What roles HBV integrant-derived RNAs could play in the life cycles of HBV and HDV?

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“Given that our previous study suggested that virus spread and superinfection could represent important determinants of the maintenance of chronic hepadnavirus infection [6], it therefore, became apparent that id-RNAs/id-HBsAg could regulate the maintenance of HBV persistence.”

First draft submitted: 25 July 2018; Accepted for publication: 6 August 2018; Published online: 7 September 2018

Keywords • HBV integrant-derived RNAs • HBV life cycle • HDV life cycle

During infection, DNA of human hepatitis B virus (HBV) randomly integrates into the host genome. It is commonly assumed that the HBV life cycle is independent of integrated HBV DNA, because the integrants cannot facilitate the production of progeny virus [1,2]. Our recent study analyzed matching liver and hepatocellular carcinoma (HCC) tissues collected from untreated individuals chronically infected with HBV. The results suggested that HBV integrant-derived RNAs (id-RNAs), which were transcribed from the viral envelope promoters and bore 5'-HBV-human-3' junction and insert of human (i.e., host) sequences immediately next to the poly(A) tail, were relatively abundant or even predominant HBV RNA species as compared to HBV replication-derived RNAs (i.e., rd-RNAs that were transcribed from covalently closed circular DNA) in majority of analyzed tissues [3]. Considering that HBV DNA integration occurs at all stages of the infection and a considerable number of hepatocytes can acquire HBV integrants over time [1,4], the observed abundance of id-RNAs has a number of interesting and important implications relevant to the life cycle and pathogenesis of HBV, and also to the life cycle of human hepatitis delta virus (HDV), which is a subviral agent of HBV that uses the envelope proteins of HBV to form its own virions and to infect susceptible hepatocytes via the HBV receptor [5].

Influence of id-RNAs on HBV life cycle

In cells that produce id-RNAs and support HBV genome replication at the same time, the id-RNAs coding for HBV envelope proteins (i.e., surface antigen or HBsAg) can potentially impact several aspects of the HBV life cycle. The extent of id-RNAs' impact will depend on the ratio of id-RNAs to rd-RNAs and on the properties of id-RNAs versus rd-RNAs. Thus, if abundant (as compared to rd-RNAs), id-RNAs could produce a significant fraction of total HBsAg that is accumulated in cells bearing HBV integrants. Although, it remains to be demonstrated whether id-RNAs that bear 3'-end inserts of the host sequences of various lengths and nucleotide sequences [3] can serve as the templates mediating efficient synthesis of HBsAg. Furthermore, id-RNA-derived HBsAg (i.e., id-HBsAg) that is produced independently of HBV genome replication can compete with rd-RNA-derived HBsAg (rd-HBsAg) in regulating HBV life cycle. First, relatively abundant id-HBsAg should participate along with rd-HBsAg in the process of HBV assembly and should influence the formation of HBV virions and their secretion. Second, the abundance of id-HBsAg (as compared to rd-HBsAg) will very likely result in significant presence of id-HBsAg in the outer envelopes of the formed HBV virions. Therefore, the properties of these virions should be mostly determined by the properties of id-HBsAg and not by the properties of rd-HBsAg. Accordingly, relatively abundant id-HBsAg would regulate the infectivity of HBV virions (i.e., for example, functional id-HBsAg can mitigate/reverse the impact of defective rd-HBsAg, etc.) and their ability to support virus spread and superinfection. The sequences coding for id-HBsAg and rd-HBsAg could differ for a number of reasons. Thus, for example, rd-RNAs could accumulate mutations associated with the influence of the host immune system or with the reverse transcription-

mediated production of the variants that could be tolerated. On the other hand, id-RNAs may reflect the integrated viral sequences that did not undergo active mutagenesis in the context of the host chromosome, or may bear the changes that reflect (i) the results of activity of host DNA repair enzymes acting on foreign sequences or (ii) the results of homologous recombination of HBV DNA into already existent HBV insert in the host genome. Given that our previous study suggested that virus spread and superinfection could represent important determinants of the maintenance of chronic hepadnavirus infection [6], it therefore, became apparent that id-RNAs/id-HBsAg could regulate the maintenance of HBV persistence. Third, given that HBsAg can reduce the levels of accumulated covalently closed circular DNA [7], it is possible that id-HBsAg may downregulate the rate of intracellular HBV genome replication.

The above described potential regulatory roles of id-RNAs/id-HBsAg that are produced independently of HBV replication suggest a need to revise the current model of the HBV life cycle that does not account for the roles of integrated HBV DNA, id-RNAs and id-HBsAg. In addition, follow-up studies need to conduct thorough comparison of the properties of id-HBsAg versus rd-HBsAg accumulated in the same infected tissues in order to better understand the mechanistic aspects of the overall influence of id-HBsAg on the HBV life cycle.

Impact of id-RNAs on HBV-related pathogenesis & anti-HBV treatments

It also became apparent that current FDA-approved anti-HBV drugs (these include interferon and nucleos(t)ide analogs) fail to achieve loss of serum HBsAg in the vast majority of cases [8], because significant amounts of HBsAg could be produced from id-RNAs regardless of HBV genome replication. Production of id-RNAs/id-HBsAg is obviously not targeted by the nucleos(t)ide analogs. Interferon is not an HBV-specific drug and does not target id-RNAs/id-HBsAg directly; while it may facilitate the killing of HBV-infected cells, including the ones bearing integrated HBV DNA. HBsAg per se can induce serious liver pathogenesis including carcinogenesis [1,9]. Therefore, if abundant, id-HBsAg can have a considerable impact on HBsAg-associated liver injury, and this impact cannot be influenced by current anti-HBV drugs. The above considerations suggest that id-RNAs should be considered as important targets for novel anti-HBV interventions, which would be able to profoundly suppress alternative (to HBV genome replication) source of HBsAg, and thus will help to achieve the loss of serum HBsAg – an outcome of anti-HBV treatment that is highly desirable according to the current guidelines on the management of chronic HBV infection [8]. There is also a possibility of a connection to HBV-associated carcinogenesis. It was suggested previously that immune-driven liver repopulation via clonal outgrowth of hepatocytes that poorly replicate HBV (and thus are not targeted by the immune system) could be a significant factor facilitating the onset of HCC [10–12]. Therefore, if id-HBsAg efficiently suppresses intracellular HBV genome replication, this could considerably increase the fraction of the hepatocytes with reduced levels of HBV replication, which could then participate in clonal expansion.

Influence of id-RNAs on HDV life cycle

HDV remains a significant human pathogen that can induce additional (to concomitant HBV infection) liver damage; facilitate faster and more frequent cirrhosis and increase HCC risk [13–15]. Our new data [3] suggesting that a significant fraction of HBsAg could be generated by id-RNAs independently of HBV genome replication strongly support the mechanism mediating HDV persistence proposed by Dr Gudima's laboratory [16]. HDV takes only HBsAg from HBV, and its life cycle is otherwise independent from the HBV life cycle [14]. Dr Gudima's laboratory showed in cell culture that HBsAg derived from naturally integrated HBV DNA can support production of infectious HDV virions in the absence of ongoing HBV replication [16], which along with relative abundance of id-RNAs during chronic HBV infection [3] strongly indicates that HDV can persist *in vivo* independently of ongoing HBV replication – if functional HBsAg is available from a source that is independent of HBV replication (i.e., id-HBsAg that is produced from id-RNAs). The notion that id-RNAs/id-HBsAg can support HDV persistence and thus, can facilitate the pathogenesis associated with ongoing HDV infection further increases the significance of id-RNAs as targets for novel antiviral interventions and at least in part explains why current anti-HBV drugs do not block HDV infection [17] and therefore, supports the development of antivirals directly targeting HDV. Currently, there are no drugs in clinical practice that target HDV directly [13,17]. In addition, the above considerations also suggest that HDV is a more significant and independent pathogen than is currently assumed, because it might persist independently of ongoing HBV genome replication.

Monitoring of anti-HBV therapy with nucleos(t)ide analogs

There is one more potentially interesting aspect related to id-RNAs and rd-RNAs that is worth mentioning. Anti-HBV therapy with nucleos(t)ide analogs (NA therapy) can control levels of HBV viremia in many patients, but HBV often rebounds after the therapy cessation [8]. Given that serum HBV DNA does not adequately reflect the levels of HBV genome replication and cannot predict the time when NA therapy can be stopped without the risk of HBV rebound [8,18] and considering that the presence of HBV RNA in sera of infected individuals has been reported (while those reports are somewhat conflicting and have not thoroughly considered discrimination of rd-RNAs from id-RNAs [19–22]), it would be interesting to find out whether both rd-RNAs and id-RNAs are measurable in sera from chronic HBV carriers. Furthermore, it would be important to determine whether serum rd-RNAs could possibly be used a biomarker, which (i) is better than serum HBV DNA in terms of reflecting the efficiency and durability of NA therapy, and (ii) could be employed to predict the time of the safe stoppage of NA therapy. This being the case would address the major unresolved question in the HBV research field, since the current guidelines on management of chronic HBV infection consider identification of the markers to predict successful discontinuation of anti-HBV therapy with nucleos(t)ide analogs as a major unmet need [8]. In addition, we would expect that serum id-RNAs would not be affected by NA therapy, which would imply that serum HBsAg remaining in sera regardless of NA therapy is produced from intrahepatic id-RNAs and therefore, cannot be eliminated by current anti-HBV drugs.

Overall, the observed abundance of id-RNAs raises a number of important research questions in the HBV/HDV field, addressing of which will considerably advance the understanding of the mechanisms of HBV and HDV infections and related pathogenesis, and could stimulate development of novel antivirals aimed at new virus-specific targets.

Financial & competing interests disclosure

SO Gudima was supported by the NIH grants (grant numbers R21AI099696 and R01CA166213) and by the School of Medicine of the University of Kansas Bridging grant. SA Weinman was supported by the NIH grants (grant numbers R01AA012863, P20GM103549 and P30GM118247). We are very grateful to Jianming Hu for constructive comments. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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