AASLD November 13-16, 2020
The Liver Meeting® Digital Experience
Poster 0818

Activity of REP 2139 in HepG2 cells expressing HBsAg isoforms

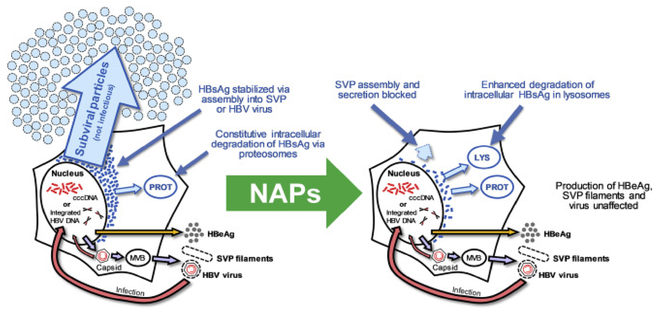
Léna Angelo[1], Matthieu Blanchet[1,2], Andrew Vaillant[2], Patrick Labonté[1]

1.Institut National de la Recherche Scientifique – Centre Armand-Frappier Santé Biotechnologies, Laval, Canada, H7V 1B7
2.Replicor Inc. Montréal, Canada, H4P 2R2

INTRODUCTION

During chronic hepatitis B (HBV) infection, spherical subviral particles (SVP) are produced in large quantity compared with infectious virions. These SVP are the major source of HBV surface antigen (HBsAg) in patient serum as well as in tissue culture supernatant. Recent *in vitro* studies in HepG2.2.15 cells[1,2] analyzed the antiviral effects of the nucleic acid polymer REP 2139 on the HBV lifecycle. These studies revealed that REP 2139 inhibits HBsAg secretion, specifically targeting the assembly and/or the secretion of spherical SVP (Figure 1). In order to assess the mechanisms leading to the effects, a novel HepG2 model system expressing only HBsAg and HBx is being developed. The HBV post-transcriptional regulatory element (HPRE) is a sequence overlapping the HBx ORF and is required for the expression of HBsAg.[3,4,5]

Figure 1. Mechanism of action of REP 2139 in HBV infection[2]



MATERIAL & METHODS

Transient transfection was used to evaluated expression constructs driving efficient expression of all HBsAg isoforms. A coding sequence for HBsAg proteins including large, medium and small isoforms and HBx from genotype D Ayw3 was cloned into a pAAVS1-puro-Tet3G plasmid (#92099 Addgene) modified with a PGK-promoter driving expression of the puromycin resistance gene to create a donor plasmid. Plasmids with various expression constructs (see Figure 2) were co-transfected with the eSpCas9 (#79888 Addgene) plasmid into HepG2 cells prior to selection with puromycin.

Endosomal release following uptake of REP 2139 is required to restore normal trafficking of NAPs in tissue culture[1] and was achieved by addition of UNC 7938 for 2 hours as previously described[1,2] (Figure 3). HBsAg was assessed using the Biorad ELISA (GS HBsAg EIA 3.0). Cell viability was assessed by BCA test (Biorad).

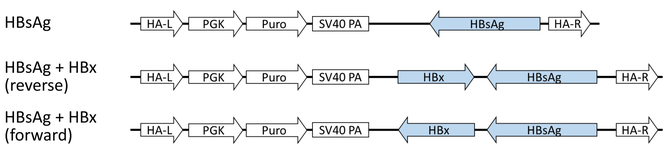


Figure 2. Expression constructs used to evaluate HBsAg secretion in HepG2 cells.

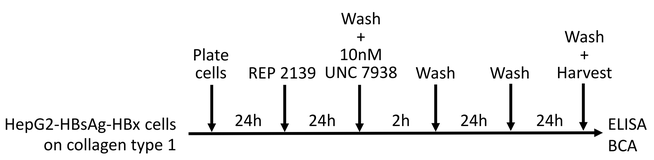
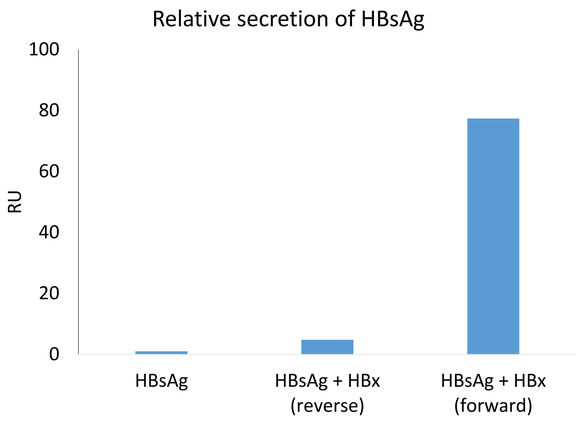
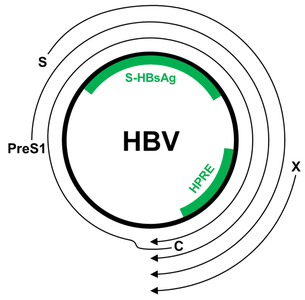


Figure 3. REP 2139 treatment paradigm in tissue culture.

RESULTS





Map of the HBV genome[5] showing the location of the HPRE. To preserve clarity only selected open reading frames are shown.

Figure 4. Correct orientation of HPRE in HBx sequence is required for efficient expression and secretion of HBsAg

Relative secretion of the extracellular HBsAg (supernatant) in three HepG2-derived transient cell lines containing either HBsAg proteins (HBsAg), HBsAg and HBx in reverse orientation (HBsAg + HBx (reverse)) or HBsAg and HBx in the correct orientation (HBsAg + HBX (forward)). See Figure 2 for donor plasmid configurations used in CRISPR/Cas9 generation of these cell lines. The experimental observations were duplicated but not presented.

Preliminary tests performed with donor plasmid coding only for HBsAg resulted in low levels of secreted HBsAg. The HBV post-transcriptional regulatory element (HPRE) overlaps the HBx ORF and is required for RNA transportation out of the nucleus[3,4,5]. This RNA element works in a position- and orientation-dependent manner.

HepG2.2.15 cells

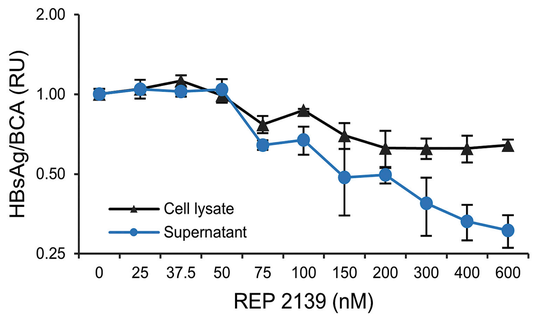


Figure 5. Previously published[1] effects on HBsAg following treatment with REP 2139 in HepG2.2.15 cells

Intracellular (lysate) and secreted (supernatant) HBsAg are indicated. In this cell line, the REP 2139 treatment at 500 nM concentration induces a ~70% diminution of secreted HBsAg. The IC_{50} was 100-150nM. RU, relative unit.

HepG2-HBsAg-HBx(F) cells

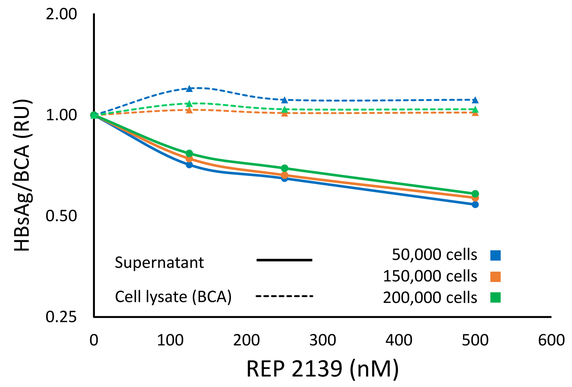


Figure 6. Effect of REP 2139 treatment on extracellular HBsAg (supernatant) at different densities of plated cells.

HBsAg response is normalized to baseline (no REP 2139). In this model, 500nM of REP 2139 induces a ~50% diminution of secreted HBsAg with a dose dependent response similar to that observed in HepG2.2.15 cells. RU, relative unit.

CONCLUSIONS

- **The HBV post-transcriptional regulatory element (HPRE) is required for efficient HBV RNA transport to the cytoplasm to drive translation of HBsAg.**
- **Effects of REP 2139 on inhibition of secreted HBsAg in HepG2-HBsAg-HBx(F) cells with enhanced HBsAg secretion are comparable to those obtained in HepG2.2.15 cells[1,2].**

REFERENCES

1. Blanchet et al., Antiviral Res 2019; 164, 97-105
2. Boulon et al., Antiviral Res 2020; 180: 104853
3. Huang, J. and Liang, T. J., Mol Cell Biol 1993; 0270-7306
4. Huang, Z. M. and Yen, T. S. , J Virol 1994; 0022-538x
5. Huang, Z. M.and Yen, T. S. , Mol Cell Biol 1995; 0270-7306

DISCLOSURES

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

Contact information:
availlant@replicor.com
patrick.labonte@inrs.ca