IMAGING AND ADVANCED TECHNOLOGY

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Myc Target miRs and Liver Cancer: Small Molecules to Get Myc Sick

MARIE–ANNICK BUENDIA,* LUDOVIC BOURRE,‡ and STEFANO CAIRO‡

**INSERM Unit U785 "Pathogenesis and Treatment of Fulminant Hepatitis and Primary Liver Cancer", Hepato-Biliary Center, Paul Brousse Hospital, Villejuif, France; ‡XenTech, Research and Development Department, Genopole Campus 3 Evry, France*

Primary liver cancer is a disease arising from malignant transformation of hepatocytes, which account for up to 80% of the liver tissue. In infants, the most common form of liver tumor is hepatoblastoma (HB), a rare childhood tumor that mostly affects kids <3 years old. In adults, hepatocellular carcinoma (HCC) is by far the most common form of liver malignancy.

HB is an embryonal tumor characterized by proliferation of immature hepatoblasts, frequently associated with malignant mesenchymal tissue, which suggests that it derives from uncommitted progenitor cells. HB occurrence has a strong genetic footprint. Deregulation of the progenitor cell differentiation program is in most cases determined by the alteration of the Wnt pathway. Activating mutation of β-catenin, which leads to sustained Wnt signaling, is observed in up to 80% of tumors. Inactivating mutation of Axin and APC, 2 proteins involved in β-catenin degradation, is also observed in HB. Children with familial adenomatous polyposis, a genetic syndrome caused by germline APC mutation, have an increased risk of developing HB. Furthermore, the Beckwith–Wiedemann syndrome, a genetic disease related to loss of genomic imprinting, is also associated with elevated HB incidence. In addition to genetic alterations, some environmental events are also associated with HB development. Eclampsia during pregnancy and very low weight at birth are the environmental factors with the strongest association with HB described so far.[1]

HCC is a world-wide emergency. In the last decade, HCC prevalence increased continuously and at present HCC is the 5th most frequent form of cancer and the 3rd leading cause of cancer-related death. Differently from HB, where primary genetic events that predispose to HCC are unknown. The etiologic factors that are most frequently associated with HCC occurrence are well described. Exposure to viral pathogens such as hepatitis B (HBV) and C (HCV) viruses, to environmental factors such as aflatoxin B1, and alcohol consumption, strongly enhance the probability of developing HCC.[2]

Despite the profound difference existing between HB and HCC in terms of etiology, genetic background, and histologic phenotype, the development of both diseases has been found tightly associated with functional hyperactivation of the Myc oncogene.

The Myc Family of Oncogenes

Myc is a transcription factor of the basic helix–loop–helix leucine zipper (bHLHZ) family. It was originally identified as the cellular homolog of v-Myc, a viral protein of the avian myelocytomatosis virus. Three Myc homologs exist in the human genome, c-Myc, N-Myc, and L-Myc, and their involvement from early steps of carcinogenesis to evolution and metastatic spread in ≥50% of human cancers is well documented. Myc binds to a 6-nucleotide DNA consensus sequence, CACGTG, called E-Box, which is present in approximately one-third of human gene promoters. Binding of Myc to this sequence requires dimerization with the Max protein, another bHLHZ protein. Myc/Max complex, induces transcriptional activation of target genes and microRNAs (miRs). Competitive binding to Max by other bHLHZ, such as transcriptional repressors of the Mad family or Mnt, antagonizes Myc activity, as binding of a Mad/Max or Mnt/Max dimer to E-box inhibits gene transcription. In addition, many other constitutive or tissue-specific bHLH or bHLHZ transcription factors can compete for binding to E-box sequences. In addition to its transactivation properties, Myc can also exert a Max-independent repressive function by interacting with several transcription factors such as Miz1, Sp1, Smad proteins, and several others to impair the recruitment of the transcriptional machinery on several gene promoters. Overall, it has been estimated that Myc participates to the regulation of ≥15% of all coding genes and of numerous miR clusters.

Beside its role as a transcription factor, Myc takes part in the modulation of chromatin status. Myc binding to DNA throughout the genome is associated with an increased amount of euchromatin regions, whereas Myc ablation leads to progressive spread of heterochromatin.

Given the pleiotropic role that Myc plays on genomic DNA and gene expression, it is not surprising that Myc activity is regulated and finely tuned by several converging upstream signaling pathways. Depending on the develop-

Abbreviations used in this paper: bHLHZ, basic helix–loop–helix leucine zipper; HB, hepatoblastoma; HCC, hepatocellular carcinoma; miRs, microRNAs; MRMs, Myc-repressed miRs.

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mental stage, the tissue type and the cell lineage, Ras, Wnt, Notch, and PI3K pathway can converge and modulate Myc activity to regulate cell growth (proliferation vs differentiation), cell metabolism (glycolysis vs citric acid cycle), and cell survival (apoptosis vs senescence).[3]

Myc in Liver Development, Homeostasis, and Carcinogenesis

In the liver, Myc plays an important role during embryonic liver development as well as in the homeostasis of adult liver. The use of animal models has provided important insights on Myc contribution to liver development and maturation. These studies showed that both N-myc and c-Myc are involved in tissue organization and in the burst of proliferation that precedes birth in rodents, with redundant as well as unique roles for each protein. In adult liver, c-Myc is involved in hepatocyte growth and is essential for achievement of polyploidy. Moreover, Myc is required to induce liver regeneration upon partial hepatectomy, as well as in response to hepatotoxic agents. These 2 properties are closely intertwined, and their relationship is well explained by the recent discovery that Myc allows cell replication under stress conditions. In this new intriguing perspective, the main functional role of Myc would be to help cells bypass the G1/S checkpoint rather than to induce proliferation.[4]

Myc overexpression is sufficient by itself to induce liver tumors. Support for this notion has been provided by several animal models. Among them, seminal studies in the field showed that when Myc is overexpressed in embryonic liver, mice succumb to liver cancer within a few days after birth, whereas if Myc overexpression is induced perinatally, the mean latency period between Myc induction and tumor occurrence is 8 weeks. By contrast, if activated in young adult mice, Myc induces HCC with an average latency period of 35 weeks. Concomitant administration of hepatotoxic molecules at the time of Myc activation dramatically drops this latency down to 1 week.[5,6] On the basis of these findings, it has been proposed that Myc oncogenic potential is fully elicited when cells are in a proliferative status. In this view, a strong requirement for HB to occur would be that aberrant Myc activation is coupled with active hepatoblast/hepatocyte proliferation at the embryonic and early postnatal stages. Accordingly, HB prevalence begins to decrease by the time children reach 3 years of age, which corresponds with the period in which most of hepatoblasts still present from fetal stages are converted into mature hepatocytes. In adults, accumulation of liver insults boosts hepatocyte regeneration and turnover. Because all these events engage Myc activation, prolonged stress increases the chances of inducing constitutive, aberrant Myc signaling, which leads to liver hyperplasia and eventually degenerates into HCC. A schematic overview of this hypothesis is depicted in Figure 1.

Expression of early hepatic precursor markers such as AFP, GPC3, KRT19, and EpCAM has been observed in poorly differentiated HBs and HCCs, supporting the view that these tumor subtypes could distinguish similar molecular features. Molecular signatures that identify Myc activation in liver cancer have been recently developed. A 16-gene signature has been established in HB that identifies an HB subclass endowed with constitutive Myc activation and poor prognosis.[7] When applied to HCC, this signature was capable of identifying invasive, poor prognosis HCC.[8] Similarly, a miR-based signature successfully stratified both HB and HCC based on the expression profile of 4 Myc target miRs, miR-100, let-7a, miR-371, and miR-373. These results suggest that, despite the fact that the modality of Myc engagement in tumorigenesis is

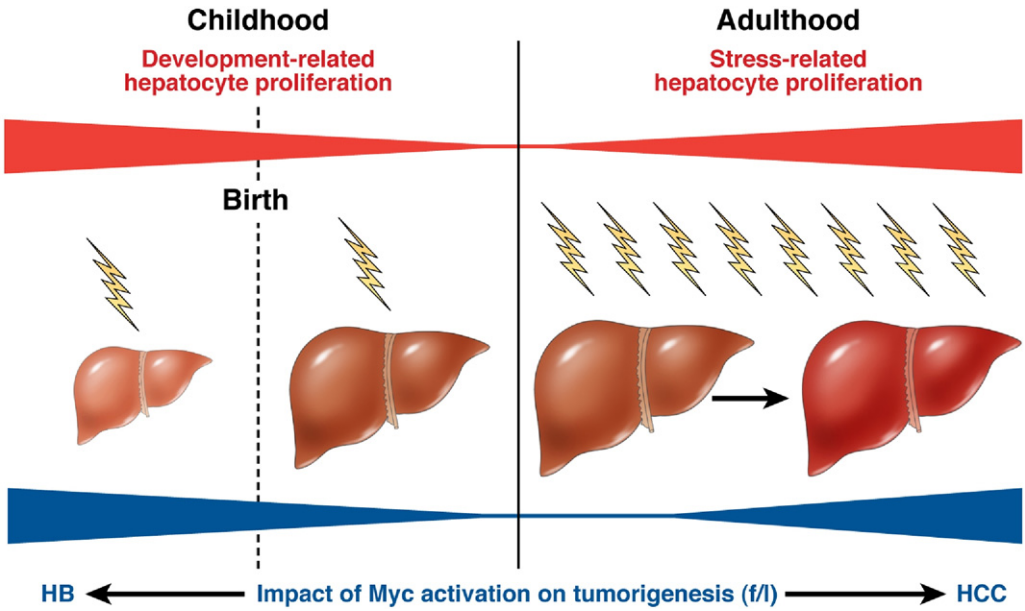

Figure 1. Schematic representation of Myc-mediated oncogenesis in developing and adult liver. The *yellow thunderbolts*, represent genetic, viral or environmental insults. The impact of Myc activation on liver tumor formation is expressed as a ratio between frequency (*f*) and latency (*l*).