Intrahepatic virology for which you need core biopsies

Ashwin Balagopal, M.D.
Center for Viral Hepatitis Research
Johns Hopkins University School of Medicine
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Disclosures

• Sanofi provided funding to JHU to study HBV using scLCM
The Burden of Viral Hepatitis
Plasma measurements do not fully uncover intrahepatic replication
Plasma measurements do not fully uncover intrahepatic replication

How can we translate viral dynamics to a more tangible understanding of replication in an organ?
How do plasma quantities relate to the intrahepatic viral burden?

**Graph:**
- **Y-Axis:** HCV RNA $\log_{10}$ IU/mL
- **X-Axis:** TIME

The graph shows a fluctuation in HCV RNA levels over time, with a decrease in viral load over the period depicted.
Hypothesis: plasma HCV RNA levels reflect the number of infected hepatocytes, and thus the time required for turnover of those hepatocytes during treatment.
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SINGLE CELL LASER CAPTURE MICRODISSECTION (scLCM)

Archived Snap-Frozen Liver Tissue

10 μm Cryosections & Hematoxylin Staining

Laser Capture Microdissection

qPCR

Kandathil et al., Gastroenterology, 2013
Graw et al., PLoS Comp Bio, 2014
Balagopal et al., JID, 2020
Viral Landscape/ Viroscape

Kandathil et al., *Gastroenterology*, 2013
Balagopal et al., *JID*, 2020
Viral Landscape/ Viroscape

Kandathil et al., *Gastroenterology*, 2013
Balagopal et al., *JID*, 2020
Intrahepatic burden of HCV before and during direct-acting antivirals

<table>
<thead>
<tr>
<th>Participant</th>
<th>1st Biopsy % (Counts)</th>
<th>2nd Biopsy % (Counts)</th>
<th>Absolute change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10.3% (67/650)</td>
<td>1.1% (3/270)</td>
<td>-7.2% pts</td>
</tr>
<tr>
<td>B</td>
<td>39.3% (112/285)</td>
<td>1.7% (5/294)</td>
<td>-37.6% pts</td>
</tr>
<tr>
<td>C</td>
<td>15.9% (63/396)</td>
<td>0.4% (1/296)</td>
<td>-15.5% pts</td>
</tr>
<tr>
<td>D</td>
<td>13.0% (35/270)</td>
<td>0.6% (2/347)</td>
<td>-12.4% pts</td>
</tr>
<tr>
<td>E</td>
<td>50.9% (180/354)</td>
<td>0.7% (2/277)</td>
<td>-50.1% pts</td>
</tr>
</tbody>
</table>

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Can we apply this strategy to Hepatitis B?
scLCM HBV Team

• Chloe L. Thio
• Hyon (John) Hwang
• Tanner Grudda
• Jeff Quinn
• Yasmeen Saad
• Michael Murphy
• Katie Ward
• Bill Osburn
• Richard Sterling (HBRN)
• Ruy M. Ribeiro
• Alan S. Perelson
• Mark S. Sulkowski
HBV replication cycle
• 20,000 droplets with individual PCR reactions
• Fraction of positive droplets analyzed using Poisson statistics to determine concentration in original sample
  • Positive control: 7SL
  • Negative control: PEN membrane
  • Previously published primers
    Werle-Lapostolle et al. Gastroenterology. 2004 Jun;126(7):1750-8

Total HBV DNA – LLOD 2-3 cp/cell
cccDNA – LLOD 2-3 cp/cell
pgRNA – LLOD 5-6 cp/cell
Total intracellular hepatitis B virus (HBV) DNA in five HIV/HBV co-infected persons receiving dually-active antiretroviral therapy (DAART)
Can we use these tools to characterize HBV longitudinally?
### Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>HB6</th>
<th>HB2</th>
<th>HB7</th>
<th>HB3</th>
<th>HB4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yrs</strong></td>
<td>53</td>
<td>46</td>
<td>47</td>
<td>28</td>
<td>51</td>
</tr>
<tr>
<td><strong>CD4+ T cell count, cells/ul</strong></td>
<td>655</td>
<td>153</td>
<td>299</td>
<td>390</td>
<td>399</td>
</tr>
<tr>
<td><strong>HIV RNA (cp/ml)</strong></td>
<td>20</td>
<td>241-358</td>
<td>UD</td>
<td>54</td>
<td>UD</td>
</tr>
<tr>
<td><strong>HBV DNA (log_{10} IU/ml) at first biopsy</strong></td>
<td>8.48 (~2 weeks prior to biopsy)</td>
<td>8.56 (~2 weeks prior to biopsy)</td>
<td>4.15 (~3 weeks prior to biopsy)</td>
<td>4.55 – 5.66 (~6 months prior to biopsy)</td>
<td>1.60</td>
</tr>
<tr>
<td><strong>HBV DNA (log_{10} IU/ml) at second biopsy</strong></td>
<td>1.63 (~2 weeks prior to biopsy)</td>
<td>UD (~2 weeks prior to biopsy)</td>
<td>2.89 (~3 weeks prior to biopsy)</td>
<td>2.93 (~2 weeks prior to biopsy)</td>
<td>UD (~3 weeks prior to biopsy)</td>
</tr>
<tr>
<td><strong>Antiretroviral therapy (ART) (HBV-active nucleos(t)ides are in bold)</strong></td>
<td>TDF/FTC/DTG/RPV/DRV/r</td>
<td>ABC/DTG/DRV/r/ETV</td>
<td>TDF/FTC/ATV/r</td>
<td>TDF/FTC/ATV/r</td>
<td>TDF/FTC/DRV/r/RAL</td>
</tr>
<tr>
<td><strong>Duration of HBV-active ART</strong></td>
<td>2 weeks</td>
<td>Stopped TDF 11 mos before bx</td>
<td>3.5 years but intermittent compliance</td>
<td>4 years but intermittent compliance. Restarted ART 7 mos before bx</td>
<td>7 years (adherent ~1 year)</td>
</tr>
<tr>
<td><strong>Interval between biopsies, yrs</strong></td>
<td>3.6</td>
<td>3.6</td>
<td>3.7</td>
<td>2.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>

All participants were male, African-American, and HBeAg+. UD – undetectable; TDF – tenofovir disoproxil fumarate; FTC – emtricitabine; DTG – dolutegravir; RPV – rilpivirine; ABC – abacavir; DRV/r – darunavir boosted with ritonavir; ARV/r – atazanavir boosted with ritonavir; RAL - raltegravir
Changes in the viroscape with time
Change in proportion of infected hepatocytes between biopsies

Years of treatment with NUCs

Balagopal, JCI Insight, 2020 (accepted)
Change in proportion of infected hepatocytes between biopsies

Years of treatment with NUCs

Balagopal, JCI Insight, 2020 (accepted)
Transcription normalized to cccDNA decreases with NUCs
cccDNA stable between biopsies
Changes in the viroscape with time
Changes in the viroscape with time

The diagram illustrates changes in the viroscape over time, with various markers indicating levels of HBV DNA and pgRNA. The data is presented for different time points (years), showing trends and fluctuations in viral load. The legend provides information on the median pgRNA [log10 copies/cell] for different groups, such as Bx1 and Bx2.
HBV replication cycle

- HBV virion (with dsiDNA)
- HBV virion (with rcDNA)
- NTCP
- Attachment
- Secretion
- Endocytosis
- Uncoating
- Nuclear import
- Double stranded linear DNA (dsiDNA)
- Relaxed circular DNA (rcDNA)
- DNA repair
- DNA integration
- Integrated HBV DNA (IDNA)
- Minichromosome (cccDNA)
- Transcription
- 0.7 kb RNA
- 3.5 kb RNA
- Pre-core/Core mRNA
- Pregenomic RNA (pgRNA)
- Capsid Formation
- Core
- Polymerase
- pgRNA
- Capsid Formation
- Reverse Transcription
- NUC
- ~10%
- ~90%
- Capsid Formation
- HBsAg
- LHBs
- MHBs
- SHBs
Viroscapes and Immunohistochemistry show similar findings of diminished HBV gene expression.
Viroscapes and Immunohistochemistry show similar findings of diminished HBV gene expression
Summary

- Hepatocytes remain infected with long-term treatment
- There is heterogeneous HBV infection and transcription, especially during treatment
- Transcriptionally regulation of HBV during NUCs
  - Proportion increases with treatment duration
  - Possible reservoir for reactivation
  - Difficult to eliminate with immunotherapy
  - Mechanism unclear
- Liver biopsies and in situ analysis are an important approach for understanding the HBV lifecycle
  - Especially during therapy (NUCs or emerging therapy)
Thank you

**Center for Viral Hepatitis Research (JHU)**
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- John Hwang
- Jeffrey Quinn
- Jaiprasath Sachithanandham
- Hyon (John) Hwang
- Tanner Grudda
- Ken Bowden
- Katie Ward

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