CLASS-A CAMs INDUCE CELL DEATH THROUGH HBV CORE PROTEIN AGGREGATION AND POTENTIALLY ACTIVATE THE INNATE IMMUNE RESPONSE

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ABSTRACT

Background & Aims

Despite a preventive vaccine, almost 300 million people suffer from a chronic hepatitis B virus (HBV) infection. Therapies controlling HBV replication exist but do not lead to functional cure of chronic hepatitis B. HBV core protein (HBc) is the building block of the HBV nucleocapsid and it modulates almost every step of the HBV life cycle. Class A capsid assembly modulators (CAM-As) represent attractive direct antiviral agents (DAAs). These compounds impair HBV replication by blocking pgRNA encapsidation and inducing HBc aggregation due to aberrant nucleocapsid structures. We previously showed that CAM-A RG7907 treatment leads to an unexpected sustained HBsAg reduction and loss of infected hepatocytes via apoptosis in an AAV-HBV mouse model. In this study, we present further insights into the mechanism of action of the CAM-A compounds.

Methods

We investigated the impact of CAM-A treatment on HBc aggregation, cell survival, and transcriptomic reprogramming in HBc-expressing hepatoma cell lines (HepG2) and primary human hepatocytes (PHH) as well as in the HBV-replicating cell line HepAD38.

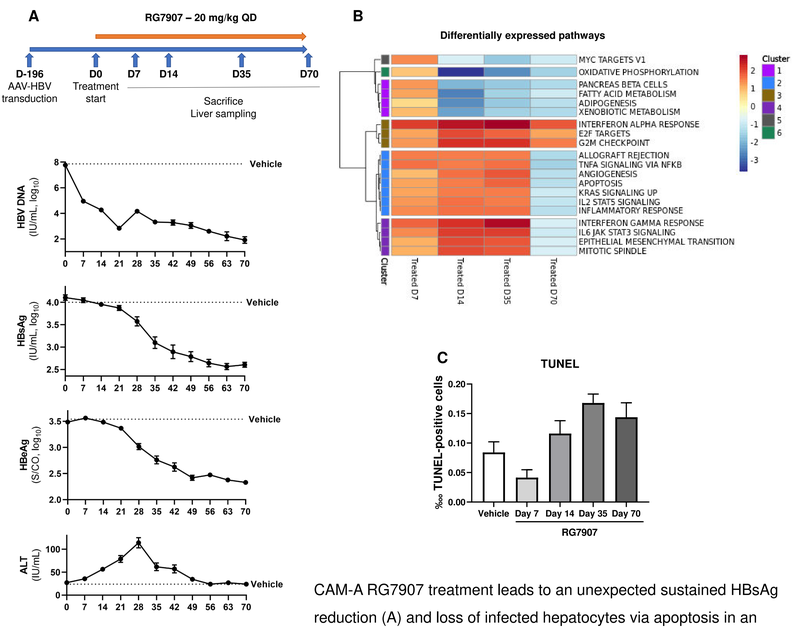
Results

CAM-A compounds induced extensive HBc-aggregation-dependent cell death both in hepatoma cells and in primary hepatocytes. Transcriptomic analyses revealed the activation of specific host pathways such as apoptosis, inflammation, and the interferon response. The induction of apoptosis-related gene expression was validated in HBc expressing HepG2 and PHH as well as in HepAD38. We also observed activation of an interferon response in HepAD38 suggesting the potential activation of the innate immunity upon CAM-A treatment.

Conclusions

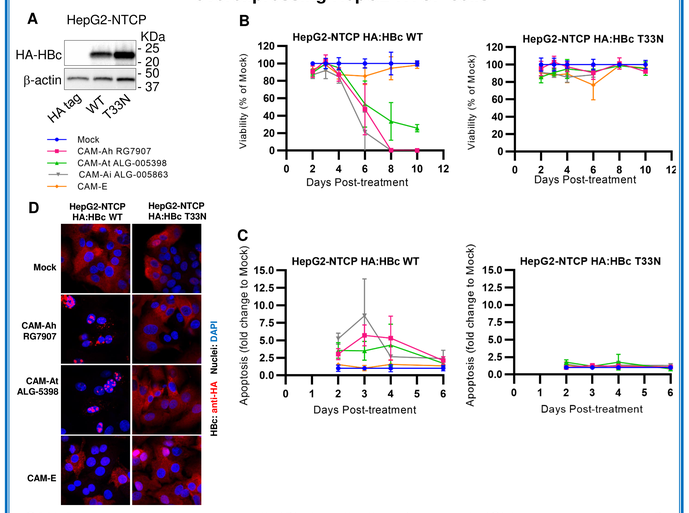
CAM-A-dependent HBc aggregation drives cell death via activation of host specific pathways such as apoptosis and the inflammatory and innate immunity responses. These results shed light on a previously unknown mechanism of action specific to CAM-A compounds.

Background: CAM-A RG7907 treatment of AAV-HBV mice



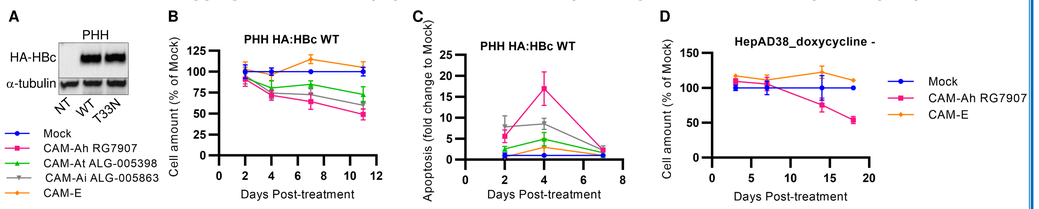
CAM-A RG7907 treatment leads to an unexpected sustained HBsAg reduction (A) and loss of infected hepatocytes via apoptosis in an AAV-HBV mouse model (B-C) [1-2].

Result 1: CAM-A-mediated HBc aggregation induces apoptosis in HBc overexpressing HepG2-NTCP cells



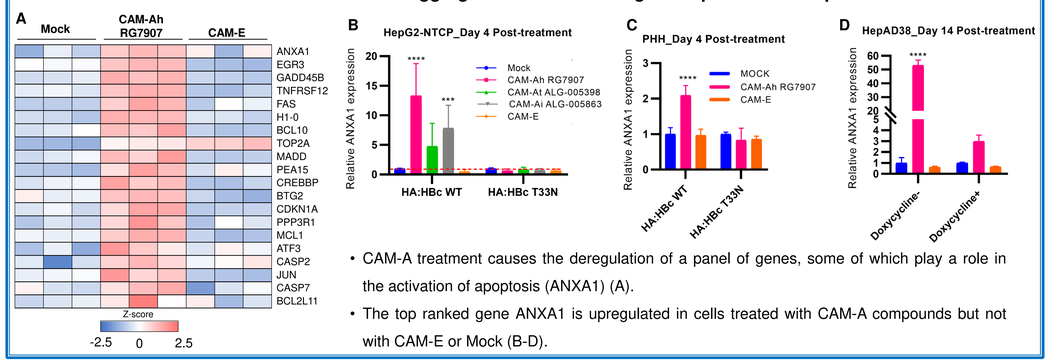
- CAM-A compounds cause cell death (B) via activation of apoptosis (C) in cells expressing HBc WT but not the T33N HBc mutant (A).
- CAM-A compounds induce the nuclear accumulation of HBc aggregates only in cells expressing HBc WT (D).

Result 2: HBc aggregation induces apoptosis in HBc-overexpressing PHH and in HBV-replicating HepAD38



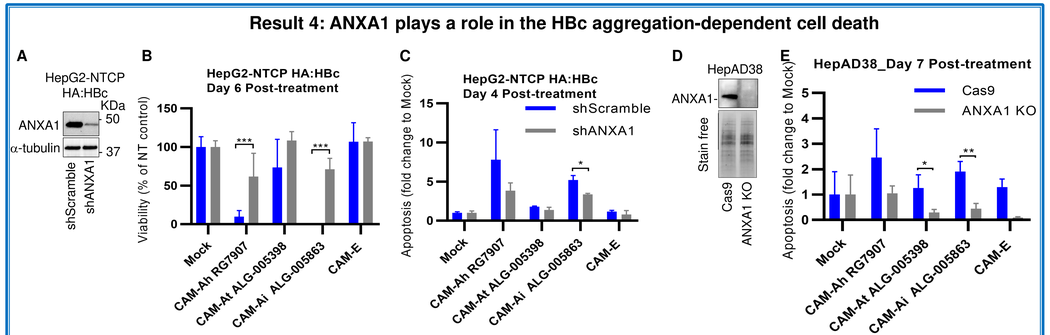
CAM-A compounds cause cell death in both PHH expressing HBc WT (A-C) and HBV-replicating HepAD38 cells (D).

Result 3: CAM-A-mediated HBc aggregation alters normal gene expression in HepG2-NTCP



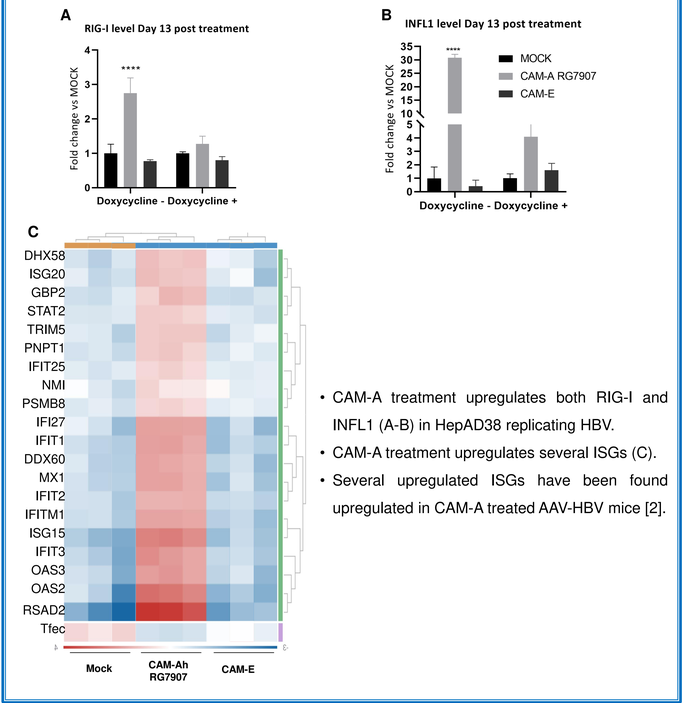
- CAM-A treatment causes the deregulation of a panel of genes, some of which play a role in the activation of apoptosis (ANXA1) (A).
- The top ranked gene ANXA1 is upregulated in cells treated with CAM-A compounds but not with CAM-E or Mock (B-D).

Result 4: ANXA1 plays a role in the HBc aggregation-dependent cell death



The loss of ANXA1 protein decreases apoptosis activation induced by HBc aggregation.

Result 5: CAM-A-mediated HBc aggregation activate an innate immune response

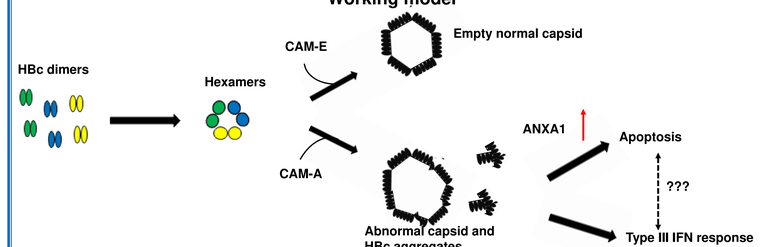


- CAM-A treatment upregulates both RIG-I and INFL1 (A-B) in HepAD38 replicating HBV.
- CAM-A treatment upregulates several ISGs (C).
- Several upregulated ISGs have been found upregulated in CAM-A treated AAV-HBV mice [2].

Conclusions

- CAM-A dependent HBc aggregation causes cell death via activation of apoptosis in HepG2-NTCP and PHH overexpressing HBc as well as in HepAD38 replicating HBV.
- ANXA1 is upregulated after CAM-A induced HBc aggregation and plays a role in the activation of apoptosis.
- CAM-A treatment of HepAD38 replicating HBV results in the activation of a type III interferon response.

Working model



References

1. Vanrusselt, H. et al. (2023) Journal of Virology. Accepted.
2. Kum, D.B, et al. (2023) Hepatology. 10.1097/HEP.0000000000000428.

Disclosures

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- Y.D. and H.V.are employees of Aligos Belgium BV..
- D.B.K. is employee of Aligos Therapeutics Inc