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# Hepatoblastoma Etiopathogenesis

**Macias RIR**[1,3*], **Armengol C**[2,3] **and Marin JJG**[1,3]

[1]*Experimental Hepatology and Drug Targeting (HEVEFARM), University of Salamanca, IBSAL, Salamanca, Spain*

[2]*Childhood Liver Oncology Group, Health Sciences Research Institute Germans Trias i Pujol (IGTP), Badalona, Spain*

[3]*Network for Biomedical Research in Hepatic and Digestive Diseases (CIBERehd), Spain*

[*]**Corresponding author:** Macias RIR, Department of Physiology and Pharmacology, Campus Miguel de Unamuno E.D. B-17, 37007-Salamanca, Spain, Tel: 34-923-294674; Fax: 34-923-294669; E-mail: rociorm@usal.es

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## Abbreviations

BWS: Beckwith-Wiedemann Syndrome; FAP: Familial Adenomatous Polyposis; HB: Hepatoblastoma; HCC: Hepatocellular Carcinoma; IGF2: Insulin-like Growth Factor 2

## Editorial

Hepatoblastoma (HB) is the predominant form of pediatric liver cancer, usually arising in young children under 3 years of age [1], but it is a rare pediatric cancer with a very low worldwide incidence (<2 cases per million children under 18 years) [2]. Hepatocellular carcinoma (HCC) can also be diagnosed in children but with much lower incidence, usually found in the teenage population in association with predisposing conditions, such as the presence of underlying liver diseases (i.e., tyrosinemia and several cholestatic syndromes). As compared with HB, the prognostic of HCC is often poorer. A third type of primary liver cancer that appears at the late childhood and adolescence was initially called transitional liver cell tumor (TLCT), because it has a mixture of histological patterns characteristic of HB and HCC [3]. This cancer has been recently classified in a new provisional category named hepatocellular neoplasm not otherwise specified [4], awaiting for a better characterization, in an international symposium aiming to develop a consensus classification system for pediatric liver malignancies.

Overall survival of patients with HB has notably improved from 30% in the 1970s to 70-80% nowadays thanks to the incorporation to the treatment of adjuvant and neoadjuvant chemotherapy using cisplatin and doxorubicin combined with efficient surgical approaches, including liver transplantation [5]. However, there are very limited treatment options for patients suffering from immature, invasive, and metastatic HB resistant to conventional first-line chemotherapeutic protocols, being the survival of these patients usually less than 3 years. The International Childhood Liver Tumor Strategy Group (SIOPEL) defined a prognostic stratification system of HB patients taking into consideration PRETEXT stage, metastatic disease, serum α-fetoprotein levels, multifocality, age and histology characterized by small undifferentiated cells [6]. Importantly, a recent study of the Children's Hepatic tumors International Collaboration (CHIC) group has confirmed these prognostic risk factors and identified new ones through a large-scale analysis using an extensive database including 1605 HB cases [7]. Patient stratification and treatment taking into account molecular biomarkers are the future challenges facing clinicians. High-throughput molecular studies of aggressive tumors highly refractory to anticancer drugs have the unique potential to provide a rational basis and new tools (biomarkers/molecular targets) for improving patient stratification and defining more specific and molecular-based therapies.

Because HB is a rare tumor, most of the few existing studies have been carried out in small groups of patients, and this fact, together with the existence of several histologic subtypes without a clear consensus for their classification, has hindered advances in the characterization of different aspects of this liver cancer. Histologically, HB is usually composed of combinations of epithelial, mesenchymal, undifferentiated and other histologic components. Moreover, the epithelial component is frequently a mixture of immature hepatocytes resembling those of embryonic and fetal livers. HB cases differ in the type and proportion of these components, showing differences in gene expression profiles, patient prognosis and response to therapy [8]. Both, the pure fetal histology in the surgical specimen and the presence of small cell undifferentiated component (SCU) have been proposed as prognostic factors [4,6]. Interestingly, based on gene expression profiling two prognosis subtypes of HB (C1 and C2) with different pathological, clinical and molecular characteristics have been identified [9]. The C2 aggressive subtype recapitulates early stages of liver development and has been characterized by a predominant embryonal histological type, with a hepatic stem-like phenotype and a specific Myc and β-catenin target gene profiling. In a similar study, deregulation of the MAPK signaling pathway and anti-apoptotic signaling was found to be preferentially upregulated in aggressive epithelial HB with a small cell component [10]. Thus, the genomic basis of the malignant phenotype is complex and dysregulation of different cell growth and survival pathways are involved.

HB is believed to arise from immature progenitor liver cells, develops in the absence of pre-existing liver diseases, and sometimes becomes detectable during pregnancy or at birth. It is thought that, as well as other embryonal malignancies, HB could be the result of an alteration of the normal developmental processes before terminal differentiation, and this is supported by the immature histology of tumors and the frequent prenatal and neonatal detection. In fact, an increasing percentage of cases are now diagnosed antenatally on routine ultrasound scans and many cases in infants aged <1 year. Whether HB occurs as a consequence of aberrant responses to external insults has caused greatest attention to be drown towards perinatal conditions. No environmental factors, parental tobacco or alcohol use, before and during pregnancy, or maternal illness or medication used during pregnancy have been clearly associated with HB development in the offspring [11,12]. Only very low birth weight (<1,500 g) has been significantly associated with HB [13], independently of gestational age. Because these premature infants are usually exposed to potentially toxic environmental agents in neonatal intensive care units

(radiation, transfusions, medications, etc) at a time when, due to their immaturity, fetal tissues are more vulnerable to procarcinogenic insults, the role of an iatrogenic etiology for some cases of HB cannot be ruled out [14]. However, the results obtained from retrospective studies have an important limitation to unambiguously reach this conclusion because the tumors were diagnosed in early childhood when it is not possible to elucidate whether the development of the tumors had occurred *in utero* or after birth.

The incidence of HB has increased over the last decades [2], and this could be related not necessarily to an increased number of new tumors per year but to the higher rate of their detection, which could be due both to improvement in the management of premature infants resulting in better outcomes and to more sensitive and accurate diagnostic tools that have permitted to overcome the underestimation of the true incidence in the past. An interesting and intriguing characteristic of HB is that, as mentioned above, this tumor is associated to low birth weight of infants, whereas for most childhood cancers, such as leukemia, central nervous system tumors, renal tumors and soft tissue sarcomas, there is a risk association between high birth weight and cancer development [15].

Heritable predisposition to HB occurs in a small proportion of cases in patients with genetic syndromes, such as Beckwith-Wiedemann syndrome (BWS), familial adenomatous polyposis (FAP), and trisomy 18 [16]. The genetics of BWS is complex, but clearly associated with alterations in the expression or function of one or more genes in the 11p15.5 imprinted gene cluster. Disturbed expression of the cell growth regulatory gene complex located in the 11p15 region results in aberrant growth during organogenesis, thus also favors tumor development. Insulin-like growth factor 2 (IGF2) and H19 (an untranslated RNA) are some of the genes located in this region. In HB, deregulation of imprinted genes, including IGF2 and H19 have been reported [17,9]. HB is also considered one of the rare extra-colonic manifestations of FAP, a disorder caused by germline mutations of the APC gene, located in the 5q21 chromosomal region, which prevents the degradation of the β-catenin. Trisomy 18 is a rare syndrome and even if some HB cases have been described in the literature, the incidence of HB among trisomy 18 patients is difficult to estimate due to the high mortality during the first year of life.

Regarding molecular abnormalities related to HB, several independent studies have shown that the frequency of mutations in this type of tumor is very low (1-7 per tumor) compared to adult HCC (35-75 per tumor) [18]. Among the mutated genes, CTNNB1, encoding β-catenin, is the most frequent one. CTNNB1 mutations are found in ≈70% of HB. Accordingly, HB stands at the first rank among human cancers endowed with constitutive activation of the Wnt signaling pathway [19]. Wnt signaling pathway regulates progenitor cell expansion and embryonic lineage decisions and its disruption has been linked to developmental defects and cancer [20]. Other causes of Wnt activation by preventing the proteosomal degradation of β-catenin are the loss-of-function mutations of the APC gene in FAP and the presence of mutations in AXIN1 and AXIN2 genes [21]. Recently, mutations in the CAPRIN2 gene have been also described to activate Wnt pathway in HB [22] and novel mutations affecting different components of several ubiquitin ligase complexes have been identified and could be considered as potential actors in the etiopathogenesis of HB [22]. Mutations in the nuclear factor "erythroid-derived 2-like 2", NFE2L2 or NRF2 have been reported to occur in 8% of HB [23,24], but also in other types of tumors, including HCC. Changes in the expression of genes involved in chemoresistance/chemoprotection in HB.

| MOC | Mechanism | Genes | Up/Down Regulation | Chemoprotection/ chemoresistance |
|---|---|---|---|---|
| 1a | Drug Uptake | SLC22A1 | Down | Increased |
| | | SLC22A5 | Up | Decreased |
| | | SLC28A1 | Down | Increased |
| 1b | Drug Export | ATP7A | Up | Increased |
| | | ABCB1 | Up | Increased |
| | | ABCC2 | Up | Increased |
| | | ABCG2 | Up | Increased |
| | | ABCC6 | Down | Decreased |
| | | MVP | Down | Decreased |
| 2 | Drug Metabolism | CES1 | Down | Decreased |
| | | CYP1A2 | Down | Decreased |
| | | GSTA1 | Down | Decreased |
| | | UPP1 | Up | Increased |
| 3 | Drug Targets | DHFR | Down | Increased |
| | | TOP1 | Up | Increased |
| 4 | DNA Repair | None | | |
| 5a | Apoptosis | BIRC3 | Up | Increased |
| | | DIABLO | Down | Decreased |
| 5b | Survival | BCL2L1 | Up | Increased |
| | | BIRC5 | Up* | Increased |
| | | MAPK1 | Up | Decreased |
| | | MYC | Up* | Increased |
| | | NAIP | Up | Increased |
| | | NFKB1 | Up | Increased |
| | | PIK3CG | Up | Increased |
| | | RPL6 | Up* | Increased |
| | | XIAP | Up | Increased |

**Table 1:** Results from the analysis in six HB of one hundred genes involved in mechanisms of chemoresistance or chemoprotection (MOC) by Taqman Low Density Arrays. Only the genes whose expression levels were significantly different ($p<0.05$) from control livers have been included in the table (modified from [28]). *Up-regulated in C2 subtype of HB [31].

The activation of the NFE2L2-KEAP1 pathway has been associated to increased chemoresistance mediated through over-expression of the target gene NQO1 encoding NADPH dehydrogenase quinone1. A high expression of this antioxidant/detoxifying enzyme has been found in

the aggressive C2 subtype of HB and is associated to significantly reduced survival compared to patients with lower expression of this gene [24]. NQO1 catalyzes the reduction of anthracyclines to less potent semiquinone radicals and it has been demonstrated that enhanced expression of NQO1 confers resistance to doxorubicin and other chemotherapeutic agents in cholangiocarcinoma cell lines [25]. Inactivating mutations have also been found in the thioredoxin-domain containing genes TXNDC15 and TXNDC16, also involved in the regulation of mechanism involved in oxidative stress status [23]. Regarding cytogenetic aberrations, few consistent chromosomal alterations have been described in HB in line with the fact that β-catenin mutations have been associated with chromosomal stability in liver cancer. Hallmark cytogenetic changes in HB include the acquisition of additional copies of whole chromosomes and a recurring unbalanced translocation involving 1q [26,27]. The copy number variation profile of HB shows more frequent gain than losses in different studies [10,22,24]. Regarding chemoresistance, we must keep in mind that changes in expression and/or the presence of mutations in an important number of genes contributing to different mechanisms of chemoresistance in liver cancers, either affecting the intracellular amount of active drugs or affecting their effectiveness can be responsible for the lack of response to chemotherapy [28-30]. Using Taqman Low Density Arrays, analysis of expression profiles in HB revealed changes in mRNA abundance for 26 genes involved in chemoprotection and chemoresistance (Table 1). Interestingly, some of these genes, such as BIRC5, RPL6 and MYC have been found up-regulated in the C2 subtype of HB [31]. Three of these changes, namely up-regulation of BIRC5 and down-regulation of SLC22A1 and CYP1A2 were also observed in HCC and cholangiocarcinoma [28]. These phenotypic traits may be involved in the susceptibility to chemically-induced carcinogenesis and/or the lack of response to chemotherapy once the tumor has already developed.

In conclusion, although important advances have been made in the last decade, there is still an urgent need for improving our understanding of the complex mechanism of etiopathogenesis of this pediatric liver cancer. The effort in collaborative investigation among several groups constitutes an important and absolutely necessary step to gain information on the etiopathogenesis of this rare tumor that to date, is not yet fully understood. New insights into the key genetic alterations that drive tumorigenesis and tumor progression will be useful both as diagnostic and prognostic markers. Finally, the characterization of changes in the mechanisms of chemoprotection and chemoresistance will contribute to predict susceptibility to develop HB and to develop novel and more effective pharmacological treatments.

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