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Significance of micro RNA-122 in chronic hepatitis C patients with serotype 1 on interferon therapy

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Abbreviations used in this paper:

WBC: White blood cells

RBC: Red blood cells

Plt: Platelets

PT: prothrombin time

AST: Aspartate aminotransferase

ALT: Alanine aminotransferase

γ-GTP: γ-glutamyltranspeptidase

Alb: Albumin

TC: Total cholesterol

TG: Triglyceride

LDL-C: low-density lipoprotein cholesterol

HDL-C: high-density lipoprotein cholesterol

FFA: free fatty acid

CHC: Chronic hepatitis C

Peg-IFN: pegylated interferon

Rib: Ribavirin

SNP: Single nucleotide polymorphism

miRNA：micro RNA

miR-122：micro RNA-122

qRT-PCR：quantitative reverse transcription polymerase chain reaction

UTR：untranslated region

ABSTRACT

Background & Aims: Peginterferon(Peg-IFN) and ribavirin combination therapy is a curative treatment for chronic hepatitis C virus (HCV) infection, and virological response to IFN therapy has been strongly associated with genetic variation in *IL28B* single nucleotide polymorphisms (SNPs). Recently, microRNA122 (miR-122), which is the most abundant miRNA in the liver, has been reported to be important for the replication of HCV RNA. Therefore, we investigated the correlation of miR-122 expression with virological response to IFN and other clinical data.

Methods: A total of 51 patients with HCV infection who were treated with IFN therapy at Nagasaki University Hospital from 2006 to 2011 were included in this study. We investigated the correlation of miR-122 expression in liver biopsy specimens with virological response to IFN therapy and other predictors of response, including IL28 SNPs.

Results: miR-122 expression did not correlate with IL28 SNPs. However, a significant difference was observed in miR-122 expression between patients who showed a sustained virological response (SVR) and those who did not ($p < 0.05$). Multivariate analysis indicated that miR-122 is an independent predictor of SVR.

Conclusions: miR-122 expression could be a marker for predicting the outcome of IFN therapy. Therapies targeting miR-122 may have positive effects not only by directly inhibiting viral propagation but also by ameliorating cholesterol and lipid abnormalities.

Key words: Micro RNA 122 Chronic hepatitis C Interferon

INTRODUCTION

Hepatitis C virus (HCV) is a positive-strand RNA virus that has infected 170 million people worldwide. Once infected, 70%-80% of patients experience persistent infection leading to repeated division of hepatocytes, and ultimately, fibrosis, cirrhosis, and occasionally, progression to hepatocelluler carcinoma[1, 2]. Therefore, the development of effective antiviral therapies against HCV is important. Treatment of chronic hepatitis C (CHC) infection has progressed from interferon (IFN) monotherapy to combination therapy with peginterferon (pegIFN) and ribavirin (Rib), and directly acting agents (DAAs) [3-7]. Such advances have improved the rate of sustained virological response (SVR) from 10% to 80% among CHC genotype 1 patients. Development of novel DAAs is expected to further improve the prognosis of CHC patients. However, because of their severe adverse effects, not all patients can adapt to DAAs. In the near future, we can use newly-developed DAAs that do not have such severe side effects. But such drugs have a problem for drug-resistance and possibility of viral mutation. So despite the low SVR, combination therapy with peg-IFN and ribavirin may remain for the one means of CHC treatment. To increase the cure rate as much as possible, factors capable of predicting SVR to CHC treatment should be identified. Numerous virus- and host-related factors are known predictors of SVR [8-13]. In 2009, a single nucleotide polymorphism (SNP) near *IL28B* was reported to be strongly associated with response to CHC treatment.[14]

MicroRNAs (miRNAs) are endogenous, small, non-coding RNAs of approximately 21–22 nucleotides that have important gene regulatory functions in animals and plants; they bind to

mRNAs of protein-coding genes to direct their post-transcriptional repression[15-17]. miRNAs are implicated in numerous biological processes and diseases, including viral infections and cancers[18]. They typically mediate their regulation by inducing mRNA destabilization or translational repression by binding to complementary sequences in the 3′ untranslated region (3′UTR) of target mRNAs.

miR-122 is a highly abundant, liver-specific miRNA that constitutes 70% of the total liver miRNA content. It positively modulates replication[19], translation, and virion production[20, 21] by HCV by binding to the complementary target sequences in the 5′UTR of HCV RNA[19, 22, 23]. Furthermore, sequestration of miR-122 in hepatoma cells by antisense oligonucleotides has been shown to decrease HCV replication and translation[24, 25].

Numerous *in vitro* studies have been performed, but few have examined the correlation of miR-122 with response to IFN therapy. A recent study of 42 patients who were seropositive for HCV RNA found that miR-122 was decreased in the livers of HCV patients, and that miR-122 correlated with clinical response to pegIFN-α but not the HCV load[26]. We investigated whether miR-122 is a contributing factor to IFN treatment of CHC patients, and determined the correlation of miR-122 expression with virological response to IFN and other clinical parameters.

PATIENTS AND METHODS

Patients and Clinical samples

Clinical data collected from the patients are listed in Table 1. Our study included 51 consecutive CHC patients who were treated with IFN therapy at Nagasaki University Hospital from January

2006 to April 2011. Forty six patients in this study received PEG-IFNα2b and ribavirin combination therapy, while 5 patients underwent PEG-IFNα2a and ribavirin combination therapy. Treatment duration was from 48 weeks to 72 weeks.

22 patients(43%) reduced IFN dose by neutopenia, and 21 patients(41%) reduced RBV dose by hemolytic anemia. Every case could continue IFN and RBV therapy. Adherence to IFN was from 42 to 170% (mean 98%) and adherence to ribavirin was from 35% to 168% (mean 98%). 21 patients(41%) could achieve IFN adherence over 80%. 48 patients(94%) could achieve RBV adherence over 60%.

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from each patient. Thereafter, a liver specimen was obtained by echo-guided liver biopsy. All liver biopsy tissue specimens were examined using hematoxylin-eosin, Azan-Mallory, and silver reticulum staining. The specimens were assessed by one reviewer blinded to patient clinical and biochemical data. Diagnosis of each case was independently and histologically confirmed by liver pathologists according to the Japanese chronic hepatitis classification (New Inuyama classification). Furthermore, we scored the degree of fat deposition in the liver. Histological characteristics of the patients are also shown in Table 1.

Methods

RNA isolation

Total RNA containing miRNA was isolated from formalin-fixed paraffin-embedded (FFPE) liver biopsy specimens using the RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Ambion®) in accordance with the manufacturer's protocol.

Quantitative reverse transcription-polymerase chain reaction

miR-122 expression was quantified using TaqMan MicroRNA assays (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's protocols. Reverse transcription was performed using 10 μg of RNA isolated from the liver FFPE specimens. Quantitative PCR was performed using the Light Cycler® 480 system (Roche Diagnostics). miR-122 expression was calculated by the relative standard curve method and normalized to RNU6B expression. The *IL28B* SNP rs8099917 was also examined. SNPs were detected by pyrosequencing analysis. The sense, antisense, and pyrosequencing primers used were **B**-TCCTCCTTTTGTTTTCCTTTCTG, AAAAAGCCAGCTACCAAACTGT, and TGGTTCCAATTTGGG, respectively, where "**B**" indicates a biotin-labeled sequence.

Statistical analysis

Data were processed on a personal computer and analyzed using StatFlex (Artech Co., Ltd, Japan). Differences in each laboratory parameter were analyzed using the Mann–Whitney U-test. $p < 0.05$ was considered statistically significant.

RESULTS

Correlation of miR-122 expression with virological response to IFN therapy

We compared miR-122 expression between *IL28B* rs8099917 TT and TG/GG. No significant difference was observed in hepatic miR-122 expression between *IL28B* SNPs TT and TG/GG (Fig. 1A). We also compared miR-122 expression and stage of fibrosis. Although there was a tendency toward a decrease in miR-122 expression as fibrosis progressed, no statistically significant difference was detected (Fig.1B). In terms of virological response to IFN therapy, a significant difference was observed in the extent of miR-122 expression and the number of patients who were classified as achieving a SVR or not ($p < 0.05$, Fig. 1C). We also investigated the correlation of miR-122 expression with SVR, transient response (TVR), and no response (NVR). Significant differences were observed between SVR and TVR ($p < 0.05$) and between SVR and NVR ($p < 0.05$) (Fig.1D). As stated above, decrease of miR-122 were associated with progression of fibrosis. Therefore, we thought to compare the correlation of miR-122 expression to viral response to INF in patients matched for stage of fibrosis. Although we found that patients achieving SVR were likely to have higher expression of miR-122 than those who failed to achieve SVR, statistical difference was not observed due limited patient numbers.

Correlation of miR-122 expression with rapid/early virological response

miR-122 expression was significantly higher in patients with a strong response to IFN and undetectable HCV RNA at week 4 (i.e., those with a rapid virological response or RVR) than in patients who did not achieve RVR (Fig. 2A, $p < 0.05$). Patients who responded to IFN therapy and who had undetectable HCV RNA at week 12 were regarded as achieving an early virological

response (EVR). miR-122 expression showed the same tendency in these patients, i.e., it was higher among those with EVR than among those without such a response (Fig. 2B, $p < 0.05$). No significant correlation was observed between miR-122 expression and several other clinical parameters including WBC, RBC, Platelets, AST, ALT, γ-GTP, albumin, pre-albumin, ferritin, type IV collagen, and hyaluronic acid (Table 2).

Fat deposition in the liver was associated with miR-122 expression

In addition, we scored the degree of fat deposition in liver biopsy specimens and examined its correlation with miR-122 expression. miR-122 expression significantly decreased as the extent of fat deposition in the liver increased (Fig. 3A, $p < 0.05$). We then divided the patient's biopsy specimens into groups based on the extent of fat deposition (0%–5% and >5%) and determined their association with non-alcoholic fatty liver disease activity score (NAS). There was a significant difference in NAS among patients in the 0%–5% and >5% groups (Fig. 3B, $p < 0.05$). Furthermore, we determined whether a viral or host factor was responsible for miR-122 expression by examining the correlation of miR-122 expression with several clinical parameters, namely the presence of hypertension and diabetes mellitus, obesity (BMI > 25), total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, fasting plasma glucose, free fatty acids, HOMA-R, HbA1c, resting energy expenditure (REE), and respiratory quotient (RQ). We found that only hypertension, fasting plasma glucose, RQ, and REE correlated with miR-122 expression (Table 2).

Factors contributing to a CHC serotype 1 SVR to IFN therapy

We further investigated factors contributing to a CHC serotype 1 SVR to IFN therapy. In

univariate analysis, more male than female patients achieved SVR. The prevalence of type 2 diabetes was also significantly higher among patients who did not achieve SVR. miR-122 expression was higher among patients with SVR than among those without such a response. Multivariate analysis indicated that miR-122 expression was an independent predictor for SVR (Table 3A).

Patients who did not achieve SVR could be divided into two further groups: those who responded to IFN therapy and momentarily had undetectable HCV RNA but who then relapsed (transient responders, TVR) and those who did not respond to IFN therapy and never had any undetectable HCV RNA (null responders, NVR). Therefore, we divided the 51patients into two groups: those who responded to IFN therapy and had undetectable HCV RNA at least once (SVR+TVR), and those who did not respond to IFN therapy and never had undetectable HCV RNA (NR). Univariate analysis indicated that patients with minor *IL28B* SNPs were less likely to achieve SVR or TVR than those with major *IL28B* SNPs. Females were also less likely to achieve SVR or TVR than males. Multivariate analysis indicated that *IL28B* SNPs were independent predictors of a null response (Table 3B).

DISCUSSION

In our study, we found that hepatic miR-122 expression correlated with virological response to IFN therapy. However, there was no significant difference in miR-122 expression between minor and major *IL28B* SNPs. We also determined whether other factors predictive of response to IFN

therapy, including *IL28B* SNPs, correlated with miR-122 expression, but no such correlation was found. These findings suggest that miR-122 is an independent factor predictive of response to IFN therapy and affects the second phase of IFN therapy.

In CHC patients, miR-122 reportedly facilitates the replication of HCV by binding to the 5′UTR of HCV RNA *in vitro* [19, 22]. However, in our study, no correlation was observed between HCV load and miR-122 expression, supporting previous findings of the lack of any such correlation. Why miR-122 expression is not correlated with the HCV load is not currently understood. Many factors have been reported to promote HCV replication and production, including cyclophilin B[27], FBL2, FK506 binding-protein 8, heat shock protein 90 [28], heat shock cognate protein 70 [29], fatty acid synthesis, geranylgeranylation [30, 31], fatty acids [32], and lipid droplets [33, 34]. Given that miR-122 is abundant in the human liver, HCV replication would likely be dependent on miR-122. However, miR-122 expression is decreased as liver injury progresses, and hence, HCV replication must be dependent on other factors.

miR-122 is reportedly associated with lipid and iron metabolism in healthy individuals[35-37]. In our study, miR-122 was inversely correlated with the extent of hepatic fat deposition. We also determined whether host or virus-related factors were responsible for fat deposition in CHC patients. We found no correlation between hepatic fat deposition and host factors such as the presence or absence of hypertension, obesity, and type 2 diabetes. Thus, it was clear that fat deposition was induced by a virus-related factor. Also, patients with low miR-122 expression had low RQ and REE. Therefore, we hypothesized that as miR-122 expression was reduced, fat was

deposited in the liver, which may have been associated with increased oxidation of fatty acids. This would lead to the use of fat as an energy source and decrease RQ.

HCV infection is associated with non-alcoholic fatty liver disease [38]. Once a host is infected with HCV, the virus begins to replicate in the host's liver using miR-122. This hijack of miR-122 may decrease lipid metabolism, which is its primary function. Indeed, it has been reported that a 4-week therapy session with an antisense nucleotide of miR-122 (miravirsen, Santaris Pharma A/S) in treatment-naïve patients with HCV genotype 1 infection resulted in lowered total cholesterol as well as suppression of viremia in chimpanzees [24]. We believe that as hepatic fat deposition progresses lipid droplets are formed and these act as sites for replication of HCV RNA. If this hypothesis is correct, then inhibition of viral propagation by targeting miR-122 using an antisense approach may have a positive effect on circulating cholesterol and HCV-associated lipid abnormalities, and hence, decrease the number of lipid droplets available for HCV replication.

In conclusion, miR-122 expression is correlated with response to IFN therapy in CHC patients with HCV serotype 1 infection and is independent of other predictors of response, including *IL28B* SNPs. miR-122 expression is also correlated with hepatic fat deposition and a patient's RQ, which may be associated with fat deposition in the liver. Hereafter, it is necessary to evaluate miR-122 expression in blood sample how fatty liver and lipid metabolism involve for the pathogenesis for chronic hepatitis.

Thus, miR-122 may be a therapeutic target as well as a predictive marker of response to IFN therapy. Targeting miR-122 may have a positive effect not only by directly inhibiting viral

propagation but also by ameliorating cholesterol and lipid abnormalities and reducing the number of sites available for HCV replication.

REFERENCES

1 Ascione A, Tartaglione T, Di Costanzo GG. Natural history of chronic hepatitis C virus infection. Dig Liver Dis. 2007 Sep;39 Suppl 1: S4-7.

2 Bruno S, Facciotto C. The natural course of HCV infection and the need for treatment. Ann Hepatol. 2008 2008 Apr-Jun;7: 114-9.

3 Forestier N, Zeuzem S. Telaprevir for the treatment of hepatitis C. Expert Opin Pharmacother. 2012 Mar;13: 593-606.

4 Vachon ML, Dieterich DT. The era of direct-acting antivirals has begun: the beginning of the end for HCV? Semin Liver Dis. 2011 Nov;31: 399-409.

5 Quer J, Buti M, Cubero M, Guardia J, Esteban R, Esteban JI. New strategies for the treatment of hepatitis C virus infection and implications of resistance to new direct-acting antiviral agents. Infect Drug Resist. 2010;3: 133-45.

6 Sarrazin C, Hézode C, Zeuzem S, Pawlotsky JM. Antiviral strategies in hepatitis C virus infection. J Hepatol. 2012;56 Suppl 1: S88-100.

7 Asselah T, Marcellin P. New direct-acting antivirals' combination for the treatment of chronic hepatitis C. Liver Int. 2011 Jan;31 Suppl 1: 68-77.

8 Mira JA, Valera-Bestard B, Arizcorreta-Yarza A, et al. Rapid virological response at week 4 predicts response to pegylated interferon plus ribavirin among HIV/HCV-coinfected patients. Antivir Ther. 2007;12: 523-9.

9 Shirakawa H, Matsumoto A, Joshita S, et al. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology. 2008 Dec;48: 1753-60.

10 Asselah T, Estrabaud E, Bieche I, et al. Hepatitis C: viral and host factors associated with non-response to pegylated interferon plus ribavirin. Liver Int. 2010 Oct;30: 1259-69.

11 Harrison SA, Rossaro L, Hu KQ, et al. Serum cholesterol and statin use predict virological response to peginterferon and ribavirin therapy. Hepatology. 2010 Sep;52: 864-74.

12 Kitamura S, Tsuge M, Hatakeyama T, et al. Amino acid substitutions in core and NS5A regions of the HCV genome can predict virological decrease with pegylated interferon plus ribavirin therapy. Antivir Ther. 2010;15: 1087-97.

13 Kurosaki M, Tanaka Y, Nishida N, et al. Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. J Hepatol. 2011 Mar;54: 439-48.

14 Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with

response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet. 2009 Oct;41: 1105-9.
15 He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet. 2004 Jul;5: 522-31.
16 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004 Jan;116: 281-97.
17 Ambros V. The functions of animal microRNAs. Nature. 2004 Sep;431: 350-5.
18 Shen J, Cho CH. miRNA in the pathogenesis and Therapy for Gastrointestinal and Hepatic Cancer. *Curr Pharm Des* 2012.
19 Jopling CL, Norman KL, Sarnow P. Positive and negative modulation of viral and cellular mRNAs by liver-specific microRNA miR-122. Cold Spring Harb Symp Quant Biol. 2006;71: 369-76.
20 Roberts AP, Lewis AP, Jopling CL. miR-122 activates hepatitis C virus translation by a specialized mechanism requiring particular RNA components. Nucleic Acids Res. 2011 Sep;39: 7716-29.
21 Jangra RK, Yi M, Lemon SM. Regulation of hepatitis C virus translation and infectious virus production by the microRNA miR-122. J Virol. 2010 Jul;84: 6615-25.
22 Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science. 2005 Sep;309: 1577-81.
23 Henke JI, Goergen D, Zheng J, et al. microRNA-122 stimulates translation of hepatitis C virus RNA. EMBO J. 2008 Dec;27: 3300-10.
24 Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science. 2010 Jan;327: 198-201.
25 Stenvang J, Petri A, Lindow M, Obad S, Kauppinen S. Inhibition of microRNA function by antimiR oligonucleotides. Silence. 2012;3: 1.
26 Sarasin-Filipowicz M, Krol J, Markiewicz I, Heim MH, Filipowicz W. Decreased levels of microRNA miR-122 in individuals with hepatitis C responding poorly to interferon therapy. Nat Med. 2009 Jan;15: 31-3.
27 Watashi K, Ishii N, Hijikata M, et al. Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. Mol Cell. 2005 Jul;19: 111-22.
28 Okamoto T, Nishimura Y, Ichimura T, et al. Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90. EMBO J. 2006 Oct;25: 5015-25.
29 Parent R, Qu X, Petit MA, Beretta L. The heat shock cognate protein 70 is associated with hepatitis C virus particles and modulates virus infectivity. Hepatology. 2009 Jun;49: 1798-809.
30 Wang C, Gale M, Keller BC, et al. Identification of FBL2 as a geranylgeranylated cellular protein required for hepatitis C virus RNA replication. Mol Cell. 2005 May;18: 425-34.
31 Kapadia SB, Chisari FV. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. Proc Natl Acad Sci U S A. 2005 Feb;102: 2561-6.
32 Yang W, Hood BL, Chadwick SL, et al. Fatty acid synthase is up-regulated during

hepatitis C virus infection and regulates hepatitis C virus entry and production. Hepatology. 2008 Nov;48: 1396-403.
33 Herker E, Ott M. Unique ties between hepatitis C virus replication and intracellular lipids. Trends Endocrinol Metab. 2011 Jun;22: 241-8.
34 Miyanari Y, Atsuzawa K, Usuda N, et al. The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol. 2007 Sep;9: 1089-97.
35 Castoldi M, Vujic Spasic M, Altamura S, et al. The liver-specific microRNA miR-122 controls systemic iron homeostasis in mice. J Clin Invest. 2011 Apr;121: 1386-96.
36 Moore KJ, Rayner KJ, Suárez Y, Fernández-Hernando C. The role of microRNAs in cholesterol efflux and hepatic lipid metabolism. Annu Rev Nutr. 2011 Aug;31: 49-63.
37 Rottiers V, Näär AM. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol. 2012 Apr;13: 239-50.
38 Cheung O, Puri P, Eicken C, et al. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. Hepatology. 2008 Dec;48: 1810-20.

Figure legend

Figure 1.

The association between hepatic microRNA - 122(miR-122) expression of serotype 1 hepatitis C virus (HCV)-infected liver and IL28B SNPs. There is no correlation with miR-122 expression between IL28B SNIP TT and TG/GG (Fig.1A). Also we compared with the fibrosis stage. Although there is a tendency miR-122 expression decreased if fibrosis progressed, there is no significant difference. (Fig.1B) According to viral response to IFN therapy , there is a significant difference between SVR and Non-SVR($p<0.05$, Fig.1C) . Furthermore, we investigate the correlation miR-122 expression between SVR and TVR, NR. There are significant difference between SVR and TVR ($p<0.05$), and SVR and NR ($p<0.05$) (Fig.1D).

Figure 2. The correlation between miR-122 expression and rapid / early virologic response.

Rapid virologic response (RVR): Patients who respond to IFN therapy with a decrease in viral load at week 4.

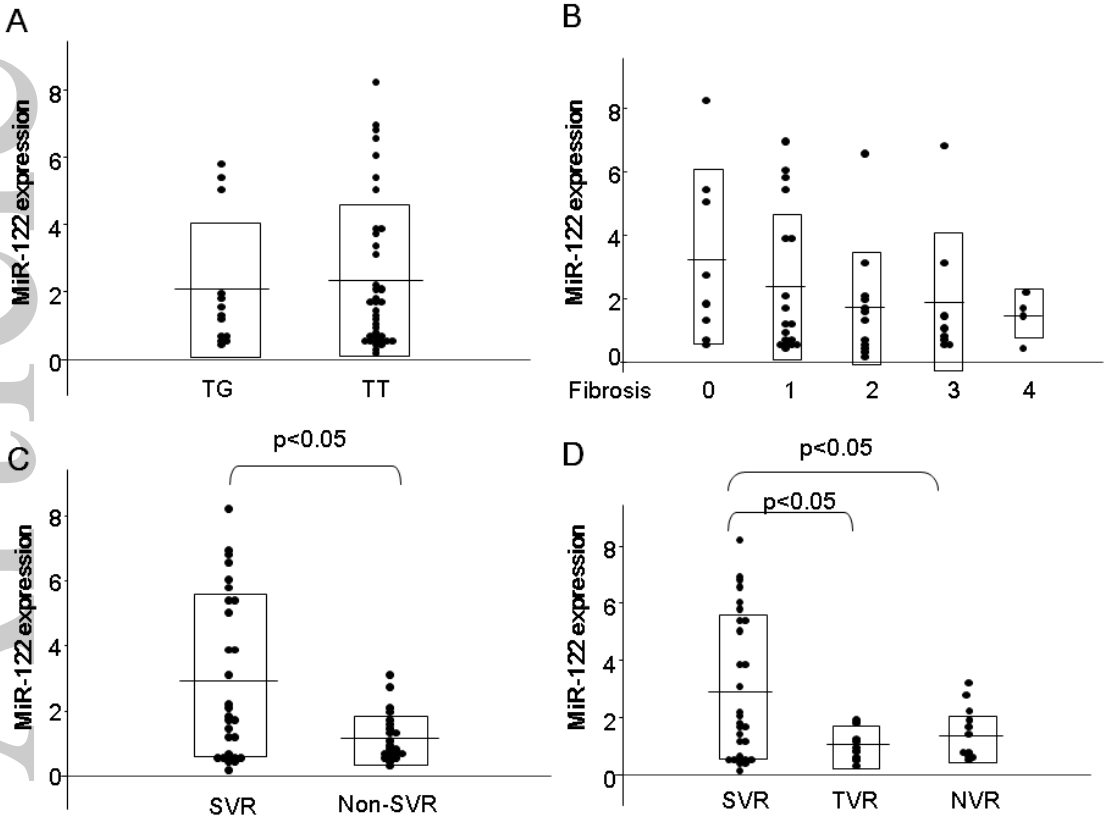
Early virologic response (EVR): Patients who respond to IFN therapy with a decrease in viral load at week 12.

According to the virologic response, patients who achieved RVR had significantly higher miR-122 expression level than did not achieved RVR (Fig.2A, $p<0.05$). The same tendency can be said

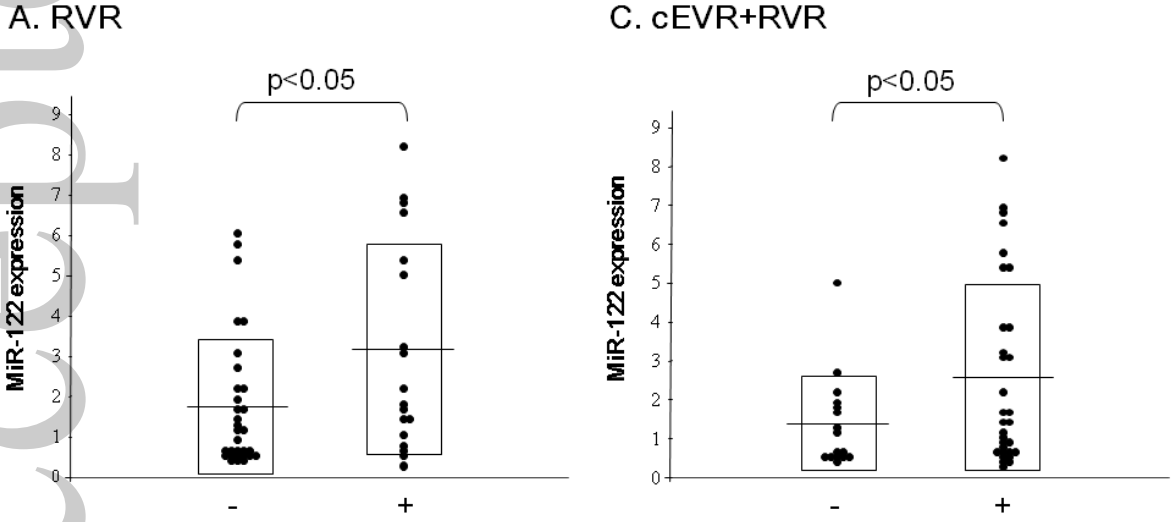
between patients who achieved and did not achieved EVR (Fig.2B, $p<0.05$). We examined the relationship between miR-122 expression and several clinical parameters (WBC, RBC, Platelet, AST, ALT, γ-GTP, albumin, pre-albumin, ferritin, Type Ⅳ collagen, Hyaluronic acid), there are no significant differences.

Figure 3. MiR-122 expression was correlated with fat deposition in liver.

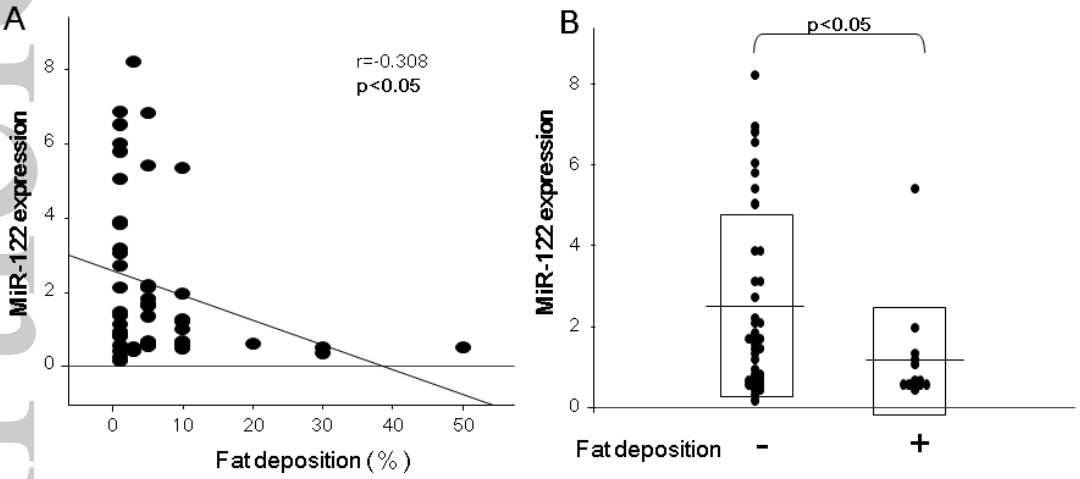
By using liver biopsy specimen, we scored the degree of fat deposition in liver and examined the relationship miR-122 expression. MiR-122 expression significantly decreased as the extent of fat deposition in the liver increased (Fig.3A, $p<0.05$). We divided patients into the patients whose fat deposition is 0~5% and 5%~ in proportion NAFLD activity score (NAS). There is a significant difference between 0~5% and 5%~(Fig.3B, $p<0.05$).



Kamo Y. et. al., figure 1



Kamo Y. et. al., figure 2



Kamo Y. et. al., figure 3

Table 1. Clinical characteristics

Characteristics	Mean,number	Standard deviation (SD)
Age(years)	57.4	9.26
Gender (F/M)	20/31	
BMI(kg/m2)	23.2	3.42
WBC(cells/ μ L)	4762	1292
Plt($\times 10^4$ platelets/ μ L)	17.41	5.765
AST(IU/L)	56.09	32.70
ALT(IU/L)	80.27	58.11
γ-GTP(IU/L)	52.51	52.57
Albumin(g/dL)	4.102	0.294
TC(mg/dL)	174.08	23.94
TG(mg/dL)	100.68	38.92
LDL-C(mg/dL)	96.43	21.10
HDL-C(mg/dL)	51.64	12.45
FFA(mEq/L)	0.438	0.409
PreAlb(mg/dL)	19.52	4.708
HbA1c(%)	5.409	0.948

HCV-RNA(LogIU/ml)	6.2	1.15
Staging (F0/1/2/3/4)	8/18/12/8/4	
Activity (A0/1/2)	2/37/7	
IFN adherence(%)	98(42-170)	
IFN adherence >80%	21(41%)	
RBV adherence(%)	98(35-168)	
RBV adherence >60%	48(94%)	
IFN response (SVR/TVR/NR)	29/12/10	
IL28B rs8099917 (TT/TG/GG)	38/13/0	

Normal values in laboratory tests: BMI calculated as follow; body weight (kg)/height $(m)^2$ WBC; cells/ μ L), 3,500-9,000; platelets (Plt; $\times 10^4$ platelets/ μ L), 12-33; aspartate aminotransferase (AST; IU/L), 10-40; alanine aminotransferase (ALT; IU/L), 5-40; γ -glutamyltranspeptidase (γ -GTP; IU/L),<70 in males,<30 in females; albumin (Alb; g/dL), 4.0-5.0; total cholesterol (TC; mg/dL), 128-220; triglyceride (TG; mg/dL), 38-150; low-density lipoprotein cholesterol (LDL-C; mg/dL), 70-139; high-density lipoprotein cholesterol (HDL-C; mg/dL), 40-80; free fatty acid (FFA; mEq/L),100-800 ; preAlbumin(preAlb), 22-40 ; HbA1c,<5.8%;

Table 2. Relation with miR-122 expression level in liver and clinical items

Items	relation	p
Age(year)	0.01	NS
BMI(kg/m2)	0.105	NS
BTR(ratio)	0.175	NS
WBC(cells/ μ L)	0.102	NS
RBC(cells/ μ L)	0.219	NS
Plt($\times 10^4$ platelets/ μ L)	0.090	NS
AST(IU/L)	0.045	NS
ALT(IU/L)	0.020	NS
γ-GTP(IU/L)	0.004	NS
Albumin(g/dL)	0.059	NS
PreAlb(mg/dL)	0.138	NS
Ferritin(ng/ml)	0.191	NS
TypeIV collagen(ng/ml)	0.214	NS
Hyaluronic acid(ng/ml)	0.178	NS
FPG(mg/dL)	0.284	<0.05
FFA(mEq/L)	0.190	NS

HOMA-R	0.08	NS
HbA1c(%)	0.167	NS
TC(mg/dL)	0.124	NS
HDL(mg/dL)	0.044	NS
LDL(mg/dL)	0.128	NS
TG(mg/dL)	0.146	NS
REE	0.352	<0.05
RQ	0.550	<0.05
IFN adherence >80%	0.222	NS
RBV adherence >60%	0.038	NS
HCV-RNA(LogIU/ml)	0.088	NS

analyzed using the Mann–Whitney U-test. $p < 0.05$ was considered statistically significant.

Normal values in laboratory tests: BMI calculated as follow; body weight (kg)/height $(m)^2$ WBC; cells/ μ L), 3,500-9,000; platelets (Plt; $\times 10^4$ platelets/ μ L), 12-33; aspartate aminotransferase (AST; IU/L), 10-40; alanine aminotransferase (ALT; IU/L), 5-40; γ -glutamyltranspeptidase (γ -GTP; IU/L),<70 in males,<30 in females; albumin (Alb; g/dL), 4.0-5.0; total cholesterol (TC; mg/dL), 128-220; triglyceride (TG; mg/dL), 38-150;

low-density lipoprotein cholesterol (LDL-C; mg/dL), 70-139; high-density lipoprotein cholesterol (HDL-C; mg/dL), 40-80; free fatty acid (FFA; mEq/L),100-800 ; preAlbumin(preAlb), 22-40 ; HbA1c,<5.8%;

Table 3. Study of predictive factors for IFN treatment in serotype 1 chronic hepatitis C patients.

A. Contributing factor to SVR in all patients

Variable	univariable analysis		multivariable analysis	
Variable	P	odds ratio(95% CI)	P	odds ratio(95% CI)
Age (years)	0.234	1.03 (0.97 - 1.10)		
Sex (F)	0.054	0.31 (0.09 – 1.02)		
Body Mass Index	0.472	0.93 (0.78 – 1.12)		
Fibrosis	0.700	1.09 (0.67 – 1.78)		
Activity	0.599	0.72 (0.22 – 2.38)		
Fat deposition	0.455	1.02 (0.96 – 1.09)		
HCV RNA(LogIU/ml)	0.892	1.04 (0.55 – 1.94)		
Albumin (g/dl)	0.897	0.88 (0.12 – 6.03)		
AST (IU/L)	0.203	0.98 (0.96 – 1.00)		
ALT (IU/L)	0.084	0.98 (0.97 – 1.00)		
γ-GTP (IU/L)	0.121	0.98 (0.96 – 1.00)		
Plt ($\times 10^4/\mu l$)	0.898	1.00 (0.91 – 1.10)		
Feritin(mEq/L)	0.569	0.99 (0.99 – 1.00)		

Pre Albumin (g/dl)	0.272	0.93 (0.82 – 1.05)		
HbA1c (%)	0.022	0.08 (0.01 – 0.71)	NS	
IFN adherence >80%	0.716	0.80 (0.25 – 2.53)		
RBV adherence >60%	0.773	1.35 (0.17 – 10.41)		
Micro RNA 122	0.012	0.55 (0.34 – 0.88)	0.029	0.401 (0.17 – 0.91)
IL28B rs8099917	0.127	0.36 (0.09 – 1.33)		

B. Contributing factor to NVR in all patients

	Univariate Analysis		Multivariate Analysis	
Variable	P	Odds ratio (95% CI)	P	Odds ratio (95% CI)
Age (years)	0.178	0.93 (0.85 - 1.02)		
Sex (Male)	0.035	5.02 (1.11 - 22.6)	0.088	4.38 (0.80 - 23.9)
BMI (kg/m2)	0.944	1.00 (0.81 - 1.24)		
Fibrosis	0.414	1.29 (0.69 - 2.41)		
Activity	0.570	1.55 (0.33 - 7.15)		
Fat deposition (%)	0.104	0.95 (0.90 - 1.00)		
HCV RNA (LogIU/ml)	0.659	1.18 (0.56 - 2.48)		
Albumin (g/dl)	0.358	3.39 (0.25 - 46.0)		
AST (IU / L)	0.656	0.99 (0.97 - 1.01)		

ALT (IU / L)	0.779	1.00 (0.98 - 1.01)		
γ-GTP (IU / L)	0.525	1.00 (0.98 - 1.02)		
Plt ($\times 10^4/\mu l$)	0.233	0.92 (0.82 - 1.04)		
Ferritin (ng / ml)	0.479	0.99 (0.99 - 1.00)		
Pre Albumin (g/dl)	0.412	1.06 (0.91 - 1.25)		
HbA1c (%)	0.406	1.83 (0.43 - 7.66)		
IFN adherence >80%	0.508	0.60 (0.13 ～ 2.68)		
RBV adherence >60%	0.778	1.40 (0.13 ～ 15.1)		
Micro RNA 122	0.141	1.51 (0.87 - 2.64)	0.239	1.42 (0.78 - 2.58)
IL28B rs8099917	0.009	7.28 (1.61 - 32.7)	0.016	7.77 (1.45 - 41.7)