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Identification of shuttle protein hnRNPA1 as a modulating factor of circulating Hepatitis B virus RNAs release in chronic hepatitis B patients

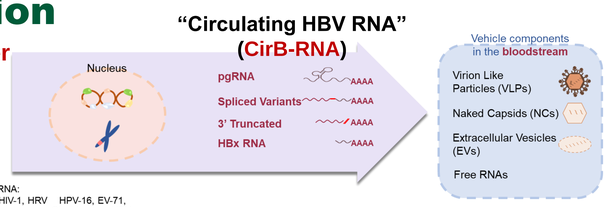
H. Tak[1*], D. Kim[1], D. Bousquet[1,2], M. Plissonnier[1], F. Berby[1], I. Bordes[1], A. Hamilton[3], M. Heil[3], M. Levrero[1,2,4], B. Testoni[1§] and F. Zoulim[1,2,5§]

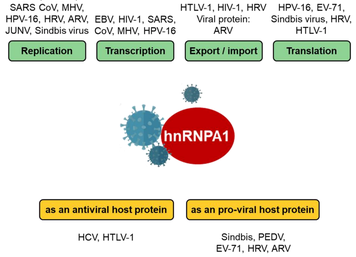
[1] INSERM U1052, CNRS UMR-5286, Cancer Research Center of Lyon (CRCL), Lyon, 69008, France; [2] University of Lyon, UMR_S1052, CRCL, 69008 Lyon, France; [3] Roche Molecular Diagnostics, Pleasanton CA; [4] Department of Internal Medicine - DMISM and the IIT Center for Life Nanoscience (CLNS), Sapienza University, Rome, Italy; [5] Department of Hepatology, Croix Rousse hospital, Hospices Civils de Lyon, France

1 Introduction

Ideal HBV biomarker

- Non-invasive
- reflects the cccDNA pool and activity
- Predicts "HBV cure"





hnRNPA1 functions in viral infections

- hnRNPA1 is known as a ubiquitously expressed and **multi-functional RBP**.
- hnRNPA1 protein levels are modulated differently, in different viruses.
- The role of hnRNPA1 has been reported both as **antiviral and proviral**.
- **hnRNPA1 overexpression in HBV-related hepatocellular carcinoma**.

2 Aim

Investigation of hnRNPA1 as shuttle protein of CirB-RNA release in chronic hepatitis B patients

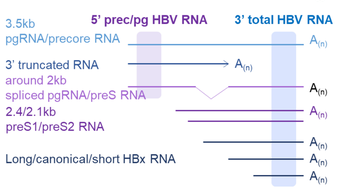
3 Patients & Methods

Patients' information

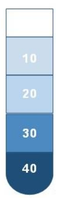
	P1	P2	P3	P4	P5	P6	P7	P8	P9
VL	H.C	H.C	8log	8log	5log	5log	5log	I.C	I.C
HBeAg	N.D	N.D	+	+	+	+	+	-	-
HBs	N.D	N.D	81000	98000	9100	7200	570	633	160

* N.D.: Not detected
* H.C.: Healthy Ctrl
* I.C.: Inactive Carrier
* VL: viral load

CirB-RNA detection primer design



Density gradient ultracentrifugation



Experimental Method

1. Ultracentrifugation for 5 hours at 35,000 rpm: serum or supernatant concentration
2. Ultracentrifugation (Density gradient ultracentrifugation) for 16 hours at 35,000 rpm with 10-40% Iodixanol/Sucrose density gradient tube
3. Total of 10 fractions collected

4 Results

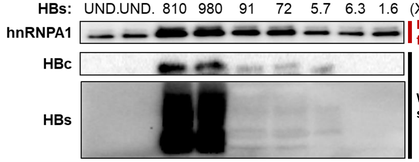
Results 1. hnRNPA1 was upregulated in HBV infected samples with high viral load

Patients sera

1/10 diluted sample

	H.C.	H.C.	8log	8log	5log	5log	5log	I.C.	I.C.
VL:									
HBeAg:	UND.	UND.	+	+	+	+	+	-	-
HBs:	UND.	UND.	810	980	91	72	5.7	6.3	1.6 (X100)

* UND.: undetected
* H.C.: Healthy Control
* I.C.: Inactive Carrier
* VL: viral load



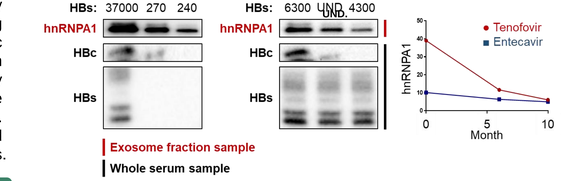
500ul of serum were ultracentrifuged on a sucrose cushion and then hnRNPA1 levels were analyzed by Western blot analysis using anti-hnRNPA1 antibody. HBc and HBs were analyzed in whole serum and only hnRNPA1 was analyzed in the exosome-enriched fraction. CHB phases are defined according to 2017 EASL CPGs.

Results 2. hnRNPA1 expression in serum was decreased during anti-viral treatment

Patients sera

	B.S.	Tenofovir			B.S.	Entecavir	
VL:	8log	5log	UND.	VL:	5log	3log	UND.
HBeAg:	+	+	+/-	HBeAg:	-	-	-
HBs:	37000	270	240	HBs:	6300	UND.	4300

* UND.: undetected
* B.S.: Baseline (before NUC-ttx)
* VL: viral load



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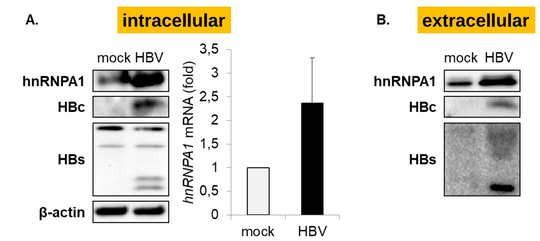
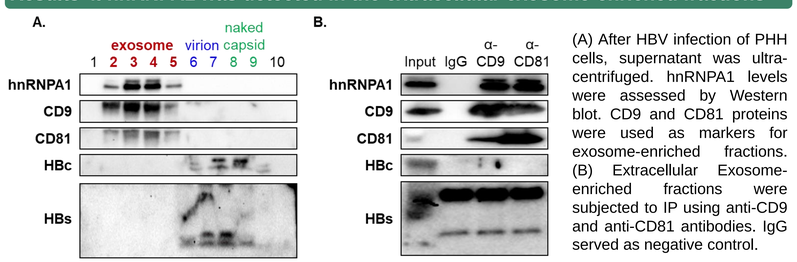
Results 3. hnRNPA1 was upregulated in HBV-infected primary human hepatocytes



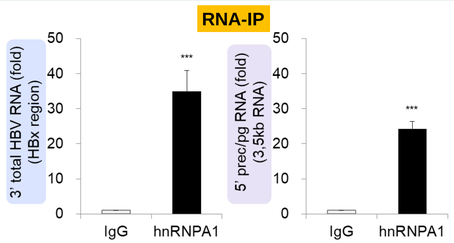
(A) 7 Days after infection of PHH cells, the levels of *hnRNPA1* mRNA were measured by RT-qPCR. The levels of HBs, HBc, hnRNPA1, and loading control β-actin by Western blotting in same lysates. (B) Supernatant collected from PHH cells infected as described in (A) were used to assess the levels of HBs, HBc and hnRNPA1. RNA levels are expressed relative to GUSb housekeeping gene.

Results 4. hnRNPA1 was detected in the extracellular exosome-enriched fractions



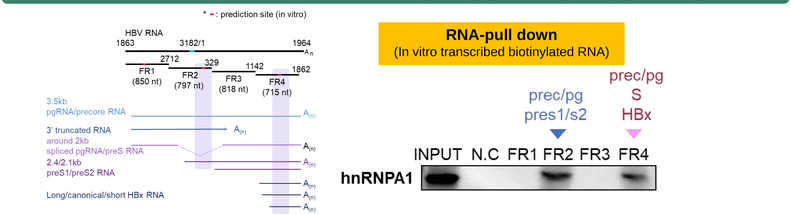
(A) After HBV infection of PHH cells, supernatant was ultra-centrifuged. hnRNPA1 levels were assessed by Western blot. CD9 and CD81 proteins were used as markers for exosome-enriched fractions. (B) Extracellular Exosome-enriched fractions were subjected to IP using anti-CD9 and anti-CD81 antibodies. IgG served as negative control.

Results 5. hnRNPA1 associated to HBV RNA at the 3' HBx and 5' preC/pg region



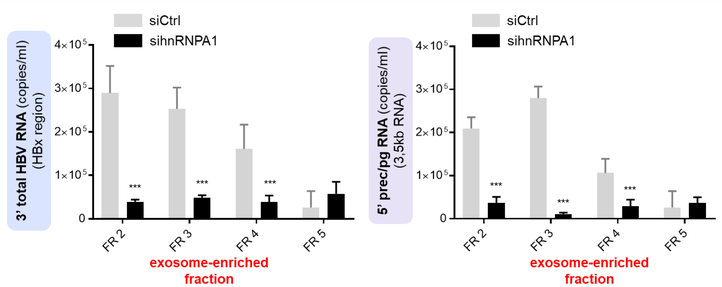
HepG2-NTCP cell lysate was subjected to RNA-IP followed by RT-qPCR analysis to measure the enriched HBV RNA in IP with anti-hnRNPA1 antibody compared to control IgG. The data represent mean ± SEM from three independent experiments. ***: p<0.005

Results 6. hnRNPA1 binds to sites located in preC/pg/S and preC/pg/S/X regions



Left, schematic location of the HBV RNA fragment1 (FR1), fragment2 (FR2), fragment3 (FR3) and fragment4 (FR4), as well as of the biotinylated in vitro generated transcripts to carry out biotin pull down analysis. *Right*, after incubation of each biotinylated transcript with HepG2-NTCP cell lysate, the interactions between the biotinylated transcript and hnRNPA1 was analyzed by Western blot using anti-hnRNPA1 antibody.

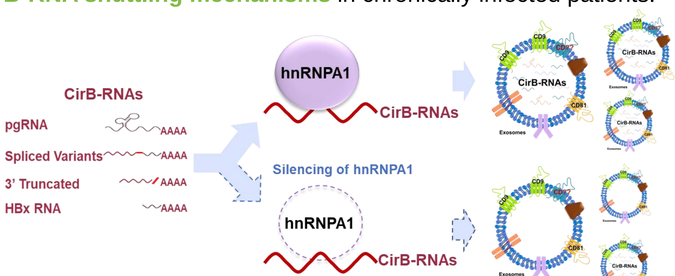
Results 7. Silencing of hnRNPA1 decreased cirB-RNA levels in exo-enriched fractions



HepG2-NTCP cells were infected for 7 days. On Day 1 and Day 4 after infection, siCtrl or sihnRNPA1 transfection was performed twice. After density ultracentrifugation, the HBV RNA in the exosome-enriched fraction was analyzed by RT-qPCR. The data represent mean ± SEM from three independent experiments. **: p<0.01, ***: p<0.005.

5 Conclusions

Altogether, our data suggest that **hnRNPA1 directly binds to HBV RNAs** and can function as a novel direct and indirect **contributor to CirB-RNA shuttling mechanisms** in chronically infected patients.



6 References

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7 Acknowledgements

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8 Contact information

Hyoseon Tak, Ph.D: hyoseon.tak@inserm.fr
Barbara Testoni, Ph.D: barbara.testoni@inserm.fr
Fabien Zoulim, M.D, Ph.D: fabien.zoulim@inserm.fr