ID No.	K3003
Project Title	Impact of human endogenous retroviruses on virus infections and human diseases
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Report

1. Regulation of human gene expression by endogenous retroviruses during mammalian evolution

APOBEC3 (A3) genes are members of the AID/APOBEC gene family that are found exclusively in mammals. A3 genes encode antiviral proteins that restrict the replication of retroviruses by inducing G-to-A mutations in their genomes, and have undergone extensive amplification and diversification during mammalian evolution. Endogenous retroviruses (ERVs) are sequences derived from ancient retroviruses that are widespread mammalian genomes. In this study we characterize the A3 repertoire and use the ERV 'fossil record' to explore the long-term history of co-evolutionary interaction between A3s and retroviruses. We examine the genomes of 160 mammalian species and identify 1,420 AID/APOBEC-related genes, including representatives of previously uncharacterized lineages. We show that A3 genes have been amplified in mammals and that amplification is positively correlated with the extent of germline colonization by ERVs. Moreover, we demonstrate that the signatures of A3-mediated mutation can be detected in ERVs found throughout mammalian genomes, and show that in mammalian species with expanded A3 repertoires, ERVs are significantly enriched for G-to-A mutations. Finally, we show

that A3 amplification occurred concurrently with prominent ERV invasions in primates. Our findings establish that conflict with retroviruses is a major driving force for the rapid evolution of mammalian A3 genes.

2. Multiomics analysis of HIV-1-infected cells in vivo

For eradication of HIV-1 infection, it is important to gain an in-depth understanding of the wide-ranging characteristics of HIV-1-infected cells in vivo. Recently developed 'omics' analyses can be a powerful tool to identify the characteristics of HIV-1-infected cells. However, it should be noted that a large majority of the CD4+ T cells in infected individuals are uninfected, and therefore, the transcriptional profiles of “bulk” CD4+ T cells in vivo do not reflect that of “pure” HIV-1-producing cells.

In this study, we used a human hematopoietic stem cell-transplanted humanized mouse model that maintains human leukopoiesis under relatively stable immunological conditions in vivo and a replication-competent reporter HIV-1 and used four recently developed techniques to investigate viral genomics and transcriptomics. First, droplet digital PCR revealed the presence of potential reservoirs in infected humanized mice. Second, ligation mediated PCR showed the preference for HIV-1 integration into open chromatin regions, particularly suggesting the association of the epigenetic modifications of integration sites with viral production. Third, digital RNA-sequencing quantified the absolute copy number of viral transcripts in the HIV-1-producing cells in vivo and further identified the differentially expressed genes between virus-infected and uninfected cells. Finally, single-cell RNA-sequencing revealed and described the heterogeneity of the HIV-1-producing cells in vivo. To our knowledge, this is the first investigation to describe multiple aspects of the characterization of HIV-1-producing cells in vivo.

To our knowledge, this study is the first comprehensive investigation of the characteristics of HIV-1-infected cells in vivo.