Deciphering Hepatocellular Carcinoma: From Bench to Bedside and Back

Hepatocellular carcinoma (HCC) is 1 of the 5 most common cancers worldwide, with >600,000 new patients diagnosed each year; it ranks 3rd in overall cancer deaths. In the United States, the incidence of HCC has almost doubled over the last 2 decades, indicating a change in the frequency of underlying etiologies. These numbers also reflect our inability to prevent, diagnose, and treat this disease in an optimal fashion, and reveal our challenges in appreciating liver cancer biology. However, many recent advances have resulted in a better understanding of disease etiology and pathogenesis, and therefore, improved therapeutic options in specific cases. Several risk factors for the development of HCC are well established, including cirrhosis, particularly when occurring as a consequence of hepatitis C (HCV) or B (HBV) virus infections or hemochromatosis. In recent years, diabetes mellitus, obesity, and nonalcoholic steatohepatitis have been identified as additional risk factors.

Exposure to an environmental risk factor, the mycotoxin aflatoxin B1 (AFB1), is linked directly to the high incidence of HCC in east Asia and sub-Saharan Africa and specific TP53 mutations in these tumors. Furthermore, several signaling pathways and genes have been associated with hepatocarcinogenesis, including WNT/β-CATENIN and transforming growth factor (TGF)-β.

HCC is challenging compared with other cancer types because of the common presence of underlying cirrhosis, which itself causes significant organ dysfunction, morbidity, and mortality, and can then prevent or limit therapeutic approaches. Hence, the best long-term treatment success occurs where resection or transplantation can be performed with curative intent in stable patients. When surgical options do not exist, locally destructive therapy, including radiofrequency ablation, transarterial chemoembolization, or direct percutaneous ethanol injection, can be used. Most recently, the tyrosine kinase inhibitor sorafenib was found to be effective in patients with Child–Pugh stage A cirrhosis and advanced disease. Typically, however, treatment options are limited and outcome remains poor for patients with advanced disease. This underscores the need for improved prevention, screening, and early diagnosis, and a rationale for treatment decisions guided by tumor biology and prognosis.

In this issue of GASTROENTEROLOGY, 2 papers shed light onto this problem from seemingly opposite angles, one originating from an in vitro cell culture system, the other starting with a large clinical retrospective analysis over 17 years. What is common to both, however, is that each will only reach their full potential impact if their observations made will ultimately reach the bedside and the bench, respectively: The in vitro results should lead to a better understanding of disease etiology and the discovery of novel therapeutics and clinical trials, whereas the clinical study could result in the evaluation of specific signaling pathways as predictors of tumor biology and outcome.

Besaratinia et al14 examine the dose-dependent effects of AFB1 on DNA adduct formation and mutation in transgenic Big Blue murine fibroblast culture in the presence of a rat liver activation system. Aflatoxin exposure is a well-established risk factor for HCC. The mycotoxin produced by Aspergillus flavus parasiticus contaminates peanuts, soy products, and corn in endemic areas. It is metabolized by the cytochrome P450 complex mainly to AFB1-8,9-epo-poxide which then forms transient AFB1-N1-guanine DNA adducts, ultimately leading to G:C-to-T:A transversion in codon 249 of TP53. This mutation of TP53 is characteristic of HCC in patients in areas endemic for AFB1. Besaratinia et al are now able to demonstrate the presence of persistent imidazole ring-opened AFB1 adducts after AFB1 exposure, and detect the presence of G:C-to-T:A transversions, typically located within an AGG codon in the inserted cII transgene, corresponding to codon 249 affected in TP53 of HCC patients in AFB1 endemic areas. This work confirms further the requirement of an activating enzyme system, namely CYP1A2 and CYP3A4, to create toxic AFB1 metabolites, as the rat liver S9 activation system needs to be present for mutations to occur. The experimental conditions allow detailed cell and genome toxicity studies and, because the mutations were assessed in the non-transcribed cII transgene, strand bias of the translated strand of DNA is not a factor.

Besaratinia et al’s work confirms many clinical observations and in vivo animal studies. It offers a chance to interrogate the sequence of AFB1 exposure, activating metabolism, adduct formation, and mutation specifically, and provides a tool to discover possibly preventative mechanisms to avoid mutagenesis. In areas endemic for AFB1 exposure, prevention could be achieved theoretically by decontamination of food sources, by inhibition of phase I AFB1 metabolism by cytochrome P450 members that pro-
duce the genotoxic AFB1 products, or by activation of phase II metabolism, through induction of glutathione S transferases and other enzymes, to reduce these AFB1 metabolites. The primary prevention of Aspergillus contamination in food seems ideal, but probably not achievable in the short term because of logistical and cost constraints in those areas of the world where it would be needed most. Clinical studies using oltipraz and chlorophyllin showed conceptually that prevention of AFB1-induced mutagenesis is possible.17–19 For the current work by Besaratinia et al to have clinical impact and relevance, this in vitro system could be used to screen for chemicals that reduce mutation frequency. Using their specific techniques to examine adduct formation and mutagenesis, one might be able to identify chemicals that specifically affect AFB1 metabolism, adduct formation or G:C-to-T:A transversion. Although it is unclear that modifiers of phase II AFB1-inactivating metabolism would be detectable also in this non-hepatocyte system, the approach could result in novel compounds that can then be brought to preclinical in vivo experiments and ultimately to the bedside in the form of clinical trials.

By contrast, the study by Roayaie et al15 starts at the bedside by analyzing pathologic data and clinical outcome, and offers the opportunity for further mechanistic insights into the biology of HCC.15 This work examines the predictive role of microvascular invasion as a risk factor for recurrence after curative resection for HCC. This retrospective study at a single institution benefits from a large and homogeneous group of patients, assessing 131 patients with Child–Pugh stage A cirrhosis with largely normal portal pressures as estimated by the absence of significant thrombocytopenia. Both macrovascular and microvascular invasion have been determined as risk factors for recurrence after resection and transplantation, and negatively impact survival,20–25 leading to their inclusion in proposed staging criteria for HCC.26 In a recent study, Nathan et al24 used the Surveillance, Epidemiology, and End Results database to examine predictors of survival after resection for HCC and devised a score based on the determined risk factors size >2 cm, multifocality, and microvascular invasion. The current study examines specific features of microvascular invasion as prognostic factors for recurrence and survival. Using multivariate analysis, the authors determined that microvascular invasion of a blood vessel with a muscle wall was a negative predictor for both freedom of recurrence and survival, whereas invasion of a blood vessel >1 cm away from the tumor border was predictive of survival alone. The size and number of vessels invaded did not impact outcome. The scoring system presented by the authors to account for these 2 parameters, invasion of a muscle-containing vessel wall and distance of invasion to primary tumor, defines 3 distinct risk categories that correlate with both disease recurrence and survival. It is striking that those patients who have none of these features exhibit recurrence rates and survival times similar to those patients who do not have any evidence of microvascular invasion. By contrast, those patients with 2 risk factors have an outcome comparable to patients with gross vascular invasion. As the authors note, this scoring system needs to be validated in future studies. Importantly, the scoring system proposed has the potential of being widely adaptable as a useful clinical tool for assessing the prognosis of HCC after resection. Because these features influence clinical outcome, the results also identify those patients with the greatest clinical need for further therapeutic strategies after resection. In addition, the authors point to the benefit of this scoring system to stratify patient populations in clinical trials.

But the true impact of these observations may go well beyond predicting clinical outcome and stratifying trial data; they could serve as indicators of differential biological behavior. Several signaling pathways have been associated with microvascular invasion: Fransvee et al27,28 implicate the TGF-β pathway and associated β-integrin signaling in this process. Cieply et al29 demonstrate a higher degree of HCCs with macro- or microvascular invasion associated with mutations in β-CATENIN.29 Wong et al30 use microarray analysis and find topoisomerase 2α expression correlated with microvascular invasion. All these results imply that the presence of microvascular invasion is not simply the advanced presentation of HCC from a previously non-invasive state, but may reflect different, and more aggressive, tumor biology. A cancer that forms microvascular invasion distant from its primary tumor might be related to one with gross vascular invasion, whereas those tumors with minimally invasive features could be similar to those that form no vascular invasion.

The present work demonstrates the opportunity to learn directly from clinical observation for cancer biology: Molecular analysis of the tumor specimen, either in the form of unbiased approaches using expression profiling strategies or a more targeted approach to look specifically for indicators of alterations in major signaling pathways, would not only reveal which pathways contribute to high-risk tumors, but may ultimately help to decide which patients would benefit from surgery. Because several major signaling pathways have been associated with microvascular invasion, further molecular characterization of those tumors included in the present study could confirm these and other pathways as predictors of outcome in their own right. Ultimately, this may lead to the identification of patients who would not benefit from resection, but rather from transplantation or systemic treatment strategies. Furthermore, this strategy could identify novel signaling pathways and molecular targets likely relevant for the treatment of HCC.

HCC remains a clinically challenging problem. Despite the identification of several risk factors and the groups of patients at risk, there are no sufficiently effective and well-
utilized strategies for disease prevention and screening. Curative and palliative treatment approaches have improved, but need to be further optimized to reflect the unique coexistence of cirrhosis and cancer, and varying disease biology. As physicians and investigators, we have to constantly bridge the gap between innovative laboratory investigation and current clinical observations, each informing the other, to achieve further progress in the detection and treatment of this devastating disease.

WOLFRAM GOESSLING, MD, PhD
Genetics and Gastroenterology Divisions
Brigham and Women’s Hospital
Gastrointestinal Cancer Center
Dana-Farber Cancer Institute
Harvard Medical School
Harvard Stem Cell Institute
Boston, Massachusetts

References


Reprint requests
Address requests for reprints to: Wolfram Goessling, MD, PhD, Genetics Division, Brigham and Women’s Hospital, Harvard Medical School, NR8458, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115. email: wgoessling@partners.org.

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