DIAGNOSTIC UTILITY OF THE HEP PAR-1 TO DIFFERENTIATE HEPATOCELLULAR CARCINOMA FROM METASTATIC CARCINOMA ON CONVENTIONAL TISSUE SECTIONS

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Summary

Hepatocellular carcinoma (HCC) is the most common primary liver neoplasm, its differentiation from other malignant tumors being sometimes difficult without a reliable immunohistochemical marker for hepatocellular differentiation. Hep Par-1 (Hepatocyte paraffin-1), an antibody specific for hepatocytes, reacts with normal and neoplastic hepatocytes. We assessed the expression of Hep Par-1 on tissue sections included in paraffin, from patients clinically diagnosed with liver tumors submitted to curative resection (22 cases), on liver tumor fragments obtained by laparoscopy (six cases) and others obtained at necropsy (five cases). We used the anti-human hepatocyte mouse monoclonal antibody, Hepatocyte Ab-1, clone OCH1E5, diluted 1:40, using the EnVision technique. We obtained: 1) positive Hep Par-1 expression in 18 of the 21 cases of HCC (85.71%), dependent of the differentiation degree (positive in all 16 G1-G2 HCCs and negative in 5 G3-G4 HCCs); 2) heterogenic or patchy Hep Par-1 distribution in tumor tissue and a uniform staining of the non-neoplastic liver surrounding the tumor. We did not note Hep Par-1 immunostaining in non-hepatocytic tumors, except one case of gastric carcinoma and cholangiocarcinoma (CC) with focal expression. Hep Par-1 is a useful marker in the differential diagnosis of HCC, but not entirely specific; its usage in association with other markers for hepatocellular differentiation can help in the correct diagnosis of HCC and its differentiation from CC and metastatic liver tumors.

Key words: hepatocellular carcinoma, metastatic carcinoma, Hep Par-1

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver neoplasm, often difficult to differentiate from cholangiocarcinoma and metastatic carcinomas (Shiran et al., 2006) on usual histological sections. Distinguishing HCC from other malignant tumors using immunohistochemistry (IHC) staining technique was limited by the lack of a reliable IHC marker for hepatocellular differentiation.

Anti-alpha fetoprotein (anti-AFP) and anti-polyclonal carcinoembryonic antigen (anti-CEAp) antibodies are used as positive markers for HCC. AFP has a low sensitivity, ranging between 17–61.5% and sometimes it can be expressed in other carcinomas, such as cholangiocarcinomas (CC), gastric and colonic carcinomas. Although it is quite specific for hepatocellular differentiation, canalicular staining with anti-pCEA or CD10 was observed in 15–80% of HCC; the canalicular staining pattern of these antibodies is often difficult to interpret, limiting their use in the diagnosis of HCC.

The recently commercially available Hep Par 1 (Hepatocyte paraffin-1) antibody (clone OCH1E5.2.10; OCH1E5) reacts with normal and neoplastic hepatocytes. This
antibody specific for hepatocytes, described in 1993 by Wennerberg et al. is considered a sensitive and specific marker for hepatocytic differentiation on HCC sections included in paraffin. It can be useful in differentiating clear cell HCC from other clear cell malignancies and embryonic-type hepatoblastoma from small cell tumors of childhood (Mivervini et al., 1997; Murakata et al., 2001; Fasano et al., 1998); it is used in distinguishing HCC with pseudoglandular differentiation and nuclear pleomorphism from CC and metastatic liver tumors.

Among the Hep Par-1 positive non-hepatocytic tumors, there are the ovarian carcinoma with hepatoid differentiation (Pitman et al., 2004) and signet-ring cell gastric carcinoma, that usually express a diffuse cytoplasmic staining, while mammary and colorectal cancers are Hep Par-1 negative (Chu and Weiss, 2004).

Various published studies affirm that Hep Par-1 antibody has a specificity of 79% and a high sensitivity for liver differentiation (Morrison et al., 2002; Lau et al., 2002; Wennerberg et al., 1993).

The antibody is directed against mitochondrial antigens from hepatocytes and is expressed through a characteristic granular cytoplasmic staining (Morrison et al., 2002; Lau et al., 2002; Wennerberg et al., 1993). The skin, smooth and skeletal muscles, mesothelium, lymph nodes, spleen, lung, breast, esophagus, stomach, intestine, pancreas, biliary tract, kidneys, urinary bladder, adrenal gland, prostate, endometrium and ovary are almost always negative. In some cases we observed an intense focal staining in the mucosa of the small bowel (Mivervini et al., 1997; Murakata et al., 2001; Fasano et al., 1998).

**Material and methods**

For defining the utility of Hep Par-1 antibody in the differentiation of HCC from cholangiocarcinoma and metastatic liver tumors, we assessed the Hep Par-1 expression (clone OCH1E5) on conventional tissue sections from paraffin blocks from 22 patients clinically diagnosed with liver tumors (primary or metastatic) who suffered curative liver resection. In our study we also included liver tumor fragments obtained by laparoscopy (six cases) and others at necropsy (five cases).

In this study we did not use biopsy material. All sections included both tumor tissue and surrounding non-tumor liver tissue. In all cases, clinical diagnosis was/or not confirmed on conventional histological sections colored with HE.

For the study of hepatocytic differentiation we used the anti-human hepatocyte mouse monoclonal antibody, Hepatocyte Ab-1, clone OCH1E5, using the EnVision technique. Sections five-micrometer-thick obtained from conventional paraffin tissue blocks were deparaffinized in xylene and rehydrated in graded series of ethanol. For epitope retrieval, deparaffinized sections were treated by microwave boiling in 10 mmol/L buffer citrate (pH 6.0) for 10-20 minutes and then incubated with primary Hepatocyte Ab-1 antibody in 1:40 dilution for 30 minutes at room temperature (clone OCH1E5, DAKO, Carpinteria, CA). Detection was made with EnVision+ (DAKO), a biotin-free detection system with a secondary antibody that links covalently to dextrose polymers that are not coated with peroxidase. The sections were stained manually, application of the chromogen (DAB-3.3 diaminobenzidine) being followed by counterstaining with hematoxylin for 2-3 minutes. Surrounding non-tumor hepatocytes were used as internal positive control. For the positive control of the reaction we included a hepatocarcinoma Hep Par-1 positive, and for the negative control the buffer substituted the primary antibody. After treating the sections with Hep Par-1 antibody, we obtained a distinct staining pattern: granular cytoplasmic staining in shades of brown and occasionally with ring-like disposition, with diffuse staining of hepatocyte cytoplasm, without canalicular accentuation. In the normal
liver, the antibody stained the hepatocytes intensely, diffusely and granular, without a preferential expression, but with a lower staining intensity in compressed hepatocytes surrounding closely the tumor. We did not observe positive staining in biliary ducts or non-hepatocytic cells. We considered as Hep Par-1 positive the cases where tumor cells presented cytoplasmic granulations in shades of brown.

Results

The 21 liver carcinomas (16 cases surgically resected and 5 cases obtained at necropsy) were grouped according to the Edmondson-Steiner classification system in well differentiated (grade I carcinomas – 2 cases), moderately differentiated (grade II carcinomas – 14 cases) and poorly differentiated (grade III or IV carcinomas – 5 cases).

All 33 liver tumors, including 21 HCCs, were evaluated for staining with Hepatocyte Ab-1, the staining pattern obtained being cytoplasmic granular (Fig. 1). We considered as focal immunostaining the cases with rare positive cells (less than 5%) and a significant clear immunoreaction if more than 5% of hepatocytes stained.

We observed positive Hep Par-1 expression in 18 of the 21 cases of HCC. Three HCCs positively stained had areas of focal immunostaining in less than 5% of hepatocytes; two of these tumors were initially negative and presented focal Hep Par-1 expression when staining was repeated on sections from the same tumor.

HCCs presented heterogenic positive staining for Hep Par-1, dependent on their degree of differentiation (Table 1). The completely negative cases were poorly differentiated/anaplastic G3-G4 HCCs (Fig. 2). All 14 moderately differentiated HCCs were positive, including three cases with multifocal staining.

Table 1. Hep Par-1 expression according to the degree of HCC.

<table>
<thead>
<tr>
<th>HCC</th>
<th>Hep Par-1 (+)</th>
<th>Hep Par-1 (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grad 1 (n=2)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Grad 2 (n=14)</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Grad 3-4 (n=5)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total (n=21)</td>
<td>18</td>
<td>3</td>
</tr>
</tbody>
</table>

We noted uniform, strong and diffuse Hep Par-1 expression in HCC with acinar/pseudoglandular (Fig. 3) and trabecular (Fig. 4) pattern, but not in areas with clear cells (Fig. 5), fat deposits and bile secretion (Fig. 6). As a whole, immunostaining of HCC with Hepatocyte Ab-1 had a heterogenic or patchy distribution, unlike the uniform staining of the non-neoplastic liver tissue surrounding the tumor (Fig. 7).
Six of the 22 cases subjected to curative liver resection proved to be metastatic liver tumors (Table 1), developed on one case of malignant melanoma, one well differentiated neuroendocrine carcinoma of the pancreas, one mucosecretory adenocarcinoma of the cecum, one pulmonary carcinoma, one leiomyosarcoma and one case of intestinal non-hodgkin malignant lymphoma. Of the six liver tumors obtained by laparoscopy, two cases were suspected to be cholangiocarcinomas and the rest were: adenocarcinomas (2 cases), one carcinoid tumor and an infiltrative ductal carcinoma.

In what concerns the non-hepatocellular tumors, only one case of cholangiocarcinoma and the gastric carcinoma presented positive Hep Par-1 expression, with focal immunostaining in less than 5% of the cells. We did not observe immunostaining in the pulmonary...
Discussion

EnVision+ is a biotin-free detection method that uses a secondary antibody that is covalently linked to dextrose polymers coated by peroxidase molecules. Tissue sections analyzed with EnVision+ presented a clear background after staining.

The results obtained after staining with Hep Par-1 can be compared with the ones reported in literature. Our results show that Hep Par-1 is a sensitive marker for hepatocytic differentiation. 18 of the 21 HCCs (85.71%) presented positive Hep Par-1 expression, with heterogenic immunostaining dependent on their degree of differentiation (Table 1). The poorly differentiated/anaplastic G3-G4 HCCs were completely negative, while all 14 moderately differentiated HCCs were positive.

In HCC with acinar/pseudoglandular and trabecular pattern we observed uniform, strong and diffuse Hep Par-1 expression, while areas with clear cells, fat deposits and bile secretion stained weakly and focally. As a whole, we obtained positive Hep Par-1 immunostaining with heterogenic or patchy distribution in tumor areas, and a uniform staining of the non-neoplastic liver surrounding the tumor.

Hepatocyte Ab-1 (Hepatocyte paraffin-1 or Hep Par-1) monoclonal antibody reacts with normal liver tissue and HCC, showing high specificity. Chu and colab. finds Hep Par-1 immunoreactivity in 92% of HCCs, with granular cytoplasmic staining pattern (Chu et al., 2002). The authors consider that Hep Par-1 is a relatively specific marker for HCC and correlate its expression with the nuclear degree (positive Hep Par-1 immunostaining in all HCCs with nuclear degree 1 and 2, in 84% of degree 3 HCCs and 50% of degree 4 tumors) and the pattern of growth (positive Hep Par-1 immunoreaction in 98% of HCCs with trabecular, pseudoglandular and sclerosing pattern and in 81% of HCCs with solid pattern).

Many authors (Minervini et al., 1997; Chu et al., 2004) noted a higher probability of finding negative Hep Par-1 immunoreaction in poorly differentiated than in well differentiated HCCs, while other studies did not observe such a correlation. In our study, the completely negative cases were poorly differentiated HCCs. All 14 moderately differentiated HCCs were positive, 3 of them presenting heterogenic, multifocal staining. These aspects support the affirmations saying that poorly differentiated HCCs tend to lose their immunoreactivity for Hep Par-1. After assessing the Hep Par-1 immunoreactivity in HCC (on pieces obtained at necropsy) according to the degree of differentiation and histological pattern, Kumagai describes the reduction of Hep Par-1 expression parallel with the decrease of tumor differentiation, suggesting that Hep Par-1 is useful as marker for HCC diagnosis and differentiation (Kumagai et al., 2001).

The heterogenic and sometimes focal staining pattern for HCC can lead to false negative results, especially on biopsy material.

Although Hep Par-1 is a marker with high specificity for HCC, it also stains frequently gastric carcinomas. Fan and colab., (Fan et al., 2003) obtains Hep Par-1 staining in 47% (16/34) of gastric carcinomas, with >5% staining in 35% of cases. Poorly differentiated carcinomas or with signet-ring cells stained more frequently and stronger than the well differentiated ones. Wennerberg analyzed 10 high degree gastric carcinomas or with signet-ring cells, obtaining immunostaining in 3 cases (Wennerberg et al., 1993). In other study, the focal Hep Par-1 staining was observed in 5 of 6 gastric carcinomas with “hepatoid” differentiation, the staining of these tumors being assigned to areas with hepatocellular differentiation, marked
through AFP and pCea positivity (Maitra et al., 2001).

Chu reported staining of 2/12 conventional gastric carcinomas, as well as 2/2 carcinomas with hepatoid differentiation (Chu et al., 2002). Other authors observed frequent Hep Par-1 staining of conventional gastric carcinomas, independent of “hepatoid differentiation”, these results limiting its utility because metastatic gastric adenocarcinoma often needs diagnostic differentiation with a liver tumor obtained by biopsy (Fan et al., 2003).

Conclusions

We support the allegation that Hep Par-1 is a useful marker in the differential diagnosis of HCC, but not entirely specific, positive Hep Par-1 immunostaining being observed occasionally in gastric carcinomas and cholangiocarcinoma. Using Hep Par-1 is association with other positive and negative markers for hepatocellular differentiation can help in the correct diagnosis of HCC.

References

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