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News and Views

Joint deletion of multifunctional *MGF505-7R* and *H240R* genes generates a safe and effective African swine fever virus attenuated live vaccine candidate

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African swine fever (ASF) caused by the ASF virus (ASFV) is a highly contagious disease of pigs and wild boars. Currently, there are no safe, effective commercial vaccines available. The genome of ASFV HLJ/18 contains more than 180 genes, including a few virulence-related genes. Identifying these key virulence genes through individual deletion and subsequent pathogenicity testing in pigs is a laborious process. In addition, some ASFV genes are indispensable for viral replication and cannot be deleted. Over the past 30 years, scientists have confirmed that certain ASFV genes particularly those host antiviral innate immune responses, are associated with the virulence of ASFV strains. Although knockout of these genes does not impact viral replication, it does attenuate the virulence of ASFV in pigs. Pigs immunized with these live attenuated vaccine candidates can provide partial or complete protection. Based on these findings, we propose a new concept that ASFV immunomodulatory genes involved in type I interferon (IFN) production, IFN-JAK-STAT signaling, inflammatory responses, cell death (involved in apoptosis, necrosis, and pyroptosis), and autophagy may be related to the pathogenicity of ASFV. An unbiased screening may identify more ASFV immunomodulatory or multifunctional genes, simplifying the confirmation of ASFV virulence-related genes. In line with these theories, we have identified *MGF505-7R* and *H240R* as key virulence genes determining the pathogenicity of the ASFV HLJ/18 strain. Deletion of *MGF505-7R*, *H240R* alone or both attenuate the virulence of ASFV HLJ/18 in pigs, providing a paradigm for developing attenuated live vaccines from basic research results.

Previous studies have shown that ASFV effectively evades the host's antiviral innate immune responses, and several members of the multigene family 360 (*MGF360*) and *MGF505* strongly inhibit IFN-β production. Recently, Li et al. and others demonstrated that *MGF-505-7R* is a multifunctional gene that inhibits the cGAS-STING signaling pathway (Li et al., 2021a; Li J et al., 2021; Yang et al., 2022), IFN-JAK-STAT signaling pathway (Li et al., 2021b), and NLRP3-dependent inflammatory responses (Li J. et al., 2021), indicating that *MGF-505-7R* is also an immunomodulatory gene involved in antiviral innate immune responses. pMGF505-7R suppresses the production of type I IFN and the expression of IFN-stimulated genes (ISGs). ASFV pMGF505-7R interacts with and inhibits the nuclear translocation of IRF3 to block type I IFN production. In addition, pMGF505-7R is found to interact with IRF7 and TBK1, promoting their degradation to inhibit type I IFN production (Yang et al., 2022). Furthermore, pMGF505-7R interacts with IRF9, leading to the inhibition of the nuclear translocation of ISGF3 and subsequent suppression of *ISGs* expression (Fig. 1, left). Recently, pMGF505-7R is found to interact with JAK1 and JAK2, mediating their degradation to inhibit IFN-γ-mediated JAK-STAT1 signaling (Fig. 1, center) (Li et al., 2021a). Previous studies have demonstrated that ASFV infection can be recognized by TLRs, which recruit MYD88 to induce the formation of the IKK complex, resulting in the phosphorylation and degradation of IκB. Finally, phosphorylated p65 transfers into the nucleus to initiate the expression of pro-interleukin-1β (pro-IL-1β) and other proinflammatory cytokines. pMGF505-7R interacts with NF-κB essential modulator (NEMO) to block the formation of the IKK complex. Subsequently, pMGF505-7R interacts with NLRP3 to inhibit NLRP3 inflammasome formation, leading to decreased IL-1β production (Fig. 1, right) (Li J. et al., 2021).

As a capsid protein, pH240R interacts with the major capsid protein p72, which is essential for ASFV icosahedral capsid formation and infectious particle production. Deletion of pH240R also affects the morphogenesis of ASFV toward the icosahedral capsid in the process of assembly. Recently, *H240R* is identified as another multifunctional gene that regulates the production of type I IFN, ISGs, and IL-1β. Mechanistically, pH240R interacts with IFN gene stimulating factor (STING) and inhibits its oligomerization and translocation from the endoplasmic reticulum to the Golgi apparatus, which inhibits the phosphorylation of TBK1 and IRF3, leading to reduced production of type I IFN (Fig. 1, left).

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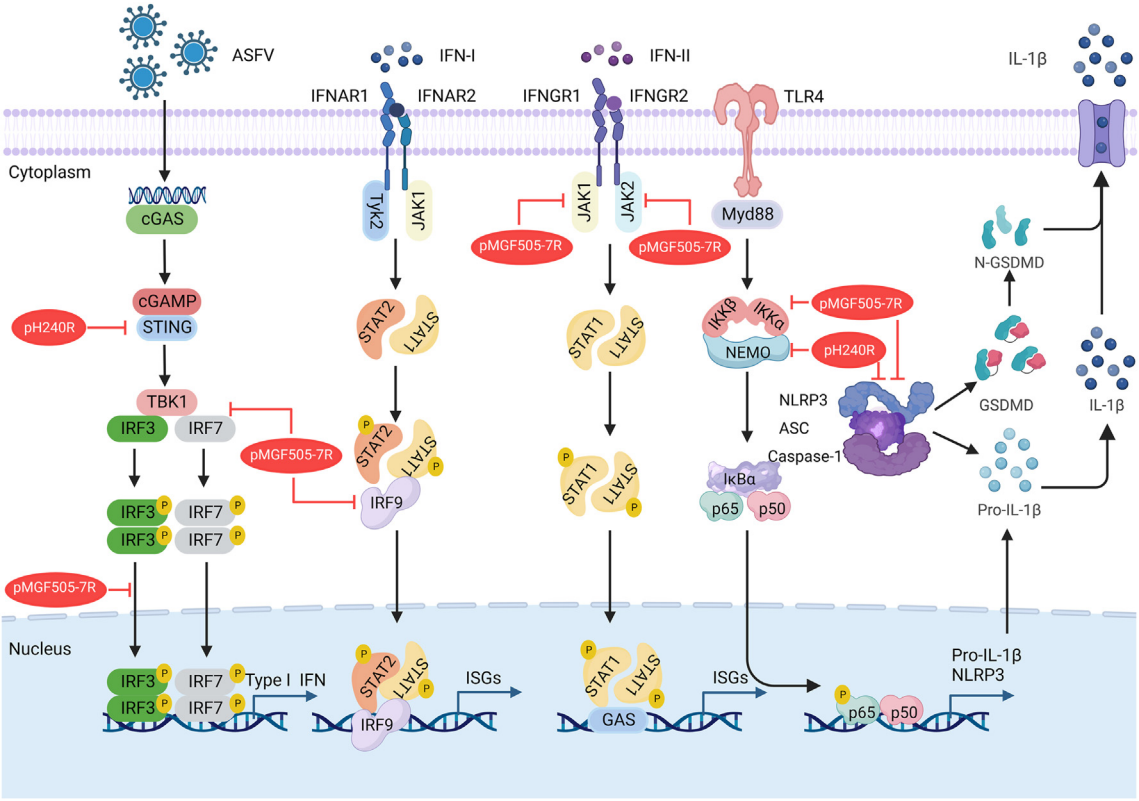

Fig. 1. MGF505-7R and H240R inhibit the production of type I IFN, ISGs and IL-1β.

pH240R interacts with NEMO, leading to inhibition of the formation of the IKK complex, thereby inhibiting the activation of the NF-κB signaling pathway and the transcription of pro-IL-1β. pH240R interacts with NLRP3 to inhibit NLRP3-dependent inflammasome assembly and oligomerization of ASC and ultimately inhibits the maturation and secretion of IL-1β (Huang et al., 2023) (Fig. 1, right). Consistently, Zhou et al. have also confirmed that pH240R inhibits the NF-κB signaling pathway by interacting with NEMO and promotes the autophagy-mediated lysosomal degradation of NEMO, resulting in a reduction in pro-IL-1β transcription. pH240R interacts with NLRP3 to inhibit its oligomerization, leading to decreased IL-1β production (Zhou et al., 2022). Taken together, *H240R* is also a multifunctional gene, regulating host innate immune responses. Interestingly, deletion of *H240R* gene alone attenuates the virulence of ASFV HLJ/18 in pigs (Huang et al., 2023; Ramirez-Medina et al., 2023).

As it is well known, development of a safe and effective vaccine is the best way to prevent and control ASF. Among all types of vaccines that have been tested, the live attenuated vaccine of ASFV can provide the best protection efficiency, but its safety is widely debated. Previous studies have indicated that the pathogenicity of ASFV is related to its ability to escape host antiviral immune responses. Consequently, some scientists have attempted to delete these multifunctional genes associated with the virulence of ASFV, providing a new strategy to develop ASF vaccines. Li et al. has generated a recombinant ASFV-GZ201801-ΔMGF505-7R by removing the *MGF505-7R* gene from the genome of the ASFV GZ201801 strain (Li et al., 2021a). Compared to its parental strain, the virulence of the recombinant ASFV-GZ201801-ΔMGF505-7R is attenuated in pigs, providing 100% protection efficiency. Recently, another recombinant ASFV lacking the *MGF505-7R* gene (ASFV-HLJ18-ΔMGF505-7R) is also generated using ASFV HLJ/18 as the parental virus. ASFV-HLJ/18-ΔMGF505-7R infection induces higher levels of IL-1β and IFN-β than its parental strain. The virulence of ASFV-HLJ/18-ΔMGF505-7R is also reduced in pigs, providing 80% protection efficiency (Li J. et al., 2021), which may be due to the increased production of IL-1β and type I IFN to suppress ASFV replication *in vivo*.

Huang et al. found that the titer of ASFV HLJ/18-ΔH240R in porcine alveolar macrophages (PAMs) has an approximately 2-log decrease compared to that of the ASFV HLJ/18 strain. Strikingly, NF-κB signaling and the NLRP3 inflammasome are markedly activated, leading to higher IL-1β secretion in PAMs upon ASFV-HLJ/18-ΔH240R infection compared to ASFV-WT. Additionally, knockdown of NLRP3 expression in PAMs inhibits ASFV-HLJ/18-ΔH240R infection-induced IL-1β secretion and caspase-1 activation (Zhou et al., 2022). Specific pathogen-free pigs infected with 10^3 HAD_{50} of ASFV-HLJ/18-ΔH240R showed normal body temperature and no obvious clinical symptoms, with all surviving at 21 days post infection (dpi); among pigs infected with 10^5 HAD_{50} of ASFV-HLJ/18-ΔH240R, two pigs (2/5) showed a transient increase in body temperature, and one pig (1/5) died at 21 days dpi. Since the *H240R gene*-encoded pH240R is a structural protein, deletion of the *H240R* gene not only weakens the virulence of ASFV but also reduces the horizontal transmission of ASFV and the production of infectious progeny viruses (Zhou et al., 2022).

We hypothesized that the recombinant of ASFV with deletion of the *MGF505-7R* and *H240R* will lose its pathogenicity, resulting in the development a safer and more effective ASF vaccine. Therefore, a new recombinant ASFV-ΔH240R-Δ7R was generated by deleting both the *H240R* and *MGF505-7R* genes. We found that the virulence of this ASFV mutant is completely attenuated. Piglets immunized with 10^3 and 10^5 HAD50 of ASFV-ΔH240R-Δ7R did not exhibit obvious clinical symptoms during the entire 28-day observation period. Additionally, piglets immunized with ASFV-ΔH240R-Δ7R are able to lethal homologous ASFV challenge without exhibiting any visible pathological damage or changes (Li et al., 2023). Overall, as an attenuated live vaccine candidate, ASFV-ΔMGF505-7R-ΔH240R demonstrated 100% protection efficiency, making it a safer and more effective vaccine compared to

ASFV-ΔMGF505-7R and ASFV-ΔH240R strains, which only exhibited 80% protection efficiency.

In clinical settings, pigs immunized with naturally attenuated ASFV strains or genetically engineered live attenuated vaccine candidates may have viremia or lower levels of viral replication in lymph nodes, providing incomplete or complete protection. It is worth noting that pigs immunized with the attenuated live vaccine are more difficult to detect than ASFV-WT. Therefore, live attenuated vaccine candidates can rapidly spread throughout the entire herd, leading to a decline in the reproductive performance of sows. Recently, several recombinant strains of genotypes I and II have been isolated in China (Zhao et al., 2023). Based on the *B646L* gene, these recombinant strains are classified as genotype I, despite having ten discrete fragments derived from genotype II ASFV strains. Notably, animal studies indicate that one of the recombinant viruses has high lethality and transmissibility in pigs. Of note, ASFV-7GD, an attenuated live vaccine derived from the genotype II ASFV strain, could not provide protection against the challenge of virulent recombinant ASFV (Zhao et al., 2023). These naturally occurring recombinant ASFV strains of genotypes I and II pose a challenge to the global pig industry; therefore, scientists should develop a safe and effective vaccine by identifying and confirming the key virulence genes of the recombinant ASFV strains of genotypes I and II.

Footnotes

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The authors declare that they have no conflict of interest with the contents of this article.

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