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Review

Molecular and Cellular Mechanisms for PRRSV Pathogenesis and Host Response to Infection

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A B S T R A C T

Porcine reproductive and respiratory syndrome virus (PRRSV) has caused tremendous amounts of economic losses to the swine industry for more than three decades, but its control is still unsatisfactory. A significant amount of information is available for host cell-virus interactions during infection, and it is evident that PRRSV has evolved to equip various strategies to disrupt the host antiviral system and provide favorable conditions for survival. The current study reviews viral strategies for modulations of cellular processes including innate immunity, apoptosis, microRNAs, inflammatory cytokines, and other cellular pathways.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) emerged three decades ago in the US and almost simultaneously but independently in Europe. The etiologic agents were identified shortly in the Netherlands and the US, and these viruses were named Lelystad and VR-2332, respectively (Benfield et al., 1992; Wensvoort et al., 1991). The genomic sequences of Lelystad and VR-2332 show only approximately 60% identity (Meulenberg et al., 1993; Nelsen et al., 1999), and thus PRRSV isolates are grouped to PRRSV-1 (formerly known as European genotype) and PRRSV-2 (formerly known as North American genotype), respectively. Since its emergence, PRRSV has been evolving greatly, and a recent variant of PRRS-2 in China shows much higher virulence compared to previous isolates (Tian et al., 2007; Zhou et al., 2008). Regardless of the genetic types of PRRSV, clinical manifestations are similar representing mainly respiratory symptoms in piglets and abortion, reproductive failure, fetal death, and congenital infections in pregnant animals (See a review in Chand et al., 2012).

PRRSV is a member of the family Arteriviridae in the order Nidovirales (https://talk.ictvonline.org/ictv-reports/ictv_9th_report/positive-sense-rna-viruses-2011/w/posrna_viruses/219/nidovirales). The viral genome is a single-stranded positive-sense RNA molecule of approximately 15 Kb in length with the 5'-cap and 3'-polyadenylation (Meulenberg et al., 1993; Nelsen et al., 1999; Wootton et al., 2000). The PRRSV genome contains 11 open reading frames (ORFs): ORF1a, ORF1b, ORF2a, ORF2b, ORFs 3 through 7, plus the newly identified ORF5a and ORF2 (TF) (Fang et al., 2012b; Firth et al., 2011; Johnson et al., 2011). ORF1a and ORF1b occupy 75% of the genome and overlap slightly. They code for two polyproteins, pp1a and pp1b, of which pp1b is translated by the -1 ribosomal frameshift in the overlapping region (den Boon et al., 1991). Pp1a contains four viral proteinase activities: two papain-like cysteine proteinases (PLP1α and PLP1β), poliovirus 3C-like cysteine proteinase (CP or PLP2), and serine proteinase (SP), residing in nonstructural proteins (nsps) 1α, nsp1β, nsp2, and nsp4, respectively. The proteolytic processing of pp1a and pp1ab is reviewed elsewhere (Fang and Snijder, 2010; Snijder et al., 2013). PLP1α, PLP1β, and PLP2 generate nsp1α, nsp1β, and nsp2 from pp1a, respectively, and SP in nsp4 generates nsp3 through nsp14. Thus, a total of 14 nsps are generated from pp1a and pp1b by autoproteolytic processing. Two additional nsps have newly been identified within nsp2, and nsp2TF and nsp2N are generated by -1/-2 frameshifting (Fang et al., 2012b). The remaining 25% of the viral genome codes for nine structural proteins; seven membrane proteins (GP2a, GP2b[E], GP3, GP4, GP5a, GP5, and M) and nucleocapsid (N) protein.

2. Molecular and cellular mechanisms for PRRSV pathogenesis

2.1. Suppression of Type I interferon response

Innate immunity is the first line of defense of host against infections, and type I interferons (IFNs-α/β) are the most potent component of the innate immunity against invading viruses (Schneider et al., 2014). IFNs-α/β are produced via the intricate signaling process in cells, and once produced, they are released from the cells and bind to their receptors on the same cell (autocrine) or neighbor cells (paracrine). The binding of IFNs to their receptors phosphorylates Janus kinase 1 (JAK1) and

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tyrosine kinase 2 (Tyk2) and activates signal transducers and activator of transcription-1 (STAT1) and STAT2, which recruits interferon response factor 9 (IRF9) to form an IFN-stimulated gene factor 3 (ISGF3) complex. ISGF3 enters the nucleus and binds to IFN-stimulated regulatory response elements (ISRE) of IFN-stimulated genes (ISGs) for expression, and ISGs are the major executors to establish an antiviral status of cells and to regulate other cellular functions (Ivashkiv and Donlin, 2014; Schneider et al., 2014).

PRRSV is sensitive to IFNs-α/β (Overend et al., 2007), and PRRSV-mediated suppression of IFNs is documented in cells and pigs (Albina et al., 1998; Calzada-Nova et al., 2011), suggesting that the virus has evolved to disarm the innate immunity of the host. The suppression of type I IFNs is a tremendous benefit for the virus to survive during infection (García-Sastre, 2017). Six proteins of PRRSV have been identified so far as IFN antagonists and, thus the IFN suppression by PRRSV is polygenic; nsp1α, nsp1β, nsp2, nsp4, nsp11, and N (Sun et al., 2012b; Yoo et al., 2010).

nsp1α: PRRSV nsp1 is cleaved to two subunits, nsp1α and nsp1β, and both subunits localize in the nucleus, implicating their potential for cell function modulation. Indeed, PRRSV nsp1 is a potent IFN antagonist (Chen et al., 2010) and also inhibits the NF-κB promoter activation (Song et al., 2010). Nsp1α degrades CREB-binding protein (CBP) in the nucleus and blocks the recruitment of CBP during the assembly of IFN enhanceosome (Han et al., 2013; Kim et al., 2010), leading to suppression of IFN gene expression. The CBP degradation by nsp1α is proteasome-dependent, suggesting involvement of the ubiquitination pathway. Since CBP degradation is a nuclear event, nsp1α is suggested to localize in the nucleus. However, nsp1α does not possess a specific nuclear localization signal (NLS), and thus, nsp1α may bind to other nuclear protein for nuclear transport. Nsp1α however contains the classical nuclear export signal (NES), and the nsp1α nuclear export is mediated by CRM-1 (Chromosomal maintenance 1, also known as Exportin 1). Further studies show that nsp1α shuttles between the nucleus and cytoplasm, and the nuclear export of NSP1α is necessary for its ability for IFN inhibition (Chen et al., 2016). Interestingly, nsp1α binds to swine leukocyte antigen class I (SLA-I) and mediates the SLA-I degradation in a ubiquitin-proteasome-dependent manner (Du et al., 2016a). It is thus tempting to speculate that nsp1α may have an ubiquitin E3 ligase activity.

nsp1β: As with PRRSV nsp1α, nsp1β is also a nuclear protein. PRRSV nsp1β inhibits the phosphorylation and nuclear translocation of interferon regulatory factor 3 (IRF3) to result in IFN suppression (Beura et al., 2010). PRRSV nsp1β degrades karyopherin-α1 and blocks the nuclear translocation of ISGF3, thereby suppressing ISGs expressions (Wang et al., 2013b). A recent study shows that PRRSV nsp1β disintegrates the nuclear pore complex (NPC) of virus-infected cells and blocks host mRNA nuclear export to the cytoplasm (Han et al., 2017). Subsequently, nucleoporin 62 (Nup62) was identified as the cellular protein binding to nsp1β, and the SAP domain in nsp1β was determined as the region for Nup62 binding (Ke et al., 2019a). A SAP mutant PRRSV generated by reverse genetics replicates at a slower rate, and its titer is also lower than wild-type virus. Notably, the production of IFNs-α/β, ISGs, IFIT-1 (IFN-induced proteins with tetratricopeptide repeats 1, IFIT-2, and IRF3 were decreased in nsp1β-mutant overexpressing-cells or Nup62-knockdown cells. This mutant virus was IFN-suppression-negative and host mRNA nuclear retention-negative, and in pigs, reduced the clinical severity and increased antibody responses (Ke et al., 2018). Evidently, PRRSV has evolved to adopt various strategies for immune evasion and enhanced replication during infection.

nsp2: PRRSV nsp2 is a papain-like proteinase, and this activity belongs to the ovarian tumor (OTU) protease family that contains the deubiquitination (DUB) and de-ISGylation activities (Frias-Staheli et al., 2007). Ubiquitination and ISGylation are important post-translational modifications involved in the retinoic acid-inducible gene I (RIG-I) and toll-like receptors (TLRs) signaling pathways, and the OTU domain interferes these pathways to antagonize type I IFN production (Makarova et al., 2000). The DUB activity of nsp2 OTU interferes with the polyubiquitination process of IκBα and prevents IκBα degradation, leading to inhibition of NF-κB activation. Nsp2 inhibits IFNβ production by suppressing the activation of the IRF-3 and NF-κB signaling (Li et al., 2010; Sun et al., 2010). Contradictory data are available that nsp2 of highly pathogenic (HP)-PRRSV activates NF-κB in cells by inducing IκBα degradation and p65 translocation to the nucleus, and thus promotes the expression of IL-6, IL-10, and COX-2. The hypervariable region of nsp2 was essential for NF-κB activation (Fang et al., 2012a). This finding was supported by Huang et al (Huang et al., 2014) that nsp2 of HP-PRRSV had no inhibitory effect on IFNβ production. The nsp2 function for the innate immune modulation needs to be clarified.

nsp4: PRRSV nsp4 is a serine proteinase (SP) and inhibits IFNβ promoter activity (Beura et al., 2010). Nsp4 did not hinder NF-kB phosphorylation and p65 nuclear translocation but antagonize IFNβ transcription in the nucleus (Chen et al., 2014b). The inhibition depends on the SP activity in nsp4. Studies also show that nsp4 cleaves NF-κB essential modulator (NEMO) at a single site (glutamate 349) and thus suppresses NF-κB activation and IFNβ production (Huang et al., 2014). However, a recent study showed that scission of NEMO by nsp4 occurred at two additional sites (glutamates 166 and 171) (Chen et al., 2019). This study further showed the NEMO fragment of residues 1-349 could activate IFN-β transcription more robustly than full-length NEMO, whereas all other NEMO cleavage products were abrogated for the IFN-β-inducing capacity. Thus, NEMO cleavage at E349 alone may not be sufficient to completely inactivate the IFN response via this signaling adaptor. These findings suggest that the multiple-site NEMO-cleavage by nsp4 is critical for disarming the innate immune response for viral survival. Additionally, nsp4 suppresses IFNβ transcription by blocking NF-κB activation (Chen et al., 2014b). PRRSV nsp4 of different pathogenic strains has a different ability to inhibit IFNβ expression. HP-PRRSV nsp4 has greater inhibitory effect on IFNβ than nsp4 of less pathogenic strains. As discussed above, nsp1α binds to and degrades SLA-I (Du et al., 2016a), A recent study supports this finding that HP-PRRSV nsp4 also downregulates the cellular level of β2-microglobulin (β2 M) (Qi et al., 2017), which forms a heterotrimeric complex with the SLA-I heavy chain and plays a critica1 role in SLA-I antigen presentation. PRRSV nsp4 binds to the B2M promoter and suppresses β2 M transcription. These findings imply that HP-PRRSV may modulate the SLA-I antigen presentation pathway, suggesting that the IFN inhibition by nsp4 is correlated with PRRSV pathogenesis.

nsp11: PRRSV nsp11 is a nidovirus-specific endoribonuclease (Nedialkova et al., 2009). The expression of nsp11 inhibits the IFNβ promoter and IRF3-dependent promoter activities, and the ribonuclease activity in nsp11 is essential for this inhibition (Shi et al., 2011). nsp11 is also able to inhibit the NF-κB promoter activation (Beura et al., 2010; Sun et al., 2016). In addition to its role for inhibition of IFN production, nsp11 participates in the IFN signaling pathway. Nsp11 induces STAT2 degradation and inhibits the production of ISGs (Yang et al., 2019). Independent from this study, nsp11 has been shown to interacts with IRF9 and impairs the formation of ISGF3 (IFN-stimulated gene factor 3) (Wang et al., 2019b), indicating the antagonism of IFN signaling by nsp11. This antagonism is independent from the NedoU activity of nsp11.

N: The PRRSV nucleocapsid (N) protein is a small basic protein of 123 amino acids. N has an RNA-binding domain and a homodimerization domain (Wootton and Yoo, 2003). As with nsp1α and nsp1β, N localizes in the nucleus in addition to its cytoplasmic distribution (Rowland et al., 1999), suggesting a nuclear role of N for modulation of host cell process. Indeed, the ability of N to suppress type I IFN induction has been shown. N inhibits IRF3 phosphorylation and nuclear translocation, and suppresses IFNβ induction (Sagong and Lee, 2011). Recently, tripartite motif protein 25 (TRIM25) has been identified as an inhibitor of PRRSV replication, and the viral N protein interferes with TRIM25-RIG-I interactions by competitive interaction with TRIM25 (Zhao et al., 2019). The N protein inhibits TRIM25

expression and TRIM25-mediated RIG-I ubiquitination to suppress IFN-β production. This study shows that TRIM25 inhibits PRRSV replication and that the N protein antagonizes the antiviral activity by interfering with TRIM25-mediated RIG-I ubiquitination, further supporting that PRRSV N inhibits innate immune responses of the host. It is unknown whether the nuclear localization of N is related to its ability for IFN modulation.

2.2. Modulation of apoptosis

Apoptosis is a programmed cell death in which two major pathways participate; the mitochondrial (intrinsic) pathway and the death receptor (extrinsic) pathway. The mitochondrial pathway is regulated by the Bcl-2 family proteins and is characterized by the disruption of mitochondrial membrane potential (MMP) and the release of cytochrome C for caspase-9 activation. The death receptor pathway is mediated by tumor necrosis factor receptor 1 (TNFR1)/Fas ligand (L) and is characterized by the formation of a death-inducing signaling complex for activation of caspase-8 and caspase-10. Other upstream regulators such as JNK (c-Jun N-terminal kinase), UPR (unfolded protein response), oxidative stress, p53, and autophagy also contribute to apoptosis (Fan, 2019).

PRRSV has been documented to induce apoptosis in cells and pigs (Costers et al., 2008; Labarque et al., 2003; Suárez et al., 1996; Sur et al., 1997). Contradictory reports are also available that PRRSV inhibits apoptosis (Huo et al., 2013; Ni et al., 2015; Wang et al., 2016). It is unclear whether such a discrepancy reflects the nature of PRRSV, for example depending on the kinetics of the viral replication, or due to different experimental conditions. Many viruses possess dual functions for apoptosis, proapoptotic function and antiapoptotic function, and further studies are needed for clarification.

The induction of apoptosis is one of the cellular mechanisms that contribute to PRRSV pathogenesis (Karniychuk et al., 2011; Novakovic et al., 2017; Suárez, 2000). PRRSV upregulates the proapoptotic protein Bax expression and causes cytochrome C release and caspase-9 activation (Lee and Kleiboeker, 2007). In addition, the decrease of antiapoptotic protein and the increase of proapoptotic protein make an additional contribution to PRRSV-induced mitochondrial apoptosis (Yin et al., 2012; Yuan et al., 2016). PRRSV also increases TNFR1/FasL, indicating the contribution of the death receptor pathway to apoptosis. Thus, a crosstalk seems to occur between the extrinsic and intrinsic pathways for PRRSV-mediated apoptosis. GP5 was initially suggested as the viral protein for inducing PRRSV apoptosis (Suárez et al., 1996), but it was not possible to confirm that finding (Lee et al., 2004), and instead, nsp4 and nsp10 were recently determined as the PRRSV apoptotic proteins (Yuan et al., 2016).

The mitogen-activated protein kinase (MAPK) pathways regulate cell proliferation, differentiation, and apoptosis (Yang et al., 2013). Three major MAPK pathways are described; ERK1/2 (extracellular signal-regulated kinase 1 and 2), JNK, and p38. The activation of MAPKs is a common event in response to viral infection (Fung and Liu, 2017; Nacken et al., 2014; Wei et al., 2009). During PRRSV infection, JNK is activated (Huo et al., 2013; Jing et al., 2014; Lee and Lee, 2012; Liu et al., 2017b; Yin et al., 2012; Yuan et al., 2016), which is attributed to ROS generation and endoplasmic reticulum (ER) stress (Huo et al., 2013; Yin et al., 2012). The JNK activation by PRRSV also contributes to virus-induced cytokine production (Lee and Lee, 2012; Liu et al., 2017b). Changes in redox balance during viral infection are linked to viral pathogenesis (Lee, 2018), and an oxidative stress is induced by PRRSV in both cells and pigs (Yan et al., 2017, 2015). The increased ROS generation by PRRSV likely attributes to the elevated inducible nitric oxide synthase (iNOS), which is associated with the changes of heat shock protein 90 and caveolin-1 expression. Inhibition of ROS generation by antioxidant protects cells from PRRSV-induced apoptosis through suppressing JNK activation (Yin et al., 2012). In addition, decreased glutathione peroxidase is observed in PRRSV-infected pigs (Štukelj et al., 2013), suggesting that inhibition of antioxidant enzyme activity may also contribute to oxidative stress by PRRSV infection.

p53 is a transcription factor that regulates apoptosis, cell cycle, and DNA repair, and its activation triggers apoptosis. p53 can activate the proapoptosis pathway in the nucleus (Schuler and Green, 2001) or can translocate to the mitochondria and activate the mitochondrial pathway by forming complexes with Bcl-2 family proteins (Mihara et al., 2003). p53 can also directly trigger Bax activation and induce apoptosis (Chipuk, 2004). Recent evidence suggests that p53 may also suppress apoptosis (Kruiswijk et al., 2015) either by inhibiting proapoptotic JNK activation (Huo et al., 2017), inducing prosurvival by p21 upregulation (Garner and Raj, 2008), or counteracting ROS-mediated apoptosis (Borrás et al., 2011). During PRRSV infection, p53 is activated and inhibits JNK-mediated apoptotic signaling and thus protects cells from apoptosis (Huo et al., 2013).

The PI3K/Akt pathway regulates cell growth, proliferation, differentiation, transcription, translation, and apoptosis (Vivanco and Sawyers, 2002). The downstream target for PI3K is the AKT kinase that exerts the antiapoptotic effects by either phosphorylating some proapoptotic Bcl-2 family proteins or activating transcription factors to upregulate antiapoptotic genes. For PRRSV, the PI3K/Akt pathway is activated at an early stage of infection (Huo et al., 2013; Ni et al., 2015; Pujhari et al., 2014; Wang et al., 2016; Wang et al., 2014a; Zhang and Wang, 2010; Zhu et al., 2013). The Akt activation by PRRSV leads to negative regulation of the JNK pathway (Huo et al., 2013) and inhibits host cell apoptosis early in infection through inhibitory phosphorylation of pro-apoptotic protein Bad (Zhu et al., 2013). Both FAK (Ni et al., 2015) and EGFR (Wang et al., 2016) are induced by PRRSV, which in turn contributes to PI3K/Akt activation. Thus, the activation of PI3K/Akt pathway inhibits PRRSV-induced apoptosis and facilitates viral replication.

2.3. MicroRNAs

MicroRNAs (miRNAs) represent small endogenous non-coding RNAs of 21-23 nucleotides in length that regulate gene expression post-transcriptionally through degradation of mRNAs or inhibition of translation by binding to mRNAs (Krol et al., 2010). Thus, miRNAs are important gene regulators for differentiation, developments, cellular proliferation and death, immune responses, and microbial infections. During viral infection, miRNAs can affect viral replication by regulating host immune responses to generate a less favorable environment for invading viruses. In turn, many viruses have evolved to produce miRNAs that can act as a negative regulator for immune response and to promote viral replication. The number of known pig miRNA genes is relatively low when compared to human and mouse genomes (877 for pig vs. 4272 for human and 2009 for mouse, ver. Ensembl release 77; https://m.ensembl.org/index.html), which is probably due to incomplete swine genome sequence and its annotation. In one study using sows, endometrial samples were collected from pregnant animals and the expression profiles of miRNAs were examined. A total of 288 miRNAs were identified in the pig endometrium (Hong et al., 2019). Studies have been conducted to determine the host cell-PRRSV interaction networks, and numerous species of miRNAs appear to be altered (Calcatera et al., 2018; Dhorne-Pollet et al., 2019; Hicks et al., 2013; Li et al., 2018; Zhang et al., 2019a), suggesting that host miRNAs indeed modulate PRRSV replication, either positively or negatively. miR-181, miR-23, miR-30c, and miR-24-3p are reported to play important roles in PRRSV replication and in modulating host antiviral responses, while other such as miR-204, miR-221, and miR-219 need more study to understand for their functions (Liu et al., 2017a). Some of the miRNAs are discussed below for their role in during PRRSV infection. At the time of writing this review, it is somewhat difficult to translate the differential expression of miRNA for meaningful implications for PRRS pathogenesis, and thus it is prepared as a systematic review with limited critical analyses.

2.3.1. miRNAs suppressing PRRSV replication

miRNA-10a: The deep sequencing of small RNAs shows an increased expression of miR-10a as the result of PRRSV infection. Overexpression of miR-10a-5p markedly reduces the expression of PRRSV N mRNA and thus N protein, and the viral titers are also reduced, demonstrating the suppression of PRRSV replication by miR-10a-5p. miR-10a-5p directly binds to the mRNA of signal recognition particle 14 (SRP14). miR-10a-5p targets the 3'-UTR of pig SRP14 mRNA in a sequence-specific manner and decreases SRP14 expression through translational inhibition (Zhao et al., 2017). Another study using a deep sequencing approach has identified six species of miRNAs in different abundance (miR-21, miR-26a, miR-140-3p, miR-185, miR-199a, and miR-505) during PRRSV infection. The copy number of endogenous miRNAs and the extent of sRNA complementarity are key factors to silence potential mRNA expression and translation, and thereby determining PRRSV viability. miR-140 induces strong suppression of viral replication.

miRNA-23 and miRNA26a: miR-23 is induced by dsRNA analogs. miR-23 promotes type I IFNs expression and suppresses viral replication. PRRSV suppresses the expression of miR-23, implying that this antagonism may be a strategy of PRRSV to evade the miRNA-mediated immune responses induced in the TLR3 pathway (Zhang et al., 2014). Similarly, miR-26a induces innate anti-viral responses, including the IFN signaling pathway and the ISG expressions, and as a result, inhibits PRRSV replication and causes the remission of the cytopathic effect in MARC-145 cells (Jia et al., 2015).

miRNA-24-3p: Heme oxygenase-1 (HO-1) is a cytoprotective enzyme. Overexpression of HO-1 inhibits PRRSV replication, and in turn, PRRSV downregulates the HO-1 expression (Xiao et al., 2015). The HO-1 mRNA and miR-24-3p interact directly, which significantly decreases the HO-1 protein production (Li et al., 2016a). PRRSV infection induces miR-24-3p expression, and collectively, miR-24-3p promotes PRRSV replication through suppression of HO-1 expression, suggesting a potential as an antiviral drug against PRRSV infection.

miRNA-c89: PRRSV infection also markedly upregulates miR-c89 expression. Both HP-PRRSV and low-pathogenic (LP)-PRRSV induce miR-c89 expression (Zhang et al., 2019c). The overexpression of miR-c89 significantly suppresses the replication of different PRRSV strains. miR-c89 targets the 3'-UTR of porcine retinoid X receptor β (RXRB) mRNA in a sequence-specific manner. Knockdown of RXRB mRNA using siRNA can suppress the replication of a variety of PRRSV strains, suggesting the potential of miR-c89 as a new antiviral drug to control PRRSV infection.

miRNA-125b: In attempts to identify and develop potential anti-PRRSV drugs for PRRS, 10 highly conserved miRNAs involved in innate immunity and antiviral function were screened, and miR-125b was identified as an inhibitor of PRRSV replication (Wang et al., 2013a). miR-125b reduces PRRSV replication and viral gene expression in a dose-dependent manner in both MARC-145 and PAMs cells. miR-125b does not target the PRRSV genome, but rather, inhibits the NF-κB activation. PRRSV, in turn, down-regulates miR-125b expression and promotes viral replication. Collectively, miR-125b is an antiviral host factor against PRRSV, which is an additional example of viral strategies to manipulate cellular miRNA for viral propagation. PRRSV down-regulates miR-125b, and miRNA-125b decreases NF-κB expression by stabilizing κB-RAS2, which is a negative regulator of NF-κB, and thus inhibits PRRSV replication (Wang et al., 2013a).

miRNA-130: The delivery of multiple miR-130 family mimics, especially miR-130b, results in inhibition of PRRSV replication in vitro (Li et al., 2015b). miR-130 is effective in inhibiting the replication of multiple PRRSV-2 strains, but not the replication of classical PRRSV-1. The overexpression of miR-130 does not induce IFN-α or TNF-α expression in PRRSV-infected PAMs. miR-130 directly targets the 5' UTR of the PRRSV genome. Intranasal inoculation of piglets with miR-130b has exhibited antiviral activity in vivo and partially protected piglets from a lethal challenge with HP-PRRSV, demonstrating the importance of the miR-130 family in inhibiting PRRSV replication.

miRNA-181 and miRNA-206: miR-181 is a positive regulator of immune response, and miR-181 can directly impair PRRSV infection. miR-181 strongly inhibits PRRSV replication in vitro by specific binding to a highly (over 96%) conserved region in the downstream of ORF4 of the viral genome (Gao et al., 2013). The inhibition of PRRSV replication is specific and dose dependent. The interactions between miR-181 and PRRSV mRNAs are specific. miR-181 and other PRRSV-targeting miRNAs, such as miR-206, are expressed much more abundantly in minimally permissive cells and tissues than in highly permissive cells and tissues. HP-PRRSV-infected pigs treated with miR-181 mimics show a substantially decrease in viral loads in blood and relief from fever compared to controls (Guo et al., 2013), indicating the important role of host miRNAs in modulating PRRSV infection and viral pathogenesis, which also supports the idea that host miRNAs may be useful for RNA interference (RNAi)-mediated antiviral therapeutic strategies.

miRNA-506: miR-506 can target porcine CD151 3'-UTR mRNA, and overexpression of miR-506 significantly decreases CD151 expression at both mRNA and protein levels (Wu et al., 2014). Furthermore, overexpression of miR-506 reduces PRRSV replication in MARC-145 cells.

miRNA-let-7f-5p: Non-muscle myosin heavy chain 9 (MYH9) is an essential factor for PRRSV infection, and MYH9 expression is regulated by miR-let-7f-5p by binding to the MYH9 mRNA 3'-UTR. The expression pattern has been examined for both miR-let-7f-5p and MYH9 in PAMs after infection with either HP-PRRSV or LP-PRRSV. The results show that both types of PRRSV reduce the let-7f-5p expression and thus MYH9 expression (Li et al., 2016a). miR-let-7f-5p significantly inhibits PRRSV replication through suppression of MYH9 expression.

Other miRNAs: miR-23, miR-378, and miR-505 have also been claimed as antiviral host factors against PRRSV. Over-expression of these miRNAs inhibits PRRSV infection, and the blockage of these endogenous miRNAs significantly enhances PRRSV replication. Different strains of PRRSV-2 harbor conserved miR-23, miR-378, and miR-505 target sites to confer miRNA-mediated repression of PRRSV replication. Of these, miR-23 was capable of inducing type I IFN expression during PRRSV infection through IRF3/IRF7 activation, which further leads to the inhibition of PRRSV infection.

2.3.2. miRNAs promoting PRRSV replication

miRNA-29: AKT3 is the target of miR-29a, and the role of miR-29 during PRRSV replication has been investigated (Zhou et al., 2016b). In PRRSV-infected PAMs, miR-29a/b-1 expression increases significantly after 6 h with the peak around 24 h. The overexpression of miR-29a reduces AKT3 expression significantly (Zhou et al., 2016b), indicating that miR-29a can target AKT3 and promote PRRSV replication during early stage of infection in cells.

miRNA-30: PRRSV upregulates miR-30c by activating NF-κB and facilitates its ability to infect pigs (Zhang et al., 2016). In that study, miR-30c was found a potent negative regulator of IFN-I signaling by targeting JAK1, resulting in the enhancement of PRRSV infection. The JAK1 expression was significantly decreased by PRRSV. Importantly, miR-30c was also upregulated by PRRSV infection in vivo, and miR-30c expression correlated positively with the viral loads in lungs and PAMs of infected pigs. miR-30c also targets IFNs-α/β receptor beta-chain (IFNAR2) and downregulates IFNAR2 expression. miR-30c and IFNAR2 mRNA co-localize in RNA-induced silencing complex (RISC), and by targeting IFNAR2, miR-30c impairs the production of ISGs and promotes PRRSV replication (Liu et al., 2018). These findings add a new strategy of PRRSV to evasion of IFN-mediated antiviral immunity by engaging miR-30c.

miRNA-146a and miRNA-373: miR-146a is also up-regulated during PRRSV infection (Chen et al., 2017a). Since miR-146a is a negative regulator of immune responses, the up-regulation of miR-146a may facilitate PRRSV infection. PRRSV upregulates the expression of miR-373 by elevating the expression of specificity protein 1 (Sp1) in MARC-145 cells (Chen et al., 2017a). miR-373 is a negative regulator for IFN-β by targeting nuclear factor IA (NFIA), NFIB, interleukin-1 receptor-

associated kinase 1 (IRAK1), IRAK4, and interferon regulatory factor 1 (IRF1), therefore miR-373 promotes PRRSV replication. PRRSV hijacks host miR-373 and impairs the production of IFN-β. Upregulation of miR-373 expression results in promoting the replication of PRRSV.

miR-339-5p and miR-181d-5p: The transcriptome analyses have mainly concentrated on PAMs, MARC-145 cells, and on the respiratory system. In swine, apoptosis of placental cells and endometrial epithelial cells (PECs) is an obvious sign linked to reproductive failure in pregnant animals, and thus, the whole transcriptome was examined in PRRSV-infected PECs (Zhang et al., 2019a). 54 differentially expressing miRNAs, 104 differentially expressing genes, 22 differentially expressing lncRNAs, and 109 isoforms were obtained, which was mainly enriched in apoptosis, necroptosis, and p53 signal pathways. Integration analysis revealed miR-339-5p and miR-181d-5p participate in apoptosis, and subsequently, pathway enrichment and functional analysis revealed that miR-339-5p played a role in regulating apoptosis of PECs after PRRSV inoculation (Zhang et al., 2019a).

2.4. Inflammatory cytokine responses

Virus-associated molecules, such as genomic RNA, DNA, or double-stranded RNA (dsRNA) that are produced in cells during infection, can be recognized by host pattern-recognition receptors (PRRs), which initiates effective and appropriate antiviral responses including production of a variety of cytokines and induction of inflammatory responses (Kawai and Akira, 2006). PRRSV infection in swine causes severe interstitial pneumonia (Morgan et al., 2016; Rossow, 1998), indicating that the inflammatory response plays an important role in infection and pathogenesis of PRRSV (van Reeth et al., 1999). Previous studies show that the expressions of IL-1β, IL-8 and TNF-α are significantly elevated in virulent PRRSV-infected swine (Thanawongnuwech et al., 2004). Similarly, HP-PRRSV generates high levels of inflammatory cytokines including IL-1, IL-6 and TNF-α in peripheral blood (Li et al., 2017), indicating that HP-PRRSV may aggravate inflammation and damage tissues and organs. In addition, in pregnant gilts that are challenged on 85 days of gestation and euthanized 21 days postinfection, cytokine gene expressions are significantly upregulated in the thymus and spleen of the fetuses (Pasternak et al., 2020). PRRSV also upregulates cytokine in PAMs (Qiao et al., 2011) and microglia (Chen et al., 2014a).

Nuclear factor-kappaB: NF-κB is an inducible transcription factor that plays a key role in inflammation and immune responses as well as in regulation of cell proliferation and survival. The inflammation in the lungs by PRRSV stimulates the recruitment of monocytes and neutrophils to affected sites, and the recruitment of fresh target cells provides favorable conditions to PRRSV for replication. PRRSV activates NF-kB during infection (Lee and Kleiboeker, 2005), and N and nsp2 have been reported as the activators, whereas nsp1α, nsp1β, nsp2, nsp4, and nsp11 are known as the suppressors. The dual roles of PRRSV for NF-κB regulation make the viral pathogenesis complicated. It seems that the suppression of NF-κB leads to the suppression of type I IFN response (see the section "2.1 Suppression of Type 1 interferon response"), and the activation of NF-κB results in the production of proinflammatory cytokines. While the bases for NF-κB suppression and IFN inhibition by PRRSV are well characterized, mechanisms for NF-κB activation is poorly studied. Ke et al (2019b) recently described a molecular basis for NF-κB activation by PRRSV. In that study, PIAS1 (protein inhibitor of activated STAT1) was identified as the cellular protein binding to N of PRRSV. Since PIAS1 can function as a repressor for NF-κB, binding of N to PIAS1 releases the p65 subunit of NF-κB. As a result, NF-κB is activated and triggers the expression of proinflammatory cytokines.

NOD-like receptor protein 3 (NLRP3): NLRP3 is an intracellular sensor that detects a broad range of microbial motifs, endogenous danger signals, and environmental irritants, resulting in the formation and activation of the NLRP3 inflammasome. The NLRP3 inflammasome is comprised of NLRP3, apoptosis-associated speck-like protein (ASC) containing a caspase activation and recruitment domain (CARD), and caspase-1 (Jacobs and Damania, 2012). Assembly of the NLRP3 inflammasome leads to caspase 1-dependent release of the proinflammatory cytokines IL-1β and IL-18 (Swanson et al., 2019). Activation of the NLRP3 inflammasome requires two steps. The first step is the priming in the expression of pro-IL-1β through the stimulation of NF-κB in response to microbial molecules, and the second step is the assembly and activation of the NLRP3 inflammasome (Jacobs and Damania, 2012). PRRSV can activate the inflammasomes in PAMs, and PRRSV induces IL-1β production dependent on TLR4/MyD88/NF-κB signaling pathway and the NLRP3 inflammasome (Bi et al., 2014). Viral RNA can be sensed by cytosolic RNA sensor DDX19A to activate NLRP3 inflammasome (Li et al., 2015a). Viral proteins can also regulate NLRP3 inflammation. The E protein of PRRSV has been shown to increase the IL-1β release from LPS-primed PAMs (Zhang et al., 2013), while PRRSV nsp11 inhibits the secretion of IL-1β in PAMs. The endoribonuclease activity of nsp11 is essential for inhibition of IL-1β production (Wang et al., 2015a) and NLRP3 inflammasome in microglia (Chen et al., 2018).

Interleukin-10: IL-10 is a crucial immunoregulatory cytokine that can inhibit the production of inflammatory cytokines and counteract adaptive immunity (Moore et al., 2001). PRRSV induces IL-10 expression in vivo and in vitro (Flores-Mendoza et al., 2008; Singleton et al., 2018; Suradhat et al., 2003), and this induction depends on NF-κB activation and p38 MAPK signaling in PAMs (Hou et al., 2012b; Song et al., 2013). By screening PRRSV structural and nonstructural proteins, GP5 was identified as an IL-10 inducer. The overexpression of GP5 induces the phosphorylation of p38 (Hou et al., 2012b), and this finding was confirmed by other investigator (Song et al., 2013). Other studies report that PRRSV N protein induces IL-10 expression in monocyte-derived dendritic cells (MoDCs) and peripheral blood mononuclear cells (PBMC) (Wongyanin et al., 2012). Besides, the integrity of N is essential for IL-10 upregulation (Yu et al., 2017). Inoculation of pigs with a recombinant adenovirus expressing nsp1 has increased IL-10 secretion and decreased IFN-γ secretion in pigs, suggesting nsp1 as an IL-10 inducer in vivo (Zhou et al., 2012). Such a study needs to be confirmed.

Interleukin-12: IL-12 is a heterodimeric cytokine that regulates NK cells, T cells secreting IFN-γ, and Th1 cells in response to viral infection (Gautier et al., 2005). Bone marrow-derived DCs produce IL-12, and this activity is tightly regulated by type I IFNs (Gautier et al., 2005). IL-12p40 is a subunit of IL-12, and PRRSV infection induces IL-12p40 production in cells and pigs. The induction of IL-12p40 is dependent on the JNK-AP-1 and NF-κB signaling pathways (Yu et al., 2016).

Interleukin-15: The role of IL-15 against viral infections is well documented. The antiviral activity of IL-15 is primarily mediated via the activation of NK cells and NKT cells (Ahmad et al., 2000). During PRRSV infection in pigs, a strong influx of NK cells and cytotoxic T cells occurs in the lungs (Lamontagne et al., 2003). In blood monocyte-derived macrophages, PAMs, and MoDCs, PRRSV upregulates IL-15 production at both the mRNA and protein levels, and the PRRSV-induced IL-15 production is likely through the N-mediated NF-κB activation (Fu et al., 2012) through the TRIF/MAVS-PKCβ1-TAK1-NF-κB signaling pathway (Du et al., 2016b).

Interleukin-17: IL-17 is a proinflammatory cytokine associated with strong inflammation. A recent study shows that HP-PRRSV induced IL-17 expression, and the PI3K and p38MAPK signaling pathways were essential for IL-17 production. The IL-17 expression was dependent on C/EBPβ and CREB binding motif in IL-17 promoter (Wang et al., 2019c). PRRSV nsp11 was the viral protein for IL-17 induction, and Ser74 and Phe76 in nsp11 was indispensable. IL-17 production by nsp11 was also dependent on the PI3K-p38MAPK-C/EBPβ/CREB pathways. A PI3K inhibitor impaired IL-17 production and alleviated lung inflammation caused by HP-PRRSV infection in pigs, indicating higher IL-17 level induced by HP-PRRSV might be associated with severe lung inflammation and pneumonia (Wang et al., 2019c).

Tumor necrosis factor-α: TNF-α is a proinflammatory cytokine

secreted by a wide range of cells including macrophages and activated T cells. TNF-α promotes an antiviral state in uninfected cells, recruitments of lymphocytes to infection sites, cytolysis of virus-infected cells, and modulation of apoptosis and cell survival (Natoli et al., 1998; Smith et al., 1994; Toews, 2001). Addition of recombinant porcine TNF-α to cultures reduces PRRSV replication (López-Fuertes et al., 2000), and thus PRRSV may have evolved to adopt an anti-TNF-α strategy. PRRSV nsp1 is involved in the down-regulation of TNF-α production. Both nsp1α and nsp1β subunits of nsp1 inhibit the TNF-α promoter activation by modulating the NF-κB and Sp1 promoter activities (Subramaniam et al., 2010). HP-PRRSV and low pathogenic-PRRSV exhibit differential TNF-α expressions in PAMs. Hou et al (2012a) also reveals that HP-PRRSV impairs TLR4- and TLR3-stimulated TNF-α release through altered ERK regulation. HP-PRRSV infection induces lower TNF-α production in PAMs, compared with low pathogenic-PRRSV, which may partially contribute to the pathogenesis of HP-PRRSV. The suppression of TNF-α by HP-PRRSV was mediated through inhibiting the ERK signaling pathway, and nsp1β and nsp11 were responsible for the inhibitory effect on TNF-α production induced by HP-PRRSV and the differential TNF-α production in PAMs (He et al., 2015; Hou et al., 2012a).

STAT3: STAT3 is a pleiotropic signaling mediator of many cytokines including IL-6 and IL-10. PRRSV nsp5 has been studied for its role for cytokine responses (Yang et al., 2017). PRRSV infection leads to reduction of STAT3 protein, and nsp5 has been identified to induce the STAT3 degradation by increasing its polyubiquitination level. These results show that PRRSV antagonizes the STAT3 signaling. This study provides an insight into the PRRSV interference with the host innate and adaptive immune responses.

2.5. Other cellular pathways involved in PRRSV proliferation

PRRSV relies on host factors to complete its replication cycle, and in turn, host immune system limits viral replication and removes invading viruses. In order to survive and propagate within the cell, PRRSV sequesters itself to limit PRRs detection, suppresses innate immunity, manipulates cytokine responses, and modulates apoptosis and autophagy, which are involved in virus proliferation.

Autophagy: Autophagy is an intracellular process to degrade damaged organelles including invading viruses (Klionsky, 2018). Autophagy regulates multiple physiological processes including apoptosis (Levine and Kroemer, 2019). Autophagy can either suppress apoptosis or promote cell death depending on the context. Viral infection can either activate or inhibit autophagy in cells. PRRSV induces autophagy to promote virus replication (Chen et al., 2017a; Chen et al., 2012; Li et al., 2016b; Liu et al., 2012; Sun et al., 2012a; Wang et al., 2015b), and the inhibition of autophagosome formation using siRNA leads to decreased yield of PRRSV (Chen et al., 2012; Sun et al., 2012a), and increased apoptosis (Zhou et al., 2016a), suggesting that the autophagy induction by PRRSV promotes virus replication and protects the host cells from cell death. In MARC-145 cells, autophagy can favor PRRSV replication by postponing apoptosis through the formation of a Bad-Beclin1 complex (Zhou et al., 2016a). For PRRSV proteins, nsp3 and nsp5 are involved in formation of autophagosome (Zhang et al., 2019b). Rab11a is a small GTPases in the Ras superfamily as a main regulator of membrane trafficking is required for PRRSV-induced autophagy to promote viral replication (Wang et al., 2017).

Unfolded protein response: UPR in the ER constitutes a critical component of host innate immunity against microbial infections. The ER is an organelle for lipid synthesis, protein synthesis, folding, and maturation. Cellular disturbances such as redox imbalance can cause accumulation of misfolded or unfolded proteins, leading to activation of an UPR. The outcome of UPR is mitigation of ER stress through blocking protein translation, increasing protein folding capacity, and promoting ubiquitination-mediated misfolded protein degradation, and re-establishing the homeostasis (Wu and Kaufman, 2006). Prolonged activation of UPR may result in cell deaths. For PRRSV, two branches of UPR pathways, IRE1-XBP1 and PERK–eIF2α, are activated. The UPR induction by PRRSV contributes to apoptosis (Huo et al., 2013; Yuan et al., 2016) and deregulates cytokine production in PAMs (Chen et al., 2017b). Further studies reveal that GP2a targets GRP78 for degradation (Gao et al., 2019). Moreover, the UPR does not inhibit but instead stimulates efficient replication of PRRSV (Gao et al., 2019). In response to PRRSV infection, induction of UPR has been found not only contributing to PRRSV-induced apoptosis in host cells (Huo et al., 2013), but also involving in the regulation of viral replication and dysregulation of cytokine production in PAMs (Chen et al., 2017b).

Other host restriction factors: Host restriction factors constitute an integral part of the host's first line of defense against the viral pathogen. PRRSV counteracts the antiviral functions of IFITM1 (Interferon Induced transmembrane protein 1) and Tetherin by the interaction of the nsp3 with IFITM1 and the E protein with Tetherin (Wang et al., 2014b). The ubiquitin-specific peptidase 18 (USP18) as a host restriction factor restricts PRRSV growth through alteration of nuclear translocation of NF-κB p65 and p50 in MARC-145 cells (Xu et al., 2012). The tripartite motif (TRIM) family proteins make up the largest group of RING domain-containing E3 ligases. TRIM proteins are key components of the innate immune system as a new class of host antiviral restriction factors (Hatakeyama, 2017). TRIM59 has been identified as a potential nsp11-binding partner to inhibit PRRSV replication in vitro (Jing et al., 2019a). TRIM25 also inhibits PRRSV replication whereas the N protein antagonizes the antiviral activity by interfering with TRIM25-mediated RIG-I ubiquitination (Zhao et al., 2019). TRIM22 can also reduce PRRSV replication through interacting with N protein nuclear localization signal (Jing et al., 2019b).

3. Genomic RNA recombination

RNA recombination may occur when two different strains of PRRSV infect the same cell such that part of their genomic sequence may exchange from each other during the process of genomic RNA synthesis. RNA recombination has experimentally been proven by co-infections of cells or swine with two different strains of PRRSV (Liu et al., 2011; Yuan et al., 1999). During the recent epidemiological investigations, recombination has been detected between two wild-type PRRSV strains (Franzo et al., 2014; Kwon et al., 2019; Zhao et al., 2015), between wild-type PRRSV and MLV vaccine (Bian et al., 2017; Wang et al., 2019a; Zhou et al., 2018) or between MLV vaccine strains (Eclercy et al., 2019). Distribution of PRRSV recombinants is wide geographically, including Asia, North America, and Europe. Recombination events are found within each genogroup of PRRSV-1 and PRRSV-2, but no recombination has been reported between two genogroups so far. Resulting recombinants contribute to genetic variations and evolution of PRRSV. Some of the PRRSV recombinants exhibit enhanced pathogenicity (Kwon et al., 2019; Zhang et al., 2017; Zhao et al., 2015). PRRSV recombinants may reduce protective efficacies of vaccines and also bring a difficulty to epidemiologic monitoring.

The recombination breakpoints seem to be diffusely distributed in the genome-wide range for each of different PRRSV recombinants. Putative recombination “hotspots” have been investigated by comparing 10 different PRRSV strains (Kapur et al., 1996), and intragenic recombination or gene conversion has been found in ORFs 2, 3, 4, 5 and 7, but not in ORF6. Liu et al. (2011) conducted plaque-cloning of 352 isolates from pigs that were coinfected experimentally with two different strains of PRRSV and investigated recombination events by sequencing the nsp2, ORF3, and ORF5 genes from each plaque-cloned virus. Of 352 cloned viruses, 133 were recombinants, representing 38%, and among the three genes, nsp2 exhibited a higher frequency of recombination with more complicated recombination patterns. In an independent study (Yu et al., 2020), possible shared recombination regions were investigated by analyzing 355 complete genome sequences of PRRSV-2 determined during 2014–2018 in the USA and China. It

appears that high-frequency inter-lineage recombination regions locate in the nsp9 and GP2a–GP3 genes. PRRSV-2 in lineage 1 (L1) are found susceptible for recombination. The analysis of recombinants between the 1991–2013 data and the 2014–2018 data show a trend moving from complex to simple. The recombination pattern has been changed from the L8 backbone to L1 backbone during 2014–2018 in China, whereas L1 is always the major parent for PRRSV in the US (Yu et al., 2020). PRRSV nsp9 codes for RNA dependent RNA polymerase (RdRp) which copies the viral genome for replication (Fang and Snijder, 2010; Snijder et al., 2013), whereas GP2a and GP4 bind to CD163 which is the cellular receptor for PRRSV (Das et al., 2010). Thus, genome recombination in these regions may contribute to an increase in replication efficiency and cellular tropism of PRRSV. Recombination breakpoints seem to be random throughout the genome, and recombination variants may have a different ability of infection or replication. These recombination variants with increased fitness may become easier to survive in pigs and spread among pig population. However, the recombination variants with decreased fitness may be harder to infect or slower replicate in pigs. Following natural selection in vivo, the recombinant PRRSVs detected in pig farms are mainly the increased fitness that the recombination hotspots located in the regions related with viral infection and replication.

Although the genetic recombination of PRRSV is rather frequent, the underlying mechanism is still unclear. In mouse hepatitis virus, a coronavirus in the order Nidovirales, RNA recombination occurs via a copy-choice mechanism (Lai, 1992; Makino et al., 1986) in which viral replicase may jump from one template to another during RNA replication. Recombination may also be associated with following factors. Firstly, sequence compositions such as sequence homologies and secondary structures nearby the recombination breakpoints may affect the position of recombination. Secondly, recombination may be related to the discontinuous transcription of PRRSV since the leader TRS (transcription regulatory sequence) jumps to the body TRS for subgenomic RNA synthesis during the transcription process (Pasternak, 2001). Overall, the mechanism of PRRSV genome recombination remains unclear and needs to be elucidated.

4. Current challenges and future research directions

Despite the tremendous research progress made in the past three decades for PRRS, the understanding of molecular and cellular mechanisms for PRRSV pathogenesis is still limited. Furthermore, some of the research data are often contradictory to each other and difficult to interpret. For example, a large body of information has recently become available for miRNAs, and some miRNAs promote PRRSV replication while others inhibit viral replication. However, it is difficult to envision their pathogenic roles for PRRSV, and further studies are needed. In addition, several challenges and difficulties are faced with PRRS research. Firstly, suitable immunological and biochemical reagents and tools are absent for swine. The reagents and tests widely available for humans and mice are often less suitable, and cellular functions of many proteins of swine are not properly characterized. Secondly, standardized experimental animal models are absent for PRRSV. Many animal studies are conducted in commercial piglets rather than in specific pathogen-free (SPF) pigs. These commercial pigs may have been infected with other infectious agents, which may affect the response to PRRSV and thus alter experimental consequences. Thirdly, strain variations of PRRSV seem to affect the host response greatly. Different strains of PRRSV can exhibit high sequence variations even in the same subtype and show different phenotypes (Rola-Łuszczak et al., 2018). Using different strains to screen common responses may be necessary to obtain unique strategies of PRRSV. High-throughput approaches including meta-transcriptomic sequencing and single-cell sequencing have recently become available, and such technologies will provide reliable and comprehensive genetic information on host cell response to infection. Whole-genome sequencing of pigs has recently been redetermined (Choi et al., 2015; Son et al., 2020), and comprehensive pig genome annotations are available (Li et al., 2020; van Son et al., 2017v). Such genetic information will help us understand protein functions and host response to infection. With the advance of CRISPR/Cas-based genome editing, specific gene-knockout pigs can be generated rapidly. The studies show that the pigs lacking either CD163 cellular receptor for PRRSV or the scavenger receptor cysteine-rich domain 5 (SRCR5) of CD163 become resistant to both PRRSV-1 and PRRSV-2 infections (Burkard et al., 2018; Prather et al., 2017; Whitworth et al., 2016; Yang et al., 2018). Such a gene-editing technology will allow generating immune-associated gene-deletion phenotypes, and genome-modified pigs will be valuable tools to study the basis for pathogenesis. Better understanding of the modulation of host response by PRRSV in combination with reverse genetics may lead to developing new generation vaccines. Such molecular approaches will fill knowledge gaps and lead us to better understand PRRSV pathogenesis.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2020.197980.

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