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 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].
To further investigate the genetic relationships and evolution of CTNCEC25 with these other selected virus strains using the N gene sequence, phylogenetic analyses 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.

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.

References