Revisiting HBV biology: perspective for cure

Fabien Zoulim
Hepatology Department, Hospices Civils de Lyon
INSERM U1052, Cancer Research Center of Lyon
Lyon University, France
What do we want to achieve?

- **HBV DNA change from baseline (log_{10} c/mL)**
  - 0.0
  - -1.0
  - -2.0
  - -3.0
  - -4.0
  - +1.0

- **Time**

- **Core-Connected Circular DNA (cccDNA)**

- **HBV DNA**

- **HBSAg**

- **Partial Cure**

- **Functional Cure**

- **Sterilizing Cure**

- **Complete Cure**

- **Anti-HBsAb**

- **Lok et al, Hepatitis B Cure: From Discovery to Regulatory Approval; Hepatology / J Hepatol joint publication; 2017**
Barriers to eradicating HBV

- Defective CD8+ responses
- Defective B cell responses
- Inefficient innate response
- Defective immune responses

cccDNA reservoir
- Long t1/2
- Continuous replenishment
- Not affected by NAs and IFN

Integrated forms
- HBV persistence

cccDNA persistence after HBs seroconversion

Maynard et al, J Hepatol 2005
Late hepatitis B reactivation following DAA-based treatment of recurrent hepatitis C in an anti-HBc-positive liver transplant recipient

Vionnet et al, Hepatology 2017
Persistence of intrahepatic viral DNA synthesis during long Tenofovir therapy (HIV-HBV cohort)

New round of infection and/or replenishment of the cccDNA pool occur despite « viral suppression »

Boyd et al, J Hepatol 2016
Targeting cccDNA, the viral minichromosome

cccDNA replenishment

cccDNA formation

cccDNA degradation

cccDNA silencing

Lucifora et al, *Science* 2014
Belloni et al, *JCI* 2012
Koeniger et al, *PNAS* 2014
Model for HBV entry in hepatocytes and development of entry inhibitors

Entry inhibitors
Myrcludex
(pre-S1 peptide)
Blank et al, J Hepatol 2016
Bogomolov et al, J Hepatol 2016

Ezetimibe
Lucifora, Antiviral Res 2013

Proanthocyanidin
Tsukuda, Hepatology 2017

Cyclosporin analogues
Shimura, J Hepatol 2017

Li et al, elife 2012; Urban et al, Gastroenterology 2014
Core inhibitors inhibit viral genome replication and prevent cccDNA formation when administered prior to HBV inoculation.
CpAMs
“Capsid inhibitors”

NUCs
“Polymerase inhibitors”
Model for cccDNA degradation

IFNalpha /Lymphotoxin beta can induce APOBEC3A/B dependent degradation of HBV cccDNA

Lucifora et al, Science 2014; Shlomai & Rice, Science 2014

Similar observation with IFNγ and TNFα – Xia et al, Gastroenterology 2015
Targeting Hepatitis B Virus With CRISPR/Cas9

- Mutations and deletions with functional inactivation of cccDNA
- Over 90% of HBV DNA cleaved by Cas9
- Cas9 cleavage 15,000 times more efficient than APOBEC-mediated cytosine deamination following IFNα treatment

In vivo proliferation of hepadnavirus-infected hepatocytes induces loss of cccDNA in mice

Dandri et al, Hepatology 2010
Reduction of nuclear DNA consistent with symmetrical distribution to daughter cells
CCC DNA formation and candidate targets

Capsid-mediated nuclear import of RC-DNA

RC-DNA

TDP2 et al.?

P-free RC-DNA

Further host repair factors

Epigenetic silencing?

HBx

RC-DNA > cccDNA conversion?
Formation of cccCNA
Relaxed circular DNA mimics damaged cellular DNA

Nassal, Gut 2015
Koeniger et al, PNAS 2014

Predictably required activities
- Tyrosyl-DNA-phosphodiesterase
- Endonuclease
- DNA polymerase
- Polynucleotide kinase / phosphatase
- DNA ligase

HBV infection in HepG2-NTCP Tdp2 knockout cells

Are DNA repair and chromatinization coupled in the formation of cccDNA?

Locatelli et al, HBV conference 2017
Chromatin structure

- H3.3 is a DNA replication-independent histone variant
- Replaces conventional H3 in a wide range of nucleosomes in active genes

### Histone Variants

<table>
<thead>
<tr>
<th>Histone</th>
<th>Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>H1.1 ; H1.5 ; H1°</td>
</tr>
<tr>
<td>H3</td>
<td>H3.1 ; H3.2 ; H3.3 ; CENP-A</td>
</tr>
<tr>
<td>H2A</td>
<td>H2A.X ; H2A.Z ; macroH2A</td>
</tr>
<tr>
<td>H2B</td>
<td>H2B1 ; H2BW</td>
</tr>
<tr>
<td>H4</td>
<td></td>
</tr>
</tbody>
</table>

- **Specific dynamics**
  - Genomic regions (centromere, telomere, genes)
  - DNA processes (replication, repair, transcription)

- H3.3 is a DNA replication-independent histone variant

- Replaces conventional H3 in a wide range of nucleosomes in active genes

- **Role of histone chaperones**

---

*Two modes of mitochondrial dysfunction lead independently to lifespan extension in Caenorhabditis elegans. Yang W., Hekimi S. Aging Cell. 2010*
HIRA: An histone chaperone involved in DNA repair

- HIRA is known to recognize naked DNA to deposit histone H3.3.
- HIRA is responsible for chromatin priming in response to DNA damage allowing transcription recovery after repair.

*Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA.* Adam S, Polo SE, Almouzni G. Cell, 2013.
Is HIRA involved in cccDNA establishment?

Locatelli et al, HBV conference 2017
Does HIRA interact with cccDNA?

Experimental approach: Chromatin Immunoprecipitation on infected cells

Locatelli et al, HBV conference 2017
HIRA and H3.3 bind to cccDNA
H3.3 binding is concomitant with the beginning of transcription

ChIP experiment – HepG2-NTCP (n=4)

HIRA binding to cccDNA

H3.3 binding on cccDNA

rcDNA enters the nucleus
2 hours P.I
Initiation of the transcription
24h P.I

Locatelli et al, HBV conference 2017
Silencing of HIRA decreases cccDNA levels

HepG2-NTCP

Hira expression post-silencing

Viral parameters post silencing

Locatelli et al, HBV conference 2017
cccDNA chromatinisation involves HIRA recruitment and deposition of H3.3 histones

Locatelli et al, HBV conference 2017
Epigenetics of covalently closed circular (ccc)DNA (Regulation by viral proteins HBc and HBx)

Silencing:
- Epigenome modifiers
- Interferon alpha
- Capsid inhibitors
- HBx inhibitors

Modulation of cccDNA epigenetic control

**In vivo**
HBV infection

**DHBV**
Tet-on cell line

**HBV-infected**
HepG2-NTCP

**Alpha-IFN:**
- inhibits cccDNA transcription (no reduction in cccDNA levels)
- reduces the acetylation of cccDNA-bound histones

---

Belloni, JCI 2012  
Liu, Plos Path 2013  
Tropberger, PNAS 2015
Chromatin proteomics of HBV minichromosome

Experimental design

363 proteins were differentially identified in either infected HepG2 or PHH respect to mock-infected cells

Gene Set Enrichment Analysis

Significant differences between transformed and primary human hepatocytes

Testoni et al, HBV conference 2017
Gene ontology analysis of cccDNA-associated proteins

Transcription/mRNA processing

Ubiquitin/Proteasome

Translation initiation

Kinases/Chromatin modification

Chromatin structure

Signaling flux in the cell

*363 proteins identified in either HepG2 or PHH

Testoni et al, HBV conference 2017
Knock-down of selected cccDNA-associated proteins in infected PHHs had a strong impact on HBV replication.
Challenges in targeting cccDNA

Further knowledge required

Specificity for cccDNA? Delivery?

Partial effect? Efficacy in vivo?

Off-target effect? Delivery?

Modified from Nassal, Gut 2015

Testoni et al, Sem Liver Dis 2017; Hong et al, Hepatology 2017
Novel biomarkers to assist drug development

Biomarkers, surrogates
van Bommel et al, Hepatology 2015
Chen et al, Sci Rep 2017

Tracking cccDNA by FISH
Zhang et al, JCI 2016; Li et al J Virol 2017

Standardization of cccDNA assays
L Allweiss et al, ANRS/DZIF/ICE-HBV concerted action

Testoni et al, Sem Liver Dis, 2017
Can we cure both the infection and the diseased liver?

Lok et al, Hepatitis B Cure: From Discovery to Regulatory Approval; Hepatology / J Hepatol joint publication; 2017
Slower HBsAg decline in HBeAg negative patients: is it linked to HBV integration?

**ETV vs ETV+TDF**

**By HBeAg status**

**Duration of treatment (weeks)**

- ETV HBeAg(-)
- ETV+TDF HBeAg(-)
- ETV HBeAg(+)
- ETV+TDF HBeAg(+)

**SiRNA**

**By HBeAg status**

- Single dose Cohort 7
- Extension Cohort 10

- 01-7981 E Pos
- 01-7982 E Pos
- 01-7985 E Pos
- 01-7988 E Neg
- 01-7974 E Neg

- First dose of extension
- Last dose of extension

---

Zoulim et al., J Hepatol 2015

Wooddell et al., Sci Transl Med 2017
Both cccDNA and viral integrants are the template for HBsAg expression.
Integration in host chromosomes and clonal expansion of hepatocytes in all disease phases

Mason et al, Gastroenterology 2016
Liver Damage and HBV infection

• Early occurrence of HBV integration

• HCC not always seen on a background of cirrhosis

• Liver damage results of immune killing of hepatocytes

• Clonal expansion of hepatocytes not supporting HBV replication occurs even before cirrhosis

• Experimental models show that clonal hepatocyte repopulation is a major risk factor for HCC

• Integration contributes to T cell exhaustion via HBsAg expression

• **Strong arguments for early treatment intervention**

Zoulim & Mason, Gut 2012; Mason et al, Gastroenterology 2016
Can we cure both the infection and the diseased liver?

**UNTREATED**

**NUCs**

**NMEs**

**“Cure”**

- Direct Antiviral Agents
- Immunomodulatory strategies

Risk of HCC reduced (after 5 yrs) but not eliminated

Zoulim & Levrero
Acknowledgements

Hepatology Unit

INSERM U1052

Collaborations

François Bailly
Samir Benmaklouf
Marie Ecochard
Kerstin Hartig
Fanny Lebossé
Massimo Leverero
Marianne Maynard
Christian Trépo

Barbara Testoni
Julie Lucifora
David Durantel
Bernd Stademeyer
Guada Martinez
Maelle Locatelli
Fleur Chapus
Aurore Inchauspé
Maud Michelet
Judith Fresquet

Marc Bonin
Thomas Lahlali
Lucyna Cova
Romain Parent
Anna Salvetti
Birke Bartosch
Eve Pecheur
Boyan Grigorov
Christophe Combet

C. Caux, Lyon CRCL
FL. Cosset, Lyon CIRI
A. Boyd, Paris
F Carrat, Paris
C Ferrari, Parma
P Lampertico, Milan
A Craxi, Palermo
JP Quivy, Institut Curie
G Almouzni, Institut Curie
M Dandri, Hamburg
XX Zhang, Shanghai