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Editorial

Unraveling the Dynamic Role of Microtubules in the HBV Life Cycle

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Acute hepatitis B virus (HBV) infection can progress to chronicity and development of cirrhosis and hepatocellular carcinoma (HCC). Current antiviral drugs offer long-term virological and biochemical response in patients with chronic HBV infection but they are unable to cure HBV.[1,2] Many viruses, including HBV, exploit the host microenvironment for their efficient replication and persistence. Although the dynamic interactions between viruses and host cytoskeletal proteins vary, the microtubular network always provides a mode of directed transport for the invading pathogens. Following viral entry, the microtubular network undergoes structural reorganization, indicating that invading pathogens not only utilize, but also alter the host cell cytoskeleton during replication and egress. The network is regulated by several post-translational modifications of tubulin along with microtubule (MT)-associated proteins (MAPs).[3] It is well known that MAP1S links autophagic cytoplasmic components to MTs and mitochondria, affecting autophagosome biogenesis and degradation. Moreover, depletion of the MAP1S gene in mice causes deficiencies of both basal autophagy for subsequent clearance of abnormal mitochondria and nutritional stress-induced autophagy for cell longevity and nutrient recycling. Autophagic defects that result from oxidative stress can subsequently either suppress tumorigenesis by triggering cell death or, support tumorigenesis if the cells survive the effects of oxidative stress. Xie *et al*.,[4] previously suggested that the high incidence of liver tumor foci and primary malignancies in MAP1S-depleted mice clearly show that MAP1S suppressed hepatocarcinogenesis by regulating autophagy.[4] Various studies have indicated that ablation of MAP1S leads to increased accumulation of lipid droplets that trigger secretion of inflammatory mediators and reactive oxygen species and cause multiple profibrotic reactions. Cycles of cell division associated with tissue repair of the fibrotic liver facilitate the increase of genome instability and can ultimately lead to tumorigenesis. Inflammatory responses triggered by autophagic deficiencies cause the loss of immune cells through the mechanism of pyroptosis and eventually lead to the failure of host immune response system and additionally in subsequent metastasis of cancer cells. Therefore, MAP1S enhances autophagic flux to suppress tumorigenesis and metastasis and prolong survival of patients probably by suppression of lytic programmed cell death because of inflammation.[4] Regarding HBV infection, it is well established that the HBx protein facilitates autophagy by different mechanisms.[5,6] Moreover, in a recent study, Wang *et al*.[7] reported that HBV infection induced endoplasmic reticulum stress and early stages of autophagy, facilitating HBV replication and secretion, but at the same time inhibition of autophagic degradation was observed in liver tissues of chronic HBV infected patients and in *in vitro* experiments.[7]

In a recent study in the *Journal of Clinical and Translational Hepatology,* Guan *et al*.,[8] describe a hypothesis that offers a novel insight on the interplay between HBV and MTs). Their primary findings are focused on the unique mechanism by which HBV promoted the formation of stable MTs resulting in enhancement of productive infection through upregulation of MAP1S. The study investigating the expression of MAP1S in both HBV stable-expressing HepG2215 cells and HBV-negative HepG2 cells and during natural infection. In HepG2215 cells, MAP1S expression was significantly upregulated compared with HepG2 control cells. To determine whether MAP1S participated in the process of HBV egress, the authors silenced or overexpressed MAP1S in HepG2215 cells and measured HBV DNA copies inside and outside the cells by real-time PCR. MAP1S silencing was accompanied by decreased acetylated MTs and an increase of the ratio of intracellular to extracellular HBV DNA, which suggested that absence of MAP1S reduced and affected the stability of MTs, impaired the cytoplasmic transport channel driven by kinesins, and led to a reduction in the release of HBV viral particles. Additional support of the role of MAP1S in the stabilization of MTs, was provided by an increase of acetylated MTs that accompanied overexpression of MAP1S, followed by a decrease of intracellular HBV accumulation, leading to a reduced ratio of intracellular to extracellular HBV DNA. The findings indicate that MAP1S was required for multiple functions including MT acetylation, HBV intracellular transport, and egress. In addition, confocal microscopy showed that HBV surface protein (HBs) in suppressed MAP1S cells was localized in the perinuclear region compared with controls, where the signal was more diffused. The results led the authors to conclude that MAP1S had a positive role in HBV transport. They also suggested that HBx was the key viral protein that significantly upregulated the expression

Abbreviations: HBV, Hepatitis B virus; MAP, microtubule-associated protein; MT, microtubule; HCC, hepatocellular carcinoma.

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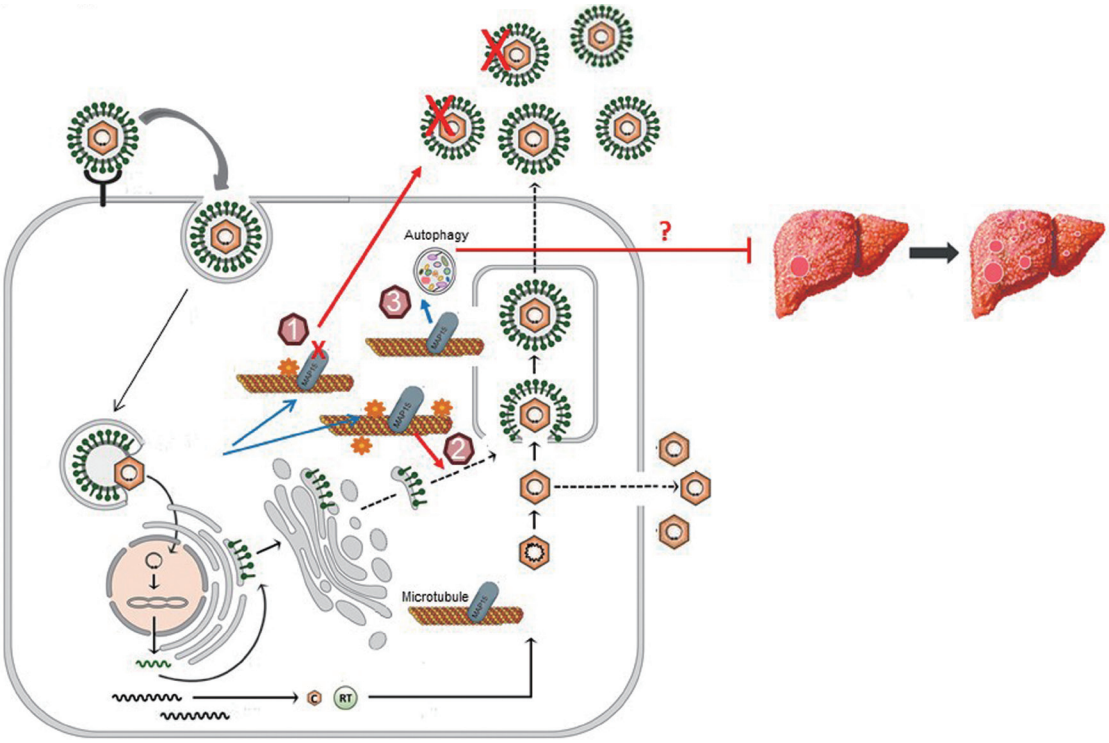

Fig. 1. Diagram of novel MT targeted therapeutics in HBV infection. Numbers indicated putative drug targets. (1) MAP1S inhibition/silencing, (2) MAP1S-dependent acetylation of MTs, and (3) MAP1S dependent autophagy. Blue lines indicate induction. Red lines indicate uppression. A red X indicates inhibition or silencing of MAP1S. Orange/yellow stars represent acetylation of MTs. HBV complete virion is shown by hexagonal capsid with green HBsAg and HBV naked capsid is shown by a brown hexagon. HBV, Hepatitis B virus; MAPs, microtubule associated proteins; MTs, microtubules; HCC, hepatocellular carcinoma.

of MAP1S protein as after transient transfection of HepG2 cell with HBx-expressing plasmids. On the basis of the results, they also performed immunohistochemical staining of liver samples from patients with chronic hepatitis B (CHB), and they observed that MAP1S expression in HBV-positive hepatocytes was much stronger than that detected in HBV-negative cells, confirming that HBV induced the upregulation of MAP1S during natural infection. Moreover, MAP1S expression observed in the same sample of liver tissue derived from patients with CHB was much stronger in HBs-positive cells than in HBs-negative cells and additionally an extensive co-localization of HBs and MAP1S was detected in the hepatocytes of the same patients. However, the mechanism by which HBx upregulated MAP1S to stabilize MTs to favor HBV intracellular transport was not clearly defined by the authors. Many viruses are able to directly interact with kinesins and dyneins, which are the essential microtubular motor proteins for transport.[9–12] For example, HCV uses the MAPs, septin 9 and PtdIns5P to support the formation of lipid droplets to enhance the assembly of HCV particles.[13] Moreover, the HCV nucleocapsid exploits the MTs lattice at late steps of the virus life cycle to facilitate virus transport and assembly and establish efficient infection.[14]

With regard to drug intervention in virus-host MT interplay, Iwamoto M. *et al*.[15] reported that HBV permissiveness was abrogated when treated with the MT inhibitor nocodazole, suggesting that HBV core impairment upon interaction with tubulin and co-localization with MT-like fibriforms disturbs capsid formation.

The above results combined with those of Guan *et al*.[8] could pave the way for the mechanism by which HBV induces MAP1S upregulation, not only to stabilize MTs and to promote HBV transport, but also to promote persistent infection, alter the host immune response, and affect HCC progression (Fig. 1). Ultimately, the development of novel drugs targeting the MTs network and MAP1S would further enrich our treatment strategies for curing chronic HBV infection.

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Conflict of interest

JK has been an editorial board member of *Journal of Clinical and Translational Hepatology* since 2018. UG has no conflict of interests related to this publication.

Author contributions

Study concept, drafting and critical revision of the manuscript (JK, UG). All authors have made a significant contribution to this study and have approved the final manuscript.

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