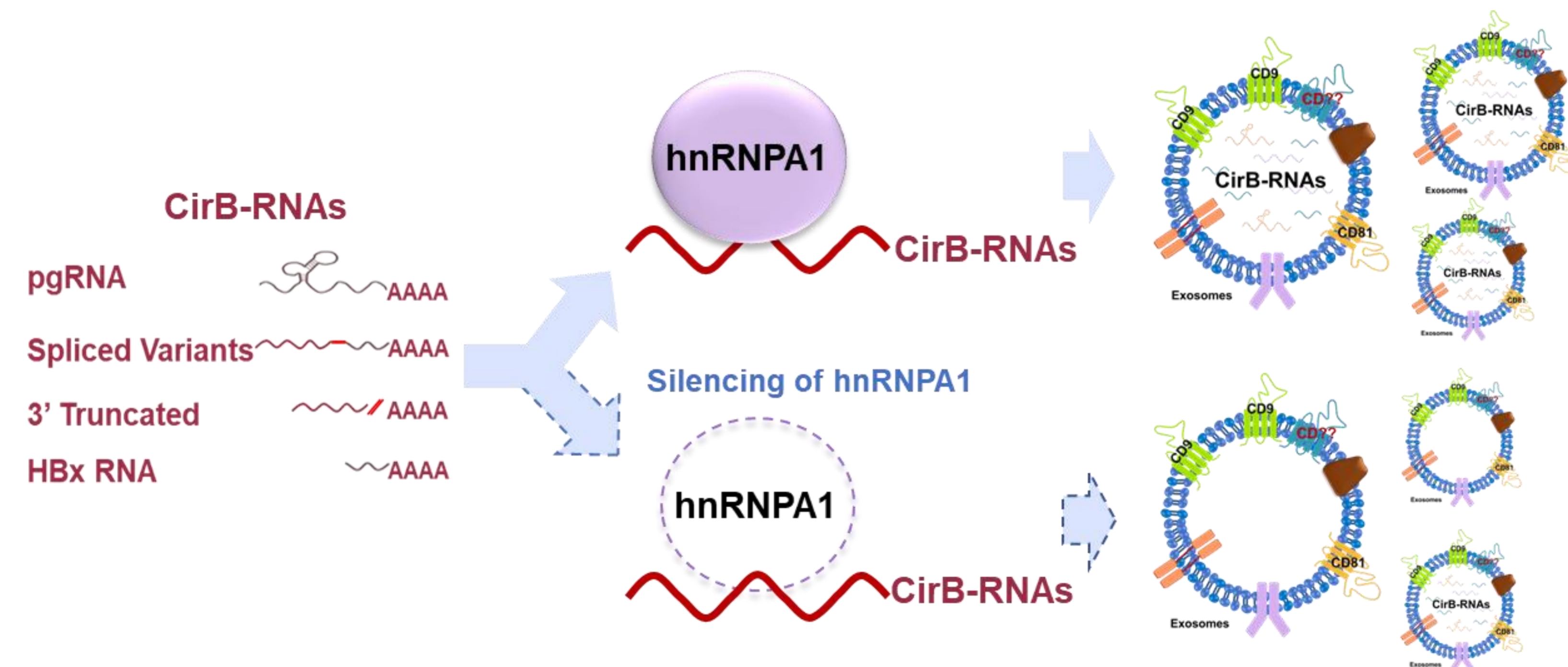


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The shuttle protein **hnRNPA1** is a modulating factor of **CirB-RNA** release



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.

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



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1 Introduction

Ideal HBV biomarker

- Non-invasive
- Reflects intrahepatic cccDNA pool and activity
- Predicts "HBV cure"

“Circulating HBV RNA” (CirB-RNA)

hnRNPA1 functions in viral infections

- hnRNPA1 is known as a ubiquitously expressed and multi-functional RBP involved in mRNA splicing, nucleo-cytoplasmic shuttling, and translation.
- hnRNPA1 protein levels are modulated differently, in different viruses, which further dictates its stability, function, and intracellular localization.
- 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 circulating HBV RNAs release in chronic hepatitis B patients

3 Method

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

RNA biotin pull down assay

Density gradient ultracentrifugation

Experimental Method

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

4 Results

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

Results 2. hnRNPA1 was decreased during anti-viral treatment

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

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

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

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

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

5 Conclusions

- hnRNPA1 is increased upon HBV infection
- hnRNPA1 is detected in the extracellular exosome-enriched fractions
- hnRNPA1 interacts with HBV RNA
- Silencing of hnRNPA1 decreases CirB-RNAs associated to exosome-enriched fraction

6 Acknowledgements

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7 References

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