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The Hsp40 chaperone DnaJB12 is involved in the morphogenesis of HBV spherical subviral particles and is selectively targeted by nucleic acid polymers

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INTRODUCTION

Nucleic acid polymers (NAPs) inhibit the assembly / secretion of HBV spherical subviral particles (SVP) without affecting the secretion of HBeAg or Dane particles. Given that > 99.99% of circulating HBsAg is derived from spherical SVP, the importance of HBsAg loss and the high rates of HBsAg loss and functional cure uniquely accompanying NAP-based therapy, the host target(s) of NAPs has been a topic of great interest. Recent validation of NAP effects in HepG2.2.15 cells[1, 2] identifies a suitable model for target identification.

MATERIALS & METHODS

A differential-interactome screen of HepG2.2.15 lysate used biotinylated NAPs which bracket the size and phosphorothioation (PS) dependent structure activity relationship of NAPs[1]. These NAPs included the clinically active 40mer PS REP 2139 and its inactive analogs: the 40mer phosphodiester REP 2147 and the short PS (20mer) REP 2179 (Figure 1).

MS/MS analysis (three experiments per NAP) identified NAP-bound proteins at pH 7.4. DNA / RNA binding proteins or proteins with interaction selectivity ratio < 2 were excluded. Selected candidates had the greatest significant ($p < 0.05$) selective interaction ratio between REP 2139 / REP 2147 (PS-dependent) and REP 2139 / REP 2179 (size-dependent). An outlier interacting protein (not size selective, see Figure 2) involved in retrograde transport (COPA) was used as a negative control.

Candidates were validated by shRNA-mediated knockdown effects on HBsAg and HBeAg secretion. Efficacy of shRNA mediated mRNA knockdown was verified by RT-qPCR. Extracellular HBsAg was detected by ELISA (GS EIA 3.0, Biorad), extracellular HBeAg was detected by ELISA (ETI-EBK PLUS N0140, Diasorin) and expressed as relative units (RU) normalized to total cellular protein (as determined by BCA assay).

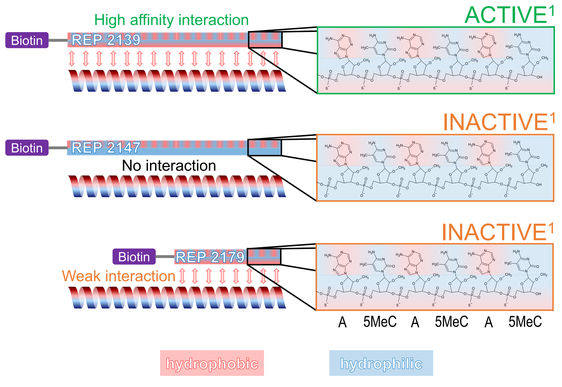

Figure 1. Antiviral and physiochemical properties of NAP ligands. Chemical structure of biotinylated NAPs bracketing their structure-activity relationship used in interactome studies. Charge distribution (hydrophobic and hydrophilic) driving interaction with target amphipathic alpha helices are identified.

REFERENCES

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9. Noordeen et al., AAC 2013; 57: 5291-5299
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RESULTS

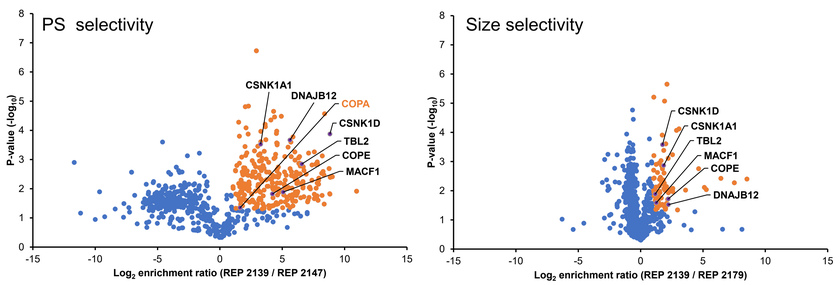

Figure 2. MS/MS identification of NAP ligands
Volcano plots for PS selective (left) and size selective (right) interaction of proteins from HepG2.2.15 cells with NAPs. No interactions with HBV proteins were observed. Candidates with the greatest PS and size selective interactions are indicated. The non-size selective control target (COPA) is identified in orange.

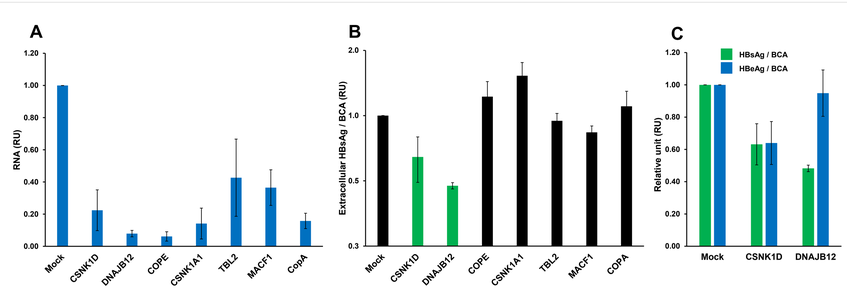

Figure 3. Validation of NAP targets
ShRNA knockdown of mRNA for candidate proteins was verified by RT-qPCR (A). Effects on inhibition of HBsAg secretion were evaluated in (B). Selective effects on HBsAg secretion for CSNK1D and DNAJB12 were validated in (C). Error bars are standard deviation from thee independent experiments.

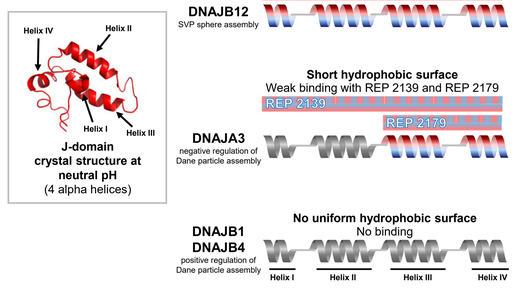

Figure 4. Model for how chemical features of DNAJ domains define the selectivity of NAP interactions and antiviral effect.

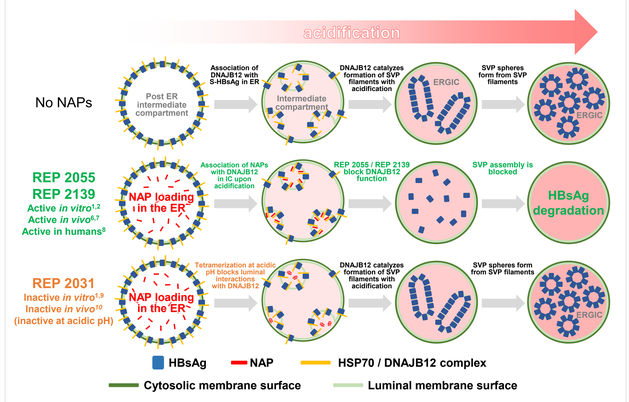

Figure 5. Model for NAP inhibition of spherical SVP assembly consistent with *in vitro*, *in vivo* and clinical data.

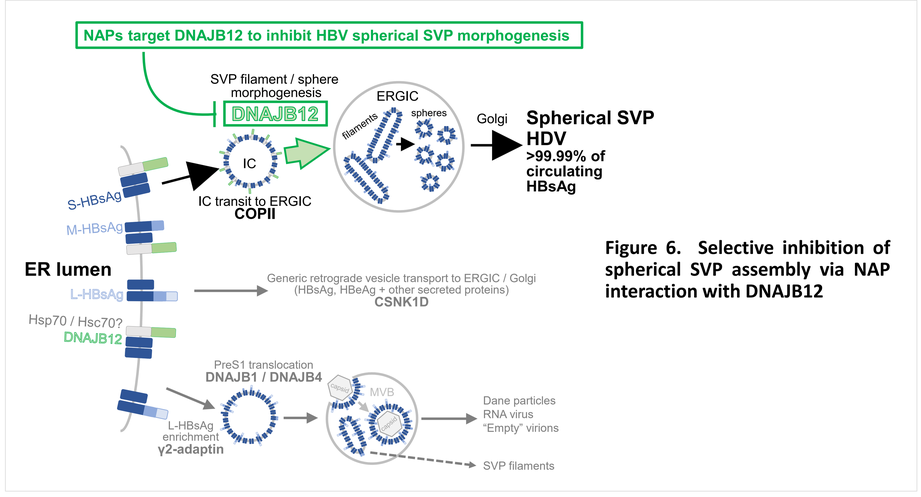

Figure 6. Selective inhibition of spherical SVP assembly via NAP interaction with DNAJB12

Table 1. Candidate NAP-interacting proteins

Candidate protein target	PS 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

Table 2. Selectivity of NAP interactions with HSP40 members

HSP40 member	Aliases	Function in HBV	NAP interaction
	Class A		
DNAJA1	HSDJ, DJ2, hDj-2, Hsj2, HSPF4, Hdj2, DjA1	unknown	None
DNAJA2	Dnj3, Dn3, HIRA-interacting protein 4, NY-REN-14, HIRIP4, DjA2	unknown	None
DNAJA3	Tid-1, hTID-1, HCA57	**HBcAg interaction, degradation of HBcAg and HBx[3]**	**Weak**
DNAJA4	Dj4, Hsj-4, MST104, MSTP104, PRO1472	unknown	None
	Class B		
DNAJB1	Hdj1, hDj-1, HSPF1, Sis1, RSPH16B	**PreS1 membrane topology[4]**	**None**
DNAJB2	HSJ1, HSPF3, Hsj1, DnaJB10, mDj8, DSMA5	unknown	None
DNAJB3	Hcg-3, Hsj-3, Msj1, MSJ-1	unknown	None
DNAJB4	HLJ-1, Hlj1, Dnajw	**PreS1 membrane topology[5]**	**None**
DNAJB5	Hsc40, Hsp40-2, Hsp40-3	unknown	None
DNAJB6	DJ4, DnaJ, HHDJ1, HSJ-2, Hsj2, LGMD1D, LGMD1E, Mrj, MSJ-1	unknown	None
DNAJB7	Dj5, HSC3, mDj5	unknown	None
DNAJB8	DJ6, mDj6	unknown	None
DNAJB9	ERdj4, Mdg1, mDj7	unknown	None
DNAJB11	ERdj3, hDj9, PWP1-interacting protein 4, ABBP-2, HEDJ	unknown	None
DNAJB12	Dj10, mDj10	SVP morphogenesis / secretion	Strong
DNAJB13	Tsarg-6/-3	unknown	None
DNAJB14	EGNR9427, PRO34683, FLJ14281	unknown	None
	Class C		
DNAJC1	ERdj1, MTJ1, Htj1, DnaJl1	unknown	None
DNAJC2	MPHOSPH11, MPP11, Zrf1	unknown	None
DNAJC3	ERdj6, p58, Prkri, protein kinase inhibitor p58	unknown	None
DNAJC4	F2, multiple endocrine neoplasia type 1 candidate protein number 18	unknown	None
DNAJC5β	Csp-beta	unknown	None
DNAJC5γ	Csp-gamma	unknown	None
DNAJC6	Auxilin, mKIAA0473, DjC6, Park19	unknown	None
DNAJC7	Dj11, mDj11, DjC7, TPR2, mTpr-2	unknown	None
DNAJC8	spf31, Hspc315, Hspc331	unknown	None
DNAJC9	DnaJ protein SB73, HdjC9	unknown	None
DNAJC10	ERdj5, MTHr, JPD1	unknown	None
DNAJC11	dJ126A5.1, FLJ10737, RP1-126A5.3	unknown	None
DNAJC12	Jdp-1, mJDP1	unknown	None
DNAJC13	RME-8, mKIAA0678, Gm1124	unknown	None
DNAJC14	Hdj3, hDj-3, LIP6, DRIP78, Drip-78	unknown	None
DNAJC15	Dnajd1, cell growth-inhibiting gene 22 protein, MCJ, Mcj	unknown	None
DNAJC16	mKIAA0962	unknown	None
DNAJC17	DnaJ homolog subfamily C member 17, C87112	unknown	None
DNAJC18	DnaJ homolog subfamily C member 18, AU041129	unknown	None
DNAJC19	TIM14, TIMM14	unknown	None
DNAJC20	Jac1, Hsc20, HscB	unknown	None
DNAJC21	Dnaja5, GS3, Jjj1	unknown	None
DNAJC22	Wus, wurst homolog (Wurst), AI506245	unknown	None
	J-like		
DNAJC23	ER-resident protein ERdj2, Sec63L, Dnajc23	unknown	None
DNAJC24	ZCSL3, JJJ3, DPH4, Dph-4, 1700030A21Rik	unknown	None
DNAJC25	bA16L21.2.1, 2010109C08Rik, 2010203O07Rik,	unknown	None
DNAJC26	GAK, auxilin-2	unknown	None
DNAJC27	RBJ, RabJs, Rap-1, Rab, DnaJ-domain containing protein	unknown	None
DNAJC28	C21orf55; C21orf78, Orf28, oculomedin	unknown	None
DNAJC29	Sacsin , SPAX6, protein phosphatase 1, regulatory subunit 138,	unknown	None
DNAJC30	Williams-Beuren syndrome chromosomal region 18 protein	unknown	None

CONCLUSIONS

1. **DNAJB12 is a HSP40 chaperone involved in the assembly of spherical SVP and is targeted by NAPs.**
2. **The interaction with and inhibition of DNAJB12-mediated chaperone function is consistent with the selective effect of NAPs to inhibit the assembly / secretion of spherical SVP.**
3. **NAP interaction with CSNK1D (and the associated inhibition of HBeAg secretion) does not appear to be physiological but likely reflects an interaction occurring *in vitro* under experimental conditions which do not mimic normal intracellular NAP trafficking.**

DISCLOSURES

Employment (Replicor, MB, AV) shareholder (Replicor, AV)

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