Reverse Genetics System for a Human Group A Rotavirus

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Abstract

Group A rotaviruses (RVs) are one of the etiological agents that cause severe diarrhea in mammals and avian host species. In humans, RVs are a leading cause of acute gastroenteritis in young children under the age of 5 years. RV infection kills approximately 215,000 infants and young children annually, particularly in developing countries. In 2017, we established an entirely plasmid-based reverse genetics system for simian RV. Although the simian RV reverse genetics system is robust enough to generate reassortant RVs and enable better understanding of the biological differences between animal and human RV strains, a complete reverse genetics system for human RV strains is desirable. Here, we established a plasmid-based reverse genetics system for human RV.

Background & Results

Recently, we developed an entirely plasmid-based reverse genetics system for simian RV strain SA11, thereby enabling manipulation of all 11 RV gene segments to expand our understanding of RV biology. However, the biological diversity among human and animal RVs means that this system is limited in terms of understanding the characteristics of human RVs. Thus, a reverse genetics system for human RV is needed. To establish the system, cDNAs encoding each of the human RV strain Odelia 11 dsRNA gene segments were introduced into plasmids at sites flanked by the T7 promoter and hepatitis delta virus ribozyme sequences. Recombinant human RV strain Odelia was generated by transfection of the 11 Odelia cDNAs and polymerase II promoter-driven expression plasmids encoding Nelson Bay reovirus FAST protein, vaccinia virus capping enzyme (D1R and D12L), and RV NSP2 and NSP5 proteins into baby hamster kidney cells expressing T7 RNA polymerase (Figure 1). This technology was used to generate a panel of monoreassortant viruses between human and simian RV strains for all of the 11 gene segments. Furthermore, we generated recombinant viruses lacking the C-terminal region of the viral nonstructural protein NSP1 and used it to define the biological function of NSP1 (Figure 2). The truncation mutant lacking the C-terminal 166 amino acids of NSP1 replicated poorly, suggesting that the C-terminal region of NSP1 plays critical roles in viral replication (Figure 2).

Significance of the research and Future perspective

Reverse genetics, an approach used to generate viruses from cloned cDNA, has increased our understanding of virus biology. Here, we developed a reverse genetics system for the human RV strain Odelia, which replicates efficiently and is suitable for *in vitro* molecular studies. The system will allow generation of engineered recombinant virus harboring desired mutations, increase our understanding of the molecular biology of human RV, and facilitate development of novel therapeutics and vaccines.

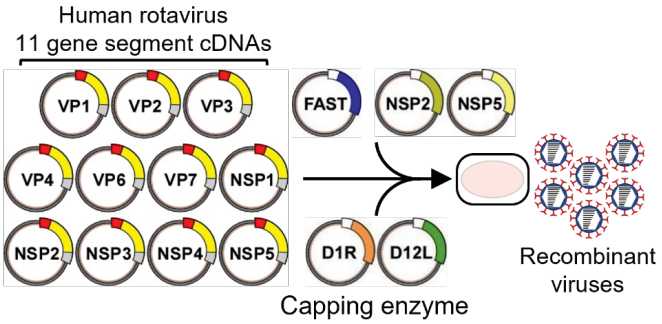

Figure 1. Human rotavirus reverse genetics system

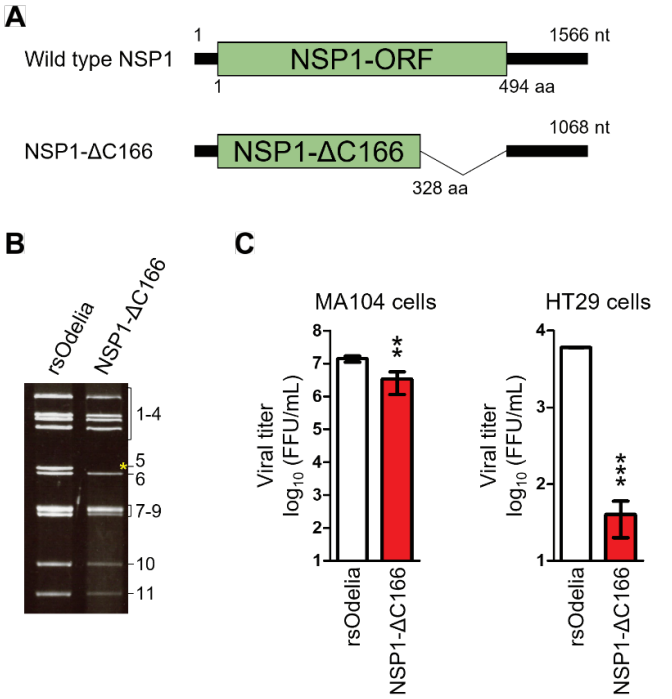

Figure 2. Generation of recombinant viruses lacking C-terminal region of NSP1. (A) Schematic presentation of the plasmid encoding the NSP1 gene used for recovery of recombinant virus. (B) Electropherotype of rsOdelia-NSP1-Δ166. (C) Viral replication of NSP1 mutant in MA104 cells and HT29 cells.

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Treatise Kawagishi, Takahiro et al. Reverse genetics system for a human group A rotavirus. J Virol. 2020 Jan 6; 94(2): e00963-19. doi: 10.1128/JVI.00963-19.
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URL

Keyword rotavirus, vaccine, reverse genetics system