-8501, Japan*
[2] *Department of Gastroenterology and Hepatology, Nissay Hospital, Itachibori 6-3-8, Nishi-ku, Osaka 550-0012, Japan*

Correspondence should be addressed to Hirayuki Enomoto; enomoto@hyo-med.ac.jp

Received 29 November 2013; Revised 7 January 2014; Accepted 7 January 2014; Published 17 February 2014

Academic Editor: Edoardo Giovanni Giannini

Background. In hepatitis B virus- (HBV-) positive patients, the relationship between the metabolic variables and histological degree of liver fibrosis has been poorly investigated. *Methods.* A total of 176 HBV-positive patients were assessed in whom the ratios of glycated albumin-to-glycated hemoglobin (GA/HbA1c) were calculated in order to investigate the relationship with the degree of liver fibrosis. *Results.* The GA/HbA1c ratio increased in association with the severity of fibrosis (METAVIR scores: F0-1: 2.61 ± 0.24, F2: 2.65 ± 0.24, F3: 2.74 ± 0.38, and F4: 2.91 ± 0.63). The GA/HbA1c ratios were inversely correlated with four variables of liver function: the prothrombin time (PT) percentage ($P < 0.0001$), platelet count ($P < 0.0001$), albumin value ($P < 0.0001$), and cholinesterase value ($P < 0.0001$). The GA/HbA1c ratio was positively correlated with two well-known markers of liver fibrosis, FIB-4 ($P < 0.0001$) and the AST-to-platelet ratio index (APRI) ($P < 0.0001$). Furthermore, the GA/HbA1c showed better correlations with two variables of liver function (PT percentage and cholinesterase value) than did FIB-4 and with all four variables than did the APRI. *Conclusion.* The GA/HbA1c ratio is associated with the degree of liver fibrosis in HBV-positive patients.

1. Introduction

In patients with chronic liver disease (CLD), liver biopsy is the gold standard method to evaluate the degree of liver fibrosis [1]. However, a liver biopsy is a costly and invasive technique associated with a risk of complications. In addition, there can be sampling errors, because only 1/50,000 of the organ is used for the analysis [1]. Furthermore, it has been reported that there are inter- and intraobserver discrepancies of 10% to 20% for biopsy samples [2, 3]. Therefore, many noninvasive markers of fibrosis available via laboratory tests have been reported, and hepatitis C virus- (HCV-) positive patients have provided a good research base in this context.

It is known that significant differences are observed between HCV-positive patients and hepatitis B virus- (HBV-) positive patients, not only in the etiology, but also in terms of many other clinical parameters, including the natural history of the disease, the laboratory parameters, and the liver histology [4, 5]. However, the number of reports regarding fibrosis markers for HBV-positive patients is much lower than that for HCV-positive patients. In particular, there have been few reports about the relationship between the metabolic parameters and histological degree of liver fibrosis in HBV-positive patients, despite the fact that the liver functions as an important metabolic organ.

The values of glycated proteins reflect the plasma glucose level, and glycated hemoglobin (HbA1c) is commonly used as a reliable index of glycemic control in diabetic patients [6, 7]. The turnover period of hemoglobin in erythrocytes is about four months, and the HbA1c level therefore reflects the plasma glucose levels for the past few months [8]. Glycated albumin (GA) is another marker of the glycemic control during the past few weeks, because the turnover of albumin is about three weeks [9, 10]. In patients with CLD, hypersplenism abbreviates the lifespan of erythrocytes, leading to lower HbA1c values relative to the degree of glycemia. In contrast, the GA levels in CLD patients are higher than those estimated based on the levels of glycemia, because the turnover of serum albumin in CLD patients is increased as a result of the compensation for the decreased albumin production in the liver [11]. Since the HbA1c shows lower values and the GA shows higher values in CLD patients, the GA/HbA1c ratio is predicted to be high in patients with CLD. Indeed, the GA/HbA1c ratio has been reported to be associated with the histological stage of liver fibrosis and portal hypertension in HCV-positive CLD and nonalcoholic steatohepatitis [12–15]. In the present study, we investigated the GA/HbA1c ratio in HBV-positive patients and its correlation with liver fibrosis.

2. Materials and Methods

2.1. Patients. We studied a total of 173 HBV-positive patients who had undergone percutaneous liver biopsies between January 2008 and March 2010 at our institution. This study was retrospective and consecutively included all patients who fulfilled the following conditions: (1) HBV infection diagnosed by positive HBsAg status for at least six months. (2) Blood samples, including samples for an analysis of the GA and HbA1c levels, were obtained on the same day as the liver biopsies. Patients with the following conditions were excluded from the study: the presence of other liver diseases, hepatocellular carcinoma, immunosuppressive therapy, HCV coinfection, and insufficient liver tissue for the staging of fibrosis (a minimum of 15 mm of liver tissue with five or more portal tracts was required for diagnosis). The present study did not include patients whose GA/HbA1c ratios could have been influenced by poorly controlled diabetes.

The characteristics of the study population are summarized in Table 1. The study conformed to the ethical guidelines of the Declaration of Helsinki, and written informed consent regarding the liver biopsy and use of clinical data was obtained from all patients on admission. This study was approved by the ethics committee of the institutional review board.

2.2. Laboratory Data and Liver Biopsy. The HbA1c was measured by high-performance liquid chromatography, with calibration using Japan Diabetes Society (JDS) Lot 2 [15, 16]. The value for HbA1c (%) was estimated as a NGSP equivalent value (%) calculated using the following formula: in the range of JDS values ≤4.9%: NGSP (%) = JDS (%) + 0.3% and in the range of JDS 5.0–9.9%: NGSP (%) = JDS (%) + 0.4% [17]. Routine laboratory studies, including platelet counts, the prothrombin time (PT) percentage, and liver function tests (ALT, AST, alkaline phosphatase, albumin, and cholinesterase), were also performed.

Table 1: The characteristics of the 173 hepatitis B virus- (HBV-) positive patients.

Age (years)	46 (25–79)
Gender (male/female)	96/77
AST (IU/L)	27 (11–269)
ALT (IU/L)	28 (7–680)
γ-GTP (IU/L)	25 (7–349)
ALP (IU/L)	203 (71–835)
Total bilirubin (mg/dL)	0.8 (0.1–2.3)
Albumin (g/dL)	3.90 ± 0.40
Hemoglobin (g/dL)	13.5 ± 3.8
Platelets ($\times 10^3/\mu$L)	178 ± 72
PT (%)	89.8 ± 12.3
Diabetes mellitus (present/absent)	6/167
Glucose (mg/dL)	91.3 ± 13.9
Triglyceride (mg/dL)	99.0 ± 45.5
Total cholesterol (mg/dL)	177 ± 32
Body mass index	22.9 ± 4.1
HBV-DNA (log copies/mL)	3.7 (n.d–over 9.1)*
HBe antigen (positive/negative)	59/114
Treatment with NAs (present/absent)	67/106
Histological stage of liver fibrosis (F0-1/F2/F3/F4)	94/38/28/13

n.d: not detectable; NAs: nucleoside/nucleotide analogues.
*HBV-DNA ranged from undetectable level in patients under treatment of NAs to over measurable level (9.1 log copies/mL) in patients without treatment.

In the present study, the values of two biomarkers associated with the progression of liver fibrosis (FIB-4 and the APRI, the AST-to-platelet count ratio index) were calculated, because these markers were previously shown to be associated with the progression of liver fibrosis [18–20]. The FIB-4 and APRI values were calculated based on formulas developed by Vallet-Pichard et al. [21] and Wai et al. [22], respectively: FIB-4 = Age [years] × AST [U/L]/(platelets [10^9/L] × (ALT [U/L])$^{1/2}$), in which the age of the patient is the age at the time of liver biopsy and APRI = 100 × (AST level/upper limit of normal)/platelets [10^9/L].

Liver biopsy examinations were carried out according to the standard techniques. All liver samples were evaluated by well-trained pathologists at our institute, with an evaluation of the fibrosis stage and activity grade. Fibrosis was staged on a scale of F0–F4 (F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; F4, liver cirrhosis) according to the METAVIR scoring system [23]. The histological findings of the biopsy tissues were also routinely evaluated in our department. All authors participated in the conferences about the histological findings, and the final results were confirmed by two authors (H. Enomoto and H. Imanishi) who received training for histological studies.

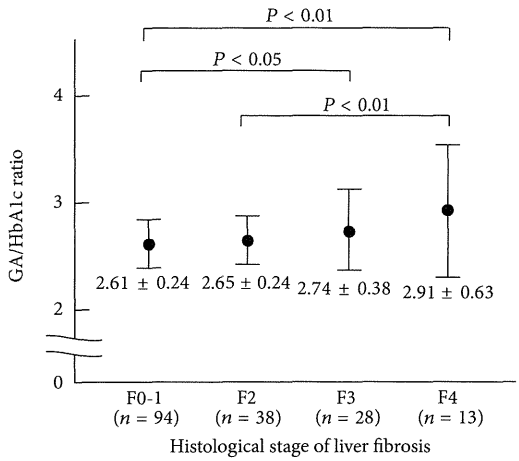

FIGURE 1: GA/HbA1c ratios in relation to the METAVIR fibrosis scores among the HBV-positive patients. The GA/HbA1c ratio increased in association with the stage of liver fibrosis. There were significant differences between the F0-F1 versus F3, F0-F1 versus F4, and F2 versus F4 groups.

2.3. Statistical Analysis. In the present study, we investigated whether the GA/HbA1c ratio is associated with the degree of liver fibrosis in HBV-positive patients. The data for the comparisons among the groups "F0-1 versus F2 versus F3 versus F4" was analyzed by a nonrepeated measurements ANOVA, and statistical significance was consequently evaluated with the Bonferroni correction. The relationships between the GA/HbA1c ratio and other variables, including the FIB-4 and APRI, were evaluated with Spearman's correlation coefficient. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. The GA/HbA1c Ratio Increases with the Histological Stage of HBV-Positive Patients. A total of 173 HBV-positive patients were included in this study. The characteristics of the study population are summarized in Table 1. The population consisted of 96 (55.5%) male patients and 77 (44.5%) female patients, and the age of patients ranged from 25 to 79 years old (median 46 years old). As shown in Figure 1, the mean value of the GA/HbA1c ratio increased in association with the histological stage of liver fibrosis in the HBV-positive patients.

3.2. The GA/HbA1c Ratio Is Associated with the Laboratory Parameters in HBV-Positive Patients. We next investigated whether the GA/HbA1c ratio was related to the laboratory parameters of the liver function, including the PT (%), platelet count, albumin level, and cholinesterase level. Table 2 shows that there is a significant reciprocal correlation of the GA/HbA1c ratio with the PT (%) ($R = -0.396$, $P < 0.0001$) and platelet count ($R = -0.421$, $P < 0.0001$) in HBV-positive patients. The GA/HbA1c ratio was also inversely correlated with the serum albumin level ($R = -0.332$, $P < 0.0001$) and the cholinesterase level ($R = -0.411$, $P < 0.0001$).

TABLE 2: The correlations of the three biomarkers with liver function parameters.

	Correlation coefficient		
	FIB-4	APRI	GA/HbA1c
Prothrombin time (%)	−0.362	−0.284	−0.396
Platelet count	−0.532	−0.372	−0.421
Albumin value	−0.372	−0.301	−0.332
Cholinesterase value	−0.344	−0.315	−0.411

GA/HbA1c: glycated albumin- (GA-) to-glycated hemoglobin (HbA1c) ratio. APRI: AST-to-platelet ratio index.

These findings showed that the GA/HbA1c ratio increased in association with changes in the levels of markers related to liver fibrosis.

3.3. The GA/HbA1c Ratio and Fibrosis-Related Markers in HBV-Positive Patients. Since we found that the GA/HbA1c ratio was associated with the stage of liver fibrosis in the HBV-positive patients, we therefore investigated the relationships of the GA/HbA1c ratio with two previously established fibrosis-related markers, FIB-4 and APRI. As shown in Figure 2, the GA/HbA1c ratio was significantly correlated with the FIB-4 ($R = 0.598$, $P < 0.0001$) and APRI ($R = 0.505$, $P < 0.0001$). We examined the correlations of these biomarkers with four parameters of liver function (PT percentage, albumin value, platelet count, and cholinesterase value) and found that the GA/HbA1c ratio showed better correlations than the FIB-4 value for two parameters (PT percentage and cholinesterase value). In addition, the correlations of the GA/HbA1c ratio with the findings of liver function tests were higher than those of the APRI for all four parameters (Table 2).

4. Discussion

Liver biopsy is the gold standard method for histologically assessing liver fibrosis. However, a liver biopsy is an invasive procedure carrying a small risk of severe complications. In addition to the FIB-4 and APRI, noninvasive biomarkers, such as the FibroTest score [24], Forns score [25], Hepascore [26], FibroMeter [27], FibroIndex [28], and Lok index [29], were previously reported to be associated with the liver fibrosis. In the present study, we showed that the GA/HbA1c ratio is associated with the histological stage of liver fibrosis in HBV-positive patients (Figure 1). We also showed that the GA/HbA1c ratio was significantly related to the laboratory variables of liver function (Table 2). Among the previously reported biomarkers for liver fibrosis, the FIB-4 and APRI are simple and useful markers that can be measured using routinely available clinical parameters without any specialized equipment. We found that the GA/HbA1c ratio significantly correlated with these well-established markers in HBV-positive patients (Figure 2). These findings suggest that there is a strong relationship between the GA/HbA1c ratio and the levels of fibrosis-related markers in HBV-positive patients.

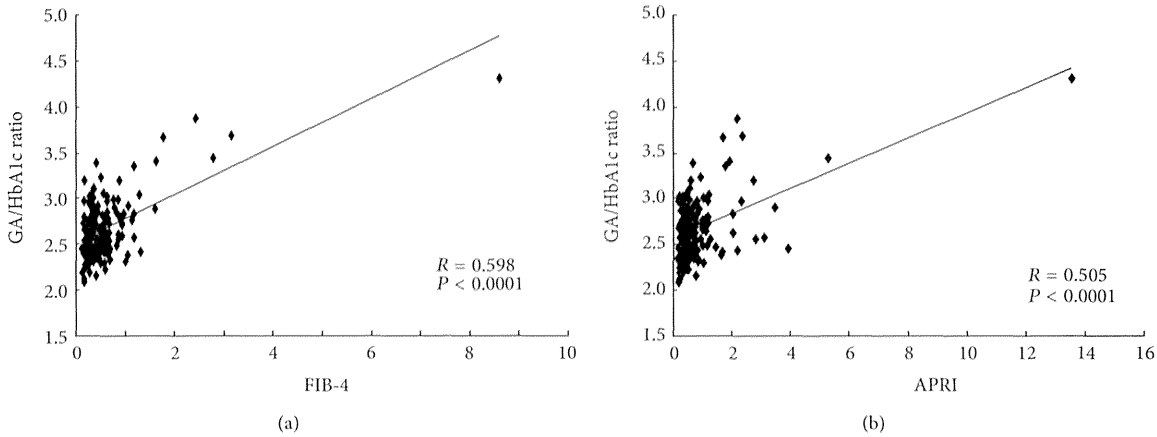

FIGURE 2: The correlation of the GA/HbA1c ratio with other fibrosis-related biomarkers. The GA/HbA1c ratio was correlated with the FIB-4 and APRI. APRI: AST-to-platelet ratio index.

When we examined the four variables of liver function, the correlation coefficients of the GA/HbA1c ratio were higher than those of the FIB-4 for two of the variables. In addition, the GA/HbA1c ratio was better correlated with all four variables examined than was the APRI (Table 2). It has been reported that the etiology of CLD influences the performance of liver fibrosis biomarkers. Unlike that observed in HCV-positive patients, noninvasive biomarkers are sometimes reported to not provide a correct evaluation of the degree of liver fibrosis in HBV-positive patients [30–32]. One major reason could be that the previously established biomarkers are obtained using calculations which include the AST and/or ALT. HBV infections sometimes show an acute liver inflammatory phase, and the AST and ALT values can therefore change from a mildly elevated level to an extremely high level in the same patient depending on the time when the patient is evaluated. In the present study, we included all HBV-positive patients without setting an upper limit for the AST and ALT levels, and some patients with remarkably elevated AST and ALT levels showed very high FIB-4 and APRI indices. The APRI is calculated using only the AST value and platelet count, while the FIB-4 calculation includes both AST and ALT values. Therefore, the acute elevation of AST and ALT in HBV-positive patients should more severely affect the value of the APRI than the FIB-4, although the ALT value was used as the $(ALT)^{1/2}$ for the FIB-4 calculation. The advantage of using the GA/HbA1c ration may therefore depend on the instability of AST and ALT values in HBV-positive patients, because the GA/HbA1c ration is calculated using only the values of two glycated proteins and is independent of the AST and ALT values.

Since the liver plays a central role in metabolism, the progression of liver disease should lead to changes in metabolic parameters. However, most of the established biomarkers for liver fibrosis depend on only nonmetabolic parameters, such as the values of AST, ALT, and the platelet count. Recently, some groups, including our group, reported that the GA/HbA1c ratio was associated with the degree of liver fibrosis in various types of CLD, such as HCV-related CLD and nonalcoholic steatohepatitis [12–15]. Furthermore, we have reported that the amino acid imbalance was associated with the degree of liver fibrosis and the severity of esophageal varices in HCV-positive patients, thus suggesting that metabolism-related parameters could be potential biomarkers for the severity of CLD [33].

We herein demonstrated that the GA/HbA1c ratio increased in association with the stage of liver fibrosis in HBV-positive patients; however, the differences among the fibrosis stages were relatively small (Figure 1). Therefore, the GA/HbA1c ratio alone is not an ideal biomarker to evaluate liver fibrosis, although its correlations with the liver functional tests were as good as the previously reported well-established markers, the FIB-4 and APRI (Table 2). In addition, the present study was a simple descriptive study and did not have a prospective or longitudinal design. Therefore, we cannot draw any conclusions regarding the relationships with the progression of liver fibrosis or clinical outcomes. Recently, there was a report that it was possible to predict portal hypertension using three metabolic parameters [34]. A new biomarker based on a combination of metabolic parameters that includes the GA/HbA1c ratio would be useful for evaluating liver fibrosis in HBV-positive patients.

5. Conclusion

In conclusion, we herein demonstrated that the GA/HbA1c ratio increases in association with the stage of liver fibrosis and is correlated with the levels of markers related to liver fibrosis in HBV-positive patients.

Abbreviations

GA: Glycated albumin
CLD: Chronic liver disease
HCV: Hepatitis C virus
HBV: Hepatitis B virus
PT: Prothrombin time.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This study was supported by a Grant-in-Aid for Health and Labor Sciences Research from the Ministry of Health, Labour, and Welfare of Japan.

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Journal of Infection (2014) xx, 1–15

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Differences in serum microRNA profiles in hepatitis B and C virus infection

Sakura Akamatsu [a,b,c,1], C. Nelson Hayes [a,b,c,1], Masataka Tsuge [c,d], Daiki Miki [a,b,c], Rie Akiyama [a,b,c], Hiromi Abe [a,b,c], Hidenori Ochi [a,b,c], Nobuhiko Hiraga [a,b,c], Michio Imamura [a,b,c], Shoichi Takahashi [e], Hiroshi Aikata [a,c], Tomokazu Kawaoka [a,b,c], Yoshiiku Kawakami [a,b,c], Waka Ohishi [f], Kazuaki Chayama [a,b,c,*]

[a] *Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan*
[b] *Laboratory for Digestive Diseases, Center for Genomic Medicine, RIKEN, Hiroshima, Japan*
[c] *Liver Research Project Center, Hiroshima University, Hiroshima, Japan*
[d] *Natural Science Center for Basic Research and Development, Hiroshima University, Hiroshima, Japan*
[e] *Koyo New Town Hospital, Hiroshima, Japan*
[f] *Department of Clinical Studies, Radiation Effects Research Foundation, Hiroshima, Japan*

Accepted 17 October 2014
Available online ■ ■ ■

KEYWORDS
Serum biomarkers;
microRNA;
miR-122;
miR-125b;
HBeAg;
Microarray

Summary *Objectives:* Patients infected with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) are at greater risk of cirrhosis and hepatocellular carcinoma. The objective of this study was to identify virus-specific serum microRNA profiles associated with liver function and disease progression. Microarray analysis of serum microRNAs was performed using the Toray 3D array system in 22 healthy subjects, 42 HBV patients, and 30 HCV patients. Selected microRNAs were then validated by qRT-PCR in 186 HBV patients, 107 HCV patients, and 22 healthy subjects.

Results: Microarray analysis showed up-regulation of a number of microRNAs in serum of both HBV and HCV patients. In qRT-PCR analysis, miR-122, miR-99a, miR-125b, miR-720, miR-22, and miR-1275 were up-regulated both in HBV patients relative to healthy subjects, and all except

List of abbreviations: HBV, Hepatitis B virus; HCV, Hepatitis C virus; HCC, hepatocellular carcinoma; qRT-PCR, quantitative real-time polymerase chain reaction; HBsAg, HBV surface antigen; HBeAg, HBe antigen; HBeAb, HBe antibody; HBcAg, HBV core antigen; γGTP, γ-glutamyl transpeptidase.

* Corresponding author. Department of Gastroenterology and Metabolism, Applied Life Sciences, Institute of Biomedical & Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Tel.: +81 82 257 5190; fax: +81 82 255 6220.
E-mail address: chayama@hiroshima-u.ac.jp (K. Chayama).
[1] Co-first authors.

http://dx.doi.org/10.1016/j.jinf.2014.10.017

Please cite this article in press as: Akamatsu S, et al., Differences in serum microRNA profiles in hepatitis B and C virus infection, J Infect (2014), http://dx.doi.org/10.1016/j.jinf.2014.10.017

miR-1275 were up-regulated in HBeAg-positive patients relative to HBeAg-negative patients. Specific microRNAs were independently associated with different aspects of HBV infection. MiR-122 was independently associated with HBV DNA level, whereas miR-125b was independently associated with levels of HBV DNA, HBsAg, and HBeAg. MiR-22 and miR-1275 were independently associated with serum γ-glutamyl transpeptidase levels.

Conclusions: Serum microRNA levels reflect differences in the etiology and stage of viral hepatitis.

Introduction

Chronic infection with hepatitis B virus (HBV), a partially double-stranded DNA virus, and hepatitis C virus (HCV), a single stranded RNA virus, increases the risk of cirrhosis and hepatocellular carcinoma (HCC). Despite improvements in antiviral therapy, many patients fail to respond to current therapies.[1–3] Therefore, non-invasive methods are needed for early detection of changes in liver function. One such approach is to measure changes in levels of small RNAs present in the serum of infected patients. In addition to messenger RNA, transfer RNA, and ribosomal RNA, there are many other classes of RNAs, many of which act to fine-tune gene expression and may play a role in disease pathogenesis. MicroRNAs are among the most important classes of non-coding RNA and consist of short linear RNA sequences that range in size from 19 to 24 nucleotides. MicroRNAs may influence gene expression by binding to a partially complementary region in the 3′ untranslated region of a targeted messenger RNA, thereby inhibiting translation or promoting degradation of the transcript. Because a single microRNA may regulate multiple genes, and a single gene may be regulated by multiple microRNAs, microRNAs may form complex regulatory networks.[4] Viral pathogenesis and inflammation may disrupt these intricate networks, resulting in changes in microRNA levels inside and outside of the cell. Given the liver's dual blood supply and central role in circulation, pathogenic changes in gene expression in the liver are likely to be reflected in changes in microRNA profiles in the serum.

Understanding the origin and function of serum microRNAs is important in the development of strategies to eradicate HCV and HBV and to monitor the degree of liver damage. Analysis of differential microRNA expression in liver tissues has revealed HCV- and HBV-specific microRNAs as well as microRNAs associated with the stage of liver disease.[5–9] MicroRNA levels in the liver have been found to be correlated with serum levels for a number of microRNAs,[10,11] suggesting that serum microRNAs might act as a surrogate measure of microRNA activity in the liver. While RNA typically has a short-half life and is quickly degraded by RNases, microRNAs tend to exist stably in serum when bound to argonaute proteins such as AGO2 as part of the RNA-induced silencing complex, the molecular scaffold that facilitates interaction of a microRNA with its target sequence.[12] Circulating microRNAs may exist in this form as vesicle-free ribonucleoprotein complexes, or they may be transported within HBV surface antigen (HBsAg) particles or contained within exosomes/microvesicles.[12–14] However, serum microRNAs are typically concentrated in exosomes.[15]

Exosomes are 30–150 nm endosome-derived microvesicles that are released from multiple cell types and are detectable in blood, urine, saliva, and other body fluids. Exosomes are involved in removal of cellular waste products as well as cell–cell communication and immune activation but may also be exploited by pathogens and contribute to tumor proliferation. Exosomes contain characteristic RNA transcripts, including microRNAs, transfer RNAs and other types of non-coding RNAs[16] and have been shown to affect gene expression in recipient cells. MiR-99a, miR128, miR-124, miR-22, and miR-99b account for 49% of identified exosome-associated microRNAs.[16] While exosomal RNA profiles vary by cell type, they do not completely mirror the RNA profile of the parent cell due to selective sorting and may change in response to cellular conditions.[16] Hepatocyte-derived exosomes are enriched for gene products involved in lipoprotein metabolism and xenobiotic processing and therefore have potential as a diagnostic tool by reflecting hepatic changes linked to disease.[17] Interferon-stimulated release of exosomes containing antiviral products and internalization by HBV-infected hepatocytes may also play a role in antiviral defense by bypassing viral interference in interferon signal transduction.[18] It is likely that analysis of serum microRNA profiles will provide insight into disease progression and antiviral activity in the liver, particularly in the case of HBV infection.

In order to investigate the relationship between serum microRNA profiles and viral hepatitis, we performed microarray and quantitative real-time polymerase chain reaction (qRT-PCR) analysis to identify host microRNAs that differ between healthy subjects and patients with chronic HBV or HCV infection as well as between HBeAg-positive and negative patients.

Methods

Study subjects

All patients had either chronic hepatitis B or C infection and were negative for HIV and HCC. No patients were co-infected with both HBV and HCV. All healthy subjects were negative for HBsAg and HCV antibody. Patient profiles are shown in Table 1. Histopathological diagnosis was determined as in Desmet et al.[19] The study was approved *a priori* by the ethical committee of Hiroshima University and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All patients provided written informed consent.

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Microarray analysis of serum microRNA expression levels

Host microRNA expression in serum samples was measured using the Toray Industries microRNA analysis system, in which serum microRNA samples were hybridized to 3D-Gene human microRNA ver17.1 chips containing 1200 microRNAs (Toray Industries, Inc., Tokyo, Japan). Serum from 42 patients with chronic HBV infection and 30 patients with chronic HCV infection were compared with serum from 12 healthy males and 10 healthy females using a separate microarray for each sample.

Quantitative RT-PCR microRNA analysis

A subset of microRNAs was selected for validation using qRT-PCR based on preliminary microarray results and a search of the literature. Expression of 7 microRNAs was measured in serum from 186 HBV patients, 107 HCV patients, and 22 healthy subjects. Circulating microRNA was extracted from 300 μl of serum samples using the mirVana PARIS Kit (Ambion Inc., Austin, TX) according to the manufacturer's instructions. RNA was eluted in 80 μl of nuclease free water and reverse transcribed using TaqMan MicroRNA Reverse Transcription Kit (Life technologies Japan Ltd, Tokyo, Japan). Each sample was spiked with *Caenorhabditis elegans* miR-238 (cel-miR-238) as a control for extraction and amplification. The reaction mixture contained 5 μl of RNA solution, 2 μl of 10x reverse transcription buffer, 0.2 μl of 100 mM dNTP mixture, 4 μl of 5x RT primer, 0.25 μl of RNase inhibitor and 7.22 μl of nuclease free water in a total volume of 20 μl. The reaction was performed at 16 °C for 30 min followed by 42 °C for 30 min. The reaction was terminated by heating the solution at 85 °C for 5 min. MicroRNAs were amplified using primers and probes provided by Applied Biosystems Inc. using TaqMan MicroRNA assays according to the manufacturer's instructions. The reaction mixture contained 12.5 μl of 2x Universal PCR Master Mix, 1.25 μl of 20x TaqMan Assay solution, 1 μl of reverse transcription product and 10.25 μl of nuclease free water in a total volume of 25 μl. Amplification conditions were 95 °C for 10 min followed by 50 denaturing cycles for 15 s at 95 °C and annealing and extension for 60 s at 60 °C in an ABI7300 thermal cycler. For the cel-miR-238 assay, a dilution series using chemically synthesized microRNA was used to generate a standard curve that permitted absolute quantification of molecules. A separate internal normalization factor was not used.

Statistical analysis

MicroRNA microarray expression data was normalized using cyclic loess and analyzed using moderated *t*-tests using the limma package in the R statistical framework (http://www.r-project.org). *P*-values were adjusted for multiple testing using the false discovery rate (P_{FDR}). qRT-PCR expression levels were compared between healthy subjects and HBV or HCV using the non-parametric Mann–Whitney *U* test. Association between qRT-PCR microRNA levels and clinical parameters such as HBsAg, HBV DNA, HBeAg, HBeAb, AST, and ALT were evaluated using multiple linear regression. Factors that were significant at 0.05 in univariate analysis were included as candidates in the multivariate model, and forward-backward stepwise selection based on Akaike information criterion (AIC) was used to identify independently associated factors.

Pathway analysis

Target genes of differentially expressed microRNAs were predicted using the miRWalk database (http://www.umm.

Table 1 Clinical characteristics of healthy controls and patients with chronic viral HBV or HCV infection. Continuous variables are shown as median and range, and categorical variables are shown as counts.

Factor	Healthy(N = 22)	Hepatitis B virus (N = 186)	Hepatitis C virus (N = 107)
Age	33 (27–45)	48 (22–79)	64 (24–85)
Sex (male/female)	12/10	122/64	47/60
Alanine aminotransferase (IU/l)	18.5 (15–22)	73.5 (10–1867)	30.5 (18–145)
Aspartate aminotransferase (IU/l)	13.5 (6–44)	47.5 (15–982)	33.5 (11–141)
γ-glutamyl transpeptidase (IU/l)	20 (11–52)	41.5 (9–459)	22 (8–161)
rs8099917 genotype (TT/GT/GG/unknown)	5/0/0/17	89/76/3/18	–
Liver fibrosis (1/2/3/4/unknown)	–	65/76/28/3/14	39/35/11/4/18
Necroinflammatory activity (1/2/3/unknown)	–	58/80/34/14	32/48/9/18
Alpha-fetoprotein (ug/l)	–	6.1 (<5.0–2510.0)	5.0 (<5.0–104.8)
Promthrombin time (s)	–	95 (35–123)	98 (71–116)
Albumin (g/dl)	–	4.4 (2.8–4.9)	4.3 (3.5–5.0)
Platelets ($x10^4/mm^3$)	–	17.4 (5.0–35.7)	17.6 (5.3–29.8)
rs8099917 genotype (TT/GT/GG/unknown)	5/0/0/17	89/76/3/18	–
HBV DNA (IU/ml)	–	6.7 (<2.1–≥9.1)	–
HBsAg (IU/l)	–	3650 (1.2–239000)	–
HBeAg (−/+)	–	82/104	–
HBeAb (−/+)	–	88/98	–
HBV genotype (A/B/C/unknown)	–	3/14/129/40	–
HCV RNA (Log IU/ml)	–	–	6.5 (1.7–7.3)
HCV genotype (1a/1b/2a/2b/3a)	–	–	5/42/18/9/1/32

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Table 2 Top up- or down-regulated serum microRNAs associated with chronic HBV or HCV infection. MicroRNAs that have been detected in exosomes are noted.

Contrast	Direction	miRNA	logFC	AveExpr	t	P	P_{FDR}	Exosome
HBV-Healthy	Up	hsa-miR-122	2.80	8.30	7.63	2.42E-11	3.23E-09	exosome
	Up	hsa-miR-3648	1.39	13.63	8.26	1.20E-12	2.14E-10	
	Up	hsa-miR-642b	1.07	9.64	9.16	1.63E-14	9.76E-12	
	Up	hsa-miR-22	1.04	8.16	5.12	1.70E-06	3.01E-05	exosome
	Up	hsa-miR-1246	1.02	10.75	5.29	8.59E-07	1.78E-05	
	Up	hsa-miR-486-3p	0.89	8.32	7.43	6.06E-11	5.66E-09	
	Up	hsa-miR-191	0.80	7.65	6.04	3.46E-08	1.30E-06	exosome
	Up	hsa-miR-1915*	0.63	7.64	4.85	5.22E-06	7.76E-05	
	Up	hsa-miR-3665	0.62	14.38	5.69	1.58E-07	4.54E-06	
	Up	hsa-miR-658	0.61	7.72	8.80	9.24E-14	3.70E-11	exosome
	Up	hsa-miR-550a	0.59	7.24	10.56	2.00E-17	2.40E-14	
	Up	hsa-miR-320b	0.57	7.22	7.13	2.43E-10	2.08E-08	
	Up	hsa-miR-320a	0.54	7.29	6.63	2.47E-09	1.41E-07	exosome
	Up	hsa-miR-320c	0.54	7.05	6.67	2.00E-09	1.24E-07	
	Up	hsa-miR-3663-3p	0.51	10.69	5.63	2.08E-07	5.67E-06	
	Up	hsa-miR-99a	0.51	6.56	5.30	8.38E-07	1.78E-05	exosome
	Down	hsa-miR-223	−0.89	7.69	−5.15	1.56E-06	2.79E-05	exosome
	Down	hsa-miR-4294	−0.86	10.91	−5.50	3.59E-07	8.98E-06	
	Down	hsa-miR-575	−0.75	7.63	−6.05	3.31E-08	1.28E-06	exosome
	Down	hsa-miR-1268	−0.57	11.77	−6.83	1.00E-09	6.66E-08	
	Down	hsa-miR-1202	−0.54	8.10	−5.40	5.51E-07	1.25E-05	
	Down	hsa-miR-1275	−0.52	8.92	−5.06	2.20E-06	3.71E-05	
HCV-Healthy	Up	hsa-miR-122	1.81	8.30	4.74	8.05E-06	7.37E-05	exosome
	Up	hsa-miR-3648	1.52	13.63	8.63	2.04E-13	2.23E-11	
	Up	hsa-miR-642b	1.42	9.64	11.67	1.12E-19	6.69E-17	
	Up	hsa-miR-24	1.11	8.80	6.58	3.06E-09	5.92E-08	exosome
	Up	hsa-miR-3925-5p	1.10	7.28	7.98	4.61E-12	2.49E-10	
	Up	hsa-miR-296-3p	1.10	7.76	7.30	1.10E-10	3.56E-09	
	Up	hsa-miR-3162-5p	1.08	8.42	8.30	9.94E-13	7.95E-11	
	Up	hsa-miR-3622b-5p	1.08	7.82	6.13	2.33E-08	3.77E-07	
	Up	hsa-miR-3665	1.06	14.38	9.27	9.51E-15	1.90E-12	
	Up	hsa-miR-3917	1.01	7.99	7.59	2.92E-11	1.11E-09	
	Up	hsa-miR-762	1.01	14.16	10.63	1.48E-17	5.93E-15	
	Up	hsa-miR-4258	0.96	8.57	7.00	4.39E-10	1.15E-08	
	Up	hsa-miR-4257	0.92	7.83	9.45	4.05E-15	9.73E-13	
	Up	hsa-miR-663	0.86	10.87	5.38	5.82E-07	7.27E-06	exosome
	Up	hsa-miR-4299	0.86	7.19	7.65	2.13E-11	9.33E-10	
	Up	hsa-miR-486-3p	0.83	8.32	6.65	2.20E-09	4.48E-08	
	Up	hsa-miR-149*	0.78	10.33	7.73	1.49E-11	6.88E-10	exosome
	Up	hsa-miR-4259	0.74	7.74	5.06	2.22E-06	2.32E-05	
	Up	hsa-miR-1469	0.74	10.93	5.28	8.83E-07	1.05E-05	
	Up	hsa-miR-3934	0.74	7.43	7.62	2.48E-11	1.03E-09	
	Up	hsa-miR-658	0.73	7.72	10.14	1.52E-16	4.57E-14	exosome
	Up	hsa-miR-3663-3p	0.73	10.69	7.65	2.18E-11	9.33E-10	
	Up	hsa-miR-671-5p	0.67	8.15	8.31	9.52E-13	7.95E-11	exosome
	Up	hsa-miR-187*	0.67	8.45	8.20	1.61E-12	1.02E-10	
	Up	hsa-miR-3131	0.66	7.71	8.40	6.21E-13	6.21E-11	
	Up	hsa-miR-3154	0.64	8.13	6.32	1.00E-08	1.77E-07	
	Up	hsa-miR-320a	0.59	7.29	6.94	5.85E-10	1.40E-08	exosome
	Up	hsa-miR-4300	0.55	6.89	6.43	6.06E-09	1.12E-07	
	Up	hsa-miR-3126-5p	0.53	6.85	7.43	6.11E-11	2.16E-09	
	Up	hsa-miR-3153	0.51	6.99	5.16	1.46E-06	1.56E-05	
	Up	hsa-miR-550a	0.51	7.24	8.70	1.50E-13	1.80E-11	
	Up	hsa-miR-3616-3p	0.50	6.87	8.18	1.78E-12	1.07E-10	
	Up	hsa-miR-371-5p	0.50	7.70	5.91	6.09E-08	9.14E-07	
	Up	hsa-miR-3147	0.50	7.60	6.20	1.68E-08	2.88E-07	

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Table 2 (*continued*)

Contrast	Direction	miRNA	logFC	AveExpr	t	P	P_{FDR}	Exosome
	Down	hsa-miR-451	−2.00	10.87	−5.76	1.16E-07	1.68E-06	exosome
	Down	hsa-miR-223	−1.42	7.69	−7.91	6.28E-12	3.14E-10	exosome
	Down	hsa-miR-92a-2*	−1.30	10.11	−7.20	1.76E-10	5.03E-09	
	Down	hsa-miR-4294	−1.22	10.91	−7.42	6.33E-11	2.17E-09	
	Down	hsa-miR-575	−1.17	7.63	−9.06	2.67E-14	4.57E-12	exosome
	Down	hsa-miR-16	−1.13	7.77	−4.99	2.96E-06	2.96E-05	exosome
	Down	hsa-miR-1275	−0.75	8.92	−7.08	3.05E-10	8.52E-09	
	Down	hsa-miR-1915	−0.75	11.10	−12.24	7.86E-21	9.44E-18	
	Down	hsa-miR-1202	−0.69	8.10	−6.61	2.67E-09	5.34E-08	
	Down	hsa-miR-887	−0.68	8.13	−8.23	1.38E-12	9.30E-11	exosome
	Down	hsa-miR-1203	−0.64	8.50	−7.05	3.48E-10	9.49E-09	
	Down	hsa-miR-125a-3p	−0.62	6.90	−7.53	3.72E-11	1.35E-09	exosome
	Down	hsa-miR-17	−0.59	6.76	−5.00	2.79E-06	2.81E-05	exosome
	Down	hsa-miR-3141	−0.59	8.72	−7.02	4.11E-10	1.10E-08	
	Down	hsa-miR-20a	−0.59	6.60	−5.65	1.91E-07	2.57E-06	exosome
	Down	hsa-miR-1268	−0.58	11.77	−6.60	2.81E-09	5.52E-08	
	Down	hsa-miR-423-5p	−0.51	7.97	−7.75	1.38E-11	6.64E-10	
HCV-HBV	Up	hsa-miR-296-3p	0.80	7.76	6.07	3.06E-08	1.67E-06	
	Up	hsa-miR-3925-5p	0.74	7.28	6.09	2.79E-08	1.59E-06	
	Up	hsa-miR-4257	0.70	7.83	8.28	1.09E-12	4.34E-10	
	Up	hsa-miR-3162-5p	0.66	8.42	5.79	1.01E-07	4.67E-06	
	Up	hsa-miR-1469	0.65	10.93	5.28	8.82E-07	2.52E-05	
	Up	hsa-miR-149*	0.64	10.33	7.23	1.54E-10	2.65E-08	exosome
	Up	hsa-miR-3917	0.57	7.99	4.91	4.01E-06	8.74E-05	
	Up	hsa-miR-4299	0.53	7.19	5.36	6.43E-07	1.98E-05	
	Up	hsa-miR-762	0.52	14.16	6.27	1.25E-08	9.35E-07	

logFC: log2 fold-change; AveExpr: The average log2 expression level; t: moderated t-statistic; P: uncorrected *P*-value for *t*-test; P_{FDR}: *P*-value adjusted for multiple testing based on the false discovery rate.

uni-heidelberg.de/apps/zmf/mirwalk/ accessed on 14 September 2014)[20] based on maximum agreement among the following tools: DIANA-mT, miRanda, miRDB, miRWalk, RNAhybrid, PICTAR5, PITA, RNA22, and TargetScan. Gene set enrichment in canonical pathways was analyzed using Ingenuity Pathway Analysis software (Ingenuity Systems, CA, USA).

Results

MicroRNA microarray results

MicroRNA microarray analysis was performed to identify differentially expressed microRNAs in serum of patients with chronic HBV or HCV compared to healthy individuals and between patients with chronic HBV compared to patients with chronic HCV. A larger number of microRNAs were significantly up- or down-regulated in serum of HCV patients compared to HBV patients (Table 2, Suppl. Table 1). MiR-122 was strongly up-regulated in both patients with HBV (logFC = 2.77) and HCV (logFC = 1.81), but the fold change was modest for other microRNAs. Several microRNAs were associated with HBV infection, including miR-22, miR-99a, miR-1246, miR-320a and miR-320b (Table 2; Fig. 1A). Serum microRNA profiles of HBeAg-positive and negative patients were compared with healthy subjects (Table 3, Fig. 1B, Suppl. Table 2). Results were similar for both HBeAg-positive and negative patients, but several microRNAs, including miR-122, miR-194, miR-125b, miR-99a, and miR-100, were up-regulated in HBeAg-positive patients compared to HBeAg-negative patients. MicroRNAs were annotated based on whether or not they have been reported to be detected within exosomes (www.exocarta.org accessed on 12 September 2014)[21,22] and/or within circulating HBsAg particles.[14] Nearly all of the significantly up-regulated microRNAs have been reported to be detected in exosomes, and miR-122, miR-30a, miR-30b, and miR-30c have been detected in HBsAg particles. However, further research is necessary to confirm in which compartments these microRNAs are present in these patients.

Quantitative RT-PCR analysis

qRT-PCR was used to validate expression of selected microRNAs (Table 4). MiR-122, miR-99a, miR-125b, miR-720, miR-22, and miR-1275 were significantly up-regulated in serum of HBV patients (n = 185) compared to healthy subjects (n = 22). MiR-122 and miR-720, but not miR-1246, were significantly up-regulated in serum of HCV patients (n = 107) relative to healthy subjects (n = 10). Microarray and qRT-PCR expression levels from the same individual were correlated ($P < 0.05$; data not shown). MiR-99a, miR-125b, miR-122, miR-720, and miR-22, but not miR-1275, were significantly elevated in HBeAg-positive versus HBeAg-negative individuals (Table 4; Fig. 2). In Fig.2, the points representing the highest

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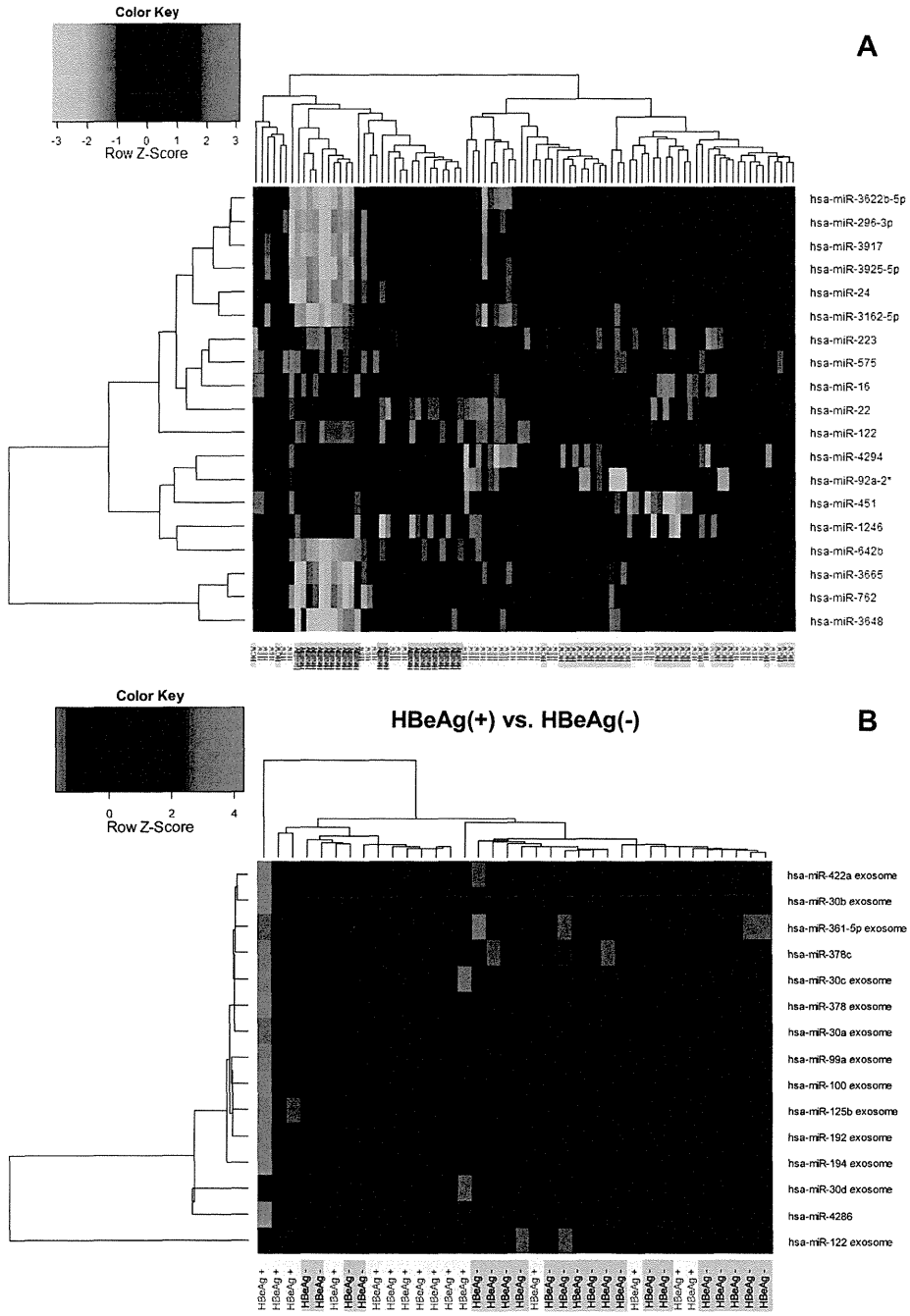

Figure 1 Heatmap of results of serum microRNA microarray analysis. Up-regulated microRNAs are shown in red, and down-regulated microRNAs are shown in green. Hierarchical clustering was performed in R using Euclidean distance and McQuitty clustering. A) Healthy (blue) versus HCV (purple) and HBV (yellow). B) HBe antigen-positive patients (yellow) versus HBe antigen-negative patients (purple). MicroRNAs that have been reported to be associated with exosomes are annotated based on a search of the Exocarta database.[21]

expression level for each of miR-122, miR-99a, and miR-125b corresponds to the same patient, who also had the highest HBsAg level (239000 IU/ml), but no other patients shared a similar rank pattern. The non-parametric Kruskal–Wallis test, based on the median instead of the mean, is robust to outliers and was used to prevent patients with high microRNA expression levels from having undo influence over the results. No internal normalization factors were selected *a priori*. However, because miR-1275 did not differ between HBeAg-positive and HbeAg-negative, it

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Table 3 Top up- or down-regulated serum microRNAs associated with HBeAg-positive or negative chronic HBV infection. MicroRNAs that have been detected in exosomes or HBsAg particles are noted.

Contrast	Direction	miRNA	logFC	AveExpr	*P*	P_{FDR}	Exosome	HBsAg
HBeAg(+) vs Healthy	Up	hsa-miR-122	3.9	8.1	3.48E-14	4.18E-11	exosome	HBsAg
	Up	hsa-miR-22	1.3	8.1	3.52E-07	3.52E-05	exosome	
	Up	hsa-miR-3648	1.2	13.0	3.47E-06	2.08E-04		
	Up	hsa-miR-1246	1.0	10.5	7.43E-06	3.43E-04		
	Up	hsa-miR-642b	1.0	9.1	3.94E-08	5.91E-06		
	Up	hsa-miR-486-3p	0.9	8.0	3.79E-06	2.15E-04		
	Up	hsa-miR-191	0.8	7.5	7.67E-07	5.76E-05	exosome	
	Up	hsa-miR-4286	0.8	7.3	3.74E-04	6.31E-03		
	Up	hsa-miR-194	0.8	6.5	1.66E-05	5.88E-04	exosome	
	Up	hsa-miR-99a	0.7	6.6	3.99E-06	2.15E-04	exosome	
	Up	hsa-miR-125b	0.7	6.7	9.17E-06	3.84E-04	exosome	
	Up	hsa-miR-30d	0.7	7.4	5.54E-06	2.66E-04	exosome	
	Up	hsa-miR-3665	0.6	14.0	5.11E-04	8.07E-03		
	Up	hsa-miR-320b	0.6	7.1	6.74E-09	1.35E-06		
	Up	hsa-miR-100	0.6	6.5	1.70E-05	5.88E-04	exosome	
	Up	hsa-miR-1915*	0.6	7.5	9.81E-04	1.39E-02		
	Up	hsa-miR-320a	0.6	7.1	8.21E-09	1.41E-06		
	Up	hsa-miR-320d	0.6	6.8	4.01E-07	3.70E-05		
	Up	hsa-miR-550a	0.6	7.1	3.38E-11	2.03E-08		
	Up	hsa-miR-320c	0.5	6.9	2.17E-07	2.61E-05		
	Up	hsa-miR-658	0.5	7.4	3.73E-09	1.00E-06	exosome	
	Down	hsa-miR-4294	−1.0	11.3	1.08E-04	2.50E-03		
	Down	hsa-miR-575	−0.7	8.0	4.65E-04	7.54E-03	exosome	
	Down	hsa-miR-92a-2*	−0.7	10.6	1.29E-03	1.69E-02		
	Down	hsa-miR-3197	−0.6	10.8	1.28E-04	2.84E-03		
	Down	hsa-miR-1268	−0.5	12.0	2.96E-05	8.89E-04		
	Down	hsa-miR-1275	−0.5	9.2	4.72E-04	7.54E-03		
HBeAg(−) vs Healthy	Up	hsa-miR-122	2.1	7.6	1.68E-06	4.39E-05	exosome	HBsAg
	Up	hsa-miR-3648	1.5	13.3	2.78E-09	2.09E-07		
	Up	hsa-miR-642b	1.2	9.3	2.15E-11	6.45E-09		
	Up	hsa-miR-1246	1.0	10.6	3.12E-05	4.31E-04		
	Up	hsa-miR-486-3p	0.9	8.1	7.30E-11	1.75E-08		
	Up	hsa-miR-22	0.8	8.0	1.07E-03	7.36E-03	exosome	
	Up	hsa-miR-191	0.8	7.5	5.11E-06	1.04E-04	exosome	
	Up	hsa-miR-3622b-5p	0.7	7.6	1.49E-03	9.54E-03		
	Up	hsa-miR-658	0.7	7.6	4.34E-10	5.21E-08	exosome	
	Up	hsa-miR-4258	0.6	8.3	3.39E-05	4.58E-04		
	Up	hsa-miR-1915*	0.6	7.5	3.79E-06	8.93E-05		
	Up	hsa-miR-24	0.6	8.5	6.50E-04	4.97E-03	exosome	HBsAg
	Up	hsa-miR-3665	0.6	14.1	3.08E-05	4.30E-04		
	Up	hsa-miR-550a	0.6	7.1	7.37E-14	8.84E-11		
	Up	hsa-miR-663b	0.6	9.3	4.31E-05	5.56E-04		
	Up	hsa-miR-3663-3p	0.6	10.5	2.75E-09	2.09E-07		
	Up	hsa-miR-320b	0.5	7.1	5.71E-07	1.90E-05		
	Up	hsa-miR-762	0.5	13.9	1.21E-05	2.02E-04		
	Up	hsa-miR-320c	0.5	7.0	1.50E-06	4.10E-05		
	Up	hsa-miR-3917	0.5	7.7	7.78E-04	5.66E-03		
	Up	hsa-miR-135a*	0.5	8.4	2.13E-04	2.09E-03	exosome	HBsAg
	Up	hsa-miR-663	0.5	10.7	1.66E-03	1.04E-02	exosome	
	Up	hsa-miR-3934	0.5	7.3	3.00E-07	1.09E-05		
	Up	hsa-miR-320a	0.5	7.1	1.58E-06	4.21E-05		
	Down	hsa-miR-451	−1.5	11.3	9.61E-06	1.72E-04	exosome	
	Down	hsa-miR-223	−1.0	8.0	7.28E-05	8.56E-04	exosome	HBsAg
	Down	hsa-miR-16	−0.8	8.0	1.39E-03	9.03E-03	exosome	
	Down	hsa-miR-4294	−0.8	11.3	7.84E-07	2.30E-05		

(*continued on next page*)

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Table 3 (*continued*)

Contrast	Direction	miRNA	logFC	AveExpr	*P*	P_{FDR}	Exosome	HBsAg
	Down	hsa-miR-575	−0.8	7.9	1.40E-06	3.89E-05	exosome	
	Down	hsa-miR-92a-2*	−0.8	10.5	9.47E-06	1.72E-04		
	Down	hsa-miR-1202	−0.6	8.3	2.12E-08	1.16E-06		
	Down	hsa-miR-1268	−0.6	11.9	1.99E-09	1.71E-07		
	Down	hsa-miR-1275	−0.5	9.1	4.35E-06	9.41E-05		
	Down	hsa-miR-17	−0.5	6.8	1.38E-05	2.24E-04	exosome	HBsAg
	Down	hsa-miR-20a	−0.5	6.7	2.58E-05	3.83E-04	exosome	
HBeAg(+) vs HBeAg(−)	Up	hsa-miR-122	2.8	8.3	1.57E-07	1.50E-04	exosome	HBsAg
	Up	hsa-miR-194	0.7	6.5	2.49E-07	1.50E-04	exosome	
	Up	hsa-miR-4286	0.6	7.3	3.97E-04	3.17E-02		
	Up	hsa-miR-30d	0.6	7.4	8.35E-06	2.01E-03	exosome	
	Up	hsa-miR-125b	0.5	6.7	1.07E-05	2.14E-03	exosome	
	Up	hsa-miR-99a	0.5	6.6	2.00E-04	1.85E-02	exosome	
	Up	hsa-miR-100	0.5	6.5	1.75E-04	1.75E-02	exosome	
	Up	hsa-miR-192	0.4	6.8	4.52E-05	6.23E-03	exosome	
	Up	hsa-miR-378	0.4	6.6	2.20E-06	6.61E-04	exosome	
	Up	hsa-miR-30a	0.3	6.5	8.66E-05	9.45E-03	exosome	HBsAg
	Up	hsa-miR-422a	0.3	6.5	1.50E-06	6.00E-04	exosome	
	Up	hsa-miR-30c	0.3	6.6	7.59E-05	9.11E-03	exosome	HBsAg
	Up	hsa-miR-378c	0.3	6.4	2.61E-04	2.23E-02		
	Up	hsa-miR-30b	0.2	6.5	4.67E-05	6.23E-03	exosome	HBsAg
	Up	hsa-miR-361-5p	0.2	6.4	3.11E-05	5.33E-03	exosome	

was used to renormalize miR-99a, miR-125b, miR-122, miR-720, and miR-22 qRT-PCR expression data. *P*-values using renormalized data decreased by approximately one order of magnitude but remained highly significant and did not affect any conclusions (data not shown).

Association between microRNA level and clinical factors in patients with chronic HBV

Multiple regression was used to identify associations among microRNA levels and clinical factors in HBV patients using

Table 4 Quantitative RT-PCR results of selected microRNAs in serum of chronic HBV or HCV patients and healthy controls and between HBeAg-positive and negative patients. Expression levels are shown as median and range and compared using the Mann–Whitney *U* test.

microRNA	Healthy (n = 22)	HBV (n = 185)	logFC	*P*	P_{FDR}
hsa-miR-122/cel-miR-238	0.021 (0.013–0.04)	0.204 (0.011–2.495)	3.31	1.54E-13	1.08E-12
hsa-miR-99a/cel-miR-238	0.014 (0.005–0.051)	0.132 (0.008–2.436)	3.24	3.64E-12	8.50E-12
hsa-miR-125b/cel-miR-238	0.023 (0.007–0.05)	0.146 (0.007–3.084)	2.70	3.36E-12	8.50E-12
hsa-miR-720/cel-miR-238	0.043 (0.024–0.123)	0.146 (0.035–3.732)	1.76	4.66E-11	8.15E-11
hsa-miR-22/cel-miR-238	0.226 (0.107–0.485)	0.335 (0.096–1.305)	0.57	4.69E-04	6.57E-04
hsa-miR-1275/cel-miR-238	0.405 (0.237–0.604)	0.517 (0.099–1.626)	0.35	4.90E-03	5.71E-03
microRNA	Healthy (n = 10)	HCV (n = 107)	logFC	*P*	P_{FDR}
hsa-miR-720/cel-miR-238	0.388 (0.232–0.749)	0.653 (0.198–1.731)	0.75	2.51E-03	7.53E-03
hsa-miR-122/cel-miR-238	0.671 (0.307–0.95)	1.096 (0.1–8.542)	0.71	1.78E-02	2.68E-02
hsa-miR-1246/cel-miR-238	2.893 (1.821–6.813)	4.360 (0.429–36.311)	0.59	7.28E-02	7.28E-02
microRNA	HBeAg-negative (n = 82)	HBeAg-positive (n = 103)	logFC	*P*	P_{FDR}
hsa-miR-99a/cel-miR-238	0.070 (0.009–0.585)	0.250 (0.008–2.436)	1.84	4.55E-11	1.59E-10
hsa-miR-125b/cel-miR-238	0.100 (0.007–0.507)	0.253 (0.012–3.084)	1.34	7.70E-10	1.80E-09
hsa-miR-122/cel-miR-238	0.143 (0.011–0.678)	0.337 (0.017–2.495)	1.24	8.60E-12	6.02E-11
hsa-miR-720/cel-miR-238	0.119 (0.035–0.517)	0.185 (0.040–3.732)	0.64	4.24E-06	7.42E-06
hsa-miR-22/cel-miR-238	0.302 (0.096–1.305)	0.391 (0.103–1.049)	0.37	2.36E-04	3.30E-04
hsa-miR-1275/cel-miR-238	0.494 (0.099–1.626)	0.541 (0.186–1.376)	0.13	1.07E-01	1.25E-01

logFC: log2 fold-change; P: uncorrected *P*-value for Mann-Whitney *U* test; P_{FDR}: *P*-value adjusted for multiple testing based on the false discovery rate.

Please cite this article in press as: Akamatsu S, et al., Differences in serum microRNA profiles in hepatitis B and C virus infection, J Infect (2014), http://dx.doi.org/10.1016/j.jinf.2014.10.017