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Introduction: Enteric caliciviruses in *Sapovirus* and *Norovirus* genera within the *Caliciviridae* family cause severe acute gastroenteritis in humans and animals. Despite its significance, studies on their life cycle have been hampered due to no reliable *in vitro* and *in vivo* systems. Currently, the porcine sapovirus (PSaV) Cowden strain remains the only cultivable member of the *Sapovirus* genus. Viral genome-linked protein (VPg), covalently linked to the 5' end of *Caliciviridae* genome, is used for viral polyprotein synthesis as a cap analog or for possibly viral genome replication as a primer [1]; however, its detailed function for PSaV translation and genome replication in association with PSaV RNA dependent RNA polymerase (RdRp) remain largely unknown. Here, we investigated roles of tyrosine residues in the VPg and RdRp to complete PSaV life cycle.

Materials and Methods: Bioinformatic analysis with the VPg from PSaV Cowden strain was performed to examine amino acid potentially involved in linkage to the PSaV genome. To determine whether tyrosine residue had a role in viral replication, we generated mutant plasmids from infectious clone pCV4A. After transfecting capped viral genome into the permissive cell line, we examined virus recovery by observing CPE and evaluated the recovered viral properties by plaque assay, qRT-PCR and $TCID_{50}$. To characterize the function of RdRp in association with other proteins, we performed 5BR assay, which is based on reporter gene activation by newly produced foreign RNA inducing IFN-β signaling [2].

Results: Bioinformatic analysis suggested that four conserved tyrosine(Y) residues, positioned at 22, 60, 76 and 110 in the VPg, could be linked to PSaV genome. From the mutagenesis study, viruses were not recovered from the mutated pCV4A at Y22 and Y76 residues but recovered from the others, indicating that like other caliciviruses, Y22 residue could link to PSaV genome and Y76 had a critical role for PSaV replication [3]. 5BR assay with PSaV RdRp alone showed significant luciferase activity, suggesting that PSaV genome could replicate in a *de novo* synthesis manner [1]. Furthermore, coexpression of RdRp with VPg or p35 proteins significantly enhanced luciferase activity, suggesting that they could increase RNA synthesis by RdRp.

Conclusions: Like other caliciviruses, tyrosine residue 22 in the VPg could be linked to PSaV genome and tyrosine residue 76 importantly functions for PSaV replication. Furthermore, PSaV RdRp could replicate viral genome in a *de novo* synthesis manner but its activity could be augmented by PSaV VPg or p35 protein.

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O-023

Variation of glycan specificity to VP8* domain of four neuraminidase sensitive animal rotaviruses

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Introduction: To initiate group A rotavirus (RVA) infections, the VP8* domains of the spike VP4 protein interact the cell surface carbohydrate moieties (glycans), terminal or internal sialic acids(SAs) or histo-blood group antigens (HBGAs) on glycoprotein or glycolipid. Some of animal rotavirus strains are neuraminidase (NA)-sensitive and most of animal and human rotavirus is NA-sensitive [1]. Although terminal SAs are attached to underlying glycans by α2,3- or α2,6-bonds, its linkage to cell surface glycans used as an attachment factor has been tested for only a limited number of NA-sensitive animal RVA strains [2]. In addition, a few NA-sensitive animal RVA strains are known to recognize both glycolipid or *N*-linked glycoprotein as attachment factors [3]. However, the nature of attachment factors recognized by VP8* domain of other NA-sensitive animal RVA strains remains largely unknown.

Materials and Methods: Four representative P genotypes (bovine G6P[1] NCDV, canine G3P[3] CU-1, and porcine G9P[7] PRG9121and G9P[23] PRG942 strains) in P[I] genogroup were selected because these strains are important pathogens for livestock and companion animal. Chemicals, metabolic inhibitors and enzymes used for infection inhibition assay included sodium periodate ($NaIO_4$), *N*-acetyl neuraminic acid (NANA), *Maackia amurensis* lectin (MAL), *Sambucus nigra* lectin (SNL), DL-*Threo*-1- phenyl-2 -decanoylamino- 3- morpholino-1-propanol (PDMP) and benzyl 4-O-β-D-galactopyranosyl-β-D-glucopyranoside (BenzylGalNAc), tunicamycin, NA and sialidase S (SS). After chemical treatment, monolayered MA104 cells were infected with each virus and its infectivity was detected by immunofluorescence assay using mouse anti-RV VP6 protein antibody. The synthetic oligosaccharide-based HBGA assay was carried out using recombinant GST-VP8* domains of four animal strains.

Results: Infectivity of P[1],P[3], P[7] and P[23] animal strains

were decreased by treatment of $NaIO_4$, NANA and NGNA, indicating usage of terminal SAs as attachment factors of these strains. P[1] and P[3] infection were reduced by treatment of NA and SNL, but P[7]and P[23] infection were decreased by treatment of NA, SS, MAL and SNL, demonstrating that for viral attachment and infection, porcine [7] and P[23] strains could recognize both α2,3-andα2,6-linked SAs, and bovine P[1]and canine P[3] strains could use α2,6-linked SAs. Pretreatment of cells with PDMP and tunicamycin inhibited infectivity of all of 4 animal strains, but pretreatment of benzylGalNAc had no effect on any RVA strains, indicating usage of glycolipid and N-linked glycoprotein as attachment factors for these strains. GST-VP8*domains of NA-sensitive RVA strains had no binding affinity to synthetic HBGAs, confirming no usage of HBGAs as attachment factors for these NA-sensitive strains.

Conclusions: Our study demonstrates that four NA-sensitive animal strains could have strain-dependent binding preference toward α2,6-linkedSAs (P[1] and P[3]) or both α2,3- and α2,6-linked SAs (P[7] and P[23])on the glycolipid and *N*-linked glycoprotein.

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O-024

Porcine sapovirus utilizes occludin as a coreceptor

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Introduction: The family of *Caliciviridae* comprises a diversity of pathogens that include the genera *Norovirus* and *Sapovirus*, which causes severe acute gastroenteritis in humans and animals [1]. Due to the lack of cell culture method for most caliciviruses, studies on the life cycle of these viruses have been quite limited. Over the past few years, there were increasing evidences that viruses hijacked different components of tight junctions (TJs) in order to complete their infectious cycle [2]. Our previous report demonstrated that both α,2,3-and α2,6-linked sialic acids (SAs) on *O*-linked glycoproteins act as functional receptors for porcine sapovirus (PSaV) entry and infection [3]. In this study, we identify that PSaV could bind the TJs in particular with occludin as a coreceptor to continue PSaV entry and infection.

Materials and Methods: In this study, we applied experiments such as confocal microscopy and immunoprecipitation (IP) assay to systematically identify the TJs that interact with PSaV**.** Other experiments such as transepithelial resistance assay, membrane lipid assay, flow cytometry, and gene silencing were also performed to further clarify the role of TJs in PSaV entry and infection.

Results: PSaV disrupted TJ integrity of LLC-PK cells, allowing PSaV entry from lateral surface. Occludin, a member of the TJ-associated marvel proteins, was found to have a strong direct interaction with PSaV by IP assay, although weak interactions with other TJs such as claudin-1 and JAM-1 were observed. Depletion of occludin prevented PSaV entry into the cytoplasm and inhibited infection, further confirming involvement of occludin for PSaV entry. Internalization of occludin and PSaV were mediated by early endosome Rab5 and then late endosome Rab7 through small GTPases.

Conclusions: Our data suggests that binding of PSaV to cell surface receptor, SAs, could disrupt TJs so that freely exposed TJ molecule, occludin, could be subsequently used as a coreceptor for PSaV entry and infection. PSaV entry with occludin could occur by processes that combine aspects of endocytosis mediated by small GTPases.

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O-025

The VP8* domain of rotavirus P[5] strains uses both α 2,6-linked sialic acid and αGal epitope as receptors

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Introduction: Group A rotaviruses (RVAs), a member of the genus *Rotavirus* within the family *Reoviridae*, are the single most important cause of severe diarrheal illness in young children and animals [1]. The VP8*of outermost layer VP4 protein of RVA particles is essential for the RVA entry. The VP8* domain of some animal RVAs is known to neuraminidase (NA)-sensitive, whereas the infectivity