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Hepatitis D virus infection revisited

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Perez-Vargas J, Amirache F, Boson B, et al. Enveloped viruses distinct from HBV induce dissemination of hepatitis D virus in vivo. *Nat Commun.* 2019;10:2098

Chronic hepatitis D is one of the most severe form of viral hepatitis ^{1,2}. It is caused by hepatitis D virus (HDV), a small virus initially characterized as a satellite of hepatitis B virus (HBV) ³. It is estimated that 10% of HBV infected patients are co-infected with HDV ^{4,5}. HDV co- and super-infections are correlated with more severe pathogenesis compared to HBV mono-infections, including a faster progression to advanced liver disease and cirrhosis and an increased risk of developing hepatocellular carcinoma (HCC) or experiencing liver failure.⁶ HDV is only poorly controlled by current interferon based therapies and nucleos(t)ide analog-based treatments are ineffective against this virus ^{1,2}. In fact, there are no treatments to date that allow complete HDV eradication. HDV is a small RNA virus, carrying a circular 1.7 kb single-stranded negative-sense RNA genome. Interestingly, HDV is the smallest known virus capable of infecting animals ¹, yet genetically, HDV is mostly related to plant viroids ⁷. HDV

does not encode any non-structural proteins and only possesses a single open reading frame encoding two structural forms of HDV antigen (HDAg), HDAg-S and HDAg-L. This unusual feature makes this virus fully dependent on host polymerases for RNA replication and transcription¹. Importantly, HDV ribonucleoproteins (RNPs), consisting of the HDV RNA genome associated with both structural forms of HDAg subvert HBV envelope proteins to assemble infectious particles¹. Therefore, HDV can only propagate in hepatocytes replicating HBV or producing HBV envelope proteins following virus integration. Consequently, HDV and HBV share the same receptor, Sodium taurocholate cotransporting polypeptide (NTCP), and entry pathways into hepatocytes⁸. While some key steps of HDV life cycle in the liver have been elucidated, fundamental aspects of HDV molecular strategies and virus-host interactions are still unclear. Notably, the origin of the virus is still open to question.

In an elegant study published in *Nature Communications*, Perez-Vargas and colleagues demonstrate for the first time that HDV is able to assemble infectious particles by subverting envelope proteins from viruses distinct from HBV. First, the authors report the accumulation of bona-fide HDV genomic RNA (gRNA) in the supernatants of hepatoma cells co-transfected with plasmids encoding the HDV genome and glycoproteins (GPs) from HBV, vesicular stomatitis virus (VSV), or hepatitis C virus (HCV). Immunoprecipitation assays confirmed that GPs of VSV and HCV were associated with HDV gRNA and HDV antigens (HDAg). Moreover, the examination by electron microscopy, of supernatants from cells producing HDV-RNPs together with HBV, VSV or HCV GPs, revealed large spheres of 35-40 nm diameters, corresponding to viral particles. Next, authors were able to show that these particles were infectious when applied to HDV replication-permissive cell lines where they observe a specific and classical HDV replication pattern, leading to the accumulation of both genomic and antigenomic RNAs. Additionally, these infections were inhibited by either ligands or neutralizing antibodies targeting the bona fide virus receptors, such as taurocholic acid (HBV), anti-LDLr- (VSV) and anti-CD81 (HCV) antibodies, confirming that envelope GPs from heterologous viruses are able to dictate GP-specific cell entry through their corresponding receptors. Authors then ask if HDV particles could be produced in hepatocytes with envelope

GPs from a broader set of enveloped viruses such as murine leukemia virus (MLV), human immunodeficiency virus (HIV), avian influenza virus (AIV), lymphocytic choriomeningitis virus (LCMV), human metapneumovirus (HMPV), dengue virus (DENV), and West Nile virus (WNV). Interestingly, only GPs from HMPV, DENV and WNV were able to package and secrete infectious HDV particles at levels similar to those of HBV GPs. Those infectious particles could also be efficiently produced in 293T human kidney cells suggesting that assembly and release of functional HDV with heterologous GP is not cell-type restricted. Importantly, the study then demonstrates for the first time that in the presence of non-HBV helper virus, like HCV or DENV, coinfecting host cells can produce fully infectious HDV particles. Moreover, authors show that when the DENV-permissive *Aedes albopictus* mosquito cells, are exposed to DENV/HDV particles, HDV RNA could replicate, assemble into RNPs, and produce infectious particles, showing for the first time that insect cells could also support HDV infection. Finally, to validate their observations *in vivo*, the authors eventually co-infected liver chimeric FRG-derived mice with HDV and HCV or HBV. Expectedly, HDV spread was observed in presence of HBV. HCV/HDV co-infection led to a dissemination of HDV, where the viral RNA could be detected several months after infection, confirming the ability of HDV to propagate *in vivo* through different virus types, including HCV.

Comment: These results have several implications regarding our understanding of HDV life cycle and tropism, its associated pathogenesis and origin. First, this study demonstrates for the first time that HDV can propagate using envelope glycoproteins from viruses distinct from HBV, which was considered to be the unique HDV helper virus so far. Even though the detailed mechanisms of HDV RNP interactions with GPs from alternative viruses is not clear yet, it is likely that farnesylation of long HDAg plays a key role in that process, as indicated by the inhibition of HCV- and VSV-coated HDV particle production in lonafarnib-treated cells. Farnesylation of cysteine 211 from the long HDAg is critical for HDV RNP interaction with cytosolic HBV GPs at the endoplasmic reticulum (ER) membrane⁹. This suggests that an assembly process involving interaction with GP and HDV RNP at the ER membrane seem to

be involved in virus production regardless of the source of viral envelopes. The use of alternative GPs for HDV production raises the question of the pathogenicity and tissue tropism of the virus. Initially considered to be a liver-specific HBV satellite virus, HDV co-infection is associated with a marked increase in liver damage and HCC risk¹. To date, the molecular mechanisms leading to the development of HDV-induced liver disease are not completely understood. However, a recent *in vivo* study suggested that HDV may persist in hepatocytes even in the absence of HBV¹⁰. The results of Perez-Vargas and colleagues suggest that HDV infection may occur in patients in the absence of HBV, with the help of an alternative virus. Particularly, chronic hepatitis D with sustained HDV replication may in theory be possible in patients carrying HCV or following HCV superinfection. So far, no study has reported the presence of HDV in chronic HCV patients, but this can be explained by the absence of a systematic screening for HDV, recommended by physicians, in HCV-infected patients in the absence of HBV. Moreover, the observation that DENV/HDV co-infections allows HDV spread suggests that DENV GP-coated HDV particles may be transmitted to cell types usually targeted by DENV, such as monocytes, macrophages, and dendritic cells¹¹, triggering HDV replication in extra-hepatic cells. More generally, these results virtually open the door for a wide range of potential HDV co-infections, which may be clinically relevant depending on the target organs or tissues of helper viruses. Eventually, this study may shed some light on the origin of this virus, which is still unknown. Structurally and genetically, HDV is related to plant viroids, sharing the same genome structure and rolling circle-based replication systems⁷. Given the specificity of its HBV-dependent infection cycle, HDV was initially proposed to be a human-specific virus originating (1) either from a plant viroid-derived structured RNA having acquired a human coding sequence during its evolution, based on the presence of a protein closely related to HDAg in the human genome (delta-interacting protein A or DIPA)¹²; (2) or from the human transcriptome, based on the identification of HDV-like ribozyme sequence and activity in the *CPEB3* gene¹³. However, the recent identification of HDV-like viruses infecting birds¹⁴ and snakes (sHDV)¹⁵ overthrow the theory of the human origin of the virus. Importantly, these newly discovered viruses do not seem to depend on hepadnaviruses for their replication

cycles, and sHDV was found in multiple animal tissues, such as neurons and epithelial cells¹⁵. Together these recent studies and the ability of HDV to replicate in mosquito cells presented by Perez-Vargas and colleagues reshuffle the cards concerning the origin and the tissue tropism of this virus. It supports a new hypothesis which posits that, in addition to an opportunistic interaction with HBV, HDV and Deltaviruses in general may take advantage of a large spectrum of helper viruses, putatively infecting a broad range of hosts and organs. This remarkable study paves the way to a better characterization and understanding of the infection cycle and pathogenicity of this unique virus.

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