Prognostic significance of metadherin overexpression in hepatitis B virus-related hepatocellular carcinoma

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Abstract. Metadherin (MTDH), which is an HIV-1 or TNF-α-inducible transcript, has a crucial role in several types of cancer by regulating multiple cellular signaling processes. However, to date, the role of MTDH in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) is still unclear. In the present study, we detected MTDH expression in normal liver, chronic hepatitis B and HBV-related HCC tissues. The data showed that MTDH expression levels were elevated in the hepatitis B tissues and especially in the HBV-related HCC tissues compared to normal liver tissues. There was a trend for gradually increased MTDH expression from normal liver tissue to hepatitis B and HBV-related HCC tissues. Furthermore, a statistical analysis revealed that MTDH expression significantly correlated with the American Joint Committee on Cancer (AJCC, 7th edition) stage (P=0.020), T classification (P=0.007), N classification (P=0.044), vascular invasion (P=0.006) and histological differentiation (P=0.020) in the HBV-related HCC patients. In addition, patients with high MTDH levels had shorter survival times compared to those with low MTDH expression (P=0.001). Taken together, these results suggest that MTDH could be a potential prognostic marker for overall survival and tumor progression and a chemotherapeutic target in HBV-related HCC patients.

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Abbreviations: AEG-1, astrocyte elevated gene-1; AFP, α-fetoprotein; AJCC, American Joint Committee on Cancer; AP-1, activator protein 1; ERK, extracellular signal-regulated kinase; GSK-3β, glycogen synthesis kinase-3β; HBV, hepatitis B virus; HBx, hepatitis B virus X protein; HCC, hepatocellular carcinoma; HIV-1, human immunodeficiency virus type 1; MTDH, metadherin; NF-κB, nuclear factor-kappa B; PI3K, phosphoinositide 3-kinase; TNF-α, tumor necrosis factor-α

Key words: metadherin, hepatocellular carcinoma, hepatitis B virus, progression, prognosis

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer mortality worldwide (1,2). Since the 1990s, it has been ranked the second leading cause of cancer deaths in China. Epidemiological studies have identified major risk factors for HCC, including infection with hepatitis B and C virus (HBV and HCV), exposure to aflatoxin B1, a high intake of alcohol, as well as metabolic diseases (2). Among these risk factors, growing evidence supports HBV infection as the most common cause of HCC. According to a recent study, 53% of HCC cases worldwide are associated with HBV infection (3). Because of endemic HBV infection, HBV-related HCC has a higher prevalence in China than in Europe and America.

HBx protein, which is coded by the HBV X gene (HBX gene), is frequently integrated with the HBV genome into cellular DNA during hepatocellular carcinogenesis and remains functionally active, regulates cell cycle progression (4), promotes cell proliferation, inhibits cell apoptosis (5), and the expression of several tumor suppressor genes including p53 (4) and senescence-related factors. However, the exact molecular mechanisms and pathways responsible for HBV-related HCC are still unclear. Therefore, it is important to elucidate the key factors that are involved in HBV-related HCC, which will help to identify HBV-related HCC patients with elevated risk, improve prognosis prediction and facilitate therapeutic intervention.

The oncoprotein metadherin (MTDH, also known as astrocyte elevated gene-1, AEG-1) was originally identified as a protein that is induced in primary human fetal astrocytes infected with human immunodeficiency virus-1 (HIV-1) or treated with HIV gp120 or tumor necrosis factor-α (TNF-α) (6). Overexpression of MTDH has been frequently observed in a variety of primary human tumors, including breast cancer (7-10), multiform glioblastoma (11-13), neuroblastoma (14,15), gastric cancer (16), colorectal carcinoma (17), osteosarcoma (18), gallbladder carcinoma (19), head and neck squamous cell carcinoma (HNSCC) (20), endometrial cancer (21), ovarian

carcinoma (22), esophageal squamous cell carcinoma (ESCC) (23), non-small cell lung cancer (24), HCC (25), melanoma (26), and prostate cancer (6,27,28). Furthermore, published studies on some cancers have revealed that MTDH is a novel and useful prognostic marker for cancer progression, and its overexpression is associated with an unfavorable prognosis (7,8,13,15-17,23,24,26,28,29). The role of MTDH in HCC has long been debated. A previous study showed that MTDH is a key regulator of multiple facets that are critical for HCC development (25). However, the role of MTDH in HBV-related HCC remains unclear. The data that have accumulated over the last few years suggest that MTDH could regulate the transformed status of some types of cancer through the activation of different signaling molecules, which overlap with the signaling pathways associated with HBx. Considering these data, we believe that MTDH may be closely linked to HBV-related HCC and should be further investigated.

In this study, we investigated the expression of MTDH in HBV-related HCC patients and the correlation between MTDH expression and clinicopathological characteristics and survival. To our knowledge, this is the first report to describe the correlation between MTDH and HBV-related HCC. The expression of MTDH was upregulated in the surgically extracted tissues of HBV-related HCC and hepatitis B patients. Moreover, the overexpression of MTDH significantly correlated with the American Joint Committee on Cancer (AJCC, 7th edition) stage, tumor-node-metastasis (TNM) classification and the histological classification of HBV-related HCC. More importantly, a multivariate analysis revealed that MTDH could be an independent biomarker for the prediction of HBV-related HCC prognosis. These results support a novel role for MTDH as an essential intermediary in HBV-related HCC and suggest that MTDH could be a useful clinical biomarker and therapeutic target in HBV-related HCC.

Materials and methods

Patients and tissue samples. Clinical samples of HBV-related HCC and hepatitis B tissues were obtained from 73 patients with HBV-related HCC and 45 patients with hepatitis B who underwent initial radical hepatectomy and liver biopsy at the Xijing Hospital of the Fourth Military Medical University (Xi'an, Shaanxi, China) from July 2004 to June 2006. Normal liver tissues were obtained from 11 patients who underwent hepatectomy due to benign liver disease. The study was approved by the Xijing Hospital Ethics Committee, and informed consent was obtained from all of the patients. The recommendations of the Declaration of Helsinki for biomedical research involving human subjects were followed. The patients with HBV-related HCC in this study included 62 male and 11 female patients with a median age of 54 years (range, 38.0-72.0 years). None of the patients had received chemotherapy or radiation therapy prior to surgery. The clinicopathological parameters of the patients are shown in Table II. All of the diagnoses of HBV-related hepatitis and HCC were confirmed by the serological detection of HBV antigens and were verified by pathologists. Tumor differentiation grades were determined according to the Edmondson-Steiner classification system. Using the AJCC (7th edition) classification, the patients with HCC were classified as stages I, II, III and IV. There were 48 patients with stage I, 9 with stage II, 8 with stage III, and 8 with stage IV. Overall survival was calculated as the time from the date of the operation until death. The follow-up of the patients was regularly performed at outpatient clinics. α-fetoprotein (AFP) assay and liver sonography were performed every 3 months, if necessary, and computerized tomography (CT) was also performed.

Immunohistochemical staining. The formalin-fixed and paraffin-embedded tissue specimens included HCC (n=73), hepatitis B (n=45) and normal liver (n=11) tissues. The procedures were carried out as described in the instruction manual. In brief, paraffin-embedded specimens were cut into 4 μm sections and baked at 60˚C for 60 min. After deparaffinization with xylene and rehydration with graded alcohol, the sections were rinsed in phosphate-buffered saline (PBS). Subsequently, the slides were placed in citrate buffer and were microwaved on the medium setting for 5 min. The sections were treated with 3% hydrogen peroxide in methanol for 20 min at room temperature to suppress the endogenous peroxidase activity and were incubated with pre-immune serum albumin to block non-specific binding. Anti-MTDH (1:400, rabbit polyclonal, Proteintech Group, Inc., Chicago, IL, USA) was incubated with the sections overnight at 4˚C. For the negative controls, the anti-MTDH antibody was replaced with goat serum in this step. After washing with TBST buffer, the tissue sections were treated with a biotinylated secondary antibody followed by an incubation with streptavidin-horseradish peroxidase complex. The tissue sections were then immersed in DAB and counterstained with 10% Mayer's hematoxylin to visualize the staining. Finally, the slides were dehydrated and mounted.

Immunohistochemical assessment. The degree of immunostaining for protein expression in the specimens was observed and scored separately by three independent investigators, who were blinded to the patient information. At x200 magnification, 10 representative fields of each section were viewed. The sections were scored by combining the proportion of positively stained cells with the intensity of the staining. The scores obtained by the three independent investigators were averaged for the comparative evaluation of MTDH expression. The cell proportion was scored as follows: 0, no positive cells; 1, <10% positive cells; 2, <50% positive cells; and 3, >50% positive cells. The staining intensity was graded using the following criteria: 0 (no staining); 1 (weak staining = light yellow); 2 (moderate staining = yellow brown); and 3 (strong staining = brown). The staining index (SI) was calculated as the product of the staining intensity score and the proportion of positive tumor cells. Using this method of assessment, we evaluated MTDH expression in the sections by calculating SI scores of 0, 1, 2, 3, 4, 6 or 9. Based on the measure of heterogeneity from the log-rank test, an optimal cut-off value for high and low expression level was identified: an SI score of ≥6 was used to define tumors with high MTDH expression, and an SI score of ≤4 was used to indicate low MTDH expression.

Western blotting. Total proteins of HCC (10 cases), hepatitis B (10 cases), and normal (10 cases) tissues were obtained from the patients previously mentioned. The protein concentrations were quantified, and 40 μg of protein per sample was separated

using sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (Immobilon-P; Millipore, Bedford, MA, USA), which had been soaked in 100% methanol for 2 min. After blocking with 5% non-fat dry milk, the membranes were incubated with anti-MTDH (1:400, rabbit polyclonal, Proteintech Group) for 1 h at room temperature. After washing with TBST buffer, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Finally, the membranes were covered with enhanced chemiluminescence reagents (Applygen Technologies Inc., Beijing, China) for 1 min and were exposed to the film to visualize the result. The experiment was performed in triplicate. Western blotting for β-actin was used as an internal loading control.

RNA extraction, reverse transcription and real-time PCR. Total RNA was extracted from the surgically obtained HCC, hepatitis B and normal liver tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cDNA was synthesized using the Primescript RT Reagent kit (Takara, Dalian, China) using the oligo(dT) primer. The qRT-PCR analysis was performed with the SYBR Premix EX Taq II (Takara) on a Bio-Rad IQ5 (Bio-Rad Laboratories, Hercules, CA, USA) machine. All of the specific primers used in the present study were designed and synthesized by Sangon Biotech (Shanghai, China). The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as a reference gene. The sequences of the primers were as follows: MTDH, 5'-AAGAGGAAAACT GAGCCATCTG-3' (forward) and 5'-CGGCTAACATCCCA CTGATAAT-3' (reverse); and GAPDH, 5'-GACTCATGAC CACAGTCCATGC-3' (forward) and 5'-AGAGGCAGGGAT GATGTTCTG-3' (reverse). Each reaction was performed in triplicate. The specific reaction conditions were as follows: initial denaturation at 95˚C for 1 min; template denaturation at 95˚C for 20 sec, annealing at 60˚C for 30 sec, and extension at 72˚C for 1 min (total of 40 cycles) and final extension at 72˚C for 10 min. In a control reaction, cDNA was substituted with water.

Statistical analysis. The χ^2 test and Fisher's exact test were used to analyze categorical variables. Bivariate correlations between study variables were calculated by Spearman's rank correlation coefficients. Survival curves were plotted by the Kaplan-Meier method and the difference was compared using the log-rank test. Prognostic relevance was evaluated using a multivariate Cox regression analysis. All of the statistical analyses were performed using the SPSS 17.0 software (SPSS Inc., Chicago, IL). P<0.05 was considered statistically significant.

Results

Differential expression of MTDH. Western blotting revealed that the MTDH protein was differentially upregulated in all of the hepatitis B and HCC tissues. In contrast, it was not detected in normal liver tissues (P<0.001, Fig. 1A and B). Similarly, qRT-PCR results showed that all of the HBV-related HCC tissues exhibited significantly (up to 10-fold) higher levels of MTDH messenger RNA compared with normal liver tissues (Fig. 1C).

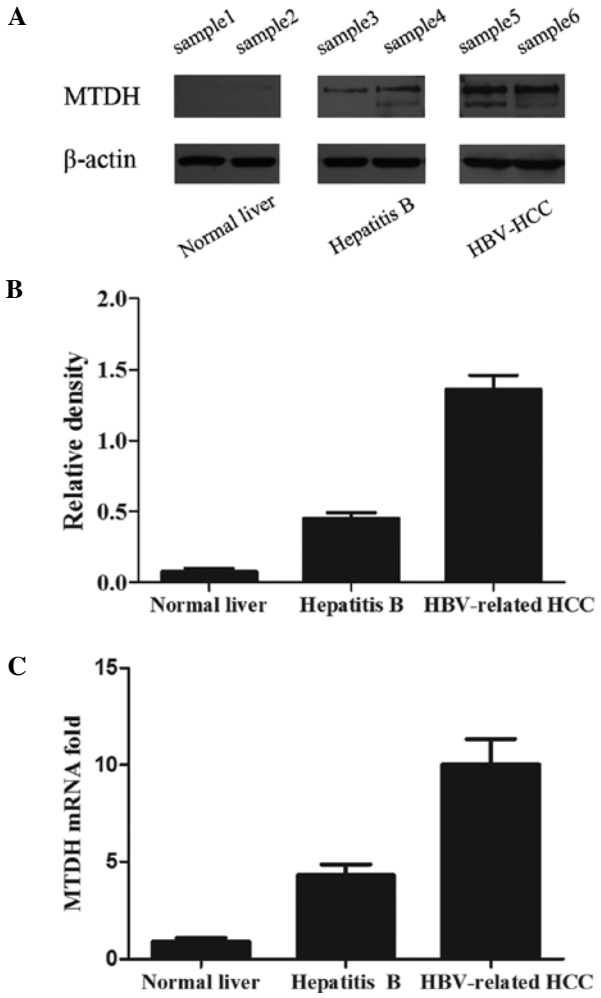

Figure 1. MTDH protein and mRNA levels in different liver tissues. (A) Western blotting shows that MTDH expression is upregulated in HBV-related hepatitis and HCC, whereas weak or no MTDH expression was observed in normal liver tissue. Expression levels were standardized against β-actin. (B) MTDH protein expression levels in the individual tissue samples was calculated as the ratio of MTDH expression relative to β-actin expression (mean ± SD, P<0.001). (C) qRT-PCR assay of MTDH mRNA expression in normal liver, HBV-related hepatitis and HCC tissues (mean ± SD, P<0.001). Expression levels were normalized to GAPDH.

Using immunohistochemical staining, very little to no MTDH was detected in normal liver specimens, whereas significant MTDH staining was observed in the hepatitis B and HBV-related HCC samples. MTDH expression was mainly localized in the cytoplasmic region, and nuclear MTDH staining was not detected in any sections. There were 2 out of 45 hepatitis B and 3 out of 73 HBV-related HCC samples that scored zero for MTDH. No significant difference was found between hepatitis B patients with cirrhosis and those without cirrhosis (P=0.139). The frequency and intensity of MTDH expression was gradually elevated from normal liver tissues to hepatitis B, with the highest indices in HBV-related HCC (Table I; Fig. 2).

Relationship between MTDH expression and the clinicopathological parameters of HBV-related HCC. As shown in Table II, MTDH expression significantly correlated with the AJCC stage (P=0.020), T classification (P=0.007), N classification (P=0.044), vascular invasion (0.006), and histological

Table I. Immunohistochemical analysis of MTDH in different liver tissues.

Categories	MTDH expression		P-value
	Low (%)	High (%)	
Normal liver tissue	11 (100)	0 (0)	<0.001
Hepatitis B	13 (53.3)	9 (46.7)	
HBV-related HCC	26 (35.6)	47 (64.4)	

P-values were calculated by the χ^2 test and Fisher's exact test.

differentiation (P=0.020) of patients with HBV-related HCC. For the M classification (P=0.083), all of the 6 patients with M1 exhibited high MTDH expression. To further confirm the correlation between MTDH expression and clinicopathological features, a Spearman correlation analysis was used. Table III shows the Spearman correlations of MTDH expression levels to histological differentiation (0.274; P=0.019), AJCC stage (0.285; P=0.015), and T classification (0.244; P=0.048).

Association between MTDH expression and the prognosis of patients with HCC. Statistical analyses showed a significant negative correlation between the MTDH staining index and the overall survival time of HBV-related HCC patients (Spearman correlation coefficient -0.385; P=0.001). A Kaplan-Meier analysis and the log-rank test were applied to calculate the effect of MTDH expression on overall patient survival. The log-rank test revealed that the MTDH expression level correlated significantly with the survival time of patients with HCC (P=0.001). Furthermore, the median survival time of patients with high MTDH expression levels was 30 months (95% confidence interval, 23.4-36.8 months), while the median survival time of those with low expression was 49 months (95% confidence interval, 42.5-52.8 months). The 5-year cumulative survival rate was 65.1% in the low MTDH expression group, whereas it was only 19.4% in the high MTDH expression group (Fig. 3).

To determine whether the MTDH expression level is an independent prognostic factor in patients with HCC, we performed a multivariate survival analysis as described in Materials and methods. Our findings revealed that MTDH expression, AJCC stage, T classification, and tumor differentiation are independent prognostic factors (Table IV). Taken together, these results indicate that MTDH expression has a significant correlation with the prognosis of HBV-related HCC.

Discussion

Despite remarkable advances in diagnosis and therapeutic techniques, only a minority of patients with HCC are diagnosed at early stages and are candidate for curative treatments, and the recurrence rate may be as high as 50% at 3 years (1).

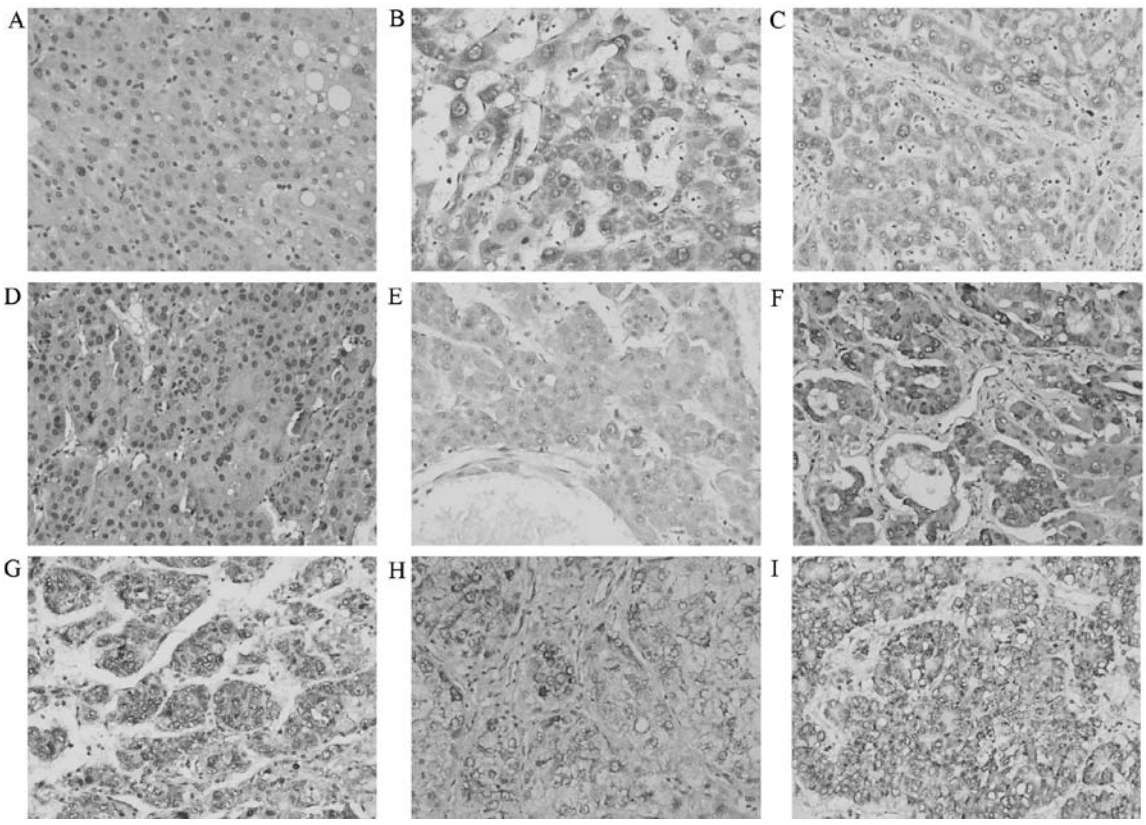

Figure 2. Immunohistochemical staining of MTDH in different liver tissues. (A) Normal liver tissues; (B) hepatitis B without cirrhosis; (C) hepatitis B with cirrhosis; (D) HBV-related HCC, scored zero; (E) HBV-related HCC, stage I, good differentiation; (F) HBV-related HCC, stage I, moderate differentiation; (G) HBV-related HCC, stage II, poor differentiation; (H) HBV-related HCC, stage III, moderate differentiation. (I) HBV-related HCC, stage IV, moderate differentiation. Original magnification, x400.

Table II. Relationship between MTDH expression and clinicopathological parameters in patients with HBV-related HCC.

Parameters	MTDH expression		n	P-value
	Low	High		
Age (years)				0.463
≤54	14	20	34	
>54	12	27	39	
Gender				0.308
Male	24	38	62	
Female	2	9	11	
Tumor size (cm)				0.204
≤5	12	15	27	
>5	13	33	46	
Tumor number				0.145
≤2	25	39	64	
>2	1	8	9	
AJCC stage				0.020[a]
I	22	26	48	
II	6	3	9	
III	2	6	8	
IV	0	8	8	
T classification				0.007[a]
T1	23	30	53	
T2	0	12	12	
T3	2	6	8	
T4	0	0	0	
N classification				0.044[a]
N1	0	8	8	
N0	26	39	65	
M classification				0.083
M1	0	6	6	
M0	26	41	67	
Histological differentiation				0.020[a]
Good	6	3	9	
Moderate	19	29	48	
Poor	2	14	16	
Vascular invasion				0.006[a]
Absence	26	36	62	
Presence	0	11	11	
Serum AFP levels (μg/l)				0.062
≤400	22	29	51	
>400	4	18	22	

[a]P<0.05 was considered as statistically significant (P-values were calculated using the χ^2 test and the Fisher's exact test).

Table III. Spearman correlation analysis between MTDH expression and clinicopathological parameters.

Values	Spearman correlation (P-value)
Histological differentiation	0.274 (0.019)
AJCC stage	0.285 (0.015)
T classification	0.244 (0.048)

Table IV. Multivariate analysis of various prognostic variables in patients with HBV-related HCC

Variable	Relative risk (95% confidence interval)	P-value
MTDH expression	7.314 (1.848-28.398)	0.004
AJCC stage	3.090 (1.468-6.505)	0.003
T classification	1.871 (0.928-3.773)	0.080
Tumor differentiation	2.321 (1.131-4.764)	0.022

The P-values were calculated using a Cox-regression analysis.

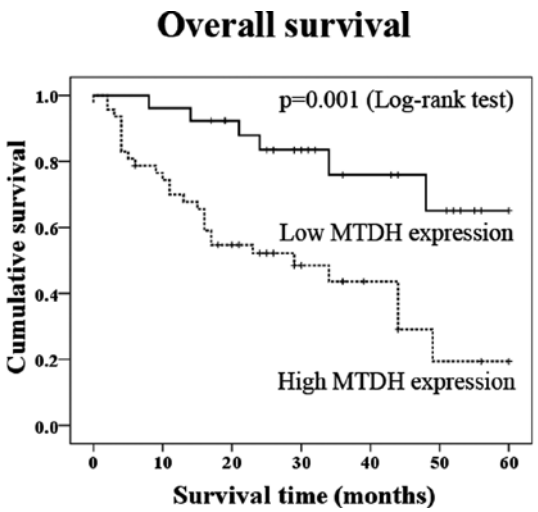

Figure 3. Kaplan-Meier curve showing the relationship between MTDH expression and the survival time of patients with HBV-related HCC. The cumulative 5-year survival rates in the high MTDH expression group and the low MTDH expression group were 19.4% and 65.1%, respectively (log-rank P=0.001).

In contrast, the remaining patients face extremely poor prognoses. It is believed that the main cause of the extremely poor prognoses for patients with HCC is the fact that the molecular mechanisms responsible for HCC are unclear. In China, chronic HBV infection has been identified as a major risk factor for HCC, and the prognosis of patients with HBV-related HCC is very poor. Therefore, elucidating the molecular mechanism and identifying prognostic and therapeutic targets are important for HBV-related HCC. In this study, we primarily focused on HBV-related HCC.

The molecular mechanisms responsible for the pathogenesis of HBV-related HCC are completely different from those of HCV-related HCC (30). Previous research has confirmed that the HBx protein of HBV has a crucial role in the pathogenesis of HBV-related HCC and activates several different

signaling pathways that are involved in HCC, including the JAK/STAT (31), Wnt/β-catenin (32,33), RAS/RAF/MAPK, and PI3K/AKT/ERK pathways and their downstream molecules, such as GSK-3β, cyclin D, A and B, and $p21^{cip1}$ (34-36). Additionally, HBx has been shown to activate the transcription factors NF-κB (37) and AP-1 (38). However, the exact molecular mechanisms responsible for HBV-mediated hepatocarcinogenesis are incompletely understood. Furthermore, the antiviral treatment of HBV infection may render patients with HBV-related HCC better able to tolerate HCC treatments and may improve prognosis. However, it is important to note that conventional treatment, such as lamivudine, inhibits HBV replication but cannot reverse HBV-related changes in downstream signaling pathways, which are closely related to the occurrence and development of HCC (39). Considering the above findings, we believe that it is important to investigate the direct molecular mechanism of the carcinogenic effect of HBV and to identify new intermediaries. The results of these studies may help to identify additional therapeutic targets for HBV-related HCC.

MTDH, which is a key regulator of multiple critical facets for the development of malignancy, has been demonstrated to inhibit cancer cell apoptosis and to increase invasiveness and metastasis. Meanwhile, the knockdown of MTDH has the inverse effects (28,40). In addition, researchers have identified a lung-homing domain of MTDH, which mediates breast cancer cell metastasis to the lung by adhering to the lung vasculature (10). Furthermore, MTDH regulates different signaling pathways that are closely related to cancer, such as NF-κB (27,41,42), Wnt/β-catenin (25), MAPK/ERK (40), PI3K/AKT (26,40), and AP-1 (28). All of these studies indicate that MTDH is potentially a key mediator of cancer and a crucial part of oncogenic signaling networks. Most importantly, many of the pathways that have been associated with MTDH overlap with the signaling pathways regulated by HBx. Therefore, we postulated that MTDH may be required for the development of HBV-related HCC.

In the present study, we found for the first time that MTDH protein was strongly overexpressed in HBV-related HCC tissue samples by western blotting. However, it was weak in hepatitis B tissues and was not detected in normal hepatic tissues, which indicates a gradient expression model. Immunohistochemical staining revealed a similar gradual elevation model. MTDH staining was mainly localized in the cytoplasm of cells, similarly to the reports of Yoo *et al* (25). Because MTDH overexpression has also been detected in colorectal adenoma (17) and breast ductal carcinoma *in situ* (43), we have reasons to believe that MTDH overexpression might be an early event during carcinogenesis. Therefore, MTDH could be used as an early-stage diagnostic marker in HBV-related HCC. Additionally, the positive expression of MTDH in the hepatitis B tissue samples suggested that one of the reasons for MTDH activation during the initiation of HBV-related HCC may be HBV infection. Of course, this conclusion should be confirmed in further studies.

A statistical analysis of the relationship between MTDH staining and the clinical parameters of patients with HBV-related HCC showed a significant correlation of MTDH expression with AJCC stage, histological differentiation, vascular invasion, T, and N classifications. MTDH expression was gradually elevated with the loss of cancer differentiation and with the elevation of AJCC stages. Moreover, the cumulative 5-year survival rate of patients in the high MTDH expression group was significantly lower than that of patients in the low MTDH expression group. A multivariate analysis verified that MTDH expression is an independent prognostic factor of patients with HBV-related HCC. MTDH expression status may be correlated with carcinogenesis, progression and poor prognosis of HBV-related HCC, similarly to published studies in other types of cancer (7,9,10,15,23,24,26,28). All of these results strongly indicated that MTDH could be utilized as a novel biomarker to identify the progression of HBV-related HCC and as a predictor for patient prognosis and survival. The data from our study partly confirmed the observation by Yoo *et al* (25), who reported a significant association between MTDH and HCC. However, their study did not reveal a significant relationship between the MTDH expression levels and HBV-related HCC. Therefore, it could be said that our data show further proof of the involvement of MTDH in HCC. We demonstrated that MTDH is a very important mediator for HBV infection to induce HCC.

It is worth noting that some studies have reported that the inhibition of MTDH may sensitize tumor cells to current antitumor treatments. It was demonstrated that MTDH enhances the expression of dihydropyrimidine dehydrogenase (DPYD), which catalyzes the initial and rate-limiting step in the catabolism of 5-FU, which is an adjuvant and palliative chemotherapeutic agent that is frequently used in the treatment of patients with HCC (44). In addition, MTDH augments the expression of the transcription factor LSF, which regulates the expression of thymidylate synthase (TS), a target of 5-FU. A similar study found that knockdown of MTDH expression in human neuroblastoma cells significantly enhanced chemosensitivity to cisplatin or doxorubicin (15,41). In addition, using lentiviral delivery of MTDH siRNA in combination with 5-FU more effectively inhibited the growth of human HCC cells xenotransplanted in athymic nude mice than either agent alone (44). These results suggest that MTDH siRNA could be a novel adjuvant therapy of HCC.

Our study is among the first to demonstrate that MTDH may be required for HBV-related HCC. However, a better understanding of the HBV-MTDH signaling pathways remains a high priority for future investigation. In conclusion, these results suggest that MTDH could be effective as both a clinical indicator of progression and a prognostic biomarker for survival in patients with HBV-related HCC. MTDH may also be a molecular target for new anticancer agents to prevent HBV-related HCC progression and metastasis. In addition, according to our findings, HBV may have a role as an initiator of MTDH activation during the initiation of HBV-related HCC. We will verify these predictions and elucidate the molecular mechanism of the effects of MTDH in the progression of HBV-related HCC in future studies.

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