Diagnosis of well-differentiated hepatocellular lesions: role of immunohistochemistry and other ancillary techniques

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The most common problems encountered in the differential diagnosis of hepatocellular lesions are: (a) adenoma vs well-differentiated hepatocellular carcinoma (HCC) in non-cirrhotic liver, (b) focal nodular hyperplasia (FNH) vs adenoma in non-cirrhotic liver, and (c) HCC vs high grade dysplastic nodules and other non-neoplastic nodules in cirrhotic liver. This discussion provides an update of immunohistochemistry and other techniques that aid in this differential diagnosis.

A. ADENOMA vs WELL-DIFFERENTIATED HEPATOCELLULAR CARCINOMA

HCC is distinguished from hepatic adenoma based on presence of wide cell plates (>3 cells thick), prominent acinar pattern, small cell change, cytologic atypia, mitotic activity, vascular invasion, absence of Kupffer cells and loss of reticulin network. However, some or most of these features are often not present in well-differentiated HCC. On the other hand, atypical features like nuclear atypia and acinar architecture can be focally present in hepatic adenomas. It has been shown that tumors that morphologically resemble adenoma can recur and metastasize, especially in males and patients over 50 years (1). The natural history and management for hepatic adenoma and HCC are different and hence it is crucial to make this distinction. The following approaches have been used in recent years to distinguish adenoma from HCC or identify adenomas that are at high risk for progression to HCC.

(1) Chromosomal analysis

HCCs show a consistent pattern of chromosomal gains and losses (2,3). The most prominent changes are gains of part or entire chromosome arms 8q (49-81%), 1q (60-79%) and 7q (40-64%), and loss of 16q (36-65%). Other common abnormalities include overrepresentation at sites Xq and 5p, and losses at 4q, 8p, 13q, 16q and 17p. These abnormalities are observed in >80% of well-differentiated HCC, but have not been observed in adenomas in women of reproductive age group.

Cytogenetic analysis can be done on paraffin-embedded tissue by comparative genomic hybridization (CGH) or fluorescence in situ hybridization (FISH). CGH is a cumbersome procedure and not presently suitable for routine diagnostic purposes. On the other hand, FISH can be easily performed on slides obtained from paraffin blocks. Gains of chromosomes 1q and 8q, the two most common abnormalities in well-differentiated HCC have been successfully used to distinguish adenoma and HCC in several studies (4-6). In one study, gains of 1q and 8q were frequently seen in adenoma-like neoplasms in men and patients over 50 years, but not in adenomas in women between 15 and 50 years (6). Some of the former also recurred to metastasized indicating that at least some of these adenoma-like neoplasms in men or patients over 50 years of age may represent well-differentiated HCC.

(2) Gene expression
Wang et al studied the expression of several thousand genes in hepatic adenoma and HCC, and showed that 53 genes were differentially expressed (7). Some of the genes were further validated by reverse transcriptase PCR and immunohistochemistry including insulin growth factor-II, clusterin, estrogen receptor and PCNA. However, this technique and the genes identified by it have not been studied more widely and its utility in needle biopsies remains to be established.

(3) Immunohistochemistry

(a) Glypican-3
Glypican-3 (GPC-3) is a membrane anchored heparin sulfate proteoglycan normally expressed in fetal liver and placenta, but not in normal adult liver. It is an oncofetal antigen that is a reliable serum and histochemical marker for hepatocellular carcinoma. GPC-3 expression has been reported in 70-90% of HCCs in most studies (8-12). Most of the hepatocellular markers used for the diagnosis of HCC like Hep Par 1 and polyclonal CEA are expressed both in benign and malignant hepatocytes. Expression of GPC-3, however, has not been observed in benign hepatocellular lesions by in situ hybridization or immunohistochemistry. However, the sensitivity of GPC-3 in well-differentiated HCC is around 50%, and is likely to be lower in needle biopsies. There are anecdotal observations of GPC-3 expression in histologically typical adenomas in young women. Although the expression of GPC-3 would strongly favor HCC, larger series with follow-up information are necessary to fully establish the utility of GPC-3 in this setting.

(b) Beta-catenin
The Wnt signaling pathway plays an important role in cell adhesion and cell proliferation. Beta-catenin, a key component of this pathway is predominantly bound to cell membranes in normal cells. Mutations in beta-catenin or abnormalities in other components of this pathway can lead to nuclear translocation of beta-catenin that can be demonstrated by immunohistochemistry. Beta-catenin mutations occur in around 20% of HCC, but have been reported in up to 40% in HCC arising in chronic hepatitis C (13).

Recent studies have shown that liver tumors that morphologically resemble adenomas and have nuclear localization of beta-catenin are at higher risk of transformation to HCC (14,15). These tumors often occur in men and show atypical features like acinar architecture and cytological abnormalities. Tumors with borderline features of adenoma and HCC often show nuclear translocation of beta-catenin. As described above, some adenoma-like neoplasms in men and patients over 50 years of age show chromosomal changes typical of HCC. These tumors often show nuclear localization of beta-catenin (16). Hence beta-catenin mutations or nuclear expression of beta-catenin in a hepatocellular neoplasm signifies a high-risk adenoma or an adenoma-like neoplasm that represents an extremely well-differentiated HCC.

(c) Glutamine synthetase
The nuclear translocation of beta-catenin as described above leads to activation of several transcription factors leading to increased expression of several genes that play a key role in cell proliferation. Glutamine synthetase (GS) is one of the genes that is upregulated as a result of nuclear translocation of beta-catenin. Hence GS shows strong and diffuse expression in tumors with beta-catenin mutations. GS is an enzyme that helps in the conversion of glutamine.

In normal liver, GS expression is seen in pericentral hepatocytes, but not by midzonal or periportal hepatocytes. In most hepatic adenomas, GS is negative, localized to the pericentral areas or shows patchy expression with no distinct pattern. In adenoma-like neoplasms and in HCC with beta-catenin
abnormalities, strong and diffuse GS expression in seen in tumor cells (15,16). The significance of strong and diffuse expression is the same as beta-catenin nuclear translocation. In some cases, strong and diffuse GS expression is seen in the absence of nuclear staining of beta-catenin. The reason for this is not clear, but it is likely that these tumors have abnormalities in the Wnt signaling pathway.

B. FNH vs. HEPATIC ADENOMA

The diagnosis of FNH can be achieved by imaging in >70% of cases. Liver biopsy is obtained in cases with atypical features on imaging. The distinction between FNH and adenoma can be challenging on liver biopsies. The presence of scar, nodular architecture, prominent ductular reaction and aberrant arterioles favors FNH. However, the telangiectatic (or variant) adenoma can show ductular reaction and can be especially difficult to separate from FNH. Some recent studies have indicated that immunohistochemistry can be helpful in this regard.

(a) Glutamine synthetase
Immunohistochemistry with glutamine synthetase (GS) demonstrates a characteristic ‘map-like’ pattern of staining in FNH. Large groups of hepatocytes are positive in a relatively continuous anastomosing fashion, often surrounding hepatic veins, whereas GS is not expressed in hepatocytes close to fibrotic bands containing arteries and ductules (17). This staining pattern has been described as very specific for FNH, and is seen in all cases irrespective of size or atypical features. In contrast, most hepatic adenomas are largely negative or show GS staining of a few pericentral, peripheral or scattered hepatocytes (16,17). As described above, adenomas with beta-catenin mutations show diffuse and strong GS expression. This pattern is different from the map-like staining seen in FNH.

(b) Beta-catenin
Mutations in beta-catenin results in nuclear staining in a subset of adenomas as described above. These adenomas show diffuse cytoplasmic staining with GS. Beta-catenin mutations have not been observed in FNH (18,19).

(c) Serum amyloid associated protein
Adenomas with telangiectatic (variant) features show sinusoidal dilatation, ductular reaction and inflammation, and hence resemble FNH. Most telangiectatic (variant) adenomas show strong and diffuse expression of serum amyloid associated (SAA) protein (15,16). Staining with SAA is not seen in FNH.

(d) Cytokeratin 7
In adenomas, CK7 highlights singly scattered or small aggregates of hepatocytes (20,21). The small and intermediate sized hepatocytes show the strongest CK7 expression, while the large mature hepatocytes show no or weak staining. Although bile ducts are typically absent in HA, occasional ductules can be identified with CK7. In contrast, CK7 highlights the ductular reaction in FNH and the hepatocytes are negative or show mild and focal staining. CK7 may not be helpful in the diagnosis of telangiectatic (variant) adenoma as the pattern of staining can overlap with FNH and conventional adenoma (20).

C. DYSPLASTIC NODULES vs EARLY HCC IN CIRRHOTIC LIVER

Small HCC are defined as tumors that are <2 cm. Based on work emanating from Japan in the last few years, small HCCs are divided into two distinct groups (22-24):
Early HCC (vaguely nodular HCC, early well-differentiated HCC) characterized by a vaguely nodular gross appearance. These are extremely well-differentiated and are difficult to distinguish from high-grade dysplastic nodules.

Progressed HCC characterized by a distinctly nodular pattern, easily identifiable thick cell plates. The diagnosis is often obvious in these cases.

Most hepatocellular nodules >2 cm in cirrhotic liver are HCC and can be diagnosed by typical imaging characteristics of arterial hypervascularity and venous washout on triphasic CT or MRI (25-27). Therapeutic approaches like resection and ablation are pursued in these cases based on clinical and radiological features and there is no need for a liver biopsy. Biopsy confirmation is necessary only if imaging features are not typical.

For hepatocellular nodules <2 cm, it is thought that the majority are HCC. However, HCCs <2 cm are often hypovascular and cannot be reliably diagnosed by imaging. Definite diagnosis of smaller nodular lesions is clinically significant for several reasons (23,25,27):

1. Small HCCs have lower propensity for vascular invasion and offer a high probability of cure.
2. Allows definite therapy like resection or ablation.
3. Small HCCs are more amenable to ablation or resection. Complete ablation is more likely to be achieved in small lesions. As per the AASLD Practice Guidelines Committee (27), it is equally important not to apply invasive treatment to lesions that do not have any malignant potential as up to 50% of these nodules can regress spontaneously.
4. Definite diagnosis of HCC enables patients to receive priority for transplantation.

Since imaging is not reliable, liver biopsy is the only modality that can achieve a reliable diagnosis. Most of the criteria of distinction between high grade dysplastic nodule (HGDN) and well-differentiated HCC (WD-HCC) are quantitative and hence very subjective. The presence of uniformly thick (>3 cells) plates, prominent pseudoglands and loss of reticulin favor HCC. However, these features are not present in all cases of HCC and are more likely to be absent in early HCC. In addition, similar features are focally present in HGDN. Vascular invasion is diagnostic of HCC, but is typically not seen in early HCC. The most reliable morphological criterion that distinguishes early HCC from HGDN is stromal invasion. However, this feature is difficult to assess in biopsies. The following approaches have been used to distinguish HGDN and early HCC.

1. Immunohistochemistry

(a) Cytokeratin 7

Stromal invasion into portal tracts, fibrous septa or adjacent parenchyma is considered diagnostic of HCC (22-25). In the setting of chronic liver disease or cirrhosis, small groups of hepatocytes (referred to as hepatocyte buds) can be surrounded by fibrous septa and has to be distinguished from true stromal invasion. The intraseptal hepatocyte buds are contiguous with ductular reaction, and is indicative of regeneration from intrabiliary progenitors (28). This regenerative ductular reaction can be highlighted by CK7 around noninvasive nodules. In areas of stromal invasion, ductular reaction is at least focally absent. Small nodular HCC has more extensive loss of CK7+ ductular reaction compared to early vaguely nodular HCC (28).

CK7+ ductular reaction is prominent in most cases of regenerative nodules and HGDN. A vast majority of HGDN show ductular reaction around >50% of the circumference of the nodule, but overlap with HCC can be seen in 5-10% of cases (28). CK19 can also be used to highlight the ductular reaction (29).
(b) CD34
Most HGDNs show focal staining at the periphery of the nodule. Early HCC (vaguely nodular HCC) typically shows multifocal sinusoidal CD34, while diffuse sinusoidal staining is typical of nodular (progressed) HCC.

(c) Heat shock protein 70
Heat shock proteins (HSP) are highly conserved proteins that are expressed in stressful conditions and play an important role in protein homeostasis, regulation of cell cycle progression and apoptosis. Different classes of HSPs are designated by their molecular weight. HSP70 is a potent anti-apoptotic and its overexpression allows cell survival. In a gene expression study comparing early HCC with other hepatocellular nodules, a set of 95 genes provided the molecular signature that distinguishes early HCC from noncancerous tissue (30). Of these, the most abundantly upregulated gene in early HCC was HSP70.

HSP was closely correlated with pathologic parameters of tumor progression like large size, vascular invasion, advanced stage and high Ki-67 index in some (31) but not all studies (32). HSP70 is immunohistochemically expressed in up to 80% of early HCC in resection specimens, but less than 50% of cases on biopsy (32-35). While regenerative cirrhotic nodules are negative, HSP70 expression has been seen in 5-10% of HGDN.

(d) Glypican-3
As described in the earlier section, glypican-3 (GPC-3) is an oncofetal antigen that is expressed in HCC but not in benign hepatocellular nodules. The sensitivity of 60-70% has been reported for GPC-3 in early HCC, but is likely to be lower in biopsies. The expression of GPC-3 in HGDN is highly variable across studies ranging from 7-22% (9,10,33) in most series and 75% in one study (11). Other pitfalls in diagnosis include patchy GPC-3 expression in hepatocytes in cases with high necroinflammatory activity (36) and in 4% of regenerative cirrhotic nodules (12).

(e) Glutamine synthetase
As described in the earlier section, diffuse cytoplasmic expression of glutamine synthetase (GS) correlates with mutations in beta-catenin. Upregulation of GS mRNA, protein and activity has been described in human HCC with stepwise increase from precancerous to early and advanced HCC (37). GS expression has been reported in 13-70% of early HCC (33,34). GS expression can be seen in 10-15% of HGDN. In contrast to diffuse expression (>50% tumor cells) in HCC, GS expression in HGDN is typically focal with involvement of <50% of tumor cells (34).

(f) Combined use of HSP70, GPC-3 and GS
Two studies from the same group, one based on resections and the other on biopsies, have evaluated the utility of the combined use of these three markers for diagnosis of HCC (33,35). The specificity and sensitivity for diagnosis of HCC was 100% and 57% respectively when two of the three markers are positive. All three stains are negative in >70% of HGDN compared to 3% of early HCC in resection specimens, while all three are positive in 43% of early HCC compared to none of the HGDN.

(g) Cyclase associated protein 2
This protein plays a role downstream of the ras pathway and is involved in cytoskeletal function through binding with actin. Cyclase associated protein 2 (CAP2) is strongly expressed in smooth muscle, but is negative in normal liver (34). upregulated in a stepwise manner in multistage
Regenerative nodules are usually negative, but can show peripheral expression. Most HGDN are negative or focally positive. CAP2 expression has been observed in 40% of early HCC in more than 70% of tumor cells (34). The tumor cells in areas of stromal invasion are often positive.

2. Gene expression

Differential expression of genes has been demonstrated by quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) in HGDN and early HCC (39,40). In one study, 12 genes were differentially expressed in early HCCs compared with dysplastic nodules. Of these, 3-gene set including GPC3, LYVE1, and survivin had a discriminative accuracy of 94% (40).

References:


