Defining the mechanism-of-action of the Hsp40 chaperones DNAJB12 and DNAJB14 in the assembly and secretion of Hepatitis B subviral particles.

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Abstract# 1225

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ABSTRACT

The hepatitis B surface antigen (HBsAg) is implicated in maintain the chronicity of HBV infection by interfering with immune function. In the blood, > 99.99% of HBsAg is in the form of non-infectious subviral particles (SVP). The molecular mechanisms underlying the morphogenesis of SVP are largely unknown. Recently, we identified Hsp40 chaperone DNAJB12 as a novel protein involved in SVP morphogenesis by interaction analysis with the nucleic acid polymer (NAP) REP 2139. DNAJB14 is a chaperone with a highly similar structure to DNAJB12, which shares some of DNAJB12's client proteins and may form heterodimers with DNAJB12. DNAJB14 may be a required co-chaperone to assist DNAJB12 in folding transmembrane protein. Using DNAJB12/DNAJB14 as starting points, the molecular machinery of HBV SVP morphogenesis is being dissected.

Immunofluorescence experiments demonstrated a strong colocalization of DNAJB12, Hsp70 and HBsAg. Using DNAJB14 and/or DNAJB12 silencing experiments, we observed that DNAJB14 is not involved in the secretion of HBsAg, in comparison to the strong implication of DNAJB12 which reaches up to 90% secretion inhibition.

DNAJB12 appears to exist in a complex with HBsAg and Hsp70, consistent with its role in the assembly of HBV SVP. DNAJB12 does not appear to require DNAJB14 as a co-chaperone in its role on SVP morphogenesis. Our results suggest that the knock-down of DNAJB12 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*.

METHODS

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 3) and for DNAJB14 (Figure 3). 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).

Confocal microscopie

Cells were fixed with 4% PFA and permeabilized with 0.2% triton. Antibodies used were an anti-HBsAg (ab9193 from Abcam), anti-DNAJB12 (HPA010642 from Sigma), anti-DNAJB14 (NBP1-82240 from Novus Bioscience) and anti-Hsp70 (ab2787 from Abcam).
Images were taken with a Zeiss confocal microscope.

RESULTS

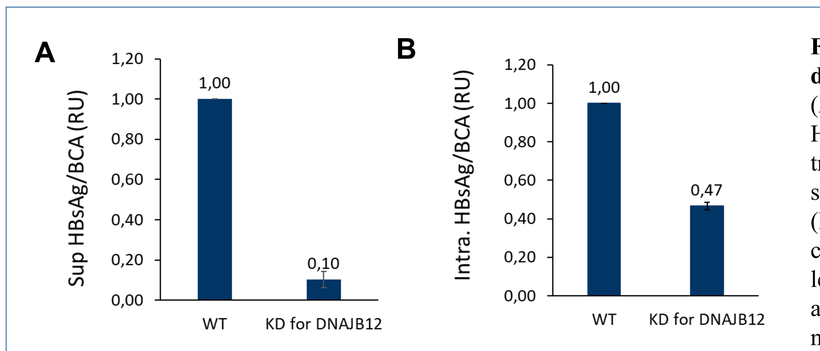

Figure 1. DNAJB12 Knock-down and effects on HBsAg.
(A) Effects on inhibition of HBsAg secretion 6 days post-transfection of DNAJB12 shRNA lentivirus.
(B) WT and DNAJB12 KD cells were lysed and HBsAg levels in the cytoplasm was assessed by ELISA and normalized on BCA.

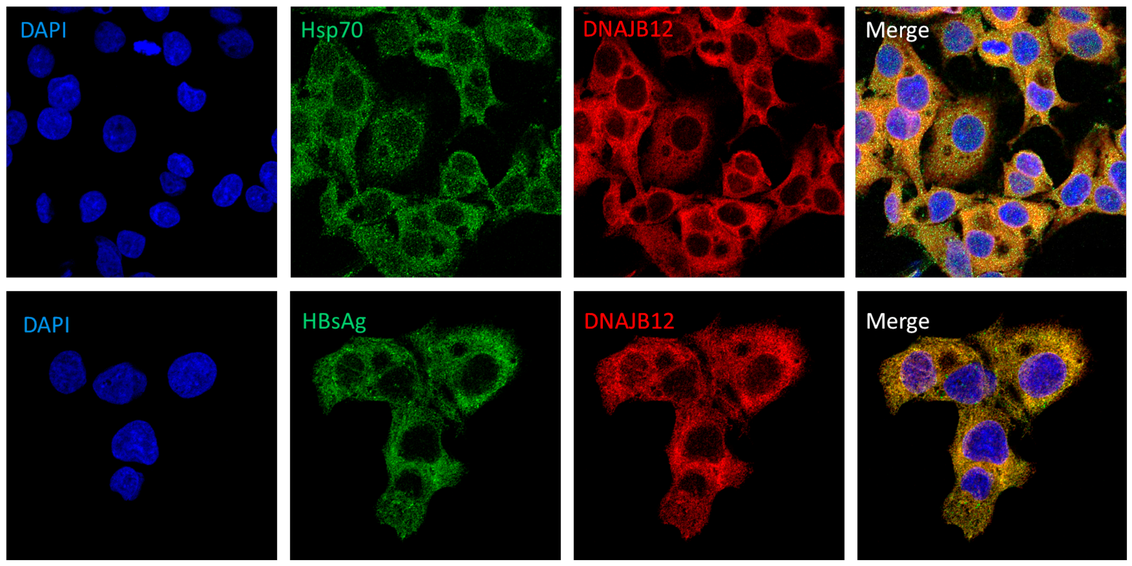

Figure 2. DNAJB12 colocalization with HBsAg and its co-chaperone Hsp70.
Immunofluorescence of DNAJB12 (red) and HBsAg or Hsp70 (green) in HepG2.2.15 cells shows a strong colocalization of these proteins.

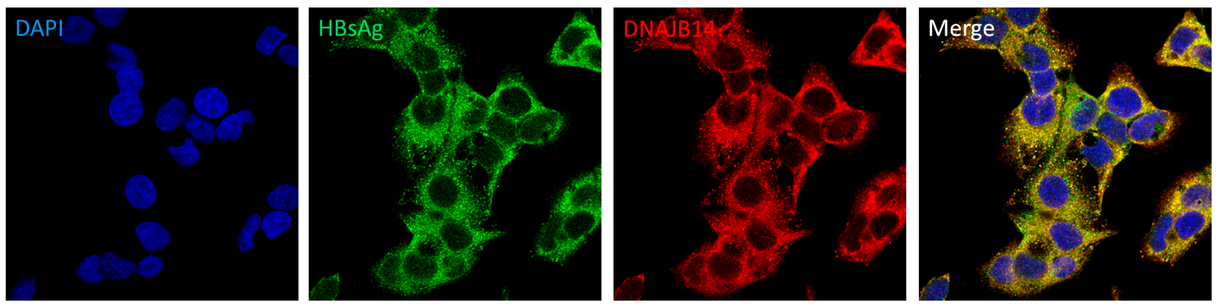

Figure 3. DNAJB14 colocalization with HBsAg.
Immunofluorescence of DNAJB14 (red) and HBsAg (green) in HepG2.2.15 cells shows a strong colocalization of these proteins.

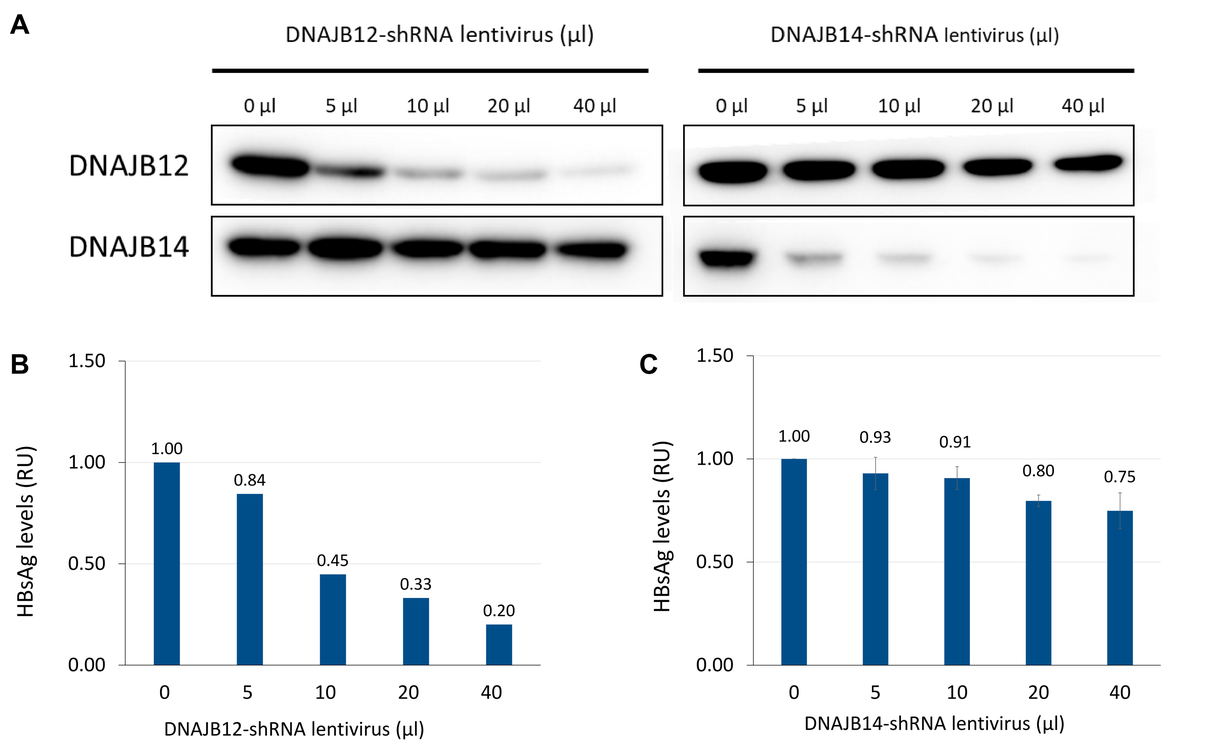

Figure 4. Effect of DNAJB12/B14 knock-down on HBsAg secretion.
(A) Knock-down dose-response effect of the DNAJB12 and DNAJB14 shRNA lentiviruses. We also confirmed there was no cross-effect between both shRNA due to the high similarity of DNAJB14 with DNAJB12.
HepG2.2.15 cells were KD for DNAJB12 (A) 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.**
- **Colocalization of DNAJB12 with Hsp70 and HBsAg is consistent with its co-chaperone function in the folding of HBsAg.**
- **DNAJB14 does not play a major function in the secretion of HBsAg.**
- **DNAJB12 knock-down does not cause any HBsAg accumulation, suggesting that HBsAg is addressed to proteasome degradation.**

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