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The utility of Hepatocyte Paraffin 1 antibody in the immunohistological distinction of hepatocellular carcinoma from cholangiocarcinoma and metastatic carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and its diagnosis on routine stains is usually straightforward, except in some cases where there may be difficulty in distinguishing HCCs from metastatic carcinomas (MC) and cholangiocarcinomas (CC). Hepatocyte Paraffin 1 antibody (Hep Par 1) is a new monoclonal antibody which reacts with normal and neoplastic hepatocytes, and this study aims to determine its specificity and sensitivity in distinguishing hepatocellular carcinoma (HCC) from cholangiocarcinoma (CC) and metastatic carcinomas (MC). Hep Par 1 antibody was applied to 28 cases of HCC, 22 cases of MC from varying sites and 8 CCs, and produced a strong, diffuse, granular, cytoplasmic staining of all benign hepatocytes. 23 out of 28 cases of HCC showed heterogeneously positive staining for Hep Par 1 irrespective of their degree of differentiation, while 2 out of 8 cases of cholangiocarcinoma were positive for Hep Par 1, and all 22 cases of metastatic carcinoma were negative. The sensitivity and specificity of Hep Par 1 for HCC was 82.1 % and 93.3 % respectively; whereby the antibody was noted to show occasional false positivity in cases of cholangiocarcinoma and non-neoplastic bowel mucosa, while its variable staining in HCC produced false negative results in some small biopsies. Thus, Hep Par 1 should be used in a panel with other antibodies to obtain useful information in distinguishing HCC from CC and MC.

Key words: Hep Par 1 antibody, hepatocellular carcinoma, metastatic carcinoma, cholangiocarcinoma.

INTRODUCTION

Carcinomas of the liver may be classified as primary and secondary tumours. The most common neoplasms occurring in the liver are metastatic carcinomas (MC), which may arise from a primary in almost any site in the body, particularly the colon, lung and breasts. Primary carcinomas of the liver are mainly of 3 types: hepatocellular carcinoma (HCC) arising from hepatocytes, cholangiocarcinoma (CC) arising from bile duct epithelium, and mixed hepato-cholangiocarcinomas. HCC is more common and accounts for approximately 90% of primary liver cancers, while cholangiocarcinomas account for nearly 10% of cases. The mixed hepato-cholangiocarcinoma type is very uncommon.[1] While the more typical histological features allow distinction between HCC and metastatic adenocarcinoma on routine stains, this can be difficult in those HCCs with pseudoglandular formation, clear cell change or poor differentiation. A number of metastatic tumours, notably from the breast, endocrine pancreas, kidney and adrenal may mimic the trabecular, liver-like pattern of HCC, and primary cholangiocarcinomas may also present with this appearance. Distinction between these 3 conditions is very important because they differ in terms of their aetiology, epidemiology, management and prognosis. [1,2,3,4]

Immunohistochemistry is sought in these difficult cases, including reactivity for alpha-fetoprotein (AFP), alpha-1-antitrypsin, alpha-HCG, carcino-embryonic antigen (CEA), factor XIIIa, ferritin, albumin, and cytokeratins 8, 18, 7, 19 and 20.[5-17] In the past, the immunohistochemical diagnosis of hepatocellular carcinoma depended

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on the identification of canalicular polyclonal carcinoembryonic antigen (pCEA) positivity, the absence of staining for markers such as monoclonal CEA (mCEA) and the finding of alpha-fetoprotein (AFP) expression.[18] The absence of cytokeratins 7, 19, and 20 has also been shown to be characteristic of hepatocellular carcinoma, in contrast to cholangiocarcinoma and most metastatic adenocarcinomas.[3,19] However, many of these markers are not readily available in many laboratories, and their use has been met with varying success and none has been proven to be sensitive or specific for HCC.

In 1993, Wennerberg *et al* reported the development of a new monoclonal antibody designated as Hepatocyte Paraffin 1 (Hep Par 1), which was produced in mice using tissue from a failed allograft liver.[4] A single clone (OCH1E5.2.10) was isolated, which was specific for adult and fetal liver tissues. Hep Par 1 reacts with normal and neoplastic hepatocytes in routine formalin-fixed, paraffin-embedded material, producing a distinct granular, cytoplasmic staining of hepatocytes. This study aims to examine the specificity and sensitivity of Hep Par 1 antibody as a marker to distinguish hepatocellular carcinoma from cholangiocarcinoma and metastatic carcinomas.

MATERIALS AND METHODS

28 cases of hepatocellular carcinoma, 22 cases of metastatic carcinoma and 8 cases of cholangiocarcinoma were obtained from the surgical pathology files of the Pathology Department, HUKM as well as the Pathology Department, Hospital Selayang. For these cases, the paraffin-embedded tissue blocks were retrieved and relevant clinical, radiological and intra-operative data were sought from the respective case files. The paraffin embedded blocks were stained with H&E (haematoxylin and eosin) and Hep Par 1, then reviewed independently by two observers.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 3-4 micrometer thickness, mounted on sialinized slides and placed in the oven for 30 minutes. Sections were then de-paraffinized with 2 changes of xylene at 3 minutes each, and subsequently rehydrated in 4 changes of decreasing concentrations of alcohol at 3 minutes for each change. They were then rinsed in water. The slides were placed in the target retrieval solution in a microwave at 100°C for 20 minutes, then left to cool at room temperature for 20 minutes. They were washed in water, then placed in 3% hydrogen peroxide for 5 to 10 minutes, and washed in water again. The slides were then placed in Tris-Buffer Solution (TBS) for 3 changes at 3 minutes each. The excess TBS was wiped from the slides.

A drop of the primary antibody in an optimized 1:50 dilution (Hep Par 1/Hepatocyte antibody, clone OCH1E5 from DAKO) was added and left for 30 minutes, then washed in 3 changes of TBS at 3 minutes each. Detection was made with EnVision + (DAKO) which was placed for 30 minutes, then washed again in 3 changes of TBS at 3 minutes each. A drop of Di-amino-benzidine (DAB) was then placed for 7 minutes, after which it was rinsed in water. Counterstaining with Haematoxylin was done for 20-30 seconds, followed by immersion in water for 3 minutes for a blueing effect. The slides were then dehydrated in increasing concentrations of alcohol, and mounted.

Normal liver tissue was used as positive control for Hep Par 1, while negative control was done using the same tissue (normal liver), omitting the primary antibody

Morphologic evaluation

All the immunostained slides were reviewed by two independent observers. Positive reaction was defined as diffuse cytoplasmic staining with moderate to strong intensity involving >10% of tumour cells.

RESULTS

Hep Par 1 staining in non-neoplastic hepatocytes

Non-neoplastic hepatocytes within the control case of normal liver (as well as all para-tumourous areas within the retrieved cases), showed diffuse cytoplasmic staining with a distinct coarse granular pattern without canalicular accentuation. This staining was uniform, strong and detected in more than 90% of the non-neoplastic hepatocytes, although individual cells showed mildly varying densities of the granular staining.

Hep Par 1 staining in hepatocellular carcinoma

23 out of 28 HCCs showed positivity for Hep Par 1, with a heterogenous staining pattern. Highly variable percentages of positive tumour

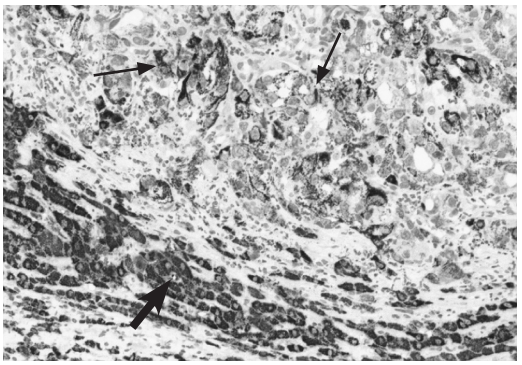

FIG. 1: Hep Par 1 staining in a case of hepatocellular carcinoma, illustrating the heterogeneity of staining with variable intensities (thin arrows). Some normal liver tissue is noted at the periphery (thick arrow) showing uniform, diffuse, strong positivity (x 100)

cells were identified in different cases (ranging from 20–100% positivity in different cases), with highly variable intensities of staining among individual tumour cells (Figure 1).

Correlation of the staining intensity vs. the degree of differentiation was attempted, but was ultimately difficult to assess. A few well-differentiated HCCs had a tendency to stain more strongly than some moderately-differentiated HCCs, which showed moderate intensity of staining, while one case of an apparently poorly-differentiated HCC was negative. However, other cases showed wide variations in intensities and percentages of positive cells, with many areas of better-differentiated HCCs showing negative reaction while lesser-differentiated adjacent areas were positive. One case of a resected, apparently well-differentiated HCC showed diffuse negativity.

Of the 5 cases that were negative, one showed positivity in <5% of tumour cells while two were from relatively small biopsy specimens exhibiting highly pleomorphic cells with areas of glandular formation in one and clear cell changes in the other. Another two resected specimens also showed negative staining for Hep Par 1, one of which showed mainly necrotic material with only small groups of viable tumour cells, while the other showed a typical appearance of HCC, albeit with many areas of glandular formations.

Hep Par 1 staining in metastatic carcinoma

All 22 cases of metastatic carcinoma (7 from colon, 6 from pancreas, 3 from gallbladder, 2 from duodenum, and 1 each from the lung, cervix, thyroid and adrenal) did not label for Hep Par 1.

Hep Par 1 staining in cholangiocarcinoma

Two of the 8 cases showed diffuse, moderate-to-strong staining for Hep Par 1 within tumour cells, with one case additionally showing strong positive staining within normal, mucin-secreting duodenal mucosa, but negative staining within adjacent Brunner's glands. Two other cases showed only focal (<10%) positivity and were subsequently designated as negative, while the remaining four cases showed negative staining throughout all the tumour cells.

All cases had confirmed primary tumour arising from the biliary tree via radiological and intra-operative findings.

DISCUSSION

While the more typical histological features such as resemblance to hepatocytes and production of bile allow the diagnosis of HCC on H&E and a few other routine stains, this may be made difficult by HCCs with pseudoglandular formation, clear cell change or poor differentiation. In addition, a number of metastatic tumours, notably from the breast, endocrine pancreas, kidney and adrenals may mimic the trabecular, liver-like pattern of HCC. Primary cholangiocarcinomas have also been known to present with this appearance.[1-4]

The primary purpose of this study was to examine the sensitivity and specificity of Hep Par 1 antibody as a marker to distinguish HCC from metastatic carcinoma and cholangiocarcinoma. Hep Par 1 gave a sensitivity of 82.1 %, labeling 23 out of 28 HCCs with moderate-to-strong intensity in more than 10% of tumour cells (Table 1). These results are comparable to those in a recent study by Fan *et al*[20] on 676 tumours using tissue microarrays and conventional tissue sections, whereby 95% of HCCs showed staining at any level, versus 79% when restricted to >5% staining. Similarly, in the original paper describing the antibody, Wennerberg *et al*[4] found 37 of 38 HCCs to be positive, while Leong *et al*[3] found 30 out of 32 HCCs to be positive, although in both these studies even cases with focal or rare positivity were considered positive.

In our study, out of the 5 HCC cases that were negative, 3 were from biopsy specimens, attesting to the possibility of false negative staining in small tissue samples. The first case showed very focal staining in less than 10% of tumor cells, and was designated as negative in

TABLE 1: Sensitivity and specificity of Hep Par 1 for Hepatocellular Carcinoma

HepPar1 immunostaining	HCC	Control (MC + CC)	Total
Positive	23	2	25
Negative	5	28	33
Total	28	30	58

Sensitivity = *TP / TP + FN = 23 / 23 + 5 = 82.1 %
Specificity = *TN / TN + FP = 28 / 28 + 2 = 93.3 %
Positive Predictive Value = *TP / TP + FP = 23 / 23 + 2 = 92.0 %

keeping with the criteria for positivity in our study, while the second case showed clear cell changes within the cytoplasm giving rise to an additional possibility of false negative staining in variants of HCC. Leong *et al*[3] and *Wennerberg et al*[4] also reported false negative staining in variants of HCC, although in both cases it was a sclerosing variant of HCC. The third negative case showed highly pleomorphic cells with areas of glandular formation, giving rise to possibility of negative staining as a result of poor differentiation. The fourth and fifth negative cases were from resection specimens. The fourth case showed extensive areas of necrosis with only a few areas of viable cells which were negative, again attesting to the possibility of false negative staining in small tissue samples, while the fifth case appeared to resemble a well-differentiated HCC on H&E staining, albeit with some areas of glandular formation.

Attempts at correlating staining intensities vs. the degree of differentiation of HCC were made, but the heterogenous staining characteristics of Hep Par 1 (Figure 1) often made this difficult to assess. In some cases, it was noted that a few well-differentiated HCCs had a tendency to stain more strongly than some moderately-differentiated HCCs, which showed moderate intensity of staining, while one case of an apparently poorly-differentiated HCC was negative. However, other cases showed many areas of better-differentiated HCCs with negative reaction while lesser-differentiated adjacent areas were positive. One case of a resected, apparently well-differentiated HCC showed diffuse negativity. These indeterminate findings correspond generally to the collective findings of previous studies, some of which observed that poorly-differentiated HCCs were more likely to be negative for Hep Par 1 than better differentiated cases[19,20,21,22] while other studies observed no such correlation[3].

8 cases of cholangiocarcinoma were evaluated for this study, out of which 2 cases showed strong, diffuse positive staining while another 2 cases showed only focal (< 10%) staining and were subsequently considered negative. All of the positivity was observed in well-differentiated mucin-secreting columnar cells, while lesser differentiated areas and glands with scanty mucin production were negative. These findings are in keeping with that of Leong *et al*[3] who found 4 positive cases out of 32 CCs (12.5%), the immunoreactivity similarly being observed in well-differentiated mucin-secreting columnar tumour cells. Wennerberg *et al*[4] also reported positivity in 2 out of 35 cases of CC (5.7%). A higher rate of positivity in cholangiocarcinoma in our study (2 out of 8, or 25%) could be due to the smaller sample size, which was due to the lack of cases of cholangiocarcinoma. However, the presence of this occasional positivity should not be surprising considering the common progenitor cell of HCCs and CCs[3].

The 2 cases of cholangiocarcinoma that were diffusely positive were composed of well-differentiated, mucin-secreting malignant glands, indicating that Hep Par 1 stains positively in cases of cholangiocarcinoma with well-differentiated, mucin-secreting glands. The other cases of cholangiocarcinoma which stained negatively for Hep Par 1 were composed mainly of irregular looking glands with scanty mucin production. One case also showed positive staining of normal mucin-secreting duodenal mucosa cells for Hep Par 1, which raises the possibility of a similar positive reaction in duodenal neoplasms. Therefore, Hep Par 1 positivity in tumours which are composed of mucin-secreting glands needs to be interpreted with caution, for they may not be HCCs, but cholangiocarcinomas or duodenal neoplasms. This finding of positive reaction for

Hep Par 1 within normal duodenal mucosa had also been noted by Leong *et al*[3] and Wennerberg *et al*[4] previously.

All 22 cases of metastatic carcinoma to the liver from various sites gave diffusely negative reactions towards Hep Par 1. Internal controls of normal liver tissue within these specimens nonetheless showed strong, uniform and diffuse positivity. These findings contrast those from other studies which showed occasional positivity for Hep Par 1 in cases of metastatic carcinomas from the pancreas, colon and stomach. However, Leong *et al*[3] also reported negativity in all of their 13 cases of metastatic carcinoma.

Despite the complete negativity of Hep Par 1 for metastatic carcinomas, a truly specific marker is extremely rare, and the finding of occasional cholangiocarcinomas and non-hepatic tissues (namely normal bowel mucosa) staining positively for Hep Par 1 is not surprising. In this study, Hep Par 1 showed a specificity rate of 93.3 %.

In summary, our study showed 82.1 % sensitivity and 93.3 % specificity of Hep Par 1 in distinguishing HCC from cholangiocarcinoma and metastatic carcinoma, with a positive predictive value of 92.0 %. The main utility of this antibody appears to be in differentiating tumours of hepatocytic origin from those of non-hepatocytic origin (whether primary or metastatic). Hep Par 1 proved useful in differentiating HCCs from metastatic carcinomas and CCs, but the heterogeneity of staining (varying percentages of positive cells and variable intensities of staining) in cases of HCC resulted in occasional cases of false negativity, most noticeably in small tissue samples. False negativity could also be attributed to cases of HCC with poor differentiation and variants of HCC. False positivity was noted in some cases of cholangiocarcinoma and non-neoplastic bowel mucosa, similar to findings from previous studies. Thus, the use of Hep Par 1 in conjunction with a cytokeratin panel (CK7, CK 18, CK19, CK20) and other positive (i.e. AFP, pCEA, CD10) and negative (i.e. EMA, monoclonal CEA, CD 15) markers of HCC is recommended for the differential diagnosis of HCC in difficult cases.

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REFERENCES

1. Robbins SL, Cotran RS, Kumar V, Collins T : Liver and biliary tract. In: Robbins Pathologic Basis of Disease. 4th edition, W.B. Saunders Co.; 1989: 958-962.
2. Juan Rosai: Liver. In: Ackerman's Surgical Pathology, Volume 1. 8th Edition. Mosby; 1996: 903-911.
3. Leong AS-Y, Sormunen RT, Tsui WMS, Liew CT. Hep Par 1 and selected antibodies in the immunohistological distinction of hepatocellular carcinoma from cholangiocarcinoma, combined tumours and metastatic carcinoma. Histopathology. 1998; 33: 318-24.
4. Wennerberg AE, Nalesnik MA, Coleman WB. Hepatocyte paraffin 1: A monoclonal antibody that reacts with hepatocytes and can be used for differential diagnosis of hepatic tumours. Am J Pathol. 1993; 143: 1050-4.
5. Thung SN, Gerber MA, Sarno E, Poper H. Distribution of 5 antigens in hepatocellular carcinoma. Lab Invest 1979; 41: 101-5.
6. Brumm C, Sholze C, Charles K et al. The significance of alpha-fetoprotein and other tumour markers in differential immunocytochemistry of primary liver tumours. Histopathology 1989; 14: 503-13.
7. Hurlimann J and Gardiol O. Immunohistochemistry in the differential diagnosis of liver carcinoma. Am J Surg Pathol 1991; 15(3): 280-8.
8. Ferrandez-Izquierdo A, Llombart-Bosch A. Immunohistochemical characterization of 130 cases of primary hepatic carcinomas. Pathol Res Pract 1987; 182: 783-91.
9. Parvin G, Muhrad N, Jooge AS, Azorides RM. Histologic markers in primary and metastatic tumours of the liver. Cancer 1988; 62: 1994-8.
10. Fucich LF, Cheles MK, Thung SN, Gerber MA, Marrogi AJ. Primary vs metastatic hepatic carcinoma. An immunohistochemical study of 34 cases. Arch Pathol. Lab. Med 1994; 118: 927-30.
11. Ma CK, Zarbo RJ, Frierson Jr., HF Lee MW. Comparative immunohistochemical study of primary and metastatic carcinomas of the liver. Am J Clin Path 1993; 99: 551-7.
12. Johnson DE, Herndier BG, Medeiros LJ, Warnke RA, Rouse RV. The diagnostic utility of the keratin profiles of hepatocellular carcinoma and cholangiocarcinoma. Am J Surg Pathol 1988; 12(3): 187-7.
13. Balaton AJ, Nehama-Sibony M, Gotheil C. Distinction between hepatocellular carcinoma, cholangiocarcinoma, and metastatic carcinoma based on immunohistochemical staining for carcinoembryonic

antigen and for cytokeratin 19 on paraffin sections. J Pathol 1988; 156: 305-10.
14. Koelman IJ, Nap M, Huitema S et al. Hepatocellular carcinoma, adenoma and focal nodular hyperplasia. Comparative histopathologic study with immunohistochemical parameters. Arch Pathol Lab Med 1986; 110: 1035-40.
15. Murukata LA, Ishak KG, Nzeako UC. Clear cell carcinoma of the liver: a comparative immunohistochemical study with renal cell carcinoma. Mod Pathol 2001; 13: 874-81.
16. Ishikura H, Kishimoto T, Andachi H, Kakuta Y, Yoshiki T. Gastrointestinal hepatoid adenocarcinoma: venous permeation and mimicry of hepatocellular carcinoma, a report of four cases. Histopathology 1997; 31: 47-54.
17. Johnson DE, Powers CN, Rupp G et al. Immunocytochemical staining of fine needle aspiration biopsies of the liver as a diagnostic tool for hepatocellular carcinoma. Mod Pathol 1992; 5: 117-23.
18. Lamps, LW, Folpe AL. The diagnostic value of hepatocyte paraffin antibody 1 in differentiating hepatocellular neoplasms from nonhepatic tumours: A review. Adv Anat Pathol 2003; 10(1): 39-43
19. Chu PG, Ishizawa S, Wu E, Weiss LM. Hepatocyte antigen as a marker of hepatocellular carcinoma. An immunohistochemical comparison to carcinoembryonic antigen, CD10, and alpha-fetoprotein. Am J Surg Pathol 2002; 26: 978-88.
20. Fan, Zhen M.D.; van de Rijn, Matt M.D., Ph.D.; Montgomery, Kelli; Rouse, Robert V. M.D. Hep Par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumours tested using tissue microarrays and conventional tissue sections. Mod Pathol 2003; 16(2): 137-44.
21. Minervini MI, Demetris AJ, Lee RG, Carr BI, Madariaga J, Nalesnik MA. Utilization of hepatocyte-specific antibody in the immunocytochemical evaluation of liver tumours. Mod Pathol 1997; 10: 686-92.
22. Kumagai I, Masuda T, Sato S, Ishikawa K. Immunoreactivity to monoclonal antibody, Hep Par 1, in human hepatocellular carcinomas according to histopathological grade and histologic pattern. Hepatol Res 2001; 20: 312-39.