Infection of Avian and Swine Cell Lines with Chimeric Porcine Deltacoronaviruses Expressing Sparrow Deltacoronavirus Spike Protein

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ABSTRACT

Porcine deltacoronavirus (PDCoV) is a newly emerged coronavirus (CoV) causing severe diarrhea and mortality in neonatal piglets. PDCoV belongs to the *Coronaviridae* family of the genus *Deltacoronavirus* that consists of avian and mammalian CoVs. Recent studies have shown that PDCoV can infect cell lines of humans, chickens, and swine via the interaction of the spike protein (S) with host aminopeptidase N (APN). Identification of new DCoV strains including sparrow DCoV (SpDCoV), coupled with close contact between sparrows and swine in production facilities may facilitate recombination of DCoVs and drive viral evolution resulting in the emergence of novel CoV variants. We utilized the chimeric viruses, in which the entire S of sparrow HKU17 (icPDCoV-SHKU17) or the receptor binding domain (RBD) of swine ISU73374 (icPDCoV-RBDISU) replaced the PDCoV S or RBD generated using the infectious cDNA clone of PDCoV OH-FD22 strain (icPDCoV) in Dr. Wang's lab, to infect cell lines of 2 species, avian fibroblast origin, DF-1, and porcine kidney origin, LLC-PK, to understand the role of the S protein. We demonstrated that DF-1 and confirmed that LLC-PK cells are susceptible to icPDCoV, icPDCoV-SHKU17, and icPDCoV-RBDISU virus infections. The results demonstrate that chimeric viruses exhibit a broad cell tropism, infecting cells derived from both chicken and swine species.

INTRODUCTION

- Coronaviruses (CoVs) are enveloped, positive sense, single-stranded RNA viruses [1].
- CoVs cause mild to lethal respiratory and intestinal infections [2] in humans, poultry, and swine.
- Porcine deltacoronavirus (PDCoV) was initially identified in pigs in Hong Kong in 2009 [3].
- In 2014, PDCoV was further detected in the United States in 2014 [4], and it contributed to significant mortality in baby piglets [5].
- PDCoV genome is closely related to sparrow coronavirus HKU17 (SpCoV HKU17) with more than 90% amino acid identity [3].
- The S protein of CoVs consists of two subunit domains, S1 and S2 [6].
- The S1 domain recognizes and binds with cell surface receptors via the receptor-binding domain (RBD) while the S2 domain mediates membrane fusion [7].
- The gene encoding S protein is found in a region at which genetic recombination occurs frequently, often leading to host and tissue tropism changes [8, 9, 10].
- Notably, the chimeric viruses icPDCoV-SHKU17 and icPDCoV-RBDISU infected the upper respiratory tract of pigs, but lost tissue tropism for the gastrointestinal tract [11].

OBJECTIVE

Compare the ability of PDCoV viruses containing S or RBD sequence from sparrow coronavirus (Figure 1) to infect and replicate in cell lines derived from avian (DF-1) or porcine (LLC-PK1) origins.

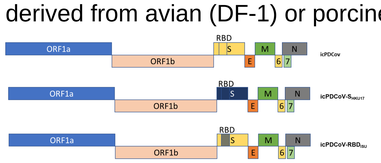

Fig. 1. Chimeric PDCoV virus genomes in which the wild type PDCoV S or RBD have been replaced by SpCoV S or RBD [11]

RESULTS

DF-1 and LLCPK1 cells are susceptible to infection with chimeric viruses

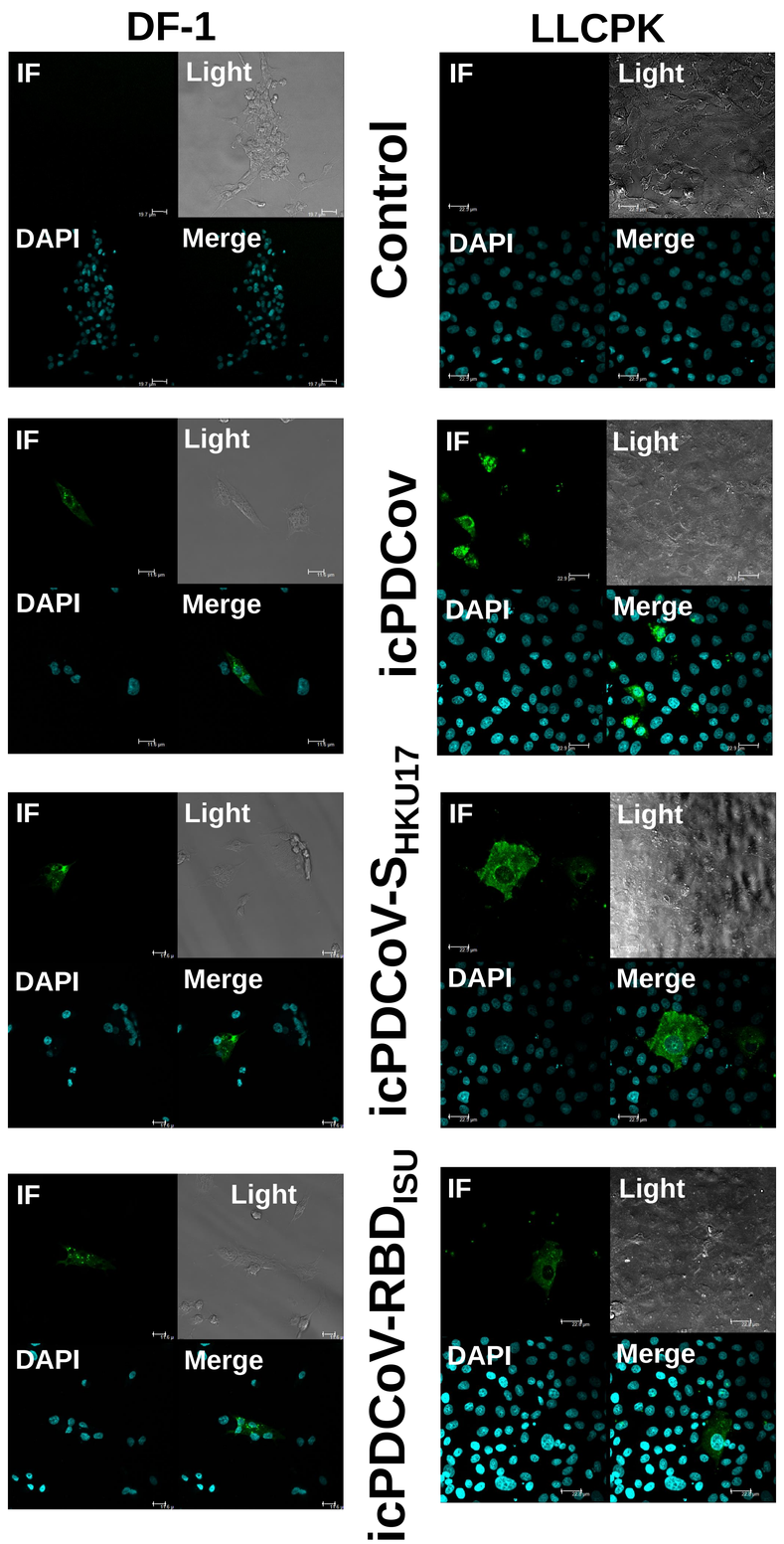

Fig. 2. Cellular tropism and localization of chimeric antigens in DF-1 and LLCPK1 cells assessed by immunofluorescent (IF) staining. Inoculated DF-1 and LLCPK1 cells were incubated with monoclonal antibody and goat anti-mouse conjugated to Alexa fluor 488. When cells were incubated with cell culture media, there were no detectable signals (control). Antibodies against N proteins (green), and the nuclei were counterstained with DAPI (blue). Original Objective lens: 40 x.

METHODS

- Cell lines: Chicken embryo fibroblasts [DF-1(ATCC CRL-12203)], and LLC porcine kidney [LLC-PK (ATCC CL-101)] were cultured in Dulbecco's Modified Eagle's Medium and Minimal Essential Media with 10 and 5% fetal bovine serum supplementation, respectively.
- Cells were infected at a multiplicity of infection (MOI) of 0.01 and fixed in 4% paraformaldehyde at 16 hours post infection.
- For immunofluorescence evaluation, cells were incubated with a mouse monoclonal antibody, SD55-197 against PDCoV N proteins (www.medgenelabs.com) at a concentration of 1:500 for 2 hours at RT, followed by incubation with 1:400 Alexa Fluor 488 conjugated goat anti-mouse IgG antibodies (Invitrogen, Thermo Fisher) .
- Cells were imaged using a Leica TCS SP6 confocal microscope.

RESULTS

- Virus derived from an infectious clone of PDCoV and chimeric viruses encoding the spike protein from SpCoV (icPDCoV-S_{HKU17}) or the RBD from SpCoV (icPDCoV-RBD_{ISU}) infect avian and porcine cell lines (Fig, 2).

CONCLUSIONS

- The full S protein or RBD from SpCoV can functionally substitute for the cognate wild type PDCoV protein sequence (this has been done by Dr. Wang's lab).
- SpCoV may pose a significant risk for recombination with PDCoV as both appear capable infecting pigs and chickens.
- Further study of replication kinetics are required to assess the ability of spike recombinant viruses to efficiently replicate in avian cells.
- In vivo experiments in poultry are important next steps to understand potential effects of spike recombinant viruses on tissue tropism and host pathology.

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