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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

A New Model to Assess Hepatitis B Virus Covalently Closed Circular DNA: A Window Into a Previously Hidden Space?

Approximately 296 million people are living with chronic hepatitis B infection with 1.5 million new infections each year.[1] Currently approved chronic hepatitis B treatment using nucleoside or nucleotide analogues inhibits hepatitis B virus (HBV) DNA replication and suppresses circulating HBV DNA levels, normalizes liver enzymes, improves histology, reduces hepatocellular carcinoma incidence, and improves overall survival. However, these therapeutic modalities fail to eliminate viral infection, in large measure because they have no meaningful impact on viral covalently closed circular DNA (cccDNA), which exists as a highly stable episomal form in the host nucleus. Moreover, the viral lifecycle replenishes cccDNA stores through recycling of unincorporated nucleocapsids to the nucleus. On the heels of the extraordinarily successful development of curative antiviral agents for treatment of hepatitis C virus, renewed attention has been directed to efforts to bring about sterilizing cure of HBV. However, this challenging task will hinge on successful elimination of cccDNA. Efforts to develop successful curative strategies will in turn rely on development of small animal models that support HBV cccDNA formation and virus production, which has until recently proved elusive.

In the past several years, several mouse HBV models supporting cccDNA formation have been constructed using adeno-associated vector (AAV)-mediated transduction of a linearized HBV genome. The AAV-HBV vectors are named by the degree to which their length exceeds the size of the native HBV genome (eg, 1.2 = 1.2-fold), and the HBV nucleotides (nt) at the 5' and 3' ends of the linearized genome, the latter designation indicating the site at which the native relaxed circular HBV genome has been linearized. These AAV vectors are AAV-HBV1.2 (nt1354 to nt1989)[2] and AAV-HBV1.3 (nt970 to nt2043).[3,4] Both the AAV-HBV linear episome and cccDNA have been consistently replicated and detected in these models, and the AAV-HBV1.2 and 1.3 mouse models have been shown to produce all HBV proteins from its linear episome and cccDNA. These models, which recapitulate the key steps of the viral lifecycle, do not, however, lend themselves to direct assessment of cccDNA, which have traditionally required direct detection of cccDNA in the liver. In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Xu et al[5] have now developed an extended and novel mouse model called AAV-HBV1.04 (nt403 to nt538), which by virtue of linearization within the polymerase and s-coding domains lacks the ability to generate hepatitis B surface antigen (HBsAg) or HBV polymerase without first generating cccDNA. Thus, the dependence of sAg secretion on cccDNA yields a simple marker for assessment of cccDNA status. This system can in turn monitor the therapeutic effects of novel agents targeting cccDNA by simply following HBsAg titers. The generation of a new HBV mouse model could now be useful for the preclinical evaluation of novel therapeutics specifically targeting HBV cccDNA.

However, this AAV-mediated mouse model does have a few clear limitations. For instance, the progeny virions cannot reenter mouse hepatocytes, resulting in an incomplete HBV lifecycle and is therefore not representative of authentic HBV infection in humans. Thus, this model cannot reliably evaluate antiviral agents for their actions on the entire lifecycle. Another issue is that the measure of HBsAg as a surrogate, although it is dependent on cccDNA formation, also relies on translation and secretion of HBsAg, so putative inhibitors of cccDNA must be excluded for their actions on these key steps. Another key issue relates to the specific mechanisms of cccDNA formation, which has previously been linked, in part, to activation of the DNA damage response.[6] Xu et al[5] proposed that the expression of ATR, a key regulator of the DNA damage response, can be activated in response to DNA damage induced by adeno-associated virus. They suggest that ATR mediates formation of HBV cccDNA and show that it is blocked in cells transfected with AAV-HBV1.04 that are treated with ATR inhibitors and speculate it may be blocked similarly in AAV-HBV mouse models. The findings by Xu et al[5] are consistent with previous work showing that HBV infection and replication activate the ATR-checkpoint kinase 1 branches of the DNA damage response through the DNA lesions on relaxed circular DNA in the HepG2-NTCP model and primary human hepatocytes.[7] However, the importance of ATR in cccDNA formation and persistence in human HBV infection remains to be established. There is no evidence at present that targeting ATR will have an effect on elimination of HBV cccDNA in patients with chronic hepatitis B. Thus, a potential limitation of the model described by Xu et al[5] for preclinical evaluation of anti-cccDNA compounds is that the effect of these compounds on cccDNA formation, degradation, or transcription will also need to be disentangled from their potential effects on ATR.

In summary, this study provides a novel and potentially important mouse model to study HBV cccDNA formation and persistence by conveniently tracking easily measurable HBsAg levels as a surrogate marker. However, this model cannot simulate the full HBV lifecycle and seems to rely on host-dependent functions to form cccDNA that may not be relevant to human infection. More studies are required to explore the mechanisms underlying HBV cccDNA formation and elimination, but this work suggests a new way forward to evaluate agents that specifically interrupt cccDNA metabolism, an important step in the systematic march toward HBV cure.

MIN XU, MD
WENYU LIN, PhD
RAYMOND T. CHUNG, MD
Liver Center, Massachusetts General Hospital
Harvard Medical School
Boston, Massachusetts

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Correspondence
Address correspondence to: Raymond T. Chung, MD, Liver Center, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114. e-mail: chung.raymond@mgh.harvard.edu.

Conflicts of interest
The authors disclose no conflicts.


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