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 2,4,6,8 and 10 were analysed for HIV integration sites by robotic processing (figure 1). HIV integration sites were called (figure 2).

Methods 2 Calling an integration site

Figure 2. The mix 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 BAli-SCUC Genome Browser (GRCm38/hg38). An HIV integration site is called if the results have 210 reads, and the frequency is determined by a length difference of 52 nucleotides.

Methods 3 Residual replication in ACH-2 cells

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

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).

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 2-J1.1 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).

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).

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.