Expression of Hepatocyte Antigen in Small Intestinal Epithelium and Adenocarcinoma

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Upon completion of this activity you will be able to:

- describe the pattern of Hep Par 1 immunoreactivity in nonneoplastic small intestinal and colonic epithelium.
- define the usefulness of Hep Par 1 immunostaining in the differential diagnosis of various hepatocellular and nonhepatocellular neoplasms.

Abstract

Hepatocyte antigen is recognized by antibody Hep Par 1, a widely used diagnostic immunomarker for hepatocellular carcinoma and tumors with hepatoid differentiation. Hepatocyte antigen expression has also been detected in nonneoplastic small intestinal epithelium, but expression in small intestinal adenocarcinoma has not been well investigated.

We immunohistochemically examined 39 nonampullary small intestinal adenocarcinomas for hepatocyte antigen expression; 34 cases contained normal-appearing nonneoplastic small intestinal mucosa on the same tissue sections. In 30 cases (88%), the nonneoplastic mucosa exhibited granular cytoplasmic Hep Par 1 staining exclusively in the epithelium. Only 9 small intestinal adenocarcinomas (23%) showed focal positive cytoplasmic staining. Nonneoplastic colonic epithelium was negative for Hep Par 1. In 31 colorectal adenocarcinomas, 3 (10%) showed positive staining for Hep Par 1 (1 diffuse, 2 focal), a frequency not different from that for small intestinal adenocarcinomas (P = .2467). Tumors expressing hepatocyte antigen did not exhibit evidence of hepatoid differentiation and were histologically indistinguishable from hepatocyte antigen-negative tumors.

Hepatocyte antigen immunoreactivity is selectively expressed in nonneoplastic small intestinal enterocytes but not in colonocytes, suggesting a potential physiologic role in intestinal biology. Whether the loss of antigen expression in a large number of small intestinal adenocarcinomas serves a role in small intestinal tumorigenesis remains to be investigated.

Hepatocyte antigen is a mitochondrial-associated epitope recognized by a monoclonal antibody, hepatocyte paraffin 1 or Hep Par 1, which was generated using tissue extracts prepared from a formalin-fixed failed allograft liver.1 Despite its diagnostic limitation in that it does not distinguish benign from malignant hepatocellular lesions, it has emerged as a frequently used immunomarker in surgical pathology to help separate hepatocellular mass lesions, particularly hepatocellular carcinoma, from nonhepatocellular neoplasms such as intrahepatic cholangiocarcinoma and metastatic carcinomas.2,3 Numerous studies have shown that Hep Par 1 antibody is highly sensitive in the detection of hepatocellular carcinoma, with a detection rate of more than 90% in most series.1,4-10 Only a few studies have reported a lower detection rate, ranging from 66% to 82%.11-13 However, Hep Par 1 is not entirely specific. In addition to being frequently detected in extrahepatic hepatoid adenocarcinomas,14 Hep Par 1 immunoreactivity has been occasionally demonstrated in nonhepatocellular neoplasms that lack overt hepatoid differentiation.1,6,7,9,10,13,15

Surprisingly, the antigen recognized by Hep Par 1 remained elusive for more than a decade until recently. By using immunoprecipitation, mass spectrometry, and database search, Butler et al16 demonstrated that the peptide sequences of the protein immunoprecipitated by Hep Par 1 matched that of carbamoyl phosphate synthetase 1 (CPS1), a rate-limiting enzyme of the urea cycle, with a very high degree of probability. While this discovery may satisfy the curiosity of pathologists and biomedical researchers, it raises a series of intriguing questions about the physiologic and pathogenetic roles of the protein.17 This is so because even in normal human tissues, Hep Par 1 immunoreactivity is not entirely restricted to hepatocytes; it is also detected in small intestinal...
Immunohistochemical Staining

Immunohistochemical staining was performed using the DAKO autostainer (DAKO, Carpinteria, CA). Briefly, 4-µm sections from formalin-fixed, paraffin-embedded tissue blocks were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide for 10 minutes to inhibit endogenous peroxidase. Following heat-induced epitope retrieval in 10 mmol/L citrate buffer at pH 6.0 for 30 minutes, the slides were incubated with a mouse monoclonal antibody specific for human hepatocyte antigen (clone OCH1E5) obtained from DAKO with a dilution of 1:200 for 45 minutes at room temperature. The immunoreaction was performed using the mouse EnVision+ detection system that contained biotin-free horseradish peroxidase–labeled polymers (DAKO). The staining was visualized using 3,3′-diaminobenzidine substrate–chromogen solution and counterstained with hematoxylin. For each batch of staining, a negative control sample was included in which the antigenicity would be preserved during small intestinal carcinogenesis similarly to that seen in hepatocarcinogenesis.

Statistical Analysis

Statistical analysis was performed by using the 2-tailed Fisher’s exact test or the χ² test with Yates continuity correction. A P value of less than .05 was considered statistically significant.

Results

The patients with primary small intestinal adenocarcinoma ranged in age from 33 to 84 years (mean, 63 years; median, 68 years). Of the patients, 25 were men and 14 were women (M/F ratio, 1.8:1). Three cases were associated with Crohn disease, 1 with Gardner syndrome, and 1 with celiac disease. No known risk factors were identified in the remaining 34 cases. At the time of surgical resection, 2 cases were stage I, 17 stage II, 8 stage III, and 12 stage IV. Nine cases were well differentiated, 20 moderately differentiated, and 10 poorly differentiated. None of the patients with small intestinal or colorectal adenocarcinoma received preoperative neoadjuvant chemoradiation therapy.

Of the 39 cases of small intestinal adenocarcinoma, 34 contained normal-appearing nonneoplastic small intestinal mucosa on the same tissue sections, of which 30 (88%) showed granular cytoplasmic staining for Hep Par 1 exclusively in the epithelium Image 1A. The staining was diffuse in 21 cases (70%) and focal in 9 cases (30%). The immunoreactivity tended to be stronger in villi and weaker in deep crypts and was not detected in microvilli Image 1B. In contrast, the normal-appearing nonneoplastic colonic epithelium present in all 31 cases of colorectal adenocarcinoma was completely negative for Hep Par 1 Image 1C. These data are summarized in Table 1.

Table 2 summarizes the findings of Hep Par 1 immunostaining in small intestinal and colorectal adenocarcinomas. Of 39 small intestinal adenocarcinomas, only 9 (23%) showed positive staining. This finding was in marked contrast with the frequency of positive Hep Par 1 staining in nonneoplastic small intestinal epithelium (P < .0001). In addition, all 9 tumors showed a focal staining pattern Image 2A, while the corresponding nonneoplastic epithelium exhibited a diffuse staining pattern in 6 cases (67%). In only 2 cases was positive Hep Par 1 staining detected in tumor cells but not in nonneoplastic epithelium. In the 30 cases that showed positive Hep Par 1 immunoreactivity, complete loss of Hep Par 1 immunoreactivity was observed in 23 corresponding adenocarcinomas (77%) Image 2B.
As shown in Table 2, loss of Hep Par 1 immunoreactivity in small intestinal adenocarcinomas did not seem to be site-specific. Although duodenal adenocarcinomas seemed more likely to preserve Hep Par 1 antigenicity, as seen in 3 of 5 cases, there was no statistically significant difference when compared with jejunal (P = .0624) and ileal (P = .5581) adenocarcinomas. Loss of Hep Par 1 immunoreactivity was noted in all 5 tumors that were associated with Crohn disease, Gardner syndrome, and celiac disease.

Of the 31 colorectal adenocarcinomas, 3 (10%) showed positive Hep Par 1 immunostaining, 1 diffuse Image 2C and 2 focal. The frequency of Hep Par 1 positivity in colorectal adenocarcinomas was similar to that in small intestinal adenocarcinomas (P = .2470). In both types of adenocarcinoma,

<table>
<thead>
<tr>
<th>Immuneactity</th>
<th>Duodenum (n = 4)</th>
<th>Jejunum (n = 19)</th>
<th>Ileum (n = 5)</th>
<th>Unspecified (n = 6)</th>
<th>Total (n = 34)</th>
<th>Colorectum (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>Focal</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Diffuse</td>
<td>3</td>
<td>11</td>
<td>3</td>
<td>4</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Positive (%)</td>
<td>100</td>
<td>89</td>
<td>80</td>
<td>83</td>
<td>88</td>
<td>0</td>
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![Image 1](https://example.com/image1.png) **A**, Diffuse immunostaining pattern for hepatocyte paraffin (Hep Par) 1 in nonneoplastic small intestinal mucosa (×100). **B**, Hep Par 1 staining in nonneoplastic enterocytes was diffusely cytoplasmic and granular (×600) and spared microvilli (arrow). **C**, The nonneoplastic colonic mucosa was completely negative for Hep Par 1 immunoreactivity (×200).
there was no discernible histologic difference between Hep Par 1–positive and Hep Par 1–negative tumors. None of the tumors exhibited histologic evidence of hepatoid differentiation.

In the control cohort, 24 (92%) of 26 hepatocellular carcinomas showed strong cytoplasmic staining for Hep Par 1. The staining was diffuse in 18 cases (75%) and focal in 6. The nonneoplastic liver tissue was diffusely and strongly positive in all cases, including the 2 cases with negative immunoreactivity in hepatocellular carcinoma.

**Discussion**

Since its discovery in 1993 by Wennerberg and colleagues, Hep Par 1 has become a widely used antibody

| Table 2 |

| Hepatocyte Paraffin 1 Expression in Small Intestinal and Colorectal Adenocarcinomas |
|-----------------------------------|------------------|------------------|------------------|------------------|
| | Small Intestine | Colorectum (n = 31) |
| | Duodenum (n = 5) | Jejunum (n = 21) | Ileum (n = 7) | Unspecified (n = 6) | Total (n = 39) |
| Immunoreactivity | Positive (%) | Focal | Diffuse | Negative | Focal | Diffuse | Negative | Focal | Diffuse | Negative | Focal | Diffuse | Negative | Focal | Diffuse | Negative | Focal | Diffuse | Negative | Focal | Diffuse | Negative |
| Negative | 60 | 14 | 29 | 30 | 28 |
| Focal | 17 | 9 | 2 |
| Diffuse | 0 | 0 | 0 |

**Image 2**

A. Focal hepatocyte paraffin (Hep Par) 1 positivity in a small intestinal adenocarcinoma (×200). B. Loss of Hep Par 1 immunoreactivity in a small intestinal adenocarcinoma (×100). Note the positive staining in nonneoplastic small intestinal mucosa overlying the tumor (upper area). C. Diffuse Hep Par 1 immunostaining in a colonic adenocarcinoma (×200). Note that the nonneoplastic colonic mucosa (right lower area) was completely negative for Hep Par 1.
in surgical pathology as a relatively sensitive and specific marker of hepatocellular differentiation. Recently, Butler et al.\(^1\) expanded our knowledge by identifying its antigen as a rate-limiting enzyme of the urea cycle, CPS1. Since it is believed that converting ammonia to urea takes place only in hepatocytes in humans, it is difficult to understand why Hep Par 1 immunoreactivity is also detected in small intestinal mucosa.\(^1,13,16,18\) It is also unclear whether this enzyme serves any role in carcinogenesis, given its occasional expression in a variety of nonhepatocellular neoplasms.\(^1,6,7,9,10,13,15\)

In this study, we immunohistochemically examined hepatocyte antigen expression in a large number of small intestinal adenocarcinomas and nonneoplastic small intestinal mucosa. Our findings extend the previous observations and confirm that Hep Par 1 antibody recognizes an epitope in nonneoplastic small intestinal epithelium that is absent in nonneoplastic colonic epithelium. In addition, our results demonstrate a frequent loss of Hep Par 1 immunoreactivity in small intestinal adenocarcinomas.

Although the presence of CPS1 in human enterocytes was convincingly demonstrated by immunoprecipitation and Western blot analysis at the protein level in the study by Butler et al.,\(^16\) it has not been examined at the functional level. It also remains to be investigated whether other critical urea cycle enzymes are present in human enterocytes. Therefore, it is unknown at present whether a functional urea cycle exists in human small intestinal epithelium, although the existence has been suggested in postweaning pigs.\(^19\) It is possible that CPS1 may serve an alternative role in small intestinal physiology, instead of participating in a nitrogen-processing pathway as in the liver. It is also possible that there is another protein or epitope unique to the small intestinal epithelium that shares enough homology with CPS1 to cross-react with Hep Par 1 antibody. This latter possibility seems unlikely, however, given the similar molecular weight (\(~165\) kDa) of the protein band on Western blot and the same granular cytoplasmic staining pattern in immunohistochemical analysis recognized by Hep Par 1 and anti-CPS1 antibodies.\(^16\) Additional studies at the messenger RNA level may be helpful in this regard.

In our study, focal and negative stains for Hep Par 1 in nonneoplastic small intestinal mucosa were noted in 9 (26%) and 4 (12%) cases, respectively. A focal staining pattern was also reported by Wennerberg et al.\(^1\) It is unclear why the staining was diffuse in the majority of the cases but focal or even negative in others. We cannot completely exclude the effect of certain technical factors, such as varied tissue fixation. We also speculate that variation in the physiologic status of the enterocytes in different people may contribute because the expression of the \(cps1\) gene has been shown to be subject to physiologic regulation.\(^20\) Understanding the physiologic function(s) of CPS1 in the small intestine may be necessary to explain the variable immunostaining patterns.

There have been only 2 studies, to our knowledge, that have examined hepatocyte antigen expression in a total of 17 small intestinal adenocarcinomas.\(^1,13\) In the study by Wennerberg et al.,\(^1\) all 6 tumors (duodenum, 3; jejunum, 2; ileum, 1) were negative for Hep Par 1. By using tissue microarray analysis, Lugli et al.\(^13\) demonstrated positive staining in 4 (36%) of 11 tumors. In line with these observations, we detected focal Hep Par 1 immunoreactivity in 9 (23%) of 39 small intestinal adenocarcinomas. The frequency of hepatocyte antigen expression in colorectal adenocarcinomas in our study (10%) is also similar to that reported in other series.\(^9,13\)

In contrast with hepatocarcinogenesis in which Hep Par 1 immunoreactivity is well preserved, the current study clearly demonstrates a frequent loss of Hep Par 1 immunoreactivity in small intestinal adenocarcinoma. While this finding may simply represent an epiphenomenon during tumor progression, it raises an intriguing question whether hepatocyte antigen (or CPS1) serves a role in small intestinal tumorigenesis. This finding also provides an additional line of evidence to substantiate previous observations that small intestinal adenocarcinoma is pathogenetically and immunophenotypically distinct from colorectal adenocarcinoma, despite their morphologic similarity.\(^21-24\) Furthermore, loss of Hep Par 1 immunoreactivity may have potential usefulness in confirming the diagnosis of small intestinal adenocarcinoma on challenging biopsy specimens.

Our study demonstrates that hepatocyte antigen is expressed in nonneoplastic small intestinal epithelium but not in nonneoplastic colonic mucosa and that the immunoreactivity is frequently lost in small intestinal adenocarcinoma. These observations may have important implications in the understanding of the physiologic, pathologic, and tumorigenic roles of CPS1 in the small intestine, which warrant additional investigations.

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