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ORIGINAL ARTICLE

# Factors Predisposing Metastatic Tumor Antigen 1 Overexpression in Hepatitis B Virus Associated Hepatocellular Carcinoma

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**Abstract**
*Background/Aim* Overexpression of metastatic tumor antigen-1 (MTA-1) is suggested to be associated with frequent postoperative recurrence and poor survival of hepatocellular carcinoma (HCC) patients. In this study, we intended to determine clinical factors predisposing the overexpression of MTA-1 in patients with hepatitis B virus (HBV)-associated HCC and also examine whether MTA-1 overexpression affects the survival periods of these patients treated with curative surgical resection.
*Methods* A total of 303 patients with HBV-associated HCC who underwent curative surgical resection were subjected. The expressions of MTA-1 in HCC and surrounding non-tumor liver tissues were evaluated using the immunohistochemical method. The clinical, radiological and histological characteristics of the patients were analyzed in relation to the expression of MTA-1 to find predisposing factors of MTA-1 overexpression.
*Results* MTA-1 was overexpressed in 104 HCC tissues (34.3 %) and none of the surrounding non-tumor tissues. Clinically, MTA-1 overexpression was significantly associated with younger age, female gender, higher serum alpha-fetoprotein level, and Child–Turcotte–Pugh class A. Also, portal vein thrombosis, microvascular invasion, capsular invasion and poorly histological differentiation were associated with overexpression of MTA-1. The cumulative survival rates were significantly lower in patients with MTA-1 overexpression compared with those in the MTA-1 negative group ($P = 0.03$). In addition to the overexpression of MTA-1, the presence of microvascular or capsular invasion was a significant factor determining the poor survival of the patients with HBV-associated HCC after curative resection.
*Conclusions* MTA-1 is overexpressed in patients with HBV-associated HCC of invasive nature. MTA-1 overexpression is associated with shorter survival periods of patients with HBV-associated HCC after curative resection.

**Keywords** Hepatocellular carcinoma · Metastatic tumor antigen-1 · Hepatitis B virus

**Abbreviations**

| | |
|---|---|
| AFP | Alpha-fetoprotein |
| CTP | Child-Turcotte-Pugh |
| CT | Computed tomography |
| E–S | Edmondson–Steiner |
| HBV | Hepatitis B virus |

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| | |
|---|---|
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |
| HIF | Hypoxia inducible factor |
| HR | Hazard ratio |
| MTA-1 | Metastatic tumor antigen-1 |
| NuRD | Nucleosome remodeling and histone deacetylation |
| PVT | Portal vein thrombosis |

## Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer worldwide and the third leading cause of cancer-associated mortality with 600,000 deaths per year globally [1, 2]. Although various etiologies are known to be involved in hepatocarcinogenesis, chronic HBV infection is mainly responsible for HCC development, especially in Far East Asia, sub-Saharan Africa, and some parts of Europe [3]. In addition, hepatitis B virus (HBV)-associated HCC shows different clinical features from HCC related to other etiologies in terms of prognosis and survival [4, 5]. Despite recent advances of techniques in diagnosis and treatment of HCC [6], the clinical outcomes of HCC patients are still disappointing because of frequent metastasis following vascular invasion and poor hepatic reserve function at the time of diagnosis [7–9].

Metastatic tumor antigen-1 (MTA-1), an angiogenic cofactor to promote neovascularization, is considered to be one of the pivotal determinants of hematogenous metastasis in various solid tumors including HCC, lung, and prostate cancers [10–15]. MTA-1 enhances the migration and invasion of tumor cells through stabilization of hypoxia inducible factor 1 alpha (HIF1-α) in angiogenic processes, which leads to activate vascular endothelial growth factor in the end [16–19]. Several previous studies suggested that MTA-1 play an important role in hepatocarcinogenesis via contributing to the process of neovascularization [10–15]. In particular, our previous study demonstrated that MTA-1 overexpression is closely associated with frequent postoperative recurrence and poor survival in patients with HCC [10]. Furthermore, MTA-1 overexpression is significantly more frequent in HBV-associated HCC than in hepatitis C virus (HCV) associated HCC [10, 18, 20]. Considering this close relationship between MTA-1 overexpression and poor survival in HCC patients, it may be necessary clinically to determine the factors predisposing MTA-1 overexpression especially in HBV-associated HCC patients.

Thus, in this study, we intended to determine clinical factors predisposing the overexpression of MTA-1 in patients with hepatitis B virus (HBV)-associated HCC and also examine whether MTA-1 overexpression affects the survival periods of these patients treated with curative surgical resection.

## Patients and Methods

### Study Population

A total of 303 patients with HBV-associated HCC who had been treated with surgical resection in a curative attempt were subjected to this study. Clinical information including laboratory and radiological data was collected from electronic medical records. The presence of cirrhosis was determined by the histological examination of surrounding liver [21, 22]. Radiological characteristics were evaluated by analyzing dynamic CT scan, magnetic resonance imaging (MRI) or angiography. Histological characteristics of HCC were analyzed by the gross and microscopical examination of the HCC tissues. This study was approved by the Institutional Review Board at the Asan Medical Center, Seoul, Korea.

### Tissue Preparation and Immunohistochemical Staining for MTA-1

MTA-1 was detected immunohistochemically in formalin-fixed, paraffin-embedded HCC and surrounding liver tissue. Microtissue samples were prepared by serially sectioning the paraffin-embedded tissues at a thickness of 4 μm using a microtome. These slides were dried in an oven at 60 °C for 60 min followed by deparaffinization with xylene and then by rehydroxylation with graded alcohols, gradually decreasing in concentration from 100 to 75 %. At this stage, antigen retrieval was performed by heating in citric acid (pH 6.0) for 20 min in a microwave oven. Tissue destruction was prevented by incubating with 3 % $H_2O_2$ at room temperature for 10 min, which blocked intrinsic peroxidase activity. Thereafter, the sections were incubated with MTA-1 monoclonal antibody (1:150) overnight at 4 °C. After avidin–biotin complex reagent was applied, slides with the substrate 3,3′-diaminobenzidine using an LSAB (Labeled Streptavidin Biotin) kit (DAKO, Carpentaria, CA, USA) were incubated and sections were counterstained with Harris hematoxylin.

### Evaluation of Immunohistochemical Staining

MTA-1 positivity was determined immunohistochemically. Negative MTA-1 was defined as being no tumor cells with positive MTA-1 staining (Fig. 1). MTA-1 overexpression was defined when at least a portion of tumor cells (>5 %) showed a positive MTA-1 staining (Fig. 1). Two independent observers, blinded to patient-identifying information,

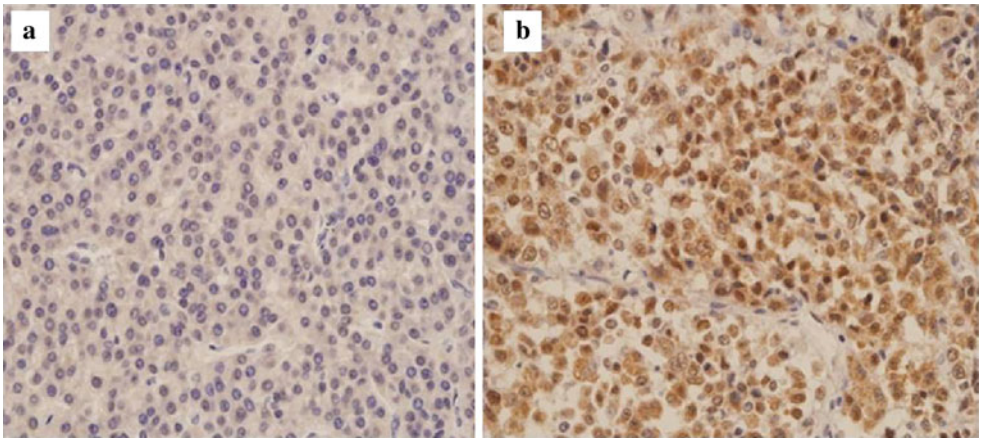


**Fig. 1** Immunohistochemical staining of MTA-1. MTA-1 overexpression was determined based on the positive staining of the nucleus of tumor cells (×200). **a** MTA-1 negative; **b** MTA-1 positive

determined the MTA-1 expression levels. Slides were reviewed again if the score was discrepant to each other.

### Statistical Analyses

Basic clinical characteristics of patients were expressed as median and range. Differences between categorical or continuous variables were analyzed using the $\chi^2$ test, Fisher's exact test, or Student's *t* test. Cumulative post-surgical survival rates were analyzed based on Kaplan–Meier survival curves, and their differences were compared using a log-rank test. A multivariate analysis was performed with Cox regression hazard model to find the predictors for postoperative survival in patients with HBV-associated HCC. A $P$ value $<0.05$ was defined as statistically significant. Statistical analyses were done using SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA).

## Results

### Baseline Characteristics of Patients

The baseline characteristics of the patients ($n = 303$) at the time of surgical resection are shown in Table 1. The median age of patients was 53 years old (range 27–74), and 241 patients (79.5 %) were male. The median serum AFP level was 5,298 ng/mL (range 0.4–2.9 $\times$ $10^5$). Cirrhosis was observed in 197 patients (65.0 %). The Child–Turcotte–Pugh (CTP) class of 239 (78.9 %) and 64 (21.1 %) patients were A and B, respectively. None of the patients was classified as CTP class C (CTP score $\geq$ 10) in the present study.

Preoperative radiological characteristics of HCC and histological features of resected HCC tissues are also shown in Table 1. Median tumor size was 4.7 cm in diameter (range 0.9–20). Portal vein thromboses were observed in 40 patients; all of them were only in the subsegmental branches of portal vein on preoperative dynamic CT scan or MRI. Most of the HCCs (96.7 %) were nodular in type. On histological examination, rather poor differentiation of HCC (Edmondson–Steiner grade III or IV) was

**Table 1** Baseline characteristics of the patients with hepatitis B virus-associated hepatocellular carcinoma

| Variables | $n = 303$ |
|---|---|
| Clinical parameters | |
| Age, years | 53 (27–74) |
| Sex, M/F, *n* (%) | 241/62 (79.5/20.5) |
| Platelet, $\times 10^3/mm^{3a}$ | 142 (20–410) |
| INR[a] | 1.1 (0.9–2.2) |
| Total bilirubin, mg/dL[a] | 1.1 (0.4–7.7) |
| Serum HBV–DNA, pg/mL[a] | 8.7 (1–2,150) |
| Serum AFP, ng/mL[a] | 5,298 (0.4–2.9 $\times$ $10^5$) |
| CTP class (A/B/C), *n* (%) | 239/64/0 (78.9/21.1/0.0) |
| Radiological parameters | |
| Tumor size, cm[a] | 4.7 (0.9–20) |
| Tumor number, 1/2 or 3, *n* (%) | 253/50 (83.5/16.5) |
| Portal vein thrombosis (+), *n* (%) | 40 (13.2) |
| Tumor type (nodular/infiltrative), *n* (%) | 293/10 (96.7/3.3) |
| Histological parameters | |
| Edmondson–Steiner grade (I, II/III, IV), *n* (%) | 91/212 (30/70) |
| Microvascular invasion (+), *n* (%) | 58 (19.2) |
| Bile duct invasion (+), *n* (%) | 8 (2.7) |
| Capsule invasion (+), *n* (%) | 109 (36.2) |
| MTA-1 (+), *n* (%) | 104 (34.3) |
| Median follow-up (month)[a] | 29 (2–50) |

*AFP* alpha-fetoprotein, *CTP* Child-Turcotte-Pugh, *HBV* hepatitis B virus, *INR* international normalized ratio, *MTA-1* metastatic tumor antigen-1

[a] Median (range)

**Table 2** Relationship between MTA-1 overexpression and clinical characteristics of patients with HBV-associated HCC

| Variables | MTA-1 expression, *n* (%) | | *P* value* |
|---|---|---|---|
| | Negative ($n = 199$) | Positive ($n = 104$) | |
| Age, years | | | 0.04 |
| <50 | 62 (57.9) | 45 (42.1) | |
| ≥50 | 137 (69.9) | 59 (30.1) | |
| Sex | | | <0.01 |
| Male | 167 (69.3) | 74 (30.7) | |
| Female | 32 (51.6) | 30 (48.4) | |
| Serum AFP, ng/mL | | | <0.01 |
| <200 | 141 (71.9) | 55 (28.1) | |
| ≥200 | 58 (54.2) | 49 (45.8) | |
| CTP | | | 0.03 |
| A | 163 (68.2) | 76 (31.8) | |
| B | 53 (82.8) | 11 (17.2) | |

*MTA-1* metastatic tumor antigen-1, *MTA-1 positive* patients who are positive for MTA-1, *MTA-1 negative* patients who are negative for MTA-1, *AFP* alpha-fetoprotein, *CTP* Child-Turcotte-Pugh, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma

* *P*-value for difference between MTA-1 positive and MTA-1 negative groups ($\chi^2$ test)

observed in 212 cases (70 %). In addition, microvascular invasion (MVI), bile duct invasion, and capsule invasion were observed in 58 (19.2 %), 8 (2.7 %) and 109 cases (36.2 %), respectively.

## Clinical Characteristics Predisposing MTA-1 Overexpression in HBV-Associated HCC

MTA-1 overexpression was more common in younger age groups (<50 years old) than in older patients ($P = 0.04$). Also, female gender was associated with MTA-1 overexpression more frequently than male patients ($P < 0.01$). Patients with higher serum alpha-fetoprotein (AFP) level over 200 ng/mL and a better CTP class (class A than B) were significantly associated with MTA-1 overexpression more frequently (both *P* values <0.01) (Table 2). Other hematological and biochemical laboratory features including preoperative serum ALT level and platelet count did not show significant association with MTA-1 overexpression.

## Radiological Characteristics Predisposing MTA-1 Overexpression in HBV-Associated HCC

There was no significant relationship between the size of HCC and MTA-1 overexpression ($P = 0.12$). However, MTA-1 was more frequently overexpressed in patients with portal vein thrombosis (PVT) compared with patients without PVT ($P = 0.04$). The infiltrative type of HCC also had a marginally significant association with MTA-1 overexpression compared with the nodular type of HCC ($P = 0.08$) (Table 3).

**Table 3** Relationship between MTA-1 overexpression and radiological characteristics of HBV-associated HCC

| Variables | MTA-1 expression, *n* (%) | | *P* value* |
|---|---|---|---|
| | Negative ($n = 199$) | Positive ($n = 104$) | |
| Tumor size (cm) | | | 0.12 |
| <5 | 123 (63.1) | 72 (36.9) | |
| ≥5 | 76 (70.4) | 32 (29.6) | |
| Tumor type | | | 0.08 |
| Nodular | 195 (66.6) | 98 (33.4) | |
| Infiltrative | 4 (40) | 6 (60) | |
| Portal vein thrombosis | | | 0.04 |
| Presence | 21 (52.5) | 19 (47.5) | |
| Absence | 178 (67.8) | 85 (32.2) | |

*MTA-1* metastatic tumor antigen-1, *MTA-1 positive* patients who are positive for MTA-1, *MTA-1 negative* patients who are negative for MTA-1, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma

* *P* value for difference between MTA-1 positive and MTA-1 negative groups ($\chi^2$ test or Fisher's exact test)

## Histological Characteristics Predisposing MTA-1 Overexpression in HBV-Associated HCC

MTA-1 overexpression was significantly more common in poorly differentiated HCC (Edmonson–Steiner grade III or IV) than in well differentiated HCC (Edmondson–Steiner grade I or II) ($P < 0.01$). Microvascular or capsular invasion were also significantly associated with MTA-1 overexpression ($P = 0.04$, $P = 0.02$, respectively). Moreover, bile duct invasion tended to be associated with MTA-1 overexpression ($P = 0.09$) (Table 4).

## Effect of MTA-1 Overexpression on Survival of Patients with HBV-Associated HCC

MTA-1 was overexpressed in 104 HCC (34.3 %) and none of the surrounding non-tumor tissues (Fig. 2). The cumulative survival rates were significantly lower in patients with MTA-1 overexpression compared with those in the MTA-1 negative group (94 vs. 95 % at 1 year, and 78 vs. 89 % at 3 years, respectively; $P = 0.03$) (Fig. 3).

## Significant Factors Determining Survival of Patients with HBV-Associated HCC After Curative Surgical Resection

Univariate analyses revealed that higher serum AFP level, the presence of PVT or MVI, and MTA-1 overexpression

**Table 4** Relationship between MTA-1 overexpression and histologic characteristics of HBV-associated HCC

| Variables | MTA-1 expression, *n* (%) | | *P* value* |
|---|---|---|---|
| | Negative ($n = 199$) | Positive ($n = 104$) | |
| E–S grade | | | <0.01 |
| I and II | 71 (78) | 20 (22) | |
| III and IV | 128 (60.4) | 84 (39.6) | |
| MVI | | | 0.04 |
| Presence | 32 (55.2) | 26 (44.8) | |
| Absence | 167 (68.2) | 78 (31.8) | |
| Bile duct invasion | | | 0.09 |
| Presence | 3 (37.5) | 5 (62.5) | |
| Absence | 196 (66.4) | 99 (33.6) | |
| Capsule invasion | | | 0.02 |
| Presence | 62 (56.9) | 47 (43.1) | |
| Absence | 137 (70.6) | 57 (29.4) | |

*MTA-1 positive* patients who are positive for MTA-1, *MTA-1* negative patients who are negative for MTA-1, *E–S grade Edmondson*–Steiner grade, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *MTA-1* metastatic tumor antigen-1, *MVI* microvascular invasion

* *P* value for difference between MTA-1 positive and MTA-1 negative groups ($\chi^2$ test or Fisher's exact test)

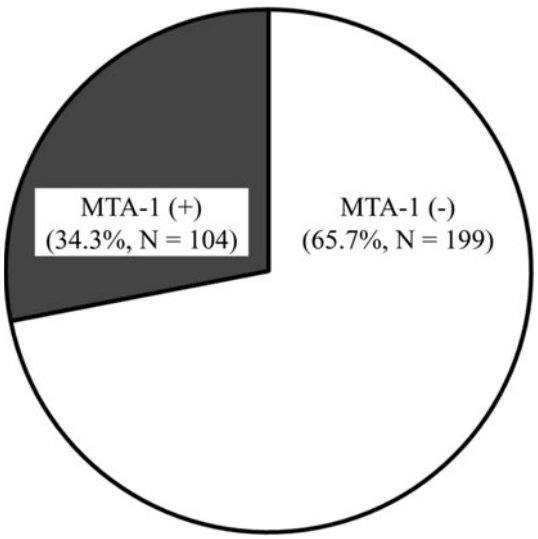


**Fig. 2** Proportion of metastatic tumor antigen-1 (MTA-1)-positive hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC). Of 303 HCCs, 104 (34.3 %) were positive for MTA-1 and 199 (65.7 %) were negative

were significant predictors for poor postoperative survival. Also, capsule invasion was marginally associated with poor survival of the patients. In multivariate analysis, however, MVI [hazard ratio (HR) 8.013, $P < 0.001$] and MTA-1 overexpression (HR 1.848, $P = 0.046$) were independent significant factors determining poor survival of patients with HBV-associated HCC after curative surgical resection. Capsule invasion had marginally significant relationship with poor survival of the patients (HR 2.121, $P = 0.054$) (Table 5).

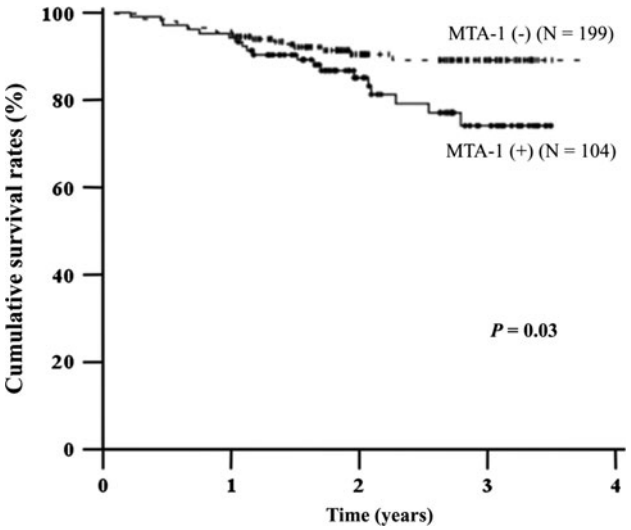


**Fig. 3** Cumulative survival rates of hepatitis B virus (HBV)-associated hepatocellular carcinoma (HCC) patients in relation to metastatic tumor antigen-1 (MTA-1) positivity. The cumulative survival rates of patients with MTA-1 overexpression were significantly lower than those with MTA-1-negative HCC. (94 vs. 95 % at 1 year, and 78 vs. 89 % at 3 years, respectively; $P = 0.03$)

## Discussion

Hepatocellular carcinoma (HCC) is a well-known hypervascular tumor. The hypervascularity contributes to rapid progression by directly invading surrounding tissues and/or blood vessels, resulting in frequent intra- and extra-hepatic metastases. Therefore, so many recent studies have focused on the hematogenously spreading features of HCC [23, 24]. Thanks to a recent dramatic development in molecular biology, MTA-1 has become a target of research, which plays an important role in migration and invasion of malignant tumor cells in vitro. Briefly, MTA-1 stabilizes the hypoxia inducible factor (HIF)-1α after forming the nucleosome remodeling and histone deacetylation (NuRD) complex, leading to enhance neovascularization and metastasis in MTA-1-overexpressing tumors under hypoxic conditions [10, 16–19]. That is, MTA-1 promotes the angiogenesis of HCC so that it is associated with poor survival of HCC patients. The results of the present study strengthen this evidence by showing that the postoperative overall survival periods of HBV-associated HCC patients with MTA-1 overexpression were significantly shorter than those of patients without MTA-1 overexpression.

Our previous study revealed that MTA-1 overexpression is closely associated with aggressiveness or invasiveness of

**Table 5** Multivariate analysis for factors predicting poor postoperative survival of patients with HBV-associated HCC

| Variables | Univariate analysis | | | Multivariate analysis[a] | | |
|---|---|---|---|---|---|---|
| | HR | 95 % CI | *P* value | HR | 95 % CI | *P* value |
| AFP, ≥200 ng/mL | 2.237 | 1.172–4.273 | 0.015 | 1.232 | 0.599–2.534 | 0.570 |
| CTP class A | 1.522 | 0.528–4.388 | 0.437 | – | – | – |
| PVT (+) | 1.414 | 1.138–5.121 | 0.022 | 1.563 | 0.657–3.717 | 0.313 |
| E–S grade, III/IV | 1.531 | 0.700–3.349 | 0.287 | – | – | – |
| MVI (+) | 7.799 | 4.038–15.061 | <0.001 | 8.013 | 3.796–16.915 | <0.001 |
| Capsule invasion (+) | 1.706 | 0.276–1.247 | 0.165 | 2.121 | 0.986–4.560 | 0.054 |
| MTA-1 (+) | 1.995 | 1.047–3.800 | 0.036 | 1.848 | 0.929–3.676 | 0.046 |

*HBV* hepatitis B virus, *CTP* Child-Turcotte-Pugh, *CI* confidence interval, *E–S grade* Edmondson–Steiner grade, *HCC* hepatocellular carcinoma, *HR* hazard ratio, *MTA-1* metastatic tumor antigen-1, *MVI* microvascular invasion, *PVT* portal vein thrombosis

[a] Cox proportional hazards model with a backward elimination method

HCC such as worse histological differentiation, and portal vein thrombosis or microvascular invasion, which results in frequent postoperative recurrence and poor patients' survival [10]. Such findings are conspicuous especially in HBV-associated HCC compared with HCV-associated HCC. In the present study, we intended to determine clinical factors predisposing the overexpression of MTA-1 in patients with HBV-associated HCC and so we tried to find a better way to manage these patients postoperatively.

We have shown here that, in patients with HBV-associated HCC, MTA-1 overexpression was significantly associated with younger age, female gender, higher serum AFP level, and CTP class A. Also, in the present study, HCC of invasive nature such as PVT, microvascular invasion, capsular invasion, and poor histological differentiation were associated with overexpression of MTA-1. Considered together with the fact that MTA-1 may be a significant poor prognostic marker of HCC patients treated with HCC, the clinical predisposing factors determined in the present study could be used for proper postoperative monitoring and management of patients with HBV-associated HCC.

Until now, a variety of prognostic factors have been suggested in patients with HCC [4, 5, 25–27]. The invasiveness of HCC has been reported to be higher in younger patients [26–28], which is consistent with the results of the present study. Previous studies showed that HCC with the wild-type estrogen receptor (ER) had a better prognosis, whereas patients with variant ERs suffered from worse survival [29, 30]. In the present study, MTA-1 overexpression is more common in female patients than male, explaining the association between gender and prognosis. MTA-1 overexpression was noted frequently also in patients with high serum AFP, which may be a marker of rapid progression or large volume of HCC [31]. In the present study, MTA-1 was overexpressed in patients with CTP class A compared with those with CTP class B. One of the possible speculations of frequent MTA-1 overexpression in patients with CTP class A is higher protein synthetic capacity of these patients. They could express biomarkers including MTA-1 much higher than those with CTP class B or C.

A variety of radiological and histological characteristics have been reported to be associated with the invasiveness of HCC [10, 18, 20]. Considering the fact that MTA-1 induces neovascularization of HCC and promotes invasion into surrounding tissues resulting in portal vein thrombosis, microvascular or capsular invasion, it is a matter of course that MTA-1 overexpression is much more frequent in HCCs with portal vein thrombosis or microvascular invasion. Especially, in this study, microvascular invasion is an independent predictor of postoperative poor survival in HBV-associated HCC patients.

The prognosis of patients with the infiltrative type of HCC has been reported to be much worse than that of patients with the nodular type of HCC, mainly due to frequent intrahepatic and extrahepatic metastasis [32]. The present study demonstrated that the infiltrative type of HCC had a tendency to be associated with MTA-1 overexpression ($P = 0.08$). Also, histologically, MTA-1 was more frequently overexpressed in poorly differentiated HBV-associated HCC, which is comparable with the results of previous reports [10, 33]. Moreover, MTA-1 overexpression was an independent predictor of poor postoperative survival of HBV-associated HCC patients (Table 5). Thus, it is suggested that MTA-1 is overexpressed especially in patients with poorly-differentiated HCC, and MTA-1 overexpression is closely associated with invasive nature of HCC resulting in poor prognosis of the patients.

In the present study, MTA-1 expression was evaluated in the liver tissues using immunohistochemical methods. It would be very useful clinically if the patients with higher risk for post-hepatectomy recurrence and/or poor clinical

outcome could be selected by simple blood tests. Hopefully, in the future, we can get simpler test kits to determine the MTA-1 overexpression in the HCC tissues, and then apply them to HCC patients to predict the clinical outcomes of them.

In conclusion, MTA-1 overexpression is significantly more frequent in HBV-associated HCC patients with younger age, female gender, a higher serum AFP level, and more invasive HCC phenotypes characterized by PVT, the infiltrative type, poor histologic differentiation, microvascular invasion, bile duct invasion, and capsular invasion. Consequently, it is necessary to monitor HCC patients with such clinical predispositions more carefully even after curative surgical resection.

**Conflict of interest** None.

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