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Peripheral blood mononuclear cells microRNA predicts treatment outcome of hepatitis C virus genotype 1 infection

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ABSTRACT

Backgrounds: Chronic hepatitis C virus (HCV) infection has been associated with induction of microRNAs (miRNAs) in peripheral blood mononuclear cells (PBMC). We aimed to evaluate the role of PBMC–miRNAs in the treatment outcome to antiviral therapy for HCV genotype 1 (HCV-1) patients.
Methods: Treatment-naive chronic HCV-1 patients, including 13 in screening phase and 48 in validation phase, were treated with 48 weeks of peginterferon/ribavirin. The primary end-point was the achievement of a sustained virological response (SVR, HCV RNA undetectable during 24 weeks post-treatment follow-up). Expression profiling of PBMC–miRNAs was performed by quantitative PCR-based array in typical responders and null-responders. Then candidate PBMC–miRNAs were validated by quantitative PCR in an independent validation set.
Results: PBMC–miR-125b was significantly predictive of an SVR, with expression levels of 5.28-fold lower in sustained responders versus null-responders ($p = 0.0163$). In multivariate analysis, PBMC–miR-125b was significantly associated with the achievement of SVR (per 2-fold decrease, odds ratio/95% confidence interval (OR/CI): 2.07/1.14-6.31) independent of sex, age and interleukin-28B genotype. In patients who did not achieve a rapid virological response (RVR, undetectable HCV RNA at treatment week 4), PBMC–miR-125b was the only predictive factor of an SVR (per 2-fold decrease, OR/CI: 2.07/1.14-6.31). However, the circulating and hepatic miR-125b did not show significant difference between responders and non-responders.
Conclusions: PBMC–miR-125b expression levels were inversely related to the achievement of an SVR in HCV-1 patients, independent of interleukin-28B genotype, and was the single predictor of SVR in non-RVR patients.

1. Introduction

Hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease, cirrhosis, and hepatocellular carcinoma in Taiwan (Yang et al., 2011), as well as worldwide (Ghany et al., 2009). The standard treatment for chronic hepatitis C (CHC) is peginterferon-α/ribavirin at 48 weeks for HCV genotype 1 or 4 (HCV-1/4) and 24 weeks for HCV-2/3 (EASL, 2011; Ghany et al., 2009; Omata et al., 2012). The goal of treatment for CHC is to achieve a sustained virological response (SVR), which is highly durable during long-term follow-up (Yu et al., 2013). With the recommended regimen of peginterferon/ribavirin, the SVR rate is approximately 50––79% and 75–94% for HCV-1/4 and HCV-2/3, respectively, in both Western and Eastern countries (Ghany et al., 2009; Yu and Chuang, 2009). Recently, two newly approved

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direct-acting antiviral agents (DAA), boceprevir and telaprevir, have much improved the treatment efficacy for HCV-1 when in combination with peginterferon/ribavirin (Ghany et al., 2011). However, peginterferon/ribavirin remains the backbone of antiviral regimen in the era of DAA currently. Several host and viral factors have been reported as significant predictors of response to anti-HCV therapy (Tanaka et al., 2009; Yu and Chuang, 2009). Of the host determinants, age, gender, race, insulin resistance, host immune response and genetic predisposition may significantly affect treatment outcome.

microRNAs (miRNAs) are evolutionarily conserved, small (~22 ribonucleotides) non-coding RNAs. MiRNAs act as translational repressors, which have important roles in the control of many biological processes, such as cellular development, differentiation, proliferation, apoptosis, and metabolism (Ambros, 2004). They are implicated in the pathogenesis, diagnosis, and therapeutic aspects of viral infections (Kloosterman and Plasterk, 2006). Several studies have shown that some specific miRNA sets are associated with the clinicopathological features of liver disease, such as cirrhosis (Jiang et al., 2008), hepatitis B and C virus infection (Ura et al., 2009), hepatocellular carcinoma metastasis, recurrence, and prognosis (Budhu et al., 2008; Xiong et al., 2010; Yao et al., 2010). HCV RNA replication is dependent on a liver-specific microRNA, miR-122 (Jopling et al., 2005). Therefore, a miR-122 antagonist, miravirsen, was designed to cure hepatitis C (Janssen et al., 2013). Furthermore, markedly decreased levels of hepatic miR-122 before treatment was associated with poor virological response to interferon therapy (Sarasin-Filipowicz et al., 2009), indicating hepatic miRNA could serve as a biomarker in predicting treatment response for CHC patients. However, liver biopsy is an invasive procedure and may have sampling bias. Non-invasive markers are needed for clinical practice.

Peripheral blood mononuclear cells (PBMC) have been reported to play an important role in HCV progression (Koziel, 1997). HCV core induces *STAT3* as a result of the alteration of inflammatory response by antigen-presenting cells via an *IL-6* autocrine pathway (Tacke et al., 2011). Moreover, HCV-specific T cells activate and express Fas ligand, which transduces the apoptotic signal to kill Fas-bearing hepatocytes (Nasir et al., 2000). There are several disease-associated miRNAs in PBMC reported in previous studies (Grek et al., 2011; Otaegui et al., 2009). Recently, induction of PBMC–miR-155 was found in patients with chronic HCV infection (Bala et al., 2012). Thus, expression levels of miRNAs in PBMC may correlate to treatment outcome to anti-HCV therapy and potentially serve as non-invasive markers.

We aimed to discover the role of PBMC-contained miRNAs on the achievement of an SVR in HCV-1 patients with peginterferon-α/ribavirin treatment. SVR-associated PBMC–miRNAs were screened by comparing expression profiles of typical responders and null responders in screening phase. Then we validated candidate SVR-associated PBMC miRNAs with an independent cohort in validation phase.

2. Methods

2.1. Patient selection

Eligible subjects were treatment-naïve HCV-1 patients who were seropositive for more than 6 months for HCV antibodies (third-generation, enzyme immunoassay; Abbott Laboratories, North Chicago, IL) and HCV RNA by polymerase chain reaction (PCR). Patients with the following concurrent diseases or conditions were excluded: hepatitis B virus infection, human immunodeficiency virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, Wilson disease, α_1-antitrypsin deficiency, decompensated cirrhosis, overt hepatic failure, current or past history of alcohol abuse ($\geqslant$20 g daily), psychiatric condition, previous liver transplantation, or with evidence of hepatocellular carcinoma or other malignancy. All the participants received 48 weeks of peginterferon-α plus weight-based ribavirin (Ghany et al., 2009; Omata et al., 2012) and maintained at least 80% of assigned treatment duration and regimens (McHutchison et al., 2002). Treatment was terminated early if patients could not achieve an early virological response (EVR) at treatment week 12. Serum HCV RNA level was determined by a real-time PCR assay (RealTime HCV; Abbott Molecular, Des Plaines IL, USA; quantification limit: 12 IU/ml) (Vermehren et al., 2011). All blood samples were collected within 2 weeks prior to antiviral therapy. The study was approved by the ethics committee at the participating hospitals and was carried out according to the guidelines of the International Conference on Harmonization for Good Clinical Practice. All patients gave written informed consent before enrollment.

2.2. Assessment of efficacy

The primary efficacy end point was SVR, defined as seronegative for HCV RNA by PCR analysis by the end of treatment and throughout the 24-week follow-up period. The others were classified as non-responders. Rapid virological response (RVR) was defined as seronegative for HCV RNA by PCR at treatment week 4. EVR was defined as serum HCV RNA decline $\geqslant$ 2 logs from baseline after 12 weeks of treatment.

2.3. Two-phase design

This study was designed prospectively. In screening phase, we aimed to explore the potential SVR-associated PBMC miRNAs. To maximize the differentiation between treatment success and treatment failure, we enrolled three typical null-responders (non-EVR patients, those considered to be "difficult-to-treat") and 10 super-responders with both RVR and SVR, the "easy-to-treat" patients. In the following validation phase, we recruited 48 consecutive patients who met the inclusion criteria as an independent cohort to validate candidate PBMC miRNAs identified in the screening phase.

2.4. MicroRNA extraction from PBMC, serum and liver tissue

PBMCs were isolated from the buffy coat of the samples by density-gradient centrifugation. Liver tissue obtained by needle biopsy were stored in RNAlater RNA Stabilization Reagent (RRSR, Qiagen, Hilden, Germany). Total RNA was isolated from PBMC, serum and liver tissue by TRIzol (Invitrogen, Carlsbad, California, USA), according to the manufacturer's recommendation. In brief, the TRIzol was supplemented with 0.2 mL of chloroform per 1 mL of reagent, mixed and centrifuged (15 min, 12,000 g, 4 °C). The supernatant was retained. To pellet the RNA, 0.5 mL of isopropanol (per 1 mL of reagent) was added and the mixture was incubated for 10 min at room temperature, followed by centrifugation (15 min, 12,000 g, 4 °C). The pellet was washed once in 0.5 mL of 75% ethanol. The final RNA products were quantified by absorbance measurements at 260 nm (A260) and 280 nm (A280). The A260/A280 values were higher than 1.6 for all of the samples.

2.5. MicroRNA profiling in the screening set

MiRNA arrays were performed to obtain miRNA profiles using the TaqMan Array Human MicroRNA Panel v3.0 (Applied Biosystems, Foster City, USA). Each array includes Cards A and B, together containing 768 TaqMan MicroRNA Assays enabling a simultaneous quantification of 754 human miRNAs and 3 endogenous controls.

The experiments were performed on an Applied Biosystems 7900 Real-time PCR System (Applied Biosystems). The data were collected and processed using SDS 2.3 (Applied Biosystems) and analyzed using RealTime StatMiner software (Integromics, Armilla, Spain). The Ct (cycle threshold) was defined as the number of PCR cycles required for the fluorescent signal to cross the threshold. Ct was inversely proportional to the amount of target nucleic acid in the sample. Relative expression numbers were calculated by the ΔCt method in which the Ct of a specific microRNA is subtracted from the Ct of the widely used housekeeping microRNA U6, U6 was duplicated in both array, cards A and B. The microRNAs that showed a Ct value greater than 34 were considered undetectable. To compare miRNA expression levels between the non-SVR and the SVR group, $\log_2$ fold change of each miRNA was calculated by the ΔΔCt method. Since the typical null responders were limited in our population, we performed power calculation to define the criteria of differential expressed miRNA. The statistical power of 13 typical subjects would be sufficient to detect an 8-fold difference in miRNA expression levels between two groups with significance of 0.05% and 80% power (Wei et al., 2004). In order to microRNAs with greater than 4-fold changes were all selected into further validation phase.

2.6. MicroRNA quantification by real-time quantitative RT-PCR in the validation set

A measure of 5 μl of RNA was reverse-transcribed using the TaqMan miRNA reverse-transcription kit and the TaqMan miRNA assay specific for microRNAs according to the instructions of the manufacturer (ABI). Real-time PCR was performed on an Applied Biosystems 7900 Real-time PCR System. Quantitative real-time PCR was performed in duplicates. The calculation of Ct and usage of internal control were the same as describing in Section 2.5.

To evaluate the role of the candidate microRNA in serum and liver, circulating and hepatic microRNA were determined by the same assay described above. The small nuclear U6 RNA and U44 were used as internal controls for normalization and quantification of the miRNA expression levels in liver and serum, respectively.

2.7. Interleukin-28B (IL28B) genotyping

IL28B rs8099917 genotype has been strongly associated with during-treatment and/or post-treatment virological responses based on genome wide association studies and replication studies in Asian cohorts (Huang et al., 2012a,b; Tanaka et al., 2009; Yu et al., 2011). Genotypes of the patients were determined by the ABI TaqMan® SNP genotyping assays (Applied Biosystems) by using the pre-designed commercial genotyping assays (ABI Assay ID: C_11710096_10).

2.8. Statistical analysis

Student's t test and χ^2 were used to analyze the patient characteristics. Multivariate logistic regression modeling was used to determine the factors associated with the outcome. The area under the curve (AUC) was calculated using receiver-operating characteristics (ROC) analysis. A suitable clinical cutoff of SVR-associated PBMC miRNA was also derived by ROC analysis. The aspartate aminotransferase (AST)-to-platelet ratio index (APRI) was calculated by the following equation: (AST level/upper limit of normal range)/platelet counts $(10^9/L) \times 100$ and used to represent the severity of liver fibrosis (Martinez et al., 2011; Wai et al., 2003; Yu et al., 2006). The independent *t*-test was used to compare the difference of gene expression between groups. All *p* values are two-sided and considered statistically significant when less than 0.05. All statistical calculations were performed using JMP software (version 9).

3. Results

3.1. Patient profiles

Table 1 demonstrated the demographical, virological, clinical features and SVR rates of the patients in the screening and validation sets. All features were similar between the sets, except that the patients in the screening set were younger than those in validation set.

3.2. Identification of SVR-associated PBMC miRNAs

3.2.1. Screening set

Among the 754 miRNA probes, the microarray detected 207 PBMC-contained miRNAs in 13 patients in the training set. Comparison of the miRNA expression levels in SVR patients to non-SVR patients showed that 3 miRNAs (miR-151-3p, miR-378 and miR-93*) were expressed 16.70, 9.85 and 7.16-fold higher, while miR-125b was 10.20-fold lower (Suppl Table 1).

3.2.2. Validation set

Subsequently, the expression levels of four candidate PBMC–miRNAs were determined by quantitative PCR in 48 patients from the validation set. miR-125b remained significantly associated with the achievement of an SVR. The expression levels of PBMC–miR-125b were 5.28-fold lower in SVR patients than in non-SVR patients ($p = 0.0163$) (Fig. 1).

Univariate analysis showed that patient gender, age, pretreatment ALT levels, baseline viral load, *IL28B* rs8099917 genotype and miR-125b expression levels in PBMC were significantly associated with the achievement of an SVR (Table 2). Further analysis using a multivariate logistic regression model demonstrated that favorable *IL28B* rs8099917 TT genotype was the most important

Table 1
Patient characteristics for screening and validation phase.

	Screening phase ($n = 13$)	Validation phase ($n = 48$)	p Value
Sex (M/F)	8/5	30/18	0.9495
Age (years, mean (SD))	41.1(9.0)	52.3(9.8)	0.0009
BMI (kg/m^2, mean (SD))	23.7(6.8)	24.4(3.5)	0.7358
High Viral loads (>400,000 IU/mL), (n, %)	7 (54%)	29 (60%)	0.6692
AST (IU/l, mean (SD))	73.7(70.3)	83.1(52.3)	0.3075
ALT (IU/l, mean (SD))	117.1(105.9)	113.6(72.6)	0.8017
APRI (mean (SD))	0.9(0.9)	1.2(0.8)	0.2981
SVR (n, %)	10(77%)	38(79%)	0.8609

SD, standard deviation; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; SVR, sustained virological response.

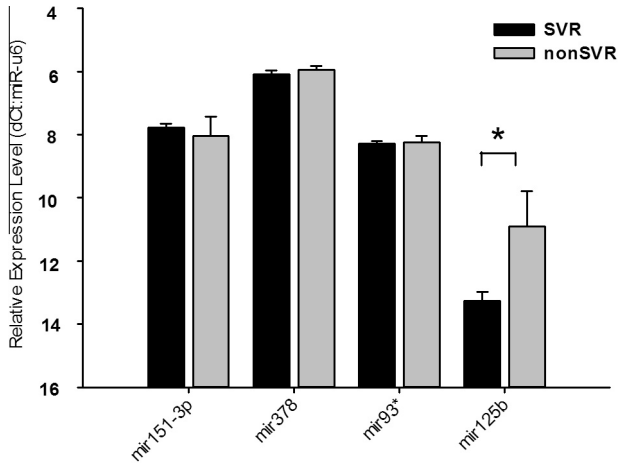

Fig. 1. Relative PBMC–miRNA expression levels in validation panel. (*$p < 0.05$).

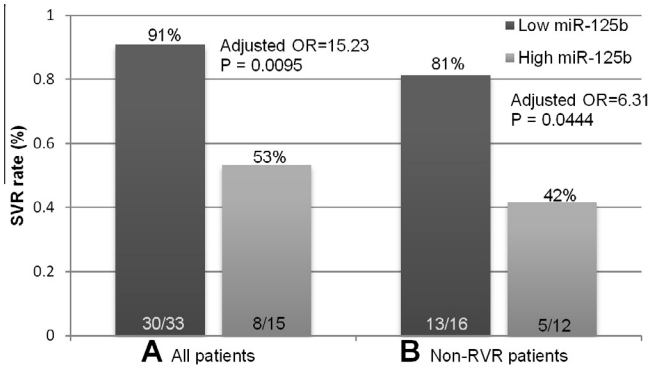

Fig. 2. The relationship of SVR rate and expression levels of PBMC–miR-125b among chronic hepatitis C patients. (A) All patients and (B) non-RVR patients. SVR, sustained virological response; high PBMC–miR-125b, expression levels of miR-125b in peripheral blood mononuclear cell < 12.3 cycles of dCt(miR125b-U6); non-RVR, did not reach rapid virological response; adjusted OR (CI), adjusted odds ratio (95% confidence intervals) by sex, age, alanine aminotransferase, and IL28B genotype.

factor significantly predictive of an SVR (odds ratio [OR]: 13.68, 95% confidence interval [CI]: 1.18-321), followed by male sex (OR: 11.65, CI: 1.16-321) and lower expressed levels of miR-125b in PBMC (per 2-fold decrease, OR: 2.74, CI: 1.26-10.3) (Table 2).

By using ROC analysis, 12.3 cycles of PBMC–miR-125b was the best cut-off value of dCt(miR125b-U6) to predict treatment response with an AUC of 0.78 ($p = 0.0004$). Ninety-one percent (30/33) of patients with low PBMC–miR-125b achieved an SVR, which was significantly higher than 53% (8/15) SVR achievement in patients with high PBMC–miR-125b (adjusted OR (CI), 15.23 (1.87–232.92), $P = 0.0095$, Fig. 2A). With the cutoff of 12.3 cycles of PBMC–miR-125b, the positive predictive value (PPV) in predicting an SVR for HCV-1 patients was 90.9%.

3.2.3. Non-RVR HCV-1 patients

Because RVR is the best single predictor of achieving an SVR (Fried et al., 2011) with a PPV of >98% in Asian treatment-naïve HCV-1 patients,(Huang et al., 2012b; Yu et al., 2008) we focused on the role of PBMC–miR125b in patients that did not achieve a RVR. Ten out of twenty-eight (35.7%) non-RVR HCV-1 patient failed to achieve an SVR. Univariate analysis showed that both

Table 2
Baseline factors associated with achievement of an SVR in validation phase.

	SVR ($n = 38$)	Non-SVR ($n = 10$)	Univariate P value	Multivariate	
				OR (95% CI)	P value
Sex (M/F)	27/11	3/7	0.0275	11.65 (1.16–321) male	0.0362
Age (years, mean (SD))	51.0(10.1)	57.1(7.1)	0.0406	0.86 (0.68–1.02) per year increased	0.0874
BMI (kg/m^2, mean (SD))	24.2(3.4)	25.3(3.8)	0.4211	–	–
High Viral loads (>400,000 IU/mL), n (%)	21(55.3)	8(80)	0.2762	–	–
APRI(mean (SD))	1.2(0.8)	1.1(1.0)	0.8381	–	–
AST (IU/l, mean (SD))	88.5(55.6)	62.7(32.6)	0.1461	–	–
ALT (IU/l, mean (SD))	123.9(76.9)	74.1(31.6)	0.0151	0.62 (0.01–183) per 10-fold increased	0.8650
IL28B rs8099917 genotype, (TT, n (%))	36(95)	6(60)	0.0128	13.68 (1.18–321)	0.0364
mir125b in PBMC (dCt, mean (SD))	13.2(1.7)	10.8(2.6)	0.0163	2.74 (1.26–10.3) per Ct increased	0.0055

SD, standard deviation; CI, confidence interval; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; SVR, sustained virological response; dCt, Delta Ct, the difference in Ct between the miRNA of interest and the endogenous control (U6snRNA); PBMC, peripheral blood mononuclear cells.

Table 3
Factors associated with achievement of an SVR in non-RVR patients in validation phase.

	SVR ($n = 18$)	Non-SVR ($n = 10$)	Univariate P value	Mltivariate	
				OR (95% CI)	P value
Sex (M/F)	11/7	3/7	0.2365	–	–
Age (years, mean (SD))	52.7(7.9)	57.1(7.1)	0.1485	–	–
BMI (kg/m^2, mean (SD))	23.3(2.6)	25.3(3.8)	0.1729	–	–
High Viral loads (>400,000 IU/mL), n (%)	14(77.8)	8(80.0)	1.0000	–	–
APRI (mean (SD))	1.0(0.6)	1.1(1.0)	0.8236	–	–
AST (IU/l, mean (SD))	73.1(47.6)	62.7(32.6)	0.6404	–	–
ALT (IU/l, mean (SD))	102.6(53.7)	74.1(31.6)	0.1478	–	–
IL28B rs8099917 genotype (TT, n (%))	17(94.0)	6(60.0)	0.0410	7.63 (0.67–189.10)	0.1021
Baseline mir125b in PBMC (dCt, mean (SD))	13.5(2.1)	10.8(2.6)	0.0133	2.07 (1.14–6.31) per Ct increased	0.0098

SVR, sustained virological response; OR, odds ratio; CI, confidence interval; BMI, body mass index; SD, standard deviation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase-to-platelet ratio index; dCt, Delta Ct, the difference in Ct between the miRNA of interest and the endogenous control (U6snRNA); PBMC, peripheral blood mononuclear cells.

Table 4
Characteristics of patients with high and low PBMC–miR-125b in validation phase.

	High miR125b (n = 15)	Low miR125b (n = 33)	Univariate P value	Multivariate	
				OR (95% CI)	P value
Sex (M/F)	9/6	21/12	0.8094	–	–
Age (years, mean (SD))	53.0(10.4)	51.9(9.7)	0.7406	–	–
BMI (kg/m^2, mean (SD))	25.4(2.6)	24.0(3.8)	0.1314	–	–
High Viral Loads (>400,000 IU/mL, n (%))	11(73.3)	18(54.6)	0.3406	–	–
APRI (mean (SD))	0.8(0.6)	1.3(0.8)	0.0182	Be removed in stepwise procedure	–
AST (IU/l, mean (SD))	52.3(24.0)	97.1(55.8)	0.0006	Be removed in stepwise procedure	–
ALT (IU/l, mean (SD))	71.9(33.2)	132.5(77.9)	0.0006	0.72 (0.53–0.90) per 10 IU increased	0.0009
IL28B (TT, n (%))	12(80.0)	30(91.0)	0.3598	–	–
Treatment outcomes					
RVR (n (%))	3(20.0)	17(51.5)	0.0344	–	–
SVR (n (%))	8(53.3)	30(90.9)	0.0040	–	–

High/low miR-125b: samples with dCt(miR125b-U6) lower/higher than 12.3 cycles. PBMC, peripheral mononuclear cell; SD, standard deviation; CI, confidence interval; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DM, diabetes mellitus; APRI, aspartate aminotransferase-to-platelet ratio index; RVR, rapid virological response. Multivariate analysis was conducted by stepwise logistic regression for using correlated covariates (APRI, AST and ALT).

PBMC–miR-125b expression levels and *IL28B* genotype were significantly associated with the achievement of an SVR in non-RVR HCV-1 patients (Table 3). Nevertheless, multivariate logistic regression modeling demonstrated that a lower expression level of PBMC–miR-125b was the only factor predictive of SVR, with an OR (CI) of 2.07 (1.14–6.31) by 2-fold decrease of PBMC–miR-125b level.

For non-RVR patients, 81% (13/16) of patients with low PBMC–miR-125b achieved an SVR, which was significantly higher than the 42% (5/12) of SVR rate in patients with high PBMC–miR-125b (adjusted OR (CI), 6.31 (1.05–53.98), P = 0.0444, Fig. 2B). With the cutoff of 12.3 cycles of PBMC–miR-125b, the PPV in predicting an SVR for non-RVR HCV-1 patients was 81.3%.

We profiled patient characteristics stratified by cutoff of 12.3 cycles of PBMC–miR-125b. High PBMC–miR-125b is significantly associated with lower levels of AST, APRI and ALT (Table 4). Multivariate analysis demonstrated that ALT level was the only variable that remained significantly inversely associated with PBMC–miR-125b (OR (CI): 0.72 (0.53–0.90), p = 0.0009, Table 4). The different ALT levels between high/low miR125b levels were shown in Fig. 3a–c. The difference is significant in all CHC patients with p value 0.0006, and the significance remains in SVR CHC patients alone with p value 0.0125.

Furthermore, we examined miR-125b expressed level in serum (13 CHC patients with SVR and 16 patients did not reach SVR) and liver (12 CHC patients with SVR and 6 patients did not reach SVR).

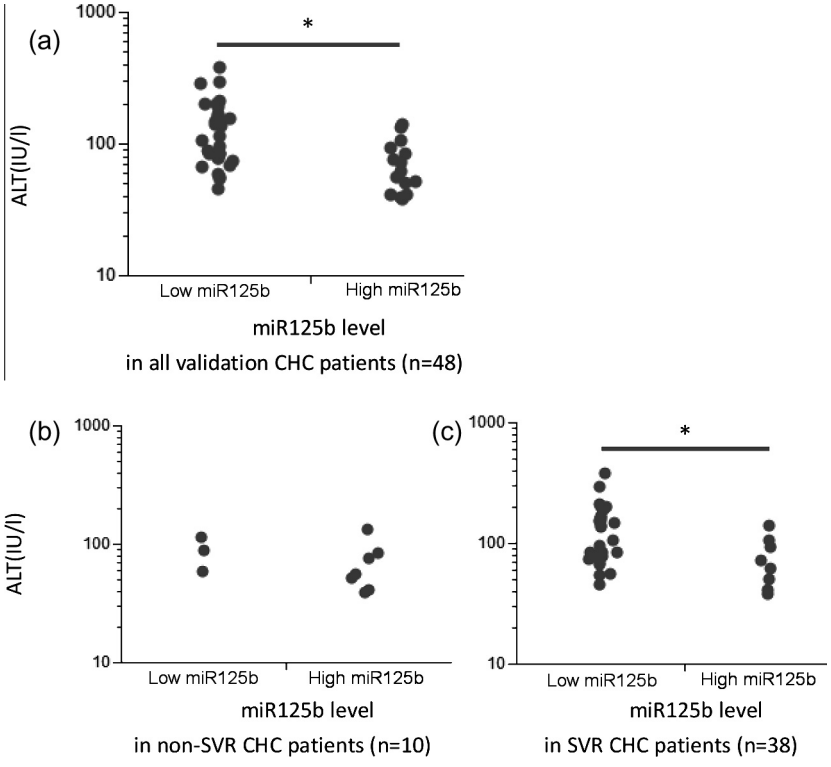

Fig. 3. The different ALT levels between high/low miR125b levels. (a) The ALT level is significantly lower in high miR125b patients in all CHC patients with p value 0.0006; (b) ALT level is not significantly different between high and low miR125b patients in non-SVR group; (c) ALT level is significantly lower in high miR125b patients in SVR group with p value 0.0125. ALT, alanine aminotransferase; High/low miR-125b: samples with dCt(miR125b-U6) lower/higher than 12.3 cycles; CHC: chronic hepatitis C; SVR, sustained virological response; $^*p < 0.05$.

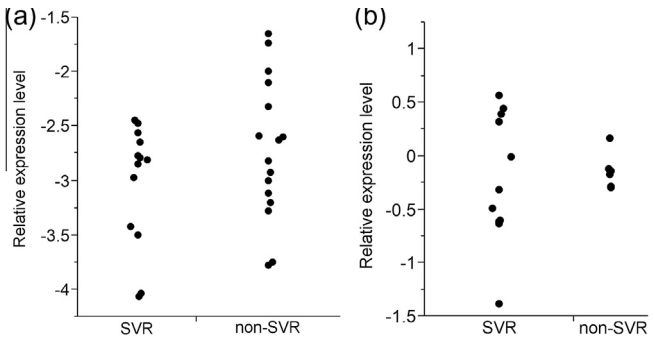

Fig. 4. Expression levels of circulating (a) and hepatic (b) miR-125b between HCV responders and HCV-non-responders. (a) No difference in serum miR125b expressed levels between patients with SVR and non-SVR (SVR $n = 13$, non-SVR $n = 16$, $p = 0.178$). (b) No significant difference of hepatic miR125b expressed levels between patients with SVR and non-SVR (SVR $n = 12$, non-SVR $n = 6$, $p = 0.584$). SVR, sustained virological response.

However, neither circulating nor hepatic miR-125b was different among patients with and without achieving an SVR (Fig. 4).

4. Discussion

We discovered that the expression levels of PBMC–miR-125b were associated with treatment outcome and may serve as a non-invasive biomarker for predicting an SVR for HCV-1 patients treated with peginterferon/ribavirin. A relatively low level of PBMC–miR-125b was associated with the achievement of an SVR, independent of the most important baseline factor, the host *IL28B* genotype (Ge et al., 2009; Huang et al., 2012a,b; Tanaka et al., 2009). For patients without achieving an RVR, miR-125b level in PBMC, but not *IL28B* genotype, was the only significant factor associated with treatment success. Our results demonstrated that PBMC–miR-125b, but not hepatic and circulating miR-125b, may play an important role in the pathogenesis and treatment response to peginterferon/ribavirin for chronic HCV-1 infection. These findings provide additional information for decision-making prior to treatment in the clinical setting.

The *IL28B* genotype has been the most powerful baseline predictor of SVR to peginterferon/ribavirin in non-RVR HCV-1 patients (Huang et al., 2012b). Interestingly, the current study revealed that PBMC–miR-125b, independent of *IL28B* genotype, was the only significant factor associated with treatment success in non-RVR HCV-1 patients. We did not observed correlation between IL28B genotype and PBMC–miR-125b levels. Further larger studies are necessary to clarify the interplay between *IL28B* genotype and PBMC–miR-125b in predicting an SVR for HCV-1 patients.

MicroRNAs may regulate pharmacogenomics-related genes and play an important role in treatment efficiency (Garofalo et al., 2008; Rukov and Shomron, 2011). Up-regulation of miR-125b has been associated with increased drug resistance in pediatric acute promyelocytic leukemia (Zhang et al., 2011). Additionally, miR-125b post-transcriptionally regulates human vitamin D receptor (*VDR*) and vitamin D3 hydroxylase (*CYP24*) and increases the anti-tumor effects of calcitriol (Komagata et al., 2009; Mohri et al., 2009). These evidences suggested that miR-125b may play a role in drug responsiveness.

Gene regulation in PBMC has been associated with the pathogenesis of CHC progression and the immune-mediated response of hepatocyte apoptosis (Koziel, 1997; Nasir et al., 2000; Tacke et al., 2011). Increased expression levels of PBMC–miR-155 has been associated with chronic HCV infection, but not in those responded to antiviral therapy (Bala et al., 2012; Zhang et al., 2012). In the current study, we reported the first PBMC–miRNA, miR-125b, in predicting the HCV response to peginterferon/ribavirin. IFNG, a target gene of miR-125b in CD4+ T cells (Rossi et al., 2011), has been associated with the achievement of an SVR to interferon for chronic HCV infection (Huang et al., 2007). Since both circulating and hepatic miR-125b were not associated with treatment response in HCV-1 patients, further study is warranted to explore the mechanism of PBMC–miR-125b in the treatment response for this clinical setting.

Interestingly, we found that serum ALT level was inversely associated with the expression level of PBMC–miR-125b. miR-125b was reported to negatively regulate *TNFα* expression in monocytes with altered proinflammatory reactions (Huang et al., 2012c), which has been positively correlated to the extent of ALT levels and necroinflammation (Ji et al., 2004). Moreover, miR-125b was reported to be down-regulated by lipopolysaccharide in mouse leukemia cell line, human macrophage and CD14+ neomonocyte (Huang et al., 2012c; Murphy et al., 2010; Tili et al., 2007). In the other hand, the number of CD14-positive Kupffer cells (specialized macrophages located in the liver) increased with an increase in necroinflammation grade (Tonan et al., 2012). Hence, lipopolysaccharide down-regulated miR-125b and increased *TNFα* in monocyte, which was associated with necroinflammation. In addition, micro RNA is also characterized of tissue-specific manner (Christodoulou et al., 2010). Our data implied that PBMC-contained miR125b might play a role in hepatic necroinflammatory activity. Moreover, only PBMC-contained miR-125b, but not hepatic nor circulating miR-125b expression, was significantly associated with SVR. These findings suggested the special role of PBMC-contained miR125b in the treatment response of HCV to IFN-based therapy, which mediating both direct antiviral and immunomodulatory effects on immune cells.

Currently approved DAAs in combination with peginterferon/ribavirin have been the new standard-of-care for HCV-1 patients (Ghany et al., 2011). Nevertheless, peginterferon/ribavirin dual therapy would remain the standard-of-care for a number of HCV-1 patients. First, the added adverse events and costs of telaprevir and boceprevir may preclude the unselected use of these agents (Barritt and Fried, 2012). Second, recent study demonstrated that HCV-1 with low viral load and RVR to peginterferon/ribavirin obviates a protease inhibitor (Pearlman and Ehleben, 2013). Third, telaprevir and boceprevir are not widely available in the majority of Asian countries currently. Furthermore, peginterferon/ribavirin remains the backbone of telaprevir or boceprevir-containing triple therapy. Therefore, our findings remain of clinical significance.

However, there are limitations in the current study. First, the limited sample size might not draw a conclusive result. Second, the current study enrolled only patients from an Asian population. Whether the findings can be translated to other ethnicities remains to be verified. Third, PBMC sampling is not included in the current routine clinical practice. Finally, approximately 85% of HCV genotype 2 or 3 patients could achieve an SVR to the current standard-of-care (Yu et al., 2007). The role of PBMC miR-125b on HCV-2/3 remains to be determined.

In conclusion, we discovered an SVR-associated PBMC microRNA, miR-125b, which expressed inversely with the achievement of an SVR to peginterferon/ribavirin treatment in HCV-1 patients. This association was independent of *IL28B* genotype, especially in non-RVR patients. Our results may provide value for decision-making prior to treatment in clinical practice and a clue for a potential target of novel therapies for treating HCV-1 patients.

5. Transparency declarations

Ming-Lung Yu and Wan-Long Chuang: consultants for Merck Sharp & Dohme and Abbott; grants support from Abbott and

Roche; on speaker's bureau from Bristol-Myers Squibb, Roche, Merck Sharp & Dohme, GlaxoSmithKline, Novartis, and Gilead Sciences. All other authors declare no competing interests.

Specific author contributions

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Statistical analysis: E. Hsi.

Analysis and interpretation of data: M.L. Yu, E. Hsi, S.H. Juo, W.L. Chuang.

Drafting of the manuscript: M.L. Yu, E. Hsi.

Obtained funding: M.L. Yu, C.Y. Dai, W.L. Chuang.

Conflict of interest

Dr. Yu and Dr. Chuang report receiving consulting fees from Merck and Abbott; grant support from Abbott and Roche; lecture or speaker fees from Bristol-Myers Squibb, Roche, Merck, GlaxoSmithKline, Novartis, and Gilead Sciences.

A portion of the results from the current study was presented at the 47th annual meeting of the European Association for the Study of the Liver, April 18–22, 2012, Barcelona, Spain.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2014.03.003.

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