Review Article
Genetics of pediatric hepatoblastoma and hepatocellular carcinoma and their clinical application

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Abstract: Hepatoblastoma (HB) and hepatocellular carcinoma (HCC) are two main primary liver malignant tumors in children arising from hepatocytes. These pediatric tumors are generally considered to be fatal because of their late diagnosis, underlying liver disease, common metastases, and refractoriness to systemic treatments. Earlier diagnosis, identifying precursor conditions, and proper monitoring and treatment of the tumors can significantly improve the survival rate for the patients with these liver malignancies. A number of unique genetic features have been identified in these tumors. These alterations include germ line mutations of certain genes which result in rare genetic disorders predisposing to pediatric HB and HCC, hallmark cytogenetic changes, and the main somatic recurrent mutations described in these tumors. Moreover, comprehensive genetic analysis of these tumors through next generation sequencing (NGS) not only confirmed previous findings of frequent mutation of CTNNB1 and p53 genes in HB and HCC, but also identified genetic alterations in new tumor associated genes. This review aims to provide an overview of genetic changes in these tumors and outlines the prospects of these findings in clinical application including screening, early diagnosis, patient classification, monitoring, prognosis prediction and treatment optimization. With improved recognition and understanding of these genetic alterations, these findings have been applied in the clinical setting at different levels for genetics-based tumor diagnosis, monitoring and paving the way to individualized cancer treatment.

Keywords: Hepatoblastoma, hepatocellular carcinoma, CTNNB1, p53, array, next generation sequencing, ctDNA

Introduction

Tumors of the liver comprise less than 4% of all pediatric solid tumors. Hepatic tumors in childhood include hepatoblastoma (HB), hepatocellular carcinoma (HCC), benign vascular tumors, mesenchymal hamartomas, focal nodular hyperplasia, adenomas, and various sarcomas and other tumors. These tumors typically present as asymptomatic abdominal masses and associated symptoms of nausea, vomiting, abdominal pain, weight loss, and jaundice are uncommon and do not appear until the disease is advanced. Lab abnormalities can be subtle, initially including mild normocytic anemia and thrombocytosis. Diagnosis is commonly made with a combination of radiographic and laboratory testing.

Two-thirds of all liver tumors are malignant and most liver tumors arise from metastases from other sites. While primary malignant liver tumors are rare in children, HB and HCC comprise most of these malignancies. Their incidence per year is only 1-1.5 case per million children under 15 years in the United States, accounting for around 1-2% of all pediatric malignances [1, 2].

HB is the most common primary liver tumor, accounting for 43% of all pediatric liver tumors or two-thirds of all liver malignancies in children [3]. The mean age at diagnosis of HB is 19 months, and only 5% of cases occur in children more than 4 years old [4]. There is a strong association between HB and prematurity especially in the extremely low birth weight infants (< 1000 g) without full understanding of the etiology or associated factors [5, 6]. HB is a highly malignant embryonal liver tumor arising from hepatocyte precursor cells called hepatoblasts. Histologically, HB can be classified into two...
major types: epithelial (56%) or mixed epithelial and mesenchymal (44%), which displays a histological pattern composed of a mixture of epithelial, mesenchymal, and teratoid components [1, 7]. Epithelial HB can be further broken down to several subtypes including pure fetal (31%), embryonal (19%), macrotrabecular (3%) and small cell undifferentiated (SCU, 3%). Clinical trials have demonstrated that, in addition to staging, the histological types are also associated with prognosis. The presence of mesenchymal elements confers a better prognosis, while SCU histology is associated with a poor prognosis [7].

HCC is less common, representing up to 20% of all pediatric liver cancers. It is more often seen in older children and adolescents and less often seen in children under five years old. Most HCC is closely correlated with viral hepatitis infections and the incidence of HCC is higher in continents such as Africa and Asia with the greatest prevalence of viral hepatitis. Pediatric HCC are traditionally divided into two distinct histopathology types, fibrolamellar carcinoma (FLC) and non-FLC. FLC HCC is a distinct clinical and histological variant representing almost one third of all pediatric HCCs. Unlike most classic HCC, this type of HCC is not preceded by cirrhosis or other underlying liver disease. Morphologically, it is characterized by the large polygonal tumor cells in cords surrounded by fibrosis in a lamellar fashion [8]. FLC exhibited greater overall survival than the non-FLC subtype [2].

Whether HCC in children and adults are the same entities, still remains an open and debatable issue. HCC in adults typically arise from conditions that cause long-term inflammation and cirrhosis, such as hepatitis C. In children, HCC may arise without long-term liver involvement. Certain unique characteristics of pediatric HCC including its early-onset, lower survival rate, and poor response to chemotherapy also suggest a different biological origin and behavior compared with adult HCC [9]. However, the macroscopic and microscopic features of non-FLC HCC in children and in adults are similar, which imply that pediatric HCC and adult HCC share some molecular mechanisms during the tumor development and progression.

Besides the difference in histological patterns between the primary hepatic tumors, HCCs are often multifocal, more likely to metastasize, and more chemoresistant, therefore carrying a poorer prognosis in comparison with HBs. In some instances, distinguishing HB and HCC can be challenging. The simultaneous presence of both HB-like and HCC-like cells within the same child has been reported and is referred to as transitional liver cell tumors (TLC) [10]. TLC develops in an age group older than that associated with the typical HB manifestation period and shows high serum Alpha-Fetoprotein (AFP) and an aggressive behavior at diagnosis [11]. AFP is a serum tumor marker increased in 90% patients with HB except a small number of them including SCU and two-thirds of patients with pediatric HCC. The detection of AFP parallels disease activity. This may indicate a unique group of primary liver cancer with different origin and pathogenesis mechanism. Recent studies have suggested HCC can also arise from fetal progenitor cells or their adult progenitor progeny besides the hepatocytes from which HCC was generally thought to arise [12]. This finding is valuable for understanding of pathology of HCC and HB.

Although primary pediatric liver tumors are rare, liver is the third-most-common site for intra-abdominal malignancies in children. Intra-abdominal tumors are typically not detected clinically until they are large and often spread within the organ or metastasize. Surgical resection, primarily or after combination chemotherapy to decrease tumor size, is essential to cure liver tumors. When chemotherapy does not allow surgical resection, orthotopic liver transplant can be considered to treat unresectable tumors. Early detection of these tumors as well as identifying precursor conditions that are at increased risk for hepatic tumors may allow for improved outcome with less advanced disease or metastases, improved resectability of the tumor, and even less need for curative liver transplants. This review aims to provide an overview of genetic changes on these tumors and outlines the clinical application of these findings including screening, early diagnosis, patient classification, monitoring, prognosis prediction, and treatment selection.

Genetic disorders predisposing to pediatric HB and HCC

Majority of pediatric hepatic malignancies are sporadic; however, there are several genetic conditions which predispose to pediatric liver malignancy (Table 1). Recognition and early diagnosis of these disorders are very important for patients and their family members.
Familial adenomatous polyposis (FAP) is a colon cancer predisposition syndrome characterized by the development of hundreds of colorectal adenomatous polyps. The lifetime risk for HB in children with FAP is about 1.6-2.5% and the majority occur prior to age 3 [13]. Although HB in FAP families often correlates with a spectrum of mutations in the 5' part of the APC gene, upstream of codon 1309, mutations in other regions of the APC gene were also observed in a large study [14]. Clear genotype-phenotype relationship has not been established. Unlike other syndromes predisposed to HB which may not affect other family members and can be identified through the associated congenital abnormalities, HB in a baby may be the first manifestation in a FAP family carrying the APC mutation, earlier than adenomas [15, 16]. Identifying such ‘hidden connection’ between APC mutation and HB is important for long-term management implications, follow-up, risk assessment, and surveillance of other family members [17].

Several overgrowth syndromes are found to be associated with HBs. Children with Beckwith-Wiedemann Syndrome (BWS) have an increased risk to develop a number of embryonal tumors, particularly Wilms tumor and HB [18]. The overall risk for tumor development in children with BWS is 7.5% and the mean age of presentation with HB is 6 months (range from birth to 30 months) [19]. These patients have variable presentations with macrosomia, hemihypertrophy, macroglossia, abdominal wall defects, and hypoglycemia and about half are born premature. Genetically, BWS patients with uniparental disomy (UPD) 11p15 or gain of methylation at imprinting center (IC) 1 carry the highest risk to develop HB [20]. In contrast, the risk of HB is lower in patients with loss of methylation at IC2 or mutations in CDKN1C [21]. Based on these observations, a more accurate guideline for the follow-up of these patients according to the molecular subtype of BWS has been suggested [22].

Simpson-Golabi-Behmel syndrome (SGBS) type 1 is another overgrowth syndrome found to be associated with HB. This is a rare X-linked inherited overgrowth syndrome mainly caused by a loss of function mutation in the GPC3 gene. About 10% of patients develop tumors in early childhood including both HB and HCC [23-25].

HB has been also reported in children with trisomy 18, trisomy 13, Li-Fraumeni syndrome (LFS), Soto's syndrome, Fanconi anaemia (FA), neurofibromatosis type I (NF1). In FA, both HB and HCC were reported. The co-occurrence of HB and HCC in these syndromes may represent the non-random association [26-30]; however, the absolute incidence and relative risk for liver malignancy in these syndromes have not been well established.

Besides HBV and HCV infection whose direct viral integration play an important role in childhood HCC as seen in adult HCC [31], several well-described syndromes resulting in non-viral liver injury and cirrhosis have been reported to be associated with the increased risks of HCCs in children. Although the underlying mechanism is not completely clear, these disorders may cause the accumulation of the toxic metabolites which can induce cell necrosis, resulting in severe damage of the liver, and subsequent regeneration of hepatocytes which are generally prone to mutations. In addition, the toxic compounds may also act as the direct mutagens, causing the defect of the processing
DNA. Hereditary tyrosinemia type I (HT1) is a typical example. This is a metabolic liver disease caused by genetic defects of fumarylacetoacetate hydrolase (FAH), which results in the accumulation of toxic tyrosine catabolic intermediates in hepatocytes, causing either acute hepatic failure in infancy or in a chronic liver disease associated with cirrhosis and HCC development. About 40% children with HT1 who survive the acute onset of liver failure beyond infancy are at high risk of developing and succumbing to HCC [32].

Glycogen storage diseases (GSD) are inherited disorders of glycogen metabolism due to intracellular enzyme deficiencies resulting in abnormal storage of glycogen in various tissues. Increased risk for HCC has been described in patients with several subtypes of GSD, primarily including GSD Ia, GSDIII, GSD IV, and GSD VI resulting from bi-allelic mutations of genes G6PC, AGL, GBE1 and PYGL respectively [33-37]. Besides HCC, HB was also reported in patients with GSD Ia [35]. It is estimated that between 22-75% patients with GSD Ia develop hepatocellular adenomas (HCA) during or after puberty and some of them may undergo malignant transformation into HCC; however, not all patients develop HCA before the appearance of HCC. How exactly the liver malignancy develops is still unclear [38] and a clear correlation between specific mutations and the development of HCC in patients with GSD Ia has not been established [33]. Age at the diagnosis of HCC in GSDs patients are generally in adulthood; however, surveillance of the liver should start from in childhood because of the earlier onset of HCA and variable duration time needed for the possible malignant transformation from adenoma to HCC. HCC as a long term complications is still unclear [38] and a clear correlation between specific mutations and the development of HCC in patients with GSD Ia has not been established [33]. Age at the diagnosis of HCC in GSDs patients are generally in adulthood; however, surveillance of the liver should start from in childhood because of the earlier onset of HCA and variable duration time needed for the possible malignant transformation from adenoma to HCC. HCC as a long term complications was reported less frequently in patients with other type of GSDs [34].

Transaldolase (taldo) deficiency is a rare genetic disease caused by homozygous mutations of TALDO1 gene resulting in inborn error of metabolism of the pentose phosphate pathway. This is a severe, early-onset multisystem disease including liver cirrhosis and early infantile hepatic failure. Although the risk of HCC in these patients has not been fully recognized, a recent study have shown that Taldo1-/- and Taldo1+/+ mice can spontaneously develop HCC [39]. The first association of taldo deficiency with early onset HCC was recently reported in a 16-month-old boy in a family with this disease [40]. Due to the lack of routine measurement of urinary polyols, identifiable storage material in hepatocytes and various clinical phenotypes in some patients, it can be challenging to establish the diagnosis for Taldo deficiency with routine clinical tests. The mutations of TALDO1 in this particular family were identified through a combination of RNA-sequencing and whole exome sequencing (WES). This disease now should be added to the list of other inborn errors of metabolism associated with increased risk of HCC.

Cholestasis resulting from the impairment of bile flow can lead to progressive conjugated hyperbilirubinemia, biliary cirrhosis, and eventual hepatic failure. Increased risk for HCC was observed in some rare genetic syndromes with cholestasis [14, 41, 42]. Progressive familial intrahepatic cholestasis Type II (PFIC2) is an autosomal recessive liver disorder caused by the mutation in ABCB11, which results in bile salt export pump (BSEP) deficiency. PFIC2 is characterized by early onset of cholestasis that progresses to hepatic fibrosis, cirrhosis, and end-stage liver disease before adulthood. Children with PFIC2 are at risk for HCC at a young age. In one study of 10 children with PFIC2 who developed HCC, the malignancy was diagnosed between the ages of 13 and 52 months [43]. Different mutations along the whole gene were identified in these patients. Because a focal lesion of HCC, especially less than 3 cm, is quite common in previously reported cases, monitoring of HCC in PFIC2 patients should be offered starting in the first year of life [44].

Alagille syndrome is a multisystem autosomal dominant disorder caused by mutations in either JAG1 or NOTCH2 gene. It is a well-recognized cause of neonatal cholestasis due to paucity of intrahepatic bile ducts. HCC has been reported in both children and adults with Alagille syndrome [45]. Most of the reported cases have cirrhosis, which presumably predisposes to the development of malignancy. The absence of cirrhosis in some patients suggests that other mechanisms not yet elucidated could be contributing to the development of HCC, as emerging data suggests that continuous activation of the Notch pathway may play a significant role in the pathogenesis of liver cancer [46].

For these well characterized genetic disorders, genetic testing is available in many clinical laboratories. Such tests can not only be used in the confirmation of the disease diagnosis for
Some analysis, which can detect both balanced and unbalanced changes at the resolution of 5-10 Mb. Chromosome abnormality including the content and number is a common characteristic of tumors which manifests at the early stage of tumorigenesis and increases throughout subsequent tumor development [47]. Previous studies have shown that HB is a tumor characterized by recurring chromosomal abnormalities (Table 2). Abnormal karyotypes were observed in almost 50% of the cases in a large cohort study [48]. The most common changes are the presence of extra copy of chromosomes 2q, 8, and 20. Chromosome loss is less common than chromosome gain, and one puzzle is the observation of relatively high frequency of monosomy 18 in HB tumor tissue considering the co-occurrence of HB and trisomy 18 in the same patient. Such aneuploidy can be single or multiple and is often seen together with the structural alterations. The most common structural abnormality is unbalanced translocations involving 1q at breakpoint of q12 - q21, resulting in trisomy of part of 1q and monosomy for the corresponding reciprocal chromosome arms, such as der(4)t(1;4)(q12;q34), der(5)t(1;5) (q21.3;q31.3), der(6)t(1;6)(q21;q26), and der(10)t(1;10)(q23;q26) [48-50].

Studies using genome wide array without establishing cell culture identified the similar changes in HB further support the importance of abnormalities in chromosomal regions 1q, 2q, 4q, 6q, 8 and 8q in the development and progression of HB [49-52]. Fine mapping through higher resolution of SNP array analysis also delineated small regions of chromosomal loss or gain and UPD in HB, which help the identification of previously undetected genetic alterations. These regions include gain of 1.3Mb on 1q32.1 harboring oncogene MDM2, loss of 1.6 Mb on 4q32.3 containing ANXA10S, a variant of the candidate

<table>
<thead>
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<th>Changes</th>
<th>Hepatic Malignant Tumors</th>
<th>Known &amp; Candidate Cancer Related Gene</th>
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<td>+2.018</td>
<td>p73</td>
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<tr>
<td>+1q</td>
<td>+1.018</td>
<td>MDM2</td>
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# Cytogenetic characterization of pediatric HB and HCC

Unraveling the genomics of pediatric liver malignancy was initiated by traditional chromosome

### Table 2. Common Chromosomal Alterations Identified in HB and HCC

<table>
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<td>-4q</td>
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<td>+18q11.2</td>
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**Notes:**

- Combined chromosome analysis and array analysis. The region identified by array analysis is included in parentheses.
- Results from several published studies, refer to reference 48, 49, 50, 52, 53. A frequency less than 10% is not listed.
- Only detected in pediatric HCC cell line, reference 11.
tumor suppressor gene (TSG), ANXA10 showing no expression in majority of tumors, as well as deletion and UPD of 11p15 where IGF-2 gene is located [51]. Multivariate analysis in these studies demonstrated that 2q gain or 4q deletion was an independent factor to predict a poor outcome.

Several specific chromosome aberrations are also detected in HCC (Table 2). The most frequently altered regions of structural aberrations observed in HCC include gain of chromosome 1q, 3q, 6p, 7p, 8q, 17q, 20, 22q and loss of 1p, 4q, 6q, 7q, 8p, 9p, 13q, 16q, 17p, 18q21qter and Y [53]. Gains of chromosome 1q and 8q which include candidate oncogenes MCL1, SHC1, MYC and COP5/JAB1 have been detected in over half of HCCs [54].

Array based analysis further confirmed these cytogenetic findings in HCC, confined the affected chromosomal loci to smaller regions, and allowed for the identification of novel loci showing nonrandom rearrangement, which may imply unknown driver genes for tumorigenesis and development of HCC. Previously reported TSGs or oncogenes are found to be in the regions with the copy number variation (CNVs) identified in HCC. Among the frequently lost loci, p73 at 1p36.1, IGF2R at 6q26, DLC1 at 8p22, p16INK4a at 9p21, RB at 13q14, AXIN1 at 16p13.3, CDH1 at 16q22.1 and p53 at 17p13 have been reported to undergo somatic mutation and/or loss of heterozygosity (LOH) in HCC. Among the frequently gained loci, MET at 7q31, EXT1 at 8q23, c-MYC at 8q24, CCND1 at 11q13, FGF4 at 11q13 and ROCK1 at 18q11.2 have been reported to show gene amplification or overexpression in HCC [55-57]. A recent investigation of possible correlation between gene expression and CNV further confirmed that part of over- or down-expressed genes are located at the regions exhibiting recurrent copy number changes [58]. An interesting finding was the detection of gain of 7q31 in HCC, where MET gene, which encodes the receptor for hepatocyte growth factor (HGF) at 7q21, is located [59]. Perinatally acquired HBV has been reported to be associated with HCC with short incubation period especially if mutations in MET/HGF occur in childhood HCC. Among all the studies, loss of 17p13.3 and gain of 8q were the most frequently detected. Increase in copy numbers of MYC was also confirmed by fluorescence in situ hybridization (FISH) in more than half of tumors showing 8q gains in array. Multivariate analysis revealed that both chromosomal loss on 17p13.3 and gain on 8q11 are independent prognostic indicators in HCC [56].

Very few cytogenetic studies have been performed on pediatric HCC. Combined chromosome and CGH analysis of a cell line derived from a pediatric HCC without the background of viral infection and liver cirrhosis characterized several aberrations, including loss of 1p, 4q, 21.22qter, 5q15q35.2, 11pterp14.1, 11q13.4qter, chromosome 21, and gain of 1q, 2q24.2-qter, 3pterq24.3, 3q29, 5qter, 9q33.1qter, 11p-13.3q12.2, 19pterq13.11, chromosome 20, and 22q12.1 [11]. Further analysis showed that this cell line resembled parts of the original pediatric epithelial liver tumor and showed some characteristics of adult HCC. The similarity between pediatric HCC and adult HCC was also detected by another study. LOHs on 8p, 13q and 17p chromosomes were found in both childhood and adult HCC [31]; however, higher frequency of LOH on 13q was observed in pediatric HCC, indicating loss of RB is an important feature of childhood HCC.

Compared with classic HCC, fewer genetic alterations have been detected in FLC, and none are specific for FLC. Pooled data from CGH studies show that many chromosome abnormalities detected in FLC were also seen in HCC. Gains of 1q, 6p, 7p, 7q, 8q and 19p, and losses of 4q, 8p, 9p, 13q, 16p, 18q and Xq have been reported in several studies. Among them, the most frequent ones were gains in 1q and 8q, and losses in 18q, occurring with a frequency of > 20% [8].

Since array techniques are able to use any sample that yields DNA to assess the genome at very high resolution and to detect both CNV and LOH changes (in SNP array), this method has been widely applied in the clinical laboratories, especially been chosen as a first-tier test for many solid tumors [60]. The American College of Medical Genetics and Genomics (ACMG) has provided the detailed technical standards and guidelines for such application [61]. Recognition of hallmark cytogenetic changes specific to HB and HCC makes it possible to apply array based technique to the early detection of tumor through biopsy, tumor classification, prognosis prediction, and improvement of therapeutics. While array test still misses some
alterations only detected by the traditional cytogenetic analysis, it has demonstrated a greater number of genomic alterations than other methodologies [60]. Although the clinical significance for some changes are still unclear, these findings can enhance our knowledge of the genomics of these tumors, leading to new discoveries involved in the liver tumor carcinogenesis and target therapy [62].

Detection of most commonly mutated genes

CTNNB1 is a key protein of Wingless (Wnt) signaling pathway which plays pivotal roles in the liver development and regeneration. Activation of Wnt signaling through mutations in CTNNB1 has been identified in the majority of pediatric hepatic malignancies including HB and HCC, suggesting its contribution in hepatic tumorigenesis in this age group [63].

Several studies have demonstrated that at least 50-90% of HBs harbored the CTNNB1 mutations, and more than half of them are the interstitial deletions in N-terminal domain involving the phosphorylation site of GSK3-β. The rest are missense mutations in the same domain within exon 3. These alterations prevent the phosphorylation of CTNNB1 at N-terminal residues by GSK3-β within a complex including APC, AXIN 1, and AXIN 2, a key step for the degradation of excess CTNNB1 by ubiquitin-proteasome pathway, and finally lead to its accumulation in cytoplasm and nuclei [63-66]. The mutations of other genes in the Wnt pathway were also detected in HB, which provided additional evidence for the contribution of this pathway in HB development. Different from the observation of increased frequency of HB in FAP patients, somatic mutations or LOH of APC gene were not the frequent findings in sporadic HBs though they were detected in several studies [64, 66-68]. Around 10% of HBs were found to have mutations of AXIN1 and AXIN2 [63]. Similar to CTNNB1 mutations, loss of function of any of these core components in Wnt signaling pathway can result in the accumulation and constitutive activation of CTNNB1, which is followed by nuclear translocation and the activation of T cell factor and various target genes, such as BIRC5, c-MYC and CYCLIN D1 [66]. Their staining has been observed in HB pretreatment biopsies but decreased after chemotherapy if the tumor cells are not chemoresistant [69]. A MYC signature has been detected in poorly differentiated, highly proliferating HBs, and it has been linked to features of aggressive tumors and poor prognosis [68, 70].

CTNNB1 mutation was also reported in around 19% of HCC [63]. Different from HB, the primary mutation type in HCC is a point mutation mainly occurring at the 5’ end of the gene (exons 2-4) [53]. The underlying mechanism for the different mutation types detected in the HB and HCC is not clear. While there was no APC mutation detected in HCC, mutations in AXIN1 and AXIN2 gene were also detected in 5-10% of HCCs. Collectively, these Wnt signaling pathway members are mutated in up to half of tumors. Biallelic mutations of CTNNB1 gene were also detected in tumor tissue from one patient with HCC in a clinical laboratory (data not published). It is unsure if CTNNB1 is functionally more activated for this patient.

The role of Wnt signal pathway in the hepatic tumorigenesis can also be confirmed by a series of in vivo experiments. It was found that in vivo RNA interference against CTNNB1 could inhibit the proliferation of pediatric liver tumor cell lines from both HB and HCC harboring mutated and overexpressed CTNNB1. The expression of CTNNB1 and downstream target genes in these cell lines were suppressed after treatment [71]. HB growth can also be inhibited by another specific inhibitor which shows strong cytotoxic effect on HB cells through blocking Wnt pathway [72]. This leads to the application of a therapeutic approach targeting this pathway in HB and HCC [73]. A recent study also found that CTNNB1 activation in a novel liver progenitor cell type is sufficient to cause both HB and HCC [74]. This finding, together with the identification of CTNNB1 accumulation within the different histotypes of the same tumor, suggested that mutational activation of CTNNB1 occurred in a common early precursor cell. The prognostic value of CTNNB1 accumulation in these pediatric tumors is controversial. While CTNNB1 mutation rate is similar among HB subtypes at different stages, the association of these mutations and distinct immunohistochemical (IHC) staining pattern with large size, poorly differentiated histology, tumor invasion, and metastases has been observed in several studies [54, 66, 75]. At least detection of mutation and accumulation of CTNNB1 can be used as an important marker not only in the differentiation between tumor cells from non-neoplastic hepatocytes but also for the indication of target treatment.
Another gene with recurrent somatic mutations identified in HCC is p53 [76]. The mutation type and frequency was found to differ according to the geographic origin and suspected etiology of HCC. Most of them were reported to be missense mutations, especially in its core DNA-binding domain (DBD at residues 93-292) [77]. A notable example is the high prevalence of G-to-T transversion at codon 249 (AGG<AGT) leading to an arginine to serine substitution of p53 in the tumors of patients with high-risk genotypes and chronically exposed to AFB1 supported the existence of genetic susceptibility in humans to the environmental carcinogen [78]. This mutation was found in 36% of tumors from Africa and 32% of tumors from China and its worldwide frequency is about 11%. Mutations in other codons of p53 gene were also observed and the overall mutation rate of this gene in HCCs is about one third.

As we know, p53 plays an important role in cell-cycle regulation and apoptosis after DNA damage to monitor genome integrity and cell homeostasis. The underlying mechanism is complicated. Increasing evidence showed that many mutant p53 proteins not only lose their normal function, but also acquire dominant-negative activities through arresting wild-type (WT) p53 in an inactive complex. In addition, some of them were found to gain new oncogenic properties independent of WT p53 possibly through the inactivation of other p53 family members, p63 and p73, or through transcriptional regulation of other genes. Instead of degradation of mutant proteins commonly observed in other TSGs, missense mutation of p53 can result in the stabilization of protein, which can be easily detected by IMHC analysis [79].

In addition to gene mutation, overexpression of WT p53 in the absence of p53 mutation was also detected in HCC [80]. The half-life of WT p53 is short; however, p53 stability is significantly increased when it binds to other molecules or various viral proteins including HBx protein encoded by the x region of HBV and HCV. Experimentally, such complex can inhibit its normal function, impact p53-mediated gene transcription, and then trigger carcinogenesis [81, 82].

P53 alterations have been associated with poorly differentiated, large tumors. Systematic review and meta-analysis to evaluate the correlation between p53 alteration and survival rate of patients with HCC concluded that both p53 mutation and overexpression of p53 in tumors had an unfavorable impact on overall survival and are associated with a poor prognosis [83, 84]. In patients with HCC, investigation of p53 through both direct sequencing and IMHC should be performed.

Because of the rarity of pediatric HCC in children, it is difficult to assemble enough patients to perform large-scale biological studies. Even with limited studies, the genetic differences between adult HCC and pediatric HCC are still noticed. Childhood HCC was found to be less dependent on cyclin D1 protein for tumor growth and progression [31]. A recent study also disclosed that epithelial cell adhesion molecule (EpCAM) was diffusely expressed in a majority of childhood HCCs but only focally expressed in small number of adult HCC [85]. Such expression pattern was also observed in HBs and most FLCs [8]. Diffuse expression pattern of EpCAM characterizes childhood HCC, making it a diagnostic marker for childhood HCCs. Such genetic difference may partially explain why HCC in childhood usually shows shorter latent period and rapid progression.

The detection of changes in these genes through IMHC or sequencing analysis have been a useful clinical tool for estimating the prognosis and selecting the target therapy for patients with pediatric liver malignancy; however, the information provided by testing single genes is obviously too limited for accurate clinical decision-making.

Application of next generation sequencing

Although many studies have uncovered genetic alterations in HB and HCC, there is still much to be determined concerning the entire genetic landscape of these tumors. Some unrecognized events may be critical for tumorigenesis of HB and HCC. The development of NGS has enabled access to the whole genomes of these tumors and substantially increased our insight into the genetic causes of these tumors.

Analysis from the first WES of HB not only identified mutations previously reported in the N-terminal domain of CTNNB1 but also the novel mutations in this gene and other genes in the Wnt pathway. Especially the novel G512V mutation near arm repeat domain of CTNNB1...
and R968H/S969C in CAPRIN2 were observed to activate the Wnt pathway in HB cells and promote the proliferation of HB cells. In addition, mutations for four genes known to be TSGs involved in the ubiquitin ligase complex, SPOP, KLHL22, TRPC4AP and RNF169, were also detected. This pathway was recently implicated in the tumorigenesis of various cancers but not in HB [86]. Specifically, this study found that mutation in SPOP resulted in loss of its tumor suppressive potential in HB cells.

While NGS study of pediatric HCC is still under way, a number of studies to identify driver genes in adult HCCs by NGS have been performed. The most frequently mutated genes detected in these studies include the genes involving in two well-known pathways, Wnt/CTNNB1 pathway and p53 pathway. Novel mutations in non-classical region of CTNNB1 were also detected and alterations in CTNNB1, AXIN1 and APC were found to be mutually exclusive in the same HCC [62]. The overall CTNNB1 mutation rate in HCC detected by NGS studies is about 30% [87]. Mutations in CDKN2A and INFB2, other components in p53 pathway were also detected, which further confirmed the previous findings [54, 58, 88, 89].

Moreover, NGS analyses are accelerating the search for additional novel and recurrently mutated genes which have not been described before. Several studies identified frequent mutations in multiple chromatin regulators in up to 50% HCCs. These genes, including ARID2 (15%), ARID1A (5%), ARID1B, MLL and MLL3, encode for chromatin remodeling complex, which constitute the third most frequent genetic alterations identified in HCC [62, 89, 90].

Mutations of these chromatin modulators often occur with CTNNB1 mutation and are largely exclusive from p53 mutations [62]. Loss of function can promote cell proliferation, indicating their role in carcinogenesis of HCC [89]. In addition, two studies also identified changes of NFE2L2 in 6% HCCs, a gene crucial in oxidative stress pathway [54, 62]. Its role in HCC still needs to be further investigated.

With the advances in high-throughput, rapid decline in NGS cost, and our increased knowledge of the genetic alterations in the tumorigenesis, this technique has quickly moved single gene mutation test to multiple gene panels, even to the entire cancer genome in different clinical setting. Target cancer gene panels specific for solid tumors by NGS have been validated for routine use in many clinical laboratories [91]. These tests allow the sensitive and simultaneous detection of variants from a list of target cancer associated genes on DNA from many types of tumor samples. Recently, WES analysis of tumor tissue has been also translated into the clinical use [92]. The comprehensive mutational profile of genes generated through these target NGS panel or WES provide the crucial information for personalized cancer medicine in the diagnosis, prognosis prediction, selection of a target therapy, establishment of eligibility for ongoing clinical trial and identification of potential therapeutic targets.

On the other hand, clinical laboratories are confronting numerous challenges for the application of this technique. Technically, many analytic variables may affect the precision and accuracy of the test. Detection of tumor heterogeneity, clonal evolution, and chromosomal structural abnormality is still difficult for most NGS platforms. More molecular diversity and complexity resulting from intratumor heterogeneity represents a unique landscape for each patient that may be ill suited to canonical clinical trials and practice paradigms but still challenges the interpretation of the result [93, 94]. Other interpretative challenge includes diverse prediction of pathogenicity or clinical actionability for variants of unknown significance. Physicians may receive different interpretations for the same variant from different laboratories or a number of variants from genes with unclear clinical utility and actionability and face the challenges on how to use the information in the clinical management. Little information is available on the post-analytic phase specific for tumor NGS tests. There is still lack of enough evidence of clinical validity showing an association between genetic variants detected with treatment response to approved molecularly targeted therapies across all solid-tumor types including pediatric liver malignancy. Whether or not reporting the germline incidental findings from normal specimen of a tumor-normal pair which is recommended by ACMG is another problem for recently launched cancer exome sequencing [95]. All of these challenges may affect the effective clinical implementation of tumor sequencing and no tumor-specific reporting guidelines have been developed.

Recently, researchers have shown considerable interest in circulating cell free tumor DNA (ctDNA) in patients with these tumors. ctDNA
are highly fragmented DNA, excessively released by cell apoptosis, death, or spontaneously released from newly synthesized DNA [96-98]. Although knowledge of the dynamic range of ctDNA in patients with cancer is still limited, higher concentrations of ctDNA have been reported in the blood and serum from cancer patients compared to healthy individuals. Because ctDNA carries the genetic and epigenetic changes characteristics of tumor tissues, tests for ctDNA provide a new clue for a noninvasive, real-time monitoring test, to assess tumor burden.

Recent studies have shown that cancer genome scanning in ctDNA by whole genome sequencing (WGS) allowed the detection of tumor-specific chromosome alterations and sequencing variants [47,99,100]. If somatic alterations specific for a tumor is well characterized, monitoring ctDNA can be performed by cancer personalized profiling through deep sequencing (CAPP-Seq) with a design covering the changes specific for a tumor [101]. This method can detect mutant allele at a very low level (0.02%) with very high specificity (96%).

Until now, most of the circulating HCC biomarkers used in clinical practice are still protein molecules and their sensitivity and specificity are low [102]. The specific mutation at codon 249 of p53 has been detected in the serum of patients with HCC [103, 104]. The disadvantage of these PCR-based assays is that they can only detect limited number of recurrent mutations which may be absent in the patients’ serum [101]. In order to further value the ctDNA in the diagnosis of HCC, researchers compared the level of ctDNA with two serum markers, a-AFP and a-AFU, in the circulation of HCC patients and in healthy individuals and found ctDNA level in HCC samples was significantly elevated [105]. This provides the great promise for the future use of NGS to test ctDNA in pediatric patients with liver malignancy.

Conclusions

In summary, HB and HCC are two common primary liver tumors of children, carrying a number of unique genetic features. Previous studies and recent large-scale, genomic studies have characterized many genetic features involved in the tumorigenesis and development of pediatric HB and HCC. These findings have been applied in the clinical setting at different levels. However, there is still much remaining to be determined, especially in NGS analysis on HB and in ctDNA of patients with these tumors. Characterizing the entire genetic landscape of these tumors is the prerequisite for individualized genetic-based tumor diagnosis, monitoring, and treatment. The gap between our knowledge about the genetic alteration of these tumors and their clinical application is still wide but is being filled in through advances in technology including NGS and the systematic integration of large scale genomic studies.

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References


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