Epigenetic control of HIV latency

IAS HIV Cure & Cancer Forum
Paris, France

Jonathan Karn
22 July 2017
Paris, France
The HIV Reservoir is in a Pseudo-Steady State

**Viral Spread**
- Sanctuary sites
- Poor drug penetration

**Latently infected cells**
- Memory T-cells
- Microglial cells
- Macrophages

**Homeostatic Proliferation**
- Chronic inflammation
- Stem cell infection

**Episodic Reactivation**
- Viral Spread
- Poor drug penetration
- Sanctuary sites
Induction of HIV transcription in clones of latently infected T-cells is strictly dependent upon NF-κB.
Epigenetic regulation of genes

**ACTIVE EUCHROMATIN**

- RNAP II

**INACTIVE HETEROCHROMATIN**

- RNAP II

Nucleosome modification

- EZH2: H3K27
- EHMT2: H3K9
Disruption of the Tat/P-TEFb feedback leads to HIV proviral latency

**Activated CD4⁺ T-cells**
- Activator complex
- RNAP II
- Tat
- Transcribed provirus
- LTR
- ENL/AF9
- ELL1/2
- AFF1/4
- CycT1
- CDK9
- pT186
- pS175

**Initiation Factors**
- NFκB ↑
- NFAT ↑
- IncRNA ↓

**P-TEFb Activation**
- CycT1 ↑
- CDK9 pT186 ↑
- CDK9 pS175 ↑

**Quiescent CD4⁺ T-cells**
- Silencing complex
- Tat
- Silenced provirus
- LTR
- 7SK snRNP complex
- CDK9
- pS175
- MEPE
- LARP7
- HEXIM
- PRC2
- EHMT
- JARID2

**Epigenetic Silencing**
- HDACs ↑
- HKMTs ↑
- IncRNA ↑

**P-TEFb Inactivation**
- CycT1 ↓
- CDK9 pT186 ↓
- CDK9 pS175 ↓
Both EZH2 and EHMT2 (G9a) HKMT are required to maintain latency in the Th17 cell model of HIV latency.
EDITS Assay: Env Detection by Induced Transcript Sequencing

HIV provirus

Location of EDITS primers

env mRNA
1st Round Primers
2nd Round Primers

M2511 Raw Data

Maximum Likelihood estimate: 58.244028
95% Confidence Interval: (23.63, 143.53)

Background Subtracted/Cell per 10^6

Maximum Likelihood estimate: 50.88 + 2.16
95% Confidence Interval: (48.95, 52.81)
Both EZH2 and H3K9MTs are required to maintain latency in primary cells.
$T_{CM}$ cells are more highly restricted by histone methyltransferases than $T_{TM}$ or $T_{EM}$ cells.
Efficient reversal of latency in $T_{CM}$ cells by treatment with histone methyltransferase inhibitors
KDM6A (UTX-1) reverses PRC-2 repression

PRC-2

Di/tri-methylation of H3K27
Chromatin compaction

K27--me K27--m-me K27--m-me

Transcription OFF

De-methylation of H3K27
Chromatin relaxation

K27--ac K27--ac

Transcription ON

B. H3K27 demethylases
KDM6A (UTX-1) knockdown drives cells deeper into latency

A

Assay Design

Day 0
Naive T-cells

Day 7
Polarize and expand

Day 8
Infect with Nef+ virus

Day 12
Induce Latent HIV

± SAHA
+ IL-15
± GSKJ4

Flow Cytometry

B

Accelerated HIV silencing mediated by GSKJ4

Day 0

Day 4 (0 nM GSKJ4)

Day 4 (500 nM GSKJ4)

Day 4 (1 μM GSKJ4)

C

GSKJ4 blocks HIV reactivation

Untreated

SAHA (500 nM) + IL15 (10 ng/ml)

SAHA (500 nM) + IL15 (10 ng/ml)

SAHA (500 nM) + IL15 (10 ng/ml)

GSKJ4 (0 nM)

GSKJ4 (500 nM)

GSKJ4 (1 μM)
KDM6A (UTX-1) knockdown enhances DNA methylation (MeDIP Assay)
Epigenetic silencing is progressive and heterogeneous.

HIV provirus → Integration into activated gene

Transcribed provirus → Histone de-acetylation

Attenuated provirus → Histone Methylation

Latent provirus → DNA methylation

Permanently silenced provirus

Epigenetic silencing
Conclusions: Latency

• From a molecular perspective latency is an integral feature of the HIV life cycle
  - NF-κB/NFAT are only needed to initiate transcription from latent proviruses by reversing epigenetic blocks
  - 10 to 20% of infections are silent integration events imposed by histone methylation
  - Non-suppressed patients have latent proviruses
• Transactivation can be thought of as a way to turn on and off transcription in response to changes in the cellular environment
• Latency is probably an escape mechanism from immune responses and thus aids virus dissemination
  - Multiple epigenetic silencers contribute to HIV latency
  - PCR-2 and H3K9MTs both contribute to latency in primary cells
  - Silencing mechanisms differ between cell types (microglial cells do not utilize PCR-2)
Special Thanks to....

Curtis Dobrowolski (Th17, EDITS)

Kien Nguyen (Epigenetics)

Biswajit Das (Screens)