Immunohistochemistry as a surrogate for molecular diagnosis in hepatic tumours

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Abstract

Molecular analysis is revolutionizing our understanding of primary hepatobiliary tumours, and many gene products are being translated into immunohistochemical stains for routine use. Hepatocellular adenomas are divided into four genotypic subclasses using a panel of immunostains against liver-fatty acid binding protein, serum amyloid A, glutamine synthetase, and beta-catenin. Glutamine synthetase also differentiates adenomas from focal nodular hyperplasia. Glypican-3 and other novel markers are improving diagnostic accuracy for well-differentiated hepatocellular carcinoma, and hepatocyte paraffin antibody and arginase aid in identifying poorly differentiated hepatocellular carcinoma. Cholangiocarcinoma remains poorly understood, but a new generation of immunohistochemical markers is beginning to highlight important subtypes. Prognostic and predictive markers are under early investigation, with some as far in development as phase II clinical trials. Here we review new molecular-based immunohistochemical markers for all of these situations and others.

Keywords cholangiocarcinoma; focal nodular hyperplasia; hepatocellular adenoma; hepatocellular carcinoma; immunohistochemistry

Introduction

Hepatobiliary tumours remain some of the least understood human neoplasms. But in recent years, molecular investigation has begun expanding our knowledge base regarding hepatobiliary disease, and many of these discoveries are being translated into immunohistochemical tools for routine clinical use. Molecular analysis has revolutionized the diagnosis of hepatocellular adenoma (HCA) and of focal nodular hyperplasia (FNH). Primary hepatobiliary carcinomas including hepatocellular carcinoma (HCC) are genetically heterogeneous, and novel immunomarkers may help identify different subtypes. Many of these markers are also beginning to shed light on the clinicopathological profile of intrahepatic cholangiocarcinoma (ICC). We review new molecular-based immunohistochemical markers for diagnostic, prognostic, and predictive use across hepatobiliary tumours.

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Subclassifying hepatocellular adenomas

Nowhere have molecular-based markers changed hepatopathology more than in the revelation of HCA subtypes. HCA is an entity with heterogeneous histological features and an uncertain malignant potential. Zucman-Rossi and coauthors' molecular investigation of HCAs was a landmark study that culminated in a molecular classification system of four HCA subtypes with strong genotype–phenotype correlation (Table 1).[1]

Hepatocyte nuclear factor 1 alpha-mutated adenoma

Hepatocyte nuclear factor 1 alpha-mutated hepatocellular adenoma (H-HCA) is defined by biallelic deactivation of the *HNF1A* gene.[1] Mutations may be either somatic or germline. Two hotspots are responsible for the majority of H–HCAs, with mutations at codon 206 causing most tumours, but those at codon 291 most frequently found in familial cases. H-HCA is often multiple, with microscopic adenomas discovered upon resection of the clinically evident mass. It is also seen in familial adenomatosis (more than 10 adenomas) and maturity onset diabetes of the young, type 3. Only exceedingly rare H–HCAs have been associated with HCC. Microscopically, H-HCA is characterized by abundant steatosis. Liver-fatty acid binding protein (L-FABP) is encoded by the *FABP1* gene, which is regulated by *HNF1A*. *HNF1A* mutation results in loss of L-FABP expression, and thus negative immunohistochemical staining for L-FABP in an HCA with positive staining in background liver parenchyma is 100% sensitive and specific for H-HCA.[2]

Beta-catenin-mutated adenoma

Beta-catenin-mutated HCA (b-HCA) is characterized by mutations in the *CTNNB1* gene. The most common mutations are in exon 3, but exons 7 and 8 can also harbour alterations.[1] B–HCAs are the most common subtype to arise in males and harbour a much greater risk of malignant transformation than other subtypes.[2] They may display cytologic atypia and often have pseudoglandular architecture. Beta-catenin immunohistochemistry shows membranous staining in non-neoplastic hepatocytes. In b-HCAs, mutations in *CTNNB1* prevent degradation of beta-catenin, leading to its accumulation in the nucleus and nuclear immunoreactivity (100% specific for b-HCA),[2] though positivity can be patchy (Figure 1). Increased nuclear beta-catenin also upregulates *GLUL*, increasing expression of its product, glutamine synthetase (GS).[1] Diffuse cytoplasmic GS positivity raises sensitivity for b-HCA to 100%. However, specificity decreases to 89%. Also of note, GS expression may be weak or focal with *CTNNB1* exon 7 or 8 mutations.[2]

Inflammatory adenoma

Inflammatory HCAs (IHCA) were previously considered a variant of FNH until molecular analysis showed that these lesions were clonal. They have abnormalities of the JAK/STAT3 pathway, with 60% containing activating mutations in *IL6ST*. Mutually exclusive mutations in related genes including *JAK1*, *STAT3*, *FRK*, and *GNAS* account for another 20% of IHCAs. No genetic defect has been identified to date in the remaining 20% of IHCA.

Summary of immunohistochemical, molecular, and morphologic findings in hepatocellular adenoma and focal nodular hyperplasia

Lesion	L-FABP	Beta-catenin	GS	SAA	Molecular abnormality	Morphology
H-HCA	Negative	Membranous	Perivenular or negative	Negative	*HNF1A* mutation	Steatosis
b-HCA	Diffuse	Nuclear or nuclear and cytoplasmic	Diffuse	Negative	*CTNNB1* mutation	Cytologic atypia, pseudoglands
IHCA	Diffuse	Membranous	Perivenular or negative	Diffuse	*IL6ST*, *JAK1*, *STAT3*, *FRK*, *GNAS* mutations	Sinusoidal dilatation, inflammation, dystrophic arterioles, ductular reaction
Beta-catenin-mutated IHCA	Diffuse	Nuclear or nuclear and cytoplasmic	Diffuse	Diffuse	*IL6ST*, *JAK1*, *STAT3*, *FRK*, *GNAS* mutations and *CTNNB1* mutation	Sinusoidal dilatation, inflammation, dystrophic arterioles, ductular reaction
Unclassifiable HCA	Diffuse	Membranous	Perivenular or negative	Negative	None	No specific features
FNH	Diffuse	Membranous	Map-like	Negative	None	Fibrous septa, ductular reaction, dystrophic arterioles

Table 1

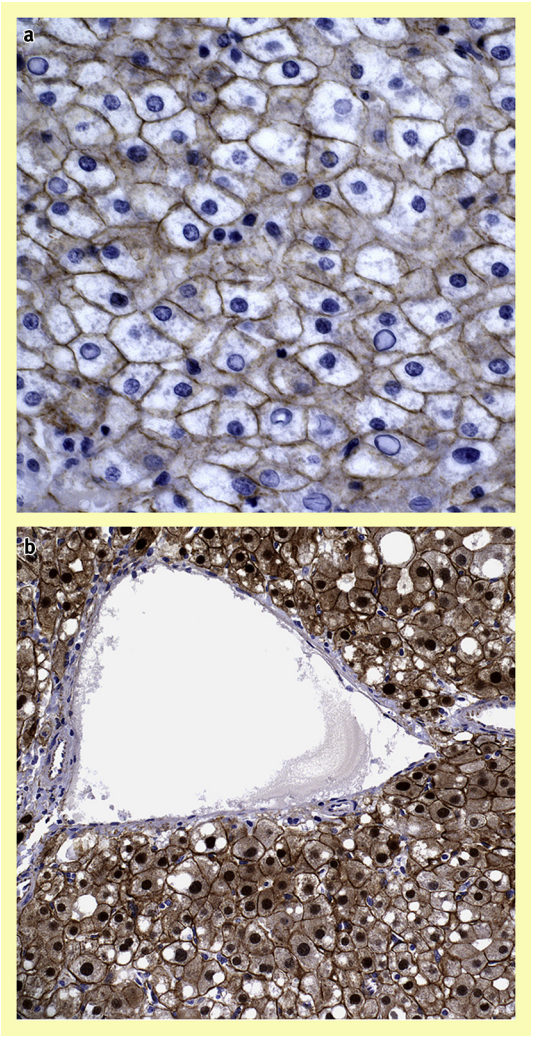

Figure 1 (**a**), Membranous beta-catenin staining in an HCA (X400). (**b**), Nuclear beta-catenin staining in a b-HCA (×200).

A subset of IHCA show concomitant *CTNNB1* mutations and are clinically and morphologically indistinguishable from other IHCA, making beta-catenin and GS testing imperative in these HCAs.[1,2] IHCA have a tendency to haemorrhage and are associated with obesity and alcohol consumption. They are often multiple, though do not typically qualify for adenomatosis. Serum gamma glutamyl-transferase, alkaline phosphatase, C-reactive protein (CRP), and fibrinogen may also be elevated. Some cases have shown a resolution of these biochemical elevations with excision of the adenoma. Morphologically, IHCA shows a variable combination of inflammatory infiltrates, pseudo-portal tracts with dystrophic arteries, ductular reaction, and sinusoidal dilatation. *CRP* and *SAA2* are upregulated, and immunostains for CRP and serum amyloid A2 (SAA) show strong, diffuse cytoplasmic staining with 94% sensitivity and specificity for IHCA (Figure 2).[2] Adjacent liver may also show microscopic foci with similar morphological and immunohistochemical features.

Unclassified adenoma

Immunohistochemical classification of HCA has been validated in numerous studies in diverse populations. Imperfections with immunohistochemistry persist, however, and molecular studies remain the gold standard.[3] For instance, CRP may be positive in non-tumour liver, rendering it unusable for diagnosing IHCA. Rare morphologically ambiguous lesions may show a GS staining pattern between that of HCA and FNH (see next section). The significance of patchy GS positivity in the absence of nuclear beta-catenin staining is also still under investigation. The proportion of HCAs unable to be classified varies in the literature, though most studies leave approximately 10% unclassified.

Differentiating between hepatocellular adenoma and focal nodular hyperplasia

Classically, differentiating between HCA and FNH lay in identifying FNH's central fibrous bands, ductular reaction, and dystrophic vessels. FNH is now understood to be a non-