In situ hybridisation for albumin RNA in paediatric liver cancers compared with common immunohistochemical markers

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ABSTRACT

Aims In situ hybridisation (ISH) for albumin mRNA is a sensitive marker of primary liver tumours in adults. However, paediatric tumours, such as hepatoblastoma (HB) and fibrolamellar hepatocellular carcinoma (FLC), have not been tested thoroughly and may require ancillary tests to diagnose with confidence. We aim to determine if albumin ISH is useful in the pathological evaluation of these malignancies and to compare it to commonly used immunohistochemical markers HepPar 1 (HEPA) and arginase-1 (ARG).

Methods Tissue microarrays of 26 HB and 10 FLC were constructed. Controls included 4 embryonal undifferentiated sarcomas of the liver, 51 neuroblastomas and 64 Wilms tumours. We evaluated a commercially available RNA ISH to detect albumin mRNA. Immunohistochemistry for HEPA and ARG was performed in the usual fashion.

Results Twenty-six of 26 HB showed positive staining by albumin ISH including 14 fetal, 8 embryonal and 4 mixed variants. All 10 FLC were diffusely positive. The sensitivity and specificity of albumin ISH were 100% for HB and FLC. ARG had 100% sensitivity and specificity for HB (26 of 26 cases) and FLC (9 of 9). HEPA stained 22 of 26 HB (85% sensitivity, 99.2% specificity) and 7 of 9 FLC (78% sensitivity, 99.1% specificity).

Conclusion Albumin RNA ISH is a useful test to determine hepatocytic origin in HB and FLC. ARG was equally sensitive and easy to interpret, while HEPA was inferior to both in HB and FLC.

INTRODUCTION

Paediatric liver malignancies are rare, and thus can be challenging for pathologists to diagnose, especially on small biopsy specimens. Staging and treatment depend on accurate classification, and thus necessitate optimisation of ancillary studies.

Hepatoblastoma (HB) is the most common malignant liver tumour in children, with an annual incidence of 1.2–1.5 per million population per year and the majority of cases occurring before age 5. HB may demonstrate epithelial and mesenchymal morphology. Currently, six variants of the epithelial subtype are recognised, including mitotically active and mitotically inactive fetal (resembling normal fetal hepatocytes), embryonal, pleomorphic, small-cell undifferentiated and macrotrabecular; mixed epithelial patterns may also occur. The embryonal component shows increased cellularity and mitotic activity with 'small blue cell' morphology.[1]

Fibrolamellar hepatocellular carcinoma (FLC), unlike other forms of hepatocellular carcinoma (HCC), tends to occur in non-cirrhotic livers in adolescents and young adults, and accounts for approximately 5% of primary liver cancers.[2] The prognosis may be better compared with other forms of HCC, though this may be related to the lack of underlying liver disease.[2]

HB may be challenging to diagnose due to its varied morphology (fetal and embryonal patterns) while FLC often resembles adenocarcinoma due to the amount of lamellated stroma (desmoplasia) and positive staining with cytokeratin 7.[3] This diagnostic dilemma is further compounded by small biopsies. Therefore, ancillary testing may be helpful to clarify hepatocellular origin in these tumours. Glypican-3 (GPC3), an oncofetal protein and one of the more useful markers for HCC, has recently been shown to be positive in a portion of other paediatric tumours, including yolk sac tumours, rhabdomyosarcoma and Wilms tumours, as well as physiologically expressed in children under 1 year of age.[4] GPC3 has also been noted to be less sensitive in FLC.[5 6] Therefore, GPC3 may not be an ideal ancillary test in the evaluation of paediatric liver malignancy (though it may certainly have utility as part of a larger panel). Additional frequently used markers of hepatocellular differentiation include HepPar (HEPA) and arginase-1 (ARG). In a series of 12 HBs,[7] HEPA was positive in 100% of cases, however, the staining was reported to be less strong in the embryonal type than in the fetal type HB. HEPA is also reported to be positive in two small series of FLCs.[3 8] To date, ARG has not systematically been evaluated in large series of HBs or FLCs, but has been shown in multiple studies to be highly sensitive and specific for both benign liver and HCC.[9–15] Nonetheless, arginase can also be negative in a subset of HCCs including those that are well differentiated.[9 16]

As it is synthesised uniquely by hepatocytes, albumin is an attractive marker of hepatocellular differentiation. However, staining for albumin protein by immunohistochemistry (IHC) is an unsuitable methodology as the protein is transported throughout the body ubiquitously. In situ hybridisation (ISH) for albumin mRNA can be used to localise the site of albumin synthesis, namely liver tissue. In adults, albumin ISH has previously been established as a sensitive marker of primary liver tumours,[17–22] including intrahepatic

cholangiocarcinomas,[23] while it is negative in most pancreatic adenocarcinomas and distal biliary tumours.[23] Previous use has been limited by lack of a simple commercially available protocol. Several albumin ISH platforms have been studied in adult primary liver tumours, including HCC[24] and intrahepatic cholangiocarcinoma[23] and initially demonstrated high sensitivity (100% and 99%, respectively) for these tumours. In contrast, non-liver primary tumours generally did not hybridise, with the exception of germ cell, pancreatic acinar cell tumours and tumours with hepatoid features.[17 24] Recently, a study by Nasir *et al*[25] from the Mayo Clinic demonstrated that albumin ISH can be positive in non-liver tumours including yolk sac tumour, acinar cell carcinoma (29%), breast invasive ductal carcinoma (18%), gallbladder adenocarcinoma (39%), gastro-oesophageal adenocarcinoma (20%), hepatoid pancreatic adenocarcinoma (33%) and lung adenocarcinoma (20%). Of these, yolk sac tumours have the most relevance in paediatric populations. To date, the utility of commercially available platforms for albumin ISH has not been fully ascertained in paediatric liver tumours.

The aim of the current study is to determine the sensitivity and specificity of albumin ISH in the pathological evaluation of childhood liver malignancies. A secondary aim is to compare albumin ISH to the common immunohistochemical markers, HEPA and ARG.

MATERIALS AND METHODS

Selection of cases

With approval from the Columbia University Medical Center Institutional Review Board, a search of departmental archives for HB over a 23-year period (1990–2013), including resection and biopsy material, was conducted. After exclusion of cases with insufficient viable tumour tissue, 34 cases of HB were identified. These included patients from 6 weeks to 7 years (average age 1.85 years) of age and were predominantly male (21 males, 13 females). Ten FLCs were included. Other paediatric malignancies including 4 embryonal undifferentiated sarcomas of the liver, 64 Wilms tumours and 51 neuroblastomas were included as controls. All tumours (plus non-lesional control tissues) were evaluated on tissue microarrays (TMAs).

Tissue microarrays

TMAs were constructed from archived formalin fixed paraffin embedded (FFPE) blocks. For the HBs, 2 mm cores were used, and when possible, at least two areas of tumour were sampled to maximise representation of epithelial subtypes (range: 1–4 per case; average: 2). Non-lesional tissue from the same specimen was used as a control. Two 2 mm cores were used for embryonal sarcoma cases. TMAs from previous studies were used for neuroblastomas and Wilms tumours. One to two 2 mm cores were selected for neuroblastoma cases. The Wilms tumour TMAs were constructed with 1 mm cores, but were more extensively sampled (between 3 and 4 cores/case).

In situ hybridiszation

ISH for albumin RNA expression was performed on fresh cuts of TMAs, using an albumin RNA ISH probe (RS7752, ACD/Leica Biosystems, UK). Tissue sections are subjected to heat retrieval with Bond ER2 solution at a pH of 9 for 15 min at 95°. RNAScope enzyme retrieval takes place at 40° for 15 min. The tissue sections are then exposed to an analytic specific reagent probe and hybridised for 2 hours at 42°. Detection of the probe is performed using the RNAScope DAB kit over the course of 4 hours.

Table 1 Staining results of ALB, ISH, ARG and HEPA in hepatoblastoma variants and FLC

		ALB ISH	ARG IHC	HEPA IHC
Hepatoblastoma	Fetal	14/14	14/14	13/14
	Embryonal	8/8	8/8	5/8
	Mixed	4/4*	4/4	4/4
	Total	26/26 (100%)	26/26 (100%)	22/26 (85%)
Fibrolamellar HCC		10/10 (100%)	9/9 (100%)†	7/9 (78%)†

*3/4 mixed subtype cases showed staining in both components; in the remaining mixed subtype case, only the fetal compartment stained.
†Tissue loss led to 9 evaluable cases.
ALB, albumin; ARG, arginase; FLC, fibrolamellar HCC; HCC, hepatocellular carcinoma; HEPA, HepPar 1; IHC, immunohistochemistry; ISH, in situ hybridisation.

Positive staining of the albumin RNA ISH was reviewed by three pathologists at ×40 (AKdG, HR and SML) and defined as red, 'dot-like' signals of varying sizes in the cytoplasm and nuclei of tumour cells.

Immunohistochemistry

HEPA IHC stain was carried out using a monoclonal mouse anti-human antibody (OCH1E5; Dako, Carpinteria, California, USA) at a dilution of 1:200. Arginase-1 (ARG) IHC staining was performed using a polyclonal rabbit antihuman antibody (Sigma-Aldrich, St. Louis, Missouri, USA) at a dilution of 1:100. IHC staining for HEPA and ARG were performed using the Ventana Benchmark ultra-automated stainer. Heat retrieval was carried out at a pH of 7.3 at 95° for 36 min and incubated at 36° for 32 min. Labelling and detection were carried out using the Ultraview DAB detection kit. Normal liver and bile duct tissues were used as a positive and negative controls, respectively, for both IHC markers. Positive staining for HEPA and ARG was defined as cytoplasmic staining of tumour cells >5%. Each stain was evaluated for diffuse or focal tumour staining patterns. A threshold of >50% tumour cells with positive staining was defined as diffuse staining. Interpretation was performed by three pathologists (AKdG, SML and HR).

RESULTS

Eight cases of HB were excluded from the analysis due to failure of RNA expression in the internal control or lack of viable tumour in the TMA blanks. Of the remaining HB which had both appreciable tumour and a positive internal control, 26 of 26 were positive for albumin RNA by ISH (table 1). All 10 FLCs were diffusely positive (table 1). Sensitivity and specificity of albumin ISH were both 100% for HB and 100% for FLC. The number of signals in the positive cells was generally very high and easily appreciated (figure 1) with the exception of one case of HB which showed only focal positivity. ARG had 100% sensitivity and specificity for HB (26/26) and FLC (9/9). Two of the 26 HB cases showed focal ARG positivity but were HEPA negative and were of embryonal type (figure 1). HEPA stained 22 of 26 HB (85% sensitivity, 99% specificity) and 7 of 9 FLC (78% sensitivity, 99% specificity). Two of the 22 HEPA positive HB cases showed only focal positivity: one embryonal and one fetal type HB. No embryonal undifferentiated sarcomas of the liver, Wilms tumour, or neuroblastoma hybridised for albumin or expressed ARG by IHC. One Wilms tumour expressed HEPA by IHC in the epithelial component of the tumour (figure 2). Sensitivity, specificity, positive predictive value and negative predictive value are summarised in table 2.

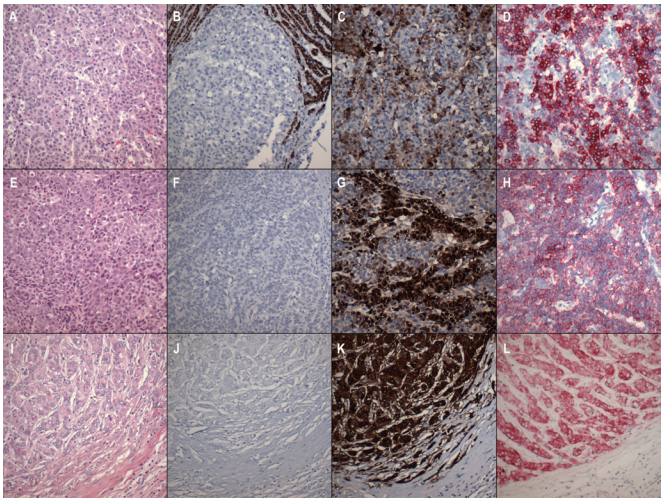

Figure 1 Fetal pattern hepatoblastoma (H&E) (A) stained negatively with HepPar (B), but positively for arginase (C) and albumin RNA ISH (D). Embryonal pattern hepatoblastoma (H&E) (E) stained negatively for HepPar1 (F), but positively for Arginase (G) and Albumin RNA ISH (H). Fibrolamellar hepatocellular carcinoma (H&E) (I) stained negatively with HepPar (J), but positively for arginase (K) and albumin RNA ISH (L) (×20 magnification). ISH, in situ hybridisation.

DISCUSSION

Albumin RNA ISH is a useful test to confirm hepatocellular differentiation in the relatively rare paediatric liver malignancies, HB and FLC. ARG offers similar sensitivity and specificity by IHC. HEPA was less sensitive and specific than both albumin ISH and ARG. HEPA was negative in the blastemal and stromal components of Wilms tumours, however, focal HEPA staining was noted in the epithelial component in one case of Wilms tumour (figure 2).

Paediatric liver malignancies are rare and can be a challenge to diagnose based on their protean histomorphological patterns. HB may recapitulate fetal liver or demonstrate the less differentiated morphology of the embryonal pattern. There may be considerable morphological heterogeneity displayed by different epithelial subtypes. Mesenchymal differentiation is also commonly encountered, particularly after therapy.[26] Wilms tumour is a triphasic tumour with stromal, epithelial and blastemal components.[27] The blastemal component may be particularly difficult to distinguish from the embryonal pattern of HB. Neuroblastoma can similarly display a range of differentiation, ranging from undifferentiated to poorly differentiated and differentiating.[28]

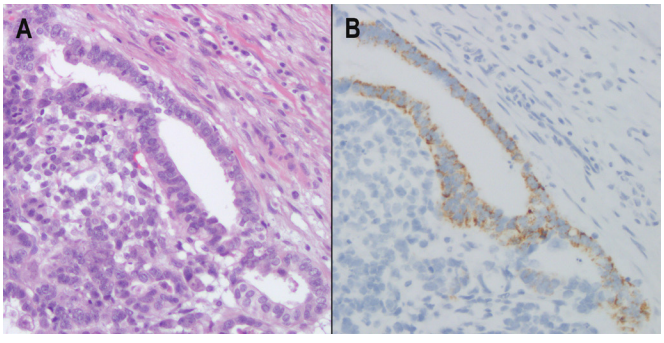

Figure 2 Epithelial and blastemal components in Wilms tumour (H&E) (A). In one case epithelial component stained positively by HepPar IHC (B) (×20 magnification). IHC, immunohistochemistry.

Table 2 Sensitivity and specificity of albumin RNA ISH compared with arginase and HepPar one IHC in hepatoblastoma

	Albumin ISH	Arginase IHC	HepPar 1 IHC
Sensitivity	100%	100%	84.6%
Specificity	100%	100%	99.2%
Positive predictive value	100%	100%	95.7%
Negative predictive value	100%	100%	96.7%)
Accuracy	100%	100%	96.6%

IHC, immunohistochemistry; ISH, in situ hybridisation.

The undifferentiated and poorly differentiated patterns can be similarly difficult to distinguish from embryonal HB by H&E morphology. Distinction between these three entities is vital for proper therapeutic selection and prognostic purposes. FLC is usually not on the differential diagnoses of these 'small blue cell tumours' and more commonly evokes consideration of adenocarcinoma. Despite these diagnostic challenges, there is a need to be mindful of tissue preservation in limited specimens, for example, needle biopsies. Molecular testing and/or tumour sequencing is commonly requested and may require significant portions of the biopsy material. Therefore, a balance must be struck between accuracy and parsimony. This balance must necessarily depend on the H&E morphology, as well as clinical features and anatomic location. Whenever a pathologist encounters a putative tumour in liver, the questions they ask are different in the context of low grade, well-differentiated lesions and high grade, poorly differentiated lesions. This study addresses the second scenario. Put another way, the situation which this study relates to is 'This is obviously a malignant tumour; what type is it?' If a needle biopsy of a liver mass shows obvious hepatoid features in a patient with clinical features suggestive of HB (eg, high alpha fetoprotein), then the pathologist may choose to omit organ-of-origin studies (like albumin ISH and ARG IHC) and focus on demonstrating malignancy (through loss of reticulin staining or abnormal positive staining by GPC3, beta-catenin and/or CD34 IHC). On the other hand, when facing a poorly differentiated or high-grade tumour in the liver of a child, a limited panel designed to determine organ of origin is appropriate. Based on this study, a panel which includes ARG IHC and/or albumin ISH is likely sufficient to demonstrate hepatocellular differentiation, particularly if the clinical and laboratory values are suggestive of such. Though all of these are relatively rare tumours, HB, Wilms tumour and neuroblastoma, may all present as abdominal masses in very young children. We did not test every type of 'small blue cell tumour,' nor every paediatric malignancy. However, among these three childhood malignancies, the positive and negative predictive value for albumin ISH and ARG was 100%.

Take home messages

- Paediatric liver malignancies can pose a diagnostic challenge due to their varied histomorphology, and often require ancillary studies for classification.
- Albumin in situ hybridisation (ISH) and arginase-1 had 100% sensitivity and specificity for confirming hepatocellular origin in hepatoblastoma and fibrolamellar hepatocellular carcinoma, while HepPar-1 was less sensitive and specific.
- Albumin ISH and arginase-1 are both useful markers for ruling in hepatocellular tumours and ruling out paediatric non-hepatocellular malignancies.

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