Immunoreactivity for Hepatocyte Paraffin 1 Antibody in Hepatoid Adenocarcinomas of the Gastrointestinal Tract

Anirban Maitra, MD,1 Linda A. Murakata, MD,2 and Jorge Albores-Saavedra, MD1

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Abstract

Hepatocyte paraffin 1 (Hep Par 1) is a monoclonal antibody considered almost specific for normal and neoplastic hepatocytes, that can be used on formalin-fixed paraffin-embedded tissues. Hep Par 1 reactivity has been demonstrated consistently in hepatocellular carcinomas and hepatoblastomas but only rarely in cholangiocarcinomas and metastatic tumors to the liver. Although its role as a marker of hepatocytic differentiation in primary liver tumors has been studied extensively, Hep Par 1 expression has not been explored in extrahepatic lesions, especially rare adenocarcinomas with hepatoid morphologic features. We studied 7 hepatoid adenocarcinomas of the gastrointestinal tract (6 gastric and 1 from the gallbladder) for Hep Par 1 immunoreactivity. Focal Hep Par 1 expression was seen in 6 of 7 tumors. These hepatoid adenocarcinomas also showed reactivity for alpha-fetoprotein and carcinoembryonic antigen. The presence of Hep Par 1 reactivity in extrahepatic hepatoid adenocarcinomas underscores the fact that Hep Par 1 expression is not unique to primary hepatocellular neoplasms. Adenocarcinomas with hepatoid features must be considered in the differential diagnosis of Hep Par 1–positive lesions.

In 1993, Wennerberg et al1 reported the development of a new monoclonal antibody designated hepatocyte paraffin 1 (Hep Par 1), which was produced in mice using tissue from a failed allograft liver. A single clone (OCH1E5.2.10) was isolated from myeloma hybridomas, 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. In the original study, bile ducts and nonparenchymal liver cells were negative, while 37 of 38 hepatocellular carcinomas, including the fibrolamellar variant, were positive.1 Two of 35 biliary tract carcinomas showed only rare positive cells, as did 3 of 10 gastric tumors, all of which were poorly differentiated signet-ring or mixed intestinal–signet-ring cell carcinomas. Sixteen other tumors from various extrahepatic organs were all negative. Subsequently, several investigators evaluated the role of Hep Par 1 in distinguishing primary hepatocellular neoplasms from cholangiocarcinomas or metastatic tumors.2-5 Based on these studies, Hep Par 1 has been shown to have very high specificity (bordering on 100%) and slightly lower sensitivity (80%-90%) for identification of the hepatocellular phenotype.5 The lower sensitivity is primarily due to the focal immunoreactivity of some liver tumors. If limited material, such as needle core biopsy specimens, is used, negative results may be obtained.

While Hep Par 1 reactivity has been evaluated in liver neoplasms, few studies document the prevalence of immunoreactivity for this antibody in extrahepatic lesions. The hepatoid adenocarcinomas of the gastrointestinal tract display a variety of cytologic features and provide an opportunity to study their hepatocytic differentiation.6 In the present study, we examined 7 hepatoid adenocarcinomas of
the gastrointestinal tract, including 6 from the stomach and 1 from the gallbladder for Hep Par 1 expression, in addition to a panel of other immunohistochemical markers.

**Materials and Methods**

Seven cases of hepatoid adenocarcinomas were obtained from the surgical pathology archives of 2 institutions: 3 gastric tumors were provided by the Armed Forces Institute of Pathology, Washington, DC (L.A.M.), while 3 gastric tumors and 1 gallbladder tumor were retrieved from the consultation files of one of us (J.A.-S.). Limited clinical information was available for most cases, since the material was largely from consultation cases. The clinical data for the 7 patients are summarized in Table 1.

H&E-stained sections were available for review in all cases. By using standard published criteria, we independently confirmed the histologic diagnoses in the study cases. Immunohistochemical staining was performed on unstained 5-µm-thick sections from formalin-fixed paraffin-embedded tissues, using an automated immunostainer (Ventana Biotek Systems, Tucson, AZ) followed by a streptavidin–biotin–labeled detection step. All cases examined were subjected to heat-induced epitope retrieval. Positive and negative controls were used in each assay.

**Results**

**Histologic Features**

A spectrum of histologic patterns and cytologic features was observed in the 6 gastric tumors. Although tubular glands predominated, variable proportions of papillary and trabecular structures were present in all tumors. Some of the glands were lined by cuboidal or columnar cells similar to those seen in intestinal adenocarcinomas. However, the majority of glands and papillary structures were lined by cells with abundant clear cytoplasm. In case 1, virtually the entire tumor was composed of clear cells. The hepatoid foci were composed of cords or trabeculae of cells with abundant eosinophilic cytoplasm, large nuclei, and prominent cherry red nucleoli. Occasionally, sinusoids were identified between the trabeculae, and these areas were indistinguishable from well-differentiated hepatocellular carcinoma. In 1 case, bile pigment was seen within the cytoplasm of scattered neoplastic cells, reflecting an advanced degree of differentiation. Occasionally, the hepatoid cells were arranged as solid nests (so-called medullary pattern), as papillary structures, or in a glandular configuration resembling hepatic acini. In 3 cases (cases 2, 4, and 5), bizarre pleomorphic cells, multinucleated giant cells, and atypical mitoses were present focally. Intracellular and intercellular hyaline droplets were found in 4 of 6 gastric tumors and psammoma bodies in 1 tumor. Extensive lymphovascular permeation was seen in 5 cases, and intraneural invasion was seen in 1 case (case 3). The nonneoplastic gastric mucosa showed intestinal metaplasia in 3 cases.

The gallbladder tumor (case 7) showed sheets, nests, and trabeculae of clear cells, with a small focus of hepatoid differentiation in the primary tumor. However, the omental and peritoneal metastases from this case showed predominantly hepatoid morphologic features, with trabeculae and acini of large eosinophilic cells, with varying degrees of differentiation and pleomorphism. Focally, large multinucleated giant cells similar to those in cases 2, 4, and 5 were present.

**Immunohistochemical Studies**

Immunohistochemical stains used in the study are summarized in Table 1, and the results for individual cases are given in Table 2. Six of 7 cases in our series, including 1 tumor from the gallbladder, focally expressed Hep Par 1. Hep Par 1 reactivity was characteristically granular and cytoplasmic and limited to the clear cells or foci of frank hepatoid differentiation. In 1 case (case 3), the primary tumor showed rare Hep Par 1 positive cells, while the omental metastasis, which consisted predominantly...
of papillary adenocarcinoma, was negative. Case 6, which had a papillary architecture with extensive clear cell features, was completely negative for Hep Par 1 reactivity. Focal alpha-fetoprotein (AFP) expression was seen in 5 of 5 cases examined, and polyclonal carcinoembryonic antigen (CEA) expression also was seen in 5 of 5 cases examined. In 2 cases, a focal canalicular pattern of CEA expression, recapitulating bile canaliculi, was seen. Cytokeratins (CKs) 7 and 20 were examined in 2 cases (cases 1 and 2), and focal CK7 was seen in both cases, while CK20 was focally present in case 2. Immunostains for synaptophysin and chromogranin were negative in the 1 case tested.

Discussion

Carcinomas that show hepatoid differentiation (“hepatoid adenocarcinomas”) have been described in a variety of anatomic sites, most commonly the stomach, but also in...
the esophagus, papilla of Vater, gallbladder, colon, lung, adrenal gland, kidney, urinary bladder, uterus, and vagina. Yolk sac tumors of the ovaries and testis also may display a focal hepatoid phenotype. The reason for the preponderance of hepatoid adenocarcinomas in the stomach is not known, but it is thought that the common embryologic derivation of the liver and the stomach from the foregut may have a contributory role. Most hepatoid adenocarcinomas have been associated with the production of AFP, but not all AFP-producing tumors demonstrate hepatoid features. For example, AFP production has been demonstrated in gastric, renal cell, lung, bladder, colorectal, and ovarian carcinomas in the absence of hepatoid features. This is an important distinction because it has been shown that carcinomas with hepatoid features, irrespective of AFP production, have a poorer prognosis than AFP-producing carcinomas without hepatoid morphologic features. The extensive venous permeation and consequent liver metastasis is thought to be the principal reason that hepatoid tumors behave aggressively, a feature not seen in AFP-producing nonhepatoid tumors. Four of 5 patients for whom follow-up data were obtained died as a result of the tumor.

Hepatoid adenocarcinomas of the stomach are distinctive neoplasms that nearly always express AFP and CEA.
and both are considered useful immunohistochemical markers for this tumor. In addition, it is not uncommon to demonstrate cytoplasmic alpha1-antitrypsin, transferrin, bile, or albumin messenger RNA in the tumor cells. Such markers of hepatocytic differentiation lend credence to the view that these hepatoid tumors arise from stem endodermal cells with a potential for dual differentiation into hepatoid and conventional intestinal cell lines. This is analogous to the development of goblet cell carcinoids of the gastrointestinal tract that show dual differentiation along epithelial and neuroendocrine lineages. We report for the first time the presence of focal Hep Par 1 reactivity, a marker of the hepatocellular phenotype, in 6 of 7 hepatoid adenocarcinomas, including 1 from the gallbladder. Unlike the oncofetal antigen AFP that can be aberrantly expressed at several sites in the gastrointestinal tract, including intestinal metaplasia, monoclonal antibody Hep Par 1 expression seems to be restricted to normal and neoplastic liver cells and is more sensitive than AFP. Hence, demonstration of Hep Par 1 in adenocarcinomas outside the liver strongly supports the previously held view that these hepatoid foci represent true hepatocellular differentiation. This finding is not surprising in the stomach and gallbladder, because both organs are foregut derivatives and probably harbor stem cells. It would be interesting to study Hep Par 1 reactivity in hepatoid neoplasms from embryologically disparate sites such as the urinary bladder or gonads.

Aside from the ontogenetic value of demonstrating Hep Par 1 reactivity in hepatoid adenocarcinomas, this finding may have diagnostic importance for the pathologist. Hepatoid adenocarcinomas have a tendency to metastasize to the liver. Thus, the mere demonstration of Hep Par 1 and/or AFP reactivity in a liver nodule should not be considered unequivocal evidence of a primary hepatocellular carcinoma but should be evaluated in the correct clinical context. When metastatic to the liver, hepatoid adenocarcinomas would generally be multiple and show characteristic central umbilication. Conversely, the presence of Hep Par 1 and/or AFP reactivity in an extrahepatic neoplasm (such as in the stomach or omentum) that otherwise resembles hepatocellular carcinoma also is not diagnostic of metastatic tumor from the liver. Most primary gastric hepatoid adenocarcinomas would be associated with foci of conventional intestinal adenocarcinoma (in situ or invasive) that merge with the hepatoid areas, and this should be a clue to the correct diagnosis. Hepatoid adenocarcinomas commonly contain a population of clear cells, which may lead to the misdiagnosis of germ cell neoplasm (especially when associated with the presence of hyaline globules) or clear cell adenocarcinoma that may arise in many anatomic sites. In such cases, demonstration of Hep Par 1 immunoreactivity would be invaluable for characterizing the tumor as a hepatoid adenocarcinoma and alerting the clinician to the substantially worse prognosis associated with these tumors.

We studied Hep Par 1 expression in 7 hepatoid adenocarcinomas of the gastrointestinal tract and demonstrated focal immunoreactivity in 6 cases. Our results underscore the fact that Hep Par 1 expression is not unique to primary hepatocellular neoplasms, and, therefore, adenocarcinomas with hepatoid features must be considered in the differential diagnosis of Hep Par 1-positive lesions.

References

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