Integration Analysis of Latently HIV Infected Cell Lines: Evidence of Ongoing Replication

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Introduction

HIV cure is limited by persistence of long lived latently infected CD4$^+$ T cells. Latently infected cell lines are widely used *in vitro* to study HIV latency. We identified and tested the stability of HIV integration sites in latently infected cell lines, obtained from NIH AIDS reagent program, using a newly developed high throughput method.

Aims

In this study we aimed to:

1. Develop a high throughput HIV integration site analysis method.
2. Determine HIV integration sites in latently infected cell lines frequently used in HIV latency studies to determine sensitivity and specificity.

Methods 1 development high through put method

HIV latently infected cells were obtained from NIH (table 1) and were passed 10 times in a 1:6 dilution. 150,000 cells from passage 0,2,4,6,8 and 10 were analysed for HIV integration sites by robotic processing (figure 1). HIV integration sites were called (figure 2).

Table 1. Cell lines obtained from NIH AIDS reagent program. Cells were divided in cells infected with a replication non-competent or replication competent virus.

Cell line	Replication competent		# copies reported
J-Lat 8.4	No	frameshift env	1
J-Lat 9.2	No	frameshift env	1
J-Lat 10.6	No	frameshift env	1
J-Lat 15.4	No	frameshift env	1
J-Lat tat-GFP 8.2	No	LTR-tat-GFP	1
J-Lat tat-GFP A1	No	LTR-tat-GFP	1
J-Lat tat-GFP A7	No	LTR-tat-GFP	1
J-Lat tat-GFP H2	No	LTR-tat-GFP	1
J-Lat tat-GFP H72	No	LTR-tat-GFP	1
U1.1	Yes	mutation in tat	2
J1.1	Yes	defective T-cell signalling	1
ACH2	Yes	mutation in tar	1

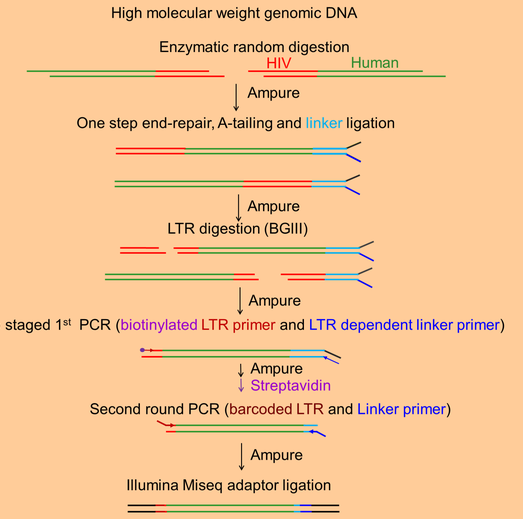


Figure 1. Schematic overview of high throughput HIV integration site analysis method suitable for robotic processing.

Method 2 Calling an integration site

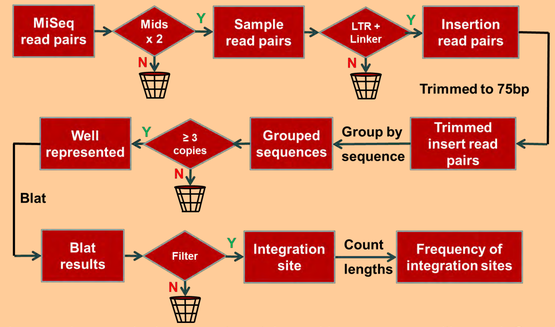


Figure 2. The miseq reads are paired and must contain a mid sequence on either end. Sample read pairs are checked for LTR sequence and linker sequence. The sequence of the insertion read pairs after LTR or linker are trimmed to 75 base pairs. The trimmed insert read pairs are grouped according to 97% sequence match with the exact same sequence length. Chromosomal alignment is determined using the Blat-UCSC Genome Browser (GRCH38/hg38). An HIV integration site is called if the results have ≥10 reads, and the frequency is determined by a length difference of ≥2 nucleotides

Methods 3 Residual replication in ACH-2 cells

Whole proviruses of ACH-2 cells were deep sequenced by a two long fragment PCR (figure 3) and sequenced by internal HIV primers. Mutations were scored if the mutation was >1% of the total reads.

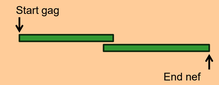


Figure 3. Schematic overview sequence strategy of ACH-2 provirus.

ACH-2 cells were passed 9 times from passage 4 to 12 with and without 1 µM integrase inhibitor in a 1:10 dilution (figure 4). 150,000 cells from each passage were assessed for HIV integration sites and a million cells were analysed for 2-LTR circles.

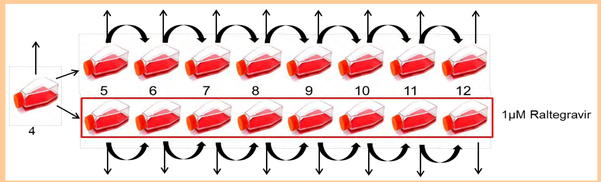


Figure 4. Schematic overview of passage experiment of ACH-2 cells.

Results 1 Stability of HIV integration sites

During the ten passages the number of unique integration sites in ACH-2 cells tended to increase, whereas it remained stable in J1.1 cells and tended to decrease in U1 cells by linear regression (figure 5).

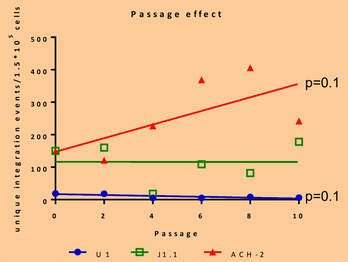


Figure 5. Passage effect of HIV integration sites in latent cell lines infected with a replication competent HIV. Unique integration sites per 150,000 cells. Statistical significance was analysed by linear regression models.

Results 2 Stability of HIV integration sites

We analysed the HIV integration sites that are always present during the ten passages. The HIV integration site in all the J-Lat cell lines remained single and stable; the detected integration sites represented 100% of the total events detected. Whereas in J1.1, U1 and ACH-2 multiple sites were continuously detected (table 2).

Table 2. HIV integration sites always present during the ten passages.

Cell line	Replication competent HIV	Chromosome	Position	Gene	Gene orientation	HIV orientation	% events
J-Lat 8.4	NO	1	77946384	FUBP1	-	-	100%
J-Lat 9.2	NO	19	46381104	PPP5C	+	+	100%
J-Lat 10.6	NO	9	136468579	SEC16A	-	+	100%
J-Lat 15.4	NO	19	34441293	UBA2	+	+	100%
J-Lat tat-GFP 82	NO	10	39936068			+	100%
J-Lat tat-GFP A1	NO	X	34073326			-	100%
J-Lat tat-GFP A72	NO	2	171821429	SLC25A12	-	+	100%
J-Lat tat-GFP H2	NO	X	45038538	KDM6A	+	-	100%
J1.1	YES	11	685243	DEAF1	-	-	~35%
		12	54257973	CBX5	-	-	~37%
U1	YES	2	48177527	AC079807.4	-	+	~35%
		X	38811467			-	~50%
		19	34452847	UBA2	+	+	~4%
ACH-2	YES	7	33019791	NT5C3A	-	-	~35%
		9	128111651	SLC25A25-AS	-	-	~11%

Results 3 Residual replication in ACH-2 cells

Deep sequencing of the provirus in ACH-2 demonstrated multiple polymorphisms indicative of reverse transcriptase activity (figure 6).

Mutation type	Number of mutations
Transition	282
Transversion	229
Insertions	820
Deletions	473

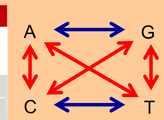


Figure 6. Number of polymorphisms that exceed >1% of the total reads at a particular nucleotide position.

ACH-2 cell cultured with raltegravir demonstrated more 2-LTR/ million cells circles in the first passages (figure 7a). During passaging the number of unique HIV integration sites reduced significantly faster with raltegravir (p = 0.008)(figure 7b).

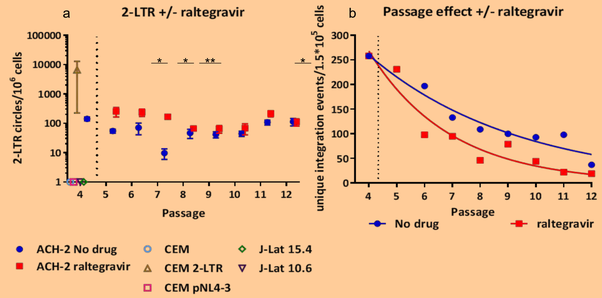


Figure 7 a. 2-LTR circles per million cells during passaging with and without 1µM raltegravir.

Figure 7 b. Unique integration sites per 150,000 ACH-2 cells .

Conclusions

- Our high throughput assay for integration sites is suitable for robotic processing.
- Cell lines infected with replication competent HIV have multiple unique HIV integration sites, not consistent with latent infection.
- The increase in 2-LTR circles in the presence of raltegravir and change in HIV integration sites observed in ACH-2 cells over time are consistent with low level replication.
- These findings have implications for the use of some latently infected cell lines as models of HIV latency.