Table 1. Clinical characteristics of patients in this study

	GWAS ($n = 303$)	Replication ($n = 391$)
Age	57.4 (9.7)	56.8 (9.9)
Sex (M/F)	151/152	209/182
Weight (kg)	60.6 (10.4)	61.3 (10.7)
Body mass index	23.5 (3.1)	23.7 (4.1)
Baseline Hb (g/dl)	14.1 (1.4)	14.1 (1.4)
Baseline platelet count (10^9/l)	151.3 (54.3)	159.7 (55.0)
Baseline ALT (IU/l)	83.5 (79.4)	86.8 (71.9)
Baseline creatinine (mg/dl)	0.70 (0.15)	0.72 (0.16)
Baseline liver fibrosis (F0–2/F3–4/ND)	153/77/73	175/59/43
rs8099917: TT/non-TT	165/138	296/95
rs1127354: AA/CA/CC	4/79/220	6/101/284
Week 4 Hb (g/dl)	11.8 (1.7)	11.9 (1.5)
Week 4 platelet count (10^9/l)	127.6 (48.2)	132.4 (51.0)
Hb reduction at week 4	−2.3 (1.4)	−2.2 (1.4)
Platelet reduction at week 4	−22.2 (38.4)	−24.7 (30.4)

located on *DDRGK1* gene and *ITPA* gene on chromosome 20 showed strong associations in the allele frequency model ($P = 3.29 \times 10^{-10}$ and $P = 2.56 \times 10^{-9}$) with Hb reduction in response to PEG-IFN plus RBV treatment (Table 3).

The above 22 SNPs were selected for the replication study and fine mapping, including rs1127354, which was reported by the US group (22) to be strongly associated with Hb reduction (Supplementary Material, Table S2). All SNPs were genotyped using the DigiTag2 assay in an independent set of 391 Japanese HCV patients with quantitative change in Hb in response to PEG-IFN/RBV treatment [137 patients with Hb reduction versus 254 patients without Hb reduction (Table 3)]. The associations of the original SNPs were replicated in the second set of 391 patients in the minor-allele-dominant model ($P = 3.86 \times 10^{-16}$, OR = 0.02 for rs11697186; $P = 6.90 \times 10^{-18}$, OR = 0.03 for rs6139030, Table 3). The combined *P*-values for both stages reached 9.43×10^{-25} (OR = 0.03; 95% CI = 0.01–0.08) and 2.12×10^{-25} (OR = 0.04; 95% CI = 0.02–0.09), respectively (Table 3). The rs1127354 was also strongly associated with a quantitative change in Hb in response to PEG-IFN/RBV treatment in a set of 694 Japanese HCV patients (303 patients from the GWAS stage plus the second set of 391 patients) with and without Hb reduction ($P = 4.58 \times 10^{-26}$, OR = 0.03; 95% CI = 0.01–0.08).

Fine mapping with 22 SNPs around *DDRGK1* and *ITPA* genes showed that four significant SNPs (rs11697186, rs6139030, rs1127354 and rs13830) at the GWAS stage had a strong linkage disequilibrium (LD) ($r^2 > 0.86$) within the 22.7 kb region (Fig. 2). As the rs1127354 is known as a functional variant in the *ITPA* gene that caused ITPase deficiency and protected against RBV-induced HA (22,25), the representative SNP was applied for the following detailed studies.

ITPA/DDRGK1 variants reflect anemia and reactive increase of the platelet count

The mean quantitative reduction of blood cells from the baseline according to the *ITPA* rs1127354 genotypes is shown in Figure 3. Patients with the rs1127354 genotypes AA and CA showed lower degree of Hb reduction at weeks 2, 4, 8 and 12 during therapy compared with those with the CC genotype ($P < 0.0001$ for weeks 2, 4, 8 and 12 in Fig. 3A). The most difference of mean Hb reduction was found at week 4 (AA/CA −1.14 versus CC −2.72). These results show that the AA and CA genotypes are significantly associated with less absolute reduction in Hb levels, especially during the early weeks of therapy, and protect against the development of severe anemia. Interestingly, the CC genotype had significantly less reduction in the mean platelet count compared with the AA/CA genotype ($P < 0.0001$ for weeks 2, 4, 8; $P = 0.019$ for week 12 in Fig. 3B), due to a reactive increase of platelet count through weeks 1–4. The most difference of mean platelet reduction was found at week 4 [AA/CA −41.2 versus CC −18.0 (10^9/l)]. There was no difference in the neutrophil leukocyte count between genotypes (Fig. 3C). We then compared the percentage of patients with platelet count reduction in the *ITPA* rs1127354 genotypes at week 4 of PEG-IFN/RBV therapy (Fig. 4). The percentage of patients with a platelet count reduction of <30 (10^9/l) at week 4 was significantly higher in the rs1127354 genotypes CC ($P < 0.0001$), indicating that the degree of platelet count reduction was less in patients with the rs1127354 genotype CC. A multivariate analysis for factors associated with a platelet reduction >30 (10^9/l) at week 4 showed that lower platelet count at the baseline and the rs1127354 genotypes AA/CA were independently associated with platelet reduction (OR = 1.15; 95% CI = 1.11–1.20; $P < 0.0001$, OR = 5.92; 95% CI = 3.82–9.17; $P < 0.0001$, respectively).

Figure 5 showed reactive increase of the platelet count through weeks 1–4 of PEG-IFN/RBV therapy. Patients with anemia (Hb reduction ≥3.0 g/dl) at week 4 had a significantly higher degree of the reactive increase of the platelet count than those without anemia ($P < 0.0001$ in Fig. 5A). Within a subgroup of patients with the rs1127354 genotypes CC, patients with anemia still had a significantly higher degree of reactive increase of the platelet count than those without anemia ($P = 0.004$ in Fig. 5B). On the other hand, patients with the rs1127354 genotypes CC had a significantly higher degree of the reactive increase of the platelet count than those with genotypes AA/CA ($P < 0.0001$ in Fig. 5C), and a similar result was obtained in a subgroup of patients without anemia (Fig. 5D). To elucidate the significant factors associated with the rs1127354 genotypes by multivariate analysis, the rs1127354 genotypes AA/CA were independently associated with protection against the reduction in Hb and more reduction in platelet counts at week 4 due to a lower degree of the reactive increase of the platelet count (OR = 0.029; 95% CI = 0.009–0.092; $P < 0.0001$, OR = 4.73; 95% CI = 3.04–7.37; $P < 0.0001$, respectively). Indeed, the reactive increase of the platelet count through weeks 1–4 was positively correlated with a high platelet count at the baseline and anemia (Hb reduction ≥3.0 g/dl) at week 4, but was negatively correlated with rs1127354 genotypes AA/CA and a platelet count reduction of ≥30 (10^9/l) at week 4 (Table 4).

Relationship between *ITPA rs1127354* genotypes and treatment outcome due to dose reduction of PEG-IFN or RBV

In this population, a multivariate analysis showed that SVR was significantly associated with *IL28B* TT-genotype [OR

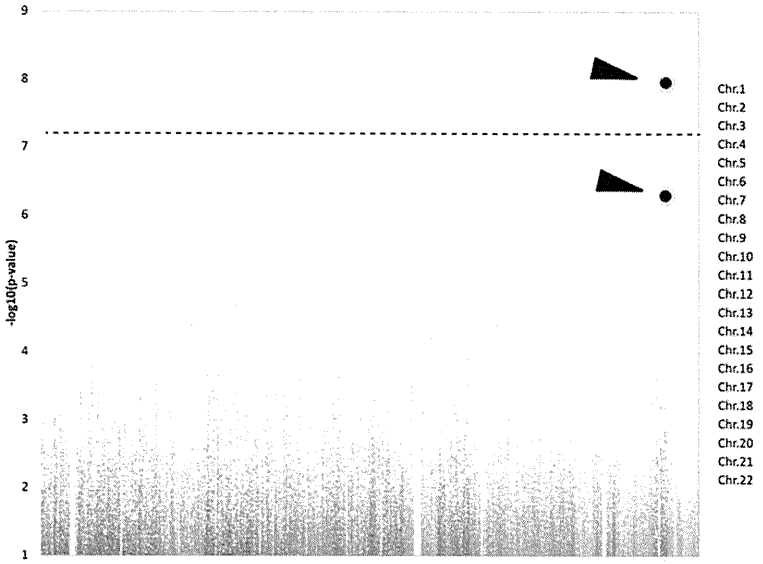

Figure 1. Genome-wide association results in 303 Japanese HCV patients with the decrease of platelets in response to PEG-IFN plus RBV treatment (107 patients with the decrease of PLT and 196 patients without the decrease of PLT). *P*-values were calculated using the χ^2 test for allele frequencies. Dots with arrow on chromosome 20 showed a significant SNP ($P = 8.17 \times 10^{-9}$ for rs11697186) and a candidate SNP with a marginal significance ($P = 4.30 \times 10^{-7}$ for rs6139030) associated with the decrease of PLT with response to PEG-IFN/RBV treatment. The dotted line indicates a genome-wide significance ($P < 8.40 \times 10^{-8}$).

Table 2. Two SNPs (rs11697186 and rs6139030) significantly associated with the decrease of PLT in response to PEG-IFN/RBV treatment

dbSNP rsID	Nearest gene	MAF[a] (allele)	Allele (1/2)	Stage	Patients with the decrease of PLT			Patients without the decrease of PLT			OR (95% CI)[b]	*P*-value[c]
					11	12	22	11	12	22		
rs11697186	*DDRGK1*	0.15 (T)	T/A	GWAS	3 (2.8)	48 (44.9)	56 (52.3)	0 (0.0)	32 (16.6)	161 (83.4)	4.6 (2.7–7.8)	8.17×10^{-9}
				Replication	3 (1.8)	65 (39.9)	95 (58.3)	3 (1.4)	25 (12.0)	181 (86.6)	4.6 (2.8–7.7)	5.88×10^{-10}
				Combined	6 (2.2)	113 (41.9)	151 (55.9)	3 (0.7)	57 (14.2)	342 (85.1)	4.5 (3.1–6.5)	5.29×10^{-17}
rs6139030	*ITPA*	0.17 (C)	T/C	GWAS	56 (52.3)	48 (44.9)	3 (2.8)	157 (80.1)	38 (19.4)	1 (0.5)	3.7 (2.2–6.1)	4.30×10^{-7}
				Replication	96 (54.9)	74 (42.3)	5 (2.9)	181 (83.8)	32 (14.8)	3 (1.4)	4.3 (2.7–6.8)	3.83×10^{-10}
				Combined	152 (53.9)	122 (43.3)	8 (2.8)	338 (82.0)	70 (17.0)	4 (1.0)	3.9 (2.8–5.5)	1.33×10^{-15}

[a]Minor allele frequency and minor allele in 184 healthy Japanese individuals.
[b]OR for the minor allele in a dominant model.
[c]*P*-value by χ^2 test for the minor allele dominant model.

6.12 (2.78–13.46), $P < 0.0001$] as well as platelet counts [OR 1.18 (1.11–1.26), $P < 0.00001$]. We analyzed whether the rs1127354 genotype could influence the treatment outcome by PEG-IFN/RBV therapy. When analyzed in the patients available for treatment outcome (172 with *ITPA*-AA/CA and 450 with *ITPA*-CC), the percentage of patients receiving >80% of the expected PEG-IFN and RBV dose at baseline and week 4 was not significantly different among the rs1127354 genotypes. However, the rate of SVR tended to be higher in patients with *ITPA*-AA/CA genotype than those with *ITPA*-CC (48.8 versus 37.3%), because the relapse rate was lower in patients with *ITPA*-AA/CA. To investigate the influence on treatment outcome by dose reduction of PEG-IFN, in a subgroup of patients with low platelet counts (<10) at baseline (19 with *ITPA*-AA/CA and 53 with *ITPA*-CC) we analyzed the treatment outcome according to rs1127354 genotypes. The SVR rate was very low in each group (21.1% in *ITPA*-AA/CA and 17.0% in *ITPA*-CC), because many patients had the initial dose reduction of PEG-IFN (<80% of standard dose)—36.8% of patients with *ITPA*-AA/CA and 44.6% of patients with *ITPA*-CC genotype. Further prospective studies are required among the pre-cirrhotic or cirrhotic patients with low platelet counts.

DISCUSSION

Recent genome-wide association studies, including our study on HCV infection, have identified two important host genetic variants: the SNP in *IL28B* gene, which is strongly associated with response to therapy for chronic genotype 1 HCV infection (16–21), and the SNP in *ITPA* gene, which precisely predicts RBV-induced anemia in

Table 3. Two SNPs (rs11697186 and rs6139030) significantly associated with quantitative change in Hb levels from baseline to week 4 of PEG-IFN/RBV treatment

dbSNP rsID	Nearest gene	MAF[a] (allele)	Allele (1/2)	Stage	Patients with quantitative change in Hb			Patients without quantitative change in Hb			OR (95% CI)[b]	*P*-value[c]
					11	12	22	11	12	22		
rs11697186	*DDRGK1*	0.15 (T)	T/A	GWAS	0 (0.0)	3 (3.3)	89 (96.7)	3 (1.5)	77 (37.0)	128 (61.5)	0.06 (0.02–0.16)	3.29×10^{-10}
				Replication	0 (0.0)	2 (1.5)	134 (98.5)	6 (2.5)	88 (37.3)	142 (60.2)	0.02 (0.01–0.09)	3.86×10^{-16}
				Combined	0 (0.0)	5 (2.2)	223 (97.8)	9 (2.0)	165 (37.2)	270 (60.8)	0.03 (0.01–0.08)	9.43×10^{-25}
rs6139030	ITPA	0.17 (C)	T/C	GWAS	88 (93.6)	6 (6.4)	0 (0.0)	125 (59.8)	80 (38.3)	4 (1.9)	0.08 (0.03–0.22)	2.56×10^{-9}
				Replication	134 (97.8)	3 (2.2)	0 (0.0)	143 (56.3)	103 (40.6)	8 (3.1)	0.03 (0.01–0.08)	6.90×10^{-18}
				Combined	222 (96.1)	9 (3.9)	0 (0.0)	268 (57.9)	183 (39.5)	12 (2.6)	0.04 (0.02–0.09)	2.12×10^{-25}

[a]Minor allele frequency and minor allele in 184 healthy Japanese individuals.
[b]OR for the minor allele in a dominant model.
[c]*P*-value by χ^2 square test for the minor allele dominant model.

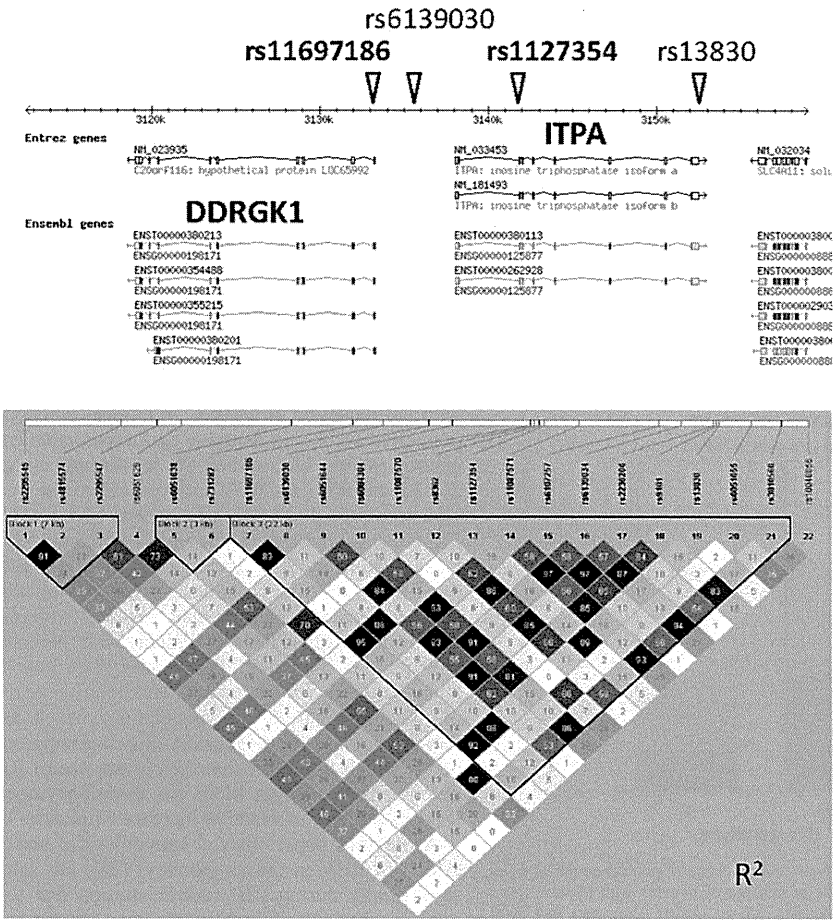

Figure 2. Pairwise LD (r^2) diagrams for *DDRGK1* and *ITPA*. Lower panel shows estimates of pairwise r^2 for 22 SNPs selected in the replication study using the second set of 391 Japanese HCV patients with and without quantitative change in PLT levels from baseline to week 4 of PEG-IFN/RBV treatment.

European-American population (22) and Japanese population (26). The genetic variation of *ITPA* causing an accumulation of inosine triphosphate (ITP) has been shown to protect patients against RBV-induced anemia during treatment for CHC infection. A recent report showed the biologic mechanism that ITP confers protection against RBV-induced ATP reduction by substituting for erythrocyte GTP, which is depleted by RBV, in the biosynthesis of ATP (25).

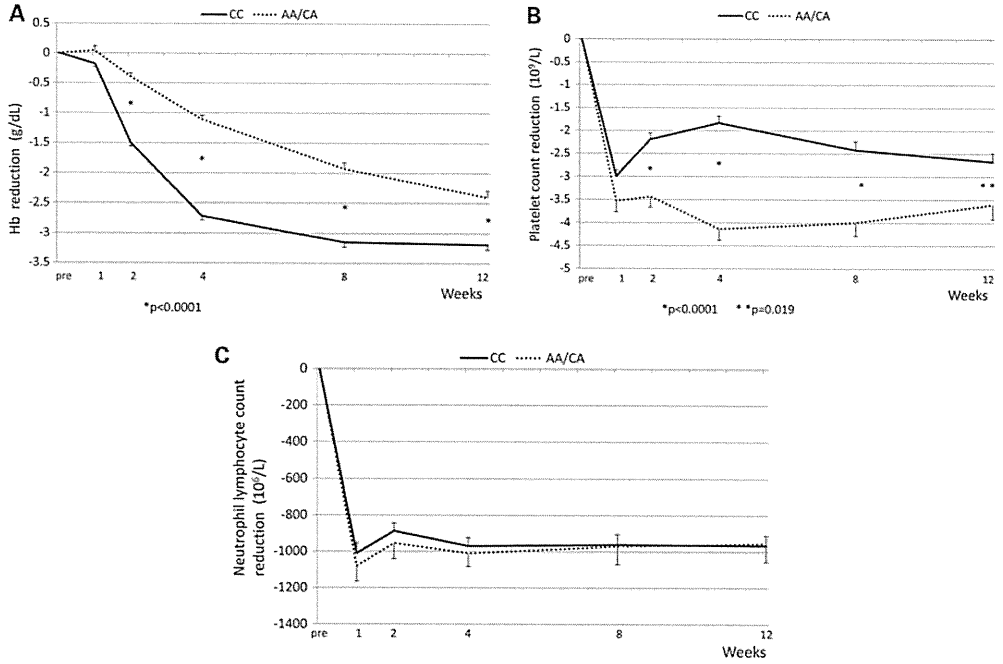

Figure 3. *ITPA* rs1127354 genotypes and the quantitative reduction of blood cells from baseline. Mean reduction of (**A**) Hb levels, (**B**) platelet counts and (**C**) neutrophil leukocyte counts during treatment according to rs1127354 genotype is shown. Solid and dotted lines indicate patients with CC and AA/CA genotypes, respectively. Error bars indicate standard error. CC genotype had more reduction in mean Hb levels during therapy compared with the AA/CA genotype ($^{*}P < 0.0001$ for weeks 2, 4, 8, 12). CC genotype had less of a reduction in mean platelet counts ($^{*}P < 0.0001$ for weeks 2, 4, 8, and $^{**}P = 0.019$ for week 12), and showed a reactive increase of platelet counts through weeks 1–4.

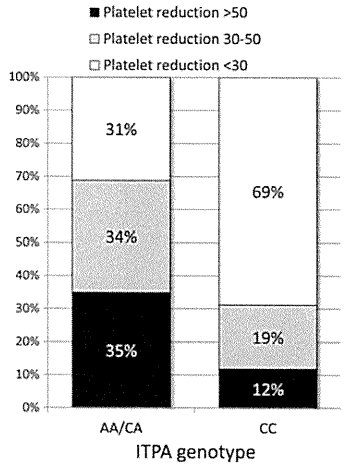

Figure 4. *ITPA* rs1127354 genotypes and reduction of platelet counts at week 4 of PEG-IFN/RBV therapy. The percentage of patients with platelet count reduction of >50 (10^9/l) (black bar), 30–50 (10^9/l) (gray bar) and <30 (10^9/l) (white bar) at week 4 is shown for rs1127354 genotypes. The incidence of platelet count reduction of >50 and <30 was significantly lower in patients with the rs1127354 genotypes CC compared with AA/CA genotypes: 12 versus 35%, $P < 0.0001$, and 69 versus 31%, $P < 0.0001$, respectively.

In this study, two SNPs, rs11697186 and rs6139030, which were within and around *DDRGK1* gene on chromosome 20, were strongly associated with thrombocytopenia as well as with Hb reduction at week 4. In clinical practice, the positive predictive value and negative predictive value by rs11697186 genotypes were 66.5 and 69.4% for thrombocytopenia, as well as 97.2 and 45% for RBV-induced anemia at week 4. As previously reported (22,26), a functional SNP (rs1127354) in the *ITPA* locus, which is in strong LD with rs11697186, was the most significant SNP associated with RBV-induced anemia and, in this study, IFN-induced thrombocytopenia in Japanese genetic populations. Note that severe Hb decline, which is mainly found in ITPA-CC patients, was inversely correlated with platelet reduction. This would contribute to an association between severe anemia and relative reactive increase of platelet count in this population, which attenuated the IFN effect on the platelet count. Our data supported a previous report which described that the current use of RBV, inducing severe anemia, might blunt the thrombocytopenic effect of IFNs as a result of reactive increase of platelet counts (27).

A previous paper showed hematological and bone marrow effects of RBV in rhesus monkeys (28). Hb values decreased significantly during RBV administration due to dose-related erythroid hypoplasia in bone marrow and returned to normal following withdrawal. On the other hand, increase of the platelet count occurred in both low- and high-dose treatment groups during RBV administration, with a fall of the platelet count to normal after drug withdrawal. The effect on platelet count was clearly dose related, with maximum counts rising to twice and three times above baseline levels in the low- and high-dose groups, respectively. This caused a significant increase of

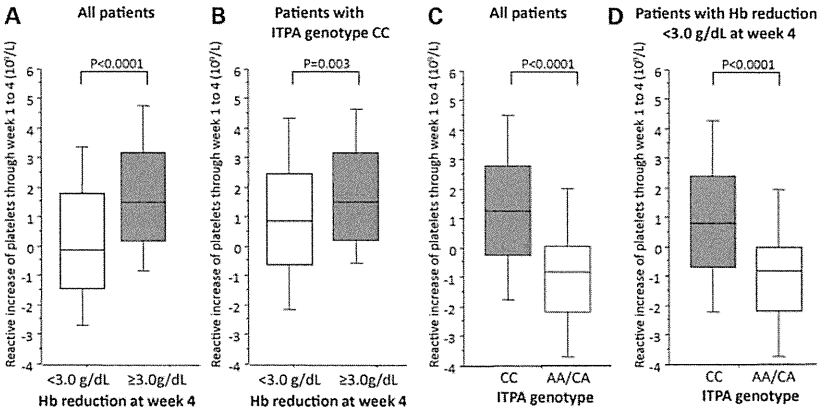

Figure 5. Reactive increase of platelet counts through weeks 1–4. Box plots of reactive increase of platelet count through weeks 1–4 according to the degree of anemia at week 4 are shown for all patients (**A**) and a subgroup of patients with the rs1127354 genotypes CC (**B**). Patients with anemia (Hb reduction ≥3.0 g/dl) at week 4 had a significantly higher degree of reactive increase of platelet count than those without anemia ($P < 0.0001$). Box plots of reactive increase of platelet counts according to the rs1127354 genotype CC are shown for all patients (**C**) and a subgroup of patients without anemia (**D**) (Hb reduction <3.0 g/dl) at week 4. Patients with the rs1127354 genotypes CC had a significantly high degree of reactive increase of platelet counts compared with those with genotypes AA/CA ($P < 0.0001$).

Table 4. Multivariate analysis of factors associated with reactive increase of platelets ≥20 (10^9/l) through weeks 1–4

	OR	95% CI	*P*-value
Baseline platelet counts	1.168	1.101–1.239	<0.0001
ITPA AA/CA	0.379	0.168–0.856	0.0196
Platelet reduction ≥30 (10^9/l) at week 4	0.051	0.021–0.120	<0.0001
Hb reduction ≥3.0 g/dl at week 4	1.602	0.914–2.809	0.0996

the platelet count associated with increased numbers of megakaryocytes. Additionally, the sequence homology of thrombopoietin (TPO) and erythropoietin (EPO) may explain the synergy of the physiologic role of TPO and EPO in platelet production. When EPO is elevated, as in iron deficiency anemia, an amino acid sequence similar to TPO may increase the platelet count (29).

Another possibility is a direct association between *ITPA* SNPs or the related SNPs with a strong LD and IFN-induced thrombocytopenia. *DDRGK1* (DDRGK domain-containing protein 1) is a novel C53/LZAP-interacting protein. C53/LZAP (also named as Cdk5rap3) is a putative tumor suppressor that plays important roles in multiple cell signaling pathways, including DNA damage response and NF-kappaB signaling (30); however, it remains largely unknown how the function of *DDRGK1* variants is regulated. Further studies are required to elucidate the possible association between *DDRGK1* variants and thrombocytopenia.

Multivariate analysis demonstrated that rs1127354 in the *ITPA* gene was independently associated with RBV-induced severe anemia and IFN-induced thrombocytopenia. This finding suggests that rs1127354 would be a useful marker to predict these hematological side effects by PEG-IFN/RBV therapy, indicating that genetic testing of *ITPA* variant might be applied to establish personalized dosages of PEG-IFN/RBV therapy. The rate of SVR tended to be higher in patients with *ITPA*-AA/CA genotype than those with *ITPA*-CC in this population. This might reflect decreased treatment efficacy (higher relapse rate) due to dose reduction of RBV in patients with *ITPA*-CC genotype. Our recent paper also demonstrated that the incidence of early dose reduction was significantly higher in *ITPA*-major (CC) patients as expected and, more importantly, that a significantly higher SVR rate was achieved in *ITPA*-hetero/minor (CA/AA) patients with HCV non-1b or low viral load strains (31) and in a subset of Japanese patients with the favorable TT genotype at rs8099917 of *IL28B* (32). Taken together, our results indicate that the *ITPA* minor variant A is not only a protective allele against PEG-IFN and RBV treatment-associated anemia in Japanese population, but also a significant predictor of SVR in certain HCV strains that show good response to IFN. The possible mechanism of protection against RBV-induced hemolysis is that ITP deficiency or low-activity variants (*ITPA* minor variant A) in turn lead to the accumulation of ITP in red blood cells (33,34), and the ITP confers protection against RBV-induced ATP reduction by substituting for erythrocyte GTP (25). On the other hand, half of the *ITPA*-major (CC) patients did not develop a significant Hb decline. This finding suggests other low-frequency *ITPA* variants or SNPs in other enzymes that are involved in erythrocyte purine nucleoside metabolism.

In Japan, the older HCV-infected patients developing liver fibrosis have been prevalent (mean age 62 years) (9). Thrombocytopenia by PEG-IFN/RBV therapy could lead to poor treatment efficiency among such Japanese patients with LC due to the initial or early dose reduction of PEG-IFN. In fact, ~40% of such population in this study had the initial dose reduction of PEG-IFN, resulting in a low SVR rate. Splenectomy or embolization of the splenic artery might be one of the options to increase the SVR rate, but a sufficient treatment outcome had not been obtained at present (35). Based on the recently accumulated SNP data, if patients had favorable *IL28B* genotype and *ITPA*-CC (lower reduction of platelet counts), a standard dose of PEG-IFN might be available for

the patients with lower platelet counts and the SVR rate might be increased due to sufficient dose of PEG-IFN.

Several STAT-C agents (specifically targeted antiviral therapies for hepatitis C) are being tested for clinical efficacy against hepatitis C (12,13,15,16). Most experts believe that when new drugs are approved to treat hepatitis C, they will be used in combination with PEG-IFN and RBV. Moreover, recent clinical trials, including NS3 protease inhibitors, have shown that PEG-IFN plus RBV would be necessary to achieve optimal treatment responses (12,13). Our present results may provide a valuable pharmacogenetic diagnostic tool for tailoring PEG-IFN and RBV dosing to minimize drug-induced adverse events and for further optimization of clinical anti-HCV chemotherapeutics.

MATERIALS AND METHODS

Patients

From April 2007 to April 2010, samples were obtained from 303 patients with chronic HCV (genotype 1) infection who were treated at 14 multi-center hospitals (liver units with hepatologists) throughout Japan. Each patient was treated with PEG-IFN-α2b (1.5 μg/kg body weight, subcutaneously once a week) or PEG-IFN-α2a (180 μg once a week) plus RBV (600–1000 mg daily according to body weight) for 48 weeks. Treatment duration was extended in some patients up to 72 weeks, according to the physicians' preferences. The dose of PEG-IFN or RBV was reduced according to the recommendations on the package inserts or the clinical conditions of the individual patients. EPO or other growth factors were not given. Written informed consent was obtained from each patient and the study protocol conformed to the ethics guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committees. HBsAg-positive and/or anti-HIV-positive patients were excluded from this study.

In the following stage of replication study, SNP genotyping in an independent set of 391 Japanese HCV patients treated with PEG-IFN plus RBV treatment was completed using the DigiTag2 or TaqMan assay (ABI) following the manufacturer's protocol. The characteristics of patients for each GWAS stage and replication stage are summarized in Table 1.

SNP genotyping and data cleaning

In the GWAS stage, we genotyped 303 Japanese HCV patients with and without the decrease of platelet counts from baseline to week 4 of PEG-IFN/RBV treatment [107 patients with a decrease of >30 (10^9/l) in platelet counts and 196 patients without a decrease of >30 (10^9/l) in platelet counts], using the Affymetrix Genome-Wide Human SNP Array 6.0 according to the manufacturer's instructions. The cut-off value was calculated to maximize the difference, which was also close to the median change. The average overall call rate of patients with and without the decrease of PLT reached 98.69 and 98.72%, respectively. We then applied the following thresholds for SNP QC in data cleaning: SNP call rate $\geq$95% for all samples, MAF $\geq$1% for all samples. A total of 595 052 SNPs on autosomal chromosomes passed the QC filters and were used for association analysis. All cluster plots of SNPs showing $P < 0.0001$ in association analyses by comparing allele frequencies in both groups with and without the decrease of PLT were checked by visual inspection, and SNPs with ambiguous genotype calls were excluded.

In the following stage of the replication study and high-density association mapping, we selected 23 tag SNPs from the 44.7 kb region, including *DDRGK1* gene and *ITPA* gene by analyzing LD and haplotype structure based on the HapMap data of Japanese, using the Haploview software. Of these tag SNPs, rs1127354 within the *ITPA* gene, which was associated with RBV-induced anemia (22), was included; however, rs7270101 was excluded because recent papers studying Japanese patients showed no variants in rs7270101 (26,31,32). The SNP genotyping in an independent set of 391 Japanese HCV patients with and without quantitative change in PLT levels from baseline to week 4 of PEG-IFN/RBV treatment (175 patients with quantitative change in PLT and 216 patients without quantitative change in PLT) was completed using the DigiTag2 assay (36). Twenty-two of the 23 SNPs were successfully analyzed and were used for SNP genotyping and data cleaning. All 22 SNPs in the replication study cleared HWE P-value >0.001.

Based on the above SNPs data obtained from 303 Japanese HCV patients, using the Affymetrix Genome-Wide Human SNP Array 6.0, we also performed GWAS between 94 patients with a quantitative change of >3 g of reduction in Hb and 209 patients without quantitative change in Hb levels from baseline to week 4 of PEG-IFN/RBV treatment. SNP genotyping in an independent set of 391 Japanese HCV patients with and without quantitative change in Hb levels from baseline to week 4 of PEG-IFN/RBV treatment (137 patients with quantitative change in Hb and 254 patients without quantitative change in Hb) was also completed using the DigiTag2 assay (36). Twenty-two of the 23 SNPs were successfully analyzed and were used for SNP genotyping and data cleaning.

An application of the Cochrane–Armitage test on all the SNPs showed the genetic inflation factor $\lambda = 1.000$ for thrombocytopenia and $\lambda = 1.006$ for anemia in the GWAS stage (Supplementary Material, Figs S1 and S2). In addition, principal component analysis was performed in 303 samples for the GWAS stage together with the HapMap samples (CEU, YRI, CHB and JPT) (Supplementary Material, Fig. S3). These results implied that the effect of population stratification was negligible, except one sample, which was excluded from further analysis.

Laboratory and histological tests

Blood samples were obtained at baseline, 1, 2, 4, 8 and 12 weeks after the start of therapy and for hematologic tests after the start of therapy and for hematologic tests, blood chemistry and HCV-RNA. Genetic polymorphism in the *IL28B* gene (rs8099917) was determined using the ABI TaqMan assay (Applied Biosystems, Carlsbad, CA, USA). Fibrosis was evaluated on a scale of 0–4 according to the METAVIR scoring system. The SVR was defined as an undetectable HCV-RNA level by qualitative PCR with a lower detection limit of 50 IU/ml (Amplicor, Roche Diagnostic Systems, CA, USA) or by Cobas Ampliprep/Cobas TaqMan assay (CAP/CTM) with a lower detection limit of

15 IU/ml (Roche Diagnostic Systems) 24 weeks after the completion of therapy.

Statistical analysis

The observed association between an SNP and the decrease of platelets/quantitative change in Hb levels with response to PEG-IFN plus RBV treatment was assessed by χ^2 test with a two-by-two contingency table in three genetic models: allele frequency model, dominant-effect model and recessive-effect model. SNPs on chromosome X were removed because gender was not matched between groups with and without the decrease of PLT and quantitative change in Hb levels. A total of 595 052 SNPs passed the quality control filters in the GWAS stage; therefore, significance levels after Bonferroni correction for multiple testing were $P = 8.40 \times 10^{-8}$ (0.05/595052) in the GWAS stage and $P = 2.27 \times 10^{-3}$ (0.05/22) in the replication stage.

The association between an SNP of the *ITPA* gene (rs1127354) and the incidence of platelet reduction at week 4 was analyzed by Fisher's exact test. The association between *ITPA* polymorphisms and the degree of reduction in platelet counts and Hb levels at each time point during therapy were analyzed by Mann–Whitney *U* test. Multivariable regression analysis was used to analyze the factors associated with *ITPA*, the rs1127354 genotype, factors associated with platelet count reductions and factors associated with the reactive increase in platelet counts. IBM-SPSS software v.15.0 (SPSS, Inc., Chicago, IL, USA) was used for these analyses.

Possible heterogeneity in allele frequencies at rs1127354 was assessed by Tarone's test. The association between the SNP and thrombocytopenia/anemia were analyzed by the Cochran–Mantel–Haenszel test. Both analyses were performed using the R (version 2.9.0) software (Supplementary Material, Table S3).

AUTHORS' CONTRIBUTIONS

Drafting of the paper, statistical analysis and approval of the final draft submitted: M.M.; drafting of the paper, statistical analysis, collecting samples and clinical data and approval of the final draft submitted: Y.T. and M.K.; statistical analysis and approval of the final draft submitted: N.N., M.S. and K.T.; collecting samples and clinical data and approval of the final draft submitted: K.M., N.S., N.E., H.Y., S.N., K.H., S.H., Y.I., E.T., S.M., M.H., Y.H., F.S., S.K. and N.I.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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The role of hepatoma-derived growth factor (HDGF) in cancer development and progression

Hirayuki Enomoto[1], Hideji Nakamura [1] and Shuhei Nishiguchi [1]

Division of Hepatobiliary and Pancreatic Medicine, Department of Internal Medicine, Hyogo College of Medicine, Mukogawa-cho 1-1, Nishinomiya, Hyogo 663-8501, Japan

Abstract

HDGF (hepatoma-derived growth factor) is a novel growth factor that belongs to a new gene family. Although HDGF was originally identified from the conditioned medium of a hepatoma cell line, HDGF is suggested to promote the proliferation of various kinds of cell via two different mechanisms: a receptor-mediated pathway followed by the activation of MAP kinase (MAPK) signaling, and direct action through its DNA binding after nuclear translocation. HDGF is a unique factor which has multiple functional characteristics, such as anti-apoptotic activity and angiogenic activity, as well its growth stimulating activities. Recent studies have shown that HDGF is considered

Correspondence: Hirayuki Enomoto, Division of Hepatobiliary and Pancreatic Medicine, Department of Internal Medicine, Hyogo College of Medicine, Mukogawa-cho 1-1, Nishinomiya, Hyogo 663-8501, Japan, E-mail:enomoto@hyo-med.ac.jp

to play significant roles in the development and progression of malignant diseases through these various activities. Furthermore, the expression level of HDGF is an independent prognostic factor for the disease-free and overall survival of various kinds of malignant disease, including hepatocellular carcinoma, pancreatic cancer, cholangiocarcinoma, esophageal cancer, gastric cancer, colorectal cancer, gastrointestinal stromal tumor, and some non-gastroenterological cancers. Therefore, HDGF is suggested to be involved in the development and progression of cancer in various organs.

Introduction

Cancer development and progression consist of many complicated processes (1, 2). One major event is an unlimited cellular proliferation associated with the escape of malignant cells from apoptotic cellular death. In addition, mutual reactions between cancer cells and surrounding stromal cells are also important, and tumor angiogenesis is considered to be essential for the progression and invasion of solid tumors. Therefore, clarifying the molecular systems involved in the anti-apoptotic/ unregulated cancer cell proliferation and tumor neovascularization processes would provide clinically important knowledge for the treatment of malignant diseases.

Hepatoma-derived growth factor (HDGF) is a novel factor that was purified from the hepatoma-derived cell line, Huh-7 (3, 4). Although HDGF was originally identified as a growth factor, HDGF has been also reported to be an anti-apoptotic factor and an angiogenic factor (5, 6), suggesting that it is involved in the development and progression of cancers through several potential mechanisms, including the stimulation of the cellular proliferation, the inhibition of the apoptosis of cancer cells and neovascularization. This article describes the characteristics of this novel factor and its possible roles in cancer development and progression, mainly focusing on hepatocelluar carcinoma (HCC).

Characteristics of HDGF

Molecular characteristics and possible signal transduction of HDGF

HDGF is a 26kDa heparin-binding acidic glycoprotein consisting of 240 amino acids. This growth factor was initially identified from the conditioned media of Huh-7 hepatoma cells. In addition, four novel genes have been identified and reported as HDGF-related proteins (HRPs) (7-9). These proteins share a highly homologous N-terminal region consisting of about 100 amino acids, which is called the HATH (homologous to the amino terminus of HDGF) region. Lens epithelium-derived growth factor (LEDGF),

which was originally reported as a survival factor for the lens epithelium, contains a HATH region, and is considered to be a member of the HDGF family (10).

Although the primary sequence of the HDGF protein does not contain the N-terminal hydrophobic sequence which is characteristic of signal peptides, it is detected in the conditioned media of various types of cells. HDGF is therefore thought to be secreted through a process which differs from the classical Golgi secretion system (5, 6). Recently, Thakar et al. (11) have reported that the N-terminal 10 amino acids of HDGF are required for the secretion of this growth factor. They also showed that phosphorylation of serine 165 in the C-terminal region of HDGF has a significant role in its secretion process.

Although the mechanism(s) in the secretion of HDGF remains unclear, the exogenous administration of HDGF significantly stimulates the proliferation of cells, including fibroblasts, endothelial cells, and fetal hepatocytes, as well as malignant cells (4, 6, 12-14). Furthermore, exogenously supplied HDGF stimulates the phosphorylation of MAP kinase (MAPK) in endothelial cells and gastric epithelial cells (15, 16). These findings strongly suggest the possibility of receptor-mediated signal transduction by HDGF. Recently, part of the HATH region (amino acids 81-100) was reported to be a possible receptor-binding site (17), and we have identified the putative receptor for HDGF (Liu et al, in submission). Based on these findings, the growth promoting effects of HDGF should occur via a receptor-mediated signal transduction pathway, and are followed by the intracellular activation of MAPK.

On the other hand, HDGF has two putative nuclear localization signals (NLSs); the first NLS (NLS1) resides in the HATH region and the second NLS (NLS2) is located in the gene-specific region. HDGF can be transported to the nucleus of cells, as demonstrated by its immunohistological detection, thus suggesting that HDGF has the characteristics of a nuclear factor (18, 19). We have shown that the nuclear translocation of HDGF is essential for the mitogenic activity of HDGF-overexpressing cells. We also found that the NLS2 is especially important for the growth stimulating effects of HDGF (18).

Although it has not been fully clarified how HDGF stimulates cellular growth after nuclear translocation, recent studies have suggested functional roles of the HATH region. The HATH regions of the HDGF family contain a PWWP motif (20, 21), which was originally reported in a candidate gene, WHSC1, for Wolf-Hirschhorn syndrome. An NMR study revealed that the PWWP domain of HDGF has a

characteristic hydrophobic cavity, thus indicating that HDGF likely binds to some component of chromatin through this cavity (22). In addition, HDGF has been suggested to bind to a conserved DNA sequence in the promoter region and suppress the transcription of its target genes, and the presumed DNA binding site is thought to reside in the PWWP domain (23). These findings suggest that the PWWP motif in the HATH region serves as a DNA binding domain for the HDGF protein.

In light of these findings, we hypothesized that HDGF would have two different mechanisms of stimulating cell growth: via a receptor-mediated pathway followed by the activation of MAPK signaling, and direct action through the DNA binding after nuclear translocation. Therefore, HDGF is a unique growth factor which has dual mechanisms for stimulating cellular proliferation.

HDGF functions as both a growth stimulating factor and an angiogenic factor

Although HDGF was originally isolated from the conditioned media of cultured hepatoma-derived cells, several studies have shown that HDGF plays important roles in organ development in the fetus and tissue repair in adults, including the liver, kidneys, lungs, and gut (5, 6, 16, 24, 25). In addition, Everett et al. (12) demonstrated that HDGF is highly expressed in proliferating fetal vascular smooth muscle cells (SMCs) and endothelial cells. They also demonstrated that HDGF expression is induced in vascular SMCs proximal to abdominal aorta constriction, and in neointimal cells after endothelial injury, suggesting functional roles for HDGF in the development and tissue repair of the cardiovascular system. Therefore, HDGF is suggested to function as an angiogenic factor, as well as a growth stimulating factor.

HDGF as a growth stimulating factor

HDGF and non-transformed hepatocyte proliferation

HDGF is highly expressed in immature fetal hepatocytes, especially in the mid-gestation stage of the liver, and its expression was dramatically decreased near birth (13). HDGF expression in hepatocytes decreases with cellular maturation, thereby suggesting that HDGF expression is related to the proliferative activity of hepatocytes. Furthermore, the exogenous administration of recombinant HDGF stimulates the proliferation of cultured fetal hepatocytes, whereas a reduction of HDGF expression severely suppresses the proliferation of these cells. These findings strongly suggest that HDGF plays a significant role in the proliferation of fetal hepatocytes during liver development (13, 14).

In the normal state, the liver is a quiescent tissue and most of mature hepatocytes are out of the replicating phase. However, the liver has the capacity to regenerate in response to cell loss, such as after hepatectomy or drug-induced hepatic injury (26, 27). In both hepatectomized and CCl_4-treated livers, the expression of HDGF was induced in hepatocytes prior to the peak of DNA synthesis. These findings indicate that the HDGF expression increases in parenchymal hepatocytes before DNA synthesis in the regenerating liver, thus suggesting that HDGF is involved in the proliferation of adult hepatocytes, as well as fetal hepatocytes (28).

Involvement of HDGF in the proliferation of various types of non-transformed cells

In the fetus, HDGF is abundantly expressed not only in the liver, but also in various other tissues, including the kidneys, heart, lungs and gut. Oliver et al. purified an endothelial growth factor from the conditioned media of a rat metanephrogenic mesenchymal cell line, and demonstrated that this purified growth factor was identical to HDGF. They have reported that HDGF should have an important role on glomerular capillary formation during nephron morphogenesis (24). HDGF was also expressed abundantly in fetal cardiovascular systems, including the heart and aorta. The HDGF protein is first detected in atrial myocytes, then its expression expands to the ventricular myocytes, endothelial and ventricular outflow cells. In addition, HDGF is strongly expressed in the proliferating vascular SMCs and endothelial cells in fetus (12). Furthermore, exogenous HDGF and endogenous overexpression of HDGF stimulated the growth of vascular SMCs. These findings suggest that HDGF can regulate vascular SMC proliferation during cardiovascular development and neointimal formation in response to vascular injury.

HDGF is highly expressed in the endothelial cells of developing blood vessels in the fetal lungs (16, 29). In a bleomycin-induced lung damage model, HDGF expression is dominantly induced in the bronchial and alveolar epithelial cells, including type II alveolar cells (30), thus suggesting that HDGF is related to the development and tissue repair of the respiratory system. With regard to the proliferation of the gut system, HDGF expression is suggested to have a suppressive role in the maturation of fetal intestinal cells and to be associated with the proliferation of these cells (25).

These research results suggest that HDGF functions as a growth stimulating factor for non-transformed cells and is involved in the development of various organs and in tissue repair processes.

HDGF as an angiogenic factor

HDGF and angiogenesis

Everett et al. (12) demonstrated that HDGF is highly expressed in the fetal cardiovascular system, and is induced during the regeneration of vascular vessels. Transplanted HDGF-overexpressing NIH3T3 cells develop large tumors in nude mice, and these tumors are histologically abundant in vasculature (31). HDGF also stimulates the proliferation and migration of human pulmonary microvascular endothelial cells *in vitro*. In addition, administration of recombinant HDGF significantly promoted blood vessel formation in the chick chorioallantoic membrane assay (16). HDGF has been demonstrated to stimulate the proliferation of human umbilical vein endothelial cells, and recombinant HDGF induces vessel formation *in vitro* (31). Interestingly, the introduction of HDGF in NIH3T3 cells induces the expression of VEGF (vascular endothelial growth factor), which is regarded as the most important angiogenic factor. The overexpression of HDGF significantly upregulates the promoter activity of VEGF, thus suggesting that HDGF promotes the transcription of the VEGF gene. Indeed, VEGF is highly expressed in the tumors developed from HDGF-overexpressing NIH3T3 cells, and growth of the HDGF-overexpressing tumors was partially suppressed by treatment with an anti-VEGF neutralizing antibody (31). Furthermore, HDGF was also reported to induce VEGF in a gastric cancer cell line (32). Therefore, the angiogenic activity of HDGF appears to occur via two mechanisms; one is its direct effect on the proliferation of endothelial cells, and the other is its induction of VEGF.

The introduction of HDGF cDNA into HepG2 hepatoma cells resulted in the formation of larger tumors in comparison to the tumors developed from the control cells (33). Tumors derived from HDGF-overexpressing HepG2 cells rapidly increased in size, although their proliferative activity *in vitro* only moderately increased. In addition, HDGF-overexpressing NIH3T3 cells show only a slight transformation capacity in soft agar, while these cells develop large tumors in nude mice, thus indicating that cells expressing high levels of HDGF had a more prominent growth activity *in vivo* than that expected from the *in vitro* studies (31). Several DNA-chip analyses demonstrated that the overexpression of HDGF upregulated several genes involved in neovascularization, including PDGF-A and Tie-1 (33). Since HDGF-overexpressing tumors are rich in vasculature and several different angiogenic growth factors can be induced by HDGF, the higher growth stimulating effects of HDGF *in vivo* may result from its angiogenic activity, in addition to its growth stimulating effects on cells.

HDGF in apoptosis

Malignant cells are sometimes able to suppress or avoid apoptotic signals, and their unregulated proliferative capacity and expression of many growth factors are suggested to contribute the tumor progression through their anti-apoptotic effects (34). However, the role of HDGF in the apoptotic pathway is still controversial.

HDGF expression has been reported to decrease in radio-resistant cells, and high HDGF expression is related to the sensitivity to irradiation in esophageal cancer cells (35). During the process of TNF/cycloheximide-induced apoptosis of endothelial cells, dephosphorylation of HDGF was shown to be an essential process for the initiation of caspase-dependent apoptosis (36), and knock-down of HDGF inhibits the apoptosis in TNF/cycloheximide-treated HeLa cells (37). In contrast, recent studies have shown that HDGF was involved in the resistance to apoptosis (REF). HDGF has also been reported to be a survival factor for CNS neurons, motor neurons and olfactory epithelium (38-40). The downregulation of HDGF induces the expression and dephosphorylation of the pro-apoptotic protein Bad, and suppresses the Erk-Akt signaling of MAPK, thus leading to the activation of an apoptotic pathway (41). In colorectal cancer cells, knockdown of HDGF induced apoptosis through the mitochondrial pathway, whereas overexpression of HDGF inhibited drug-induced apoptosis, suggesting that HDGF is associated with the resistance of these cells to chemotherapy (42, 43). Furthermore, the blockage of HDGF activated both the Fas-mediated extrinsic and Bad-mediated intrinsic apoptotic pathways in hepatoma cells (41, 44). Therefore, HDGF is also thought to function as a survival factor in hepatoma cells by exerting multiple anti-apoptotic effects.

Based on these recent reports, HDGF can be regarded as an anti-apoptotic survival factor in various cancer cells, although additional studies are required to elucidate the precise roles of HDGF in apoptosis.

HDGF in cancer

HDGF in hepatocellular carcinoma

HDGF is expressed in various hepatoma cell lines, including Huh-7, HepG2, Hep3B, PLC/PLF/5, SK-Hep1, and Mahlavu (5, 6, 18, 33). In addition, the endogenous overexpression of HDGF significantly increases the proliferation and DNA synthesis in hepatoma cells (18), whereas antisense treatment targeting HDGF reduces the cellular proliferation (45). Moreover, HDGF-overexpressing HepG2 hepatoma cells developed larger tumors in a xenograft model using nude mice in comparison to the control tumors (33).

These *in vitro* and *in vivo* experimental studies strongly suggest that HDGF acts as a growth factor for hepatoma cells.

We examined the HDGF expression in the livers of two rodent HCC models. Fisher F344 rats fed with a choline-deficient amino acid (CDAA) diet develop steatohepatitis with the progression to liver fibrosis, and HCC is observed beginning after 52 weeks of age. The Fatty Liver Shionogi (FLS) mouse is an inbred mouse strain that spontaneously develops fatty changes, and at 52 weeks, 90% of male FLS mice develop liver tumors that are histologically confirmed to be hepatocellular adenoma and carcinoma (46). HDGF was expressed more highly in HCC than in the adjacent cirrhotic liver in CDAA-fed rats, and it was also expressed more strongly in HCC than in the adjacent liver with steatohepatitis in the FLS mice. Of note, the HDGF expression increased in the liver of FLS mice before the development of visible solid tumors, suggesting a growth stimulating function of HDGF during the early stage of hepatocarcinogenesis as well as during the progression of HCC (47).

In the previous reports, the protein expression of HDGF in human HCC tissue samples was evaluated by immunostaining, and the levels of the HDGF protein were found to be higher in human HCC tissues than in the adjacent tissues (47). Moreover, the expression level of HDGF is strongly associated with the prognosis of HCC after surgery, and a higher expression of HDGF was found to be related to poorer prognosis (48). In fact, three independent groups (including our group) have demonstrated that HCC patients with a higher HDGF expression level showed an earlier recurrence and an unfavorable overall survival rate compared to those with lower expression levels of HDGF (48-50), and that the HDGF expression was found to be an independent prognostic factor for the disease-free and overall survival in patients after curative resection of HCC. These findings suggest that HDGF plays a significant role in the progression of human HCC.

HDGF in pancreatic cancer

Pancreatic ductal carcinoma is one of the most fatal cancers, and shows a high proliferative and invasive activity, with a poor prognosis. HDGF is strongly expressed in pancreatic cancer cells, as well as hepatoma cells, including various pancreatic ductal carcinoma cell lines such as MIA PaCa-2, PANC-1, PL45 and KP-4 (51). We examined the HDGF expression by immunohistochemistry for 50 patients with primary ductal pancreatic carcinoma who received surgical treatment, and reported that the univariate and multivariate analyses showed nuclear HDGF expression to be an independent prognostic factor for pancreatic

ductal carcinoma after curative resection (51). Our findings suggest that HDGF expression, as evaluated by immunohistochemistry, can be used as a new prognostic factor for pancreatic cancer.

HDGF in cholangiocarcinoma

The expression levels of HDGF and VEGF were examined in patients with human hilar cholangiocarcinoma (52). HDGF and VEGF had a positive correlation, and patients with positive HDGF expression had a significantly poorer overall survival rate than those with negative HDGF expression. A multivariate analysis showed that HDGF expression was an independent prognostic factor. These findings suggested that a high expression of HDGF plays an important role in the development and progression of human hilar cholangiocarcinoma and that HDGF expression can be a valuable prognostic factor for this cancer.

HDGF in esophageal cancer

As described in the "HDGF in apoptosis" section, a previous study suggested that there is a relationship between the expression of HDGF and the radiosensitivity of esophageal cancer cells. HDGF is highly expressed in radiosensitive esophageal cancer cells, and radiotherapy is more effective in patients with a high expression of HDGF than in those with low expression (35). In addition, the induction of apoptosis in cancer cells is thought to be one of the important mechanisms involved in the anti-tumor effects of radiotherapy, and HDGF expression is suggested to be involved in the resistance to apoptotic signals (38-44). Therefore, the mechanism(s) underlying how HDGF expression is associated with the sensitivity to radiation still remains to be clarified.

Although an increased expression of HDGF leads to a higher effect of radiotherapy, HDGF expression is associated with the recurrence and prognosis of cancer in patients with esophageal cancer (53). Patients with a higher expression of HDGF had a poorer disease-free and overall survival compared with those with a lower expression. Further studies are therefore necessary to determine the functional role of HDGF in esophageal cancer.

HDGF in gastric cancer

HDGF is expressed in gastric cancer cells (15, 32). Transfection of HDGF stimulated cellular proliferation via the activation of Erk1/2 signaling, and promoted the anchorage-independent growth of gastric cancer cells (15). Furthermore, a reduction of HDGF in gastric cancer cells induced Bad-mediated apoptotic signaling, and decreased the invasive activity of the cells (32). Interestingly, HGF (hepatocyte growth factor) induced HDGF in a dose-dependent manner, and HDGF induced VEGF expression, thus suggesting that HDGF may be involved in tumor growth by means of its cooperation with these growth factors. In

patients with gastric cancer, higher expression levels of HDGF are significantly associated with higher rates of infiltrative tumor growth, as well as vascular and lymphatic invasion (54). Thus, HDGF is suggested to have a significant role in the development and progression of gastric cancer.

HDGF in colorectal cancer

HDGF has been reported to be involved in the development and progression of colorectal cancer cells (42, 43). HDGF expression is remarkably high in human colorectal cancers, especially in tumors proficient in DNA mismatch repair (25). We have documented that recombinant HDGF stimulated the proliferation of colonic HT-29 cells, whereas a polyclonal antibody against recombinant HDGF significantly suppressed their proliferation (55). Knockdown of HDGF induced apoptosis through the activation of the mitochondrial pathway (42), whereas the overexpression of HDGF resulted in the resistance of colorectal cancer cells to drug-induced apoptosis (43). These findings suggest that HDGF plays an important role in gut epithelial cell proliferation, including that of colorectal cancer cells.

HDGF in gastrointestinal stromal tumors (GIST)

The HDGF protein was detected in GIST tissues (56, 57). An immunohistochemical evaluation suggested that there is a significant relationship between HDGF expression and tumor growth. HDGF expression correlates with tumor mitosis and tumor size. A high expression of HDGF in patients with GIST was related to an early recurrence and poor prognosis, and HDGF expression was reported to be an independent prognostic factor for the disease-free and overall survival of patients after surgical resection (56). Furthermore, with regard to the surgically resected colorectal stromal tumors, it was reported that patients with high HDGF expression had shorter disease-free survival than patients with low HDGF levels, and that HDGF was an independent prognostic factor for patients with colorectal stromal tumors (57).

HDGF in malignant diseases of non-gastroenterological tissues

In addition to the gastroenterological cancers, HDGF is thought to be associated with the development and progression of various malignant diseases in non-gastroenterological tissues. HDGF has been shown to function as a mitogenic factor for lung epithelial cells both *in vitro* and *in vivo*, and previous studies have shown the high HDGF expression correlates with the aggressive biological behavior and poor clinical outcomes reported for non-small cell lung cancer (NSCLC) (5, 16, 58-60). Patients with a high expression level of HDGF show a poorer overall and disease-free survival than those with a low HDGF expression level, thus indicating that HDGF

is a significant independent prognostic factor in NSCLC (58, 59). Furthermore, HDGF has been also reported to be involved in the unfavorable clinical features of many other malignant diseases, including malignant melanoma, nasopharyngeal carcinoma, breast cancer and prostate cancer (61-63). Although HDGF was originally identified as a growth factor for hepatoma cells, HDGF also appears to have significant roles in many kinds of malignant disease, including non-gastroenterological cancers.

Conclusion

HDGF is a novel growth factor belonging to a new gene family. It is a unique molecule with multiple malignant characteristics, such as its ability to act as a growth stimulating factor, an angiogenic factor and a possible anti-apoptotic factor. Numerous studies have demonstrated that the overexpression of HDGF correlates with poor outcomes in various types of malignant diseases. HDGF is considered to play significant roles in the proliferation and survival of cancer cells and also in the induction of angiogenesis. HDGF is therefore suggested to be a potential therapeutic target for many malignant diseases.

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