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Type I interferon suppression-negative and host mRNA nuclear retention-negative mutation in nsp1β confers attenuation of porcine reproductive and respiratory syndrome virus in pigs

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) has the ability to suppress the type I interferons (IFNs-α/β) induction to facilitate its survival during infection, and the nsp1 protein of PRRSV has been identified as the potent IFN antagonist. The nsp1β subunit of nsp1 has also been shown to block the host mRNA nuclear export as one of the mechanisms to suppress host antiviral protein production. The SAP motif in nsp1β is the functional motif for both IFN suppression and host mRNA nuclear retention, and using infectious clones, two mutant viruses vL126A and vL135A have been generated. These mutants retain the infectivity, but the phenotype is negative for both IFN suppression and host mRNA nuclear retention due to the loss of the SAP motif. To examine the pathogenic role of IFN suppression in pigs, 40 piglets were allotted to four groups and each group was intramuscularly infected with vL126A, vL135A, wild-type (WT) PRRSV, and placebo. Pigs infected with vL126A or vL135A exhibited mild clinical signs with low viral titers and short duration of viremia. The levels of PRRSV-specific antibody remained comparable in all infected groups but the neutralizing antibody titers were high in vL126A-infected or vL135A-infected pigs. The IFN-α concentration was also high in pigs infected with the SAP mutants. Reversion to WT sequence was observed in the SAP motif in some animals, and the revertants regained the function to suppress IFN production and host mRNA nuclear export, indicating strong selection pressure in the SAP motif of nsp1β. Together, our data demonstrate that the IFN antagonism and host mRNA nuclear retention mediated by nsp1β contributes to viral virulence, and loss of these functions confers PRRSV attenuation.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) emerged in the United States in 1987 and almost simultaneously but independently in Europe. PRRS has since become endemic in most pig producing countries with a significant economic impact. The etiologic agent is porcine reproductive and respiratory syndrome virus (PRRSV) of the family *Arteriviridae* in the order *Nidovirales* (https://talk.ictvonline.org/taxonomy). PRRSV genome is a single-strand positive-sense RNA of 15 Kb in length with the 5′-cap and 3′-polyadenylated tail. According to their genomic similarity of approximately 60%, PRRSVs are grouped into two distinct species: PRRSV-1 and PRRSV-2. Eleven functional open reading frames (ORFs) are found in the PRRSV genome. Among them, ORF1a and ORF1b take up three-quarters of the genome in the 5′-proximity. They are coding for two large polyproteins pp1a and pp1ab of which the latter is produced by the -1 ribosomal frame-shifting mechanism. The polyproteins are further processed to generate 14 nonstructural proteins (nsps). A -2/-1 ribosomal frame-shifting has been identified in the nsp2 gene for nsp2TF and nsp2N expression (Fang et al., 2012). The remaining one-quarter in the 3′-proximity of the genome codes for eight structural proteins: E, GP2, GP3, GP4, GP5, ORF5a, M, and N proteins (Firth et al., 2011; Johnson et al., 2011; Nelsen et al., 1999; Wootton et al., 2000).

Host-virus interactions determine the infection outcome, and the innate immunity is the first line of host defense against viral infection. Type I interferons (IFNs-α/β) are the most potent antiviral cytokines produced by hosts against invading viruses (Schneider et al., 2014). Type I IFNs in pigs are comprised of at least 39 functional subtypes

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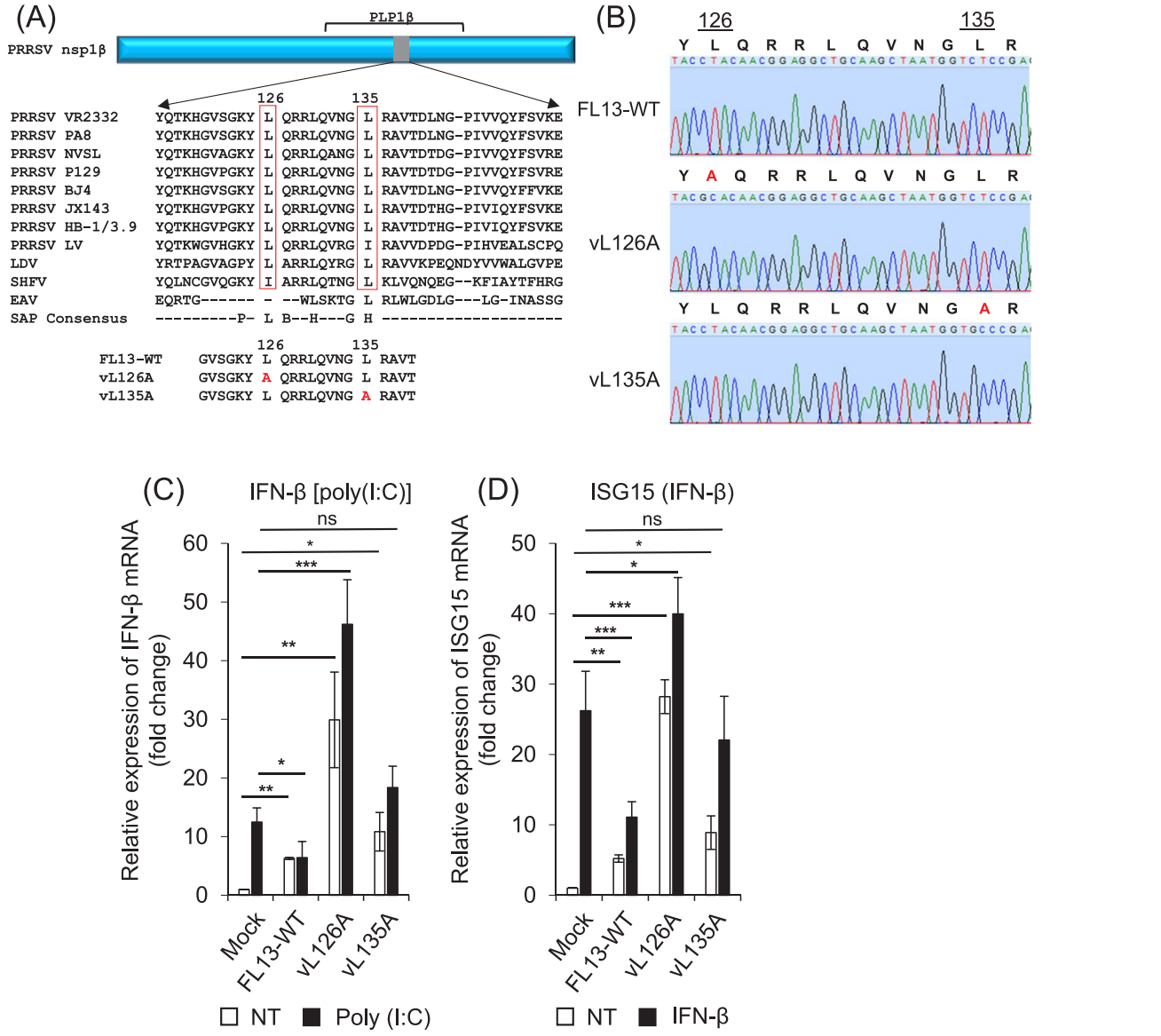

Fig. 1. (A), The SAP motif conserved in the nsp1β protein of various arteriviruses and the mutation of SAP to generate L126A and L135A. Red boxes indicate crucial amino acids at positions 126 and 135. (B), Electrophoregram of the mutated sequence. Leucine (Leu) at position 126 was mutated to alanine (Ala) by changing CTA to GCA, and Leu at 135 was mutated to Ala by changing CTC to GCC. SAP mutant viruses were generated by the reverse genetics using the FL13 PRRSV infectious clones. (C) and (D), RT-qPCR for mRNAs for IFN-β gene expression (C) and ISG15 gene expression (D). MARC-145 cells were mock-infected, or infected with FL13-WT, vL126A, or vL135A at a multiplicity of infection (MOI) of 5 for 24 h, followed by poly(I:C) stimulation (black bars in panel C) or recombinant IFN-β treatment (black bars in panel D) for 12 h. A total RNA was isolated, and RT-qPCR was performed to detect the transcripts for IFN-β or ISG15. Relative fold increases were compared to mock-infected or untreated groups, and mRNA levels were normalized using β-actin mRNA. NT, non-treated; PIC, poly(I:C)-treated. A statistical analysis was carried out by comparing the FL13-WT, vL126A, or vL135A groups with the mock-infection group, respectively. Statistical significance (*P*-value) was calculated by two-tailed Student's *t*-test. ns, not significant, *, $P < .05$; **, $P < .01$; ***, $P < .001$.

which are more than double of humans plus 16 pseudogenes (Groenen et al., 2012; Sang et al., 2010), suggesting that pigs have a more complicated IFN system than humans. Nevertheless, the induction of type I IFNs is unusually poor in PRRSV-infected cells and pigs (Albina et al., 1998; Buddaert et al., 1998; Miller et al., 2004; Van Reeth et al., 1999), suggesting that PRRSV may actively modulate type I IFNs response during infection. Since type I IFNs also play pleiotropic roles in the regulation of adaptive immunity in addition to their antiviral activity, the adaptive responses of pigs to PRRSV are also perturbed. It seems that the suppression of type I IFN production is an important strategy of PRRSV to modulate host antiviral defense and to facilitate its own replication. So far, at least six PRRSV proteins (nsp1α, nsp1β, nsp2, nsp4, nsp11, and N) have been identified as IFN antagonists, and nsp1α and nsp1β are two potent proteins (Han and Yoo, 2014; Ke and Yoo, 2017). The mechanisms for PRRSV to suppress type I IFNs production vary. The PRRSV nsp1α protein travels to the nucleus and binds to the CREB (cyclic AMP-responsive element binding)-binding protein (CBP), leading to its degradation. Thus, the IFN enhanceosome is disrupted, and IFN production is suppressed (Chen et al., 2016; Han et al., 2013; Kim et al., 2010). The PRRSV nsp1β protein also suppresses type I IFNs production (Chen et al., 2010; Han and Yoo, 2014; Patel et al., 2010).

The papain-like cysteine protease (PLP1β) domain in nsp1β is believed to play crucial roles for viral genome replication (Kroese et al., 2008), nsp2TF gene expression (Fang et al., 2012), and type I IFN suppression (Li et al., 2013, 2016). We have recently shown that the nsp1β protein blocks the host mRNAs export from the nucleus to the cytoplasm, which allows PRRSV to utilize the cellular translational machinery exclusively for viral protein synthesis and thus to promote progeny production (Han et al., 2017b). Furthermore, the nsp1β-

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