Hepatitis B: Clinical Relevance of HBV cccDNA

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What is cccDNA?

- Covalently closed circular DNA
- Repair of RC DNA by cellular enzymes
- Wrapped by nuclear chromatin
- « viral minichromosome »
- Template for viral gene expression
- Genetic archiving of mutations
- Responsible for viral persistence at the single cell level
- Replenishment by intracellular recycling of viral NC
- Long half-life
Epigenetics of cccDNA

**CLOSED**

- repressive DNA methylation
- repressive nucleosome spacing
- nucleosomes with repressive histone PTMs
- nucleosomes with activating histone PTMs
- activating nucleosome spacing

**OPEN**

- RNA pol II
- RNA

blocked by HBx

repulsive histone modifiers & chromatin remodellers et al.

activating histone modifiers & chromatin remodellers et al.
Mapping of histone modifications in cccDNA

Tropberger et al, PNAS 2015
cccDNA and natural history of CHB
cccDNA levels in the different phases of chronic HBV infection

- HBeAg+ patients had significantly higher cccDNA (90-fold) and total HBV DNA (147-fold) levels compared to HBeAg- patients. (p<0.001, Wilcoxon tests)

*Werle et al, Gastroenterology 2004*
cccDNA epigenetics in the different phases of chronic HBV infection

Low-replicative or latent infection

*Epigenetic control*

LOW-REPLICATIVE STATE  \[\rightarrow\]  HIGH-REPLICATIVE STATE

- Spontaneously
- Immunosuppression

Pollicino et al. Gastroenteroplogy 2006
Levrero et al. J Hepatol, 2009
cccDNA and antiviral therapy of CHB
48 weeks of ADV resulted in significant reductions in:
- serum HBV DNA > total intrahepatic HBV DNA > cccDNA
- > 14 years of therapy to clear completely viral cccDNA

Werle et al, Gastroenterology 2004
0.8 log10 (84%) decline in cccDNA, not paralleled by a similar decline in the number of HBcAg+ cells

Suggests cccDNA depleted primarily by non-cytopathic mechanisms or new rounds of hepatocyte infection occurred during therapy

*Werle et al, Gastroenterology 2004*
Slow decay of cccDNA and HBsAg during NUC therapy

Wong et al, Clin Gastroenterol Hepatol 2013
Werle et al, Gastroenterology 2004
Persistence of cccDNA after HBs seroconversion

Maynard et al, J Hepatol 2005
Long-term therapy is required to maintain viral suppression.

**SERUM**

- HBsAg: Red line with a steep decline, indicating a significant decrease in viral load.
- HBV DNA: Dashed blue line remaining relatively stable, suggesting persistent viral replication.

**LIVER**

- cccDNA: Orange line showing a gradual decrease, indicative of reduced viral persistence.

**Time**

- The graph illustrates the time course of viral load changes post-therapy, emphasizing the importance of continuous treatment for viral suppression.
Stopping TDF therapy after long-term viral suppression

High rates of viral replase & ALT elevations

3 patients with HBsAg loss out of 41

24-week TFFU Completers (n=41)

HBeAg positive (n=4)

HBeAg negative (n=37)

Buti et al AASLD 2015
Challenges with cccDNA (1)

- Harmonization of the detection and quantification methods
  - Southern blot analysis
  - dPCR assays

- Analysis of cccDNA epigenome
  - Need for sensitive methodologies

- Surrogate markers for non invasive cccDNA evaluation in patients
  - qHBsAg: relevant in cell culture systems
  - But not in vivo: many confounding factors (cccDNA levels, epigenetic status, number of infected cells, integration etc...
Methods for the detection of cccDNA

- 1990ies and before: Southern Blot analysis of HBV replicative intermediates and cccDNA selectively extracted from liver tissues or HBV-transfected cells

(Dandri, Hepatology 2000)

Pollicino et al., Gastroenterology, 2006

Pro: you see it
Cons: detection limits

- real-time PCR method for the detection of relaxed circular and cccDNA in frozen liver biopsies


The method was validated in 3 different labs: Zoulim (France)
Locarnini (Australia)
Petersen (Germany)
cccDNA quantification by real time PCR

- Many studies have been published!
- cccDNA measurements both using liver tissues and cell culture systems
- But no real standardization of the methods: DNA extraction, cccDNA enrichment, primer sequences etc…
- Need for harmonization of the technologies
Correlation between HBcAg and cccDNA

Matzusaki et al, Journal of Gastroenterology and Hepatology 2013
Challenges with cccDNA (2)

- Can we target cccDNA to improve the rate of functional cure with antiviral therapy?
  - Improved viral suppression to deplete the cccDNA pool
  - cccDNA degradation: is the whole pool of cccDNA susceptible to degradation? will all infected cells be susceptible?
  - cccDNA silencing: targeting virus-specific mechanisms to avoid safety issues, i.e. HBx and/or HBc
  - Hepatocyte turn-over to dilute cccDNA but exposes to the risk of clonal selection of hepatocytes
Persistence of intrahepatic viral DNA synthesis during Tenofovir therapy (HIV-HBV cohort)

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

*Boyd et al, in revision*
Targeting cccDNA

Lucifora et al, Science 2014
Belloni et al, JCI 2012
Koeniger et al, PNAS 2014
Tropberger et al, PNAS 2015
New treatment concepts for a functional cure of HBV infection

- Therapy
  - Antivirals
  - Immune restoration

Decay or epigenetic control

SERUM

LIVER

Antivirals

Immune restoration

HBsAg

HBVDNA

cccDNA
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