Current concepts in the immunohistochemical evaluation of liver tumors

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Abstract

Immunohistochemistry often plays an important role in the evaluation of liver tumors. Recent advances have established a classification system for hepatocellular adenomas (HCAs) based on morphology, molecular alterations, and immunohistochemistry. Specifically, loss of liver fatty acid binding protein is seen in HNF1α-inactivated HCA, staining with serum amyloid A is seen in inflammatory HCA, and diffuse staining with glutamine synthetase (GS) is seen in β-catenin activated HCA. A panel of immunohistochemical stains including glypican-3 (GPC-3), heat shock protein 70, and GS are useful in distinguishing HCC from non-malignant dysplastic nodules. Immunohistochemistry is also useful to determine whether a liver tumor is of primary hepatocellular or metastatic origin. Recently described markers useful for this purpose include arginase-1, GPC-3, and bile salt export pump. These newer markers may offer superior utility when compared to traditional markers of hepatocellular differentiation such as alpha-fetoprotein, hepatocyte paraffin-1, polyclonal carcinoembryonic antigen, and CD10. This paper will review recent advances in the immunohistochemical evaluation of liver tumors.

Key words: Immunohistochemistry; Hepatocellular adenoma; Focal nodular hyperplasia; Hepatocellular carcinoma

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Core tip: Immunohistochemical stains may be an important complement to morphology in the characterization of liver tumors. Immunohistochemical stains can now be used to subtype hepatocellular adenomas. A panel of immunohistochemical stains can help distinguish hepatocellular carcinoma from dysplastic nodules and hepatocellular adenomas. Several new markers of hepatocellular differentiation have been described. These advances are reviewed.
INTRODUCTION

Although the global incidence of hepatocellular carcinoma (HCC) varies from region to region, incidence in Europe and North America has been increasing[1]. The majority of these cancers arise in the setting of chronic liver disease, especially chronic infection by hepatitis B virus (HBV) and HCV or cirrhosis of any cause. There is a male predominance of approximately 3:1[1]. With improvements in imaging, specifically four phase multi-detector computed tomography and dynamic, contrast-enhanced magnetic resonance imaging, there has been a concomitant increase in the detection of small liver nodules. While many of these lesions can be diagnosed on imaging, histologic diagnosis remains the gold standard, especially for small nodules (< 1-2 cm), with the goal of diagnosing cancers at an early stage where treatment may be curative. Nonetheless, it can be challenging to distinguish HCC from other hepatocellular proliferations, such as focal nodular hyperplasia (FNH), hepatocellular adenoma (HCA), and dysplastic nodules, particularly when presented with small samples (e.g., needle biopsy). Other primary tumors (e.g., cholangiocarcinoma) and metastases may also enter the differential depending on morphology and history. In recent years, there have been a number of advances reported employing immunohistochemistry to answer such questions. These advances are reviewed herein.

FNH

FNH is a benign hepatocellular lesion thought to develop in response to localized hyperperfusion relating to the presence of an anomalous artery[2,3] with a female predominance of 8:1 and a median age of 38[4]. Histologically, the classical type is a hyperplastic nodular lesion with a central scar containing the anomalous vessel, and a ductular reaction. Most cases are asymptomatic and are often incidentally discovered[5]. Frequently, this entity can be reliably diagnosed on imaging and no treatment is required; however, some cases may be difficult to confirm with imaging, and may require biopsy to rule out HCA and HCC which could require surgical excision. In challenging cases, immunohistochemistry for glutamine synthetase (GS, an enzyme that catalyzes the synthesis of glutamine from glutamate and ammonia, important in nitrogen metabolism) is useful and shows a characteristic geographic “map-like” pattern of staining[6] (Figure 1).

HCA

In contrast to FNH, HCAs are neoplastic clonal proliferations. Resection of adenomas larger than 5 cm is recommended due to the risk of hemorrhage and potential malignant transformation in up to 7% of cases[6]. Risk factors for HCA are female gender, steroid sex hormone exposure (oral contraceptives, anabolic steroids, pregnancy), glycogen storage disease types I and III, maturity onset diabetes of the young type 3 (MODY3), and familial adenomatosis coli[7,8]. Adenomas can often be diagnosed on imaging, but if the differential diagnosis includes FNH or HCC, the lesion may be biopsied. Based on molecular and immunohistochemical studies, Bioulac-Sage et al[9] have identified 4 types of hepatocellular adenomas, now recognized by the World Health Organization. Immunohistochemical stains are therefore useful for both diagnosis and sub-classification.

HNF1α-inactivated HCA

In HNF1α-inactivated HCA (H-HCA), inactivation of both alleles of the HNF1α gene, which encodes hepatocyte nuclear factor 1 (a transcription factor related to hepatocyte differentiation), results in increased production of fatty acids, steatosis in hepatocytes, and loss of liver fatty acid binding protein expression, which can be appreciated by a negative immunohistochemical stain (Figure 2A and B). H-HCA accounts for 35%-40% of HCAs and is associated with MODY3 and adenomatosis[2,4,9] but is not thought to be associated with higher risk of transformation to HCC.

Inflammatory HCA

In inflammatory HCA (IHCA), activating mutations in genes (IL6ST, STAT3, GNAS, FRK) along the JAK-STAT pathway result in increased expression of inflammatory markers including serum amyloid A (SAA)[9,10]. Histologically, IHCA are characterized by an inflammatory infiltrate, vascular anomalies, and may exhibit a ductular reaction. In the past, this lesion was known as “telangiectatic FNH,” but has now been shown by molecular and immunohistochemical analysis to be IHCA[11]. They stain with SAA and C reactive protein by immunohistochemistry (Figure 2C and D) and account for 40%-55% of HCAs[5,9,10].

β-catenin-activated HCA

β-catenin-activated HCAs (β-HCAs) are the subtype of HCA with the highest risk (4%)[12] for transformation to HCC, and account for 10%-15% of HCAs. Histologically, β-HCAs may show cholestasis and both architectural and cytologic atypia including pseudoacinar structures. In β-HCA, activating mutations (predominantly in exons 3, 7, or 8) in the CTNNB1 gene, which encodes β-catenin, cause activation of the WNT/β-catenin pathway. This is the most commonly mutated pathway in HCC[13]. The mutations may lead to upregulation of the gene coding for GS; consequently, this subtype is expected to exhibit abnormal nuclear staining with β-catenin and diffuse GS staining by immunohistochemistry (Figure 2E and F). Staining for β-catenin is less sensitive than staining for GS, though GS is much less specific than nuclear β-catenin. The purported sensitivity and specificity of GS in this setting is 100% and 89%[9]. However, in our experience, GS may diffusely stain many adenomas which do not exhibit atypical morphologic or clinical signs of atypia[14]. Furthermore, when our group sequenced GS overexpressing HCA, we could identify β-catenin mutations in only 1 OF 8 HCAs (unpublished...
data). In our opinion, GS overexpression is an imperfect surrogate for β catenin mutation, and should not be a definitional characteristic.

**Unclassified HCA**

Unclassified HCAs represent the remaining 10% of HCAs, and lack characteristic histology, immunohistochemistry, or molecular changes.

**DYSPLASTIC NODULES**

The pathogenesis of HCC is thought to be a stepwise accumulation of mutations arising in a small clonal population (dysplastic nodules and a small proportion
of adenomas\cite{13,15}. The background liver is most often cirrhotic, with HBV as the most common underlying cause worldwide, especially in Sub-Saharan Africa and Asia where HBV is endemic, and HCV and the most common underlying cause in the United States\cite{16}. In the cirrhotic liver, it is important to distinguish large regenerative nodules, which are benign, from low- and high-grade dysplastic nodules (H-DN), which precede HCC in a stepwise manner, and to distinguish these from early and progressed HCC itself. Histologic criteria were established by the International Consensus Group for Hepatocellular Neoplasia in 2009, but the differences between these entities may be subtle as they lie on a continuum. The best criteria to distinguish H-DN from early HCC is the presence of invasion into portal tracts\cite{17}. This feature may not be identifiable on biopsy material, however. Historically, thickened portal plates and subsequently a diminished reticulin framework were noted to be markers of progression from H-DN to early HCC, although an intact reticulin framework could not exclude HCC\cite{18}. In this case, immunostains are a useful aid to histomorphology.

### CD34

Normal sinusoidal endothelium does not express CD34. However, since capillarization of sinusoidal endothelium occurs during the progression of dysplastic nodules to HCC (which corresponds to the enhancement seen in HCC on the arterial phase of dynamic imaging modalities), immunostaining for the vascular marker CD34 has been found to be a useful marker of malignant transformation (since it does mark capillarized endothelial cells)\cite{19,20}. However, while increased to diffuse vascular markings with CD34 is a suspicious finding in a liver tumor, no specific cutoff has yet been established in distinguishing between H-DN and early HCC\cite{18-21}.

In studies of biopsy and resection specimens\cite{22,23}, a panel of 3 immunostains including glypican-3 (GPC-3), heat shock protein 70 (HSP70), and GS was found to be useful in distinguishing dysplastic nodules from HCC, with a combination of at least any two positive stains shown to be 72% sensitive (resection specimens; 57% on biopsy specimens) and 100% specific in distinguishing early HCC from dysplastic nodules. Overall, it was found that positive staining in at least 2/3 markers supported a diagnosis of HCC, but lack of staining was not sufficient to rule out HCC especially on biopsy specimens.

### GPC-3

GPC-3 is a heparan-sulfate cell surface oncofetal proteoglycan noted to be expressed in HCC, but generally not in benign liver (normal or cirrhotic) or in metastatic carcinomas\cite{24,25}. Immunostaining with GPC-3 in HCC may be cytoplasmic and/or membranous. Some authors have found that the sensitivity increases as the tumor becomes less differentiated\cite{25,26}. Other studies have not found higher GPC-3 expression in poorly differentiated tumors, however, so confirmation in additional studies would be helpful\cite{27}. GPC-3 is also useful in distinguishing HCC from HCA\cite{14}. Anecdotally, we have seen strong positivity in a case of scirrhous variant HCC which could easily have been mistaken for metastatic adenocarcinoma (Figure 3). On the other hand, GPC-3 may be less helpful in the fibrolamellar variant of HCC\cite{28}.

GPC-3 is known to stain a few other malignancies, such as yolk sac tumor and melanoma\cite{29}. Another caveat is the reported expression of GPC-3 in cirrhotic nodules in cases of hepatitis C infection\cite{30}.

### HSP70

HSP70 is an anti-apoptotic regulator promoting cell survival and has been implicated in tumorigenesis. HSP70 expression increases stepwise as a lesion progresses from precancerous to advanced HCC\cite{31}. Immunohistochemistry marks HCC, but not dysplastic nodules or HCA\cite{14,23}. Staining is nucleoplasmic and may be focal or diffuse, and is 74% sensitive and 98% specific for HCC on resection specimens, and 48% and 94% on biopsy specimens, respectively when evaluating HCC vs dysplastic nodules\cite{22,23}. However, a potential pitfall of HSP70 is that it reacts commonly with metastatic adenocarcinomas and cholangiocarcinomas\cite{14}. Therefore,
the utility is restricted to tumors which are clearly hepatocytic in differentiation.

**GS**

GS catalyzes the conversion of glutamate and ammonia to glutamine in the liver. As noted in the discussion of b-HCA, GS is a target of β-catenin, and is upregulated when this pathway is constitutively activated. In normal liver, GS expression is restricted to perivenular hepatocytes. In neoplasms GS expression should be strong, homogenous, and diffuse (not map-like), and should stain > 50% of the cells in question. Given these conditions, on resection specimens, the sensitivity and specificity were 70% and 94%, respectively (59% and 98% on biopsy specimens) when the consideration was HCC vs dysplastic nodule. GS is frequently positive in HCA, however, and therefore not useful in the distinction of HCA from HCC. An important caveat when using these 3 markers is that GS and HSP70 are frequently positive in cholangiocarcinoma and metastases, thus highlighting that the utility of this panel is restricted to the specific contexts in which evidence supports their use. GPC-3 is the only one of the three markers above which is useful in overtly malignant tumors requiring evaluation of differentiation.

**ESTABLISHING HEPATOCELLULAR ORIGIN**

Since poorly differentiated HCC may have histologic overlap with poorly differentiated metastatic tumors and intra-hepatic cholangiocarcinomas, there has always been interest in reliable immunohistochemical markers of hepatocytic differentiation. At a basic level, the cytokeratin profile can be helpful but in most cases of ambiguous morphology, not definitive. Hepatocytes are generally positive for CK8 and 18 and negative for both CK7 and CK20, although HCC may acquire CK7 and or CK20 positivity in some cases. One caveat is fibrolamellar carcinoma, which tends to be found in younger patients without cirrhosis, and has been found to be positive for CK7 (as well as CD68). Some HCCs may acquire biliary features (CK19 positivity) by immunohistochemistry; in one study, these patients had a higher recurrence rate of HCC after transplantation, indicating a worse prognosis in these lesions. Therefore, it is often necessary to go beyond cytokeratin analysis.

**MARKERS OF PRIMARY HCC**

Traditionally, the most commonly used markers for this purpose include hepatocyte paraffin 1 (HepPar-1), alphafetoprotein (AFP), CD10, and polyclonal carcinoembryonic antigen (p-CEA). Each marker has drawbacks including limitations of sensitivity and specificity, as well as the requirement of a canalicular staining pattern for hepatocellular specificity in CD10 and p-CEA.

**HepPar-1**

HepPar-1 is an antibody to carbamoyl phosphate synthetase 1, a urea cycle enzyme in hepatocellular mitochondria, which is expressed predominantly in the liver, but also in other organs such as small intestine. Developed in 1993 from a failed liver allograft, this antibody has been found to be relatively sensitive (70%, although some authors report higher sensitivity) and specific (84%) for hepatocellular differentiation, in both normal tissue and HCC, as well as hepatoblastoma. Caveats with this marker include the reported loss of sensitivity as tumors become less differentiated, and frequent negativity in the scirrhous variant. Through many years of use, many of the pitfalls of HepPar-1 have been elucidated. For one, it marks hepatoid tumors of any organ. Furthermore, because of the expression of carbamoyl phosphate synthetase in small bowel, it marks many small intestinal adenocarcinomas, as well as adenocarcinomas of the ampulla with intestinal morphology. Additionally, a large study by Lugli et al. suggested that other tumors (notably gastric, lung, small intestinal, colonic, and pancreatic adenocarcinomas, cholangiocarcinoma, and melanoma) may have low (generally less than 15%) rates of positive staining with HepPar-1. Ovarian and neuroendocrine carcinoma have also been reported to show occasional positivity. However, staining in non-hepatocellular tissues has generally been reported as weak, whereas staining in tissues of hepatocellular origin tends to be strong and cytoplasmic. Thus, despite these many well-established pitfalls, HepPar-1 remains a very useful marker.

**AFP**

AFP is an oncofetal glycoprotein that has been used as a tumor marker both in serum and in tissue by immunohistochemistry for some time. Although also positive in yolk sac tumors, specificity by immunohistochemistry is high (97%) with very few metastatic adenocarcinomas or cholangiocarcinomas showing positive staining. However, sensitivity is low, around 30%, limiting its utility.

**p-CEA**

In normal liver, p-CEA stains a biliary glycoprotein similar to CEA (a fetal glycoprotein), present in the bile canaliculi and ductal epithelium. The staining pattern is characteristic: a delicate branching canalicular pattern, which has been reported as 70% sensitive and 100% specific for hepatocellular differentiation. However, this pattern may be lost as HCC dedifferentiates, and in general, the staining pattern may be difficult to distinguish from non-specific membranous or cytoplasmic staining, which can be seen in some HCC, bile duct epithelium, and metastatic adenocarcinoma and cholangiocarcinoma.

**CD10**

CD10 is a membrane metallo-endopeptidase which
cleaves the amino group of hydrophobic residues, and is expressed in multiple tissues. Although cytoplasmic, membranous, and apical staining may be seen in adenocarcinomas from multiple other primary sites, the same canalicular pattern described above for p-CEA when seen with anti-CD10 is specific for both normal and neoplastic liver, with a reported sensitivity of 68% and specificity of 100%[39]. As above, however, canalicular staining may be difficult to establish. Recently, additional markers have been described.

**GPC-3**

As mentioned previously, some investigators have reported a higher GPC-3 sensitivity in poorly differentiated HCC than in well differentiated tumors[26]. This could be an important finding, as HepPar-1 expression becomes less sensitive in poorly differentiated lesions. The reported overall sensitivity of GPC-3 for HCC is 75% (39% for well-differentiated, 89% for poorly differentiated)[50] and the specificity is 86%. GPC-3 staining has been reported in extragonadal germ cell tumors, melanoma, ovarian carcinoma,
and squamous cell cancer of the lung, and more recently, in pancreatic acinar cell carcinoma, esophageal squamous cell carcinoma and adenocarcinoma. Still, most of these are rare in the liver, and so GPC-3 is now commonly used as part of the immunohistochemical panel used to establish the histogenesis of a tumor in liver.

Arginase-1

Another novel marker of hepatocellular differentiation is Arginase-1 (ARG-1). ARG-1 is a manganese urea cycle metalloenzyme isoform expressed primarily in the liver. In two series, it was found to be more sensitive and specific (84% and 96%, respectively) than HepPar-1. As with many other markers of hepatocellular differentiation, expression decreased as tumor grade increased, but ARG-1 was more sensitive grade for grade than HepPar-1 in both series. In terms of specificity, ARG-1 expression has been reported in a small subset of pancreatic, colon, gastric, and pulmonary cancers, but when HepPar-1 was also positive, specificity rose to 100%. ARG-1 does not stain non-neoplastic small intestinal and ampullary mucosa, and only rarely stains adenocarcinomas of these sites.

Bile salt export pump

Bile salt export pump (BSEP) is another recently described immunohistochemical marker for hepatocellular differentiation. BSEP is a membrane-bound ATP-binding cassette transporter expressed only in hepatocytes, and functions to transport bile out of the hepatocyte. A study by Lagana et al. reported 90% sensitivity and 100% specificity for HCC. Furthermore, since it is expressed exclusively in hepatocytes, there is no requirement for a canicular pattern of staining as there is with CD10 and p-CEA (though canicular staining was present in 33 of 43 positive cases).

Cholangiocarcinoma

Intrahepatic cholangiocarcinoma is generally unlikely to be histologically confused with HCC. The morphology can, however, be identical to metastatic carcinomas. In the rare cases where a pathologist is entertaining the diagnosis of HCC and cholangiocarcinoma, the markers listed above, along with the addition of CK7 and CK19, should suffice.

CONCLUSION

Diagnosing liver tumors can be challenging, especially on needle biopsy specimens which may only minimally sample a lesion. Proper identification and classification is essential, as some lesions require no treatment at all, whereas in others, resection, chemotherapy, and transplantation may be offered. Recent advances in immunohistochemistry have furthered our ability to accurately characterize these lesions (summarized in Table 1).

REFERENCES

1 El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011; 365: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra101668]
2 Rebuissou S, Bioulac-Sage P, Zucman-Rossi J. Molecular pathogenesis of focal nodular hyperplasia and hepatocellular adenoma. J Hepatol 2008; 48: 163-170 [PMID: 17997499 DOI: 10.1016/j.jhep.2007.10.003]
3 Wanless IR, Mawdsley C, Adams R. On the pathogenesis of focal nodular hyperplasia of the liver. Hepatology 1985; 5: 1194-1200 [PMID: 4065824 DOI: 10.1002/hep.184005022]
4 Nguyen BN, Flejou JF, Terris B, Belghiti J, Degott C. Focal nodular hyperplasia of the liver: a comprehensive pathologic study of 305 lesions and recognition of new histologic forms. Am J Surg Pathol 1999; 23: 1441-1454 [PMID: 10584697 DOI: 10.1097/00004013-199912000-00001]
5 Bioulac-Sage P, Launomer H, Rullier A, Cubel G, Laurent C, Zucman-Rossi J. Over-expression of glutamine synthetase in focal nodular hyperplasia: a novel easy diagnostic tool in surgical pathology. Liver Int 2009; 29: 459-465 [PMID: 18803590 DOI: 10.1111/j.1477-3231.2008.01849.x]
6 Balabaud C, Al-Rabih WR, Chen PJ, Evasion K, Ferrell L, Hernandez-Prera JC, Huang SF, Longerich T, Park YN, Quaglia A, Schirmacher A, Sempoux C, Thung SN, Torbenson M, Wee A, Yeh MM, Yeh SH, Le Bail B, Zucman-Rossi J, Bioulac-Sage P. Focal Nodular Hyperplasia and Hepatocellular Adenoma around the World Viewed through the Scope of the Immunopathological Classification. Int J Hepatol 2013; 2013: 268625 [PMID: 23691331 DOI: 10.1155/2013/268625]
7 Sempoux C, Chang C, Gouw A, Chiche L, Zucman-Rossi J, Balabaud C, Bioulac-Sage P. Benign hepatocellular nodules: what have we learned using the patho-molecular classification. Clin Res Hepatol Gastroenterol 2013; 37: 322-327 [PMID: 23876350 DOI: 10.1016/j.clinre.2013.04.008]
8 Bioulac-Sage P, Rebuissou S, Thomas C, Blanc JF, Sarr JE, Cunha A, Rullier A, Cubel G, Coachu G, Imbeaud S, Balabaud C, Zucman-Rossi J. Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. Hepatology 2007; 46: 740-748 [PMID: 17663417 DOI: 10.1002/hep.21743]
9 Nault JC, Zucman Rossi J. Molecular classification of hepatocellular adenomas. Int J Hepatol 2013; 2013: 315947 [PMID: 23404783 DOI: 10.1155/2013/315947]
10 Pilati C, Letouzé E, Nault JC, Imbeaud S, Boulai A, Calderano J, Poussin K, Franchon A, Couchu G, Morecette G, Mallet M, Tauso S, Balabaud C, Terris B, Canal F, Paradis V, Scoazec JY, de Muret Nault JC, Zucman Rossi J. Genomic profiling of hepatocellular adenomas reveals recurrent FRK-activating mutations and the mechanisms of malignant transformation. Cancer Cell 2014; 25: 428-442 [PMID: 24735922 DOI: 10.1016/j.ccr.2014.03.005]
11 Bioulac-Sage P, Rebuissou S, Sa Cunha A, Jeannot E, Lepreux S, Blanc JF, Blanche H, Le Bail B, Saric J, Laurent-Puig P, Balabaud C, Zucman-Rossi J. Clinical, morphologic, and molecular features defining so-called telangiectatic focal nodular hyperplasias of the liver. Gastroenterology 2005; 128: 1211-1218 [PMID: 15887105 DOI: 10.1016/j.gastro.2003.07.034]
12 Stoot JH, Coelen RJ, De Jong MC, Dejong CH. Malignant transformation of hepatocellular adenomas into hepatocellular carcinomas: a systematic review including more than 1600 adenoma cases. HPB (Oxford) 2010; 12: 509-522 [PMID: 20887318 DOI: 10.1111/j.1477-2578.2010.00222.x]
13 Nault JC, Zucman-Rossi J. Genetics of hepatobiliary carcinogenesis. Semin Liver Dis 2011; 31: 173-187 [PMID: 21538283 DOI: 10.1055/s-0031-1276646]
14 Lagana SM, Salomao M, Bao F, Moreira RK, Lefkowitch JH, Remotti HE. Utility of an immunohistochemical panel consisting of glypican-3, heat-shock protein-70, and glutamine synthetase in the distinction of low-grade hepatocellular carcinoma from hepatocellular adenoma. Appl Immunohistochem Mol Morphol 2013; 21: 170-176 [PMID: 22914605 DOI: 10.1097/PAI.0b013e318254527]
15 Roskams T, Kojiro M. Pathology of early hepatocellular carcinoma: conventional and molecular diagnosis. Semin Liver Dis 2010; 30: 17-25 [PMID: 2075030 DOI: 10.1055/s-0030-1247129]

16 El-Serag HB, Kanwal F. Epidemiology of hepatocellular carcinoma in the United States: where are we? Where do we go? Hepatolgy 2014; 60: 1767-1775 [PMID: 24839253 DOI: 10.1002/hep.27222]

17 International Consensus Group for Hepatocellular Neoplasia The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology 2009; 49: 658-664 [PMID: 19177576 DOI: 10.1002/hep.22709]

18 Roncalli M, Borzio M, Di Tommaso L. Hepatocellular dysplastic nodules. Hepatol Res 2007; 37 Suppl 2: S125-S134 [PMID: 17877473 DOI: 10.1111/j.1872-034X.2007.00175.x]

19 Park YN, Yang CP, Fernandez GJ, Cubukcu O, Thung SN, Theise ND. Neangiogenesis and sinusoidal “capillarization” in dysplastic nodules of the liver. Am J Surg Pathol 1998; 22: 656-662 [PMID: 9630172 DOI: 10.1097/00000478-199806000-00002]

20 Costom WM, Loera L, Lau SK, Ishizawa S, Jiang Z, Wu CL, Yen Y, Weiss LM, Chu PG. Distinction of hepatocellular carcinoma from benign hepatic mimickers using Glypican-3 and CD34 immunohistochemistry. Am J Surg Pathol 2008; 32: 433-444 [PMID: 18308086 DOI: 10.1097/PAS.0b013e3181581422]

21 Roncalli M, Rze E, Coggi G, Di Rocco MG, Bossi P, Minola E, Gambacorta M, Borzio M. The vascular profile of regenerative and dysplastic nodules of the cirrhotic liver: implications for diagnosis and classification. Hepatology 1999; 30: 1174-1178 [PMID: 10534338 DOI: 10.1002/hep.510300507]

22 Di Tommaso L, Franchi G, Park YN, Fiamengo B, Destro A, Morenghi E, Montorsi M, Torazzini M, Terracciano L, Tornillo L, Vecchione R, Roncalli M. The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. J Hepatol 2009; 50: 746-754 [PMID: 19231003 DOI: 10.1016/j.jhep.2008.11.014]

23 Libbrecht L, Severi T, Cissman D, Vander Borght S, Pirenne J, Nevens F, Verslype C, van Pelt J, Roskams T. Glypican-3 expression distinguishes small hepatocellular carcinomas from dysplastic nodules and focal nodular hyperplasia-like nodules. Am J Surg Pathol 2006; 30: 1405-1411 [PMID: 17063081 DOI: 10.1097/01.pas.0000213323.92794.9a]

24 Chan ES, Yeh MM. The use of immunohistochemistry in liver tumors. Clin Liver Dis 2010; 14: 687-703 [PMID: 21055690 DOI: 10.1016/j.cld.2010.10.001]

25 Shirakawa H, Suzuki H, Shimomura M, Kojima M, Gotohda N, Takahashi S, Nakagohri T, Konishi M, Kobayashi N, Kinoshita T, Nakatsura T. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. Cancer Sci 2009; 100: 1403-1407 [PMID: 19496787 DOI: 10.1111/j.1349-7006.2009.01206.x]

26 Yamauchi N, Watanabe A, Hishimura M, Ohashi K, Mizokawa T, Sakurazawa A, Shikama T, Shiba H, Nakagawa T, Yano T, Kitakata Y, Kodama T, Fukayama M. Glypican-3 expression in clear cell adenocarcinoma of the ovary. Mod Pathol 2009; 22: 824-832 [PMID: 19329941 DOI: 10.1038/modpathol.2009.40]

27 Abdul-Al HM, Makhloof HR, Wang G, Goodman ZD. Glypican-3 expression in benign liver tissue with active hepatitis C: implications for the diagnosis of hepatocellular carcinoma. Hepatol Pathol 2008; 30: 209-212 [PMID: 17949778 DOI: 10.1016/j.hepan.2007.06.004]

28 Chuma M, Sakamoto M, Yamazaki K, Ohta T, Okhi M, Asaka M, Hirohashi S. Expression profiling in multistage hepatocarcinogenesis: identification of HSP70 as a molecular marker of early hepatocellular carcinoma. Hepatology 2003; 37: 198-207 [PMID: 12550205 DOI: 10.1053/jhep.2003.50022]

29 Reitzer LJ, Wicke BM, Kennell D. Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells. J Biol Chem 1979; 254: 2669-2676 [PMID: 429069]

30 Durrez A, Verslype C, Nevens F, Favery J, Aerts R, Pirenne J, Lesaffre E, Libbrecht L, Desmet V, Roskams T. The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. Histopathology 2006; 49: 138-151 [PMID: 16879391 DOI: 10.1111/j.1365-2559.2006.02486.x]

31 Ward SC, Huang J, Tickoo SK, Thung SN, Ladaisy M, Kimstra DS. Fibrolamellar carcinoma of the liver exhibits immunohistochemical evidence of both hepatocyte and bile duct differentiation. Mod Pathol 2010; 23: 1180-1190 [PMID: 20495535 DOI: 10.1038/modpathol.2010.105]

32 Ross HM, Daniel HD, Vivekanandan P, Kannangai R, Yeh MM, Wu TT, Makhloof HR, Torbenson M. Fibrolamellar carcinomas are positive for CD68. Mod Pathol 2011; 24: 390-395 [PMID: 2111339 DOI: 10.1038/modpathol.2010.207]

33 Wenerherberg AE, Nalesnik MA, Coleman WB. Hepatocyte paraffin 1: a monoclonal antibody that reacts with hepatocytes and can be used for differential diagnosis of hepatic tumors. Am J Pathol 1993; 143: 1050-1054 [PMID: 7692729]

34 Carrozza MJ, Calafati SA, Edmonds PR. Immunocytochemical localization of polyclonal carcinoembryonic antigen in hepatocellular carcinomas. Acta Cytol 1991; 35: 221-224 [PMID: 1851357]

35 Tsuji M, Kashiwara T, Terada N, Mori H. An immunohistochemical study of hepatic atypical adenomatous hyperplasia, hepatocellular carcinoma, and cholangiocarcinoma with alpha-fetoprotein, neprilysin (CD10) as a diagnostic marker for hepatocellular carcinomas. Lab Invest 2003; 88: 78-88 [PMID: 18026163 DOI: 10.1038/labinvest.3700699]

36 Radwan NA, Ahmed NS. The diagnostic value of arginase-1 immunostaining in differentiating hepatocellular carcinoma from metastatic carcinoma and cholangiocarcinoma as compared to HepPar-1. Diagn Pathol 2012; 7: 149 [PMID: 23111165 DOI: 10.1186/1746-1596-7-149]

37 Chu PG, Ishizawa S, Wu E, Weiss LM. Hepatocyte antigen as a marker of hepatocellular carcinoma: an immunohistochemical comparison to carcinoembryonic antigen, CD10, and alpha-fetoprotein. Am J Surg Pathol 2002; 26: 978-988 [PMID: 12170084 DOI: 10.1097/00000478-200208000-00002]

38 Lau SK, Prakash S, Geller SA, Alsabeh R. Comparative immunochemical profile of hepatocellular carcinoma, cholangiocarcinoma, and metastatic adenocarcinoma. Hum Pathol 2002; 33: 1175-1181 [PMID: 1254785 DOI: 10.1053/hupa.2002.130104]

39 Krings G, Ramachandran R, Jain D, Wu TT, Yeh MM, Torbenson M, Kakar S. Immunohistochemical pitfalls and the importance of diagnostic confidence in the diagnosis of scirrhous hepatocellular carcinoma. Mod Pathol 2013; 22: 782-791 [PMID: 23348905 DOI: 10.1038/modpathol.2012.243]

40 Maitra A, Murakata LA, Albores-Saavedra J. Immunoreactivity for hepatocyte paraffin 1 antibody in hepatoid adenocarcinomas of the gastrointestinal tract. Am J Clin Pathol 2001; 115: 689-694
Fan Z, van de Rijn M, Montgomery K, Rouse RV. Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. *Mod Pathol* 2003; 16: 137-144 [PMID: 12591966 DOI: 10.1097/01.mp.0000052103.13730.20]

Lugli A, Tornillo L, Mirlacher M, Bundi M, Sauter G, Terracciano LM. Hepatocyte paraffin 1 expression in human normal and neoplastic tissues: tissue microarray analysis on 3,940 tissue samples. *Am J Clin Pathol* 2004; 122: 721-727 [PMID: 15491968 DOI: 10.1309/ke69-ytf2-m4dl-uq6]

Knisely A. Hepatocellular carcinoma, renal cell carcinoma metastatic to liver, and adrenal carcinoma metastatic to liver: immunostaining for bile salt export pump as a marker of hepatocellular differentiation. United States and Canadian Academy of Pathology 98th Annual Meeting Abstracts; March 7-13, 2009; Boston, Massachusetts. *Laboratory Investigation* 2009; 89 Supp 1s: 312A-313A [DOI: 10.1038/labinvest.2008.147]

Lagana SM, Salomao M, Remotti HE, Knisely AS, Moreira RK. Bile salt export pump: a sensitive and specific immunohistochemical marker of hepatocellular carcinoma. *Histopathology* 2015; 66: 598-602 [DOI: 10.1111/his.12601]
