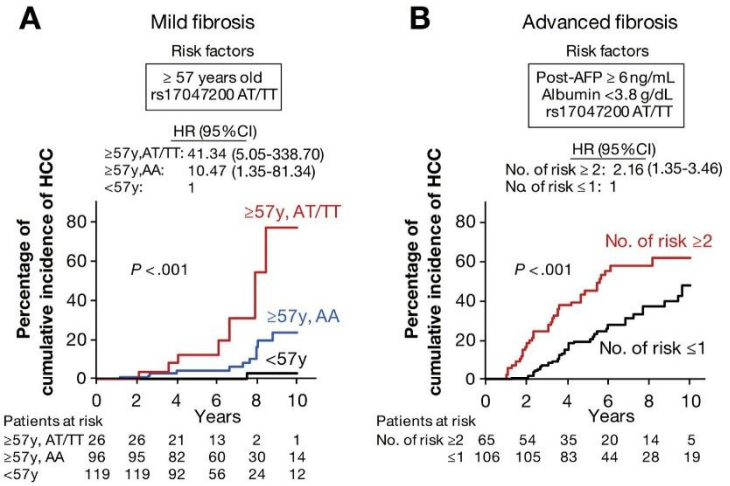

Figure 2. Cumulative incidence of HCC after the eradication of HCV by interferon-based therapy according to risk factors in patients with mild (*A*) and advanced hepatic fibrosis (*B*). HR (95% CI) up to 10 years after the end of treatment were calculated by the Cox proportional hazard method. *P*-values were calculated by log-rank testing. Data of subjects whose genotypes were not determined were excluded. Post, post-treatment (24 weeks after the end of treatment).

either *TLL1* or *BMP1* mRNA levels in the surrounding nontumorous tissues (Figure 4*B* and Supplementary Figure 11*B*). Despite these findings, there were no differences in levels of *TLL1* mRNA according to the rs17047200 genotype in the liver tissues of CHC patients or the tumorous and nontumorous tissues of patients developing HCC after achieving SVR (Supplementary Figure 12). We hypothesized that rs17047200 or other intronic SNPs in strong LD with rs17047200 may affect splicing of *TLL1* mRNA. So far, 1 short variant, *TLL1* isoform 2, consisting of

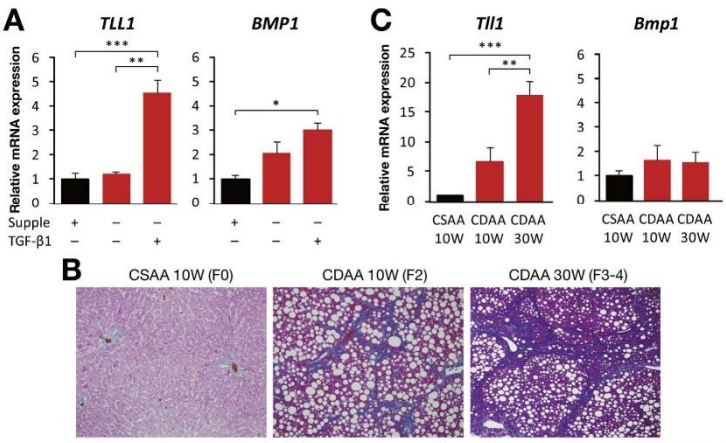

Figure 3. Gene expression levels of *TLL1/BMP1* (*Tll1/Bmp1*) in human hepatic stellate cells and CDAA-fed hepatic fibrosis model rats. (*A*) Human stellate cells (HHSteC) were treated with human recombinant TGF-β1 (5 ng/mL) or mock-treated 6 hours after seeding, then harvested 72 hours after seeding (Supplementary Figure 9). The relative levels of *TLL1/BMP1* mRNA were normalized to those of cells cultured in media with supplement (black bar). Three independent experiments were carried out in triplicate. Data represent mean ± SEM (bars: n = 3 per group). (*B*) Pathologic hepatic fibrosis stage by Heidenhain's azan staining in rats. CSAA 10W: a CSAA diet was fed for 10 weeks, showed METAVIR fibrosis stage F0; CDAA 10W/CDAA 30W: CDAA diet was fed for 10/30 weeks, showed F2/F3–4. (*C*) Relative *Tll1/Bmp1* mRNA levels were normalized to those in CSAA 10W. Data represent mean ± SEM (*bars*: n = 5 per group). *P* values were calculated by 1-way analysis of variance, followed by Tukey multiple comparison testing (*$P < .05$; **$P < .01$; ***$P < .001$).

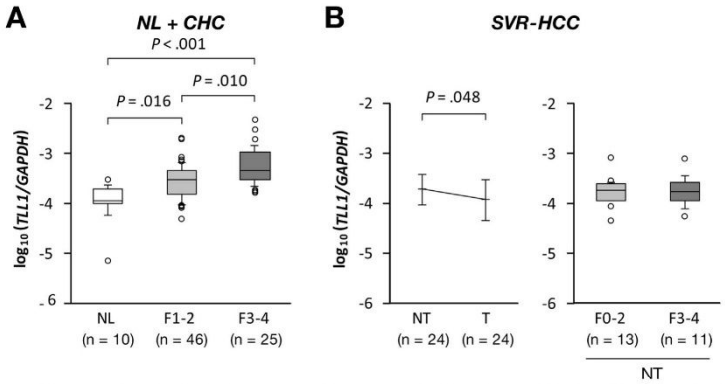

Figure 4. *TLL1* mRNA expression levels in liver tissues of patients with chronic hepatitis C and in those developing hepatocellular carcinoma after achieving SVR. (*A*) *TLL1* mRNA expression levels in normal liver (NL) and liver tissues of chronic hepatitis C (CHC) patients according to METAVIR fibrosis stage. *Boxes* represent the interquartile range of the data. The *lines across boxes* and the numbers indicate the median values. The *hash marks* above and *below boxes* indicate the 90th and 10th percentiles for each group, respectively. *P* values were calculated by Kruskal–Wallis test, followed by Steel-Dwass' multiple comparison testing. (*B*) *Left panel: TLL1* mRNA expression levels in tumor (T) and the surrounding nontumor (NT) tissues in patients who developed HCC after achieving SVR. Data represent mean ± SD. *P* values were calculated by paired *t* testing. *Right panel: TLL1* mRNA expression levels in NT tissues according to METAVIR fibrosis stage. Data represent in the same manner as (*A*). *P* values were calculated by Mann–Whitney U testing.

exon 1–10, has been identified (GeneBank Accession: BC016922.2). We further examined *TLL1* mRNA levels of exon 20–21 span in addition to those of exon 5–6 span, and indirectly compared relative expression of short variants including isoform 2 by analyzing the proportion of mRNA of exon 5–6/exon 20–21 (Supplementary Figure 13*A*). For this, we used liver tissues of patients who developed HCC after achieving SVR (Supplementary Table 8). These results indicated that the proportions of short splice variants in nontumor and tumor tissues were higher in patients with rs17047200 AT/TT than in those with AA (Supplementary Figure 13*B*). Next, we designed the TaqMan probe and primers specific for *TLL1* isoform 2 (Supplementary Methods). We compared *TLL1* isoform 2 mRNA levels in different rs17047200 genotypes using these tissue samples. This showed that *TLL1* isoform 2 expression was also higher in patients with rs17047200 AT/TT (Supplementary Figure 13*C*).

Discussion

This is the first report that showed a strong association at a genome-wide level of significance between a genetic variant and the development of HCC after the eradication of HCV by IFN-based therapy. Notably, the OR for this *TLL1* variant of rs17047200 was markedly higher (OR, 2.37) than found in previous investigations applying GWAS for HCV- or HBV-related HCC (OR, 1.19–1.75).[22–27] In further clinical investigations, the *TLL1* variant was found to be an independent risk factor for developing HCC after eradication of HCV, in addition to other risk factors, such as male sex, older age, advanced hepatic fibrosis stage, presence of diabetes, lower albumin level, and higher post-treatment AFP level, which have been reported previously.[5–7,11–13] Notably, the *TLL1* variant was a strong predictor for developing HCC even in patients with mild hepatic fibrosis (Supplementary Table 6). When we constructed different models for predicting HCC in patients with mild as opposed to advanced hepatic fibrosis by combining this *TLL1* variant with other distinct risk factors, these proposed models could be useful for predicting the occurrence of HCC after achieving SVR in the clinical practice (Figure 2).

As is well known, lower platelet count, higher pre- and post-treatment aspartate aminotransferase-to-platelet ratio index and fibrosis-4 score were also strong HCC risk factors in univariate analysis (Table 3). However, we did not include these factors as covariates in the multivariate analysis because they were strong confounders for the pathologic stage of hepatic fibrosis. Antibody to hepatitis B core antigen (anti-HBc) status was also significantly associated with developing HCC by univariate analysis, but not by multivariate analysis (Table 3). Further analyses indicated that anti-HBc status strongly correlated with age and sex; thus, anti-HBc–positive rates were higher in elderly and male patients (data not shown). Because older age and male sex were strong risk factors for developing HCC in the multivariate analysis (Table 3), we concluded that anti-HBc status was not directly associated with developing HCC after achieving SVR.

In addition, this would be the first evidence that showed the relationship between *TLL1/Tll1* expression and HSC activation or hepatic fibrosis progression using in vitro and in vivo models as well as human samples. Interestingly, these results indicated that TLL1 would specifically play a

role in hepatic fibrogenesis, although previous studies had shown that *Tll1* and *Bmp1* are co-expressed in various tissues.[38,39] Although the molecular mechanisms of hepatocarcinogenesis have not been fully elucidated, the cirrhotic tissue microenvironment is thought to cause initiation and promotion of neoplastic clones by facilitating genetic aberrations and cellular transformation, resulting in HCC development.[40] Indeed, Sakaida et al[41] showed that pre-existing hepatic fibrosis with activated HSCs accelerated the development of pre-neoplastic lesions in a CDAA-fed rat model. Therefore, we presume that TLL1 may contribute to HCC development mainly via hepatic fibrogenesis. Independently of this scenario of cirrhosis-driven HCC development caused by TLL1, TGF-β has been reported to be involved in hepatocarcinogenesis, behaving as an anti-oncogenic factor at the early stage of tumor development, while over-activation of TGF-β signaling may act as a pro-oncogenic factor at the later stage by promoting epithelial to mesenchymal transition and cancer dissemination.[42] Intriguingly, BMP1/TLD-like proteinases are potently up-regulated by TGF-β1,[43] implying the existence of a positive feedback loop between these proteinases and TGF-β signaling (Supplementary Figure 8). On the other hand, BMP1/TLD-like proteinases have been reported to activate insulin-like growth factors by cleavage of their binding proteins[44]; and insulin-like growth factor signaling is also involved in hepatocarcinogenesis.[45] Furthermore, Amann et al[46] showed that activated HSCs promoted HCC progression potentially via the activation of nuclear factor−κB and extracellular signal–regulated kinase. Given these findings, TLL1 might contribute to the development of HCC via activation of these signaling pathways. Regarding relationships between *TLL1* and other cancers, Odagiri et al[47] reported that full-length ANGPTL2 was extracellularly cleaved by TLL1 into an inactive form lacking the capacity to enhance tumor aggressiveness. TLL1 also might be involved in hepatocarcinogenesis through the cleavage of cancer-related gene products. Intriguingly, our data indicated that the induction of *Tll1* mRNA in the liver was stronger in CDAA-fed rats than in CCl_4-treated mice (Figure 3*C* and Supplementary Figure 10). Previous studies have shown that the CDAA-fed NASH model causes HCC more frequently than the CCl_4-treated model.[48,49] These findings strengthen our proposal that TLL1 may directly contribute to hepatocarcinogenesis independently of the progression of hepatic fibrosis. Taken together, we hypothesize that TLL1 may contribute to HCC development mainly via cirrhosis-driven carcinogenesis and probably via pro-oncogenic roles as well (Supplementary Figure 14). Future studies using overexpression or knockout of the *TLL1* gene in animal models might provide valuable information to clarify the mechanism of hepatic fibrogenesis and carcinogenesis.

In the present study, variants near or within *MICA* and *DEPDC5*, which have been identified as related to hepatocarcinogenesis under persistent HCV infection,[22,23] were not replicated. We hypothesize that the mechanisms of hepatocarcinogenesis after achieving SVR might be different, at least in part, from those related to persistent HCV infection. One possibility is that viral carcinogenesis, for example, via interaction of HCV proteins with cellular pathways, cancer-related genes, and innate immunity,[50] decreases after achieving SVR, which could result in cirrhosis-driven carcinogenesis becoming dominant. Therefore, we presume that TLL1 is involved in hepatic fibrogenesis and carcinogenesis in patients not only after achieving SVR, but also those with other etiologies, such as NASH. Indeed we showed that *Tll1* mRNA was up-regulated in step with the progression of hepatic fibrosis in NASH model rats. It might be interesting to evaluate the association between the *TLL1* SNP and the development of HCC during chronic HCV infection including non-SVR or NASH.

Despite our findings described, the distribution of rs17047200 genotypes did not correlate with hepatic fibrosis stage either in HCC or non-HCC patients (Supplementary Tables 4 and 5). As is well-known, several factors influence hepatic fibrogenesis in chronic hepatitis C. Therefore, it would be difficult to evaluate the association between *TLL1* genotype and stage of hepatic fibrosis in these cross-sectional groups. Furthermore, we found no differences in the levels of *TLL1* mRNA according to the rs17047200 genotype in the liver tissues of patients with CHC and HCC after achieving SVR. Our results based on SNP imputation and pairwise LD diagrams in the HapMap data showed no SNP in strong LD with rs17047200 in the exon or the promoter region of *TLL1*, but several intronic SNPs within *TLL1* were in strong LD with rs17047200. BMP1 has the highest catalytic activity, its longer splice variant mammalian TLD has much lower activity, and TLL1 has intermediate activity. Berry et al[51] demonstrated that truncated TLL1 molecules have higher catalytic activity than not only full-length TLL1, but also BMP1. Our investigation indicated that the levels of *TLL1* short variants including isoform 2 were higher in patients with rs17047200 AT/TT (Supplementary Figure 13). On the basis of these findings, we hypothesize that rs17047200 or other intronic SNPs in strong LD with rs17047200 may affect splicing of *TLL1* mRNA, yielding short variants (isoform 2 or unknown variants) with high catalytic activity especially in patients with rs17047200 AT/TT, which might accelerate hepatic fibrogenesis and carcinogenesis (Supplementary Figure 14). Further studies are necessary to elucidate the association between rs17047200 genotypes and *TLL1* mRNA, splicing forms, and protein expression as well as proteinase activity using more samples in the future.

There are several limitations in the present study. First, the number of patients in our study was not large, and only Japanese were included. Genotype and allele frequencies at rs17047200 are different among ethnicities: especially the risk allele frequency is higher in African populations (Supplementary Table 9). Second, we designed a retrospective case-control study using the original inclusion criteria of patients deriving from an exploratory study. Third, we evaluated risk factors for developing HCC and cumulative incidence of HCC using the stepwise Cox proportional hazard model and the Kaplan−Meier method in the biased population: selection bias for the inclusion criteria and the patients were overlapped in the GWAS and the replication analyses. Prospective validation studies that

follow patients until a fixed end point after EOT in non-biased populations would be an ideal way to validate our results in the future.

IFN-free oral treatment regimens combining direct-acting antiviral agents are becoming the standard of care for anti-HCV therapy in developed countries. Previous studies have revealed that IFN-based therapy inhibits development of HCC in CHC patients, especially those achieving SVR.[52–54] However, it is uncertain whether IFN-free therapy can inhibit this to the same extent as IFN-based therapy after eradication of HCV. Future studies are necessary to evaluate whether the *TLL1* variant is associated with HCC development after achieving SVR by IFN-free therapy.

In conclusion, we identified a genome-wide level of significance in the association of a *TLL1* variant and hepatocarcinogenesis in CHC patients after eradication of HCV. Therefore, genetic testing of the *TLL1* variant would be useful for implementing personalized surveillance of HCC in patients who have achieved SVR. Additionally, our results might contribute to the elucidation of the mechanism of hepatic fibrogenesis and carcinogenesis.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2017.01.041.

References

1. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380:2095–2128.
2. Davis GL, Alter MJ, El-Serag H, et al. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. Gastroenterology 2010;138:513–521, 521 e1–e6.
3. **Reddy KR, Bourliere M**, Sulkowski M, et al. Ledipasvir and sofosbuvir in patients with genotype 1 hepatitis C virus infection and compensated cirrhosis: an integrated safety and efficacy analysis. Hepatology 2015;62:79–86.
4. Hiramatsu N, Oze T, Takehara T. Suppression of hepatocellular carcinoma development in hepatitis C patients given interferon-based antiviral therapy. Hepatol Res 2014;45:152–161.
5. Makiyama A, Itoh Y, Kasahara A, et al. Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. Cancer 2004;101:1616–1622.
6. Chang KC, Hung CH, Lu SN, et al. A novel predictive score for hepatocellular carcinoma development in patients with chronic hepatitis C after sustained response to pegylated interferon and ribavirin combination therapy. J Antimicrob Chemother 2012;67:2766–2772.
7. Sato A, Sata M, Ikeda K, et al. Clinical characteristics of patients who developed hepatocellular carcinoma after hepatitis C virus eradication with interferon therapy: current status in Japan. Intern Med 2013; 52:2701–2706.
8. Arase Y, Kobayashi M, Suzuki F, et al. Effect of type 2 diabetes on risk for malignancies includes hepatocellular carcinoma in chronic hepatitis C. Hepatology 2013; 57:964–973.
9. El-Serag HB, Kanwal F, Richardson P, et al. Risk of hepatocellular carcinoma after sustained virologic response in veterans with HCV-infection. Hepatology 2016; 64:130–137.
10. Ogawa E, Furusyo N, Kajiwara E, et al. Efficacy of pegylated interferon alpha-2b and ribavirin treatment on the risk of hepatocellular carcinoma in patients with chronic hepatitis C: a prospective, multicenter study. J Hepatol 2013;58:495–501.
11. Asahina Y, Tsuchiya K, Nishimura T, et al. alpha-fetoprotein levels after interferon therapy and risk of hepatocarcinogenesis in chronic hepatitis C. Hepatology 2013;58:1253–1262.
12. **Oze T, Hiramatsu N**, Yakushijin T, et al. Post-treatment levels of alpha-fetoprotein predict incidence of hepatocellular carcinoma after interferon therapy. Clin Gastroenterol Hepatol 2014;12:1186–1195.
13. Huang CF, Yeh ML, Huang CY, et al. Pretreatment glucose status determines HCC development in HCV patients with mild liver disease after curative antiviral therapy. Medicine (Baltimore) 2016;95:e4157.
14. Yu ML, Lin SM, Lee CM, et al. A simple noninvasive index for predicting long-term outcome of chronic hepatitis C after interferon-based therapy. Hepatology 2006; 44:1086–1097.
15. Huang CF, Yeh ML, Tsai PC, et al. Baseline gamma-glutamyl transferase levels strongly correlate with hepatocellular carcinoma development in non-cirrhotic patients with successful hepatitis C virus eradication. J Hepatol 2014;61:67–74.
16. Akuta N, Suzuki F, Hirakawa M, et al. Amino acid substitutions in hepatitis C virus core region predict hepatocarcinogenesis following eradication of HCV RNA by antiviral therapy. J Med Virol 2011;83:1016–1022.
17. **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;41:1105–1109.
18. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
19. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009; 41:1100–1104.
20. Patin E, Kutalik Z, Guergnon J, et al. Genome-wide association study identifies variants associated with progression of liver fibrosis from HCV infection. Gastroenterology 2012;143:1244–1252 e1–e12.
21. Urabe Y, Ochi H, Kato N, et al. A genome-wide association study of HCV-induced liver cirrhosis in the

Japanese population identifies novel susceptibility loci at the MHC region. J Hepatol 2013;58:875–882.
22. Kumar V, Kato N, Urabe Y, et al. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nat Genet 2011; 43:455–458.
23. **Miki D, Ochi H**, Hayes CN, 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. Zhang H, Zhai Y, Hu Z, et al. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. Nat Genet 2010;42:755–758.
25. **Chan KY, Wong CM, Kwan JS**, et al. Genome-wide association study of hepatocellular carcinoma in Southern Chinese patients with chronic hepatitis B virus infection. PLoS One 2011;6:e28798.
26. **Li S, Qian J, Yang Y**, et al. GWAS identifies novel susceptibility loci on 6p21.32 and 21q21.3 for hepatocellular carcinoma in chronic hepatitis B virus carriers. PLoS Genet 2012;8:e1002791.
27. **Jiang DK, Sun J, Cao G**, et al. Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus-related hepatocellular carcinoma. Nat Genet 2013; 45:72–75.
28. Noureddin M, Wright EC, Alter HJ, et al. Association of IL28B genotype with fibrosis progression and clinical outcomes in patients with chronic hepatitis C: a longitudinal analysis. Hepatology 2013;58:1548–1557.
29. Trepo E, Pradat P, Potthoff A, et al. Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. Hepatology 2011;54:60–69.
30. Rattenholl A, Pappano WN, Koch M, et al. Proteinases of the bone morphogenetic protein-1 family convert procollagen VII to mature anchoring fibril collagen. J Biol Chem 2002;277:26372–26378.
31. Veitch DP, Nokelainen P, McGowan KA, et al. Mammalian tolloid metalloproteinase, and not matrix metalloprotease 2 or membrane type 1 metalloprotease, processes laminin-5 in keratinocytes and skin. J Biol Chem 2003;278:15661–15668.
32. **Ge G, Seo NS**, Liang X, et al. Bone morphogenetic protein-1/tolloid-related metalloproteinases process osteoglycin and enhance its ability to regulate collagen fibrillogenesis. J Biol Chem 2004;279:41626–41633.
33. **Gonzalez EM, Reed CC**, Bix G, et al. BMP-1/Tolloid-like metalloproteases process endorepellin, the angiostatic C-terminal fragment of perlecan. J Biol Chem 2005; 280:7080–7087.
34. Kessler E, Takahara K, Biniaminov L, et al. Bone morphogenetic protein-1: the type I procollagen C-proteinase. Science 1996;271:360–362.
35. Ge G, Greenspan DS. BMP1 controls TGFbeta1 activation via cleavage of latent TGFbeta-binding protein. J Cell Biol 2006;175:111–120.
36. Schuppan D, Krebs A, Bauer M, et al. Hepatitis C and liver fibrosis. Cell Death Differ 2003;10(Suppl 1): S59–S67.
37. Benyon RC, Iredale JP. Is liver fibrosis reversible? Gut 2000;46:443–446.
38. Scott IC, Blitz IL, Pappano WN, et al. Mammalian BMP-1/Tolloid-related metalloproteinases, including novel family member mammalian Tolloid-like 2, have differential enzymatic activities and distributions of expression relevant to patterning and skeletogenesis. Dev Biol 1999; 213:283–300.
39. Takahara K, Brevard R, Hoffman GG, et al. Characterization of a novel gene product (mammalian tolloid-like) with high sequence similarity to mammalian tolloid/bone morphogenetic protein-1. Genomics 1996;34: 157–165.
40. Aihara T, Noguchi S, Sasaki Y, et al. Clonal analysis of regenerative nodules in hepatitis C virus-induced liver cirrhosis. Gastroenterology 1994;107:1805–1811.
41. Sakaida I, Hironaka K, Uchida K, et al. Fibrosis accelerates the development of enzyme-altered lesions in the rat liver. Hepatology 1998;28:1247–1252.
42. Massague J. TGFbeta in cancer. Cell 2008;134:215–230.
43. Lee S, Solow-Cordero DE, Kessler E, et al. Transforming growth factor-beta regulation of bone morphogenetic protein-1/procollagen C-proteinase and related proteins in fibrogenic cells and keratinocytes. J Biol Chem 1997; 272:19059–19066.
44. Kim B, Huang G, Ho WB, et al. Bone morphogenetic protein-1 processes insulin-like growth factor-binding protein 3. J Biol Chem 2011;286:29014–29025.
45. Nalesnik MA, Michalopoulos GK. Growth factor pathways in development and progression of hepatocellular carcinoma. Front Biosci (Schol Ed) 2012; 4:1487–1515.
46. Amann T, Bataille F, Spruss T, et al. Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. Cancer Sci 2009;100:646–653.
47. **Odagiri H, Kadomatsu T**, Endo M, et al. The secreted protein ANGPTL2 promotes metastasis of osteosarcoma cells through integrin alpha5beta1, p38 MAPK, and matrix metalloproteinases. Sci Signal 2014;7:ra7.
48. Nakae D, Yoshiji H, Mizumoto Y, et al. High incidence of hepatocellular carcinomas induced by a choline deficient L-amino acid defined diet in rats. Cancer Res 1992; 52:5042–5045.
49. Perez Tamayo R. Is cirrhosis of the liver experimentally produced by CCl4 and adequate model of human cirrhosis? Hepatology 1983;3:112–120.
50. Goossens N, Hoshida Y. Hepatitis C virus-induced hepatocellular carcinoma. Clin Mol Hepatol 2015; 21:105–114.
51. Berry R, Jowitt TA, Garrigue-Antar L, et al. Structural and functional evidence for a substrate exclusion mechanism in mammalian tolloid like-1 (TLL-1) proteinase. FEBS Lett 2010;584:657–661.
52. Ikeda K, Saitoh S, Arase Y, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: A long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124–1130.
53. Kasahara A, Hayashi N, Mochizuki K, et al. Risk factors for hepatocellular carcinoma and its incidence after

interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. Hepatology 1998; 27:1394–1402.

54. Yoshida H, Shiratori Y, Moriyama M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and non-cirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med 1999;131:174–181.

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Conflicts of interest
These authors disclose the following: Yasuhito Tanaka is currently conducting research sponsored by Merck Sharp & Dohme, Corp, Chugai Pharmaceutical Co, Ltd, Bristol-Myers Squibb, and AbbVie Inc. Takashi Kumada received remuneration for lectures at meetings from Bristol-Myers Squibb. Yasuhiro Asahina belongs to a donation-funded Department funded by Toray Industries Inc., Chugai Pharmaceutical Co. Ltd, Merck Sharp & Dohme, Gilead Sciences Co. Ltd, and AbbVie GK. The remaining authors disclose no conflicts.

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