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Original Research

PD1 gene polymorphism is associated with a poor prognosis in hepatocellular carcinoma following liver resection, cohort study

Masateru Yamamoto[a,c], Tsuyoshi Kobayashi[a,c,*], Hiroaki Mashima[a,c], Daiki Miki[b,c], Shintaro Kuroda[a,c], Michinori Hamaoka[a,c], Hiroshi Aikata[b,c], Kazuaki Chayama[b,c], Hideki Ohdan[a,c]

[a] Department of Gastroenterological and Transplant Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[b] Department of Gastroenterology and Metabolism, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[c] Research Center for Hepatology and Gastroenterology, Hiroshima University, Hiroshima, Japan

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ABSTRACT

Background: This study examined whether single nucleotide polymorphism (SNP) in programmed cell death protein (PD)-1 is related to the postoperative prognosis of patients with hepatocellular carcinoma (HCC). The immune checkpoint protein PD-1 is an important inhibitor of T cell responses. SNP in the promoter region of PD-1 -606 G/A has been reported to result in high activation and expression of PD-1 associated with cancer risk.
Materials and methods: We analyzed 321 patients with HCC who underwent hepatectomy between 2010 and 2015. PD-1 SNP was analyzed by polymerase chain reaction, and the prognosis after surgical treatment of patients with HCC was analyzed.
Results: The PD-1 SNP statuses were as follows: 90 AA (28.1%), 163 GA (50.8%), 68 GG (21.2%). The baseline parameters did not statistically differ between the three groups. The overall survival (OS) of patients with the GG genotype was significantly lower than that of those with the other genotypes (P = 0.031). The GG genotype was an independent risk factor for OS (P = 0.009; HR 2.201). There was no significant difference between the GG genotype and other genotypes in recurrent-free survival. The extrahepatic recurrence (EHR) rate of those with the GG genotype was significantly higher than that of those with the other genotypes (P = 0.036). The GG genotype was an independent risk factor for EHR (P = 0.008; HR 2.037).
Conclusions: The PD-1 SNP GG genotype is associated with poor survival and increased EHR in HCC. Furthermore, the GG genotype is an independent predictive factor for OS and EHR.

1. Introduction

The pathogenesis of cancer is unclear but is widely recognized to result from gene-environment interactions. The human immune system plays an important role in combating and eliminating cancer cells, and influences the onset of cancer. Immune cells identify and eliminate certain incipient cancer cells. However, some of these cells escape surveillance and cell death mediated by the immune system [1]. The understanding of the molecular basis of this phenomenon has increased and new anti-cancer approaches have been developed in recent researches. Immune system activation alters tumor-specific T cell immunity in the cancer microenvironment and modulates tumor progression and metastasis [2]. Many receptor-ligand interactions have been shown to trigger anti-apoptotic pathways that prevent T cell activation and induce T cell death [3,4].

Programmed cell death-1 (PD-1, also called CD279), is a member of the CD28-B7 superfamily of costimulatory molecules for T lymphocyte activation [5,6], well known as an immunoinhibitory receptor that negatively regulates T cells through inhibitory signals. The human PD-1 gene is on chromosome 2q27.3 and encodes a 50–55 kDa type I transmembrane glycoprotein. PD-1 protein consists of an extracellular immunoglobulin V domain, intracellular domain containing an immune receptor tyrosine-based inhibitory motif, and immune receptor tyrosine-based switch motif [7]. The interaction between PD-1 and PD-L1 activates the immune receptor tyrosine-based inhibitory motif of PD-1 and provokes the inhibitory signal to attenuate T lymphocyte activation and proliferation to suppresses cytokine secretion. T cell apoptosis occurs, and peripheral tolerance is established [8–10].

* Corresponding author. Department of Gastroenterological and Transplant Surgery, Graduate School of Biomedical and Health Science, Hiroshima University, Hiroshima, Japan, 1-2-3, Kasumi, Minami-ku, Hiroshima City, Hiroshima, 734-8551, Japan.
E-mail address: tsukoba@hiroshima-u.ac.jp (T. Kobayashi).

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Previous studies showed that a single nucleotide polymorphism (SNP) in PD-1, rs36084323 A > G, is associated with disease progression and cancer development [11,12]. The binding of transcription factors may be affected by mutations in the promoter region of functional genes, and such mutations could alter the activation of genes and initiation of gene transcription [13]. rs36084323 resides in the putative binding site for UCE-2 transcription regulators (GGCCG at position −610 to −606). The SNP can influence PD-1 gene transcription by increasing the promoter activity, thereby promoting the development of cancers and progression of human diseases [7].

Genetic diversity can affect gene function and alter disease phenotypes. Therefore, polymorphisms of the gene-related immune response regulating T lymphocyte activation and proliferation may contribute to the progression of malignant disease. SNPs are among the most common genetic variations. Although many studies have assessed the association of the SNP of PD-1 (rs36084323) and risk of various types of diseases [7], the function of this SNP remains controversial in hepatocellular carcinoma (HCC). Thus, this study was performed to further assess the role of this SNP in HCC.

2. Materials and Methods

2.1. Patients

A total of 321 Japanese patients (245 men, 76.3% and 76 women, 23.7%) with a median age of 70 years (range, 31–91) who underwent hepatectomy consecutively at our institution between January 2010 and December 2015 were enrolled in this study. The number of patients with hepatitis B virus (HBV) positive was 57 (17.8%), and the number of patients with hepatitis C virus (HCV) was 176 (54.8%). The median follow-up time in this study was 3.4 years (range, 0.24–8.78). The baseline characteristics are summarized in Table 1. The inclusion criteria were as follows: the tumor was histologically diagnosed as HCC; no distant metastasis was detected in the preoperative image; hepatectomy for HCC was conducted for the first time; and absence of any other malignancies. The baseline clinicopathological findings were retrieved from the hospital database and reviewed. The primary end point is overall survival (OS) and OS is defined from the date of operation to the date of the last follow-up before the data were analyzed, or the date of death. Hepatectomy and liver function were classified according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer [14]. The hepatectomy procedure was performed as previously reported [15,16]. After being discharged from the hospital, all patients were screened for tumor recurrence and metastasis by measuring tumor markers every 3 months, as well as by abdominal ultrasound, computed tomography and magnetic resonance imaging every 6 months. The duration of follow-up was defined from the date of operation to the date of the last follow-up before the data were analyzed, or the date of death. This study was the approved by the Institutional Review Board (Provided ID Number: Hi-202) on the Ethical Guidelines for Clinical Research of the Ministry of Health, Labour and Welfare in Japan. All patients gave written informed consent to participate according to the Declaration of Helsinki. The data that support the findings of this study are available upon request to the corresponding author. The data are not publicly available due to privacy or ethical restrictions. The work has been reported in line with the STROCSS criteria [17].

2.2. Genotyping of PD-1 polymorphism

Genomic DNA was isolated from whole blood collected from patients using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, German). DNA was amplified via polymerase chain reaction (PCR) using a Quick Taq HS DyeMix (Toyobo, Osaka, Japan). The following primers were designed: forward 5′-tggaagatctggaactgtgg-3′ and reverse 5′-attctgtcggagcctctgg-3′. PCR was performed as follows: 94 °C for 5 min; 40 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s; and 72 °C for 7 min. PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) and sequenced with the forward primer using a BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Table 1
Baseline characteristics.

	AA N = 90 (28.1%)	AG N = 163 (50.8%)	GG N = 68 (21.2%)	P value
Age (years)	72 (40–87)	69 (31–91)	70 (31–87)	0.282
Sex				
Male	71 (78.9)	125 (76.7)	49 (72.1)	0.599
Female	19 (21.1)	38 (23.3)	19 (27.9)	
HBV positive	13 (14.4)	34 (20.9)	10 (14.7)	0.335
HCV positve	47 (52.2)	88 (53.9)	41 (60.3)	0.573
Alb (g/dL)	4 (2.9–5.1)	4 (2.3–5.4)	4 (2.9–4.9)	0.808
Plt (x10^4/mm^3)	13.2 (4.6–239)	14.4 (4.3–240)	14.4 (3.1–31.4)	0.264
PT (%)	85 (27–116)	85 (54–119)	85 (33–112)	0.952
T-Bil (mg/dL)	0.8 (0.2–2.3)	0.7 (0.3–2.9)	0.7 (0.3–1.9)	0.107
AST (IU/L)	34 (14–151)	30 (11–296)	34 (12–130)	0.789
ALT (IU/L)	28 (10–144)	28 (10–204)	31 (10–148)	0.799
ICGR15 (%)	12.3 (2.1–40)	12.4 (2.6–66)	13.8 (3.5–50)	0.671
AFP (ng/mL)	8.4 (1–290,700)	12.6 (0.5–57410)	15.1 (0.5–6050)	0.339
DCP (mAU/mL)	49 (0–71992)	61 (0–147,910)	37.5 (10–124,310)	0.316
Child-Pugh (A/B)				0.719
A	85 (94.4)	150 (92.1)	64 (94.1)	
B	5 (5.6)	13 (7.9)	4 (5.9)	
Tumor number	1 (1–9)	1 (1–20)	1 (1–13)	0.894
Tumor size (mm)	20 (6–355)	18 (9–160)	25 (7–170)	0.101
Anatomical resection	67 (74.4)	109 (66.5)	47 (70.2)	0.417
Operation time (min)	318 (127–644)	315 (76–760)	323 (130–531)	0.475
Blood loss (mL)	387 (10–7798)	327 (20–4470)	275 (20–2750)	0.451
MVI	14 (15.9)	41 (25.3)	10 (14.9)	0.083
LC	15 (17.4)	34 (22.8)	18 (26.1)	0.371

AFP alpha-fetoprotein levels, *Alb* albumin, *AST* asparate aminotransferase, *ALT* alanine aminotransferase, *DCP* des-gamma-carboxyprothorombin, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *ICGR15* indocyanine green retention rate at 15 min, *LC* liver cirrhosis, *MVI* microvascular invasion, *Plt* platelet count, *PT* prothrombin time, *T. Bil* total bilirubin.

2.3. Statistical analysis

Median and range were considered continuous variables and compared by Mann-Whitney *U* test. Categorical variables were expressed as numbers and percentages and compared using Fisher's exact test. A multivariate Cox proportional hazards model was used to determine independent risk factors associated with survival. The optimal cut-off points for the OS were determined by receiver operating characteristic curve analysis. Survival curves were generated using the Kaplan-Meier method and compared between different groups using the log-rank test. Statistically significant variables in univariate analysis were evaluated by multivariate Cox regression analysis. Statistical analyses were performed using JMP Pro (version 14; SAS Institute, Cary, NC, USA). A P-value less than 0.05 was considered as significant.

3. Results

A total of 321 patients were included in the study. The PD-1 -606 SNP statuses in the promoter region (rs36084323) were 90 AA (28.1%), 163 GA (50.8%), 68 GG (21.1%). All the following baseline clinical characteristics were compared between the AA, AG, and GG genotype groups. The baseline parameters did not statistically differ in age, sex, HBV, HCV, albumin level, platelet count, prothrombin time, total

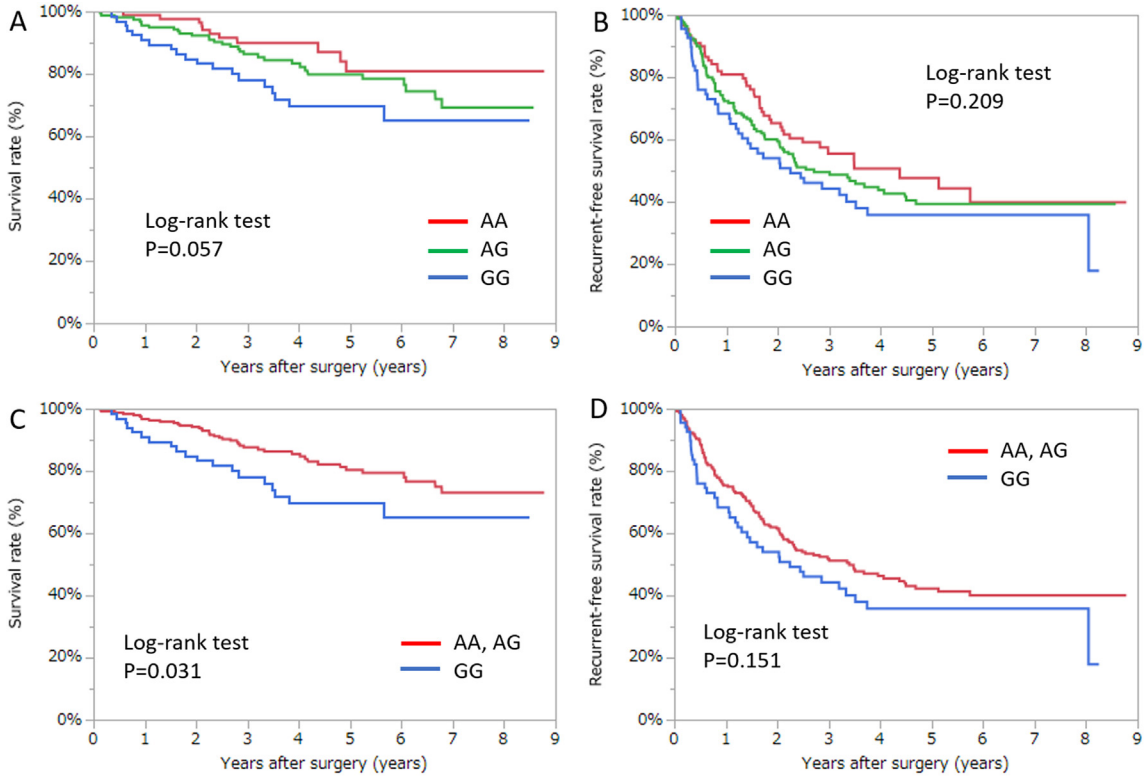

Fig. 1. Overall survival (A) and disease-free survival (B) of HCC patients between three groups according to the programmed cell death 1 single-nucleotide polymorphism rs36084323 genotype. Overall survival (C) and disease-free survival (D) of HCC patients between two groups according to the programmed cell death 1 single-nucleotide polymorphism rs36084323 genotype.

bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), indocyanine green retention rate at 15 min (ICGR15), alpha-fetoprotein (AFP), des-gamma-carboxyprothrombin (DCP), Child-Pugh grade, number of tumors, tumor size, anatomical resection, operation time, blood loss, microvascular invasion (MVI), and liver cirrhosis (Table 1).

The results of Kaplan-Meier analyses to determine OS and recurrent-free survival (RFS) using the PD-1 -606 SNP genotype are shown in Fig. 1A and B. Although there were no significant differences between the three groups in both OS and RFS according to the log-rank test, the groups were clearly stratified by the SNP genotype. Therefore, the background was compared between the GG group and the other groups. There was no difference in patient background (Table 2). The OS of the GG group was significantly lower than that of the other groups in Kaplan-Meier analyses (P = 0.031) (Fig. 1C). However, Fig. 1D shows that the GG group was not correlated with RFS. The cumulative extrahepatic recurrence (EHR) rate of GG was significantly higher than that in the other groups (P = 0.011) (Fig. 2).

In the univariate analysis, significant prognostic factors contributing to the poor OS rate included HCV, AST level > 35 IU/L, ICGR15 level > 15%, AFP level > 10 ng/mL, Child-Pugh grade B, multiple tumors, tumor size > 50 mm, blood loss > 1000 mL, MVI, and GG genotype. Multivariate analysis identified five indicators of poor OS (HCV, Child-Pugh grade B, multiple tumors, tumor size > 50 mm, and GG genotype; Table 3). Univariate analysis identified the following significant prognostic factors for EHR; albumin level < 3.5 g/dL, AST level > 35 IU/L, AFP level > 10 ng/mL, DCP level > 100 mAU/mL, Child-Pugh grade B, multiple tumors, tumor size > 50 mm, operation time > 300 min, blood loss > 1000 mL, MVI, and GG genotype. Multivariate analysis revealed three factors resulting in a high EHR (multiple tumors, tumor size > 50 mm, and GG genotype; Table 4).

Table 2
Baseline characteristics between GG genotype and other groups.

	AA + AG N = 253 (78.8%)	GG N = 68 (21.2%)	P value
Age (years)	70 (31–91)	70 (31–87)	0.925
Sex			
Male	196 (77.5)	49 (72.1)	0.341
Female	57 (22.5)	19 (27.9)	
HBV positive	47 (18.7)	10 (14.3)	0.591
HCV positive	135 (53.4)	41 (60.3)	0.338
Alb (g/dL)	4 (2.3–29)	4 (2.9–4.9)	0.537
Plt (x10^4/mm^3)	14.3 (4.3–45.2)	14.4 (3.1–31.4)	0.309
PT (%)	85 (27–119)	85 (33–112)	0.821
T-Bil (mg/dL)	0.8 (0.2–2.9)	0.7 (0.3–1.9)	0.275
AST (IU/L)	31 (11–296)	34 (12–130)	0.651
ALT (IU/L)	28 (10–204)	31 (10–148)	0.518
ICGR15 (%)	12.3 (1–66)	13.8 (3.5–50)	0.386
AFP (ng/mL)	9.8 (0.5–290,700)	15.1 (0.5–6050)	0.233
DCP (mAU/mL)	58 (5–147,910)	37.5 (10–124,310)	0.378
Child-Pugh (A/B)			1
A	235 (92.9)	64 (94.1)	
B	18 (7.1)	4 (5.9)	
Tumor number	1 (1–20)	1 (1–13)	0.949
Tumor size (mm)	25 (6–355)	25 (7–170)	0.838
Anatomical resection	176 (68.3)	47 (70.2)	1
Operation time (min)	317 (76–760)	323 (130–531)	0.241
Blood loss (mL)	360 (10–4470)	275 (20–2750)	0.298
MVI	55 (22.0)	10 (14.9)	0.235
LC	49 (20.6)	18 (27.2)	0.317

AFP alpha-fetoprotein levels, *Alb* albumin, *AST* asparate aminotransferase, *ALT* alanine aminotransferase, *DCP* des-gamma-carboxyprothorombin, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *ICGR15* indocyanine green retention rate at 15 min, *LC* liver cirrhosis, *MVI* microvascular invasion, *Plt* platelet count, *PT* prothrombin time, *T. Bil* total bilirubin.

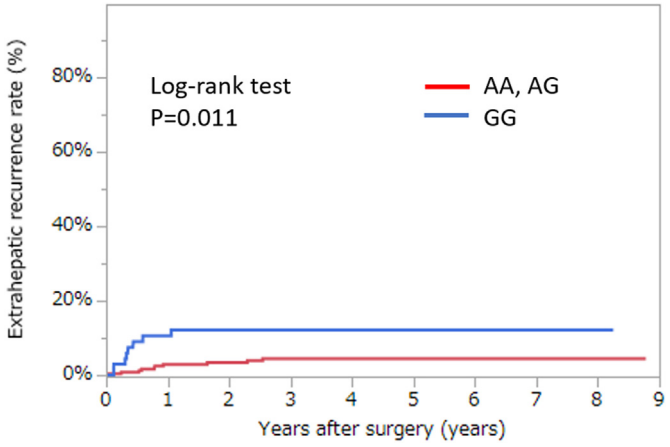

Fig. 2. Extrahepatic recurrence of HCC patients between two groups according to the programmed cell death 1 single-nucleotide polymorphism rs36084323 genotype.

4. Discussion

In this study, we investigated the association of an SNP in PD-1 genes and the prognosis of patients with HCC following hepatectomy. Our data revealed that the GG genotype for the PD-1 SNP (rs36084323) was significantly associated with an unfavorable prognosis and an independent risk factor in patients with HCC following hepatectomy. Moreover, the GG genotype was significantly associated with an increased risk of EHR and was an independent risk factor for EHR. This is the first study to demonstrate a relationship between PD-1 SNP -606 G/A and OS that is due of high EHR.

Human cancers are affected by numerous genetic and epigenetic factors. Changes in these factors can produce neoantigens that are potentially recognized by the immune system [18]. However, tumors acquire multistep resistance mechanisms, including local immunosuppression, acquisition of resistance, and T cell dysfunction [19–22]. In addition, tumors utilize numerous pathways to escape immune-mediated destruction. Various checkpoints allow the tumor to modulate the nascent immune response and to evade the antitumor immune responses, one of which includes PD-1. PD-1, a T cell suppressor, causes T cell dysfunction through increased interactions with its ligand, PD-L1 [23]. Suppression of the immune system alters the tumor-specific T cell immunity in the cancer microenvironment, and promotes tumor progression and metastasis.

Regarding the PD-1 SNPs, it is worth considering the PD-1 functional SNPs, rs36084323, rs11568821, rs2227981, and rs2227982 in different cancers. Since rs2227981 has already been reported to be related to HCC and is absent in the Japanese population [11], we analyzed the others (rs36084323, rs11568821, and rs2227982). rs11568821 is located in intron 4, alters the binding of transcription factor, and modifies the translational regulation [24]. rs2227982 is located in exon 5 and involved in transcription splicing [24]. We focused on rs36084323 because, of the three, it was the only one found to be correlated with the prognosis (Supplementary figure 1).

A haplotype of the −606 G allele with a high promoter activity was shown to be correlated with the development of subacute sclerosing panencephalitis caused by persistent infection with measles virus [11]. The relative PD-1 expression was higher in patients with subacute sclerosing panencephalitis compared to that in the controls. This PD-1 gene promoter SNP was found to be correlated with a poor prognosis in surgically-resected non-small cell lung cancer [12]. The OS of the patients with GG genotype of PD-1 was significantly lower compared with patients having other genotypes (Fig. 1A). This may mean the haplotype with the G allele has a reduced ability to eliminate cancer cells. However, the AA genotype and the A allele in PD-1 -606 G/A polymorphism have been reported to occur frequently in p53 mutations [25], suggesting that other genetic and environmental factors are involved.

The PD-1 gene −606 G/A polymorphism may modify the activity of the promoter and is Asian-specific [7]. However, the correlation between this Asian-specific PD-1 SNP and HCC is unclear. Because the prognosis of HCC is largely due to liver function, its influence is considered greater than that of the gene. This can also be seen from the fact that Child-Pugh grade B is an independent prognostic factor of OS in the present study. Analyzing the effects of genes on the prognosis of HCC may be more accurate because of the large number of cases of Child-Pugh grade A. In addition, HCV was an independent prognostic factor, and the key determinant of HCC is HBV in East Asia, except Japan [26]. HCV is more common in Japan than in other countries, and the background may be responsible for the differing results from studies performed in other countries [27,28].

The significantly higher EHR in patients with the GG genotype caused the poor OS in the GG genotype in this study. Some studies [29–31] have described MVI as risk factors for EHR following hepatectomy that agrees with our results. In addition, the GG group was considered as a strong prognostic factor for EHR.

There were some limitations to this study. First, this is a retrospective cohort study with a relatively small number of cases. Although HCC is a common disease in Asia, its epidemiology in other world regions is different, and this study population is limited to Japanese patients. Additional validation is required to overcome selection bias in the population and management of HCC patients. Second, the effect of PD-1 SNP on diseases remains controversial. There was no correlation between the SNP and RFS. The poor prognosis for RFS is usually considered to be the cause of the poor prognosis for OS, but in this study, high EHR due to SNP was considered to be the cause. Although there was no significant difference among the three groups, they were clearly stratified, suggesting that the haplotype with the G allele is involved in PD-1 expression. The small sample size may have contributed to this result, therefore a larger sample size is necessary to confirm finding in replicative studies. Third, cancer is a multifactorial disease resulting from complex interactions between the environment and genetic factors. The subjects in this study were limited to Japanese patients, whose backgrounds differ from other patients worldwide.

In conclusion, this study demonstrated that the PD-1 rs36084323 -606 A > G polymorphism is associated with poor survival and is an independent risk factor following initial hepatectomy for HCC. In addition, the PD-1 SNP is associated with a high EHR rate and is an independent risk factor.

Ethical approval

This study was the approved by the Institutional Review Board of Hiroshima University (Provided ID Number: Hi-202) on the basis of the Ethical Guidelines for Clinical Research of the Ministry of Health, Labour and Welfare in Japan.

Sources of funding

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Author contribution

Masateru Yamamoto, Tsuyoshi Kobayashi, and Hideki Ohdan were involved in the study concept and design, data acquisition and analysis, drafting of the manuscript. Hiroaki Mashima, Daiki Miki, Shintaro Kuroda, Michinori Hamaoka, Hiroshi Aikata, and Kazuaki Chayama contributed to perform data analysis and interpretation and to draft the manuscript.

Table 3
Univariate and multivariate analyses of prognostic factors for OS.

	N = 321	univariate analysis			multivariate analysis		
		P value	HR	95%CI	P value	HR	95%CI
Age (years)							
≤70	170						
> 70	151	0.897	0.967	0.578–1.601			
Sex							
Male	76	0.313	0.739	0.427–1.351			
Female	245						
HBV positive	57	0.074	0.516	0.214–1.061			
HCV positive	176	0.049	1.681	1.002–2.903	0.015	2.045	1.144–3.755
Alb (g/dL)							
≥3.5	276						
< 3.5	44	0.109	1.767	0.871–3.275			
AST (IU/L)							
≤35	190						
> 35	130	0.005	2.046	1.234–3.417	0.177	1.449	0.845–2.509
ALT (IU/L)							
≤34	198						
> 34	123	0.423	1.232	0.734–2.041			
Plt ($x10^4/mm^3$)							
≥14	166						
< 14	155	0.091	1.543	0.932–2.579			
PT (%)							
≥80	228						
< 80	92	0.946	0.981	0.528–1.716			
T-Bil (mg/dL)							
≤1	258						
> 1	63	0.727	1.125	0.554–2.086			
ICGR15 (%)							
≤15	186						
> 15	129	0.025	1.779	1.074–2.958	0.151	1.502	0.862–2.613
AFP (ng/mL)							
≤10	154						
> 10	163	0.011	1.988	1.173–3.476	0.061	1.691	0.978–3.013
DCP (mAU/mL)							
≤100	191						
> 100	127	0.236	1.362	0.813–2.264			
Child-Pugh							
A	299						
B	22	0.011	3.015	1.317–6.032	0.037	2.501	1.058–5.237
Tumor number							
Solitary	212						
Multiple	109	< 0.001	2.667	1.611–4.447	< 0.001	2.521	1.473–4.327
Tumor size (mm)							
≤50	263						
> 50	55	0.004	2.353	1.323–4.016	< 0.001	2.834	1.459–5.324
Anatomical resection	223	0.651	1.131	0.654–1.896			
Operation time (min)							
≤300	145						
> 300	176	0.132	1.479	0.889–2.508			
Blood loss (mL)							
≤1000	213						
> 1000	108	0.028	1.782	1.065–2.948	0.109	1.584	0.901–2.761
LC	67	0.074	1.715	0.946–2.981			
MVI	65	0.015	2.024	1.151–3.431	0.119	1.656	0.874–3.045
rs36084323							
AA genotype	90	0.084	0.579				
AG genotype	163	0.803	0.938				
GG genotype	68	0.042	1.799	1.023–3.051	0.009	2.201	1.221–3.848
Rs11568821							
AA genotype	56	0.732	1.128				
GG genotype	265						
Rs2227982							
CC genotype	152	0.333	1.287				
CT genotype	92	0.176	0.675				
TT genotype	77	0.738	1.108				
GG genotype	68	0.042	1.799	1.023–3.051	0.009	2.201	1.221–3.848

AFP alpha-fetoprotein levels, Alb albumin, *AST* asparate aminotransferase, *ALT* alanine aminotransferase, *BMI* body mass index, *CAR* C-reactive protein to albumin ratio, *CRP* C-reactive protein, *DCP* des-gamma-carboxyprothorombin, *GPS* Glasgow prognostic score, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *ICGR15* indocyanine green retention rate at 15 min, *LC* liver cirrhosis, *LMR* lymphocyte to monocyte ratio, *MVI* microvascular invasion, *NLR* neutrophil to lymphocyte ratio, *PI* prognostic index, *PLR* platelet to lymphocyte ratio, *Plt* platelet count, *PNI* prognostic nutritional index, *PT* prothrombin time, *T. Bil* total bilirubin.

Table 4
Univariate and multivariate analyses of prognostic factors for EHR.

		univariate analysis			multivariate analysis		
	N = 321	P value	HR	95%CI	P value	HR	95%CI
Age (years)							
≤70	170						
> 70	151	0.491	1.389	0.547–3.776			
Sex							
Male	76	0.968	1.023	0.366–3.609			
Female	245						
HBV positive	57	0.841	0.881	0.204–2.673			
HCV positive	176	0.381	1.512	0.596–3.962			
Alb (g/dL)							
≥3.5	276						
< 3.5	44	0.799	1.204	0.342–7.619			
AST (IU/L)							
≤35	190						
> 35	130	0.571	1.322	0.513–3.798			
ALT (IU/L)							
≤34	198						
> 34	123	0.135	0.452	0.128–1.261			
Plt ($x10^4/mm^3$)							
≥14	166						
< 14	155	0.078	0.415	0.133–1.101			
PT (%)							
≥80	228						
< 80	92	0.301	1.667	0.613–4.234			
T-Bil (mg/dL)							
≤1	258						
> 1	63	0.781	1.188	0.391–5.128			
ICGR15 (%)							
≤15	186						
> 15	129	0.462	0.696	0.242–1.794			
AFP (ng/mL)							
≤10	154						
> 10	163	0.347	1.566	0.617–4.258			
DCP (mAU/mL)							
≤100	191						
> 100	127	0.148	1.981	0.781–5.189			
Child-Pugh							
A	299						
B	22	0.872	0.851	0.047–4.145			
Tumor number							
Solitary	212						
Multiple	109	0.141	2.014	0.786–5.161			
Tumor size (mm)							
≤50	263						
> 50	55	0.011	3.791	1.375–9.881	0.085	2.521	0.873–6.881
Anatomical resection	223	0.393	1.591	0.571–5.615			
Operation time (min)							
≤300	145						
> 300	176	0.263	1.725	0.671–4.956			
Blood loss (mL)							
≤1000	213						
> 1000	108	0.036	2.712	1.069–7.106	0.162	2.006	0.751–5.439
LC	67	0.211	2.072	0.636–6.001			
MVI	65	< 0.001	5.711	2.249–14.97	0.002	5.209	1.847–14.82
GG genotype	68	0.021	3.144	1.201–7.973	0.006	4.521	1.552–12.71

AFP alpha-fetoprotein levels, Alb albumin, *AST* asparate aminotransferase, *ALT* alanine aminotransferase, *BMI* body mass index, *CAR* C-reactive protein to albumin ratio, *CRP* C-reactive protein, *DCP* des-gamma-carboxyprothorombin, *GPS* Glasgow prognostic score, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *ICGR15* indocyanine green retention rate at 15 min, *LC* liver cirrhosis, *LMR* lymphocyte to monocyte ratio, *MVI* microvascular invasion, *NLR* neutrophil to lymphocyte ratio, *PI* prognostic index, *PLR* platelet to lymphocyte ratio, *Plt* platelet count, *PNI* prognostic nutritional index, *PT* prothrombin time, *T. Bil* total bilirubin.

Research registration Unique Identifying number (UIN)

1 Name of the registry: UMIN Clinical Trials Registry (UMIN-CTR)
2 Unique Identifying number or registration ID: UMIN000039950
3 Hyperlink to your specific registration (must be publicly accessible and will be checked): https://www.umin.ac.jp/ctr/index.htm

Guarantor

Tsuyoshi Kobayashi, MD, PhD.

Department of Gastroenterological and Transplant Surgery, Graduate School of Biomedical and Health Science, Hiroshima University, Hiroshima, Japan.

1-2-3, Kasumi, Minami-ku, Hiroshima City, Hiroshima 734–8551, Japan.

Telephone number: 082-257-5222.

E-mail: tsukoba@hiroshima-u.ac.jp.

Fax number: 082-257-5224.

Declaration of competing interest

All author declared no conflicts of interest.

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Appendix A. Supplementary data

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Provenance and peer review

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