Infection, Genetics and Evolution 43 (2016) 347–353

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Characterization and pathogenicity of a novel mammalian orthoreovirus from wild short-nosed fruit bats

Zhijie Li [1], Dafei Liu [1], Xuhua Ran, Chunguo Liu, Dongchun Guo, Xiaoliang Hu, Jin Tian, Xiaozhan Zhang, Yuhao Shao, Shengwang Liu *, Liandong Qu *

State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China

ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 30 May 2016
Accepted 30 May 2016
Available online 31 May 2016

Keywords:
Mammalian orthoreoviruses
Genome sequencing
Pathogenicity
Reassortment
Bat

ABSTRACT

Mammalian orthoreoviruses (MRVs) have a wide range of geographic distribution and have been isolated from humans and various animals. This study describes the isolation, molecular characterization and analysis of pathogenicity of MRV variant B/03 from wild short-nosed fruit bats. Negative stain electron microscopy illustrated that the B/03 strain is a non-enveloped icosahedral virus with a diameter of 70 nm. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) migration patterns showed that the B/03 viral genome contains 10 segments in a 3:3:4 arrangement. The isolate belongs to MRV serotype 1 based on S1 gene nucleotide sequence data. BALB/c mice experimentally infected with B/03 virus by intranasal inoculation developed severe respiratory distress with tissue damage and inflammation. Lastly, B/03 virus has an increased transmission risk between bats and humans or animals.

1. Introduction

Mammalian orthoreovirus (MRV) belongs to the genus Orthoreovirus, which includes non-enveloped double-stranded RNA viruses, each with a genome comprising 10 genetic segments divided into three size classes (Attoui et al., 2011). Four major MRV serotypes have been characterized by neutralization assays, and all inhibit hemagglutination: type 1 Lang (T1L), type 2 Jones (T2J), type 3 Dearing (T3D) and type 4 Ndelle (T4N) (Kohl et al., 2012; Attoui et al., 2001a, 2001b). MRV isolates were obtained from hosts with or without clinical signs of disease, and the virus can infect a broad range of mammals (Dermody et al., 2013). MRVs are ubiquitous mammalian pathogens, infecting nearly all mammalian hosts, including humans and other animal species (Steyer et al., 2013; Decaro et al., 2005; Attoui et al., 2011).

Infected bats are associated with an increasing number of emerging and re-emerging viruses, including the Hendra virus (HeV), Nipah virus (NiV), Ebola virus (EBOV) and SARS coronavirus. Infected bats threaten public health because they exist in large populations and travel across wide geographical distances (Wong et al., 2007; Calisher et al., 2006). However, reports on the detection and isolation of orthoreovirus from bats are limited. In 1968, the first orthoreovirus in bats, Nelson Bay virus (NBV), was isolated from the blood of fruit bats in Australia. In 1999, the second bat-borne orthoreovirus, Pulau virus (PulV), was isolated from fruit bat urine collected on Tioman Island, Malaysia. Since then, bat-borne orthoreoviruses have received much attention. Additional orthoreoviruses (MelV, KamV, Xi-River, Broome viruses, Kampar, Sikamat, HK23629/07, RpMRV-YN2012, Cangyuan virus) have been isolated from or detected in bats and in humans who were likely in contact with bats (Chua et al., 2007; Chua et al., 2008; Du et al., 2010; Thalmann et al., 2010; Cheng et al., 2009; Chua et al., 2011; Wang et al., 2015; Hu et al., 2014).

Recently, several groups have reported MRV infection in bats that resulted in visible pathology within tissues (Kohl et al., 2012; Lelli et al., 2013). The authors speculated that bat-to-human interspecies transmission was possible, but no substantial evidence to support this hypothesis was provided. In this study, we report the characterization of a novel MRV strain (called "B/03") isolated from healthy, wild short-nosed fruit bats in Guangdong province, China. The whole genome sequence of strain B/03 was determined. Its evolution and evidence of genetic reassortment were analyzed by sequence comparison using phylogenetic analysis. Furthermore, we evaluated the pathogenicity of B/03 virus using four-week-old female BALB/c mice.

2. Materials and methods

2.1. Virus, cells and animals

MRV strain MPC/04 was isolated from masked palm civets in Guangdong Province in southern China by our laboratory and caused a

* Corresponding authors at: State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, CAAS, 427 Maduan Street, Harbin 150001, China.
E-mail addresses: SWLIU@hvri.ac.cn (S. Liu), qld@hvri.ac.cn (L. Qu).
[1] Zhijie Li and Dafei Liu have contributed equally to this work.

http://dx.doi.org/10.1016/j.meegid.2016.05.039
1567-1348/

potentially fatal infection of the inoculated host mouse (Li et al., 2015). Vero E6 cells were obtained from the ATCC (ATCC® CRL-1586™) and grown at 37 °C in 5% CO_2 in DMEM supplemented with 2 mM glutamine, 5% fetal calf serum and antibiotics. Four-week-old female BALB/c mice were obtained from the experimental animal center of Harbin Veterinary Research Institute (HVRI). All animals were housed in the animal facility at HVRI under standard conditions in accordance with institutional guidelines.

2.2. Viral isolation and identification

Thirty tissue samples from short-nosed fruit bats were collected from Shaoguan city of China's Guangdong province and homogenized. The homogenate was filtered through a 0.22 μm pore-size filter and used to inoculate confluent monolayers of Vero E6 cells. Blind passages were performed until a cytopathic effect (CPE) was observed. The infected cells were plaque purified, and the virus was propagated in Vero E6 cultures. Virus was collected from infected cells by three freeze-thaw cycles. Aliquots were stored at −80 °C. One aliquot was titrated on Vero E6 cells to estimate a titer by plaque assay. If CPE was not observed after 4 passages, the result of virus isolation was considered negative. The infected cells were prepared for negative stain and thin section examination by electron microscopy (EM).

In addition, an indirect immunofluorescence assay (IFA) was used to detect MRV proteins in infected cell cultures. Briefly, after washing with PBS, cells were fixed with 4% paraformaldehyde and incubated with 1% BSA for 1 h. Then, the cells were incubated with a mouse anti-MRV (T3D) antibody, followed by a goat anti-mouse IgG-FITC secondary antibody (SANTA CRUZ, USA). After washing, fluorescence was observed under an AMG EVOS F1 inverted microscope. Normal mouse sera, diluted 1:50, was used as a negative control.

2.3. Electropherotype

Viral dsRNA was extracted from purified virus particles using TRIZOL Reagent according to the manufacturer's protocol. Double strand RNA (dsRNA) segments were separated by electrophoresis in 8% (*w*/*v*) polyacrylamide slab gels. Approximately 30 μl of each sample was loaded

Table 1
Primers used in this study for amplification of the full-length genome of B/03 strain.

Gene	Primer	Start	Sequence (5′-3′)
L1	L1a	9	TTCCACGACAATGTCATCCA
	L1b	1019	AGTTCGCGCGCTTTCTTATC
	L1c	951	GGGAGTCATGCCATTGTCCA
	L1d	1964	TGAATCATGTTCTGCATTCC
	L1e	1886	CTGCATCCATTGTAAATGACGAGTC
	L1f	2339	GCTATGTCATATTTCCATCCGAATTC
	L1g	1930	GCTAGGCCGATATCGGGAATGCAG
	L1h	2278	CTTGAGATTAGCTCTAGCATCTTCTG
	L1i	2212	CCAAGGTGACGACGGACTGA
	L1j	2901	CGCTCGTCCAGATTTCGTAG
	L1k	2806	AACGCAGATTATCGCAGGTG
	L1l	3839	CACGACCCATGGTAGACTCA
L2	L2a	13	ATGGCGAACGTY(T/C)TGGGGR(A/G)GTGAG
	L2b	817	GGACGTTGGCTGAGAATTGCTCTA
	L2c	735	GGTCAGY(T/C)GCR(A/G)CCTCCTTATATT
	L2d	1684	CACCACGGGCAACATCATAAC
	L2e	1577	CCGTTAGTCATAGAGCCCTGGATT
	L2f	2681	CGGCCCCCAAAGACAACATA
	L2g	2578	CAGCCGAGTGGM(A/C)TGY(T/C)TGGAA
	L2h	3346	CTGGATTAGCGTTAGACCCGAC
	L2i	3301	GGAATCTAY(C/T)ACM(A/C)ATGCAGGC
	L2j	3895	GAGGGACRR(A/G)TGAGTTACAGAGG
L3	L3a	13	GATGAAGCGGATTCCAAGGA
	L3b	1148	AAAACCCCGTGTGCCTATTC
	L3c	1054	GGGGGCTAATCCGCTAATGTT
	L3d	2179	GCGCCATAACGAATCTGAGAG
	L3e	2125	GTGGGCTGAAATTATTCATAGATACTGG
	L3f	3489	GCATTAGCGTACTGACGTGGATCATA
	L3g	3439	CGCTTACCCATACATGCTGC
	L3h	3901	GATGAATCGGCCCAACTAGC
M1	M1a	1	GCTATTCGCGGTCATGGC
	M1b	1485	CCTGTCATCATGCGGAATGAG
	M1c	1382	GAGCAK(T/G)GCGGTTATGGAR(G/A)AT
	M1d	1764	TGCGCR(G/A)CTAGTR(A/G)GCATACAT
	M1e	1590	CATTCGCTCATGCCGATAGTG
	M1f	2304	GATGAAGCGCGTACGTAGTCTTAG
M2	M2a	2	GCTAATCTGCTGACCGTCACTC
	M2b	2199	TGTGCCTGCATCCCTTAACC
M3	M3a	1	GCGGTCGGTCGACGCTAAAGTGACCGTGGTCATGGCTTCATTCAAGGG
	M3b	2241	GCAGGGGATCCGATGAATGGGGGTCGGGAAGGCTTAAGGG
S1	S1a	9	CGCCTATGGATGCATCTCTCA
	S1b	400	CAAAGTGGATGTTCGTCCAGTGA
	S1c	340	ACCACGAGTTGACAGTCTGGAT
	S1d	1436	CGCGCTAGATTCACCTCACATT
S2	S2a	1	GCTATTCGCTGGTCAGTTATGGC
	S2b	1331	GATGAATGTGTGGTCAGTCGTGAG
S3	S3a	1	GCTAAAGTCACACCTGTCGTCGTC
	S3b	1198	GATGATTAGGCGTCACCCACCAC
S4	S4a	1	GCGAATTCGCTATTTTTGCCTCTTCCCAGA
	S4b	1215	CAS4TGCCTGCAGATGAATGAAGCCTGTCCCACGTC