HIV Integration Sites in Cellular Models of Viral Persistence

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Background

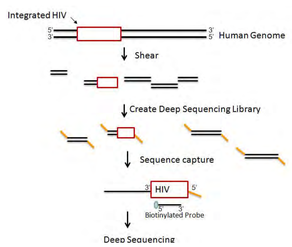
- The location of HIV integration is thought to play a critical role in the clonal expansion of infected cells and HIV persistence.
- Cell culture models are often used to study and assess HIV latency and latency-reversing agents, but it remains unclear how these models reflect HIV integration *in vivo.*
- We have developed a high-throughput sequence-capture assay to evaluate the integration site characteristics of four cellular models.

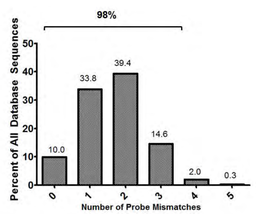
Objectives

- Develop sequence-capture assay for integration site analysis.
- Evaluate HIV integration site characteristics in four cellular models.
- Compare integration site characteristics between cellular models and in comparison to previously published datasets.

Methods

- Integration site analysis was performed on ACH-2 cells, J-Lat cells, Bcl-2 transduced primary CD4+ cells, and cells from the cultured T_{CM} model.
- A clonal population of ACH-2 cells was also included after limiting dilution and expansion with ART.
- Sequence enrichment was performed with a diverse biotinylated probe library (52 consensus probe sequences designed using LANL).

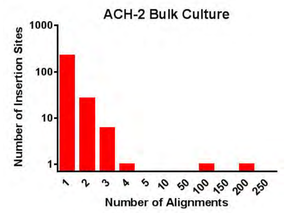


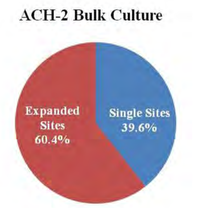


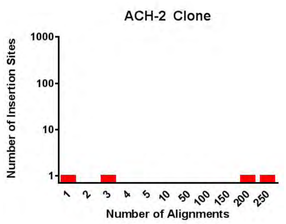
- Libraries were sequenced on the MiSeq (Illumina).
- Sites were annotated with the UCSC canonical gene annotations and gene ontology analysis performed using the Gene SeT Analysis Toolkit.
- Genes with viral integrants from the cellular models were compared to highly represented genes from two previously published patient datasets (Maldarelli et al. and Wagner et al.).
- Median and range values of integration site characteristics (e.g. percent intron) were determined using all applicable reads for each participant.

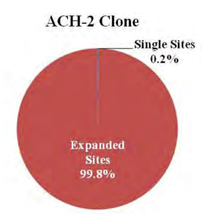
Results

Result #1: Integration Site Analysis of ACH-2 Bulk and Clonal Populations



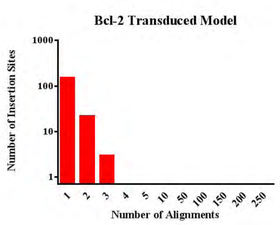


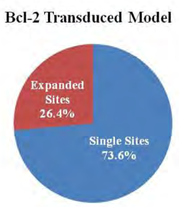


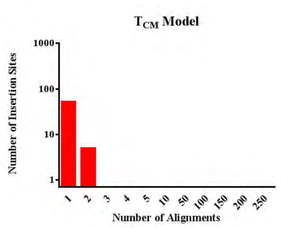


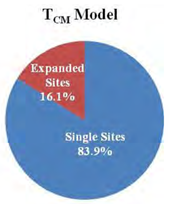
- We identified two major viral integration sites (chr7 and chr9) in ACH-2 cells.
  - 47% of reads were outside of these two major integration sites.
- Integration site analysis of the clonal ACH-2 population showed near elimination of single integration sites (ACH-2 bulk culture vs. ACH-2 clone: 39.6% vs. 0.2%, Fisher's P <0.01).

Result #2: Integration Site Analysis of the Bcl-2 Transduced CD4+ Model and the T_{CM} Model









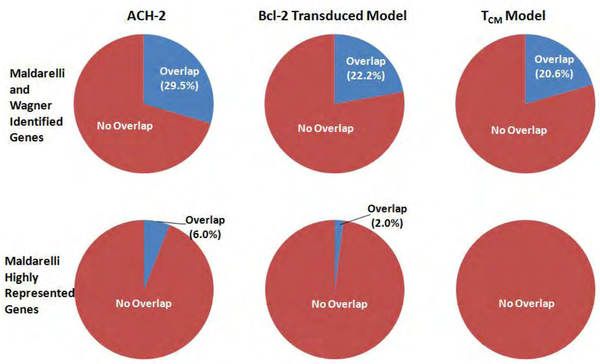
- Both models contained no dominant integration sites.

Table 1: Integration Site Analysis

Samples	Unique Integration Sites		Genomic Region		Genomic Annotation		HIV Integration Orientation Relative to Human Genes	
	% Single Sites	% Expanded Sites	% Intergenic	% Genic	% Intron	% Exon	% Same Orientation	% Opposite Orientation
ACH-2	40	60	12	88	86	14	64	36
Bcl-2 Transduced Model	74	26	18	82	98	2	52	48
T_{CM} Model	84	16	39	61	97	3	38	62
Patients*	70 (53-94)	30 (6-47)	18 (13-56)	82 (44-87)	93 (58-98)	7 (2-42)	35 (23-56)	65 (44-77)

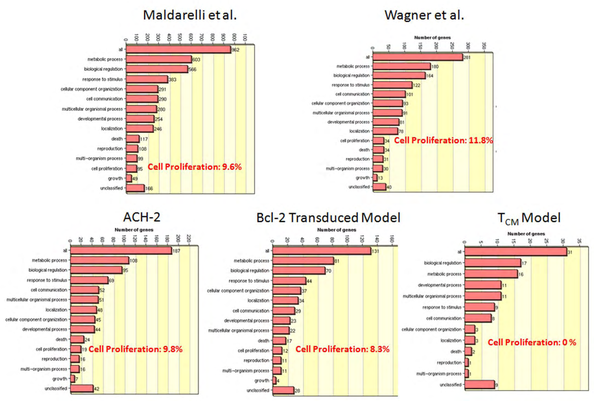
- There were significant differences between the cellular models in the proportion of clonally expanded integration sites identified within the human genome (X^2 P <0.01).
- The ACH-2 model had the highest percentage of integration sites located in exons and in the same orientation as the host gene (X^2 P<0.01 for both comparisons).
- ACH-2 and Bcl-2 models had a similar proportion of integration sites in genic regions, but a lower proportion was detected in the T_{CM} model (X^2 P<0.01).

Result #3: Gene Overlap Between Previously Described Patient Samples and Cellular Models



- Compared to all of the genes identified in patient samples from published datasets, approximately 20-30% of genes were also found in ACH-2, the Bcl-2 transduced model and the T_{CM} model.
- When comparing the genes with viral integrants in these cellular models to highly represented genes from the human studies, there were relatively few genes that overlapped.

Result #4: Gene Ontology Analysis



- ACH-2 and the Bcl-2 transduced model cells had viral integration in genes associated with cellular proliferation at similar percentages to the previously published patient datasets; T_{CM} model cells revealed no viral integrants in these genes.

Conclusions

- Using ACH-2 cells, we demonstrated that this sequence-capture assay has an extremely low false positive rate.
- Large numbers of minority integration sites in the ACH-2 cell line likely represents low-level viral replication.
- Integration site differences exist amongst commonly used cellular models of HIV persistence and in comparison to integration sites found in patient samples.
- Gene ontology analysis of highly represented genes from the patient samples found little overlap with HIV-containing genes from the cell lines.
- These results may contribute to differences seen in response to latency-reversing agents amongst cellular models and patient-derived cells.

Acknowledgements

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