9 Markers and Immunoprofile of Hepatobiliary Tumors

Contents

9.1 Hepatocellular Tumors

9.1.1 Diagnostic Antibody Panel for Hepatocytes and Hepatocellular Tumors

Hep Par 1, Arginase-1, CD10, Alpha-fetoprotein, Glutamine synthetase, BSEP, MDR-3, Glypican-3, HSP70, CD34, and cytokeratin profile [1–3].

9.1.1.1 Hepatocyte Specific Antigen (Hep Par1)

Hepatocyte specific antigen (Hep Par1)		
Expression pattern: Cytoplasmic (granular)		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Hepatocellular carcinoma/ hepatoblastoma	Adrenal gland tumors, intestinal metaplasia and small intestinal adenocarcinoma, signet ring cell carcinoma, tumors with hepatoid differentiation, yolk sac tumor	Hepatocytes, intestinal enterocytes
Positive control: Liver tissue		

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Diagnostic Approach Antibodies to Hep Par-1 (Hepatocyte paraffin-1) react with the carbamoyl-phosphate synthetase-1, a urea cycle enzyme located on the mitochondrial membrane of hepatocytes that is also found in the mitochondria of the intestinal epithelium and cells of renal tubules. Hep Par-1 is a specific marker for hepatocytes and hepatocellular tumors; however, it also labels the epithelium of small intestinal mucosa and small intestinal adenocarcinomas in addition to gastric and esophageal intestinal metaplasia, including Barrett's mucosa [4–8].

Diagnostic Pitfalls Generally, extrahepatic tumors with hepatoid differentiation have the same immunoprofile as hepatocellular tumors, being positive for Hep Par-1, AFP, and CD10 [9]. The expression of Hep Par1 is also reported in tumors of the adrenal cortex and adenocarcinomas of the stomach and small intestine, but these tumors are negative for Arginase [10].

False positive results in the immunostaining of liver tissue can be caused by the high biotin activity of the hepatocytes; thus, the inactivation of endogenous biotin is recommended to eliminate the biotin background. The use of a biotin-free polymer detection system is recommended for immunohistochemistry on liver tissue.

9.1.1.2 Arginase-1

Arginase-1 is a manganese urea cycle metalloenzyme that catalyzes the conversion of arginine to ornithine and urea. In the hepatobiliary and gastrointestinal systems, the expression of Arginase-1 is limited to hepatocytes, while bile duct epithelium, sinusoidal endothelial cells, and gastrointestinal mucosa lack the expression of this enzyme. Arginase-1 is more specific for hepatocytes and hepatocellular carcinomas than Hep-Par-1 and is found in 85–100% of primary and metastatic hepatocellular carcinoma, whereas the expression intensity correlates with the differentiation grade of the tumor (Fig. 9.1) [11, 12].

Diagnostic Pitfalls Similar to Hep Par-1, hepatoid carcinomas of different origins may be positive for Arginase-1. Various expression levels of Arginase-1 are also found in myeloid cells and macrophages.

9.1.1.3 Alpha Fetoprotein

Alpha fetoprotein (AFP)		
Expression pattern: Cytoplasmic/membranous		
Main diagnostic use	**Expression in other tumors**	**Expression in normal cells**
– Hepatocellular carcinoma – Yolk sac tumor	Tumors with hepatoid differentiation, pancreatic-acinar cell carcinoma, pancreatoblastoma	Fetal liver
Positive control: Fetal liver		

Diagnostic Approach Alpha-fetoprotein (AFP) is an oncofetal glycoprotein found in the fetal liver, fetal gastrointestinal tract, yolk sac, and fetal plasma. AFP is also present in a very low concentration in adult plasma. In the majority of cases, hepatocellular carcinoma reveals a high expression level of AFP, and a lesser expression degree is also found in germ cell tumors, that is, yolk sac tumor (Fig. 9.2).

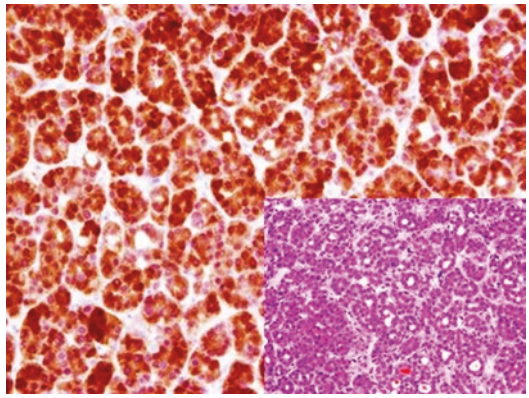

Fig. 9.1 Well-differentiated hepatocellular carcinoma. Arginase-1 staining the cytoplasm of neoplastic hepatocytes

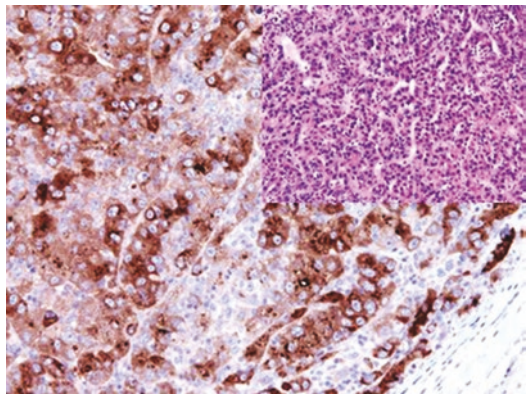

Fig. 9.2 Hepatocellular carcinoma showing strong cytoplasmic AFP expression in neoplastic hepatocytes

Diagnostic Pitfalls It is important to consider that about 5% of all hepatocellular carcinomas are negative for AFP. Due to formalin fixation and tissue processing, up to 50% of hepatocellular carcinoma turns negative for AFP in paraffin immunohistochemistry. The low expression level of AFP is reported in pancreatic acinar cell carcinoma, pancreatoblastoma, and renal cell carcinoma. AFP is also expressed by hepatoid tumors of different origins.

9.1.1.4 Bile Salt Export Pump and Multidrug-Resistance Protein 3

Bile salt export pump (BSEP) is a member of the adenosine-triphosphate-binding cassette transporter family encoded by the ABCB11 gene. BSEP is a membrane-associated ATP-dependent bile salt transporter protein localized on the canalicular microvilli and subcanalicular vesicles of hepatocytes and responsible for the transport of bile-conjugated salts out of hepatocytes into the canaliculus system [13].

The multidrug-resistance protein 3 (MDR-3) is another member of the same transporter family and a transmembrane protein involved in the transport of bile salts from hepatocytes.

Both BSEP and MDR-3 are expressed exclusively on the membrane of hepatocytes and used as sensitive and specific markers for hepatocytes and hepatocellular tumors. These markers can also be used to differentiate between hepatocellular and bile duct tumors [14].

9.1.1.5 Glypican-3

Glypican-3 is a membrane and extracellular heparan sulfate glycoprotein that regulates signaling during embryogenesis, acting as a receptor for several heparin-binding growth factors. Glypican-3 is normally expressed in fetal tissue and trophoblasts. In adult tissue, the expression of glypican-3 is restricted to a few tissue types, namely gastric glands and renal tubules. In neoplastic tissue, Glypican-3 is expressed in a wide range of epithelial and mesenchymal tumors, including pulmonary squamous cell carcinoma and small cell carcinoma, hepatocellular carcinoma and hepatoblastoma, acinar carcinoma of the pancreas, neuroblastoma, Wilms tumor, ovarian clear cell carcinoma, endometrial carcinoma, yolk sac tumor, choriocarcinoma, placental site nodule, liposarcoma, rhabdomyosarcoma, and undifferentiated pleomorphic sarcoma. Glypican-3 is a helpful marker to discriminate

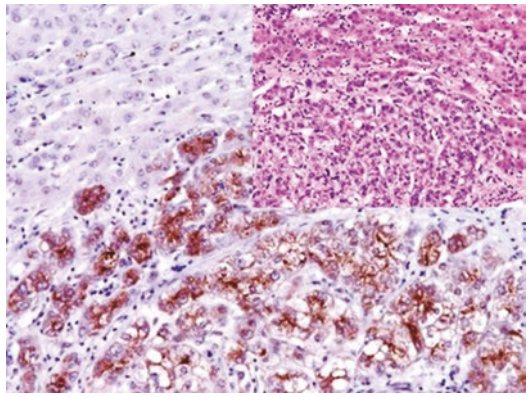

Fig. 9.3 Hepatocellular carcinoma exhibiting cytoplasmic Glypican-3 expression in neoplastic hepatocytes. Note negative reaction in non-neoplastic liver tissue

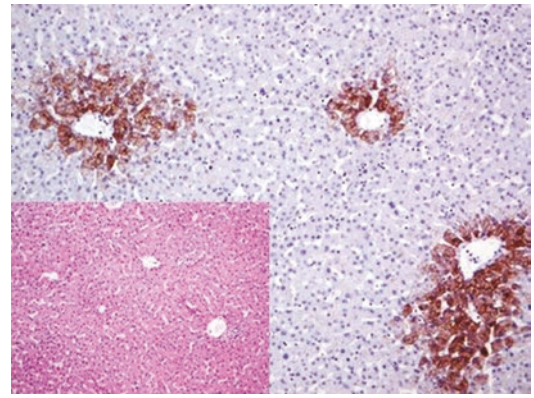

Fig. 9.4 Centrilobular expression of Glutamine synthetase in normal liver parenchyma

between hepatocellular carcinoma (overexpressed in 70–80% of the cases) and benign liver tissue, which is consistently negative for Glypican-3. Nevertheless, it is essential to consider that Glypican-3 may be focally positive in cirrhotic liver tissue, active chronic hepatitis C, and dysplastic liver nodules (Fig. 9.3). In germ cell tumors, embryonal carcinoma and seminoma lack the expression of Glypican-3.

9.1.1.6 Glutamine Synthetase

Glutamine synthetase (GS) is an enzyme that catalyzes the synthesis of glutamine from glutamate and ammonia in the hepatocytes. GS is involved in the regulation of pH and nitrogen balance in the liver. The expression of GS is activated by β-catenin, and mutations causing the activation of this transcriptional factor cause the overexpression of GS. Glutamine synthetase is normally expressed in hepatocytes, proximal renal tubules, and the brain, in addition to the solid pseudopapillary neoplasm of the pancreas [3, 15].

In normal and pathologic liver parenchyma, GS shows the following different expression patterns:

- In normal liver parenchyma, the expression of GS is limited to centrilobular hepatocytes around the central hepatic venules, whereas periportal and mid zones lack the expression of GS (Fig. 9.4).

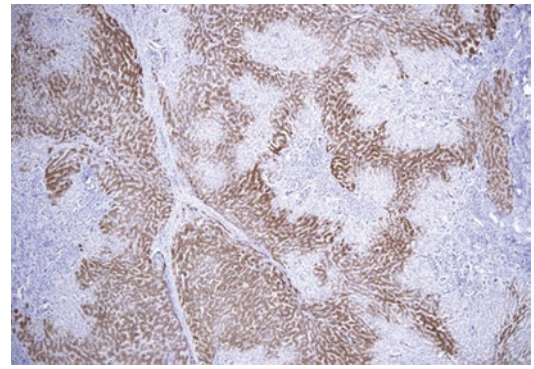

Fig. 9.5 Focal nodular hyperplasia (FNH) with centrilobular GS expression in an anastomosing pattern

- Focal nodular hyperplasia (FNH) shows centrilobular expression in an anastomosing pattern (Fig. 9.5).
- In hepatocellular adenoma (HCA), the GS expression pattern depends on the mutation associated with the adenoma type: In inflammatory and HNF-1α- inactivated HCA, the GS expression is similar to that in normal liver parenchyma with a centrilobular distribution pattern in addition to a weak patchy positivity at the periphery of the lobules. HCA associated with exon 3 mutation or β-catenin-activated HCA shows diffuse GS expression similar to hepatocellular carcinoma.
- High intracytoplasmic accumulation of GS with diffuse expression is characteristic for up to 70% of hepatocellular carcinoma (HCC), whereas the expression intensity correlates

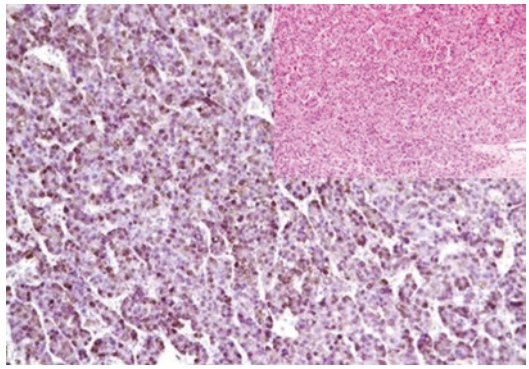

Fig. 9.6 Hepatocellular carcinoma with strong diffuse GS expression in the hepatocytes

with the differentiation grade of the HCC (Fig. 9.6).

– High-grade dysplastic nodules (HGDN) usually lack the expression of GS or show weak focal expression.

9.1.1.7 Heat-Shock Protein-70

Heat-shock protein-70 (HSP70) is an anti-apoptotic regulator expressed in different malignant tumors. In routine immunohistochemistry, HSP70 can be used as a marker to discriminate between hepatocellular carcinoma positive for HSP70 (nuclear/cytoplasmic stain pattern) and dysplastic nodules or hepatocellular adenoma negative for HSP70.

Diagnostic Pitfalls ~ 30% of hepatocellular carcinomas are negative for HSP70 and a subset of cholangiocarcinomas may also be positive for HSP70. Since HSP70 is expressed in different malignant tumors, including gastrointestinal adenocarcinomas, it cannot be used to discriminate between hepatocellular carcinoma and metastatic carcinoma [16, 17].

9.1.1.8 Immunoprofile of Liver Sinusoidal Endothelial Cells

In normal liver parenchyma, the sinusoidal cells are positive for CD4, CD14, CD16, and CD31

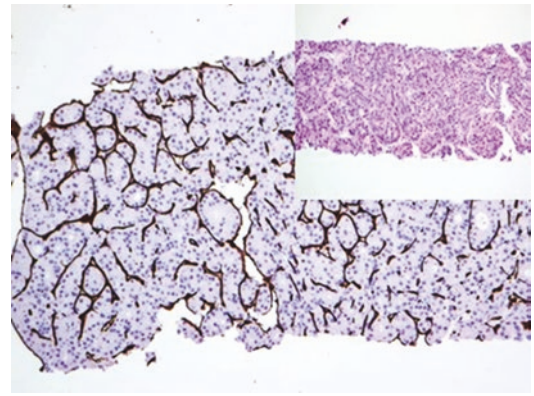

Fig. 9.7 Liver core biopsy. CD34 highlighting sinusoidal cells in hepatocellular carcinoma (sinusoidal capillarization)

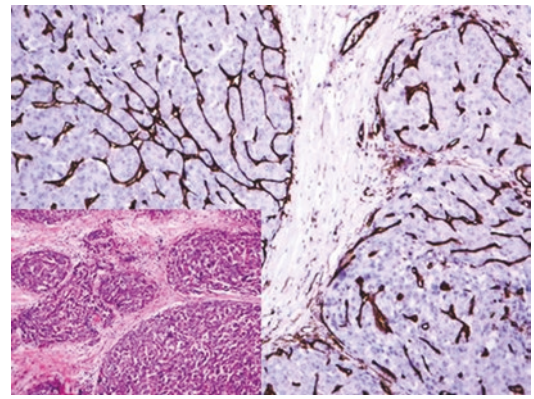

Fig. 9.8 Hepatocellular carcinoma with sinusoidal capillarization labeled by CD34

but negative for CD34. During malignant transformation and remodeling of the normal liver histological architecture, the sinusoidal cells undergo capillarization and exhibit the immunophenotype of vascular endothelial cells with strong CD31 and CD34 expression. This pattern is characteristic for hepatocellular carcinoma (Figs. 9.7 and 9.8).

Finally, a panel of Glypican-3, Glutamine synthetase, heat shock protein 70, BSEP, and CD34 is highly effective in differentiating between benign, dysplastic, and malignant liver nodules.

9.2 Cholangiocarcinoma

9.2.1 Diagnostic Antibody Panel for Intra- and Extrahepatic Cholangiocarcinoma and Gallbladder Carcinoma

Cytokeratin profile, hepatocellular markers, CEA, PDX-1, TTF-1, CD56, S100P, MUC-5 AC, and MUC-6 [18].

All the abovementioned markers are listed in detail in previous sections. PDX-1 is a specific marker for primary intra- and extrahepatic cholangiocarcinoma; nevertheless, it is also expressed in a subset of hepatocellular carcinomas. Although TTF-1 is a specific marker for pulmonary adenocarcinoma and thyroid carcinomas, a weak to moderate nuclear expression is also found in a subset of cholangiocarcinomas, which is to be considered in the differential diagnosis between primary and metastatic liver tumors [19]. The TTF-1 expression in cholangiocarcinoma is most characteristic for large duct-type cholangiocarcinoma and carcinomas of extrahepatic bile ducts (Fig. 9.9).

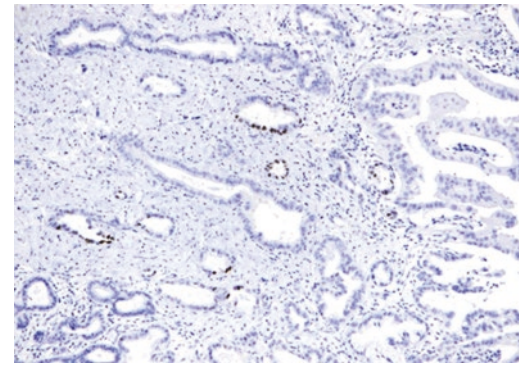

Fig. 9.9 Nuclear TTF-1 expression in the cholangiocarcinoma cells of the common bile duct

Immunoprofile of hepatobiliary tumors				
Tumor type	+ in > 90% (+)	+ in 50–90% (±)	+ in 10–50% (∓)	+ in < 10% (−)
A. Intrahepatic tumors				
Hepatocellular adenoma: – HNF-1α-inactivated HCA – Inflammatory HCA – β-catenin-activated HCA – β-catenin-activated inflammatory HCA – Unclassified HCA	Arginase-1, Hep Par-1, **CD34**[a]	ER, PgR	**Glutamine synthetase**, [b]	**Glypican-3**, **AFP**, HSP70
Hepatocellular carcinoma:	**Arginase-1**[c], Hep Par-1, **Glutamine synthetase**[d], **BSEP**, **MDR-3**, **CD34**[a], CK8/18	**Glypican-3**, **AFP**, Cadherin 17, SATB-2, EMA, CD138, CD10[e], CK7[f] CEA[g], HSP70, PDX-1, TTF-1, [h] ER, AR, MAGE-1, Osteonectin, CD66a, CD56, CD68[i]	CK19[j] CK20[j], BER-EP4, PgR, Vimentin	CK5/6, CK14, EMA, Inhibin, Melan A, EPCAM[k]
Hepatoblastoma:	**Hep Par-1**, Pan-CK, **Glypican-3**, β-catenin	CK18, AFP, CEA, CD34, CD56, EMA, Chromogranin, Vimentin	S100	
Intrahepatic Cholangiocarcinoma: – Small duct type	**CK7**, CK8, CK18, CK19, CEA, EMA, CK17	**PDX-1**, CDH17, N-Cadherin, CD56, CD5	CDX-2	AFP, CK5/6, CK20, TTF-1

Intrahepatic Cholangiocarcinoma: – Large duct type	**CK7**, CK8, CK18, CK19, CEA, EMA, CK17	**PDX-1**, CDH17, S100P, TTF-1; MUC-5AC, MUC-6	CDX-2, CK20, Vimentin	AFP, CK5/6
Angiomyolipoma:	**HMB45**, HMB50, **Melan-A**, Actin, CD63 (NK1-C3), Calponin, PgR	CD117	MIFT, ER	EMA, Pan-CK
B. Extrahepatic and gallbladder tumors				
Biliary intraepithelial neoplasia (BilIN- 1/2/3):	CK7, CK19	CK20, **Cyclin D1**[l], **p21**[l], **MUC5AC**[l], **MUC-6**, **S100p**[l], p16[m]		
Extrahepatic Cholangiocarcinoma	CK7, CK8, CK18, CK19, CEA, EMA, CK17, PDX-1, S100P, DPC-4	CK20, CDX-2, TTF-1		CD56
Biliary mucinous cystic neoplasm: – Cystadenoma – Cystadenocarcinoma	CK7, CA125, CA19.9, CEA *Ovarian type stroma*: ER, PgR, CD10	CK20		
Adenocarcinoma of the gallbladder:	**CK7**, CK18, CK19, EMA, CEA, S100P		CK20	Arginase-1, BSEP, MDR-3, CK5/6

[a] Labels sinusoidal endothelium lining neoplastic trabeculae (sinusoidal capillarization), which are absent or rare in normal liver parenchyma
[b]The diffuse expression only in β-catenin-activated type of HCA
[c] The intensity of arginase expression correlates with the differentiation of HCC
[d] In HCC, diffuse GS expression in the three zones of liver parenchyma
[e] Apical canalicular stain pattern
[f] CK7 strong expression in the majority of fibrolamellar hepatocellular carcinoma and in up to 30 in conventional hepatocellular carcinoma but is usually negative in normal hepatocytes
[g] Only polyclonal CEA antibodies show a canalicular staining pattern, whereas monoclonal antibodies are negative
[h] Granular cytoplasmic TTF-1 expression due to cross-reaction in hepatocellular carcinoma (Fig. 9.10)
[i] Positive in fibrolamellar hepatocellular carcinoma, negative in conventional HHC and normal hepatocytes.
[j] CK19/20 shows aberrant expression in a subset of neoplastic hepatocytes, usually negative in normal hepatocytes
[k] EPCAM (BerEp-4) is usually positive in hepatoid carcinomas but negative in hepatocellular carcinoma
[l] The expression increases with the grade of dysplasia
[m] The expression decreases with the grade of dysplasia

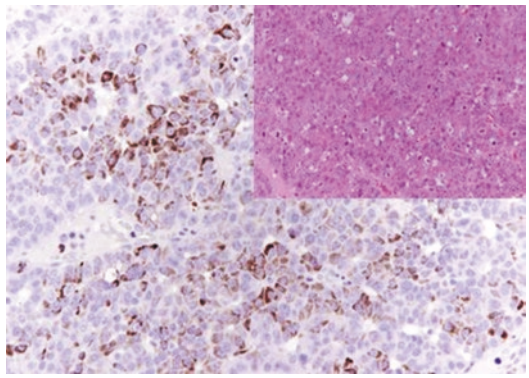

Fig. 9.10 Cytoplasmic TTF-1 expression in hepatocellular carcinoma

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