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Investigation of expression level of MTDH, CEACAM1 and HBx in HBV-associated hepatocarcinoma tissues and its clinical significance.

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Abstract

Background: To analyze the expression of Metadherin (MTDH), Carcinoembryonic Antigen related Cell adhesion Molecule 1 (CEACAM1) and Hepatitis B virus X protein (HBx) in HBV-associated Hepatocellular Carcinoma (HCC) tissues and clinical significance.

Methods: Total of 65 specimens of HBV-associated HCC tissues (the observation group) and 65 specimens of para-carcinoma tissues (the control group), which derived from 65 HBV-associated HCC patients in the pathologic department of our hospital from Jan 2015 to Dec 2016. The differences of expression levels of MTDH, CEACAM1 and HBx in tissues and its associations with the clinical pathological characteristics of HBV-associated HCC were analysed.

Results: The positive expression rates of MTDH and HBx in HBV-associated HCC tissues were significantly higher than those in the para-carcinoma tissues (60.00% *vs.* 32.31%, P<0.05; 43.08% *vs.* 64.62%, P<0.05). The positive expression rates of CEACAM1 in the HBV-associated HCC tissues was significantly lower than those in the para-carcinoma tissues (43.08% *vs.* 64.62%, P<0.05). The expressions of MTDH, CEACAM1 and HBx in the HBV-associated HCC tissues were significantly correlated with the TNM stage, portal vein invasion, lymph node metastasis and tissue differentiation (P<0.05). CEACAM1 and HBx were closely associated with the tumor diameter and size (P<0.05).

Conclusion: The high expression of MTDH and HBx, the low expression of CEACAM1 was closely associated with TNM stage, portal vein invasion, lymph node metastasis and tissue differentiation degree in HBV-associated HCC tissues, MTDH and HBx might promote the development and occurrence of HBV-associated HCC by inhibiting the expression of CEACAM1.

Keywords: HBV-associated hepatocarcinoma, Metadherin (MTDH), Carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM1), Hepatitis B virus X (HBx), Clinical significance.

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Introduction

Hepatocellular Carcinoma (HCC) is one of the commonest malignant tumors in China and even all over the world and its morbidity rate and mortality rate are on a gradual rise on a year-to-year basis worldwide [1,2]. HCC often occurs to the population aged from 40 to 49 y, the ratio between male patients and female patients is about 2~5:1 and material shows that, the annual morbidity rate is as high as 40% [3]. HCC has the clinical characteristics of a high malignancy and rapid progression of patient's conditions; patients' life cycle is often less than 6 months after the onset. The occurrence and development of HCC are subject to the regulation of multiple cytological factors. As is shown in literature report, *hepatitis B virus X* gene (HBx) is probably the principal reason to induces HCC, especially the hepatitis B virus X region reads the X protein encoded by an open reading frame, and transactivate viral and cellular promoters and enhancers through protein-protein interactions with well-defined targets, which is also called Hepatitis B virus X protein (HBx) [4-6].

Previous studies have shown that, Carcinoembryonic Antigen related Cell adhesion Molecule 1 (CEACAM1) is a kind of transmembrane glycoproteins, which falls in the category of immunoglobulin superfamily gene, its expression is generally reduced in malignant tumor, and it is characterized with tumor-suppressing effects [7]. Numerous recent studies have shown that Metaherin (MTDH) has the capacity to induce overexpression in multiple tumors, and the detection of their overexpression is of help to the early clinical diagnosis and the judgment of tumor progression and prognosis [8,9]. Due to the complexity in pathogenesis of HBV-associated HCC, the main clinical approaches for the HCC treatment include hepatectomy, liver transplantation, radiation therapy, local ablative therapy and interventional therapy, but they have the disadvantages of great harms to patients as well as unsatisfying prognosis and efficacy [10,11].

Therefore, the current key to the treatment of HBV-associated HCC lies in the exploration of new treatment targets. The authors of this study aim to investigate the expression levels of MTDH, CEACAM1 and HBx in HBV-associated HCC tissues as well as the clinical significance for the attempt to provide new targets for the treatment of HBV-associated HCC.

Materials and Methods

Clinical materials

65 patients that derived from the pathologic department of our hospital from Jan 2015 to Dec 2016 were enrolled and 65 specimens of tissues at the center of the HBV-associated HCC were collected from them and divided into the observation group. All patients had had a history of HBV infection for ten years or above when they were diagnosed with HCC and the result of qualitative test of serum HBsAg was positive or the HBVcDNA in tissue specimens>1000 cps/ml. 65 specimens of the corresponding para-carcinoma tissues were collected and divided into the control group and the para-carcinoma tissues were the non-tumorous tissues at the site 2 cm or more away from the margins of the hepatocarcinoma foci. The specimens collected in this study were approved by the Ethic Committee of this Hospital and agreed by National Health and Family Planning Commission in China. All patients should provide complete clinical materials, none of them received therapies such as chemoradiotherapy and hormones within one month before the operation, and patients concomitant with other malignant tumors were ruled out. Of them, 41 were males and 27 were females, aged from 30 to 39 y, with a mean of 47.22 ± 7.31 y. The tumor had a diameter of ranging from 2 to 7 cm, with a mean of 4.13 ± 1.58 cm. In terms of TMN stage, 10 cases were at stage II, 20 were at stage IIIA, 27 were at stage IIIB and 8 were at stage IIIC.

Main reagents

S-P kit and DAB color kit were purchased from Beijing ZS-BIO Company (China). Rabbit anti-human MTDH monoclonal antibody, rabbit, anti-human CEACAM1 monoclonal antibody and mouse anti-human HBx monoclonal antibody were purchased from Abcam Company (US). Other relevant reagents such as PBS buffer solution were provided by Pathology Department of our hospital.

Methods

The specimens were instantly freezed after being exsomatized and preserved in a -80°C refrigerator for later use. All the collected specimens were fixed by 10% formaldehyde and embedded by paraffin, and then they were prepared into the paraffin section with a thickness of 4 μm. The immunohistochemistry (streptavidin-perosidase, S-P) method was adopted for the detection, diaminobenzidine (DAB) was used for color development and S-P kit was used to detect the expression of MTDH, CEACAM1 and HBx in paraffin sections; the known standard positive sections were used as the positive control, and the PBS buffer solution was used as the negative control. All the steps were performed in strict accordance with the kit instructions.

A positive expression was confirmed if the cell membrane/cytoplasm/karyon staining turned brownish yellow. A negative expression was confirmed if only the karyon staining turned blue. 10 fields were randomly selected under the high power lens for observation. A statistical analysis was made on the total sum of the cell staining intensity scores and the percentage of positive cells: if the result was 0 to 3, it was negative and marked as -; if the result was ≥ 3, it was positive and marked as +. The scoring method was based on the immunohistochemical semi-quantitative scoring criteria and the details are shown in Table 1 [12,13].

Table 1. *Scoring standard immunohistochemistry semi quantitative.*

Staining strength score		Proportion of positive cells	
Intensity	**Score**	**%**	**Score**
Colourless	0	0	0
Light yellow	1	<50%	1
Brownish yellow	2	50%~75%	2
Dark brown	3	>75%	3

Statistical analysis

The statistical software SPSS 21.0 was used for the statistical analysis of data. The enumeration data were analyzed using the chi-square (χ^2) test and $P<0.05$ means that the difference was of statistical significance.

Results

Intergroup comparison between the observation group and the control group in terms of the expression level of MTDH, CEACAM1 and HBx

The positive signals of MTDH in HBV-associated HCC tissues were mainly located in the cytoplasm, and were partially located in karyon. The positive expression rate of MTDH in the observation group was significantly higher than that in the control group (60.00% *vs.* 32.31%, $P<0.05$). The positive expression rate of CEACAM1 in the observation was significantly lower than that in the para-carcinoma tissues (43.08% *vs.* 64.62%, $P<0.05$). The positive expression rate of HBx in the observation was significantly higher than that in the para-carcinoma tissues (73.85% *vs.* 46.15%, $P<0.05$). The results are shown in Table 2.

Table 2. *Intergroup comparison in terms of MTDH expression.*

Characteristics	Observation group (65)	Control group (65)	χ^2	P
MTDH			10.029	0.002
-	26	44		

+	39	21		
Positive rate (%)	0.6	0.3231		
CEACAM1			6.067	0.014
-	37	23		
+	28	42		
Positive rate (%)	0.4308	0.6462		
HBx			10.385	0.001
-	17	35		
+	48	30		
Positive rate (%)	0.7385	0.4615		

Relation between the expression of MTDH, CEACAM1 and HBx and the clinical pathology of HBV-associated HCC

The expression of MTDH, CEACAM1 and HBx in the HBV-associated HCC tissues was irrelevant to the patient's age and gender (P>0.05).

(1) The positive expression rate of MTDH in the patients with a tumor diameter of less than 5 cm was significantly lower than that in the patients with a tumor diameter of more than 5 cm (44.74% (17/38) *vs.* 74.07% (20/27), P<0.05). Its positive expression rate in the tissues at TNM stage of I to II was significantly lower than that in the tissues at TNM stage of III to IV (44.12% (15/34) *vs.* 70.97% (22/31), P<0.05). Its positive expression rate (77.78%, 28/36) in the tissues with portal vein invasion was significantly higher than that (51.72%, 15/29) in the tissues without portal vein invasion (77.78% (28/36) *vs.* 51.72% (15/29), P<0.05). Its positive expression rate in the tissues with lymph node metastasis was significantly higher than that in the tissues without lymph node metastasis (79.49% (31/39) *vs.* 46.15% (12/26), P<0.05). Its positive expression rates in the highly-differentiated and medium-differentiated tissues were significantly higher than those in the poorly-differentiated tissues (68.75% (22/32) *vs.* 65.00% (13/20) *vs.* 23.08% (3/13), P<0.05).

(2) The positive expression rate of CEACAM1 in the patients with a tumor diameter of less than 5 cm was significantly higher than that in the patients with a tumor diameter of more than 5 cm (57.89% (22/38) *vs.* 29.63% (8/27), P<0.05); its positive expression rate in the tissues at TNM stage of I to II was significantly higher than that in the tissues at TNM stage of III to IV (58.82% (20/34) *vs.* 32.26% (10/31), P<0.05); its positive expression rate in the tissues with portal vein invasion was significantly lower than that without portal vein invasion (33.33% (12/36) *vs.* 68.97% (20/29), P<0.05); its positive expression rate in the tissues with lymph node metastasis was significantly lower than that in the tissues without lymph node metastasis (30.77% (12/39) *vs.* 57.69% (15/26), P<0.05); its positive expression rates in the highly-differentiated tissues and medium-differentiated tissues were significantly lower than those in the poorly-differentiated tissues (28.13% (9/32) *vs.* 40.00% (8/20) *vs.* 69.23% (9/13), P<0.05).

(3) Its positive expression rate in the tissues at TNM stage of I to II was significantly lower than that in the tissues at TNM stage of III to IV (67.65% (23/34) *vs.* 93.55% (29/31), P<0.05); its positive expression rate in the tissues with portal vein invasion was significantly higher than that in the tissues with portal vein invasion (86.11% (31/36) *vs.* 55.17% (16/29), P<0.05); its positive expression rate in the tissues with lymph node metastasis was significantly higher than that in the tissues without lymph node metastasis (84.62% (333/39) *vs.* 61.54% (16/26), P<0.05); its positive expression rates in the highly-differentiated and medium-differentiated tissues were significantly higher than those in the poorly-differentiated tissues (87.50% (28/32) *vs.* 85.00% (17/20) *vs.* 53.85% (7/13), P<0.05) and the details are shown in Table 3.

Table 3. *Relationship between the expression of MTDH, CEACAM1 and HBx and the clinical pathology of HBV-associated HCC.*

Characteristics	**Case (n)**	**MTDH**		**χ^2**	**P**	**CEACAM1**		**χ^2**	**P**	**HBx**		**χ^2**	**P**
		-	**+**	**-**		**+**	**-**	**+**					
Age (y)													
≥ 50	37	14	23	3.344	0.067	22	15	0.098	0.755	10	27	0.269	0.604
<50	28	17	11			15	12			6	22		
Gender													
Male	43	17	26	0.21	0.647	24	19	1.759	0.185	6	37	2.903	0.088
Female	22	10	12			16	6			7	15		
Tumor diameter (cm)													
≤ 5	38	21	17	5.54	0.019	16	22	5.074	0.024	11	27	1.289	0.256
>5	27	7	20			19	8			12	15		
TNM stage													

Stage I to II	34	19	15	4.767	0.029	14	20	4.605	0.032	11	23	6.799	0.009
Stage III to IV	31	9	22			21	10			2	29		
Portal vein invasion													
Yes	36	8	28	4.869	0.027	24	12	8.159	0.004	5	31	7.678	0.006
No	29	14	15			9	20			13	16		
Lymph node metastasis													
Yes	39	8	31	7.741	0.005	27	12	4.656	0.031	6	33	4.477	0.034
No	26	14	12			11	15			10	16		
Differentiation degree													
High differentiation	32	10	22	8.45	0.015	23	9	6.508	0.039	4	28	6.995	0.03
Middle differentiation	20	7	13			12	8			3	17		
Low differentiation	13	10	3			4	9			6	7		

Discussion

HCC is a hepatic malignant tumor derived from hepatic epithelial cells or mesenchymal tissues. At the early stage, patients present with no specific symptom, and at the intermediate and advanced stages, patients present with symptoms such as pain at hepatic region, weakness, abdominal swelling, athrepsia and progressive hepatomegaly [14]. Currently, China has a relatively high incidence rate of HBV and the long-term HBV infection is the primary cause that leads to the onset of HBV-associated HCC [15]. Currently, the HBV-associated pathogenesis has not been entirely clear; seeking new specific and sensitive biological markers for HBV-associated HCC is of important significance to the pathogenesis, clinical diagnosis and treatment of HBV-associated HCC.

MTDH is the first gene cloned from the neuroastrocytoma and located at chromosome 8q22 [16,17]. MTDH is involved in the regulation of multiple biological processes such as PI3K/AKT and transcriptional activator-1 signal pathway and mitogen activated protein kinase signal pathway; it plays an important role in tumor cell proliferation, epithelial-mesenchymal transition, invasion and metastasis [18]. Studies have revealed that, the expression of MTDH is up-regulated in multiple tumors such as breast cancer and gastric adenocarcinoma, indicating that it is involved in the occurrence and development of multiple tumors [19]. The studies done by some scholars have indicated that, inhibiting MTDH overexpression plays an important role in inducing tumor cell apoptosis and inhibiting tumor cell invasion and metastasis, and the knockout or inhibition of MTDH gene and protein expression is of some vital significance to the treatment of malignant tumor [20]. CEACAM1 is a member of immunoglobulin gene superfamily; as a type of transmembrane glycoprotein, it has a relative molecular weight of 1050 and is located at 19q13.2 [21]. The expression of CEACAM1 differs in different types of tumors. For example, the expression of some epithelial tissue derived malignant tumors such as urinary bladder cancer and hepatocarcinoma is down-regulated, but the expression in pancreatic adenocarcinoma and pulmonary carcinoma is up-regulated, especially in the melanoma cells where it mediates immunologic escape, indicating that it may become a new target for melanoma treatment [22-26]. HBx is a protein expressed by *HBVx* gene, containing 154 amino acids, it has wide effects of non-specific trans-activation and transcriptional activation. During the recent years, massive studies have validated, HBx can mutually interact with the hepatic intranuclear and extranuclear protein molecules, leading to hepatocellular regulation disorder, impacting the activity and expression of oncogene and anti-oncogene and finally causing canceration. Therefore, MTDH, CEACAM1 and HBx were chosen as three indicators for this study to investigate their expression in HBV-associated HCC tissues and the clinical significance and to attempt to provide new targets for the clinical treatment of HBV-associated HCC.

The results of this study indicated that, MTDH had a significantly higher positive expression rate in the HBV-associated HCC tissues than in the para-carcinoma tissues (60.00% *vs.* 32.31%, $P<0.05$). The CEACAM1 had a significantly lower positive expression rate in the HBV-associated HCC tissues than in the para-carcinoma tissues (43.08% *vs.* 64.62%, $P<0.05$). HBx had a significantly higher positive expression rate in the HBV-associated HCC tissues than in the para-carcinoma tissues (73.85% *vs.* 46.15%, $P<0.05$), this indicated that, MTDH had a high expression in the HBV-associated HCC tissues, CEACAM1 had a low expression in the HBV-associated HCC tissues, HBx had a high expression in the HBV-associated HCC tissues and the three indicators were involved in the disease occurrence and development. The expression of MTDH, CEACAM1 and HBx in the HBV-associated HCC tissues was irrelevant to the patient's age and gender, the three indicators were significantly correlated with TNM stage, portal vein invasion, lymph node metastasis, and tissue differentiation, and CEACAM1 and HBx were also closely associated with the tumor diameter and size ($P<0.05$), indicating that MTDH, CEACAM1 and HBx was

closely associated with the severity of HCC disease. The high expression of MTDH and HBx in the HBV-associated HCC indicated that they played a promoting role in the HCC occurrence and development as well as the invasion and metastasis. CEACAM1 had a down-regulated expression in the HBV-associated HCC, based on that we assumed that CEACAM1 plays the role of anti-oncogene in the HBV-associated HCC. The three indicators are expected to become the new targets for the treatment of HBV-associated HCC and further profound studies should be carried out.

In conclusion, the expression of MTDH, CEACAM1 and HBx in HBV-associated HCC tissues was closely associated with TNM stage, portal vein invasion, lymph node metastasis and tissue differentiation degree, the three indicators are expected to become the new targets for the treatment of HBV-associated HCC, but there is a room to confirm in further studies.

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