No.	23-3031	
Project Title	全てのパラミクソウイルスに対応する弱毒ワクチン開発機構と新規ワクチンベクターへの応用	
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共同研究報告　（研究完了）

In this study, to utilize paramyxoviruses that do not conform to the rule of six as vaccine platforms, we aimed to establish an experimental system capable of detecting whether genome length conforms to multiples of six nucleotides.

Paramyxoviruses are classified into three major subfamilies: *Orthoparamyxovirinae*, *Rubulavirinae*, and *Avulavirinae*. Among these, *Orthoparamyxovirinae* includes the genera *Respirovirus*, *Morbillivirus*, and *Henipavirus*, which include many viruses that infect humans and animals. Sendai virus (SeV), a member of the genus *Respirovirus*, infects rodents and has been widely used as a model virus for studying paramyxovirus replication. Canine distemper virus (CDV), belonging to the genus *Morbillivirus*, infects canids and causes systemic disease.

Paramyxoviruses share a common mechanism of genome replication. In SeV, the negative-sense single-stranded RNA genome encodes six genes in the order 3′–N–P/V–M–F–HN–L–5′, separated by gene junctions. The genome is encapsidated by the nucleoprotein (N) and serves as a template for the RNA-dependent RNA polymerase (RdRp) complex, composed of the large protein (L) and phosphoprotein (P). This complex synthesizes mRNAs sequentially from the genome and also produces a positive-sense antigenome, which serves as a template for generating progeny genomes. In addition, paramyxoviruses follow the “rule of six,” whereby genome length must be a multiple of six nucleotides for efficient replication.

A notable feature of paramyxoviruses is RNA editing in the P/V gene region. During this process, the RdRp inserts guanine nucleotide(s) at a specific site in a template-independent manner. This insertion causes a frameshift in the mRNA, resulting in the production of different proteins. In Orthoparamyxovirinae, the P protein is produced without editing, whereas insertion of a single nucleotide leads to the production of the V protein. The P protein functions as an essential component of the polymerase complex, while the V protein acts as an accessory factor that interacts with host proteins and suppresses interferon responses. Thus, RNA editing influences the balance between P and V protein expression and affects viral replication. Conventional methods for measuring RNA editing efficiency, such as primer extension assays or sequencing of cloned cDNAs, have limitations in accurately quantifying diverse editing products.

Here, we developed a method to quantify RNA editing rates in infected cells by combining reverse transcription-PCR with nanopore sequencing using the MinION platform (Oxford Nanopore Technologies). By optimizing reverse transcription primers, RNA editing efficiencies were measured in mRNA as well as in antigenome and genome RNA. Using this approach, differences in RNA editing efficiency between SeV and CDV were identified. In addition, RNA editing was detected in the antigenome of CDV, suggesting that edited genomes can be generated in this virus and may give rise to genomes that do not conform to the rule of six. The method established in this study is expected to be useful for detailed characterization of viral properties. It will also support our ongoing vaccine development using paramyxoviruses that do not conform to the rule of six.