

HBV Core-Directed Antivirals and Importin β Can Synergistically Disrupt Capsids

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260 million people suffer from chronic Hepatitis B Virus (HBV) infection, and current therapies are not curative [1]. HAPs are a class of antivirals that have been shown to reduce HBV replication by misdirecting virus assembly [2]. However, HAPs affect other aspects of the HBV lifecycle, but the mechanisms are poorly understood [2,3]. HBV core protein (Cp) is involved in almost every stage of the viral lifecycle, including nuclear trafficking [4]. During nuclear transport, HBV capsids bind to host importin α and β [5]. The C-terminal domain (CTD) of the Cp, which carries nuclear localization signals and an importin β -binding sequence, must externalize to the capsid exterior so that capsids can bind to importins [4,6]. After binding, the viral cargo is shuttled to the nucleus. Our previous work showed that importin β can directly bind to capsids and Cp *in vitro* [6]. In a parallel story, the small molecule HAP12 has been shown to stimulate Cp assembly, produce aberrant structures, and bind to HBV capsids [7]. Here we investigated how HAP12-binding influenced the interaction between HBV capsids and importin β *in vitro*. Proteolysis of HAP12-bound empty, pre-genomic RNA, and E. coli RNA-filled capsids showed increased CTD externalization rates. E. coli RNA-filled capsids were more resistant to proteolysis and required higher [HAP12] to increase sensitivity to proteolysis than compared to empty capsids. Transmission electron microscopy (TEM) showed that HAP12-bound empty capsids adopted faceted, elongated, and broken structures. Charge detection mass spectrometry (CDMS) revealed that HAP12 increased the amount of importin β bound to empty capsids, consistent with increased CTD exposure. Our study also indicated that HAP12 treatment of empty capsids led to the formation of broken capsids and heterogenous importin β -Cp complexes, both small and large. However, E. coli RNA-filled capsids exhibited neither increased importin β -binding nor capsid disruption with addition of HAP12 and importin β . For empty capsids, the formation of disrupted capsids indicates that HAP12 and importin β act synergistically to destabilize capsids. Our work showed that core protein-targeted antivirals can be used to perturb viral-host protein interactions. Catalyzing capsid disruption is an unexpected additional mechanism of action for antiviral molecules like HAPs. Untimely capsid disassembly not only can hamper important HBV lifecycle steps, but it can also cause the virus to become vulnerable to host innate immune responses.

References

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