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Short communication

Characterization of a wild rabies virus isolate of porcine origin in China

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ABSTRACT

Rabies virus (RABV) that circulates worldwide in a variety of mammals can cause fatal encephalomyelitis. GD-SH-01, a street rabies virus, was isolated from a rabid pig in China. We investigated the pathogenicity of GD-SH-01 in suckling and adult mice, and compared the susceptibility of NA and BHK-21 cells in the culture to infection by GD-SH-01 and CVS-24. The complete GD-SH-01 genome sequence was determined and compared with known RABV wild strains to understand the mutations and genetic diversity that allow RABV to spread and adapt in new hosts, such as pigs. Our results suggest that GD-SH-01 possesses the characteristics of a virulent strain in Southern China and shows higher pathogenicity index than that of CVS-24 regardless of its lower level of replication in mouse brain. Up to 47 unique nucleotide substitutions were found in the genome, including five missense mutations. These data provide useful information for further understanding the transmission mechanism and the genetic variation of RABV in dissimilar hosts.

1. Introduction

Rabies virus (RABV), a member of the genus *Lyssavirus* in the family of *Rhabdoviridae*, is the causative agent of rabies. RABV is a neurotropic virus that causes fatal encephalitis in warm-blooded animals (Jackson, 2003). RABV has a non-segmented, single-strand negative-sense RNA genome that is approximately 12 kb; it encodes five proteins, namely, nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase (L) in the order of 3′-N-P-M-G-L-5′ (Schnell et al., 2010; Tordo et al., 1986). RABV is distributed in all continents, except Antarctica. Various mammals serve as major hosts in different parts of the world, primarily in the orders Carnivora and Chiroptera (Rupprecht et al., 2002). Domestic dog is the primary reservoir and vector of rabies transmission in China, although RABV has been isolated or detected in other animal species, such as cat, ferret badger, fox, pig, cattle, and donkey (Hu et al., 2009; Jiang et al., 2008; Xie et al., 2012; Zhang et al., 2011, 2010).

RABV spreads among the same species as well as in dissimilar taxa. Some specific virus variants within a genotype tend to persist among particular hosts in different geographic areas (Smith, 1996). In general, lyssaviruses are relatively stable, although the opportunity for selection and adaptation may change such stasis. Several factors may be involved in generating sequence heterogeneity in rabies virus, including infection duration, transmission route, virus load, host immune response and virus and host protein interaction (Kissi et al., 1999). In the absence of proofreading enzymes to correct errors in replication, genetic drift gradually occurs over time from a limited accumulation of spontaneous mutations, rather than recombination. Certain geographic features, such as mountains and rivers, may create physical barriers to animal movement and promote localized viral evolution in specialized hosts (Bourhy et al., 1999). Movements of infected animals to new unaffected areas have the potential to produce explosive, sustainable outbreaks (Childs et al., 2000). Occasionally, although unpredictably, less frequent but more rapid emergence of viral variants may occur, thereby possibly extending the host range (Kissi et al., 1999). Virus circulation is an equilibrium of multiple components, including genetic and antigenic properties of the pathogen, pathobiology of infection on the individual host level and ecological properties of the host on the population level (Rupprecht et al., 2011). To date, little is known about the relationship between the potential diversity available for selection and evolution observed in nature, especially the colonization of new animal vector species by RABV.

In March 2011, an epidemic of rabies in pigs occurred in a rural pig farm in Sihui of Guangdong Province in Southern China, resulting in the death of 14 pigs. Pigs are occasionally infected by rabies viruses (Jiang et al., 2008), but in this incident, the rabies virus seemed to spread in the herd through bites. Such phenomenon has not been reported, although it is likely to occur. Therefore, we isolated the swine-origin RABV strain, tested the pathogenicity in mice, and determined and analyzed the complete genome

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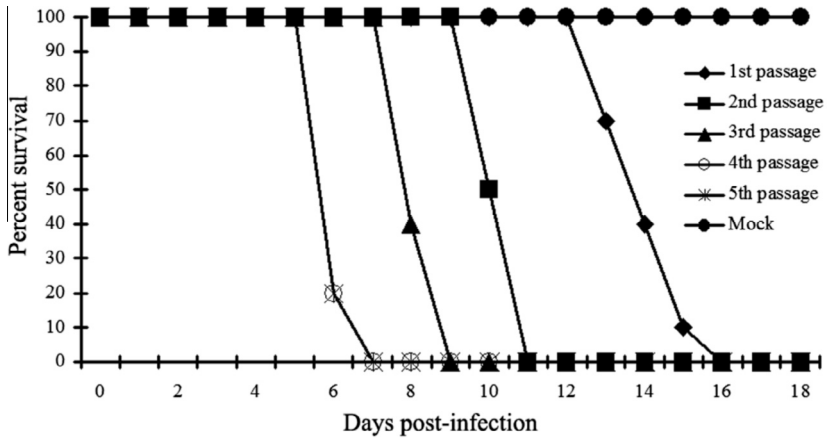

Fig. 1. Survivorship of suckling mouse intracranial inoculation with 10^4 FFU/mouse GD-SH-01 in five consecutive passages.

Table 1
Virus titers and pathogenicity of GD-SH-01.

Strain	Virus titer (Log FFU/ml)		Neurotropism Index[a]	LD_{50}/ml		Pathogenicity index[b]	
	NA	BHK-21		Suckling mice	Adult mice	Suckling mice	Adult mice
GD-SH-01	5.92	4.31	1.61	$10^{-5.34}$	$10^{-4.9}$	0.26	0.1
CVS-24	8.78	7.21	1.57	$10^{-7.98}$	$10^{-7.5}$	0.16	0.05

[a] The neurotropism index is the logarithm of the titer in NA cells minus the logarithm of the titer in BHK cells.
[b] Pathogenicity index was calculated by dividing the LD_{50} value per ml determined after inoculation by the virus titer (FFU/ml) in NA cells.

sequence to study the viral genetic variation and pathogenic mechanism for adapting to the new host. This research would help establish a strategic plan for rabies prevention and control based on an understanding of the ecology and dynamics of RABVs in nature.

2. Materials and methods

2.1. Cells and virus

Neuroblastoma NA cells of A/J mouse were grown at 37 °C in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). BHK-21 cells were grown at 37 °C in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 10% FBS. A virulent wild RABV isolate, GD-SH-01, was obtained from the brain of a naturally infected pig from Guangdong Province in South China. A stock of virus [10% brain suspension in phosphate-buffered saline (PBS)] was prepared following a single passage of the primary isolates in suckling mouse brains. Virulent standard rabies virus strain CVS-24 was propagated in the brain of the suckling mice.

2.2. Titration of virus

The virus was titrated by a focus assay on confluent monolayers of NA or BHK-21 cells in 24-well plates. At 48 h post-infection (hpi), cells were fixed in 80% acetone and stained with FITC-labeled RABV N protein-specific antibody (Centocor, Inc). Foci were counted using a fluorescence microscope, and the virus titers were calculated in focus-forming units (FFU).

2.3. Pathogenicity studies in mice

After five successive passages in suckling mouse brains (10^4 FFU/mouse), the GD-SH-01 virulence for suckling and adult mice was measured in 2-day-old and 6-week-old female Kunming mice, respectively. Groups of 10 suckling or adult mice were intracranially inoculated with 10 and 25 μl of serial 10-fold dilutions of virus, respectively. The animals were observed for four weeks, and the 50% lethal dose (LD_{50}) was calculated using virulence of the method of Reed and Muench (Reed and Muench, 1938).

2.4. Genome sequencing and analysis

Total RNA from the brain tissues of the rabid pig was extracted with TRIzol reagent (Invitrogen, USA) according to the instructions of the manufacturer. First-strand cDNA was synthesized using random primers and Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (TaKaRa, Japan). The entire genome was generated with PCR using 10 pairs of primers (Supplementary Table S1) amplifying 10 overlapped RV fragments. The forward primer for the 5′ end and the reverse primer for the 3′ end were designed complementary to the 11 bases of both UTRs to amplify both ends of the genomic sequence. The two UTRs were considered highly conserved in RABV (Marston et al., 2007; Ming et al., 2009). The PCR products were purified and cloned into a pMD18-T vector (TaKaRa, Japan) and sequenced on a 3730 DNA Analyzer (Applied Biosystems). The genome was assembled using DNAStar (version 7.0). The complete genome sequence of strain GD-SH-01 was submitted to GenBank under Accession No. JX088694. A brief report of the complete genome was published in the Genome Announcement (Luo et al., 2012). Multiple sequence alignment and identity were ascertained using CLUSTALX version 1.83 (Thompson et al., 1997). The phylogenic tree was constructed with MEGA v5.1 (Tamura et al., 2011) using the neighbor-joining (NJ) method. Branching pattern was statistically evaluated by bootstrap analysis of 1000 replicates.

3. Results and discussion

3.1. Pathogenicity of GD-SH-01

GD-SH-01 was isolated from the brain of a rabid pig, and five consecutive passages were performed in suckling mouse brains.