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Harnessing Gene Editing Tools to Study ASFV Pathogenesis

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Abstract The study utilizes advanced gene editing tools, specifically the CRISPR/Cas9 system, to investigate the pathogenesis of African Swine Fever Virus (ASFV) by creating recombinant virus strains with targeted gene deletions. The study successfully demonstrated the application of CRISPR/Cas9 to delete key immune response modulation genes (A238L, EP402R, and 9GL) in ASFV. The reconstituted virus exhibited similar replication kinetics to the parent virus, indicating that these genes can be modified with low frequency. Additionally, the use of CRISPR/Cas9 significantly accelerated the production of recombinant ASFV strains, reducing the time required from several months to less than two months. The study also highlighted the potential of CRISPR/Cas12a for sensitive and specific detection of ASFV, which could be crucial for on-site diagnostics and control of ASF outbreaks. The findings underscore the utility of CRISPR/Cas9 and CRISPR/Cas12a systems in both the study of ASFV pathogenesis and the development of rapid diagnostic tools. These advancements could pave the way for more effective control measures and the potential development of live-attenuated vaccines for ASFV.

Keywords African Swine Fever Virus; CRISPR/Cas9; Gene editing; Pathogenesis; Diagnostic tools; Recombinant virus; ASFV detection

1 Introduction

African Swine Fever Virus (ASFV) is a highly contagious and often lethal virus that causes hemorrhagic fever in domestic pigs and wild boar. The disease is characterized by high mortality rates, leading to severe economic consequences for the global swine industry. ASFV has been identified as one of the most significant threats to animal farming, with major outbreaks reported in Eastern Europe, Asia, and recently in India (Cackett et al., 2020; Senthilkumar et al., 2022). The virus's ability to spread rapidly and the lack of effective vaccines or antiviral treatments exacerbate its impact, making it a critical concern for animal health and food security worldwide (O'Donnell et al., 2016; Cackett et al., 2020).

Controlling ASFV outbreaks is challenging due to several factors. Firstly, there are no commercially available vaccines or antiviral drugs to combat the virus, necessitating the culling of infected animals as the primary control measure (Cackett et al., 2020; Gladue et al., 2020). Secondly, the virus's complex genome and its ability to evade the host immune response complicate the development of effective vaccines (Gallardo et al., 2018; Bosch-Camós et al., 2021). Additionally, the virus's persistence in the environment and its ability to infect wild boar populations make eradication efforts difficult (Gallardo et al., 2023). These limitations highlight the urgent need for advanced research to understand the virus's biology and develop novel strategies for its control (O'Donnell et al., 2016; Cackett et al., 2020).

Gene editing tools, such as CRISPR/Cas9, have revolutionized the field of virology by enabling precise modifications of viral genomes. These tools are particularly relevant for studying ASFV pathogenesis, as they allow researchers to investigate the functions of specific viral genes and their roles in virulence and immune evasion (O'Donnell et al., 2015; 2016). For instance, the deletion of certain ASFV genes has been shown to attenuate the virus, providing insights into potential vaccine candidates (O'Donnell et al., 2015; 2016; Gladue et al., 2020). By leveraging gene editing technologies, researchers can dissect the molecular mechanisms underlying ASFV infection and identify novel targets for therapeutic intervention (O'Donnell et al., 2016; Teklue et al., 2020).

This research aims to explore the potential of gene editing tools to enhance our understanding of ASFV pathogenesis and aid in the development of new treatments. By utilizing CRISPR/Cas9 and other gene editing technologies, we seek to identify key viral genes involved in virulence and immune evasion, and to develop attenuated virus strains that could serve as vaccine candidates. Additionally, we aim to investigate the molecular interactions between ASFV and host cells to uncover novel therapeutic targets. Ultimately, this research will contribute to the development of effective strategies to control and prevent ASFV outbreaks, thereby safeguarding global swine populations and the swine industry.

2 ASFV Genomic Structure and Key Genes

2.1 Overview of ASFV genome

African swine fever virus (ASFV) is a large, double-stranded DNA virus with a genome size ranging from approximately 170 to 193 kilobase pairs (kbp), depending on the isolate (Dixon et al., 2013; Wang et al., 2021). The ASFV genome is characterized by its complex structure, which includes covalently closed termini with imperfectly base-paired hairpin loops and inverted arrays of tandem repeats adjacent to these termini. The genome contains between 150 and 167 open reading frames (ORFs) that are closely spaced and read from both DNA strands (Dixon et al., 2013). ASFV's genome encodes a variety of enzymes necessary for transcription and replication, as well as structural proteins and factors involved in evading host defense mechanisms (Dixon et al., 2013; Wang et al., 2021). The virus replicates predominantly in the cytoplasm of infected cells, forming perinuclear factory areas where DNA replication begins approximately 6 hours post-infection (Dixon et al., 2013).

2.2 Identification of key genes involved in pathogenesis

ASFV encodes several key genes that play crucial roles in its replication and pathogenesis. Notable among these are genes involved in immune evasion, such as A238L, which regulates NF-κB and NFAT pathways, and A224L, an apoptosis inhibitor. The EP153R gene modulates MHC-I antigen presentation, while A276R is involved in modulating type I interferon (IFN) responses (Correia et al., 2013; Gallardo et al., 2018). Additionally, genes from multigene families (MGFs) such as MGF360 and MGF530 have been identified as important for the virus's ability to evade the host's innate immune response. The A276R gene from MGF360, for example, inhibits the induction of IFN-β by targeting IRF3 (Correia et al., 2013). These genes collectively contribute to the virus's ability to replicate efficiently and evade host immune defenses, thereby enhancing its virulence.

2.3 Challenges in targeting ASFV genes

Editing the ASFV genome presents significant challenges due to its large size and complex structure. The virus's genome contains numerous closely spaced ORFs and repetitive sequences, which complicate the precise targeting of specific genes (Dixon et al., 2013; Wang et al., 2021). Additionally, the use of genome editing tools such as CRISPR-Cas9 in ASFV is hindered by potential off-target effects, which can lead to unintended mutations and affect the virus's overall fitness and pathogenicity (Chen and Gonçalves, 2015). The development of effective genome editing strategies for ASFV requires careful consideration of these factors to minimize off-target effects and ensure the accurate modification of target genes. Furthermore, the high genetic diversity and variability among different ASFV isolates add another layer of complexity to genome editing efforts (Malogolovkin and Kolbasov, 2019; Wang et al., 2020).

3 Application of CRISPR/Cas9 in ASFV Research

3.1 Mechanism of CRISPR/Cas9

The CRISPR/Cas9 system, originally derived from the adaptive immune system of bacteria, has revolutionized genome editing due to its simplicity, efficiency, and precision. The mechanism involves two key components: the Cas9 nuclease and a single-guide RNA (sgRNA). The sgRNA is designed to match a specific DNA sequence in the target genome. When introduced into a cell, the sgRNA guides the Cas9 enzyme to the complementary DNA sequence, where Cas9 induces a double-strand break (DSB) (Manghwar et al., 2019; Rodriguez-Rodriguez et al., 2019). The cell's natural repair mechanisms then attempt to repair the DSB, often resulting in insertions or deletions (indels) that can disrupt the gene, or precise modifications if a repair template is provided (Bortesi and Fischer, 2015; Manghwar et al., 2020).

3.2 Targeting ASFV genes with CRISPR/Cas9

In the context of African Swine Fever Virus (ASFV) research, CRISPR/Cas9 has been employed to target several key viral genes. These include genes responsible for viral replication, virulence, and immune evasion. For instance, targeting the p72 gene, which encodes a major capsid protein, has shown promise in disrupting viral assembly and replication (Lin et al., 2021). Another target is the B646L gene, which encodes the ASFV major capsid protein p72, crucial for virus infectivity (Adli, 2018).

Recent studies have demonstrated the successful application of CRISPR/Cas9 in editing ASFV genes, leading to attenuated viral strains that could serve as potential vaccine candidates. For example, the deletion of the DP71L gene, which is involved in inhibiting host immune responses, resulted in a virus with reduced virulence, providing insights into ASFV pathogenesis and potential vaccine development (Li et al., 2021; Lin et al., 2021). These advancements highlight the potential of CRISPR/Cas9 not only in understanding ASFV biology but also in developing effective control measures against the virus.

3.3 Limitations and challenges

Despite its potential, the application of CRISPR/Cas9 to ASFV research faces several technical challenges. One major issue is the delivery of the CRISPR/Cas9 components into ASFV-infected cells, which can be complicated by the virus's ability to evade host defenses and the lack of efficient delivery systems (Lino et al., 2018). Additionally, off-target effects, where the Cas9 enzyme cuts at unintended sites, can lead to unwanted mutations and complicate the interpretation of results (Liu et al., 2016; Manghwar et al., 2020) (Figure 1).

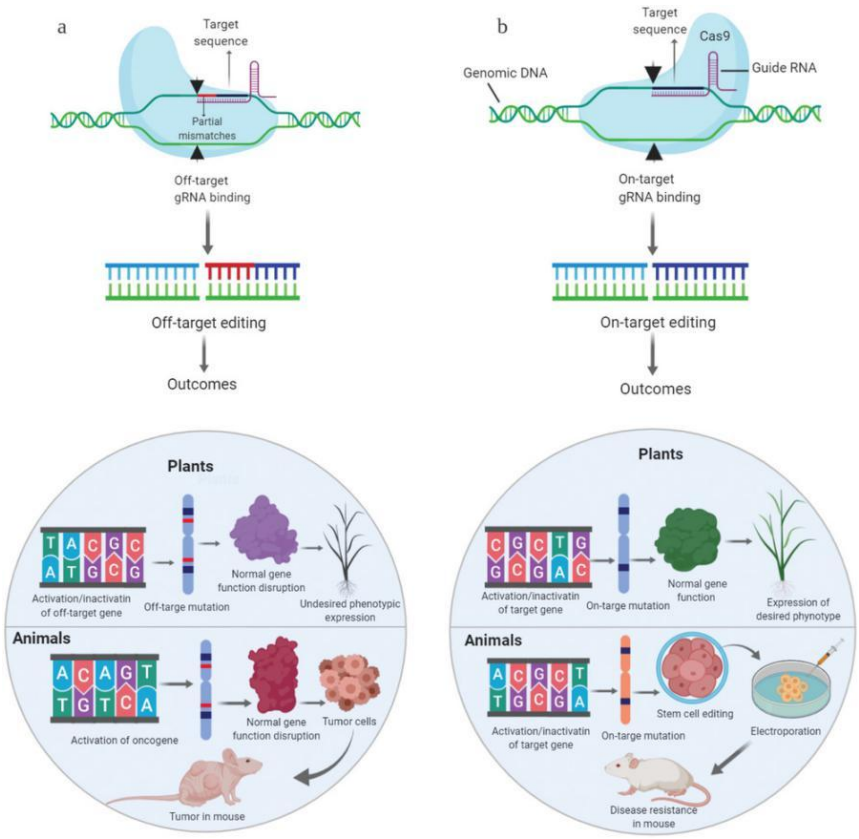

Figure 1 Major concerns/outcomes of off-target effects (Adopted from Manghwar et al., 2020)

Image caption: CRISPR/Cas systems usually offer great potential in genome editing, but off-target activity, causing unintended consequences, is limiting its applications for therapeutic and agricultural purposes. a) CRISPR/Cas9 with a specific sgRNA may sometimes bind and edit at a site other than its target sequence, known as off-target editing. This may result in unexpected serious consequences, such as the activation/inactivation of off-target genes which can result in lethal or undesired phenotypes, or the activation of oncogenes causing cancer in animals. b) CRISPR/Cas9 that accurately edits its target gene is termed on-target editing. CRISPR/Cas9 has been used in wide range of plants and animals due to its robust on-target editing efficiency. On-target editing leads to desired targeted phenotypes. Created with BioRender.com (Adopted from Manghwar et al., 2020)

The use of CRISPR/Cas9 in ASFV research also raises ethical and biosafety concerns. The potential for creating genetically modified viruses necessitates stringent biosafety protocols to prevent accidental release and ensure that modified viruses do not pose a greater threat than the wild-type strains (Sharma et al., 2020). Moreover, ethical considerations regarding the use of gene editing in animals and the potential long-term impacts on ecosystems and biodiversity must be carefully evaluated (Lino et al., 2018; Li et al., 2021).

4 Use of TALENs and Other Gene Editing Tools

4.1 TALENs (transcription activator-like effector nucleases)

4.1.1 How TALENS differ from CRISPR/Cas9 in ASFV research

TALENs and CRISPR/Cas9 are both powerful tools for genome editing, but they have distinct mechanisms and applications. TALENs are composed of a DNA-binding domain derived from transcription activator-like effectors and a nuclease domain, typically FokI, which induces double-strand breaks at specific DNA sequences. In contrast, CRISPR/Cas9 uses a guide RNA to direct the Cas9 nuclease to the target DNA sequence, where it creates double-strand breaks (Demirci et al., 2018; Gupta et al., 2019; Janik et al., 2020).

One of the key differences in ASFV research is the specificity and off-target effects. TALENs are known for their high specificity due to the modular nature of their DNA-binding domains, which can be customized to recognize long DNA sequences, reducing off-target effects. CRISPR/Cas9, while easier to design and implement, can have higher off-target effects, although various strategies have been developed to mitigate these (Manghwar et al., 2020; Naeem et al., 2020). Additionally, TALENs can target mitochondrial DNA, which is challenging for CRISPR/Cas9 due to difficulties in importing guide RNA into mitochondria (Bhardwaj and Nain, 2021).

4.1.2 Case studies of TALENs in ASFV gene editing

TALENs have been successfully used in various studies to edit genes in ASFV, demonstrating their utility in understanding the virus's pathogenesis. For instance, TALENs have been employed to knock out specific genes in ASFV to study their roles in viral replication and virulence. These studies have provided insights into the genetic factors that contribute to the virus's ability to infect and cause disease in swine (Bhardwaj and Nain, 2021). Moreover, TALENs have been used to create ASFV-resistant pig models by targeting and modifying genes associated with susceptibility to the virus, showcasing their potential in developing disease-resistant livestock (Li et al., 2020).

4.2 Other emerging gene editing technologies

4.2.1 Overview of other gene editing tools like ZFNs and base editors

In addition to TALENs and CRISPR/Cas9, other gene editing technologies such as Zinc Finger Nucleases (ZFNs) and base editors have emerged. ZFNs are engineered proteins that combine a zinc finger DNA-binding domain with a FokI nuclease domain, similar to TALENs. They are highly specific but more challenging to design and construct compared to CRISPR/Cas9 (Eş et al., 2019; Gupta et al., 2019).

Base editors, on the other hand, are a newer class of gene editing tools that enable precise nucleotide changes without inducing double-strand breaks. They use a modified Cas protein fused to a deaminase enzyme to convert specific bases (e.g., cytosine to thymine) at targeted sites. This technology offers a high degree of precision and is particularly useful for correcting point mutations (Naeem et al., 2020).

4.2.2 Potential applications in ASFV research

The potential applications of these emerging gene editing technologies in ASFV research are vast. ZFNs can be used to create specific gene knockouts or insertions in the ASFV genome, aiding in the identification of viral genes critical for infection and replication. This can lead to the development of targeted antiviral therapies (Gupta et al., 2019; Li et al., 2020).

Base editors offer the possibility of correcting point mutations in the ASFV genome, which could be used to study the effects of specific genetic changes on the virus's behavior and pathogenicity. This precise editing capability can also be applied to develop ASFV-resistant pig models by correcting susceptibility-related mutations in the host genome (Naeem et al., 2020).

5 Insights Gained from Gene Editing Studies

5.1 Understanding ASFV pathogenesis

Gene editing tools, particularly CRISPR/Cas systems, have significantly advanced our understanding of African swine fever virus (ASFV) pathogenesis. By enabling precise manipulation of the ASFV genome, researchers have been able to identify and characterize the functions of various viral genes. For instance, the use of CRISPR/Cas9 has facilitated the identification of ASFV genes involved in immune evasion, such as those that inhibit the interferon response, which is crucial for the host's antiviral defense (Correia et al., 2013). Additionally, high-throughput proteomic analyses have revealed the interactome of key ASFV proteins, shedding light on the molecular pathways exploited by the virus during infection, such as intracellular and Golgi vesicle transport, endoplasmic reticulum organization, and lipid metabolism (García-Dorival et al., 2023) (Figure 2). These insights are pivotal for understanding the complex mechanisms of ASFV pathogenesis and identifying potential therapeutic targets.

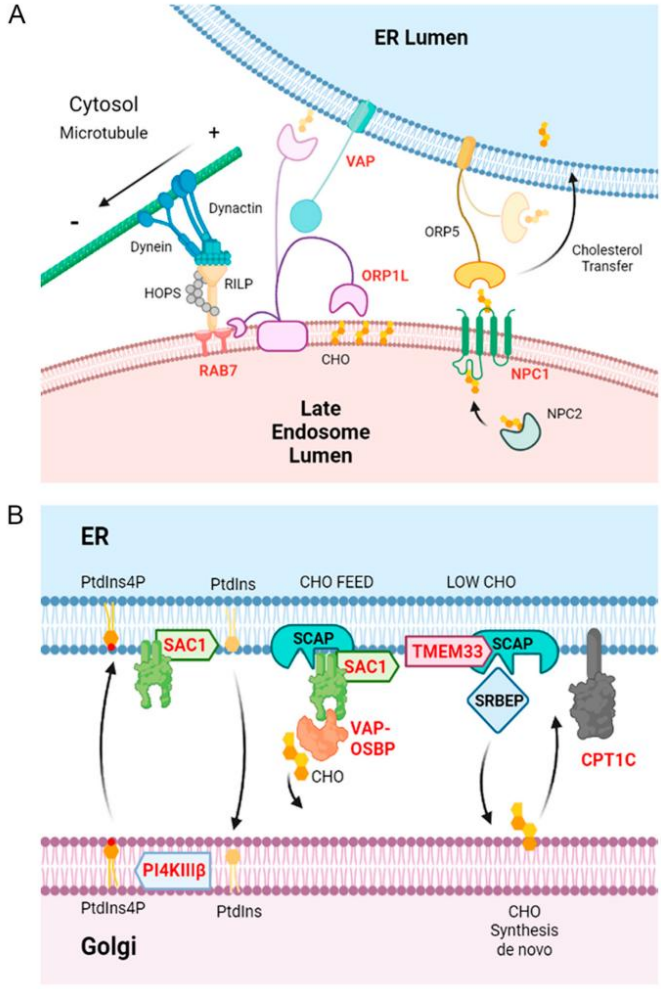

Figure 2 ASFV interactome significant hits related to Golgi transport and ER pathways (Adopted from García-Dorival et al., 2023)

Image caption: ER membrane contacts are important in the control of membrane trafficking and regulation of intracellular organelles. ASFV interactome significant hits are highlighted in bold letters in the schematics. (A). Upon stress and high cholesterol content, endosomes and lysosomes move to a perinuclear location clustered around the microtubule-organizing center together with vesicles of the trans-Golgi network (TGN). This movement is orchestrated by ER VAP that regulates the association (or dissociation) of ORP1L, in complex with RILP, Rab7, and ORP1L and the HOPS complex through microtubule motor dynein, as shown in panel (A). (B). Lipid transfer proteins regulate cholesterol transfer between ER and Golgi membranes, mediated by SACM1, OSBP and VAP. SACM1 is an ER-resident phosphatase that dephosphorylates PtdIns4P in the ER. It is also regulated by the carnitine palmitoyltransferase (CPT) (Adopted from García-Dorival et al., 2023)

5.2 Identification of potential drug targets

Gene editing has been instrumental in pinpointing specific ASFV proteins and pathways that can serve as potential drug targets. For example, the identification of ASFV proteins such as P34, E199L, MGF360-15R, and E248R, which are involved in critical steps of the viral life cycle, has opened new avenues for antiviral drug development. These proteins interact with host cellular machinery, including Rab proteins that regulate the endocytic pathway, making them attractive targets for therapeutic intervention (García-Dorival et al., 2023). Furthermore, the discovery of ASFV genes that manipulate the interferon response, such as A276R, A528R, and I329L, highlights their potential as targets for antiviral drugs aimed at enhancing the host immune response (Correia et al., 2013). These findings underscore the power of gene editing in identifying and validating novel drug targets for ASFV.

5.3 Implications for vaccine development

Gene editing technologies hold significant promise for informing the design and development of effective ASFV vaccines. By enabling the precise deletion or modification of specific viral genes, researchers can create attenuated virus strains that elicit a robust immune response without causing disease. For instance, the deletion of ASFV genes involved in immune evasion, such as those that inhibit the interferon response, could lead to the development of attenuated virus strains that are more immunogenic and capable of inducing protective immunity (Correia et al., 2013). Additionally, the identification of key antigenic regions on ASFV structural proteins, such as p54, through epitope mapping studies, provides valuable information for the design of subunit vaccines that target these critical regions (Petrovan et al., 2020). These advancements highlight the potential of gene editing to revolutionize ASFV vaccine development and contribute to the control and eradication of this devastating disease.

6 Case Studies and Current Research

6.1 Case study 1: CRISPR/Cas9 and ASFV resistance in pigs

Recent advancements in gene editing technologies, particularly the CRISPR/Cas9 system, have shown promise in developing resistance to African swine fever virus (ASFV) in pigs. One notable study attempted to create a recombinant ASFV strain by deleting specific genes associated with the virus's ability to evade the host immune response. The genes A238L, EP402R, and 9GL were targeted using the CRISPR/Cas9 system. The modified virus demonstrated similar replication kinetics to the parent virus, indicating the potential of CRISPR/Cas9 in developing ASFV-resistant strains (Woźniakowski et al., 2020). Another study successfully generated a vaccine prototype by deleting the EP402R (CD2v) and A238L genes from the ASFV Arm/07/CBM/c2 strain using CRISPR/Cas9, which proved to be safe and fully protective against a virulent Korean Paju strain (Pérez-Núñez et al., 2022).

6.2 Case study 2: gene editing to study ASFV-host interactions

Gene editing tools have also been instrumental in elucidating the interactions between ASFV and its host. For instance, the deletion of ASFV genes DP148R, DP71L, and DP96R from the highly virulent ASFV CN/GS/2018 strain resulted in significant attenuation of the virus. This mutant strain not only provided complete protection against homologous challenges in pigs but also revealed important insights into host-virus interactions. RNA sequencing analysis showed that the deletion of these genes led to the upregulation of the host histone H3.1 gene and downregulation of the ASFV MGF110-7L gene, highlighting potential targets for future therapeutic strategies (Qi et al., 2023).

6.3 Case study 3: high-throughput screening of ASFV genes

High-throughput screening methods have been employed to identify critical ASFV genes that could serve as targets for vaccine development. One study developed a new live-attenuated vaccine candidate by deleting the H240R and MGF505-7R genes from the ASFV HLJ/18 strain. This mutant strain exhibited decreased viral titers in porcine alveolar macrophages and provided 100% protection in piglets against a virulent ASFV challenge, demonstrating the effectiveness of high-throughput gene screening in identifying potential vaccine candidates (Li et al., 2023) (Figure 3). Additionally, the use of CRISPR-Cas12a coupled with nucleic acid amplification has been optimized for the sensitive detection of ASFV, showcasing the potential of high-throughput screening in diagnostic applications (Tao et al., 2020).

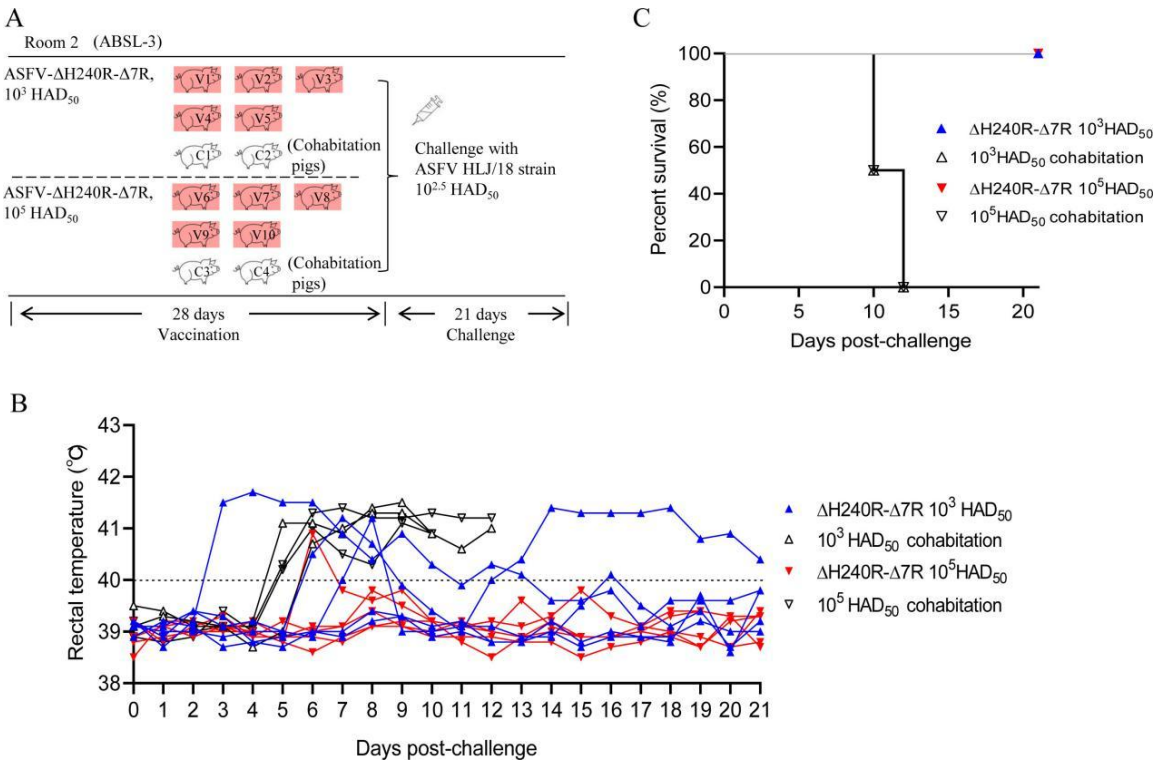

Figure 3 Piglets immunized with ASFV-ΔH240R-Δ7R provide protection against lethal challenge of ASFV HLJ/18 (Adopted from Li et al., 2023)

Image caption: (A) Schematic diagram of animal experiment design. The ASFV-ΔH240R-Δ7R-immunized piglets and cohabiting piglets (C1, C2, C3, and C4) were challenged with a virulent parental ASFV HLJ/18 as indicated. (B) Rectal temperatures of piglets in the virulent challenge. (C) Survival rate of piglets in the virulent challenge (Adopted from Li et al., 2023)

7 Future Directions and Potential of Gene Editing in ASFV Research

7.1 Advancements in gene editing technologies

Recent advancements in gene editing technologies, particularly the development of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR/Cas9 system, have revolutionized the field of genetic research. These tools allow for precise and targeted modifications of the genome, which is crucial for studying the pathogenesis of African swine fever virus (ASFV). The CRISPR/Cas9 system, in particular, has gained widespread popularity due to its simplicity, efficiency, and cost-effectiveness (Gupta and Shukla, 2017; Li et al., 2020; Hawsawi et al., 2022). The ability to introduce site-specific double-stranded DNA breaks has enabled researchers to create more accurate models of ASFV infection and to identify key viral and host factors involved in the disease process (Mahas et al., 2017; Manghwar et al., 2019). Future advancements in these technologies, such as improved delivery methods and reduced off-target effects, will further enhance their utility in ASFV research (Yin et al., 2017; Serajian et al., 2021).

7.2 Integration of multi-omics approaches

The integration of multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, with gene editing technologies holds great promise for advancing our understanding of ASFV pathogenesis. By combining these approaches, researchers can obtain a comprehensive view of the molecular mechanisms underlying ASFV infection and identify potential therapeutic targets. For instance, CRISPR/Cas9-mediated gene knockouts can be used to study the function of specific genes in the context of ASFV infection, while transcriptomic and proteomic analyses can provide insights into the changes in gene expression and protein levels that occur during infection (Rui et al., 2019; Pavlovic et al., 2020). This holistic approach will enable the identification of novel biomarkers and therapeutic targets, paving the way for the development of more effective ASFV treatments (Ma and Liu, 2015; Manghwar et al., 2019).

7.3 Towards personalized ASFV therapies

The ultimate goal of ASFV research is to develop personalized therapies that can effectively combat the virus. Gene editing technologies offer a powerful tool for achieving this goal by enabling the precise modification of the host genome to enhance resistance to ASFV infection. For example, CRISPR/Cas9 can be used to disrupt viral entry receptors or to enhance the expression of antiviral genes in pigs, thereby conferring resistance to ASFV (Mahas et al., 2017; Li et al., 2020). Additionally, the use of gene editing to create transgenic pigs with enhanced immune responses to ASFV is a promising avenue for future research (Gupta and Shukla, 2017; Yin et al., 2017). Personalized ASFV therapies will require a deep understanding of the genetic and molecular factors that influence susceptibility to the virus, which can be achieved through the integration of gene editing technologies with multi-omics approaches (Rui et al., 2019; Pavlovic et al., 2020). As these technologies continue to advance, the development of personalized ASFV therapies will become increasingly feasible, offering new hope for the control and eradication of this devastating disease.

8 Ethical, Regulatory, and Biosafety Considerations

8.1 Ethical concerns in gene editing

The advent of gene editing technologies, particularly CRISPR/Cas9, has revolutionized the field of genetic research, offering unprecedented precision and efficiency. However, these advancements come with significant ethical concerns, especially when applied to editing viral and host genomes. One primary ethical issue is the potential for dual-use research, where the same technology used for beneficial purposes could be misused to create harmful biological agents. The modification of viral genomes, such as ASFV, raises concerns about the potential creation of more virulent or transmissible strains, which could pose significant risks to animal and possibly human health (DiEuliis and Giordano, 2017).

Moreover, editing the host genome to enhance resistance to viruses like ASFV involves altering the genetic makeup of animals, which raises questions about animal welfare and the long-term ecological impacts. Ethical considerations must also address the potential for unintended off-target effects, which could lead to unforeseen health issues in the modified organisms (Zhang et al., 2020). The international community has called for stringent ethical standards and guidelines to govern the use of gene editing technologies, emphasizing the need for responsible research practices and the prevention of misuse (DiEuliis and Giordano, 2017; Zhang et al., 2020).

8.2 Regulatory framework for gene editing

The regulatory landscape for gene editing is complex and varies significantly across different regions. In the context of ASFV research, regulatory frameworks must balance the need for scientific advancement with the imperative to ensure safety and ethical compliance. For instance, the European Union's regulatory approach to genome editing in agriculture, which could extend to ASFV research, is characterized by stringent guidelines that aim to prevent potential risks associated with genetic modifications (Jones, 2015). These regulations impact the pace and scope of research by imposing rigorous safety assessments and approval processes.

In contrast, countries like Argentina have adopted more flexible regulatory frameworks that facilitate the rapid development and deployment of gene-edited organisms, provided they do not introduce novel genetic combinations. This approach has been praised for its efficiency and predictability, which could be beneficial for ASFV research by enabling quicker development of gene-edited solutions to combat the virus (Lema, 2019). However, the global disparity in regulatory standards underscores the need for international harmonization to ensure that gene editing technologies are safely and effectively integrated into ASFV research and other applications (Zhang et al., 2020; Pillai and Raybould, 2023).

8.3 Biosafety challenges

Biosafety is a critical consideration in gene editing experiments, particularly when dealing with pathogens like ASFV. Ensuring the safety of such experiments involves multiple layers of oversight and risk management. One of the primary challenges is preventing the accidental release of genetically modified viruses, which could have severe consequences for animal health and the agricultural industry. Laboratories conducting ASFV research must adhere to high biosafety level (BSL) standards, including stringent containment measures and regular safety audits (Pillai and Raybould, 2023).

Additionally, the potential for off-target effects in gene editing necessitates thorough preclinical testing and validation to ensure that unintended genetic changes do not compromise the safety or efficacy of the modified organisms (Li et al., 2019). The involvement of biosafety and biosecurity communities in the regulatory process is essential to address these challenges and develop comprehensive guidelines that mitigate risks while enabling scientific progress. International collaboration and the establishment of best practices are crucial to fostering a safe research environment and preventing the misuse of gene editing technologies (DiEuliis and Giordano, 2017; Pillai and Raybould, 2023).

9 Concluding Remarks

Gene editing tools, particularly CRISPR/Cas9, have significantly advanced our understanding of African swine fever virus (ASFV) pathogenesis. These tools have enabled precise modifications of the ASFV genome, facilitating the study of viral gene functions and interactions. For instance, the use of CRISPR/Cas9 has allowed for the efficient deletion of specific ASFV genes, such as those involved in immune evasion and virulence, providing insights into their roles in the virus's life cycle and pathogenicity. Additionally, the development of multiplex-crRNA strategies has enhanced the sensitivity of ASFV detection, demonstrating the potential of gene editing tools in diagnostic applications.

The future of ASFV research through gene editing holds promising potential for several breakthroughs. One of the primary goals is the development of effective vaccines. By identifying and deleting virulence genes, researchers aim to create attenuated virus strains that can serve as vaccine candidates. For example, the deletion of the CD2v and C-type lectin-like genes from ASFV has been explored, although it highlighted the complexity and unpredictability of genetic manipulations. Another long-term goal is to enhance the precision and efficiency of gene editing techniques to minimize off-target effects, as seen in other applications of gene editing technologies. Furthermore, the integration of CRISPR/Cas systems in diagnostic platforms could revolutionize ASFV detection, making it faster and more accurate.

Continued research and collaboration are crucial in the fight against ASFV. The complexity of the virus and its significant impact on global swine production necessitate a multidisciplinary approach, combining virology, immunology, and biotechnology. Collaborative efforts can accelerate the development of innovative solutions, such as gene-edited vaccines and advanced diagnostic tools. The insights gained from gene editing studies not only enhance our understanding of ASFV but also contribute to the broader field of viral pathogenesis and gene therapy. As we move forward, it is essential to maintain a focus on safety, efficacy, and ethical considerations in the application of gene editing technologies.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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