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CRISPR/Cas9 Screening Identifies Genes Mediating Porcine Epidemic Diarrhea Virus Replication

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Abstract

Recently, porcine epidemic diarrhea virus (PEDV) is still identified as the main pathogen causing severe diarrhea in pig farms of many countries. Porcine epidemic diarrhea which is caused by this virus results in substantial economic losses for pig farmers all over the world. Resistance breeding has proven one of the effective strategies to control and prevent the spread of PEDV. Identification of crucial host factors and genetic variants associated with PEDV infection is the prerequisite for implementing resistance breeding. Genetic perturbation enables the generation of marked phenotypes related to PEDV infection, which will advance the identification of host factors crucial for host and PEDV interactions. In this review, we describe the status of PEDV spread in pig farms in recent years and discuss the findings on genes involved in host-PEDV interactions. We also discuss the advantages of genetic screens in identifying host factors that are important for virus replication and how it has been used to expand our understanding of viral pathogenesis. Further studies on host and PEDV interactions using new genetic technologies will advance identifications of key host factors involved in mediating PEDV infections and further contribute to genetic resistance breeding for porcine epidemic diarrhea.

Porcine epidemic diarrhea disease in pig farms

Porcine epidemic diarrhea virus (PEDV) was first recognized in the United Kingdom in 1971 and had spread throughout the world by 2013. PEDV is an enveloped, single-stranded, positive-sense RNA virus that belongs to the family *Coronaviridae*, genus *alphacoronavirus*. The 28 kb genome of PEDV encodes four structural proteins including the spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) and three non-structural proteins (ORF1a/1b and ORF3) (**Fig. 1A**). Among these proteins, the S protein is responsible for the attachment of virus particles to cell surface receptor and for the fusion to host cells (Li, 2015). The PEDV propagates through fecal–oral and nasal cavity pathways to enter the intestinal epithelium (Lin *et al*., 2016; Li *et al*., 2018). PEDV replicates in the cytoplasm of villus epithelial cells and causes villi atrophy, shortening and fusion (**Fig. 1B**), which leads to watery diarrhea, vomiting, and dehydration of infected animals. PEDV can infect pigs at all ages and result in up to 80–100% mortality for suckling pigs, and cause production losses for breeding adult pigs. In recent years, PEDV is still identified as the main pathogen causing severe diarrhea in pig farms (Su *et al*., 2020). Due to the high morbidity and mortality in suckling pigs, porcine epidemic diarrhea disease leads to substantial economic losses to the pig industry of all the world.

Genes involved in mediating host-PEDV interactions

Cell surface receptor is the pivotal determinant for the PEDV to bind and enter host cells. Previous studies suggested that porcine aminopeptidase N (APN) acts as a receptor for PEDV entry into target cells (Li *et al*., 2007; Park *et al*., 2015). However, it is a controversial issue whether APN is

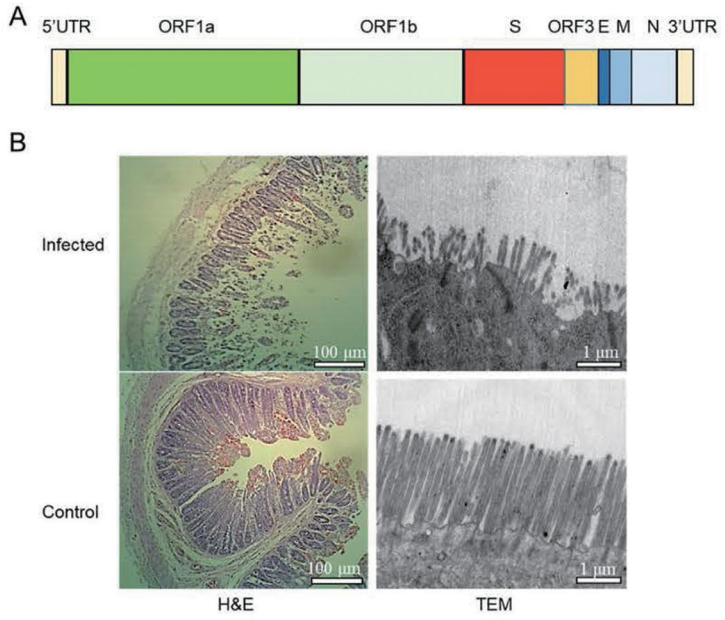

Fig. 1. Genomic structure of PEDV and intestinal pathologies induced by PEDV. (A) Diagram of the PEDV genome. (B) Histopathological analyses of the jejunum tissues derived from PEDV-infected and control animals. H&E: hematoxylin and eosin staining, TEM: transmission electron microscopic.

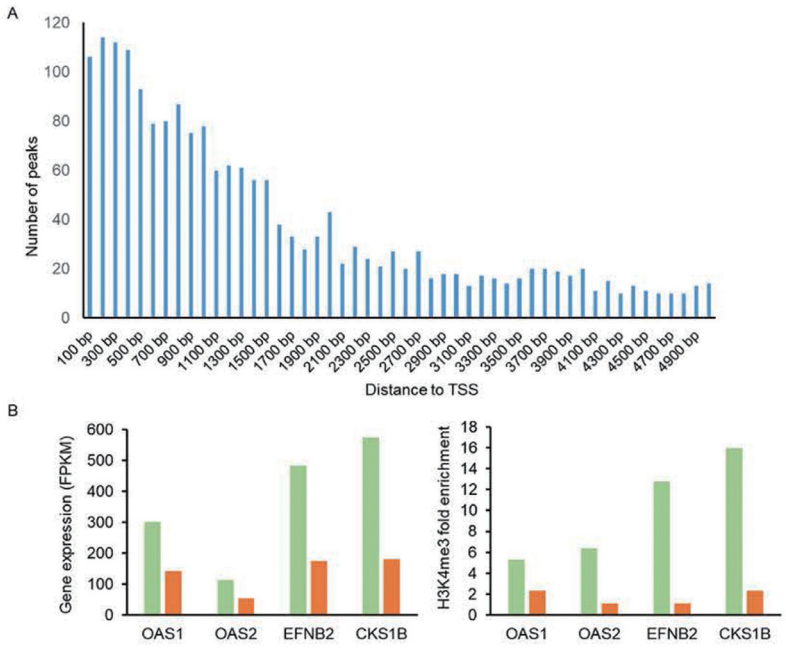

Fig. 2. H3K4me3 peak distribution and associations with gene expression. (A) Distribution of H3K4me3 peaks with the distance to transcription start site. (B) Expression and H3K4me3 fold enrichment of the OAS1, OAS2, EFNB2, and CKS1B genes in PEDV-infected and control animals. Green and oranges bars represent PEDV-infected and control samples, respectively.

the functional receptor for PEDV due to the recent reports that APN is not required for PEDV cell entry (Shirato *et al*., 2016; Ji *et al*., 2018). Therefore, identification of the functional receptor for PEDV cell entry is worthy to be further explored. In addition, researchers have explored the gene expression changes on transcriptomic and proteomic levels and non-coding RNA expression alterations induced by PEDV infection and identified a group of genes such as OAS1, IFIT, and Mx1 potential involved in regulating the interactions between PEDV and host cells (Li *et al*., 2016; Chen *et al*., 2019). Genetic divergence and association analyses on piglet resilience found that the EBI3, MUC16, and TCF3 genes can be related to PEDV infections (Bertolini *et al*., 2017). Mechanistic studies further unraveled that PEDV could avoid the innate antiviral immune responses by restricting production of interferons (Guo *et al*., 2016; Zhang *et al*., 2018). Our studies revealed changes in the patterns of H3K4me3 histone modifications related to PEDV infection (**Fig. 2A**), providing novel insights into PEDV infection from epigenetic layers (Wang *et al*., 2019). Several genes including OAS1, OAS2, EFNB2, and CKS1B demonstrated higher H3K4me3 enrichment and expression levels in PEDV-

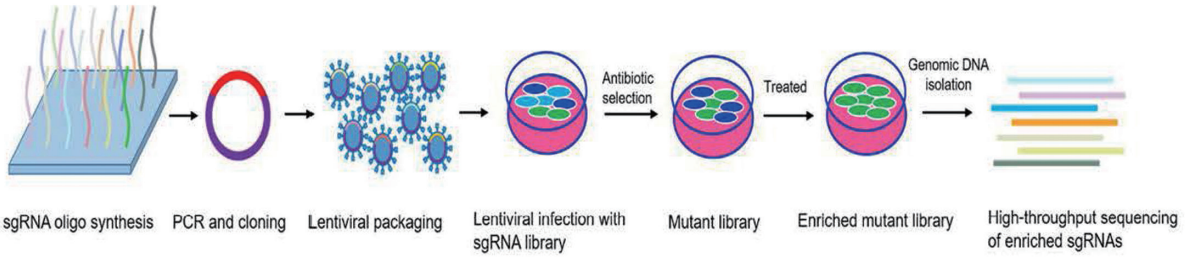

Fig. 3. Schematic of CRISPR/Cas9 screening workflow.

Table 1. sgRNA sequence targeting porcine genes

sgRNA ID	Gene	Gene ID	Chromosome	Sequences
sgTCOF1_1	TCOF1	100516425	chr2	TGGCAGAGGCCAGGAAGCGG
sgTCOF1_2	TCOF1	100516425	chr2	CTACCAGCATCTGCTGCAGG
sgTCOF1_3	TCOF1	100516425	chr2	TACCAGCATCTGCTGCAGGC
sgTCOF1_4	TCOF1	100516425	chr2	GCAGGCGGGCTATGTGCGCG
sgNSRP1_1	NSRP1	100517122	chr12	CGTGAGTGAAAGCCTTCAGA
sgNSRP1_2	NSRP1	100517122	chr12	CCTCTGAAGGCTTTCACTCA
sgNSRP1_3	NSRP1	100517122	chr12	CAGCCCAGATTCTAGGGCAA
sgNSRP1_4	NSRP1	100517122	chr12	CCCAGAGGAGTGTCAAGAGA
sgNSRP1_5	NSRP1	100517122	chr12	CAGATACTTAGCCCGGCAGA
sgSPPL3_1	SPPL3	100154995	chr14	ACACCTGACTGGAATCCACC
sgSPPL3_2	SPPL3	100154995	chr14	CACCTGACTGGAATCCACCA
sgSPPL3_3	SPPL3	100154995	chr14	ACTGGAATCCACCAGGGAAT
sgSPPL3_4	SPPL3	100154995	chr14	CAGGCCCTGTTCCTTCCAAT

infected samples (**Fig. 2B**), suggesting the potential roles of H3K4me3 deposition in promoting their expression. Recent reports have preliminarily revealed the host factors potentially interacting with PEDV, while the key determinant for PEDV cell entry and replication remain poorly understood. Further investigations on the detection of key regulators of host-PEDV interactions and on the underlying molecular mechanisms are required.

Application of CRISPR/Cas9 Screening in virus-host interactions

Loss-of-function genetic detection is an effective strategy in functional genomic studies via stably suppressing or disrupting gene expression in a cell or organism. Programmable CRISPR-associated nuclease Cas9 provides an effective way to cause targeted loss-of-function mutations at specific genomic sites of interest (Cong *et al*., 2013). Cas9 is guided by short RNAs to the specific sites and precisely recognizes and cleaves the target DNA, producing frameshifting indels that results in loss-of-function mutations. Feasibility of genome-wide CRISPR/Cas9 screening was firstly proved and applied in mammal cells (Shalem *et al*., 2014; Wang *et al*., 2014). Emerging studies have proven CRISPR/Cas9 screening as a reliable strategy to identify host factors that are paramount for virus replication (Ma *et al*., 2015; Zhang *et al*., 2016; Savidis *et al*., 2016). Protocols and practical considerations for this strategy can refer to the previous reports (Joung *et al*., 2014). The schematic of CRISPR/Cas9 screening workflow is shown in **Fig. 3**. To unravel host factors that are crucial for PEDV replication, we established a mutant cell library for CRISPR/Cas9 screening. An sgRNA library (~92,000 sgRNAs) targeting the porcine genomic genes (~20,000 genes) was designed, with 3~5 sgRNAs per gene. Examples of sgRNA sequence targeting porcine genes are shown in **Table 1**. A mutant cell library was then obtained after lentiviral infection and antibiotic selection. The mutant cell library was infected with PEDV and survival cells were collected for genomic DNA isolation and high-throughput sequencing. After comparison of sgRNA abundance derived from the PEDV-treated cells with the untreated controls by using the MAGeCK software (Li *et al*., 2014), top ranked genes including ERN1, THEM19, and KDM2B with high potential to repress PEDV replication were screened out. Considering the possible noisy during the screening process, it is required to verify that knockout of the candidate genes confers the phenotype of inhibition of PEDV replication.

Perspectives

With the increased pressure induced by the application of antiviral drugs and vaccine inoculation, frequently appearing mutations bring about variabilities in the viral genome and further alter the pathogenicity of the new PEDV variants. It is difficult to implement selective breeding for disease resistance, as outbreaks are often sporadic and resistant/resilient animals are difficult to identify. Genetic screen technology that can generate marked phenotypes of interest enables identification of host factors crucial for virus replication and it will substantially contribute to our understanding of viral pathogenesis and the development of antiviral therapeutics. Our limited knowledge about the host factors involved in

interaction between PEDV and host cells hinders the control and prevention of porcine epidemic diarrhea. Therefore, investigations on the mechanisms underlying the PEDV-host interactions and detection of crucial genes for PEDV infection to establish strategies to prevent the spread of PEDV.

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References

Bertolini, F., J. C. Harding, B. Mote, A. Ladinig, G. S. Plastow, and M. F. Rothschild (2017) Genomic investigation of piglet resilience following porcine epidemic diarrhea outbreaks. Animal genetics, 48(2), 228–232.

Chen, J., C. Zhang, N. Zhang, and G. Liu (2019) Porcine endemic diarrhea virus infection regulates long noncoding RNA expression. Virology, 527, 89–97.

Cong, L., F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini, and F. Zhang (2013) Multiplex genome engineering using CRISPR/Cas systems. Science, 339(6121), 819–823.

Guo, L., X. Luo, R. Li, Y. Xu, J. Zhang, J. Ge, Z. Bu, L. Feng, and Y. Wang (2016) Porcine epidemic diarrhea virus infection inhibits interferon aignaling by targeted degradation of STAT1. Journal of Virology, 90(18), 8281–8292.

Ji, C. M., B. Wang, J. Zhou, and Y. W. Huang (2018) Aminopeptidase-N-independent entry of porcine epidemic diarrhea virus into Vero or porcine small intestine epithelial cells. Virology, 517, 16–23.

Joung, J., S. Konermann, J. S. Gootenberg, O. O. Abudayyeh, R. J. Platt, M. D. Brigham, N. E. Sanjana and F. Zhang (2017) Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. Nature Protocols, 12(4), 828–863.

Li, F. (2015) Receptor recognition mechanisms of coronaviruses: a decade of structural studies. Journal of Virology, 89(4), 1954–1964.

Li, B. X., J. W. Ge, and Y. J. Li (2007) Porcine aminopeptidase N is a functional receptor for the PEDV coronavirus. Virology, 365(1), 166–172.

Li, W., H. Xu, T. Xiao, L. Cong, M. I. Love, F. Zhang, R. A. Irizarry, J. S. Liu, M. Brown, and X. S. Liu (2014) MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. Genome Biology, 15(12), 554.

Li, Y., Q. Wu, L. Huang, C. Yuan, J. Wang, and Q. Yang (2018) An alternative pathway of enteric PEDV dissemination from nasal cavity to intestinal mucosa in swine. Nature Communications, 9(1), 3811.

Li, Z., F. Chen, S. Ye, X. Guo, A. Muhanmmad Memon, M. Wu, and Q. He (2016) Comparative proteome analysis of porcine jejunum tissues in response to a virulent strain of porcine epidemic diarrhea virus and its attenuated Strain. Viruses, 8(12), 323.

Lin, C. M., L. J. Saif, D. Marthaler, and Q. Wang (2016) Evolution, antigenicity and pathogenicity of global porcine epidemic diarrhea virus strains. Virus research, 226, 20–39.

Ma, H., Y. Dang, Y. Wu, G. Jia, E. Anaya, J. Zhang, S. Abraham, J. G. Choi, G. Shi, L. Qi, N. Manjunath, and H. Wu (2015) A CRISPR-based screen identifies genes essential for West-Nile-virus-induced cell death. Cell Reports, 12(4), 673–683.

Park, J. E., E. S. Park, J. E. Yu, J. Rho, S. Paudel, B. H. Hyun, D. K. Yang, H. J. Shin, (2015) Development of transgenic mouse model expressing porcine aminopeptidase N and its susceptibility to porcine epidemic diarrhea virus. Virus research, 197, 108–115.

Savidis, G., W. M. McDougall, P. Meraner, J. M. Perreira, J. M. Portmann, G. Trincucci, S. P. John, A. M. Aker, N. Renzette, D. R. Robbins, Z. Guo, S. Green, T. F. Kowalik, and A. L. Brass (2016) Identification of Zika virus and Dengue virus dependency factors using functional genomics. Cell Reports, 16(1), 232–246.

Shalem, O., N. E. Sanjana, E. Hartenian, X. Shi, D. A. Scott, T. Mikkelson, D. Heckl, B. L. Ebert, D. E. Root, J. G. Doench, F. Zhang (2014) Genome-scale CRISPR-Cas9 knockout screening in human cells. Science, 343(6166), 84–87.

Shirato, K., M. Maejima, M. T. Islam, A. Miyazaki, M. Kawase, S. Matsuyama, and F. Taguchi (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.

Su, M., C. Li, S. Qi, D. Yang, N. Jiang, B. Yin, D. Guo, F. Kong, D. Yuan, L. Feng, and D. Sun (2020) A molecular epidemiological investigation of PEDV in China: Characterization of co-infection and genetic diversity of S1-based genes. Transboundary and Emerging Diseases, 67(3), 1129–1140.

T. Wang, J. J. Wei, D. M. Sabatini, and E. S. Lander (2014) Genetic screens in human cells using the CRISPR-Cas9 system. Science, 343(6166), 80–84.

Wang, H., L. Yang, H. Qu, H. Feng, S. Wu, and W. Bao (2019) Global mapping of H3K4 Trimethylation (H3K4me3) and transcriptome analysis reveal genes involved in the response to epidemic diarrhea virus infections in pigs. Animals, 9(8), 523.

Zhang, Q., H. Ke, A. Blikslager, T. Fujita, and D. Yoo (2018) Type III interferon restriction by porcine epidemic diarrhea virus and the role of viral protein nsp1 in IRF1 signaling. Journal of Virology, 92(4), e01677-17.

Zhang, R., J. J. Miner, M. J. Gorman, K. Rausch, H. Ramage, J. P. White, A. Zuiani, P. Zhang, E. Fernandez, Q. Zhang, K. A. Dowd, T. C. Pierson, S. Cherry, and M. S. Diamond (2016) A CRISPR screen defines a signal peptide processing pathway required by flaviviruses. Nature, 535(7610), 164–168.