Foamy Virus in the Tree Shrew *Tupaia Belangeri* Is Highly Related to Simian Foamy Virus in *Macaca Mulatta*

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Abstract

Foamy viruses (FVs) are ancient retrovirus that infect most nonhuman primates and several animals, but are rarely reported in tree shrew *Tupaia belangeri*. In the present study, foamy virus was detected in tree shrew. Phylogenetic analysis indicated that FV_{tup} shared the highest homology with SFV_{mac} (99.3%) in China. The discovery of FV_{tup} indicated that the tree shrew is a new host of foamy virus. FV_{tup} is highly prevalent in *Tupaia* in China and there is the possibility of cross-species transmission from nonhuman primate to *Tupaia*.

Introduction

FOAMY VIRUSES (FVs) OF THE retrovirus family are the most ancient of all retroviruses, since it was discovered in the coelacanth genome.^{1,2} FVs are ubiquitous in nonhuman primates (NHP)³ and infect cattle, cats, and horse.⁴ In contrast to complex orthoretroviruses, which are sometimes highly pathogenic, FV infections are persistent and asymptomatic in their natural hosts.^{3,5} FVs are transmitted zoonotically to humans, who are natural habitats for occupational exposure to NHP.^{5,6} Despite the lack of pathogenicity of FV infection, the potential risk for the emergence of new strains of FV transmission to humans cannot be neglected.

The tree shrew (*Tupaia belangeri*), belonging to the order Scandentia, is a small mammal that has a wide distribution in Southeast Asia and Southwest China.^{7,8} Phylogenomic analysis of the tree shrew and other mammals highly supports its close affinity to primates.⁸ The tree shrew has been thought to be a viable animal model that can serve as an alternative to primates in biomedical research.^{8–11} FV infections in monkeys are highly endemic in Asia.^{6,12} However, epidemiological and genotypic data on FVs in *Tupaia* are unknown. In the present study, a molecular epidemiological study on FV infection in *Tupaia* was conducted.

Materials and Methods

A total of 24 peripheral blood specimens were collected from tree shrew captive in the wild of Southwest China. All fresh samples were stored at –80°C until use. DNA was isolated from blood using a DNA extraction kit (Axygen) according to the manufacturer's instructions. The polymerase gene of FV, which is highly conserved among Asian, African, and New World monkeys and apes,³ was amplified by nested polymerase chain reaction (nPCR) using conserved primers. The outer primers

consisted of F1: 5'-CTCCAGGGTATCCAAAAC-3' and R1: 5'-AAACAAGAGTTGATGAGGAG-3', and the inner primers consisted of F2: 5'-ACCGGACGAG ACGTACTTTTC-3' and R2: 5'-ATACCTTTTCTTTAGCC CAATC-3'. The expected nPCR product was 411 base pairs (bp). The nPCR protocol was performed at 94°C for 3 min, 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, and 72°C for 7 min. The PCR products were detected by electrophoresis on an agarose gel containing 0.5 µg/ml ethidium bromide and sequenced in a DNA analyzer (Applied Biosystems 3730 DNA Analyzer, USA).

Results

Among the 24 blood samples from *Tupaia*, 62.5% (15/24) were positive for FV proviral DNA. This value was higher than that in *Macaca mulatta* (39.19%) trapped in the same region 2 years ago.⁶ Five FV strains isolated from *Tupaia* were unique and were submitted to GenBank with accession numbers KC196051 to KC196055.

The FV strains isolated from *Tupaia* shared 94.2–98.3% homology with each other. Blast analysis of the 411-bp polymerase gene sequences from the tree shrew showed maximum nucleotide identity of 90.1–99.3% to SFV_{mac} from *M. mulatta* isolated in the same area (KC196056 to KC196060) and 90.8–94.9% with SFV_{mac} isolated from Germany (JN801175). The homology was 88.2–92.5% for SFV-1 (X54482 and X58484), 87.9–92.5% for SFV_{mac} (NC_010819), 83.7–87% for SFV-3 (M74895), 83.6–87.4% for SFV_{wrc} (FN860006), 73.9–78% for SFV_{cpz} (U04327), 79.5–76.1% for SFV_{gor} (HM245790), 66.9–71% for SFV spider monkey (EU010385), and 72.9–77.1% for human spumaretrovirus (M54978 and U21247).

Phylogenetic analysis of the *pol* sequences of the five *Tupaia* FV clearly illustrated that FV_{tup} clustered together with SFV_{mac} (Fig. 1). This result suggests that *Tupaia* FV was highly related to SFV_{mac}.

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FIG. 1. Phylogenetic analysis based on the polymerase (411 bp) sequence of five *Tupaia* foamy viruses (FVs) (FVtup) isolated in this study and another 19 references of foamy viruses with complete sequences using the neighbor-joining method. The tree was evaluated using the interior branch test method with Mega 4 software. Percent bootstrap support is indicated at each node. The scale bar represents nucleotide substitutions per base. GenBank accession numbers and species are indicated. The FVs isolates from *Tupaia* and *M. mulatta* in the same area are marked with a triangle and circle, respectively.

Discussion

Approximately 75% of emerging infectious diseases in humans are of zoonotic origin, the majority of which originates in wildlife.¹³ Simian retroviruses are precursors of all human retroviral pathogens.⁹ There are no reports of human specific FVs, but zoonotic transmission from NHPs to humans has been identified.^{6,12} People requiring close contact with NHPs may be at high risk for FV infection.^{5,6,12} In the present study, FVs were detected in the tree shrew and shared the highest homology with *M. Mulatta* (99.3%) captive in the same area 2 years ago. This result indicates the possibility of transmission from NHPs to tree shrews.

Similarly, tree shrews have not shown any symptoms specifically associated with FV infection, as established in previous reports on humans or NHPs.⁵ However, a persistent foamy virus infection reported induces immunosuppression in rabbits.¹⁴ Clinical information on infected individuals and the pathogenic mechanism of FV warrants further study.

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Author Disclosure Statement

No competing financial interests exist.

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