Observational Study

Medicine®

OPEN

Expression and significance of urinary microRNA in patients with chronic hepatitis B

Jia-Wei Shang, BS[a], Xiu-Li Yan, PhD[b], Hui Zhang, PhD[a,*], Shi-Bing Su, PhD[a,*]

Abstract

The aim of this study was to investigate the alterations of urinary microRNA (miRNA) expression and explore its clinical significance in patients with chronic hepatitis B (CHB).

The expression levels of urinary miRNA were detected by miRNA microarray and quantitative reverse transcription polymerase chain reaction (qRT-PCR) from 106 CHB and 40 healthy controls (Ctrl) subjects. The correlation between the levels of miRNA expression and clinical characteristics were analyzed. Receiver-operator characteristic (ROC) curves were generated to determine the specificity and sensitivity of each individual miRNA. MiRNAs expression were further measured by PCR from exosomes, which were isolated from urine samples. LX2 cells were transfected with miRNA inhibitor and accumulation of cytoplasmic lipid droplets was analyzed by Oil Red O staining.

miRNA expression profile analysis showed that 22 miRNAs were upregulated and 55 miRNAs were downregulated in CHB patients compared with Ctrl subjects (fold-change>1.5 and $P < .05$). miR-92b-3p, miR-770-5p, miR-5196-5p, and miR-7855-5p were significantly higher ($P < .0001$) in CHB subjects than in Ctrl subjects. ROC curve analysis showed that these four miRNAs were sensitive and specific enough to distinguish CHB and Ctrl subjects. The levels of miR-92b-3p expression were negatively correlated with total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and APOA-1. Moreover, in vitro experiments indicated that inhibition of miR-92b-3p increased lipid droplet formation in LX2 cells.

Aberrant expression of miRNAs has been observed in urine of CHB patients. Our findings may provide novel insights into the pathogenesis of CHB and may assist in the diagnosis of patients with CHB.

Abbreviations: CHB = chronic hepatitis B, Ctrl = healthy controls, LDL-C = low-density lipoprotein cholesterol, miRNA = microRNA, qRT-PCR = quantitative reverse transcription polymerase chain reaction, ROC = receiver-operator characteristic (ROC), TC = total cholesterol.

Keywords: chronic hepatitis B, expression, significance, urinary microRNA

1. Introduction

Chronic hepatitis B virus (HBV) infection represents a serious public health problem and is a leading cause of liver cirrhosis and hepatocellular carcinoma (HCC).[1,2] Up to 40% of patients with chronic hepatitis B (CHB) progress to chronic end-stage liver disease or HCC during their lifetime.[3] In China, HBV infection is a predominant etiological factor of liver disease, whereby 7.8% of the population are HBV carriers.[4] Additionally, China is an area with the highest prevalence of HCC due to the significant number of chronic HBV carriers.[5] Thus, prevention, diagnosis and treatment progress of CHB needed to be investigated and monitored.

MicroRNAs (miRNAs) are small, noncoding RNAs that play an important role in regulating various biological processes through their interaction with cellular messenger RNAs. It has been reported that miRNAs are expressed in all animal cells and have fundamental roles in cellular activities, such as the development, cellular differentiation, proliferation, apoptosis, cell-cycle control, metabolism, and cancer. Extracellular miRNAs in circulation, saliva, and urine have recently been shown to be associated with various pathological conditions,[6–10] including liver disease.[10–13] For example, a serum miRNA signature is associated with the immune control of HBV infection.[11] Serum miR-210 can be used as an indicator of HBV replication and translation, and a potential marker of necroinflammation in patients with CHB.[12] Expression of miR-155 is downregulated in peripheral blood mononuclear cells of patients with CHB.[13]

Editor: Josephine Kam Tai Dermawan.

J-WS and X-LY contributed equally to this research.

This study was supported by the National Natural Science Foundation of China (Grant numbers 81773979 and 81373518).

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

[a] Research Center for Traditional Chinese Medicine Complexity System, Institute of Interdisciplinary Integrative Medicine Research, [b] Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, PR China.

[*] Correspondence: Hui Zhang, Research Center for Traditional Chinese Medicine Complexity System, Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, Shanghai, 201203, PR China (e-mail: zhanghuiman@126.com); Shi-Bing Su, Research Center for Traditional Chinese Medicine Complexity System, Institute of Interdisciplinary Integrative Medicine Research, Shanghai University of Traditional Chinese Medicine, Shanghai, 201203, PR China (shibingsu07@163.com).

How to cite this article: Shang JW, Yan XL, Zhang H, Su SB. Expression and significance of urinary microRNA in patients with chronic hepatitis B. Medicine 2019;98:37(e17143).

Received: 21 February 2019 / Received in final form: 17 June 2019 / Accepted: 20 August 2019

http://dx.doi.org/10.1097/MD.0000000000017143

Our previous study revealed a five-miRNA profile (miR-122, -572, -575, -638, and -744) in serum that was deregulated in patients with CHB. The levels of these miRNAs can specifically predict liver injury in CHB.[6] Comprehensiveness of those factors will help diagnosis, select optimal therapy and shed light on the underlying mechanism of CHB.

Urine is one of the most readily accessible biological fluids, and it harbors numerous biologically significant miRNAs. Recent studies have shown that urinary miRNAs exist in a stable form and are associated with liver disease.[9,10,14] For example, urine and plasma miRNAs appear to be used as biomarkers of kidney or liver damage.[9] miR-192 and -21 can be used as potential indicators for liver fluke-associated cholangiocarcinoma risk group.[10] Urinary miRNAs (miR-625, -532, -618, -516–5p, and -650) may serve as biomarkers for screening high-risk patients for the early detection of hepatocellular carcinoma.[14]

Blood and urine are the most common types of samples employed for monitoring disease-related biomarkers.[6–14] However, urine sample is more preferable as it enables non-invasive monitoring of physiological or pathological conditions.[9,10,14] So far, however, miRNA profiling of the urine of CHB patients is incomplete. The goal of this study is to investigate whether urinary miRNAs can be used as molecular biomarkers to monitor the pathological development of CHB. In this study, we collected urine samples from patients with CHB and from healthy volunteers. We described the global miRNA expression profile in urine and identified a urinary miRNA signature for CHB. Our study revealed that a number of urinary miRNAs were differentially expressed in patients with CHB and underscored the potential importance of miRNAs in the pathogenesis of CHB.

2. Material and methods

2.1. Study subjects and clinical parameters

Urine samples were collected from subjects, including 106 CHB and 40 healthy controls (Ctrl). The profiles of urinary miRNA expression were first generated with urine samples from 6 patients with CHB and 5 Ctrl subjects by microarray analysis. The levels of several miRNAs were further quantitated by real-time reverse transcription polymerase chain reaction (qRT-PCR) with urine samples from another 100 CHB patients and 35 Ctrl subjects. The diagnostic criteria for CHB followed the guidelines defined by the Chinese Society of Hepatology and Chinese Society of Infectious Diseases in 2005.[15] The diagnostic criteria for CHB include a history of hepatitis B or positive HBsAg for at least 6 months and the continuing presence of HBsAg and/or HBV DNA in the serum. The diagnosis of HBV infection is based on serologic markers, virologic markers, liver function test, biochemical assays, as well as other clinical and supportive findings. Urinary Ctrl samples were randomly selected from a collection of 40 individuals who underwent an annual physical examination at Shanghai Shuguang Hospital in Shanghai, China. Normal Ctrls were healthy subjects without medical disease. Samples of CHB were from patients seeking treatment in Shanghai Shuguang Hospital. The clinical parameters of these patients are given in Table 1. This study was subject to approval by the Institutional Review Board of Shanghai Shuguang Hospital (2012–206-22-02). An informed consent form was issued and signed by each of the participants, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki (1964).

Table 1

Clinical parameters of participants.

Parameters	CHB	Healthy
Individuals (n)	100	35
Male	84	25
Female	16	10
Age (years)	30.0 ± 15.3	25.4 ± 10.4
ALT (IU/L)	64.2 ± 84.0	21.6 ± 19.3
AST (IU/L)	48.5 ± 39.2	23.4 ± 20.6
GGT (IU/L)	47.7 ± 53.8	21.0 ± 11.7
ALP (IU/L)	86.0 ± 23.1	59.4 ± 20.3
TBIL (μm/L)	17.0 ± 7.6	16.2 ± 5.8
HBV DNA	11726454.3	
(0–44888788)	0	
HBV status (n)		
HBsAg+	100	0
HBsAg–	0	35

The values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), and hepatitis B virus (HBV) DNA are shown as medians (range).
CHB = chronic hepatitis B, HBsAg = hepatitis B surface antigen.

2.2. Urine samples collection and RNA isolation

All urine samples were collected in the morning and stored at−-80°C. Urine total RNA was extracted from 800 μl urine samples using a mirVana PARIS kit (Ambion, Austin, TX) according to the manufacturer's protocol and then eluted from the membrane into a collection tube with 35 μl elution buffer.

2.3. Urine miRNA profiling and data analysis

The profiles of urine miRNAs from 6 CHB patients and 5 Ctrl cases were generated using Agilent Human miRNA microarray V21.0 (Agilent Technologies, Santa Clara, CA). The microarray chip consists of 2549 different probes for human miRNAs. One hundred ng RNA was used for each array. The arrays were read using an Agilent microarray scanner, and the data were extracted using Feature Extraction V10.7 (Agilent Technologies, Santa Clara, CA). All data were converted to Log Base 2. Statistical analysis of the microarray data was carried out using the Shanghai Biochip Co., Ltd. (Shanghai, China) Analysis System (SAS) (http://www.ebioservice.com). The core arithmetic of the SAS system is R, which is an open source programming language and software environment for statistical computing and graphics. Only those miRNAs with a fold difference ≥1.5 and a P-value $< .05$ were considered significant.

2.4. Quantitative real-time RT-PCR analysis

qRT-PCR-based quantification of miRNAs was performed with Bulge-Loop miRNA qPCR Primer and SYBR Green PCR Master Mixture (Toyobo, Ltd., Osaka, Japan), according to the manufacturer's instructions, using the ABI StepOne Plus Real-Time PCR System with results normalized to U6 expression.[9,16] The ΔΔCt method was used to calculate relative expression. Primer sequences used for RT-PCR are shown in Table 2.

2.5. Urinary exosome isolation and electron microscopy

Overnight urine collections (~50 mL/subject) were obtained from all recruited subjects. Urine was precleared by both centrifugation

Table 2

Primer sequences for qRT-PCR.

Name	Sequence
miR-92b-3p-RTP	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGGAGGCCG
miR-92b-3p-FP	ACACTCCAGCTGGGTATTGCACTCGTCCCG
miR-770-5p-RTP	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGGCCCTG
miR-770-5p-RP	ACACTCCAGCTGGGTCCAGTACCACGTGTC
miR-5196-5p-RTP	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCCAACCC
miR-5196-5p-FP	ACACTCCAGCTGGGAGGGAAGGGGACGAGG
miR-7855-5p-RTP	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCGAGCTT
miR-7855-5p-FP	ACACTCCAGCTGGGTTGGTGAGGACCCCAA
URP	TGGTGTCGTGGAGTCG
U6-RP	CTCGCTTCGGCAGCACA
U6-FP	AACGCTTCACGAATTTGCGT

FP = forward primer, RP = reverse primer, RTP = RT primer, URP = universal reverse primer.

(300 g 10 min at 4°C and 17,000 g 20 min at 4°C) and filtration (0.8 μm) to remove cellular debris. Then, exosomes were isolated by two consecutive ultracentrifugation steps (200,000 g 75 min at 4°C; 70.1 Ti rotor, Beckman Instruments, Fullerton, CA), as previously described.[17] Pellets were suspended in phosphate buffer, and exosome quality and purity were assessed by electron microscopy.

2.6. Cell culture

Human LX2 cells (a generous gift from Dr. Xu L[18]) were maintained in plastic culture plates in DMEM supplemented with 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA), 100 U/mL penicillin G sodium salt and 100 U/mL streptomycin sulfate (Gibco, Carlsbad, CA), and incubated at 37°C under an atmosphere of 5% CO_2.

2.7. Transfection of miRNA inhibitor and Oil Red O staining

The miR-92b-3p inhibitor (anti-miR-92b-3p) and miRNA inhibitor negative control (anti-miR-Con) were transfected into cells at 100 nmol/L concentrations with Lipofectamine 3000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After transfection for 24 hours, cells were treated with 10 ng/mL TGF-β1 (R&D Systems, Shanghai, China) for 48 hours. The cells were washed with phosphate-buffered saline and fixed with 4% paraformaldehyde. Oil Red O (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was added, washed away, and lipid droplets were photographed. Photographs were taken at 200× magnification.

2.8. miRNA target prediction and enrichment information

The miRNA potential target genes were shown as the intersection of TargetScan (http://www.targetscan.org/), PITA (https://genie.weizmann.ac.il/pubs/mir07/mir07_data.html), and microRNA.org (http://www.microrna.org/microrna/home.do) databases (GeneSpring software version 12.5). These putative target genes were subjected to Gene Ontology (GO) biological process annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the DAVID online analysis tool (http://david.abcc.ncifcrf.gov/). The *P*-value was calculated with a unilateral Fisher's exact test and corrected by false discovery rate (FDR). GOs and pathways with *P*-value < .05 and FDR < 0.05 were considered significant.

2.9. Statistical analysis

Comparisons between groups were analyzed using the Mann–Whitney *U* test or Pearson correlation analysis wherever appropriate. Receiver-operator characteristic (ROC) curves were generated to evaluate the difference in the levels of urinary miRNAs between CHB and Ctrl subjects. All tests were two-tailed, and $P < .05$ was considered statistically significant.

3. Results

3.1. Aberrant miRNA expression profiling in CHB urine

To assess differential urinary miRNA expression profiles between CHB and Ctrl, miRNA microarray experiments were conducted on the total RNA obtained from urinary samples from 6 CHB and 5 Ctrl cases. Among the 2549 miRNAs analyzed, 77 were differentially expressed between CHB and Ctrl subjects. Compared with Ctrl subjects, CHB patients showed 22 upregulated miRNAs and 55 downregulated miRNAs (fold-change > 1.5 and *P*-value < .05) (Figure 1 and Table 3). For example, miR-7855-5p, -770-5p, -5196-5p, and -92b-3p were upregulated in CHB patients; miR-642a-3p, -320c, and -3138 were downregulated in CHB patients (Table 3).

3.2. Validation of urinary miRNA expression by qRT-PCR

To validate these microarray-generated results, we prepared RNA from urinary samples of another 100 CHB patients and 35 Ctrl and were subsequently subjected to qRT-PCR to measure the levels of miR-7855-5p, -770-5p, -5196-5p, and -92b-3p. We selected these four miRNAs for further analysis due to its elevated miRNA expression with a low *P*-value in CHB patients compared with Ctrl subjects (Table 3), and its high expression abundance in the urine based on microarray data. Identical to what we observed with microarray analysis, the levels of miR-7855-5p, -770-5p, -5196-5p, and -92b-3p were significantly higher in the urine of CHB patients than in Ctrl subjects ($P < .0001$, respectively) (Figure 2).

3.3. Levels of urinary miRNAs can be used to distinguish CHB patients from Ctrl subjects

To determine whether the levels of urinary miRNAs can be used to distinguish patients with CHB from Ctrl, we generated ROC curves to analyze the difference in the levels of urinary miR-92b-3p, -770-5p, -5196-5p, -7855-5p between groups. Comparing CHB subjects with Ctrl, ROC curve areas of miR-92b-3p, -770-5p, -5196-5p, and -7855-5p were found to be 0.89 (95% CI: 0.84–0.94), 0.92 (95% CI: 0.88–0.97), 0.76 (95% CI: 0.67–0.85), and 0.82 (95% CI: 0.72–0.91), respectively. The sensitivity and the specificity of each of these miRNAs were 86% and 91.4%, 93% and 90.9%, 57% and 91.4%, 83% and 80% in CHB and Ctrl subjects, respectively (Figure 3). These results clearly showed that the levels of urinary miR-92b-3p, -770-5p, -5196-5p, and -7855-5p can distinguish patients with CHB from Ctrl.

3.4. Expression of urinary miRNAs in exosomes

To investigate the distribution of the urinary miRNAs, we isolated exosomes from urine samples of 6 patients with CHB and 6 Ctrl subjects. A typical electron micrograph of negatively stained urinary vesicles (Figure 4A and B) illustrates that the distribution of vesicle size shows a range of 30–100 nm. The levels of miR-92b-3p,

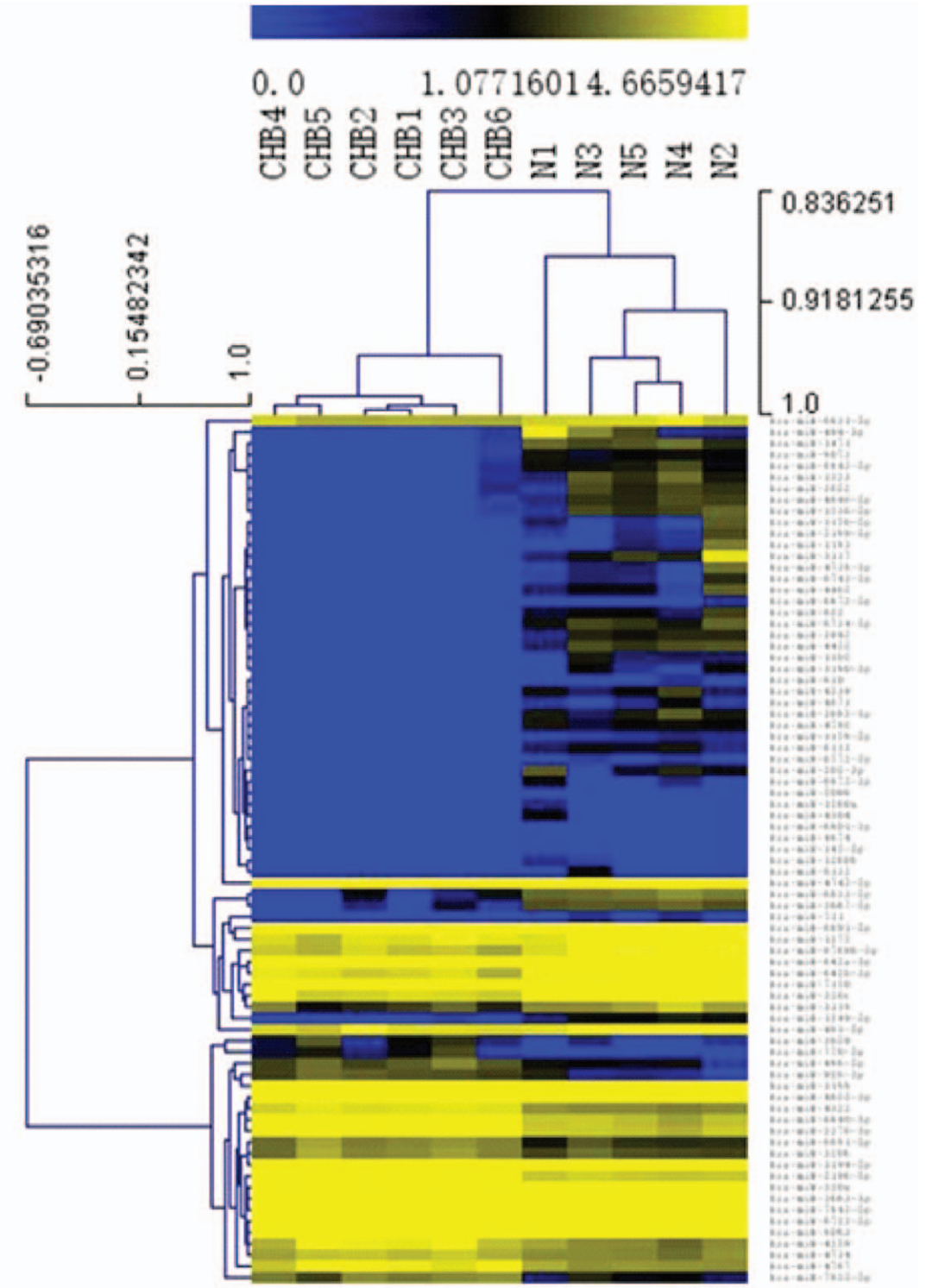

Figure 1. Heatmap of miRNA expression in urine for patients with CHB and Ctrl.

-770-5p, -5196-5p, and -7855-5p were further measured by PCR. We concluded that miR-92b-3p, -770-5p, -5196-5p, and -7855-5p were observed at high abundance in urinary exosomes. These results indicate that urine miRNAs such as miR-92b-3p, -770-5p, -5196-5p, and -7855-5p are likely to be mainly contained in exosomes. Urinary miRNAs could possibly be applied in the diagnosis of CHB as a novel urine-based biomarker.

3.5. Aberrant levels of urinary miR-92b-3p, -770-5p, -5196-5p, and -7855-5p correlate with clinical pathological parameters

We analyzed the potential correlations between each of these four miRNAs (miR-92b-3p, -770-5p, -5196-5p, and -7855-5p) and each of the clinical pathological parameters evaluated. We correlated the expression levels of these four miRNAs with

Table 3

Differentially expressed miRNAs in CHB and Ctrl subjects.

miRNAs	Fold-change (CHB/Ctrl)	*P*-Value	Regulation
miR-2276-3p	2.10	.00	up
miR-7855-5p	5.97	.01	up
miR-3659	5.42	.02	up
miR-770-5p	4.37	.01	up
miR-5196-5p	3.19	.03	up
miR-92b-3p	2.56	.04	up
miR-4259	2.53	.00	up
miR-4655-3p	2.49	.00	up
miR-486-5p	2.47	.00	up
miR-4322	2.11	.00	up
miR-4734	2.07	.02	up
miR-6840-3p	2.06	.00	up
miR-6881-5p	2.03	.00	up
miR-3663-3p	2.03	.01	up
miR-4767	1.98	.03	up
miR-320a	1.97	.03	up
miR-8063	1.94	.02	up
miR-3194-5p	1.93	.02	up
miR-6723-5p	1.91	.01	up
miR-7845-5p	1.84	.00	up
miR-320b	1.66	.01	up
miR-3188	1.60	.00	up
miR-1305	16.44	.00	down
miR-3137	16.03	.01	down
miR-6734-5p	14.70	.00	down
miR-4455	14.57	.00	down
miR-3682-3p	12.12	.00	down
miR-1471	11.91	.02	down
miR-3945	11.35	.00	down
miR-494-3p	11.01	.04	down
miR-4792	10.27	.00	down
miR-622	10.23	.00	down
miR-4539	10.11	.00	down
miR-4465	9.83	.01	down
miR-6833-5p	9.47	.01	down
miR-6133	9.23	.00	down
miR-202-3p	9.03	.01	down
miR-1236-5p	8.98	.03	down
miR-1323	8.86	.03	down
miR-4646-5p	8.44	.03	down
miR-3652	7.91	.04	down
miR-3667-5p	7.83	.01	down
miR-4738-3p	7.72	.01	down
miR-1183	7.62	.01	down
miR-6845-5p	7.43	.03	down
miR-6743-5p	7.35	.01	down
miR-711	7.31	.01	down
miR-5189-5p	7.22	.01	down
miR-1260a	6.98	.00	down
miR-3156-5p	6.86	.01	down
miR-3180-3p	6.84	.01	down
miR-4673	6.70	.00	down
miR-1249-5p	6.69	.03	down
miR-3158-5p	6.57	.00	down
miR-8073	6.45	.03	down
miR-6772-5p	5.29	.00	down
miR-1260b	4.99	.01	down
miR-6872-3p	4.72	.01	down
miR-6872-5p	4.72	.00	down
miR-6132	4.51	.01	down
miR-610	4.10	.01	down
miR-4304	3.73	.02	down
miR-4674	3.04	.00	down
miR-345-5p	2.99	.01	down
miR-6801-3p	2.67	.01	down
miR-5096	2.57	.01	down
miR-3138	2.45	.01	down
miR-320c	2.34	.00	down
miR-642a-3p	2.23	.00	down
miR-642b-3p	1.91	.01	down
miR-7150	1.78	.00	down
miR-6831-5p	1.70	.03	down
miR-4745-5p	1.69	.04	down
miR-483-5p	1.67	.02	down
miR-1275	1.65	.02	down
miR-6769b-5p	1.56	.02	down
miR-6891-5p	1.52	.01	down

(*continued*)

Table 3

(continued).

clinical indicators of HBV infection, including serum ALT, AST, HBsAg, HBeAg level, and HBV DNA level. However, no significant correlation was discovered (data not shown). A Pearson correlation analysis showed that correlation only existed between selected miRNAs and selected liver function parameters (Table 4). For example, miR-770-5p was significantly correlated with serum Total protein (TP) ($r=-0.339$, $P=.001$). miR-92b-3p was significantly correlated with Total cholesterol (TC) ($r=-0.263$, $P=.008$), low-density lipoprotein cholesterol (LDL-C) ($r=-0.241$, $P=.016$), and APOA-1 ($r=-0.221$, $P=.028$) (Table 4). In this study, we found that serum levels of TP were higher in CHB group than in the Ctrl group; TC, LDL-C, and ApoA-1 were lower in CHB patients than in matched Ctrl (Table 5).

3.6. Inhibition of miR-92b-3p increases lipid droplet formation in LX2 cells

Hepatic stellate cells (HSCs) play an important role in liver physiology and under healthy conditions they have a quiescent and lipid-storing phenotype. To further investigate whether miR-92b-3p is involved in the lipid metabolism, Human HSC (LX2) cells were transfected with miR-92b-3p inhibitor and miRNA negative control for 24 hours and treated with TGF-β1 (10 ng/mL) for 48 hours. Oil Red O staining analysis demonstrated that downregulation of miR-92b-3p allows HSCs to restore their ability to accumulate cytoplasmic lipid droplets (Figure 4C and D). Our results suggest that miR-92b-3p contribute to the regulation of lipid metabolism.

3.7. GO terms and KEGG pathway annotation of miR-92b-3p targets

To investigate the potential function of miR-92b-3p in lipid metabolism, we predicted its targets using TargetScan, PITA, and microRNA.org databases. There were 2430 putative targets for TargetScan, 325 putative targets for PITA, and 2466 putative targets for microRNA.org. We calculated 144 target genes by computing the intersection of the three databases (Figure 5A, Supplementary Table 1, http://links.lww.com/MD/D232). Gene ontology analysis showed that the target genes of miR-92b-3p played a part in lipid metabolism, such as negative regulation of lipid storage (ITGB3; ITGAV) and liver development (MKL2; NOTCH1; AACS; MAN2A1; JARID2) (Figure 5B). Pathway enrichment analysis showed that the target genes of miR-92b-3p

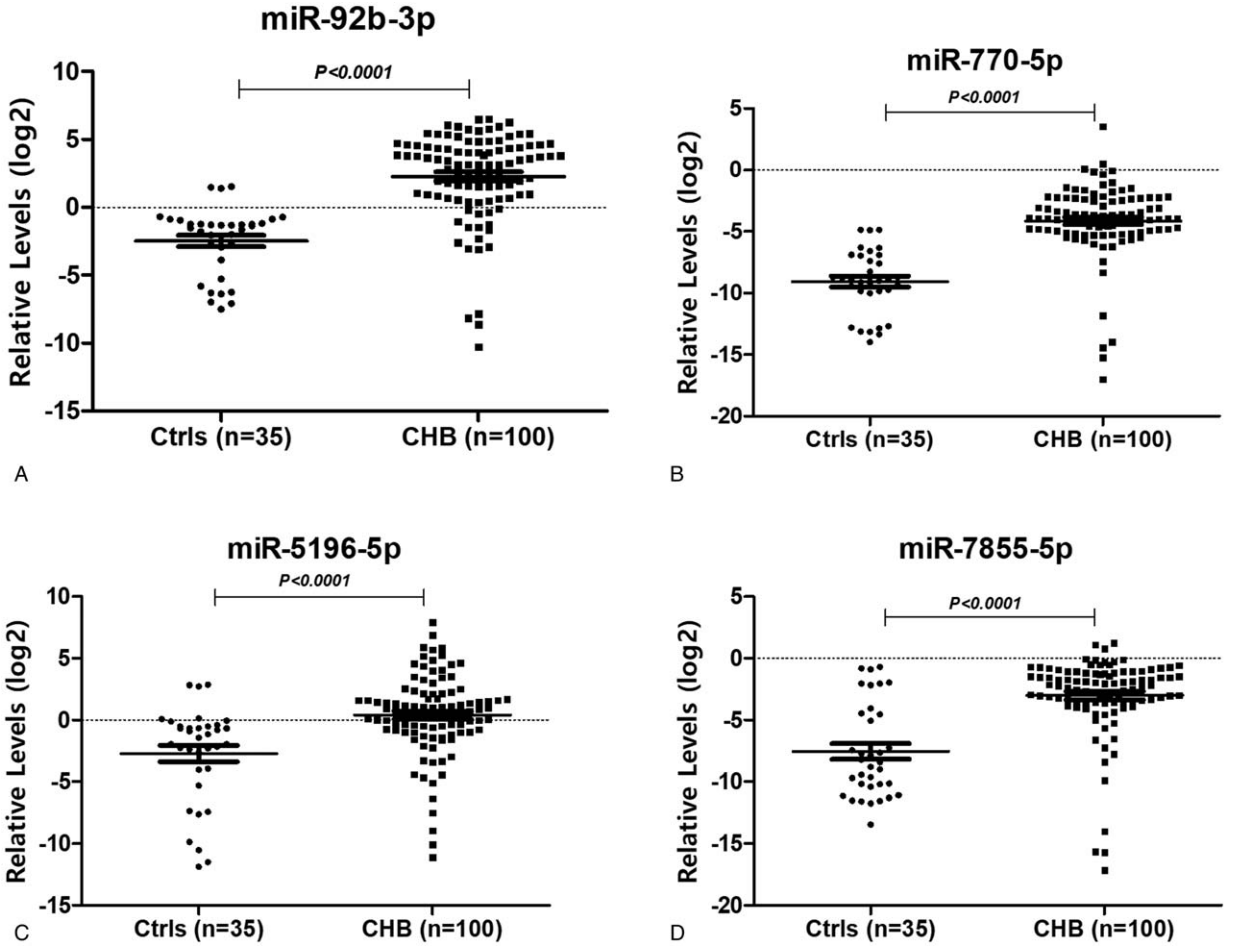

Figure 2. Expression levels of miRNAs in urine samples of CHB and Ctrl subjects. The levels of urinary miR-92b-3p (A); miR-770-5p (B); miR-5196-5p (C); miR-7855-5p (D) in patients with CHB (n = 100) and Ctrl subjects (n = 35) were measured by qRT-PCR. The line for each group represents the median value of the indicated miRNA. The values were normalized to U6 and displayed in log2 scale on the Y-axis. CHB = chronic hepatitis B, Ctrl = healthy control.

involved in pathways related to lipid metabolism, such as the Thyroid hormone signaling pathway (ITGB3; NOTCH1; NCOA1; ITGAV), Notch signaling pathway (NOTCH1; DTX2), Hedgehog signaling pathway (BTRC; RAB23), and FoxO signaling pathway (NLK; FOXG1; RAG1) (Figure 5C). This finding clearly indicates that these miRNAs may be actively secreted and involved in CHB pathogenesis.

4. Discussion

Recent studies have shown that miRNA expression patterns are disease and tissue specific. MiRNAs are abundant in the liver and modulate a diverse spectrum of cellular processes associated with liver injury such as inflammation, apoptosis, and hepatocyte regeneration. Deregulation of miRNA expression may be a key pathogenic factor in various liver diseases, including viral hepatitis,[6,11–13] liver fibrosis/cirrhosis,[19,20] HCC,[21] metabolic,[22] and acute liver diseases.[23] A clearer understanding of the mechanisms involved in miRNA deregulation would offer new diagnostic and therapeutic strategies to address liver diseases.

Cell-free miRNAs are shielded from degradation of RNase through packaging in exosomes, microvesicles and apoptotic bodies[24] or formation of protein-miR complexes with argonaute 2 (Ago2) or high-density lipoprotein (HDL)-associated protein.[25,26] Cell-free miRNAs in serum[27] and urine[28] are a novel class of noninvasive disease biomarker with specificity, stability and reproducibility. miRNA expression profiles are altered in various hepatic diseases compared with those in healthy conditions.[6,11–13,19–23,29–45] For example, miR-122 was previously described to be an abundant, liver-specific miRNA.[29] In rodents, liver injury induced by alcohol or chemicals increases the level of serum or plasma miR-122, and this increase occurs earlier than the increase in ALT, a commonly used marker.[6,30] Moreover, the level of plasma miR-122 exhibits an excellent correlation with the necro-inflammatory activity of HBV[31] and HCV infection.[32,33] Circulating miR-122 is a predictor for virological response in CHB patients with high viral load treated with nucleos(t)ide analogs.[34] MiR-122 inhibits HBV replication through NDRG3[35] and CCNG1-modulated P53 activity.[36] Serum miR-210 can be used as an indicator of HBV replication and translation, and a potential marker of necroinflammation in patients with CHB.[12] HBV infection suppresses the expression of inflammatory macrophage miR-210.[37] Circulating miR-210 and -22 combined with ALT can predict the virological response to interferon-alpha therapy of CHB.[38] Zhang et al found that miR-210 negatively regulates HBV replication by targeting the HBV pre-S1 region in HepG2 2.2.15 cells under normoxic condition.[39] Serum miR-125b was correlated with HBV

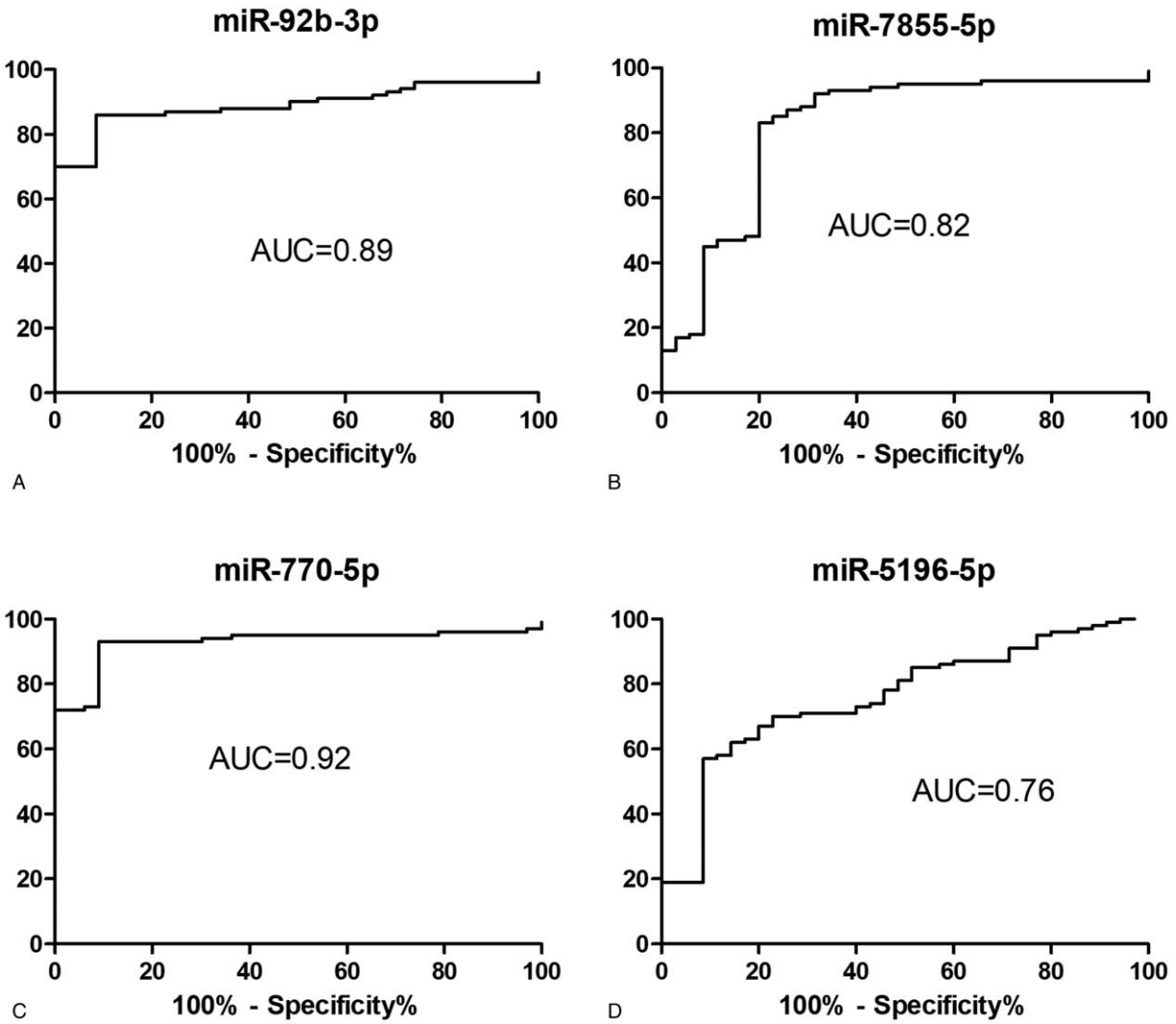

Figure 3. Receiver-operating characteristic (ROC) curves for miR-92b-3p, -770-5p, -5196-5p, and -7855-5p to discriminate CHB patients from Ctrl subjects. (A) miR-92b-3p (CHB/Ctrl); (B) miR-770-5p (CHB/Ctrl); (C) miR-5196-5p (CHB/Ctrl); (D) miR-7855-5p (CHB/Ctrl). AUC = area under the ROC curve, CHB = chronic hepatitis B, Ctrl = healthy control.

replication and liver necroinflammation,[40] and miR-125b levels predict virologic response to nucleos(t)ide analogue treatment in patients with HBeAg-positive CHB.[41] MiR-125b inhibits HBV expression through targeting of the SCNN1A[42] and miR-125b also inhibits the secretion of HBsAg and HBeAg.[43] Serum miR-146a-5p was highly expressed in HBV-infected patients. In HBV-infected hepatocytes, NF-κB-mediated miR-146a-5p induced autophagy via the miR-146a-5p-XIAP-MDM2/p53 pathway, leading to HBV propagation.[44] Wang et al demonstrates that miR-146a upregulation in CHB causes impaired T cell function by targeting Stat1, which may contribute to immune defects and immunopathogenesis during chronic viral infection.[45] Our previous study has revealed that serum levels of miR-122, -572, -575, -638, and -744 are deregulated in patients with CHB or nonalcoholic steatohepatitis (NASH). The levels of these miRNAs may be used as potential biomarkers for liver injury caused by CHB and NASH.[6]

However, the complete urinary miRNA profile of CHB remains largely unknown. In the present study, using miRNA microarray, we investigated the global expression profiles in urine from CHB patients. The results revealed significant differences in expression profiles of miRNAs between CHB and Ctrl in which 22 were upregulated and 55 downregulated in CHB subjects (fold-change>1.5 and $P<.05$) (Figure 1 and Table 3). Overall miRNA expression levels were downregulated in CHB patients. This change may be attributed to inhibition of miRNA expression and dysfunction of organism-specific immune response resulting from HBV infection. Subsequent validation work found that the median levels of miR-92b-3p, -770-5p, -5196-5p, and -7855-5p were significantly higher ($P<.0001$) in CHB than in Ctrl (Figure 2). ROC curve analysis showed that the levels of miR-92b-3p, -770-5p, -5196-5p, and -7855-5p in urine were sensitive and specific enough to distinguish CHB and Ctrl subjects. Therefore, this study has revealed a novel phenomenon of the aberrant expression levels of miRNA in urine of CHB patients.

Urine has very interesting features as a source of biomarkers; it is easy to collect, contains both proteins and metabolites, and contains extracellular vesicles (uEVs). These biomarkers are mostly derived from renal and urethral cells but may also be

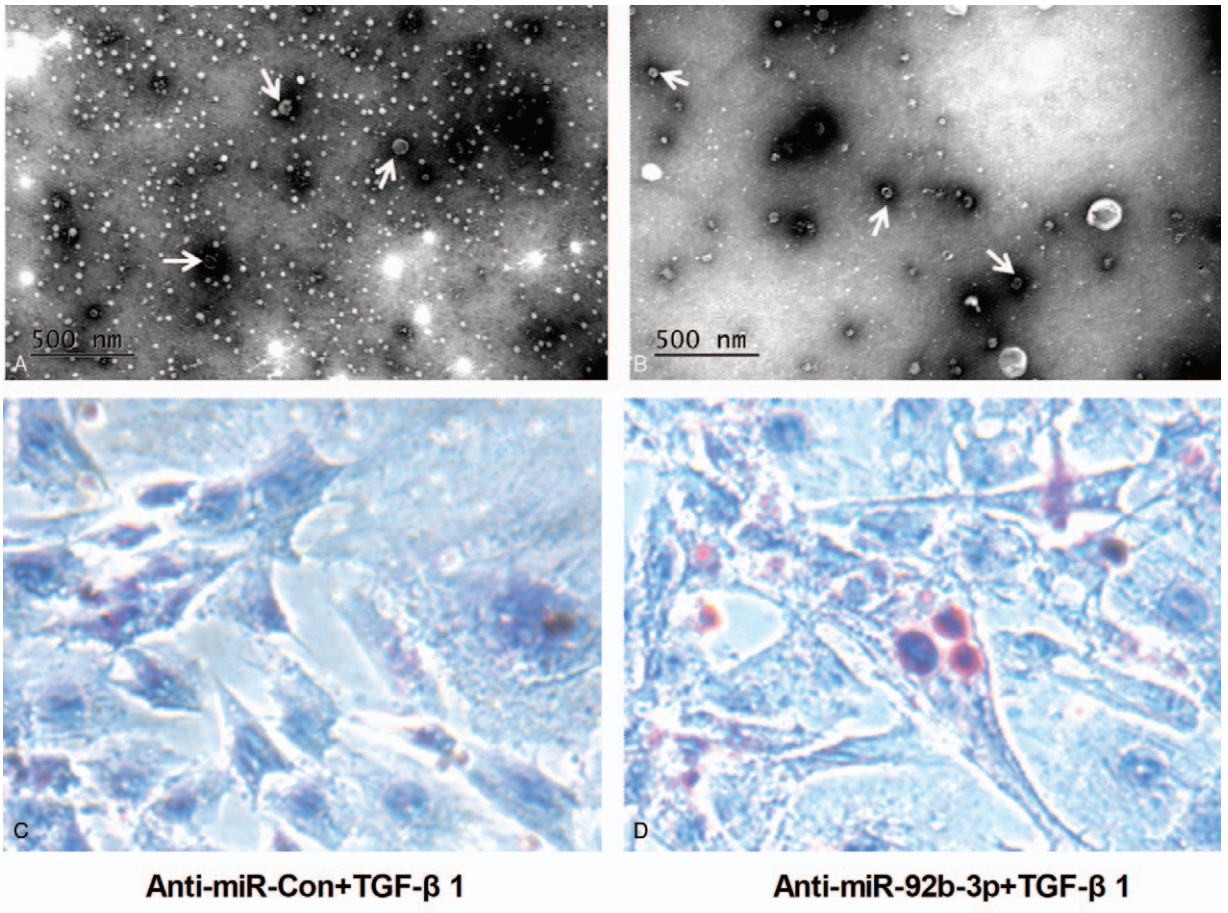

Figure 4. Electron microscope image of urinary vesicles and down-regulation of miR-92b-3p allows HSCs to restore their ability to accumulate cytoplasmic lipid droplets. (A) CHB patients; (B) Ctrl. Scale bar: 500 nm. CHB = chronic hepatitis B, Ctrl = healthy control. Human HSC (LX2) cells were transfected with miRNA inhibitor negative control (C) and miR-92b-3p inhibitor (D) for 24 h and then treated with TGF-β1 (10 ng/mL) for 48 h. The cells were washed with phosphate-buffered saline and fixed with 4% paraformaldehyde. Oil Red O was added, washed away, and lipid droplets were photographed. Photographs were taken at 200× magnification.

directly filtered from systemic circulation. MiRNAs released from the liver of patients with CHB are likely to be affected by a variety of different mechanisms. First, miRNA levels of the liver cells could be altered, leading to changes in the number of miRNAs released via normal processes. Second, normal miRNA release from cells via microvesicles, exosomes, or protein-bound complexes could be altered. Third, cell injury and death (e.g., necrosis or apoptosis) could lead to gross leakage of miRNA. All of these processes could affect the number of miRNAs released from the liver into the plasma and, subsequently, into other cell-free body fluids, such as urine. To determine the distribution of miR-92b-3p, -770-5p, -5196-5p, and -7855-5p in urine, we also measured the levels of total RNA in the urinary exosomal enrichment fraction in 6 CHB patients and 6 Ctrl subjects. miR-92b-3p, -770-5p, -5196-5p, and -7855-5p were observed at high abundance in urinary exosomes. These results indicate that urine miRNAs, such as miR-92b-3p, -770-5p, -5196-5p, and -7855-5p, are likely to be mainly contained in exosomes. Therefore, we speculate that these miRNAs may be actively secreted and play important roles in process of chronic HBV infection.

Table 5

TP, TC, LDL-C, and APOA-1 in patients with CHB and Ctrl.

Parameter	CHB (n = 100)	Control (n = 35)	*P*-value
TP (g/L)	76.82 ± 4.80	71.71 ± 6.33	<.0001
TC (mmol/L)	4.50 ± 1.21	4.60 ± 0.33	.0225
LDL-C (mmol/L)	2.59 ± 0.84	2.55 ± 0.64	.6532
APOA-1 (g/L)	1.24 ± 0.31	1.33 ± 0.16	.0081

Data are shown as the mean ± SD.
APOA-1 = apolipoprotein A1, CHB = chronic hepatitis B, Ctrl = Healthy volunteers, LDL-C = low density lipoprotein cholesterol, TC = total cholesterol, TP = total protein.

Table 4

Coefficient of Pearson correlation between miRNA variables and clinical parameter variables (all CHB samples).

Variables	miR-92b-3p	miR-770-5p
miR-92b-3p	1.000	–.027
miR-770-5p	–0.027	1.000
TP	0.088	–0.339**
TC	–0.263**	–0.145
LDL-C	–0.241*	–0.106
APOA-1	–0.221*	–0.186

** $P < .01$.
* $P < .05$ miRNA vs miRNA, miRNA vs clinical parameter (2-tailed test). LDL-C = low-density lipoprotein cholesterol, TC = total cholesterol, TP = total protein.

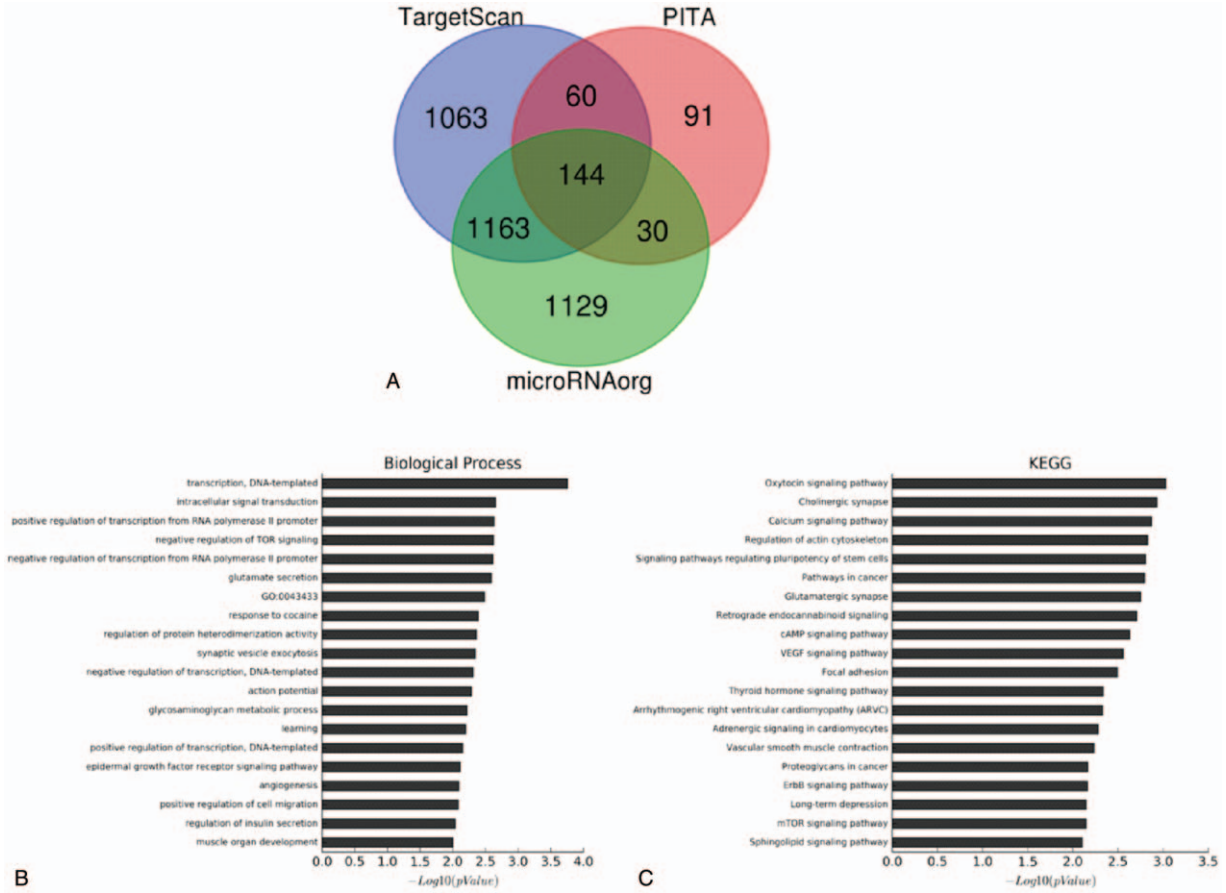

Figure 5. GO biological process enrichment and KEGG pathway annotation of differentially expressed miR-92b-3p potential targets. (A) Venn diagrams of the number of miR-92b-3p potential targets in each database. (B) GO biological process enrichment of miR-92b-3p potential targets. (C) KEGG pathway annotation of miR-92b-3p potential targets. GO and Pathway enrichment score provided by David software as -Log10 *P*-values ($P < .05$).

We analyzed the potential correlations between miR-92b-3p, -770-5p, -5196-5p, -7855-5p and each of the clinical pathological parameters. However, no significant correlation was found between HBV infection and these miRNAs (data not shown). This study shows that the expression level of urinary miR-770-5p is significantly negatively correlated with serum total protein (TP) in patients with CHB (Table 4). Serum levels of TP were higher in the CHB group than in the Ctrl group (Table 5). Notably, Zhang et al reported that serum levels of TP were lower in the CHB group than the healthy control group,[46] but were higher in the HBV-associated cirrhosis group than in the HBV-associated HCC group.[47]

The liver plays a vital role in lipid metabolism; hence, its consequent degeneration following chronic hepatitis B infection could potentially provoke dyslipidemia.[48–51] Our study revealed significantly lower TC and ApoA-1 in CHB patients than in matched Ctrl (Table 5). Some studies have also reported significantly lower TC in CHB patients than in Ctrl.[27,46,47,52] Expression of ApoA-1 mRNA and protein levels, as well as serum ApoA-1 levels, were lower in CHB patients than in correspondin Ctrl.[47,53] Recent evidence suggests that miRNAs are critical regulators of lipid synthesis, fatty acid oxidation, and lipoprotein formation and secretion. For example, miR-122-deletion in the whole body or specifically in the liver resulted in a marked decrease in total serum cholesterol and triglyceride (TG) levels. Anti-miR-122 therapy resulted in a significant reduction (25%–30%) of circulating cholesterol levels.[54–56] miR-27a and -27b have been shown to target RXRα and regulate fat metabolism.[57]

A growing body of evidence suggests that miR-92b-3p is involved in various human diseases.[58–61] miR-92b-3p promotes neurite growth and functional recovery via the PTEN/AKT pathway in acute spinal cord injury.[58] miR-92b-3p has been reported to act as an oncogene that promotes the colorectal carcinoma, pancreatic cancer, and gastric cancer by targeting FBXW7,[59] Gabra3[60] and HOXD10.[61] Uotani et al reported that circulating mir-92b-3p as a novel biomarker for monitoring of synovial sarcoma.[62] However, the relationship between mir-92b-3p and CHB remains unclear. We found, for the first time, that the expression level of urinary miR-92b-3p is significantly negatively correlated with serum TC, LDL-C, and APOA-1 (Table 4), implicating regulatory functions in lipid homeostasis.

HSCs play an important role in liver physiology and under healthy conditions they have a quiescent and lipid-storing phenotype. Interestingly, the inhibition of miR-92b-3p in human hepatic stellate LX2 cells displayed an increased number of Oil red O-stained lipid droplets (Figure 4C and D), suggesting that miR-92b-3p may be involved in the regulation of fat metabolism. To further investigate the function of miR-92b-3p, we predicted its potential targets using bioinformatics tools. Gene Ontology and pathway enrichment analysis showed that the target genes of miR-92b-3p played a part in lipid metabolism, such as negative

regulation of lipid storage (ITGB3; ITGAV), liver development (MKL2; NOTCH1; AACS; MAN2A1; JARID2), Thyroid hormone signaling pathway (ITGB3; NOTCH1; NCOA1; ITGAV), Notch signaling pathway (NOTCH1; DTX2), Hedgehog signaling pathway (BTRC; RAB23) and FoxO signaling pathway (NLK; FOXG1; RAG1) (Figure 5B and C). This result clearly indicates that these miRNAs might play important roles in maintaining the lipid homeostatic mechanisms of chronic HBV infection and shows that they may be actively secreted. However, their mechanism of action needs to be further elucidated in order to fully understand this phenotype.

In summary, our studies have shown aberrant expression of miRNAs in urine of CHB patients. In addition, the expression levels of urinary miR-92b-3p are negatively correlated with TC, LDL-C and APOA-1, implicating regulatory functions in lipid homeostasis. Our findings may provide novel insights into the pathogenesis of CHB and may assist in the diagnosis of patients with CHB. Nevertheless, this approach should be further validated in larger cohorts of patients, especially those with non-CHB-related disease, to assess their efficacy and potential applicability in a screening setting.

Acknowledgments

We are grateful to Dr Xu L (Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, China) for the generous gift of human LX2 cells.

Author contributions

Conceptualization: Hui Zhang.
Data curation: Jia-Wei Shang, Xiu-Li Yan, Hui Zhang, Shi-Bing Su.
Formal analysis: Shi-Bing Su.
Funding acquisition: Hui Zhang.
Methodology: Jia-Wei Shang, Xiu-Li Yan.
Resources: Hui Zhang.
Supervision: Jia-Wei Shang, Xiu-Li Yan, Hui Zhang, Shi-Bing Su.
Validation: Xiu-Li Yan.
Writing – original draft: Xiu-Li Yan.
Writing – review & editing: Hui Zhang.
Hui Zhang: 0000-0002-5867-1478.

References

[1] Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet 2015;386:1546–55.
[2] de Martel C, Maucort-Boulch D, Plummer M, et al. World-wide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. Hepatology 2015;62:1190–200.
[3] Weisberg IS, Brown RSJr, Sigal SH. Hepatitis B and end-stage liver disease. Clin Liver Dis 2007;11:893–916.
[4] Custer B, Sullivan SD, Hazlet TK, et al. Global epidemiology of hepatitis B virus. J Clin Gastroenterol 2004;38(10 Suppl 3):S158–68.
[5] Tiollais P, Pourcel C, Dejean A. The hepatitis B virus. Nature 1985;317:489–95.
[6] Zhang H, Li QY, Guo ZZ, et al. Serum levels of microRNAs can specifically predict liver injury of chronic hepatitis B. World J Gastroenterol 2012;18:5188–96.
[7] Zhou J, Yu L, Gao X, et al. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. J Clin Oncol 2011;29:4781–8.
[8] Konstantinidou A, Mougios V, Sidossis LS. Acute exercise alters the levels of human saliva miRNAs involved in lipid metabolism. Int J Sports Med 2016;37:584–8.
[9] Wolenski FS, Shah P, Sano T, et al. Identification of microRNA biomarker candidates in urine and plasma from rats with kidney or liver damage. J Appl Toxicol 2017;37:278–86.
[10] Silakit R, Loilome W, Yongvanit P, et al. Urinary microRNA-192 and microRNA-21 as potential indicators for liver fluke-associated cholangiocarcinoma risk group. Parasitol Int 2017;66:479–85.
[11] Brunetto MR, Cavallone D, Oliveri F, et al. A serum microRNA signature is associated with the immune control of chronic hepatitis B virus infection. PLoS One 2014;9:e110782.
[12] Yu F, Yang J, Ouyang J, et al. Serum microRNA-210 levels in different groups of chronic hepatitis B patients. Clin Chim Acta 2015;450:203–9.
[13] Yu SL, Deng H, Li XH, et al. Expression of microRNA-155 is downregulated in peripheral blood mononuclear cells of chronic hepatitis B patients. Hepat Mon 2016;16:e34483.
[14] Abdalla MA, Haj-Ahmad Y. Promising candidate urinary microRNA biomarkers for the early detection of hepatocellular carcinoma among high-risk hepatitis C virus Egyptian patients. J Cancer 2012;3:19–31.
[15] Chinese Society of Hepatology, Chinese Medical Association; Chinese Society of Infectious Diseases, Chinese Medical AssociationGuideline on prevention and treatment of chronic hepatitis B in China. Chin Med J (Engl) 2007;120:2159–73.
[16] Kao HW, Pan CY, Lai CH, et al. Urine miR-21-5p as a potential non-invasive biomarker for gastric cancer. Oncotarget 2017;8:56389–97.
[17] Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A 2004;101:13368–73.
[18] Xu L, Hui AY, Albanis E, et al. Human hepatic stellate cell lines, LX-1 and LX-2: new tools for analysis of hepatic fibrosis. Gut 2005;54:142–51.
[19] Zheng J, Wang W, Yu F, et al. MicroRNA-30a suppresses the activation of hepatic stellate cells by inhibiting epithelial-to-mesenchymal transition. Cell Physiol Biochem 2018;46:82–92.
[20] Zhang H, Yan XL, Guo XX, et al. MiR-27a as a predictor for the activation of hepatic stellate cells and hepatitis B virus-induced liver cirrhosis. Oncotarget 2017;9:1075–90.
[21] Yang P, Li QJ, Feng Y, et al. TGF-β-miR-34a-CCL22 signaling-induced Treg cell recruitment promotesvenous metastases of HBV-positive hepatocellular carcinoma. Cancer Cell 2012;22:291–303.
[22] Singaravelu R, Quan C, Powdrill MH, et al. MicroRNA-7 mediates cross-talk between metabolic signaling pathways in the liver. Sci Rep 2018;8:361.
[23] Feng Z, Li Z, Zhu D, et al. Mir-24 regulates hepatocyte apoptosis via BIM during acute liver failure. Am J Transl Res 2017;9:4925–35.
[24] Wang K, Zhang S, Weber J, et al. Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res 2010;38:7248–59.
[25] Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A 2011;108:5003–8.
[26] Vickers KC, Palmisano BT, Shoucri BM, et al. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011;13:423–33.
[27] Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18:997–1006.
[28] Ramachandran K, Saikumar J, Bijol V, et al. Human miRNome profiling identifies microRNAs differentially present in the urine after kidney injury. Clin Chem 2013;59:1742–52.
[29] Lagos-Quintana M, Rauhut R, Yalcin A, et al. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002;12:735–9.
[30] Wang K, Zhang S, Marzolf B, et al. Circulating microRNAs, potential biomarkers for drug-induced liver injury. Proc Natl Acad Sci U S A 2009;106:4402–7.
[31] Zhang Y, Jia Y, Zheng R, et al. Plasma microRNA-122 as a biomarker for viral-, alcohol-, and chemical-related hepatic diseases. Clin Chem 2010;56:1830–8.
[32] Bihrer V, Friedrich-Rust M, Kronenberger B, et al. Serum miR-122 as a biomarker of necroinflammation in patients with chronic hepatitis C virus infection. Am J Gastroenterol 2011;106:1663–9.
[33] Vickers KC, Palmisano BT, Shoucri BM, et al. MicroRNAs are transported in plasma and delivered to recipient cells by highdensity lipoproteins. Nat Cell Biol 2011;13:423–33.
[34] Wu Y, Gao C, Cai S, et al. Circulating miR-122 is a predictor for virological response in CHB patients with high viral load treated with nucleos(t)ide analogs. Front Genet 2019;10:243.

[35] Fan CG, Wang CM, Tian C, et al. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. Oncol Rep 2011;26:1281–6.
[36] Wang S, Qiu L, Yan X, et al. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1) -modulated P53 activity. Hepatology 2012;55:730–41.
[37] Li F, Bian H, Wang W, et al. HBV infection suppresses the expression of inflammatory macrophage miR-210. Mol Med Rep 2019;19:1833–9.
[38] Li J, Zhang X, Chen L, et al. Circulating miR-210 and miR-22 combined with ALT predict the virological response to interferon-alpha therapy of CHB patients. Sci Rep 2017;7:15658.
[39] Zhang GL, Li YX, Zheng SQ, et al. Suppression of hepatitis B virus replication by microRNA-199a-3p and microRNA-210. Antiviral Res 2010;88:169–75.
[40] Li F, Zhou P, Deng W, et al. Serum microRNA-125b correlates with hepatitis B viral replication and liver necroinflammation. Clin Microbiol Infect Dis 2016;22:384.e1–0.
[41] Zhou P, Dong M, Wang J, et al. Baseline serum miR-125b levels predict virologic response to nucleos(t)ide analogue treatment in patients with HBeAg-positive chronic hepatitis B. Exp Ther Med 2018;16:3805–12.
[42] Zhang Z, Chen J, He Y, et al. miR-125b inhibits hepatitis B virus expression in vitro through targeting of the SCNN1A gene. Arch Virol 2014;159:3335–43.
[43] Ninomiya M, Kondo Y, Kimura O, et al. The expression of miR-125b-5p is increased in the serum of patients with chronic hepatitis B infection and inhibits the detection of hepatitis B virus surface antigen. J Viral Hepat 2016;23:330–9.
[44] Fu L, Fu X, Mo J, et al. miR-146a-5p enhances hepatitis B virus replication through autophagy to promote aggravation of chronic hepatitis B. IUBMB Life 2019;71:1336–46.
[45] Wang S, Zhang X, Ju Y, et al. MicroRNA-146a feedback suppresses T cell immune function by targeting Stat1 in patients with chronic hepatitis B. J Immunol 2013;191:293–301.
[46] Zhang J, Feng G, Zhao Y, et al. Association between lymphocyte-to-monocyte ratio (LMR) and the mortality of HBV-related liver cirrhosis: a retrospective cohort study. BMJ Open 2015;5:e008033.
[47] Wu T, Zheng X, Yang M, et al. Serum lipid alterations identified in chronic hepatitis B, hepatitis B virus-associated cirrhosis and carcinoma patients. Sci Rep 2017;7:42710.
[48] Liu PT, Hwang AC, Chen JD. Combined effects of hepatitis B virus infection and elevated alanine aminotransferase levels on dyslipidemia. Metabolism 2013;62:220–5.
[49] Janicko M, Senajová G, Drazilová S, et al. Association between metabolic syndrome and hepatitis B virus infection in the Roma population in eastern Slovakia: a population-based study. Cent Eur J Public Health 2014;22:S37–42.
[50] Jarčuška P, Janičko M, Kružliak P, et al. Hepatitis B virus infection in patients with metabolic syndrome: a complicated relationship. Results of a population based study. Eur J Intern Med 2014;25:286–91.
[51] Hsu CS, Liu CH, Wang CC, et al. Impact of hepatitis B virus infection on metabolic profiles and modifying factors. J Viral Hepat 2012;19: e48–57.
[52] Wang Y, Hao J, Liu X, et al. The mechanism of apoliprotein A1 down-regulated by hepatitis B virus. Lipids Health Dis 2016;15:64.
[53] Jiang W, Zheng L, Yang Q, et al. Investigation into the effect of hepatitis B virus on apoliprotein A1 expression and its mechanism. Lipids Health Dis 2014;13:130.
[54] Esau C, Davis S, Murray SF, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006;3: 87–98.
[55] Tsai WC, Hsu SD, Hsu CS, et al. microRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest 2012;122: 2884–97.
[56] Hsu SH, Wang B, Kota J, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest 2012;122:2871–83.
[57] Ji J, Zhang J, Huang G, et al. Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation. FEBS Lett 2009;583:759–66.
[58] Chen Z, Li Z, Jiang C, et al. MiR-92b-3p promotes neurite growth and functional recovery via the PTEN/AKT pathway in acute spinal cord injury. J Cell Physiol 2019;234:3043–23052.
[59] Gong L, Ren M, Lv Z, et al. miR-92b-3p promotes colorectal carcinoma cell proliferation, invasion, and migration by inhibiting FBXW7 in vitro and in vivo. DNA Cell Biol 2018;37:501–11.
[60] Long M, Zhan M, Xu S, et al. miR-92b-3p acts as a tumor suppressor by targeting Gabra3 in pancreatic cancer. Mol Cancer 2017;16:167.
[61] Li C, Huo B, Wang Y, et al. Downregulation of microRNA-92b-3p suppresses proliferation, migration, and invasion of gastric cancer SGC-7901 cells by targeting Homeobox D10. J Cell Biochem 2019;120: 17405–12.
[62] Uotani K, Fujiwara T, Yoshida A, et al. Circulating microRNA-92b-3p as a novel biomarker for monitoring of synovial sarcoma. Sci Rep 2017;7:14634.