Value of Glypican 3 Immunostaining in the Diagnosis of Hepatocellular Carcinoma on Needle Biopsy

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CME

Upon completion of this activity you will be able to:
- list 5 immunohistochemical markers used in differential diagnosis of hepatocellular nodules.
- describe the expected pattern of staining seen with antibodies recognizing glypican 3 staining patterns and comparing benign liver nodules to hepatocellular carcinoma.
- discuss the diagnostic specificity of an absence of staining of glypican 3 when diagnosing biopsies from liver nodules.

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Questions appear on p 312. Exam is located at www.ascp.org/ajcpcme.

Abstract

The diagnostic value of glypican 3 (GPC3) immunostaining on needle biopsy specimens has not been well assessed. In this study, 120 liver needle biopsy specimens, including 46 from cirrhotic livers and 74 hepatocellular carcinomas (HCCs), were immunohistochemically examined for expression of GPC3. The results showed strong cytoplasmic and membranous staining in 36 HCCs (49%), among which 20 cases (56%) showed diffuse immunoreactivity. None of the 46 cirrhotic livers exhibited positive GPC3 immunostaining. The nonneoplastic liver tissues (cirrhotic or noncirrhotic) that were present in the majority of the HCC cases were also completely negative for GPC3 expression. These data demonstrate that GPC3 is a reliable immunohistochemical marker for the diagnosis of HCC on needle biopsy specimens when positive. However, the detection rate in our series seems lower than that reported in studies using resection specimens as the study materials. Our findings emphasize that GPC3 immunoreactivity can be focal and that negative staining should not be viewed as evidence to exclude the diagnosis of HCC in challenging needle biopsy specimens.

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related death worldwide.[1] Estimates of HCC burden in the United States, mainly related to hepatitis C virus infection, suggest that the incidence will increase within 2 decades, probably to equal that currently reported in Japan.[2] Early detection is critically important because the most effective treatment for HCC is surgical resection or ablation therapy when the tumor is small. Continuing advances in technology have facilitated radiologic and imaging detection of small lesions in the liver, but the findings are frequently nonspecific and nondiscriminating between small HCCs and benign conditions.[3,4] Therefore, a diagnostic challenge often faced by pathologists is the interpretation of needle biopsy specimens of small liver lesions. The currently available immunomarkers, such as α-fetoprotein, hepatocyte antigen (Hep Par 1), polyclonal carcinoembryonic antigen, CD10, and CD34, have significant diagnostic limitations.[5,6]

Glypican 3 (GPC3) is a member of the heparan sulfate proteoglycan family, which is linked to the cell surface through a glycosylphosphatidylinositol anchor.[7] It is an oncofetal protein that is expressed in the embryo and involved in morphogenesis and growth control during development.[8] GPC3 expression is silenced in adult tissues, and loss-of-function mutation is responsible for Simpson-Golabi-Behmel syndrome, a rare X-linked prenatal and postnatal overgrowth with multiple congenital anomalies and increased risk of neoplasias in infancy.[9] Down-regulation of GPC3 has also been observed in several human malignancies, including mesothelioma and ovarian, breast, and lung cancers.[10-13] These observations indicate that GPC3 is an inhibitor of cell proliferation and a tumor suppressor in a tissue-specific manner.

GPC3 is expressed in fetal livers but not in adult livers. There have been a number of studies showing that GPC3 expression is frequently up-regulated in HCCs at the messenger RNA and protein levels when compared with normal livers and benign hepatic lesions,[14-25] although its role in hepatocarcinogenesis is unclear. The results of immunohistochemical studies have convincingly shown that GPC3 is a novel diagnostic marker for HCC.[16,21,22] However, these studies were performed primarily on resection specimens in which the diagnosis of HCC is usually straightforward and ancillary studies are only occasionally needed. The diagnostic value of GPC3 immunostaining on needle biopsy specimens, which more frequently pose diagnostic challenges to pathologists, has not been well assessed.

Materials and Methods

A total of 120 needle biopsies of liver lesions were retrieved from surgical pathology archives of Washington University Barnes-Jewish Hospital, St Louis, MO, and Northwestern Memorial Hospital of Northwestern University, Chicago, IL. These included 74 HCCs and 46 cirrhotic livers. The clinical history, pathology reports, and H&E-stained slides were reviewed to confirm the diagnoses and to determine the underlying etiologies. In fact, the diagnosis of HCC in the majority of cases was confirmed by histologic examination of subsequent liver resections.

Immunohistochemical staining for GPC3 was performed following the protocol described previously.[26] Briefly, 4-μm sections from formalin-fixed, paraffin-embedded tissue blocks were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide for 15 minutes to inhibit endogenous peroxidase. Following heat-induced epitope retrieval in 0.1 mol/L of citrate buffer at pH 6.0 in a microwave for 20 minutes, the slides were incubated with a mouse monoclonal antibody specific for GPC3 (clone 1G12, BioMosaics, Burlington, VT) with a dilution of 1:200 for 1 hour at room temperature. After incubation with a rabbit antimouse secondary antibody, a reaction was performed using the EnVision plus detection system that contained biotin-free horseradish peroxidase–labeled polymers (DAKO, Carpinteria, CA). The staining was visualized using 3,3′-diaminobenzidine substrate-chromogen solution and counterstained with hematoxylin.

Immunohistochemically stained slides were independently evaluated by 2 observers (F.A. and H.L.W.). Cases with significantly discrepant interpretation were resolved by review together by the 2 observers. A case was considered negative if fewer than 5% of the cells of interest exhibited immunoreactivity. Positive stains were further stratified as focal (5%-50% of cells stained) or diffuse (>50% of the cells stained). Different staining patterns (cytoplasmic and/or membranous) were recorded.

Statistical analysis was performed by using the 2-tailed Fisher exact test or the χ^2 test with Yates continuity correction. A P value of less than .05 was considered statistically significant.

Results

The underlying causes and demographic data for HCC and non-HCC cases included in the study are summarized in **Table 1** and **Table 2**. The ages of patients with HCCs ranged from 36 to 87 years (mean, 62.4 years), and the male/female ratio was 1.96:1. Based on clinical history, imaging studies, and histopathologic examination, 43 HCCs (58%) occurred in cirrhotic livers. In 39 cases (53%), the underlying cause for HCC could not be determined, particularly for cases arising in noncirrhotic livers. Histopathologically, 34 HCCs (46%) were well differentiated, 32 (43%) were moderately differentiated, and 8 (11%) were poorly differentiated.

Strong cytoplasmic and membranous staining for GPC3 was observed in 36 HCCs (49%), among which 20 cases (56%) showed diffuse immunoreactivity **Image**

Table 1
Underlying Cause and Demographic Data for 74 Cases of Hepatocellular Carcinoma

Underlying Cause	No. (%) of Cases	Mean Age (Range), y	Sex (M/F)
Cirrhosis (n = 43)			
HCV hepatitis	12 (28)	66 (38-76)	11/1
HBV hepatitis	4 (9)	61 (43-78)	2/2
Hemochromatosis	2 (5)	63 (61-66)	1/1
Alcoholic hepatitis	7 (16)	64 (45-76)	7/0
Cryptogenic	18 (42)	61 (39-73)	13/5
Noncirrhotic (n = 31)			
HCV hepatitis	7 (23)	69 (59-85)	5/2
HBV hepatitis	1 (3)	56	1/0
Hemochromatosis	1 (3)	72	0/1
Autoimmune hepatitis	1 (3)	36	0/1
Unknown	21 (68)	67 (43-87)	9/12

HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 2
Underlying Cause and Demographic Data for 46 Cases of Liver Cirrhosis Without Hepatocellular Carcinoma

Underlying Cause	No. (%) of Cases	Mean Age (Range), y	Sex (M/F)
HCV hepatitis	30 (65)	50 (37-66)	24/6
HBV hepatitis	3 (7)	56 (48-67)	3/0
Alcoholic hepatitis	2 (4)	52 (51-54)	2/0
Autoimmune hepatitis	4 (9)	49 (44-58)	3/1
Cystic fibrosis	1 (2)	12	1/0
Cryptogenic	6 (13)	68 (52-80)	1/5

HBV, hepatitis B virus; HCV, hepatitis C virus.

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1 and the remaining 16 cases (44%) showed focal GPC3 expression **Image 2**. In contrast, none of the 46 cirrhotic livers exhibited positive GPC3 immunostaining **Table 3**. The nonneoplastic liver tissues (cirrhotic or noncirrhotic) that were present in the majority of the HCC biopsy specimens were also completely negative for GPC3 expression **Image 3**. No statistically significant differences in GPC3 expression were detected among the different grades of HCCs **Table 4** or between tumors with and without a cirrhotic background.

Discussion

Distinction between benign and malignant hepatocellular mass lesions or between hepatocellular and nonhepatocellular neoplasms can be challenging on liver needle biopsy specimens. A number of immunomarkers have been widely utilized to facilitate the distinction. However, the currently available immunomarkers have significant diagnostic limitations.[5,6] For example, Hep Par 1 does not discriminate benign from malignant hepatocytes and tends to be nonimmunoreactive in

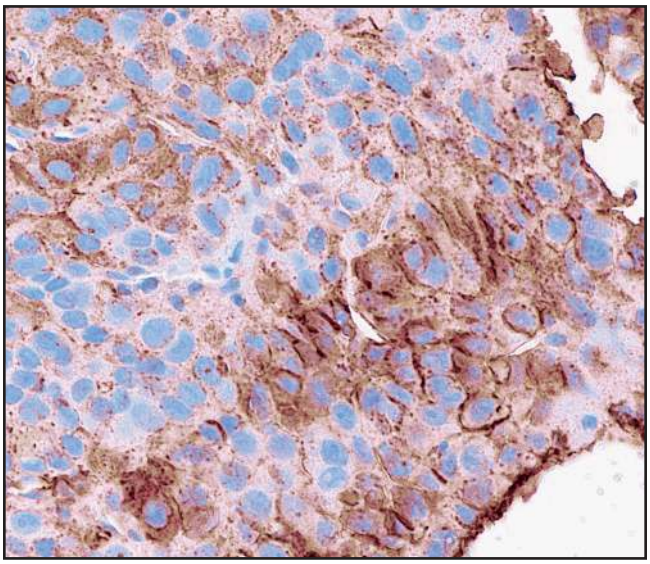

Image 1 Diffuse glypican 3 immunostaining in hepatocellular carcinoma in a needle biopsy specimen (×400).

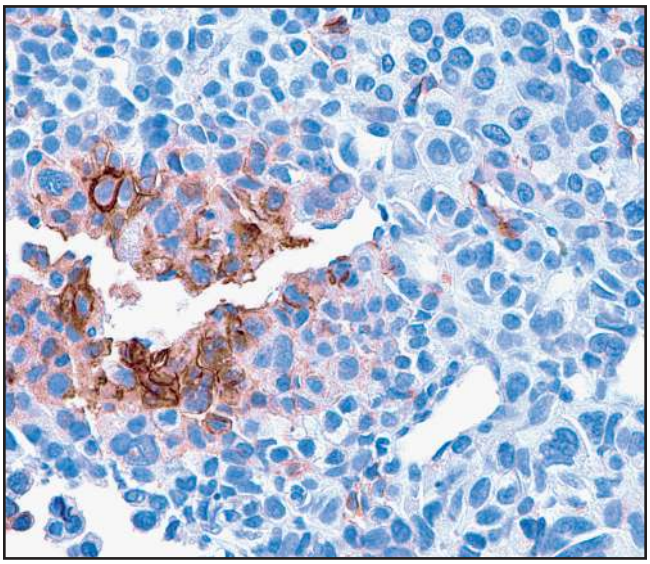

Image 2 Focal glypican 3 immunostaining in hepatocellular carcinoma in a needle biopsy specimen (×400).

Table 3
Summary of Immunohistochemical Findings of Glypican 3 Expression in HCC and Cirrhotic Livers in Needle Biopsy Specimens*

Immunoreactivity	HCC (n = 74)	Cirrhosis (n = 46)
Negative (<5%)	38 (51)	46 (100)
Focal (5%-50%)	16 (22)	0 (0)
Diffuse (>50%)	20 (27)	0 (0)

HCC, hepatocellular carcinoma.
* Data are given as number (percentage).

Table 4
Correlation of Glypican 3 Expression With Tumor Differentiation in Hepatocellular Carcinoma in Needle Biopsy Specimens

Tumor Differentiation	No. (%) of Cases			
	Negative	Focal	Diffuse	Total
Well	21 (62)	6 (18)	7 (21)	34
Moderate	13 (41)	8 (25)	11 (34)	32
Poor	4 (50)	2 (25)	2 (25)	8
Total	38 (51)	16 (22)	20 (27)	74

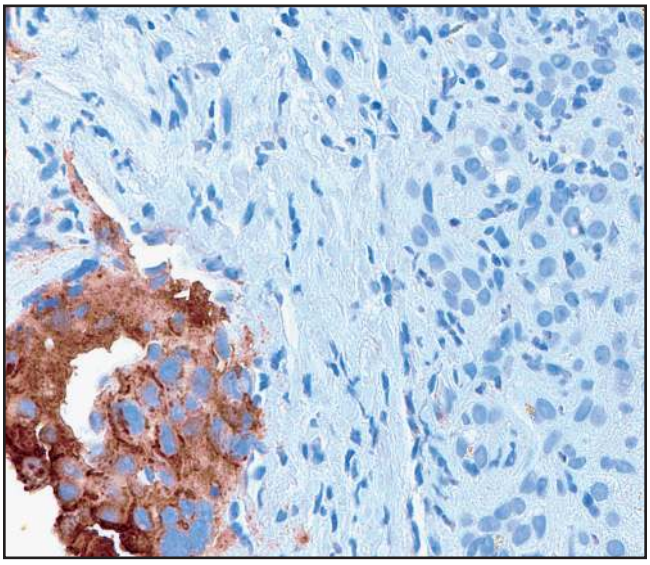

Image 3 Negative glypican 3 (GPC3) immunostaining in nonneoplastic hepatocytes (right). Note that the adjacent hepatocellular carcinoma cells (left) were strongly positive for GPC3 expression (×400).

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poorly differentiated HCCs. It is also occasionally expressed in nonhepatocellular neoplasms.[27,28] Similarly, polyclonal carcinoembryonic antigen does not discriminate benign from malignant hepatocytes and shows low sensitivity in poorly differentiated HCCs. In addition, its concurrent cytoplasmic immunoreactivity may obscure the interpretation of the canalicular staining pattern unique to HCCs.[6] In this study, we evaluated the diagnostic value of GPC3 immunostaining in a large number of needle biopsy specimens of HCCs and cirrhotic livers. Our data confirmed high specificity of GPC3 as a diagnostic marker of HCC on needle biopsy specimens.

There has been only 1 study that has examined GPC3 expression in liver lesions on needle biopsy specimens. In the study by Libbrecht et al,[23] 22 diagnostic needle biopsy specimens from focal liver lesions and 8 from nonlesional livers from 18 patients with cirrhosis were studied, and GPC3 expression was detected in 10 (83%) of 12 HCCs. None of the 8 biopsy specimens of nonlesional cirrhotic livers and none of the 6 dysplastic nodules and 2 focal nodular hyperplasias expressed GPC3. It is interesting that among the 10 GPC3-expressing HCCs, 7 exhibited focal immunoreactivity, including 3 biopsy specimens with fewer than 5% of tumor cells positively stained. Only 3 biopsy specimens showed positive staining in more than 66% of the tumor cells. These results are in accordance with our observations described in this report.

Our study showed that only one half of HCC needle biopsy specimens exhibited positive GPC3 immunoreactivity. This detection rate seems to be lower than rates reported by others using surgical resection specimens as the study materials. For example, Yamauchi et al[21] reported diffuse GPC3 staining in 47 (84%) of 56 HCCs. Di Tommaso et al[25] detected GPC3 expression in 39 (74%) of 53 HCCs. Even with 1-mm tissue microarray, which might simulate needle core biopsy specimens in some way, a 70% detection rate was reported by Wang et al.[22] One explanation is that GPC3 expression can be highly heterogeneous, even within the same tumor. This has been evidenced by the focal staining observed in this study and some previous studies by others.[16,23,24] In our own experience, focal GPC3 expression is not uncommon when immunostaining is performed on resection specimens. Specifically, among 111 HCCs we examined, a total of 84 tumors (75.7%) showed GPC3 immunoreactivity, but only 61 tumors (73%) exhibited a diffuse staining pattern with more than 50% of the tumor cells positively stained.[29]

It has been unclear whether GPC3 expression in HCC correlates with tumor differentiation. In the studies by Yamauchi et al[21] and Libbrecht et al,[23] the presence and the extent of GPC3 expression were not significantly associated with differentiation grade. However, the study by Di Tommaso et al[25] showed that the number of GPC3-immunoreactive cells statistically increased with HCC dedifferentiation. In addition, Wang et al[22] reported that GPC3 expression was more frequently detected in HCCs arising in cirrhotic livers than in livers without cirrhosis, but this difference became insignificant when tumor differentiation status was taken into consideration. Our data presented in this report failed to demonstrate a correlation of GPC3 expression with the degree of HCC differentiation or the presence of cirrhosis. Although it is conceivable that the differentiation status is difficult to determine on needle biopsy specimens and that poorly differentiated HCC is underrepresented with only 8 poorly differentiated tumors included in this study, lack of correlation with differentiation and cirrhosis was also observed in our study performed on resection specimens.[29]

Our data demonstrate that GPC3 is a useful diagnostic immunomarker to distinguish HCC from nonneoplastic hepatocytes, which can be reliably used in combination with other already available immunomarkers on liver needle biopsy specimens. Our findings emphasize the fact that GPC3 immunoreactivity can be focal, and thus negative immunostaining should not exclude the diagnosis of HCC in challenging biopsy specimens.

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