Characterization of circulating Hepatitis B virus RNA *in vitro* and in chronic hepatitis B patients

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1 Introduction

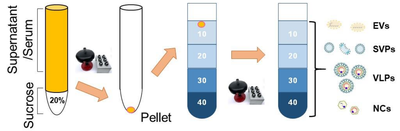
Circulating HBV RNA (cirB-RNA) is emerging as a promising non-invasive biomarker for cccDNA transcriptional activity. However, the molecular characteristics and circulating particles containing cirB-RNA *in vitro* and *in vivo* remain to be fully defined.

2 Aim

Identification of circulating HBV RNA species and associated particles in HBV-infected HepG2-NTCP cells supernatant and CHB patients

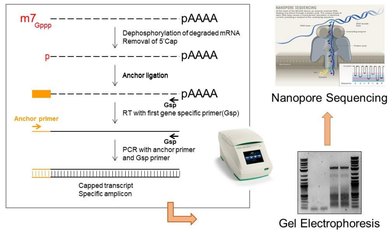
3 Method

Sample preparation



1. Serum concentration: Ultracentrifugation for 5 hours at 35,000rpm
2. Density gradient ultracentrifugation: 10-40% Iodixanol/Sucrose, for 16h at 35,000rpm
3. Total of 10 fractions collected, 500ul each

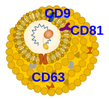
5' RACE PCR analysis



Analysis volume

Experiment	Vol.
DNA/RNA extraction	100ul
HBe/HBs ELISA	100ul
Western blotting	80ul
Density	20ul
5' RACE analysis	200ul

EVs

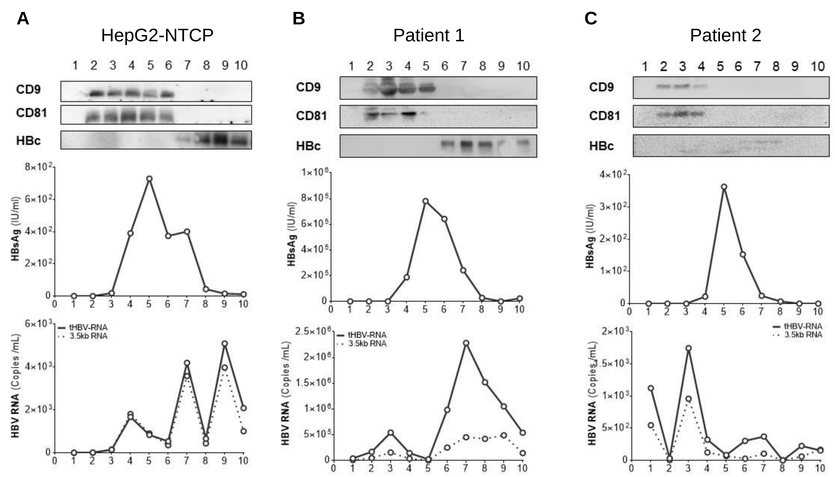


Function: Cell communicator
Markers: CD9 and CD81

Patients' information

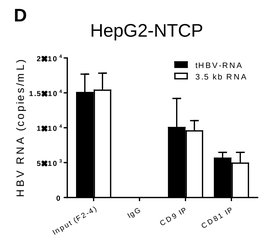
Sample Number	Serum HBV DNA (log IU/ml)	HBeAg	HBsAg (log IU/ml)	ALT (U/L)	Anti-HBV Treatment
Patient 1	8.5	Positive	4.9	36	NO
Patient 2	5.6	Negative	2.7	158	NO
Patient 3	2.9	Negative	2.8	27	NO

4 Results



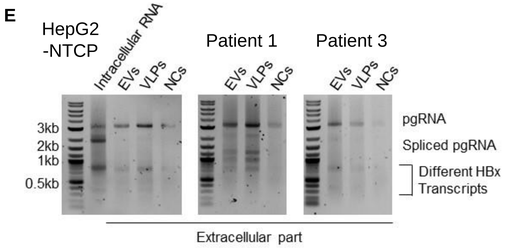
Circulating HBV RNA is found in EVs-enriched fractions after CHB patients' serum density gradient fractionation.

Gradient fractions were obtained according to the protocol detailed in Method section. A) HBV (250 MOI) infected HepG2-NTCP cell, Top: Representative Western Blot analysis using anti-extracellular vesicle (EVs) markers (anti-CD9 and anti-CD81), and anti-HBV core (HBc) antibody. Middle: HBsAg ELISA kit (Autobio Diagnostic). Bottom: HBV RNA quantification by RT-qPCR/ddPCR. HBx ORF common region was used to quantify total HBV RNA. pre-Core/pgRNA 5' unique region was used to quantify 3.5kb HBV RNA . B) Patient 1, Using the same analytical technique as panel A. C) Patient 2, Using the same analytical technique as panel A.



Circulating HBV RNA is associated to EVs.

HBV (1000 MOI) infected HepG2-NTCP cell supernatant was immunoprecipitated with antibodies against EVs markers (anti-CD9 and anti-CD81) and IgG as a negative control.



Circulating HBV RNA composition is similar across gradient fractions but differs among CHB patients.

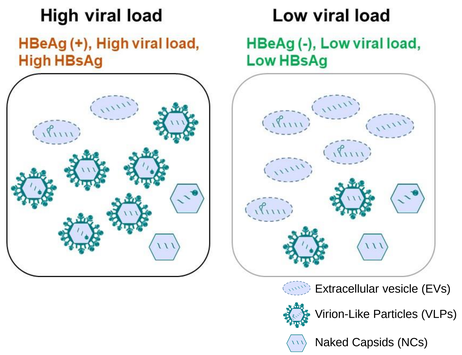
Serum was ultracentrifuged on a sucrose cushion and then RNA was extracted using Trizol reagent according to manufacturer's instructions. RNA was then subjected to 5' RACE protocol followed by agarose gel migration (Stadelmayer et al. J Hep 2020). Extracellular Vesicles (EVs), subviral particles (SVPs), virion-like particles (VLPs), and naked capsids (NCs)

5 Conclusions

- In HBV infected HepG2-NTCP and Patient 1 {HBeAg(+) and high HBsAg expression}, CirB-RNA was mainly detected in VLPs.
- In patients 2 and 3 {HBeAg(-) and low HBsAg expression}, cirB-RNA was mainly detected in the EVs.
- CirB-RNAs are mostly composed by pgRNA (3.5kb RNA), spliced pgRNA and HBx RNAs of various sizes.

Take home message

Circulating HBV RNAs are secreted through extracellular vesicles mainly in HBeAg(-) CHB patients with low viral load and low HBsAg levels



6 Acknowledgements

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7 References

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- Stadelmayer B et al. Full-length 5'RACE identifies all major HBV transcripts in HBV-infected hepatocytes and patient serum. J Hepatol 2020; 73:40-51

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