Ph-dependent interaction of NAPs with the HSP40 chaperone DNAJB12

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INTRODUCTION

Nucleic acid polymers (NAPs) inhibit the assembly and secretion of HBV spherical subviral particles (SVP)[1,2]. Several studies have suggested that this activity occurs inside acidified intracellular compartments[1,3,4]. NAPs have no effect on cccDNA transcription, HBV RNA translation, or the production and secretion of HBeAg or Dane particles[2] (Figure 1).

Given the highly potent clinical effects of NAP-based combination therapy in achieving HBsAg loss, immune reconstitution in the liver and periphery, silencing of cccDNA and high rates of functional cure of HBV[5-10], the host target(s) of NAPs has been a topic of great interest.

Recent experimental evidence[11] identified the HSP40 chaperone DNAJB12 (previously reported to be involved in protein turnover within the ER[12]) as a novel chaperone involved in the assembly of SVP which is targeted by NAPs. Analysis of NAP interactions with putative targets at acidic pH (simulating the luminal pH of the ERGIC) was carried out to establish the physiological relevance of NAP-target interactions.

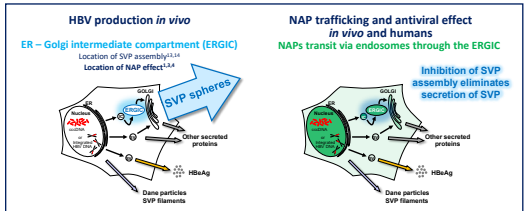


Figure 1. Antiviral effects of NAPs in HBV infection.

METHODS

A MS/MS interactome analysis in HepG2.2.15 lysates was conducted in triplicate at pH 7.4 and 6.5. Hydrophobic (antiviral) protein interactions with REP 2139 were validated with REP 2179 (size control)[1], REP 2147 (polyanionic control)[1] and REP 2031 (inactive at acidic pH)[1,11,12] (see Figure 2). Proteins with DNA / RNA binding activity were excluded. Secretion of HBsAg (GS EIA 3.0, Biorad) and HBeAg (ETI-EBK PLUS N0140, Diasorin) was monitored by ELISA and normalized to total cellular protein (as determined by BCA assay).

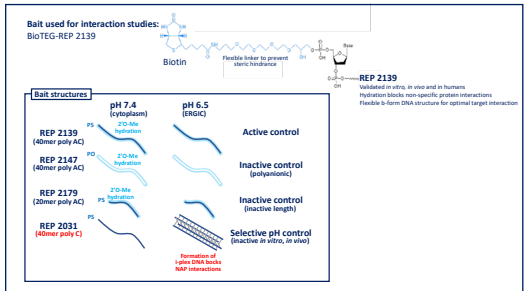
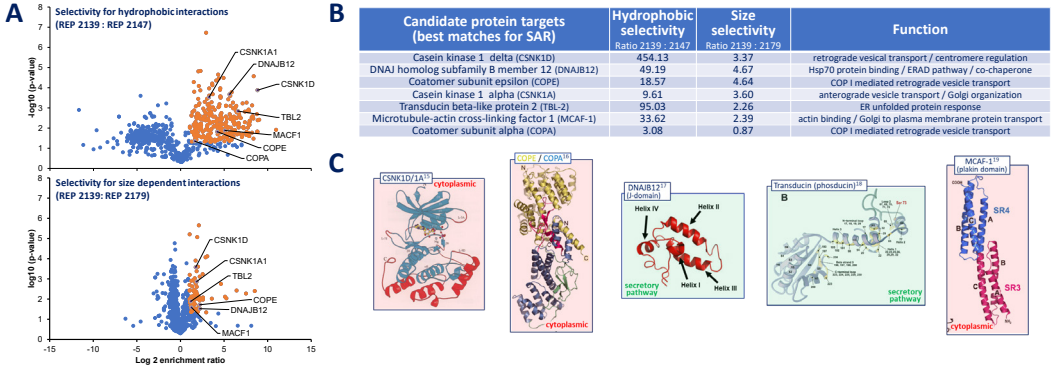


Figure 2. NAP bait design for MS/MS interactome analysis

RESULTS



Candidate protein targets (best matches for SAR)	Hydrophobic selectivity Ratio 2139 : 2147	Size selectivity Ratio 2139 : 2179	Function
Casein kinase 1 delta (CSNK1D)	454.13	3.37	retrograde vesical transport / centromere regulation
DNAJ homolog subfamily B member 12 (DNAJB12)	49.19	4.67	Hsp70 protein binding / ERAD pathway / co-chaperone
Coatomer subunit epsilon (COPE)	18.57	4.64	COP I mediated retrograde vesicle transport
Casein kinase 1 alpha (CSNK1A)	9.61	3.60	anterograde vesicle transport / Golgi organization
Transducin beta-like protein 2 (TBL-2)	95.03	2.26	ER unfolded protein response
Microtubule-actin cross-linking factor 1 (MCAF-1)	33.62	2.39	actin binding / Golgi to plasma membrane protein transport
Coatomer subunit alpha (COPA)	3.08	0.87	COP I mediated retrograde vesicle transport

Figure 3. MS/MS identification of NAP interactors

A) Volcano plots derived from MS/MS interaction analysis for hydrophobic selective (top) and size selective (bottom) interaction of proteins at pH 7.4 from HepG2.2.15 cells with NAPs. No interactions with HBV proteins were observed. Candidates with the greatest hydrophobic and size selective interactions are indicated. Intracellular function of candidate targets (B) and crystal structures (C) are indicated. Targets with subcellular localization consistent with NAP antiviral effect are indicated in green. All candidate proteins contained domains of amphipathic alpha helices with potentially exposed hydrophobic surfaces consistent with the documented target interface for NAPs in diverse infectious systems[20,21].

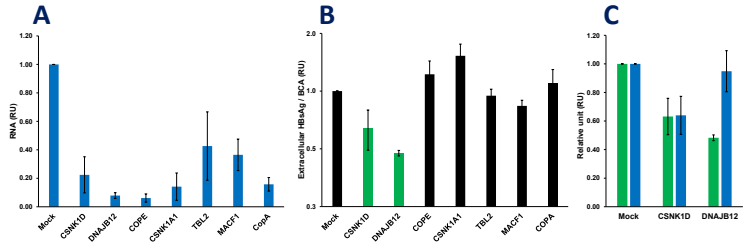


Figure 4. Validation of NAP targets

ShRNA knockdown of mRNA for candidate proteins was verified by RT-qPCR (A). Effects on inhibition of HBsAg secretion were evaluated in (B). Selective effects on HBsAg secretion for CSNK1D and DNAJB12 were validated in (C). Error bars are standard deviation from thee independent experiments.

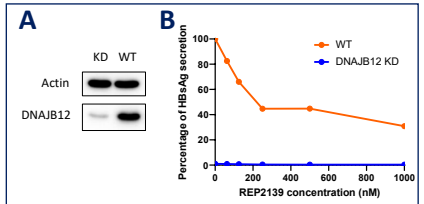


Figure 5. Relative effect of REP 2139 in WT and DNAJB12 knockdown HepG2.2.15 cells

A) Reduction in expression of DNAJB12 by shRNA in HepG2.2.15 cells as determined by western blotting. B) Effect of REP 2139 on secreted HBsAg in WT and DNAJB12 shRNA knockdown (DNAJB12 KD) cells demonstrated that most HBsAg secretion was blocked by DNAJB12 KD with REP 2139 having negligible additional effect.

Table 1. Effect of pH on NAP interactions

Target	Hydrophobic selectivity (REP 2139 : REP 2147)		Size selectivity (REP 2139 : REP 2179)		Acidic interaction selectivity (REP 2139: REP 2031)		
	pH 7.4	pH 6.5	pH 7.4	pH 6.5	pH 7.4	pH 6.5	
DNAJB12	27.21*	1071.48*	3.10*	1.23	5.29*	10.83*	Secretory pathway
TBL2	19.38*	2.45*	2.06*	1.31	0.22*	0.58*	
CSNK1D	1416.00*	26.82	3.23*	1.21*	0.80*	2.38*	
COPE	23.83*	1.18*	5.76*	1.44	0.19*	13.47	
CNSK1A	12.75*	2.70*	3.83*	1.25	0.64*	0.50*	
MCAF1	149.59*	0.99	0.96	1.17	0.84	0.88	Cytoplasm
COPA	0.37	120.12	0.98	3.14	0.13	144.50	

A second MS/MS interactome analysis was conducted at pH 7.4 and 6.5 and also included the pH selective NAP REP 2031. Enrichment ratios for identified targets are presented. * = $p \leq 0.05$. Expected parameters for antiviral targets are 1. location within the secretory pathway, 2. enhanced hydrophobic (antiviral) interaction at acidic pH and increased interaction of REP 2139 vs REP 2031 at acidic pH. Antiviral NAP interaction with DNAJB12 was enhanced 40-fold and with CNSK1D was decreased 53-fold at acidic pH. Moreover, REP 2139: REP 2031 enrichment ratio with DNAJB12 was increased at acidic pH.

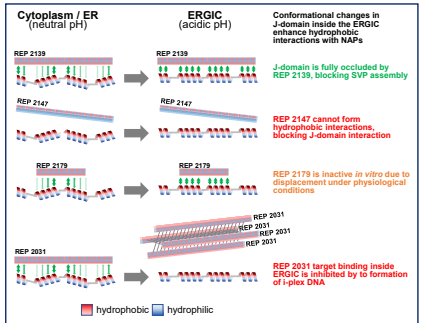


Figure 6. Biochemical basis for NAP interaction with the J-domain of DNAJB12

Molecular interactions of NAPs with exposed amphipathic alpha helices in the J-domain in DNAJB12 require the presence of phosphorothioation (REP 2139 vs REP 2147) and only functional efficiently to block SVP assembly when the entire J-domain is occluded by REP 2139 (vs REP 2179). The formation of i-plex DNA by REP 2031 inside the ERGIC prevents antiviral effect of NAPs.

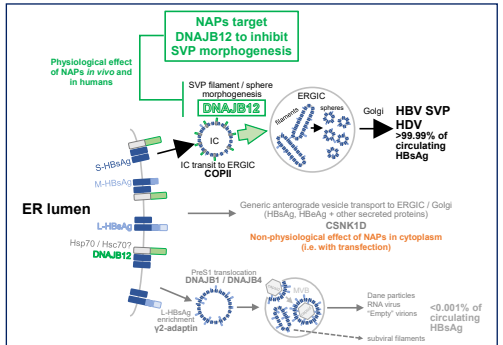


Figure 7. Proposed model for the molecular basis for the inhibition of SVP assembly by NAPs.

CONCLUSIONS

1. **DNAJB12 is a HSP40 chaperone required for the assembly of spherical subviral particles and is targeted by NAPs.**
2. **The NAP-DNAJB12 antiviral interaction with REP 2139 is enhanced at acidic pH but reduced at acidic pH with REP 2031, suggesting that NAP antiviral effects may occur within the acidified ERGIC.**
3. **NAP interactions with cytoplasmic proteins (i.e. CNSK1D) appear non-physiologic. The associated inhibition of HBsAg and HBeAg secretion following CNSK1D knockdown is likely driven by broad inhibition of anterograde transport of secretory vesicles not effected by NAPs under physiological conditions.**

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