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Opinion Article

A Retrospective Study of Porcine Epidemic Diarrhea Virus Interactome with Nucleocapsid Protein

Paolo Colomba*

Department of Pathology, University of Houston, Calhoun, Houston, Texas, United States

ABOUT THE STUDY

Porcine Epidemic Diarrhoea (PED) is an acute and highly contagious enteric disease characterized by watery diarrhoea, vomiting, dehydration, severe enteritis, and weight loss. For many years, inactivated and live-attenuated CV777-based vaccines were used as a major strategy to control PED until the outbreak in China in October 2010. This outbreak occurred on both vaccinated and non-vaccinated pig farms and resulted in nearly 100% morbidity and mortality rates among suckling piglets, resulting in significant economic losses for the swine industry. In April 2013, a PED outbreak erupted in the United States, resulting in high piglet mortality and massive economic loss. These re-emerging outbreaks demonstrated that PED is a serious threat to the global swine industry.

The Porcine Epidemic Diarrhea Virus (PEDV) is a large-enveloped RNA virus that belongs to the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*, and genus *Alpha coronavirus*. Its genome is approximately 28 kb long, with a 5' cap and a 3' polyadenylated tail, and includes a 5' and a 3' Untranslated Region (UTR) encoding two replicase polyproteins (pp1a and pp1ab), spike (S), envelope (E), membrane (M), and nucleocapsid (N), four structural proteins, and one hypothetical accessory protein. N protein is a multifunctional viral protein that functions as an RNA-binding protein, viral RNA synthesis, and modulator of host cell processes during PEDV infection. It can suppress innate immunity by inhibiting beta and lambda interferon production, extending the S phase of host cells, inducing endoplasmic reticulum stress, and increasing interleukin-8 expression.

As obligate intracellular parasites, viral pathogen replication in a host is a complex process that involves numerous interactions to achieve viral invasion, replication, and packaging processes. Proteome analysis is a powerful tool for discovering the cellular proteins that participate in the viral life cycle by interacting with specific viral proteins, as well as for discovering new therapeutics against virus infection. To investigate the biological function of Porcine Epidemic Diarrhea Virus Nucleocapsid Protein (PEDV N-protein) and the role of N protein in viral replication, the interactome of N protein was discovered in this study, which provided useful information for further research into the function of N proteins as well as hints for antiviral drug targets.

Viral proteins frequently interact with cellular proteins in order to complete the viral life cycle or to create a favorable environment for viral replication. The study of these interactions will aid in the understanding of viral pathogenesis and the function of viral proteins, revealing the viral infection mechanism and providing more antiviral targets. The interactome of the PEDV N protein was discovered in this study, which would provide a great platform for studying the role of N protein in PEDV infection and the selection of anti-PEDV therapies.

We used an EGFP-trap combined with a label-free LC-MS/MS approach to elucidate the N protein interactomes, which has previously been used to successfully elucidate viral proteins such as human respiratory syncytial virus, infectious bronchitis virus, porcine reproductive and respiratory syndrome virus, and Ebola viral protein 24 (EVP24). The pEGFP-N samples would be selectively enriched for N's specific interaction partners. Pull downs with both the pEGFP control and pEGFP-N were performed independently in triplicate to provide a statistically robust data set.

The STRING algorithm was used to analyze the interaction of these proteins, and it was discovered that the majority of these proteins were in the nucleus. The function of these proteins was mostly related to RNA, including mRNA metabolic process, translation, RNA binding, and so on, which was consistent with the reported function of RNA-binding protein, viral RNA synthesis, and the characteristic of nucleocytoplasmic trafficking of N protein. This interactome also hints at a novel role for N protein and cellular proteins. For example, a UniProt database search revealed that 107 of 125 cellular proteins were related to acetylation. This provided new insight into the epigenetic changes that occur during N protein expression and PEDV infection.

Correspondence to: Paolo Colomba, Department of Pathology, University of Houston, Calhoun, Houston, Texas, United States, E-mail: paolo@ahri.gov.edu

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