ELSEVIER

European Journal of Cancer 41 (2005) 1503–1514

European Journal of Cancer

www.ejconline.com

Review

The emerging family of hepatoblastoma tumours: From ontogenesis to oncogenesis

Arthur Zimmermann *

SIOPEL Liver Tumour Study Group, Institute of Pathology of the University, Murtenstrasse 31, CH-3010 Berne, Switzerland

Received 6 September 2004; received in revised form 13 December 2004; accepted 25 February 2005
Available online 27 June 2005

Abstract

The identification of distinct types and subtypes of hepatoblastoma has led to a successful classification of these lesions. In recent years, and particularly within large tumour trials, the spectrum of paediatric epithelial liver tumours has increased. This, together with the need for defining clinically relevant risk groups, will require a new approach to defining and classifying these cancers. Furthermore, an impressive amount of molecular biological information on liver ontogenesis and growth regulation of hepatic tumours has recently accumulated, which will allow the development of a comprehensive classification system with particular emphasis on prognostics. In this review, novel findings relating to these issues are discussed.

Keywords: Hepatoblastoma; Liver tumours; Liver development; Ontogenesis; β-Catenin; Stromal tumours; Ductal plate

1. Introduction

Hepatoblastoma (HB) is a rare solid tumour in infancy and childhood that accounts for approximately 1% of all paediatric malignancies and represents one of the most common malignant paediatric liver neoplasms [1]. Between 1973 and 1997, 271 primary hepatic malignancy cases were reported in the US to Surveillance, Epidemiology and End Results (SEER). All the cases were from those less than 20 years of age and 67% and 31% were HB and hepatocellular carcinoma (HCC), respectively. In the group less than 5 years of age, HB accounted for 91%, whereas among those 15–19 years of age, HCC represented 87% of the cases [2]. From the reported cases, it can be seen that HB and HCC (of the adult type) display characteristic age prevalence, albeit with an overlap with the older paediatric age group.

The discovery of morphology distinction between HB and HCC was a major leap forward [3] and provided the baseline for later attempts at histological classifications. The identification of specific histological HB phenotypes ranges from the late sixties to the early nineties. The foetal/embryonal subtypes were the first to be identified, while the macrotrabecular subtype was the last. The description of these morphotypes (or patterns) resulted in the now widely used HB classification system [4] and the system currently employed in SIOPEL (International Childhood Liver Tumours Strategy Group)-3 as shown in Table 1. The criteria for HB typing and subtyping, and matters of HB classification, have recently been reviewed, including a compilation of pertinent literature [5]. One of the main merits of the classification now in use is that apart from separating HB from childhood HCC, it recognises that the histopathology of HB reflects distinct phases of liver cell development and maturation.

* Tel.: +41 31 632 32 22; fax: +41 31 632 49 95.
E-mail address: zimmerma@patho.unibe.ch.
URL: www.pathology.unibe.ch.

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.02.035

Table 1
Classification of hepatoblastomas (HBL) according to the SIOPEL Liver Study Group

Wholly epithelial type
Foetal ('purely foetal') subtype
Embryonal/mixed foetal and embryonal subtype
Macrotrabecular subtype
Small cell undifferentiated subtype (SCUD; formerly anaplastic)
Mixed epithelial and mesenchymal type
Without teratoid features
With teratoid features
Hepatoblastoma, not otherwise specified (HBL-NOS)

However, this appears to be only true for the embryonal and foetal subtypes, as the last two identified forms, *i.e.*, small cell undifferentiated HB and macrotrabecular HB, do not seem to fit into this concept. A major issue in the classification of HB is the reliable identification of risk groups within the clinical setting, similar to what has been achieved for other blastomatous tumours, in particular neuro and nephroblastoma. The recognition of novel phenotypes, together with the progress in detecting distinct molecular features of neoplasms, may now open the doors for extensive classification of what can now be defined as the hepatoblastoma tumour family. Important considerations for such an approach are reviewed here and more specifically, a new preliminary working formulation of HB and related tumours that also refers to risk is proposed for future discussions in both academic and clinical settings. Similar to previous classifications, the feasibility and significance of this working formulation will require its assessment within ongoing and future studies. In addition, lessons obtained from liver molecular embryology are discussed in light of potential pathways leading from ontogenesis to oncogenesis in the hepatoblastoma family.

2. Undifferentiated epithelial hepatoblastomas: a complex spectrum of lesions

Small cell undifferentiated HB was originally designated 'anaplastic', however, Haas *et al*. proposed to replace 'anaplasia' by 'small cell undifferentiated' (SCUD) in 1989 to give HB-SCUD [6]. HB-SCUD is composed of nests and sheets of usually small, loosely cohesive and mostly monotypic cells with scant cytoplasm and numerous mitotic figures that is reminiscent of neuroblastoma or other 'small cell blue tumours' [7]. Round or ovoid cells predominate (Fig. 1A) in HB-SCUD with the occasional presence of spindle or stellate cells within the mucoid matrix. HB-SCUD is generally regarded as high-risk morphology [7], which relates to its high proliferative activity that is linked to p27/KIP 1 down regulation [8]. In a 1989 study that reported 10 HB-SCUD cases, the estimated 24-month patient survival probability was 0% [6]. Similar to macrotrabecular hepatoblastoma (see below), very few studies have systematically analysed the SCUD phenotype with regard to its prognostic impact. In a study of completely resected HB, the 38% recurrence rate in lesions with SCUD histology compared unfavourably with the overall estimated event free survival rate of 91% for the entire group [9]. In addition, a recent study has demonstrated that a focal (partial or predominant) expression of small cell histology in completely resected HB may have an unfavourable effect on outcome [10], similar to that which has been found in nephroblastoma [11].

The cell composition of undifferentiated HB tumours is another area that requires further clarification: are undifferentiated HB tumours always small cell? In the on going SIOPEL-3 pathology review, we have encountered HB-undifferentiated (HB-UD) tumours exhibiting intermediate or large rather than small cells. As a working method, we currently propose to denote the two lesion types HB-intermediate cell undifferentiated (HB-ICUD) and large cell undifferentiated (HB-LCUD), respectively. An example of HB-LCUD is depicted in Fig. 1B. Without immunohistochemistry, large cell lesions might be confounded with other large cell tumours, including large B cell lymphoma. It is important therefore to recognise such lesions in the diagnostic setting. The difficulties arising from attempts to classify these lesions are underlined by the finding that CD99 antibody reactivity is found in some of the tumours without evidence of PNET (Fig. 1C). Large cell features are also known for other blastomas, specifically large cell anaplastic medulloblastoma [12] and large cell neuroblastoma [13], where it confers highly aggressive tumour behaviour. Medium-sized cells in undifferentiated HB has also been previously reported [7] and earlier observations of large cells occurring in HB has been summarised elsewhere [3].

Another topic that requires further study is the cellular source of undifferentiated epithelial HB. It has been suggested that stem or pluripotent cells may be the origin of small cells. This hypothesis is in part based on an ultrastructural and immunohistochemical similarity between small and oval cells in rat and injured human livers. One group proposed a role for hepatic stem-like cells in HB based on immunoreactivity for cytokeratin-7, albumin, and the oval cell-associated antigens OV-1 and OV-6 [14]. However, a more recent study failed to detect an oval cell phenotype in HB small cells [15]. Therefore, the question as to the source of these undifferentiated cells remains to be solved. If hepatic stem cells are not involved in the pathogenesis of HB-SCUD, an alternative pathway might involve

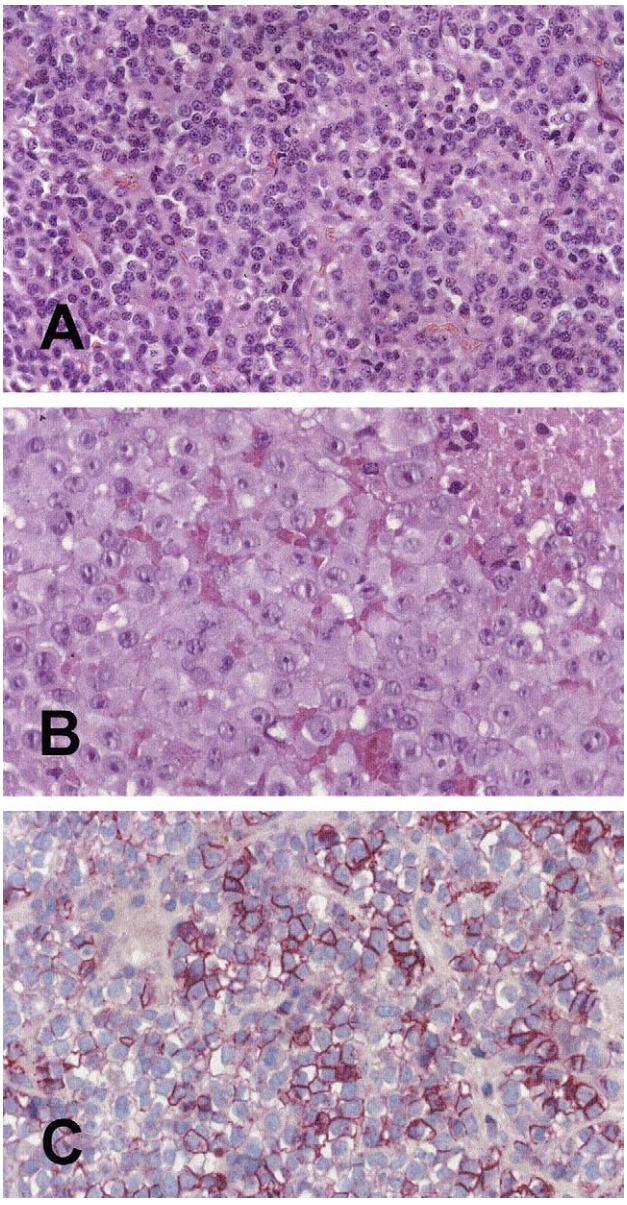

Fig. 1. (A) Hepatoblastoma, small cell undifferentiated subtype (HB-SCUD). Note that the small cells growing in a diffuse pattern display scant cytoplasm only, with an increased nucleus-to-cytoplasm ratio. This results in the features of a "small round blue cell tumour (SRBC)" (hematoxylin and eosin stain). (B) Undifferentiated hepatoblastoma, using the same magnification as for the lesion in (A). In contrast to HB-SCUD shown in (A), this large cell undifferentiated hepatoblastoma consists of large cells with abundant cytoplasm, however, not reflecting the phenotype of hepatocytes. The vesicular nuclei exhibit prominent nucleoli. Necrosis is seen at the top right corner (hematoxylin and eosin stain). (C) Hepatoblastoma, small to intermediate cell undifferentiated subtype, immunostained for CD99. Numerous tumour cells display typical membranous staining (red reaction product) (CD99 immunostain; MIC2 antibody).

regression to a primitive cell lineage of the hepatogenic foregut endoderm. This view is briefly outlined in later paragraphs.

3. Paediatric liver cell tumours with a mature hepatocellular phenotype: macrotrabecular hepatoblastomas and transitional liver cell tumour

In contrast to embryonal and foetal HBs that consist of hepatocyte lineage cells with immature 'blastic' features, several other types of liver cell tumours that develope in infants and children display a morphologically more mature phenotype. Tumours with cells resembling differentiated hepatocytes comprises macrotrabecular hepatoblastoma, hepatocellular carcinoma ('adult-type' HCC), and the recently described highly malignant transitional liver cell tumour (TLCT) that occurrs in older children and young adolescents. Irrespective of the mature-looking features of their cell composition, most of these tumours are clinically very aggressive and behave differently from foetal and mixed HBs with regard to their response to chemotherapy. The specific disease biology that is common to these hepatocytic lesions suggests that they form a distinct pathological and clinical group.

Macrotrabecular hepatoblastoma (HB-MT) is an uncommon tumour and is currently classified as one of wholly epithelial 'subtypes' of HB. However, the macrotrabecular morphology, characterised by cellular plates that are 5 to more than 20 cells thick, refers to a growth pattern rather than a distinct subtype. In fact, the term, 'macrotrabecular pattern' has been already been employed in paediatric liver tumour literature [16]. The reason for this is that large cell plates (the macrotrabecula) consist of either foetal or embryonal type cells, or a third cell type resembling neoplastic hepatocytes of HCC (Fig. 2A). In fact, the presence of foetal cells was regarded as requisite for the diagnosis of HB. Whereas HB-MT containing foetal and/or embryonal cells fits with the concept of hepatoblastoma, tumours with a trabecular growth pattern but composed of hepatocyte-like cells does not and suggests that HB-MT lesions form a heterogeneous group. The hepatocytic HB-MT phenotype is distinguished from HCC with great difficulties only, also with fine needle aspiration cytology.

Owing to its rarity, reliable information on the survival outcome from MT lesions is sparse, even though an unfavourable biological effect from this morphology is to be expected. In HB studies reported so far, only few analyses concerning prognostic impact of tumour histology are available [6,17–21]. In some studies, the authors did not employ types/subtypes for the definition of standard *vs*. high-risk tumours [9,22,23] and only two therapy studies have specifically referred to HB-MT. In the first study on 168 patients with HB, 18 patients had HB-MT, and their estimated 24-month survival probability was 50%, in comparison with 92%, 63%, and 0% for the purely foetal, embryonal, and SCUD histologies, respectively [6]. In a later second investigation

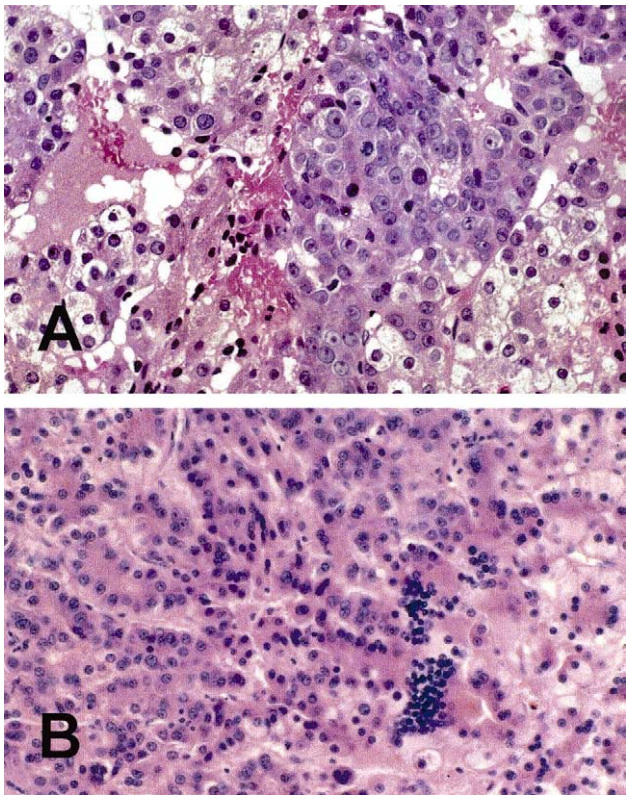

Fig. 2. (A) Hepatoblastoma, macrotrabecular subtype (HB-MT). Medium-sized to large cells resembling hepatocytes have formed a plate-like structure more than 10 cells thick (right to the middle of the figure). In addition, clusters of foetal-type tumour cells with clear or dark cytoplasm are in evidence (hematoxylin and eosin stain). (B) Transitional liver cell tumour (TLCT). The phenotype of this neoplasm shares features of hepatoblastoma and hepatocellular carcinoma, suggesting a transition between the two. Few multinuclear tumour giant cells are seen (right half of the figure) (hematoxylin and eosin stain).

that also classified tumours as HB-MT, the histology of any type/subtype did not have a significant prognostic effect [17].

The answer relating to the potential prognostic significance of the MT phenotype has, therefore, to await the analysis of larger trials, including SIOPEL-3, with more evaluable lesions with this histology. We propose to break down the MT growth pattern tumours into two categories: MT-1 consisting of HB-MT composed of hepatocyte/HCC-like cells (the expected high-risk group) and MT-2 composed of foetal and/or embryonal cells (the expected standard risk group), both occurring in either focal or diffuse pattern.

A novel liver cell tumour that develops in older children and young adolescents that has hepatocyte-like phenotype which is distinct from HCC, has recently been classified as transitional liver cell tumour (TLCT [24]). TLCT is a highly aggressive lesion that usually presents with large neoplasms and high or very high serum α-fetoprotein. Most of the lesions reported so far are based on biopsies that were initially diagnosed as HB. The tumour histology was reviewed owing to the observation that the biology of disease under an HB chemotherapy regimen was very unfavourable. It was found that the morphotype of these lesions was apparently situated between hepatoblasts and mature hepatocytes (Fig. 2B). Hypothetically, the neoplastic cell lineage involved may reflect a differentiation window in the transition between immature and mature liver cells. Similar to many HBs, TLCT in part express β-catenin [24]. The criteria for defining TLCT, with regard to histology and in a biological/clinical setting, have to await further studies. In particular, the differentiations between TLCT and HCC and between TLCT and HB-MT1 that develops in older children have yet to be worked out in more detail.

4. Aspects of growth and growth regulation: evidence for microheterogeneity in epithelial hepatoblastomas

Several biochemical and molecular biological abnormalities recently detected in hepatoblastomas are related to growth and the regulation of the cell cycle. They include the up regulation of growth factors, alterations in the expression of factors directly affecting cell cycle progression and cycle checkpoints and also cytokinesis proteins such as Polo-like kinase-1 (the PLK1 oncogene) that is highly expressed in HB to indicate poor prognosis [25]. A small number of putative tumour suppressor genes may also be altered in HB, *e.g.*, the paternally imprinted polyspecific transporter gene BWR1A (Beckwith–Wiedemann-related gene 1A/ORCTL2, organic cation transporter-like2) on chromosome 11p15.5 that is implicated in BWS [26]. By use of full length cDNA libraries and expression profiling, screening of HB has recently uncovered a host of other genes frequently appearing in HB with or without secretion of AFP (alpha-fetoprotein) in comparison with normal infant liver [25]. HBs are also characterised by complex alterations in signal transduction pathways. They show inactivation of the SOCS-1 gene, a JAK-binding inhibitor of the JAK/STAT signaling pathway [27]. However, more prominent in HBs are alterations of the β-catenin signalling pathway. In fact, mutations in the β-catenin gene represent the most frequent molecular alteration in sporadic HB detected so far. β-Catenin is central to the convergence of the Wnt, β-catenin, and cadherin pathways, where it forms a signalling complex with axins, APC (adenomatous polyposis coli) tumour suppressor protein, glycogen synthase kinase 3β, and other proteins (review in reference [28]). Simply put, the Wnt signalling pathway acting through frizzled receptors activates the dishevelled (Dsh) protein, which in turn uncouples β-catenin from its proteasomal degradation

pathway and results in its entry into the cell nucleus to effect distinct gene transcription. The Wnt pathway is affected by extracellular factors that bind Wnt, such as the co-receptor LRP (Dickkopf). Mutations in the β-catenin gene in HBs, in particular in the gene degradation targeting box, favour the by passing of the proteasomal degradation of β-catenin and a preferential shift of the protein into the nucleus, where it can then easily be detected by immunohistochemistry. Activating mutations of the β-catenin gene occur in at least 50% of HB, both in epithelial and mixed types (see review [29]). Mutations in β-catenin gene causes the protein to localise to the nucleus (a prognosticator in HB [30]) and are associated with poorly differentiated histology [31]. Furthermore, stabilised β-catenin can inhibit TNFα-induced apoptosis [32] and thereby probably affect growth responses. Interestingly, other members of the β-catenin signalling complex have been shown to be altered in HB also, including axin I/II [33] and APC [34]. Uncontrolled wingless/WNT signalling in HB is furthermore indicated by the over expression of a secreted inhibitor of WNT signalling, hDkk-1/human Dickkopf-1, suggesting a negative feedback mechanism [35].

It has been surmised that, in addition to the regulation of growth, mutations in the Wnt/β-catenin system may also be involved in carcinogenic pathways and may represent an early event in HB. The host of genetic alterations detectable in HB is impressive, and one may hope to employ these findings to arrive at a future molecular classification of HB and related tumours. However, the significance of these changes in regard to carcinogenic pathways still remains open. Many of the molecular aberrations may be late events in tumour evolution and could indicate the result of progressive genomic instability with subsequent clonal selection rather than primary events. This view is favoured when the spatial distribution of altered gene product expression in HBs is more closely examined. Even at lower magnification, part of the epithelial HBs display few or sometimes numerous circumscribed spheroid foci of smaller cells (Fig. 3A). The cells forming these foci partly resemble the small cell phenotype shown in a recent work on

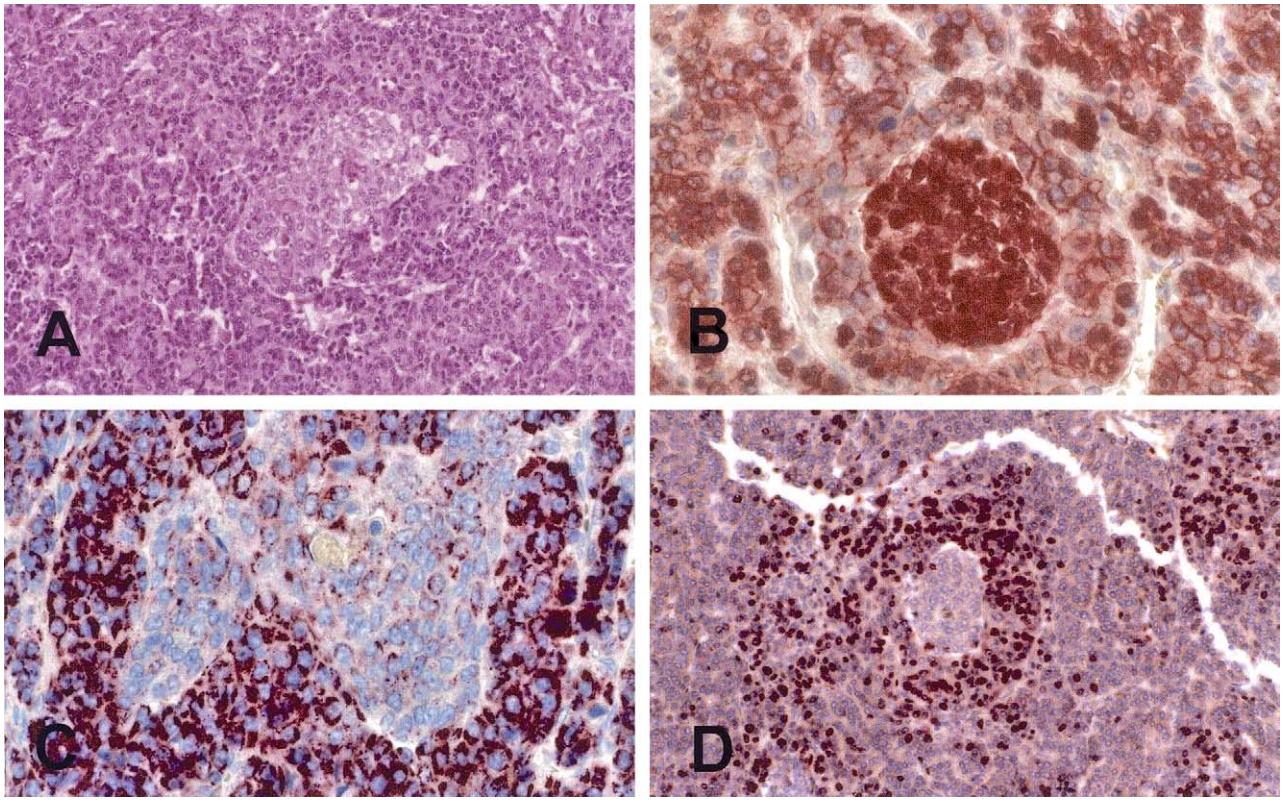

Fig. 3. (A) This heptoblastoma shows, in the middle of the figure, an ovoid, sharply delineated focus of paler cells. Numerous such foci were detected in this tumour (microheterogeneity) (hematoxylin and eosin stain). (B) A focus similar to that shown in (A), immunostained for β-catenin (red reaction product). It is seen that almost all cells of the focus reveal nuclear β-catenin staining, whereas adjacent tumour cells also display membranous staining (β-catenin immunostain). (C) Hepatoblastoma with a focus, stained for a mitochondrial antigen (red granular cytoplasmic reaction product). The cells of the focus show relatively few mitochondria, indicating poor cytoplasmic differentiation. In contrast, adjacent tumour cells encircling the focus are very rich in these organelles (mitochondrial immunostain, 113-3 antibody). (D) Density and distribution of proliferating tumour cells in a focus-containing hepatoblastoma; cells in proliferation have red nuclei. While the focus has a very low proliferation fraction, similar to more remote areas of the neoplasm, a rim of tumour cells encircling the focus discloses a very high proliferative activity (Ki-67 immunostain; MIB1 antibody).

focal SCUD morphology [10], though larger cells may also be involved. Immunohistochemically, these cells express β-catenin in an exclusively nuclear pattern, indicating β-catenin gene mutations, whereas neighboring foetal HB cells show more frequently membranous or cytoplasmic staining (Fig. 3B). This finding suggests that β-catenin alterations exhibit a distinct spatial distribution pattern within the tumours, possibly reflecting (later) selection of β-catenin gene-mutated subclones. This view is underlined by the observation that nuclear β-catenin reactivity is sometimes found in cells of the peripheral invasion front, and is absent or less in the more central parts of tumour nodules. In immunostains for mitochondrial antigens, the cells in the foci are low in mitochondria in comparison to adjacent foetal cells and suggest that they are poorly differentiated and may be rapidly growing (Fig. 3C). However, in the Ki-67 immunostain (Fig. 3D), few cells in the foci are in cycle and are encircled by a rather thin rim of proliferating cells. Taken together these observations might suggest that at least part of HBs display microheterogeneity and could contain complex cellular units consisting of poorly differentiated but weakly proliferating cell clusters with β-catenin gene mutations. The mutated cells could also be affecting the growth response of adjacent cells lacking the mutations. The identification of such foci/units and their perifocal area offers the opportunity to selectively isolate distinct cell populations (*e.g.*, through laser capture) for more detailed molecular analyses.

5. Stromal–epithelial and stromal tumours of the liver

One group of HB, the mixed epithelial and mesenchymal type, is histologically characterised by the variable development of stromal components, chiefly immature-looking fibroblastoid/myofibroblastoid tissue and osteoid. These features seem to have a prevalence ranging from about 20% to 50% [3,6]. However, these figures have to be interpreted with caution as they could be modified by differences in biopsy sampling techniques. For resections, the observation is that osteoid stromal component is both more frequent and extended in tumour tissue following chemotherapy [36,37]. As recent therapy studies have not stratified according to this type of HB, the prognostic significance of the presence of stromal elements have not been sufficiently clarified so far. In one study, the presence of osteoid or chondroid components was associated with improved prognosis for survival [6], whereas other investigations failed to show an association between survival and the presence of these elements [37].

Notwithstanding the morphological differences in mixed HBs between epithelial components of any kind and the stromal components, there is evidence that both have a common lineage. This is suggested by the observation that β-catenin mutations visualised by nuclear reactivity occur in epithelial and mesenchymal components. The pathogenic pathways causing the development of both epithelial and mesenchymal/stromal lineages within the same tumour are not yet known. A neoplastic disorder of mesenchymal-to-epithelial transition (MTET) or epitheliomesenchymal transdifferentiation (EMT) regulated by several factors, including members of the TGFβ family, cell-to-cell, and cell-to-matrix adhesion molecules has been discussed in this context.

HBs that are examined after resection sometimes reveal such a degree of stromal tissue predominance that the detection of epithelial components is difficult. But there are also rare instances, where the epithelial part is apparently lacking over larger parts of the tumour, and mostly a mesenchymal/stromal tissue is in evidence (Fig. 4A). Such lesions may be termed, paediatric hepatic stromal tumours (PHST), which in some way resemble lesions that have been described in the childhood kidney cancer, *i.e.*, metanephric stromal tumour/MST. We recently encountered a further childhood liver tumour characterised by an intricate relationship between epithelial and stromal components. In this lesion, bud-like structures consisting of epithelial cells of a hepatoid lineage were encircled by a distinct stromal spindle cell cap blending into foci of calcification and ossification of the mature type, *i.e.*, not only osteoid (Fig. 4B; ''ossifying hepatic tumour of infancy''). The centrally placed cells were reactive for a hepatocyte marker (Fig. 4C), and a fraction of their nuclei was reactive for β-catenin (Fig. 4D). Infantile stromal tumours with distinct ossification resemble those recognised for the kidney (ossifying renal tumour of infancy). Recently, another type of stromal–epithelial tumour of the liver that developed in three older children has been reported. These were large and encapsulated lesions, displaying an organoid arrangement of spindled and epithelioid cellular nests, with focal areas of calcification in one case. Two of the patients presented with Cushing's syndrome resolving after resection, and the tumours exhibited features of ACTH (adrenocorticotropic hormone) production [38].

6. Hepatoblastomas with cholangioblastic features and 'ductal plate tumours': evidence of a bimodal differentiation in liver cell tumours?

Cholangiocytes and duct-like structures consisting of them are recognised to occur in two distinct paediatric liver tumours: mesenchymal hamartoma and undifferentiated (embryonal) sarcoma. However, their contribution to other hepatic tumours in this age group is not well established. The generation of the intrahepatic

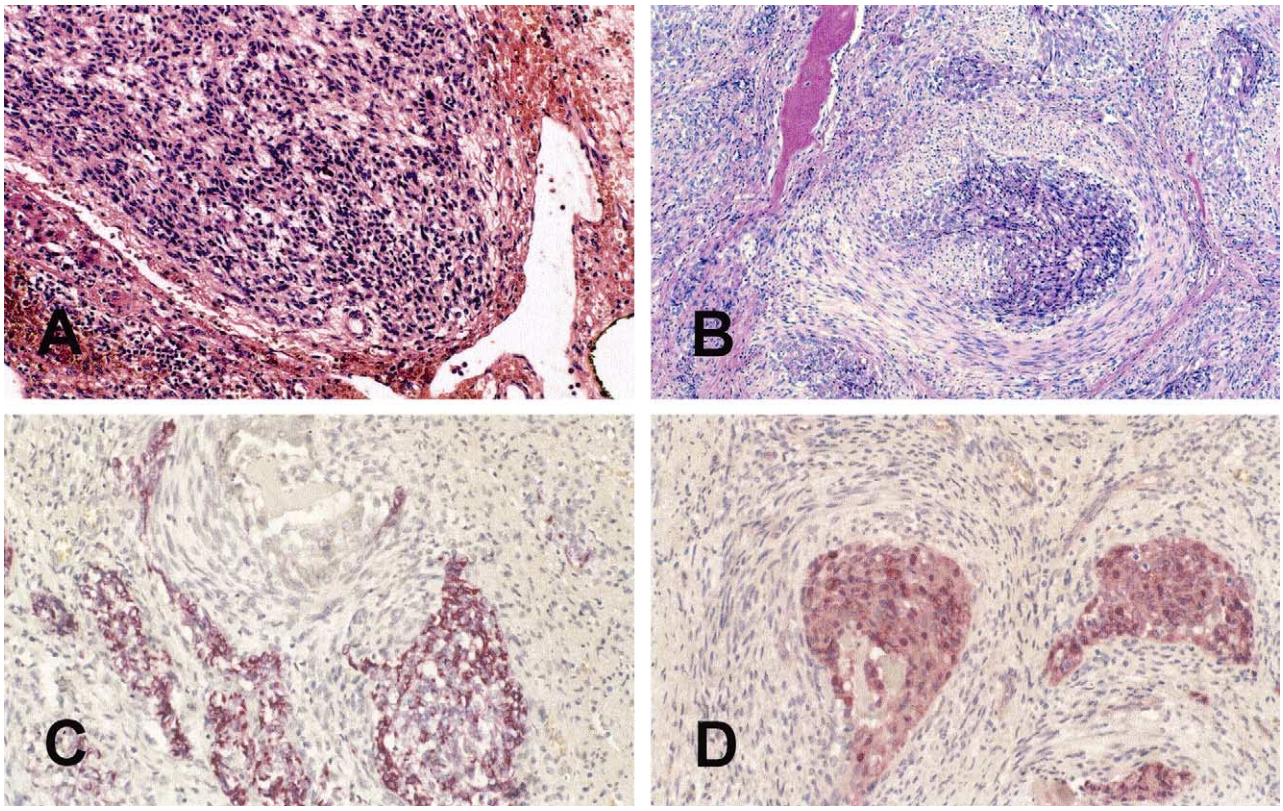

Fig. 4. (A) Hepatic tumour composed of densely packed spindle cells resulting in a stromal tumour pattern lacking cells of an epithelial lineage (hematoxylin and eosin stain). (B) This liver tumour consists of numerous bud-like structures with an epithelial core (centre of the figure). The epithelial bud is completely encircled by a cap of mesenchymal spindle cells, some of them are of a myoid phenotype. Focal ossification is seen (to the left upper corner) (hematoxylin and eosin stain). (C) Same tumour as in (B). The epithelial cell clusters forming the core of the buds are markedly reactive for a hepatocyte antigen (in red) (hepatocyte antigen immunostain; OCH1E5 antibody). (D) Same tumour as in (B). The cells of the epithelial buds express β-catenin in a mixed pattern (in red), with both nuclear and cytoplasmic reactivity (β-catenin immunostain).

biliary tree is critically dependent on the construction of the ductal plate, morphologically characterised by a distinct plate-like cellular structure at the border between parenchyma and future portal tract spaces, and composed of cells fated to become cholangiocytes (Fig. 5A). We have observed few HBs that display, at the periphery of hepatoblastic nodules of either foetal or embryonal morphology, a thin rim of atypical cholangiocyte-like cells and sometimes with slits resulting in a double layer of cells that in some way mimics an abnormal ductal plate (Fig. 5B; "ductal plate tumour" [39]). Moreover, it has recently been reported that HBs and related tumours may contain bile duct cells and even duct-like profiles in a focal distribution pattern (HBs with so-called cholangioblastic features, or cholangioblastic HB [39,40]). HBs with a contribution of a cholangiocyte-like lineage show clusters of complete or incomplete ductular profiles, frequently at the border of other epithelial components (Fig. 5C). The features may be missed in conventional preparations, but immunostaining for a cholangiocyte lineage marker, cytokeratin-19, should uncover the cells of interest (Fig. 5D). It is important to emphasise that there is no evidence that these cell formations are pre-existing ductules (the ductular reaction [41]), which are frequently encountered in the vicinity of liver tumours, but rather represent a distinct part of the neoplastic process itself. The proportion of HB displaying this change is not yet known neither is the biological significance of cholangioblastic features. These issues are to be assessed within large on going studies.

7. Organoid tumours of the liver: mimicry of normal hepatogenesis?

The last group of HBs and HB-like tumours to be discussed is characterised by a higher order of tissue organisation. The more usual growth patterns of HBs are either mass-forming or multinodular, the latter is frequently encountered post-chemotherapy and in the case of mixed HBs is sometimes related to the growth of epithelial tumour tissue around larger foci of osteoid. However, some neoplasms deviate from these patterns, forming organoid structures suggesting a distinct pathway for cell and tissue differentiation. There are HBs

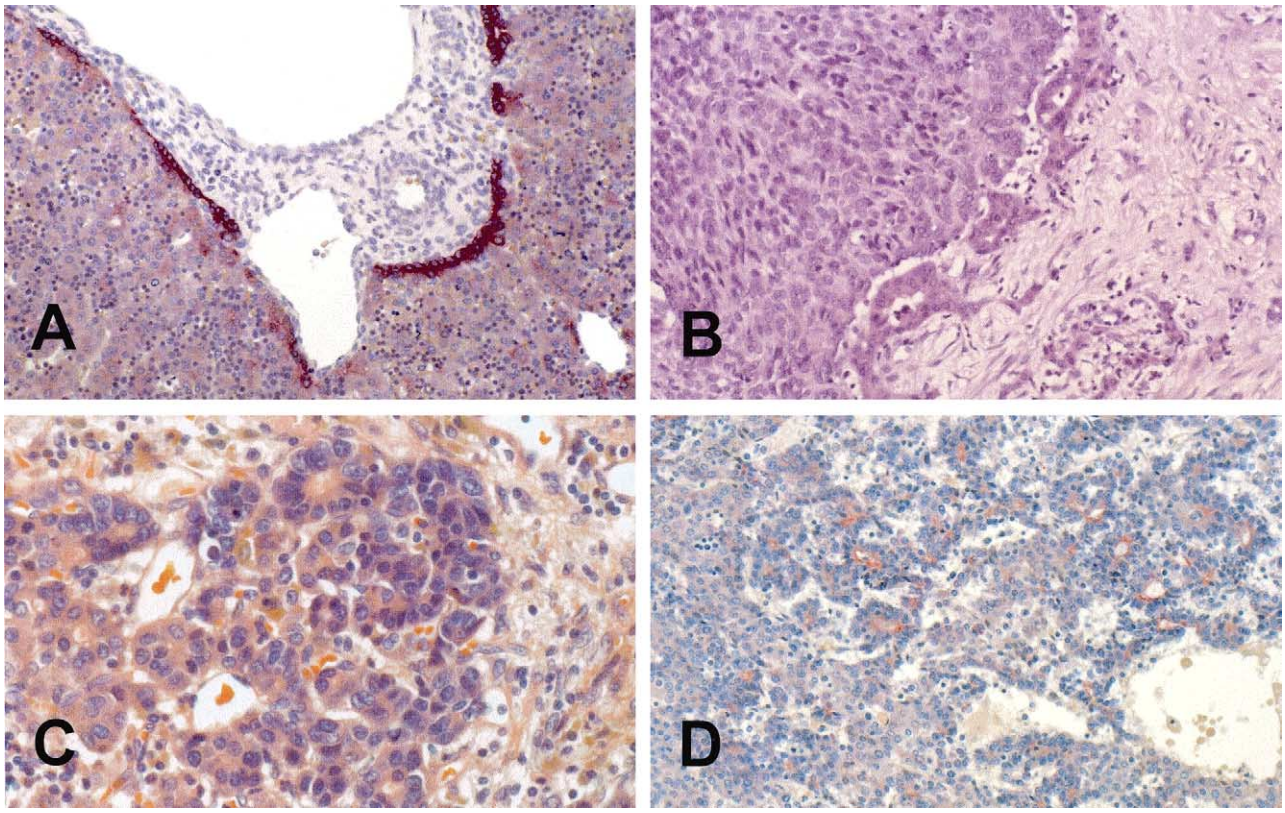

Fig. 5. (A) Normal foetal liver, immunostained for cytokeratin-19, a cholangiocyte marker (shown in red). Whereas the parenchymal cells and the cells of the future portal tract (containing vein and artery) are negative, a markedly positive band-like structure, split at few places, is seen between parenchyma and portal fields. This is the ductal plate that will give rise to intrahepatic bile ducts (cytokeratin-19 immunostain). (B) In this epithelial hepatoblastoma, a rim of cholangiocyte-like cells is interposed between the tumour nodule and the adjacent stroma, forming a structure reminiscent of an abnormal ductal plate (hematoxylin and eosin stain). (C) Epithelial hepatoblastoma with development of duct-like profiles in a focal distribution (right to the middle of the figure) (hematoxylin and eosin stain). (D) Immunostaining for the cholangiocyte marker, cytokeratin-19, reveals that these profiles are positive (''cholangioblastic hepatoblastoma'') (cytokeratin-19 immunostain).

apparently constructing a lobule-like architecture, the lobuloid units being arranged around an arterial vascular core. Within the lobule-like components, the HB cells seem to be non-randomly placed within a stromal bed, in some way reflecting the phenotype of a gland (Fig. 6A). This might result in a diagnostically intriguing situation, because the organoid configuration may mimick a hamartoma (''hamartoma-like HB''). This histological presentation of HB has already been described in older literature [42].

Another hepatic tumour with an organoid organisational pattern with an immature hepatic tumour of bimodal differentiation, has recently been described by us [43]. This novel type of hepatic neoplasm is characterised by numerous and small nodules consisting of an immature hepatocyte cell population, the nodules themselves being encircled by atypical duct profiles sometimes forming a double plate (Fig. 6B). The unique pattern of the ductal plate-like cholangiocyte component, far remote from pre-existing ducts and ductules, is best visualised by use of cytokeratin-19 immunohistochemistry (Fig. 6C). The authors proposed that this organoid phenotype, with formation of numerous ''liverlets'' could mimic a distinct phase of hepatogenesis [43].

8. Pathways from ontogenesis to oncogenesis: the potential impact of molecular embryology for future molecular classification of the hepatoblastoma family

Basic knowledge regarding normal hepatic ontogenesis (hepatogenesis) chiefly goes back to seminal investigations in the 1960s and 1970s, and has since been refined to considerable detail (see reviews [44,45]). Specifically, it has been found that the hepatic organogenesis proceeds in a series of distinct phases, encompassing a priming phase, a phase of increasing specification, the growth of a liver bud, migration of hepatoblasts into the mesenchyme of the transverse septum, a phase of specific hepatic vascularisation, the expansion phase, terminal differentiation of the hepatocyte lineage, and the construction of the biliary tree.

The parenchymal component of the liver is of endodermal origin. In the foregut endoderm, domains of competence (DOCs, the so-called hepatic field) develop

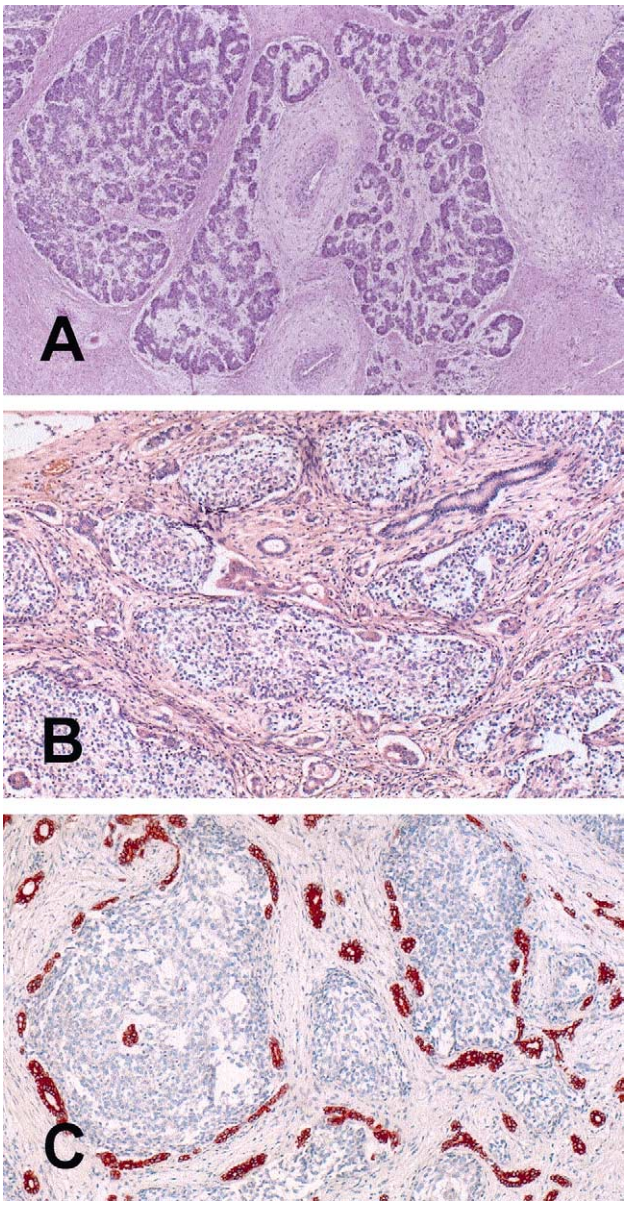

Fig. 6. (A) The cells of this epithelial hepatoblastoma form lobular structures centred around a vascular core. This organoid pattern results in hamartoma-like features of such tumours (hematoxylin and eosin stain). (B) This unique liver neoplasm is composed of numerous nodules consisting of immature hepatoid cells. The nodules are encircled by abnormal cholangiocyte-like profiles, together forming organoid units. Non-neoplastic bile ducts are seen in the upper half of the figure (hematoxylin and eosin stain). (C) Same tumour as in (B); cytokeratin-19 immunostain (reaction product red). It is seen that the cytokeratin-positive cholangiocyte profiles closely follow the tumour nodules, mimicking ductal plate-like structures. This unique tumour seems to produce ''liverlets'' (cytokeratin-19 immunostain).

where immature progenitor cells are fated to become liver. Within DOCs, commitment or priming of precursor cells to enter future hepatocyte and cholangiocyte lineages results in what is called specification and depends on a complex interaction between endodermal cells. A major step ahead was the recognition of the molecular mechanisms underlying this distinct sequence of organogenetic events (see review [46]). Specifically, early steps of liver development require an intricate network of several proliferation-specific transcription factors and a complex mesodermal–epithelial cell cross talk (see review [47]). The significance of an early mesodermal influence is illustrated by the production of the distinct pro-hepatogenic mesodermal signals, bone morphogenetic proteins and fibroblast growth factor isoforms by the septum transversum and cardiogenic mesenchyme, respectively. Priming for a hepatocyte lineage is a very early event and is associated with mesenchymal contacts, in that liver cell differentiation is initiated during endodermal–mesenchymal interactions prior to liver formation. The achievement of a hepatocyte fate (or hepatocyte lineage competence) markedly depends on the interaction with cardiogenic mesenchyme which activates crucial transcription factors (sox17α, HNF3β and GATA4) that are required for hepatocyte development. These developmental steps start with a still immature cell that rapidly expresses AFP but which will only later acquire the morphological features of hepatoblasts. Future analyses employing, *e.g.*, array technologies are probably required to discern if undifferentiated HBs reflect this early, endoderm-derived cellular phenotype.

Specification includes differentiation of cells to hepatoblasts that will form a liver bud that then invades the mesenchyme of the transverse septum. This is a highly critical step of hepatogenesis and is controlled by several factors, including factors regulating the NF-κB signalling pathway, Hex, Notch signalling, and probably Jumonji [47,48]. Immigration of hepatoblasts into the transverse septum mesenchyme leads to the characteristic patterning of these cells and later results in plate formation together with sinusoidalisation. This migrational step seems to depend on the remodeling factor, Prox1. Prox-1 deficient mice fail to accomplish septal hepatoblast migration and hepatoblasts remain clustered near the liver diverticulum [49]. Hepatoblast migration and differentiation is furthermore connected with an interaction of these cells with mesenchymal cells. Future hepatic cords intermingle with a distinct, desmin-positive subset of septum transversum mesenchyme cells that express the LIM-homeobox gene *Lhx2* and these cells later become an integral part of the liver [50]. The complex features of the mesenchymal bed for immigrating hepatoblasts is furthermore underlined by the recent indentification of a foetal liver stroma consisting of cells in epithelial–mesenchymal transition (EMT). EMT stromal cells generated from primary cultures exhibit a hybrid phenotype expressing both mesenchymal and epithelial markers. They have a haematopoietic supportive capacity that is lost after hepatocyte maturation, and this novel stromal cell type may be derived from endodermal–mesodermal stem cells or from circulating stem cells seeding the liver primordium [51]. It may be surmised that paediatric liver tumours with a significant

stromal component (stromal–epithelial and stromal tumours) take their origin from cell systems reflecting this distinct phase of liver development. The eventual reinstallment of a foetal-type EMT stroma with haematopoietic supportive capacity by HBs may also be a reason for the typical haematopoiesis seen in these lesions.

So what determines the terminal differentiation of hepatoblasts to a mature hepatocyte phenotype? A central regulator of this crucial step is the transcription factor, HNF4, belonging to the nuclear hormone receptor family. It is expressed in the hepatic diverticulum, is involved in endodermal development and is essential for final hepatocyte differentiation and also for mature hepatocyte function [52]. A differentiated phenotype detected in tumours such as HB-MT1 and TLCT may depend on the differential expression of this factor.

As already discussed in the paragraph on cholangioblastic HBs, the generation of intrahepatic bile ducts critically depends in the construction of a ductal plate [53]. Recently, gene products regulating the development of a cholangiocyte lineage and the tissues derived thereof have been identified. One key transcription factor involved in cholangiogenesis is HNF6 that is expressed in hepatocytes and cholangiocytes of intra- and extrahepatic bile ducts [47]. Hnf6 knockout mouse embryos fail to develop a gallbladder and both extra- and intrahepatic bile ducts and is associated with a diminished expression of a further transcription factor, Hnf1β [54,55]. The development of the ductal plate itself is linked to Jagged1 (JAG1)/Notch ligand/receptor signalling, as shown by a Jag1/Notch2 doubly heterozygous mouse model [56] and the human disorder Alagille syndrome. Further genes and products affecting the ductal plate/bile duct system comprise fibrocystin/polyductin, which is localised to cilia, and inversin [57–59]. We surmise that HBs with cholangioblastic features and tumours mimicking the formation of ductal plate may exhibit changes in the expression patterns of these genes.

9. Has the time come for a new classification of hepatoblastomas and related tumours?

The analysis of large numbers of tumours within clinical trials has uncovered an increasing spectrum of lesions deviating from those classified so far. In addition, investigating HBs and related tumours that mimic hepatic ontogenetic steps for modified or aberrant expressions of genes, which are now recognised to play a central role in normal hepatogenesis, may in the future lead to approaches for a molecular classification of these neoplasms. But any attempt at novel classifications should, at the end, have an impact within a clinical and biological setting. In particular, future classifications of HB must aim at defining reliable risk groups that, together with what has been achieved so far by use of pre-treatment staging systems, will offer improved stratification systems for therapy.

A refined histological classification of tumours affects the construction of efficient risk algorithms and has been convincingly demonstrated for other paediatric blastomatous tumours. The International Neuroblastoma Pathology Classification employs an intricate combination of growth pattern types and levels of tissue differentiation along the neuroblast maturation lineage, resulting in the definition of favourable and unfavourable prognostic subsets [60,61]. Along a similar line of thinking, distinct histological features have entered the classifications of renal tumours in childhood [62–64]. For the case of nephroblastoma, histology phenotypes proposed by the SIOP group have allowed the generation of three pre-treatment risk groups [64].

As briefly outlined above, comparable histologic risk groups for HB and related tumours await improved definitions and revised identifications of (novel) phenotypes to be tested within large trials. A preliminary attempt for such a risk-related classification is given in Table 2.

Table 2
Proposal of a new working classification for tumours of the hepatoblastoma family

Tumour category	Risk (expected)[a]
Wholly epithelial hepatoblastomas	
Foetal subtype (including 'purely foetal HB')	SR
Embryonal and mixed embryonal/foetal subtype	SR
Macrotrabecular subtype 2 (MT-2)	SR
Undifferentiated subtype (diffusely or focally 'anaplastic')	
Small cell undifferentiated (SCUD)	HR
Intermediate cell undifferentiated (ICUD)	HR
Large cell undifferentiated (LCUD)	HR
Liver cell tumours with a mature hepatocellular phenotype	
Macrotrabecular HB type 1 (MT-1)	pHR
Transitional liver cell tumours (TLCT)	HR
Stromal–epithelial and stromal tumours	
Mixed epithelial and mesenchymal hepatoblastoma	pSR
Stromal–epithelial tumour of the liver, ACTH-producing	IR
Paediatric hepatic stromal tumours (PHST)	IR
Ossifying hepatic tumour of infancy (OHTI)	IR
Hepatoblastomas with cholangioblastic features	
Hepatoblastoma, cholangioblastic	IR
So-called 'ductal plate tumours'	IR
Hepatoblastoma family tumours with organoid features	
Hepatoblastoma, hamartoma-like	SR
Immature hepatic tumour of bimodal differentiation	HR

[a] In current HB studies, the estimation of risk mostly refers to pre-treatment staging (*e.g.*, by use of the Pretext system). In this Table, the expected risk is based on the histopathology of the tumours listed. The term, expected, is used because knowledge regarding the biological significance of a given tumour type or subtype is in part still incomplete or even lacking. SR: standard risk; IR: indeterminate risk; HR: high risk; p as a prefix: probable. MT-1: refers to tumours with a macrotrabecular growth pattern consisting of hepatocyte-like cells. MT-2 refers to tumours with a macrotrabecular growth pattern consisting of foetal and/or embryonal-type cells.

Furthermore, the availability of an increasing number of neoplasms with novel phenotypes entering tumour banks will allow more detailed analyses of molecular genetic alterations. These may in the future be suitable to create additional molecular classifications to define risk groups at the biological level.

10. Conclusions

Subsequent to the recognition of distinct types of hepatoblastomas and the construction of baseline classifications, an increasing spectrum of paediatric liver cell tumours seems to be emerging to form a hepatoblastoma family of lesions, which requires a new classification approach. The significance of any such classification will have to be measured in relation to their prognostic impact within a clinical setting. In addition, the feasibility of any new working formulation/classification will have to be tested in a circle of specialised pathologists, in order to find agreement for later clinical use in ongoing and future studies. Apart from a detailed morphological description and the reproducible definition of the phenotypes, novel findings of molecular biology should be employed in order to identify future risk groups and to arrive at molecular classifications complementing the classifications based on pathology.

Conflict of interest statement

None declared.

References

1. Perilongo G, Plaschkes J, Zimmermann A. Hepatic tumours. 2nd ed. In Souhami RL, Tannock I, Hohenberger P, Horiot JC, eds. *Oxford textbook of oncology*, vol. 2. Oxford, Oxford University Press, 2002. pp. 2657–2668.
2. Darbari A, Sabin KM, Shapiro CN, *et al*. Epidemiology of primary hepatic malignancies in US children. *Hepatology* 2003, **38**, 560–566.
3. Weinberg AG, Finegold MJ. Primary hepatic tumours of childhood. *Hum Pathol* 1983, **14**, 512–537.
4. Stocker JT. Hepatoblastoma. *Semin Diagn Pathol* 1994, **11**, 136–143.
5. Rowland JM. Hepatoblastoma: assessment of criteria for histologic classification. *Med Pediatr Oncol* 2002, **39**, 478–483.
6. Haas JE, Muczynski KA, Krailo M, *et al*. Histopathology and prognosis in childhood hepatoblastoma and hepatocarcinoma. *Cancer* 1989, **64**, 1082–1095.
7. Lack EE, Neave C, Vawter GF. Hepatoblastoma. A clinical and pathologic study of 54 cases. *Am J Surg Pathol* 1982, **6**, 693–705.
8. Brotto M, Finegold MJ. Distinct patterns of p27/KIP1 gene expression in hepatoblastoma and prognostic implications with correlation before and after chemotherapy. *Hum Pathol* 2002, **33**, 198–205.
9. Ortega JA, Douglass EC, Feusner JH, *et al*. Randomized comparison of cisplatin/vincristine/fluorouracil and cisplatin/continuous infusion doxorubicin for treatment of paediatric hepatoblastoma: a report from the Children's Cancer Group and the Paediatric Oncology Group. *J Clin Oncol* 2000, **18**, 2665–2675.
10. Haas JE, Feusner JH, Finegold MJ. Small cell undifferentiated histology in hepatoblastoma may be unfavorable. *Cancer* 2001, **92**, 3130–3134.
11. Faria P, Beckwith JB, Mishra K, *et al*. Focal versus diffuse anaplasia in Wilms tumour-new definitions with prognostic significance: a report from the National Wilms Tumour Study Group. *Am J Surg Pathol* 1996, **20**, 909–920.
12. Giangaspero F, Rigobello L, Badiali M, *et al*. Large-cell medulloblastoma. A distinct variant with highly aggressive behavior. *Am J Surg Pathol* 1992, **16**, 687–693.
13. Tornoczky T, Kalman E, Kajtar PG, *et al*. Large cell neuroblastoma: a distinct phenotype of neuroblastoma with aggressive clinical behavior. *Cancer* 2004, **100**, 390–397.
14. Ruck P, Xiao JC, Pietsch T, *et al*. Hepatic stem-like cells in hepatoblastoma: expression of cytokeratin 7, albumin and oval cell associated antigens detected by OV-1 and OV-6. *Histopathology* 1997, **31**, 324–329.
15. Badve S, Logdberg L, Lal A, *et al*. Small cells in hepatoblastoma lack "oval" cell phenotype. *Mod Pathol* 2003, **16**, 930–936.
16. Stocker JT, Conran RM, Selby DM. Tumours and pseudotumours of the liver. In Stocker JT, Askin FB, eds. *Pathology of solid tumours in children*. London, Chapman & Hall Medical, 1998. pp. 97–99.
17. Conran RM, Hitchcock CL, Waclawiw MA, *et al*. Hepatoblastoma: the prognostic significance of histologic type. *Pediatr Pathol* 1992, **12**, 167–183.
18. Von Schweinitz D, Wischmeyer P, Leuschner I, *et al*. Clinicopathlogical criteria with prognostic relevance in hepatoblastoma. *Eur J Cancer* 1994, **30A**, 1052–1058.
19. Von Schweinitz D, Hecker H, Schmidt-von Arndt G, *et al*. Prognostic factors and staging systems in childhood hepatoblastoma. *Int J Cancer* 1997, **74**, 593–599.
20. Brown J, Perilongo G, Shafford E, *et al*. Pretreatment prognostic factors for children with hepatoblastoma – results from the International Society of Paediatric Oncology (SIOP) Study SIOPEL 1. *Eur J Cancer* 2000, **36**, 1418–1425.
21. Fuchs J, Rydzynski J, Von Schweinitz D, *et al*. Pretreatment prognostic factors and treatment results in children with hepatoblastoma: a report from the German Cooperative Paediatric Liver Tumour Study HB 94. *Cancer* 2002, **95**, 172–182.
22. Perilongo G, Shafford E, Maibach R, *et al*. Risk-adapted treatment for childhood hepatoblastoma. Final report of the second study of the International Society of Paediatric Oncology – SIOPEL 2. *Eur J Cancer* 2004, **40**, 411–421.
23. Suita S, Tajiri T, Takamatsu H, *et al*. Improved survival outcome for hepatoblastoma based on an optimal chemotherapeutic regimen – a report from the study group for paediatric solid malignant tumours in the Kyushu area. *J Pediatr Surg* 2004, **39**, 195–198.
24. Prokurat A, Kluge P, Kosciesza A, *et al*. Transitional liver cell tumours (TLCT) in older children and adolescents: a novel group of aggressive hepatic tumours expressing beta-catenin. *Med Pediatr Oncol* 2002, **39**, 510–518.
25. Yamada S, Ohira M, Horie H, *et al*. Expression profiling and differential screening between hepatoblastomas and the corresponding normal livers: identification of high expression of the PLK1 oncogene as a poor-prognostic indicator of hepatoblastomas. *Oncogene* 2004, **23**, 5901–5911.
26. Albrecht S, Hartmann W, Houshdaran F, *et al*. Allelic loss but absence of mutations in the polyspecific transporter gene *BWR1A* on 11p15.5 in hepatoblastoma. *Int J Cancer* 2004, **111**, 627–632.
27. Nagai H, Naka T, Terada Y, *et al*. Hypermethylation associated with inactivation of the SOCS-1 gene, a JAK/STAT inhibitor, in human hepatoblastomas. *J Hum Genet* 2003, **48**, 65–69.

28. Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* 2004, **303**, 1483–1487.
29. Buendia MA. Genetic alterations in hepatoblastoma and hepatocellular carcinoma: common and distinctive aspects. *Med Pediatr Oncol* 2002, **39**, 530–535.
30. Park WS, Oh RR, Park JY, *et al*. Nuclear localization of beta-catenin is an important prognostic factor in hepatoblastoma. *J Pathol* 2001, **193**, 483–490.
31. Takayasu H, Horie H, Hiyama E, *et al*. Frequent deletions and mutations of the beta-catenin gene are associated with overexpression of cyclin D1 and fibronectin and poorly differentiated histology in childhood hepatoblastoma. *Clin Cancer Res* 2001, **7**, 901–908.
32. Shang XZ, Zhu H, Lin K, *et al*. Stabilized beta-catenin promotes hepatocyte proliferation and inhibits TNFalpha-induced apoptosis. *Lab Invest* 2004, **84**, 332–341.
33. Taniguchi K, Roberts LR, Aderca IN, *et al*. Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. *Oncogene* 2002, **21**, 4863–4871.
34. Thomas D, Pritchard J, Davidson R, *et al*. Familial hepatoblastoma and APC gene mutations: renewed call for molecular research. *Eur J Cancer* 2003, **39**, 2200–2204.
35. Wirths O, Waha A, Weggen S, *et al*. Overexpression of human Dickkopf-1, an antagonist of wingless/WNT signaling, in human hepatoblastomas and Wilms' tumours. *Lab Invest* 2003, **83**, 429–434.
36. Saxena R, Leake JL, Shafford EA, *et al*. Chemotherapy effects on hepatoblastoma. A histological study. *Am J Surg Pathol* 1993, **17**, 1266–1271.
37. Heifetz SA, French M, Correa M, *et al*. Hepatoblastoma: the Indiana experience with preoperative chemotherapy for inoperable tumours: clinicopathological considerations. *Pediatr Pathol Lab Med* 1997, **17**, 857–874.
38. Heerema A, Finegold M. ACTH-producing stromal–epithelial tumours of the liver. *Lab Invest* 2004, **84**, 270. [Abstract].
39. Zimmermann A. Hepatoblastoma with cholangioblastic features ('cholangioblastic hepatoblastoma') and other liver tumours with a bimodal differentiation in young patients. *Med Pediatr Oncol* 2002, **39**, 487–491.
40. Libbrecht L, Desmet V, Roskams T. Stages of normal and aberrant intrahepatic bile duct development in a mixed hepatoblastoma. *Histopathology* 2003, **42**, 618–620.
41. Roskams TA, Theise ND, Balabaud C, *et al*. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology* 2004, **39**, 1739–1745.
42. Allison RM, Willis RA. An ossifying embryonic mixed tumour of an infant's liver. *J Pathol Bacteriol* 1956, **72**, 155–159.
43. Gornicka B, Ziarkiewicz-Wroblewska B, Michalowicz B, *et al*. Immature hepatic tumour of bimodal differentiation in a young adult patient: a novel lesion expressing beta-catenin and mimicking a distinct phase of hepatogenesis. *J Hepatol* 2001, **34**, 955–961.
44. Zaret KS. Hepatocyte differentiation: from the endoderm and beyond. *Curr Opin Genet Dev* 2000, **11**, 568–574.
45. Zaret KS. Regulatory phases of early liver development: paradigms of organogenesis. *Nat Rev Genet* 2000, **3**, 499–512.
46. Duncan SA. Mechanisms controlling early development of the liver. *Mech Dev* 2003, **120**, 19–33.
47. Costa RH, Kalinichenko VV, Holterman AX, *et al*. Transcription factors in liver development, differentiation, and regeneration. *Hepatology* 2003, **38**, 1331–1347.
48. Tanimizu N, Miyajima A. Notch signaling controls hepatoblast differentiation by altering the expression of liver-enriched transcription factors. *J Cell Sci* 2004, **117**, 3165–3174.
49. Sosa-Pineda B, Wigle JT, Oliver G. Hepatocyte migration during liver development requires Prox1. *Nat Genet* 2002, **25**, 254–255.
50. Kolterud A, Wandzioch E, Carlsson L. Lhx2 is expressed in the septum transversum mesenchyme that becomes an integral part of the liver and the formation of these cells is independent of functional Lhx2. *Gene Expr Patterns* 2004, **4**, 521–528.
51. Chagraoui J, Lepage-Noll A, Anjo A, *et al*. Foetal liver stroma consists of cells in epithelial-to-mesenchymal transition. *Blood* 2003, **101**, 2973–2982.
52. Watt AJ, Garrison WD, Duncan SA. HNF4: a central regulator of hepatocyte differentiation and function. *Hepatology* 2003, **37**, 1249–1253.
53. Crawford JM. Development of the intrahepatic biliary tree. *Semin Liver Dis* 2002, **22**, 213–226.
54. Clotman F, Lannoy VJ, Reber M, *et al*. The onecut transcription factor HNF6 is required for normal development of the biliary tract. *Development* 2002, **129**, 1819–1828.
55. Coffinier C, Gresh L, Fiette L, *et al*. Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1b. *Development* 2002, **129**, 1829–1838.
56. McCright B, Lozier J, Gridley T. A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. *Development* 2002, **129**, 1075–1082.
57. Ong AC, Wheatley DN. Polycystic kidney disease – the ciliary connection. *Lancet* 2003, **361**, 774–776.
58. Masyuk TV, Huang BQ, Ward CJ, *et al*. Defects in cholangiocyte fibrocystin expression and ciliary structure in the PCK rat. *Gastroenterology* 2003, **125**, 1303–1310.
59. Harris PC, Rossetti S. Molecular genetics of autosomal recessive polycystic kidney disease. *Mol Genet Metab* 2004, **81**, 75–85.
60. Shimada H, Ambros IM, Dehner LP, *et al*. The International Neuroblastoma Pathology Classification (the Shimada System). *Cancer* 1999, **86**, 364–372.
61. Peuchmaur M, d'Amore ESG, Hata J, *et al*. Revision of the International Neuroblastoma Pathology Classification. Confirmation of favorableand unfavorable prognostic subsets in ganglioneuroblastoma, nodular. *Cancer* 2003, **98**, 2274–2281.
62. Beckwith JB, Zuppan CE, Browning NG, *et al*. Histological analysis of aggressiveness and responsiveness in Wilms' tumour. *Med Pediatr Oncol* 1996, **27**, 422–428.
63. Boccon-Gibod LA. Pathological evaluation of renal tumours in children: International Society of Paediatric Oncology approach. *Pediatr Dev Pathol* 1998, **1**, 243–248.
64. Vujanic GM, Sandstedt B, Harms D, *et al*. On behalf of the SIOP Nephroblastoma Scientific Committee. Revised International Society of Paediatric Oncology (SIOP) working classification of renal tumours of childhood. *Med Pediatr Oncol* 2002, **38**, 79–82.