Analysis of HIV Proviruses in Clonally Expanded Cells In Vivo

Francesco R Simonetti[1-2], Jonathan Spindler[1], Xiaolin Wu[3], Shawn Hill[1], Wei Shao[4], Stephen Hughes[1], Mary F Kearney[1], Frank Maldarelli[1], John Mellors[5], John Coffin[6].

1. HIV Dynamics and Replication Program, National Cancer Institute, National Institutes of Health, Frederick, MD; 2. Department of Biomedical and Clinical Sciences Luigi Sacco, University of Milan, Italy; 3. Leidos Biomedical Research, Frederick, MD; 4. Advanced Biomedical Computing Center SAIC Frederick, Frederick National Laboratories for Cancer Research, Frederick, MD; 5. University of Pittsburgh, Division of Infectious Diseases, Pittsburgh, PA; 6. Tufts University, Department of Molecular Biology and Microbiology, Boston, MA.

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ABSTRACT #337

BACKGROUND

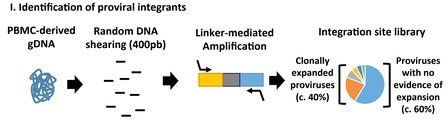
The mechanisms of HIV persistence during cART remain only partially understood. Recently, we and others demonstrated that clonally expanded populations of HIV infected cells persist during prolonged cART, and that many proviruses were integrated in genes associated with cell growth, suggesting integration may strongly influence both clonal expansion and cell persistence during cART (1, 2). Most of HIV proviruses are defective, due to the integration of HIV genomes containing errors occurred during reverse transcription (3, 4). As current techniques that detect insertion sites only yield short sequences of HIV LTRs, methods aimed to characterize individual proviral structures require detailed amplification and sequencing strategies. Here we describe characterization of a series of patient-derived HIV proviruses, in order to investigate whether they could affect gene expression due to the presence of intact promoters, splicing donors and intact *tat/rev*.

MATERIALS AND METHODS

Longitudinal PBMC samples were obtained from participants with chronic HIV infection enrolled on a long term study of HIV persistence during cART (NIH study 08-I-0221). Genomic DNA was extracted from total PBMCs or from CD4+ cells enriched by negative selection. Integration sites were determined from randomly sheared DNA as previously described (1). Proviruses were selectively amplified using specific primers designed on the human genome sequence surrounding the integration site (below).

Sequence were analyzed in order to identify APOBEC3 G/F hypermutation, stop codons, frameshifts and deletion. LTR and *gag* leader sequences were aligned to study promoter and splicing donor regions.

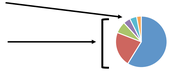
I. Identification of proviral integrants



Identifies insertion site, host gene, but only SHORT HIV LTR sequence

II. Selection of expanded proviral integrants for analysis:

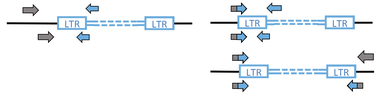
Orientation-dependent *MKL2* Highly expanded *AMBI-1* *HORMAD-2*



III. Amplification Strategy

A. Host primer: HIV Primer

B. Hybrid Host/HIV primer: HIV Primer



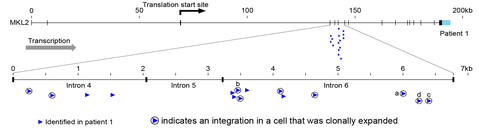
IV. Determination of provirus sequence

HIV Proviruses in Clonally Expanded Populations

Patient 1

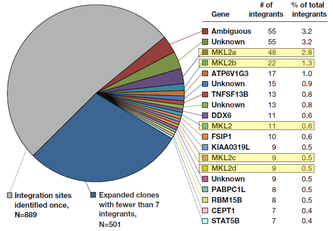
Highly Expanded Clones in *MKL2* gene (CHR 16): Orientation Dependent and Defective BUT LTR Promoter and Major Splice Donor Elements INTACT

MKL2 Insertions

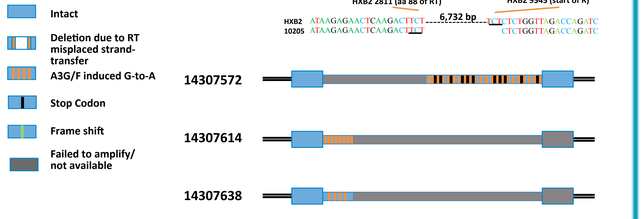


IS position Chr 16	MKL2 hits	LTR	GAG	POL	ENV	NEF	Replication Competent	Tat/rev
14307572	1	Intact Promoter	NA	Hyper Mut	Hyper Mut	Hyper Mut	NO	NA
14307614	1	Intact Promoter	Hyper Mut	NA	NA	NA	NO	Stop codons
14307638	26	Intact Promoter	Hyper Mut	FAILED	FAILED	FAILED	NO	NA
14307781	1	Intact Promoter	OK	FAILED	FAILED	FAILED	?	NA
14308320	6	Intact Promoter	Hyper Mut	FAILED	Hyper Mut	Hyper Mut	NO	Stop codons
14310205	49	Intact Promoter	Frame Shift	PARTIAL	MISSING	MISSING	NO	DELETED
14310594	11	Intact Promoter	OK	FAILED	FAILED	FAILED	?	NA

MKL2 Integration site Frequency



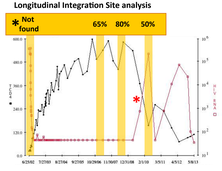
Gene	# of integrants	% of total integrants
Ambiguous	55	3.2
Unknown	55	3.2
MKL2a	48	2.8
MKL2b	22	1.3
ATP6V1G3	17	1.0
Unknown	15	0.9
TNFSF13B	13	0.8
Unknown	13	0.8
DDX6	11	0.6
MKL2	11	0.6
FSIP1	10	0.6
KIAA0319L	9	0.5
MKL2c	9	0.5
MKL2d	9	0.5
Unknown	9	0.5
PABPC1L	8	0.5
RBM15B	8	0.5
CEPT1	7	0.4
STAT5B	7	0.4



Patient 2

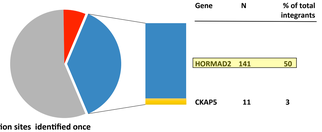
Highly expanded clone with a solo LTR in *HORMAD2* gene

Longitudinal Integration Site analysis

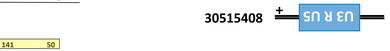


Rebound in viremia due to cART interruption

Integration sites



Chr22: nt position (*HORMAD2* intron)

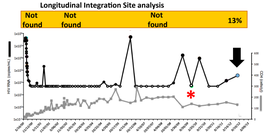


∗The solo LTR was identified in pre-therapy samples, suggesting early initiation of clonal expansion

Patient 1

Intact and infectious Provirus

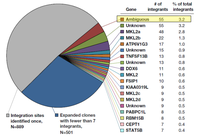
Longitudinal Integration Site analysis



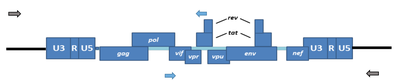
Single copy AMBI-1 detected in plasma

- Low level viremia despite adequate cART
- Highly expanded clones detected
- Most highly expanded clone integrated in ambiguous site in human genome

Integration sites

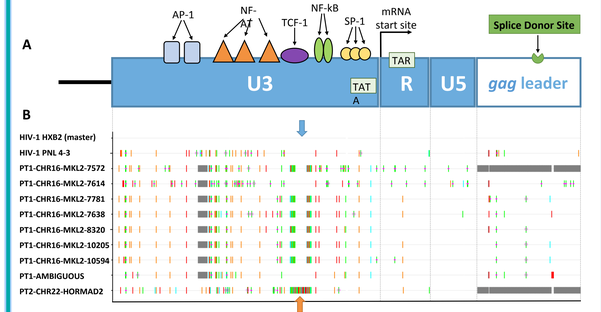


AMBI-1 provirus



- Intact provirus detected
- HIV reconstructed from overlapping PCR-amplified fragments
- Infectious in *in vitro* assays
- Identical clone recovered by in vitro cultivation of patient derived PBMC

LTR and *Gag* Leader Sequences from HIV Integrants in Expanded Clones



TCF-1 duplication in 6 out of 7 *MKL2* integrants

TCF-1 duplication followed by a 25bp insertion

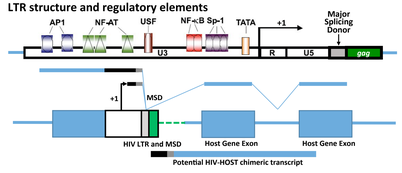
A. LTR map showing promoter region and major splicing site; **B**. Highlighter plot showing LTR sequences from integrants. HIV-1 HXB2 used as master sequence; PNL 4-3 used a reference. A: Green, T: Red, G: Orange, C: Light blue, Gaps: Gray, Circle: Apobec, Diamond: G>A

RESULTS

Eleven integrants were characterized

- A highly expanded clone in an ambiguous region of the human genome showed a full-length intact sequence and was proved to be replication competent by transfection and viral out-growth experiments (5).
- One of the proviruses had a large *pol*-U3 internal deletion, likely being the result of reverse transcriptase misplaced jumping before integration.
- Seven integrants in *MKL2* gene were characterized.
  - 4 proviruses showed evidence G-to-A mutation leading to multiple stop codons
  - 3 were found to lack intact *tat* or *rev*. All seven, however, had intact LTR promoter elements and retained the major splice donor sequence.
- Proviral sequence analysis of the HORMAD2 integrant revealed a 675 nt single LTR with intact promoter elements.
  - The HORMAD2 integrant was present both pre- and on-cART
  - accounted for 50-80% of all of the infected cells after 7-8 y on cART, implying that expansion of the clone started early in infection, likely long before cART initiation.
  - HORMAD2 integrant was already present pre-therapy as a solo-LTR in an expanded clone.
- Another solo LTR was characterized in patient 1 in the X chromosome, integrated in another highly expanded clone (3% of IS).

LTR structure and regulatory elements



SUMMARY

- HIV infected cells undergo clonal expansion in vivo
- Proviruses in expanded clones may be:
  - Intact
  - Hypermutated
  - Partially deleted
  - Consisting of a solo LTR.
- MKL2 proviruses, have intact LTR regulatory elements but some lack *tat*, which is normally required for HIV transcription.
- Further studies will address how the presence of the LTR promoter region and MSD can alter the expression of genes, affecting the half-life, proliferation and persistence of infected cells.

REFERENCES

1. Maldarelli F et al., Science 2014
2. Wagner T et al., Science 2014
3. Ho Y-C et al., Cell 2013
4. Cohn L et al., Cell 2015
5. Simonetti FR et al., PNAS, 2016

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