The Role of HIV Integration Sites in Extensive Clonal Expansion of Infected Cells in Patients

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Background
Despite complete suppression of HIV-1 by combinations of antiretroviral therapy (ART), infected cells persist for many years in patients. Long-term control of viruses by ART reveals clinical and genetic evidence of virus-infected cells in the blood of most patients, implying clonal expansion of some HIV-infected cells. The mechanisms driving clonal expansion of unknown but are central to HIV persistence. To address this issue, we determined the distribution of proviral integrations in HIV-infected patients using a specific and highly sensitive amplification strategy that yields large numbers of virus-host junctions, identifies their exact location, and measures the relative depth of clonal expansion.

Methodology
DNA was prepared from TH295s from 5 patients, randomly selected, and linker-mediated PCR was used to specifically amplify both the 5’ and 3’ LTR integration site junctions, whose sequences, as well as those of the shared breakpoints in the host DNA, were determined by Illumina paired-end sequencing. Clonal expansion of infected cells was demonstrated by the presence of DNA fragments with exactly the same integration site and different host DNA breakpoints.

Results
Analysis of integration site libraries comprising ≥500 independent integration events revealed clonal expansion of HIV-infected cells in all patients studied. In one patient, about half of the infected PBMCs were derived from a single infected cell. Infected clones persisted in patients for at least 11 years. Integrations in the same orientation of two different genes (MKL2 and BACH2) were seen in independent clones from more than one patient. In one patient, we detected 919 unique sites, of which 14 were in different expanded clones derived from independent integration events in a single 3.5 kb intron of MKL2, and 12 were in a single intron of BACH2. These data show that the expression of these genes must have been affected by these integrations in ways that make a critical contribution to the clonal expansion and persistence of the infected cells. Both these genes have been linked to the control of cell growth and human cancers. Other genes that have been identified in clonal expansions include PAK1, PAK2, and CYTH1, which are involved in the regulation of cell growth and/or apoptosis.

Conclusions
There is extensive clonal expansion of HIV-infected cells in the first 5 patients we analyzed, in one patient a single clone represented approximately half of the infected cells in the blood.

Clonally expanded cells can persist for >11 years.

In a substantial number of instances, the integration site plays an important role in the clonal expansion and persistence of the infected cells.

The figure shows the number of integrations in clonal expansions in one patient. The 919 unique sites detected in patient 5, as derived from independent integration events in a single 3.5 kb intron of MKL2, and 12 were in a single intron of BACH2. These data show that the expression of these genes must have been affected by these integrations in ways that make a critical contribution to the clonal expansion and persistence of the infected cells. Both these genes have been linked to the control of cell growth and human cancers.