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Subclassification of hepatocellular adenomas: practical considerations in the implementation of the Bordeaux criteria

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Summary

Hepatocellular adenomas are benign liver lesions with a risk of rupture and malignant transformation. Various molecular subgroups have been identified which appear to have characteristic morphological and immunohistochemical features. We examined the morphology and immunohistochemical profile of a series of 121 HCA from 97 patients to identify the HCA subtypes present and determine the number at risk for malignant transformation according to the World Health Organization (WHO) criteria for hepatocellular adenomas. There were 34 HNF1α inactivated HCA (28%), 61 inflammatory HCA (50%), 15 β-catenin activated HCA (12%) and 11 unclassified adenomas (9%). This proportion of cases was similar to that seen in other series utilising molecular classification. The morphological features of the adenomas were suggestive but not definite indicators of the subtypes present. Morphological features that showed overlap between the subtypes included steatosis within the lesion, a ductular reaction and focal atypia, so that immunohistochemical typing was required for accurate classification. In conclusion, immunohistochemistry is a clinically useful surrogate for identifying underlying molecular changes in the HCA subtypes.

Key words: Liver neoplasms; adenoma; liver cell; hepatocellular adenoma; immunohistochemistry.

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INTRODUCTION

Hepatocellular adenomas (HCA) are uncommon benign liver tumours usually seen in women of reproductive age who are using the oral contraceptive pill.[1] Other known risk factors include obesity, androgenic steroid use and various metabolic syndromes such as glycogen storage disease type 1 and mature onset diabetes of the young type 3 (MODY3).[2–4] The clinical significance of these lesions is related to their potential for rupture when larger than 5 cm in size and the 4–8% risk of malignant transformation to hepatocellular carcinoma (HCC).[5,6] For pathological diagnosis, the resemblance of some HCA to focal nodular hyperplasia is also important.[7]

The molecular subclassification of HCA was first published in 2007 and has since been incorporated into the World Health Organization (WHO) classification of liver tumours.[8,9] It separates HCA into four subgroups with distinctive morphological, immunohistochemical (IHC) and molecular features:

1. H-HCA: adenomas with inactivating mutations in the gene hepatocyte nuclear factor 1-alpha (*HNF1A*), characterised morphologically by steatosis and loss of staining of liver fatty acid binding protein (LFABP) within the tumour by IHC.
2. I-HCA: inflammatory adenomas with activating mutations affecting the Jak-Stat pathway, commonly the gp130 subunit of the IL-6 receptor, characterised morphologically by telangiectasia, inflammation and a ductular reaction and showing IHC staining with serum amyloid A (SAA) and C-reactive protein (CRP).
3. B-HCA: β-catenin-activated adenomas with mutations in the *CTNNB1* (β-catenin) gene, characterised by variable morphology ranging from no specific differentiating features to architectural and cytological atypia and showing staining with glutamine synthetase (GS) and/or nuclear expression of β-catenin (BC). Approximately 10% of I-HCA, expressing SAA and CRP, also show evidence of BC activation and are termed BI-HCA.
4. U-HCA: unclassified adenomas (U-HCA) with no specific histological features and an absence of characteristic IHC staining allowing subcategorisation into another group.

Since the original WHO classification, a further subtype of HCA, comprising adenomas originally classified as U-HCA, has been described based on mutations in the sonic Hedgehog pathway.[10] The identification of the different molecular pathways involved in the pathogenesis of the HCA subtypes and their morphological features has enabled the identification of distinctive radiological features of many of the adenoma subtypes. This allows for the non-invasive stratification of some of the subtypes according to their risk of rupture and malignant potential. Cohorts reporting on the prevalence of the various subtypes have been published from Europe, United States and Japan.[11–15] By far, the largest case series is the multi-institutional study from France.[10]

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The aim of this study was to subclassify a large cohort of HCA diagnosed in an Australian population based on an analysis of morphological features and the IHC profile as available to most community pathologists and to highlight issues around the application of diagnostic criteria.

METHODS

Patients

We reviewed the morphology and IHC profile of 121 consecutive HCA, from 97 patients, diagnosed at three institutions in Brisbane, Australia (Envoi Specialist Pathologists, Princess Alexandra Hospital and Royal Brisbane and Women's Hospital) from 1999 to 2016. Six cases interpreted as HCC were excluded.

Pathological review

All cases were reviewed, and the diagnosis of HCA was confirmed by two pathologists (GCM and CMC) with expertise in liver pathology. The following morphological features were evaluated in the lesion. Steatosis was scored according to the following criteria (0, <5%; 1, 6–33%; 2, 34–66%; 3, >66%), while inflammation, ductular reaction and telangiectasia were scored as present or absent. Atypia characterised by the presence of small cell change, pseudogland formation, nuclear atypia or loss of reticulin staining was recorded as absent or focal (involving less than 5% of the tumour volume) and the type of atypia was recorded. Cases with more than 5% of the tumour volume showing any of these changes were labelled HCC and were excluded. In resection specimens for HCA, the adjacent non-adenomatous liver was analysed for the presence of fibrosis (staged 0–4 as per METAVIR staging), steatosis (0–3), and portal or lobular inflammation.[16]

Immunohistochemical analysis

Immunohistochemistry was performed for BC (clone CAT-5H10; ready to use; Dako, Denmark), SAA (clone MC1; ready to use; Dako), LFABP (clone 2G4; dilution 1:3200; Abcam, UK), GS (clone GS6; dilution 1:1000; Becton Dickinson, USA) and CRP (clone Y284; dilution 1:1500; Abcam).

Interpretation of IHC staining

Liver fatty acid binding protein IHC was scored as present or absent (loss of the normal staining within the lesion). SAA was interpreted as negative (no staining) or positive (moderate granular or diffuse cytoplasmic staining in >10% of adenoma cells). CRP was interpreted as negative or positive (at least moderate cytoplasmic staining in >50% of cells). For BC, membranous (negative) or nuclear (positive) staining was recorded. GS staining was recorded as negative, patchy or diffuse with the percentage, intensity of staining and location of positive cells recorded.

HCA subgroup classification

Tumours were assigned to the following categories based on the immunohistochemical profile: H-HCA, loss of staining of LFABP in the tumour; I-HCA, presence of positive threshold staining for either CRP or SAA in the tumour; B-HCA, presence of either BC nuclear staining or positive staining with GS (the B-HCA group included those lesions showing both BC/GS and CRP/SAA); U-HCA, no specific staining pattern.

Ethics approval

This study was approved by the Royal Brisbane and Women's Hospital Low and Negligible Risk Review Board (HREC/12/QRBW/27).

Statistical analysis

Statistical analysis was performed using SPSS version 23. Parametric data were analysed using Pearson's chi-squared test. Non-parametric data were analysed using Kruskall–Wallis test. A p value of <0.05 was regarded as significant.

RESULTS

A total of 121 HCA were analysed from 97 separate patients (95 resections, 26 biopsies). There were 92 females and five males, with most cases of child-bearing age (median 38 years; range 18–74). The mean size was 52 mm (range 2–150 mm). Eighteen patients had multiple adenomas (18.6%). After interpretation of the immunohistochemical staining profile, 61 HCA (50.4%) showed positive staining with either SAA or CRP, consistent with inflammatory HCA (I-HCA); 34 HCA (28.0%) showed loss of LFABP, consistent with HNF1α inactivated HCA (H-HCA) and 15 HCA (12.4%) showed evidence of BC activation characterised by either nuclear staining with BC or any positive staining with GS (B-HCA) (Fig. 1, Table 1). Within this group of 15 B-HCA, 11 also showed positive staining for either SAA or CRP (BI-HCA). In patients with HCA diagnosed on biopsy, seven (26.9%) were H-HCA, 13 (50%) were I-HCA, four (15.3%) showed β-catenin activation and two (7.7%) were U-HCA.

In the classification of I-HCA, CRP staining was more often positive and more extensive than SAA, improving the diagnostic yield (Fig. 2). There were 11 cases (9.1%) with no specific immunophenotype consistent with the unclassified HCA subgroup (U-HCA). When compared with HCA without IHC staining for BC or GS, B-HCA were found at a younger median age (34 vs 38 years, p=0.031) and were larger on average (87 vs 52 mm, p=0.019).

In 18 patients with multiple adenomas, 13 had 2–4 lesions, four had 5–10 lesions and one had >10 adenomas. In five of the 18 patients (28%), more than one adenoma subtype was seen. These were two cases with both I-HCA and U-HCA, two cases with both I-HCA and H-HCA, and one case with I-HCA and B-HCA.

No morphological feature was specific for an adenoma subgroup and, to a varying degree, there was overlap in the morphological features between the groups, emphasising the centrality of the immunohistochemical profile to the diagnosis. Steatosis was seen in all subtypes of HCA (Fig. 3) but was statistically more likely to be seen in H-HCA (p<0.0001) (Table 1) where it was also more likely to be severe (Table 2). I-HCA were most likely to show changes of telangiectasia (p<0.001), a ductular reaction (p<0.001) and inflammation within the lesion (p<0.001) (Fig. 4). There were no morphological features identified specific to U-HCA.

Atypia was seen in all subtypes except I-HCA and pure B-HCA (Fig. 5). In all 10 cases the atypical changes, which were either small cell change, pseudogland formation or loss of reticulin, were only focal within the lesion. Some lesions contained more than one atypical feature but overall they did not occur in more than 5% of the tumour volume. Pseudoglands were seen in all subtypes other than I-HCA, small cell change was seen in two cases of H-HCA and three cases of BI-HCA and there was focal loss of reticulin in two cases of BI-HCA and in one U-HCA. The reticulin loss was not in an area of steatosis.

In the adjacent, non-adenomatous liver, steatosis was much more likely to occur in cases of I-HCA (p<0.001). There were no cases showing significant peritumoural inflammation or fibrosis.

DISCUSSION

The molecular subtyping of HCA has led to an appreciation of the different and distinct pathogenesis, clinical and pathological features, and natural history of these lesions.[10] The correlation between genotype and phenotype allows a practice without access to advanced molecular technology, to subclassify these lesions based on the use of an

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