Poster Abstract P1

Ph-dependent interaction of NAPs with the HSP40 chaperone DnaJB12

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Background
Nucleic acid polymers (NAPs) inhibit assembly and secretion of HBV subviral particles (SVP) but not HBeAg or Dane particles. This oligonucleotide effect is driven by length- and phosphorothioate (PS)-dependent hydrophobic interactions independent of sequence or base / sugar modification. NAPs act in the acidified lumen of post-ER, pre-Golgi vesicles (ERGIC) where SVP morphogenesis occurs. A differential NAP interactome screen in HepG2.2.15 cell lysates at pH 7.4 identified the HSP40 chaperone DnaJB12 as a putative host target for NAPs inhibiting SVP assembly. DnaJB12's involvement in SVP assembly was confirmed by DnaJB12-knockdown in HepG2.2.15 cells inhibiting HBsAg but not HBeAg secretion. Continued analysis of NAP interactions at acidic pH was performed to examine physiologically relevant binding activity within the ERGIC.

Methods
MS/MS interactome analysis with biotinylated NAPs in HepG2.2.15 lysates was conducted at pH 7.4 and 6.5. Protein interactions were validated by interaction ratios between the active REP 2139 (40mer PS, hydrophobic) and REP 2179 (20mer PS), REP 2147 (40mer phosphodiester, non-hydrophobic) and REP 2031 (40mer PS poly C – hydrophobic but inactivated at acidic pH by homo tetramerization). shRNA mediated DNAJB12 knockdown was confirmed by western blotting. Effects on secretion of HBsAg (GS EIA 3.0, Biorad) and HBeAg (ETI-EBK PLUS N0140, Diasorin) were monitored by ELSIA and normalized to total cellular protein (as determined by BCA assay).

Results
shRNA knockdown of the previously identified casein kinase 1D (CSNK1D) inhibited secretion of HBsAg and HBeAg. A 53-fold reduction in the ratio of REP 2139 : REP 2147 interaction with CSNK1D was observed pH 6.5 vs 7.4, indicating a loss of hydrophobic interaction. Moreover, the CSNK1D interaction of REP 2139 at pH 6.5 was comparable to the inactive REP 2031. In contrast, a 40-fold increase in the ratio of REP 2139 : REP 2147 interaction of REP 2139 to DNAJB12 occurred at pH 6.4 vs 7.4, indicating an increased hydrophobic interaction. At acidic pH, the interaction of REP 2139 with DNAJB12 was 10-fold greater than REP 2031. REP 2139 : REP 2179 ratios declined ~2 fold at pH 6.5 vs 7.4 with both targets, suggesting similar binding affinities.
Previous 48h shRNA knockdown of DNAJB12 resulted in 40-50% decline in secreted HBsAg. Extending this knockdown to 6 days increased inhibition of secreted HBsAg to 90%, consistent with the inhibition of HBsAg secretion with REP 2139.

Conclusions
Hydrophobic (antiviral) interactions of REP 2139 with DNAJB12 are strongly enhanced at acidic pH. This is consistent with the location of DNAJB12 within the acidified ERGIC and the assembly of SVP and its inhibition by NAPs within the ERGIC. The loss of hydrophobic interactions of NAPs with the cytoplasmic CSNK1D at pH 6.5 is consistent with a non-physiological interaction and the knockdown of CSNK1D driving the non-physiological inhibition of HBeAg.