Prognostic Value And Morphological Findings Of Overexpression Of Glypican-3 In Hepatocellular Carcinoma

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Abstract

Purpose: Hepatocellular carcinoma (HCC) is the seventh most common cancer all over the world and is second in cancer-related deaths. . In HCC, whose prognosis is still not good despite current treatments, there is a need for prognostic markers as well as early diagnosis. Glypican (GPC)-3 has been proposed as a potential serologic and histochemical marker specific to HCC. The aim of this study was to determine the relationship between GPC3 overexpression and HCC prognosis and clinicomorphologic features.

Patients and methods: In total 152 patients patients who were diagnosed as a result of hepatectomy, lobectomy, or liver transplantation were enrolled. The patients were divided into two groups as GPC3-positive (>10%) and GPC3-negative (<10%). The demographic data of the patients ,tumor characteristics , survival times were recorded.

Results: Survival was significantly lower in the GPC3+ group. In the multivariate analysis, hepatitis C, AFP, tumor number, tumor focality, portal vein tumor thrombosis, and GLP3 positivity were found to be independent risk factors on survival.

Conclusion: Our study showing that GPC3 overexpression is a poor prognostic factor in HCC.GLP3 positivity were found to be independent risk factors on survival.

Introduction

Even if hepatocellular carcinoma (HCC) is not the most common cancers, it is the second most common cancer-related death. [1]. In many societies, the incidence of HCC is directly related to age until the age of 75 years, and it is seen 2–4 times more in men than in women [2]. Hepatitis B and C viruses are still considered the most important factors for the development of HCC. Their prevalence is decreasing thanks to effective treatments in HCV and HBV and neonatal vaccination programs for HBV. Non-alcoholic fatty liver disease (NAFLD) is another important cause of HCC with an increasing prevalence. In HCC, whose prognosis is still not good despite current treatments, there is a need for prognostic markers as well as early diagnosis.

Glypican (GPC)-3 has been considered as a potential HCC-specific marker. GPC3 is a heparin sulfate proteoglycan, a member of the heparan sulfate proteoglycan family. It plays an important role in cell modulation, proliferation, and differentiation [3–6]. It has been shown in many studies in the literature that GPC3 expression is minimal or not expressed in normal tissue, whereas its expression is increased in malignant tissue. In addition, it is known that GPC3 is expressed as high as 70% in HCC [7–10]. There are also studies showing that GPC3 overexpression is a poor prognostic factor in HCC [11, 12].

In this study, we aimed to define the relationship between GPC3 overexpression and HCC prognosis and morphological features.

Patients And Methods

Two hundred fifty-eight patients aged over 18 years who were histologically diagnosed as having HCC between 2017 and 2021 were included in our study. Thirty-eight patients were excluded due to a pathologic diagnosis of cholangiocellular cancer. Seventy patients were excluded from the needle biopsy specimens. One hundred fifty-two patients who were diagnosed as a result of hepatectomy, lobectomy, or liver transplantation were included in the study. Data about the patients were obtained from the electronic information system. All follow-up of the patients was performed in a single center.

The patients were divided into two groups as GPC3-positive ($> 10\%$) and GPC3-negative ($< 10\%$).

The demographic data of the patients were analyzed.

Etiologic data were recorded, including HBV, HCV, hepatitis D virus (HBV + HDV), NAFLD, alcohol-induced liver disease (ALD), and any It was recorded as no risk factor (cryptogenic).

NAFLD/non-alcoholic steatohepatitis (NASH) diagnoses were determined by excluding other liver diseases and with alcohol intake < 30 g/day in men and < 20 g/day in women with histologic or radiologic signs of fat [13, 14].

Alcohol consumption for ALD was defined as an average of > 210 g per week for men or > 140 g per week for women over at least two years [15].

Tumor characteristics and morphologic features: maximal tumor diameter (MTD), number of lesions (1 = solitary, > 1 = multifocal), and portal vein invasion were recorded.

Portal vein invasion: In patients with portal thrombosis, those with wash-out findings on dynamic magnetic resonance imaging (MRI) and/or other malignant criteria in areas with portal involvement and 2-fluoro-2-deoxy on positron emission tomography/computed tomography (PET-CT). Those with D-glucose (FDG) uptake were accepted [16–18].

Patient survival times were evaluated from the time of HCC diagnosis.

This study was conducted in accordance with the ethical rules of the Declaration of Helsinki. The study was approved by University Ethics Committee (116/2021).

Pathologic Samples

It was prepared from paraffin embedded blocks. After the preparations were prepared, primary monoclonal anti-GPC3 antibody was added and incubated for 2 hours at room temperature. Necessary washings were made on the slides. Secondary antibody-treated slides were washed three times every 5 minutes. Slides were washed again, stained with hematoxylin and dried.

For immunohistochemical analysis of GPC3, It was evaluated in each patient, including the HCC lesion and the adjacent noncancerous lesion. GPC3 expression was classified according to areas of

immunohistochemical staining results. GPC3-positive staining cells were as follows: GPC3-negative (< 10%) and GPC3-positive (> 10%).

STATISTICAL ANALYSIS

The assessment of the normality of data distribution of continuous variables was performed using the Shapiro-Wilk test. According to GPC3 positivity, the mean age was compared using Student's t-test, and the survival time was compared using the Mann-Whitney U test. The relationship with categorical variables was evaluated using the Chi-square test. Factors that affected the survival time were examined using Cox regression as univariate and multivariate. The difference between the median survival times according to GPC 3 positivity was evaluated using the log-rank test and shown with a Kaplan-Meier graph. Data analysis was performed using the IBM SPSS 21 program.

Results

The demographic and clinicimorphologic findings of all patients are shown in Table 1. Of the 152 patients included in our study, 56.6% were in the GPC3-negative group, and 43.4% were in the GPC3-positive group. There was no difference between the groups in terms of age and sex ($p > 0.05$). When we compared the groups in terms of tumor morphology, in the GPC3 + group, the tumors were larger and more multifocal ($p < 0.001$). Alpha-fetoprotein (AFP) levels tended to be higher in the GPC3 + group ($p < 0.001$). GPC3 expression was observed less frequently in well-differentiated HCC than in moderately or poorly differentiated HCC; the frequency difference was statistically significant ($p < 0.001$). Survival was significantly lower in the GPC3 + group ($p = 0.003$) (Fig. 1).

Table 1
Demographic and clinicimorphologic findings of all patients

Variables	GPC3-negative		GPC3-positive		P-value
	86 (56.6%)		66 (43.4%)		
Age	61.92 ± 12.75		64.53 ± 11.27		0.282
mean ± SD	64 [58–67]		64.5 [59-72.25]		
median [IQR]	(17–84)		(31–86)		
(min-max)					
Sex					
Male	76	88.4%	51	77.3%	0.067
Female	10	11.6%	15	22.7%	
Hepatitis B (+/-)					
-	38	44.2%	33	50.0%	0.476
+	48	55.8%	33	50.0%	
Hepatitis C (+/-)					
-	70	81.4%	56	84.8%	0.575
+	16	18.6%	10	15.2%	
AFP					
AFP (< 400)	71	82.6%	33	50.0%	< 0.001
AFP (> 400)	15	17.4%	33	50.0%	
Number of tumors					
Solitary	48	55.8%	25	37.9%	0.028
Multiple	38	44.2%	41	62.1%	
Tumor size					
< 5	44	51.2%	18	27.3%	0.003
≥ 5	42	48.8%	48	72.7%	
Portal vein tumor thrombosis					
-	65	75.6%	42	63.6%	0.110
+	21	24.4%	24	36.4%	
Cirrhosis background					

Variables	GPC3-negative		GPC3-positive		P-value
-	32	37.2%	33	50.0%	0.114
+	54	62.8%	33	50.0%	
Differentiation					
Well	46	53.5%*	15	22.7%	< 0.001
Moderate	28	32.6%*	10	15.2%	
Poor	12	14.0%	41	62.1%*	
Survival (months)	13.5 [8-28.5]		6 [6–16]		0.003
median [IQR]	(3–50)		(1–60)		
(min-max)					

The factors that affected the survey are shown in Table 2. As a result of the univariate analysis, it was observed that hepatitis C, AFP, tumor size, tumor focality, portal vein tumor thrombosis, tumor differentiation, and GLP3 positivity were effective on survival. In the multivariate analysis, hepatitis C, AFP, tumor number, tumor focality, portal vein tumor thrombosis, and GLP3 positivity were found to be independent risk factors (Table 2).

Table 2
Factors affecting the survey

	Univariate analysis	Multivariate analysis
Age	1.010 (0.986–1.034) (p = 0.433)	1.018 (0.991–1.045) (p = 0.197)
Sex	1.688 (0.880–3.239) (p = 0.115)	0.998 (0.465–2.144) (p = 0.996)
Hepatitis B	1.205 (0.681–2.131) (p = 0.521)	0.831 (0.421–1.638) (p = 0.592)
Hepatitis C	0.355 (0.127–0.988) (p = 0.047)	0.293 (0.090–0.955) (p = 0.042)
AFP (> = 400)	4.332 (2.444–7.677) (p < 0.001)	2.822 (1.398–5.698) (p = 0.004)
Number of tumor (multiple)	4.870 (2.466–9.618) (p < 0.001)	4.080 (1.713–9.721) (p = 0.002)
Tumor size (> = 5cm)	3.385 (1.713–6.690) (p < 0.001)	0.600 (0.223–1.616) (p = 0.312)
Portal vein tumor thrombosis (+)	3.126 (1.767–5.530) (p < 0.001)	2.045 (1.023–4.089) (p = 0.043)
Cirrhosis background	0.777 (0.442–1.366) (p = 0.381)	1.094 (0.593–2.016) (p = 0.774)
Differentiation		
Moderately/Well	2.979 (1.172–7.573) (p = 0.022)	2.382 (0.848–6.690) (p = 0.100)
Poorly/Well	7.427 (3.242–17.015) (p < 0.001)	1.423 (0.451–4.483) (p = 0.547)
GLP-3 positive	3.515 (1.930–6.403) (p < 0.001)	2.490 (1.172–5.290) (p = 0.018)
p: Cox Regression		

Discussion

In this study, we examined the relationship between the expression level of GPC3 and the morphologic and histologic features of tumors and the prognostic value of GPC3 in HCC. We found that poor tumor morphologic findings (multifocality, increased tumor size, AFP elevation, portal vein tumor thrombosis) were significantly more frequent in the GLP3 + group. In the GLP3 + group, the tumor was larger, tended to be multifocal, the incidence of PVTT was significantly higher, and AFP values were higher. When we looked at the histologic features of the tumor, we observed that tumors tended to be significantly poorly

differentiated in the GLP3 + group. As a result of the univariate and multivariate analysis, we determined that the GLP3 + lignin was an independent risk factor for survival.

When we evaluated the literature, we observed poor consistency between studies and meta-analyses. Studies were showing that GLP3 overexpression was associated with both poor and good prognoses.

In a study of 300 patients, patients were evaluated in terms of postoperative recurrence and survival according to GLP3 levels, and it was shown that postoperative recurrence was higher in the group with low GLP3 levels, and survival was shorter in the group with low GLP levels (19). In another study consisting of 129 patients, the patients were divided into low and high GLP3 levels, and the risk of postoperative recurrence was found to be high in the group with low GLP3 levels, and this situation was associated with poor prognosis (20). In a meta-analysis of 1070 patients, GLP3 was evaluated in terms of ovarian survival and disease-free survival, and it was stated that GLP3 expression was associated with poor prognosis and might have predictive value in terms of HCC invasion and metastasis [21]. In another meta-analysis in which 14 studies and 2364 patients were evaluated, it was reported that GLP3 overexpression was associated with poor tumor differentiation, advanced tumor stage, and vascular invasion. However, although all studies in this meta-analysis were conducted in the Asian population, they were highly heterogeneous and the mean follow-up times of the patients were different. Cut-off values for GLP3 were also not the same in all studies [22]. In a meta-analysis of 2336 patients in which 15 studies were evaluated, GLP3 was shown to be a significant poor prognostic factor. In this meta-analysis, there were some limitations of the studies such as small sample size, publication bias, insignificant GLP3 cut-off value, and inability to perform subgroup analyses [23].

Our study also had some limitations. Our study was tissue-based in terms of GCP3 levels. GCP3 levels could also have been measured from blood. Thus, GCP3 comparisons could be made in tissue and blood. Our study is a single-center study and it is not known whether GCP3 levels are related to regions and genetic diversity. We set the cut-off limit as 10% for GPC3. However, there are various cut-offs in the literature.

As a result, in our study, we showed that GLP3 overexpression was significantly associated with poor prognosis. We found that GLP3 overexpression was an independent risk factor for survival. We observed in the literature that there are difficulties in determining both the prognostic value and the optimal cut-off values. It is necessary to determine whether GLP3 levels in both blood and tissue should be associated with prognosis in larger studies, if it is more valuable in tissue or blood, or if both should be evaluated together. Studies with larger samples are needed to determine the cut-off with high specificity and sensitivity.

Declarations

Conflict of interest: The authors declare that there is no conflict of interest

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There is no financial disclosures of the authors.

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Figures

Figure 1

Correlation between GPC3 expression and patient survival