Virus Research 143 (2009) 6–14

Contents lists available at ScienceDirect

Virus Research

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

Molecular characterization of the complete genome of a street rabies virus isolated in China

Pinggang Ming[a,b], Jialiang Du[a], Qing Tang[a,*], Jiaxin Yan[b], Susan A. Nadin-Davis[c], Hao Li[a], Xiaoyan Tao[a], Ying Huang[d], Rongliang Hu[d], Guodong Liang[e]

[a] State Key Laboratory for Molecular Virology and Genetic Engineering, National Institute for Viral Disease Control and Prevention, China CDC, 100 Yingxin St., Xuan Wu District, Beijing 100052, China
[b] Department of Genetic Engineering, Wuhan Institute of Biological Product, 9 Linjiang St., Wu Chang District, Wuhan 443006, China
[c] Rabies Center of Expertise, Ottawa Laboratory-Fallowfield, Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, ON, Canada K2H 8P9
[d] Laboratory of Epidemiology, Veterinary Institute, Academy of Military Medical Sciences, 1068 Qinglong Road, Changchun 130062, China
[e] State Key Laboratory for Infectious Disease Prevention and Control, Institute for Viral Disease Control and Prevention, China CDC, Beijing 100052, China

ARTICLE INFO

Article history:
Received 24 November 2008
Received in revised form 17 February 2009
Accepted 25 February 2009
Available online 13 March 2009

Keywords:
Complete genome sequence
Street virus
Rabies
China

ABSTRACT

In this study, the complete genomic sequence of a rabies virus isolate HN10, recovered from brain tissue of a rabid patient in China, was determined. This is the first Chinese street isolate that has been fully characterized. The overall organization of this virus is typical of that observed for all other rabies viruses. Alignments of amino acid sequences of the phosphoprotein, glycoprotein and large protein of HN10 with those of other rabies viruses were used to examine the extent of conservation of known functional regions. Phylogenetic analysis using either the complete or partial genomic sequence of HN10 determined that this isolate is most closely associated with viruses previously shown to circulate in Guangxi and Hunan provinces. In addition, of all vaccine strains used for comparison, the attenuated Chinese vaccine strain CTN181 is most closely related to HN10.

1. Introduction

Rabies is a widespread zoonotic disease which is acute and virtually universally fatal once clinical signs present. In countries of Asia and Africa, where rabies has become an important public health problem, it is estimated that this disease is responsible for over 50,000 human deaths annually (WHO, 2005). In China, which now has the second highest number of human cases in the world after India (Tang et al., 2005), the human death toll has been increasing since 1998 (Zhang et al., 2006); in the year 2007 alone total case numbers were 3302 with particularly high incidence in southern and eastern regions.

The causative agent of this disease, rabies virus (RABV), comprises genotype 1 of the *Lyssavirus* genus in the family *Rhabdoviridae*. It is a non-segmented, single-stranded negative-sense RNA virus. The RABV genome is approximately 12 kb, comprising five genes that encode the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase (large protein, L) (Tordo et al., 1986; Wunner, 2007). Several of these genes, particularly the N (Kissi et al., 1995), G (Badrane et al., 2001) and P (Nadin-Davis et al., 2002) have been targeted for detailed phylogenetic analysis of rabies viruses recovered from around the world. These studies have defined several genetically distinct viral strains that circulate in geographically restricted areas in particular host species. In China, N (Zhang et al., 2006) and G gene (Meng et al., 2007) sequence analysis identified three main groupings of street viruses that clustered respectively with strains from Indonesia, Thailand/Malaysia and the cosmopolitan lineage that includes many vaccine strains. Although closely related viruses were often recovered from particular regions, some provinces, such as Hunan, Guizhou and Guangxi (Zhang et al., 2006; Liu et al., 2007) yielded isolates from two or more main groupings indicating substantial movement and mixing of strains at least in certain parts of the country. Extensive migration of humans and their associated animals in recent times is likely to have contributed to this pattern.

Currently, many kinds of rabies virus strains are used for vaccine production in different countries and several of these have been genetically characterized (Conzelmann et al., 1990; Du et al., 2008; Ito et al., 2001; Geue et al., 2008; Metlin et al., 2008). There are known biological differences between street and attenuated strains of rabies (Yu, 2006) which can influence the host's ability to initiate apoptosis of infected cells (Morimoto et al., 1999) and mount innate immune responses (Wang et al., 2005) but in most cases the molecular basis for these differences is not clear. In a previous study the complete genomic sequence of the vaccine strain CTN181 was determined (Du et al., 2008). To facilitate a detailed compari-

* Corresponding author. Tel.: +86 1063539777; fax: +86 1063532053.
E-mail address: qtang04@sina.com (Q. Tang).

0168-1702/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2009.02.014

Table 2
The rabies viruses for which complete genome sequence is available that were used in this study.

Accession no.	Strain	Country of isolation	Length (bp)	Note	References
Vaccine strains					
EF564174	CTN181	China	11923	Attenuated vaccine strain, derived from strain CTN-1, which is used for production of human rabies vaccine in China	Du et al. (2008)
AB044824	Nishigahara	Japan	11926	The parental strain of RC-HL	Ito et al. (2001)
AB085828	HEP-Flury	America	11615	Isolated from a rabid patient in 1940; used as attenuated vaccine strain after 136 passages in chicken embryo fibroblast cells	Inoue et al. (2003)
AB128149	Ni-CE	Japan	11926	Established from the Nishigahara strain after 100 passages in chicken embryo fibroblast cells	Shimizu et al. (2007)
AF499686	SRV9	China	11928	Avirulent vaccine strain; maintained in BHK-21 cells	Unpublished (2004)
EF206707	ERA	America	11931	Attenuated rabies vaccine strain closely related to the SAD strain	Geue et al. (2008)
M13215	PV	France	11932	Pasteur vaccine strain	Tordo et al. (1986, 1988)
M31046	SAD B19	America	11928	Attenuated vaccine strain	Conzelmann et al. (1990)
EF542830	RV-97	Russia	11932	Russian vaccine strain	Metlin et al. (2008)
AB009663	RC-HL	Japan	11926	Attenuated strain used for production of animal vaccine in Japan	Ito et al. (2001)
EF206719	SAG 2	America	11928	Attenuated vaccine strain Street Alabama Gif; derived from SAD field virus	Geue et al. (2008)
Street strains					
EU643590	HN10	China	11923	A street virus isolated from a rabid patient	This study
EU549783	BD06	China	11924	A street virus isolated in China	Unpublished (2008)
AY705373	SHBRV-18	America	11923	A silver-haired bat-associated virus isolated in the United States	Faber et al. (2004)
EU311738	RRV ON-99-2	Canada	11923	A raccoon strain virus isolated in Canada in 1999	Szanto et al. (2008)
AY956319	Hum-Trans-IND	Germany	11928	A street virus transmitted by solid organ transplantation; imported from India	Unpublished (2005)
EF437215	NNV-RAB-H	India	11928	An Indian street virus isolated from human brain tissue	Unpublished (2007)
EU293121	8743THA	Thailand	11923	An Thailand street virus isolated from human	Delmas et al. (2008)
EU293111	8764THA	Thailand	11925	An Thailand street virus isolated from human	Delmas et al. (2008)

son of the sequence characteristics of this attenuated vaccine strain with a phylogenetically related pathogenic street isolate, the complete genomic nucleotide sequence of a Chinese rabies virus street isolate is now described.

2. Materials and methods

2.1. Virus strain

The HN10 strain was isolated from the brain tissue of an 18-month-old boy with rabies in Yongzhou, Hunan province, China. Touch impressions of the brain were taken from the regions of the hippocampus, cerebellum, and medulla for direct fluorescent assay (DFA) using rabies DFA reagent (Millipore, UK), according to the manufacturer's instructions.

2.2. Primer design

In total, 24 pairs of primers spanning the entire HN10 genome were designed using the Primer Premier, version 5 software (PREMIER Biosoft International, CA, USA) based on the full-length genome sequence of the PV strain (GenBank accession no. M13215) (Table 1 in the supplementary material). All of the primers were designed within the conserved regions of the genome, determined from an alignment of all of the full genomic sequences of the reference strains, except Mokola virus (see Table 2). To amplify both ends of the genomic sequence, the forward primer for the 5′ end and the reverse primer for the 3′ end were designed to be complementary to the 11 bases of both UTRs. These two UTRs are considered to be highly conserved in rabies virus (Bourhy et al., 1989; Marston et al., 2007; Szanto et al., 2008). All primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.

2.3. Reverse transcription-PCR and direct sequencing

Total RNA from the brain tissues of the rabid patient was extracted with TRIzol Reagent (Invitrogen, USA) according to the manufacturer's instructions. The RNA pellet was resuspended in 70 μl of DNAse-, RNase-free sterile water (Promega, USA) and stored at −70 °C. For reverse transcription, 32 μl of total RNA was heated at 60 °C for 10 min, then quickly chilled on ice for at least 2 min, and then transferred to a reaction tube of Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences, USA) with 1 μl of random primer pd$(N)_6$ (0.2 μg/μl) (TaKaRa, Japan). After incubation at 37 °C for 60 min, the synthesized cDNA product was used for PCR. Twenty-four overlapping fragments were amplified by PCR using the Platinum Pfx DNA Polymerase kit (Invitrogen, USA) and the primers in Table 1. Briefly, 5 μl of cDNA was amplified with 20 pmol of each primer and 2.5 U of Pfx DNA polymerase. The cycling parameters were one cycle at 94 °C for 2 min for an initial denaturation, then 35 cycles at 94 °C for 15 s, 52 °C for 30 s, and 68 °C for 60 s, and a final extention at 68 °C for 10 min. The PCR products were identified on 1% Ultrapure™ Agarose (Invitrogen, USA) gel and visualized by ethidium bromide staining under UV illumination with 2 kb DNA markers (TaKaRa, Japan). All of the PCR products of the expected size were then excised from the agarose gel and purified with the QIAquick Gel Extraction Kit (Qiagen, Germany), following the manufacturer's instructions. The purified products were then directly sequenced commercially (Shanghai Sangon Biological Engineering