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Molecular Characteristics of the N Gene of the Chicken Embryo Cell-Adapted Rabies Virus Strain CTNCEC25

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Abstract

Rabies virus is the prototypical species of neurotropic viruses and is the main causative agent of rabies, an ancient central nervous system disease that is almost invariably fatal. Recently, CTNCEC25, the China vaccine strain CTN-1 adapted to chicken embryo cells, was obtained and its complete genome was sequenced. Previous studies have demonstrated that CTNCEC25 possessed high immunogenicity and induced high levels of anti-rabies antibodies in animals. In the present study, the molecular characteristics and bioinformatic analysis of CTNCEC25 N gene was investigated. Sequence alignment showed that a single synonymous mutation was occurred in the CTNCEC25 N gene compared to the parent CTN-1 strain and all the important motifs and antigenic sites were conserved in CTNCEC25 N. The percentage homology of CTNCEC25 N gene with other rabies virus strains ranged from 99.9% to 84.8%. Phylogenetic analysis demonstrated that CTNCEC25 was closely related and clustered into the same group with most of those rabies virus street strains isolated in different regions in China. These results provide fundamental data to the characteristics of the CTNCEC25 N gene and pave the way for future application of CTNCEC25 for rabies control in China.

Keywords: Rabies virus; CTNCEC25; N gene; Phylogenetic analysis

Introduction

Rabies is an ancient acute central encephalomyelitis that affects almost all kinds of mammals, including humans [1]. It causes one of the most fatal zoonotic diseases and the mortality is almost 100%. Approximately 55,000 human lives are estimated to be lost annually due to bites inflicted by rabid animals and more than 15 million people undergo post-exposure prophylaxis every year around the world [2]. Most of those human deaths occur in the developing world such as Asia and Africa where rabies is endemic and resources are limited [3]. China has the second highest incidence of rabies after India and rabies is still considered as a great threat to the public health in China [4].

The causative agents of rabies are viruses belonging to the *Lyssavirus* genus in the family *Rhabdoviridae* of which the type species rabies virus (RABV) is responsible for the majority of cases. RABV has a non-segmented, single-stranded negative-sense RNA genome of approximately 12 kb which encodes five structural proteins (in the order 3' to 5'): nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (large protein, L) [5]. The N, P and L together with the viral genomic RNA form a ribonucleoprotein (RNP) complex in the virion core while the M and G are associated with the viral envelope and wrap the RNP complex [5].

The N protein is most abundant protein in the RNP complex and is involved in the encapsidation of viral genome [6]. Besides, the N gene is also the most conserved gene in the viral genome, making it an ideal candidate for phylogenetic analysis [7]. It was found that the N protein was phosphorylated in RABV possibly by a cellular casein kinase II and different phosphorylation states of N played an important in role in viral transcription and replication [8,9]. Furthermore, previous studies have mapped several functional regions to the N protein. It has been shown that in addition to the G protein, the N protein also possessed antigenic and immunogenic properties. A number of antigenic sites have been identified in the N protein, including antigenic site I (aa 358-367), antigenic site II (aa 373-383) and antigenic site IV (aa 359=366 and aa 375-383) [10,11], and a phosphorylation site, Ser389, was also involved in the N antigenicity [11,12]. In addition, several RABV-specific Th cell epitopes were identified in the N protein, comparing aa 21-35 and the so-called 31D (aa 404-418), the latter of which was shown to be an immunodominant epitope to stimulate RABV-specific Th cell production *in vitro* [13,14]. Finally, the RNA-binding site on N for interaction with viral RNA has been localized to region aa 298-352 of the N protein [15].

Results and Discussion

Sequencing of the CTNCEC25 N gene identified that only a synonymous mutation was occurred at 461 nt from a G in CTN-1 to a A in the CTNCEC25 genome [16]. Comparison of the N protein sequence of CTNCEC25 with those of other selected RABV strains indicated that virtually all of the above important functional motifs were retained in CTNCEC25 N protein (Figure 1). To further investigate the N gene homology between the CTNCEC25 with other RABV strains selected, the comparison of complete coding sequence of the N gene was performed. As can be seen in Table 1, the percentage homology of CTNCEC25 N gene with other strains ranged from 99.9% with the parent CTN-1 strain to 84.8% with the SHBRV18 strain isolated in America.

Our previous study using the complete genome nucleotide or the mature G protein amino acid sequence has shown that CTNCEC25 was more closely related with China indigenous RABV strains than other vaccine strains commonly used in China [17].

Strains	Accession no.	Similarity (%)	Strains	Accession no.	Similarity (%)
CTN-1	FJ959397	99.9	HN10	EU643590	94.6
SRV9	AF499686	88.3	BD06	EU549783	90
SAG2	EF206719	88.3	DRV	DQ875051	86
ERA	EF206707	88.2	CQ92	EU159388	90.3
SAD B19	M31046	88.3	MRV	DQ875050	88.8
HEP-Flury	AB085828	88.5	SHBRV18	AY705373	84.8
FluryLEP	DQ099524	88.5	FJ008	FJ866835	89.9
PM	DQ099525	89	D01	FJ712193	90.1
RC-HL	AB009663	86.6	Yue1	EU159385	88.2
CVS	D42112	88.8	N11	FJ594278	89.1
PV	M13215	88	GX4	EU159386	95.4
FY1	EU159362	90	HNDB11	EU008919	90.1
Jiangsu_Wx1	DQ666321	89.4	NC	EU159389	89.9
JSL26	EU159381	90	QC	EU159377	89.5
Guangxi Y166	DQ666287	89.5	Henan_Sq59	DQ666306	89.7
HuNAN16	DQ515993	89.8	Yunnan QJ07	EU275245	89.9
Guizhou Qx5	DQ666296	89.7	JX08-45	GU647092	94.8

Table 1: Homologies of the CTNCEC25 N Gene Sequence with Those of Other RABV Strains.

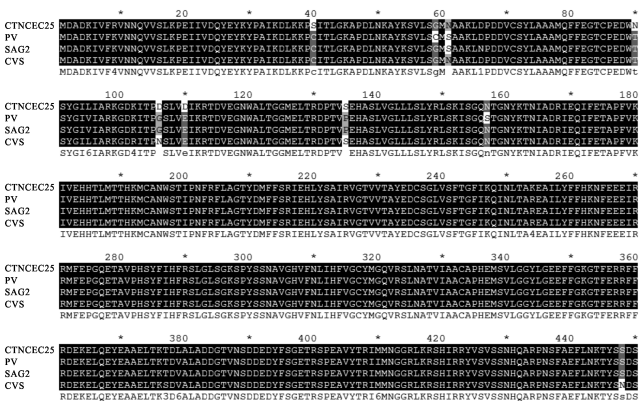

Figure 1: Amino acid sequence alignment of the amino acid sequence of CTNCEC25 N protein with other RABV N proteins. Four RABV N protein sequences were selected and aligned using Clustal X 1.83 and edited with GeneDoc software. Black shading denotes 100% conservation. Dark gray and light gray shading represents 80% and 60% conservation, respectively. Rules used to assign conservation are denoted as follows: A=G=S=T, V=L=I =M=F=Y=W, N=Q=D=E, and R=K=H.

To further investigate the genetic relationships and evolution of CTNCEC25 with these other selected virus strains using the N gene sequence, phylogenetic analysis were performed based on nucleotide sequence of the N gene (Figure 2). The results showed that similar to the parent CTN-1 strain, the CTNCEC25 strain was clustered with most of the RABV strains isolated in different regions in China. In contrast, the vaccine strains commonly used in China, PM and PV, clustered into another group with strains isolated in other countries. The above results were consistent with data from our previous study.

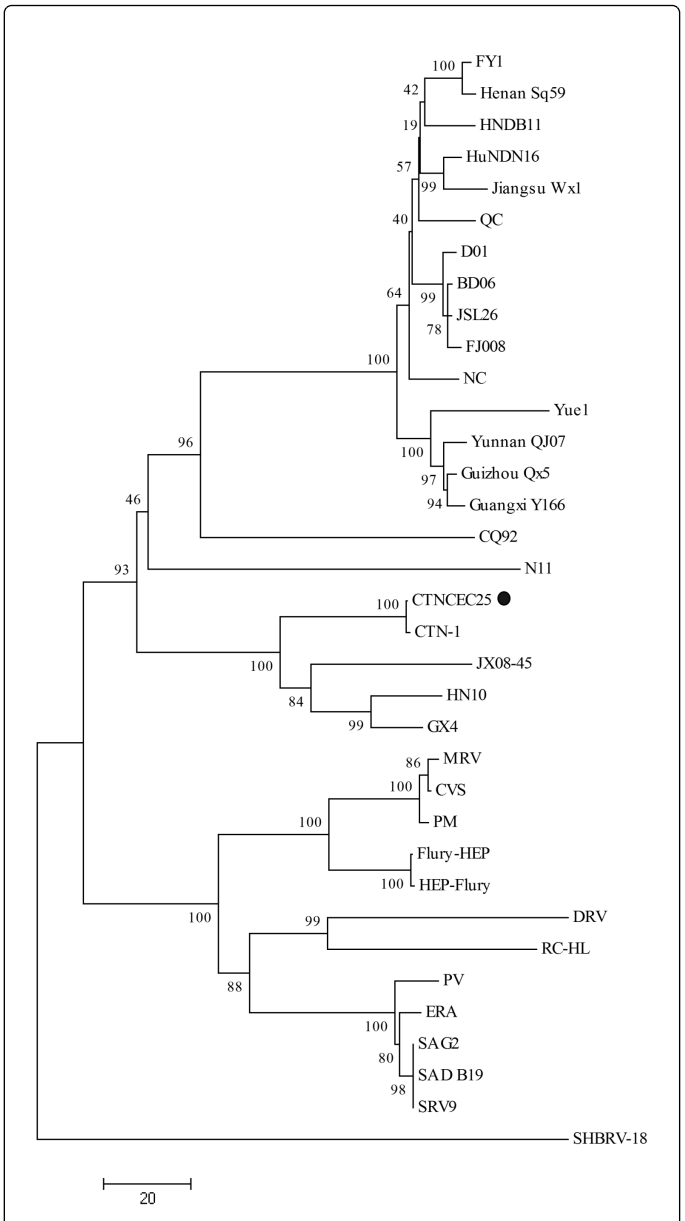

Figure 2: Phylogenetic analysis of the N gene sequence of CTNCEC25 compared with other RABV strains. The tree was constructed using the Neighbor-Joining algorithm in MEGA 4.0 software. The numbers below the branches are bootstrap values for 1000 replicates. CTNCEC25 was marked with a black solid circle.

In summary, the present study described the characterization of the N gene of the chicken embryo cell-adapted CTNCEC25 strain and the

results demonstrated that the N gene is highly conserved in different RABV strains. Importantly, phylogenetic analysis using the N gene sequence demonstrated that CTNCEC25 was more closely related to China indigenous RABV strains than other vaccine strains used in China using the complete genome sequence, the mature G protein amino acid sequence and the N gene sequence. As it has been shown that variations existed in virus-neutralizing antibody titer values when heterologous virus strains other than homologous virus strains were used as the challenge virus [18,19], it is therefore reasonable to assume that the best vaccine strain should be the one most closely related to the circulating strains within a target area. Further studies are undertaken to unravel the potential application of CTNCEC25 in rabies prevention and control in China.

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