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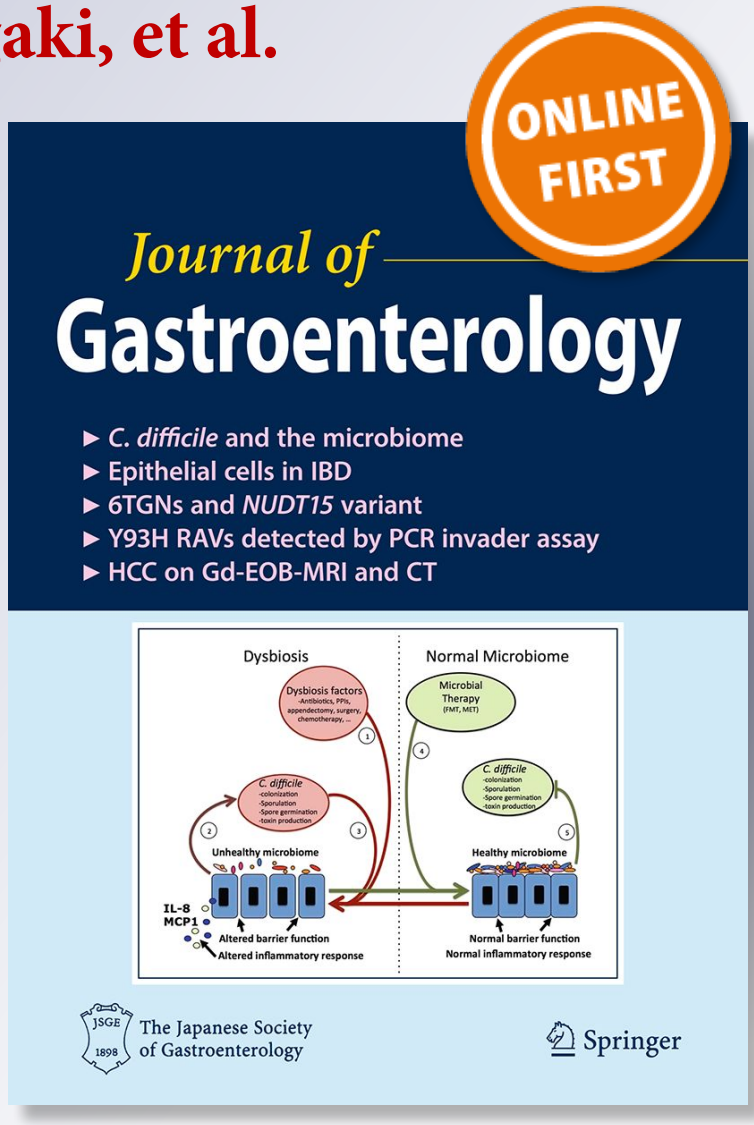

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Serum HMGB1 concentrations at 4 weeks is a useful predictor of extreme poor prognosis for advanced hepatocellular carcinoma treated with sorafenib and hepatic arterial infusion chemotherapy

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Abstract

Background Biomarkers predicting the response to the anticancer treatment and prognosis in patients with advanced hepatocellular carcinoma (HCC) are required. Recently, high mobility group box 1 (HMGB1) was reported to promote HCC progression and be associated with poor prognosis for patients with HCC. The purpose of this study was to assess serum HMGB1 concentrations before and during sorafenib treatment or hepatic arterial infusion chemotherapy (HAIC) and to explore the ability of serum HMGB1 concentrations to predict prognosis.

Methods Serum HMGB1 concentrations were measured in 71 and 72 patients with advanced HCC treated with sorafenib and HAIC, respectively, to assess their usefulness for prediction of the response to the treatment and prognosis.

Electronic supplementary material The online version of this article (doi:10.1007/s00535-017-1348-8) contains supplementary material, which is available to authorized users.

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Results Multivariate analysis identified high HMGB1 at 4 weeks ($P = 0.001$), high α-fetoprotein (AFP) at baseline ($P = 0.025$), tumor liver occupying rate ($P = 0.009$) and modified RECIST (mRECIST, $P < 0.0001$) as independent predictors of poor overall survival in sorafenib treatment. High HMGB1 at 4 weeks ($P = 0.025$), vascular invasion to the hepatic vein (Vv) ($P < 0.0001$), mRECIST ($P < 0.0001$) and Child-Pugh B were identified as independent predictors of poor overall survival in HAIC treatment. The concentrations of HMGB1 at baseline and 4 weeks were not correlated with conventional tumor markers and progressive disease assessed by mRECIST at 8 weeks.

Conclusions These results suggest that serum HMGB1 at 4 weeks after the start of treatment might be a useful biomarker with added value to the conventional tumor marker and radiologic responses to predict poor overall survival in patients with advanced HCC treated with sorafenib or HAIC.

Keywords Biomarker · Liver cancer · HCC · High mobility group box 1 · Sorafenib · Hepatic arterial infusion chemotherapy

Abbreviations

HMGB1	High mobility group box 1
HCC	Hepatocellular carcinoma
HAIC	Hepatic arterial infusion chemotherapy
AFP	A-fetoprotein
mRECIST	Modified response evaluation criteria in solid tumors
Vv	Hepatic vein invasion
MVI	Macroscopic vascular invasion
DCP	Des-gamma-carboxy prothrombin
VEGF	Vascular endothelial growth factor
PDGF	Platelet-derived growth factor
MST	Median survival time
DNA	Deoxyribonucleic acid
RAGE	Receptor for advanced glycation end products
MAPK	MAP kinase
TLR	Toll-like receptor
Ang2	Angiopoietin-2
TACE	Trans catheter arterial chemo-embolization
ECOG PS	Eastern Cooperative Oncology Group performance status
PS	Propensity score
SHARP	Sorafenib hepatocellular carcinoma assessment randomized protocol
TMN	Tumor node metastasis staging system of the Liver Cancer Study Group of Japan
CDDP	Cis-diamminedichloroplatinum
5-FU	5-fluorouracil
IFN	Interferon
HCV	Hepatitis C virus
HBV	Hepatitis B virus
mAU	Milli Arbitrary Unit
OS	Overall survival
HR	Hazard ratio
CI	Confidence interval
PD	Progressive disease
Vp	Portal vein invasion
EGF	Epidermal growth factor
bFGF	Basic fibroblast growth factor
HGF	Hepatocyte growth factor
IGF	Insulin-like growth factor
sVEGFR	Soluble VEGF receptor
PI3K	Phosphatidylinositol 3-kinase
PDK1	Phosphoinositide-dependent kinase 1
cPKC	Classical protein kinase C

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Introduction

Primary liver cancer is the second leading cause of cancer-related deaths worldwide [1]. HCC constitutes 70–90% of primary liver cancers occurring worldwide [2]. Sorafenib, an oral multikinase inhibitor used to treat patients with HCC, targets Raf-1, VEGFR1-3, PDGFR, KIT, RET, and other tyrosine kinases and has both antiproliferative and antiangiogenic effects [3]. Sorafenib has demonstrated survival benefits and is the current standard drug for systemic treatment in patients with advanced unresectable HCC [4, 5]. HAIC has been widely applied for advanced HCC in Southeast and East Asian countries. Several studies have indicated that HAIC improves survival in patients with advanced HCC in the absence of distant metastasis, with an increase in response rate from 20.8% up to 52% [6–13]. The median survival time (MST) is 40 and 17 months among complete and partial responders, respectively [6–13]. Several studies show the effectiveness of sorafenib in Japanese patients [14, 15].

However, not all HCC patients respond to these treatments. Survival in patients treated with sorafenib is prolonged by only about 3 months [4], and benefits of the drug are offset by the high cost of the drug and occasional incidence of severe adverse events [4, 5]. Some studies have reported that the MST was significantly shorter in non-responders than responders in HAIC treatment [7–10, 12, 15–17].

Therefore, biomarkers predicting response and prognosis to these treatments in patients with advanced HCC are required.

High mobility group box 1 (HMGB1) is a nuclear deoxyribonucleic acid (DNA)-binding protein that loosely binds to chromatin and is present in almost all eukaryotic cells [18]. Recent studies have shown that HMGB1 promotes HCC progression and invasiveness [19–23]. High expression of HMGB1 in HCC tissue was reported to be associated with poor prognosis for patients with HCC after curative hepatectomy [24].

HMGB1 localizes to both the nucleus and the cytosol and is secreted into the extracellular space [25]. Nuclear HMGB1 binds to DNA and interacts with various transcription factors, including NF-κB, p53, and TATA-binding proteins [26–28]. Cytoplasmic HMGB1 was found to bind to a number of molecules related to cancer progression via promoting cell cycle progression, cell proliferation, and anti-apoptosis [29, 30]. Extracellular HMGB1 binds to several receptors, including the receptor for advanced glycation end products (RAGE), Toll-like receptor (TLR)-2, TLR-4, TLR-9, activating Ras-MAP kinase (MAPK) and the NF-κB/MAPK pathway [31, 32]. A recent study reported that HMGB1 translocates to the cytoplasm and is then actively secreted by HCC cells; extracellular HMGB1 can then promote cancer invasion and metastasis through TLR-4 signaling [19]. Recent studies have also suggested that targeting HMGB1 production or release might be potential approaches for HCC treatment [19, 32, 33].

It has been reported that plasma angiopoietin-2 (Ang2) and vascular endothelial growth factor (VEGF) were predictors of survival [34] and that changes in plasma VEGF [35] and AFP [18] were useful post-treatment biomarkers associated with the prognosis of the HCC patients treated with sorafenib. However, the role of HMGB1 in patients treated with sorafenib or HAIC is unknown.

The aims of this cohort study were (1) to explore factors including serum HMGB1 associated with overall survival of patients with advanced HCC treated with sorafenib and HAIC, (2) to investigate changes in HMGB1 levels during sorafenib and HAIC treatment, and (3) to reveal whether the changes in HMGB1 levels were associated with the response to treatment and prognosis in these patients.

Patients and methods

This study was approved by the Hiroshima University ethical committee.

Patients

Sorafenib

Patients with advanced HCC who underwent sorafenib treatment between August 2009 and December 2014 at Hiroshima University Hospital were included in this study. The following patients were excluded: (1) patients who received other treatments less than 4 weeks prior to starting sorafenib treatment, (2) patients who received other treatments during sorafenib treatment, and (3) patients who received treatment with sorafenib for less than 8 weeks. In total, 71 patients met these criteria and were included in the study. Sorafenib (standard dose: 800 mg/day) was administered for as long as possible and was withheld or its dose was tapered to 400 mg/day in the event of and depending on the severity of adverse events. The preliminary criteria for the selection of sorafenib treatment is Child-Pugh liver function class A and considered unfit for surgery, liver transplantation, repeat loco regional therapy, repeat trans catheter arterial chemo-embolization (TACE) or repeat HAIC. The more detailed inclusion criteria for treatment with sorafenib are as follows: an Eastern Cooperative Oncology Group performance status (ECOG PS) score of 2 or less; Child-Pugh liver function class A or B; adequate hematologic function (platelet count $\geq 5\times10^4/\mu L$, hemoglobin $\geq$8.5 g/dL); adequate hepatic function (albumin $\geq$2.8 g/dL, total bilirubin $\leq$3 mg/dL and alanine amino-transferase and aspartate aminotransferase $\leq$5 times the upper limit of the

normal range); and adequate renal function (serum creatinine ≤1.5 times the upper limit of the normal range), according to the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) study [17]. Treatment was discontinued for one or more of the following reasons: severe side effects, worsening of Eastern Cooperative Oncology Group performance status to 4, aggravation of liver dysfunction or refusal to continue participating in the study.

Haic

In the advanced HCC patients treated with HAIC started in February 2007 and August 2016 at Hiroshima University Hospital, the patients for whom stored serum samples from pretreatment and 4 weeks after treatment were enrolled. The following patients were excluded: (1) patients who received other treatments less than 4 weeks prior to starting HAIC treatment, (2) patients who received other treatments during HAIC treatment, and (3) patients who could not complete one course of HAIC treatment. In total 72 patients met these criteria and were included in the study.

Serum HMGB1, AFP, DCP, HBsAg, and HCV-Ab were analyzed at baseline in all patients, and all patients were classified according to the Child-Pugh and TNM classification before starting treatment.

Serum HMGB1 concentrations and AFP and DCP levels at 4 weeks were analyzed in 136 and 124 of the 143 patients, respectively.

Radiologic responses to therapy were evaluated according to modified RECIST at 8 weeks of treatments in all patients.

Treatment regimens

Sorafenib

All patients commenced treatment with sorafenib at a standard dose of 400 mg twice daily. Patients continued therapy until death or until one of the following criteria for cessation of therapy was met: (1) adverse events that required termination of treatment, (2) deterioration of ECOG PS to 4, (3) worsening liver function, or (4) withdrawal of consent. Other palliative treatments or best supportive care were provided subsequently.

Haic

Patients received repeated arterial infusions of anticancer agents via the injection port. Two drug regimens were used in this study. Intra-arterial low-dose cisplatin (CDDP, Nihonkayaku, Tokyo, Japan) combined with 5-fluorouracil (5FU, Kyowa Hakko, Tokyo) therapy (FP) or intra-arterial 5FU with subcutaneous interferon (IFN) combination therapy (5FU + IFN). One course of chemotherapy lasted 2 weeks. A 5FU (300 mg body weight/day) was administered over 24 h using a mechanical infusion pump from day 1 to day 5 of the first and second weeks in both regimens. CDDP was injected intra-arterially at 6 mg/body weight/day on days 1–5 and 8–12. The IFN used in the 5FU + IFN regimen was recombinant IFNα-2b (Intron A, Schering-Plough Pharmaceuticals, Osaka, Japan, 3×10^6 Pharmaceuticals, Tokyo, 5×10^6 U [3 MU]) or natural IFN-α (OIF, Otsuka U [5 MU]) administered intramuscularly on days 1, 3, and 5 of each week (total dose, 36 and 60 MU, respectively). FP and 5FU + IFN were provided to 46 and 26 patients, respectively.

Serum HMGB1 measurements

Serum samples obtained by venipuncture using 5 mL serum separating tubes (P1; SRL, Tokyo, Japan) were centrifuged at 3500 rpm for 10 min, and the supernatant was kept frozen at −80 °C for later use in HMGB1 measurements. Serum HMGB1 levels were measured quantitatively using an enzyme-linked immunosorbent assay kit (HMGB1 ELISA Kit II, Shino-Test Corporation, Kanagawa, Japan) according to the manufacturer's instructions. The measurement range was from 2.5 to 80 ng/mL.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences version 11.0.1 J or 22 (IBM SPSS, Chicago, IL). Differences between groups were examined for statistical significance using the χ^2 test where appropriate. Continuous variables are expressed as the median and range, while categorical variables are expressed as counts. Correlations between HMGB1 and AFP or DCP were evaluated with Spearman's rank correlation coefficient. Kaplan–Meier survival curves with the log–rank test were used for analysis of overall survival. Prognostic factors for overall survival were analyzed by Cox's proportional hazards model. A P value less than 0.05 was regarded as statistically significant.

The Mann–Whitney U test was used for the following comparison: age, AFP, DCP and duration of the treatment between the patients with different serum HMGB1 concentrations at baseline and at 4 weeks.

Result

Patient characteristics

In total, 143 patients, including 71 patients treated with sorafenib and 72 patients treated with HAIC were enrolled

Table 1 Clinical characteristics of HCC patients treated with Sorafenib or HAIC

	All	Sorafenib ($n = 71$)	HAIC ($n = 72$)
Age	66 (20–87)	66 (20–87)	66 (32–85)
Sex (male/female)	117/26	57/14	60/12
Etiology (HBV/HCV/NBNC/HBV + HCV)	50/53/38/2	30/25/14/2	20/28/24/0
AFP	457.4 (<5–1895000)	85.2 (5 <–1503000)	2548.6 (<5–1,895,000)
DCP	2139 (11–1170900)	988 (11–490160)	3527 (24–1,170,900)
HMGB1	11.1 (<2.5–86.0)	9.1 (<2.5–65.2)	13.3 (<2.5–86.0)
Child-Pugh (A/B)	122/21	71/0	51/21
Main tumor size	50 (0–194)	26 (0–194)	70 (10–180)
Tumor liver occupying rate (<50%/>50%)	113/30	57/14	56/16
Vp (0/>1)	69/74	52/19	17/55
Vv (0/>1)	119/24	58/13	61/11
TNM stage (II/III/IVa/IVb)	8/31/52/52	5/12/13/41	3/19/39/11

HCC hepatocellular carcinoma, *HAIC* hepatic arterial infusion chemotherapy, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *NBNC* non-HBV and non-HCV, *HMGB1* high mobility group box 1, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *TNM stage* tumor node metastasis staging system of the Liver Cancer Study Group of Japan

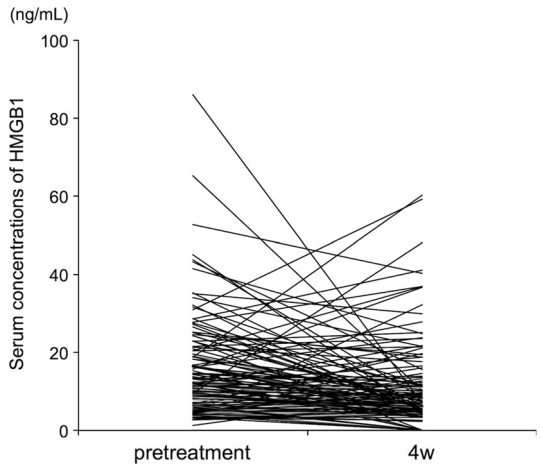

Fig. 1 Transition of the serum HMGB1 concentration at pretreatment and 4 weeks. The transition of the serum HMGB1 concentration at pretreatment and 4 weeks in the 136 patients for whom HMGB1 concentrations were analyzed at both time points. HMGB1 concentration decreased in 67% (91/136) of the patients and increased in 33% (45/136) of the patients

in this study, and their characteristics are listed in Table 1. The study subjects included 117 males and 26 females with a median age of 66 years. The background liver diseases were hepatitis C viral (HCV) infection ($n = 53$), hepatitis B viral (HBV) infection ($n = 52$), HBV-HCV co-infection ($n = 2$) and non-HCV-non-HBV hepatitis ($n = 38$). The median AFP was 457.4 ng/mL, and DCP was 2139 mAU/mL. Liver function was evaluated with the Child-Pugh classification system, with 122 patients classified as Child-Pugh A and 21 patients classified as Child-Pugh B (Table 1).

Serum HMGB1 concentration at baseline and at 4 weeks of treatment

The serum HMGB1 concentrations in all of the 143 patients with the serum of pretreatment and 4 weeks, patients treated with sorafenib and HAIC are shown in Fig. 1, respectively. The HMGB1 concentration decreased in 67% (91/136) of the patients and increased in 33% (45/136) of the patients.

In this study, we classified patients with serum HMGB1 >11.1 ng/mL, which was the median at baseline in all patients, and <11.1 ng/mL at baseline as the HMGB1-high and HMGB1-low groups, respectively. We also classified patients with serum HMGB1 >11.1 ng/mL and <11.1 ng/mL at 4 weeks of treatment as the HMGB-non-suppression (HMGB1-ns) and HMGB1-suppression (HMGB1-s) groups, respectively (Fig. 1).

The characteristics of the patients in the HMGB1-high and HMGB1-low groups

The frequency of patients with HBV ($P = 0.037$) and portal vein invasion ($P = 0.012$) were significantly higher and the frequency of patients with HCV ($P = 0.001$) was significantly lower in the HMGB1-high group (Table 2).

The characteristics of the patients in HMGB1-ns and HMGB1-s groups

The baseline HMGB1 concentration was significantly higher in the HMGB1-ns group than in the suppression group ($P < 0.0001$). The rate of the patients with Child-

Table 2 The characteristics of the patients in HMGB1-high and HMGB1-low groups

	HMGB1-high	HMGB1-low	*P*
Age	64 (85–32)	67 (20–87)	0.14
Sex (male/female)	60/11	57/15	0.271
Etiology (HBV/HCV/NBNC/HBV + HCV)	30/18/23/0	20/35/15/2	0.011
AFP	773.6 (<5–1,503,000)	287.7 (<5–1,895,000)	0.267
DCP	2321 (11–1,170,900)	2025.5 (16–281,280)	0.31
Tumor liver occupying rate(<50%/>50%)	54/17	59/13	0.255
TNM stage(II/III/Iva/IVb)	4/17/31/19	4/14/21/33	0.113
Vp (0/>1)	27/44	42/30	0.012
Vv (0/>1)	59/12	60/12	0.574
Child-Pugh (A/B)	57/14	65/7	0.113

HBV hepatitis B virus, *HCV* hepatitis C virus, *NBNC* non-HBV and non-HCV, *HMGB1* High mobility group box 1, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *TNM stage* tumor node metastasis staging system of the Liver Cancer Study Group of Japan, *Vp* portal vein invasion, *Vv* hepatic vein invasion

Table 3 The characteristics of the patients in HMGB1-ns and HMGB1-s groups

	HMGB1-ns	HMGB1-s	*P*
Age	65 (32–77)	66 (20–87)	0.084
Sex (male/female)	32/8	78/18	0.52
Etiology (HBV/nonHBV)	17/23	27/69	0.055
HMGB1	16 (4–53)	10 (<2.5–86)	<0.0001
AFP	1386 (<5–1,895,000)	430 (<5–958,000)	0.052
DCP	3417 (22–1,170,900)	2026 (11–634,690)	0.285
Tumor liver occupying rate (≥50%/<50%)	10/30	18/78	0.274
TNM stage(II–IVa/IVb)	15/25	64/32	0.392
Vp (0/>1)	13/27	54/42	0.009
Vv (0/>1)	32/8	81/15	0.349
Child-Pugh (A/B)	28/12	86/10	0.025

HBV hepatitis B virus, *HCV* hepatitis C virus, *NBNC* non-HBV and non-HCV, *HMGB1* High mobility group box 1, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *TNM stage* tumor node metastasis staging system of the Liver Cancer Study Group of Japan, *Vp* portal vein invasion, *Vv* hepatic vein invasion

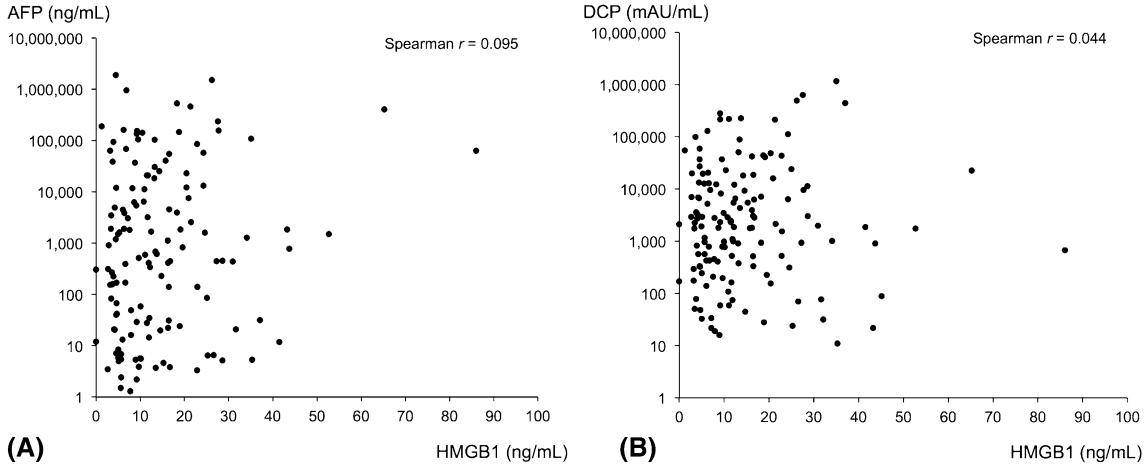

Fig. 2 Correlation between serum HMGB1 and AFP or DCP concentrations. **a** There was no significant correlation between serum HMGB1 and AFP concentrations at baseline ($r = 0.095$). **b** There was no significant correlation between serum HMGB1 and DCP concentrations at baseline ($r = 0.044$)

Pugh B ($P = 0.025$) and Vp ($P = 0.009$) was significantly higher in the HMGB1-ns group (Table 3).

Correlation analysis between HMGB1 and AFP or DCP

There was no significant correlation between either serum HMGB1 and AFP ($r = 0.095$; Fig. 2a) or HMGB1 and DCP ($r = 0.044$, Fig. 2b) levels at pretreatment. There was no significant correlation between either serum HMGB1 and AFP ($r = 0.095$) or HMGB1 and DCP ($r = 0.431$) levels after 4 weeks of sorafenib treatment. No correlation was observed between the trend of serum HMGB1 and AFP ($P = 0.216$) nor HMGB1 and DCP ($P = 0.446$) at 4 weeks (Supplementary Table 1).

Overall survival according to the serum HMGB1 concentration and trends of AFP and DCP at 4 weeks

HMGB1-ns vs. HMGB1-s

The MST of the HMGB1-ns group and the HMGB1-s group was 7.9 and 15.2 months, respectively. Overall survival (OS) in the HMGB1-ns group was significantly lower than in the HMGB1-s group ($P = 0.007$, Fig. 3a).

Patients with AFP increase vs. patients with AFP decrease

The MST of the patients with AFP increase and with AFP decrease was 10.4 and 21.0 months, respectively. OS among the former was significantly lower than the latter ($P = 0.003$, Fig. 3b).

Patients with DCP increase vs. patients with DCP decrease

The MST of the patients with DCP increase and with DCP decrease was 10.4 and 26.6 months, respectively. OS of the former was significantly lower than the latter ($P = 0.0012$, Fig. 3c).

HMGB1-ns with AFP increased vs. others (HMGB1-s or AFP decrease)

The MST of the HMGB1-ns with AFP increase and HMGB1-s or AFP decrease was 4.7 and 13.6 months, respectively. OS of the former was significantly lower than the latter ($P = 0.0005$, Fig. 3d).

HMGB1-ns with DCP increased vs. others (HMGB1-s or DCP decrease)

The MST of the HMGB1-ns with DCP increase and HMGB1-s or DCP decrease was 4.9 and 15.0 months, respectively. OS of the former was significantly lower than the latter ($P = 0.0002$, Fig. 3e).

Correlation analysis between HMGB1 at 4 weeks and response to therapy assessed by mRECIST

There was no significant difference in progressive disease assessed by mRECIST at 8 weeks between the HMGB1-ns and HMGB1-s groups in all patients ($P = 0.095$), in patients treated with sorafenib ($P = 0.389$), nor in patients treated with HAIC ($P = 0.15$) (Supplementary Table 2).

Prognostic factors for overall survival

The following parameters were analyzed by univariate analysis and multivariate analysis: Baseline parameters: age, sex, AFP, DCP, HMGB1, tumor liver occupying rate, Vp, hepatic vein invasion (Vv), Child-Pugh class; Parameters assessed at 4 weeks: HMGB1-ns/s classification, increase or decrease of AFP and DCP; Parameters assessed at 8 weeks: mRECIST. For the continuous values, the median value was used as a cut off value.

All patients

Univariate analysis identified the following pretreatment factors associated with poor overall survival: AFP (high, $P = 0.0004$), DCP (high, $P = 0.0247$), tumor liver occupying rate (>50%, $P = 0.0002$), Vp (presence, $P = 0.0021$), Child-Pugh class (B, $P = 0.0158$) HMGB1-ns ($P = 0.007$), AFP increase ($P = 0.003$), DCP increase ($P = 0.0012$), and mRECIST (PD, $P < 0.0001$). Multiple Cox proportional hazard model analysis identified HMGB1-ns (HR 3.0, 95% confidence interval [95% CI] 1.8–5.1, $P < 0.0001$), tumor liver occupying rate (HR 2.8, 95% CI 1.6–4.7, $P < 0.0001$), Vv (HR 1.8, 95% CI 1.0–3.1, $P = 0.036$), and mRECIST (HR 5.0, 95% CI 3.0–8.3, $P < 0.0001$) as independent factors associated with poor OS (Table 4).

Patients treated with sorafenib

In the patients treated with sorafenib, univariate analysis identified the following pretreatment factors associated with poor overall survival: AFP (high, $P = 0.0014$), DCP (high, $P = 0.0336$), Vp (presence, $P = 0.0043$), Vv

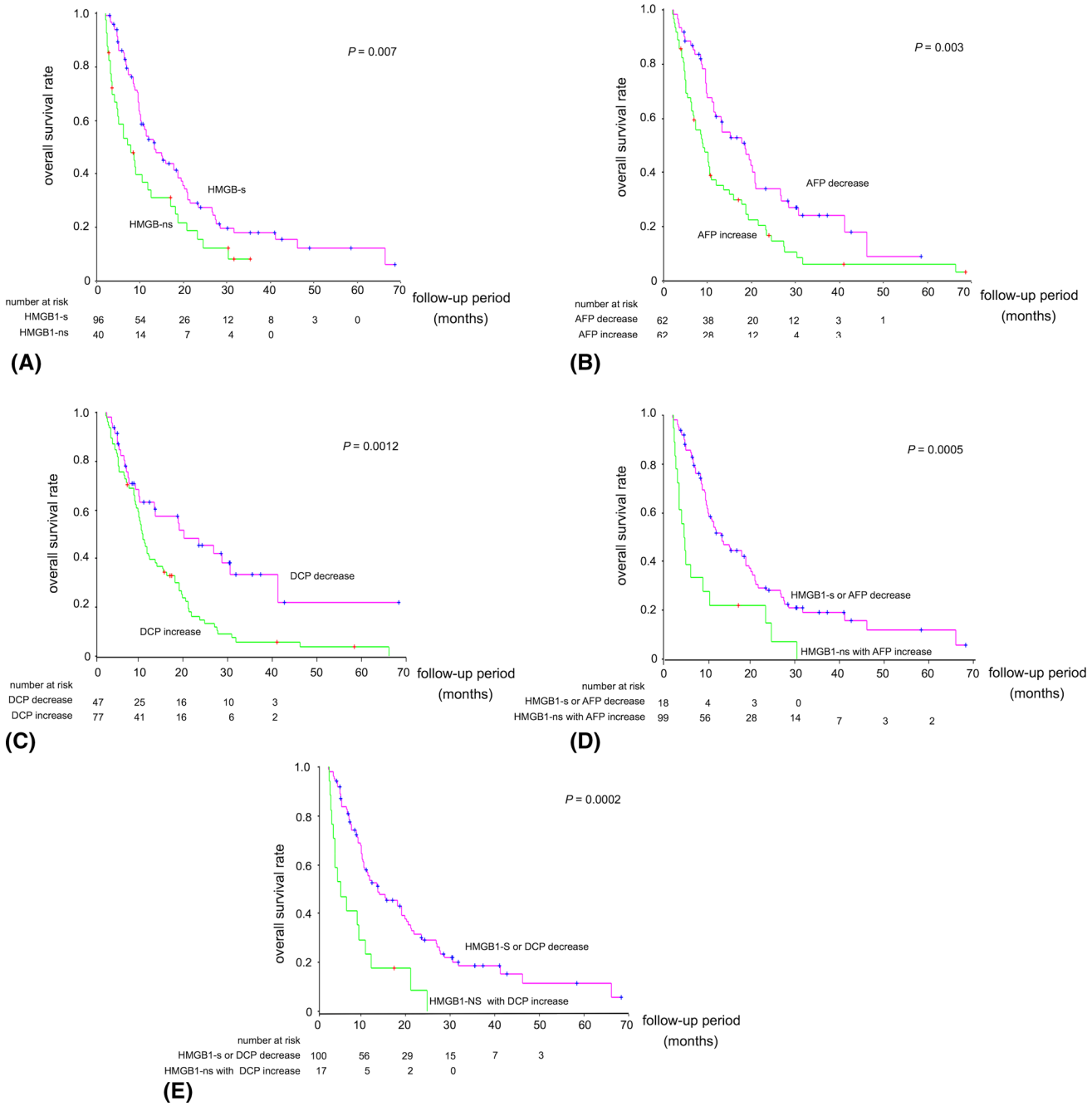

Fig. 3 Overall survival according to the serum HMGB1 concentrations and the trend of AFP and DCP at 4 weeks. The Kaplan–Meier plot illustrates the overall survival according to (**a**) the classification of HMGB1-s and HMGB1-ns, the trend of (**b**) AFP, **c** DCP and combination of HMGB1 concentration and trend of tumor markers, such as (**d**) AFP and **e** DCP at 4 weeks

(presence, $P = 0.0258$), HMGB1-ns ($P = 0.0363$), AFP increase ($P = 0.0482$), and mRECIST (PD, $P < 0.0001$). Multiple Cox proportional hazard model analysis identified high AFP (HR 1.9, 95% CI 1.1–3.4, $P = 0.0025$) at baseline and HMGB1-ns (HR 3.6, 95% CI 1.8–7.5, $P = 0.001$), tumor liver occupying rate (HR 2.7, 95% CI 1.3–5.5, $P = 0.009$) and mRECIST (HR 3.9, 95% CI 2.1–7.5, $P < 0.0001$) as independent factors associated with poor OS (Table 4).

Patients treated with HAIC treatment

In the patients treated with HAIC, univariate analysis identified the following pretreatment factors associated

Table 4 Prognostic factors for overall survival

Parameter	All			Sorafenib			HAIC		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
	p value	OR (95% CI)	*p* value	*p* value	OR (95% CI)	*p* value	*p* value	OR (95% CI)	*p* value
Age, high[a]	0.9947			0.6145			0.6188		
Sex, female	0.1007			0.6332			0.0025		
AFP, high[a]	0.0004			0.0014	1.9 (1.1–3.4)	0.025	0.0254		
AFP increase (4 week)	0.003			0.0482			0.0478		
DCP, high[a]	0.0247			0.0336			0.0775		
DCP increase (4 week)	0.0012	1.5 (0.9–2.6)	0.101	0.4815			0.001		
HMGB1, high[a]	0.7648			0.0799			0.4302	0.475 (0.2–1.1)	0.097
HMGB1-ns (4 week)	0.007	3.0 (1.8–5.1)	<0.0001	0.0363	3.6 (1.8–7.5)	0.001	0.0219	2.7 (1.1–6.3)	0.025
Tumor liver occupying rate (≥50%)	0.0002	2.8 (1.6–4.7)	<0.0001	0.0502	2.7 (1.3–5.5)	0.009	0.0022		
Vp, presence	0.2688			0.0043			0.6159		
Vv, presence	0.0021	1.8 (1.0–3.1)	0.036	0.0258			0.008	3.6 (1.4–9.3)	0.009
TNM stage, IVb	0.2141			0.2179			0.00025		
mRECIST, PD (8 week)	<0.0001	5.0 (3.0–8.3)	<0.0001	0.0006	3.9 (2.1–7.5)	<0.0001	<0.0001	9.3 (3.9–22.2)	<0.0001
Child-Pugh class, B	0.0158						0.0025	3.4 (1.5–7.7)	0.004

HAIC hepatic arterial infusion chemotherapy, *OR* odds ratio, *CI* confidence interval, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *HMGB1* high mobility group box 1, *TNM stage* tumor node metastasis staging system of the Liver Cancer Study Group of Japan, *Vp* portal vein invasion, *Vv* hepatic vein invasion

[a] Cut off value is median of the all patients

with poor overall survival: sex (female, $P = 0.0025$), AFP (high, $P = 0.0254$), Vv (presence, $P = 0.008$), tumor liver occupying rate (>50%, $P = 0.0022$), TNM stage (IVb, $P = 0.00025$), mRECIST (PD, $P < 0.0001$), and Child-Pugh class (B, $P = 0.0025$) in baseline parameters and HMGB1-ns ($P = 0.0219$) and mRECIST ($P < 0.0001$) in the parameters assessed after the start of treatment. Multiple Cox proportional hazard model analysis identified HMGB1-ns (HR 2.7, 95% CI 1.1–6.3, $P = 0.025$) and Vv (HR 3.6, 95% CI 1.4–9.3, $P = 0.009$), mRECIST (HR 9.3, 95% CI 3.9–22.2, $P < 0.0001$), and Child-Pugh B (HR 3.4, 95% CI 1.5–7.7, $P = 0.004$) as independent factors associated with poor OS (Table 4).

The correlation between HMGB1 concentrations at 4 weeks and adverse events

Patients treated with sorafenib Discontinuation of treatment due to adverse events was observed in 30 of 64 patients treated with sorafenib for whom HMGB1 concentrations at 4 weeks were analyzed. The discontinuation was observed in 6 of 14 and 24 of 51 patients in the HMGB1-ns and HMGB1-s groups. There was no significant difference between the rates of the discontinuation ($P = 0.601$). The median duration of the treatment in HMGB1-ns and HMGB1-s groups was 6.8 and 7.2 months, respectively, and there was no significant difference between the two groups ($P = 0.09$).

Patients treated with HAIC The HMGB1-ns and HMGB1-s groups received a median of two courses of HAIC. There was no significant difference in the duration of HAIC between the two groups ($P = 0.115$). HAIC treatment was discontinued due to adverse events as follows in nine patients (six patients in the HMGB1-s group and three patients in the HMGB1-ns group): vasculitis, infection of port-catheter system and arterial occlusion in descending order.

Discussion

Llovet et al. analyzed plasma biomarkers to identify predictors of outcome of sorafenib treatment for advanced HCC. They analyzed plasma biomarkers, such as Ang2, EGF, bFGF, VEGF, sVEGFR-2, sVEGFR-3, HGF, s-c-KIT, IGF-2, and all forms of circulating Ras in 491 patients at baseline and in 305 patients after 12 weeks of treatment in a phase 3, randomized controlled trial—the SHARP trial—and revealed that Ang2 and VEGF were independent predictors of survival and that none of the tested biomarkers significantly predicted response to sorafenib [34]. Recently, regorafenib, an inhibitor of a broad range of kinases (including VEGFR, PDGFR, FGFR, TIE2, KIT, RET, and RAF), was reported to provide benefit in HCC patients progressing on sorafenib treatment in a phase 3 trial [36]. Tsuchiya et al. analyzed plasma VEGF monthly and demonstrated that changes 8 weeks after starting sorafenib were important for predicting OS [35]. In the current study, we did not analyze plasma VEGF, so we cannot comment on the correlation between plasma VEGF and serum HMGB1. However, there is little doubt that use of several different biomarkers would help guide prediction and decision-making. Furthermore, the current study shows that the serum combination of HMGB1 and tumor marker at 4 weeks after the start of treatment can predict poor prognosis. Biomarkers that can help predict prognosis earlier can help to avoid excessive progression caused by a delay in the decision to initiate second line therapy.

HAIC is widely used in Asia, especially Japan. Several studies have reported survival benefits of HAIC for advanced HCC [6–13]. Identification of responders (and non-responders) at an early stage of HAIC is advantageous so that non-responders can be switched to other more effective treatments. In fact, we previously reported that switching to other therapies, including sorafenib, could improve the prognosis in HAIC-refractory patients [37]. The response to HAIC is generally assessed by RECIST based on imaging studies. However, evaluation of treatment outcome by imaging studies alone is limited in the early stage of such treatment. We have also reported that the decrease of AFP and DCP in the early stage treatment with HAIC was identified as significant and independent factors associated with survival [16].

For this reason, we used two tumor markers in addition to imaging studies. The present study analyzed prognosis according to the results of imaging studies and AFP/DCP tumor marker ratio after one course of HAIC.

Previous clinical studies have reported that serum HMGB1 levels were significantly higher in patients with HCC than in patients with chronic hepatitis or liver cirrhosis or in healthy controls. A higher level of serum HMGB1 was reported to be correlated with larger tumor size, worse tumor stage, and pathological differentiation grades in these patients [32, 38]. However, there has been no report investigating the usefulness of serum HMGB1 as a biomarker for advanced HCC patients who received treatment with sorafenib. The present study demonstrated that high HMGB1 concentration at 4 weeks was significantly associated with poor OS in both the sorafenib and HAIC cohort. Interestingly, the concentrations of HMGB1 were not correlated with the concentrations of conventional tumor markers such as AFP and DCP at base line. Furthermore, the HMGB1 trend during the therapy was independent of the trend of these tumor markers. These results suggest that HMGB1 could add value to use of the tumor markers alone in a complementary fashion. We demonstrated that the combination of HMGB1 levels at 4 weeks and the trend of tumor markers could predict an extremely poor prognosis phenotype. Phosphatidylinositol 3-kinase (PI3 K), its downstream target phosphoinositide-dependent kinase 1 (PDK1), and classical protein kinase C (cPKC) signal pathways were reported to act in concert to control HMGB1 secretion [39]. Young et al. reported that HMGB1 secretion was inhibited through the inhibition of HMGB1 phosphorylation by inhibiting the PI3 K-PKC signaling pathway [40]. Tang et al. reported that 5-FU induced autophagic cell death in HepG2 hepatocarcinoma cells by inhibiting the PI3 K/AKT/mTOR pathway [41]. Therefore, we consider it possible that sorafenib and HAIC might inhibit HMGB1 secretion through inhibition of the PI3 K pathway.

Furthermore, it is interesting to note that serum concentration of HMGB1 at 4 weeks was associated with poor prognosis but not with early response as assessed by mRECIST. This result suggests that HMGB1 itself has a function related to tumor promotion, as reported in previous studies [19–21, 25, 32], so that the patients with a decrease of HMGB1 via therapy might have a worse OS than those without such a decrease. Therefore, HMGB1 could be a potential target for advanced HCC.

To our knowledge, this is the first demonstration of the usefulness of HMGB1 as a predictor of outcome of sorafenib and HAIC treatment for advanced HCC. Further studies with a larger number of patients are needed to confirm these findings. We also hope to explore the mechanism by which sorafenib and HAIC treatment induces decrease in HMGB1 levels in future research.

The frequency of patients with Child-Pugh class B was significantly higher in the HMGB1-ns group. We consider that this was because HMGB1-ns was associated with HMGB1 high at baseline. All of the patients with Child-Pugh class B received HAIC, and the HMGB1 at baseline was higher in the HAIC treatment group. Patients who

received sorafenib and HAIC, and for whom frozen serum was available, were not artificially enrolled in this study. However, the frequency of patients with HBV was higher than the usual case for HCC patients in Japan [42, 43].

In conclusion, serum HMGB1 concentrations at 4 weeks could be a useful predictor of poor OS for advanced HCC treated with sorafenib or HAIC. In particular, the combination of serum HMGB1 concentration and conventional tumor markers such as AFP and DCP could predict an extremely poor phenotype.

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Compliance with ethical standards

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