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REVIEW

Molecular Basis of the Pathogenicity of the Foot-and-Mouth Disease Virus Isolated in Japan

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Abstract

Foot-and-mouth disease (FMD), the most contagious mammalian disease, causes severe economic damage to the livestock industry. Animals infected with FMD virus (FMDV) typically present with vesicles in the mouth, nostrils, and around the breasts and feet. The pathogenicity and infectivity of FMDV in cattle and swine are strain dependent. To investigate the molecular mechanisms underlying these differences, we examined the molecular basis of the pathogenicity of FMDV using two viral strains isolated from two outbreaks of differing severities in Japan. We demonstrated that the two strains have completely different transmissibility in inoculated cattle and virulence in suckling mice. Our study also indicated that VP1 and 3D proteins are independent genetic determinants of this difference. The selectivity of VP1 for host cell receptors and the fidelity of viral RNA during replication were considered important individual factors in the induction of differences in pathogenicity in the host and the severity of outbreaks in the field. These findings provide new insights into the molecular mechanisms underlying the pathogenicity of FMDV.

Discipline: Animal Science
Additional key words: full-length infectious cDNA clone, genetic determinant, molecular characterization

Introduction

Foot-and-mouth disease (FMD), the most contagious mammalian disease, causes severe economic damage to the livestock industry. The FMD virus (FMDV) belongs to the genus *Aphthovirus* in the family *Picornaviridae* (Knowles et al. 2012). Its genome comprises a single-stranded positive-sense RNA approximately 8.4 kilobases in length, divided into an S-fragment and L-fragment by a poly(C) sequence at the 5′ untranslated region (UTR) of the genome. Translation of viral mRNA is initiated at the internal ribosomal entry site (IRES) by a cap-independent mechanism (Mason et al. 2003). RNA is translated into a polyprotein within a single, long open reading frame. A series of post-translational proteolytic cleavages generate 12 proteins as follows: L, viral protein (VP) 1-4, 2A-2C, and 3A-3D. The FMDV capsid surface is covered by VP1, 2, 3, and held by VP4, buried within the virion (Mason et al. 2003). The loop connecting the βG and βH strands of the protein (G-H loop) of VP1, including the Arg-Gly-Asp (RGD) amino acid sequence motif, binds to RGD-binding integrins as receptors of FMDVs in susceptible animals (Acharya et al. 1989). To date, five primary antigenic sites have been identified in capsids (Crowther et al. 1993, Kitson et al. 1990). The nonstructural proteins 2A-2C and 3A-3D are involved in protein processing and genome replication (Mason et al. 2003). During the infection cycle, 3C protein is responsible for most of the cleavage of viral polyproteins. The 3D protein is a virus-encoded RNA-dependent RNA polymerase that catalyzes RNA replication. The replication cycle is finalized by encapsidating nascent positive-sense viral RNA and maturation cleavage of

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VP0 into VP2 and VP4.

FMDV isolates comprise the following immunologically distinct serotypes: O, A, C, Asia 1, and South African territories 1-3 (Knowles et al. 2016). Each serotype is divided into genetically and geographically distinct topotypes based on the comparison of its VP1 sequence (Knowles et al. 2016). The FMDV serotypes O and A have the broadest distribution and occur in many parts of Africa, Asia, and South America. Regular outbreaks of FMD have been reported in India and mainland Southeast Asia (Knowles & Samuel 2003). Sporadic introduction of viruses into FMD-free countries may cause devastating outbreaks. In 1999-2002, the serotype O Middle East-South Asia (ME-SA) topotype, the PanAsia lineage, caused widespread outbreaks in East Asia, including China, Russia, Mongolia, South Korea, and Japan, before those in South Africa and Europe (Knowles & Samuel 2003). In 2010 and 2011, incursions of the FMDV serotype O, Southeast Asia (SEA) topotype, and Mya-98 lineage, which are normally restricted to mainland Southeast Asia, caused extensive outbreaks across East Asian countries (Valdazo-González et al. 2013).

Domesticated cloven-hoofed animals, including cattle, swine, sheep, and goats; and wild animals, including deer and wild boars; are susceptible to FMD. FMDV-infected animals typically exhibit vesicles on the mouth, nostrils, and around the breasts and feet. The pathogenicity and infectivity of FMDV in cattle and swine are strain-dependent; for example, FMDV with atypical pathogenicity, showing high morbidity and mortality in swine but not in cattle, was confirmed to cause a devastating outbreak in Taiwan (Beard & Mason 2000). In contrast, FMDVs with limited pathogenicity in cattle have been isolated in South Korea and Argentina (García-Nuñez et al. 2010, Oem et al. 2008). Several previous studies have reported that pathogenicity in hosts and viral growth in cell culture are related, and the factors responsible are the two substitutions on the 133rd amino acid in VP2 and the 56th amino acid in VP3, a deletion of L^{pro}, a partial deletion in 3A, and IRES-3′-UTR modulation (Chinsangaram et al. 1999, García-Nuñez et al. 2014, Li et al. 2010, Morioka et al. 2008, Pacheco et al. 2013). However, few studies have described the genes responsible for the pathogenicity of FMDV among multiple topotypes.

FMDV O/JPN/2000 and O/JPN/2010 were isolated from outbreaks of markedly different scales

Japan has experienced two FMD outbreaks in the past century. In 2000, an FMD outbreak caused by the serotype O virus ME-SA/PanAsia occurred in Japan (Sakamoto et al. 2002). The FMDV serotype O, SEA/Mya-98, was introduced in Japan in 2010. The outbreaks in 2000 and 2010 differed notably in severity. The 2000 outbreak was limited to four cattle farms, whereas the 2010 outbreak spread to 292 farms and slaughtering approximately three hundred thousand animals (Muroga et al. 2012). In 2000, infected cattle showed only fever and salivation; typical clinical signs including vesicular development were not confirmed. In 2010, the typical clinical signs of fever, excessive salivation, and vesicular development were confirmed. This difference in pathogenicity in the field might be one factor causing the difference in the severities of the two outbreaks. Efforts to understand the molecular characteristics of these strains may provide new insights into the molecular mechanisms underlying differences in pathogenicity beyond the multiple topotypes of FMDV.

The infectivities of O/JPN/2000 and O/JPN/2010 in Holstein cattle have been estimated (Nishi et al. 2019, Onozato et al. 2014). Two of the two Holstein cattle inoculated intradermally with O/JPN/2000 exhibited unexpected vesicular development in all four limbs. However, only one of the two contact cattle was infected 5 days after viral excretion from the inoculated cattle and showed only pyrexia, while no vesicular lesions were confirmed. In contrast, two of the two cattle inoculated with O/JPN/2010 showed fever, salivation, lameness, and vesicular development. Furthermore, two of the two contact cows were confirmed to be infected within two days of virus excretion from the inoculated cattle. These data demonstrate that O/JPN/2000 has a very low transmissibility and pathogenicity when in contact with cattle.

Molecular characterizations of the two FMDV strains isolated in Japan

Several studies using wild-type and mutant strains have reported that viral growth in cell culture and host pathogenicity are closely related. For example, a mutant with a partial deletion of the 3A protein does not replicate efficiently in bovine cells in vitro, and is attenuated in cattle (Li et al. 2010, Pacheco et al. 2013). In contrast, a remarkable difference in viral growth in primary bovine kidney and LFPK-αvβ6 cells was not observed between O/JPN/2000 and O/JPN/2010, which belong to different genetic topotypes (Nishi et al. 2019). In suckling mice, which have been widely used as practical models of FMDV pathogenicity (Platt 1956, Salguero et al. 2005, Skinner 1951), O/JPN/2000, and O/JPN/2010 showed definite differences in pathogenicity.

The 50% lethal doses (LD_{50}s) of O/JPN/2000 and O/JPN/2010 were determined to be $10^{2.2}$ and $10^{0.1}$ 50% tissue culture infectious dose ($TCID_{50}$), respectively, indicating that O/JPN/2010 had higher pathogenicity in suckling mice. Based on this result, the mortality rates of suckling mice inoculated intraperitoneally with 10 $TCID_{50}$ of O/JPN/2000 and O/JPN/2010 were 0% and 100%, respectively (Nishi et al. 2019). The present data supports the idea that mortality in infected suckling mice is an effective index for comparing the infectivity of FMDVs, particularly those belonging to different genetic topotypes.

Although the one-step growth in the cell monolayers of the two strains was not remarkably different, they showed significantly different viral features after serial passages in cells. O/JPN/2000 and O/JPN/2010 were serially passaged 10 times in BHK-21 cells and inoculated into suckling mice (Nishi et al. 2019). In the O/JPN/2000 virus stock at the primary stage, two types of viruses were observed: one showed small plaques and avirulent pathogenicity in suckling mice, whereas the other showed large plaques and higher pathogenicity (Morioka et al. 2008). Similar to a previous study, O/JPN/2000 after the passages showed two substitutions on the 133rd amino acid in VP2 and the 56th amino acid in VP3, which are known as heparin sulfate-binding sites that influence plaque size and pathogenicity in cattle (Sa-Carvalho et al. 1997), and significantly decreased mortality in suckling mice (LD_{50}, $>10^{3.0}$ $TCID_{50}$). In contrast, no nonsynonymous substitutions or changes in mortality in suckling mice were confirmed in serially passaged O/JPN/2010.

Construction of a full-length infectious cDNA clone of FMDV O/JPN/2010

Infectious cDNA clones of FMDV have been constructed to understand viral pathogenicity at the molecular level and for vaccine development (Falk et al. 1992, Hema et al. 2009, Liu et al. 2004, Rajasekhar et al. 2013, Rieder et al. 1993, Seago et al. 2013, Xin et al. 2009, Zibert et al. 1990). They can be used to construct genetic recombinants and site-directed mutants for genomic studies on FMDV (Ellard et al. 1999, Pacheco et al. 2003, Piccone et al. 1995, van Rensburg et al. 2004, Xin et al. 2014).

The genome of an FMDV isolated during the 2010 epidemic in Japan, O/JPN/2010-290/1E, was determined to be 8,171 nucleotides in length, excluding the poly(C) tract between the S- and L-fragments and the poly(A) tail at the 3′ end (GenBank Accession No. LC036265). A full-length infectious cDNA clone of the O/JPN/2010-290/1E genome was constructed and named as pSVL-f02 (Nishi et al. 2016). The recovered virus had the same in vitro characteristics as the parental virus, following antigenicity in neutralization and indirect immunofluorescence tests, plaque size, and one-step growth. Viruses recovered from transfected cells also retained their in vivo pathogenicity in pigs compared to their parental strain (Nishi et al. 2016).

Genetic determinants of pathogenicity between two FMDV isolates

To identify the genes responsible for the differences in pathogenicity, the genetic regions of full-length O/JPN/2010 cDNA were replaced with the corresponding fragments of O/JPN/2000 (Nishi et al. 2019). Eight recombinant viruses were recovered from the transfected cells, regardless of the number of amino acid differences in each recombinant fragment. The pathogenicity of the parental viruses and recombinant FMDV in suckling mice indicated that VP1 and 3D proteins were individually responsible for the pathogenicity of O/JPN/2010.

The outermost component of the viral particle, VP1, is responsible for receptor binding (Azuma & Yoneda 2009). Analysis of the three-dimensional structure of the viral protein showed that the amino acid differences between the two strains were mainly located near the G-H loop, indicating that the two strains had different selectivities or affinities for host cell receptors. After serial passaging of the cells described above, the data on viral features support the hypothesis that the two strains have different selectivities for host cell receptors. The finding that capsid coding sequences are determinants of FMDV pathogenicity is consistent with a previous study using interserotypically recombined chimeric viruses (Lohse et al. 2012).

Previous reports have indicated that FMDV infection induces cell death via apoptosis mediated by interactions with the integrin receptor (Jin et al. 2007, Ku et al. 2005). Programmed dead cells are processed into small apoptotic bodies, which are subsequently phagocytosed by macrophages. Although FMDV replication in macrophages has not yet been confirmed, macrophages would play a role in the transport of infectious FMDV to different sites in the body, where it can be released to infect other cells for replication (Baxt et al. 1995, Rigden et al. 2002). Therefore, the selectivity or affinity of a virus for its receptors is likely related to its infectivity in susceptible animals. Yamada et al. (2018) demonstrated that terminal deoxynucleotidyl transferase-mediated dUTP-nick end labeling (TUNEL)-

positive labeling in pigs inoculated with O/JPN/2000 of the small plaque phenotype was weaker than that in pigs inoculated with O/JPN/2010. This finding suggests that the viral function inducing apoptosis differs between O/JPN/2000 and O/JPN/2010. Further studies on the relationship between viral selectivity or affinity for receptors and viral function to induce apoptosis would help to elucidate the mechanism of FMDV pathogenicity.

The 3D protein of FMDV functions as an RNA polymerase and has a right-handed structure comprising the finger, palm, and thumb domains (Ferrer-Orta et al. 2004). According to three-dimensional structural analysis of the protein, three, three, and one amino acid differences between O/JPN/2000 and O/JPN/2010 were found in the finger, palm, and thumb domains, respectively (Nishi et al. 2019). Previous reports have suggested that mutations located at the top of the finger domain affect elongation rates, whereas mutations in the palm domain have the greatest effect on mutation frequency (Campagnola et al. 2015). Interestingly, FMDV with a high-fidelity polymerase has been reported to be attenuated in hosts (Li et al. 2018, Xie et al. 2014, Zeng et al. 2014). Higher replication fidelity can restrict quasispecies diversity and affect the adaptability and pathogenicity of the strain. In their study, mutants with 1.51- to 1.88-fold higher replication fidelity exhibited 10- to 100-fold lower virulence in suckling mice than in wild-type mice (Zeng et al. 2014). In our previous study (Nishi et al. 2019), O/JPN/2000 showed approximately 1.53-fold higher fidelity than O/JPN/2010, and $10^{2.1}$-fold lower virulence in suckling mice. These data demonstrated that replication fidelity is a factor that accounts for the adaptability and virulence of the virus in the host. Further studies on the correlation between FMDV polymerase fidelity and pathogenicity will aid in developing live attenuated FMDV vaccine candidates, as enhanced replication fidelity promises high stability and safety.

Conclusion

Individual FMDV strains have different degrees of infectivity and pathogenicity in host animals. The differences in severity among outbreaks may be attributed to differences in pathogenicity among FMDV strains. Our study demonstrated that O/JPN/2000 and O/JPN/2010 had completely different transmissibilities in inoculated cattle and virulence in suckling mice, and this difference was independent of the differences in VP1 and 3D proteins. The selectivity of VP1 for receptors and the replication fidelity of the polymerase may be the individual factors responsible for the differences in infectivity and pathogenicity in the host. These findings will aid in the development of safe live vaccines and antivirals that prevent viral infections in natural hosts.

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