Apolipoproteins are not required for HBsAg secretion *in vitro*.

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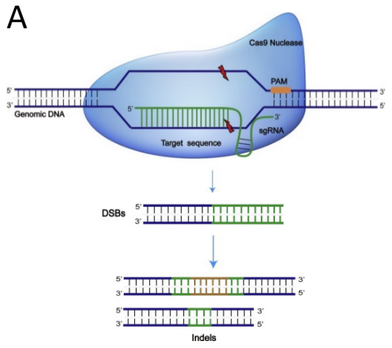
BACKGROUND & AIMS

- Apolipoproteins are crucial for the assembly and stability of lipoprotein particles and in maintaining cellular and plasma lipid homeostasis.
- The HBV lifecycle depends on cellular lipid metabolism and is also able to stimulate lipogenic pathways, mainly through the HBx regulatory protein (1-4).
- Cholesterol has been shown to be implicated in the secretion of subviral and Dane particles (5,6) and several studies have reported an interaction between HBsAg and Apolipoprotein H (7).
- HBV subviral particles share biochemical features with HDL (similar buoyant density, miRNA content and protein / cholesterol / cholesterol ester / triglyceride / phospholipid content) (8-10).
- Nucleic acid polymers have been previously shown to interact with apolipoproteins B and E and to interfere with the assembly and or secretion of HBsAg *in vitro and in vivo* (11-12).

AIM → **To explore the role of various apolipoproteins on HBsAg secretion *in vitro* using CRISPR/Cas9 based knock outs (KO) in HepG2.2.15 cells.**

MATERIALS & METHODS

A

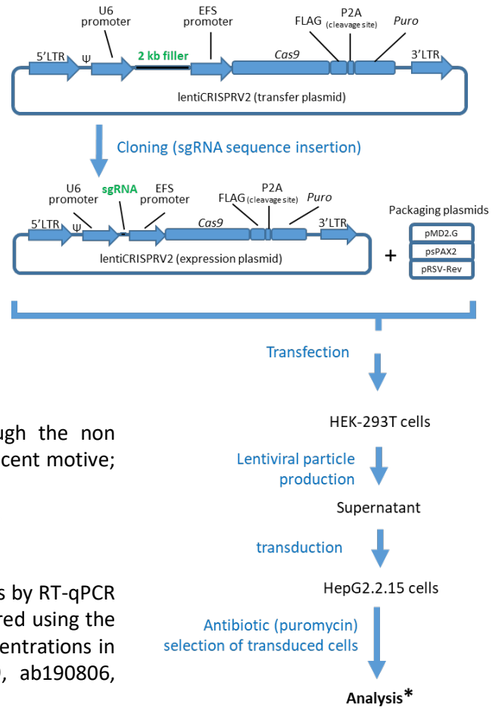


Derived from Yi L, Li J. 2016. Biochim Biophys Acta **1866:**197-207.

B

Target gene information	
Name	**ApoA1**
Accession number	M27875.1
Target cDNA length (bp)	801
SgRNA target sequence	5' GTTTAGCTGTTTTCCCAAGG 3'
Name	**ApoA2**
Accession number	NM_001643.1
Target cDNA length (bp)	303
SgRNA target sequence	5' GTCCTTGCCATAGTCAGTCA 3'
Name	**ApoB**
Accession number	NM_000384.2
Target cDNA length (bp)	13692
SgRNA target sequence	5' GAGAGATCTTCAACATGGCG 3'
Name	**ApoC3**
Accession number	NM_000040.1
Target cDNA length (bp)	300
SgRNA target sequence	5' GCAACAACAAGGAGTACCCG 3'
Name	**ApoE**
Accession number	NM_001302688.1
Target cDNA length (bp)	954
SgRNA target sequence	5' GGAGGAACAACTGACCCCGG 3'
name	**ApoH**
Accession number	NM_000042.2
Target cDNA length (bp)	1038
SgRNA target sequence	5' GTACATTTCAGAGTGTTGAT 3'

C

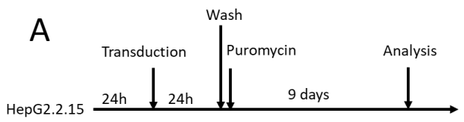


A. General principle of production of KO cells using the CRISPR/Cas9 technology through the non homologous end joining (NHEJ) pathway. sgRNA, small guide RNA; PAM, protospacer adjacent motive; DSB, double strand break; Indels, random insertions and deletion at the site of DSB.
B. Targeted gene information and sequences used for the cloning of sgRNA encoding plasmid.
C. Map of the donor plasmid lentiCRISPRv2 and protocol for the production of KO cell lines

* KOs were verified by titration of secreted proteins in the supernatant using ELISA, as well as by RT-qPCR (SYBRGreen) on specific intracellular mRNAs. Effect of KO on HBsAg secretion was measured using the Murex HBsAg Version 3 Kit (Diasorin). Apolipoprotein A1, A2, B, C3, E, H and TIMP2 concentrations in the supernatant were monitored using ELISA kit from Abcam (ab189576, ab184859, ab190806, ab154131, ab108813, ab108814, and ab188395, respectively)

RESULTS (SINGLE KO)

A



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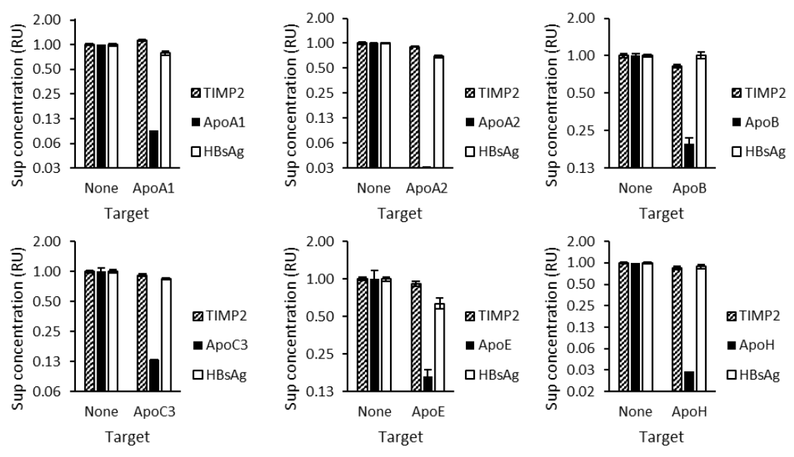
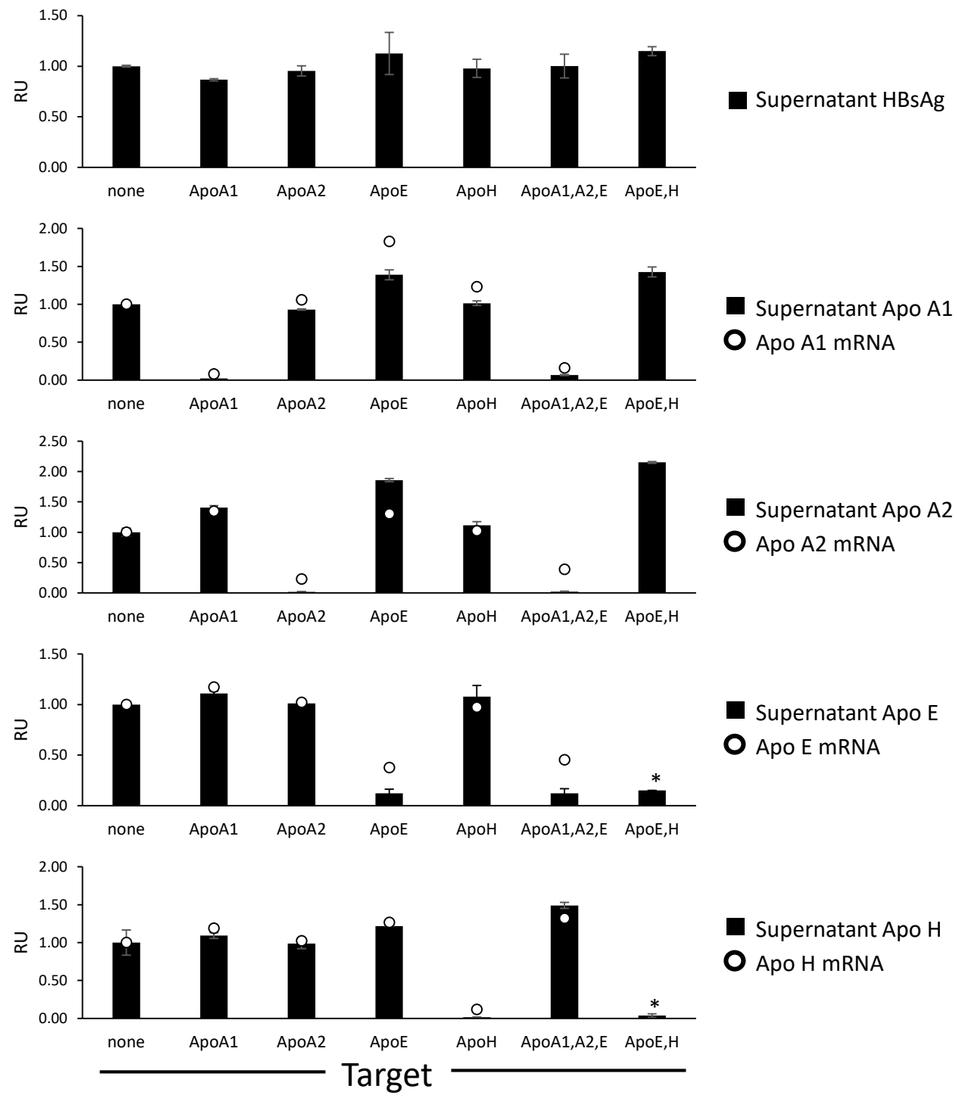


Single apolipoprotein knock out efficiency and effect on the secretion of HBsAg. Cells were treated as described in (A). At the indicated time, supernatants were harvested and tested for the presence of specific apolipoproteins (knock out efficiency) and TIMP2 (control secreted protein) and HBsAg (B). Results are presented relative to the mock (no target). RU, relative unit.

RESULTS (COMBINATION KO)



Combination of apolipoprotein knock out efficiency and consequences on the secretion of HBsAg. Experimental setup was as for single KO experiment. Results from ELISA tests were normalized to cellular protein content (BCA). Specific intracellular mRNA concentrations were monitored after total RNA normalization (via nanodrop). Results are presented relative to the mock (no target). RU, relative unit; W, wash; *, mRNA not tested.

CONCLUSIONS

- Potent single and multiple apolipoprotein KO (>80%) have been obtained at the protein (supernatant) level.
- Corresponding mRNA levels follow a similar trend.
- The single and multiple apolipoprotein KOs performed do not trigger significant alterations in HBsAg concentration in the supernatant.
- HBsAg may be secreted independently of apolipoprotein metabolism *in vitro* but additional combinations of apolipoprotein KOs are required to confirm this.

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