



Characterization of circulating HBV RNAs in CHB patients and mechanism of cellular export

International Workshop on Viral Biomarkers

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Doohyun Kim, PhD

CONFIDENTIAL























Which vehicle carries CirB-RNAs?



HepG2-NTCP

D Bousquet's results



CirB-RNAs are detected in sEVs, virion-like particles (VLPs), and naked nucleocapsids (NCs)



Which vehicle carries CirB-RNAs?

RB-RNA 🖄

Patient serum: HBeAg(+) chronic infection VL: 8.9 log₁₀ IU/mL HBsAg: 4.7 log₁₀ IU/mL ALT: 27 U/L







CirB-RNAs are protected in sEVs



Longitudinal study during NUC treatment





HBV RNAs are still found in EVs during TDF treatment



Which CirB-RNA Species are detected?







Which CirB-RNA Species are detected?



X Grand's results



preS2/S RNA

Spliced variants

HBx RNA











mechanism?

New potential viral serum biomarkers

CirB-RNAs

Vehicles?

- High viral load & HBsAg: ٠ VLPs, sEVs, and NCs
- Low viral load & HBsAg: ٠ sEVs



Small Extracellular Vesicles (sEVs)



Sub-Viral Particles (SVPs)



Virion Like Particles (VLPs)



Naked Capsids (NCs)

| Di | stribution? | |
|----|-------------|--|
| | NUCs | |

sEVs

treatment

VLPs

| pgRNA | |
|---------------|--|
| Spliced pgRNA | |
| HBs RNA | |
| HBx RNA | |

Species?

Differ according to CHB stage



Is hnRNPA1 involved in HBV RNA release mechanism?





U/G-RBPs that interact with Exo-RNA or Exo-miRNA

HNRNP** HNRNP** HNRNP** HNRNP** HNRNP**

hnRNPA1 is..

- an multi-functional RBP
- involved in sEV sorting mechanism
- binds specifically to 5'-A,U,G-3'
- overexpressed in HBV-related HCC

CRCL hnRNPA1 downregulation reduces secretion of HBV RNAs in EVs fractions



H Tak's results



and statistics were performed using Student's T test (***: <p=0.005)

















New potential viral serum biomarkers

CirB-RNAs

Vehicles?

- High viral load & HBsAg: ٠ VLPs, EVs, and NCs
- Low viral load & HBsAg: • **sEVs**



Small Extracellular Vesicles (sEVs)



Sub-Viral Particles (SVPs)



Virion Like Particles (VLPs)



Naked Capsids (NCs)



treatment

VLPs



Differ according to CHB stage

Species?

Altogether, our results significantly contribute to the characterization of cirB-RNAs

in EVs/VLPs/NCs as viral biomarker







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INSERM U1052- RHU's Team

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Pr. Massimo Levrero Dr. Marie-Laure Plissonnier Dr. Francesca Casuscelli di Tocco Dr. Alexia Paturel

Roche Team

Dr. Marintha Heil Dr. Aaron Hamilton

<u>Clinic</u>

Dr. Caroline Scholtes Carrie-lynn Newsom

Françoise Berby Isabelle Bordes

Project Manager

Bernadette Vaz Octavie Paris







HCL HOSPICES CIVILS DE LYON

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| Table.1 CHB patients and Longitudir | al study of CHB patients during NUCs |
|-------------------------------------|--------------------------------------|
| treatment information | |

| Patient's No. | DNA (log ₁₀) | HBe status | HBsAg (IU/mL) | ALT | CHB phase | Geno- type | Anti-HBV Treatment | Figure No. |
|----------------------------|------------------------------------|---------------|------------------|-----|--------------|---------------|----------------------------------|---------------------------------|
| P1 | 8.9 | Р | 51000 | 27 | CI | В | NO | Fig 2 A-D, Fig 3B and Fig 4A |
| P2 | 8 | Р | 12000 | 42 | CI | ND | NO | Fig S2 |
| P 3 | 8.5 | Р | 81000 | 36 | CI | D | NO | Fig S3 |
| P4 | 8.7 | Р | 98000 | 30 | CI | E | NO | Fig S4 |
| P5 | 5.6 | Ν | 570 | 158 | СН | D | NO | Fig S8 |
| P6 | 4.1 | Ν | 9100 | 30 | СН | D | NO | Fig S6 |
| P 7 | 4.5 | Ν | 7200 | 40 | СН | D | NO | Fig 4A and Fig S7 |
| P 8 | 2.94 | Ν | 633 | 27 | CI | D | NO | Fig 2E-H and 4A |
| P 9 | 2.13 | Ν | 160 | 30 | CI | В | NO | Fig S5 |
| P10 | 4.5 | Р | 16000 | 123 | СН | D | Entecavir | Fig S13 |
| HC1 | - | - | - | 17 | | - | - | Fig S9 |
| HC2 | - | - | - | 32 | | - | - | Fig S9 |
| P11 (19/3/2019) | 5.7 | Ν | 6300 | 43 | СН | D | NO | Fig 5 |
| P11 (19/7/2019) | 2.4 | Ν | ND | 43 | | D | Entecavir (After 4 month) | Fig 5 |
| P11 (14/11/2019) | LoQ | N | 4300 | 41 | | D | Entecavir (After 8 month) | Fig 5 |
| P12 (4/7/2019) | 8.3 | Ρ | 37000 | 201 | СН | С | NO | Fig 6 |
| P12 (9/1/2020) | 3.1 | Р | 270 | 27 | | С | Tenofovir (After 6 month) | Fig 6 |
| P12 (7/5/2020) | LoQ | Ρ | 240 | 24 | | С | Tenofovir (After 10 month) | Fig 6 |

P1-10; patients number HC1-2; Healthy control P11 and 12: (Day/Month/Year); Follow up date Lo Q; DNA detectable but below Low quantity P; HBeAg Positive N; HBeAg Negative ND; not determined



P1(HBeAg +, CI) and P8(HBeAg -, CI)







P 1-4 (HBeAg +, CI)







P5 (HBeAg -, CH) and P8-9 (HBeAg -, CI)







P 6 and 7 (HBeAg +, CH)







Hildt's group protocol







EV fraction validation









HBV splicing variants map







Longitudinal study during NUCs treatment





hnRNPA1 is upregulated in HBV-infected samples with high viral load





Patient's serum



CRCL^{CENTRE DE} RECHERCHE EN CANCÉROLOGIE DE LYON

| | H1 | H2 | P1 | P2 | P 3 | P4 | P5 | P6 | P7 |
|-------|-----|-----|-------|-------|------------|------|------|------|------|
| VL | H.C | H.C | 8log | 8log | 5log | 5log | 5log | 2log | 2log |
| 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.: HBeAg(-) Chronic Infection

* VL: viral load



hnRNPA1 is upregulated upon HBV infection of hepatocytes





All graphs are the results of three independent experiments and statistics were performed using Student's T test (***: <p=0.005, *:<p=0,5)



HBx increases *hnRNPA1* mRNA transcription





All graphs are the results of three independent experiments and statistics were performed using Student's T test (***: <p=0.005)



hnRNPA1 was detected in the extracellular EVs-enriched fractions











Patient's serum

| | Whole serum | c IgG Cl | х- D9 С | α- CD81 |
|------|----------------|-------------|------------|------------|
| NPA1 | | | | - |
| CD9 | - | - | | |
| CD81 | 10 | • | | |
| HBc | | 0.00 | | |
| HBs | | 1 | | |

| Serum information | | | | | | |
|-------------------|--|--|--|--|--|--|
| 8log | | | | | | |
| + | | | | | | |
| 81000 | | | | | | |
| | | | | | | |

sEVs fractions

CRC hnRNPA1 downregulation reduces secretion of HBV RNAs in EVs fractions





All graphs are the results of three independent experiments and statistics were performed using Student's T test (***: <p=0.005, **:<p=0,05, n.s: not significant)

CRCL hnRNPA1 downregulation reduces secretion of HBV RNAs in EVs fractions





A total of 42 genotype sequences data were analyzed. Anna L McNaughton, J.Gen.Virol., 2020

5%

FR4

95%

non-consensus

consensus

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