The Transition From Inflammation to Cancer in the Liver

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Liver cancer represents the second leading cause of cancer-related death worldwide and has an approximate incidence of 850,000 new cases annually. Despite numerous large clinical trials, sorafenib and regorafenib have remained the only effective systemic treatment option for advanced hepatocellular carcinoma (HCC), the most frequent form of liver cancer. This underscores the need to identify novel molecular targets and develop new

therapeutic approaches. However, rather than waiting to treat patients with advanced cancer, the highest potential to reduce the disease burden of HCC might lie in improving early detection and thereby instituting chemoprevention of HCC.

In contrast with most other malignancies, HCC arises almost exclusively in chronically inflamed livers, because of

Abbreviations: DAMP, damage-associated molecular pattern; FADD, Fas-associated protein with death domain; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HMGB1, high-mobility group box 1; IL, interleukin; MDSC, myeloid-derived suppressor cell; NASH, nonalcoholic steatohepatitis; NF- κ B, nuclear factor- κ B; PD-1, programmed cell death protein 1; RAGE, receptor for advanced glycation end products; RIPK, receptor-interacting protein kinase; RNS, reactive nitrogen species; ROS, reactive oxygen species; STAT-3, signal transducer and activator of transcription 3; TGF- β , transforming growth factor- β ; TNF, tumor necrosis factor.

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FIG 1 Basic molecular events driving inflammatory hepatocarcinogenesis. Different forms of programmed cell death exist that are activated in a pathogen-specific manner. Dying hepatocytes activate the immune system by the release of danger molecules termed DAMPs such as HMGB1 or S100 proteins and activate immune cell via specific receptors like RAGE. Inflammation is a key promoter driving the maladaptive wound healing response toward HCC. Type I macrophages emerge in the very early stage of the tumor microenvironment, recruiting inflammatory cells by a complex network of chemokines. These cells mediate the Th1 and Th17 response and promote hepatic compensatory proliferation by cytokines, such as tumor necrosis factor (TNF) and IL-6, which activate antiapoptotic, proproliferative signals like NF- κ B or signal transducer and activator of transcription 3 (STAT-3) in hepatocytes. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by activated inflammatory/immune cells induce mutations and genomic alterations resulting in the appearance of premalignant cells that proliferate and survive. Type II macrophages emerge in the late stage of the tumor and are in charge of Th2 response by recruiting Th2 cells that contributed to immunosuppression. MDSCs contribute an immunosuppressive effect by secreting IL-10 and transforming growth factor- β (TGF- β), and also promote angiogenesis caused by the secretion of cytokines such as VEGF. Because of immunosuppressive factors in the tumor microenvironment, antitumor immunity exerted by, for example, cytotoxic T cells cannot be activated unless the immunosuppressive factors are neutralized or eliminated.

the various known risk factors. These include mainly chronic viral hepatitis [hepatitis B virus (HBV) and hepatitis C virus (HCV)], chronic alcohol abuse, and hemochromatosis, but also the metabolic syndrome, caused by diabetes and obesity, and the associated nonalcoholic fatty liver disease and nonalcoholic steatohepatitis (NASH).¹ Therefore, the population at risk for HCC development can be identified, a fact that offers great potential to decrease HCC-related mortality through early detection and prevention. In terms of prevention, the greatest achievements have been made in viral hepatitis-related HCC, both by universal vaccination against HBV and through the implementation of effective antiviral treatment against HBV and HCV.¹ Unfortunately, effective antiviral suppression, even with the newly available antiviral agents, does not fully eliminate HCC risk.^{2,3}

Moreover, in nonviral chronic liver diseases, the elimination of the underlying damaging agents (e.g., in NASH) often is not possible. Therefore, a better understanding of the basic molecular processes that link chronic liver damage with inflammation, fibrosis, and HCC development might help, not only in detection of early HCC, but also through development of new drugs to efficiently prevent *de novo* hepatocarcinogenesis or by guiding new adjuvant strategies for use after resection or transplantation.

In this article, we summarize the common molecular events that link chronic liver injury and cell death with inflammation and HCC development, with a focus on the clinical implications for risk stratification and chemopreventive strategies that might delineate present and future clinical practice.

FIG 2 Activation of necroptosis in human NASH. (A) For years, the term *apoptosis*, relying on the activation of caspases, was used synonymously with programmed cell death. However, it became evident that another form of programmed necrosis exists that is termed *necroptosis* and relies on the activation of RIPK1 and RIPK3. (B) In livers of human NASH patients, the necroptosis executor RIPK3 is strongly upregulated, suggesting that necroptosis might be a central regulator of liver injury and inflammation in NASH. FADD, Fas-associated protein with death domain. Reproduced with permission from Gautheron et al.⁵ Copyright 2015, Jérémie Gautheron, Mihael Vucur, and Tom Luedde. Abbreviations: GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; IKK, I-kappa-B-Kinase; NEMO, NF-kappaB-essential modulator; TAB, TAK1-binding protein; TAK1, TGF-beta-activated Kinase 1.

CHANGES IN THE MICROENVIRONMENT ARE DRIVING HCC DEVELOPMENT

The liver has the unique ability to rapidly regenerate upon acute injury and hepatocyte cell death. However, this means that in the setting of chronic, unresolved injury, the molecular mechanisms driving liver regeneration can similarly lead to the initiation and promotion of hepatocarcinogenesis, which explains why HCC mostly arises on the background of chronic liver damage and has been termed by some authors as “a wound that never heals” (Fig. 1).⁴

Different modes of cell death have been identified that trigger specific cell death responses and promote progression of liver disease through distinct mechanisms. However, it is not yet fully understood which forms of cell death are specifically activated by certain pathogenic factors. In viral hepatitis, apoptosis of virally infected hepatocytes, mediated by specific enzymes called *caspases*, represents a key step in viral clearance. Similarly, apoptotic death of hepatocytes has been proposed to play a role in patients with NASH fibrosis.⁴ Recently, another form of cell death called *necroptosis* has been discovered, which represents a highly regulated form of necrosis mediated through receptor-interacting protein kinases

3 (RIPK3).⁴ Necroptosis is strongly activated in livers of patients with NASH fibrosis, where RIPK3 expression was much more evident than cleavage of the apoptosis mediator caspase-3 (Fig. 2),⁵ suggesting it might be a major additional cell death pathway in NASH.

In most chronic liver diseases, only a small percentage of hepatocytes die at the same time. In this setting, cell death has no significant impact on liver function. Instead, hepatic responses to cell death, often persisting over decades, dictate the development of long-term consequences and clinical outcomes. The so-called damage-associated molecular patterns (DAMPs), such as high-mobility group box 1 (HMGB1), are factors that normally remain intracellularly, where they influence functions not necessarily linked with inflammation. However, when they get released from dying cells, they trigger sterile inflammation and macrophage activation occurring after tissue injury via receptors such as receptor for advanced glycation end products (RAGE) or Toll-like receptors⁶ (Fig. 1).

Inflammation is key to promoting malignant transformation. Figure 1 illustrates the complex interplay among immune cells (i.e., type I macrophages, TH1/TH17 cells), cytokines, and chemokines that have a central function

in tumor initiation. As such, the mitogenic cytokine interleukin-6 (IL-6) is released from macrophages and triggers the compensatory proliferation of other hepatocytes, which in a setting of increased oxidative stress and subsequent DNA damage promotes the accumulation of mutations as basis for carcinogenesis.⁷ Moreover, it was shown that inflammatory microniches constituting immune cells and a complex cytokine milieu help malignant hepatocyte progenitor cells to thrive and gain self-sufficiency until they egress from these niches and form tumors, a process associated with the autocrine production of cytokines previously provided by the niche and depending on nuclear factor- κ B (NF- κ B) activation and the presence of T cells.⁸ However, other immune cells emerge at later stages of tumor promotion [i.e., type II macrophages, TH2 cells, myeloid-derived suppressor cells (MDSC)], which provide an immunosuppressive effect. Therefore, antitumor immunity exerted by cytotoxic T cells cannot be activated unless the immunosuppressive factors are neutralized or eliminated.⁹ Overcoming anti-tumor immunity by immune checkpoint inhibition represents one of the most promising future strategies for the treatment of HCC, as recently demonstrated by a clinical trial that tested the anti-PD-1 antibody nivolumab in patients with advanced HCC.¹⁰

CLINICAL IMPLICATIONS

HCC Risk Stratification

Most HCC research has been focused on the treatment of full-blown tumors, such as the use of systemic tyrosine kinase inhibitors like sorafenib.¹ However, the emerging knowledge on the sequential process involving cell death, cell death responses, and inflammation in the pathogenesis of liver cancer might provide new cues to improve early detection and help stratify HCC risk. As a general rule, cancer cure is more feasible in the context of early-stage disease, so any initiative that is successful in increasing early detection is likely to improve patients' survival. There is robust evidence suggesting that molecular data can improve prognosis prediction and risk stratification in HCC.¹¹ For instance, a 186-gene signature from the tumor microenvironment was able to predict not only HCC recurrence in patients who underwent surgical tumor resection, but also the risk for *de novo* hepatocarcinogenesis in HCV-related early-stage cirrhosis.^{12,13} Marker genes that defined the signature were enriched

FIG 3 Gene expression–based risk stratification in HCC. Robust data suggest that gene expression signatures from cirrhotic tissue enable accurate stratification of patients based on their risk for HCC development. These technologies would allow identifying those patients at higher risk, enabling chemoprevention clinical trials using enriched populations. Here, the likelihood of events is higher, and hence the time that is required to complete the trial decreases.

in inflammation signals, including the NF- κ B pathway and IL-6 signaling.

In terms of clinical implementation, gene signatures could be very useful to identify cirrhotic patients at higher risk for HCC development, thereby enriching the study population with patients most likely to respond to

the therapy under study (Fig. 3). Similarly, specific DAMP-signatures and chemokines involved in the cell death-inflammation sequence might be measured in patients' serum, which could act as proxies for the occurrence of specific forms of cell death in the liver and eventually correlate with the risk for development of HCC.

HCC Chemoprevention

Besides viral eradication in patients with viral hepatitis, there are no effective chemoprevention strategies in patients at high risk for HCC development as per clinical practice guidelines. Biannual surveillance with abdominal ultrasound remains as the current best option for these patients to detect HCCs at the earliest stages, where potentially curative treatments are applicable. To date, no therapeutic intervention has been shown to decrease recurrence in patients with early HCC treated with surgical resection. The latest attempt included 1100 patients in the largest clinical trial ever reported in HCC that evaluated sorafenib as an adjuvant therapy in patients treated with either resection or ablation (STORM trial). However, this trial was negative,¹⁴ underscoring the need for a better understanding of the factors that mediate the transition from cell death to inflammation and cancer. Ultimately, this will provide numerous opportunities to develop pharmacological strategies and prevent HCC development and recurrence. For instance, the caspase inhibitor emricasan was shown to decrease liver injury and fibrosis in a murine NASH model¹⁵ and is currently being tested in a multicenter clinical trial in patients with NASH fibrosis (NCT02686762). Given the importance of caspase-dependent apoptosis in murine models of hepatocarcinogenesis,¹⁶ it might also influence HCC chemoprevention in patients with NASH and other liver diseases.

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