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Characterization and comparative analysis of a simian foamy virus complete genome isolated from Brazilian capuchin monkeys

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ABSTRACT

Foamy viruses infect a wide range of placental mammals, including primates. However, despite of great diversity of New World primates, only three strains of neotropical simian foamy viruses (SFV) have been described. Only after 40 years since serological characterization, the complete sequence of an SFVcap strain infecting a family of six capuchin monkeys (*Sapajus xanthosternos*) was obtained. Co-culture of primate peripheral blood mononuclear cells with Cf2Th canine cells was established and monitored for the appearance of cytopathic effects, PCR amplification of integrated SFV proviral genome and viral reverse transcriptase activity. The novel SFVcap was fully sequenced through a next-generation sequencing protocol. Phylogenetic analysis of the complete genome grouped SFVcap and SFVmar, both infecting primate species of the Cebidae family with a genetic similarity of approximately 85%. Similar ORF sizes were observed among SFV from neotropical primates, and *env* and *pol* genes were the most conserved. Neotropical SFV presented the smallest LTRs among exogenous mammalians. The novel SFVcap strain provides a valuable research tool for the FV community.

1. Introduction

Foamy viruses (FVs) are complex retrovirus that belong to the *Spumavirus* genus and are found in a wide range of mammals including felines, bovines, sheep, bats, equines, prosimians and non-human primates (NHP) (Materniak et al., 2013; Muniz et al., 2013; Wu et al., 2012). FVs induce cytopathic effects *in vitro*, such as cellular syncytia, formation of multiple vesicles in the cytoplasm of infected cells and cell death. Simian foamy viruses (SFV) cause a life-long infection in their natural hosts, but no disease has so far been associated with infection. FV proviral DNA has been found in many tissues, including lung, liver, brain and lymphocytes of infected NHP (Murray et al., 2006), while FV RNA was found in superficial epithelial cells of the oral mucosa (Murray et al., 2006, 2008) and in fecal samples (Liu et al., 2008). Transmission occurs mainly by contact with saliva from infected animals, usually *via* bites. SFV has been detected in humans, but all infections have so far resulted from zoonotic transmissions of viruses from Old World primates (OWP) (Gessain et al., 2013; Peeters et al., 2014).

SFV strains have been identified in a wide range of primate species from Africa and Asia and complete genomes were obtained from a variety of OWM, including chimpanzees, gorillas, African green monkeys and Asian macaques. On the other hand, SFV from New World primates (NWP) are currently represented by only three complete genomes available (Pacheco et al., 2010; Thumer et al., 2007), despite the wide diversity of these primates that contain eight subfamilies, at least 18 genera and over 120 species (Wilson and Reeder, 2005). Recently, our group evidenced the presence of SFV in 23 different NWP species, comprising all three families of neotropical primates, with further characterization of two novel SFV strains, SFVcap and SFVhow from capuchins and howler monkeys, respectively (Muniz et al., 2013).

SFVmar and SFVsqu infect primates of the same family (Cebidae), but in different subfamilies (Callitrichinae and Saimirinae, respectively), while SFVspm infects a genus of the Atelidae family. Katzourakis et al. showed that a host-virus co-speciation hypothesis is strongly supported in FV from OWP, but inconsistent in NWP, raising the alternative hypothesis of interspecies transmission to explain their results (Katzourakis et al., 2014).

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Here, we isolated a strain of SFVcap infecting a family of Brazilian yellow-breasted capuchin monkeys (*Cebus xanthosternos*, now *Sapajus xanthosternos*) from a third distinct subfamily of Cebidae through cell culture, and obtained the complete genome viral sequence by next generation sequencing. Comparative analyses with the other three NWP SFV sequences available and with those of other primates evidenced unique genomic and structural features in viruses from this group and further support the host-virus co-speciation hypothesis in NWP SFV.

2. Materials and methods

2.1. Ethics statement

NWP samples have been collected from six specimens of *S. xanthosternos* of the same family housed in a single vivarium at Fundação RIOZOO (Fundação Jardim Zoológico da Cidade do Rio de Janeiro). Approximately 3 ml of whole blood were collected from each animal by venous puncture following national guidelines and provisions of IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis), Brazil (permanent license no. 11375-1), which included animal welfare standard operational procedures. All samples have been collected by veterinarians from Fundação RIOZOO. This study was submitted and has been approved by the Ethics Committee on Animal Use (CEUA) of Universidade Federal do Rio de Janeiro (no. 037/14).

2.2. SFV status and species confirmation

All six *S. xanthosternos* had been previously sampled in 2012 and had their SFV status confirmed by PCR and sequencing as described previously (Muniz et al., 2013). All animals shared the same vivarium and belonged to the same family group, comprising one alpha male, one alpha female and their offspring: two males and two females. All animals carried the same virus as confirmed by phylogenetic analysis, and their taxonomic classification was confirmed by PCR and sequencing of a 975-bp fragment of cytochrome b mitochondrial sequence as previously described (Muniz et al., 2013).

2.3. NWP sample processing and cell co-culture

Peripheral blood mononuclear cells (PMBC) from all six capuchins were isolated from whole blood with Ficoll-Paque^{TM} Plus within 2 h after blood collection. PBMC were pooled and activated with 1 μg/ml phytohemagglutinin (PHA) and 10 U/ml interleukin 2 in RPMI medium supplemented with 20% FBS, 1% penicillin and 0.5% fungizone for 48 h at 37 °C and 5% CO_2. After 48 h of activation, 3×10^6 PBMC were co-cultivated with 3×10^6 Cf2Th cells in DMEM medium (20% FBS, 1% penicillin and 0.5% fungizone) at 37 °C and 5% CO_2. Supernatant was collected each 3–4 days and analyzed for reverse transcriptase (RT) activity with the Reverse Transcriptase Assay, colorimetric kit (Roche Diagnostics, São Paulo, Brazil), and cells were trypisinized. Half of the cells were used to extract genomic DNA (gDNA) and the other half was kept in co-culture for up to 60 days. As an infection control, a Cf2Th culture was infected with an SFVspm viral stock isolated from a NW spider monkey (kindly provided by William Switzer, CDC, Atlanta, USA) for 10 days and the supernatant was used to test for viral RT activity. The purified HIV-1 RT supplied by kit was used as a positive control and a standard curve was plotted to estimate the concentration of FV RT (ng/μl) by interpolation.

2.4. Preparation of gDNA and PCR amplification of viral sequences

Cf2Th cells collected from the co-culture had their gDNA extracted using the Illustra Blood Genomic Prep Mini Spin kit (GE Healthcare, São Paulo, Brazil). DNA integrity was evaluated by amplification of a 975-bp fragment of the mitocondrial cytochrome b gene as described above. Nested and semi-nested PCR were conducted to amplify portions of the SFV integrase and LTR-gag genes (Muniz et al., 2013). After sequencing of capuchin SFV (SFVcap) DNA fragments, sequences were used to design specific primers to amplify the complete genome in two parts: 5′LTR to *pol* and *pol* to 3′LTR. Primers LTRCebus-Fow1 (5′ CCA CAA CAG TTT GGC GCC CAA CG 3′) and Cebus-rev (5′ CCC TTC TGG AGG TCT AAT GGG T 3′) were used to amplify the first part (a product of 3945 bp). The second part (*pol*-3′LTR; 7410 bp) was amplified using primers Cebus-fow (5′ TGC CTC TCC GAA AAC AGG ATA 3′) and LTRCebus-rev1 (5′ CGT TGG GCG CAA AAC TGT TGT GG 3′). Both fragments had an overlap of 676 bp to enable full-length genome assembly.

2.5. Next-generation sequencing (NGS) library preparation and sequencing

PCR products were quantified in a spectrophotometer and libraries were prepared with the Nextera DNA Sample Preparation kit (Illumina Inc., USA). Approximately 50 ng of purified DNA were fragmented into sizes of ~1200 bp and tagged with the transposome kit. Index sequences were added at both ends of the DNA through a PCR and libraries were quantified by qPCR with the KAPA Library Quantification kit (KAPA Biosystems, MA, USA). Libraries were loaded onto a HiSeq flowcell and clonal clusters were generated with cBot (Illumina) using the adaptor sequence tags. Paired-end sequencing by synthesis was conducted in an Illumina® HiSeq 2500 platform (Illumina) and 2× 100 cycles of sequencing were performed.

2.6. NGS sequence analysis

Sequencing data were converted with CASAVA v.1.8.2 (Illumina) into Fastq files containing reads' sequences. Read quality and quantity were analyzed with FastQC (Babraham Bioinformatics, Cambridge, UK). Sequence tails with a Phred quality score lower than 28 (base calling accuracy of approximately 99.9%) and reads with a length lower than 70 bp were discarded with Sickle (Joshi and Fass, 2011). Reads were mapped onto the SFV complete genome from the common marmoset (GenBank acc.# GU356395) with Stampy v.0.1.19 (Lunter and Goodson, 2011). A consensus sequence was extracted with the SAMtools suite v.0.1.18 (Li et al., 2009) using the commands 'mpileup', 'bcftools view' and 'vcf2fq vcfutils.pl'. Reads were then reassembled with BWA v.0.6.1 (Takehisa et al., 2009) onto the consensus extracted from the first assembly. Finally, the consensus from this alignment was extracted as described above and indels were analyzed manually by visual inspection using IGV (Robinson et al., 2011). The full-length consensus sequence of SFVcap has been submitted to the GenBank database and has been assigned the accession number KP143760.

2.7. SFVcap genome analysis

The generated SFVcap sequence was aligned with 25 SFV genomes available from the GenBank using MUSCLE into MEGA v.6.05 (Hall, 2013). A phylogenetic tree was made using the neighbor-joining method and the Kimura 2-p model. The robustness of the phylogenetic clusters was assessed using 1000 *bootstrap* replicates. With the same alignment, a maximum likelihood (ML) tree was inferred in Phyml 3 (Guindon et al., 2010) using the GTR + G + I model of sequence evolution, which was chosen using the likelihood ratio statistic available in HyPhy (Pond et al., 2005). ML tree search was implemented using the best of both NNI and SPR algorithms. Clade support was assessed with the adjusted likelihood ratio test (aLRT) statistics (Anisimova and Gascuel, 2006).