Potential Newer Therapeutic Targets for Hepatitis B Virus Drug Development

Abstract 1

Christian Königer^{a,b}, Ida Wingert^a, Moritz Marsmann^a, Christine Rösler^a, Jürgen Beck^a, and Michael Nassal^a. Involvement of the host DNA repair enzyme TDP2 in formation of the covalently closed circular DNA persistence reservoir of hepatitis B viruses. *Proc Natl Acad Sci U S A*. 2014 Oct 7;111(40):E4244–53.

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Hepatitis B virus (HBV), the causative agent of chronic hepatitis B and prototypic hepadnavirus, is a small DNA virus that replicates by protein-primed reverse transcription. The product is a 3-kb relaxed circular DNA (RC-DNA) in which one strand is linked to the viral polymerase (P protein) through a tyrosyl-DNA phosphodiester bond. Upon infection, the incoming RC-DNA is converted into covalently closed circular (ccc) DNA, which serves as a viral persistence reservoir that is refractory to current anti-HBV treatments. The mechanism of cccDNA formation is unknown, but the release of P protein is one mandatory step. Structural similarities between RC-DNA and cellular topoisomerase-DNA adducts and their known repair by tyrosyl-DNA-phosphodiesterase (TDP) 1 or TDP2 suggested that HBV may usurp these enzymes for its own purpose. Here we demonstrate that human and chicken TDP2, but only the yeast ortholog of TDP1, can specifically cleave the Tyr–DNA bond in virus-adapted model substrates and release P protein from authentic HBV and duck HBV (DHBV) RC-DNA in vitro, without prior proteolysis of the large P proteins. Consistent with TDP2's having a physiological role in cccDNA formation; RNAi-mediated TDP2 depletion in human cells significantly slowed the conversion of RC-DNA to cccDNA. Ectopic TDP2 expression in the same cells restored faster conversion kinetics. These data strongly suggest that TDP2 is a first, although likely not the only, host DNA repair factor involved in HBV

cccDNA biogenesis. In addition to establishing a functional link between hepadnaviruses and DNA repair, our results open new prospects for directly targeting HBV persistence.

Abstract 2

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Current antiviral agents can control but not eliminate hepatitis B virus (HBV), because HBV establishes a stable nuclear covalently closed circular DNA (cccDNA). Interferon- α treatment can clear HBV but is limited by systemic side effects. We describe how interferon- α can induce specific degradation of the nuclear viral DNA without hepatotoxicity and propose lymphotoxin- β receptor activation as a therapeutic alternative. Interferon- α and lymphotoxin- β receptor activation up-regulated APOBEC3A and APOBEC3B cytidine deaminases, respectively, in HBV-infected cells, primary hepatocytes, and human liver needle biopsies. HBV core protein mediated the interaction with nuclear cccDNA, resulting in cytidine deamination, apurinic/

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Abbreviations: AP: apurinic/apyrimidinic; APOBEC3: apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3; ccc: covalently closed circular; DHBV: duck HBV; dHEPSRG: differentiated HepaRG; HBV: Hepatitis B virus; HCC: hepatocellular carcinoma; LT β R: lymphotoxin- β receptor; pgRNA: pregenomic RNA; PHH: primary human hepatocytes; RC-DNA: relaxed circular DNA; TDP: tyrosyl-DNA-phosphodiesterase

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aprimidinic site formation, and finally cccDNA degradation that prevented HBV reactivation. Genomic DNA was not affected. Thus, inducing nuclear deaminases—for example, by lymphotoxin- β receptor activation—allows the development of new therapeutics that, in combination with existing antivirals, may cure hepatitis B.

COMMENT

Hepatitis C treatment has undergone a paradigm shift with the availability of multiple oral antiviral drugs. The pace of drug development and the results with newer drugs have changed the overall treatment outcome in terms of response rates. The cure rates with these drugs have been reported to be up to 90–100% in different subgroups including cirrhosis.¹ Complications of cirrhosis and development of hepatocellular carcinoma (HCC) due to hepatitis C are expected to decrease with time. In fact, eradication may be possible in the near future.

Similar to hepatitis C virus, lot of research is currently ongoing for the development of potential new therapeutic targets for hepatitis B virus (HBV). Across the world, more than 350 million individuals are infected with HBV. They are at risk of developing end stage liver disease and HCC.

Hepatitis B virus is a DNA virus having partial double stranded circular DNA genome also called relaxed circular DNA (RC-DNA). It codes for four proteins, namely—polymerase (P), core (C), surface (S) and X proteins. HBV replicates within the host through a pregenomic RNA (pgRNA) intermediate. For this, HBV RC-DNA is end repaired to produce closed covalent circular DNA (cccDNA) by host DNA repair enzyme. The HBV RNA is further transcribed from viral cccDNA genome using host DNA dependent RNA polymerase in cell nucleus and translated to individual proteins in cell cytosol. HBV DNA polymerase or P protein has reverse transcriptase activity that helps in production of viral genomic DNA from pgRNA intermediate. During the process of viral replication, HBV produces three DNA phases, namely—RC-DNA, cccDNA and linear DNA phase. RC-DNA is required for mature virion formation, cccDNA gets archived in host cell nucleus in episomal form and linear HBV DNA may integrate into host chromosome. The current oral antiviral drugs or nucleos(t)ide analogs available for HBV therapy mostly target HBV DNA polymerase by interfering with viral replication or formation of RC-DNA from pgRNA. But they have no effect on viral RNA transcription from genomic cccDNA. These drugs control HBV only by reducing the viral load and are not helpful for eradication or cure. Therefore, there is a need for development of drugs with novel mechanisms of action to achieve complete cure of HBV. Recently, such strategies have been proposed for development of drugs that can potentially cure HBV. This novel strategy mostly targets clearance of HBV cccDNA by the use of novel drugs. Persistence of cccDNA is the major hindrance for HBV cure

and clearance of the cccDNA may take up to five decades with current available nucleos(t)ide analog therapy.² Two possible proposed mechanisms are elucidated by Koniger et al³ and Lucifora et al⁴ for clearance of cccDNA. This includes development of drugs that can inhibit formation of cccDNA from RC-DNA and development of drugs that favor direct degradation of cccDNA in host.

FORMATION OF cccDNA FROM RC-DNA

HBV genomic DNA or RC-DNA is bound to viral DNA polymerase (P) within the virion. Upon infection, RC-DNA is released inside cell cytoplasm and translocated to cell nucleus for cccDNA conversion. Recently, Koniger et al³ have identified a host tyrosyl-DNA-phosphodiesterase (TDP) protein that releases P protein from RC-DNA which favors cccDNA formation. For experimental evidence, they have carried out series of experiments using chicken and human hepatoma cell lines to study the effect of host specific (avian and mammalian) TDP on release of P protein from both avian (DHBV) and human HBV. They have confirmed role of TDP on release of P protein from RC-DNA, which is a critical step in cccDNA formation. In another experiment, they have also proven that depletion of TDP by gene knockdown or gene silencing can significantly reduce the conversion of RC-DNA to cccDNA. These results suggest the physiologically relevant role of the host TDP2 in the formation of cccDNA.

Though it appears simplistic, this proposed mechanism may be one of the critical steps and could be a potential site for development of future target therapy.

DEGRADATION OF cccDNA

The other proposed mechanism for drug development is degradation of cccDNA. The proposed mechanism for cccDNA degradation is cytidine deamination of cccDNA by host enzymes cytidine deaminases. This deaminated cccDNA is further recognized by DNA glycosylases and N-glycosidic bonds are cleaved to create apurinic/aprimidinic site (AP site). These AP sites are recognized and cleaved by endonucleases resulting in degradation of cccDNA. The experimental evidence for virus specific deamination of cccDNA sparing host genomic DNA and degradation of cccDNA over repair of cccDNA has been proposed by Lucifora et al. They have described the mechanism of interferon- α action on HBV DNA and proposed alternative mechanisms including lymphotoxin- β receptor (LT β R) activation as a novel way for degradation of cccDNA. HBV-infected, differentiated HepaRG (dHEPSRG) cells and primary human hepatocytes (PHH) were used to study the effects of IFN- α . The use of IFN- α resulted in 80% reduction in cccDNA after 10 days of treatment. In contrast, oral antivirals (lamivudine and entecavir) did not have an effect on the cccDNA.

The effect of LT β R activation was studied using super-agonist tetravalent bispecific antibodies (BS1) and bivalent anti-LT β R monoclonal antibody (CBE11). The IFN- α and LT β R activation resulted in deamination and apurinic/aprimidine (AP) site formation in the cccDNA. This was studied by differential DNA denaturation PCR. Sequencing analysis revealed G $\rightarrow$ A transitions. These foci of deamination and mutation were specifically seen in the viral cccDNA and no effect was seen on the host genome. This highlights the specific actions of these drugs.

HBV is inhibited by apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 (APOBEC3), the mechanism proposed being cytidine deamination.⁵ IFN- α and LT β R result in upregulation of APOBEC3A and APOBEC3B cytidine deaminases respectively. In this study, A3A and A3B were seen specifically in the nucleus as compared to the other members of APOBEC family.

These two studies highlight the newer mechanisms which can be used to target cccDNA, and generate hope that a possible cure may be achievable. In the near future, we may see newer combination therapies for hepatitis B, as there are for hepatitis C virus. However, though theoretically it sounds interesting, the development of new drugs with specific activities will be a huge task and their associated side effects on hosts need to be evaluated in future studies. All in all, the future may bring hope for hepatitis B virus infected patients.

CONFLICTS OF INTEREST

The author has none to declare.

REFERENCES

1. Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. *N Engl J Med*. 2013;368:1907–1917.
2. Chevaliez S, Hézode C, Bahrami S, Grare M, Pawlotsky JM. Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: finite treatment duration unlikely. *J Hepatol*. 2013;58:676–683.
3. Königer C, Wingert I, Marsmann M, Rösler C, Beck J, Nassal M. Involvement of the host DNA-repair enzyme TDP2 in formation of the covalently closed circular DNA persistence reservoir of hepatitis B viruses. *Proc Natl Acad Sci U S A*. 2014;111:E4244–E4253.
4. Lucifora J, Xia Y, Reisinger F, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science*. 2014;343:1221–1228.
5. Suspène R, Guétard D, Henry M, Sommer P, Wain-Hobson S, Vartanian JP. Extensive editing of both hepatitis B virus DNA strands by APOBEC3 cytidine deaminases in vitro and in vivo. *Proc Natl Acad Sci U S A*. 2005;102:8321–8326.

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