PE20 LB

Assay to measure the latent reservoir of replication-competent HIV-1 in suppressed patients based on ultra deep sequencing

S.-K. Lee[1], S. Zhou[1], N. Archin[2], D. Margolis[2], R. Swanstrom[1]
[1]University of North Carolina, Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, Chapel Hill, United States, [2]University of North Carolina, Department of Medicine, Chapel Hill, United States

Background: Viral outgrowth assay (VOA) is a widely used culture assay to measure the latent HIV-1 reservoir harboring replication-competent HIV-1 in resting CD4+ T cells in patients on HAART. However, the assay is costly, and both labor and resource intensive. To overcome some of these issues with the VOA, we designed an assay using ultra deep sequencing (UDS), which directly analyzes the number of different sequences of the induced viruses to score the number of latently HIV-infected resting CD4+ T cells. In this study, we tested the premise whether the viral sequences derived from two different proviruses are genetically distinct, since the assay involves a bulk culture.

Methods: To analyze viruses derived from different VOA culture wells scored as p24 positive, the viral samples derived from different culture wells were assigned with a specific Barcode and subjected to sequence analysis of the V1-V3 region of env sequences using the Primer ID-based paired-end MiSeq platform. A total of nine patient samples, two acute and seven chronic, were analyzed by UDS. Phylogenetic trees were generated by using consensus sequences created from sequences with the identical Primer ID and were used to detect distinct viral lineages present in the individual culture supernatant. For chronic patient samples, IUPM values were determined by using distinct viral lineages detected and the adjusted number of patient-derived resting CD4+ T cells used for VOA.

Results: Approximately 50% of the viral lineages derived from each chronic patient were distinct. In contrast, all viral lineages derived from each acute patient were homogeneous. When IUPM values determined by UDS analysis were compared to the IUPM values obtained from VOA, we observed approximately 2-fold higher IUPM values than the IUPM values determined by VOA. We also observed a significant positive correlation between the number of viral lineages observed per well and the number of resting T cells present per well.

Conclusions: The results suggest that approximately 50% of the viral lineages induced from different cells derived from chronic patients were distinct. Thus, the UDS assay is applicable for samples derived from chronic patients. The multiplexing ability of the assay improves the efficiency for the throughput capacity.