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Original Article

Correlation between HBV protein preS2 and tumor markers of hepatocellular carcinoma

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ABSTRACT

Background: Alpha-fetoprotein (AFP) and Glypican 3 (GPC3) are both oncogenes and reactivated in hepatocellular carcinoma (HCC). PreS2 has been proved to be an important transactivator in HCC. In this study, we aim to provide evidence that HBV protein preS2 is responsible for AFP and GPC3's reactivation in HCC.
Methods: Totally Sixty-three cases of HCC, aged 34–79, who were surgically treated and pathologically confirmed were enrolled. The levels of AFP in peripheral serum were detected with electrochemical luminescence method before surgery. Levels of GPC3 in HCC samples were evaluated by immunohistochemistry. Luciferase reporter assays were used to measure the effect of preS2 on AFP and GPC3 promoters.
Results: AFP level and GPC3 but not albumin were significantly higher in preS2-positive HCC samples than preS2-negative HCC samples. And the preS2 protein expression was positively related with serum AFP level and GPC3 expression. Furtherly, dual luciferase assay showed that preS2 activated AFP and GPC3 promoter activity.
Conclusion: The expression of preS2 protein relates closely to HCC markers AFP and GPC3.

1. Introduction

Hepatocellular carcinoma (HCC) is characterized by short disease course, fast progression and high mortality, with more than 600 thousands of people die of hepatocarcinoma each year worldwide [1]. The report from International Agency for Research on Cancer (IARC) has showed that the morbidity rate of hepatocarcinoma ranks third and the mortality rate of it ranks second in China [2]. Alpha-fetoprotein (AFP) has been known as the "gold standard" for the diagnosis of hepatocarcinoma, with a specificity up to 97.4%, however, its sensitivity is only 58.8% [3]; Glypican 3 (GPC3) is the most potential hepatocarcinoma marker newly-found in recent years, with specific over-expression in hepatocarcinoma tissues, and the positive detection rate of GPC3 in AFP-negative HCC specimen is 72% [4,5].

Chronic hepatitis B virus (HBV) infection has always been identified as an important risk factor for HCC [6,9]. Clinical studies have found that the Hepatitis B surface antigen is positive in 63.2% of the patients with hepatocarcinoma and almost all of the HBV-associated HCCs harbor chromosomally integrated HBV DNA [6]. In 1990, Keelhaul et al. reported that MHBs^t (containing preS2 domain) coded by c-terminally truncated middle surface protein of HBV had *trans*-activation function [7]. Subsequent research has verified that preS2 domain (aa 1–55) is the minimal functional unit and can promote HCC development by activating various genes including hTERT, Foxp3, TAZ and so on [8–11].

Both AFP and GPC3 are reactivated in liver cancer and involve in HCC development as oncogenes [5,12]. There have been reports demonstrating that the expression of AFP is closely correlated with HBV infection in patients with HCC [13]. Therefore, it is reasonable to speculate that HBV protein preS2 with the effects of promoting tumor growth in the process of HCC development may have correlation with reactivated expression of AFP and GPC3. To address our hypothesis, the expression of preS2 and GPC3 in HCC tissues and serum AFP were examined by immunohistochemical staining and electrochemiluminescence analysis respectively. We have found that AFP and GPC3 expression in preS2-positive HCC patients are both higher than that in preS2-negative HCC patients. Spearman analysis showed that the expression of AFP and GPC3 were respectively correlated with HBV protein preS2 in HCC patients. Cotransfection and dual-luciferase assay further confirmed that preS2 transactivated AFP and GPC3 core promoter.

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Table 1
Details of the 63 patients (22 negative for hepatitis B virus (HBV) and 41 positive for HBV; age 34–79 years) involved in the study.

Tissue no	Gender	Age (yr)	HBsAg	HBV DNA (copies/ml)	ALT (U/L)	Cirrhosis	Differentiation grade	preS2 level (IHC score)	AFP levels (ng/ml)	GPC3 levels (IHC score)	Albumin (g/l)
1.	M	79	–	/	18	No	I	0	1210	8	38.5
2	M	60	–	/	419	No	I	0	2.1	8	44.9
3	M	59	–	/	16	Yes	I	0	2.2	0	37.9
4	M	65	–	/	27	No	I	0	7.48	0	45.1
5	M	41	–	/	28	No	I	0	3.8	0	42.1
6	M	57	–	/	26	No	I-II	0	1.85	0	36.3
7	M	64	–	/	29	No	II	0	1210	8	38.7
8	M	48	–	/	240	No	II	0	10.89	8	41.9
9	M	40	–	/	31	No	II-III	0	2.5	2	40.3
10	M	70	–	/	25	No	II-III	0	1210	0	47.7
11	M	58	–	/	494	No	III	0	1210	2	38.4
12	M	50	–	/	36	Yes	II	0	8.82	8	40.1
13	M	51	–	/	37	Yes	II	0	350.1	3	39.5
14	F	60	–	/	35	Yes	I	0	2.72	7	38.2
15	M	50	–	/	29	Yes	II	0	502.7	3	36.9
16	F	65	–	/	19	Yes	II	0	1.85	3	35.9
17	M	58	–	/	48	No	II	0	2	5	37.8
18	F	60	–	/	40	Yes	I	0	3.09	1	40.4
19	M	60	–	/	42	Yes	I	0	1.2	3	40.8
20	F	48	–	/	96	No	I	0	8.83	5	39.5
21	M	56	–	/	327	Yes	I	0	3.2	3	39.6
22	M	66	–	/	34	Yes	I	0	2.3	8	41.4
23	F	41	+	< 1000	155	Yes	I	3	43.28	0	39.5
24	M	49	+	4.1×10^3	52	Yes	I-II	9	2.25	3	43.9
25	M	65	+	< 1000	82	No	I-II	2	61.55	12	38.9
26	F	56	+	< 1000	41	No	I-II	1	3.11	0	44.1
27	M	52	+	5.21×10^5	69	Yes	II	9	1210	12	45.1
28	M	34	+	6.35×10^3	29	Yes	II	9	1210	12	35.3
29	M	49	+	1.32×10^5	41	Yes	II	8	161.6	8	39.7
30	M	48	+	2.33×10^4	90	Yes	II	6	1210	6	40.9
31	M	60	+	< 1000	20	Yes	II	6	116	8	43.3
32	M	46	+	3.1×10^3	67	No	II	3	61.47	8	45.7
33	M	43	+	2.42×10^5	50	No	II	9	31.96	12	40.4
34	M	64	+	4.46×10^5	55	No	II	9	71.21	0	43.1
35	M	53	+	2.42×10^3	13	Yes	II	12	1210	4	37.5
36	M	51	+	< 1000	71	Yes	II-III	8	482.5	1	36.2
37	M	74	+	< 1000	1213	Yes	II-III	6	1210	8	40.9
38	M	66	+	< 1000	40	Yes	II-III	4	1210	12	37.9
39	M	40	+	1.86×10^4	32	No	II-III	8	30.46	12	36.8
40	M	49	+	< 1000	74	No	II-III	9	25.55	6	41.4
41	M	49	+	< 1000	127	No	II-III	12	1210	9	41.8
42	F	63	+	4.23×10^6	18	No	II-III	4	35.13	6	37.5
43	M	56	+	5.56×10^5	251	Yes	II	8	1210	9	36.6
44	M	51	+	1.86×10^4	28	No	II	3	22.44	3	40.4
45	M	57	+	3.54×10^5	42	No	II-III	12	1200	9	37.5
46	M	40	+	5.63×10^7	65	Yes	III	8	1210	9	43.9
47	M	39	+	3.69×10^5	37	Yes	III-IV	1	4.3	1	36.9
48	M	62	+	1.52×10^7	43	Yes	II-III	2	2.83	8	42.1
49	M	68	+	1.56×10^6	50	Yes	III	3	33.77	2	40.1
50	M	55	+	1.26×10^8	25	Yes	III	2	3.21	5	36.3
51	M	49	+	< 1000	33	No	II	3	17.28	4	38.7
52	M	65	+	1.71×10^6	23	No	II	12	155.1	12	42.9
53	M	52	+	2.0×10^6	28	No	II-III	2	1.48	3	40.3
54	M	67	+	1.78×10^9	51	No	II-III	2	3.89	2	45.7
55	F	42	+	4.23×10^6	40	Yes	II	1	4	3	38.4
56	M	39	+	1.50×10^5	164	Yes	II	8	1210	6	43.1
57	M	60	+	3.76×10^6	22	Yes	I	3	16.91	1	39.5
58	M	74	+	2.31×10^6	36	No	II	8	1210	6	38.2
59	M	70	+	< 1000	43	Yes	II	12	1940.3	9	36.9
60	M	49	+	2.22×10^5	34	Yes	II	1	2.26	5	39.9
61	M	53	+	9.58×10^5	66	No	II-III	8	1210	6	37.8
62	M	50	+	1.46×10^5	89	No	II-III	8	1210	8	40.4
63	M	50	+	1.45×10^5	39	Yes	II-III	8	1210	4	41.8

2. Patients and methods

2.1. Patients, specimen collection and tissue preparation

Sixty-three surgical samples of HCC were collected from Shandong Provincial Hospital and Qianfoshan hospital, affiliated to Shandong University (Table 1). The patients consisted of 57 men and 6 women, ranging in age from 34 to 79 years (mean ± SD, 55.32 ± 10.83 years). The diagnosis was confirmed histologically in all cases, based mainly on the examination of sections stained with H & E. All tumors were histologically diagnosed as HCC according to the Edmondson-Steiner classification system [13]. Serologic examinations indicated that 41 of the patients were positive for hepatitis B antigen. None of the patients was positive for hepatitis C antigen or human immunodeficiency virus antigen, which was determined using a standard serologic test. None of them consumed excessive quantities of