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Analysis of GP73 in patients with HCC as a function of anti-cancer treatment

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Abstract

In this study, we examined the level of Golgi protein 73 (GP73) in the serum of 9 patients as a function of anti-liver cancer treatment. Although the numbers are small, a clear trend was observed. Patients who remained tumor free (up to 6 years post-treatment) showed reductions in GP73 at the first time point available post-treatment. In contrast, patients who had high levels GP73 post treatment all had re-occurrence within a 5 year period. These data are preliminary but dramatically imply that this marker may have value in the monitoring of HCC patients and may be elevated even when small, undetectable tumors are present.

In our previous studies we have examined the ability of GP73 to differentiate patients with cirrhosis from those with hepatocellular carcinoma (HCC) [3, 1, 6]. In this study, we have performed a retrospective analysis of GP73 as a function of treatment in 11 patients chronically infected with HBV and who developed HCC. In all cases samples were coded and the status (disease stage) unknown at the time of analysis. GP73 was measured by immunoblotting and AFP was measured by ELISA as before[3, 6]. Nine out of the eleven patients were positive for GP73 at the time of HCC detection (see table 1) and were treated for their cancer using several different methods. The time and type of treatment are indicated in table 1.

Figure 1 shows the results of patients H1–H3, who were diagnosed with HCC, received treatment shortly after diagnosis and remained tumor free for a period of time after treatment. In figure 1, white bars represent GP73 levels relative to the amount detected in commercial healthy serum and the solid black bars represent the log transformed AFP levels. Log transformed AFP levels are utilized to allow for a direct comparison between AFP and GP73. For both GP73 and AFP, the cut-off values are indicated with the horizontal line and were 5 relative units for GP73 and 100 ng/mL for AFP. As this figure shows, patients in which GP73 levels decreased after treatment, had long survival times following treatment for their HCC. As figure 1A shows, patient H1 had 7 relative units of GP73 at the two time points prior to the diagnosis of HCC in December of 2003. Following the radiofrequency ablation (RFA) of the HCC in December of 2003, the level of GP73 decreased at the next time point in August of 2004 to 3 relative units and has remained low at the last time point in 2008. A similar trend was also seen with AFP, which decreased following treatment and remained low at the subsequent time points. Figures 1B&C show

the result from two other patients (H2 and H3) in whom the levels of serum GP73 also were reduced following treatment. Of particular interest is patient H3, who received treatment in October of 2000 and yet showed no reduction of GP73 following this initial treatment in (see figure 1C and table 1). This patient subsequently had another lesion detected in August 2004, and only after further treatment in August of 2004 was there a reduction in GP73. It is noted that Patient H3 had lower levels of AFP (less than 100 ng/ml) at the time of diagnosis and this decreased slightly following treatment.

Figure 2 shows the level of GP73 in patients whose tumors recurred following the initial treatment. These patients had either no change in the level of GP73 after treatment or an increase in the level of the protein. As figure 2A and table 1 show, patient H4 was diagnosed with HCC in November of 2007, and was treated in December of 2007. The level of GP73 dropped from 12 relative units to 9 relative units in this time period, which is still considered highly elevated[6]. Similarly, the AFP level decreased significantly but was still at a high level (100 ng/mL) following treatment. This patient had a recurrence of HCC in June of 2008. As figures 2B&2C show, patients H5 and H6 followed a similar trend. Patient H6 was diagnosed with HCC in November of 2007 and received treatment in January of 2008 (table 1). GP73 values dropped slightly from 9 relative units to 6 relative units and increased to 8 relative units in May of 2008 (Figure 2C). A 2cm HCC lesion was diagnosed in this patient in June of 2008. This patient had an AFP negative tumor and hence AFP did not change significantly.

The level of these biomarkers was similar in patients who succumb to their cancer. Patient H7 was diagnosed with HCC in August of 2007 and received both RFA and transarterial chemoembolization (TACE) treatment between August and December of 2007 (table 1). The level of GP73 did not decrease in this patient but rather increased over this time. The same was true of AFP. In contrast, as figure 3B shows, patient H8 had significant reductions in AFP following treatment in December of 2004. However GP73 levels remained elevated in patient H8. HCC was detected in 2006 and treated again. GP73 levels remained elevated and this patient died of a lung metastasis in 2008. Similar results were observed for patients H9 (see figure 3C). In patient H9, HCC was first detected in 2004 and treated over a period of time from 2004 to 2006 (Table 1). During this period, GP73 levels remained elevated. In contrast, AFP levels were decreased after a second treatment in 2006. GP73 levels remained elevated in 2007 and 2008 when the patient died from HCC in June of 2008.

In this study, we examined the level of a potential biomarker of HCC as a function of HCC treatment and patient outcome. Although the sample numbers are small, a clear trend was observed. Patients who remain tumor free (up to 6 years post-treatment) show decreased levels in GP73. In contrast, all patients who maintained high levels of GP73 had multiple lesions detected on subsequent follow up. This was not the case with AFP, as it was elevated in only one patient who had re-occurrence. More work is needed, but our results clearly indicate that these markers may have value in monitoring of patients and may be elevated even when small, undetectable tumors are present.

These data imply that (a) the biomarkers are produced by the HCC cells and that (b) comparing the levels of these markers, before and after treatment, may have value in monitoring of HCC patients. We, and others, have now reported that GP73 protein is upregulated in patients with HCC[3, 5, 4, 2, 7]. Taken together with the results of the study reported here, it seems likely that the “source” cells for the elevated secreted levels of GP73 observed in HCC patients is the cancer, itself. Finally, given the observation, albeit in a small number of patients, when GP73 “rebounded” to above baseline levels following tumor ablation, multiple small lesions recurred. It appears that GP73 may become elevated even when small, undetectable tumors are present. This finding would imply that markers such as

GP73 may be elevated in patients well before tumor mass becomes visible on imaging study and thus may have value in the monitoring of patients with liver disease.

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Figure 1. The change in GP73 and AFP as a function of HCC treatment in patients with no sign of re-occurrence or secondary tumors

The change in GP73 and AFP patients H1, H2 and H3 who were successfully treated for HCC. For graphs, the white bar represents GP73, the solid black bar represents the natural log (Ln) of AFP (ng/mL). The solid vertical line represents the time of treatment. The solid horizontal line represents the cut-off for GP73 (5 relative Units) and (Ln) of AFP (100 ng/mL). Dates are given as month and year. For example, 12/03 is December, 2003. Dates of initial diagnosis are in table 1.

Figure 2. The change in GP73 and AFP as a function of HCC treatment in patients with HCC relapse

The change in GP73 and AFP in patients H4 (A), H5 (B) and H6 (C) who had secondary tumors following initial tumor therapy. Graphs are as in figure 1.

Figure 3. The change in GP73 AFP as a function of HCC treatment in patients who died as a result of a secondary or malignant HCC

The change in GP73 and AFP in patients H7 (A), H8 (B) and H9 (C) who had secondary tumors following initial tumor therapy. Graphs are as in figure 1.

Table 1

Time of diagnosis and Treatment for patients utilized in this study.

Patient ¹	Date of diagnosis ²	Age and Gender ³	ALT ⁴	AST ⁵	HBV DNA ⁶	Date of treatment ⁷	Treatment ⁸	Re-occurrence ⁹
H1	12/03	56-M	28	28	neg	12/03	RFA	>5 years
H2	3/00	64-M	41	36	neg	5/00 and 3/01	Not defined	>5 years
H3	10/00	58-M	65	44	12X10 ⁶	10/00 and 8/04	RFA (10/00) and TACE (8/04)	>5 years
H4	11/07 6/08	20-F	37	26	neg	12/07	PCEI+RFA	<2 years
H5	4/07 6/08	54-M	95	119	neg	7/07	RFA	<2 years
H6	11/8/07 6/08	64-M	54	48	neg	1/08	RFA	<2 years
H7	8/07	54-F	74	104	1200*	8/07, 9/07 11/07, 12/07	TACE on 8/07, 9/07 11/07, RFA12/07	<2 years and deceased
H8	12/04 2/08 (lung metastasis)	64-F	58	179	neg	12/04 3/06	TACE	<5 years and deceased
H9	10/04 4/05 3/06	69-M	31	40	neg	10/04 5/05 3/06	RFA in 10/04 and 5/05 TACE on 3/06	<4 years and deceased

¹ Patient identifier as shown in figure 1.

² Date of HCC diagnosis. Dates are given as month and year. For example, 12/03 is December, 2003.

³ Age at time of initial diagnosis.

⁴ Alanine transaminase (IU/ml) level at time of HCC

⁵ Aspartate transaminase (IU/ml).

⁶ HBV DNA level. Either in total viral particle load or in the case of copies/mL*.

⁷ Date of treatment for HCC.

⁸ The type of treatment and times if multiple treatments. RFA, radio-frequency ablation; PCEI, percutaneous ethanol injections; TACE, transarterial chemoembolization.

⁹ Time period for re-occurrence.