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Technical Advances

Hepatocyte Paraffin 1: A Monoclonal Antibody that Reacts with Hepatocytes and Can Be Used for Differential Diagnosis of Hepatic Tumors

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Hepatocyte paraffin 1 is a monoclonal antibody that has been developed specifically to react with hepatocytes in routine formalin-fixed and paraffin-embedded surgical pathology tissues. It results in a distinct, granular cytoplasmic staining of hepatocytes but fails to react with bile ducts and nonparenchymal liver cells. The antibody decorates a majority of hepatocellular carcinomas, including fibrolamellar variants. It fails to react with a wide variety of other adult malignancies, with the exception of focal staining in a few gastrointestinal malignancies, including a subpopulation of gastric carcinomas. (Am J Pathol 1993, 143:1050–1054)

Panels of antibodies useful in evaluating primary and secondary liver tumors include α-fetoprotein, α-1-antitrypsin, monoclonal carcinoembryonic antigen, polyclonal carcinoembryonic antigen cross-reactive for biliary glycoprotein, antibodies to cytokeratins, and neuroendocrine markers such as chromogranin.[1–3] We have developed a new monoclonal antibody, hepatocyte paraffin 1 (Hep Par 1), that reacts with both normal and neoplastic hepatocytes in routine formalin-fixed, paraffin-embedded tissues. We have evaluated this antibody in a retrospective analysis of adult human tumors, with and without metastases, and find that it is conserved in a majority of hepatocellular carcinomas (HCCs).

Materials and Methods

Immunizations and Fusion

The immunogen was obtained from a 10% neutral buffered, formalin-fixed, failed allograft liver that was mechanically disrupted. Six-week-old female Balb/C mice received serial injections of the immunogen. Animal use protocols had prior approval of the Institutional Animal Care and Use Committee of the University of Pittsburgh. Fusion was by a standard method and based on a modification of Kohler and Milstein.[4] The myeloma cells (P3X63-Ag8.653) and fusion protocols were a generous gift from Dr. Howard Reisner (UNC Chapel Hill, NC).[5–7]

Screening

Positive wells were screened by evaluating staining patterns of supernatants on microscope slides, containing multiple sections of nondigested, formalin-fixed human tissues, by immunoperoxidase using a mouse immunoglobulin G (IgG) ABC kit (Vector Laboratories, Burlingame, CA). One well that was highly specific for adult and fetal liver was subjected to single cell cloning and is the subject of this report.

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Cloning, Class, and Isotyping

The antibody was classed as an IgG1 κ. Single cell cloning by limiting dilution and antibody classifying was performed by Drs. Albert DeLeo and Lina Lu of the Hybridoma Facility of the Pittsburgh Cancer Institute.

Supernatant

Clone OCH1E5.2.10 (Hep Par 1) supernatant was collected in batch for the study described herein. For control of nonspecific binding of IgG in tissue sections, the supernatant from the parental myeloma cell line was spiked with purified mouse IgG (Chemicon, El Segundo, CA).

Selection and Immunoperoxidase Evaluation of Tissues

All cases were routine surgical pathology tissues that had been fixed in 10% neutral buffered formalin and paraffin-embedded. Metastases were those that came at the time of the original resection or biopsy with the exception of second-stage lymph node dissections. Staining times for primary and control supernatants were for 90 minutes, and staining times for the biotinylated secondary and avidin-biotin complex reagents were for 60 minutes each. 3-amino-9-ethylcarbazole (AEC) (Biomeda, Foster City, CA) was used as the chromogen for 10 minutes.

Results

In histologically normal adult human livers, Hep Par 1 detects an antigen that is localized to the hepatocyte cytoplasm. No staining of bile ducts or other nonparenchymal cells is seen. The pattern is distinctly granular, occasionally ringlike, and is present diffusely throughout the hepatocyte cytoplasm without canalicular accentuation (Figure 1A). A similar pattern was seen in a 17-week gestation human fetal liver. There does not seem to be any zonal preference in normal liver; however, in the liver immediately adjacent to tumors decreased staining in compressed hepatocytes may be seen (Figure 1B and Figure 2D). Many of the HCCs arose in cirrhotic

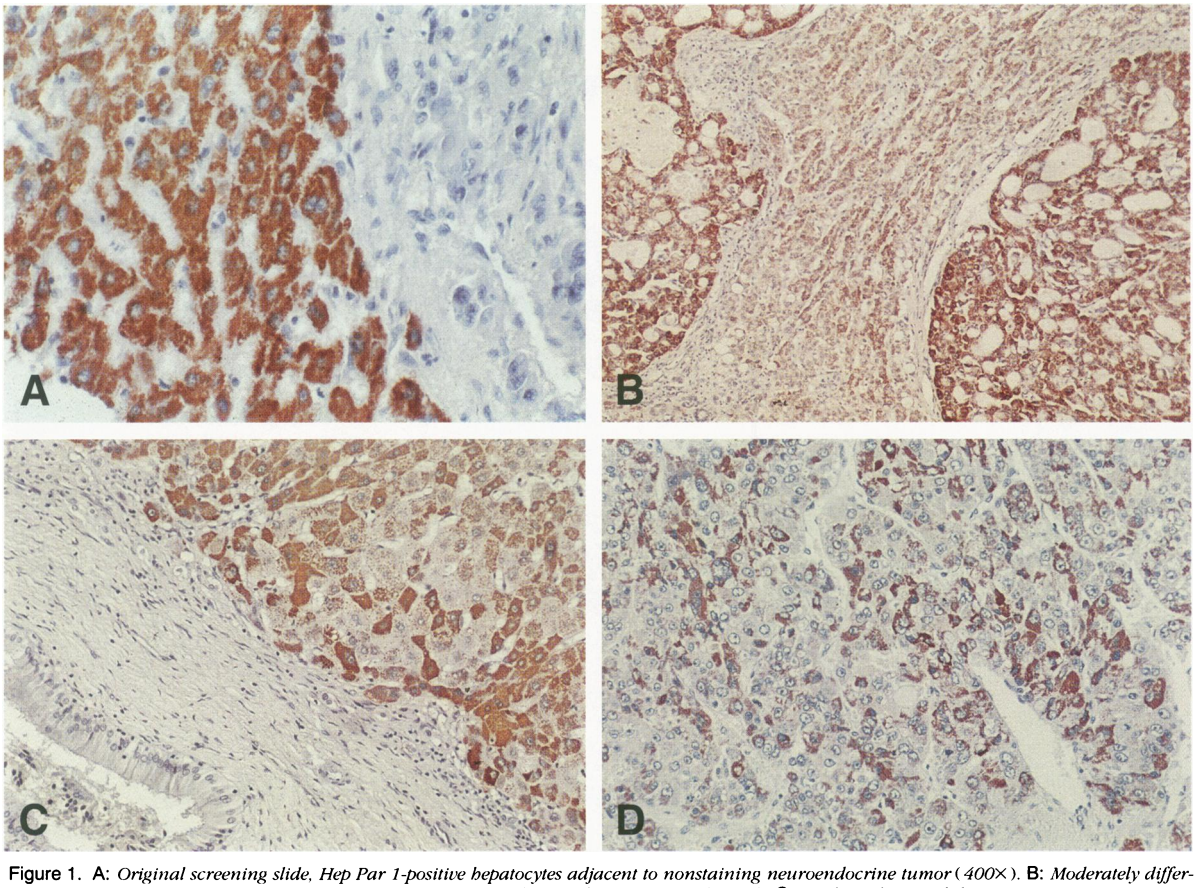

Figure 1. A: *Original screening slide, Hep Par 1-positive hepatocytes adjacent to nonstaining neuroendocrine tumor (400×).* B: *Moderately differentiated HCC nodules, strongly Hep Par 1-positive. Intervening liver with compression (100×).* C: *Cirrhotic liver with heterogenous Hep Par 1 positivity. Bile ducts Hep Par 1-negative (200×).* D: *Positive Hep Par 1 staining in HCC (200×).*

livers and often regenerative nodules showed heterogeneous Hep Par 1 expression (Figure 1C). In fatty livers, Hep Par 1 positivity was maintained in the cytoplasm adjacent to fat vacuoles. Other normal adult human tissues, including skin, smooth and skeletal muscle, mesothelium, lymph nodes, spleen, lung, breast, esophagus, stomach, intestine, pancreas, biliary tract, kidney, urinary bladder, adrenal, prostate, endometrium, and ovary, were almost all negative for Hep Par 1 reactivity. The rare exceptions are noted below.

Table 1 gives the results of Hep Par 1 staining in a variety of adult human malignancies. The majority of HCCs were positive for Hep Par 1 expression (Figure 1, B and D). The staining pattern within individual tumor cells maintained its granular cytoplasmic distribution; however, there could be considerable variability from one area to another, best demonstrated in larger samples. Four HCCs had only rare positivity, and of these, one had a negative lymph node metastasis. Occasionally, accentuated staining was seen in tumor areas adjacent to vascularized fibrous septa and in the edges of tumor trabeculae. All five fibrolamellar HCCs were Hep Par 1-positive. Only one sclerosing HCC was available for inclusion in this study, and it was entirely Hep Par 1-negative.

A large number of non-HCC tumors were selected to include biliary tract and gall bladder, pancreas, intestinal, and neuroendocrine tumors because these tumors frequently enter into the pathological differential diagnosis of liver cancer. The normal biliary tract does not show immunohistologically demonstrable Hep Par 1 expression. Cholangiocarcinomas and bile duct tumors were predominately negative (Figure 2A). Only two of 31 cholangiocarcinomas and bile duct tumors showed any Hep Par 1 positivity; this positivity was a minor, focal component seen in one postirradiation cholangiocarcinoma arising in an intrahepatic duct and in one intraductal papillary adenocarcinoma. Focal staining of inflammed, but nonmalignant, hilar bile ducts was an exceptional finding in a case of cholangiocarcinoma arising in a patient with Caroli's disease. Three of 12 pancreatic adenocarcinomas showed rare cells with Hep Par 1 positivity (two mucinous cystadenocarcinomas and one adenocarci-

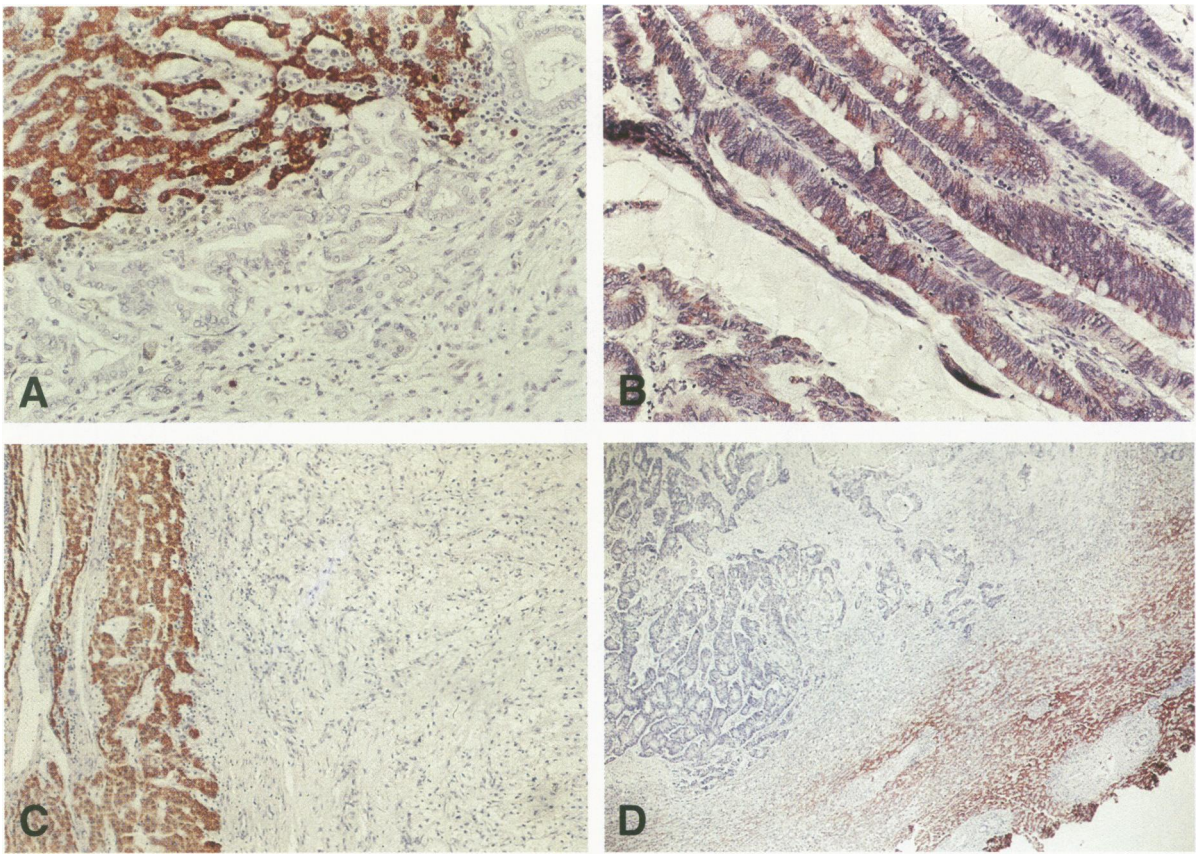

Figure 2. A: *A typical cholangiocarcinoma (Hep Par 1-negative) and liver interface (200×).* B: *Hep Par 1 positivity seen in villous fronds of right colon polyp yet underlying infiltrating adenocarcinoma Hep Par 1-negative (200×).* C: *Hep Par 1-positive liver with negatively stained metastatic renal cell carcinoma (100×).* D: *Wedge biopsy of Hep Par 1-positive liver with negatively stained metastatic adenocarcinoma of the colon (40×).*

Table 1. *Hep Par 1 Staining*

Tumor	Primaries: HepPar+/ (total)	Metastases: HepPar+/ (total)	Tumor subtypes	Comments
Biliary tract	2*/(35)	0/(17)	Intrahepatic (24); bile duct (7); gallbladder (4)	*Rare (+) in 1 intrahepatic chol. s/p radiation; *rare (+) cell in intraductal papillary cholangio-carcinoma
Breast	0/(9)	0/(6)	Infiltrating duct (4); lobular (3); medullary (2)	
Colon	0–1*/(8)	0/(8)	Well-diff. (3); moderate (4); poorly (1)	*Overlying villous polyp strong (+), underlying tumor (−)
Endometrial	0/(3)	0/(1)	Moderate adenoca. (1); well-diff. papillary (1); adenoca NOS (1)	
Esophageal	0/(7)	0/(2)	Moderate squamous (4); poor squamous (2)	
Gastric	3/(10)	1/(7)	All poorly diff. signet or mixed intestinal/signet	
Hepatocellular	37*/(38)	4/(5)	Sclerosing HCC (1); fibrolamellar (5); HCC (32); of nonfibrolamellar, 22/32 livers cirrhotic	*Sclerosing HCC negative *4 HCC only rare positive cells
Liver (vascular)	0/(5)	0/(2)	Epithelioid hemangio-endothelioma (3); angiosarc. (2)	
Lung	0/(5)	0/(2)	Adenoca. (4); squamous (1)	
Melanoma	0/(3)	0/(1)		
Mesothelioma	0/(3)	0		
Neuroendocrine (non-lung)	0/(14)	0/(6)		
Ovarian	0/(7)	0/(2)	Clear (1); serous (2); mucinous (2); endometrioid (2)	
Pancreatic	3*/(12)	0/(2)	Mucinous cystadeno. (2); adenoca poorwell (10)	*Rare (+) cells in 2/2 mucinous cystadeno; *rare (+) cell in 1 adeno. head of pancreas
Pheochromocytoma	0/(2)	0		
Prostate	0/(4)	0		
Renal	0/(5)	0/(4)	Clear, sarcomatoid, oncocytoma, NOS	
Small bowel	0/(6)	0/(5)	Jejunum (2); duodenum (3); ileum (1)	
Testicular	0/(2)	0/(1)	Embryonal, choriocarcinoma	
Urinary bladder	0/(1)	0		

noma of the head of the pancreas). Nonpulmonary neuroendocrine tumors were negative, including 14 specimens from liver, pancreas, and small intestine. Gastric adenocarcinomas (all were poorly differentiated, signet ring, or mixed intestinal/signet ring) were negative, with the exception of three cases showing focal, but strong, Hep Par 1 expression. The residual gastric mucosa, including mucosa with intestinal metaplasia, was Hep Par 1-negative. Whereas all small bowel adenocarcinomas, including those appearing to arise in villous polyps, were Hep Par 1-negative, there does seem to be a propensity for nonmalignant small bowel mucosa to stain focally, but often intensely, in a minority of cases. Four of the cases in this series displayed focal Hep Par 1 expression in benign small intestinal mucosa. The Hep Par 1-positive pattern in the small bowel appeared as cytoplasmic granules in absorptive enterocytes of the surface and neck region. One poorly differentiated right-sided colon adenocarcinoma, seeming to arise in an overlying villous tumor, had strong Hep Par 1 expression in the overlying villous tumor but lacked expression in the infiltrating tumor (Figure plate 2, B).

Other tumors included in this study were Hep Par 1-negative. Adjacent non-neoplastic tissues, when present, were also negative. The sole exception was the presence of Hep Par 1-positive hepatocytes adjacent to secondary, metastatic tumors (Figure 2, C and D).

Discussion

Unlike α-feto protein, the antigen detected by Hep Par 1 does not seem to be an oncofetal antigen as it has been immunohistologically localized in both fetal and adult liver. As it is present in normal human liver, it is also not a tumor-associated antigen. Hep Par 1 does, however, seem to be conserved in most adult HCCs, including fibrolamellar variants. For the evaluation of primary and secondary liver tumors, Hep Par 1 expression may be detected in a larger proportion of HCCs than is seen with AFP. Like AFP, it can be seen occasionally in other endodermally derived tissues.[8]

The monoclonal antibody, Hep Par 1, is highly, but not absolutely, specific for hepatocytes and results in a very distinct granular cytoplasmic staining in these cells. Occasionally, within the granules, a ringlike staining pattern is noted, suggesting that the antigen may be localized to the membrane of a cytoplasmic organelle. The fact that they are diffusely distributed throughout the cytoplasm and do not cluster near the canaliculus would argue against this being an endoplasmic reticulum/microsome-associated antigen. Their presence in such large numbers and their size (approximately 0.5 to 2 μ), would make them unlikely to be peroxisomes. A likely candidate would be a mitochondrial-associated antigen, although the lack of staining of mitochondrial-rich kidney tubules and skeletal muscle would suggest that it is not a ubiquitious mitochondrial antigen. Preliminary data (results not shown) have localized the antigen to the mitochondrial fraction of rat liver homogenates.

The decreased Hep Par 1 expression seen as a compression rim in the liver adjacent to some tumors and the perivascular accentuation seen in some HCCs may represent a probable effect of blood shunting. A similar vascular shunting in cirrhotic livers may account for the heterogeneous expression seen in regenerative nodules. Staining with Hep Par 1 is very crisp and does not result in background staining of serum. We have not yet attempted to determine whether the Hep Par 1-detected antigen is present in human sera. Hep Par 1 does give similar staining patterns in formalin-fixed liver as well as in acetone-permeabilized frozen cryostat sections of liver, suggesting that the antibody can be used to detect native, nondenatured antigen. Work to isolate the antigen is currently underway.

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