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Identification of novel simian endogenous retroviruses that are indistinguishable from simian retrovirus (SRV) on current SRV diagnostic assays

Richard Grant¹, Brandon Keele², LaRene Kuller¹, Robin Watanabe¹, Alex Perret¹, and Jeremy Smedley¹

¹Washington National Primate Research Center, University of Washington, Seattle, WA, USA

²AIDS and Cancer Virus Program, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD, USA

Abstract

Simian betaretroviruses include the well-known exogenous simian retroviruses (SRV-1 through SRV-8), and some closely related simian endogenous retroviruses (SERV). Here, we characterized two new viral genomes, which appear to represent novel SERVs but have characteristics of both SRV and SERV highlighting the need to develop new assays providing molecular and serologic differentiation of SERV and SRV to avoid false positives.

Keywords

Macaque; PCR; sequencing; Specific Pathogen-Free (SPF); western blot

1 | INTRODUCTION

Like the related endogenous–exogenous paired betaretroviruses in other species, macaques harbor copies of simian endogenous retrovirus (Ma-SERV) within their genomes that are highly homologous to pathogenic exogenous simian retrovirus (SRV).[–] The exogenous version of SRV can spread quickly and cause a wasting disease especially in young macaques, but the Ma-SERV and their possible interactions with SRV in macaques are not well understood. It has been widely reported widely that endogenous retroviral genomes, including Ma-SERVs, can produce viral proteins and immune responses can be detected.[–] The presence of various Ma-SERVs now known to exist in pigtailed can be indistinguishable from some serotypes of exogenous SRV using current diagnostic technology. While new exogenous SRV serotypes are still being discovered[–] and new endogenous SERVs are still being defined[–] it is important that we make our diagnostic assays more specific so as to distinguish exogenous from endogenous viruses.

2 | MATERIALS AND METHODS

All animals were maintained in fully AAALAC-accredited facilities in accordance with the Animal Welfare Act, Regulations, and the Guide for the Care and Use of Laboratory Animals.² All procedures involving animals in this study were approved by the University of Washington's IACUC.

Animals reported were *Macaca nemestrina* imported from the Indonesian Primate Center, Bogor, Indonesia as follows: 9227 – 1998; 0324 – 2003; 0460 – 2004; 1241 – 2012; 1271 – 2012.

SRV preparations were produced in A549 cells, supernatant concentrated through TFF and purified on sucrose gradients, and virus pelleted through glycerol cushion as previously described.³

Immunoblots are prepared by running stock virus preps on denaturing SDS-PAGE, transferring onto nitrocellulose, cutting the nitrocellulose into strip and reacting the strips with plasma or sera and a secondary anti-monkey conjugate.

Standard SRV diagnostic ELISA or Luminex, immunoblot serology, and PCR methods were used for identification and initial detection of the new Ma-SERV genomes and antibody responses in animals imported from 1998 to 2012. The PCR was run with SYBR mastermix to allow detection of the melt temperature (T_m) of the PCR products.

Complete genome sequencing and phylogenetic analysis was done at The AIDS and Cancer Virus Program, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research (Frederick, MD, USA).

3 | RESULTS

The five animals found to contain the new Ma-SERV DNA sequence have all been *M. nemestrina* born in Indonesia and imported to WaNPRC in the years 1998, 2003, 2004, and 2012. The animals found in 2012 were considered at the time to be infected with SRV and were humanely euthanized prior to release from international quarantine.

Serological testing of animals imported in 1998 and 2003 found they had robust antibody responses that cross-reacted with SRV-5 and SRV-2, respectively, and reacted with the major core protein, p27, and the major Env glycoproteins, gp70 and gp20, similar to reactivity seen in experimentally infected *M.n.* (Figure 1).

PCR for SRV serotypes can be grouped into two melt temperatures (T_m), serotypes that melt at T_m of 79-79.5°C, and serotypes that melt at 80-81.5°C (Figure 2). The PCR product of all five animals with the new Ma-SERVs melted at 81.5°C. In addition, the Ma-SERVs all react with extremely high copy numbers ($C_q < 24$) in PBMC DNA, as compared to PBMCs from experimentally infected animals with moderate to low copy numbers ($C_q > 30-35$). The PBMCs from 1998 and 2003 and plasma from 2012 all failed to yield infectious virus in vitro when cultured with Raji cells for >4 weeks (data not shown).

Upon nucleotide sequencing of full-length genomes from the three animals with samples obtained from one animal in 1998 (9227) and from two animals from 2012 (1242 and 1271), we found the genome from 9227 (ca. 1998) was identical (100%) to the genome of animal 1242 (ca. 2012). The two identical genomes from two separate animals sampled 14 years apart were also 99% identical to the genome of animal 1271 sampled in circa 2012 (Figure 2).

4 | DISCUSSION

New SRV-like viral sequences were first recognized in a 2012 group imported from Indonesia in which some animals were diagnosed as SRV positive based on PCR and/or serology. Subsequently, we found that the two PCR-positive animals in this group had a distinct sequence that appeared to be consistent with a new endogenous genome that most resembled SRV in genome sequence and organization. The same sequence was found in three Indonesian-origin animals from 1998, 2003, and 2004 shipments as well as the animals imported in 2012. The sequences, however, look most like endogenous sequences because they existed without sequence heterogeneity even in the highly variable portions of the genome in different animals separated by many years, and they exist in high copy number similar to housekeeping genes. Within one animal, the sequences are virtually identical through hundreds of sequencing events and all attempts to culture viruses have failed (data not shown). From archived DNA samples from 1998 to 2004 and from 2012 to 2016 routine diagnostic testing samples, we found only these five animals with extremely high copy numbers and the same characteristic DNA melt temperature. All animals with this new genome contained high copy number with PCR C_q numbers of 23 indicating approximately 100,000 copies per ng of DNA from whole blood compared to C_q of 32-35 indicating approximately 10-100 copies/ng from our SRV-2 and SRV-5 experimentally infected animals. Sequence analysis revealed that this new virus is 100% identical in four of five animals and one animal is 99% identical across the entire genome. Important characteristics of the new macaque (Ma-SERV) genome are an intact betaretrovirus organization with Gag-Pro-Pol-Env. Despite an apparent intact full-length genome, multiple attempts to culture replication competent virus in several different cell lines have proven unsuccessful (data not shown). Despite the high copy number of the Ma-SERV genome, the affected animals demonstrated no evidence of SRV-related disease clinically or upon necropsy.

Overall, the data support a previously unidentified endogenous virus present in a subset of macaques from Indonesia. In light of the potential for false-positive results in SRV testing, these data have implications for management of macaque colonies and selection of animals to be used in other viral studies. Diagnostic assays to differentiate this and potentially other SERVs from exogenous SRV need to be developed.

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Funding information

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FIGURE 1.

SRV immunoblot antibody reactivity in two pigtailed macaques in 2003, later found to be endogenous betaretrovirus compared to two pigtaills with exogenous SRV-1 infections in 2016. (A) *M.n.* 0324; (B) *M.n.* 9227

FIGURE 2. (A) Comparison of PCR product melt temperatures T_m (left graph) and (B) C_q values (right graph) of the new SERV genomes and exogenous SRV-infected animals. (C) Phylogenetic tree of whole-genome DNA sequences