Longitudinal proviral sequencing provides a window into selection pressures

Una O’Doherty
July 20 2019

HIV HBV pre IAS meeting
Successful antiviral therapy revealed the HIV reservoir

How can we measure the reservoir when viral loads are undetectable?
Near full-length single genome proviral sequencing

1. PCR #1
2. Screen for Positives by qPCR
3. PCR #2
4. Screen for Positives and purify DNA
5. DNA fragmentation/ligation of adapters at limiting dilution, library preparation
6. Sequence on Illumina MiniSeq
7. De novo Assembly
8. Determine **Intact Sequences** by presence of:
   - psi packaging element
   - 9 complete ORFs
   - critical splice sites

Ho Cell 2013
Bruner Nat Med 2016
Imamichi PNAS 2016
Heiner Cell Reports 2017
Lee JCI 2017
Decay estimates by proviral sequencing

Cannon (in preparation)

**Absolute number of proviruses/CD4**
* the % intact
Analysis of defective proviruses suggests two forces

Pinzone Nat.Comm. 2019
Why might D1+ proviruses contract over time?

D1 splicing may enhance protein expression by placing the 5’UTR next to an ORF

<table>
<thead>
<tr>
<th>Provirus</th>
<th>HIV RNA Copies/Cell</th>
<th>GFP+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>36000</td>
<td>42</td>
</tr>
<tr>
<td>D1+D4-</td>
<td>13000</td>
<td>31</td>
</tr>
<tr>
<td>D1-D4+</td>
<td>7800</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Weissman and Pinzone
Why would D1-D4+ proviruses expand more than others?

![Diagram showing steps: Infect, Cultivate 7 d, RNA sequence]

Table 1: Aberrant splice occurs more often from D4 splice site than D1

<table>
<thead>
<tr>
<th>Donor splice site</th>
<th>D1</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proper splicing</td>
<td>568</td>
<td>27</td>
</tr>
<tr>
<td>Aberrant splicing</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>
Selection against proviral DNA is stronger in first years.
Clonal contraction and expansion in Tem

Why do D1-D4+ increase?

"Contribution of naïve CD4+ T cells to the intact HIV reservoir”
Tuesday en Casa Montejo 4:30 pm. Venanzi-Rullo
Conclusions

Proviral contraction and expansion occur behind the scenes of the “stable” reservoir

Aberrant splicing may have a role in persistence

D1-D4+ proviruses capable of expression expands in Tem

Negative selection is more apparent during the first 6-7 years; this mirrors CD4 and CD4/CD8 constitution
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