Natural variation in HIV transcription in HIV-infected individuals on antiretroviral therapy

Professor Sharon R Lewin, FRACP, PhD, FAHMS
Global Virology Network, Annecy, France. Nov 28-30., 2018
Doctors raise alarm about ancient HTLV-1 virus: 'Prevalence is off the charts' in Australia

By Jacqueline Howard, CNN

Updated 0819 GMT (1619 HKT) May 8, 2018
Rapid rebound of HIV when ART is stopped
HIV persists on ART in multiple forms

Latent infection

T cell survival

T cell proliferation

Residual virus production

“active reservoir”
There is a spectrum of HIV transcriptional activity on ART: why?

**Productive infection**
- DNA positive
- RNA positive
- HIV protein positive
- DEATH

**Latent infection**
- DNA positive
- RNA negative
- HIV protein negative
- SURVIVAL
Variation in cell associated unspliced HIV RNA in HIV-infected individuals on ART

N=30 participants in a randomised clinical trial. B1 = screening; B2 = between screening and enrolment; B3 = day of administration of disulfiram

Elliott et al., Lancet HIV 2015
Does HIV transcription have a circadian rhythm?
Circadian Cycles

- CLOCK and BMAL1 heterodimers bind to an E-box to drive transcription
- Control is mediated through *Per* and *Cry* genes

Gekakis et al., Science 1998; Hogenesch et al., PNAS 1998; Kume et al., Cell 1999; Jin et al., Cell 1999
Why would this be relevant to HIV transcription?

• The HIV LTR has 4 E-box binding sites with two flanking the TATA bindings site

• E-boxes are palindromic sequence motifs (CANNTG) for basic helix-loop-helix (bHLH) class of DNA-binding proteins

• Important for the regulation of transcription of multiple retroviruses including HIV-1 and HTLV-1

• CLOCK proteins have intrinsic histone acetyl transferase activity and can mediate chromatin re-modelling through histone acetylation

• In individuals off ART, plasma HIV RNA has circadian variation

Terme et al., Retrovirology 2009; Ou et al., J Virol 1994; Doi et al., Cell 2006; Zeichner et al., Pathobiology 1996;
Visit related changes in expression of genes controlling circadian rhythms.
Increased cortisol and thyroid stimulating hormone at B3

Measurement of T-cell numbers, T-cell subsets, activation markers and H3 acetylation showed no change at the three baseline timepoints

Chang et al., AIDS 2018
Major effect of “visit” and modest effect of “time” on unspliced HIV RNA

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$, Intercept</td>
<td>0.80</td>
<td>0.33</td>
<td>0.016</td>
</tr>
<tr>
<td>$\gamma$, Time of blood collection</td>
<td>0.051</td>
<td>0.026</td>
<td>0.046</td>
</tr>
<tr>
<td>(per hour of day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_2$, Visit 2</td>
<td>0.10</td>
<td>0.07</td>
<td>0.119</td>
</tr>
<tr>
<td>$\beta_3$, Visit 3</td>
<td>0.62</td>
<td>0.10</td>
<td>$9 \times 10^{-10}$</td>
</tr>
</tbody>
</table>

Using a path analysis, we replaced the time of blood draw in the mixed effects model with the expression levels of the CLOCK-associated genes. BMAL1 was the only gene that had a statistically significant effect on log CA-US HIV RNA, with an effect size of 8.508 (SE 3.777, p-value = 0.028).
Evaluation of changes in HIV transcription over 24 hours

Prospective study of HIV-infected individuals on ART (n=17). Strict attention to lights, diet and other stimulants (University of Wisconsin - Madison)
Circadian variation of HIV RNA over a 24 hour period in individuals on ART

Circadian Activators

US HIV RNA

HIV DNA

Circadian Repressor
CLOCK/BMAL-1 together increase HIV transcription

Chang et al., AIDS 2018
Increase in HIV transcription initiation with CLOCK/BMAL-1 in J-Lat cell line
E-box2 is required for CLOCK/BMAL-1 mediated HIV transcription

Chang et al., AIDS 2018
Summary

- Natural variation in CA-US HIV RNA in individuals on ART with a clear relationship to time over a 24 hour period

- BMAL-1 was the only circadian gene that was associated with both CA-US HIV RNA and time.

- BMAL-1/CLOCK forms a heterodimer and activated HIV transcription through binding to the second E-box

- The CLOCK/BMAL-1 pathways could be exploited to identify new latency reversing agents (LRA) or potentially enhance activity of LRAs based on time of administration
Effect of stress on HIV transcription
Evaluation of stress: control and stress day

Control day

0-10 min | 11-25 m | 25-30 m | 31-65 m

Rest | Rest | Recovery 1 | Recovery 2

Trier Social Stress Test - Stress day

0-10 min | 11-15 m | 16-20 m | 21-25 m | 25-30 m | 31-65 m

Rest | Speech prep | Speech task | Math task | Recovery 1 | Recovery 2

N=25 HIV-infected individuals on ART, CD4 current = 637 cells/ul; nadir = 240 cells/ul
Evaluation of stress: Trier Social Stress Test

Physiological evaluation:
Heart rate variability = parasympathetic
• Respiratory Sinus Arrhythmia (RSA) = vagal tone

Impedance cardiography = sympathetic
• Pre-ejection period (PEP)
• Cardiac output

HIV evaluation
Virology:
• HIV DNA and US RNA
Immunology
• T-cell subsets
• Activation markers
TSST induces physiological stress

- **Salivary cortisol, mmol/L**
  - TSST Visit: Increases significantly at t=20 and t=30 with p<0.001.
  - Control Visit: No significant change.

- **Respiratory sinus arrhythmia, ln(ms)**
  - TSST Visit: Decreases significantly from Baseline to Speech with p=0.001.
  - Control Visit: No significant change.

- **Cardiac Output, L/min**
  - TSST Visit: Increases significantly from Baseline to Math with p=0.002.
  - Control Visit: No significant change.

- **Pre-ejection period, ms**
  - TSST Visit: Increases significantly from Baseline to Late Recovery with p=0.21.
  - Control Visit: No significant change.
Significant increase in unspliced HIV RNA with stress

No significant changes in T-cell subsets or activation following stress
Autonomic nervous system (ANS) changes associated with changes in unspliced HIV RNA but not HIV DNA

![Graph showing correlation between fold change in unspliced HIV RNA and pre-ejection period](image)

<table>
<thead>
<tr>
<th>ANS Measure</th>
<th>US RNA Spearman rho</th>
<th>US RNA P-value</th>
<th>DNA Spearman rho</th>
<th>DNA P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ejection Period (PEP)</td>
<td>-0.59</td>
<td>0.002</td>
<td>-0.09</td>
<td>0.68</td>
</tr>
<tr>
<td>Respiratory Sinus Arrhythmia (RSA)</td>
<td>-0.05</td>
<td>0.81</td>
<td>-0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>Cardiac Output (CO)</td>
<td>0.60</td>
<td>0.003</td>
<td>-0.02</td>
<td>0.94</td>
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Summary and implications

• Significant natural variation of cell associated unspliced HIV RNA in individuals living with HIV on ART with a clear effect of time and stress. This needs to be considered in the design of clinical trials of LRAs.

• The circadian transcription factors, CLOCK and BMAL1, upregulate HIV LTR-mediated transcription initiation and this upregulation requires an intact E-box 2 motif.

• Circadian proteins and or stress represent pathways that could potentially be exploited for latency reversal through development of novel drugs or optimising the timing of administration of LRAs.

• Other cell associated markers of RNA transcription such as multiply spliced RNAs may be better biomarkers of latency reversal in clinical trials.
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DARE to find a cure
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DARE
Delaney AIDS Research Enterprise
to find a cure