A comprehensive view on the host factors and viral proteins associated with PEDV infection

Yi Hu[1], Xiaohong Xie[2], Lincheng Yang[1], and Aibng Wang[1]

[1]Hunan Agricultural University
[2]Changsha Lvye Bio-Technology Co., Ltd

August 28, 2020

Abstract

Porcine epidemic diarrhea virus (PEDV), a coronavirus pathogen of the pig intestinal tract, can cause fatal watery diarrhea in piglets, thereby leading huge economic losses to swine industry around the world. The pathogenesis of PEDV has been intensively studied, however, the viral proteins of PEDV and the host factors in target cells, as well as their interactions, which underly the molecular mechanisms of viral infection, remains to be illustrated and summarized. PEDV has multiple important structural and functional proteins which play various roles in the process of virus infection. Among them, the S and N proteins play vital roles in biological processes related to PEDV survival via interacting with the host cell proteins. Vice versa, a number of host factors including receptors are required for the infection of PEDV per the interactions with the viral proteins, thereby affecting the reproduction and contributing to the life cycle of PEDV. In this review, we intend to provide an update understanding of the PEDV viral proteins and host factors and illustrate the interactions between them. Additionally, the effects of cellular factors or events or signaling pathways on PEDV infection are also discussed. Thus, these comprehensive and creative insights should facilitate to guide the future research, control and prevention of PEDV infection.

A comprehensive view on the host factors and viral proteins associated with PEDV infection

Running title: Factors associated with PEDV infection

Yi Hu[1], Xiaohong Xie[2], Lingchen Yang[1*], Aibing Wang[1,3*]

[1]Lab of Animal Disease Prevention & Control and Animal model, Hunan Provincial Key Laboratory of Protein Engineering in Animal Vaccines, College of Veterinary Medicine, Hunan Agricultural University (HUNAU), Changsha, Hunan, 410128 China.

[2]Changsha Lvye Bio-Technology Co., Ltd, Changsha City, 410199, China.

[3] PCB Biotechnology LLC, Rockville, Maryland, USA 20852.

*Corresponding author: Aibing Wang,*bingaiwang@hunau.edu.cn*

Lingchen Yang, *lcyang@hunau.edu.cn*

College of Veterinary Medicine, Hunan Agricultural University

No.1 Nongda road, Furong District, Changsha, 410128, People's Republic of China

Summary

Porcine epidemic diarrhea virus (PEDV), a coronavirus pathogen of the pig intestinal tract, can cause fatal watery diarrhea in piglets, thereby leading huge economic losses to swine industry around the world. The pathogenesis of PEDV has been intensively studied, however, the viral proteins of PEDV and the host

factors in target cells, as well as their interactions, which underly the molecular mechanisms of viral infection, remains to be illustrated and summarized. PEDV has multiple important structural and functional proteins which play various roles in the process of virus infection. Among them, the S and N proteins play vital roles in biological processes related to PEDV survival via interacting with the host cell proteins. Vice versa, a number of host factors including receptors are required for the infection of PEDV per the interactions with the viral proteins, thereby affecting the reproduction and contributing to the life cycle of PEDV. In this review, we intend to provide an update understanding of the PEDV viral proteins and host factors and illustrate the interactions between them. Additionally, the effects of cellular factors or events or signaling pathways on PEDV infection are also discussed. Thus, these comprehensive and creative insights should facilitate to guide the future research, control and prevention of PEDV infection.

Keywords: Porcine epidemic diarrhea virus (PEDV); Host factors; Viral proteins; Viral infection; Interaction

Introduction

Porcine epidemic diarrhea (PED) is an acute and highly contagious intestinal infectious disease mainly caused by porcine epidemic diarrhea virus (PEDV) (OV, 2009). This disease can cause morbidity in pigs of all ages, with the most harmful to piglets. Clinically, it is characterized by vomiting, diarrhea and dehydration of piglets, with a mortality rate even reaching to 100% (XL, LY, & J, 2007). During the period of 2013 to 2015 when the rapid spread and great epidemic of PEDV occurred, the U.S. pig industry suffered serious economic losses, with a loss of nearly 7 million pigs (A. M & G, 2019). Similarly, the largest pig raising country China also has long time been persecuted by this virus. A epidemiological investigation conducted in the period of Feb, 2011 to Mar, 2014 indicated the presence of PEDV epidemics in 29 provinces, with the PEDV-positive rates of 61.10%–78.49% and 71.43%–83.47% in collected samples and in surveyed pig farms, respectively (Wang D, L, & S, 2016).

As a member of the Nidovirales order and Coronaviridae family, PEDV has a typically corolla-shaped, mostly spherical morphology, with a diameter ranging from 95 to 190 nm (including spikes) and an average of 130 nm, as similar to those of other members of the coronavirus family. This enveloped virus has a single-stranded, positive-sense RNA genome of 28 kb with a 5' cap and a 3'-polyadenylated tail. The DNA sequences located at the 3' side of PEDV genome encodes 4 structural proteins, namely the spike protein (S, 150-220 kDa), membrane protein (M, 20-30 kDa), envelop protein (E, 7 kDa), and nucleocapsid protein (N, 58 kDa) (K. R, A, M, & K, 2001).

PEDV-caused diarrhea firstly broke out in England in 1971, with the clinical symptoms resembling transmissible gastroenteritis virus (TGEV). It was firstly reported in Asia in 1982 and has thereafter had a growing economic impact on pig farming in this region as well. Especially, China has witnessed a rapid increase of the PED incidence in pig population since 2010. PEDV has only one serotype, while it can still be divided into genotype 1 (G1a and G1b) and 2 (G2a and G2b) based on the amino acid differences in the N-terminal domain of the S gene (K. R et al., 2001). Several large outbreaks of this disease have occurred in Asia, with a higher mortality rate in suckling piglets than previously described in this area, which was dominated by the G2b isolates of PEDV 2010 later. According to the epidemiological survey conducted by Li et al in 2011, more severe PEDV infection had occurred in immunized pig farms in China, suggesting the emergence of highly pathogenic PEDV strains. Furthermore, the S genes of PEDV from isolated strains in China had large variations compared with these of the vaccine strains, as mainly reflected by the presence of mutations in the core region of neutralization epitope (COE) in these newly emerging and highly virulent PEDV strains . For instance, compared to the vaccine strain CV777, 5 strains of PEDV with high virulence detected in Gansu province of China have 8 mutations in the COE (A517S, S523G, V527I, T549S, G594S, A605E, L612F and I635V) (MZ et al., 2016). Likewise, 10 PEDV strains, including one classical strain GDS03 isolated in Guangdong province, China from 2011 to 2013, show different mutations in the COE, with larger sequence diversity as well (J, C, L, Y, & Y, 2014). The results of genetic evolution analysis revealed a separate branch of them, suggesting the prevalence of variant strains in this country (L. W et al., 2012). Furthermore, PED still remains one of the most serious diseases of swine especially in winter, mainly because of the failure of

effectively prevention and control. Due to strict biosecurity measures and feeding, the prevalence of PEDV in North America exhibited a declined trend, while the outbreaks of PEDV in Asia presented highly complex variability as a result of the continuous occurrence and emergence of recombination or new isolates in recent years (S. Y et al., 2019).

Similar to those of other coronaviruses (SR & S, 2005), the infection or replication processes of PEDV consist of several main steps, such as virus attachment and entry, viral replication enzyme translation, genome transcription and replication, structural protein translation, and virion assembly and release. Besides viral proteins, many host factors are required for these processes, which are mainly mediated by an interacting way. Therefore, understanding these viral proteins and host factors as well as their interactions not only facilitates the elucidation of the pathogenic mechanisms of PEDV, but also benefits the development of drugs or vaccines against this virus. In this review, we firstly characterize these viral proteins and host factors documented in the literatures, then discuss those cellular events or signaling pathways involved and the interactions among them, finally highlight the directions for future efforts.

The viral proteins involved in PEDV infection

The S protein of PEDV

This S gene encoded spike (S) protein is a type I glycoprotein, while three S proteins of PEDV forming a rod-shaped functional spike trimer located in the outermost layer of virus particles, are the foundation for its multi-function, such as the binding of PEDV to other molecules, the entry of virus into target cells (L. C et al., 2015; F. F et al., 2017). Based on the homology analysis of its counterparts of other coronaviruses, the S protein of PEDV can be divided into two parts: S1 (1-789 aa) and S2 (790-1383 aa). The function of the S1 protein is to mediate the viruses to adsorb the viral receptors on the host cells, while the role of the S2 is to induce membrane fusion, thereby facilitating the viruses to invade the host cells (Wrapp D & JS, 2019; L. F, 2012, 2015). Moreover, the binding of the S1 with receptors on the host cell surface can incur the conformation change and fusion of the S2 with the cell membrane. Indeed, previous results have confirmed the interaction of the S protein with the target cell surface receptor(s), which is mediated by the S1 domain NTD (L. C et al., 2015; D. F et al., 2016). Besides these, the involvement of the S protein in trypsin-dependent PEDV propagation in cultured cells has been suggested (L. W, FJM, Q, PJM, & BJ, 2016). This effect of the S protein is considered to be exerted by proteolytic activation by trypsin or other proteases following its binding to receptor(s) (L. C et al., 2016; O et al., 2014), which is necessary for the membrane fusion, formation of syncytium, cell entry of the virus, thereby enhancing infectivity of PEDV (JE, DJ, & HJ, 2011). This is also supported by the S protein mutations influencing the proteolytic cleave of it (L. W et al., 2015; K. Y, C, V, RA, & KO, 2017). Additionally, a determinant role for the S protein in efficient viral release from target cells has also been proposed, while compelling evidence are required to substantiate this concept (O et al., 2014). It becomes clear that the receptor binding capability and the role in viral entry allow the S protein to determine PEDV invasion and release, tissue tropism, host range and cross-species transmission, even to affect trypsin-dependent PEDV proliferation (L. C et al., 2016; O et al., 2014; L. W et al., 2015). Therefore, the property of the S gene prone to mutation often leads to pathogenic alterations of this virus (C. F et al., 2015; H. Y et al., 2017). The S protein contains a variety of antigenic epitopes and plays an important role in inducing the body to produce neutralizing antibodies (Song D & B, 2012). In view of this vital role of the S protein, it is widely used for the development of PEDV subunit vaccine, genetic engineering vaccine and PEDV antibody detection kit (CY et al., 2019; L. H et al., 2018).

The M protein of PEDV

The M protein encoded by PEDV is an important membrane glycoprotein in the envelope and consists of 226 amino acids. The studies on the M protein have revealed that it has at least several functions. Firstly, it can be utilized as an important antigen of diagnostic reagent. Indirect (ELISA) using the recombinant PEDV M protein as antigen displays high sensitivity and specificity in detecting PEDV antibody (F. JH, YZ, XQ, WY, & JM, 2015). Secondly, during the course of virus infection, the M protein of PEDV is required for the binding with each other or other viral proteins. For instance, the M protein can not only play a key role

in the process of virus assembly by binding with the N protein and other membrane proteins through its carboxyl terminal, but also participate in the process of virus envelope assembly together with the E protein. Additionally, the important role of the M protein is also reflected in virus packaging and budding (CA, L, PS, H, & PJ, 1998; V. H, RJ, DA, MC, & WJ, 1991).

The N protein of PEDV

The N protein of PEDV, consisting of 441 amino acids, is the only phosphorylated nucleocapsid protein among the known structural proteins of coronaviruses. Previous investigation on the cellular distribution of the N protein indicated that it is mainly located in the cytoplasm, with some N proteins in the nucleus of PEDV infected Vero cells (Shi D et al., 2014). The multifunctional property of the N protein manifests in several aspects: 1) it is bound by viral RNA to provide a structural basis for the helix nucleocapsid, while the resultant product is recognized by M protein through the interaction between the N protein and C-terminal domain of the M protein (aa237-252), and then packaged into virus particles to form the core of coronavirus (Shi D et al., 2017); 2) as an alkaline phosphoprotein, the N protein is also associated with virus replication and transcription (YW, S, H, J, & DX, 2006); 3) its participation in the biological processes related to PEDV survival has been affirmed (Shi D et al., 2017; K. N et al., 2019); 4) its localization in the nucleus may be associated with the regulation of host cell cycle and the promotion of PEDV replication process (X et al., 2013). For instance, the interaction of it with Nucleophosmin 1 (NPM1) protects NPM1 from proteolysis, thereby improving cell survival rate and affecting the replication process of PEDV (Shi D et al., 2017).

The ORF3 of PEDV

As a unique feature to other coronaviruses such as TGEV and SeCoV, the sequence located between the PEDV S and E genes is responsible for encoding the nonstructural ORF3 protein. It consisting of 224 amino acids contains 4 transmembrane regions and forms a homologous tetramer structure. Notably, the ORF3 gene remains relatively conservative in wild strains, while the in vitro transmission of PEDV incurs deletion or mutation of it, thereby resulting in incomplete expression of the ORF3 protein (C. F et al., 2015; SJ, HK, DS, HJ, & BK, 2011). As an accessory protein, the ORF3 is generally believed not to play a role in the reproduction process of PEDV, therefore, the ORF3 gene is often replaced by a marker gene and other genes in reverse genetics. However, a series of investigations on the ORF3 have revealed that this is not the case. The bioinformatic analysis indicated that the complete ORF3 protein can form ion channels, the evidence supporting this stems from the fact that silencing the ORF3 gene would lead to a decrease of virus titer, suggesting that it may play a regulatory role in the process of PEDV infection (W. K et al., 2012). Moreover, Ye et al found that the ORF3 gene of PEDV can promote the formation of vesicle structure, prolong the S-phase of target cells, and enhance the proliferation of the attenuated strain expressing a truncated ORF3, indicating its implication in the replication process of PEDV (Y. S et al., 2015). Furthermore, Challika et al provided more direct evidence to demonstrate that the interaction of the PEDV ORF3 with the cellular vacuolar protein-sorting-associated protein 36 (VPS36) inhibits virus replication (K. C, Y, S, & A, 2019). These studies highlight the important roles of the ORF3 gene/protein in the PEDV infection.

The nonstructural proteins of PEDV

The translated products poly-proteins pp1a and pp1ab of the ORF1a and ORF1b transcripts are cleaved into 16 mature replicase proteins nsp1-nsp16 per the action of the Papain -like protease (PLpro is encoded by nsp3) and 3C like protease (EJ et al., 2003; T. V et al., 2003). Previous study indicated that the nsp1 protein has the most pronounced effect on the host innate immune response among all encoded proteins of PEDV. Furthermore, the action mechanism of it is mediated by the degradation of transcription factor binding protein (CREB-binding protein, CBP) and inhibition of interferon (IFN) stimulated gene (IFN stimulated genes, ISGs) expression to suppress the expression of IFN-β, as markedly different from other proteins with interferon antagonistic activity (Q, K, & D, 2016). Meanwhile, the nsp1 is a potential virulence factor and a target for vaccine development since it can disrupt host gene expression and stimulate an antiviral response, thereby blunting the innate immune response of the host to the coronavirus pathogens (N. K, SI, KG, & S, 2015). The nsp5 protein, also called 3CLpro, functions to split proproteins between the nsp5 and nsp16, thus

turning them into mature proteins which participate in various stages of virus replication (T. S et al., 2015). The functional experiments on the nsp5 also revealed that it plays an antagonistic role of interferon through shearing NEMO (NF-κB essential modulator), moreover, the cystine protease activity at the catalytic site of the nsp5 is a key factor for achieving this through cleaving Gln231 in the NEMO protein (Wang D, L, Y, et al., 2016). Likewise, intensive studies on the nsp9 of PEDV suggested that it may also play a vital role in virus replication in the form of homodimers, as similar to those of SARS-CoV and MERS-CoV (S. G et al., 2004). Moreover, other study has also demonstrated the presence of different dimerized forms of the nsp9 protein, which may enhance its nucleic acid binding affinity (Z. Z et al., 2018). Since no or few studies addressing the roles of other PEDV non-structural proteins in terms of viral infection are present and further investigations on these initially characterized ones are required, these should be the future emphases.

Host factors involved in PEDV infection

It is well known that viral infection is a multi-step process including adsorption, cell entry, dehulling, biosynthesis, assembly, and release, etc. Besides the viral proteins of PEDV, the implication of numerous host factors including receptors in these processes has been confirmed, which is described as following in accordance with the infection stages.

Attachment and Entry

Adsorption of viruses to the host cells is the first and decisive step for determining the virus tropism, namely the ability of different viruses to infect various cell types. Correspondingly, virus-specific receptors on the host cell surface are tightly related to the tissue tropism and host range, and mediate the entrance of viruses including PEDV into the host cells.

Porcine aminopeptidase N

Porcine aminopeptidase N (pAPN), a protein of 963 aa widely distributed in the small intestine and kidney of pigs, can be decomposed into 2 subunits by trypsin, a 95 kDa of N-terminal and a 50 kDa of C- terminus, while the positions of 717-813aa contain 3 antigen neutralizing sites. The functions or roles of pAPN in the pathogenic mechanisms of PEDV or TGEV infection have been extensively studied in recent years. Through screening and identification, the main antigen functional regions of pAPN are determined to be located at the positions of 36-153aa, 349-591aa and 592-963aa, respectively (Bo-qi, Guang-xing, & Xiao-feng, 2009). Although the conclusion of the receptor role for pAPN in PEDV infection remains elusive, this suggestion has been supported by the facts that pAPN can bind to the S1 region of the S PEDV protein (BX, JW, & YJ, 2007), and the ST cells efficiently expressing pAPN through gene recombination can support the PEDV infection and proliferation, which also is closely associated with the distributed density of pAPN (N. E & C, 2010). However, other studies indicated that the presence of pAPN does not render Vero cells susceptible to PEDV infection (JS, DS, & BK, 2003) and the capability of pAPN to enhance the infectivity of PEDV is related to its aminopeptidase activity rather than its receptor role (S. K et al., 2016). Whether this discrepancy is only due to various cell lines used in these studies since the results obtained in ST cells can not be replicated in Hela cells, or whether the existence of other routes of PEDV infection results in variable consequences, remains to be further classified.

Sialic acid

It is recognized that a large number of the main receptors for many viruses belong to glycoprotein or glycolipid, with sialic acid or sialic acid derivatives at the end, such as those of pathogenic influenza A, B, C and parainfluenza virus (S. H et al., 2016). In this regard, the ability of PEDV binding to sialic acid, such as carbohydrates (Neu5Ac) and proteins especially glycoproteins or glycolipid molecules located at the cell surface, has also been confirmed in previous studies (K. F & G, 1993; P. G et al., 2012). Moreover, using glycoproteins as receptors is important strategy for viral intestinal pathogenicity because the properties of glycoproteins facilitate viral binding to mucin on the surface of epithelial cells in animal viscera (W. Jh, 2002). The effort of Daniel Wrapp et al. to parse the prefusion conformation of the PEDV S protein using cryo-electron microscopy at a resolution of 3.1 Å revealed the presence of the sialic acid-binding domain at

the N terminal of the S1 subunit (Wrapp D & JS, 2019). Recognition of sugars as co-receptors for PEDV appears to be a strategy for adapting organisms to this class of diarrhea-causing viruses, suggesting that the binding of PEDV to sialic acid facilitates to survive in adverse intestinal conditions. Further in-depth investigation indicated that the S1-434, S253-533 fragments of PEDV weakly while the S19-638 fragment strongly binds to the pAPN expressing cells, suggesting the occurrence of recognition of pAPN as a receptor by the C-terminal region of the PEDV S1 protein and the capability of recognition of other cellular molecules like sugars by the N-terminal region of the S1 subunit (D. F et al., 2016). Thus, PEDV may use sugars as receptors or co-receptors, as similar to TGEV using Neu5Gc and Neu5Ac as co-receptors (P, CF, & S, 2006). The next works focusing on what exact sugar molecules are utilized by PEDV as co-receptors are of significance.

Heparan sulfate

Heparin sulfate (HS) is known for its role as an attachment factor by many viruses to enter cells (B. H et al., 2003). Similar function of it in mediating the attachment/absorption of PEDV to its host cells has been suggested (CC et al., 2015), as confirmed by series of observations. First, HS is a complex polysaccharide located on the cell surface and extracellular matrix (S. S, WC, & JD, 2011). Second, Glycosaminoglycans (GAGs), composed of several covalently attached heparan sulfate (HS) chains, can provide sites for various viruses to bind to eukaryotic cells (B. H et al., 2003). Third, it has demonstrated that PEDV utilizes HS for its attachment to Vero cells, while pretreatment with heparin can inhibit PEDV infection. Moreover, both N- and O-linked sulfate groups within the heparan sulfate carbohydrate structure are functionally important for PEDV binding to cells (CC et al., 2015). Last, the binding ability of PEDV to Vero cells is reduced following the enzymatic removal of cell-surface heparan sulfate or the inhibition of heparan sulfate biosynthesis by treatment with chlorate (CC et al., 2015). These results robustly suggest that HS is at least an adsorbing factor for the infection of PEDV in Vero cells.

Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR) belonging to type I epidermal growth factor family, is widely distributed on the membrane surface of mammalian epithelial cells and fibroblasts (AB & RC, 2004). Importantly, EGFR-mediated signaling pathway plays an important role in cell proliferation, differentiation and apoptosis, even viral infection. In this regard, the activation of EGFR occurring in the early stage of PEDV infection has been found in a recent study. Furthermore, this EGFR activation by PEDV infection may be mediated by the direct interaction between EGFR and the S protein, which in turn enhances PEDV infectivity. Mechanistically, the effect can be associated with the suppression of type I interferon antiviral activity following PEDV induced EGFR-STAT3 signaling pathway activation (Y. L et al., 2018), suggesting that PEDV can effectively utilize EGFR to inhibit cellular antiviral defenses. Despite these, more details about the events related to this signaling remains to be further elucidated.

DC-SIGN (CD209)

DC-SIGN (also called CD209) specifically expressed on the surface of DCs, is a C-type lectin-like cell-surface receptor with multiple functions. Its expression is also found in gastric and intestinal mucosa and other epithelial cells. Initially, DC-SIGN was proved to only be a pattern recognition receptor (PRR) and adhesion molecule for dendritic cells to recognize pathogenic infections and participate in innate immunity of organisms. Accumulating evidence has indicated that DC-SIGN also act as the receptor of many viruses for infecting hosts and the mediator of virus immune escape. Therefore, DC-SIGN has increasingly become a research hotspot in the field of virology. Importantly, DC-SIGN, together with other PRRs, can identify and capture viruses, further swallow and store viruses to evade lysosome degradation, then participate in antigen presentation, thereby achieving the mediation of virus infection and dissemination in vivo. For instance, compared with natural 3T3 cells, transfection of 3T3 cells with plasmids expressing DC-SIGN renders the infection of infectious bronchitis virus (IBV) to be significantly enhanced, while pretreatment with anti-DC-SIGN monoclonal antibody inhibits IBV infection (Zhang, Buckles, & Whittaker). Therefore, it has been speculated that the mannose carbohydrate residues on the surface of coronavirus spike protein can bind

to DC-SIGN receptor and play an important role in the process of coronavirus infection (Z. Y, E, & GR, 2012). Human aminopeptidase N (hAPN) has been confirmed to be a cell receptor of HCoV-229E which is a common coronavirus of upper respiratory tract. Similarly, a series of experiments have also confirmed that HCoV-229E can use CD209L as one of its receptors (Jeffers, Hemmila, & Holmes, 2006). Based on the similarity of the sequence of HCoV-229E with that of PEDV, CD209L may be a receptor of PEDV as well. However, substantial evidence is required to confirm this concept in the future.

Type II transmembrane serine proteases (TTSPs)

TTSPs being a family with more than 20 members can be mainly divided into four subgroups: HAT/DESC, hepsin/TMPRSS, matriptase and corin (S. R & TH, 2008). TTSPs consisting of several functional domains are expressed in many tissues and cell mucosal epithelia, moreover, the localization of them in respiratory mucosal epithelium often facilitates respiratory virus infection (B.-F. E et al., 2010). This role of TTSPs is closely associated with the protease activity of them. Intriguingly, the serine protease inhibitor ABESF-HCl could significantly inhibit the replication of PEDV in Vero cells (JE, DJ, & HJ, 2014). Furthermore, the culture of PEDV could be achieved in stably transmembrane protease serine 2 (TMPRSS2) expressing Vero cells even in the absence of trypsin. Meanwhile, indirect immunofluorescence revealed TMPRSS2-induced cell fusion in virus-infected cells (S. K, S, M, & F, 2011). Likewise, similar role for mosaic serine protease large-form (MSPL) in enhancing the in vitro proliferation of PEDV has been described (S. W et al., 2017). These suggest that like trypsin, the promoting effect of TMPRSS2 and MSPL on PEDV proliferation may be ascribed to their ability of catalyzing the cleavage of the S protein, thus enhancing the entry and release of viral particles during PEDV infection. Additionally, Dipeptidyl Peptidase 4 (DPP4), also called CD26 with the activity of protease and the wide distribution in many tissues and cells, is very conserved among various species and play an important role in the infection of Middle East Respiratory Syndrome virus and other emerging human coronaviruses (O. K et al., 2013). Though robust evidence remains to be provided, CRISPR/Cas9 technology-mediated ablation of CD26/DPP4 gene in target cells should help to illustrate this issue.

Tight junction proteins

Tight junction proteins widely present between the epithelial cells and endothelial cells, are responsible for closing the cell gap and preventing the free entry and exit of substances inside and outside the epithelial layer. Meanwhile, paracellular transport of bacteria, toxins and other substances in the intestinal cavity is also required to maintain the integrity of epithelial barrier function, which is achieved by regulating the role of tight junction proteins in accordance with physiological state (JA & BB, 2009). Tight junction proteins composed of transmembrane proteins and cytoplasmic proteins, are complex structures formed by the interaction of various proteins. They are linked to microfilaments by cytoplasmic binding proteins, which can be divided into transmembrane proteins (e.g. occludin, claudin) and cytoplasmic proteins (e.g. ZO-1, ZO-2, ZO-3) (G.-M. L, R, & D, 2008). The extracellular components of the transmembrane proteins between adjacent cells interact to form intercellular spaces, while the intracellular components are bound to cytoplasmic scaffold proteins ZO-1 and ZO-3, and then directly linked to the prejunctional ring structure composed of actin filaments and myosins (T. S, M, K, & MS, 2010). Importantly, several tightly linked proteins including occludin, claudin, CAR and JAM, have been found to act as receptors for viruses (JM & CF, 2015; M. M, A, PL, & R, 2015). Moreover, it has been regarded that viruses invade epithelium by binding and destroying these tight junction proteins. For instance, the invasion of HCV into hepatic epithelial cells is mediated by tight junction proteins claudin-1 and occludin. Similarly, claudins-6/9 has also been identified to be an invasive co-receptor in endothelial cells (P. A et al., 2009; MJ et al., 2007). Notably, PEDV has been confirmed to cause structural alterations in the barrier integrity both in vitro and in vivo through modulating related proteins of the tight junction and adhesion junction in the early stage of infection (Z. S, J, L, & Q, 2014). Furthermore, this effect of PEDV on cell junction is achieved by affecting the MAPK pathway, since inhibition of MAPK pathway could regulate the changes in tight junction of cells (Z. S et al., 2014). In particular, the essential role of the tight junction protein occludin in PEDV infection during late entry events has been suggested and characterized (L. X et al., 2017). The tight junctional distribution

of occludin is pronouncedly affected by PEDV infection. Furthermore, overexpression or downregulation of occludin promotes or alleviates the susceptibility of target cells to PEDV infection, respectively (L. X et al., 2017). Interestingly, although of PEDV and occludin are mutually influenced by each other, their direct interaction is absent (L. X et al., 2017). Additionally, it remains undetermined whether other tight junction proteins play similar or various roles in the infection of PEDV. Future works are warranted to elucidate these issues.

Genome Replication and Transcription

When PEDV enters the cytoplasmic exfoliation, the RNA genome in the virion is released. The infected cells generally contain 7 to 9 virus-specific mRNAs, which carry the same 3' mRNA, whereas the longest one is viral genomic RNA. The PEDV replicase synthesizes full-length negative stranded RNAs using genomic RNA as a template, and then these newly synthesized RNAs function as a template for the synthesis of new genomic RNA (Masters, 2006). Although genome replication/transcription is regarded to be mediated primarily by viral replicating enzymes, multiple host factors are also involved in these processes. Moreover, the N protein of PEDV, as an RNA companion, plays a key role in the interaction with host factors.

Nucleophosmin 1 (NPM1)

The NPM1(B23.1) protein derived from the main transcription form and the longest transcript of the NPM gene, is mainly located in the granular region of nucleolus. It has several functional domains responsible for various actions, such as oligomerization, chaperone activity, histone binding, ribonuclease activity (H. K, A, & MO, 2000). As a nuclear shuttle protein, NPM1 plays multiple roles in the nucleolus including centrosome duplication, ribosome biogenesis, intracellular transport, apoptosis and mRNA splicing (MS, 2011). Importantly, a series of evidence have demonstrated the interaction/interplay of the PEDV N protein with the NPM1 protein, as well as their contribution to PEDV infection. Firstly, the interaction and co-localization of those two proteins has been confirmed by both immunoprecipitation (IP) and GST-pull down assay, as well as confocal microscopy, respectively. Moreover, the region of aa147-294 of the PEDV N protein and the region of aa189-294 of NPM1 was verified to be essential for their interaction. Secondly, the infection of PEDV was found to cause considerable upregulation of the NPM1 expression. Especially, the NPM1 overexpression of could promote the expression of the N protein and the proliferation of PEDV, while its downregulation led to converse consequences. Thirdly, mechanically the interaction between the N and NPM1 proteins could prevent the cells from being cut off, increase the cell resistance to apoptosis, avoid premature cell death, thus enhancing virus replication (Shi D et al., 2017).

Heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1)

Heterogeneous nuclear ribonucleoprotein (hnRNP) is a general term designated for the members of the RNA binding protein family, which consists of at least twenty members, referred as to from A to U, with molecular weight ranging from 34kDa to 120kDa. HnRNPA1, one of the most abundant members of this family (CK et al., 2017), can be divided into two different parts: the N-terminal functional region with two closely linked RNA recognition motifs mainly responsible for binding to RNA, and the C-terminal glycine enrichment region with RNA binding sites and localization sequence M9 principally involved in RNA binding, cell localization and protein-protein interaction (U & H, 2013). As a multifunctional protein, HnRNPA1 widely participates in the regulation of RNA transcription, splicing, nuclear shuttle and the translation of cellular and viral proteins. Similarly, the N protein, as an RNA binding protein, is implicated in forming the replication and transcription complex of coronaviruses, and plays a crucial role in the replication of viruses. Especially, the binding of hnRNPA1 to the N proteins of other coronaviruses like MHV and SARS-CoV, has been confirmed by a series of experiments (W. Y & X, 1999). Likewise, the binding and co-localization of hnRNPA1 with the N protein of PEDV have been verified, implying the involvement of hnRNPA1 in the formation of PEDV replication-transcription complex (L. Z et al., 2018). It remains to be determined whether the same binding site of the PEDV N protein is used to interact with hnRNPA1 as that of other coronaviruses such as MHV and SARS-CoV [75, 76].

Assembly and release

Successful PEDV replication requires the coordinated production, processing and assembly of each protein and nucleic acid of the virus, as well as the release of progeny viruses capable of infecting new cells from infected cells. Initially, the interaction of the same type M proteins provides a scaffold for the morphogenesis of the virus, while the interaction between the M-S and M-N facilitates the recruitment of the structural components of the virus to the assembly site (Y. Y & BG, 2007). Finally, the newly generated virus particles are transported in the smooth vesicles and released by the exocytic pathway of exocytosis. In this aspect, multiple host factors also take part in these processes of coronavirus including PEDV.

Bone marrow stromal cell antigen 2 (BST2)

As the first defense line against pathogenic microorganism invasion, natural immune response plays a vital antiviral role in the early stage of virus infection. The critical role of interferon (IFN) in the process of virus infection and proliferation has long been recognized. As a IFN-induced common natural immune limiting factor (Sauter D, 2014; K. S et al., 2003), BST2 can suppresses viral production through affecting the release of viruses from infected cells (V. D. N et al., 2008). By analyzing the distribution of BST2 in tissues, Kong et al. found that in spite of its expression in almost all tissues and organs, BST2 exhibited high level in immune tissues and organs, large intestine, small intestine and lungs, hinting a key role of BST2 in early natural immune response. Indeed, the BST2 overexpressing Vero cells had much less virus content than that of the control cells, similarly, the viral titer in the cell supernatant was also pronouncedly reduced. On the contrary, PEDV proliferation was remarkably enhanced in Vero cells upon downregulating BST2 gene level, suggesting that BST2 protein could restrain PEDV proliferation in Vero cells. Furthermore, this function of BST2 might be achieved by binding and degrading the N protein of PEDV (K. N et al., 2019).

Other factors in the host cells affecting PEDV infection

Activity of cytokines and regulation of signaling pathways

As the invasions, viruses should have the capability to adjust the activities of cytokines and regulate intracellular signaling pathways of the host cells following invasion, thereby facilitating the replication and proliferation of viral particles. Indeed, pronounced changes of the expression of numerous proteins occur, as revealed by proteomic analysis in PEDV infected Vero cells. Moreover, these altered proteins are identified to participated in various biologic processes such as apoptosis, signal transduction and stress response (Z. S et al., 2015). For instance, PEDV induced apoptosis is mainly mediated by the activation of caspase-dependent mitochondrial apoptotic inducible factor (AIF) signaling pathway in target cells (K. Y & C, 2014). Likewise, PEDV infection can also activate the components of intracellular MAPK signaling pathway including ERK (extracellular signaling-regulated kinase), p38 MAPK and JNK (c-Jun N-terminal kinase) (K. Y & C, 2015). In addition, PEDV infection induced endoplasmic reticulum (ER) stress response and the activation of NF-κB signaling, have been found and described as well (W. K et al., 2012; Xu et al., 2013). Therefore, it is reasonable and logical to believe that the PEDV induced alterations of protein expression, cellular response and signaling collectively create a conducive microenvironment for its proliferation in the host cells.

Cell autophagy

It has been recognized that autophagy is not only a lysosome-dependent degradation pathway, but also a defense mechanism. Growing studies have demonstrated the fundamental functions of autophagy in the process of virus infection. On the one hand, autophagy can induce innate immune response to suppress the proliferation of viruses; on the other hand, viruses evolve various strategies to defend against and escape the destructive effects of autophagy, and even use it to promote their own proliferation (S. Y et al., 2014). So far, four main forms of the interaction between virus infection and autophagy have been characterized. Autophagy is utilized by different viruses to boost their proliferation, such as foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV). On the contrary, the inhibition of autophagy leads to a decrease of viral proliferation titer (D. M, P, SF, & FV, 2009; O. D. V et al., 2011). More relevantly, TGEV induced autophagy in the ST and PK-15 cell lines has been observed. Interestingly, silencing the three main autophagic proteins could considerably increase viral load, indicating inhibitory role of autophagy in TGEV replication (G. L et al., 2016). Similarly, suppressing the production of autophagosome is one of the ways used by viral

infection. In the regard, herpes simplex virus type I (HSV-1) encoded ICP34.5 protein curbs autophagy by affecting Beclin-1 or eIF2α dephosphorylation, thus promoting self-replication (O. A et al., 2007). Besides these, interfering with autophagy related downstream pathways can be an effective approach to influence autophagy, as well exemplified by influenza A virus (IAV). Although there was no significant change in the IAV replication in the ATG5-deficient MEFs, sharply increased autophagosomes were observed in the IVA infected wild-type MEFs. Further evidence revealed that the IVA encoded M2 protein could prevent the fusion of autophagosomes and lysosomes, thereby confirming inhibited formation of autophagic lysosomes by viral infection (G. M et al., 2009). More importantly, the specific role of autophagy in PEDV proliferation has been verified in a previous study. The viral titer of PEDV was considerably decreased following the inhibition of cellular autophagy with its inhibitors (i.e. 3-MA or CQ), while increased proliferation of PEDV was observed upon the induction of cellular autophagy by its inducer (e.g. rapamycin). Decreased viral titer of PEDV was similarly achieved by silencing the expression of the key autophagy genes Beclin1 and ATG5. These robustly demonstrate the implication of cell autophagy in the replication of PEDV (G. X et al., 2017).

The interaction of viral proteins and host factors

As described above, intensive investigations have not only confirmed the involvement of some structural or non-structural proteins of PEDV, but also verified the important contribution of some identified host factors in the forms of proteins, signaling pathways or physiological processes in target cells to the infection of PEDV. Two features can therefore be deduced: different viral proteins and host factors participate in various stages of PEDV infection, and the contribution of these proteins/factors is mainly achieved by their interaction. To clearly illustrate these, we summarize the major functions of viral proteins of PEDV and host factors in Table 1, and provide an overall view of the participation and interaction of them during PEDV infection in Fig 1. Briefly, at the initial stage of PEDV infection, identified host factors so far, including pAPN, sialic acid, HS, TMPRSS2, MSPL and occludin, are confirmed to interact with the S protein, thereby facilitating the attachment and entry of the PEDV viral particles into target cells. Next, the host factors hnRNPA1 and NPM1 interact with the N protein of PEDV to promote viral transcription and replication. Subsequently, the host factor BST2 inhibits PEDV replication by binding and degrading the N protein of PEDV, while TMPRSS2 plays a role in the release of PEDV. Additionally, intracellular signaling pathways of host cells are regulated to promote the replication and proliferation of virus particles following the invasion of PEDV. For instance, it utilizes p38 MAPK and JNK signaling pathways for optimal replication, while NF-ϰB may contribute to translocate from the cytoplasm to the nucleus. Similarly, autophagy is beneficial to PEDV replication through autophagy regulatory factors and RNA interference (Table 1, Fig 1).

Perspectives

The circulation of PEDV has caused huge economic damages to the pig industry in the world, while the emergence of its variant strains aggravates the challenge. An increased prevalence of PEDV has been witnessed in those years. For example, an outbreak of PEDV caused PED in China in 2010 was characterized by high mortality in suckling pigs (RQ et al., 2012). Though progress in the understanding of PEDV including its genome, viral structure, has been made by the efforts of numerous investigations during the past years, it seems we still stand a little far away from completely revealing the pathogenic mechanisms of PEDV. In addition, the development of effective and preventive measures like medicines and vaccines remains on the way.

To establish a productive infection, invading viruses need to overcome their host's first line of defense, the innate immune response. Although this reaction can largely protect the host from attacking by most microorganisms, it is still susceptible to antagonism and destruction of pathogenic viruses. As an important porcine pathogen emerging in recent years, PEDV has evolved strategies to overcome host innate immunity, including avoiding recognition by PRRs, inhibiting IFN production, and/or antagonizing IFN signal transduction and antiviral action mechanism of the host. Relative to extensive studies and in-depth information on other coronaviruses like SARS-CoV, the host-PEDV interactions just receive increased attention recently. One typical example is that PEDV targets intestinal epithelial cells (IECs) in the intestinal villi of pigs, while the most common in vitro cell culture system used to study PEDV does not derive from IECs of pigs. The

development of new pig IEC derived cell lines will undoubtedly provide an alternative, more physiologically relevant model for future studies of the PEDV-host interactions.

After an outbreak of PED in the United States in 2014, Zoetis has developed PEDV inactivated whole virus vaccine, which can provide passive immunity to piglets by immunizing sows before delivery. Before the new PED outbreak, the attenuated virus vaccine has played an effective role in the prevention and control of PED (CH, BJ, JG, GO, & YB, 1999; DS et al., 2007). Besides this, PEDV genetic engineering vaccines based on the S protein which can induce the body to produce neutralizing antibodies, also display broad prospects. As the most ideal vaccine for oral immunization, transgenic plant vaccine similarly have great development space (TJ, SC, MS, & YS, 2006). The numerous properties of nanoparticles, including possessing immunoadjuvant activity, generating natural immune response of antigens, easily reaching antigen-presenting cells to regulate the immune response through a variety of ways, targeting to present antigen, and releasing slowly, allow it to be an ideal candidate for preparing new vaccines against the occurrence of PED (DM, JK, & JR, 2013; T et al., 2020). Notably, although vaccine immunization is an effective way to prevent and control PED, for piglets infected with PEDV, it often cannot solve all of the issues. Therefore, some therapeutic drugs are urgently needed as well. In this regard, the validly inhibitory effect of IFN-L on the proliferation of PEDV in pig intestinal epithelial cells together with better action than type I interferon, empowers it to receive more attention (L. L et al., 2017). Likewise, PEDV specific yolk antibody has also been reported to increase the survival rate of infected piglets (DH et al., 2015). Besides these, prokaryotic expression of single stranded variable region of PEDV monoclonal antibody or small peptides identified by phage screening, which is able to bind to PEDV receptor, can also prevent PEDV from invading host cells (M. F et al., 2014). Additionally, the key role of proteases in virus release from cell surface and enhancement of PEDV infection, render them to be important drug targets. Therefore, protease inhibitors may also be good candidates for developing anti-PEDV compounds to fight this infectious disease (S. K et al., 2011). Similarly, some Chinese herbal extracts, such as quercetin and ginkgo peel extract, can effectively suppress the infection process of PEDV in vitro (HJ et al., 2009; L. JH et al., 2015; S. JH, JK, & HJ, 2011; T et al., 2020). Although some drugs have been proved to have anti-PEDV activity, they have not been used in clinical treatment due to its cost and safety. Therefore, it is of far-reaching significance to deeply study the pathogenesis of PEDV, identify PEDV receptors and related host factors, thereby providing more druggable targets for the prevention and treatment of PEDV.

Funding

This work was supported by General Program of National Natural Science Foundation of China (Grants No. 31571432/31802252), Hunan Provincial Natural Science Foundation of China (Grant No. 2015JC3097/2020JJ4041). Support was also provided by "Shennong" Scholar funding to Aibing Wang.

Competing interests

The authors declare that they have no competing interests.

Ethics Statement

No ethical approval was required as this is a review article with no original research data.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

References

A, O., D, A., Z, T., Q, S., Y, W., W, Z., . . . B, L. (2007). HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell host & microbe, 1* (1), 23-35.

A, P., MJ, E., VA, G., M, P., H, Y., YP, d. J., & CM, R. (2009). Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature, 457* (7231), 882-886.

AB, S., & RC, H. (2004). Epidermal growth factor receptor activation differentially regulates claudin expression and enhances transepithelial resistance in Madin-Darby canine kidney cells. *The Journal of biological chemistry, 279* (5), 3543-3552. doi:10.1074/jbc.M308682200

Bo-qi, L., Guang-xing, L., & Xiao-feng, R. (2009). Initial identification of the domain of TGEV specific receptor APN.*heilongjiang animal science and veterinary medicine* .

BX, L., JW, G., & YJ, L. (2007). Porcine aminopeptidase N is a functional receptor for the PEDV coronavirus. *Virology, 365* (1), 166-172.

C, K., Y, Y., S, R., & A, J. (2019). Porcine Epidemic Diarrhea Virus (PEDV) ORF3 Interactome Reveals Inhibition of Virus Replication by Cellular VPS36 Protein. *Viruses, 11* (4), undefined.

C, L., J, T., Y, M., X, L., Y, Y., G, P., . . . F, L. (2015). Receptor usage and cell entry of porcine epidemic diarrhea coronavirus.*Journal of virology, 89* (11), 6121-6125.

C, L., Y, M., Y, Y., Y, Z., J, S., Y, Z., . . . F, L. (2016). Cell Entry of Porcine Epidemic Diarrhea Coronavirus Is Activated by Lysosomal Proteases. *The Journal of biological chemistry, 291* (47), 24779-24786. doi:10.1074/jbc.M116.740746

CA, d. H., L, K., PS, M., H, V., & PJ, R. (1998). Coronavirus particle assembly: primary structure requirements of the membrane protein.*Journal of virology, 72* (8), 6838-6850.

CC, H., Y, W., B, N., R, W., L, H., XF, R., . . . X, M. (2015). Porcine epidemic diarrhea virus uses cell-surface heparan sulfate as an attachment factor. *Archives of virology, 160* (7), 1621-1628.

CH, K., BJ, K., JG, L., GO, K., & YB, K. (1999). Derivation of attenuated porcine epidemic diarrhea virus (PEDV) as vaccine candidate. *Vaccine, 17* (null), 2546-2553.

CK, C., CJ, C., CC, W., SW, C., SR, S., & RL, K. (2017). Cellular hnRNP A2/B1 interacts with the NP of influenza A virus and impacts viral replication. *PloS one, 12* (11), e0188214.

CY, C., JY, P., YH, C., YC, C., YT, W., PS, T., . . . HW, C. (2019). Development and comparison of enzyme-linked immunosorbent assays based on recombinant trimeric full-length and truncated spike proteins for detecting antibodies against porcine epidemic diarrhea virus. *BMC veterinary research, 15* (1), 421. doi:10.1186/s12917-019-2171-7

D, S. (2014). Counteraction of the multifunctional restriction factor tetherin. *Frontiers in microbiology, 5* , 163. doi:10.3389/fmicb.2014.00163

D, S., & B, P. (2012). Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. *Virus genes, 44* (2), 167-175. doi:10.1007/s11262-012-0713-1

D, S., H, S., D, S., J, C., X, Z., X, W., . . . L, F. (2017). Nucleocapsid Interacts with NPM1 and Protects it from Proteolytic Cleavage, Enhancing Cell Survival, and is Involved in PEDV Growth.*Scientific reports, 7* (undefined), 39700.

D, S., M, L., J, C., H, S., S, Z., X, Z., & L, F. (2014). Molecular characterizations of subcellular localization signals in the nucleocapsid protein of porcine epidemic diarrhea virus. *Viruses, 6* (3), 1253-1273.

D, W., & JS, M. (2019). The 3.1-Angstrom Cryo-electron Microscopy Structure of the Porcine Epidemic Diarrhea Virus Spike Protein in the Prefusion Conformation. *Journal of virology, 93* (23). doi:10.1128/jvi.00923-19

D, W., L, F., & S, X. (2016). Porcine epidemic diarrhea in China. *Virus research, 226* , 7-13. doi:10.1016/j.virusres.2016.05.026

D, W., L, F., Y, S., H, Z., L, G., G, P., . . . S, X. (2016). Porcine Epidemic Diarrhea Virus 3C-Like Protease Regulates Its Interferon Antagonism by Cleaving NEMO. *Journal of virology, 90* (4), 2090-2101.

DH, L., YS, J., CK, P., S, K., DS, L., & C, L. (2015). Immunoprophylactic effect of chicken egg yolk antibody (IgY) against a recombinant S1 domain of the porcine epidemic diarrhea virus spike protein in piglets. *Archives of virology, 160* (9), 2197-2207.

DM, S., JK, S., & JR, B. (2013). Applications of nanotechnology for immunology. *Nature reviews. Immunology, 13* (8), 592-605. doi:10.1038/nri3488

DS, S., JS, O., BK, K., JS, Y., HJ, M., HS, Y., . . . BK, P. (2007). Oral efficacy of Vero cell attenuated porcine epidemic diarrhea virus DR13 strain. *Research in veterinary science, 82* (1), 134-140.

E, B.-F., C, F., F, S., S, S., M, E., J, U., . . . W, G. (2010). Cleavage of influenza virus hemagglutinin by airway proteases TMPRSS2 and HAT differs in subcellular localization and susceptibility to protease inhibitors. *Journal of virology, 84* (11), 5605-5614.

E, N., & C, L. (2010). Contribution of the porcine aminopeptidase N (CD13) receptor density to porcine epidemic diarrhea virus infection. *Veterinary microbiology, 144* (null), 41-50.

EJ, S., PJ, B., JC, D., V, T., J, Z., LL, P., . . . AE, G. (2003). Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *Journal of molecular biology, 331* (5), 991-1004.

F, C., Y, Z., M, W., X, K., S, Y., Z, L., . . . Q, H. (2015). Comparative Genomic Analysis of Classical and Variant Virulent Parental/Attenuated Strains of Porcine Epidemic Diarrhea Virus. *Viruses, 7* (10), 5525-5538.

F, D., G, Y., Q, L., MT, N., X, Z., Y, L., . . . G, P. (2016). Identification and Comparison of Receptor Binding Characteristics of the Spike Protein of Two Porcine Epidemic Diarrhea Virus Strains. *Viruses, 8* (3), 55.

F, F., L, L., L, S., B, Y., H, S., J, Z., . . . P, L. (2017). A spike-specific whole-porcine antibody isolated from a porcine B cell that neutralizes both genogroup 1 and 2 PEDV strains. *Veterinary microbiology, 205* (undefined), 99-105.

F, K., & G, H. (1993). Structural and functional analysis of the surface protein of human coronavirus OC43. *Virology, 195* (1), 195-202.

F, L. (2012). Evidence for a common evolutionary origin of coronavirus spike protein receptor-binding subunits. *Journal of virology, 86* (5), 2856-2858.

F, L. (2015). Receptor recognition mechanisms of coronaviruses: a decade of structural studies. *Journal of virology, 89* (4), 1954-1964.

F, M., S, S., DS, Z., Y, C., X, M., Q, Z., & X, R. (2014). A phage-displayed peptide recognizing porcine aminopeptidase N is a potent small molecule inhibitor of PEDV entry. *Virology, null* (undefined), 20-27.

G, P., L, X., YL, L., L, C., JR, P., KV, H., & F, L. (2012). Crystal structure of bovine coronavirus spike protein lectin domain. *The Journal of biological chemistry, 287* (50), 41931-41938.

G, S., E, F., L, C., S, S., T, W., J, N., . . . DI, S. (2004). The nsp9 replicase protein of SARS-coronavirus, structure and functional insights. *Structure (London, England : 1993), 12* (2), 341-353.

H, B., C, S., MI, A., F, Z., RJ, L., H, T., . . . TF, B. (2003). Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate. *The Journal of biological chemistry, 278* (42), 41003-41012.

H, L., H, Z., L, G., B, L., K, H., & H, F. (2018). Development and application of an indirect ELISA for the detection of antibodies to porcine epidemic diarrhea virus based on a recombinant spike protein. *BMC veterinary research, 14* (1), 243. doi:10.1186/s12917-018-1570-5

H, S., J, P., Y, W., Y, S., J, H., L, L., . . . J, L. (2016). Highly Pathogenic Avian Influenza H5N6 Viruses Exhibit Enhanced Affinity for Human Type Sialic Acid Receptor and In-Contact Transmission in Model Ferrets. *Journal of virology, 90* (14), 6235-6243. doi:10.1128/jvi.00127-16

H, V., RJ, d. G., DA, H., MC, H., & WJ, S. (1991). Primary structure of the membrane and nucleocapsid protein genes of feline infectious peritonitis virus and immunogenicity of recombinant vaccinia viruses in kittens. *Virology, 181* (1), 327-335.

HJ, C., JH, K., CH, L., YJ, A., JH, S., SH, B., & DH, K. (2009). Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. *Antiviral research, 81* (1), 77-81.

J, H., C, X., L, H., Y, W., & Y, C. (2014). Bioinformatics insight into the spike glycoprotein gene of field porcine epidemic diarrhea strains during 2011-2013 in Guangdong, China. *Virus genes, 49* (1), 58-67.

JA, G., & BB, F. (2009). Tight junctions as targets of infectious agents. *Biochimica et biophysica acta, 1788* (4), 832-841.

JE, P., DJ, C., & HJ, S. (2011). Receptor-bound porcine epidemic diarrhea virus spike protein cleaved by trypsin induces membrane fusion.*Archives of virology, 156* (10), 1749-1756. doi:10.1007/s00705-011-1044-6

JE, P., DJ, C., & HJ, S. (2014). Clathrin- and serine proteases-dependent uptake of porcine epidemic diarrhea virus into Vero cells. *Virus research, 191* (undefined), 21-29.

Jeffers, S. A., Hemmila, E. M., & Holmes, K. V. (2006). Human coronavirus 229E can use CD209L (L-SIGN) to enter cells. *Adv Exp Med Biol, 581* , 265-269. doi:10.1007/978-0-387-33012-9_44

JH, F., YZ, Z., XQ, S., WY, G., & JM, D. (2015). Development of an enzyme-linked immunosorbent assay for the monitoring and surveillance of antibodies to porcine epidemic diarrhea virus based on a recombinant membrane protein. *Journal of virological methods, 225* (undefined), 90-94.

JH, L., JS, P., SW, L., SY, H., BE, Y., & HJ, C. (2015). Porcine epidemic diarrhea virus infection: inhibition by polysaccharide from Ginkgo biloba exocarp and mode of its action. *Virus research, 195* (undefined), 148-152.

JH, S., JK, S., & HJ, C. (2011). Quercetin 7-rhamnoside reduces porcine epidemic diarrhea virus replication via independent pathway of viral induced reactive oxygen species. *Virol J, 8* (undefined), 460.

Jh, W. (2002). Protein recognition by cell surface receptors: physiological receptors versus virus interactions. *Trends in biochemical sciences, 27* (3), 122-126. doi:10.1016/s0968-0004(01)02038-2

JM, T.-F., & CF, A. (2015). Tight Junctions Go Viral! *Viruses, 7* (9), 5145-5154. doi:10.3390/v7092865

JS, O., DS, S., & BK, P. (2003). Identification of a putative cellular receptor 150 kDa polypeptide for porcine epidemic diarrhea virus in porcine enterocytes. *Journal of veterinary science, 4* (3), 269-275.

K, H., A, S., & MO, O. (2000). Mapping the functional domains of nucleolar protein B23. *The Journal of biological chemistry, 275* (32), 24451-24457.

K, N., SI, R., KG, L., & S, M. (2015). Coronavirus nonstructural protein 1: Common and distinct functions in the regulation of host and viral gene expression. *Virus research, 202* (undefined), 89-100.

K, O., BL, H., R, H., VS, R., H, M., S, I., . . . C, M. (2013). Inhibition of Middle East respiratory syndrome coronavirus infection by anti-CD26 monoclonal antibody. *Journal of virology, 87* (24), 13892-13899.

K, S., M, M., MT, I., A, M., M, K., S, M., & F, T. (2016). Porcine aminopeptidase N is not a cellular receptor of porcine epidemic diarrhea virus, but promotes its infectivity via aminopeptidase activity. *The Journal of general virology, 97* (10), 2528-2539. doi:10.1099/jgv.0.000563

K, S., S, M., M, U., & F, T. (2011). Role of proteases in the release of porcine epidemic diarrhea virus from infected cells. *Journal of virology, 85* (15), 7872-7880.

K, W., W, L., J, C., S, X., H, S., H, H., . . . B, S. (2012). PEDV ORF3 encodes an ion channel protein and regulates virus production.*FEBS letters, 586* (4), 384-391.

L, G.-M., R, T., & D, C. (2008). Crosstalk of tight junction components with signaling pathways. *Biochimica et biophysica acta, 1778* (3), 729-756.

L, G., H, Y., W, G., X, L., R, L., J, Z., . . . Y, W. (2016). Autophagy Negatively Regulates Transmissible Gastroenteritis Virus Replication.*Scientific reports, 6* (undefined), 23864.

L, L., F, F., M, X., W, C., J, L., H, S., . . . P, L. (2017). IFN-lambda preferably inhibits PEDV infection of porcine intestinal epithelial cells compared with IFN-alpha. *Antiviral research, 140* (undefined), 76-82.

L, Y., J, X., L, G., T, G., L, Z., L, F., . . . Y, W. (2018). Porcine Epidemic Diarrhea Virus-Induced Epidermal Growth Factor Receptor Activation Impairs the Antiviral Activity of Type I Interferon.*Journal of virology, 92* (8), undefined.

M, A., & G, W. (2019). Current Status of Porcine Epidemic Diarrhoea (PED) in European Pigs. *Journal of veterinary research, 63* (4), 465-470. doi:10.2478/jvetres-2019-0064

M, D., P, G., SF, W., & FV, C. (2009). The autophagy machinery is required to initiate hepatitis C virus replication. *Proceedings of the National Academy of Sciences of the United States of America, 106* (33), 14046-14051.

M, G., D, D., R, A., J, D., T, T., PC, R., . . . C, M. (2009). Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. *Cell host & microbe, 6* (4), 367-380.

M, M., A, G., PL, S., & R, C. (2015). Connections matter–how viruses use cell–cell adhesion components. *Journal of cell science, 128* (3), 431-439. doi:10.1242/jcs.159400

Masters, P. S. (2006). The molecular biology of coronaviruses.*Advances in Virus Research, 66* (1), 193.

MJ, E., T, v. H., DM, T., AJ, S., M, P., B, W., . . . CM, R. (2007). Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature, 446* (7137), 801-805.

MS, L. (2011). NPM1/B23: A Multifunctional Chaperone in Ribosome Biogenesis and Chromatin Remodeling. *Biochemistry research international, 2011* (undefined), 195209.

MZ, H., H, W., SY, W., DA, C., X, T., & YM, L. (2016). Molecular characterization and phylogenetic analysis of porcine epidemic diarrhea virus samples obtained from farms in Gansu, China. *Genetics and molecular research : GMR, 15* (1), undefined.

N, K., T, S., H, W., Y, J., Y, Z., L, L., . . . G, T. (2019). BST2 suppresses porcine epidemic diarrhea virus replication by targeting and degrading virus nucleocapsid protein with selective autophagy.*Autophagy* , 1-16. doi:10.1080/15548627.2019.1707487

N, V. D., D, G., C, K., RL, J., R, M., MC, J., . . . J, G. (2008). The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. *Cell host & microbe, 3* (4), 245-252. doi:10.1016/j.chom.2008.03.001

O, W., W, L., L, W., TJ, M., RW, W., FJ, v. K., . . . BJ, B. (2014). Proteolytic activation of the porcine epidemic diarrhea coronavirus spike fusion protein by trypsin in cell culture. *Journal of virology, 88* (14), 7952-7961. doi:10.1128/jvi.00297-14

OV, S. (2009). [Porcine epidemic diarrhea]. *Voprosy virusologii, 54* (2), 4-8.

P, I., CF, A., & S, L. (2006). Role of sialic acids in rotavirus infection. *Glycoconjugate journal, 23* , 27-37. doi:10.1007/s10719-006-5435-y

Q, Z., K, S., & D, Y. (2016). Suppression of type I interferon production by porcine epidemic diarrhea virus and degradation of CREB-binding protein by nsp1. *Virology, 489* (undefined), 252-268.

R, K., A, B., M, A., & K, T. (2001). Completion of the porcine epidemic diarrhoea coronavirus (PEDV) genome sequence. *Virus genes, 23* (2), 137-144.

R, S., & TH, B. (2008). Type II transmembrane serine proteases in development and disease. *The international journal of biochemistry & cell biology, 40* (null), 1297-1316.

RQ, S., RJ, C., YQ, C., PS, L., DK, C., & CX, S. (2012). Outbreak of porcine epidemic diarrhea in suckling piglets, China. *Emerging infectious diseases, 18* (1), 161-163. doi:10.3201/eid1801.111259

S, K., V, K., R, R., A, S., A, W., & G, B. (2003). Bst-2/HM1.24 is a raft-associated apical membrane protein with an unusual topology. *Traffic (Copenhagen, Denmark), 4* (10), 694-709. doi:10.1034/j.1600-0854.2003.00129.x

S, S., WC, L., & JD, E. (2011). Heparan sulfate proteoglycans. *Cold Spring Harbor perspectives in biology, 3* (7), undefined.

S, T., M, N., K, M., & MS, B. (2010). Rho signaling and tight junction functions. *Physiology (Bethesda, Md.), 25* (1), 16-26.

S, T., ML, J., SE, S. J., HL, O., PR, N., LN, P., . . . AD, M. (2015). Ligand-induced Dimerization of Middle East Respiratory Syndrome (MERS) Coronavirus nsp5 Protease (3CLpro): IMPLICATIONS FOR nsp5 REGULATION AND THE DEVELOPMENT OF ANTIVIRALS. *The Journal of biological chemistry, 290* (32), 19403-19422.

S, Y., Z, L., F, C., W, L., X, G., H, H., & Q, H. (2015). Porcine epidemic diarrhea virus ORF3 gene prolongs S-phase, facilitates formation of vesicles and promotes the proliferation of attenuated PEDV. *Virus genes, 51* (3), 385-392.

S, Z., H, Z., Z, D., R, L., K, A., L, L., . . . L, F. (2015). Proteome analysis of porcine epidemic diarrhea virus (PEDV)-infected Vero cells. *Proteomics, 15* (11), 1819-1828.

S, Z., J, G., L, Z., & Q, Y. (2014). Transmissible gastroenteritis virus and porcine epidemic diarrhoea virus infection induces dramatic changes in the tight junctions and microfilaments of polarized IPEC-J2 cells. *Virus research, 192* (undefined), 34-45.

SJ, P., HK, K., DS, S., HJ, M., & BK, P. (2011). Molecular characterization and phylogenetic analysis of porcine epidemic diarrhea virus (PEDV) field isolates in Korea. *Archives of virology, 156* (4), 577-585.

SR, W., & S, N.-M. (2005). Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. *Microbiology and molecular biology reviews : MMBR, 69* (4), 635-664.

T, T., H, H., J, Z., S, D., X, Z., W, T., . . . J, L. (2020). Glycyrrhizic-Acid-Based Carbon Dots with High Antiviral Activity by Multisite Inhibition Mechanisms. *Small (Weinheim an der Bergstrasse, Germany), 16* (13), e1906206. doi:10.1002/smll.201906206

TJ, K., SC, H., MS, Y., & YS, J. (2006). Expression of synthetic neutralizing epitope of porcine epidemic diarrhea virus fused with synthetic B subunit of Escherichia coli heat-labile enterotoxin in tobacco plants. *Protein expression and purification, 46* (1), 16-22. doi:10.1016/j.pep.2005.07.026

U, B., & H, S. (2013). Heterogeneous nuclear ribonucleoprotein A1 in health and neurodegenerative disease: from structural insights to post-transcriptional regulatory roles. *Molecular and cellular neurosciences, 56* (undefined), 436-446.

V, O. D., JM, P., M, L., T, B., W, J., LL, R., . . . B, B. (2011). Foot-and-mouth disease virus utilizes an autophagic pathway during viral replication. *Virology, 410* (1), 142-150.

V, T., KA, I., A, P., T, H., B, S., S, B., . . . J, Z. (2003). Mechanisms and enzymes involved in SARS coronavirus genome expression. *The Journal of general virology, 84* (null), 2305-2315.

W, L., FJM, v. K., Q, H., PJM, R., & BJ, B. (2016). Cellular entry of the porcine epidemic diarrhea virus. *Virus research, 226* , 117-127. doi:10.1016/j.virusres.2016.05.031

W, L., H, L., Y, L., Y, P., F, D., Y, S., . . . Q, H. (2012). New variants of porcine epidemic diarrhea virus, China, 2011. *Emerging infectious diseases, 18* (8), 1350-1353.

W, L., O, W., FJ, v. K., Q, H., PJ, R., & BJ, B. (2015). A Single Point Mutation Creating a Furin Cleavage Site in the Spike Protein Renders Porcine Epidemic Diarrhea Coronavirus Trypsin Independent for Cell Entry and Fusion. *Journal of virology, 89* (15), 8077-8081. doi:10.1128/jvi.00356-15

W, S., W, F., J, B., Y, T., L, W., Y, J., . . . Y, L. (2017). TMPRSS2 and MSPL Facilitate Trypsin-Independent Porcine Epidemic Diarrhea Virus Replication in Vero Cells. *Viruses, 9* (5), undefined.

X, G., M, Z., X, Z., X, T., H, G., W, Z., . . . Q, H. (2017). Porcine Epidemic Diarrhea Virus Induces Autophagy to Benefit Its Replication. *Viruses, 9* (3), undefined.

X, L., L, G., J, Z., Y, X., W, G., L, F., & Y, W. (2017). Tight Junction Protein Occludin Is a Porcine Epidemic Diarrhea Virus Entry Factor. *Journal of virology, 91* (10), undefined.

X, X., H, Z., Q, Z., Y, H., J, D., Y, L., . . . D, T. (2013). Porcine epidemic diarrhea virus N protein prolongs S-phase cell cycle, induces endoplasmic reticulum stress, and up-regulates interleukin-8 expression. *Veterinary microbiology, 164* (null), 212-221.

XL, H., LY, Y., & J, L. (2007). Development and evaluation of enzyme-linked immunosorbent assay based on recombinant nucleocapsid protein for detection of porcine epidemic diarrhea (PEDV) antibodies. *Veterinary microbiology, 123* (null), 86-92.

Xu, X., Zhang, H., Zhang, Q., Dong, J., Liang, Y., Huang, Y., . . . Tong, D. (2013). Porcine epidemic diarrhea virus E protein causes endoplasmic reticulum stress and up-regulates interleukin-8 expression. *Virol J, 10* , 26. doi:10.1186/1743-422x-10-26

Y, H., CM, L., M, Y., BL, Y., D, M., AL, D., . . . Q, W. (2017). Deletion of a 197-Amino-Acid Region in the N-Terminal Domain of Spike Protein Attenuates Porcine Epidemic Diarrhea Virus in Piglets. *Journal of virology, 91* (14). doi:10.1128/jvi.00227-17

Y, K., & C, L. (2014). Porcine epidemic diarrhea virus induces caspase-independent apoptosis through activation of mitochondrial apoptosis-inducing factor. *Virology, null* (undefined), 180-193.

Y, K., & C, L. (2015). Extracellular signal-regulated kinase (ERK) activation is required for porcine epidemic diarrhea virus replication. *Virology, 484* (undefined), 181-193.

Y, K., C, O., V, S., RA, H., & KO, C. (2017). Trypsin-independent porcine epidemic diarrhea virus US strain with altered virus entry mechanism. *BMC veterinary research, 13* (1), 356. doi:10.1186/s12917-017-1283-1

Y, S., S, Y., N, D., C, M., S, M., S, Z., . . . C, D. (2014). Autophagy benefits the replication of Newcastle disease virus in chicken cells and tissues. *Journal of virology, 88* (1), 525-537. doi:10.1128/jvi.01849-13

Y, S., Y, C., X, H., Z, Y., Y, W., & G, Z. (2019). Porcine epidemic diarrhea virus in Asia: An alarming threat to the global pig industry. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases, 70* , 24-26. doi:10.1016/j.meegid.2019.02.013

Y, W., & X, Z. (1999). The nucleocapsid protein of coronavirus mouse hepatitis virus interacts with the cellular heterogeneous nuclear ribonucleoprotein A1 in vitro and in vivo. *Virology, 265* (1), 96-109.

Y, Y., & BG, H. (2007). Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. *Journal of virology, 81* (7), 3597-3607. doi:10.1128/jvi.01472-06

Y, Z., E, B., & GR, W. (2012). Expression of the C-type lectins DC-SIGN or L-SIGN alters host cell susceptibility for the avian coronavirus, infectious bronchitis virus. *Veterinary microbiology, 157* (null), 285-293.

YW, T., S, F., H, F., J, L., & DX, L. (2006). Amino acid residues critical for RNA-binding in the N-terminal domain of the nucleocapsid protein are essential determinants for the infectivity of coronavirus in cultured cells. *Nucleic acids research, 34* (17), 4816-4825.

Z, L., W, Z., S, Y., J, L., A, N., B, Z., . . . Q, H. (2018). Cellular hnRNP A1 Interacts with Nucleocapsid Protein of Porcine Epidemic Diarrhea Virus and Impairs Viral Replication. *Viruses, 10* (3), undefined.

Z, Z., F, D., K, S., G, Y., G, W., L, F., . . . G, P. (2018). Dimerization of Coronavirus nsp9 with Diverse Modes Enhances Its Nucleic Acid Binding Affinity. *Journal of virology, 92* (17), undefined.

Zhang, Y., Buckles, E., & Whittaker, G. R. Expression of the C-type lectins DC-SIGN or L-SIGN alters host cell susceptibility for the avian coronavirus, infectious bronchitis virus. *157* (3-4), 285-293.

Figure legend:

Fig 1: The involvement of numerous host factors and viral proteins in the different processes of PEDV infection . During the process of virus attachment and entry into cells, the host factors pAPN, HS, sialic acid, EGFR, TMPRSS2, MSPL and occludin are found to promote PEDV infection by interacting with the S protein. DC-SIGN and DPP4 may play a role in this process as well, while more robust evidence is needed. HnRNPA1 and NPM1 are verified to interact with the N protein of PEDV, thus participating in the transcription and replication stages. Finally, TMPRSS2 and BST2 are identified to take part in the assembly and release of the virus. PEDV also utilizes the p38 MAPK and JNK signaling pathways for optimal replication. Similarly, PEDV infection induces endoplasmic reticulum (ER) stress response and activation of NF-ϰB signal also contributes to PEDV replication. Additionally, PEDV can facilitate its replication by affecting autophagy. Note: —, — in the figure denote promotion or inhibition, respectively.

Hosted file

`Figure 1.docx` available at https://authorea.com/users/353471/articles/477335-a-comprehensive-view-on-the-host-factors-and-viral-proteins-associated-with-pedv-infection

Hosted file

`Table 1.docx` available at https://authorea.com/users/353471/articles/477335-a-comprehensive-view-on-the-host-factors-and-viral-proteins-associated-with-pedv-infection