

ORIGINAL ARTICLE

Glypican-3 is useful but not superior to Hep Par 1 in differentiating hepatocellular carcinoma from other liver tumours

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Abstract

To assess the diagnostic utility of glypican-3 (GPC-3) in comparison to Hep Par 1 in the diagnosis of liver tumours, a cross-sectional study involving 66 resected liver tumours were tested for the protein expression of these markers by immunohistochemistry using monoclonal antibodies. Of the 66 cases, 26 (39.4%) were hepatocellular carcinoma (HCC), 4 (6.1%) were intrahepatic cholangiocarcinoma and 36 (54.5%) were metastatic tumours. Hep Par 1 and GPC-3 expressions in HCC were 24/26 (92.3%) and 19/26 (73.1%) respectively. In contrast, of non-HCC cases, only 2/40 cases (5.0%) expressed Hep Par 1, including a metastatic colorectal adenocarcinoma and a metastatic gastric adenocarcinoma. GPC-3 was expressed in 3/40 cases (7.5%), i.e. a metastatic adenocarcinoma of unknown origin, a metastatic gastric adenocarcinoma and an intrahepatic cholangiocarcinoma. The sensitivity and specificity for Hep Par 1 were 92.3% and 95% respectively while that of GPC-3 was 73.1% and 92.5% respectively. GPC-3 is a useful marker in the diagnosis of HCC. However it is not superior to Hep Par 1 in its sensitivity and specificity. We recommend that it is utilized together with Hep Par 1 as a panel in the diagnosis of HCC.

Keywords: Hep Par 1, glypican-3, primary liver tumour, immunohistochemical marker

INTRODUCTION

Liver cancer is an increasingly prevalent malignancy which is associated with high mortality.^{1,2} It is diagnosed twice more frequently in men than in women.^{1,2} It is the fifth most frequently diagnosed cancer and second most frequent cause of death due to cancer in men globally.¹ The most common type of liver cancer is hepatocellular carcinoma (HCC), which is related to hepatitis B and hepatitis C infection.¹⁻³ Most cases of liver cancer are seen in the East and South-East Asia as well as in Middle and Western Africa, where the incidence of hepatitis B infection is also high.^{1,4} In Malaysia, liver cancer is the tenth most frequently diagnosed cancer regardless of gender and is associated with chronic hepatitis B and C infections and liver cirrhosis.⁵ The accurate diagnosis of HCC is important but distinguishing it from metastatic tumours may be challenging and pathologists frequently resort to specific ancillary techniques and tumour markers in their assessment.

Primary liver cancers are classified into

hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and combined hepatocellular and cholangiocarcinoma (CHC).⁶ Differentiating HCC from other types of liver cancer is important for prognosis and management.^{7,8} Several markers have been suggested to distinguish HCC from other liver tumours. These include Glypican-3 (GPC-3), Arginase-1 and Hepatocyte paraffin 1 (Hep Par 1).⁹⁻¹² However, past reports have shown conflicting results. While some studies reported that GPC-3 is a sensitive marker for HCC, others believed that Arginase-1 and Hep Par 1 are better markers for detection of HCC.⁹⁻¹³ In this study we investigated the expression, sensitivity and specificity of GPC-3 in differentiating HCC from other types of liver tumour in comparison to Hep Par 1.

MATERIALS AND METHODS

This is a cross-sectional study that was conducted on archived formalin-fixed paraffin-embedded tissue blocks from resected liver tumours at our

institution over a four-year period. This study was approved by the Ethical Committee of the National University of Malaysia.

The histopathological characteristics of each specimen were reviewed by two pathologists to confirm the diagnosis. The HCC cases were graded from grade I to IV based on the histological differentiation.¹⁴ Grade I is best differentiated, and consists of small tumour cells arranged in thin trabeculae.¹⁴ Cells in grade II are larger with abnormal nuclei and glandular structures may be present.¹⁴ In grade III, the nuclei are larger and more hyperchromatic than grade II cells and cytoplasm is granular and acidophilic, but less than grade II.¹⁴ In grade IV, tumour cells are less differentiated, displaying hyperchromatic nuclei and loss of the trabecular pattern.¹⁴

Any discrepancy in diagnosis was resolved through reassessment over a multi-viewer microscope.

Tissue microarray

Tissue microarrays (TMA) were constructed using Alphelys Tissue Arrayer Minicore 3 (ALPHELYS, Plaisir, France) by the method of re-locating tissue from individual paraffin blocks to a recipient block. A hollow needle was used to remove 0.6 mm tissue cores in duplicate, from regions of interest and inserted into a recipient block in a precisely spaced, arrayed pattern. Three micron thick sections were made from the TMA blocks for immunostaining.

Antibodies

GPC-3 mouse monoclonal immunoglobulin 12 (Biomosaics, USA) and Hep Par 1 monoclonal mouse anti-human hepatocyte, Clone OCH1 (Dako, Denmark) were used for immunohistochemistry.

Immunohistochemistry

GPC-3 antibody was used at a dilution of 1:500 using Dako Envision Plus detection system. The sections were also incubated with primary Hep Par 1 antibody at a dilution of 1:50 using the same detection system. Positive control tissues included sections of placenta for GPC-3 and normal hepatocytes for Hep Par 1 both with cytoplasmic staining pattern.

Analysis of Glypican-3 and Hep Par 1

GPC-3 was considered positive when at least one TMA tissue core was stained or more than 10% of tumour cells showed membrane

or cytoplasmic labelling. Negative GPC-3 was defined as absence of staining in both TMA tissue cores for each sample. Hep Par 1 was considered positive when at least 10% of the tumour cells were stained and was considered negative when both cores were not stained.¹⁵

Statistical analysis

Data were analyzed using the statistical package for social sciences (SPSS) software 20.0 (IBM Inc, Chicago, IL, USA). Data were presented as frequency and percentage. Specificity and sensitivity was assessed for each marker. Fisher's exact test was used to assess the association between each tumour marker positivity and HCC diagnosis. A p value of less than 0.05 was considered statistically significant.

RESULTS

A total of 66 cases of primary and secondary malignant liver tumours were investigated. Forty-six (69.7%) tumours were from male and 20 (30.3%) from female patients. The mean age at diagnosis was 58.7 ± 10.7 years, ranging from 36 to 76 years old. Ethnically, 35 (53%) were Chinese, 25 (38%) were Malay, 2 (3%) were Indian and 4 (6%) were from other ethnicities.

Among primary tumours, 26 (39.4%) were identified as HCC and four (6.1%) were intrahepatic cholangiocarcinoma. Thirty-six (54.5%) cases were metastatic tumours comprising 21 (31.8%) metastatic colorectal adenocarcinoma and 15 (22.7%) from other primary sites (Table 1).

Of 26 HCC cases, 9 (34.6%) were grade I, 10 (38.4%) grade II and 7 (26.9%) were grade III (Table 2). There were no grade IV cases.

Hep Par 1 immunostaining

Twenty-four of 26 HCC (92.3%) were positive for Hep Par 1 (Table 1). In contrast, only two non-HCC cases (2/40, 5%), i.e. a metastatic gastric adenocarcinoma of intestinal type and a metastatic adenocarcinoma of colon, were Hep Par 1-positive.

Glypican-3 immunostaining

Nineteen cases of 26 HCC (73.1%) were positive for GPC-3 (Table 1), expressed as strong membranous, cytoplasmic and pericanalicular labelling patterns (Fig. 1). Among non-HCC cases, GPC-3 was expressed in three cases (one case of metastatic adenocarcinoma of unknown origin, one case of intrahepatic cholangiocarcinoma and one case of metastatic

TABLE 1: Distribution of histological diagnosis of primary and secondary liver tumours according to gender and their expression for Hep Par 1 and glypican-3

	Total	Male	Female	Hep Par 1	GPC-3
				No. positive/tested (%)	No. positive/tested (%)
Hepatocellular carcinoma	26	21	5	24/26 (92.3%)	19/26(73.07%)
Intrahepatic cholangiocarcinoma	4	3	1	0/4 (0.00%)	1/4 (25.00%)
Metastatic colorectal adenocarcinoma	21	16	5	1/21 (4.76%)	0/21 (0.00%)
Metastatic pancreatic adenocarcinoma	1	1	0	0/1 (0.00%)	0/1 (0.00%)
Metastatic adenocarcinoma of unknown origin	8	4	4	0/8 (0.00%)	1/8 (12.50%)
Metastatic breast adenocarcinoma	1	1	0	0/1 (0.00%)	0/1 (0.00%)
Metastatic periampullary adenocarcinoma	1	0	1	0/1 (0.00%)	0/1 (0.00%)
Metastatic gastric adenocarcinoma (intestinal type)	1	1	0	1/1 (100%)	1/1 (100%)
Metastatic ovarian adenocarcinoma	1	0	1	0/1 (0.00%)	0/1 (0.00%)
Diffuse large B-cell lymphoma	1	1	0	0/1 (0.00%)	0/1 (0.00%)
Metastatic gastrointestinal tumour (GIST)	1	0	1	0/1 (0.00%)	0/1 (0.00%)

gastric adenocarcinoma of intestinal type) (Table 1, Fig. 1).

Of the seven GPC-3 negative HCCs, four were grade I, two grade II and one showed clear cell pattern where the grading could not be determined. None of the 20 cases of colorectal adenocarcinoma expressed GPC-3 (rendering it suitable for differentiating HCC from colorectal adenocarcinoma). The expression of Hep Par 1 and GPC-3 according to grade is summarised in Table 2.

The expression of GPC-3 and Hep Par 1 was independently associated with diagnosis of HCC ($p < 0.001$) (Table 3). The sensitivity and specificity of Hep Par 1 in detecting HCC was 92.3% and 95% respectively, whereas the sensitivity and specificity of GPC-3 was 73.1% and 95.2% respectively. These results suggest that Hep Par 1 is associated with detection of true HCC tumours 1.3 times more than GPC-3. The positive and negative predictive value for Hep Par 1 in detecting HCC were 92.3% and 95.0% respectively, while positive predictive value for

GPC-3 in detection of HCC was 86.4% and the negative predictive value was 84.1%.

DISCUSSION

The primary purpose of this study was to compare the usefulness of GPC-3 with Hep Par 1 in the diagnosis of HCC. We were prompted to conduct these investigations seeing that previous reports have shown conflicting results.⁹⁻¹³

In our study, Hep Par 1 proved to be a sensitive (92.3%) and specific (95.0%) marker for HCC, comparable to the findings of a previous study which showed 83.3% sensitivity and 96.6% specificity.¹³ Also, in the study by Kakar *et al* (2003) on immunoreactivity of Hep Par 1, 93% of HCCs were positive for this marker.¹⁶

Studies using different clones of Hep Par 1 antibodies have demonstrated that Hep Par 1 is consistently sensitive and specific in the diagnosis of HCC.¹⁷⁻¹⁹ Our findings are consistent with previous reports showing high sensitivity and specificity for Hep Par 1 in comparison to GPC3.

TABLE 2: Expression of Hep Par 1 and Glypican-3 according to the tumour grade

Grade	Grade I n=9	Grade II n=10	Grade III n=7	Total
Hep Par 1 (No. of positive cases)	8	9	7	24
GPC-3 (No. of positive cases)	6	7	6	19

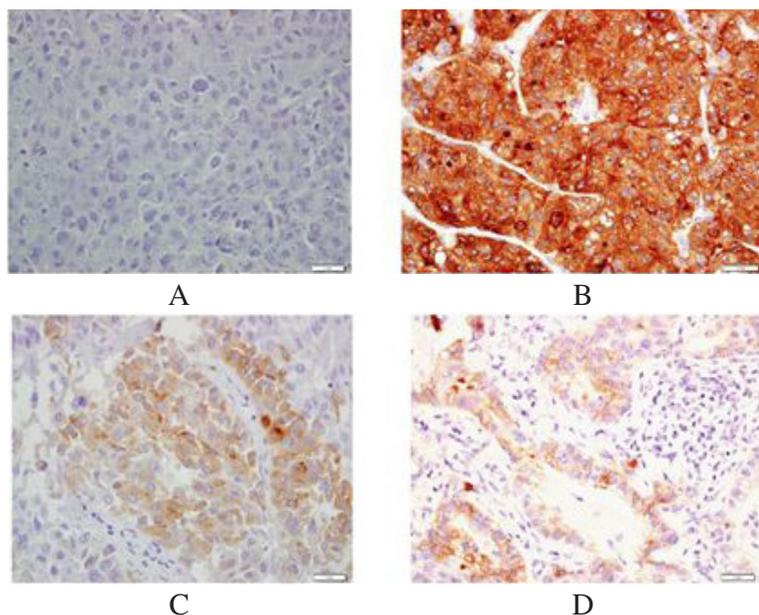


FIG. 1: Cytoplasmic and membranous staining of Hep Par 1 and Glypican-3 in liver tumours. (A) Negative Hep Par 1 staining in a case of cholangiocarcinoma (x400), (B) Strong Glypican-3 cytoplasmic staining in a case of HCC (x400), (C) Positive cytoplasmic staining for Glypican-3 in a case of cholangiocarcinoma (x400) and (D) Cytoplasmic and membrane staining of Glypican-3 in metastatic adenocarcinoma (intestinal type) (x400)

In this study, GPC3 was positive in 19/26 (73.01%) of HCC cases and in only 3/40 (7.5%) non-HCC tumours: a metastatic adenocarcinoma of unknown origin, a metastatic gastric adenocarcinoma of intestinal-type and an intrahepatic cholangiocarcinoma. This low number of GPC3-positivity in non-HCC tumours were also observed by Baumhoer *et al* (2008), who found that three out of 24 (13%) cases of ICC were GPC3-positive (13%).²⁰ On the other hand in another study on FNA samples of HCC and metastatic tumours, GPC-3 was reported positive only in HCC tumours.²¹ The difference in the findings of our study and the latter is most likely due to the difference in sampling method where smaller amounts of tumoral cell concentrations are found in FNA sampling.

The correlation between Hep Par 1 and GPC-3 expression and tumour grades is of some dispute. In our study, the two Hep Par 1-negative HCCs were low-grade tumours; i.e. a grade I and grade II. However previous reports showed that Hep Par-1 negativity is associated with high grade HCC while Hep Par 1 positivity is seen in well-differentiated HCC tumours. Most of the GPC-3-negative HCC (5/7) were low grade and this is in keeping with previous findings where strong positivity for GPC-3 is associated with poorly-differentiated HCC tumours.^{22,23}

The current study showed that GPC3 and Hep Par 1 are expressed in the majority of HCC cases with high specificity and sensitivity but in non-HCC cases the expression is low or negative indicating that both markers are excellent for

TABLE 3: Hep Par 1 and Glypican-3 positivity in HCC and non-HCC tumours

		HCC	Non-HCC	p
Hep Par 1	Positive	24	2	<0.001*
	Negative	2	38	
Glypican-3	Positive	19	3	<0.001*
	Negative	7	37	

* Statistically significant association based on Fisher’s exact test

differentiating HCC from ICC or metastatic carcinoma. This is in line with the findings of a previous study that reported Hep Par 1 and GPC-3 as better markers in differentiating HCC from non-HCC tumours.²⁴

In addition, our results showed that GPC-3 is highly specific, comparable to Hep Par 1 in detecting HCC (95.2% vs 95.0%) but it is not as sensitive as Hep Par 1 as a tumour marker (73.1% vs 92.3%).

Based on the results of this study, it can be concluded that GPC3 is a useful marker in the diagnosis of HCC but found not to be as sensitive as Hep Par 1. From these findings we proposed that GPC3 is to be used together with Hep Par 1 within a panel of antibodies in the diagnosis of HCC.

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REFERENCES

- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011; 61: 69-90.
- Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin.* 2014; 64: 9-29.
- El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology.* 2012; 142: 1264-73.
- Fallot G, Neuveut C, Buendia MA. Diverse roles of hepatitis B virus in liver cancer. *Curr Opin Virol.* 2012; 2: 467-73.
- Omar ZA, Ibrahim Tamin NS. National cancer registry report: Malaysia cancer statistics-data and figure. Malaysia: National Cancer Registry; 2011. p. 85-7.
- Clavien PA. Malignant Liver Tumors: Current and Emerging Therapies. 3rd ed. West Sussex, UK: Wiley-Blackwell; 2010. p. 1006.
- Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol.* 2009; 27: 1485-91.
- Gomaa AI, Khan SA, Leen ELS, Waked I, Taylor-Robinson SD. Diagnosis of hepatocellular carcinoma. *World J Gastroenterol.* 2009; 15: 1301-14.
- Shirakawa H, Kuronuma T, Nishimura Y, *et al.* Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. *Int J Oncol.* 2009; 34: 649-56.
- Shafizadeh N, Ferrell LD, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Mod Pathol.* 2008; 21: 1011-8.
- Timek DT, Shi J, Liu H, Lin F. Arginase-1, HepPar-1, and Glypican-3 are the most effective panel of markers in distinguishing hepatocellular carcinoma from metastatic tumor on fine-needle aspiration specimens. *Am J Clin Pathol.* 2012; 138: 203-10.
- Fujiwara M, Kwok S, Yano H, Pai RK. Arginase-1 is a more sensitive marker of hepatic differentiation than HepPar-1 and glypican-3 in fine-needle aspiration biopsies. *Cancer Cytopathol.* 2012; 120: 230-7.
- Hanif R, Mansoor S. Hep par-1: a novel immunohistochemical marker for differentiating hepatocellular carcinoma from metastatic carcinoma. *J Coll Physicians Surg Pak.* 2014; 24: 186-9.
- Vauthey JN, Brouquet A. Multidisciplinary Treatment of Hepatocellular Carcinoma. Heidelberg, Berlin: Springer-Verlag Berlin Heidelberg; 2013.
- Shirakawa H, Suzuki H, Shimomura M, *et al.* Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. *Cancer Sci.* 2009; 100: 1403-7.
- Kakar S, Muir T, Murphy LM, Lloyd RV, Burgart LJ. Immunoreactivity of Hep Par 1 in hepatic and extrahepatic tumors and its correlation with albumin in situ hybridization in hepatocellular carcinoma. *Am J Clin Pathol.* 2003; 119: 361-6.
- Lamps LW, Folpe AL. The diagnostic value of hepatocyte paraffin antibody 1 in differentiating hepatocellular neoplasms from nonhepatic tumors: a review. *Adv Anat Pathol.* 2003; 10: 39-43.
- Kanitakis J, Chouvet B, Claudy A, Scoazec JY. Immunoreactivity of hepatocyte paraffin 1 monoclonal antibody in cutaneous metastatic tumors. *Am J Clin Pathol.* 2004; 122: 85-9.
- Amarapurkar AD, Rege JD, Joshi AS, Vaiphei K, Amarapurkar DN. Utilization of antihepatocyte clone OCH1E5 (Hep Par 1) in histological evaluation of liver tumors. *Indian J Pathol Microbiol.* 2006; 49: 341-4.
- Baumhoer D, Tornillo L, Stadlmann S, Roncalli M, Diamantis EK, Terracciano LM. Glypican 3 expression in human nonneoplastic, preneoplastic, and neoplastic tissues: a tissue microarray analysis of 4,387 tissue samples. *Am J Clin Pathol.* 2008; 129: 899-906.
- Ibrahim TR, Abdel-Raouf SM. Immunohistochemical Study of Glypican-3 and HepPar-1 in Differentiating Hepatocellular Carcinoma from Metastatic Carcinomas in FNA of the Liver. *Pathol Oncol Res.* 2015; 21: 379-87.
- Fan Z, van de Rijn M, Montgomery K, Rouse RV. Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. *Mod Pathol.* 2003; 16: 137-44.
- Nguyen T, Phillips D, Jain D, *et al.* Comparison of 5 Immunohistochemical Markers of Hepatocellular Differentiation for the Diagnosis of Hepatocellular Carcinoma. *Arch Pathol Lab Med.* 2015; 139: 1028-34.
- Saleh HA, Aulicino M, Zaidi SY, Khan AZ, Masood S. Discriminating hepatocellular carcinoma from metastatic carcinoma on fine-needle aspiration biopsy of the liver: the utility of immunocytochemical panel. *Diagn Cytopathol.* 2009; 37: 184-90.