Biology of HBV infection

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Chronic Infection with hepatitis B Virus

- 95% Acute self-limiting infection
- 5% Persistent infection (but high rates in perinatal age)

- Prevention of HBV infection is achievable (effective vaccine)
- ca. 250 million people are chronically infected with HBV worldwide
- HBV polymerase inhibitors efficiently suppress viral replication

But no cure for CHB due to persistence of cccDNA & weak immune responses

Limited knowledge about:
- mechanisms leading to infection establishment
- cccDNA formation, stability & regulation
- virus-host interactions (evasion mechanisms)

Invisible or active evasion?
Hepatitis B Virus: structure

- **High species-specific:** humans, chimpanzees (Tupaia)
- **High tissue-specific:** liver tropism

HBV extremely adapted to its host!

- smallest DNA enveloped animal virus (3,2 Kbp) (4 overlapping ORFs)
- **Genome** in circulating virions:
  - relaxed circular partially double-stranded DNA (rcDNA)
  - covalently linked to **viral polymerase (RT)**
- **Capsid:** core protein (HBcAg), icosahedral arrangement
- **Envelope:** Large (L), Middle (M), Small (S) proteins (host derived-lipids)

(adapted from Neuvet et al., J. Hepatol. 2010)
Hepatitis B Virus: structure

(S. Urban, R. Bartenschlager, R. Kubitz, F. Zoulim, Gastroenterol. 2014)
HBV infection: early entry steps

Attachment:
1) Reversible interaction with HSPGs
   (Schulze, Hepatol. 2007)
2) Irreversible binding to NTCP
   (Yan, Elife 2012)

NTCP: sodium taurocholate co-transporting polypeptide
- is localized to the basolateral membrane of highly differentiated primary hepatocytes
- it mediates most of the hepatocellular uptake of bile salts
- Binding is mediated by the preS1 domain of the HBV envelope protein
The recognition of NTCP as the *bona fide* HBV and HDV entry receptor has enabled the establishment of human cell lines supporting HBV and HDV infection.

**Pre-screening of new antiviral compounds & elucidation of unclear infection steps** 😊
Implications of HBV receptor discovery

**Improved in vitro infection systems**

HBV-HBsAg in HepG2-hNTCP

HDV-δAg in HepG2-hNTCP

(Yan et al, Elife 2012)

(Bhadra et al. unpublished)

HDV displays higher infection efficacy!

**Improved in vivo infection systems?**

h-NTCP

m-NTCP

aa 84-87

hNTCP Transgenic mice

(immune competent HBV/HDV infection models)
Implications of HBV receptor discovery

hNTCP transgenic primary murine hepatocytes (in vivo & in vitro) are not **HBV** permissive

**Low efficiency and Temporary** **HDV** infection could be shown in hNTCP transgenic mice
(He et al. *PLOS Pathogens* 2015)

**Unknown species-specific factors hinder HBV infection establishment in mice**

A mouse liver cell line (AML12) supports HBV entry upon hNTCP expression
(HBV RNA and proteins production, rcDNA – virion productivity – barely detectable)

(Lempp *J Virol* 2016)
Steps involved in HBV infection establishment

Attachment:
1) Interaction with HSPGs (Schulze, Hepatol. 2007)
2) Binding to NTCP (Yan, Elife 2012)

Internalization:
By endocytosis mediated by host factors
- caveolin-1 (Macovei, J.Virol.2010)
- clathrin
- Rab proteins (GTPases) (Macovei, J.Virol.2013)

Uncoating

Transport to the nucleus along microtubules

(Urban et al. Gastroenterology 2014)
Steps involved in HBV infection establishment

Intracellular transport

Intracytoplasmic transport via microtubules

capsids dissociate from microtubules (unknown mechanisms)
attach to importin α/β
pass the Nuclear Pore (NPC)
Capsids dissociate to core protein dimers
Genome is released
cccDNA formation

(Blondot, Bruss, Kann, J Hepatol. 2016)
cccDNA formation

Conversion of rcDNA to cccDNA is a largely unknown multistep process involving different host enzymes

- HBV Polymerase removal mediated by host DNA repair enzymes like TDP2 (tyrosyl-DNA-phosphodiesterase)
- Removal of a short terminal redundancy & small RNA primer on + strand
- Completion of the plus strand
- Ligation

(adapted from Königer et al. PNAS, 2014)

The cccDNA assembles with histone and non-histone proteins to form a stable minichromosome in hepatocyte nuclei

(acetylated histones  core protein)

(Newbold; Levrero et al. J.Hepatol.2009; Lucifora et al. J.Hepatol. 2011; Tropberger PNAS 2015)
HBV productive infection = cccDNA establishment & transcription

*The cccDNA minichromosome serves as transcription template for all HBV RNAs*
NUCs efficiently suppress HBV replication but not cccDNA-driven RNA transcription (no reduction of viral proteins and circulating HBsAg)
cccDNA transcription can be reduced by IFNα

<table>
<thead>
<tr>
<th>No treatment</th>
<th>IFN-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>H4K5/8/12/16</td>
<td>H3K27</td>
</tr>
<tr>
<td>CBP</td>
<td>P300</td>
</tr>
<tr>
<td>TFn2</td>
<td>TFn1</td>
</tr>
<tr>
<td>PICAF</td>
<td></td>
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<td>Ac</td>
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<td>Ac</td>
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</tr>
<tr>
<td>Active transcription (pgRNA; subgenomic RNAs)</td>
<td>Transcription repression (↓ pgRNA; ↓ subgenomic RNAs)</td>
</tr>
<tr>
<td>High replication</td>
<td>Low replication</td>
</tr>
</tbody>
</table>

(Belloni et al., J.Clin.Invest. 2012)

human IFNα

HBV-infected human liver chimeric uPA/SCID mice

Ac-Histone H4 on cccDNA

Rel Fold induction

NT  +IFNα

IFNα can inhibit cccDNA transcription by reducing the acetylation status of histones bound to the cccDNA

12 weeks of peg-IFNα treatment in humanized mice lacking adaptive immune responses led to significant suppression of viremia and antigen production

(Allweiss et al., J. Hepatology 2014)
IFNα & LTβR activation can promote cccDNA destabilization

**IFN-α induced epigenetic suppression of the cccDNA and strong reduction of viral antigen production**

(Belloni, JCI 2012; Allweiss, J.Hepatology 2014)

(Adapted from Thimme & Dandri, J.Hepatol. 2012)

**IFNα-treatment & activation of the lymphotoxin β receptor can induce APOBEC-mediated cccDNA deamination and partial cccDNA degradation**

(Lucifora, et al. Science 2014)
Studies aiming at understanding cccDNA biology / dynamics & HBV-host interactions

Use of preS1-derived Lipopeptide Myr-B

(Modified from Dandri, Locarnini, Gut, 2012)
Preclinical studies with HBV entry inhibitors

Chemically synthesized lipopeptides derived from the preS1 domain of HBV envelope block de novo HBV infection in vitro and in vivo

(Myrcludex B: Myr-GQNLSSTSSNPMDQFLDPAPRANTPDNENPHKDTWPDANKVG)

(Myrcludex-B efficiently blocked HBV spreading post-infection in the setting of constant viral exposure)

(Volz et al. J. Hepatology 2013)

(Petersen, Dandri, Urban, Nature Biotech. 2008)
Antiviral efficacy of Myrcludex-B administration on intrahepatic cccDNA amplification

Myrcludex-B hindered the increase of intrahepatic / intracellular cccDNA loads

(Volz et al. J.Hepatol 2013)

Intracellular cccDNA amplification seems to be inefficient in PHHs, explaining lower cccDNA/cell found in patients

Virus-specific differences among hepadnaviruses (DHBV vs. HBV)

(Kock et al. PloS Pathogens 2010)
Chronic HBV infection has been associated with alterations in lipid and cholesterol metabolism.

(Bar-Yishay Liver Int 2011; Hsu et al., J. Viral Hepatitis 2012)

**Use of preS1-derived lipopeptide to explore virus-host interactions**

**NTCP mediates 80% of the hepatocellular uptake of bile acids**

(Stieger, Handb. Exp. Pharmacol. 2011)

Studies in humanized mice indicated that HBV alters the profile of various genes related to host metabolism.

(Oehler, et al. Hepatol. 2014)
CYP7A1 is strongly enhanced in HBV-infected mice

Strong enhancement of CYP7A1, the rate-limiting enzyme promoting conversion of cholesterol to bile acids

CYP7A1 increase was confirmed also in liver biopsies from CHB patients

Myrcludex-B enhanced CYP7A1 expression in uninfected mice, thus identifying the preS1-domain as the viral component triggering CYP7A1 induction

(Oehler, et al. Hepatol.2014)
HBV and host metabolism

- Nuclear localization of the bile acid sensor FXR was strongly reduced in HBV-infected cells

  (Oehler, et al. Hepatology 2014)

- Binding of HBV to NTCP limits the uptake of bile salts, HBV hinders FXR nuclear translocation and alters the expression of bile acid metabolic genes

HBV can alter the profile of various genes related to host metabolism.

Entry inhibition strategies (Myr-B) block HBV spreading & cccDNA accumulation.

Interference with innate immune responses?

cccDNA targeting: Silencing / destabilization.

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