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In vitro model for studying T cell proliferation/survival driven by HIV-1 integration

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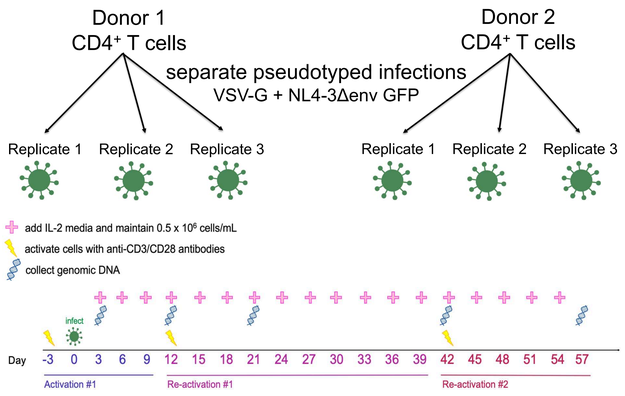
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BACKGROUND

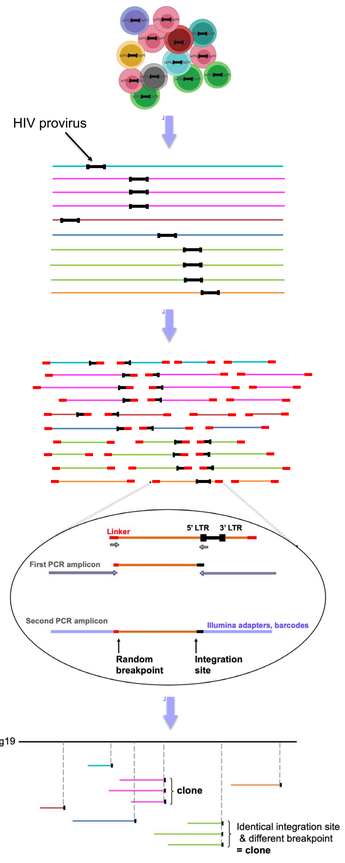
Recent evidence indicates the latent reservoir can be maintained through clonal expansion of T cells containing proviruses in specific introns [1-2]. Prominent clusters of integration sites in some of these introns imply that HIV integration can regulate gene expression to drive cell survival or proliferation.

METHODS

In vitro system for primary cell infection and expansion



Integration site analysis reveals clonal expansion



Extract genomic DNA from infected cell cultures.

Fragment DNA and ligate linker sequences.

PCR enriches for fragments containing linker and HIV LTR.

Nested PCR adds adaptors and barcodes for sequencing.

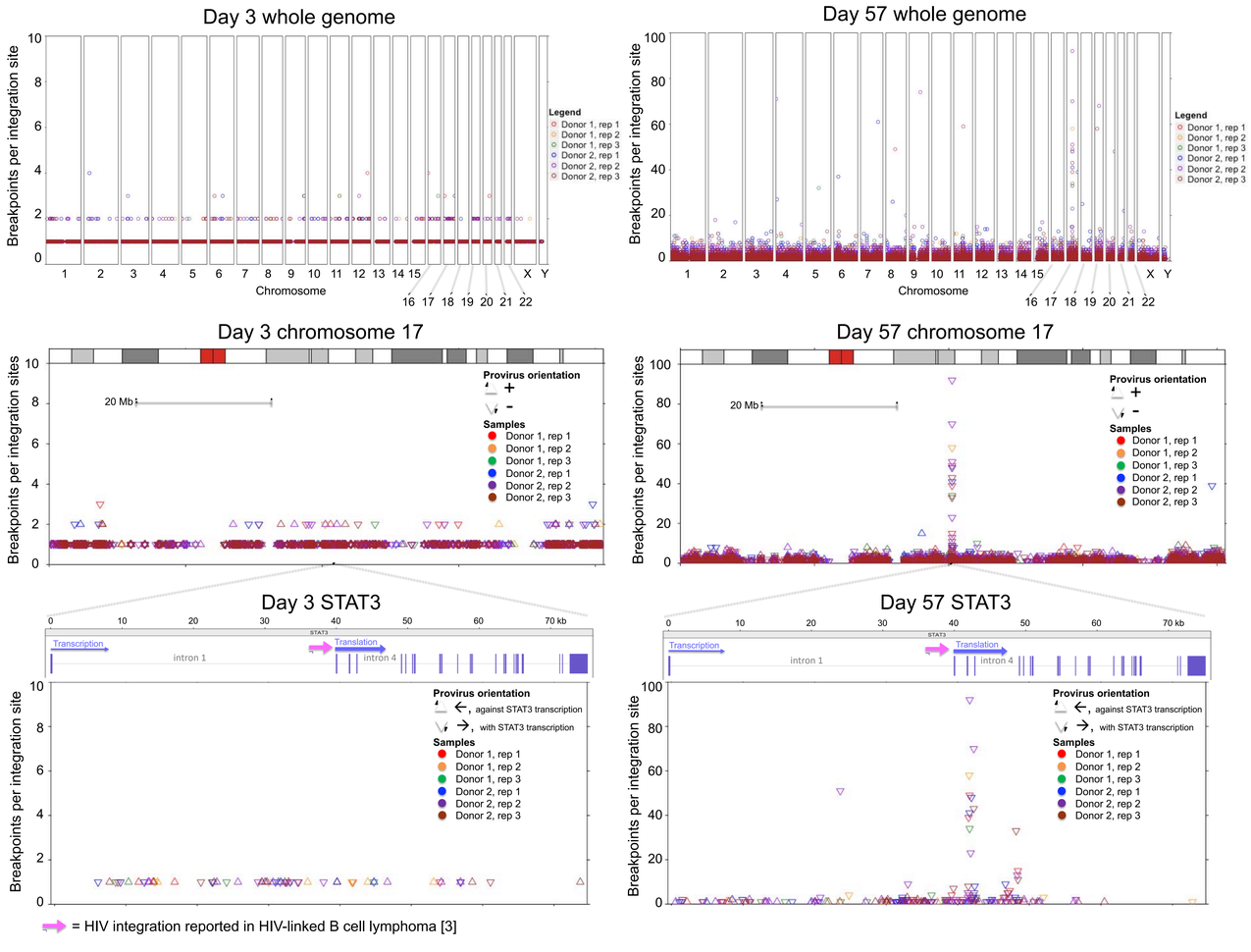
Collapse PCR amplicons, align to reference, and quantify clonal expansion.

Does HIV integration site play a role in infected cell persistence?

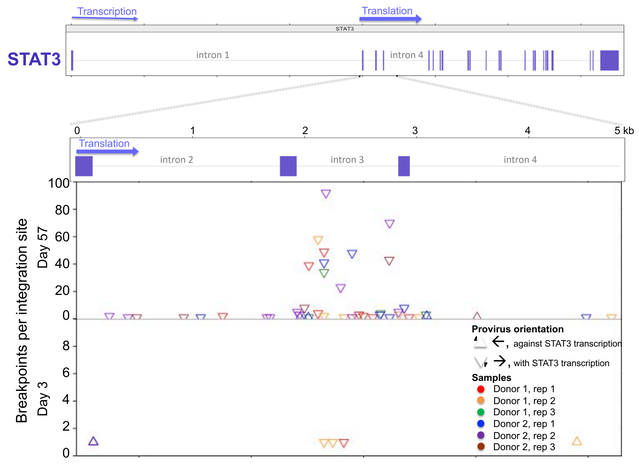
- HIV can drive cellular proliferation and/or survival in primary cell cultures.
- HIV provirus perturbation of STAT3 expression is associated with selective clonal outgrowth.

RESULTS

Prominent clusters of integration sites in STAT3 intron 3 associated with extensive clonal expansion at day 57 compared to the broadly distributed integrations from day 3. All 6 replicates from 2 donors contained HIV integrations in the 1kb intron 3 region that significantly clonally expanded, with strong selection for proviral orientation in the direction of STAT3 transcription. From our *in vitro* system we detected 54,511 total unique integration sites in the day 3 samples, and 226,355 total unique integration sites in the day 57 samples.



HIV integrations associated with clonal expansion cluster in intron 3 of STAT3



CONCLUSIONS

- Primary cells harboring specific HIV proviruses can selectively expand in culture and be tracked with integration site analysis.
- HIV integrations in BACH2 and MKL2 did not associate with clonal expansion.
- HIV integrations in intron 3 of STAT3 strongly associated with clonal expansion of repeatedly activated T cells.

FUTURE WORK

- Confirm STAT3 as a hot spot for HIV integrations associated with clonal expansion with more donors.
- Perform this *in vitro* infection and expansion experiment with other cell models such as B cells.
- Investigate the effect on STAT3 expression that the observed HIV integrations in intron 3 have.
- Study the connection between altered STAT3 expression and cellular proliferation/survival.
- Explore the possibility of HIV provirus association with lymphoma.

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