ORIGINAL ARTICLE

Gut and Liver, Vol. 7, No. 2, March 2013, pp. 206-212

Metadherin Is a Prognostic Predictor of Hepatocellular Carcinoma after Curative Hepatectomy

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Background/Aims: The prognosis after surgical resection of hepatocellular carcinoma (HCC) remains poor because of a high rate of recurrence. Thus, it is crucial to identify patients with a high risk of recurrence after curative hepatectomy and to develop more effective and targeted treatment strategies to improve disease outcomes. In this study, we investigated the roles of metadherin (MTDH) in the prognosis of HCC. **Methods:** We investigated MTDH expression using immunohistochemistry in tumor tissue microarrays of 288 primary HCC patients who underwent curative surgical resection. **Results:** High MTDH expression was observed in 138 of the 288 HCC cases (47.9%). High MTDH expression was associated with a younger age (p<0.001), higher Edmondson grade (p<0.001), microvascular invasion (p<0.001), higher American Joint Committee on Cancer T stage (p=0.001), and higher α-fetoprotein level (p=0.003). Multivariate analyses revealed that high MTDH expression (p=0.014), higher Barcelona-Clinic Liver Cancer (BCLC) stage (p<0.001), and Edmondson grade III (p=0.042) were independent predictors of shorter disease-free survival (DFS). Higher BCLC stage (p<0.001) and Edmondson grade III (p=0.047) were also independent predictors of shorter disease-specific survival. **Conclusions:** High MTDH expression may be a prognostic predictor of shorter DFS in HCC patients after curative hepatectomy. **(Gut Liver 2013;7:206-212)**

Key Words: Metadherin; Hepatocellular carcinoma; Survival

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world.[1] With continued surveillance and advances in imaging, the detection rate of localized HCC has increased, resulting in an increase in the curative surgical resection rate. However, the prognosis after surgical resection of HCC remains poor because of a high rate of recurrence and lack of effective adjuvant therapy.[2] Tumor recurrence complicates more than 70% of cases at 5 years,[3] and the 5-year survival rate is 60% to 70%.[2] Cancer classification using prognostic biomarkers can identify patients with a high risk of recurrence after curative hepatectomy.[4] Further investigation of these biomarkers would provide personalized therapy according to the predicted risk of recurrence.

Metadherin (MTDH) is a single-pass transmembrane protein with a gene located at chromosome 8q22.[5] MTDH inhibits cancer cell apoptosis and increases invasiveness and metastasis.[6-8] It regulates different signaling pathways that are closely related to cancer, such as nuclear factor-kappaB, Wnt/β-catenin, MAPK/ERK, PI3K/AKT, and AP-1.[8-11] Clinical studies have linked MTDH with tumor progression and poor clinical outcomes in several cancer types, including breast cancer, prostate cancer, esophageal cancer, colorectal carcinoma, and HCC.[11-15] Song *et al*.[14] reported that high MTDH expression was observed in 16.1% of colorectal low-grade adenoma, 46.7% of high-grade adenoma, and 70.7% of carcinoma, and hypothesized that high MTDH expression might be an early warning sign of malignant transformation of colorectal mucosa, especially in the adenoma-carcinoma sequence. It had been reported that MTDH mRNA expression in hepatitis C virus-related HCCs using a gene expression microarray was significantly increased in comparison with normal liver, and this overexpression was associated with elevated copy numbers of MTDH, predominantly due to gains of large regions of chromosome 8q.[9] Recent studies showed that HCC patients with high MTDH expression had shorter overall survival times compared to those with low MTDH expression.[15,16] However, the prognostic significance of MTDH in HCC remains uncertain. In this study, we investigated the roles of MTDH in HCC prognosis in 288 HCC patients with long-term follow-up

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Received on May 8, 2012. Revised on July 4, 2012. Accepted on July 26, 2012. Published online on February 7, 2013.
pISSN 1976-2283 eISSN 2005-1212 http://dx.doi.org/10.5009/gnl.2013.7.2.206

using tissue microarrays (TMAs).

MATERIALS AND METHODS

1. Patients and histopathology

A total of 288 consecutive primary HCCs were collected from patients who were treated with curative hepatectomy at the Samsung Medical Center, Seoul, Korea from July 2000 to May 2006. Patient ages ranged from 17 to 76 years with an average of 52.6 years. The male to female ratio was 237 to 51. Two hundred and eighteen (75.7%) patients were infected with hepatitis B and 30 (10.4%) with hepatitis C. We defined curative resection as complete resection of all tumor nodules with clear microscopic resection margins and no residual tumors as indicated by a computed tomography scan at 1 month after surgery. None of the patients received preoperative chemotherapy. This study was approved by the Institutional Review Board of Samsung Medical Center. Clinical parameters, including age, gender, date of surgery, and tumor size were obtained from pathology reports. Histopathologic features of HCCs examined by two pathologists (C.K.P and S.A) were histological differentiation, microvascular invasion, major portal vein invasion, intrahepatic metastasis, multicentric occurrence, and nontumor liver pathology. HCCs were graded histologically according to the criteria of Edmondson and Steiner.[17] Microvascular invasion was considered present when at least one or more endothelial cells or the tunica media of the vessel surrounded a neoplastic cell group. Intrahepatic metastasis and multicentric occurrence were matched to the criteria of the Liver Cancer Study Group of Japan.[18]

Serum α-fetoprotein serum levels and computed tomography scans were performed at least once every 3 months after surgery until December 31, 2010. When tumor recurrence was suspected, precise diagnostic imaging was performed using magnetic resonance imaging. Disease-free survival (DFS) was defined from the date of resection until the detection of tumor recurrence. While HCC is the cause of death in most patients with the disease, some patients die of liver failure or other causes in the absence of progressive HCC (30 of the 129 deaths in this study died of non-HCC causes). We chose HCC-related mortality (disease-specific death) as the clinical endpoint for survival analysis, defined as : 1) tumor occupying more than 80% of the liver, 2) portal venous tumor thrombus (PVTT) proximal to the second bifurcation, 3) obstructive jaundice due to the tumor, 4) distant metastases, or 5) variceal hemorrhage with PVTT proximal to the first bifurcation.[19] At the time of analysis, the median follow-up period was 97.1 months (range, 40 to 126 months), tumor recurrence was detected in 189 patients (65.6%), and 99 patients (34.4%) died of HCC.

Tissues with dysplastic nodule (DN), a precancerous lesion of HCC, (n=28) were included, and DNs were subdivided into low-grade DN and high-grade DN according to the guideline of the International Working Party.[20]

2. Preparation of TMA

All histologic sections were examined by two pathologists (C.K. Park and S. Ahn) and representative tumor areas free from necrosis or hemorrhage were pre-marked in formalin-fixed paraffin-embedded blocks. Two, 2.0-mm-diameter tissue cores were taken from the donor blocks and transferred to the recipient paraffin block at defined array positions. Consecutive sections of 4-μm-thickness were mounted onto silane-coated slides (Sigma, St. Louis, MO, USA). As controls, we used uninvolved normal liver tissue from 12 patients with metastatic colonic carcinoma of the liver.

3. Immunohistochemical staining

Immunostaining was performed using rabbit polyclonal antibody to MTDH (NBP1-51585, 1:400; Novus Bio, Littleton, CO, USA). Consecutive 4-μm tissue sections embedded in the microslides were deparaffinized with xylene, hydrated in serial dilutions of alcohol, and immersed in peroxidase-blocking solution (Dako, Glostrup, Denmark) to quench endogenous peroxidase activity. Sections were microwaved in 0.01 mol/L citrate buffer (pH 6.0) for 30 minutes. Incubation with the primary antibody was performed overnight at 4°C. After washing, sections were incubated in DakoREAL EnVision/HRP rabbit/mouse detection reagent (Dako) for 20 minutes at room temperature, followed by an additional washing. 3,3'-diaminobenzidine tetrahydrochloride was used as the chromogen, and Mayer's hematoxylin counterstain was applied. Negative controls (isotype-matched irrelevant antibody) were run simultaneously.

To validate the concordance between TMAs and whole tumor sections, we used immunohistochemistry to detect the expression of MTDH in 40 corresponding whole tumor sections randomly chosen from the 288 cases.

4. Evaluation of immunohistochemical staining

We used a scoring method to evaluate both the intensity of immunohistochemical staining and the proportion of stained epithelial cells. Staining intensity was scored first (0, negative; 1, weak; 2, moderate; and 3, strong), followed by the percentage of positive cells (0, <21%; 1, 21% to 40%; 2, 41% to 60%; 3, 61% to 80%; and 4, 81% to 100%). The final score of each tumor was obtained by multiplying the score for staining intensity by the score for percentage of positive cells. For categorical analyses, the immunoreactivity of tumor cells was graded as low (total score, 0 to 6) or high (total score, 7 to 12). The results of staining were evaluated by two independent pathologists (C.K. Park and S. Ahn) without knowledge of the clinicopathologic features, and any difference in interpretation was resolved by consensual agreement. Duplicate tissue cores for each tumor showed high levels of homogeneity for staining intensity and percentage of positive cells. The higher score was taken as the final score in cases of a difference between duplicate tissue cores.

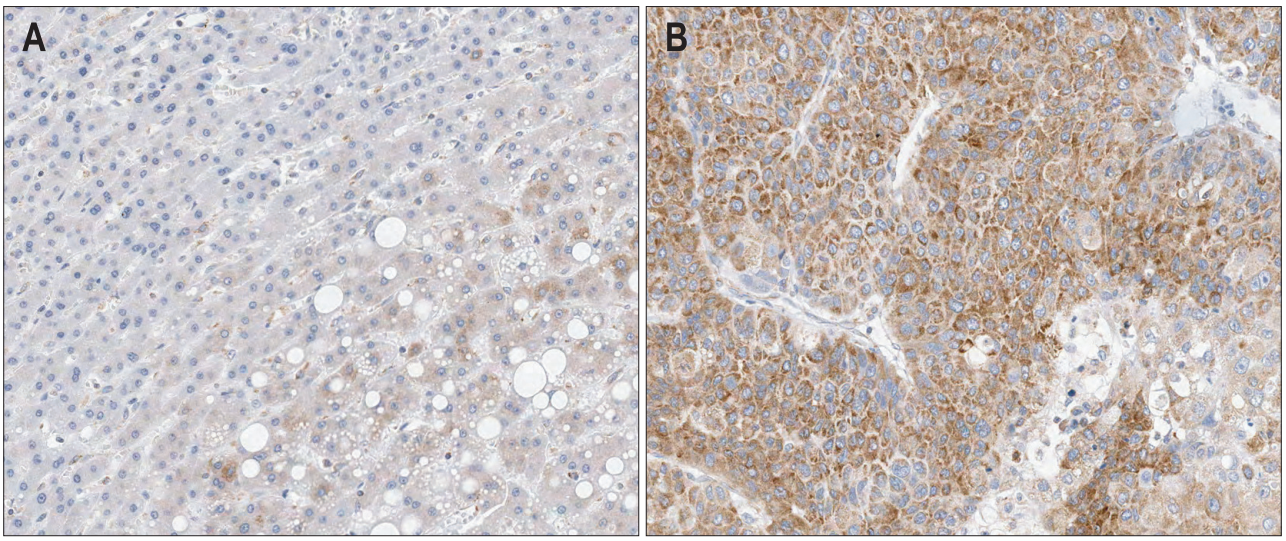

Fig. 1. Immunostaining of metadherin showing (A) low expression in the dysplastic nodule and (B) high expression in the hepatocellular carcinoma (horseradish peroxidase stain, ×200).

Table 1. Correlation between Metadherin Expression and the Clinicopathologic Features of 288 Hepatocellular Carcinomas

Variable	No.	High metadherin expression	p-value
Age, yr			<0.001
≤55	165	94 (57.0)	
>55	123	44 (35.8)	
Gender			0.862
Male	237	113 (47.7)	
Female	51	25 (49.0)	
Tumor size, cm			0.811
≤5.0	190	92 (48.4)	
>5.0	98	46 (46.9)	
Edmondson grade			<0.001
I	30	3 (10.0)	
II	195	95 (48.7)	
III	63	40 (63.5)	
Microvascular invasion			<0.001
-	129	47 (36.4)	
+	159	91 (57.2)	
Major portal vein invasion			0.314
-	275	130 (47.3)	
+	13	8 (61.5)	
Intrahepatic metastasis			0.132
-	220	100 (45.5)	
+	68	38 (55.9)	
Multicentric occurrence			0.368
-	269	127 (47.2)	
+	19	11 (57.9)	
AJCC T stage			0.001
1	121	42 (34.7)	
2	117	70 (59.8)	
3	44	22 (50.0)	
4	6	4 (66.7)	
BCLC stage			0.797
0–A	164	76 (46.3)	
B	109	54 (49.5)	
C	15	8 (53.3)	
Albumin level			0.193
>3.5	258	127 (49.2)	
≤3.5	30	11 (36.7)	
AFP level, ng/mL			0.003
≤200	173	71 (41.0)	
>200	104	62 (59.6)	
Etiology			0.016
Nonviral	40	19 (47.5)	
HBV	218	112 (51.4)	
HCV	30	7 (23.3)	
Liver cirrhosis			1
-	144	69 (47.9)	
+	144	69 (47.9)	

Data are presented as number (%).
AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus.

5. Statistical analysis

Statistical analyses were performed using IBM SPSS version 18 software (IBM, Armonk, NY, USA). Fisher's exact probability, Pearson's chi-square test, and analysis of variance (ANOVA) were used for comparison among groups. The log-rank test was applied to compare survival between different groups. Univariate and multivariate analyses were based on the Cox proportional hazards regression model. A p-value less than 0.05 was regarded as statistically significant.

RESULTS

1. MTDH protein expression in HCC

Immunoreactivity for MTDH was observed only in the cytoplasm of tumor cells and hepatocytes in 12 control normal livers. In all control normal livers, weak MTDH immunoreactivity was observed in less than 20% of hepatocytes. Among the 28 DNs, 4 (14.3%) showed weak MTDH immunoreactivity in less than 20% of hepatocytes. No high MTDH expression was observed in any DNs (Fig. 1A). Among the 288 HCCs, 21 (7.3%) showed weak MTDH immunoreactivity in less than 20% of hepatocytes. High MTDH expression was observed in 138 of the 288 HCCs (47.9%) (Fig. 1B). Correlations of MTDH expression with various clinicopathologic parameters, including age, gender, tumor size, Edmondson grade, microvascular invasion, major portal vein invasion, intrahepatic metastasis, multicentric occurrence, American Joint Committee on Cancer (AJCC) T stage,[21] Barcelona Clinic Liver Cancer (BCLC) stage,[22] serum albumin level, serum fetoprotein level, etiology, and nontumor liver pathology, are shown in Table 1. A high MTDH expression was associated with a younger age (p<0.001), higher Edmondson grade (p<0.001), microvascular invasion (p<0.001), higher AJCC T stage (p=0.001), and higher α-fetoprotein level (p=0.003).

2. Survival analysis

The DFS and disease-specific survival (DSS) rates for 288 HCCs were 42.7% and 78.2% at 3 years, 36.3% and 71.4% at 5 years, 30.1% and 67.1% at 7 years, and 27.9% and 60.8% at 9 years, respectively. On univariate analyses, larger tumor size, Edmondson grade III, microvascular invasion, major portal vein invasion, intrahepatic metastasis, higher AJCC T stage, higher BCLC stage, higher α-fetoprotein level, and lower albumin level showed unfavorable influences on DFS and DSS (Table 2). The 5-year DFS rate of the low MTDH expression group was significantly higher than that of the high MTDH expression group (42.1% vs 30.1%, p=0.013) (Fig. 2A). The median DFS was 35.3 months for the low MTDH expression group compared with 15.0 months for the high expression group. However, high MTDH expression was not a prognostic factor for DSS (p=0.593) (Fig. 2B). The 5-year DSS rate was 74.5% for the low expression group and 68.0% for the high expression group.

As tumor size, vascular invasion, intrahepatic metastasis, AJCC stage, and serum albumin level were associated with BCLC stage, we did not enter these into multiple analyses with the indices to avoid potential bias. On multivariate analyses, Edmondson grade III (p=0.042), higher BCLC stage (p<0.001), and high MTDH expression (p=0.014) were defined as independent predictors of shorter DFS. High MTDH expression patients were more likely to suffer from recurrence than low MTDH expression patients (hazard ratio, 1.451). Edmondson grade III (p=0.047) and higher BCLC stage (p<0.001) were defined as independent predictors of shorter DSS. However, high MTDH expression was not an independent predictor for DSS (p=0.589) (Table 3).

DISCUSSION

MTDH promotes HCC carcinogenesis through activation of the Wnt/β-catenin signaling pathway via activation of ERK42/44 and upregulation of lymphoid-enhancing factor 1/T cell factor 1, the ultimate executor of the Wnt pathway.[9]

In this study, most of the HCC tissues expressed higher levels of MTDH than normal liver or DN tissues, with high MTDH expression in 47.9% (138 of 288) of HCCs. High MTDH expression was not observed in normal liver or DN tissues. It is reported that high MTDH expression was observed in 0% of normal liver, 46.7% of hepatitis B, and 64.4% of hepatitis B virus (HBV)-related HCC.[16] MTDH expression might not be an early event in HCC carcinogenesis.

High MTDH expression in HCC was significantly associated with higher Edmondson grade, microvascular invasion, and higher AJCC T stage. These findings were consistent with previous reports.[15,16] Many of the pathways associated with MTDH overlap with the signaling pathways associated with HBV X protein. However, there was no significant difference in MTDH expression between nonviral etiology and HBV infection (nonviral, 47.5%; HBV, 51.4%). High MTDH expression, Edmondson grade III, and higher BCLC stage were independent predictors of shorter DFS. MTDH expression status might be correlated with progression and poor prognosis of HCC. Zhu *et al*.[15] reported that the clinical outcome was consistently poorer for the high MTDH expression group than for the low MTDH expression group in the 1-, 3-, and 5-year cumulative recurrence rates and in the 1-, 3-, and 5-year overall survival rates. In this study, high MTDH expression was not an independent predictor for DSS. Different clinical variables (e.g., overall survival rate vs DSS) and racial difference may have resulted in the discrepancy between previous study and this study. Our findings indicated that MTDH is a potential new prognostic marker for HCC after curative hepatectomy, and could help clinicians identify patients at high risk of recurrence and enable them to administer adjuvant therapy after surgery. Disruption of the fundamental signaling pathways that enable tumors to grow and invade would represent an elegant therapeutic approach. The activation of

Table 2. Univariate Analyses of Disease-Free Survival and Disease-Specific Survival in 288 Hepatocellular Carcinomas

Variable	No.	Disease-free survival		Disease-specific survival	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Age, yr					
≤55	165		0.524		0.544
>55	123	0.910 (0.680–1.218)		0.883 (0.591–1.320)	
Tumor size, cm					
≤5.0	190		<0.001		<0.001
>5.0	98	1.740 (1.298–2.332)		2.955 (1.989–4.391)	
Edmondson grade					
I+II	225		<0.001		0.001
III	63	1.856 (1.340–2.570)		2.047 (1.338–3.132)	
Microvascular invasion					
-	129		<0.001		<0.001
+	159	2.137 (1.589–2.874)		3.058 (1.953–4.789)	
Major portal vein invasion					
-	275		<0.001		<0.001
+	13	3.914 (2.170–7.060)		5.537 (2.859–10.725)	
Intrahepatic metastasis					
-	220		<0.001		<0.001
+	68	4.640 (3.371–6.385)		5.586 (3.733–8.358)	
Multicentric occurrence					
-	269		0.326		0.318
+	19	1.342 (0.746–2.412)		0.601 (0.221–1.634)	
AJCC T stage					
1	121		<0.001		<0.001
2+3+4	167	2.177 (1.612–2.938)		3.092 (1.950–4.900)	
BCLC stage					
0+A	164		<0.001		<0.001
B+C	124	2.141 (1.606–2.853)		3.735 (2.462–5.665)	
AFP level, ng/mL					
≤200	173		0.002		0.033
>200	104	1.605 (1.198–2.152)		1.553 (1.037–2.326)	
Etiology					
Nonviral	40		0.899		0.847
Viral	248	0.974 (0.648–1.464)		0.946 (0.537–1.665)	
Liver cirrhosis					
-	144		0.839		0.780
+	144	1.030 (0.774–1.370)		0.945 (0.638–1.402)	
Albumin					
>3.5	258		0.007		0.001
≤3.5	30	1.817 (1.173–2.815)		2.433 (1.420–4.168)	
Metadherin					
Low	150		0.013		0.593
High	138	1.437 (1.080–1.913)		1.114 (0.751–1.652)	

HR, hazard ratio; CI, confidence interval; AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; AFP, α-fetoprotein.

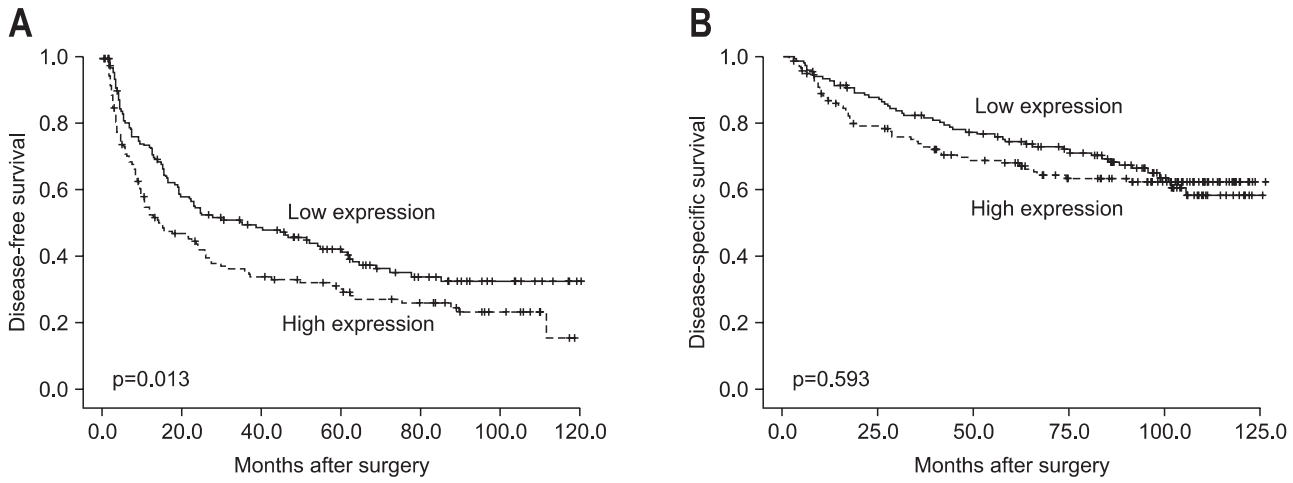

Fig. 2. Kaplan-Meier analysis of (A) disease-free survival and (B) disease-specific survival for metadherin expression in 288 hepatocellular carcinomas.

Table 3. Multivariate Analyses of Disease-Free Survival and Disease-Specific Survival in 288 Hepatocellular Carcinomas

Variable	No.	Disease-free survival		Disease-specific survival	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Edmondson grade					
I+II	225		0.042		0.047
III	63	1.42 (1.013–2.002)		1.551 (1.005–2.392)	
Etiology					
Nonviral	40		0.676		0.755
Viral	248	1.904 (0.718–1.667)		1.104 (0.594–2.052)	
Liver cirrhosis					
-	144		0.971		0.459
+	144	1.006 (0.742–1.363)		0.861 (0.580–1.278)	
BCLC stage					
0+A	164		<0.001		<0.001
B+C	124	2.067 (1.535–2.785)		3.467 (2.268–5.301)	
Metadherin					
Low	150		0.014		0.589
High	138	1.451 (1.082–1.944)		1.118 (0.746–1.673)	

HR, hazard ratio; CI, confidence interval; BCLC, Barcelona Clinic Liver Cancer.

multiple signaling pathways in different HCCs makes it difficult to develop effective alternative therapies using small molecules. MTDH that contributes to the activation of some of these pathways might be a molecular target for therapeutic intervention for HCC.

To our knowledge, this is the first report showing high MTDH expression as an independent predictor of shorter DFS after curative hepatectomy in a large number of HCC patients with long-term follow-up. MTDH could be used as an immunohistochemical biomarker to detect patients with a high risk of recurrence. Prospective studies with larger patient populations are needed to further investigate the value of MTDH as a prognostic predictor.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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