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Short communication

Mutation in the cytoplasmic retrieval signal of porcine epidemic diarrhea virus spike (S) protein is responsible for enhanced fusion activity

Kazuya Shirato[a], Madoka Maejima[a], Shutoku Matsuyama[a], Makoto Ujike[b], Ayako Miyazaki[c], Natsumi Takeyama[d], Hidetoshi Ikeda[b], Fumihiro Taguchi[a,b,*]

[a] Laboratory of Acute Respiratory Viral Diseases and Cytokines, Department of Virology III, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan
[b] Laboratory of Virology and Viral Infections, Faculty of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan
[c] Research Team for Viral Diseases, National Institute of Animal Health, 3-1-5 Kannondai, Tuskuba, Ibaraki 305-0856, Japan
[d] Nippon Institute for Biological Science, 9-2222-1 Shinmachi, Ome, Tokyo 198-0024, Japan

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ABSTRACT

Murine-adapted porcine epidemic diarrhea virus (PEDV), MK-p10, shows high neurovirulence and increased fusion activity compared with a non-adapted MK strain. MK-p10 S protein had four mutations relative to the original virus S, and one of these (H → R at position 1381, H1381R) in the cytoplasmic tail (CT) was suggested to be responsible for the increased fusion activity. To explore this, we examined fusion activity using recombinant S proteins. We expressed and compared the fusion activity of MK-p10 S, S with the H1381R mutation, S with the three other mutations that were not thought to be involved in high fusion activity, and the original S protein. The MK-p10 and MK-H1381R S proteins induced larger cell fusions than others. We also examined the distribution of these S proteins; the MK-p10 and MK-H1381R S proteins were transported onto the cell surface more efficiently than others. These findings suggest that the H1381R mutation is responsible for enhanced fusion activity, which may be attributed to the efficient transfer of S onto the cell surface. H1381 is a component of the KxHxx motif in the CT region, which is a retrieval signal of the S protein for the endoplasmic reticulum–Golgi intermediate compartment (ERGIC). Loss of this motif could allow for the efficient transfer of S proteins from ERGIC onto the cell surface and subsequent increased fusion activity.

Porcine epidemic diarrhea virus (PEDV) is a causative agent for pig diarrhea and induces inappetence and weight loss in adult pigs, while it can induce lethal infection in piglets (Pensaert and de Bouck, 1978). PEDV is a group I coronavirus (CoV), enveloped by a viral membrane. Membrane fusion with the host cell is an important event allowing injection of the viral RNA genome into the host cell. CoV membrane fusion is mediated by the spike (S) protein, which consists of two functional subunits: the N-terminal S1 subunit, responsible for receptor binding activity, and the C-terminal transmembrane (TM) S2 subunit, responsible for fusion activity. The S2 subunit can be further structurally divided into three distinct domains: a large ectodomain, a TM anchoring region, and a cytoplasmic tail (CT) region. The role of the ectodomain in fusion activity has been well documented; this domain contains a protease cleavage site, a putative fusion peptide, and two heptad repeat (HR) regions, which have been shown to be the principal players in membrane fusion (Bosch et al., 2003; Li et al., 2006; Matsuyama and Taguchi, 2009; Tripet et al., 2004; Watanabe et al., 2008). On the other hand, the role(s) of the TM and CT regions in fusion activity is not well understood, although a few studies have reported that the aromatic domain of the TM region (Howard et al., 2008; Jeetendra et al., 2003; Sainz et al., 2005) and the cysteine-rich domain of the CT region have regulatory effects on fusogenic activity (Broer et al., 2006; Chang et al., 2000). Another important feature of the CT of the S protein is localization in the endoplasmic reticulum–Golgi intermediate compartment (ERGIC), for which a signal composed of KxHxx or KKxx motif is believed to be critical; this dibasic motif of the S protein of SARS-CoV, and infectious bronchitis virus (IBV) was responsible for retrieving the S protein in the ERGIC, and the lack of this motif resulted in increased surface expression of S protein (Lontok et al., 2004; McBride et al., 2007). The region of S

Abbreviations: CoV, coronavirus; CT, cytoplasmic tail; ERGIC, endoplasmic reticulum–Golgi intermediate compartment; DMEM, Dulbecco's modified Eagle's medium; HR, heptad repeat; PEDV, porcine epidemic diarrhea virus; PBS, phosphate-buffered saline; S, spike; SARS, severe acute respiratory syndrome; TPB, tryptose phosphate broth; TM, transmembrane.

* Corresponding author at: Laboratory of Virology and Viral Infections, Faculty of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan. Tel.: +81 422 31 4151; fax: +81 422 33 2094.
E-mail address: ftaguchi@nvlu.ac.jp (F. Taguchi).

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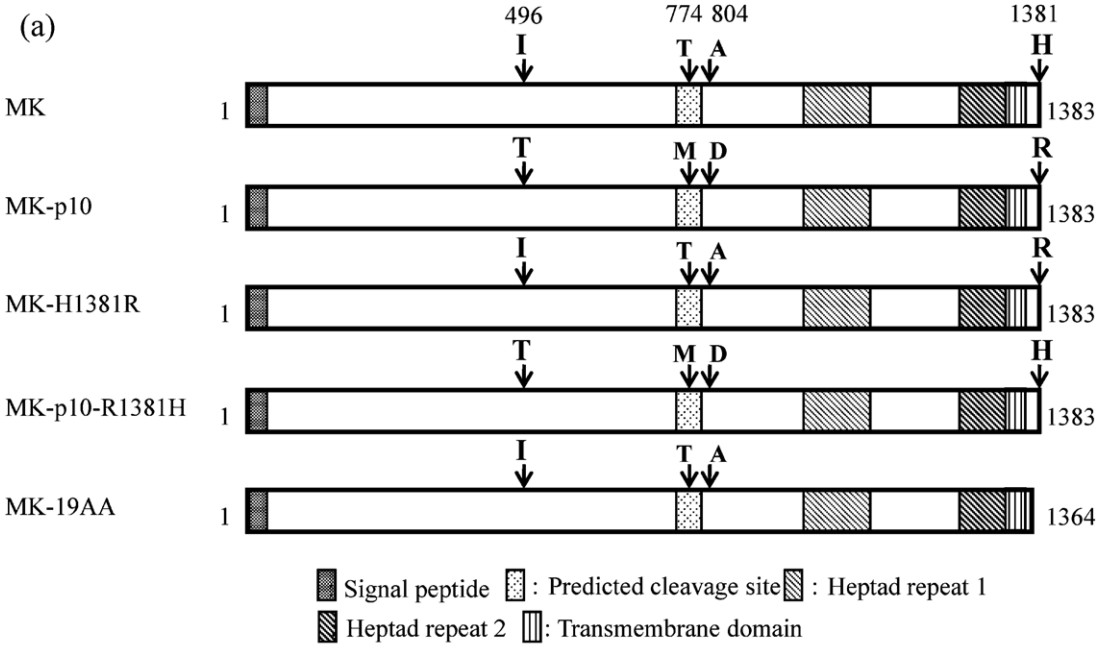
protein located in the ERGIC facilitates an efficient interaction of with M and E proteins in the ERGIC, which is critical for virus budding into those intracellular vesicles. Although some S proteins are transported onto the cell surface, M and E proteins are restricted to those vesicles and are not transported to the cell plasma membrane (Corse and Machamer, 2000; Kapke et al., 1988; Weisz et al., 1993). Thus, maturation of CoVs by budding takes place only into the ERGIC, not from the plasma membrane.

In our previous report, the murine-adapted variant of PEDV, MK-p10, which was isolated by 10 sequential passages through suckling mice brains, showed higher virulence for suckling mice when inoculated intracerebrally (Shirato et al., 2010). One interesting features of MK-p10 was increased fusogenic activity compared with the original MK strain. The S protein of MK-p10 had four amino acid mutations, and one of these (H → R at position 1381, H1381R) in the CT region seemed to be involved in the increased fusiogenic activity because fusion enhancement of S protein was coincident with H1381R mutation. The histidine at a position 1381 (H1381) is a constituent of the KxHxx motif. Thus, there is a possibility that the lack of this motif caused by the H1381R mutation enhanced surface expression of MK-p10 S protein, resulting in increased fusion activity. There is another possibility that the mutation reduces virion budding into the ERGIC because reduced localization of S protein at ERGIC decreases the chance of its interaction with M and E proteins at the ERGIC. In this study, to determine the mechanisms of the increased fusogenic activity of the MK-p10 S protein, we generated several recombinant S mutants and analyzed their fusion activities. Our study showed that H1381R mutation damaged the KxHxx motif, allowing for the efficient transport of the S proteins from the ERGIC onto the cell surface, inducing increased fusion activity. The present study in combination with our previous study [17] that MK-p10 multiplies more-or-less similarly to the original virus suggest that the localization of the S at the ERGIC is not critical for virus replication.

We have reported that the S protein of MK-p10 had four amino acid mutations, as depicted in Fig. 1a and the replication kinetics



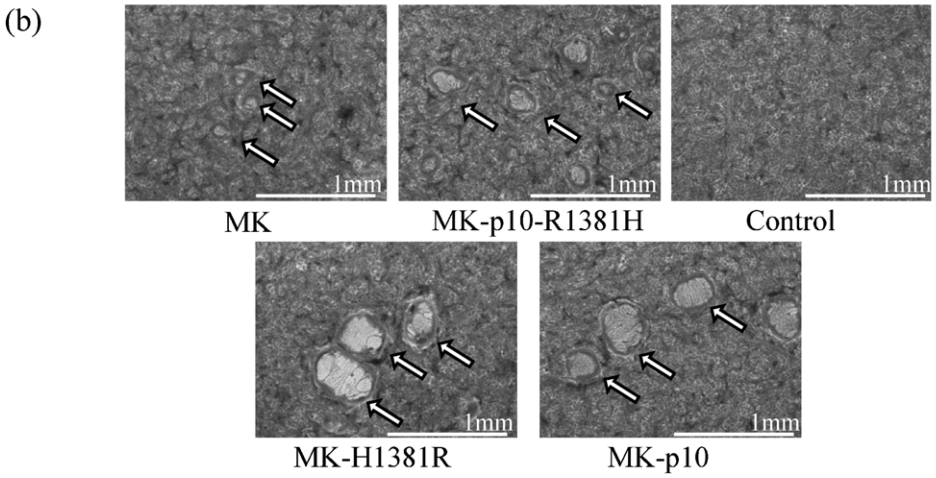


Fig. 1. Cell fusion induced by recombinant S proteins of PEDV MK and MK-p10. (a) A schematic presentation of amino acid substitutions in the S protein of MK-p10 compared with the parental MK S protein and of constructed plasmids. (b) Cell fusion induced by recombinant PEDV S proteins. The S protein expression plasmids were transfected into Vero cells using the DMRIE-C reagent. After 3 days of incubation, cells were washed with PBS, added to DMEM containing 10% TPB and 1.25 μg/mL of trypsin, and incubated for 15 h. These cells were fixed and stained with PBS containing 20% formalin and 0.1% crystal violet and observed by microscopy. Fused cells are indicated by white arrows.