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Preview

Measuring the size and decay dynamics of the HIV-1 latent reservoir

Runxia Liu,[1] Allison A. Catalano,[1,2] and Ya-Chi Ho[1,*]
[1]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06519, USA
[2]Department of Epidemiology of Microbial Diseases, Yale University School of Public Health, New Haven, CT 06519, USA
*Correspondence: ya-chi.ho@yale.edu
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Measuring HIV-1 latent reservoir is essential for HIV-1 cure strategies. Levy et al.[1] developed a multiplex droplet digital PCR (ddPCR) assay—5-target intact proviral DNA assay—to detect multiple regions of HIV-1 proviral genome and increase accuracy.

HIV persists in memory CD4$^+$ T lymphocytes as a stably integrated provirus in the genome. Although antiretroviral therapy (ART) prevents new rounds of infection, ART does not kill existing infected cells, allowing HIV-1 latent reservoirs to persist lifelong. HIV-1-infected cells hide from immune recognition during latency and contribute to high levels of viremia once ART is discontinued. The HIV-1 latent reservoir is the major barrier to cure.

Accurate measurement of the size of the latent reservoir is essential for developing HIV-1 cure strategies. The latent reservoir is defined as cells harboring intact HIV-1 proviruses while cells harboring defective proviruses are not latent reservoirs. HIV-1 defective proviruses outnumber intact proviruses: ~1–100 per million CD4$^+$ T cells harbor intact HIV-1 proviruses, while ~1,000–10,000 per million harbor defective proviruses.[2] HIV-1 DNA qPCR amplifies both intact and defective proviruses and therefore reflects the frequency of defective proviruses, not the latent reservoir.

HIV-1 RNA-based assays measure inducible HIV-1 but not necessarily intact HIV-1

Previously, the gold standard of HIV-1 latent reservoir measurement was the quantitative viral outgrowth assay (QVOA), which measures the frequency of CD4$^+$ T cells harboring replication-competent HIV-1 that can generate exponential viral outgrowth upon *ex vivo* maximum T cell activation.[3] However, QVOA is labor-intensive, low-throughput, and time-consuming. Assays that measure HIV-1 RNA expression upon *ex vivo* latency reversal significantly reduce the assay duration and labor.[4,5] However, because defective proviruses can also express HIV-1 RNA,[6] RNA-based assays measure inducible but not necessarily intact HIV-1 (Figure 1A). Measuring unspliced, cell-associated HIV-1 RNA captures intact and some defective proviruses.[5] Measuring spliced cell-associated HIV-1 RNA[4,5] (suggesting the presence of 5′ and 3′ splice elements) provides a closer estimate of intact HIV-1. Measuring HIV-1 RNA in the culture supernatant (suggesting the presence of functional HIV-1 proteins sufficient for viral budding) provides an even closer estimate of intact HIV-1. Still, proviruses that have packaging signal (ψ) deletions can be spliced through cryptic splice sites and produce HIV-1 RNA and proteins in the supernatant.[2,6] Therefore, RNA-based measurements may overestimate the size of the latent reservoir by counting some defective HIV-1 as being intact. Another caveat of inducible HIV-1 measurements is the stochasticity of virus reactivation: despite maximum T cell activation, only a proportion of the latent reservoir are activated, leading to an under-estimation of latent reservoir by 64-fold.[2]

HIV-1 DNA-based assays that measure HIV-1 genome integrity overcome the caveat of insufficient HIV-1 reactivation due to stochasticity

HIV-1 near full-length proviral sequencing profiles HIV-1 genome integrity, overcomes caveats of inducible HIV-1 assays, and examines HIV-1 proviral landscape as a footprint of immune selection pressure.[6] However, near full-length proviral sequencing is labor-intensive.[2,7] To examine HIV-1 genomic DNA integrity in a clinically feasible assay, the Siliciano group developed intact proviral DNA assay (IPDA),[8] a duplex ddPCR-based method that detects two HIV-1 genome regions of the same HIV-1 provirus in the same droplet. The two probes targeting HIV-1 packaging signal and Rev response element (RRE) are chosen because the presence of both predicts HIV-1 genome integrity at ~97%.[8] The 3′ probe design excludes binding to hypermutated regions. Like all PCR-based methods, HIV-1 sequence diversity prevents 100% match with the probe, and 6.3%–28%[9,10] of HIV-1 sequences may not be captured.

5T-IPDA multiplex ddPCR detects 5 HIV-1 proviral genomic regions to increase accuracy

Here, Levy et al. developed a new ddPCR-based method called five-target IPDA (5T-IPDA) to increase accuracy by increasing HIV-1 targeting probes from two to five.[1] By leveraging different fluorescent intensity, 5T-IPDA uses two fluorescent probes in two triplex assays to measure five HIV-1 genomic regions. The five HIV-1 probes are divided into two assays with three targets each—with one target overlapping between the two assays as an inter-assay control. Presumably, targeting five HIV-1 genomic regions can provide a better capture of HIV-1 genome. Of note, defective proviruses having packaging signal deletions may not be captured because of the probe design. Another advancement of 5T-IPDA is using a T cell-specific DNA copy

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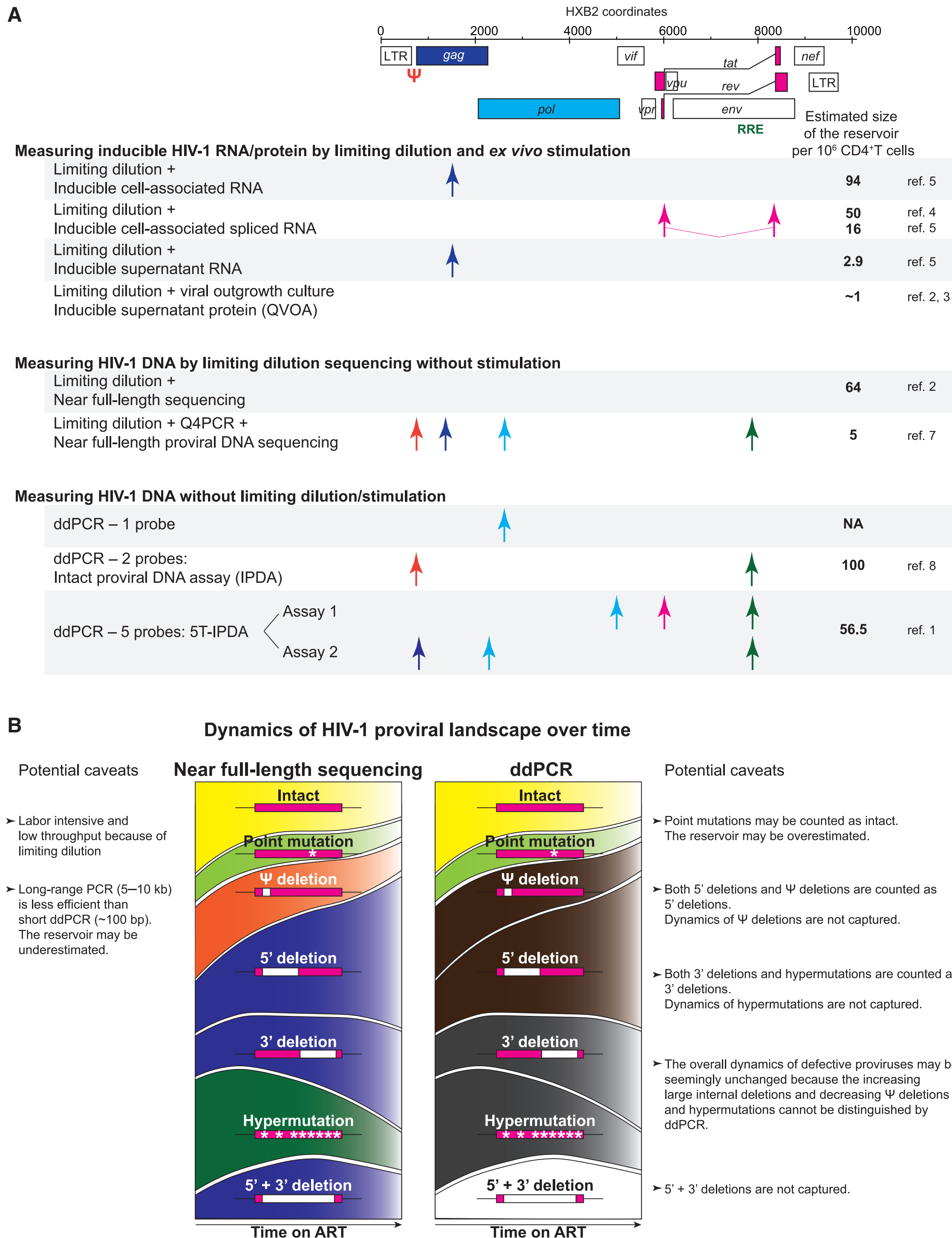

Figure 1. Measuring the size of the HIV-1 latent reservoir and the dynamics of HIV-1 proviral landscape

(A) Comparison of inducible RNA-based, proviral sequencing-based, and DNA ddPCR-based methods that measure the size of HIV-1 latent reservoir.
(B) Caveats of using HIV-1 near full-length proviral sequencing and multiplex ddPCR to profile the dynamics of intact versus defective HIV-1 proviral landscape. Ψ, packaging signal. RRE, Rev-response element.

reference to measure the frequency of T cells without isolating them. Typically, the cell number in ddPCR assays is measured by the frequency of *RPP30* that is present as one copy per cell. The frequency of HIV-1 DNA in T cells can be calculated using a genomic target in the T cell receptor D gene that is not present in mature T cells.

The decay dynamics of defective HIV-1 proviruses may be masked by ddPCR-based profiling

There are four types of defective proviruses: point mutations, packaging signal deletions, hypermutations, and large internal deletions[2,6] (Figure 1B). Intact proviruses and defective proviruses having point mutations, packaging signal deletion, and hypermutations can express HIV-1 RNA, induce cytotoxic T lymphocyte (CTL) activation, and decrease over time due to immune selection pressure.[6] In contrast, defective proviruses that have large internal deletions may not express HIV-1 RNA, do not induce CTL activation, and increase over time due to the lack of immune selection pressure.[6] The ddPCR-based methods, both IPDA and 5T-IPDA, can measure the frequency of intact HIV-1 proviruses and examine the decay dynamics of intact proviruses over time. However, the probe design cannot distinguish different subsets of defective proviruses: packaging signal deletion proviruses are counted as 5′ deletion, and hypermutated proviruses are counted as 3′ deletion. Mixed detection of the decreasing packaging signal deletion proviruses with the increasing 5′ large internal deletion proviruses and, similarly, the mixed detection of the decreasing hypermutated proviruses and the increasing 3′ large internal deletion proviruses creates a seemingly unchanged landscape of defective HIV-1 proviruses over time. Therefore, using ddPCR-based methods to examine defective proviral landscape may be inaccurate and should be interpretated with caution.

Overall, 5T-IPDA developed by Levy et al. targets more HIV-1 genomic regions than previous methods and may potentially increase the sensitivity and specificity of intact provirus detection and benefit HIV-1 cure clinical trials.

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