The use of HIV-1 integration site analysis information in clinical studies aiming at HIV cure

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Abstract

The mechanisms for the establishment and the persistence of the latent HIV-1 reservoir remain to be completely defined. HIV-1 infection is characterised by the integration of the reverse transcribed proviral DNA into the host's genome. This integrated proviral DNA can remain replication silent, but a small part of it is fully competent to restart viral replication when treatment is interrupted. Hence, this replication-competent provirus is the cause of viral rebound and is called the viral reservoir. The exact site of proviral integration within the host's cellular chromosome may affect the transcriptional activity of HIV. Thanks to recent technological advances, HIV-1 integration site analysis has been used to assess HIV-1 reservoirs in HIV-infected individuals. Analysis of HIV-1 integration sites in infected individuals undergoing suppressive ART led to identification of expanded clonal cell populations, indicating that clonal proliferation of the proviral reservoir may contribute to the long-term persistence of viral reservoirs. Here we describe the findings of several clinical studies, where a comprehensive HIV-1 integration site analysis was performed.

Keywords: integration site, HIV-1, HIV-1 persistence

Introduction

The most crucial step in the life cycle of a retrovirus is the integration of the viral DNA into the host genome of an infected cell. After integration, the provirus becomes an integral part of the cell genome. Hence, its fate is closely linked to that of the infected cell. Retroviruses persist in a host for the lifetime of the infected cells and in their daughter cells during cycles of cell division. Thus, the integration site is critical for both the virus and its host, since it can affect the transcriptions of the integrated provirus and of the host genes. The integration of the proviral DNA is a unique event. Several studies show that the integration of HIV-1 occurs randomly but it is biased towards transcriptionally active genes, which in turn may promote efficient viral gene expression [1].

A recent study by Marini and colleagues, offers an explanation for HIV-1 integration site preferences [2]. The study results show that the three-dimensional composition of the cell nucleus dictates the gene regions that are most rapidly accessible to HIV-1. Upon entry into the nucleus, the virus was shown to choose the nearest options, which are areas located on the periphery of the nucleus. Also, a strong bias against integration into genes located more centrally was reported. In addition to the location, the authors' suggestion is that the virus integrates into the first open chromatin regions it meets along its route into the nucleus, i.e. into actively transcribed genes. The authors also confirm previous findings that the functional viral integrase and the presence of the cellular Nup153 and LEDGF/p75 integration co-factors are indispensable for the peripheral integration of the virus [2–4].

In the past, it was thought that HIV-1 latency might reflect HIV-1 integration into chromosomal sites that repress viral transcription or that HIV-1 integration itself may result with transcriptional repression. Later on, it was shown that the absence of virus production in resting CD4+ T cells with integrated HIV-1 could not be attributed to integration into chromosomal regions that intrinsically repress transcription [5].

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The early research on HIV integration sites and HIV persistence focused more on HIV-1 integration sites and the effects on the viral transcriptional activity and fate of the virus itself. Conversely, the latest research examines the effect of HIV-1 integration on the fate of the infected cells, the cells' machinery and survival mechanisms, and the contribution to the maintenance of the latent reservoir [6,7].

HIV-1 integration site analysis using patient material

The early research performed by Ikeda and colleagues showed that infected resting CD4+ T cells from two HIV-1 patients on long-term successful ART harboured clusters of integration sites in cancer-related genes [5]. More interestingly, in the same study, HIV-1-infected cells with multiple identical integration sites were shown in longitudinal analysis of samples from three patients. These results led the authors to suggest that a plausible clonal T cell expansion may be occurring.

Maldarelli and colleagues recently confirmed these findings. Using a deep sequencing integration site sequencing method, they identified 2410 HIV-1 integration sites in CD4+ T cells from five patients receiving ART. Remarkably, 40% of HIV-1 integrations were found to be identical, hence, coming from clonally proliferated cells. Some of the cells were shown to have HIV-1 integration sites in specific genes, which are known to promote cell survival and expansion. One patient stood out, as approximately 50% of the infected cells had the same integration site, indicating that all sequences come from a single clone [6].

Concurrently with the report of Maldarelli and colleagues, a parallel pivotal study from Wagner and colleagues came to similar conclusions, using the integration site loop amplification technique, which provides a method to link integration sites with Env sequences of single proviral clones. This study demonstrated that the chromosomal HIV-1 integration site in an infected cell's genome may promote cell growth and contribute to persistence in the absence of viral replication [7]. In this study, a total of 534 HIV-1 integration sites from three patients were investigated, and identical viral sequences integrated at the same position in multiple cells were found in each participant. HIV-1 integrations were overrepresented in genes associated with cancer and 12 genes were identified across multiple participants. The analysis was

performed on longitudinal samples, where a greater proportion of persisting proviruses were detected in proliferating cells [7].

The latest study by Cohn and colleagues added another piece to the puzzle on HIV-1 reservoir maintenance in patients on ART by comparing HIV-1 integration in viraemic controllers, untreated and treated progressors, with longitudinal samples before and after ART [8]. Cohn *et al.* reported that the majority of integration sites were derived from proliferating clones of infected cells and their number increased with time on ART. More interestingly, none of the expanded T cell clones contained intact HIV-1 sequences. In contrast, they report a decrease over time of the number of cells bearing unique integration sites that were found to produce replication-competent virus. The study results further showed that the surviving cells were enriched for HIV-1 integration in silent regions of the genome. Based on these findings, the authors suggest that dividing, clonally expanded T cells contain defective proviruses, and that the replication-competent HIV-1 reservoir is primarily found in CD4+ T cells that remain relatively quiescent [8]. In contrast, a recent study on longitudinal blood and tissue samples from one HIV-1 positive patient by Simonetti and colleagues, revealed that clonally expanded cells contained an intact, infectious provirus that persisted and was able to produce replication-competent virus. Because of these conflicting findings, studies including higher numbers of patients will be necessary to estimate the frequency of clonally expanded populations that carry replication-competent HIV-1 to better understand the latent reservoirs [9].

HIV-1 persistence mechanism: the obstacle to a cure for HIV-1

The mechanism of HIV-1 reservoir persistence remains an important topic of a constant debate. It remains to be confirmed whether the maintenance of HIV is a result of ongoing low-level replication in sanctuary sites, of clonal T cell proliferation, or of both mechanisms combined. The three latest integration site studies are the most pivotal studies that identify and describe HIV-1 integration sites in patients on ART [6–8]. These studies provided proof of concept for the maintenance of reservoirs in patients under optimal ART, in the absence of ongoing low-level HIV-1 replication.

On one hand, ongoing HIV-1 replication in ART-adherent HIV-infected individuals has been considered unlikely by many authors because of a lack of viral genetic divergence over time, and the lack of emerging drug resistance mutations [10]. This is supported by studies showing that even after long-term ART, identical variants of HIV-1 are found in the blood [11] and in the lymph node tissue in virally suppressed patients [12], suggesting that clonal expansion is the main mechanism of viral persistence, with no evidence for low-level viral replication in tissue sites.

On the other hand, most studies only examined viral sequences derived from the blood compartment [13]. The findings from the blood compartment might not necessarily be applicable to other body compartments, particularly to lymphoid tissue where the frequency of infection per cell is mostly higher [14] and the intracellular drug concentrations are lower compared to the blood [15]. Under low drug concentrations, the virus can continue to replicate and evolve in these sanctuary sites within the reservoir of cells in lymphoid tissue, and remain undetectable in the bloodstream for a time, depending on the viral population migration dynamics between these two compartments. Indeed, a recent mathematical modelling of HIV-1 spatial dynamics between blood and lymph node tissues, based on *pol* sequencing data from three patients, showed that production of partially drug-resistant strains cannot become dominant in the sanctuary sites, because of the competition with the drug-sensitive strains in the absence of the drugs [14].

Conclusions

The new knowledge that HIV-1 integration site analyses have provided combined with the anticipated research, will guide the design of optimal HIV-1 cure approaches. Hence, HIV-1 cure strategies may need to focus on two major aspects: (1) to prevent ongoing low-level HIV-1 replication in presence of ART; and (2) to prevent clonal T cell expansion of already-infected cells.

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