Parallelized Genome-Wide Detection of Intact HIV Provirus with Bimodal Phenotyping in Single Cells

Yi Qi[1,4,5], Leena Babiker[2,4], Hanzhi Wang[1,7], Sam Barnett Dubensky[2,3,4], Hunter Courtney[8], Jonathan Tedesco[4], Parker Wilson[7], Michael R. Betts[5,6], Laura Vella[2,4,5]

[1] Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania , [2] Institute for Immunology and Immune Health, Perelman School of Medicine, University of Pennsylvania, [3] Medical Scientist Training Program, Perelman School of Medicine, University of Pennsylvania, [4] Division of Infectious Diseases, Children's Hospital of Philadelphia, [5] Center for AIDS Research, University of Pennsylvania, [6] Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, [7] Division of Diagnostic Innovation, Department of Pathology and Laboratory Medicine, University of Pennsylvania, [8] UCLA-Caltech Medical Scientist Training Program, David Geffen School of Medicine, University of California

Prolonged cART is associated with a greater proportion of provirus in closed chromatin and shifted reservoir landscape. However, current single cell reservoir approaches are unable to detect a heterochromatic provirus while maintaining the ability to simultaneously define the features of its host CD4 T cell. To enable rational targeting of the reservoir, we hypothesized that chemically relaxing the heterochromatin would enable detection of heterochromatic provirus as cell identity was maintained. Further, we hypothesized that full-length sequencing would allow detailed virologic information of these proviruses, including estimates of intactness and integration site (IS).

To broaden the range of proviral detection across heterochromatin while defining cellular identity, we developed SCRIPT-seq: Single Cell detection of Retroviral Integration and cellular PhenoTyping. We applied SCRIPT-seq to JLat 6.3 and primary memory CD4+ T cells from people with HIV. Cells were permeabilized by digitonin and treated with lithium 3,5-diiodosalicylate (LIS) to remove nucleosomes prior to Tn5 tagmentation. Transcriptome and surface proteins of each CD4 T cell were captured using the 10X Genomics Multiome protocol. Biotinylated probes were applied to libraries to enrich for proviral sequences before full-length sequencing.
SCRIPT-seq resulted in marked expansion of genomic and proviral read coverage. In primary $CD4^+$ T cells, LIS enabled capture of centromeric loci previously reported as IS in PWH after long-term cART. In activated JLat, LIS increased the percent HIV DNA+ cells (78.6% vs 20.9%), correct integration site calls (44.1% vs 4.2%), capture of IPDA regions (66.4% vs 28%) and calling of intact genomes (35.8% vs 20.9%). While activation is known to induce proviral transcription, the marked increase in IS detection after LIS suggests activation alone may not fully open chromatin across IS. In both JLat and primary cells, the addition of LIS did not alter the transcriptional cell identity.

We demonstrate that SCRIPT-seq enables pan-chromatin assessment of proviral IS and intactness, in parallel with multimodal phenotyping of the host cell. Future work based on SCRIPT-seq will allow precise definition of reservoir features in various therapeutic settings to delineate the host-viral interplay that promotes persistence.