

or not), and alcoholic consumption (<50 g/day or not). For continuous data, the univariate associations were evaluated using the Student's *t* test or nonparametric Wilcoxon rank-sum test as appropriate. Since the age at onset of HCC (the primary endpoint of this study) satisfied the assumption of normal distribution (Kolmogorov–Smirnov test, $P > 0.05$), we used stepwise regression analysis to adjust the influence of IL28B genotype by sex, BMI (<25 or not), and alcoholic consumption (<50 g/day or not). All statistical analyses were two-sided, and the threshold of the reported *P* values for significance was accepted as <0.05. All statistical analyses were performed using R 2.13.1 software (<http://www.r-project.org>).

Results

Patient characteristics

Patient characteristics are shown in Table 1. Frequencies of the rs8099917 TT, TG, and GG genotype were 74.3 % (261/351), 24.8 % (87/351), and 0.9 % (3/351), respectively. The SNP genotype distribution was in Hardy–Weinberg equilibrium (*P* value was not significant). We defined the IL28B major genotype as homozygous for the major sequence (TT) and the IL28B minor genotype as homozygous (GG) or heterozygous (TG) for the minor sequence. The mean age at onset of the HCC patients was 69.3 years, and approximately 60 % were male. The mean age at the time of enrollment was 67.2 years and the follow-up period was 27.9 months in average.

Table 1 Clinical characteristics and genotype distributions in the study cohort ($n = 351$)

Parameter	Values
Mean age at onset of HCC, in years	69.26 ± 8.07
Mean age at the time of enrollment, in years	67.16 ± 8.32
Male sex	200 (57.0 %)
BMI >25	70 (20.0 %)
Alcohol consumption (>50 g/day)	75 (21.4 %)
IL28B genotype	
TT	261 (74.3 %)
TG	87 (24.8 %)
GG	3 (0.9 %)
T allele frequency	0.87
HCV genotype	
Genotype 1	240 (68.4 %)
Genotype 2	91 (25.9 %)
Not tested	20 (5.7 %)

Continuous variables were represented as the mean ± standard deviation (SD) and categorical variables were as number and frequencies (%)

Primary endpoint

Table 2 shows the age at onset of patients with HCC and the associations among IL28B genotypes, sex, BMI, alcohol consumption, and HCV genotype. The mean age at onset in patients with HCC for the IL28B major and minor genotypes were 69.88 ± 7.97 and 67.48 ± 8.17, respectively, and significantly higher in patients with the IL28B major genotype than in those with the minor genotype ($P = 0.02$). In multivariate analysis, the age at onset of HCC was significantly younger in patients with the IL28B minor genotype ($P = 0.02$, Fig. 1), independently of male sex ($P < 0.001$) and higher BMI ($P = 0.009$). The characters of HCC, such as sizes (2.56 vs. 2.40 cm, $P = 0.41$) or the numbers (1.94 vs. 2.23, $P = 0.54$) at diagnosis were not significantly different between IL28B major and minor genotypes. We also analyzed the interval between blood transfusion and the onset of HCC in 161 patients who have histories of blood transfusion which had been the major cause of HCV infection in Japan [20]. The mean interval between blood transfusion and the onset of HCC for the IL28B major and minor genotypes were 39.09 ± 9.99 and 38.86 ± 9.27 years, respectively ($P = 0.9$; data not shown).

Secondary endpoint

Table 3 shows the clinical findings and associations between the IL28B genotypes at the time of enrollment in our cohort. The IL28B major genotype was significantly associated with a higher probability of having an APRI >1.5 (58.62 vs. 46.67 %, $P = 0.01$; Fig. 2), a lower platelet count (11.15 vs. 12.80 × 10⁴/μL, $P = 0.002$), a higher AST level (77.69 vs. 69.12 IU/L, $P = 0.02$), a higher ALT level (80.92 vs. 67.79 IU/L, $P = 0.002$), and a lower prothrombin time (75.40 vs. 79.27 %, $P = 0.002$) compared to the IL28B minor genotype after adjustment for sex, BMI, alcoholic consumption, and the age at enrollment of our cohort. A lower γ-GTP level was significantly associated with the IL28B major genotype in univariate analysis, and alcoholic consumption, sex, and age were stronger factors associated with the γ-GTP level. Thus, after adjustment for these factors, the IL28B genotype was not extracted as a significant factor associated with the γ-GTP level. Histological assessments of liver fibrosis were performed in 248 patients at the time of initial therapy. The prevalence of histologically proved liver cirrhosis (F4) was 65.6 % (118/180) in patients with major genotype and 51.5 % (35/68) in those with minor genotype. The prevalence of liver cirrhosis was significantly higher in patients with major genotype after adjustment for sex, BMI, alcoholic consumption, and the age at the time of initial therapy for HCC ($P = 0.045$, data not shown).

Table 2 Factors associated with the age at onset of HCC

Variable	Mean	Standard deviation (SD)	P value	
			Univariate	Multivariate ^a
IL28B genotype			0.02	0.02
Major (TT)	69.88	7.97		
Minor (TG/GG)	67.48	8.17		
Sex			<0.001	<0.001
Male	67.94	8.48		
Female	71.02	7.16		
BMI			0.01	0.009
>25	66.87	9.11		
≤25	69.86	7.70		
Alcohol consumption			0.11	–
>50 (g/day)	67.78	9.37		
≤50 (g/day)	69.67	7.65		
HCV genotype			0.29	–
Genotype 1	69.65	7.59		
Genotype 2	68.22	8.79		

^a Stepwise regression analysis for the age at onset of HCC (the dependent variable) using IL28B genotype, sex, BMI, alcohol consumption, and HCV genotype as independent variables

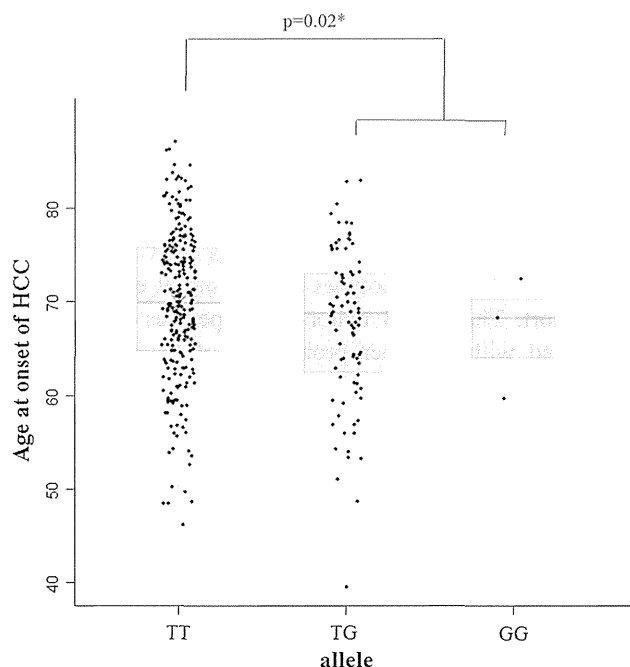


Fig. 1 Box and whisker and dot plot distributions of the age at onset of HCC in each genotype. The mean age at onset of HCC for the IL28B major and minor genotypes were 69.88 ± 7.97 and 67.48 ± 8.17 , respectively, and was significantly higher in patients with the IL28B major genotype than in those with the minor genotype ($P = 0.02$). * P values after adjustment for sex, BMI, and alcoholic consumption

Discussion

In the present study, we evaluated the association between the IL28B polymorphism and the age at onset of HCC in patients with CHC. The IL28B minor genotype was

significantly associated with younger age at onset of HCC with well known risk factors for the development of HCC such as male gender and higher BMI [21] without prior IFN-based treatment. Our previous study analyzing a susceptibility locus for HCV-induced HCC using a genome-wide association study (GWAS) could not detect the significant association between IL28B genotypes and the development of HCC in a cross-sectional distribution analysis between patients with and without HCC in more than 3,000 samples [22]. Also, IL28B alleles were not identified as a susceptibility locus for HCV-induced HCC in another GWAS study [23]. The cross-sectional distribution analyses may have underestimated the susceptibility to HCC because it could not take into consideration the future development of HCC and the duration after the past onset of HCC. Moreover, although GWAS would provide an effective and unbiased approach for revealing risk alleles for genetically complex non-Mendelian disorders, the risk of multiple comparisons made in a GWAS have resulted in reports of false positive results (Type 1 errors), and if the correction is overly conservative or the power is inadequate, false negative results (Type 2 errors) [24–26]. The relation between IL28B polymorphism and the susceptibility to HCC is still controversial. A previous study from Japan reported that the rs8099917 TT genotype was associated with a lower incidence of HCC even in non-responders to IFN based treatment [27] that was in agreement with the present study. Another study from Italy evaluating the association between genome frequency and the presence of cirrhosis due to hepatitis C, hepatitis B, alcohol use, and other factors also showed a higher prevalence of the IL28B minor allele in patients with HCC

Table 3 Associations between the IL28B genotype and clinical findings at the time of enrollment in our cohort

Variable	Mean/proportion (standard deviation; SD)		P values	
	Major (TT)	Minor (TG/GG)	P value	Adjusted P value [¶]
APRI >1.5 ^a	58.62 % (52.38–64.66)	46.67 % (36.07–57.69)	0.07	0.01
Platelet count ($\times 10^4/\mu\text{L}$)	11.15 (5.00)	12.80 (5.43)	0.01	0.002**
AST (IU/L)	77.69 (45.14)	69.12 (38.16)	0.12	0.02**
ALT (IU/L)	80.92 (60.45)	67.79 (41.78)	0.17	0.002**
T.B (mg/dL)	0.90 (0.40)	0.83 (0.39)	0.02	–
Alb (g/dL)	3.69 (0.46)	3.71 (0.46)	0.9	–
ALP (IU/L) ^b	236.4 (81.75)	216.4 (58.96)	0.08	0.11**
γ GTP (IU/L) ^c	76.83 (65.34)	87.23 (42.92)	0.005	–
PT (%) ^d	75.40 (13.36)	79.27 (13.13)	0.02	0.002**

[¶] Adjusted for sex, BMI, alcoholic consumption, and the age at enrollment (independent variables). The dependent variables of each P values are the items in the leftmost fields of corresponding rows (the proportion of having APRI >1.5, platelet count, AST, ALT and so on)

^{||} P value by stepwise logistic regression analysis

** P value by stepwise regression analysis

^a Odds ratio (95 % CI) for major allele was 1.88 (1.13–3.11), and 95 % confidence interval (CI) of each proportion is parenthesized for this outcome

^b Missing in 115 patients

^c Missing in 112 patients

^d Missing in 4 patients

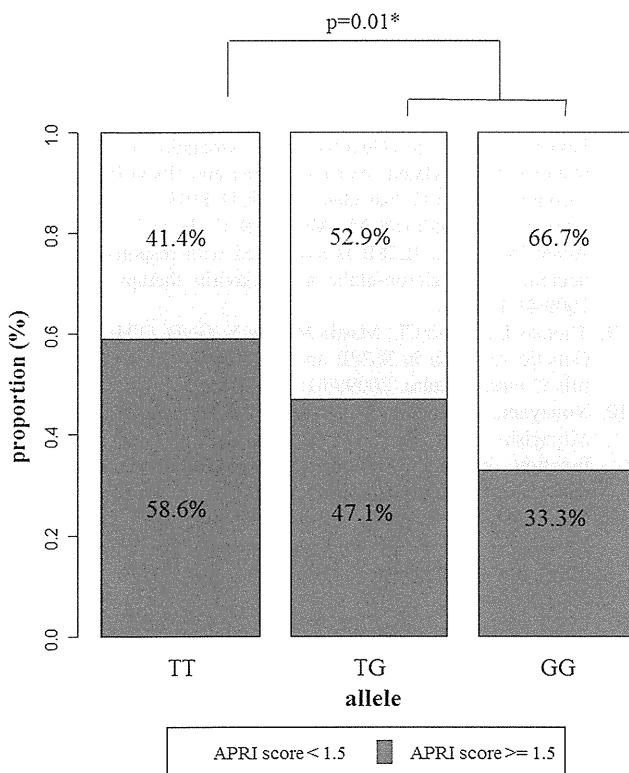


Fig. 2 Bar plot the proportion of having an AST-to-platelet ratio (APRI) score >1.5 in each allele. *P values after adjustment for sex, BMI, alcoholic consumption, and the age at enrollment

compared to those without HCC [28]. However, other studies showed no relation between IL28B polymorphism and the susceptibility to HCC [29–32]. Some studies have reported the HCV genotype 1 as a risk factor associated with HCC in patients who had CHC [33–35]; however, we could not find a significant association between the HCV genotype and hepatocarcinogenesis in the present study. Our data showed no relationship between the duration of HCV infection in the patients with a history of blood transfusion. The mean age of blood transfusion was not significantly different between patients with major and minor genotypes (28.99 in major genotype vs. 27.60 in minor genotype, $P = 0.18$). Moreover, older age at HCV infection was reported to be associated with more rapid disease progression [36]. Thus, the difference in the duration of HCV infection may have little effect on the result of the present study. The IL28B genotype may have a critical role in the onset of HCC. Moreover, only about 45 % of all patients in the present study have the history of blood transfusion; hence, further analysis with larger samples may be indicated.

Previous studies evaluating patients with chronic HCV infection showed severer histological inflammatory activity and fibrosis, as well as higher ALT levels and APRI scores in patients homozygous for the IL28B major alleles [29, 32, 37, 38]. Similarly, in the present study, the IL28B

major genotype was significantly associated with a higher probability of having an APRI >1.5 and a higher ALT level; and the prevalence of histologically proved liver cirrhosis (F4) was significantly higher in patients with major genotype at the age at the time of initial therapy for HCC. Given the association between the IL28B major allele and the severe inflammatory activity or progressed fibrosis, the IL28B allele is thought to be associated with the susceptibility to HCC via a mechanism that is independent of controlling an activity of HCV infection.

Recent experimental studies have suggested that IFN- λ has an antitumor activity. In esophageal cancer cell lines expressing IFN- λ receptor complexes, IFN- λ 1 suppressed growth via the induction of the G1 phase arrest or apoptosis [39]. An antitumor activity of IFN- λ was also shown in the B16 melanoma, BNL hepatoma, Colon 26, and neuroendocrine BON1 tumor cells [40–43]. One probable explanation for the paradoxical result of the present study is that the more aggressive inflammatory activity of patients with IL28B major genotype may reflect a stronger immune response to the virus, which may also have anti-tumor effects. However, the innate immune responses and anti-tumor activity via IFN- λ , as well as the mechanism underlying the association of the IL28B genotype, have not been elucidated. Further studies are needed to determine the functional role of the IL28B gene in relation to the course of chronic HCV infection, including hepatocarcinogenesis.

Because of the retrospective design, this study is limited by the absence of some important clinical details such as information about the histological findings of fibrosis and inflammation. Although the APRI is a useful index for the prediction of fibrosis, the limitation of this score has been reported in previous studies [44, 45]. Prospectively designed studies are needed to confirm our findings. However, observing chronic HCV-infected patients without antiviral treatment would be nearly impossible in the future. In this regard, the present study may have important implications.

In conclusion, the IL28B minor genotype was associated with a younger age of onset of HCC in patients with CHC, and this association was completely independent of the response to IFN-based treatment. Hepatocarcinogenesis appeared to be suppressed in patients who had CHC with the IL28B major genotype, despite higher inflammatory activity and progressed fibrosis of liver. The current findings may provide a clinically important information in the follow-up or HCC screening of cirrhotic patients.

Acknowledgments This study was supported by the Global COE Program, “Center of Education and Research for Advanced Genome-Based Medicine: For personalized medicine and the control of worldwide infectious diseases”; the Ministry of Education, Culture, Sports, Science and Technology, Japan; by grants from the Leading Project of the Ministry of Education, Culture, Sports, Science and

Technology, Japan; and by Health and Labor Sciences Research Grants for Research on Hepatitis from the Ministry of Health, Labor and Welfare, Japan.

Conflict of interest None of the authors have any conflicts of interest.

References

- Barrera JM, Bruguera M, Ercilla MG, Gil C, Celis R, Gil MP, et al. Persistent hepatitis C viremia after acute self-limiting posttransfusion hepatitis C. *Hepatology*. 1995;21:639–44.
- Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med*. 2004;140:346–55.
- Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*. 2001;358:958–65.
- McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med*. 2009;360:1827–38.
- Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med*. 2011;364:1195–206.
- Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*. 2009;461:399–401.
- Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet*. 2009;41:1105–9.
- Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet*. 2009;41:1100–4.
- Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O’Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature*. 2009;461:798–801.
- Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol*. 2004;5:730–7.
- Moriyama M, Kato N, Otsuka M, Shao RX, Taniguchi H, Kawabe T, et al. Interferon-beta is activated by hepatitis C virus NS5B and inhibited by NS4A, NS4B, and NS5A. *Hepatology*. 2007;45:302–10.
- Li CZ, Kato N, Chang JH, Muroyama R, Shao RX, Dharel N, et al. Polymorphism of OAS-1 determines liver fibrosis progression in hepatitis C by reduced ability to inhibit viral replication. *Liver Int*. 2009;29:1413–21.
- Li W, Lewis-Antes A, Huang J, Balan M, Kotenko SV. Regulation of apoptosis by type III interferons. *Cell Prolif*. 2008;41:960–79.
- Numasaki M, Tagawa M, Iwata F, Suzuki T, Nakamura A, Okada M, et al. IL-28 elicits antitumor responses against murine fibrosarcoma. *J Immunol*. 2007;178:5086–98.
- Li M, Liu X, Zhou Y, Su SB. Interferon-lambdas: the modulators of antiviral, antitumor, and immune responses. *J Leukoc Biol*. 2009;86:23–32.

16. Maher SG, Sheikh F, Scarzello AJ, Romero-Weaver AL, Baker DP, Donnelly RP, et al. IFNalpha and IFNlambda differ in their antiproliferative effects and duration of JAK/STAT signaling activity. *Cancer Biol Ther*. 2008;7:1109–15.
17. Tateishi R, Shiina S, Teratani T, Obi S, Sato S, Koike Y, et al. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer*. 2005;2005(103):1201–9.
18. Masuzaki R, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, et al. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology*. 2009;49:1954–61.
19. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38:518–26.
20. Kiyosawa K, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, et al. Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology*. 2004;127:S17–26.
21. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*. 2007;132:2557–76.
22. Kumar V, Kato N, Urabe Y, Takahashi A, Muroyama R, Hosono N, et al. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. *Nat Genet*. 2011;43:455–8.
23. Miki D, Ochi H, Hayes CN, Abe H, Yoshima T, Aikata H, et al. Variation in the DEPDC5 locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers. *Nat Genet*. 2011;43:797–800.
24. McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet*. 2008;9:356–69.
25. Cantor RM, Lange K, Sinsheimer JS. Prioritizing GWAS results: a review of statistical methods and recommendations for their application. *Am J Hum Genet*. 2010;86:6–22.
26. Johnson RC, Nelson GW, Troyer JL, Lautenberger JA, Kessing BD, Winkler CA, et al. Accounting for multiple comparisons in a genome-wide association study (GWAS). *BMC Genomics*. 2010;11:724.
27. Asahina Y, Tanaka K, Suzuki Y, Tamaki N, Hoshioka T, Kato T, et al. Association between IL28B gene variation and development of hepatocellular carcinoma after interferon therapy in patients with chronic hepatitis C. *J Hepatol*. 2011;54:S37.
28. Fabris C, Falletti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, et al. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol*. 2011;54:716–22.
29. Bochud PY, Bibert S, Kutalik Z, Patin E, Guergnon J, Nalpas B, et al. IL28B alleles associated with poor hepatitis C virus (HCV) clearance protect against inflammation and fibrosis in patients infected with non-1 HCV genotypes. *Hepatology*. 2012;55:384–94.
30. Joshita S, Umemura T, Katsuyama Y, Ichikawa Y, Kimura T, Morita S, et al. Association of IL28B gene polymorphism with development of hepatocellular carcinoma in Japanese patients with chronic hepatitis C virus infection. *Hum Immunol*. 2012;73:298–300.
31. Miura M, Maekawa S, Kadokura M, Sueki R, Komase K, Shindo H, et al. Analysis of viral amino acids sequences and the IL28B SNP influencing the development of hepatocellular carcinoma in chronic hepatitis C. *Hepatol Int*. 2012;6:386–96.
32. Agundez JA, Garcia-Martin E, Maestro ML, Cuenca F, Martinez C, Ortega L, et al. Relation of IL28B gene polymorphism with biochemical and histological features in hepatitis C virus-induced liver disease. *PLoS ONE*. 2012;7:e37998.
33. Bruno S, Crosignani A, Maisonneuve P, Rossi S, Silini E, Mondelli MU. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: a seventeen-year prospective cohort study. *Hepatology*. 2007;46:1350–6.
34. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. *Hepatology*. 1997;25:754–8.
35. Silini E, Bottelli R, Asti M, Bruno S, Candusso ME, Brambilla S, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a case-control study. *Gastroenterology*. 1996;111:199–205.
36. Freeman AJ, Dore GJ, Law MG, Thorpe M, Von Overbeck J, Lloyd AR, et al. Estimating progression to cirrhosis in chronic hepatitis C virus infection. *Hepatology*. 2001;34:809–16.
37. Moghaddam A, Melum E, Reinton N, Ring-Larsen H, Verbaan H, Bjoro K, et al. IL28B genetic variation and treatment response in patients with hepatitis C virus genotype 3 infection. *Hepatology*. 2011;53:746–54.
38. Abe H, Ochi H, Maekawa T, Hayes CN, Tsuge M, Miki D, et al. Common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol*. 2010;53:439–43.
39. Li Q, Kawamura K, Ma G, Iwata F, Numasaki M, Suzuki N, et al. Interferon-lambda induces G1 phase arrest or apoptosis in oesophageal carcinoma cells and produces anti-tumour effects in combination with anti-cancer agents. *Eur J Cancer*. 2010;46:180–90.
40. Lasfar A, Lewis-Antes A, Smirnov SV, Anantha S, Abushahba W, Tian B, et al. Characterization of the mouse IFN-lambda ligand-receptor system: IFN-lambdas exhibit antitumor activity against B16 melanoma. *Cancer Res*. 2006;66:4468–77.
41. Abushahba W, Balan M, Castaneda I, Yuan Y, Reuhl K, Raveche E, et al. Antitumor activity of type I and type III interferons in BNL hepatoma model. *Cancer Immunol Immunother*. 2010;59:1059–71.
42. Sato A, Ohtsuki M, Hata M, Kobayashi E, Murakami T. Antitumor activity of IFN-lambda in murine tumor models. *J Immunol*. 2006;176:7686–94.
43. Zitzmann K, Brand S, Baehs S, Goke B, Meinecke J, Spottl G, et al. Novel interferon-lambdas induce antiproliferative effects in neuroendocrine tumor cells. *Biochem Biophys Res Commun*. 2006;344:1334–41.
44. Khan DA, Fatima Tuz Z, Khan FA, Mubarak A. Evaluation of diagnostic accuracy of APRI for prediction of fibrosis in hepatitis C patients. *J Ayub Med Coll Abbottabad*. 2008;20:122–6.
45. Sebastiani G, Vario A, Guido M, Noventa F, Plebani M, Pistis R, et al. Stepwise combination algorithms of non-invasive markers to diagnose significant fibrosis in chronic hepatitis C. *J Hepatol*. 2006;44:686–93.

Impact of IL28B Genetic Variation on HCV-Induced Liver Fibrosis, Inflammation, and Steatosis: A Meta-Analysis

Masaya Sato¹, Mayuko Kondo¹, Ryosuke Tateishi^{1*}, Naoto Fujiwara¹, Naoya Kato², Haruhiko Yoshida¹, Masataka Taguri³, Kazuhiko Koike¹

1 Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan, **2** Unit of Disease Control Genome Medicine, Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo, Japan, **3** Department of Biostatistics and Epidemiology, Yokohama City University Medical Center, Yokohama, Kanagawa, Japan

Abstract

Background & Aims: IL28B polymorphisms were shown to be strongly associated with the response to interferon therapy in chronic hepatitis C (CHC) and spontaneous viral clearance. However, little is known about how these polymorphisms affect the natural course of the disease. Thus, we conducted the present meta-analysis to assess the impact of IL28B polymorphisms on disease progression.

Methods: A literature search was conducted using MEDLINE, EMBASE, and the Cochrane Library. Integrated odds ratios (OR) were calculated with a fixed-effects or random-effects model based on heterogeneity analyses.

Results: We identified 28 studies that included 10,024 patients. The pooled results indicated that the rs12979860 genotype CC was significantly associated (vs. genotype CT/TT; OR, 1.122; 95%CI, 1.003–1.254; $P=0.044$), and that the rs8099917 genotype TT tended to be (vs. genotype TG/GG; OR, 1.126; 95%CI, 0.988–1.284; $P=0.076$) associated, with an increased possibility of severe fibrosis. Both rs12979860 CC (vs. CT/TT; OR, 1.288; 95%CI, 1.050–1.581; $P=0.015$) and rs8099917 TT (vs. TG/GG; OR, 1.324; 95%CI, 1.110–1.579; $P=0.002$) were significantly associated with a higher possibility of severe inflammation activity. Rs8099917 TT was also significantly associated with a lower possibility of severe steatosis (vs. TG/GG; OR, 0.580; 95%CI, 0.351–0.959; $P=0.034$), whereas rs12979860 CC was not associated with hepatic steatosis (vs. CT/TT; OR, 1.062; 95%CI, 0.415–2.717; $P=0.901$).

Conclusions: IL28B polymorphisms appeared to modify the natural course of disease in patients with CHC. Disease progression seems to be promoted in patients with the rs12979860 CC and rs8099917 TT genotypes.

Citation: Sato M, Kondo M, Tateishi R, Fujiwara N, Kato N, et al. (2014) Impact of IL28B Genetic Variation on HCV-Induced Liver Fibrosis, Inflammation, and Steatosis: A Meta-Analysis. PLoS ONE 9(3): e91822. doi:10.1371/journal.pone.0091822

Editor: Ming-Lung Yu, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Taiwan

Received: October 3, 2013; **Accepted:** February 15, 2014; **Published:** March 17, 2014

Copyright: © 2014 Sato et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by the Global COE Program, “Center of Education and Research for Advanced Genome-Based Medicine: For personalized medicine and the control of worldwide infectious diseases”; the Ministry of Education, Culture, Sports, Science and Technology, Japan; by grants from the Leading Project of the Ministry of Education, Culture, Sports, Science and Technology, Japan; and by Health and Labor Sciences Research Grants for Research on Hepatitis from the Ministry of Health, Labor and Welfare, Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: tateishi-ky@umin.ac.jp

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1]. In epidemiological studies of chronic HCV infection, age, duration of infection, alcohol consumption, coinfection with human immune deficiency virus, low CD4 count, male gender, and HCV genotype 3 have been shown to be associated with histological activity [2–7]. Although these factors explain part of the extreme variability seen in the progression of fibrosis among HCV-infected patients, they do not completely account for the differences. Genetic host factors have long been suspected to play a role in chronic hepatitis C (CHC) [8–10]. Two genome-wide association studies recently reported the susceptible loci for the progression of liver cirrhosis [11,12].

Currently, patients with CHC are treated with a combination of peg-interferon (peg-IFN) and ribavirin [13,14]. Telaprevir and boceprevir, two protease inhibitors, were recently approved for patients with genotype 1 in combination with peg-IFN and ribavirin. This combination has been shown to lead to substantial improvement in the sustained virologic response rate [15,16]. Genetic variations near the interleukin 28B (IL28B) gene, encoding type III IFN- λ 3, were shown to be strongly associated with the response to peg-IFN and ribavirin treatment in patients with CHC [17–20] and with spontaneous clearance of HCV [21]. Host immune cells produce IFN and other cytokines in response to viral infection. In response to HCV, cellular sensors detect the double-stranded RNA via retinoic acid-inducible gene-I and toll-like receptor 3 and activate a pathway to produce antiviral cytokines, including alpha and beta IFNs that trigger an antiviral response to eradicate the virus [22,23].

Polymorphisms of genes involved in innate immunity are likely to influence the strength and nature of this defense system [24]. Moreover, IL28B polymorphisms were shown to be associated with lipid metabolism [25]. Thus, this genetic factor is thought to influence the natural course of HCV infection including liver fibrosis, inflammation activity, or steatosis. However, associations between IL28B polymorphisms and the state of background liver disease (fibrosis, inflammation activity, or steatosis) in patients with CHC remain controversial. Single studies may have limited statistical power to detect the modest effects of IL28B polymorphisms on disease progression.

Thus, we conducted the present meta-analysis to integrate the results of eligible studies and provide statistically reliable evidence of the role of IL28B polymorphisms in patients with CHC.

Materials and Methods

2.1 Search strategy

An electronic search was conducted in MEDLINE, EMBASE, and the Cochrane Library for articles published prior to 30 April, 2012. Search terms included *IL28B*, *IL28*, *IL-28B*, *interleukin-28B*, *interleukin 28B*, *rs12979860*, and *rs8099917*. The search was limited to the English language.

2.2 Inclusion criteria

A study was included in the current analysis if it satisfied the following criteria: (1) It evaluated the associations between IL28B polymorphisms (rs12979860 or rs8099917) and liver fibrosis, inflammation activity, or steatosis. We also included studies that evaluated fibrosis or inflammation activity using the aminotransferase platelet ratio index or ALT. (2) It provided sufficient published data for estimating odds ratios (OR) with 95% confidence intervals (CIs). In case of multiple studies based on the same population, we selected the study with the largest number of participants. A study was excluded if (1) it dealt only with coinfection of HCV and human immunodeficiency virus, (2) it dealt only with patients with a specific condition such as a comorbid disease (e.g., thalassemia) or status after liver transplantation, or (3) it only used a recessive hereditary model (rs12979860 CC + CT vs. TT, or rs8099917 TT +TG vs. GG).

2.3 Data extraction

Two authors (M.S. and M.K.) independently screened titles and abstracts for potential eligibility and full texts for final eligibility. Disagreements were resolved by consultation with a third author (R.T.). The following information was extracted or calculated from each study: first author, year of publication, country of origin, ethnicity, sex, HCV genotype, and background liver information (fibrosis, inflammation activity, or steatosis) for each genotype. The analysis was based on the dominant model (CC vs. CT and TT in rs12979860; TT vs. TG and GG in rs8099917).

2.4 Definition

In some studies, mild or severe fibrosis or inflammation activity was not defined. To compare results among studies on these outcomes, we defined Ishak level F4 to F6; METAVIR, Ludwig Batts, and Inuyama level F3 to F4; and Knodell histology activity index as severe fibrosis. We also defined METAVIR A2 to A3 as severe inflammation activity.

2.5 Statistical analysis

The association of liver fibrosis, inflammation activity, or steatosis with the IL28B genotype in patients with CHC was assessed by summary ORs and corresponding 95% CIs. Hetero-

geneity among studies was examined with I^2 statistics interpreted as the proportion of total variation contributed by between-study variation [26]. If there was no or low statistical heterogeneity among studies ($I^2 < 50\%$ and $P > 0.05$), the ORs and 95% CIs were calculated by the fixed-effects model. Otherwise, the random-effects model was adopted. When significant heterogeneity was observed, we performed a meta-regression analysis to investigate relationships between the effect of IL28B polymorphisms on liver fibrosis, inflammation activity, or steatosis; and continuous variables (proportion of patients with genotype 1 or 4 virus infection, proportion of males; and proportion of Caucasian, African-American, and Asian patients) to explore the possible reason for heterogeneity between studies [27,28]. To check for publication bias, we used the linear regression approach described by Egger et al. [29]. All calculations were performed using Comprehensive Meta-Analysis software (Biostat, Englewood, NJ).

Results

3.1 Characteristics of articles

Figure 1 shows the literature search and study selection procedures. A total of 471 potentially relevant publications up to 30 April, 2012, were initially identified through MEDLINE, EMBASE, and the Cochrane Library, 443 of which were excluded because they did not meet our inclusion criteria. Therefore, 28 studies involving a total number of 10,024 patients were included in the meta-analysis. Study characteristics are shown in Table 1. There were 5616 males and 3974 females, and the sex was not reported in the remaining 434 patients (1 study). Nineteen studies (7542 patients) evaluated liver fibrosis according to rs12979860 polymorphism and 16 studies (5052 patients) according to rs8099917 polymorphism; four studies (2301 patients) evaluated inflammation activity according to rs12979860 polymorphism and eight studies (2904 patients) according to rs8099917 polymorphism; and four studies (962 patients) evaluated steatosis according to rs12979860 polymorphism and five studies (1308 patients) according to rs8099917 polymorphism.

3.2 Fibrosis

For rs12979860, the between-study heterogeneity was not significant ($I^2 = 25\%$, $P = 0.147$); thus, the fixed-effects model was applied. The pooled results indicated that IL28B rs12979860 genotype CC was associated with an increased possibility of severe fibrosis (OR, 1.122; 95%CI, 1.003–1.254; $P = 0.044$) (Fig. 2-a). For rs8099917, there was no or low heterogeneity ($I^2 = 31\%$, $P = 0.111$), and IL28B rs8099917 genotype TT tended to be associated with a higher possibility of severe fibrosis; however, the difference did not reach statistical significance (OR, 1.126; 95%CI, 0.988–1.284; $P = 0.076$) (Fig. 2-b). Egger's test showed no evidence for publication biases for either rs12979860 ($P = 0.839$) or rs8099917 ($P = 0.342$). When restricted to studies in which only treatment-naïve patients were included, 12 studies (5865 patients) according to rs12979860 polymorphism and eight studies (3333 patients) according to rs8099917 polymorphism were extracted. The between-study heterogeneities were not significant for rs12979860 ($I^2 = 0\%$, $P = 0.615$) and rs8099917 ($I^2 = 16\%$, $P = 0.304$). For rs12979860, fixed-effect model analyses showed a higher probability of severe fibrosis in genotype CC (OR, 1.184; 95%CI, 1.040–1.348; $P = 0.010$) (Fig. 2-c), and for rs8099917, genotype TT tended to be associated with a higher possibility of severe fibrosis; however, the difference was not statistically significant (OR, 1.154; 95%CI, 0.985–1.351; $P = 0.076$) (Fig. 2-d). Egger's test showed no evidence of publication bias ($P = 0.394$ for rs12979860 and $P = 0.295$ for rs8099917).

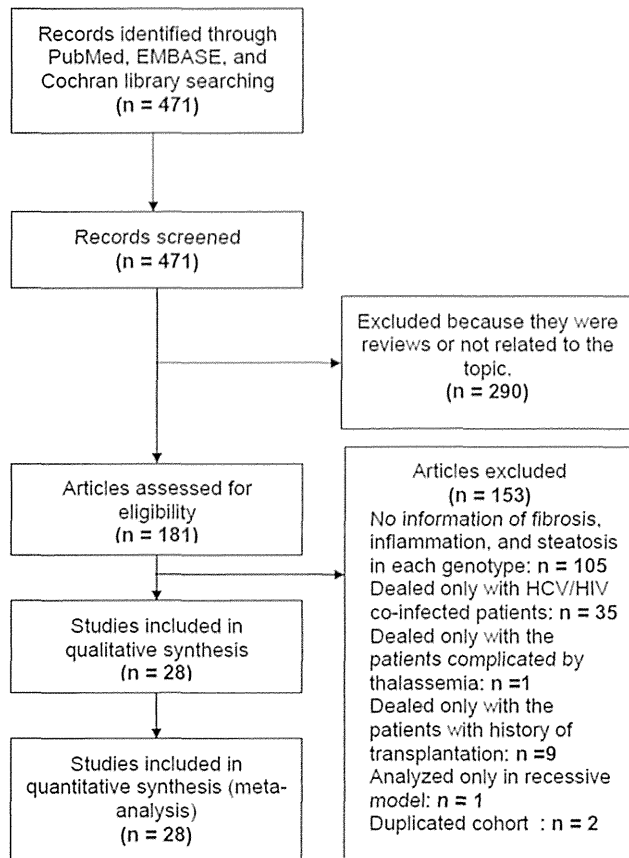


Figure 1. Literature search and study selection process. Twenty-eight individual studies that met all of the inclusion and exclusion criteria.

doi:10.1371/journal.pone.0091822.g001

3.3 Inflammation activity

The between-study heterogeneity was not significant ($I^2 = 35\%$, $P = 0.204$) for rs12979860. In the fixed-effects model, the pooled results indicated that IL28B rs12979860 genotype CC was associated with a higher possibility of severe inflammation activity (OR, 1.288; 95%CI, 1.050–1.581; $P = 0.015$) (Fig. 3-a). For rs8099917, there was no or low heterogeneity ($I^2 = 0\%$, $P = 0.598$), and IL28B rs8099917 genotype TT was also associated with a higher possibility of severe inflammation activity (OR, 1.324; 95%CI, 1.110–1.579; $P = 0.002$) (Fig. 3-b). Egger's test showed no evidence of publication biases for rs12979860 ($P = 0.448$) and rs8099917 ($P = 0.531$). When restricted to studies in which only treatment-naïve patients were included, three studies (2192 patients) according to rs12979860 polymorphism and two studies (1769 patients) according to rs8099917 polymorphism were extracted. Significant heterogeneities were found for rs12979860 ($I^2 = 53\%$, $P = 0.120$); thus, the random-effect model was applied. The pooled results indicated that IL28B rs12979860 genotype was not associated with inflammatory activity (OR, 1.340; 95%CI, 0.938–1.916; $P = 0.108$) (Fig. 3-c). For rs8099917, the between-study heterogeneity was not significant ($I^2 = 0\%$, $P = 0.585$). In the fixed-effects model, genotype TT tended to be associated with a higher possibility of severe inflammation activity (OR, 1.217; 95%CI, 0.978–1.515; $P = 0.079$) (Fig. 3-d). Egger's test showed no evidence of publication bias in rs12979860 ($P = 0.646$). For rs8099917, Egger's test was not applicable because only 2 studies were included. We also performed a meta-regression analysis for

rs12979860 because significant heterogeneities were observed. Table 2 shows the results of these meta-regression analyses. Significant correlation was observed between rs12979860 polymorphisms and the proportion of patients with genotype 1 or 4 virus (slope, 2.992 ± 1.497 ; $P = 0.046$).

3.4 Steatosis

Significant heterogeneities were found for rs12979860 ($I^2 = 86\%$, $P < 0.001$) and rs8099917 ($I^2 = 52\%$, $P = 0.082$); thus, we applied the random-effects model for this outcome. The pooled results indicated that IL28B rs12979860 genotype CC was not associated with hepatic steatosis (OR, 1.062; 95%CI, 0.415–2.717, $P = 0.901$) (Fig. 4-a), whereas rs8099917 TT was significantly associated with a lower possibility of severe steatosis (OR, 0.580; 95%CI, 0.351–0.959; $P = 0.034$) (Fig. 4-b). Egger's test showed no evidence of publication biases for rs12979860 ($P = 0.238$) or rs8099917 ($P = 0.182$). We also performed a meta-regression analysis because significant heterogeneities were observed. Table 3 shows the results of these meta-regression analyses. In terms of the effect of rs12979860 on steatosis, significant correlations were observed between the proportion of patients with genotype 1 or 4 virus (slope, -4.947 ± 1.086 ; $P < 0.001$), the proportion of Caucasian patients (slope, 7.361 ± 1.569 ; $P < 0.001$), and the proportion of African-American patients (slope, -8.996 ± 1.918 ; $P < 0.001$). We also observed a significant correlation between the effect of rs8099917 polymorphism on steatosis and the proportion of male patients (slope, 6.225 ± 2.530 ; $P = 0.014$) (Fig. 5). Finally, we observed significant correlations between rs8099917 polymorphisms and the proportion of patients with genotype 1 or 4 virus (slope, -2.704 ± 1.277 ; $P = 0.034$), the proportion of Caucasian patients (slope, 1.168 ± 0.422 ; $P = 0.006$), and the proportion of Asian patients (slope, -1.049 ± 0.398 ; $P = 0.008$). When restricted to studies in which only treatment-naïve patients were included, two studies (495 patients) according to rs12979860 polymorphism and four studies (812 patients) according to rs8099917 polymorphism were extracted. The between-study heterogeneities were not significant for rs12979860 ($I^2 = 0\%$, $P = 0.823$) and rs8099917 ($I^2 = 41\%$, $P = 0.166$). For rs12979860, fixed-effect model analyses showed that rs12979860 genotype CC was significantly associated with a higher possibility of severe steatosis (OR, 1.708; 95%CI, 1.047–2.787; $P = 0.032$) (Fig. 4-c), whereas rs8099917 TT was significantly associated with a lower possibility of severe steatosis (OR, 0.675; 95%CI, 0.474–0.960; $P = 0.026$) (Fig. 4-d). Egger's test showed no evidence of publication bias in rs8099917 ($P = 0.554$). For rs12979860, Egger's test was not applicable because only 2 studies were included.

Discussion

In the present study, we evaluated the association between IL28B polymorphisms and the background liver disease (fibrosis, inflammation activity, or steatosis) in patients with CHC. The rs12979860 CC genotype was significantly associated with a higher probability of severe fibrosis (Fig. 2-c), and the rs8099917 TT genotype tended to be associated with a higher possibility of severe fibrosis (Fig. 2-d). The accumulation of liver inflammation promotes liver fibrosis, and these polymorphisms are associated with the effect of IFN-based treatment; therefore, past treatment might alter the results. Thus, we also analyzed studies involving only patients without a history of IFN-based treatment; however, the results were not changed.

The rs12979860 CC and rs8099917 TT genotypes were also associated with a higher possibility of severe inflammation activity. Genetic variations near the IL28B gene were originally reported as

Table 1. Main characteristics of all studies included in the meta-analysis.

First author (year)	Ref.	Population ethnicity, region	IL-28B SNP rsID, Allele	Outcome measure F(Fibrosis) A(Activity) S(Steatosis)	Patients*			HCV genotype	Genotype for patients rs12979860		Genotype for patients rs8099917	
					Male	Female	Total		CC	CT/TT	TT	TG/GG
					Abe (2010)	[48]	Asian, Japan		rs8099917 T/G	F, A: Inuyama	212	152
Honda (2010)	[49]	Asian, Japan	rs8099917 T/G	F, A: Inuyama	58	33	91	1			60	31
Lotrich (2010)	[50]	Mixed (African-American/Caucasian), USA	rs12979860 C/T	F: Ishak	101	32	133	1/2	57	76		
Monte (2010)	[51]	Caucasian, Spain	rs12979860 C/T	F: Scheuer	166	117	283	1–4	129	154		
Thompson (2010)	[52]	Mixed (African-American/Caucasian/Asian/Hispanic), USA	rs12979860 C/T	F: METAVIR	986	642	1628	1	538	1090		
Bochud (2011)	[53]	Caucasian, Switzerland	rs12979860 C/T rs8099917 T/G	F: Ishak, A: ALT S: 163 Histological finding		79	242	1–3	90	150	150	92
Dill MT (2011)	[54]	Caucasian, Switzerland	rs12979860 C/T rs8099917 T/G	F, A: METAVIR	30	79	109	1–4	33	96	52	57
Fabris (2011)	[44]	Caucasian, Italy	rs12979860 C/T	F: Ishak	N.A	N.A	434	1–4	133	301		
Falletti (2011)	[55]	Caucasian, Italy	rs12979860 C/T	F: Ishak	357	272	629	1–4	205	424		
Kurosaki (2011)	[56]	Asian, Japan	rs8099917 T/G	F: METAVIR S: Histological finding	250	246	496	1			269	106
Lagging (2011)	[57]	Caucasian, Sweden	rs12979860 C/T rs8099917 T/G	F: Ishak S: Histological finding	169	83	252	1–4	93	159	153	99
Lin (2011)	[58]	Asian, Taiwan	rs12979860 C/T rs8099917 T/G	F: METAVIR	123	68	191	1	171	20	170	21
Lindh (2011)-1	[59]	Mixed (Caucasian/Asian), Sweden	rs12979860 C/T rs8099917 T/G	F: Batts Ludwig	67	43	110	1	38	72	66	44
Lindh (2011)-2	[60]	Caucasian, Sweden	rs12979860 C/T	F: Ishak	204	137	341	2/3	150	191		
Marabita (2011)	[61]	Caucasian, Italy	rs12979860 C/T rs8099917 T/G	F: Ishak	129	118	247	1–4	88	159	131	116
Miyamura (2011)	[62]	Asian, Japan	rs8099917 T/G	F, A: Inuyama	37	42	79	1			53	26
Moghaddam(2011)	[63]	Caucasian, Norway	rs12979860 C/T rs8099917 T/G	F: APRI score	166	115	281	3	129	152	201	80
Rueda (2011)	[64]	Caucasian, Spain	rs12979860 C/T	F, A: Scheuer	246	177	423	1–4	83	184		
Tillman (2011)	[35]	Mixed (African-American/Caucasian/Asian), USA	rs12979860 C/T rs8099917 T/G	S: Histological finding	215	110	325	1	88	237	97	67
Yu (2011)	[65]	Asian, Taiwan	rs8099917 T/G	F: Knodell and Scheuer	264	218	482	2			315	34
Asahina (2011)	[66]	Asian, Japan	rs12979860 C/T rs8099917 T/G	F: Inuyama	28	60	88	1	54	34	54	34

Table 1. Cont.

First author (year)	Ref.	Population ethnicity, region	IL-28B SNP rsID, Allele	Outcome measure F(Fibrosis) A(Activity) S(Steatosis)	Patients*			HCV genotype	Genotype for patients rs12979860		Genotype for patients rs8099917	
					Male	Female	Total		CC	CT/TT	TT	TG/GG
Bochud (2012)	[47]	Caucasian, Switzerland	rs12979860 C/T rs8099917 T/G	F, A: METAVIR	870	657	1527	1–4	534	993	855	672
Mach (2012)	[67]	Slav: Poland	rs12979860 C/T	F: Batts Ludwig	82	60	142	1	38	104		
Miyashita (2012)	[68]	Asian, Japan	rs8099917 T/G	F, A: Desmet	88	132	220	1/2			155	63
Ohnishi (2012)	[69]	Asian, Japan	rs8099917 T/G	S: Histological finding	83	70	153	1			116	37
Rembeck (2012)	[70]	Caucasian, Sweden	rs12979860 C/T	F: Ishak	199	140	339	2/3	144	179		
Tolmane (2012)	[71]	Caucasian, Latvia	rs12979860 C/T	F: Knodell histology activity index S: Histological finding	84	58	142	1–3	41	80		
Toyoda (2012)	[72]	Asian, Japan	rs8099917 T/G	F, A: METAVIR	139	133	272	1			187	59

*Patients included in the original study.

Thus, patients without information regarding IL28B polymorphism were also included.

APRI, aminotransferase platelet ratio index.

doi:10.1371/journal.pone.0091822.t001

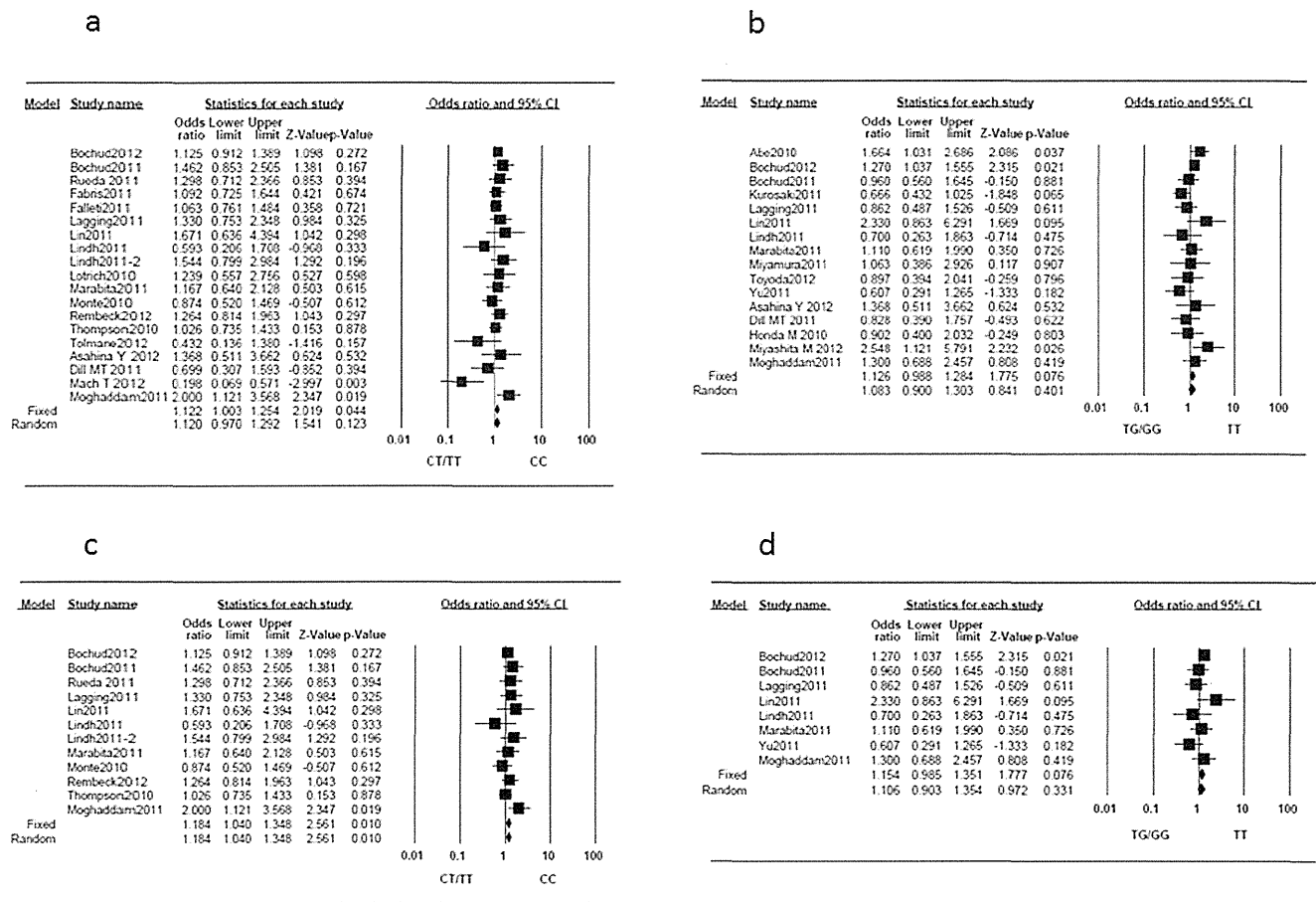


Figure 2. Forest plot of the IL28B genotypes and the risk of severe fibrosis. (a) rs12979860 in all patients, (b) rs8099917 in all patients, (c) rs12979860 in treatment-naïve patients, and (d) rs8099917 in treatment-naïve patients. doi:10.1371/journal.pone.0091822.g002

strong predictors of a sustained viral response [17–20] or spontaneous clearance of HCV [21]. The level of IL28B gene transcripts is reportedly higher in patients homozygous for the IFN responsive allele [18,19]. Therefore, in patients with the rs12979860 CC and rs8099917 TT genotype, IL28B production, which induces expression of interferon-stimulated genes, including some inflammatory cytokines, was thought to be increased. This may be the underlying cause of the higher inflammation activity and progressed fibrosis in patients with the IFN responsive allele. In analysis with the studies involving only patients without a history of IFN-based treatment, rs12979860 CC and rs8099917 TT genotypes were associated with higher possibility of having severe inflammation activity; however, the differences did not reach to the significant level. Only three studies according to rs12979860 polymorphism and two studies according to rs8099917 polymorphism were included when restricted to studies with only treatment-naïve patients, and may be underpowered to detect the effects of IL28B polymorphisms on inflammation activity. The further analyses with larger sample are needed to confirm this association. Additionally, meta-regression analysis showed that the effect of the rs12979860 polymorphism was influenced by viral genotype distribution. This result may imply a different influence of rs12979860 polymorphism on immune response according to viral genotype in treatment-naïve patients.

IL28B polymorphisms were also shown to be associated with lipid metabolism [25]. In the present study, the rs8099917 TT

genotype was significantly associated with a lower possibility of severe steatosis. This association still remained statistically significant after we restricted to studies in which only treatment-naïve patients were included. The lower hepatic steatosis in patients with the IFN responsive allele could be explained by a more efficient export of lipids from hepatocytes. Higher interferon expression was shown to lead to suppression of lipoprotein lipase, which would result in decreased conversion of VLDL to LDL and subsequent higher steatosis [30–33]. The difference in IL28B expression might cause an aberration of lipid metabolism in patients with CHC. We found no significant association of rs12979860 with steatosis. And when we restricted to treatment-naïve patients, rs12979860 CC genotype was significantly associated with a higher possibility of severe steatosis. Previous studies have shown that racial differences or viral genotypes make a difference in the effects of rs12979860 and rs8099917 polymorphisms [34,35]. This may explain the discrepancy between the effect of rs12979860 and rs8099917 on hepatic steatosis. However, only four studies (962 patients) were included in the analysis of rs12979860; or when it comes to the studies with only treatment-naïve patients, only two studies (495 patients) were extracted. Thus, we should not make any definite conclusion on this matter right now. Further studies with larger sample sizes are needed to identify their exact correlation.

According to the meta-regression analysis, the effect of rs8099917 polymorphisms on steatosis became smaller with the

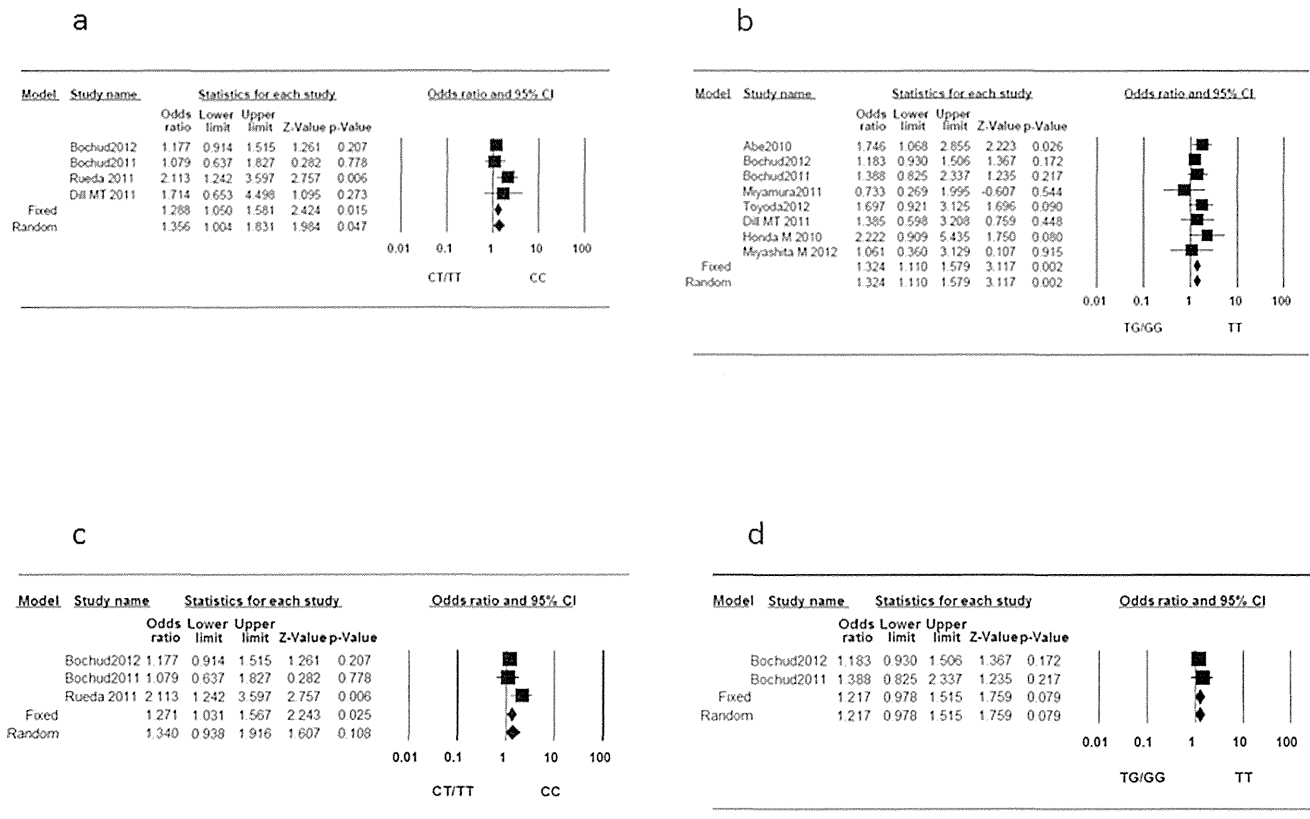


Figure 3. Forest plot of the IL28B genotypes and the risk of severe inflammation activity. (a) rs12979860 and (b) rs8099917. (c) rs12979860 in treatment-naïve patients, and (d) rs8099917 in treatment-naïve patients. doi:10.1371/journal.pone.0091822.g003

increase in the male proportion (Fig. 5), suggesting that a sexual dimorphism might be involved in the effect of rs8099917 polymorphisms on the liver fat content. Although the present study cannot explain the interaction between the polymorphism and sex, immune systems responding to IFN are reportedly controlled by estrogenic sex hormones [36,37]. Differences in IL28B expression mediated by sex hormones could be a possible

mechanism for the sexual dimorphism in the effect of rs8099917 polymorphisms on liver steatosis.

The rs738409 genotype within the patatin-like phospholipase domain containing 3 locus was also reported to be associated with hepatic steatosis in patients with CHC [38–40]. Notably, previous meta-analysis evaluating the effect of patatin-like phospholipase domain containing 3 polymorphisms on steatosis also reported a

Table 2. Meta-regression analysis between each continuous variable among the studies (only treatment-naïve patients were included) and the effect (log odds ratio) of IL28B polymorphisms on inflammation activity.

Variables	Slope*	Standard error	P-value
Proportion of patients with genotype 1 or 4 virus, per 1% increase			
rs12979860	2.992	1.497	0.046
Proportion of male patients, per 1% increase			
rs12979860	-2.963	5.802	0.610
Proportion of Caucasian patients, per 1% increase			
rs12979860†	—	—	—
Proportion of African-American patients, per 1% increase			
rs12979860†	—	—	—
Proportion of Asian patients, per 1% increase			
rs12979860†	—	—	—

*Positive (negative) slope values indicate that the proportions of patients with the rs12979860 CC genotype with severe inflammation activity are increasing (decreasing) as the values of each contentious variable (proportions of genotype 1 or 4 virus, male, or each race) is increasing.

†We could not perform meta-regression analyses for these outcomes because only caucasian patients were included in all 3 studies included in this analysis. doi:10.1371/journal.pone.0091822.t002

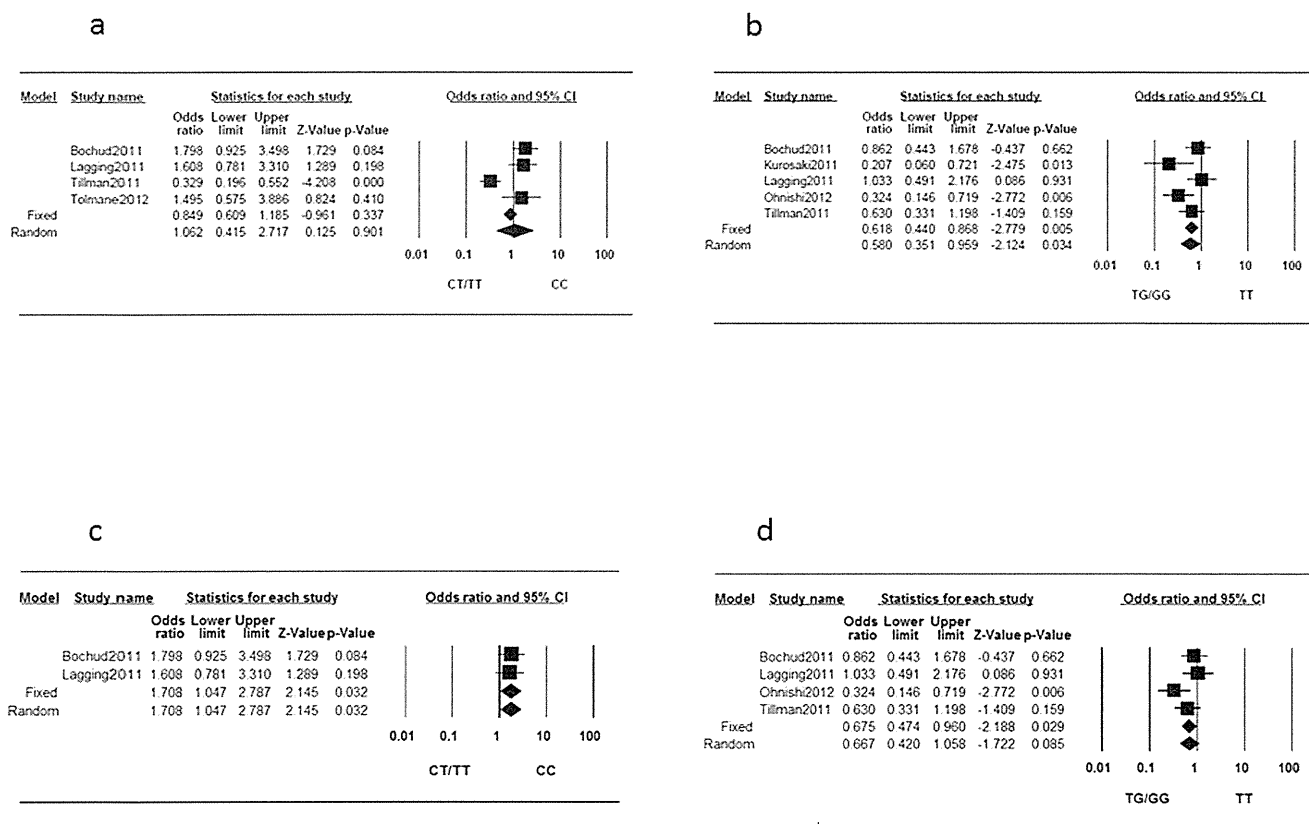


Figure 4. Forest plot of the IL28B genotypes and the risk of hepatic steatosis. (a) rs12979860 and (b) rs8099917. (c) rs12979860 in treatment-naïve patients, and (d) rs8099917 in treatment-naïve patients. doi:10.1371/journal.pone.0091822.g004

Table 3. Meta-regression analysis between each continuous variable among the studies and the effect (log odds ratio) of IL28B polymorphisms on steatosis.

Variables	Slope*	Standard error	P-value
Proportion of patients with genotype 1 or 4 virus, per 1% increase			
rs12979860	-4.947	1.086	<0.001
rs8099917	-2.704	1.277	0.034
Proportion of male patients, per 1% increase			
rs12979860	-2.899	16.577	0.861
rs8099917	6.225	2.530	0.014
Proportion of Caucasian patients, per 1% increase			
rs12979860	7.361	1.569	<0.001
rs8099917	1.168	0.422	0.006
Proportion of African-American patients, per 1% increase			
rs12979860	-8.996	1.918	<0.001
rs8099917	0.142	2.147	0.947
Proportion of Asian patients, per 1% increase			
rs12979860†	-	-	-
rs8099917	-1.049	0.398	0.008

*Positive (negative) slope values indicate that the proportions of patients with the rs12979860 CC or rs8099917 TT genotypes with severe steatosis are increasing (decreasing) as the values of each contentious variable (proportions of genotype 1 or 4 virus, male, or each race) is increasing.

†We could not perform a meta-regression analysis for this outcome because only one patient was included in the corresponding studies.

doi:10.1371/journal.pone.0091822.t003

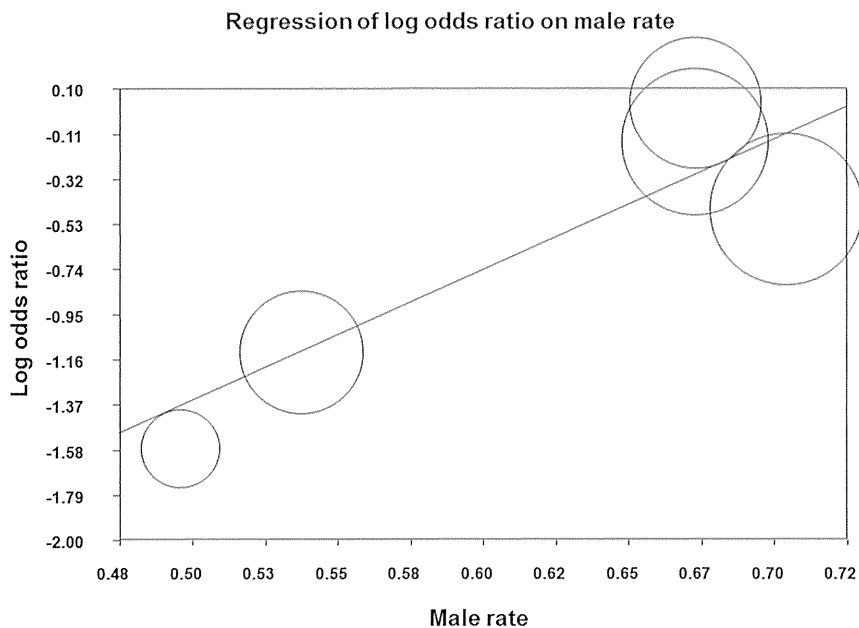


Figure 5. Meta-regression plot for log odds ratios in rates of patients with severe hepatic steatosis by proportion of males (%) in rs8099917.

doi:10.1371/journal.pone.0091822.g005

negative correlation between the male proportion and the effect of rs738409 on the liver fat content in nonalcoholic fatty liver disease [41]. Interestingly, the meta-regression analysis in the present study showed that the effect of the IL28B (rs12979860 and rs8099917) polymorphisms on steatosis was also influenced by racial and viral genotype distributions.

In the present study, we included studies that did not report the associations between IL28B genotypes and background liver diseases as study outcomes, but provided raw data that allowed us to calculate the OR for each outcome, which may have minimized potential publication bias. In fact, no publication bias was observed in the present study. The Human Genome Epidemiology Network highlighted the necessity of meta-analysis before evidence for a particular association can be regarded as strong [42]. The impact of IL28B genotypes on the disease progression found in the present meta-analysis may provide clinically important information in the follow-up of patients with CHC. The effect of IL28B polymorphisms on hepatocarcinogenesis, which is also crucial information in the HCC screening of patients with CHC, remains controversial [43–47]. Further analysis with larger sample sizes may be needed to elucidate the exact effect of IL28B polymorphisms on hepatocarcinogenesis.

A potential limitation of this study is inter-study variability in the outcome measure and the definition of “severe” among studies, where some discrepancies among studies exist. The studies without a pathological diagnosis, using laboratory data as

surrogates, were also included. These studies may have diminished the accuracy of our research results concerning liver disease severity.

In conclusion, the present study highlighted the impact of IL28B polymorphisms on liver fibrosis, inflammation activity, and steatosis in patients with CHC. Disease progression appeared to be promoted in patients with rs12979860 CC or rs8099917 TT genotypes. The current findings may provide clinically important information in the follow-up of patients with CHC.

Supporting Information

Checklist S1 PRISMA 2009 Checklist.
(DOC)

Acknowledgments

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: <http://www.textcheck.com/certificate/IWcYpT>.

Author Contributions

Conceived and designed the experiments: MS RT NK. Performed the experiments: MS MK RT. Analyzed the data: MS RT. Contributed reagents/materials/analysis tools: MS. Wrote the paper: MS RT HY. Critical revision of manuscript: NF MT KK.

References

- Barrera JM, Bruguera M, Ercilla MG, Gil C, Celis R, et al. (1995) Persistent hepatitis C viremia after acute self-limiting posttransfusion hepatitis C. *Hepatology* 21: 639–644.
- Poynard T, Bedossa P, Opolon P (1997) Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 349: 825–832.
- Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, et al. (1999) Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 29: 1215–1219.
- Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, et al. (2000) Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* 31: 828–833.
- Massard J, Ratzu V, Thabut D, Moussalli J, Lebray P, et al. (2006) Natural history and predictors of disease severity in chronic hepatitis C. *J Hepatol* 44: S19–24.
- Bochud PY, Cai T, Overbeck K, Bochud M, Dufour JF, et al. (2009) Genotype 3 is associated with accelerated fibrosis progression in chronic hepatitis C. *J Hepatol* 51: 655–666.

7. De Nicola S, Aghemo A, Rumi MG, Colombo M (2009) HCV genotype 3: an independent predictor of fibrosis progression in chronic hepatitis C. *J Hepatol* 51: 964–966.
8. Thursz M, Yallop R, Goldin R, Trepo C, Thomas HC (1999) Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. *Lancet* 354: 2119–2124.
9. Pradat P, Tillmann HL, Sauleda S, Braconier JH, Saracco G, et al. (2007) Long-term follow-up of the hepatitis C HENCORE cohort: response to therapy and occurrence of liver-related complications. *J Viral Hepat* 14: 556–563.
10. Kato N, Ji G, Wang Y, Baba M, Hoshida Y, et al. (2005) Large-scale search of single nucleotide polymorphisms for hepatocellular carcinoma susceptibility genes in patients with hepatitis C. *Hepatology* 42: 846–853.
11. Urabe Y, Ochi H, Kato N, Kumar V, Takahashi A, et al. (2013) A genome-wide association study of HCV-induced liver cirrhosis in the Japanese population identifies novel susceptibility loci at the MHC region. *J Hepatol*; 58 (5): 875–82
12. Patin E, Kutalik Z, Guernon J, Bibert S, Nalpas B, et al. (2012) Genome-Wide Association Study Identifies Variants Associated with Progression of Liver Fibrosis from HCV Infection. *Gastroenterology*; 143 (5): 1244–52
13. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, et al. (2004) Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 140: 346–355.
14. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, et al. (2001) Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 358: 958–965.
15. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, et al. (2009) Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 360: 1827–1838.
16. Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, et al. (2011) Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 364: 1195–1206.
17. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, et al. (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 461: 399–401.
18. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, et al. (2009) Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 41: 1105–1109.
19. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, et al. (2009) IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 41: 1100–1104.
20. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, et al. (2010) Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 138: 1338–1345. [e1331–1337](https://doi.org/10.1053/j.gastro.2010.03.031).
21. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, et al. (2009) Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 461: 798–801.
22. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, et al. (2004) The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 5: 730–737.
23. Moriyama M, Kato N, Otsuka M, Shao RX, Taniguchi H, et al. (2007) Interferon-beta is activated by hepatitis C virus NS5B and inhibited by NS4A, NS4B, and NS5A. *Hepatology* 45: 302–310.
24. Li CZ, Kato N, Chang JH, Muroyama R, Shao RX, et al. (2009) Polymorphism of OAS-1 determines liver fibrosis progression in hepatitis C by reduced ability to inhibit viral replication. *Liver Int* 29: 1413–1421.
25. Li JH, Lao XQ, Tillmann HL, Rowell J, Patel K, et al. (2010) Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. *Hepatology* 51: 1904–1911.
26. Borenstein M, Hedges LV, Higgins JP, Rothstein HR (2009) *Introduction to Meta-analysis*. West Sussex: John Wiley & Sons Ltd.
27. Baker WL, White CM, Cappelleri JC, Kluger J, Coleman CI (2009) Understanding heterogeneity in meta-analysis: the role of meta-regression. *Int J Clin Pract* 63: 1426–1434.
28. Thompson SG, Sharp SJ (1999) Explaining heterogeneity in meta-analysis: a comparison of methods. *Stat Med* 18: 2693–2708.
29. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315: 629–634.
30. Schectman G, Kaul S, Mueller RA, Borden EC, Kissebah AH (1992) The effect of interferon on the metabolism of LDLs. *Arterioscler Thromb* 12: 1053–1062.
31. Ehnholm C, Aho K, Huttunen JK, Kostianen E, Mattila K, et al. (1982) Effect of interferon on plasma lipoproteins and on the activity of postheparin plasma lipases. *Arteriosclerosis* 2: 68–73.
32. Shimohara E, Yamashita S, Kihara S, Hirano K, Ishigami M, et al. (1997) Interferon alpha induces disorder of lipid metabolism by lowering postheparin lipases and cholesteryl ester transfer protein activities in patients with chronic hepatitis C. *Hepatology* 25: 1502–1506.
33. Andrade RJ, Garcia-Escano MD, Valdivielso P, Alcantara R, Sanchez-Chaparro MA, et al. (2000) Effects of interferon-beta on plasma lipid and lipoprotein composition and post-heparin lipase activities in patients with chronic hepatitis C. *Aliment Pharmacol Ther* 14: 929–935.
34. Sarrazin C, Susser S, Doehring A, Lange CM, Muller T, et al. (2011) Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. *J Hepatol* 54: 415–421.
35. Tillmann HL, Patel K, Muir AJ, Guy CD, Li JH, et al. (2011) Beneficial IL28B genotype associated with lower frequency of hepatic steatosis in patients with chronic hepatitis C. *J Hepatol*; 55 (6): 1195–200
36. Nakaya M, Tachibana H, Yamada K (2006) Effect of estrogens on the interferon-gamma producing cell population of mouse splenocytes. *Biosci Biotechnol Biochem* 70: 47–53.
37. Siracusa MC, Overstreet MG, Housseau F, Scott AL, Klein SL (2008) 17beta-estradiol alters the activity of conventional and IFN-producing killer dendritic cells. *J Immunol* 180: 1423–1431.
38. Cai T, Dufour JF, Muellhaupt B, Gerlach T, Heim M, et al. (2011) Viral Genotype-Specific Role of PNPLA3, PPARG, MBOAT2 and IL28B in Hepatitis C Virus-Associated Steatosis. *J Hepatol*.
39. Trepo E, Pradat P, Pothoff A, Momozawa Y, Quertinmont E, et al. (2011) Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. *Hepatology* 54: 60–69.
40. Valenti L, Rumi M, Galmozzi E, Aghemo A, Del Menico B, et al. (2011) Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 53: 791–799.
41. Sookoian S, Pirola CJ (2011) Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 53: 1883–1894.
42. Ioannidis JP, Boffetta P, Little J, O'Brien TR, Uitterlinden AG, et al. (2008) Assessment of cumulative evidence on genetic associations: interim guidelines. *Int J Epidemiol* 37: 120–132.
43. Asahina Y, Tanaka K, Suzuki Y, Tamaki N, Hoshioka T, et al. (2011) Association between IL28B gene variation and development of hepatocellular carcinoma after interferon therapy in patients with chronic hepatitis c. *Journal of Hepatology* 54: S37.
44. Fabris C, Falletti E, Cussigh A, Bitetto D, Fontanini E, et al. (2011) IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol* 54: 716–722.
45. Joshita S, Umemura T, Katsuyama Y, Ichikawa Y, Kimura T, et al. (2011) Association of IL28B gene polymorphism with development of hepatocellular carcinoma in Japanese patients with chronic hepatitis C virus infection. *Hum Immunol*; 73 (3): 298–300
46. Miura M, Maekawa S, Kadokura M, Sueki R, Komase K, et al. (2011) Analysis of viral amino acids sequences and the IL28B SNP influencing the development of hepatocellular carcinoma in chronic hepatitis C. *Hepatology* 54: 17 [Epub ahead of print].
47. Bochud PY, Bibert S, Kutalik Z, Patin E, Guernon J, et al. (2011) IL28B alleles associated with poor hepatitis C virus (HCV) clearance protect against inflammation and fibrosis in patients infected with non-1 HCV genotypes. *Hepatology*; 55 (2): 384–94
48. Abe H, Ochi H, Maekawa T, Hayes CN, Tsuge M, et al. (2010) Common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. *J Hepatol* 53: 439–443.
49. Honda M, Sakai A, Yamashita T, Nakamoto Y, Mizukoshi E, et al. (2010) Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. *Gastroenterology* 139: 499–509.
50. Lotrich FE, Loftis JM, Ferrell RE, Rabinovitz M, Hauser P (2010) IL28B Polymorphism Is Associated with Both Side Effects and Clearance of Hepatitis C During Interferon-Alpha Therapy. *J Interferon Cytokine Res*; Dec 6 [Epub ahead of print].
51. Montes-Cano MA, Garcia-Lozano JR, Abad-Molina C, Romero-Gomez M, Barroso N, et al. (2010) Interleukin-28B genetic variants and hepatitis virus infection by different viral genotypes. *Hepatology* 52: 33–37.
52. Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, et al. (2010) Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology* 139: 120–129. [e118](https://doi.org/10.1053/j.gastro.2010.03.031).
53. Bochud PY, Bibert S, Negro F, Haegmans B, Soulier A, et al. (2011) IL28B polymorphisms predict reduction of HCV RNA from the first day of therapy in chronic hepatitis C. *J Hepatol*; 55 (5): 980–8.
54. Dill MT, Duong FH, Vogt JE, Bibert S, Bochud PY, et al. (2011) Interferon-induced gene expression is a stronger predictor of treatment response than IL28B genotype in patients with hepatitis C. *Gastroenterology* 140: 1021–1031.
55. Falletti E, Bitetto D, Fabris C, Cussigh A, Fornasiere E, et al. (2011) Role of Interleukin 28B rs12979860 C/T Polymorphism on the Histological Outcome of Chronic Hepatitis C: Relationship with Gender and Viral Genotype. *J Clin Immunol*; 31 (5): 891–9.
56. Kurosaki M, Tanaka Y, Nishida N, Sakamoto N, Enomoto N, et al. (2011) Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. *J Hepatol* 54: 439–448.
57. Lagging M, Askariieh G, Negro F, Bibert S, Soderholm J, et al. (2011) Response prediction in chronic hepatitis C by assessment of IP-10 and IL28B-related single nucleotide polymorphisms. *PLoS One* 6: e17232.

58. Lin CY, Chen JY, Lin TN, Jeng WJ, Huang CH, et al. (2011) IL28B SNP rs12979860 is a critical predictor for on-treatment and sustained virologic response in patients with hepatitis C virus genotype-1 infection. *PLoS One* 6: e18322.
59. Lindh M, Lagging M, Arnholm B, Eilard A, Nilsson S, et al. (2011) IL28B polymorphisms determine early viral kinetics and treatment outcome in patients receiving peginterferon/ribavirin for chronic hepatitis C genotype 1. *J Viral Hepat* 18: e325–331.
60. Lindh M, Lagging M, Farkkila M, Langeland N, Morch K, et al. (2011) Interleukin 28B gene variation at rs12979860 determines early viral kinetics during treatment in patients carrying genotypes 2 or 3 of hepatitis C virus. *J Infect Dis* 203: 1748–1752.
61. Marabita F, Aghemo A, De Nicola S, Rumi MG, Cheroni C, et al. (2011) Genetic variation in the interleukin-28B gene is not associated with fibrosis progression in patients with chronic hepatitis C and known date of infection. *Hepatology* 54: 1127–1134.
62. Miyamura T, Kanda T, Nakamoto S, Wu S, Fujiwara K, et al. (2011) Hepatic STAT1-nuclear translocation and interleukin 28B polymorphisms predict treatment outcomes in hepatitis C virus genotype 1-infected patients. *PLoS ONE*; 6 (12): e28617.
63. Moghaddam A, Melum E, Reinton N, Ring-Larsen H, Verbaan H, et al. (2011) IL28B genetic variation and treatment response in patients with hepatitis C virus genotype 3 infection. *Hepatology* 53: 746–754.
64. de Rueda PM, Lopez-Nevot MA, Saenz-Lopez P, Casado J, Martin-Casares A, et al. (2011) Importance of Host Genetic Factors HLA and IL28B as Predictors of Response to Pegylated Interferon and Ribavirin. *Am J Gastroenterol*.
65. Yu ML, Huang CF, Huang JF, Chang NC, Yang JF, et al. (2011) Role of interleukin-28B polymorphisms in the treatment of hepatitis C virus genotype 2 infection in Asian patients. *Hepatology* 53: 7–13.
66. Asahina Y, Tsuchiya K, Muraoka M, Tanaka K, Suzuki Y, et al. (2011) Association of gene expression involving innate immunity and genetic variation in IL28B with antiviral response. *Hepatology*.
67. Mach T, Ciesla A, Sanak M, Golwacki M, Warunek W, et al. (2012) The importance of IL28B polymorphism in response to pegylated interferon (alpha) and ribavirin in chronic hepatitis caused by HCV genotype 1b. *Przegląd Gastroenterologiczny* 7: 38–42.
68. Miyashita M, Ito T, Sakaki M, Kajiwara A, Nozawa H, et al. (2012) Genetic polymorphism in cyclooxygenase-2 promoter affects hepatic inflammation and fibrosis in patients with chronic hepatitis C. *J Viral Hepat* 19: 608–614.
69. Ohnishi M, Tsuge M, Kohno T, Zhang Y, Abe H, et al. (2012) IL28B polymorphism is associated with fatty change in the liver of chronic hepatitis C patients. *J Gastroenterol* 47: 834–844.
70. Rembeck K, Alsio A, Christensen PB, Farkkila M, Langeland N, et al. (2012) Impact of IL28B-related single nucleotide polymorphisms on liver histopathology in chronic hepatitis C genotype 2 and 3. *PLoS One* 7: e29370.
71. Tolmane I, Rozentale B, Keiss J, Ivancenko L, Subnikova N, et al. (2012) Interleukin 28B Gene Polymorphism and Association with Chronic Hepatitis C Therapy Results in Latvia. *Hepat Res Treat*: 324090.
72. Toyoda H, Kumada T, Tada T, Hayashi K, Honda T, et al. (2012) Predictive value of early viral dynamics during peginterferon and ribavirin combination therapy based on genetic polymorphisms near the IL28B gene in patients infected with HCV genotype 1b. *J Med Virol* 84: 61–70.

The role of microRNAs in hepatocarcinogenesis: current knowledge and future prospects

Motoyuki Otsuka · Takahiro Kishikawa ·
Takeshi Yoshikawa · Motoko Ohno ·
Akemi Takata · Chikako Shibata · Kazuhiko Koike

Received: 17 October 2013 / Accepted: 4 November 2013 / Published online: 21 November 2013
© Springer Japan 2013

Abstract MicroRNAs (miRNAs) are small, noncoding RNA molecules that regulate gene expression post-transcriptionally through complementary base pairing with thousands of messenger RNAs. Although the precise biological functions of individual miRNAs are still unknown, miRNAs are speculated to play important roles in diverse biological processes through fine regulation of their target gene expression. A growing body of data indicates the deregulation of miRNAs during hepatocarcinogenesis. In this review, we summarize recent findings regarding deregulated miRNA expression and their possible target genes in hepatocarcinogenesis, with emphasis on inflammation-related hepatocarcinogenesis. Because miRNA-based strategies are being applied to clinical therapeutics, precise knowledge of miRNA functions is crucial both scientifically and clinically. We discuss the current open questions from these points of view, which must be clarified in the near future.

Keywords MicroRNA · Hepatocarcinogenesis · Inflammation

Introduction

MicroRNAs (miRNAs) are short, single-stranded, non-coding RNAs, which are expressed in most organisms, from plants to vertebrates [1]. Since the discovery of the miRNA *lin-4* in *Caenorhabditis elegans* [2, 3], 1,872 miRNA precursors and 2,578 mature miRNA sequences in humans have been deposited in miRBase, a public repository hosted by the Sanger Institute, as of November 2013 [4]. Bioinformatic predictions suggest that miRNAs regulate more than 30 % of human protein-coding genes [5–7]. Through the regulation of gene expression, miRNAs are involved in various physiological and pathological processes, including cell proliferation, apoptosis, differentiation, metabolism, oncogenesis and oncogenic suppression [8, 9]. Thus, it is not surprising that deregulation of miRNAs is linked closely to various human pathological conditions. In this review, we will describe the crucial role of miRNAs in liver carcinogenesis, especially inflammation-related hepatocarcinogenesis.

Biogenesis and functions of miRNAs

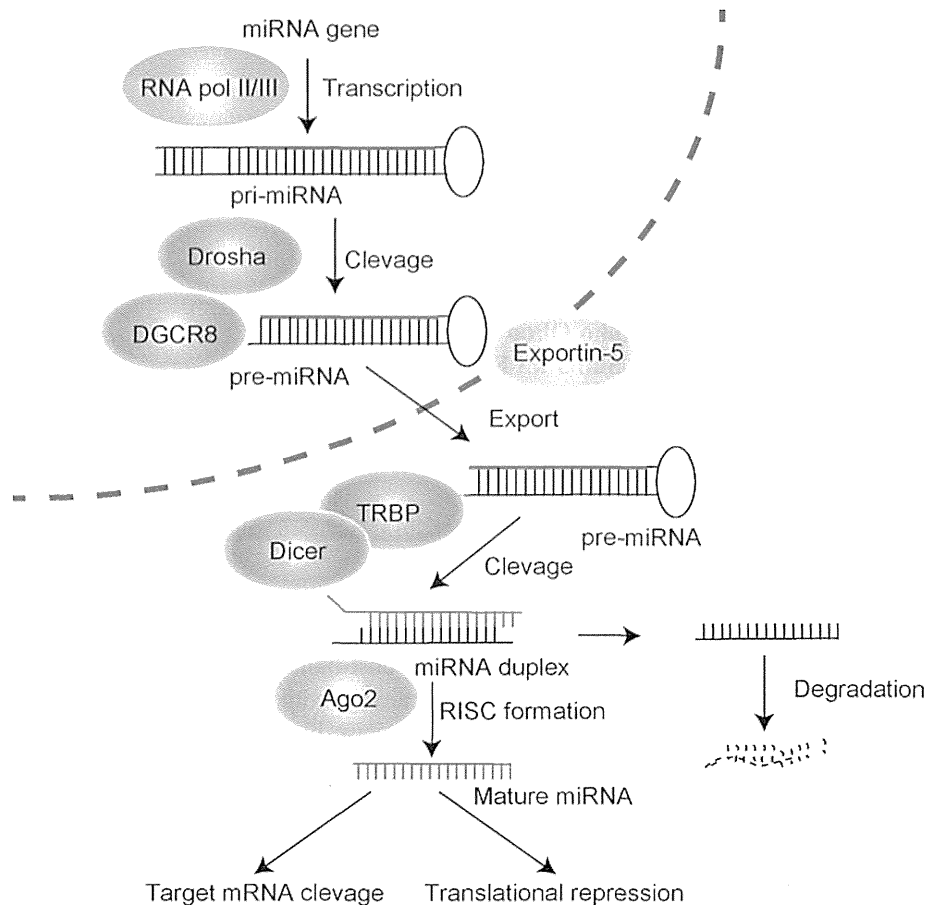
Transcription is the first step in miRNA expression (Fig. 1). Similar to most protein-coding genes, transcriptional factors, enhancers and silencers are involved in miRNA transcription [10–12]. Epigenetic mechanisms, such as promoter methylation or histone modification, also regulate miRNA transcription, and it was shown that histone deacetylase (HDAC) inhibition results in transcriptional changes in ~40 % of miRNAs [13].

Primary miRNAs, which possess stem-loop structures, are transcribed by RNA polymerase II [8]. These pri-miRNAs are processed by a microprocessor complex

M. Otsuka (✉) · T. Kishikawa · T. Yoshikawa · M. Ohno ·
A. Takata · C. Shibata · K. Koike
Department of Gastroenterology, Graduate School of Medicine,
The University of Tokyo, 5-3-1 Hongo, Bunkyo-ku,
Tokyo 113-8655, Japan
e-mail: otsukamo-ky@umin.ac.jp

M. Otsuka
Japan Science and Technology Agency,
PRESTO, Kawaguchi, Saitama 332-0012, Japan

Fig. 1 Biogenesis of miRNAs. The primary miRNA transcript (pri-miRNA) is transcribed from the genome by RNA polymerase II or III. The microprocessor complex Drosha–DGCR8 cleaves the pri-miRNA into the precursor hairpin, pre-miRNA in the nucleus. The pre-miRNA is exported from the nucleus by exportin-5–Ran-GTP. In the cytoplasm, the RNase Dicer in complex with the double-stranded RNA-binding protein, TRBP, cleaves the pre-miRNA hairpin to its mature length. The functional strand of the mature miRNA is loaded together with Argonaute (Ago2) proteins into the RNA-induced silencing complex (RISC), where it guides RISC to silence target mRNAs through mRNA cleavage or translational repression. The passenger strand (black) is degraded



comprising Drosha (RNAase III) [14] and DGCR8/Pasha [15] in the nucleus [16]. The processed products are approximately 65-nucleotide hairpin-shaped precursors (pre-miRNAs) that are transported to the cytoplasm via exportin-5 [17, 18]. Pre-miRNAs are further cleaved into mature miRNAs by Drosha and Dicer RNA polymerase III. Mature miRNA duplexes are loaded onto an RNA-induced silencing complex (RISC) and are unwound into the single-stranded mature form [19–21]. The resulting co-complex directly targets the 3'-untranslated regions (3'-UTRs) of target mRNAs, depending on the sequence similarities, to negatively regulate their expression by enhancing mRNA cleavage or inhibiting translation (Fig. 1) [8, 22]. Because most miRNAs guide the recognition of imperfect matches of target mRNAs, individual miRNAs have multiple (probably hundreds) of mRNA targets. In addition, multiple miRNAs can cooperate to regulate the expression of the same transcript [6]. Thus, depending upon the identity of the target mRNAs, miRNAs play roles as “fine-tuners of gene expression” in the control of various biological functions.

Identifying functionally important miRNA target genes is crucial for understanding the impact of specific miRNAs on cellular function. However, this is challenging because

miRNAs usually have imperfect complementarity with their targets [22]. In mammals, the most consistent requirement for miRNA-target interaction, although not always essential, is a contiguous and perfect pairing of the miRNA (nt 2–8), representing the “seed” sequence [22]. In many cases, the seed sequences determine this recognition, but in other cases, additional determinants are required, such as reasonable complementarity to the miRNA 3' half to stabilize the interaction. In addition, target pairing to the center of some miRNAs has also been reported [23]. Although public miRNA target prediction algorithms, such as TargetScan [24] and PicTar [25], have facilitated the rapid identification of miRNA target genes [22], candidates should be validated experimentally.

miRNAs and cancer

The involvement of miRNAs in cancer pathogenesis is well established. miRNAs can affect six hallmarks of malignant cells, which are (1) self-sufficiency in growth signals, (2) insensitivity to anti-growth signals, (3) evasion of apoptosis, (4) limitless replicative potential, (5) angiogenesis, and (6) invasion and metastasis [26]. miRNAs are frequently

up- or downregulated in malignant tissues and can be considered oncogenes or tumor suppressors, respectively. However, it is essential to test experimentally whether the deregulated miRNAs are actually causative to carcinogenesis, since miRNAs have a very restricted tissue-specific expression and the apparent miRNA modulation in cancer tissues may only reflect the different constituents of a cell population as compared to normal tissues. Extensive analyses have confirmed the causative roles of miRNAs in cancer by using either human cancer cells or genetically engineered animal models, such as transgenic expression of miR-155, miR-21 and miR-15-a/16-1, which are sufficient to initiate lymphomagenesis in mice [27–29]. These results suggest the potential role of miRNAs in the pathogenesis of carcinogenesis and as therapeutic targets.

miRNAs and hepatocarcinogenesis

Numerous reports regarding the deregulated expression of miRNAs in human hepatocellular carcinoma (HCC) are extant. Most studies compared the miRNA expression levels between cancer tissues and background non-tumorous tissues, selected candidate miRNA(s) and revealed their target genes, which may be involved in carcinogenesis. As shown in Tables 1 and 2, many miRNAs have been identified as downregulated or upregulated in recent studies (Tables 1, 2). However, these numerous results are not always superimposable due to the large variances in the results. These significant differences may be due to several reasons, such as the use of different techniques or different samples as controls, normal liver tissues versus peritumoral non-neoplastic tissues. In addition, one may need to take into consideration the fact that HCCs arise in background livers with different etiologies, such as hepatitis B, hepatitis C or steatohepatitis, and also the age or sex of the tissue-derived patients and background liver condition, such as fibrosis staging or inflammation activity, which may result in differences in the expression status of miRNAs. Despite these considerable limitations, the list suggests that diverse miRNAs play crucial roles in hepatocarcinogenesis. We will briefly describe some of them below.

The expression levels of miRNAs have restricted tissue specificities. In the liver, miR-122, miR-192 and miR-199a/b-3p are the three most expressed miRNAs, accounting for 52, 17 and 5 % of all mRNAs in the tissues, respectively [30]. The tumorigenic role of the loss of miR-122 was confirmed in gene-knockout mice [31, 32] and its expression is indeed decreased in half of the HCCs, especially non-viral HCCs [30]. We also reported that decreased expression of miR-122 is linked with poor prognosis of HCC [33]. While miR-192 does not appear to

be deregulated in HCC samples in previous studies, miR-199a/b-3p is decreased with high frequency in HCC, which is closely linked to a poor prognosis of HCC [30]. In contrast, miR-21, whose expression is increased following rat hepatectomy [34], is upregulated as a known oncomiRNA and represses PTEN signaling, resulting in promotion of HCC development [35]. Although individual miRNAs may be involved in hepatocarcinogenesis, because miRNAs often function co-operatively, the extent of their involvement remains to be determined.

As described above, miRNAs usually have multiple mRNA targets. Thus, it is not practical to describe only a few genes as being responsible for the phenotypes by deregulation of specific miRNAs, while many studies identify specific genes as targets of specific miRNAs. Nonetheless, the identified targeted genes are generally related to at least one of the hallmarks of cancer, such as cell growth, apoptosis, invasion, and so on. These results suggest that the deregulation of miRNA expression might mediate hepatocarcinogenesis through deregulating the expression of their target genes.

The miRNAs identified as deregulated in hepatocarcinogenesis may be useful as diagnostic and prognostic markers [36], because miRNAs in the circulation are reported to be relatively stable [37]. Also, deregulated miRNAs may be candidate therapeutic and preventive targets against HCC. However, to include the obtained results in clinical interventional applications, it is necessary to confirm if the deregulated miRNAs are truly drivers or are simply passive in hepatocarcinogenesis. To this end, genetically modified mice may provide some information. In addition, to correctly interpret the data, a standard method of normalizing the microRNAome data between studies may also be crucial. Since there are multiple target genes of miRNAs and, conversely, one transcript can be targeted by multiple miRNAs, a more systematic comparison using miRNA data, transcriptome data and proteome data would increase our understanding of the consequences of the deregulation of miRNAs during hepatocarcinogenesis. From this point of view, systematic and comprehensive target gene analyses for *in silico* systems biology models may be one option to resolve these issues.

miRNAs linked to inflammation-mediated hepatocarcinogenesis

Inflammation is considered to be a major cause of cancer [38, 39]. In the liver, hepatocarcinogenesis frequently occurs in persistently inflamed liver tissues caused by chronic hepatitis viral infection or non-alcoholic steatohepatitis. However, the molecular linkage between chronic inflammation and carcinogenesis is not well characterized.

Table 1 Upregulated miRNAs in hepatocarcinogenesis

miRNA	Expression levels	Targets	Main tested samples	References
miR-17-5p	Upregulated	p38 pathway	Cultured cells, human tissues	[52]
miR-18a	Upregulated	ER1a	Human tissues, cultured cells	[53]
miR-21	Upregulated	C/EBP β	Mouse CDAA model	[54]
	Upregulated	PTEN	Human tissues, cultured cells	[35]
miR-22	Upregulated	ERa, IL-1a	Human tissues, cultured cells, DEN model	[55]
miR-23a	Upregulated	PGC-1a, G6PC	Human tissues, cultured cells	[56]
miR-26a	Upregulated	Lin28B, Zcchc11	Human tissues, xenograft model	[57]
	Upregulated	NF- κ B, IL-6 pathways	Human tissues	[58]
miR-30d	Upregulated	GNAI2	Human tissues, cultured cells	[59]
miR-100	Upregulated		Human tissues	[60]
miR-106b	Upregulated	APC	Human tissues, cultured cells	[61]
miR-122	Upregulated		Human tissues	[60]
miR-130b	Upregulated	TP53INP1	Human tissues, xenograft model	[62]
miR-135a	Upregulated	FOXM1, MTSS1	Human tissues, cultured cells, xenograft	[63]
miR-143	Upregulated	FNDC3B	Human tissues, HBX transgenic mouse	[64]
miR-146a	Upregulated in endothelial cells	BRCA, PDGFRA	Cultured cells	[65]
miR-151	Upregulated	FAK	Human tissues, cultured cells	[66]
	Upregulated	FAK, RhoGDI A	Human tissues, cultured cells	[67]
miR-155	Upregulated	SOCS1	Orthotropic transplant model	[68]
	Upregulated	DKK1, APC	Human tissues, cultured cells	[69]
	Upregulated	PTEN	Mouse CDAA model	[54]
miR-181	Upregulated	TIMP3	Mouse CDAA model	[70]
	Upregulated	CDX2, GATA6, NLK	Cultured cells	[71]
miR-183	Upregulated	AKAP12	Human tissues	[72]
miR-186	Upregulated	AKAP12	Human tissues	[72]
miR-200	Upregulated	NRF2 pathway	Rat HCC model,	[73]
miR-210	Upregulated	VMP1	Human tissues, cultured cells	[74]
miR-216a	Upregulated	TSLC1	Human tissues, cultured cells	[75]
miR-216a/217	Upregulated	PTEN, SMAD7	Cultured cells, Human tissues	[76]
miR-221	Upregulated	CDK inhibitors	Transgenic mouse	[77]
	Upregulated	p27, p57, Arnt	Primary hepatocytes	[78]
	Upregulated	Bmf	Cultured cells, human tissues	[79]
	Upregulated	p27, p57	Cultured cells, human tissues	[80]
miR-221/222	Upregulated	p27, DDIT4	Human tissues, mouse model	[81]
miR-224	Upregulated		Human tissues	[82]
	Upregulated	Atg5, Smad4, autophagy	Human tissues, HBV X transgenic mice	[83]
	Upregulated	API-5	Cultured cells, human tissues	[84]
	Upregulated		Human tissues	[85]
	Upregulated	API-5	Human tissues	[86]
miR-423	Upregulated	p21/waf1	Human tissues, cultured cells	[87]
miR-485-3p	Upregulated	MAT1, LIN28B	Human tissues, xenograft model	[88]
miR-490-3p	Upregulated	ERCIC3	Human tissues, cultured cells	[89]
miR-494	Upregulated	MCC	Human tissue, mouse liver cancer model	[90]
miR-495	Upregulated	MAT1, LIN28B	Human tissues, xenograft model	[88]
miR-517a	Upregulated		Human tissues, cultured cells	[91]
miR-657	Upregulated	TLE1, NF- κ B	Human tissues, cultured cells	[92]
miR-664	Upregulated	MAT1, LIN28B	Human tissues, xenograft model	[88]
miR-1323	Upregulated		Human tissues	[93]