Identification of the Hsp40 chaperone DNAJB12 in the functional mechanism of the antiviral nucleic acid polymer REP 2139 and establishment of its mechanism-of-action in the secretion of HBsAg.

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ABSTRACT

HBV subviral particles (SVP) constitute >99.99 of circulating HBsAg. The molecular mechanisms of SVP morphogenesis/secretion are largely unknown. Nucleic acid polymers (NAPs) interfere with the assembly/secretion of SVP without affecting the release of HBeAg or Dane particles (1,2) and in combination with peg-interferon and TDF, achieve high rates of HBsAg loss, and functional cure (5).

Pull-down with NAP biotinylated REP 2139 followed with mass-spectrometry identified the Hsp40 chaperone DNAJB12 as a novel protein involved in SVP morphogenesis (3). DNAJB12 is located at the endoplasmic reticulum membrane where it determines the fate of nascent transmembrane proteins, leading to the degradation of misfolded proteins. DNAJB14 is a Hsp40 family member structurally similar to DNAJB12 which may form heterodimers with DNAJB12 and share some of its client proteins, which wasn't found in the pull-down, was also investigated regarding the HBsAg secretion.

We hypothesize that the interaction of REP 2139 with DNAJB12 prevents correct folding of nascent HBsAg, leading to its proteasomal/lysosomal degradation. Investigations of the molecular mechanism of NAPs and DNAJB12 involvement in SVP assembly and secretion are presented.

Our results suggest that the REP 2139 targets DNAJB12 and prevents the recruitment of Hsc70/Hsp70 to properly fold HBsAg, leading to its proteasomal degradation. Those results are consistant with the absence of intracellular HBsAg accumulation *in vitro* in the presence of REP 2139 (2) and the clearance of HBsAg from the liver (5, 9).

METHODS

Co-immunoprecipation / Pull-down assays of REP 2139

We have conducted a MS/MS analysis data identified DNAJB12 as the potential target for the REP 2139 (3). In order to validate this interaction, co-immunoprecipation and pull-down assays were performed on biotinylated REP 2139 (REP 2139-B). HepG2.2.15 cells were lysed with NP40 lysis buffer and incubated overnight with 50 nM of either REP 2139 or REP 2139-B. Magnetic protein G beads were coupled overnight with anti-biotin antibody. After several washes, HepG2.2.15 lysate incubated with NAPs were incubated with either the protein G bead coupled with anti-biotin (co-immunoprecipation) or with streptavidin beads (pull-down). The supernatants (S) of the beads were kept (figure 2) and both the supernatant and eluates (IP) were analyzed by western blot.

DNAJB12 and DNAJB14 knock-downs (KD)

Plasmid pLKO.1 containing shRNA for DNAJB12 and DNAJB14 were purchased from Sigma (MISSION® shRNA). Lentiviruses were produced in HEK-293T by transfection of the pLKO.1 donor plasmid and the envelope plasmid pMD2.G and the packaging plasmids pRSV-REV and psPAX2. Supernatant were collected after 48h.

HepG2.2.15 cells were innoculated with various shRNA lentiviruses and supernatants were collected 6 days post-transduction. Efficacy of shRNA mediated mRNA knock-down was verified for various lentivirus quantity by western blot for DNAJB12 (Figure 1 and 4) and for DNAJB14 (Figure 4). Effects on the KD on HBsAg secretion were measured by ELISA (GS EIA 3.0, Biorad) and expressed as relative units (RU) normalized to total cellular protein (as determined by BCA assay).

RESULTS

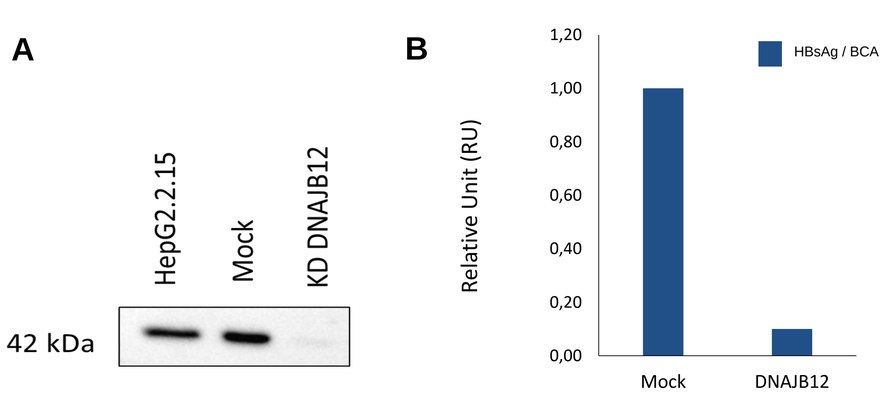

Figure 1. DNAJB12 Knock-down and effects on HBsAg secretion.
(A) Western-blot for DNAJB12. DNAJB12 level was determined in HepG2.2.15 cells, in shRNA negative cell line (mock) or in knockdown DNAJB12 cell line (KD DNAJB12) at day 6.
(B) Effects on inhibition of HBsAg secretion 6 days post-transfection of DNAJB12 shRNA lentivirus.

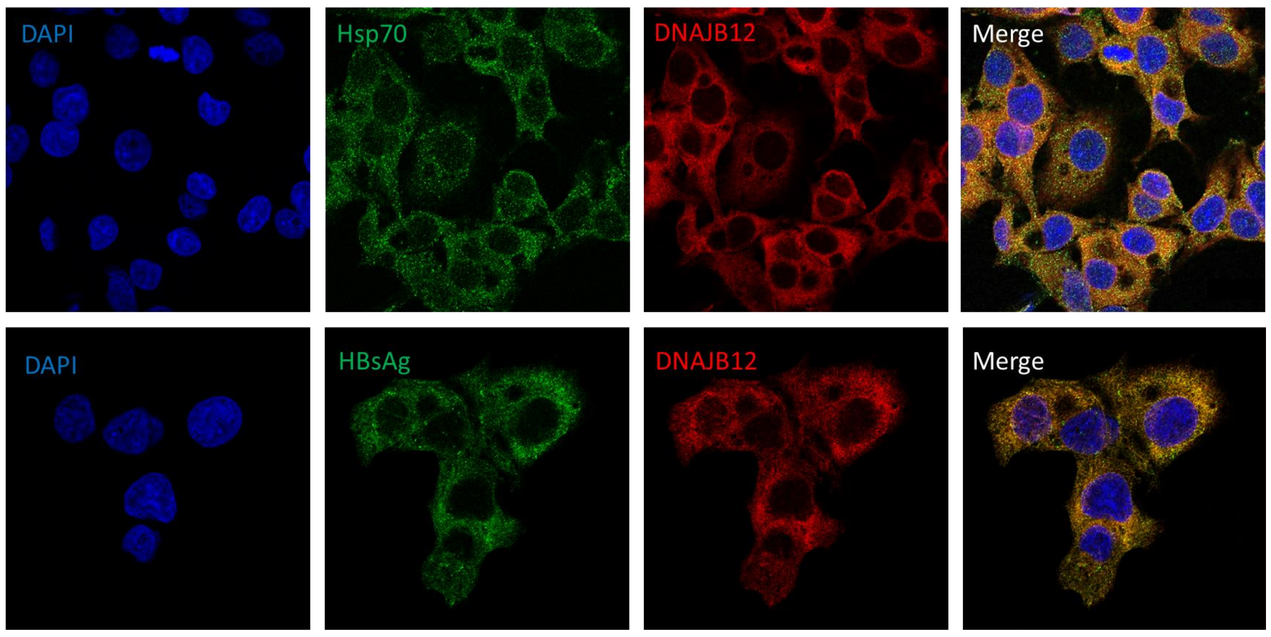

Figure 3. DNAJB12 colocalization with HBsAg and its co-chaperone Hsp70.
Immunofluorescence of DNAJB12 (red) and HBsAg or Hsp70 (green) in HepG2.2.15 cells shows a strong colocalization of these proteins.

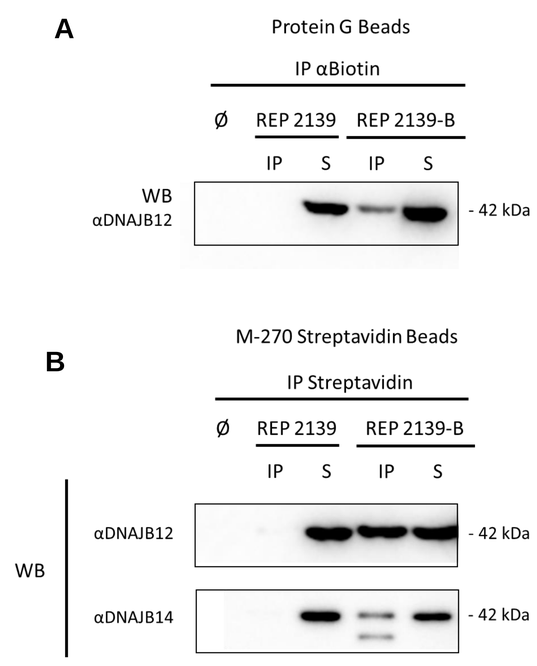

Figure 2. Interaction of REP 2139 with DNAJB12
(A) HepG2.2.15 cells were lysed and incubated with Protein G beads coupled with anti-biotin antibody and REP 2139 +/- biotin.
(B) HepG2.2.15 cells were lysed and incubated with M-270 streptavidin beads and REP 2139 +/- biotin. For both experiment, DNAJB12 was detected in the eluate (IP) and in the remaining supernatant (S). DNAJB14 was also pull-downed with the DNAJB12 - REP 2139-B complex.

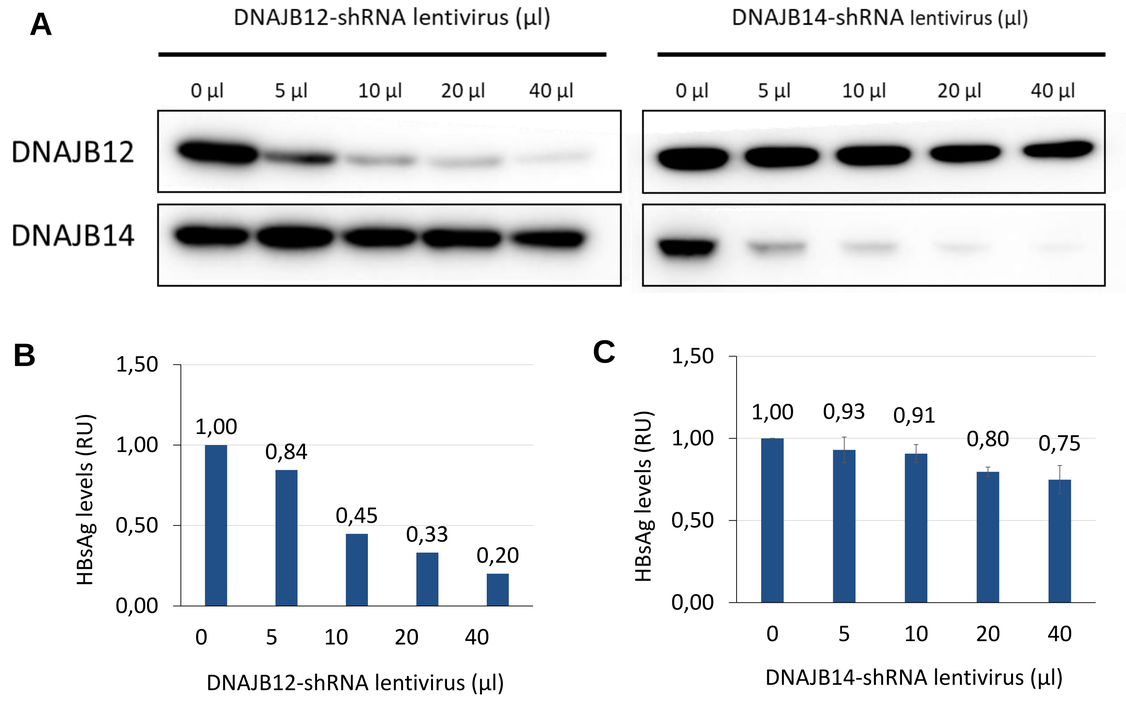

Figure 4. Effect of DNAJB12/B14 knock-down on HBsAg secretion.
(A) Knock-down dose-response effect of the DNAJB12 and DNAJB14 shRNA lentiviruses. We also confirmed there was no cross-effect between both shRNA due to the high similarity of DNAJB14 with DNAJB12.
HepG2.2.15 cells were KD for DNAJB12 (A) or DNAJB14 (B) with various quantities of shRNA lentiviruses. Extracellular levels of HBsAg were measured by ELISA and normalized on BCA.

CONCLUSIONS

- **DNAJB12, a Hsp40 chaperone, is crucial for the HBsAg secretion and is targeted by REP 2139.**
- **Colocalization of DNAJB12 with Hsp70 and HBsAg is consistent with its co-chaperone function in the folding of HBsAg.**
- **DNAJB14 can be precipitated by REP 2139 but does not play a major function in the secretion of HBsAg.**

DISCUSSIONS / PERSPECTIVES

- **We hypothesized that REP 2139 interacts with DNAJB12 via its J-domain based on the presence of exposed α-helix in the J-domain and the amphipaticity of REP 2139. Further experiments need to be done to confirm this interaction.**
- **Hsc70/Hsp70 interaction with DNAJB12 in the presence or absence of REP 2139 will be assessed by PLA and pull-down assays.**

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