Prognostic value and morphological findings of overexpression of glypican-3 in hepatocellular carcinoma

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Objectives Hepatocellular carcinoma (HCC) is the seventh most common cancer all worldwide 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. This study aimed to determine the relationship between GPC3 overexpression and HCC prognosis and clinicomorphologic features.

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

Conclusion Our study shows that GPC3 overexpression is a poor prognostic factor in HCC. GPC3 positivity were found to be an independent risk factor for survival.

European Journal of Gastroenterology & Hepatology 2023, 35:89–93

Keywords: hepatocellular carcinoma, glypican-3, prognosis, survival, tumor features

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Received 26 May 2022 Accepted 18 August 2022

Introduction

Hepatocellular carcinoma (HCC) is not the most common cancer, but it is the second-highest cause of 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. Nonalcoholic 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 is considered 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 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 clinicomorphologic features.

Material 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-six patients were excluded due to a pathologic diagnosis of cholangiocellular carcinoma. 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. Patient data 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, 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: Patients with portal thrombosis, those with wash-out findings on dynamic MRI and/or other malignant criteria in areas with portal involvement and 2-fluoro-2-deoxy on PET/computed tomography. 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 the University Ethics Committee (116/2021).

**Pathologic samples**

Samples were prepared from paraffin-embedded blocks and immunohistochemistry (IHC) was performed. After the preparations were prepared, a primary mouse monoclonal anti-GPC3 antibody was added and incubated for 2 hours at room temperature. Necessary washes were made on the slides. Secondary antibody-treated slides were washed three times every 5 minutes. The slides were washed again, stained with hematoxylin and dried.

Immunohistochemical analyses of GPC3 were performed in each patient, including the HCC lesion and the adjacent noncancerous lesion. GPC3 expression was classified according to areas of immunohistochemical staining results.

GPC3 shows membranous and/or cytoplasmic staining. When we searched the literature, we found that the cut-off GPC3 was taken as >10% staining for overexpression in many studies.

In our study, we planned according to membranous Gp3 staining. GPC3-positive stained cells were evaluated as follows: GPC3-negative (<10%) and GPC3-positive (overexpression) (>10%) (Figs. 1(a, b) and 2).

**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 using a Kaplan–Meier graph.

We did not want to restrict it to those that were significant in univariate analyses. Instead, clinical variables that might affect the survey were included in the multivariate model. When variables were evaluated together, the variables that are insignificant in univariate models, we wanted to observe how they affected the survey in multiple models. Data analysis was performed using the IBM SPSS 21 program.

Results

The demographic and clinicomorphologic 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 and P < 0.05, respectively). 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).

When evaluated in terms of cirrhotic background and portal vein thrombosis, no significant difference was observed between the groups (P > 0.05). Survival was significantly lower in the GPC3+ group (P = 0.003) (Table 1). There was a negative correlation between GPC3 expression and survival (Fig. 3).

Factors affecting the survey were evaluated using univariate and multivariate analyses. 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 foci, portal vein thrombosis, tumor differentiation and GPC3 positivity were effective in survival. In the multivariate analysis, hepatitis C, AFP, tumor foci, portal vein thrombosis and GPC3 positivity were found to be independent risk factors (Table 2).

Discussion

In this study, we examined the relationship between the expression level of GPC3 and the morphologic and

| Variables                        | GPC3-negative | GPC3-positive | P-value |
|---------------------------------|---------------|---------------|---------|
| Age mean±SD median(IQR)(min–max)| 61.92 ± 12.75 | 64.53 ± 11.27 | 0.282a |
| Sex                             |               |               |         |
| Male                            | 76            | 61            | 0.067b |
| Female                          | 10            | 25            |
| B(±)                            | 38            | 44.2%         |         |
| +                               | 48            | 55.8%         |         |
| C(±)                            | 70            | 81.4%         |         |
| +                               | 16            | 18.6%         |         |
| AFP                             |               |               |         |
| AFP(<400)                       | 71            | 50.0%         | <0.001b |
| AFP(>400)                       | 15            | 50.0%         |         |
| Number of tumors                |               |               |         |
| Solitary                        | 48            | 55.8%         |         |
| Multiple                        | 38            | 44.2%         |         |
| Tumor size                      |               |               |         |
| <5                              | 44            | 51.2%         |         |
| ≥5                              | 42            | 48.8%         |         |
| PVT                             |               |               |         |
| –                               | 65            | 75.6%         |         |
| +                               | 21            | 24.4%         |         |
| Cirrhosis background            |               |               |         |
| –                               | 32            | 37.2%         |         |
| +                               | 54            | 62.8%         |         |
| Histological differentiation    |               |               |         |
| Well                             | 46            | 53.5%*        | <0.001b |
| Moderate                        | 28            | 32.6%*        |         |
| Poor                            | 12            | 14.0%         |         |
| Survival (months) median(IQR)(min–max)| 13.5 [8-28.5] | 6 [6-16] | 0.003c |

B, hepatitis B; C, hepatitis C; D, hepatitis D; AFP, alpha-feto protein; PVT, Portal vein thrombosis.

aStudent’s t test.
bChi-squared test.
cMann–Whitney U test.
*Statistically significant and high rate (P < 0.05).
histologic features of tumors and the prognostic value of GPC3 in HCC. We found that poor tumor morphologic findings (multifocality, increased tumor size, high level of AFP, and portal vein tumor thrombosis) were significantly more frequent in the GPC3+ group. In the GPC3+ group, tumors were 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 tumors, we observed that tumors tended to be significantly poorly differentiated in the GPC3+ group. As a result of the univariate and multivariate analysis, we determined that the GPC3+ 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 GPC3 overexpression was associated with both poor and good prognoses.

In a study, 300 patients were evaluated in terms of postoperative recurrence and survival according to GPC3 levels. It was shown that postoperative recurrence was higher in the group with low GPC3 levels, and survival was shorter in the group with low GLP levels [19,22]. In another study consisting of 129 patients, the patients were divided into low and high GPC3 levels, and the risk of postoperative recurrence was found to be high in the group with low GPC3 levels, and this situation was associated with poor prognosis [20,23]. In a meta-analysis of 1070 patients, GPC3 was evaluated in terms of overall survival and disease-free survival, and it was stated that GPC3 expression was associated with poor prognosis and might have predictive value in terms of HCC invasion and metastasis [21,24]. In another meta-analysis in which 14 studies and 2364 patients were evaluated, it was reported that GPC3 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 GPC3 were also not the same in all studies [22,25]. In a meta-analysis of 2336 patients in which 15 studies were evaluated, GPC3 was shown to be a significantly poor prognostic factor. There were some limitations of the studies in this meta-analysis, such as small sample size, publication bias, insignificant GPC3 cut-off value, and an inability to perform subgroup analyses [23,26].

These inconsistencies in the literature may be related to the standardization of the GPC3 staining pattern. In particular, membranous staining is more valuable than cytoplasmic staining, and in our study, classification was made according to membranous staining.

Our study also had some limitations. Our study was tissue-based in terms of GPC3 levels. GPC3 levels could also have been measured from blood. Thus, GPC3 comparisons could be made in tissue and blood. Our study is a single-center study and it is not known whether GPC3 levels are related to regions and genetic diversity. We set the cut-off limit as 10% for GPC3. However, there are also have been measured from blood. Thus, GPC3 cross-sectional studies and meta-analyses. Studies were showing that GPC3 overexpression was associated with both poor and good prognoses.

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Table 2. Factors affecting the survey

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

P: cox regression analysis.
B, hepatitis B; C, hepatitis C; D, hepatitis D; AFP, alpha-feto protein; PVT, Portal vein tumor thrombosis.

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As a result, in our study, we showed that GPC3 overexpression (positive) was significantly associated with a poor prognosis. We found that GPC3 overexpression (positive) was an independent risk factor for survival. We observed that GPC3 overexpression (positive) was significantly associated with a poor prognosis. We found that GPC3 overexpression (positive) was significantly associated with a poor prognosis.

There are no conflicts of interest.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

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